2.3.1: Student centric methods, such as experiential learning, participative learning and problem solving methodologies are used for enhancing learning experiences using ICT tools

A) Experiential learning

I) Pharm-D Students Internship
II) B.Pharm Project work
III) M.Pharm Project work

The practical/tutorial sessions focus on student requirement and learn from experiences during various learning activities, like lab-postings, seminars, assignments, preparation of posters, Internships, Industrial and hospital visits. Being a professional-course flexibility to move from one discipline to another is not available in undergraduate course.

The B.Pharm and Pharm-D courses are basically interdisciplinary in nature. All the PG departments are involved in teaching B.Pharm subjects. The institute promotes and encourages multi/interdisciplinary research between the departments and institutions. Most of the PG dissertations works are inter-departmental.

Patient-centric and Evidence-Based Learning

The curriculum is designed that student gets his/her professional potency in the subject through expertise teaching. Practical training in labs, patient-encounters, and industrial-visits are some of the initiatives taken for making the student professionally competent. Students undergo

professional-training in clinical-postings in attached hospital. Students are trained in standard clinical practice guidelines and practice in evidence-based medicine.

Project-based learning

The students' projects as part of curriculum are mandatory for their successful completion of the course as per the PCI/University norms.

UG Courses:

B.Pharm

B.Pharm Students carry out project for a duration of 6 months in a team of 3/4 students during final-year as part of their curriculum.

PG Course:

Pharm-D

As per the regulations of PCI and affiliating University Pharm-D students are required to carry out their project work during their V year.

Project work is generally carried out as a team of 2/3 students with the guidance from faculty at college and doctors at attached hospital.

M.Pharm

It is mandatory for all the M.Pharmacy students of all the specializations to carry out a one-year project work as part of their curriculum.

Role Play: Students participate in role play like patient-counseling during their practical hours and also allowed to participate at inter college competitions.

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This is to certify that the dissertation entitled *DESIGN,PREPARE AND CHARACTERIZATION OF TADALAFIL FAST* is a bonafide research work carried out by ASSELLA.SWAPANA Reg. No. 16VMIS0302 under the supervision of **Mr.GUNTURU.NATESH,M.Pham,(Ph.D).** in partial fulfillment of the award for the degree of "Master Of Pharmacy"by the Jawaharlal Nehru Technological University, Hyderabad, Telangana, Hyderabad, AP. This work has not been submitted earlier to any university either in part or in full the award of any degree.

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This is to certify that the investigations described in this thesis entitled **"*FORMULATION AND EVALUATION OF BUCCAL MUCOADHESIVE TABLETS OF REPAGLINIDE*"** submitted to the **Jawaharlal Nehru Technological University - Hyderabad** in partial fulfillment for the award of **MASTER OF PHARMACY** in **PHARMACEUTICS**, by **MOGILI PRAVEEN (Reg. No.15VM1S0313)** is a bonafide research work done under the guidance of **Mrs.N.SHANTHI**,Assistant Professor, Vikas College of Pharmaceutical sciences, Rayanigudem, Suryapet, Nalgonda.

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This is to certify that this dissertation work entitled **"DEVELOPMENT AND CHARACTREIZATION OF PROPRANOLOL GASTRO,RETENTIVE DRUG DELIVERY SYSTEM"** Submitted in partial fulfillment for the award of degree in Master of Pharmacy of JNT University, Hyderabad. is a bonafide work carried out by **N.SARITHA, Reg.No.15VM1S0315** under the guidance of **Y.KRIN KUMAR** during the academic year 2015-2017 . This is original and has not been submitted for any other degree or diploma to this or any other university.

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This is to certify that the dissertation entitled **FORMULATION AND EVALUATION OF NIFEDIPINE ORAL DISPERSIBLE TABLETS** '' submitted in partial fulfillment for the award of degree in **"MASTER OF PHARMACY"** of Jawaharlal Nehru Technological University, Hyderabad, Telangana This is a Bonafide research work carried out by **ALUVALA NAGALAXMI 19VM1S1302**.Under the supervision of **Mr.NATESH.G M-Pharm, (Ph.D)**during the academic year 2019-2021. This is original and has not been submited earlier to any university either in part or in full the award of any degree.

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This is to certify that the dissertation entitled **METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE DETERMINATION OF PITAVASTATIN IN BULK FORM AND TABLET DOSAGE BY RP-HPLC "** submitted in partial fulfillment for the award of degree in **"MASTER OF PHARMACY"** of Jawaharlal Nehru Technological University, Hyderabad, Telangana This is a Bonafide research work carried out by **PANNALA VENNELA 19VM1S1208**.Under the supervision of **P.VENKATESHWARLU M-Pharm, (Ph.D)**during the academic year 2019-2021. This is original and has not been submited earlier to any university either in part or in full the award of any degree.

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This is to certify that the investigations described in this thesis entitled **"FORMULATION AND EVALUATION OF CURCUMINPERIODONTAL FILMS"** submitted to the **Jawaharlal Nehru Technological University - Hyderabad** in partial fulfillment for the award of **MASTER OF PHARMACY** in **PHARMACEUTICS**, by **MD.KARISHMA**, **(Reg. No.16VM1S0315**is a bonafide research work done under the guidance of **Dr.Y. KIRAN KUMAR**, Vikas College of Pharmaceutical sciences, Rayanigudem, Suryapet.

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This is to certify that this dissertation work entitled "**DESIGN,PREPARE AND CHARACTERIZATION OF CARVEDILOL BUCCAL PATCHES.**' Submitted in partial fulfillment for the award of degree in **MASTER OF PHARMACY** of JNT University, Hyderabad, is a bonafide work carried out by **T.PRATHYUSHA, REG.NO : 17VM1S0309,.** under the guidance of **Dr. Y. KIRAN KUMAR** during the academic year 2017-19. This is Original and has not been submitted for any other degree of diploma to this of any Other University.

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This is to certify that the investigations described in this thesis entitled **"DESIGN AND INVITRO CHARACTERIZATION OF MEBENDAZOLE TABLETS FOR COLON DRUG DELIVERY SYSTEM"** submitted to the **Jawaharlal Nehru Technological University - Hyderabad** in partial fulfillment for the award of **MASTER OF PHARMACY** in **INDUSTRIAL PHARMACY**, by **Mr. T.RAMESH, (Reg. No. : 14VM1S0813)** is a bonafide research work done under the guidance of **Dr.Y.Kiran kumar M.pharm.,Ph.D.** Vikas College of Pharmaceutical sciences, Rayanigudem, Suryapet..

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This is to certify that the dissertation entitled **EXTRACTION AND QUANTITAVE ESTIMATION OF PARACETAMOL REMAINED IN EXPERIED PARACETAMOL TABLET I.P** submitted in partial fulfillment for the award of degree of bachelor pharmacy under JNTUH is a bonafied work carriedout by G.LINGARAJU HT.NO: 14VM1R0015 B.KALYANI HT.NO: 14VM1R0004 J.SABITHA HT.NO: 147N1R0010, P.MADHU HT.NO: 14VM1R0034 Under the guidance of **Dr.VENKATESWHWAR RAO,Ph.D** during the academic year 2017-2018.The work is original and has not been submitted in any other university.

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This is to certify that the dissertation entitled "A COMPARATIVE EVALUATION OF QUALITY OF GENERIC AND BRANDED DRUGS OF METOPROLOL TARTRATE" submitted in partial fulfillment for the award of degree in "Bachelor Of Pharmacy" of Jawaharlal Nehru Technological University, Hyderabad, Telangana. This is a Bonafide research work carried out by P.VENNELA - (15VM1R0052), G.ASHISH - (15VM1R0024), A.UMA - (15VM1R0001), V.CHETAN - (15VM1R0080), S.NAGAMANI – (15VM1R0065).Under my supervision of Mrs.NIMMALA SHANTHI Mpharm (ph.d)during the academic year 2018-2019. This is original and has not been submited earlier to any university either in part or in full the award of any degree.

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This is to certificate that the declaration entitle"**PHYTOCHEMICAL SCREENING AND IDENTIFICATION OF ANTI-OBESE PRINCIPLES FROM COCUS NUCIFERA (ARECACEAE) (COCONUT TREE)** " is bonafied research work carried out of by **P.RADHA (15VM1R0053), M.KEERTHANA (15VM1R0042), T.KALYAN (15VM1R0072), B.SUMA (16VM5R0001),** under supervision **Dr. P. VENKATESWAR RAO M.pharm PH.D & Assistant professor** in partial fulfillment for the award of degree of academic year 2018 to 2019 of Pharmacy in jawaharlal Nehreu technological University, Hyderabad and work has been not submitted earlier to any University either in part and full the degree.

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This is to certify that the project work entitled a study on ASSESSMENT OF PHARMACEUTICAL CARE PROVIDED IN CKD PATIENTS AT KIMS HOSPITAL is a bonafied project work done by Ms. Swathi sai Marri, Ms. Bhavani Meesala, Ms. Sravani Mukota in partial fulfilment to the requirement for the degree of **Pharm .D** (Doctor of pharmacy)

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METHOD DEVELOPMENT ,VALIDATION & DEGRADATION STUDIES OF IBUPROFEN BY UV-VISIBLE SPECTROPHOTOMETRIC METHOD

This is submitted to

FACULTY OF PHARMACY

JAWAHARLAL NEHRU TECHNOLOGICAL UNIVERSITY
HYDERABAD (T.S)

In partial fulfillment for the Award of degree of

B.Pharmacy

By

M.LAXMI, 18VM1R0020
M.ARUN, 18VM1R0024
N.VENNELA 18VM1R0027

Under the guidence of

P.VENKATESHWARLU M.Pharm.(Ph.D)
DEPT.OF PHARMACEUTICAL ANALYSIS

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RAYANIGUDEM, SURYAPET
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March 2018-2022

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CERTIFICATE BY PRINCIPAL /HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled " SYNTHESIS CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF CHALCONES BASED ON GREEN CHEMISTRY PRINCIPLES "submitted in partial fulfillment for the award of degree in "Bachelor Of Pharmacy" of Jawaharlal Nehru Technological University, Hyderabad, Telangana. This is a Bonafide research work carried out by CH.ROOPA(18VM1R0008)K.KOMALA(18VM1R0015)A.SAIDEEPIKA(17VM1R0004) Under my supervision of Dr. ADEPU **RAMESH, M.Pharm, Ph.D.** during the academic year 2019-2020. This is original and has not been submited earlier to any university either in part or in full the award of any degree.

Date: 8/7/22

Place: suryapet

Principal

Dr. ADEPU **RAMESH,M.Pharm,Ph.D.**

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CERTIFICATE BY PRINCIPAL/HEAD OF THE INSTITUTION

This is to certify that the thesis entitled **"Analytical Method Development And Validation Of Amlodipine Besylate By UV Spectroscopy"** that is being submitted by K.RAGHAVI(18VM1R0013),B.RAMU(18VM1R0001),K.VINEELA(18VM1R0018)., in partial fulfillment for the award of B.Pharm. In the department of pharmaceutical sciences, Telangana, India, is a record of bonafide work carried out by him under my guidance and supervision. He has carried out the work at the Department of pharmaceutical analysis, Vikas College of Pharmaceutical Sciences Suryapet, Telangana, India., Affiliated to Jawaharlal Nehru Technological University Hyderabad, Telangana. The results embodied in this thesis have not been submitted to any other University or Institute for the award of any degree.

Date

Place

Principal

Dr. Adepu Ramesh M.Pharm Ph.D.,

Vikas College of Pharmaceutical Sciences

Suryapet, Telangana.

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CERTIFICATE OF THE PRINCIPAL

This is to certify that the thesis entitled **REGULATORY REQUIREMENTS OF MEDICAL DEVICES IN INDIA** has been carried out by **BODA SWETHA (189R1R0030), SHAIK SAMI UDDIN (18VM1R0038), KHAJA MUZAMMIL AHMED (18VM1R0016)**, under the guidance of **NATESH .G M.pharm (Ph.D)** for the partial fulfillment of degree Bachelor of Pharmacy. This work has not been submitted earlier in part or full to any other university or college for the award of other degree.

Date: 02/04/2022
Place Suryapet

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CERTIFICATE BY THE HEAD OF THE DEPARTMENT

This is to certify that the dissertation entitled "MI RISK ASSESSMENT IN PATIENTS USING EZ-CVD RISK ASSESSMENT TOOL" submitted in partial fulfillment for the award of degree in "Bachelor Of Pharmacy" of Jawaharlal Nehru Technological University, Hyderabad, Telangana. P.VIVEK KUMAR (18VM1R0034), J.RAJESH (18VM1R0012),B.NAVEEN (18VM1R0004).This is a Bonafide research work carried out by under my supervision of **Dr.ADEPU RAMESH M Pharm, Ph.D.** during the academic year 2021-2022. This is original and has not been submitting for any other degree or diploma to this or any other university.

DATE : 02/07/2022

PLACE : Suryapet

Dr.Adepu Ramesh M Pharm. Ph.D
Principal cum Director
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This is to certify that the thesis entitled **"Analytical Method Development And Validation Of Amlodipine Besylate By UV Spectroscopy"** that is being submitted by K.RAGHAVI(18VM1R0013),B.RAMU(18VM1R0001),K.VINEELA(18VM1R0018)., in partial fulfillment for the award of B.Pharm. In the department of pharmaceutical sciences, Telangana, India, is a record of bonafide work carried out by him under my guidance and supervision. He has carried out the work at the Department of pharmaceutical analysis, Vikas College of Pharmaceutical Sciences Suryapet, Telangana, India., Affiliated to Jawaharlal Nehru Technological University Hyderabad, Telangana. The results embodied in this thesis have not been submitted to any other University or Institute for the award of any degree.

Date

Place

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Dr. Adepu Ramesh M.Pharm Ph.D.,

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CERTIFICATE BY PRINCIPAL /HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled "SYNTHESIS AND BIOLOGICAL EVALUATION OF 4-AMINO BENZAMIDE DERIVATIVES AS ANTIBACTERIAL AGENTS" submitted in partial fulfillment for the award of degree in "Bachelor of Pharmacy" of Jawaharlal Nehru Technological University, Hyderabad, Telangana. This is a Bonafide research work carried out by NAKKALA ANIL KUMAR (18VM1R0028) TRACHULA PRASANNA (18VM1R0041) BYROJU SANDEEP (18VM1R0045) Under the supervision of Dr.P. Naresh., M. Pharm., PhD., SEFM, during the academic year 2018-2022. This is original and has not been submitted earlier to any university either in part or in full the award of any degree.

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Date: 09-07-2022

Place: Suryapet

Dr. ADEPU RAMESH., M. Pharm., PhD.

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This is to certify that the thesis entitled **REGULATORY REQUIREMENTS OF MEDICAL DEVICES IN INDIA** has been carried out by **BODA SWETHA (189R1R0030), SHAIK SAMI UDDIN (18VM1R0038), KHAJA MUZAMMIL AHMED (18VM1R0016)**, under the guidance of **NATESH .G M.pharm (Ph.D)** for the partial fulfillment of degree Bachelor of Pharmacy. This work has not been submitted earlier in part or full to any other university or college for the award of other degree.

Date: 02/09/2022
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CERTIFICATE BY PRINCIPAL / HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled"EFFECT OF NATURAL PENETRATION ENHANCERS ON *IN-VITRO* PENETRATION OF DICLOFENAC SODIUM PATCH" submitted in partial fulfillment for the award of degree in "Bachelor Of Pharmacy" of Jawaharlal Nehru Technological University, Hyderabad, Telangana. B.SRIKANTH(18VMIR005) ,M.KAVYA(18VMIR0021), P.ANUSHA(18VMIR0032).This is a Bonafide research work carried out by Under my supervision of **N.SHANTHI M.pharm,** (Ph.D) during the academic year 2021-2022. This is original and has not been submited earlier to any university either in part or in full the award of any degree.

DATE : 02/7/2022
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CERTIFICATE BY PRINCIPAL /HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled "SOLVENT-FREE RICE HUSK AND LACTIC ACID MEADIATED ONE-POT GREEN SYNTHESIS OF NOVEL IMIDAZOLE AND EVALUATION OF ANTIOXIDANT ACTIVITY" submitted in partial fulfillment for the award of degree in "Bachelor Of Pharmacy" of Jawaharlal Nehru Technological University, Hyderabad, Telangana. Tis is a Bonafide research work carried out by K.PRAVALIKA(18VM1R0014), K.SHIRISHA(18VM1R0017) S.VINAY(18VM1R0039) Under my supervision of **Dr.ADEPU RAMESH, M.Pharm,Ph.D.** during the academic year 2018-2022. This is original and has not been submited earlier to any university either in part or in full the award of any degree.

Date:

Place: suryapet

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CERTIFICATE BY THE PRINCIPAL /HEAD OF THE INSTITUTION

This is to certify that the thesis entitled **METHOD DEVELOPMENT , VALIDATION & DEGRADATION STUDIES OF IBUPROFEN BY UV-VISIBLE SPECTROPHOTOMETRIC METHOD** has been carried out by **M.Laxmi (18VM1R0020),M.Arun (18VM1R0024),N.Vennela (18VM1R0027)**,under the guidance of **P.Venkateshwarlu m.pharm(Ph.D) for the partial fulfillment of degree** Bachelor of Pharmacy. This work has not been submitted earlier in part or full to any other university or college for the award of other degree.

Date: 02/07/22

Place:Suryapet

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CERTIFICATE BY PRINCIPAL / HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled "M.I RISK ASSESSMENT IN PATIENTS USING EZ-CVD RISK ASSESSMENT TOOL" submitted in partial fulfillment for the award of degree in "Bachelor Of Pharmacy" of Jawaharlal Nehru Technological University,Hyderabad, Telangana. P.VIVEK KUMAR (18VM1R0034), J.RAJESH (18VM1R0012), B.NAVEEN (18VM1R0004). This is a Bonafide research work carried out by Under my supervision of Dr.ADEPU RAMESH M Pharm , Ph.D. During the academic year 2021-2022. This is original and has not been submitted earlier to any university either in part or in full the award of any degree.

DA
TE : 02/07/22
PLA
CE : Suryapet

Principal

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DATE : 02/7/2022
PLACE : suryapet.

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CERTIFICATE BY PRINCIPAL /HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled INVITRO COMPARATIVE EVALUATION OF EFFICACY OF ANTI-OXIDANT ACTIVITY OF PLANT EXTRACTS submitted in partial fulfillment for the award of degree in "Bachelor Of Pharmacy" of Jawaharlal Nehru Technological University,Hyderabad, Telangana. This is a Bonafide research work carried out by **P.DEVIKA (18VM1R0029), V. INDU (18VM1R0044)** Under the supervision **Mrs. Shiva Shanmukhi** M.Pharm, during the academic year 2018-2022. This is original and has not been submitted earlier to any university either in part or in full the award of any degree

Date: 8/7/2022
Place:suryapet

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Date: 02/07/22

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2.3.1: Student centric methods, such as experiential learning, participative learning and problem solving methodologies are used for enhancing learning experiences using ICT tools

B) Participative learning

I) Seminar

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34							
35	19VM1R0035	MAMIDI RAMYA	P	P	P	P	P
36	19VM1R0036	MANDA RAJESH	P	P	P	P	P
37	19VM1R0037	MEESALA KALYANI	P	P	P	P	P
38	19VM1R0038	MIDIDDODI SRILEKHA	P	P	P	P	P
39	19VM1R0039	MITTAGADUPU LA SANDEEP	P	P	P	P	P
40	19VM1R0040	MITTAGADUPU LA VARSHITHA	P	A	P	P	P
41	19VM1R0041	MUDIMANIKYA M SWETHA	P	P	P	P	P
42	19VM1R0042	MUTHINENI GOWTHAMI	P	P	P	P	P
43	19VM1R0043	NAKIREKANTI CHAITANYA	P	P	P	P	P
44	19VM1R0044	NALGONDA NAGAMANI	P	P	P	P	P
45	19VM1R0045	NEPPARLA SAI	P	P	P	P	P
46	19VM1R0046	PATHI RAKESH	P	P	P	P	P
47	19VM1R0047	PITTALA ANAND KUMAR	P	P	P	P	P
48	19VM1R0048	RAMISETTY YASHWITHA	P	P	P	P	P
49	19VM1R0049	RAMPALLY KAVYA	P	P	P	P	P
50	19VM1R0050	RAVIPUDI VIJAYALAXMI	P	P	P	P	P
51	19VM1R0051	RESU DHANUSH	P	P	P	P	P
52	19VM1R0052	SARAGANDLA CHANDANA	P	P	P	P	P
53	19VM1R0053	SAYYAD SANA	P	P	P	P	P
54	19VM1R0054	SHAIK SHAHIN	P	P	P	P	P
55	19VM1R0055	SIRA UMA	P	P	P	P	P
56	19VM1R0056	SIVARATHRI SAI KUMAR	P	P	P	P	P
57	19VM1R0057	SUNKARABOIN A MOUNIKA	P	P	P	P	P
58	19VM1R0058	SURA MOUNIKA	P	P	P	P	P
59	19VM1R0059	SURA SRUTHI	P	P	P	P	P
60	19VM1R0060	THADOJU KRISHNAVENI	P	P	P	P	P
61	19VM1R0061	THANGELLA SNEHA	P	P	P	P	P
62	19VM1R0062	VANAM ASHOK	P	P	P	P	P
63	19VM1R0063	VEMPATI MAHESH	P	P	P	P	P
64	19VM1R0064	VEMULA NAVEEN	P	P	P	P	P
65	19VM1R0065	VENNAMALLA VIJAY CHANDAN	P	P	P	P	P
66	19VM1R0066	VOGGU SOWJANYA	P	P	P	P	P
67	19VM1R0067	YARRANAGULA KALAVATHI	P	P	P	P	P
68	19VM1R0068	BANDARI ARCHANA	P	P	P	P	P
69	19VM1R0069	YELLAVULA SRAVANI	P	P	A	P	P
70	19VM1R0070	VUDUGU VENKATESH	P	P	P	P	P

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2.3.1: Student centric methods, such as experiential learning, participative learning and problem solving methodologies are used for enhancing learning experiences using ICT tools

B) Participative learning

I) Seminar

II) Quiz

III) Group-discussion

IV) Industrial visit

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Email : vikassuryapet@gmail.com

2.3.1: Student centric methods, such as experiential learning, participative learning and problem solving methodologies are used for enhancing learning experiences using ICT tools

B) Participative learning

III) Group-discussion

For B.Pharm conducted a group discussion on "Simple and Sensitive method development" for IV year students. 40 members have participated in the event. This program was organized by the concern faculty Dr.Adepu Ramesh. Principal cum director of Vikas College of pharmaceutical sciences.

The total strength was divided into 4 groups. The event was started with an introduction given by the concern faculty as the term" Simple and Sensitive method development" refers to the main difference between UV and HPLC is that the HPLC provides less time of analysis and good results than UV.

During the session all the students were actively involved and gained knowledge about the above said topic. Marks were awarded out of 10 by the concern faculty and the best team was selected as the winner. This was done to motivate the students and make ready for the next challenge.

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Email : vikassuryapet@gmail.com

2.3.1: Student centric methods, such as experiential learning, participative learning and problem solving methodologies are used for enhancing learning experiences using ICT tools

B) **Participative learning**

I) Seminar

II) Quiz

III) Group-discussion

IV) Industrial visit

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C) **Problem solving methodologies**

- Problem-based learning is a regular practice adopted.
- A problem is posed to a group of students and its diagnosis and treatment is invited.
- The students are made to think based on their theoretical and clinical knowledge to arrive at a probable solution.
- This practice gives the students the capabilities of thinking and analyzing various types of cases.
- Conducting tutorial classes for the slow learners
- For the slow learners giving case study to understand the problems.
- Giving assignments and quizzes at the end of instruction of each unit.
- Case Study Analysis and Discussion.
- Product Design and development.

2.3.1. Case studies:

Case study method is adopted in teaching learning process to make the students have logical thinking and practical knowledge to develop problem solving ability.

This is commonly adopted in management programmes as well as while teaching Business law, cyber laws etc.

2.3.1. Analysis and Reasoning:

All questions in examination are based on analysis and reasoning.• Free internet access in the library and wifi facilities in campus promotes the habit of• self learning and discussion.

2.3.1 Discussion

University follows the discussions methods in many of the subjects as it makes the• students to think wide and participate in coming up with the opinions & suggestions to check their current knowledge. Discussions are held basically in soft skills, managerial communications, business adoptions etc.

2.3.1 Quizzes

Quizzes are conducted by subject teachers in all UG and PG programmes.

2.3.1 Research Activities:

Research activities are conducted in each Department under the guidance of senior faculty where the students of different semester get knowledge about emerging area and help them to promote in Research aptitude.

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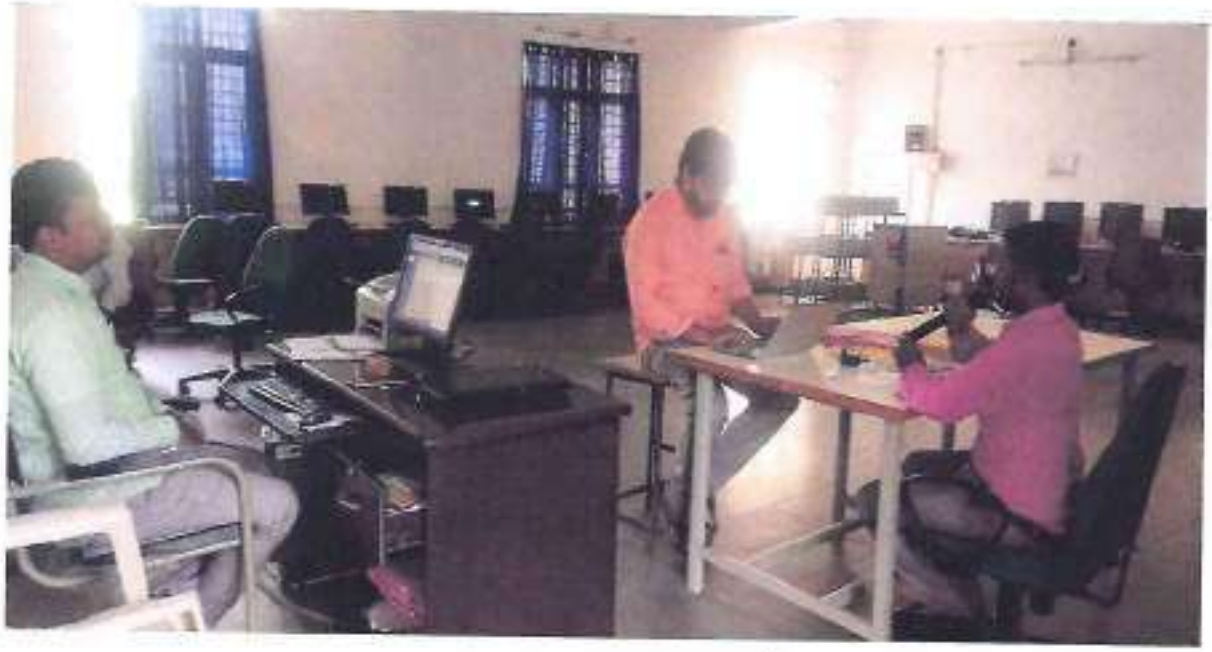
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Computer Lab





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PHARMACEUTICAL CHEMISTRY LAB



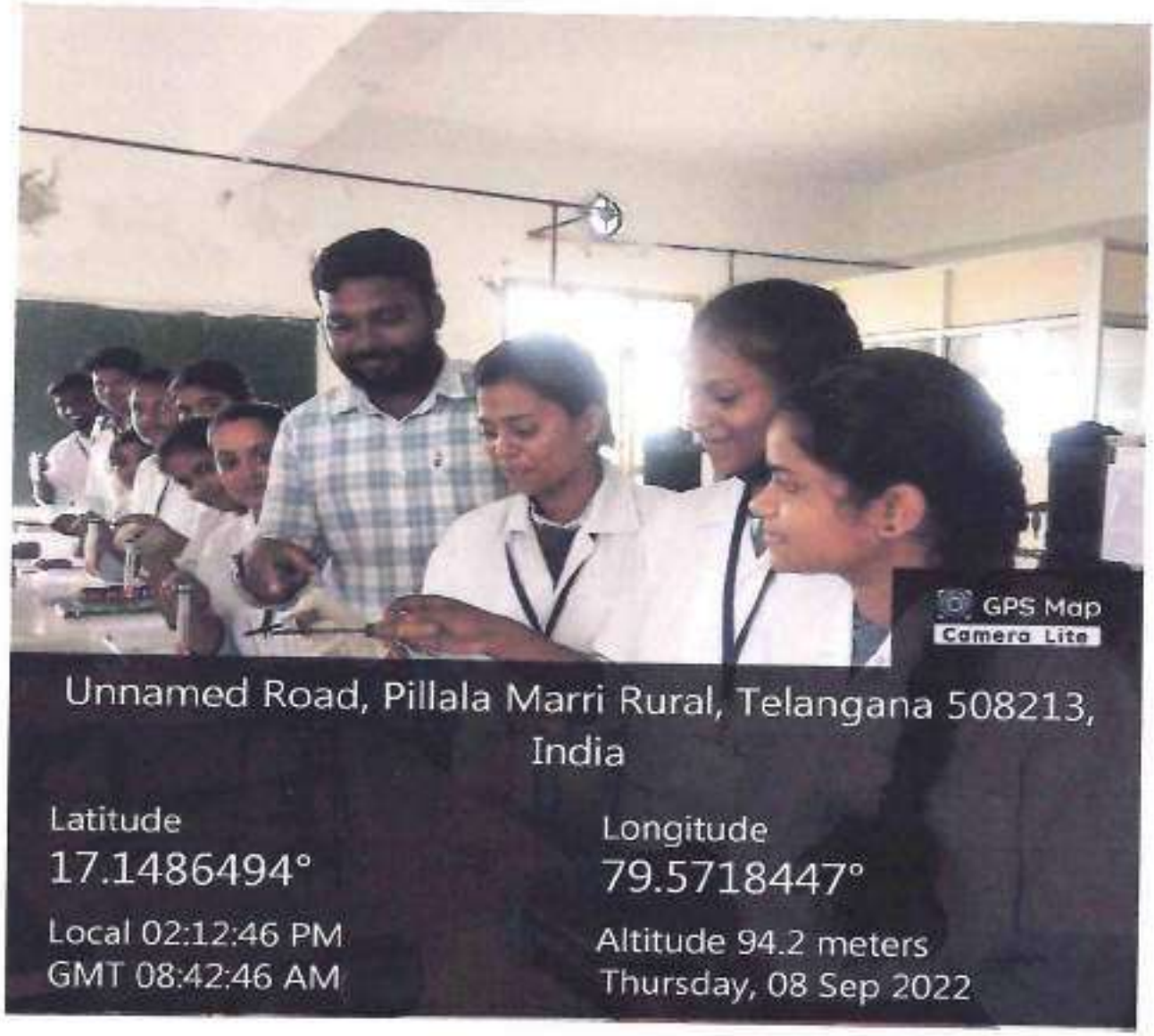
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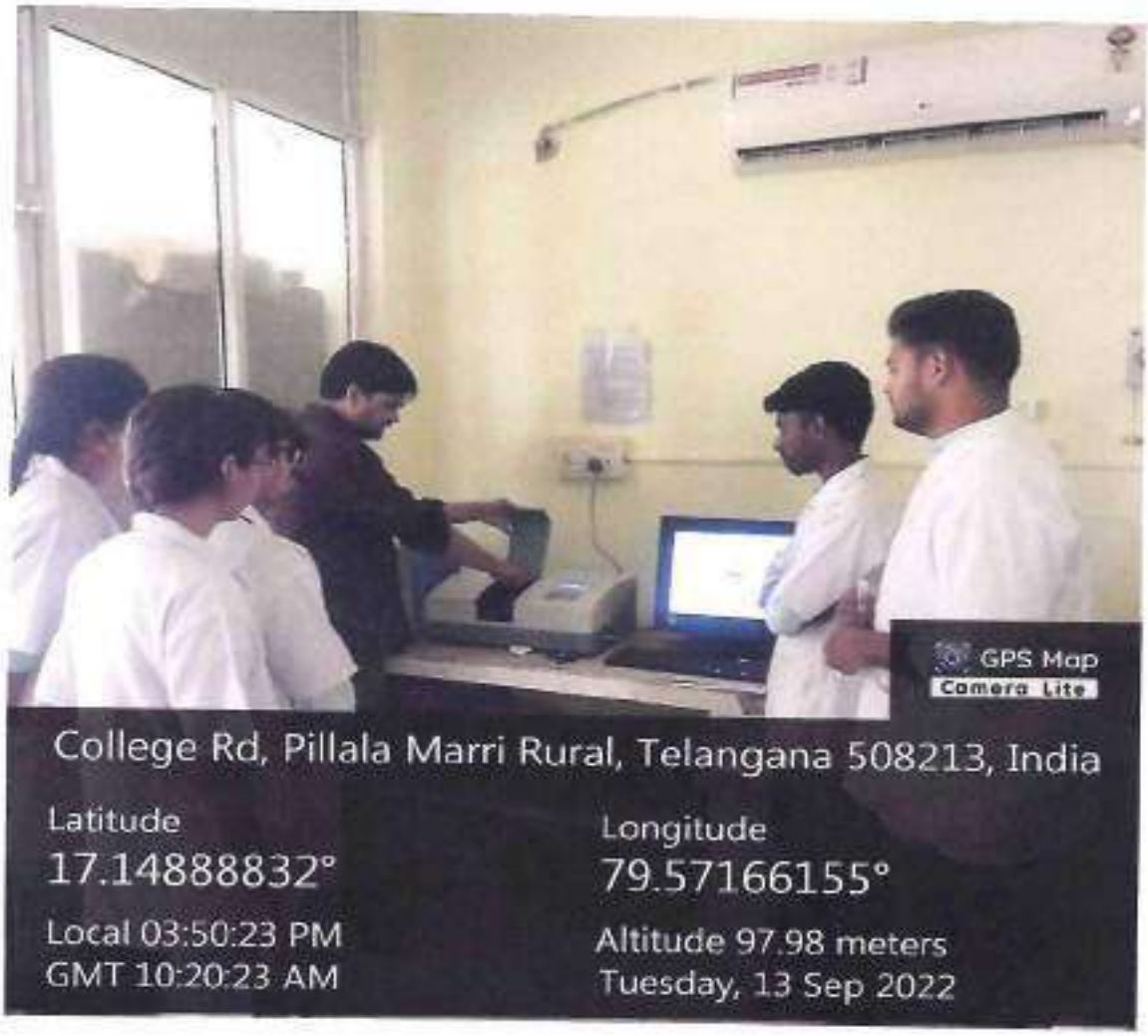
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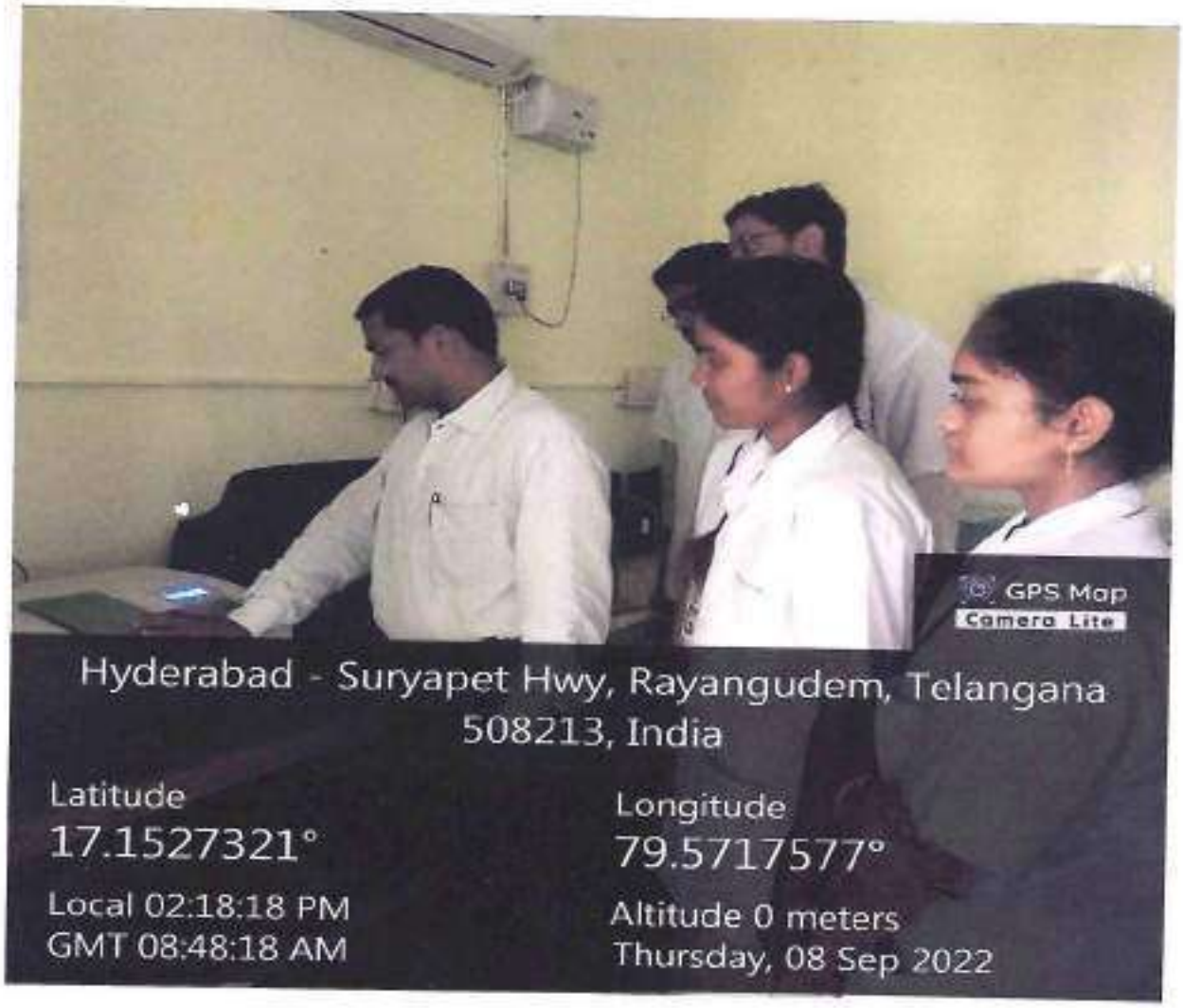
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PHARMACEUTIAL ANALYSIS LAB



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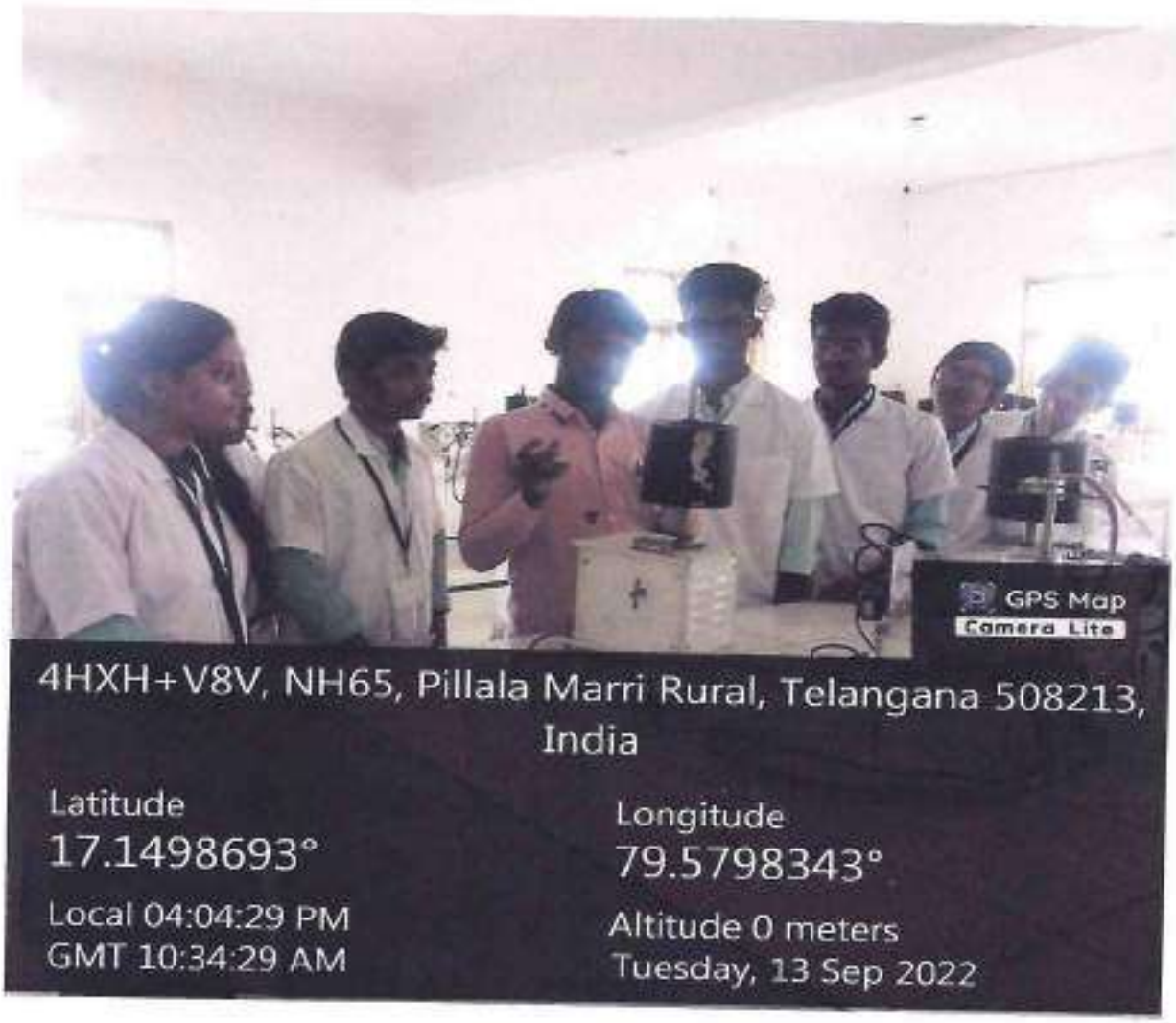
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PHARMACEUTICS LAB



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PHARMACOLOGY LAB



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PHARMACEUTICAL MICROBIOLOGY LAB



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LIBRARY



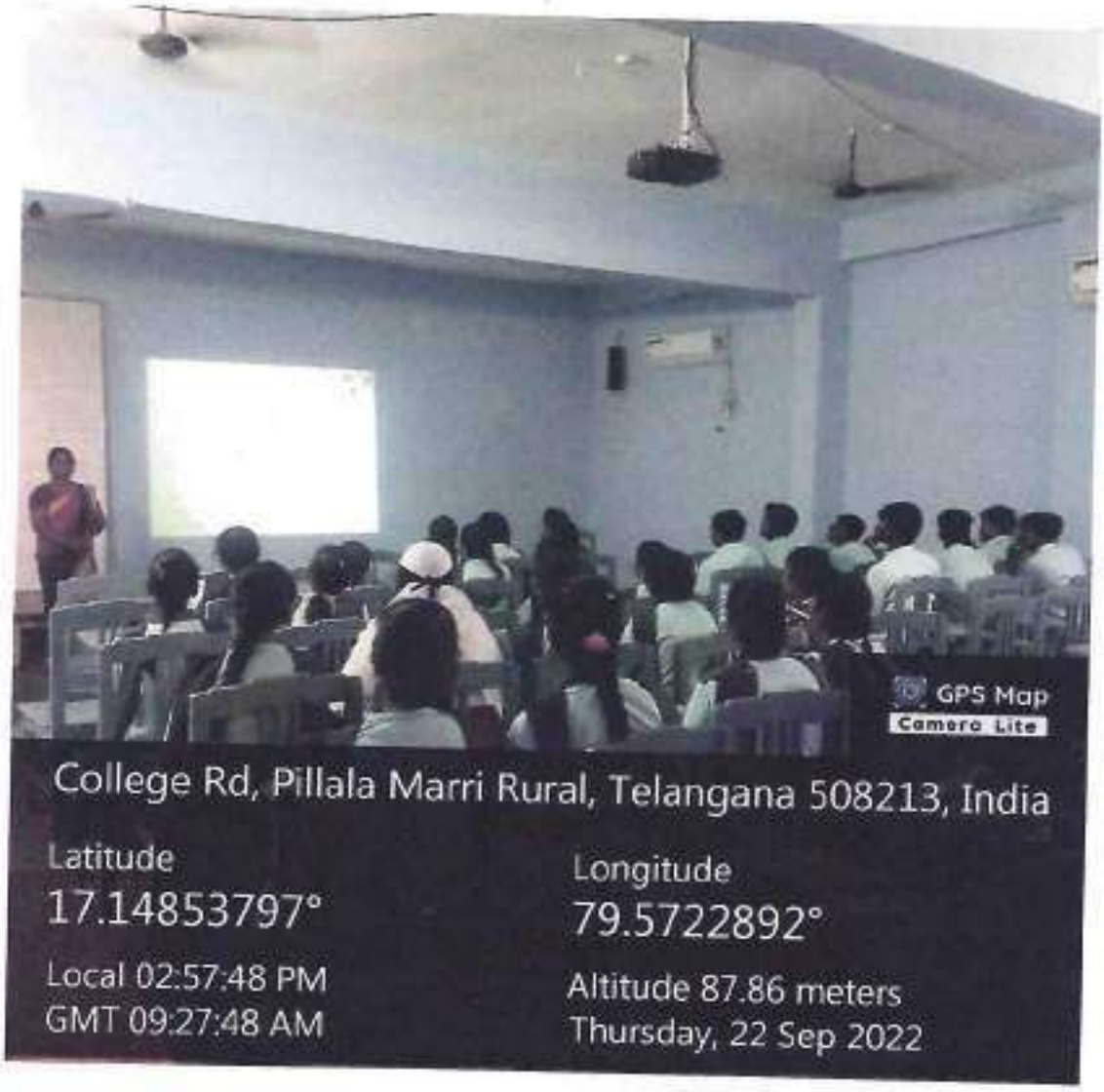
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ICT Seminar Hall



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- ICT enabled teaching practices are given thrust by all the faculty members of the institute. All the lecture classes are ICT enabled for interactive learning. In addition virtual class room facility available in the institute is fully utilized as a learning system and helps students acquire knowledge and skills from experts working in corporate hospitals and pharma institutions.

- Various steps are taken by the institute to transition from conventional/traditional classroom into an e-learning environment. This is achieved by regularly organizing webinars and encouraging them to utilize e-learning resources by providing Wi-Fi facility, ICT enabled e-classrooms.

- All kinds of teaching aids including MOOCS and modern teaching and learning methods available in the institute and are regularly used by the teaching faculty to have the student's better learning experience. The following e-learning resources are extensively used.

- Internet facility is provided to all the departments and faculty is encouraged to use and download latest information in the respective subjects so as to use ICT enabled teaching practices by all the faculty members

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List of ICT –enabled Tools for Teaching &Learning

1. Digital Corner in Library
2. Webinars
3. CLINIREX (Software)
4. CAL PHARMA Frog(Software)
5. DELL NET(E-LIBRARY)
6. E-contents in College Website
7. Computers
8. OHP /LCD Presentations
9. Charts & Diagrams
10. Specimens
11. Models
12. Poster presentations
13. Audio visual presentations




GPS Map
Camera Lite
College Rd, Pillala Marri Rural, Telangana 508213, India
Latitude
17.148623333333333°
Longitude
79.57169°
Local 12:40:59 PM
GMT 07:10:59 AM
Altitude 101.8 meters
Wednesday, 26 Oct 2022


GPS Map
Camera Lite
College Rd, Pillala Marri Rural, Telangana 508213, India
Latitude
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Longitude
79.57185333333334°
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GMT 07:16:51 AM
Altitude 101 meters
Wednesday, 26 Oct 2022

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LIST OF TEACHERS USING ICT TOOLS DURING ACADEMIC YEAR 2021-2022

S.No	Name of the Teachers	Designation
1	ADEPU RAMESH	Professor
2	BANOTHU BHADRU	Associate Professor
3	DEEKONDA MOUNIKA	Assistant Professor
4	ERIGIDINDLA RENUKA	Assistant Professor
5	MORE KISHORE	Professor
6	GAJULA SAMATHA	Associate Professor
7	GOLI SANTHOSHI	Assistant Professor
8	GUNDALA MAHESH	Associate Professor
9	ITTAMALLA SOWMYA	Assistant Professor
10	JAYANTHIMALA SAMMETA	Associate Professor
11	KONYALA SAISREE	Assistant Professor
12	KOPPULA MAHESH	Assistant Professor
13	LAKSHMI PRASANNA D	Assistant Professor
14	MADDY SHAILAJA	Assistant Professor
15	MARIYA KAUSAR	Assistant Professor
16	MARPU KRANTHI REKHA	Assistant Professor
17	MOHAMMED RASHEEDUDDIN	Assistant Professor
18	MUKKALA SHIVA SHANMUKHI	Associate Professor
19	NATESH GUNTURU	Associate Professor
20	NIMMALA SHANTHI	Associate Professor
21	PALLELA MAMATHA	Assistant Professor
22	PANDIRI SUSHMITHA ANIDU	Assistant Professor
23	PARALA VENKATESHWARA RAO	Professor
24	PASUPULAETI LAXMI PRASANNA	Assistant Professor
25	PEDAPANGA SANDHYA	Assistant Professor
26	PEDDINTI NAVEEN KUMAR	Assistant Professor
27	PODILA NARESH	Associate Professor
28	PRIYATHAM KUMAR D	Assistant Professor
29	PUNNA VENKATESHWARLU	Associate Professor
30	SAI PAWAN A R	Associate Professor
31	SATISH GUNDA	Associate Professor
32	SWETHA YERROJU	Associate Professor

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21	M VENKATA SUNIL KUMAR	Professor
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14	SWAROOPA RANI GURRAM	Associate Professor
15	Y.KIRAN KUMAR	Professor
16	VEMULA GEETHANJALI GOUD	Associate Professor
17	G. NEELAMMA	Associate Professor
18	THOUDOJU SWETHA	Assistant Professor
19	BHONGIRI BHARGAV	Assistant Professor
20	B.PRAVEEN KUMAR	Assistant Professor
21	RAVITEJA BANDLA	Professor
22	A.KEERTHANA	Assistant Professor
23	Y.YASHASWINI	Assistant Professor
24	THATI SWATHI	Assistant Professor
25	D.NAGARAJU	Assistant Professor
26	VELUPULA NARESH	Assistant Professor

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

P.Venkateswarlu
Associate Professor

Vikas college of Pharmaceutical Sciences

Definition:

- **Chromatography** is a physical process of separation in which the components to be separated are distributed between 2 immiscible phases -a **stationary phase** which has a large surface area and **mobile phase** which is in constant motion through the stationary phase.



Introduction:

- HPTLC is the improved method of TLC which utilizes the conventional technique of TLC in more optimized way.
- It is also known as **planar chromatography** or **Flat-bed chromatography.**
- The basic difference between conventional TLC & HPTLC is only in the particle size & pore size of the sorbents.
- It is very useful in quantitative & qualitative analysis of pharmaceuticals.



PRINCIPLE :

- *The principle of separation is adsorption.*

  Stationary phase - solid

  Mobile phase - liquid

ADVANTAGES OF HPTLC OVER OTHER CHROMATOGRAPHIC METHODS

HPTLC is the most simple separation technique today available to the analyst due to-

- Simultaneously analysis of sample and standard
- Lower analysis time and less cost per analysis.
- It is very simple to learn and the instrumentation is very easy to operate.
- The sample preparation is very simple.
- It involves very low maintenance cost.
- Solvents used in HPTLC needs no prior treatment like filtration and degassing.
- HPTLC allows the use of corrosive and UV absorbing mobile phases.
- In HPTLC ,the mobile phase consumption for sample is extremely low.

DIFFERENCES BETWEEN TLC AND HPTLC

Parameters	TLC	HPTLC
TYPE OF CHROMATOGRAPHIC PLATE	HAND MADE / PRECOATED	PRECOATED
PARTICAL SIZE DISTRIBUTION	WIDE	NARROW
PARTICAL SIZE RANGE	5 – 20 µm	4 – 8 µm
SHAPE	SPOT	SPOT / **BAND**
SPOT SIZE	2 – 4 mm	0.5 – 1 mm
LAYER THIKNESS	250 µm	100 – 200 µm
SOLVENT CONSUMPTION	50 ml	5 – 10 ml
NO. OF SAMPLES	MAXIMUM 12	36 – 72
OPTIMUM DEVELOPMENT DISTANCE	10 - 15 cm	5 – 7cm
SAMPLE VOLUME	1-10 µl	0.1-2µl
NO. OF SAMPLE PER PLATE	15 – 20	40 - 50

INSTRUMENTATION :

- ***Steps involved***

1. Selection of the HPTLC plates & sorbents
2. Sample preparation including prewashing & pre chromatographic derivatization
3. Application of sample
4. Pre-conditioning
5. Development techniques(separation)
6. Detection including post chromatographic derivatization
7. Quantitation
8. Documentation



PROCEDURE:

SELECTION OF CHROMATOGRAPHIC PLATES

↓

PLATES PRE-WASHING

↓

PLATES PRE-CONDITIONING

SAMPLE AND STANDARD PREPARATION

↓

APPLICATION OF SAMPLE

↓

CHROMATOGRAPIC DEVELOPMENT

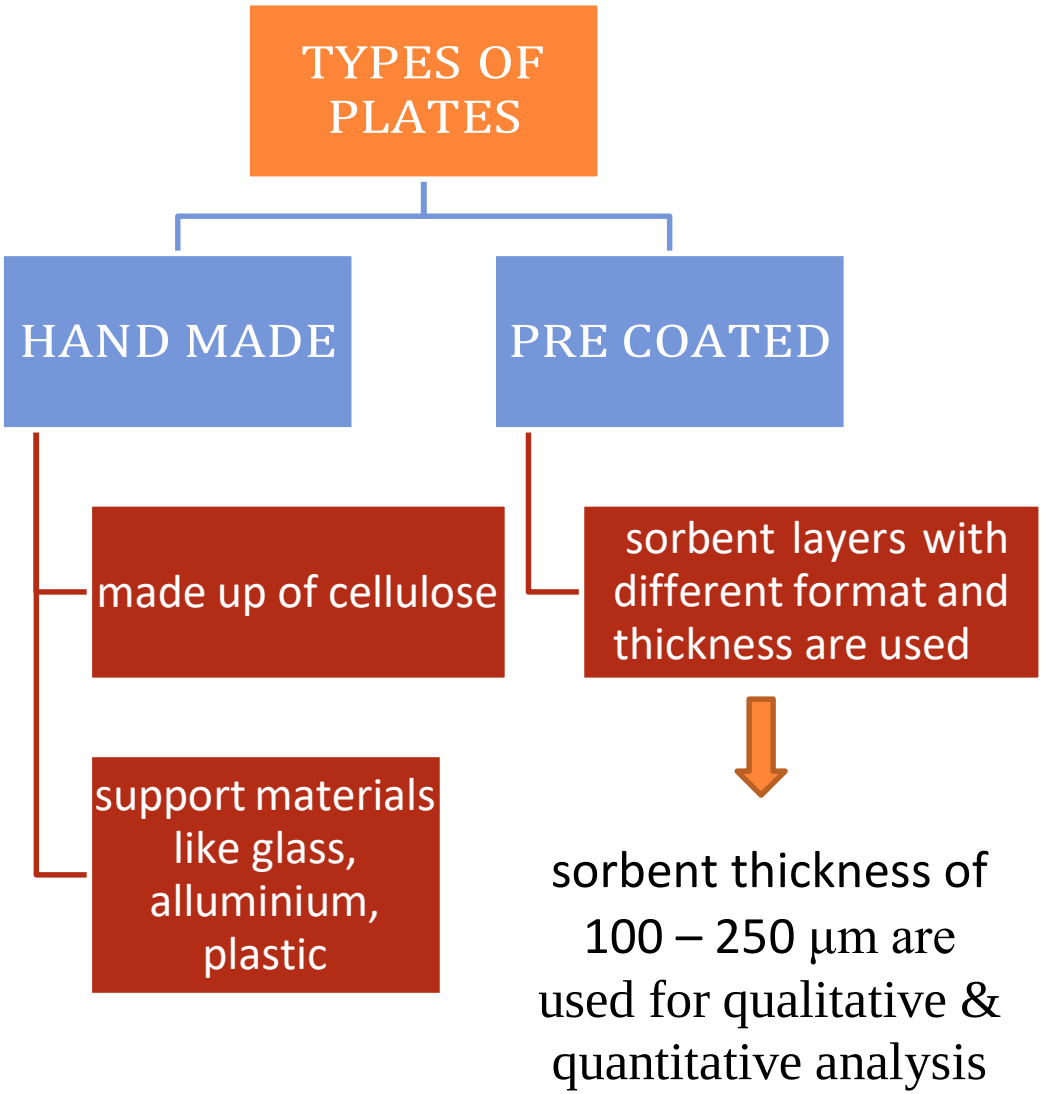
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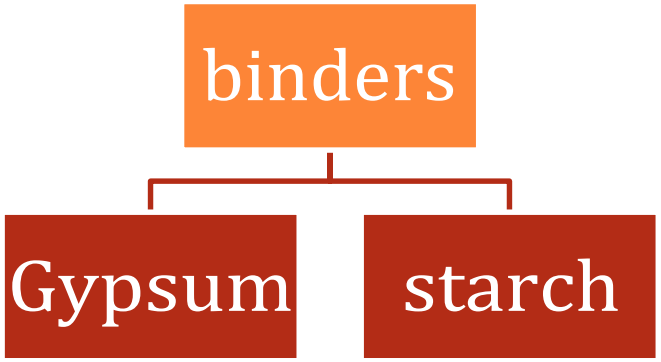
DETECTION OF SPOTS

↓

SCANNING AND DOCUMENTATION OF CHROMOPLATE USING PC CATS SOFTWARE

1.SELECTION OF HPTLC PLATES:





Pre washing of pre coated plates

The main purpose of the pre-washing is to remove impurities which include water vapours and other volatile substances from the atmosphere when they get exposed in the lab environment.

Common methods involved pre washing are as follows

1. Ascending method
2. Dipping method
3. Continuous method

SOLVENTS USED FOR PRE-WASHING

- Methanol
- Chloroform: methanol (1:1)
- Chloroform: Methanol: Ammonia (90:10:1)
- Methylene chloride: Methanol (1:1)
- Ammonia solution (1%)

ACTIVATION OF PLATES:

- Plates exposed to high humidity.
- Plates are placed in oven at 110°-120°c for 30 min prior to the sample application.
- If the plates are not dried properly, the moisture is present on the plates & the principle changes to **partition chromatography**. (stationary phase- liquid, mobile phase- liquid)

2. Sample Preparation:

- For normal chromatography: Solvent should be non-polar and volatile.
- For reversed chromatography: Polar solvent is used for dissolving the sample
- Sample and reference substances should be dissolved in the same solvent to ensure comparable distribution at starting zones.



3. Application of sample

1. Sample application is the most critical step for obtaining good resolution for quantification by HPTLC.
2. Some applicators used for spotting are:
   a) Capillary tubes
   b) Micro bulb pipettes
   c) Micro syringes,
   d) Automatic sample applicator.

The major criteria is that they shouldn't damage the surface while applying sample.



AUTOMATIC APPLICATORS USED:

1) CAMAG Nanomat:

Samples applied in the form of spots. The volume is controlled by disposable platinum iridium of glass capillary which has volume of 0.1-0.2µl.



2) CAMAG Linomat:

Automated sample application device. Sample is loaded in micro syringe (Hamilton Syringe) 1µl capacity. Sample can apply either as spot or band by programming the instrument with parameters like spotting volume ,band length etc.





Glass: Borosilicate

MOBILE PHASE:

- Mobile phase should be of high graded.
- Chemical properties, analytes and sorbent layer factors should be considered while selection of mobile phase.
- Mobile phase optimization is necessary while performing HPTLC.
- Twin trough chambers are used in which smaller volumes of Mobile phase usually 10-15 ml is required.

- Various components of Mobile phase should be measured separately and then placed in mixing vessel and then transferred to developing chambers.. This prevents contamination of solvents and also error arising from volumes expansion or contraction on mixing.

Development Chambers:

1. Twin trough chamber
2. Rectangular chambers
3. V-shaped chambers
4. Sandwitch chamber
5. Horizontal development chamber
6. Automatic development chamber (ADC)

DEVELOPMENT CHAMBERS:

1. *Twin trough chamber:*







Rectangular chambers



Automatic development chamber (ADC)



4.Pre-conditioning (Chamber Saturation):

- Preconditioning of HPTLC chamber is required for efficient separations.
- It is the process in which the chamber is saturated with the mobile phase components used for development. This ensures equilibrium is established in the atmosphere within the chamber with the mobile phase components.
- The chamber is lined with filter paper, moistened with the mobile phase before keeping the HPTLC plate. Alternatively pour mobile phase in the twin trough chamber, set a side for 30 min, place the HPTLC plate quickly and close with the lid immediately, to avoid mobile phase vapours leaving the chamber.

5. DEVELOPMENT TECHNIQUES:

- The different methods used for development of chambers are like- Ascending, descending, 2-dimentional, horizontal, multiple overrun, gradient & forced flow planar chromatography.
- Plates are spotted with sample and air dried then placed in the developing chambers.
- After the development, plate is removed from chamber and mobile phase is removed under fume cup-board to avoid contamination of laboratory atmosphere.
- The plates should be always laid horizontally because when mobile phase evaporates the separated components will migrate evenly to the surface where it can be easily detected.

Drying:

- Drying of chromatogram should be done in vacuum desiccators with protection from heat and light.
- If hand dryer is used there may be chances of getting contamination of plates, evaporation of essential volatile oils if any present in the spot or compounds sensitive to oxygen may get destroyed due to the rise in temperature.



Factors influencing separation and resolution of spots:

- Type of stationary phase
- Type of pre-coated plates
- Layer thickness
- Binder in the layer
- Mobile phase composition
- Solvent purity
- Size of developing chamber
- Sample volume to be spotted
- Size of spot diameter
- Solvent level in chamber
- Gradient elution
- Relative humidity
- Temperature
- Separation distance
- Mode of development

Greater the difference between two spots and smaller the spot diameter of sample and better will be the **resolution**.

6. Detection and visualization

Detection are of two types:

- Qualitative
- Quantitative

- *Qualitative detection:*

  HPTLC is routinely used for qualitative analysis of raw materials, finished products, plant extracts etc. It involves the identification of unknown sample from mixture by comparing the Rf values of the sample components with the standards.

- *Quantitative detection:*

  Quantitative of the chromatogram by HPTLC basically involves direct and indirect methods.

Indirect method:

It involves removal of analyte from the plate followed by quantitation.

Eg :

Scrapping and elution which is followed by analysis of eluant by convenient methods like

1) Spectrophotometry

2) Flourimetry

3) Colorimetry

Collection of samples from scrapping will results in the loss of sample, so vaccum devices and elution chamber are used.

Densitometry



•Visualization of spots/bands done directly on plate.

•Amount of compound unknown is measured by comparing standard curve from reference chromatogram with same condition.

❖**Densitometer basically consists of :**

Light source

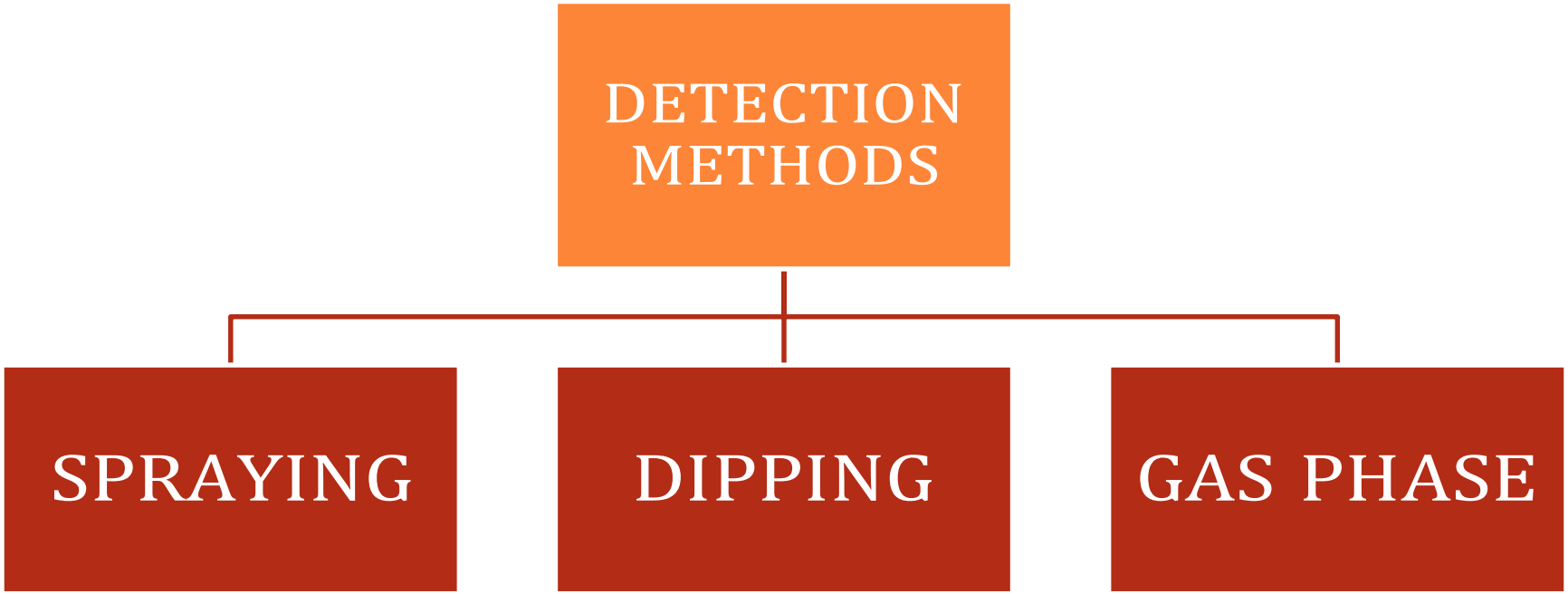
Visible Radiation

UV Radiation

Wave length selection device.

Condensing and focusing system.

Photo-sensing detectors.


DETECTION METHODS
SPRAYING
DIPPING
GAS PHASE

Derivatization by Spraying:

- It comes with a rubber pump but may also be operated from a compressed air or nitrogen supply.
- It also consists of two kinds of spray heads.
- Spray head type A is for spray solutions of normal viscosity , e.g. lower alcohol solution.
- Spray head type B is for liquids of higher viscosity. Eg: sulphuric acid reagent



Derivatization by Dipping

- For proper execution of the dipping technique, the chromatogram must be immersed in reagent and withdrawn at a controlled uniform speed.



Derivatization through gas phase:

- It offers rapid and uniform transfer of the reagent.
- Only few reagents are suitable they include I,Br,Cl, as well as volatile acids, bases and some other gases like H_2S , NO.
- In gas phase derivatization can be easily accomplished in twin trough chambers where the reagent is placed or generated in the rear trough , while the plate facing the inside of the chamber is positioned in the front trough.

DOCUMENTATION

HPTLC plates have been evaluated quantitatively and qualitatively should be documented as per guidelines of GLP.

Common methods of documentation are:-

- PHOTO DOCUMENTATION.
- VIDEO DOCUMENTATION.

APPLICATIONS

* Food analysis
* Pharmaceutical industry
* Clinical applications
* Industrial applications
* Forensic applications
* Cosmetics analysis



Food analysis:

Estimation of vitamins, pesticides ,food substances.

Curcumin-curcuminoids in turmeric powder.

Sudan-1,2,3 etc in chilli powder.

Cholesterol in edible oil.

Saffron in food.

Forensic applications:

Detection and estimation of traces of drugs or poisonous compounds.

Cosmetic analysis:

Presence of colouring agents, preservatives, trace materials and prohibited substances

Features	HPLC	HPTLC
Stationary phase	Liquid/solid	Solid
Mobile phase	Liquid	Liquid
Conditioning phase	None	Gas
Sample should be		Non volatile
Results	By detector	Detector + eyes
Analysis	On-line	Off-line
Resolution	Very high	Moderate to high
Chromatographic sys	Closed	Open
Seperating medium	Tubular column	Planar layer(plate)
Analysis in parallel	No	Yes
High pressure	Yes	Up to 100 samples
Time per sample	2-60min	1-3min
Data obtained	Little to high	Very high
Post-chromatography	Limited possibilities	Simple,possible for every sample
Fraction collection	Requires prep.scale	Simple no special

PARAMETERS:

- **Separation Efficiency**:
- Position of the mobile phase at time t as it moves through a sorbent layer

$$(Z_f)^2 = \kappa t$$

- Zf - distance moved by the mobile phase from the sample origin
- k - velocity constant.

k is dependent on:

$$\kappa = 2K_o d_p (\gamma/\eta) \cos\theta$$

- K_o - permeability constant, d_p -average particle diameter,
- g -surface tension of the mobile phase,
- η- viscosity of the mobile phase, and
- θ- contact angle.

Plate Height:

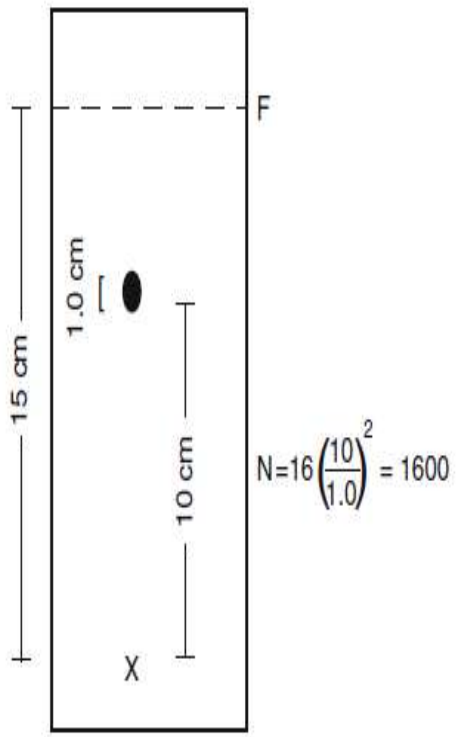
- Broadening of a chromatographic spot can be expressed in terms of the theoretical plate number 'N' of the given chromatographic system:

$$N = 16Z_fZ_s/W_s$$

- Z_f and Z_s are the migration lengths of the mobile phase and solute, and W_s is the chromatographic spot width in the direction of the mobile phase migration.

$$N = 16(Z_s/W_s)^2$$

- N is proportional to the migration length of the mobile phase Z_f, when Zs /Ws ratio remains constant.
- Increase in Z_f -increase of N and better separatio



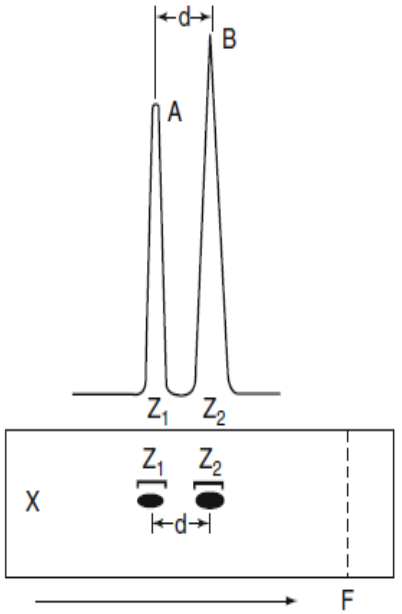
- **Resolution:**
- The separation between two spots is measured by the quantity R_s, and is called resolution.
- The R_s of two adjacent chromatographic spots 1 and 2 is defined as the distance between the two spot centers divided by the mean spot widths.

$$R_s = z_2 - z_1 / 0.5(w_1 + w_2)$$

- When R_s=1, the two spots are reasonably well separated.
- R_s values larger than 1 indicates better separation and smaller than 1 suggest a poor separation.
- R_s is also given by the equation

$$R_s = R_{f(2)} - R_{f(1)} / 0.5(w_1 + w_2)$$

$R_f(1)$ and $R_f(2)$ are the R_f values of chromatographic spots 1 and 2



CAPACITY FACTOR/ RETENTION FACTOR

- The ratio of its retention time in the stationary phase to that in the mobile phase.

$$k = t_s / t_m$$

- Measures the degree of retention

$$R_f = 1/k + 1 \text{ or } k = (1 - R_f)/R_f$$

- Ratio of total number of moles of analytes in each phase.
- If a substance doesn't migrate, its R_f =0 and k=1
- If a substance migrates with solvent front, its R_f=1 and k=0
- If k< 1, more faster elution (lesser r_t)
- If k > 5, Lesser elution (Longer r_t)
- Ideally k for an analyte **between 1 & 5**.

SPOT CAPACITY

- Separation number or spot capacity is defined as the maximum number of substances, which are completely separated between R_f 0 and 1, provided that the separation conditions are isocratic
- measures the efficiency of separation.

$$SN = (Z_f/b_0 + b_1) - 1$$

- Separation number is given by the expression
- Z_f - migration distance of the front
- b_o - width of the spot at Rf =0
- b_l - width of the spot with Rf = 1

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Thank You!

IR SPECTROSCOPY

M.KISHORE

VIKAS COLLEGE OF PHARMACEUTICAL SCIENCES

THE ELECTROMAGNETIC SPECTRUM

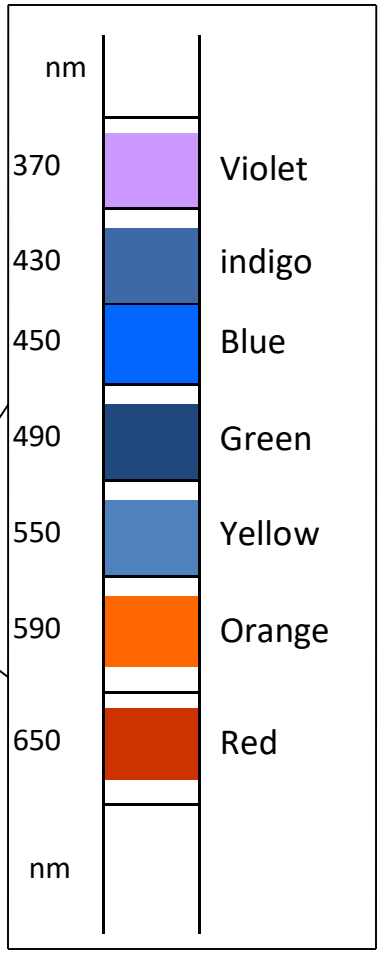
Characteristics of radiations

Absorbing radiations	Energy Kcal/mol	Λ ^{0}A	Frequency (Hz)	Type of spectroscopy
Gamma rays	9.4×10^{7}	1	10^{21}	Emission
X rays	9.4×10^{3}	15 0	10^{17}	Both E & Abs
UV	9.4×10^{1}	3 8 0 0	10^{15}	Absorption
Visible	9.4×10^{-1}	7 6 0 0	10^{13}	Absorption
Infra-Red	**9.4×10^{-3}**	**6×10^{6}**	**10^{11}**	**Absorption**
Micro waves	9.4×10^{-5}	3×10^{9}	10^{09}	Absorption
Radio waves	9.4×10^{-7}	3×10^{13}	10^{07}	NMR Abs

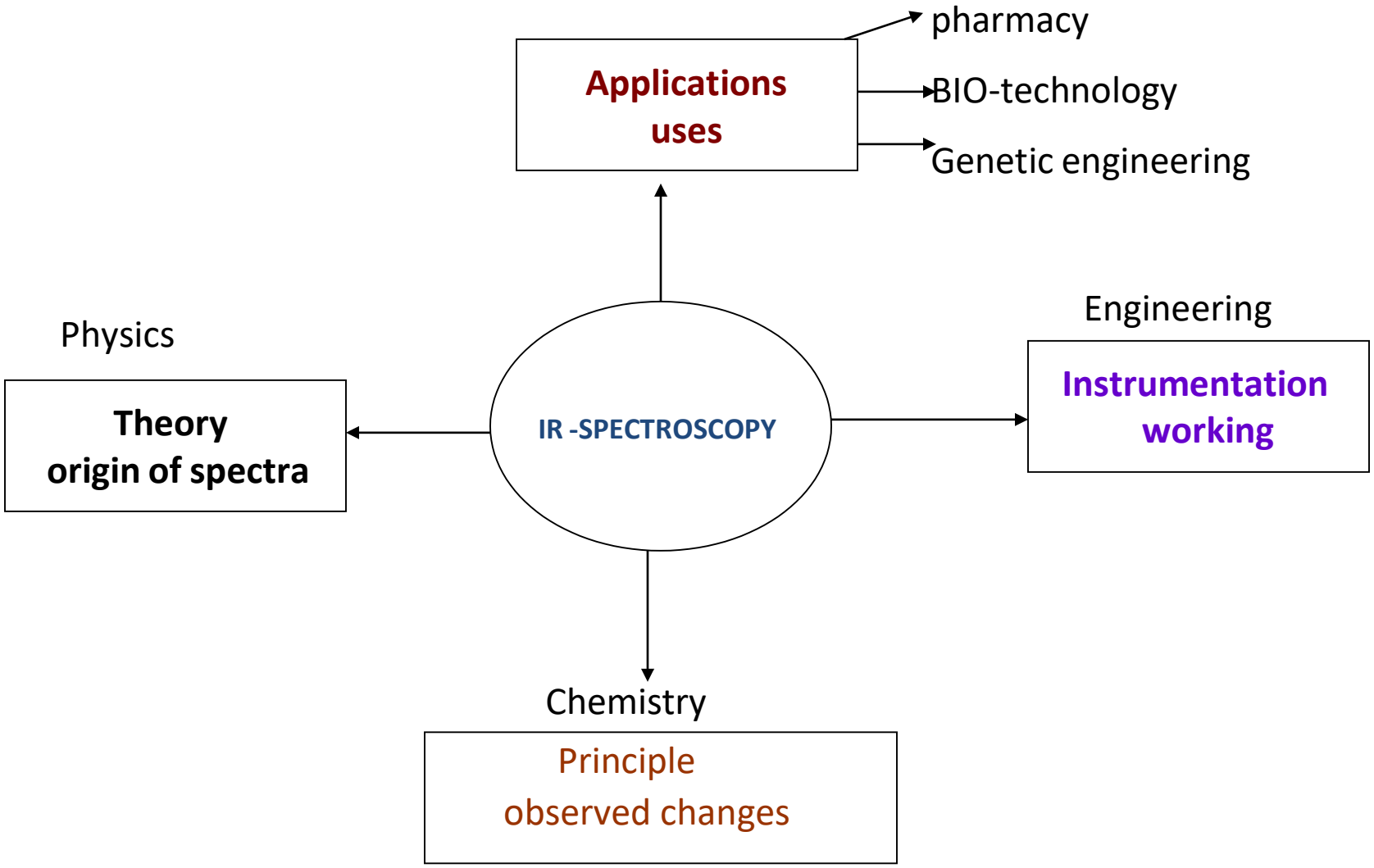
EMR → Drug substance



Resulting spectrum



Multidisciplinary of IR spectroscopy



IR-REGION: 12,800 - 10 cm^{-1}

REGION	WAVE LENGTH λ (μm)	WAVE NUMBER υ (cm^{-1})	FREQUENCY RANGE Hz
NEAR	0.78 - 2.5	12800 - 4000	$3.8x10^{14}$-$1.2x10^{14}$
MIDDLE	2.5 - 50	4000 - 200	$1.2x10^{14}$ - $6x1^{12}$
FAR	50 - 1000	200 -10	$6x10^{12}$- $30x10^{11}$
MOST USED	2.5 - 15	4000 - 670	$1.2x10^{14}$-$2x10^{13}$

1. Near IR----carbohydrates and proteins
2. Middle IR-----organic molecules—functional groups
3. Far IR—in-organic –co-ordination bonds& quaternary ammonium compounds

Differences between various types of IR spectra

Character	Electronic band spectra [a]	Vibration- rotational spectra [b]	Rotational spectra [c]
1. IR region	Near IR	Middle IR	Far IR
2.Energy required	Higher	less	very less
3.Dipole moment	less induced	Definite dipole	Intense dipole
4.Sample state	Solids	Liquids / gases	Only gases
5.Thoery supporting	Frank codon principle	Harmonic oscillator principle	Rigid rotor principle
6.Changes observed	Excitation, vibration	Vibration , rotation	Only rotation
7.Highly feasible for	single bonds	double bonds	Triple bonds

INFRARED THEORY

1. **Selection rules**
   - Matching of Frequency
   - Dipole moment
   - Vibrational Quantum Number
2. **Types of vibrations**
3. **Number of possible vibrational modes**
   - Translational motion
   - Rotational motion
   - Vibrational motion
4. **Vibrational frequency**
5. **Factors influencing vibrational modes**
   - A. Phase and solvents used
   - B. Coupled interactions
   - C. Hydrogen bonding
   - D. Fermi resonance
   - E. Electronic effects

- For a molecule to absorb IR, the vibrations within a molecule must cause a net change in the dipole moment of the molecule. The alternating electrical field of the radiation (remember that electromagnetic radiation consists of an oscillating electrical field and an oscillating magnetic field, perpendicular to each other) interacts with fluctuations in the dipole moment of the molecule.
- If the frequency of the radiation matches the vibrational frequency of the molecule then radiation will be absorbed, causing a change in the amplitude of molecular vibration.

Molecular vibration induced by IR adsorption

The stretching frequency of a bond can be approximated by Hooke's Law. Two atoms and the connecting bond are treated as a simple harmonic oscillator composed of 2 masses (atoms) joined by a spring:

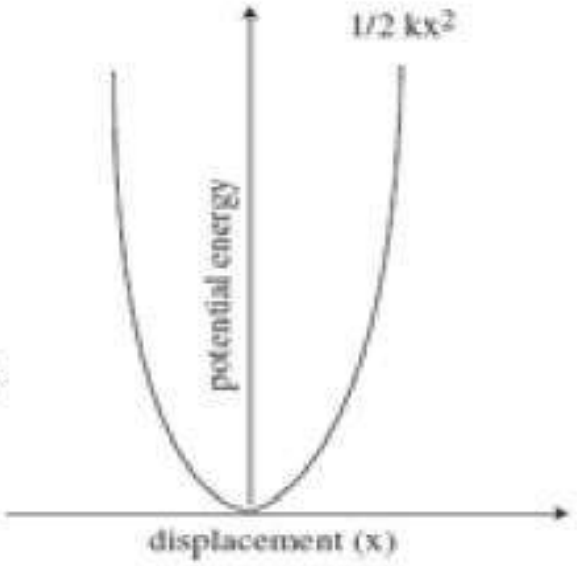


According to Hooke's law, the vibration frequency of the spring is is expressed by:

$$\nu = \frac{1}{2\pi}\sqrt{\frac{k}{m}}$$

where ***k*** is the force constant, ***m*** is the mass, *v* is the vibration frequency

In the classical harmonic oscillator, $E = 1/2kx^2 = hv$, where *x* is the displacement of the spring. Thus, the energy or frequency is dependent on how far one stretches or compresses the spring, which can be any value. If this simple model were true, a molecule could absorb energy of any wavelength.



Molecular vibration induced by IR adsorption

The following formula has been derived from Hooke's law. For the case of a diatomic molecule,

$$\bar{\nu} = \frac{1}{2\pi c}\sqrt{\frac{f(m_1 + m_2)}{m_1 m_2}}$$



recall: $\nu = \frac{c}{\lambda}$ and $\lambda = \frac{c}{\nu}$

$\bar{\nu} = \frac{1}{\lambda}$ $\quad \nu = c\bar{\nu}$

where $\bar{\nu}$ is the wavenumber (cm^{-1}), m_1 and m_2 are the mass of atoms 1 and 2, respectively, c is the velocity of light (cm/s), f is the force constant of the bond (dyne/cm)

Equation shows the relationship of bond strength and atomic mass to the wavenumber (vibration frequency) at which a molecule will absorb IR radiation. As the force constant increases, the wavenumber increases.

$$V(r) = \frac{1}{2}k \cdot \Delta r^2 = 2\pi^2 \cdot \mu\nu_{osc}\Delta r^2$$

$$\mu = \frac{m_1 \cdot m_2}{m_1 + m_2} \quad \text{(reduced mass)}$$

$$\nu_{osc} = \frac{1}{2\pi} \cdot \sqrt{\frac{f}{\mu}}$$

Vibrations – relative positions of the atoms change while the average position and orientation of the molecule remain fixed.

Fundamental Vibrations

Degrees of freedom	linear	non-linear
Translational	3	3
Rotational	2	3
Vibrational	$3N–5$	$3N–6$
Total	$3N$	$3N$

N = number of atoms in molecule

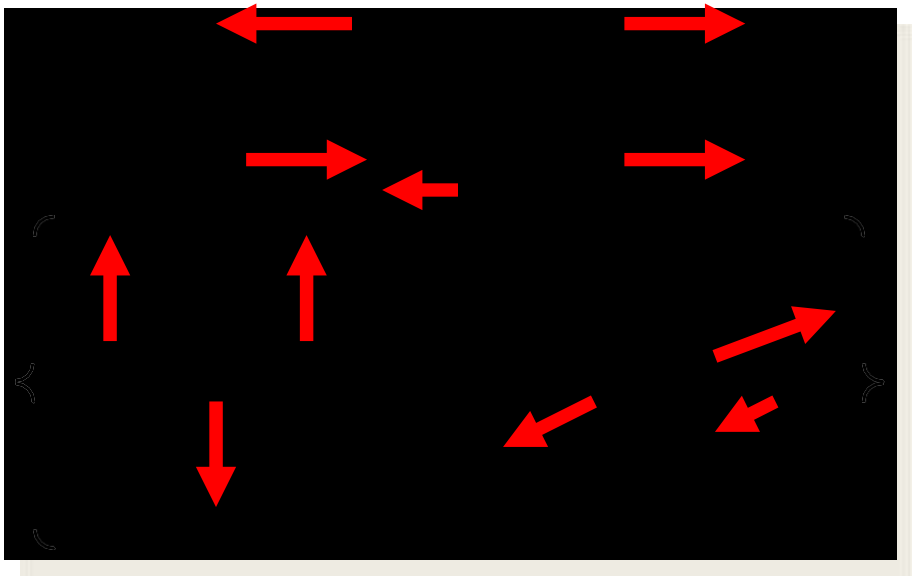
prerequisites for Infrared active

1) The frequency of the infrared light must be identical to the frequency of the vibration (resonance).
2) The dipole of the molecule must change during vibration.
3) The direction of the dipole change must be the same as the direction of the electric filed vector

- That is, the molecules can interact with light and lead to infrared absorption only when all the following requirements are met:

INFRARED SPECTROSCOPY

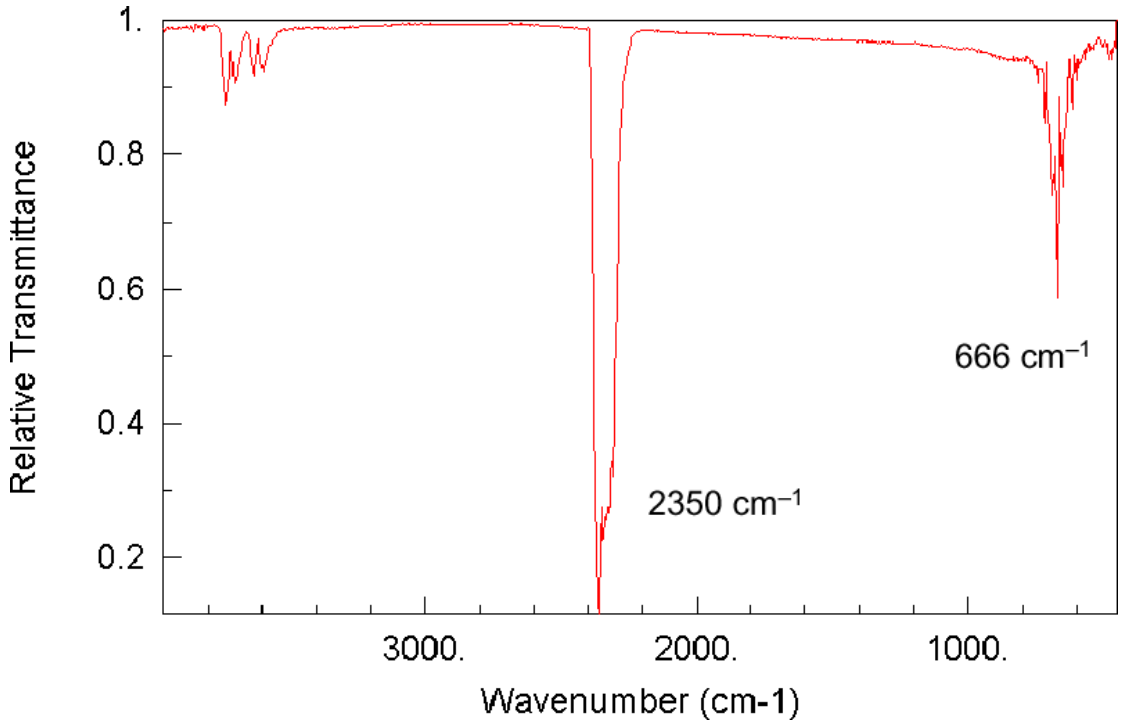
- **Only vibrations that cause a change in 'polarity' give rise to bands in IR spectra – which of the vibrations for CO_2 are infrared active?**



Symmetric stretch

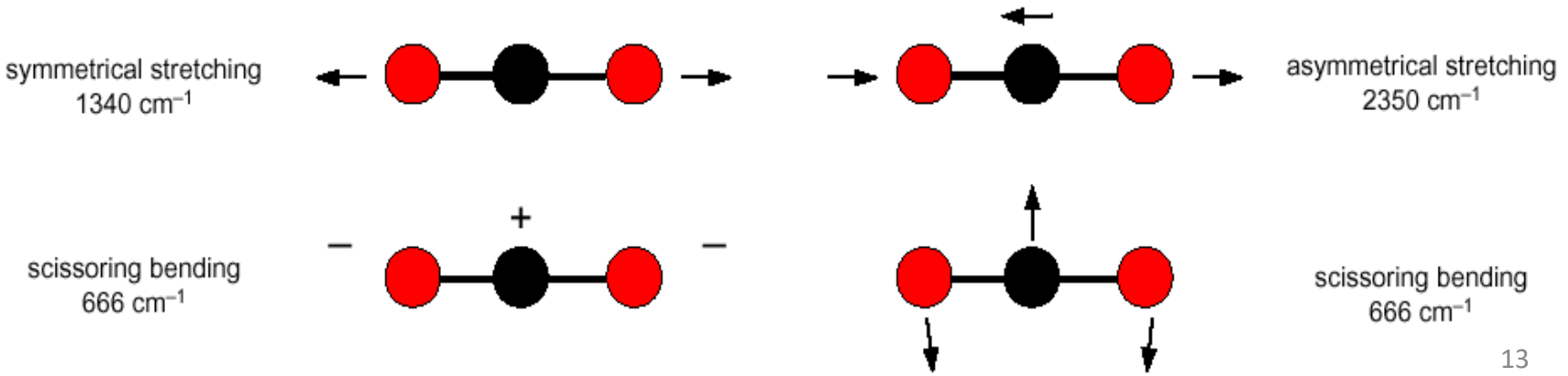
Asymmetric stretch

Bending (doubly degenerate)



Infrared Spectrum of Carbon Dioxide

Example: CO_2



Selection Rules

The energy associated with a quantum of light may be transferred to the molecule if work can be performed on the molecule in the form of displacement of charge.

Selection rule:

A molecule will absorb infrared radiation if the change in vibrational states is associated with a change in the dipole moment (μ) of the molecule.

$$\boldsymbol{\mu} = q\mathbf{r}$$

q: electrical charge, **r**: directed distance of that charge from some defined origin of coordinates from the molecule.

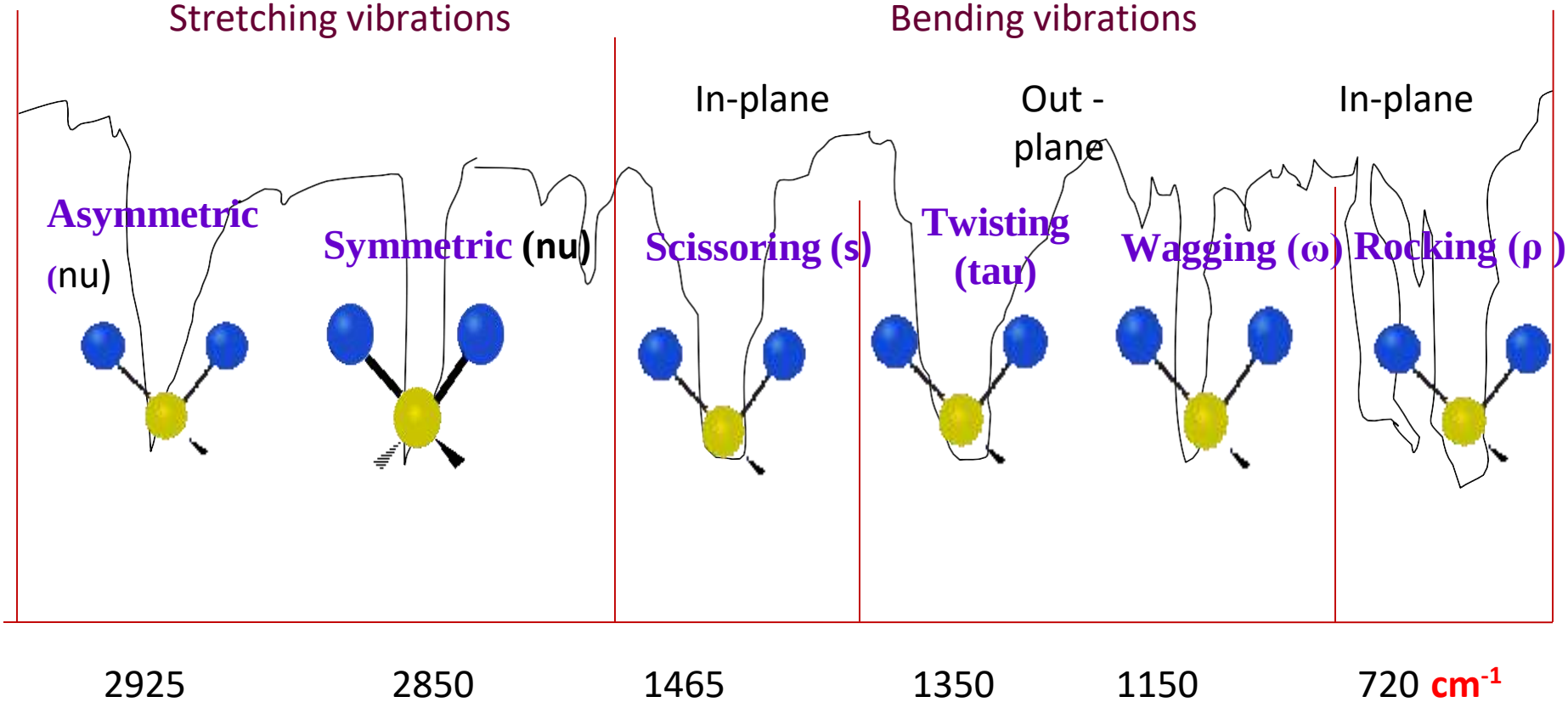
Dipole moment is greater when electronegativity difference between the atoms in a bond is greater. Some electronegativity values are:

H 2.2; C 2.55; N 3.04; O 3.44; F 3.98; P 2.19; S 2.58; Cl 3.16

• Vibrations which do not change the dipole moment are *Infrared Inactive* (homonuclear diatomics).

Types of vibrations

For stretching vibration = N -1
For bending vibration
[(3N - 6)-(N -1)]=2N -5 for non-linear
[(3N - 5)-(N -1)] =2N – 4 for linear 'N' is the number of atoms in the bond.

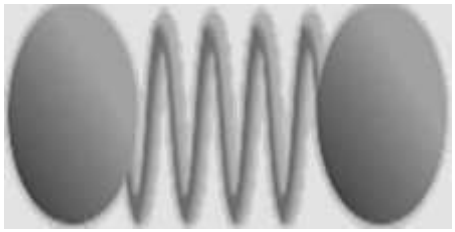


Vibrational energy depends on :-

1. masses of the atoms 2. strength of bonds

3. arrangement of atoms within the molecule

Vibrational frequency



M1 Force constant, k M2

- Ball and spring representation of 2 atom of molecule vibrating in the direction of bond

Hooke's law :

$$\bar{\nu} = \frac{1}{2\pi c}\sqrt{\frac{K}{\mu}}$$

$\bar{\nu}$: Frequency in cm^{-1}

c : Velocity of light => $3 * 10^{10}$ cm/s

K : Force constant => dynes /cm

$$\mu = \frac{m_1 m_2}{m_1 + m_2} = \frac{M_1 M_2}{M_1 + M_2 (6.02 * 10^{23})}$$

Factors influencing absorption frequency

- **Masses of attached atoms.** As masses increase, wave number decreases.

C-H	C-C	C-O	C-Cl	C-Br	C-I
3000 cm^{-1}	1200 cm^{-1}	1100 cm^{-1}	750 cm^{-1}	6[illegible] cm^{-1}	500 cm^{-1}

- **Strength of chemical bond.** As bond strength increases, wave number increases.

C≡C	C=C	C-C
2150 cm^{-1}	1650 cm^{-1}	1200 cm^{-1}

- **Hybridization.** Bonds are stronger in the order $sp > sp^2 > sp^3$.

C-H (sp)	C-H (sp^2)	C-H (sp^3)
3300 cm^{-1}	3100 cm^{-1}	2900 cm^{-1}

- **Resonance.** Conjugation lowers the energy to vibrate bond.

isolated ketones	α,β-unsaturated ketone	α,β,γ,δ-unsaturated ketone
1715 cm^{-1}	1690 cm^{-1}	1675 cm^{-1}

Factors influencing vibrational modes

A. Phase and solvents used

Phase and solvents may bring the changes in IR in the aspects of

1.Band frequency shifts

2. Band splitting

e.g.;- the effect of phase and solvents in Acetone.

>c=o in acetone ----------1742 **cm^{-1} in vapor state**

----------1718 cm^{-1} in liquid state

Dipole-dipole lowers wave number

Acetone interactions with some solvents

----------1726 cm^{-1} in a solution of Hexane

----------1713 cm^{-1} in chloroform

----------1709 cm^{-1} in ethanol

B. Coupled interactions

Extent of coupling influenced by

1.stretching vibrations with two vibrations have common atom

2. bending vibrations with a common bond b/t vibrating groups.

3. coupled groups of identical energies.

4. groups separated by two/more bonds, little or no interaction occur.

6. vibrations of symmetrical species.

Factors influencing vibrational modes

C.. Hydrogen bonding

Strength of H-bond effected by

1. **ring strain**
2. **molecular geometry**
3. **relative acidity and basicity of proton donor and acceptor**

Types of hydrogen bonding :-

1. **intermolecular hydrogen bonding** — **extent of bonding depends on Temp.**
2. **intramolecular hydrogen bonding**

D. Fermi resonance

Factors leads to Fermi resonance

a) vibrational levels are same for symmetrical compounds.

b) interacting groups located in the molecule for an appreciable mechanical coupling to occur.

e.g.:-

1. co_2 actual absorption frequencies at 1286,1388 cm-1 the splitting caused by coupling b/t fundamental c=o stre. near 1340 cm^{-1} and 667 cm^{-1} -----1344 cm^{-1} 1st overtone
2. lactones, lactims, lactums, aldehydes.

Factors influencing vibrational modes

E. Electronic effects

1. Inductive effect—introduction of alkyl group → length ↑ → bond strength ↓ → force constant ↓ → vibrational frequency ↓

2. Mesomeric effect

3. Field effect.

HCHO----1750 cm-1

CH_3CHO---1745 cm-1

CH_3COCH_3---1715 cm-1

- Lone pair of electrons
- conjugation lowers absorption
- Mesomeric effect dominate inductive effect for some time and vice versa

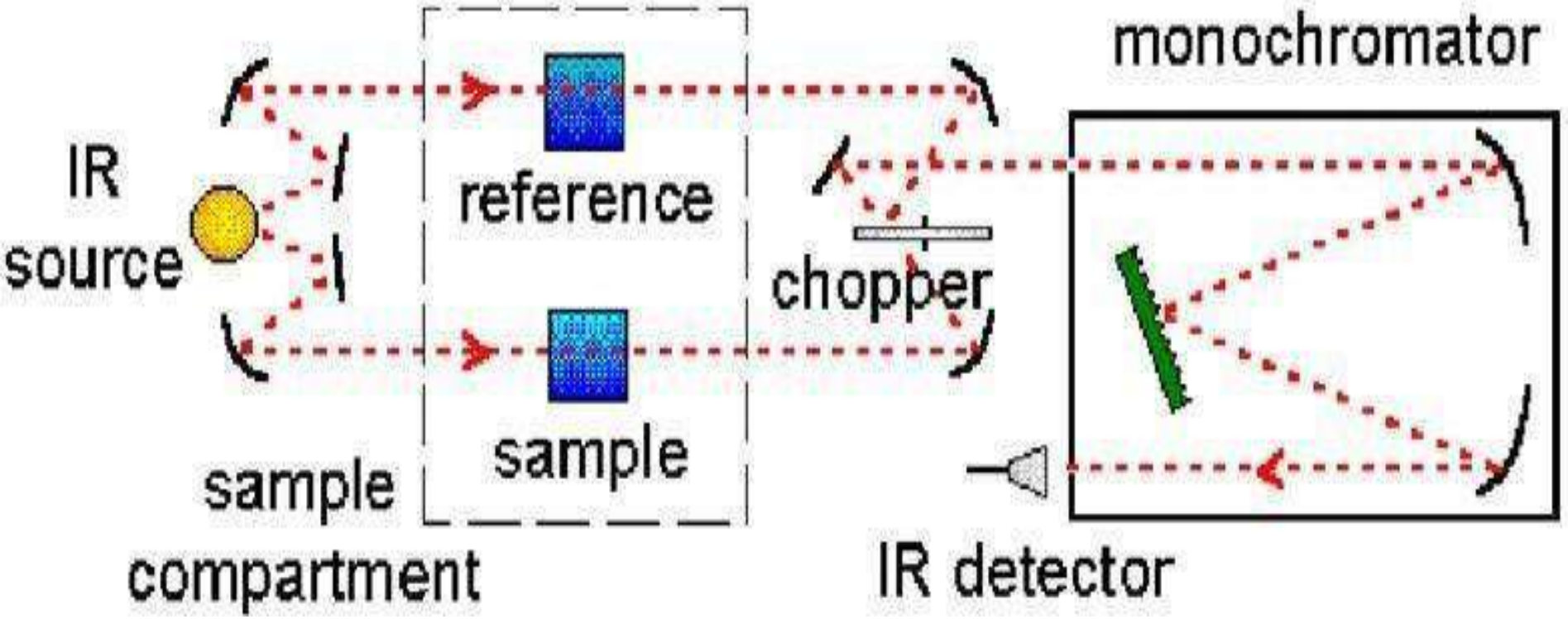
Introduction of electronegative atoms → ↑ Bond strength → ↑ Force constant → ↑ Vibrational frequency

CH_3COCH_3---1715 cm-1

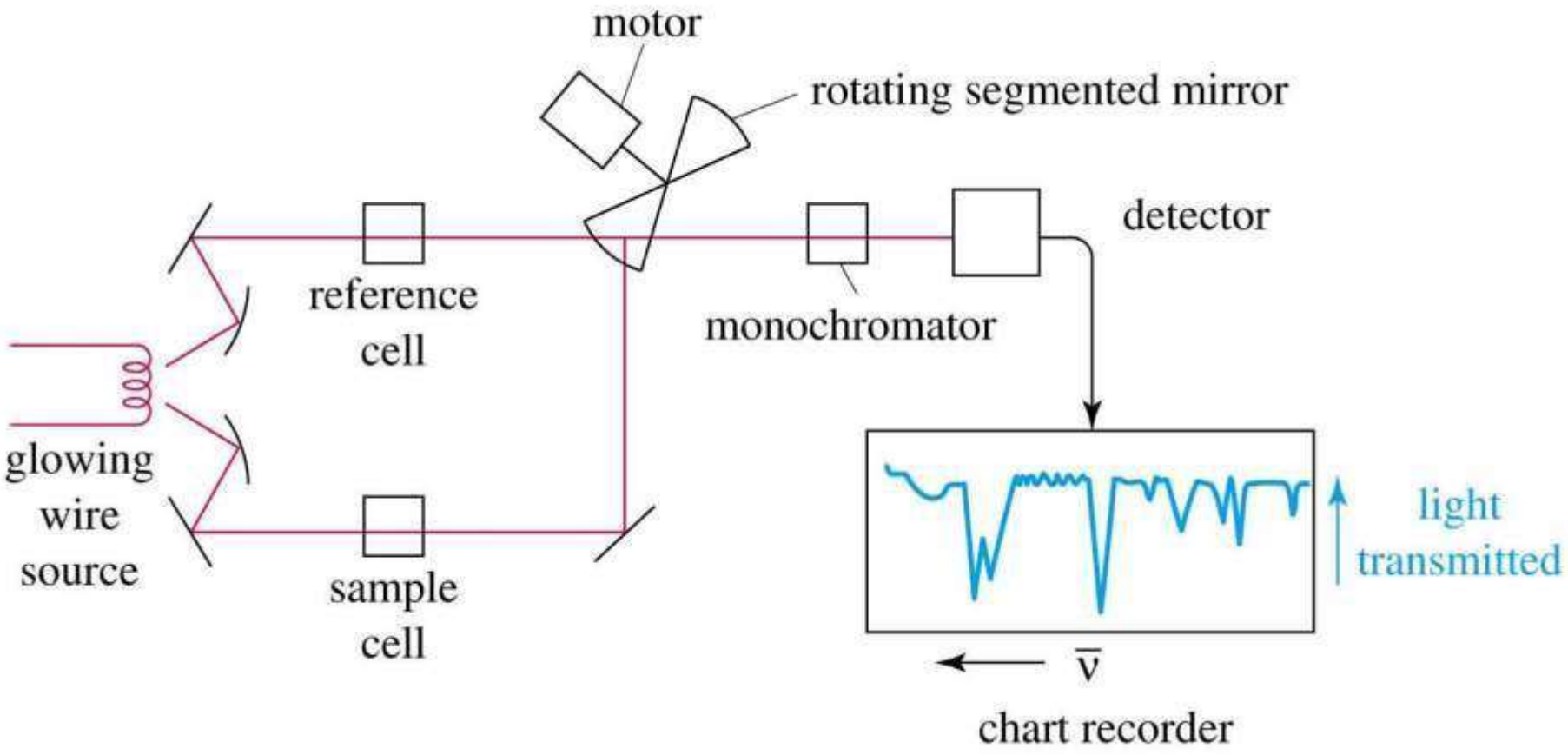
$ClCH_2COCH_3$--1725 cm-1

$Cl_2CHCOCH_3$----1740 cm-1

INSTRUMENTATION



An Infrared Spectrometer



=>

INFRARED SOURCES

S.NO	Character	Nernst glower	Globar	Incandescent	Mercury arc	Tungsten lamp	Co_2 laser
1.	Composition	Rare earth oxides	Silicone carbide	Nichrome wire	High (Hg) pressure	Tungsten – Halogen	Tunable Co_2 laser.
2.	Operating temp.	1200 — 2200K	1300 --- 1500 K	1100K	1000K	3500K	-------
3.	Radiations produced O.P	cm-1	cm-1	cm-1	cm-1	cm-1	cm-1
4.	IR region used	Near / visible	Middle	Near	Far	Middle	Middle /near
5.	Intensity of radiation	More intense	As equal to Nernst	Less but longer life.	Greater	Mild	More effective
6.	Out put significant (λ)	>2µm	>5µm	2-4µm	10µm	2-4µm	5 µm
7.	Used for	Carbohydrate , protein	Simple Functional groups	complex organic molecules.	In- organic complexes.	Most organic functional groups	NH_3 C_6H_6, C_2H_5OH

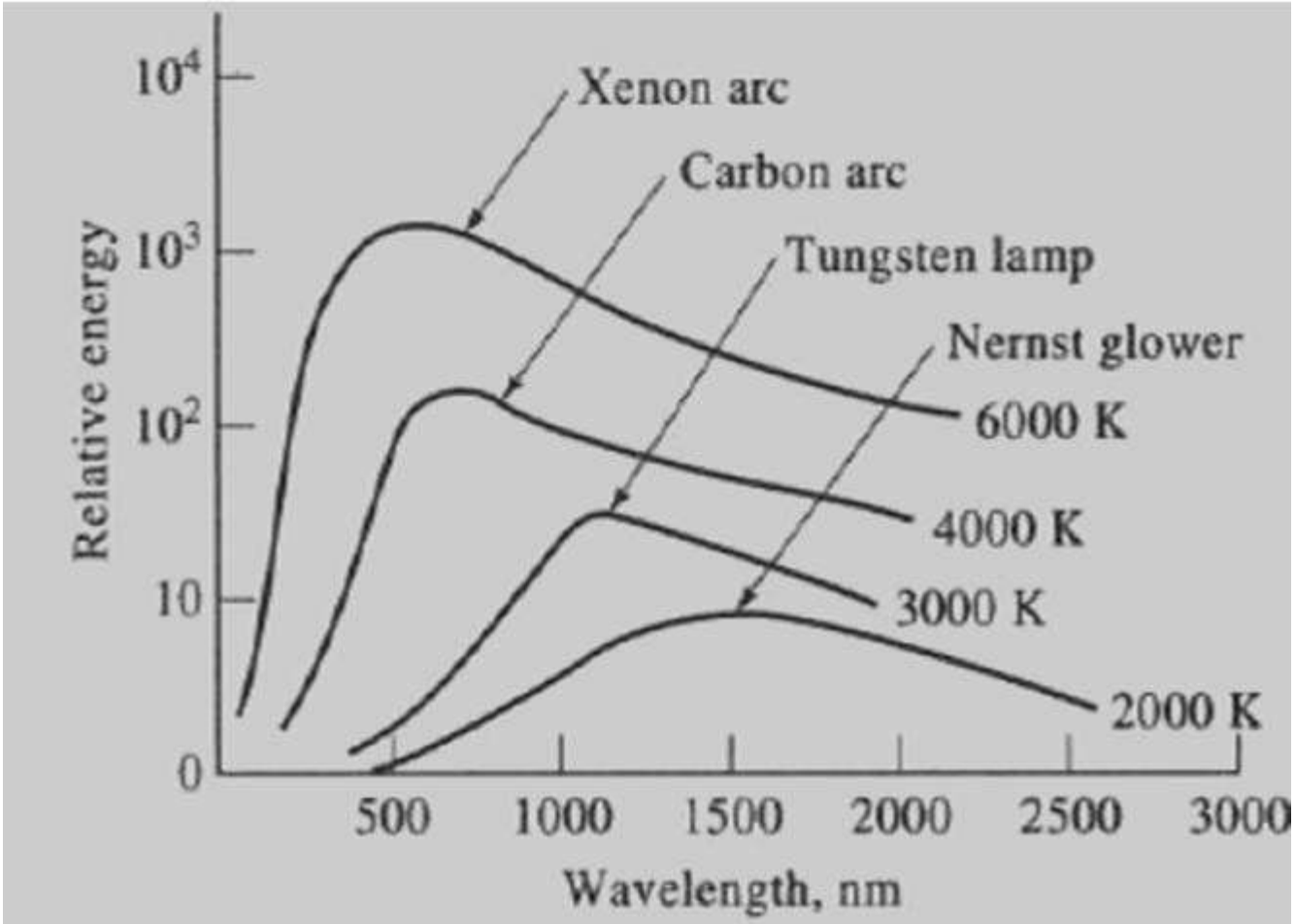
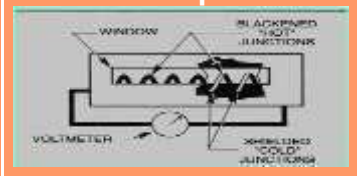
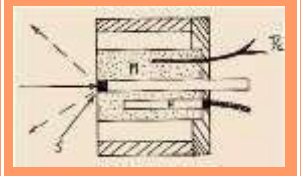
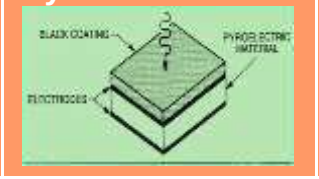
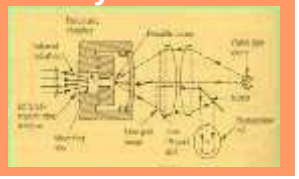


Figure 6-18 Blackbody radiation curves.

DETECTORS or TRANSDUCERS

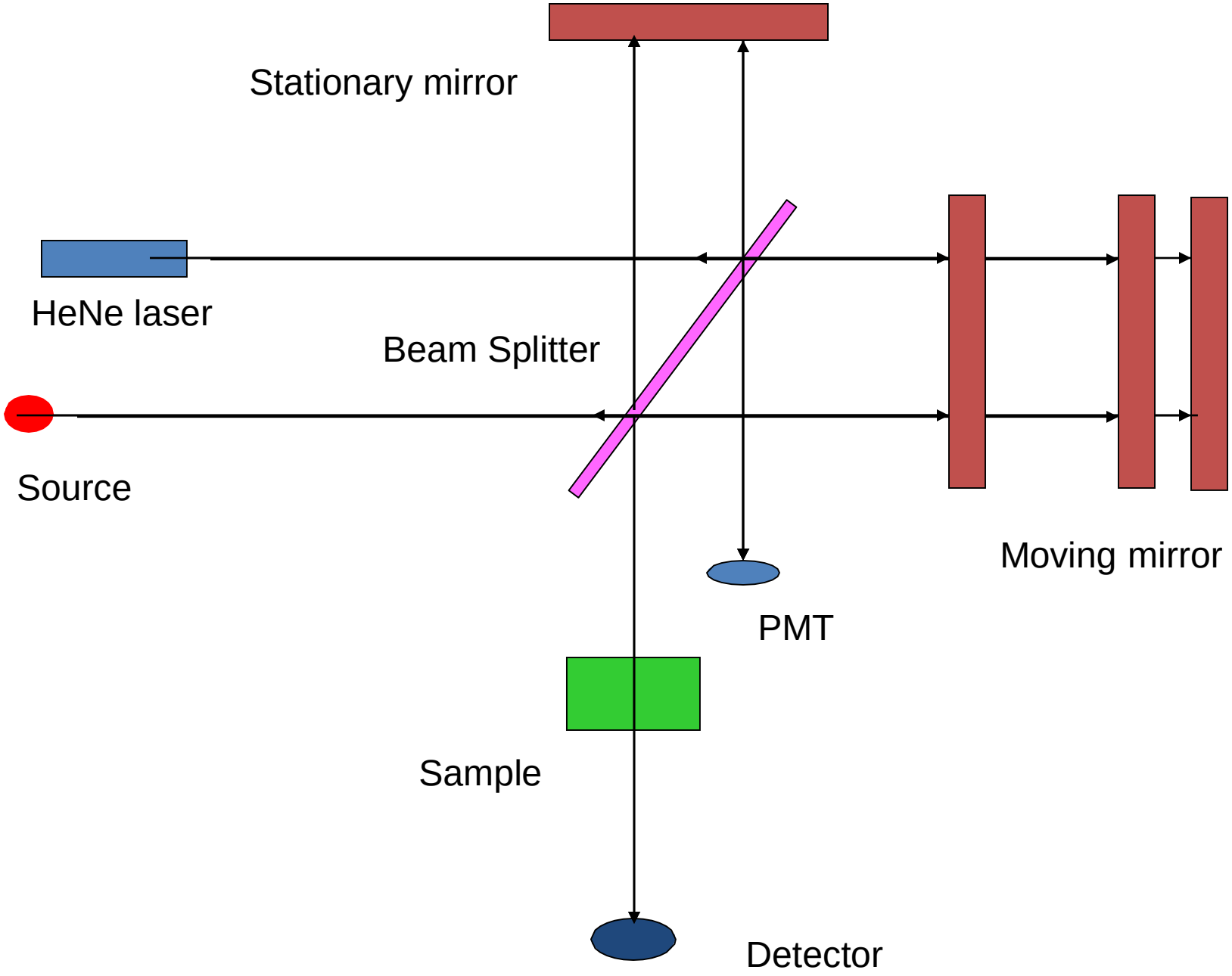
S.No	Character	Thermocouple	Thermister	Pyroelectric	Golay
					
1.	Principle	Pelletier effect	Whetstone bridge	Electric polarization	Expanction of gases
2.	Materials used	Bismuth & Antimony, coated by metal oxides	Sintered oxides of Mn, co, Ni	TGS, DTGS, $LiTGO_3$, $LiTubO_3$	generally CO_2
3.	Material should be	Thermally active	Thermally sensitive resistors	Non-center symmetric crystal	Inert nature
4.	Description	Half -junction- hot Alternate -junction -cold	----------	----------	Metal cylinder closed in b/t metal plate & Ag
5.	Conversion unit	Radiant to Electric signal ---measured	Change in resistance - Q	Thermal alteration to E.polarization	Expanction of gas to pressure to e.signal
6.	Used	Photocuastic spectroscopy	Diffusive reflectance	FTIR	Non –dispersive IR
7.	Response time	30 sec	4 sec	multiple scanning	0.01sec

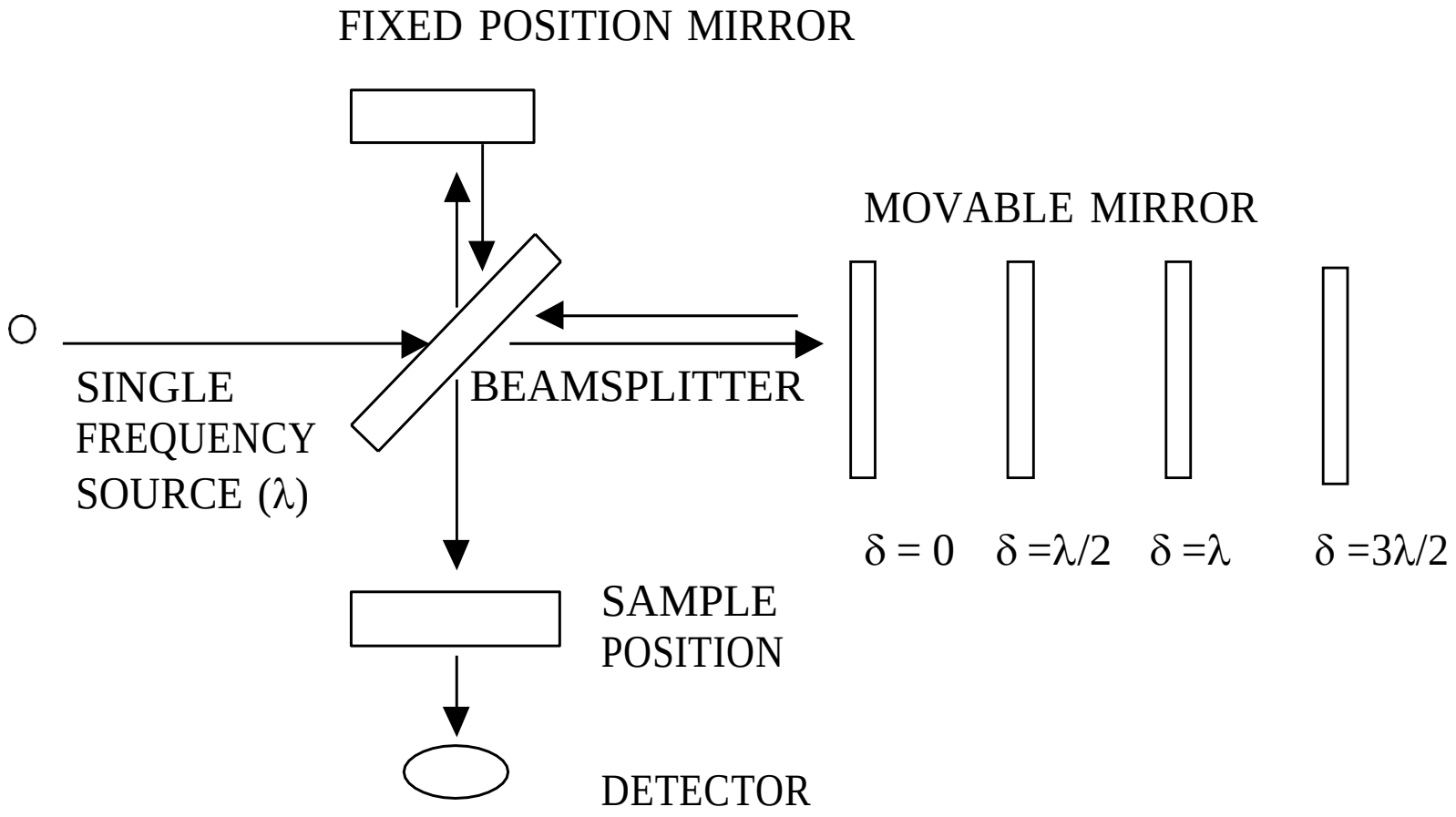
FT – IR spectrophotometer

- *Fourier transform infrared spectroscopy is preferred over dispersive or filter methods of infrared spectral analysis for several reasons:*
- It is a non-destructive technique
- It provides a precise measurement method which requires no external calibration
- It can increase speed, collecting a scan every second
- It can increase sensitivity – one second scans can be co-added together to ratio out random noise
- It has greater optical throughput
- It is mechanically simple with only one moving part

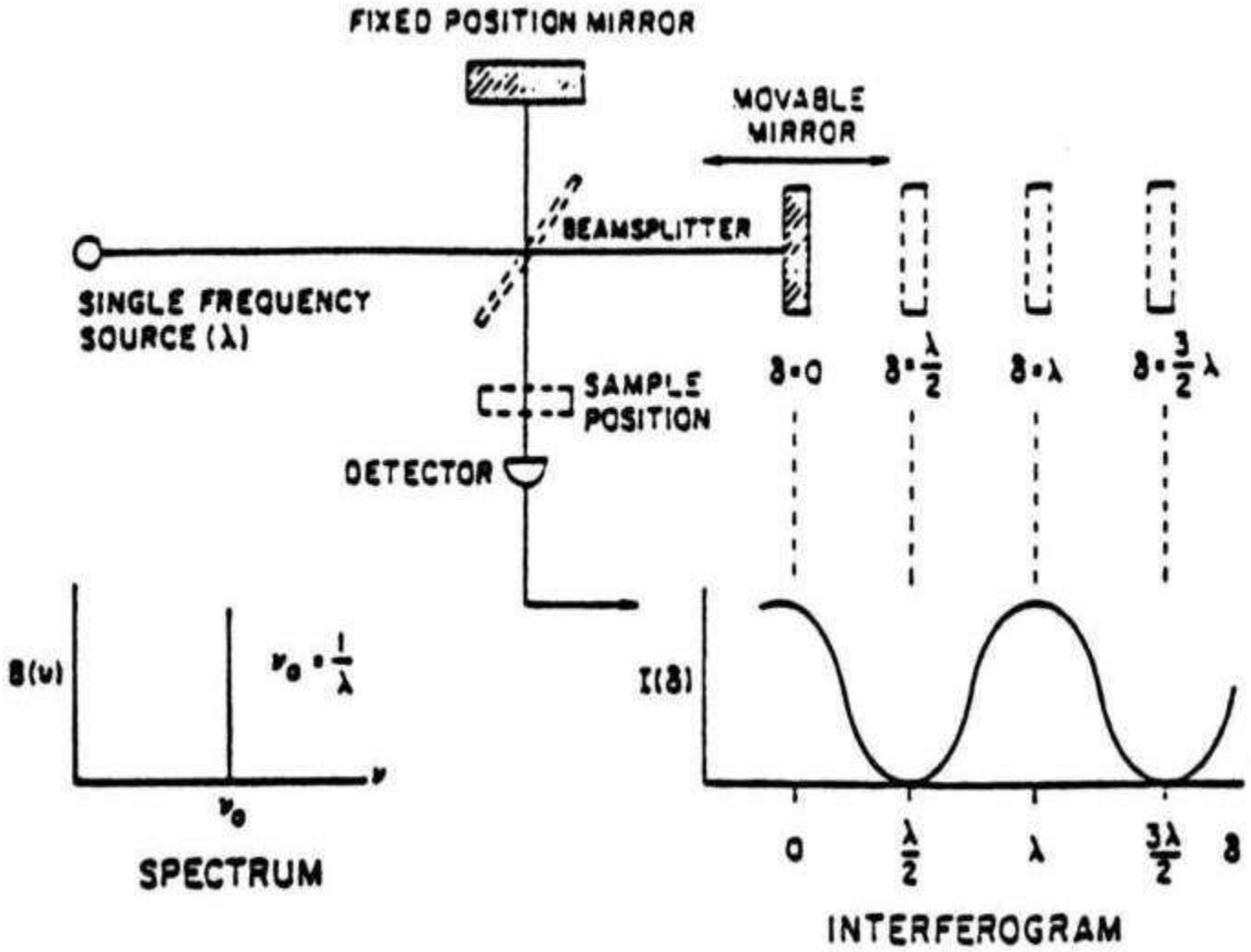
Why FT-IR?

- Fourier Transform Infrared (FT-IR) spectrometry was developed in order to overcome the limitations encountered with dispersive instruments. The main difficulty was the slow scanning process. A method for measuring all of the infrared frequencies **simultaneously**, rather than individually, was needed.
- A solution was developed which employed a very simple optical device called an **interferometer**.
- The interferometer produces a unique type of signal which has all of the infrared frequencies “encoded”into it.
- The signal can be measured very quickly, usually on the order of **one second** or so.
- Thus,the time element per sample is reduced to a matter of a few seconds rather than several minutes.


Stationary mirror
HeNe laser
Beam Splitter
Source
Moving mirror
PMT
Sample
Detector



THE MICHELSON INTERFEROMETER



Schematic of a Michelson Interferometer.

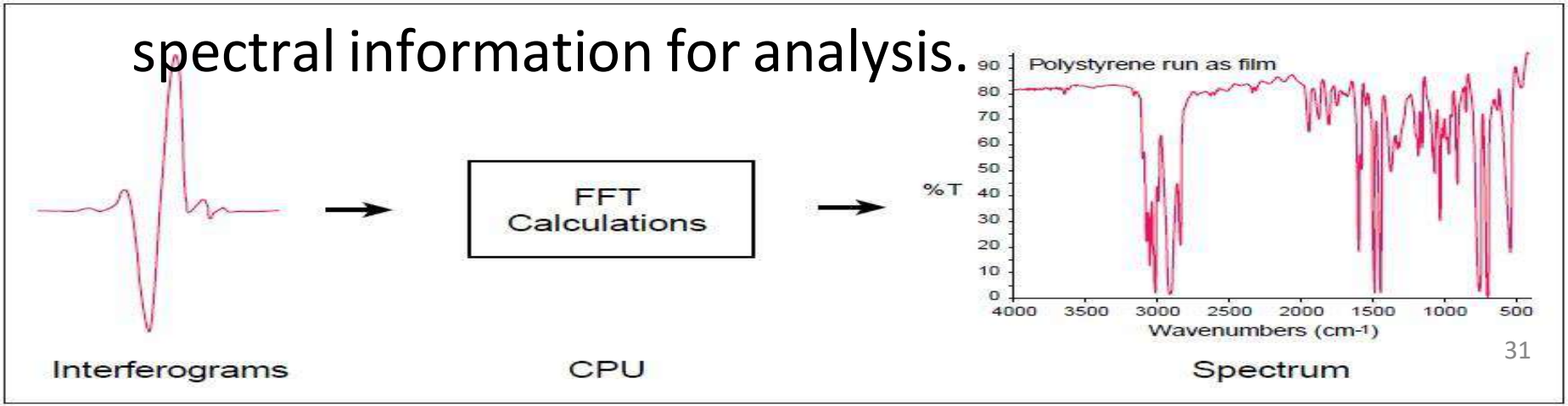
- Most interferometers employ a **beamsplitter** which takes the incoming infrared beam and divides it into two optical beams. One beam reflects off of a flat mirror which is fixed in place.

- The other beam reflects off of a flat mirror which is on a mechanism which allows this mirror to move a very short distance (typically a few millimeters) away from the beamsplitter.

- The two beams reflect off of their respective mirrors and are recombined when they meet back at the beamsplitter. Because the path that one beam travels is a fixed length and the other is constantly changing as its mirror moves, the signal which exits the interferometer is the result of these two beams "interfering" with each other. The resulting signal is called an **interferogram** which has the unique property that every data point (a function of the moving mirror position) which makes up the signal has information about every infrared frequency which comes from the source.

- This means that as the interferogram is measured, all frequencies are being measured **simultaneously**. Thus, the use of the interferometer results in extremely fast measurements.

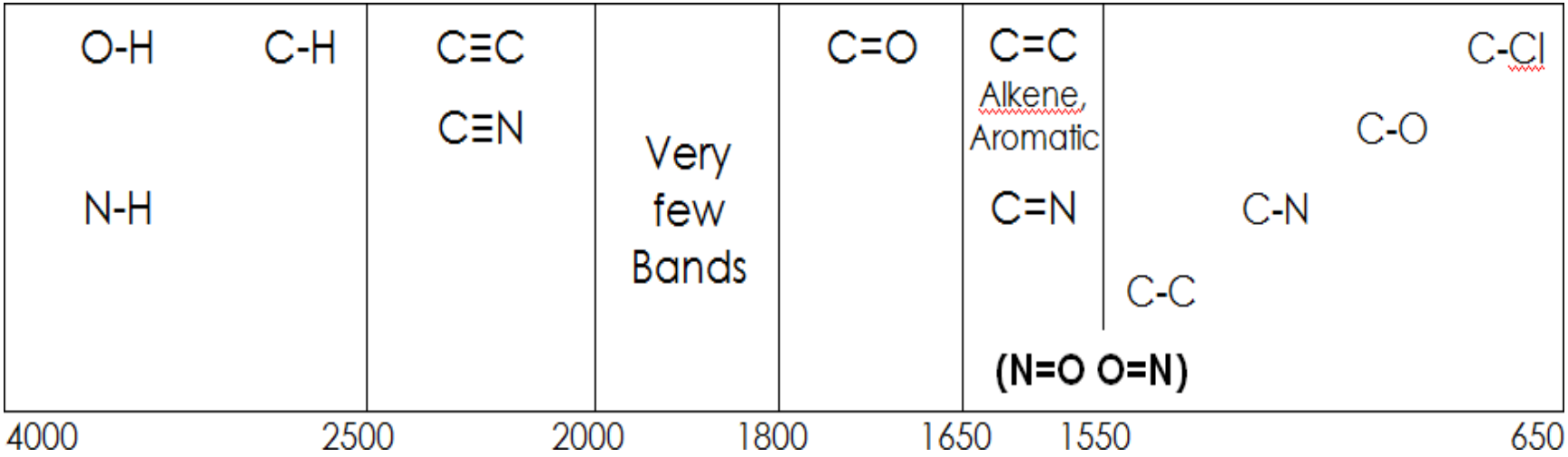
- Because the analyst requires a **frequency spectrum** (a plot of the intensity at each individual frequency) in order to make an identification, the measured interferogram signal can not be interpreted directly.
- A means of “decoding” the individual frequencies is required. This can be accomplished via a well-known mathematical technique called the **Fourier transformation**.
- This transformation isperformed by the computer which then presents the user with the desired spectral information for analysis.



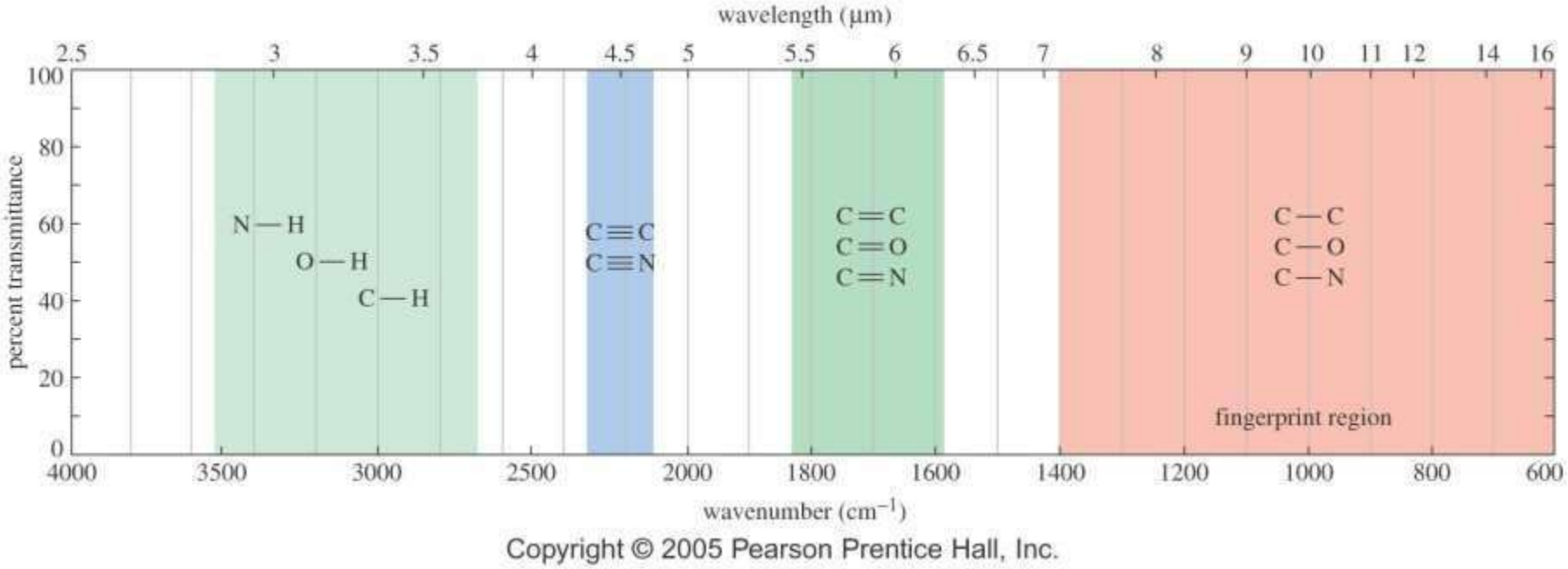
Advantages of FT-IR

- Some of the major advantages of FT-IR over the dispersive technique include:
- • **Speed:** Because all of the frequencies are measured simultaneously, most measurements by FT-IRare made in a matter of seconds rather than several minutes. This is sometimes referred to as the**Felgett Advantage**.
- • **Sensitivity:** Sensitivity is dramatically improved with FT-IR for many reasons. The detectors employed are much more sensitive, the optical throughput is much higher (referred to as the **Jacquinot Advantage**) which results in much lower noise levels, and the fast scans enable thecoaddition of several scans in order to reduce the random measurement noise to any desired level (referred to as **signal averaging**).
- • **Mechanical Simplicity:** The moving mirror in the interferometer is the only continuously moving part in the instrument. Thus, there is very little possibility of mechanical breakdown.
- • **Internally Calibrated:** These instruments employ a HeNe laser as an internal wavelength calibration standard (referred to as the **Connes Advantage**). These instruments are self-calibrating and never need to be calibrated by the user.

Ranges of IR


O-H
C-H
C≡C
C≡N
Very few Bands
C=O
C=C
Alkene, Aromatic
C=N
C-Cl
C-O
C-N
C-C
(N=O O=N)
4000
2500
2000
1800
1650
1550
650

Summary of IR Absorptions



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TABLE 17-3 Abbreviated Table of Group Frequencies for Organic Functional Groups

Bond	Type of Compound	Frequency Range, cm^{-1}	Intensity
C—H	Alkanes	2850–2970	Strong
		1340–1470	Strong
C—H	Alkenes (>C=C<H)	3010–3095	Medium
		675–995	Strong
C—H	Alkynes (—C≡C—H)	3300	Strong
C—H	Aromatic rings	3010–3100	Medium
		690–900	Strong
O—H	Monomeric alcohols, phenols	3590–3650	Variable
	Hydrogen-bonded alcohols, phenols	3200–3600	Variable, sometimes broad
	Monomeric carboxylic acids	3500–3650	Medium
	Hydrogen-bonded carboxylic acids	2500–2700	Broad
N—H	Amines, amides	3300–3500	Medium
C=C	Alkenes	1610–1680	Variable
C=C	Aromatic rings	1500–1600	Variable
C≡C	Alkynes	2100–2260	Variable
C—N	Amines, amides	1180–1360	Strong
C≡N	Nitriles	2210–2280	Strong
C—O	Alcohols, ethers, carboxylic acids, esters	1050–1300	Strong
C=O	Aldehydes, ketones, carboxylic acids, esters	1690–1760	Strong
NO_2	Nitro compounds	1500–1570	Strong
		1300–1370	Strong

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Carbon-Carbon Bond Stretching

- Stronger bonds absorb at higher frequencies:
  - C-C 1200 cm^{-1}
  - C=C 1660 cm^{-1}
  - C≡C 2200 cm^{-1} (weak or absent if internal)
- Conjugation lowers the frequency:
  - isolated C=C 1640-1680 cm^{-1}
  - conjugated C=C 1620-1640 cm^{-1}
  - aromatic C=C approx. 1600 cm^{-1} =>

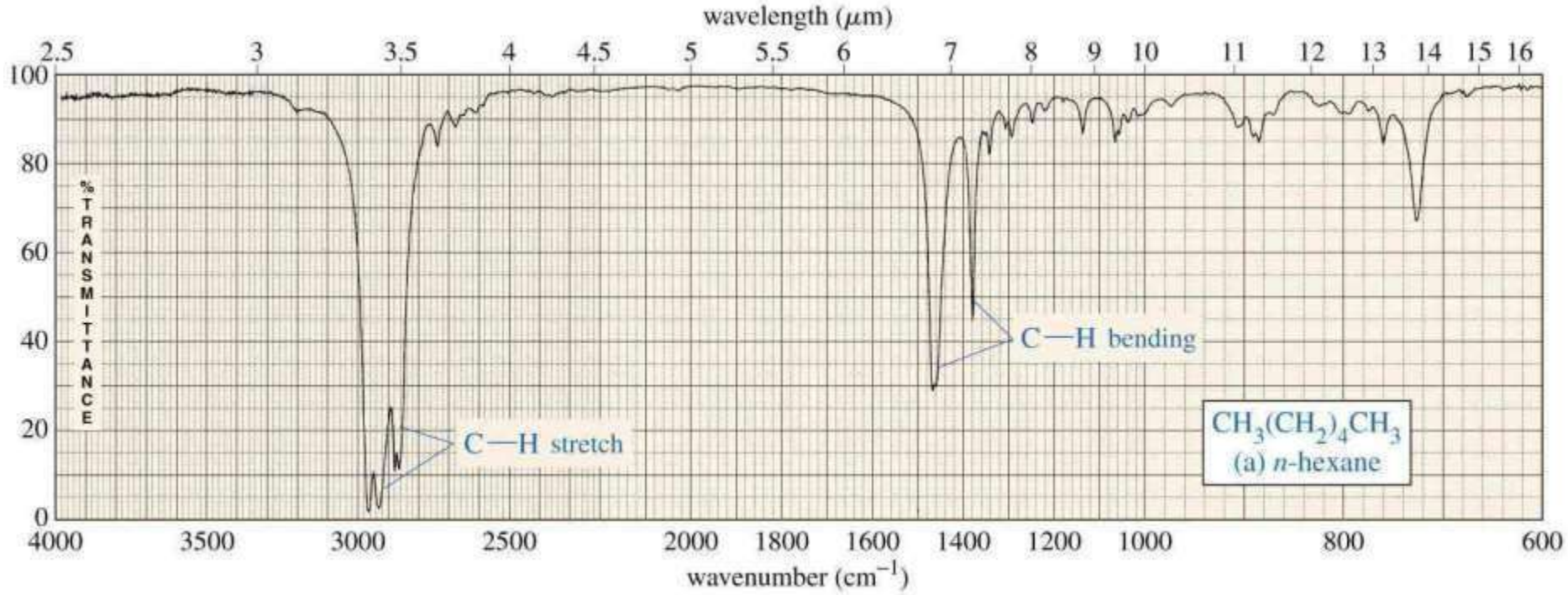
Carbon-Hydrogen Stretching

Bonds with more *s* character absorb at a higher frequency.

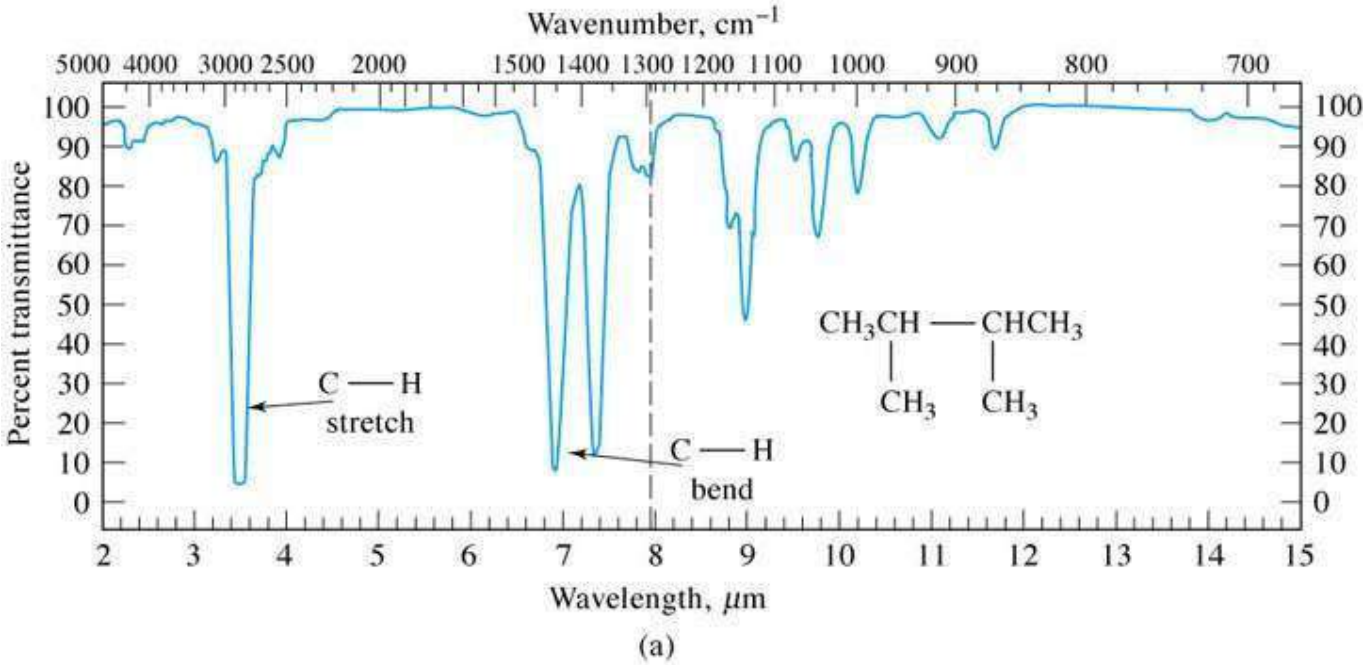
- sp^3 C-H, just below 3000 cm^{-1} (to the right)
- sp^2 C-H, just above 3000 cm^{-1} (to the left)
- *sp* C-H, at 3300 cm^{-1}

=>

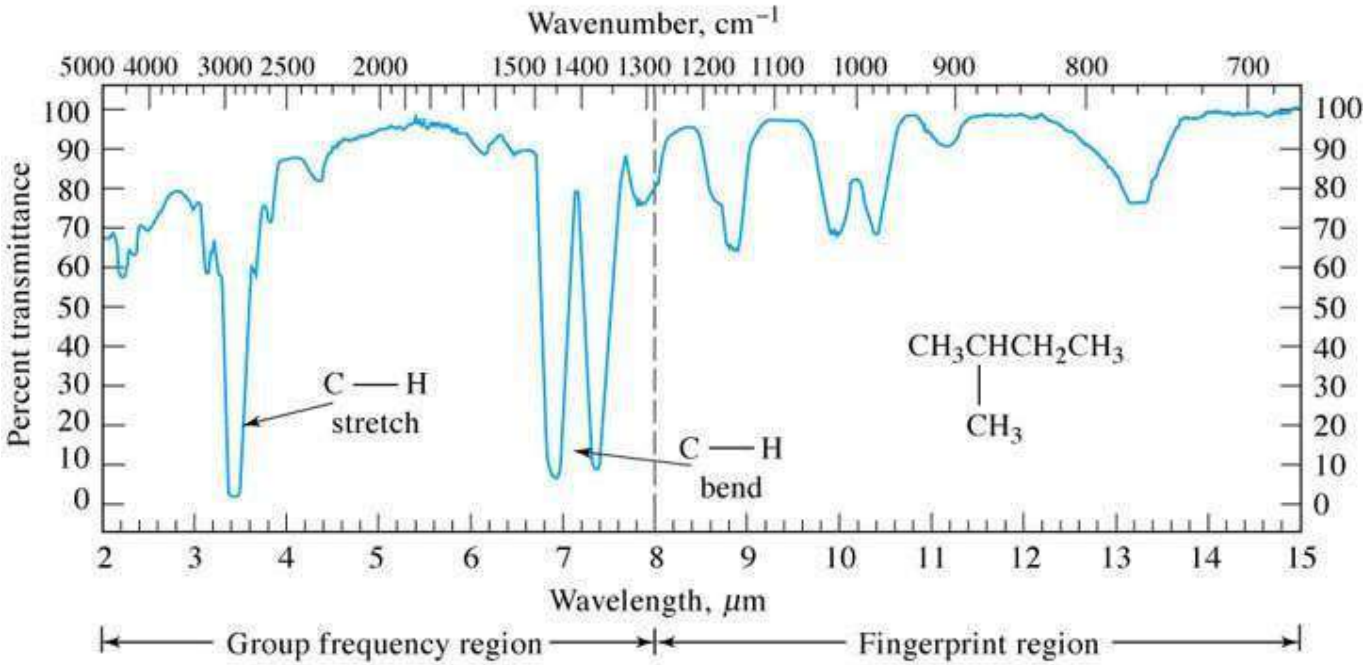
An Alkane IR Spectrum



=>



(a)



(b)

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N-Alkanes

- interpreted in terms of 4 vibrations:
  - stretching and bending of C–H and C–C bonds
    - C–C bends: ca. 500 cm^{-1} (out of spectral window)
    - C–C stretches: 1200–800 cm^{-1}, weak bands
      not of value for interpretation (fingerprint)

more characteristic

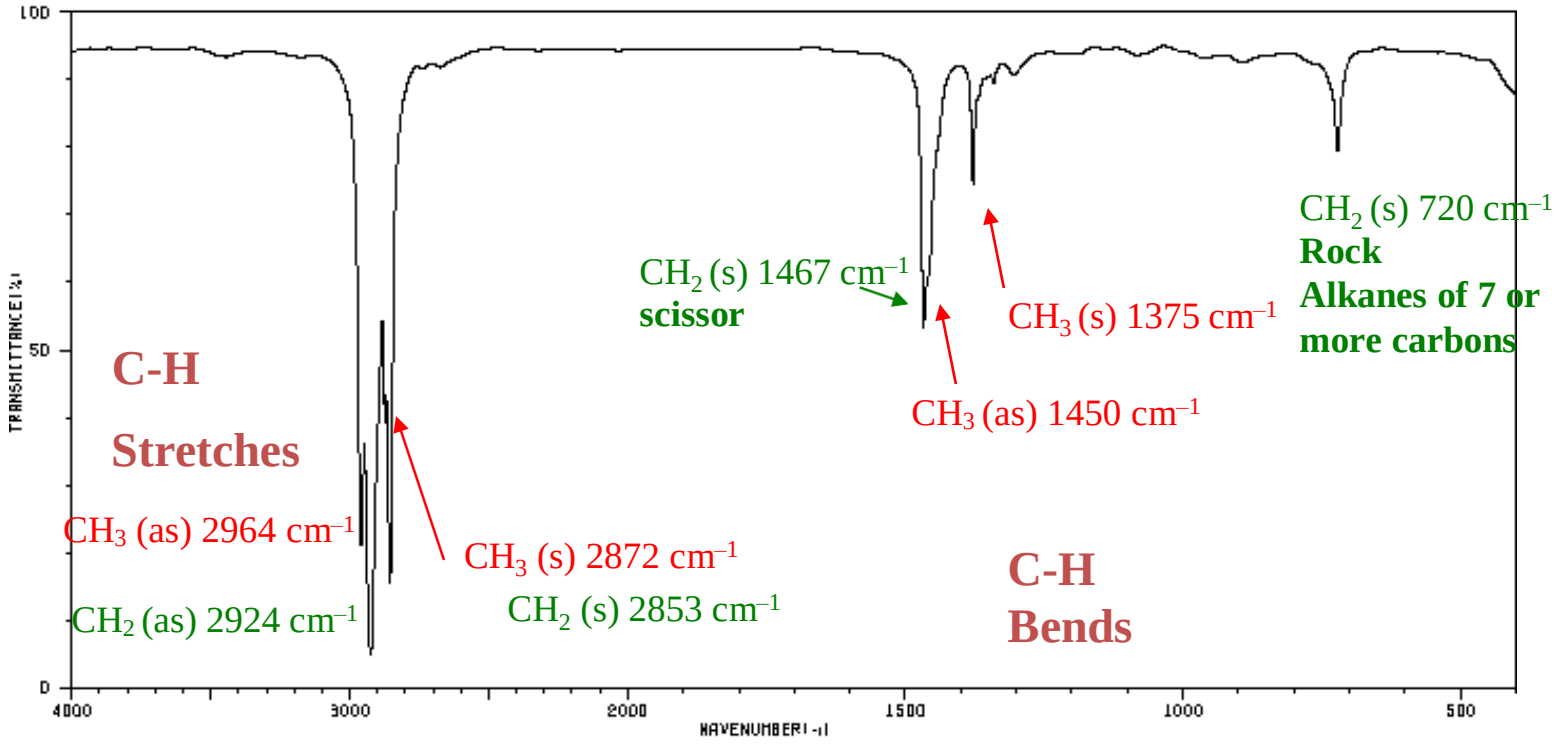
- C–H stretches: occurs from 3000 - 2840 cm^{-1}

CH_3: 2962 cm^{-1}, asymmetrical stretch
2872 cm^{-1}, symmetrical stretch

CH_2: 2926 cm^{-1}, asymmetrical stretch
2853 cm^{-1}, symmetrical stretch

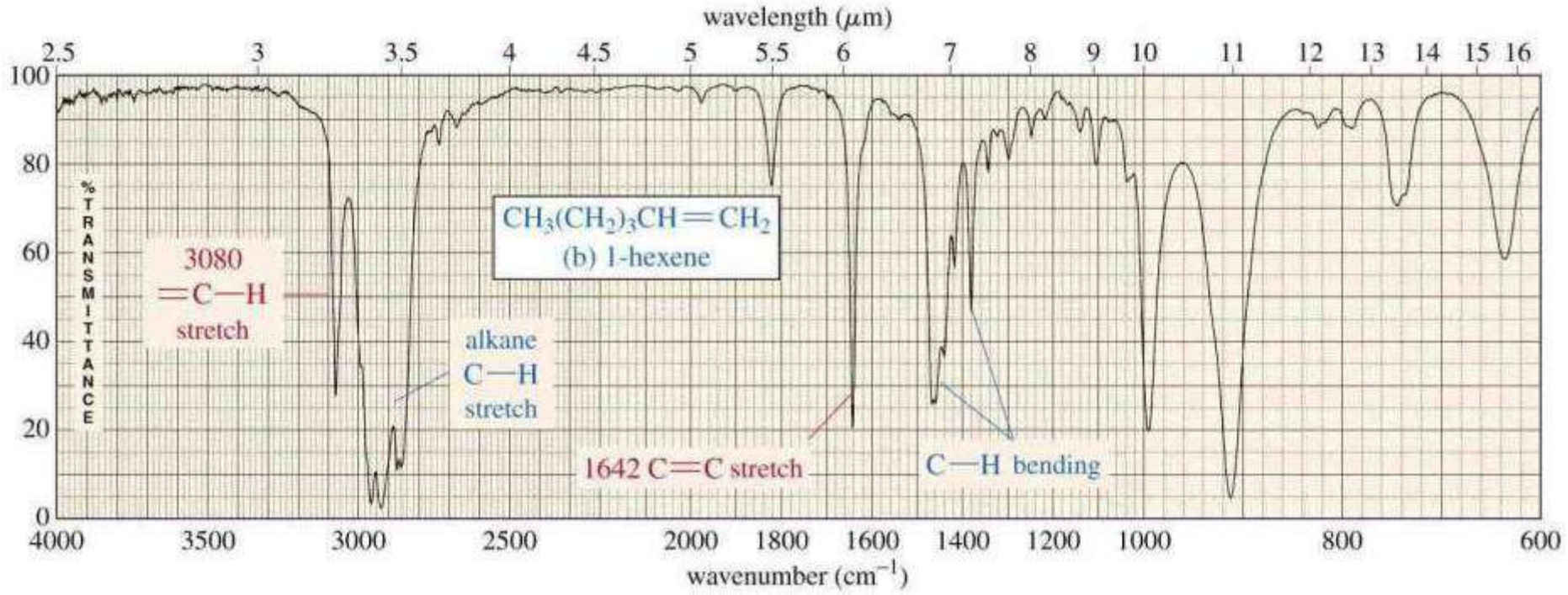
Note precision!

n-alkanes



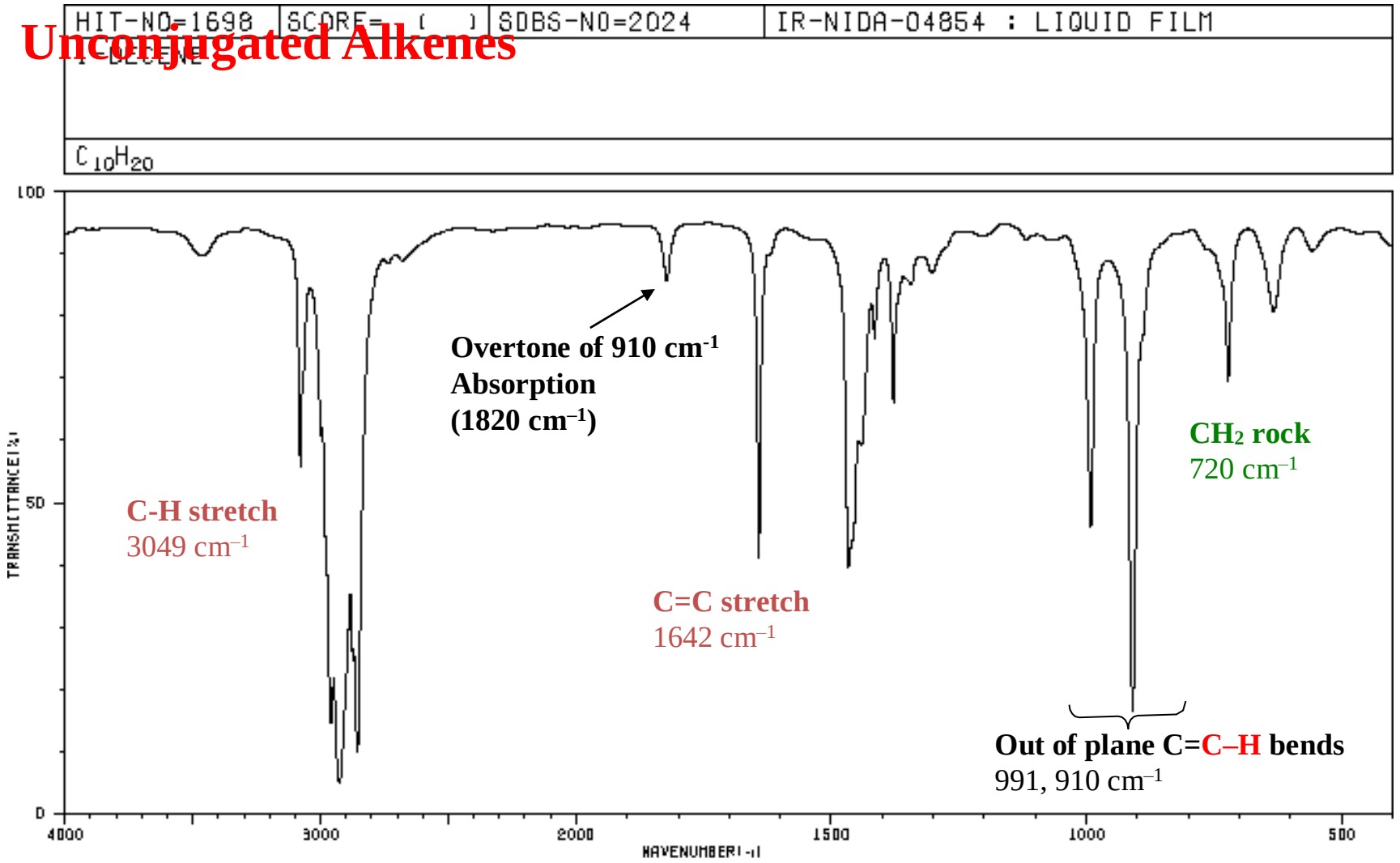
$C_{12}H_{25}$

An Alkene IR Spectrum

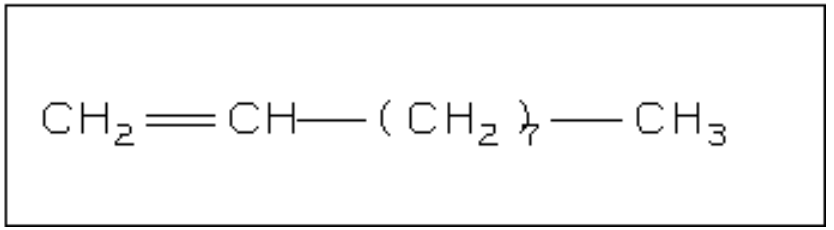


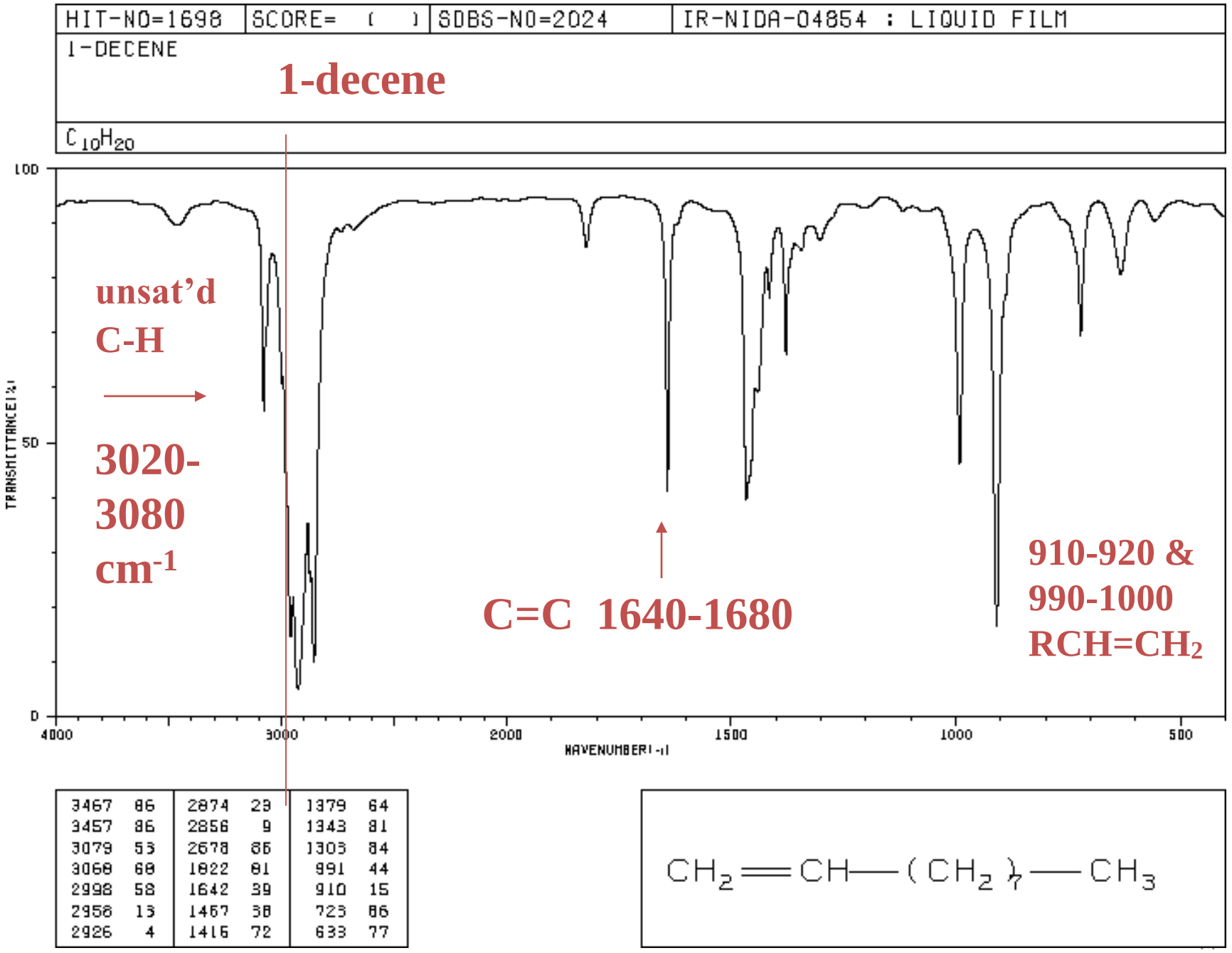
=>

Unconjugated Alkenes



1-decene

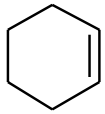




3467	86	2874	29	1379	64
3457	86	2856	9	1343	81
3079	53	2678	86	1303	84
3068	68	1822	81	991	44
2998	58	1642	39	910	15
2958	13	1467	38	723	86
2926	4	1416	72	633	77

• cyclic alkenes:

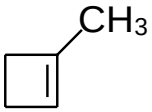
- C=C stretch: sensitive to ring strain



1650 cm^{-1}



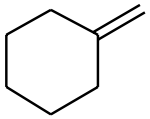
1566 cm^{-1}



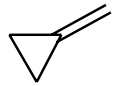
1641 cm^{-1}



1640 cm^{-1}



1650 cm^{-1}



1781 cm^{-1}

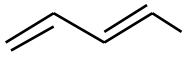
• cumulated alkenes:

- C=C=C stretch (asymmetric): 2000–1900 cm^{-1}

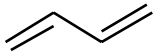
• conjugated alkenes:

-the alkene bond stretching vibrations in alkenes w/o a center of symmetry give rise to two C=C stretches

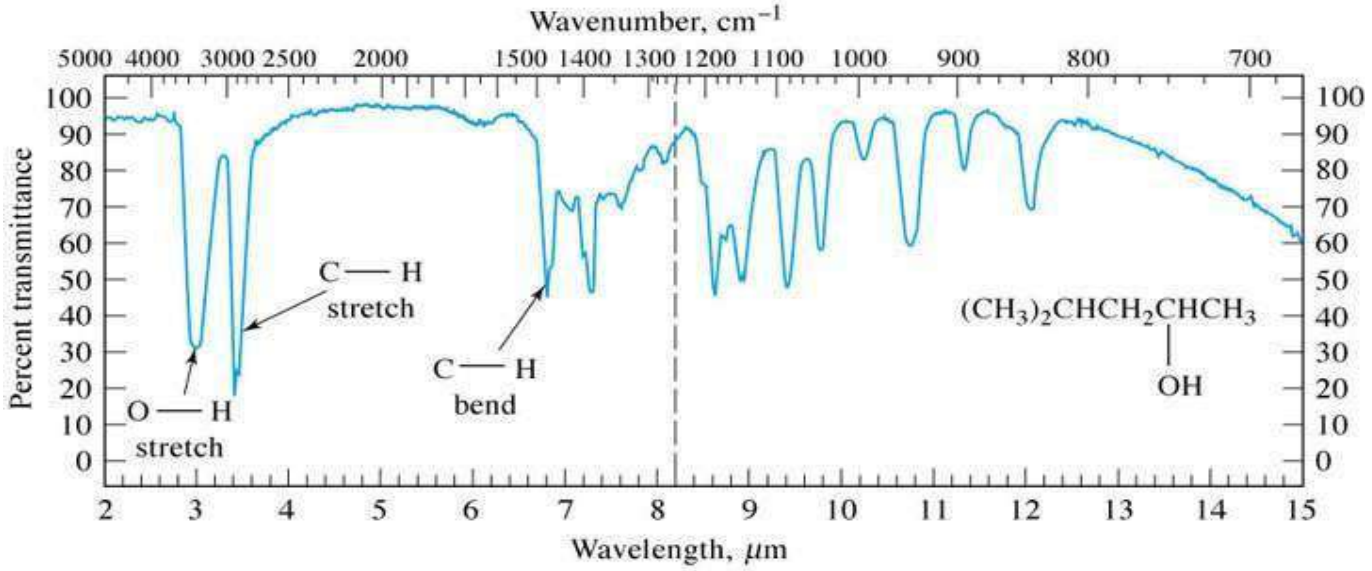
-for symmetrical molecules, e.g. butadiene, only the asymmetric stretch is observed



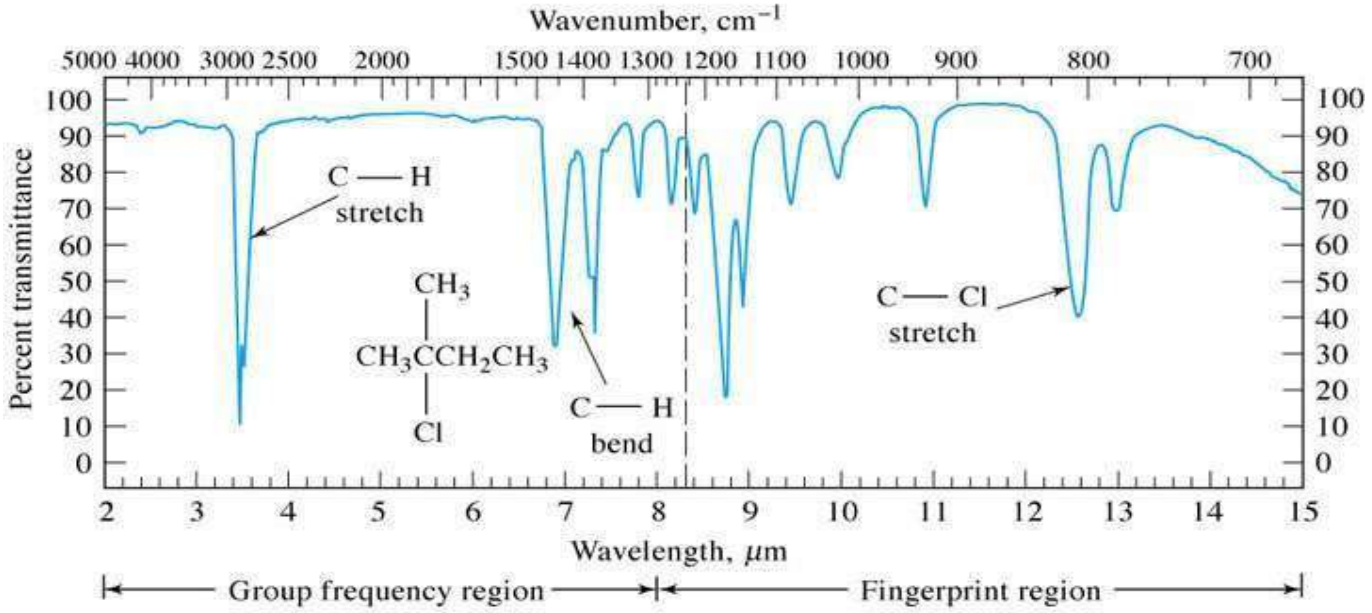
1650 cm^{-1} (as)
1600 cm^{-1} (s)



1600 cm^{-1} (as)

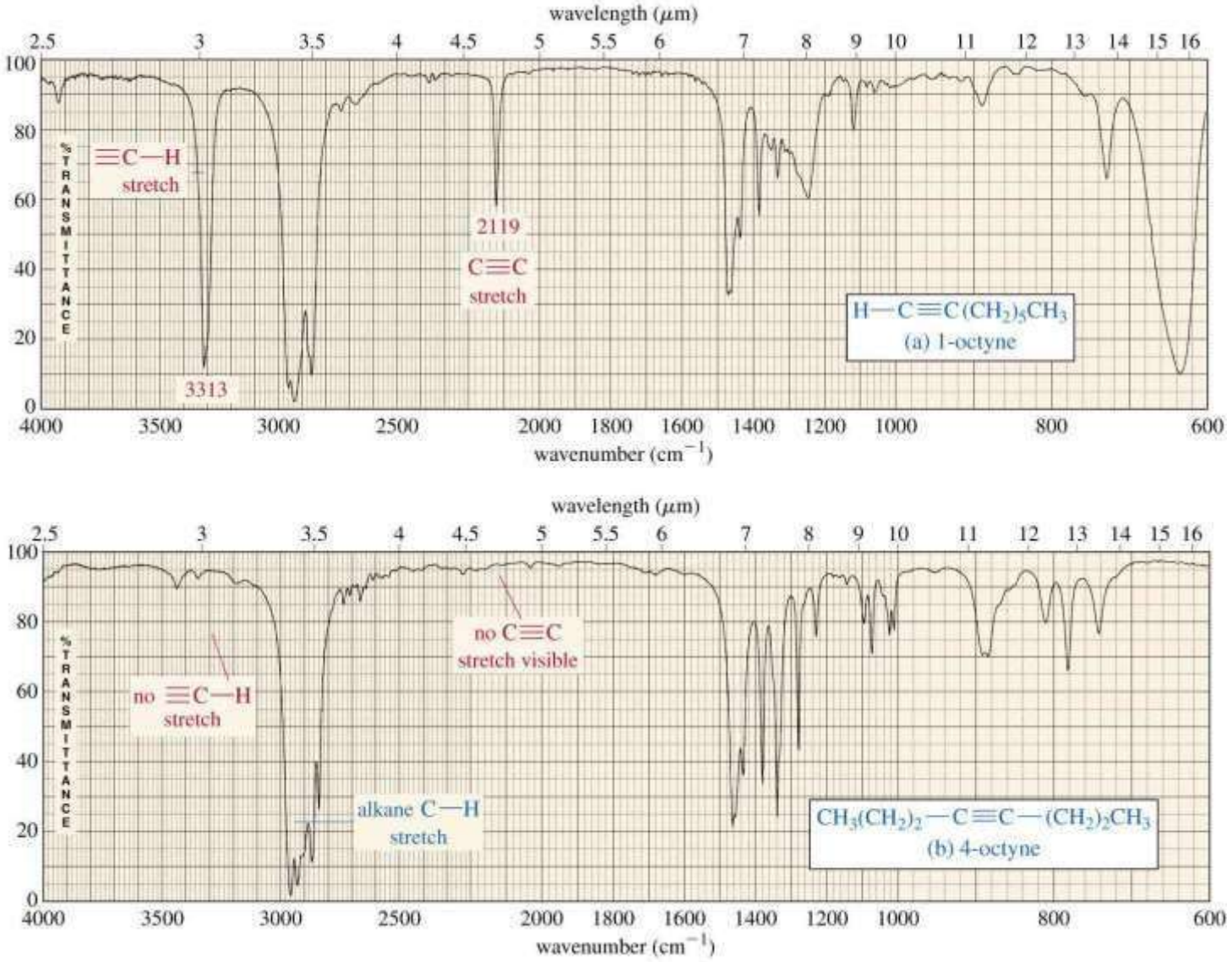

Wavenumber, cm^{-1}
5000 4000 3000 2500 2000 1500 1400 1300 1200 1100 1000 900 800 700
Percent transmittance
C — H stretch
C — H bend
O — H stretch
$(CH_3)_2CHCH_2CHCH_3$
OH
Wavelength, μm
2 3 4 5 6 7 8 9 10 11 12 13 14 15

(c)


Wavenumber, cm^{-1}
5000 4000 3000 2500 2000 1500 1400 1300 1200 1100 1000 900 800 700
Percent transmittance
C — H stretch
CH_3
$CH_3CCH_2CH_3$
Cl
C — H bend
C — Cl stretch
Wavelength, μm
2 3 4 5 6 7 8 9 10 11 12 13 14 15
Group frequency region
Fingerprint region

(d)

An Alkyne IR Spectrum

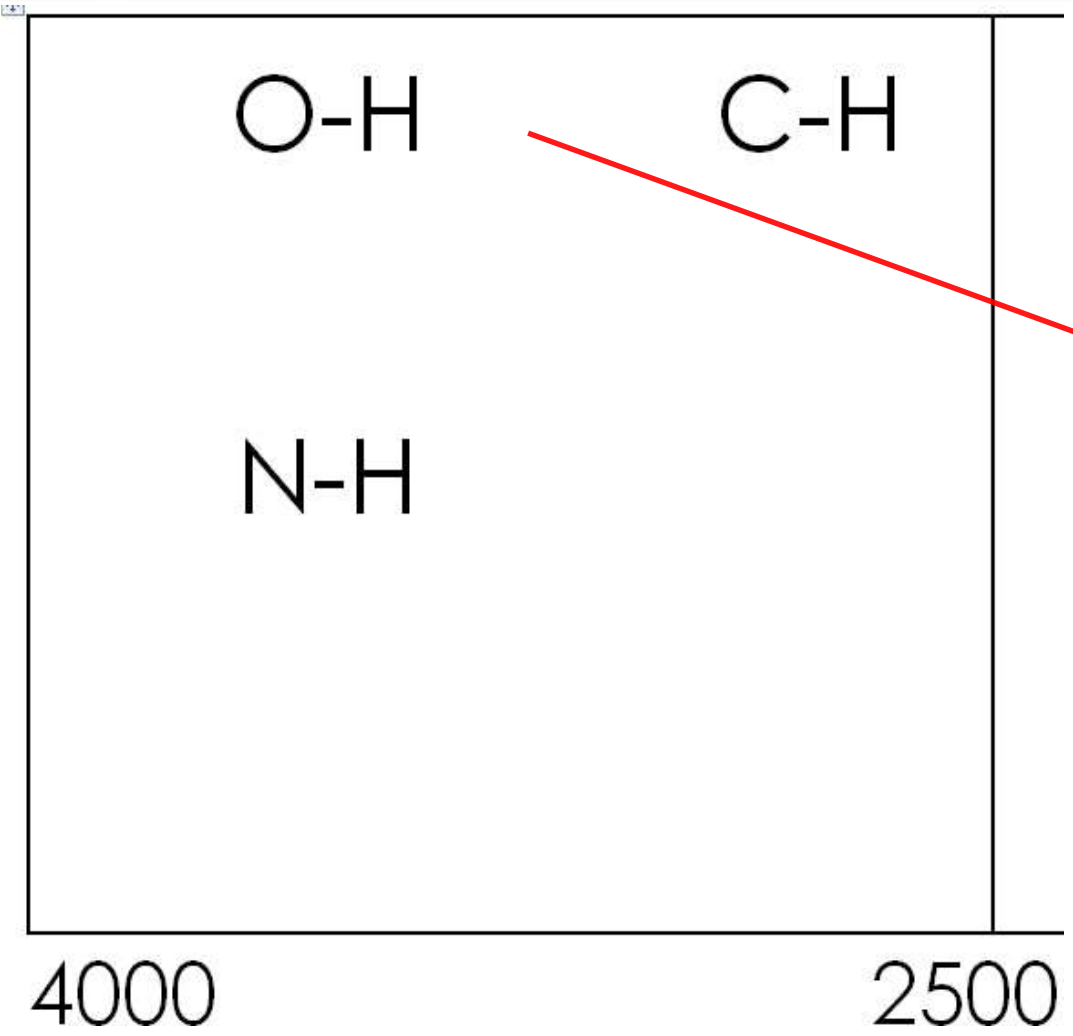


=>

O-H and N-H Stretching

- Both of these occur around 3300 cm^{-1}, but they look different.
  - Alcohol O-H, broad with rounded tip.
  - Secondary amine (R_2NH), broad with one sharp spike.
  - Primary amine (RNH_2), broad with two sharp spikes.
  - No signal for a tertiary amine (R_3N) =>

Region 4000-2500 cm-1



O-H *(alcohols and phenols)*

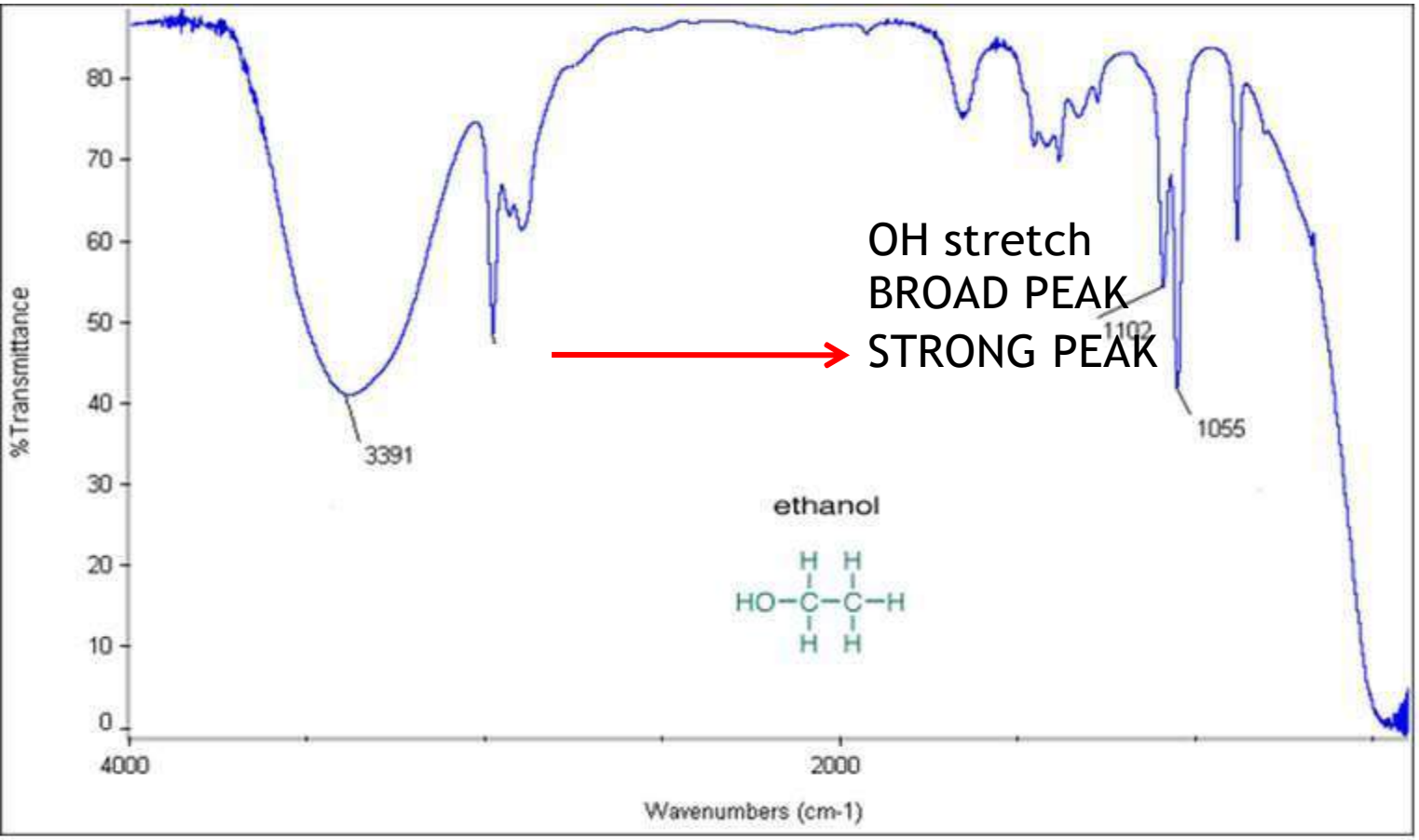
3650-3200 cm^{-1}

Strong, Broad Peak in the range around 3400

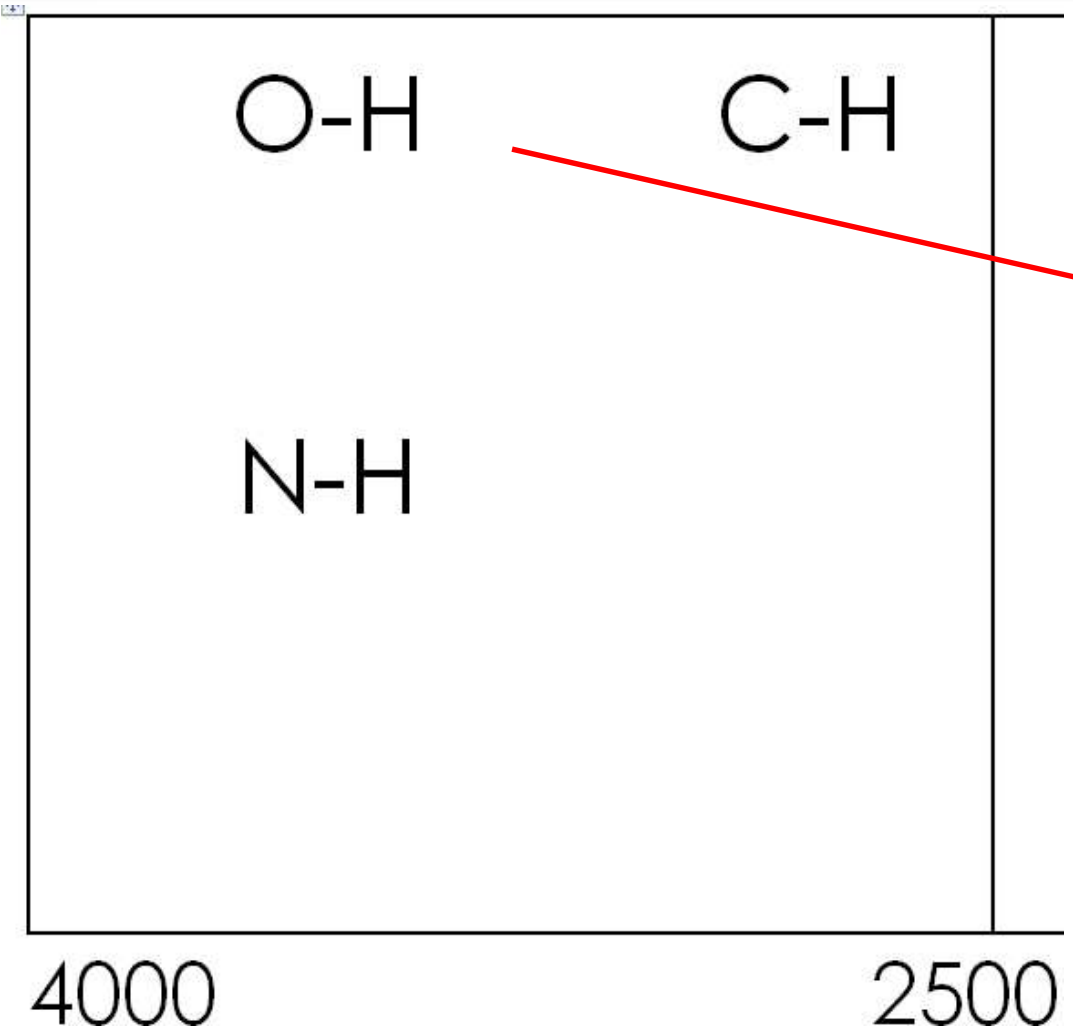
..

Could **NOT** be confused with another peak

Alcoholic and phenolic OH



Region 4000-2500 cm-1

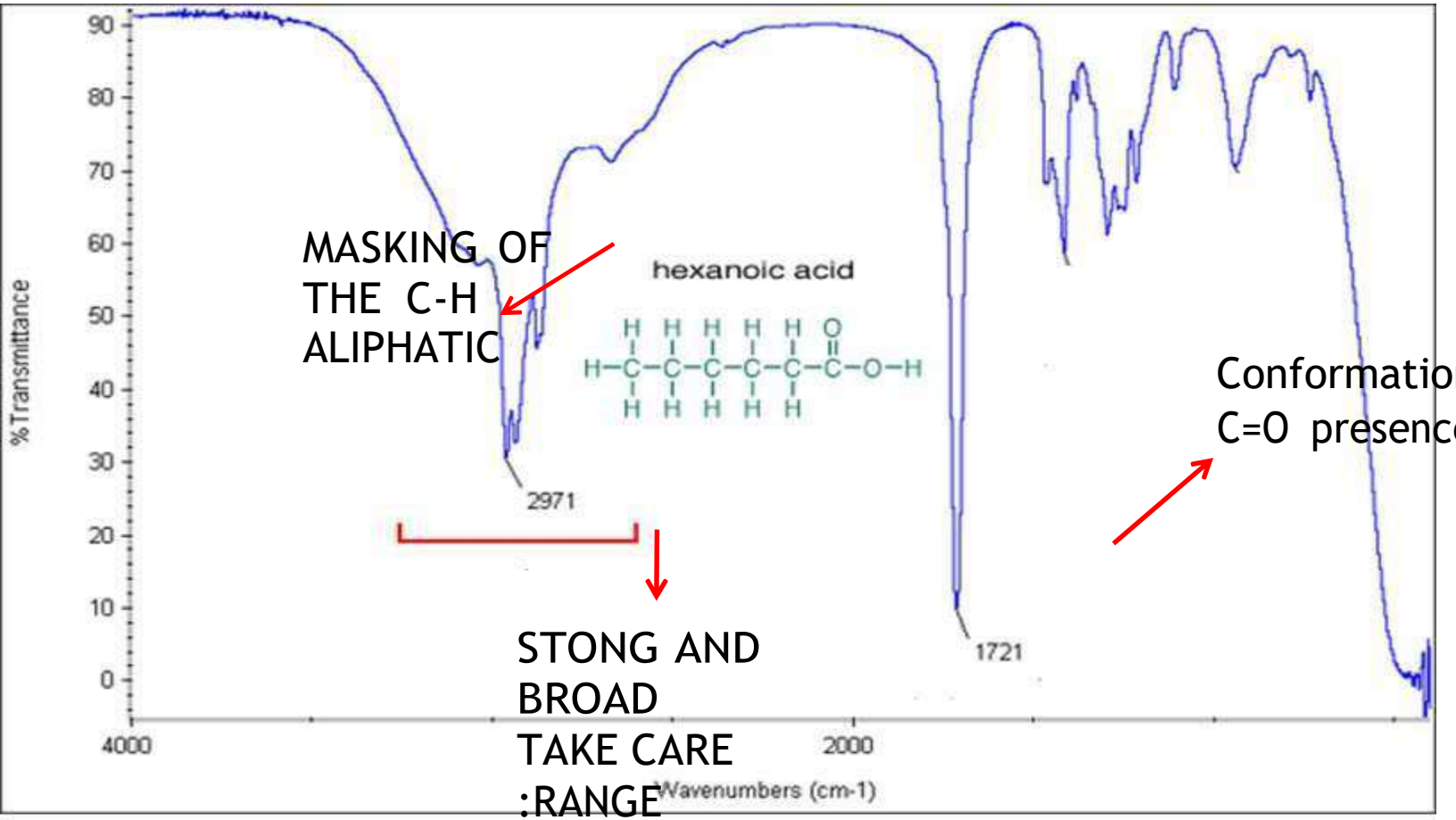


- Strong .. but **MUCH BROADER** than O-H of alcohols and phenols
- Notice the range > 400 cm^{-1}

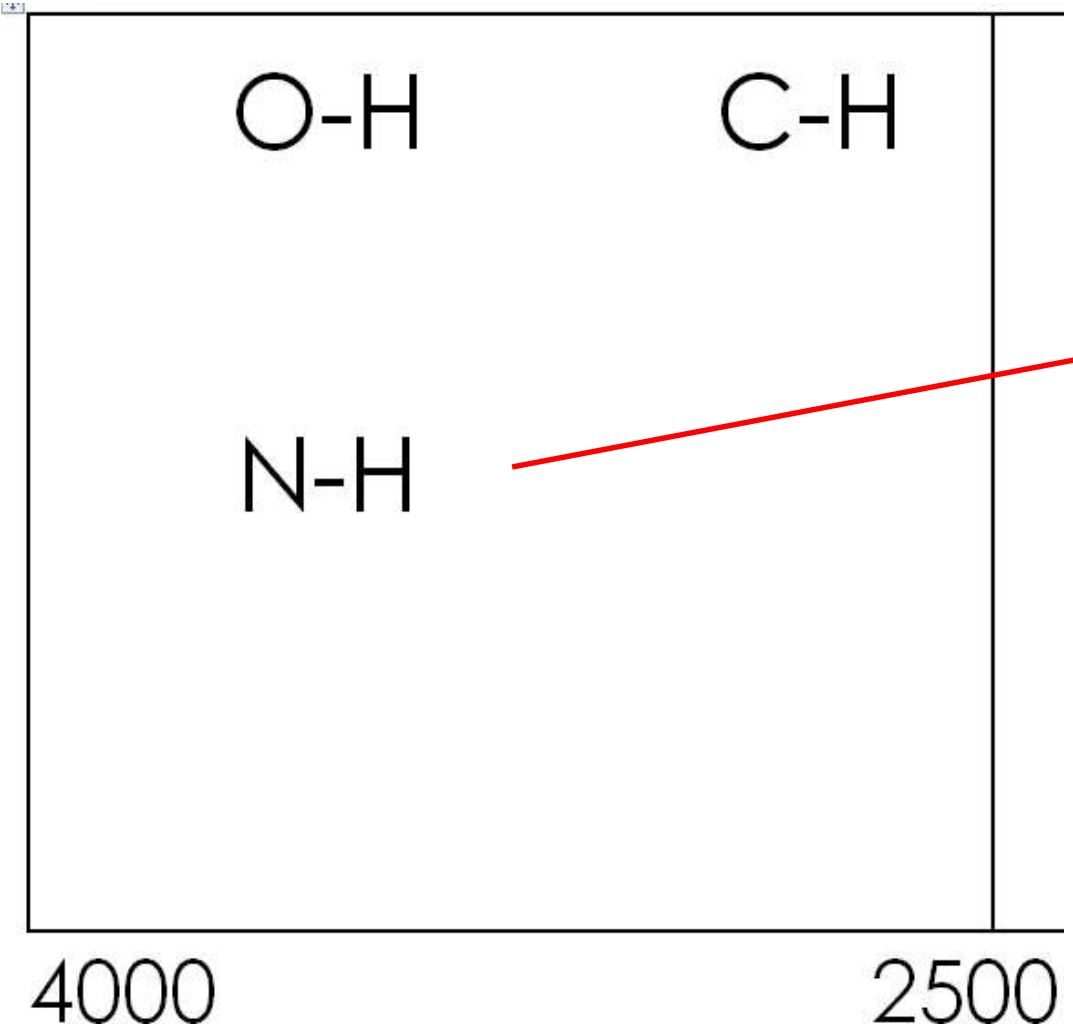
O-H *(carboxylic acids)*

- Could **NOT** be confused with O-H alcoholic or phenolic
- Easy to confirm by looking for C=O of acid

Carboxylic acid OH



Region 4000-2500 cm^{-1}



3500-3100 cm^{-1}

N-H *(stretching)*

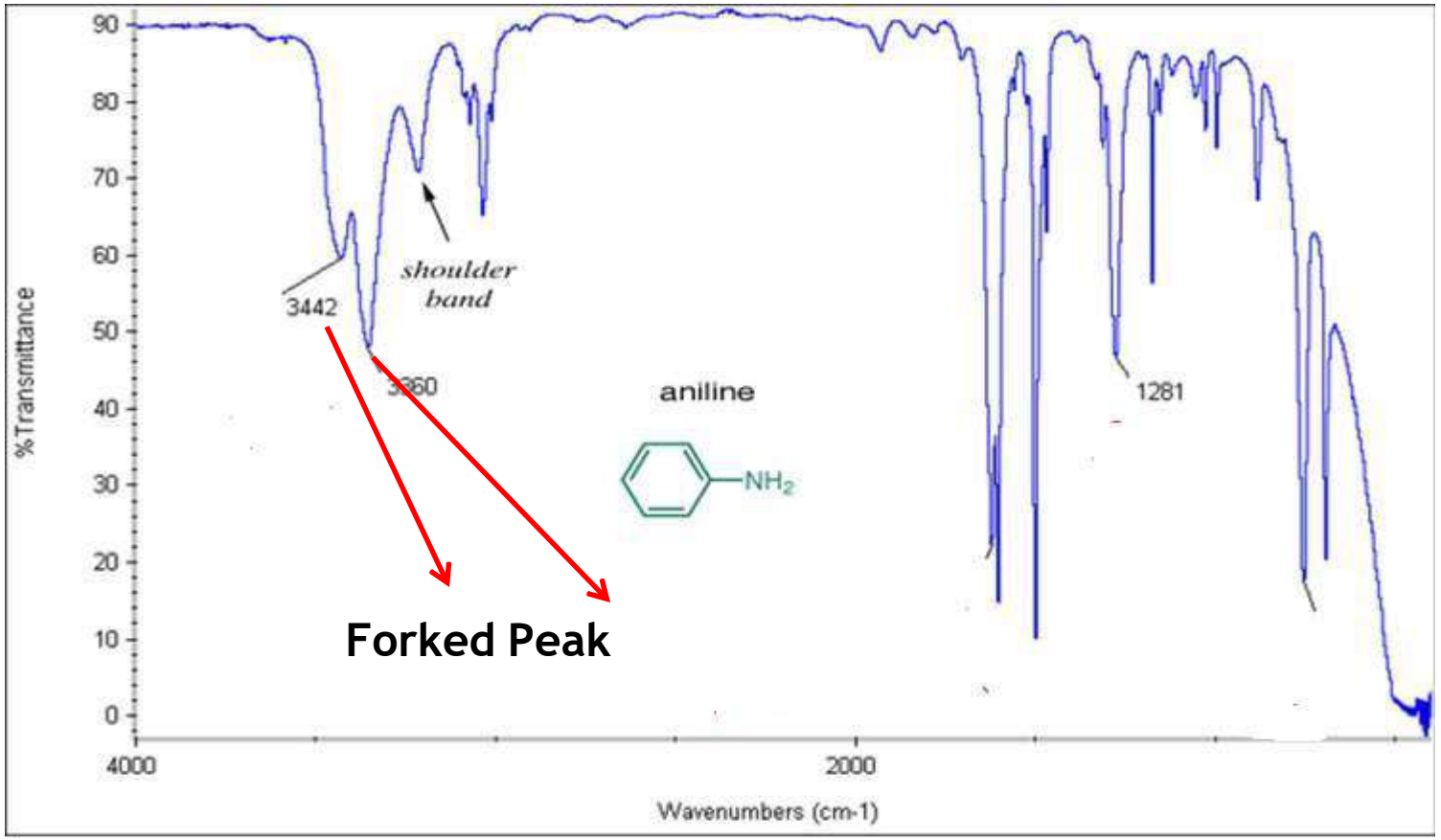
LESS strong .. LESS broad than O-H, ***although in the same range!!***

Primary amines (R-NH_2) give **Forked Peak**

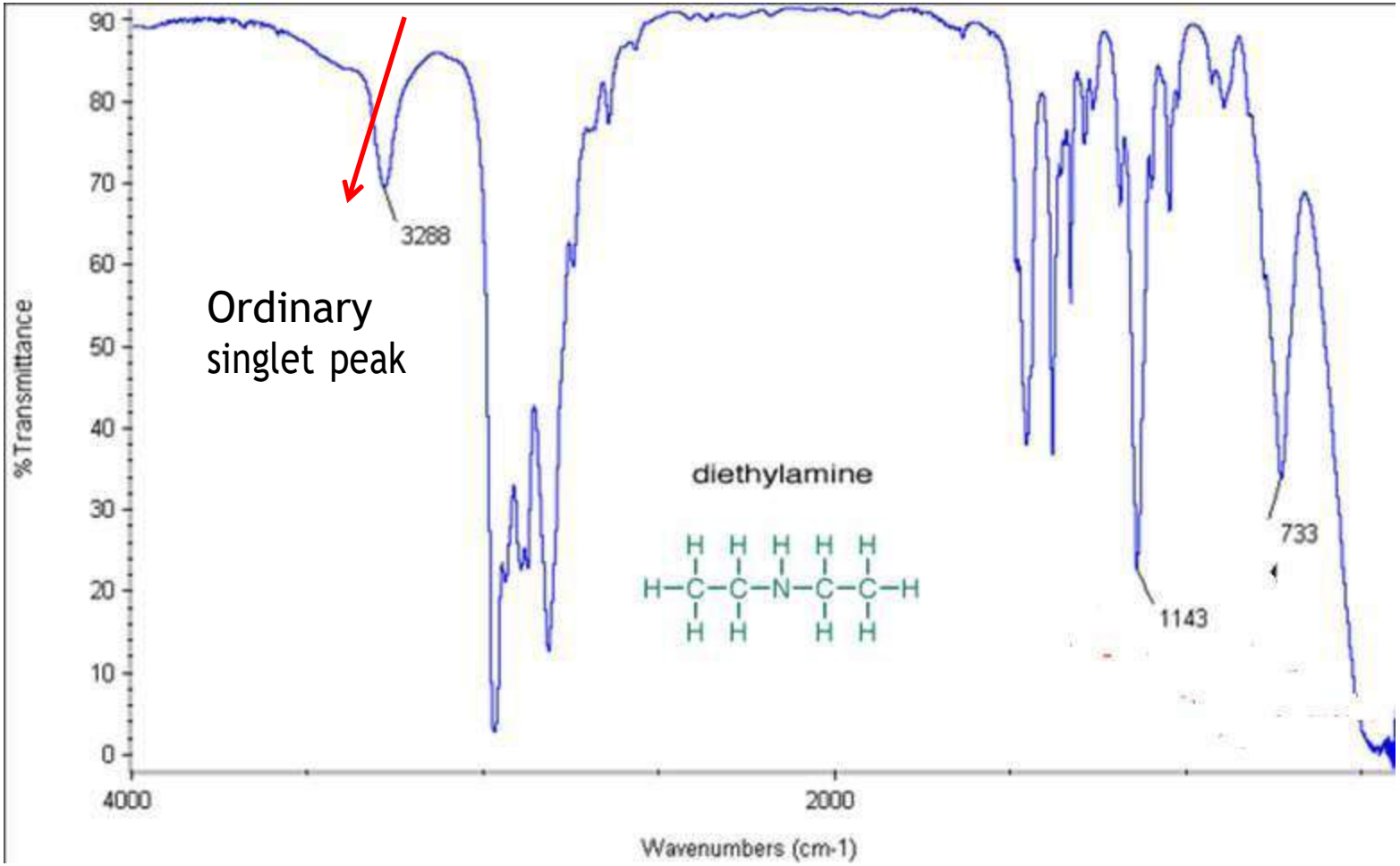
Secondary amines (R-NH-R') give and ordinary singlet peak

Tertiary amines (R_3-N) show no peaks in this region .. simply because they donNOT possess any N-H ***!!!***

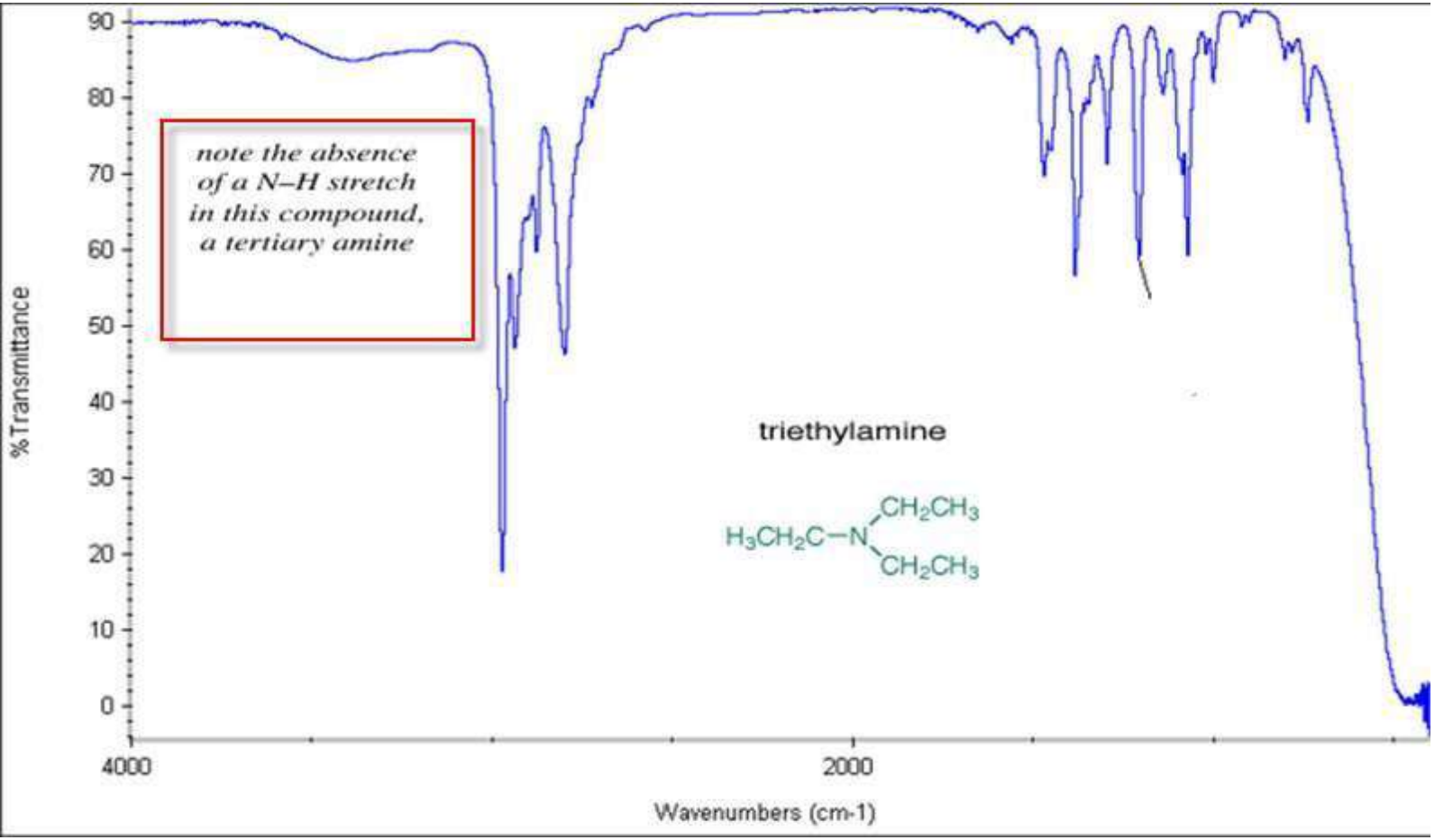
Amines (primary amines)



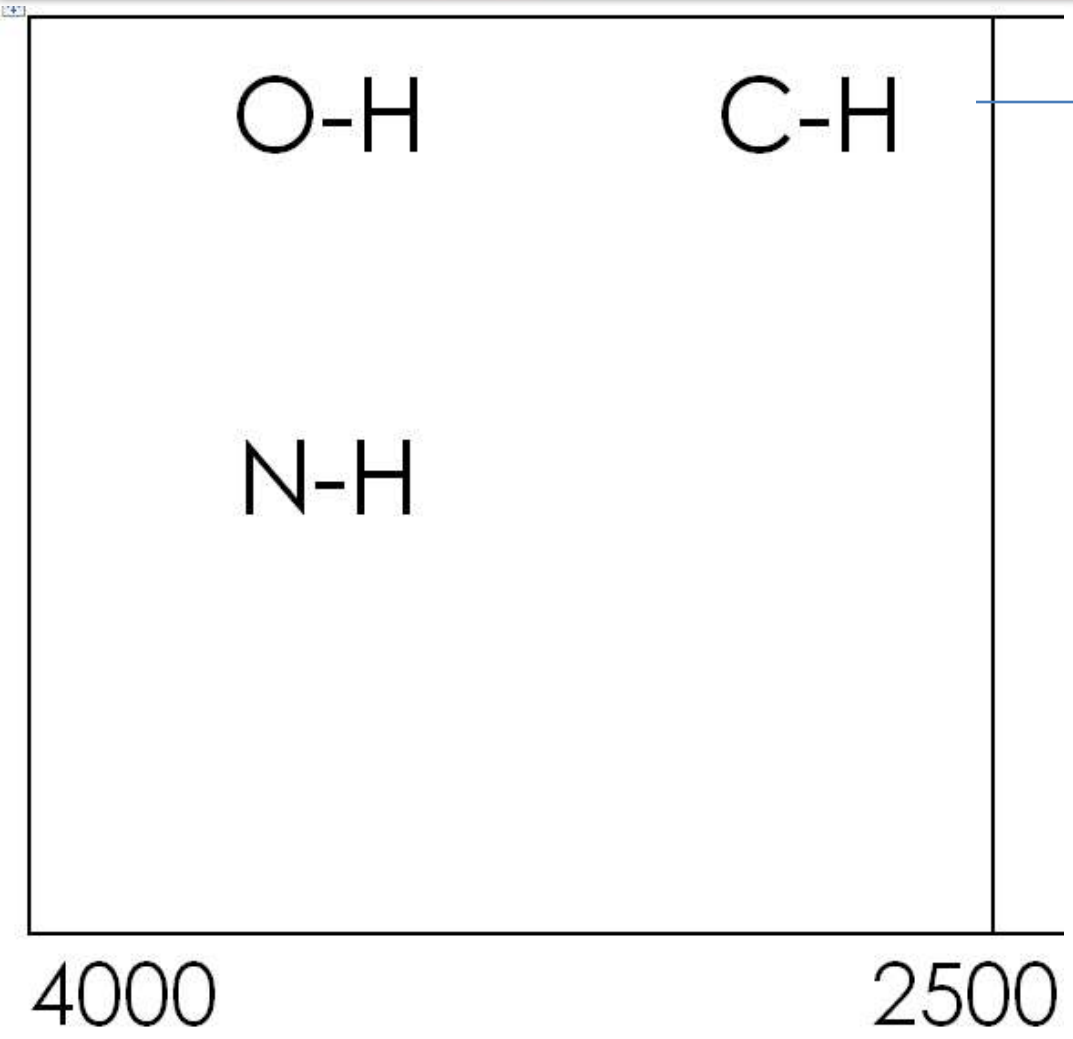
Amine (secondary amines)



Amines (Tertiary amines)

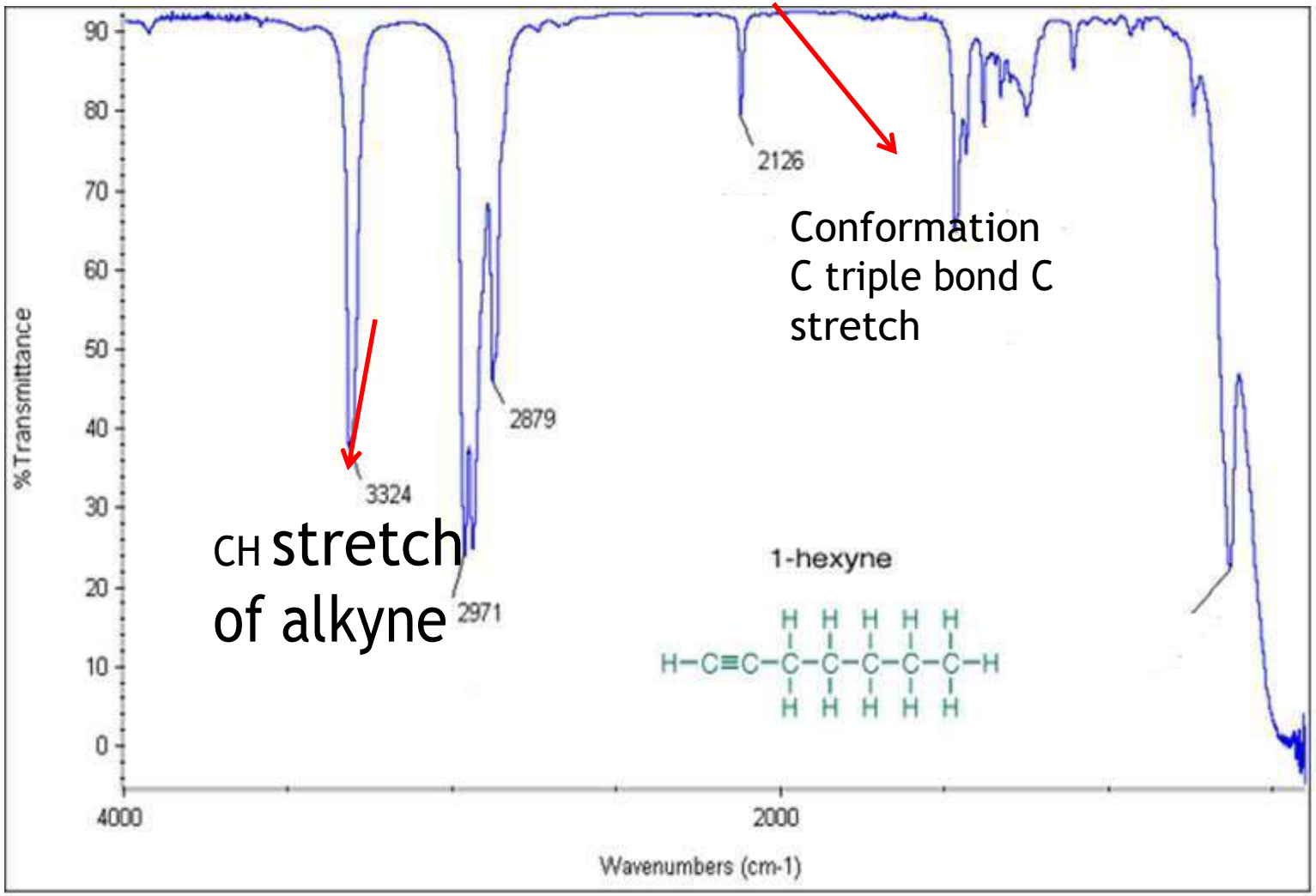


Region 4000-2500 cm^{-1}

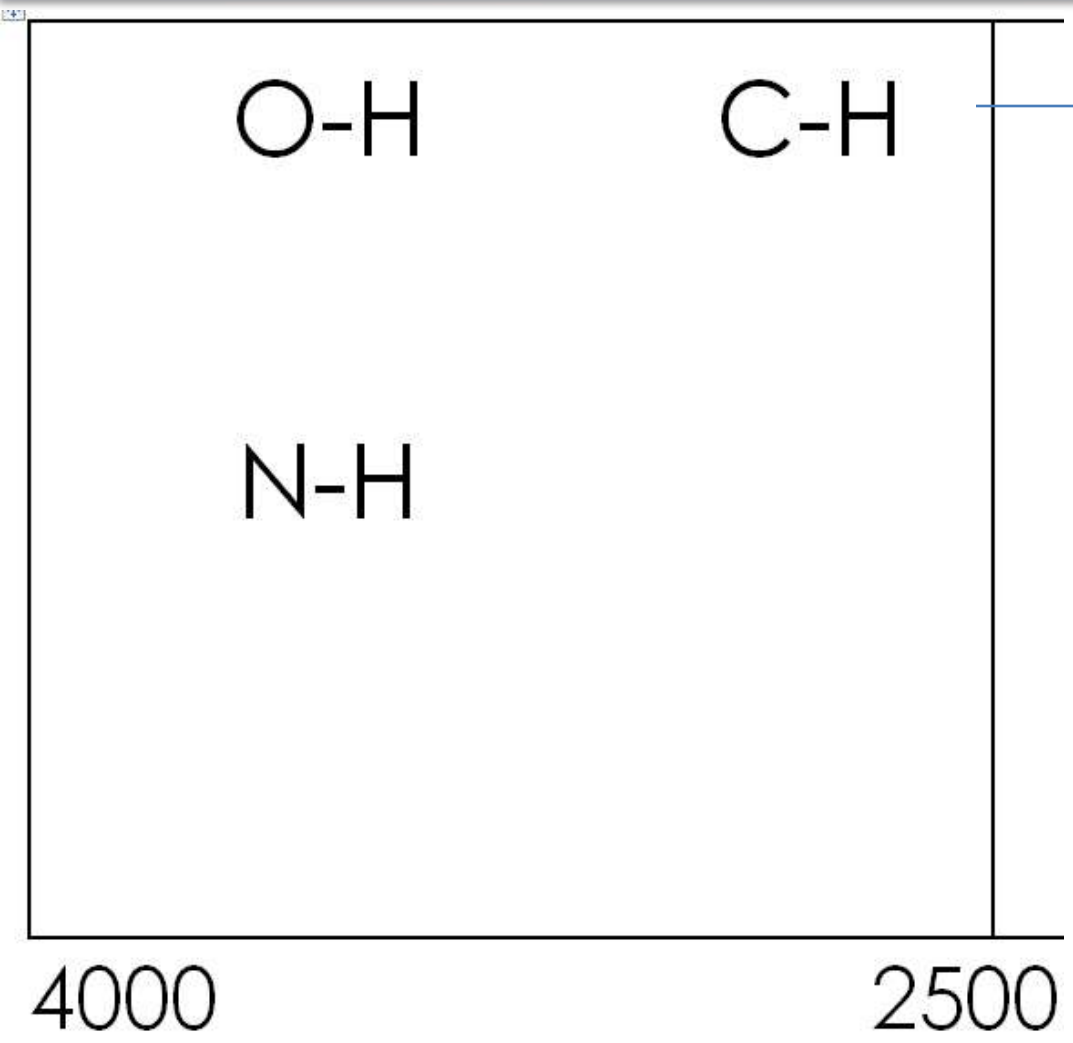


ALKYNE
1-ALKYNE
SHARP, medium peak around 3300 cm^{-1}
2-ALKENE
AND
AROMATIC
3-ALKANE
4-ALDEHYDIC

C-H ALKYNE


% Transmittance
90
80
70
60
50
40
30
20
10
0
4000
2000
Wavenumbers (cm-1)
3324
2879
2971
2126
Conformation
C triple bond C
stretch
CH stretch
of alkyne
1-hexyne

Region 4000-2500 cm^{-1}



1-Alkyne

2-Alkene and Aromatic 3100-3000 cm^{-1}

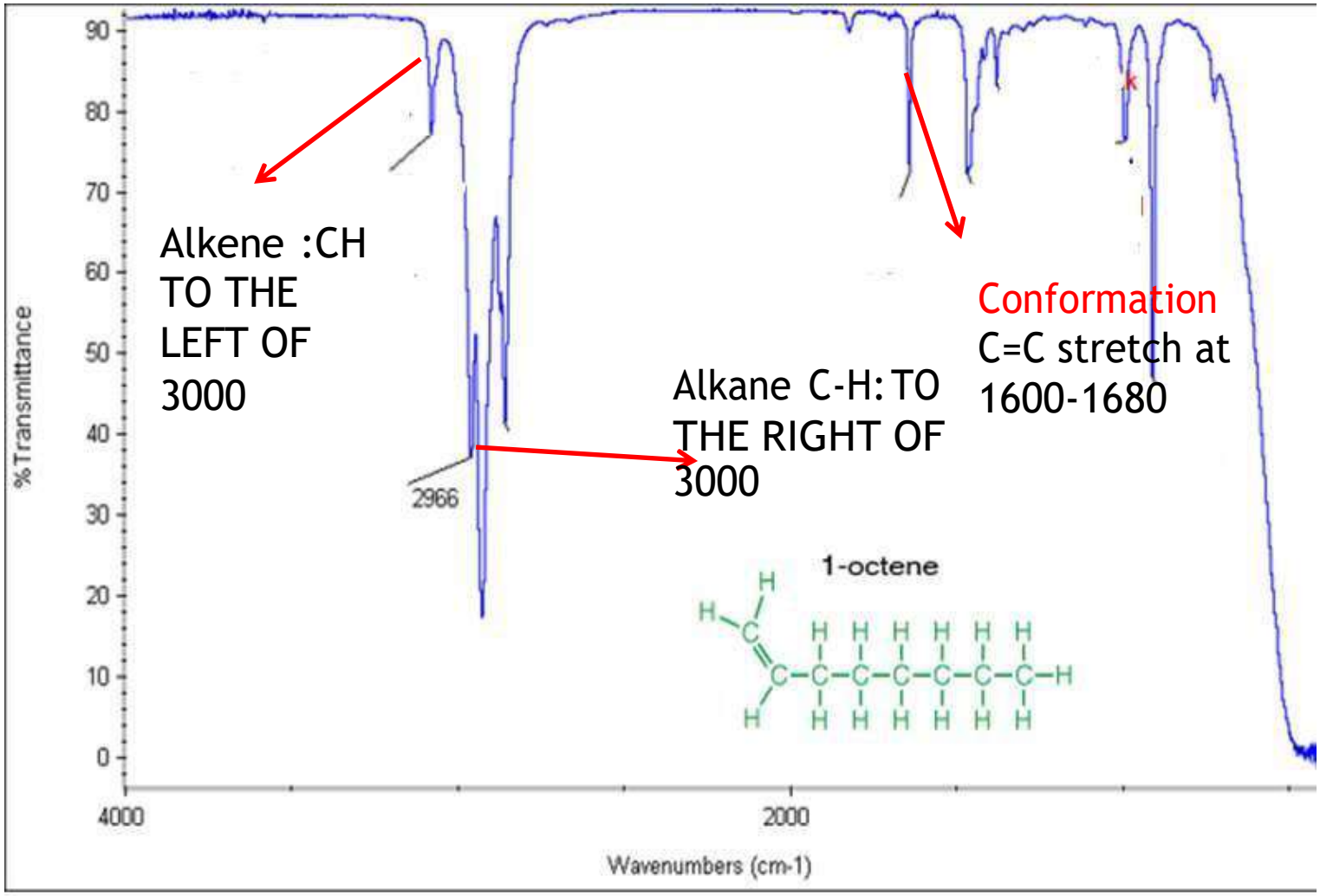
Simply ... to the LEFT of 3000 cm^{-1}

2-Alkene and Aromatic

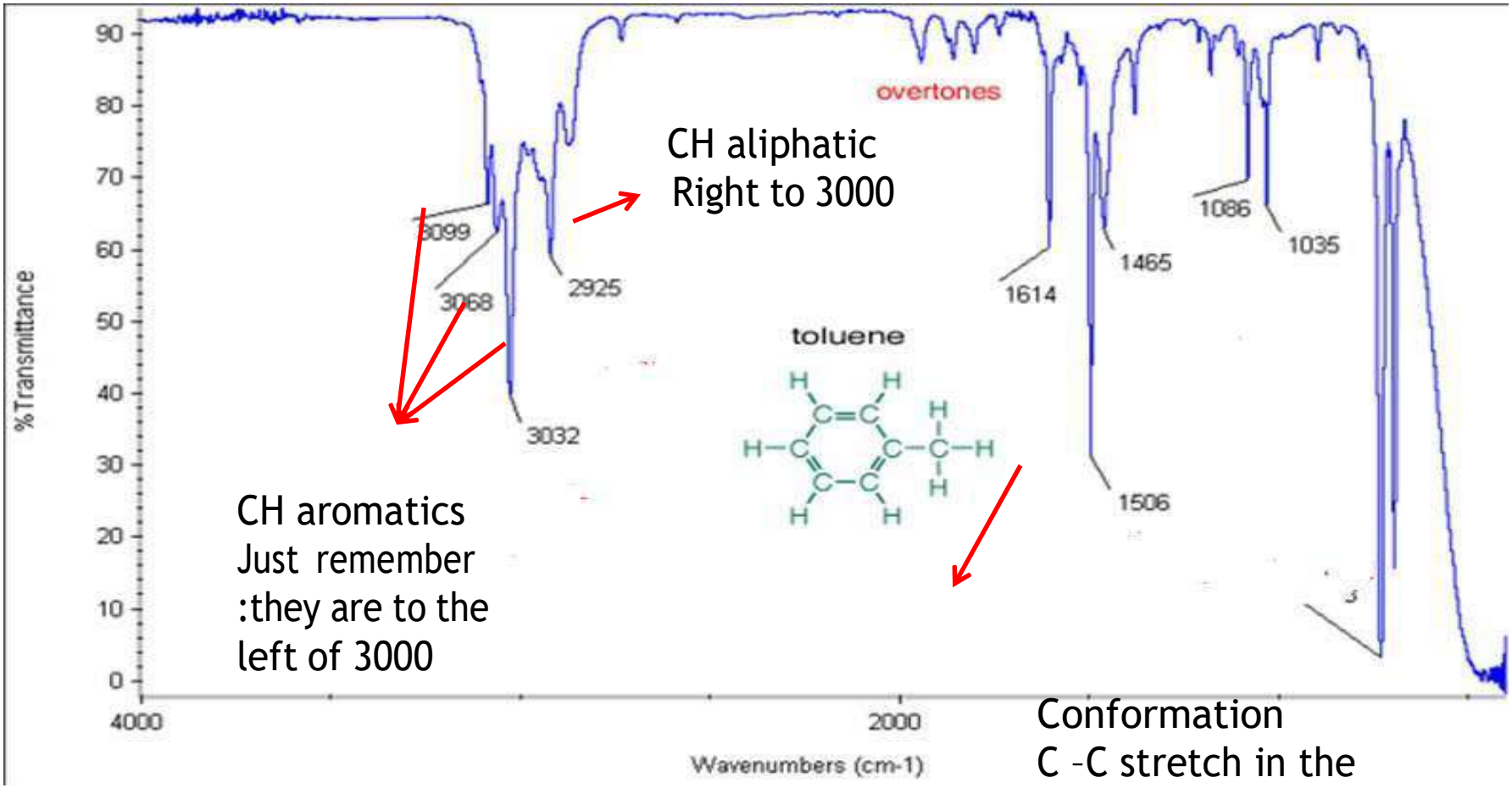
3-Alkane

4-Aldehydic

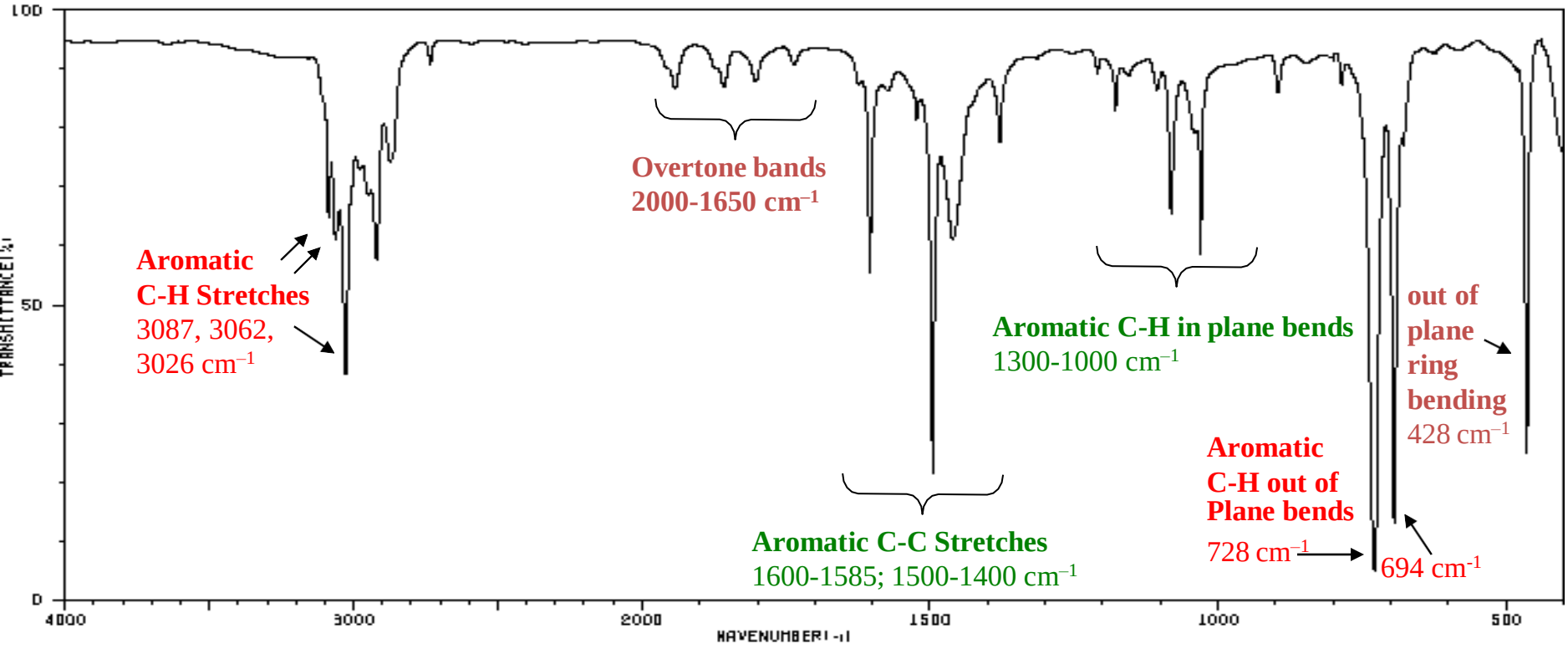
C-H Alkene


Alkene :CH TO THE LEFT OF 3000
Alkane C-H: TO THE RIGHT OF 3000
Conformation
C=C stretch at 1600-1680
2966
1-octene
%Transmittance
Wavenumbers (cm-1)
4000
2000
90
80
70
60
50
40
30
20
10
0

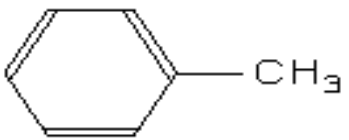
C-H aromatic


overtones
CH aliphatic
Right to 3000
3099
3068
2925
3032
1614
1465
1086
1035
1506
toluene
CH aromatics
Just remember
:they are to the
left of 3000
Conformation
C –C stretch in the
ring (two peaks)
1600 and 1475
%Transmittance
Wavenumbers (cm-1)
4000
2000
90
80
70
60
50
40
30
20
10
0

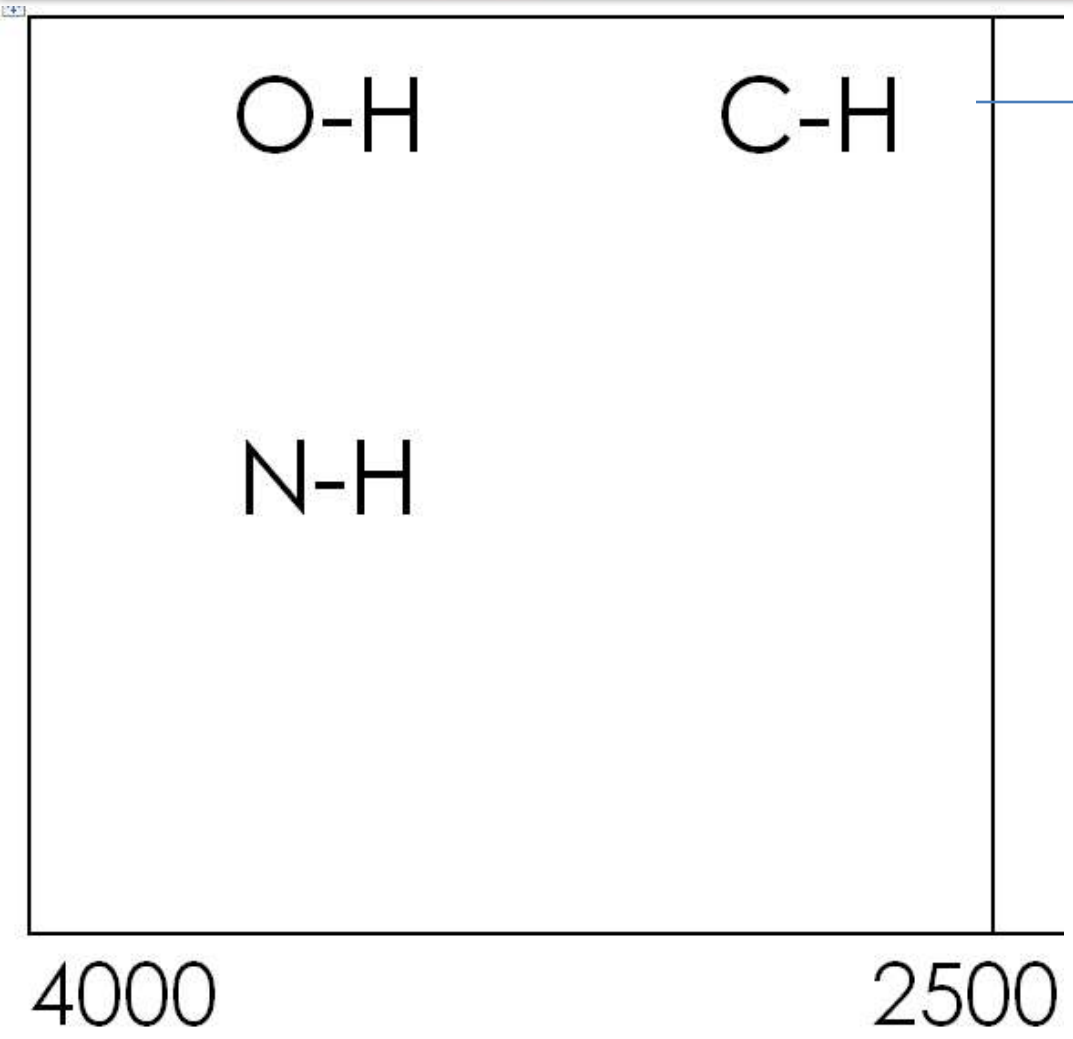
Mononuclear Aromatics



3087	62	1868	84	1210	86	895	81
3062	58	1803	84	1179	79	786	84
3028	37	1605	55	1156	86	729	4
2948	66	1524	79	1107	84	696	12
2920	55	1496	20	1082	62	678	74
2873	70	1461	58	1042	77	465	29
1942	84	1379	74	1030	67		



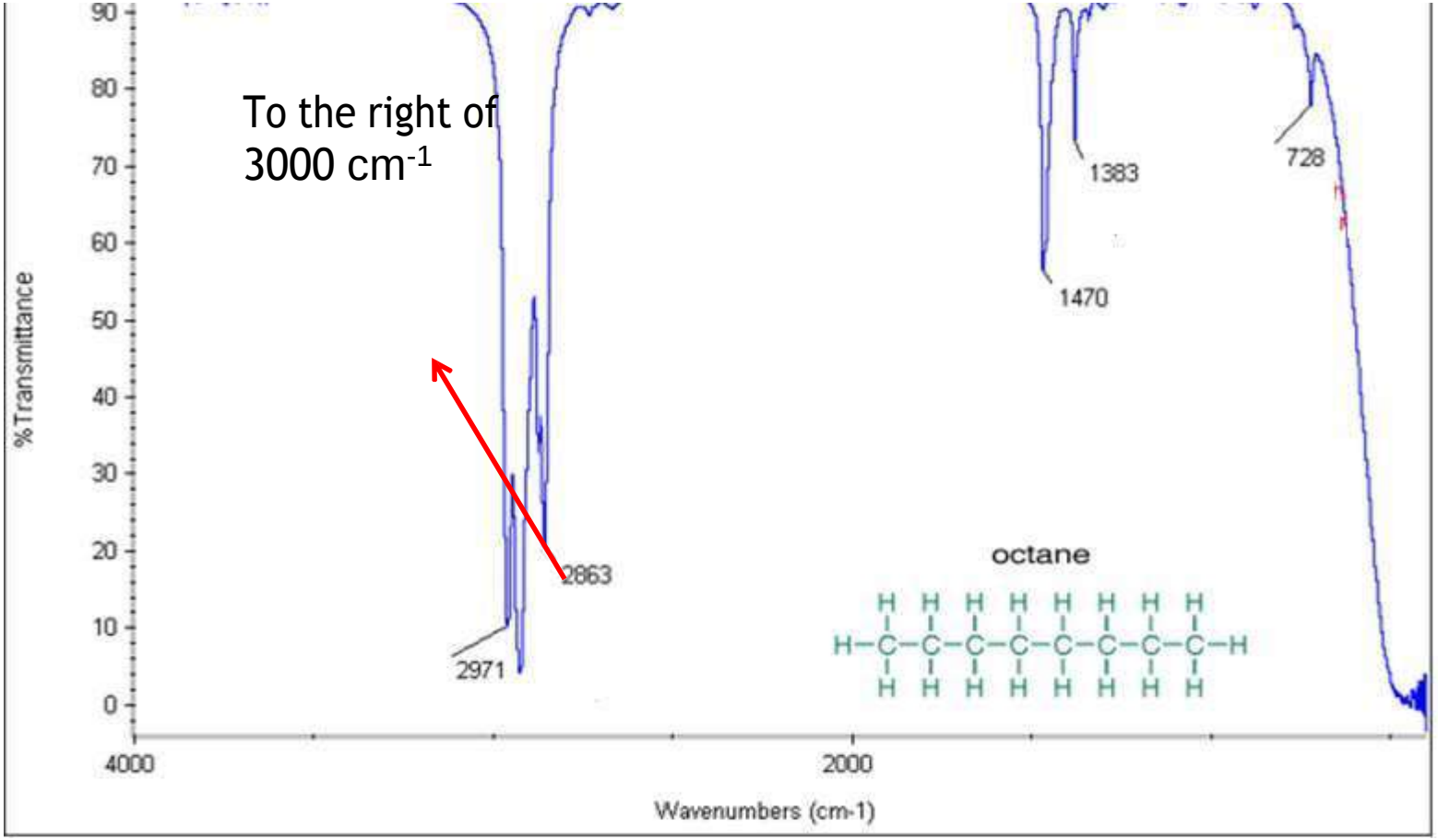
Region 4000-2500 cm^{-1}



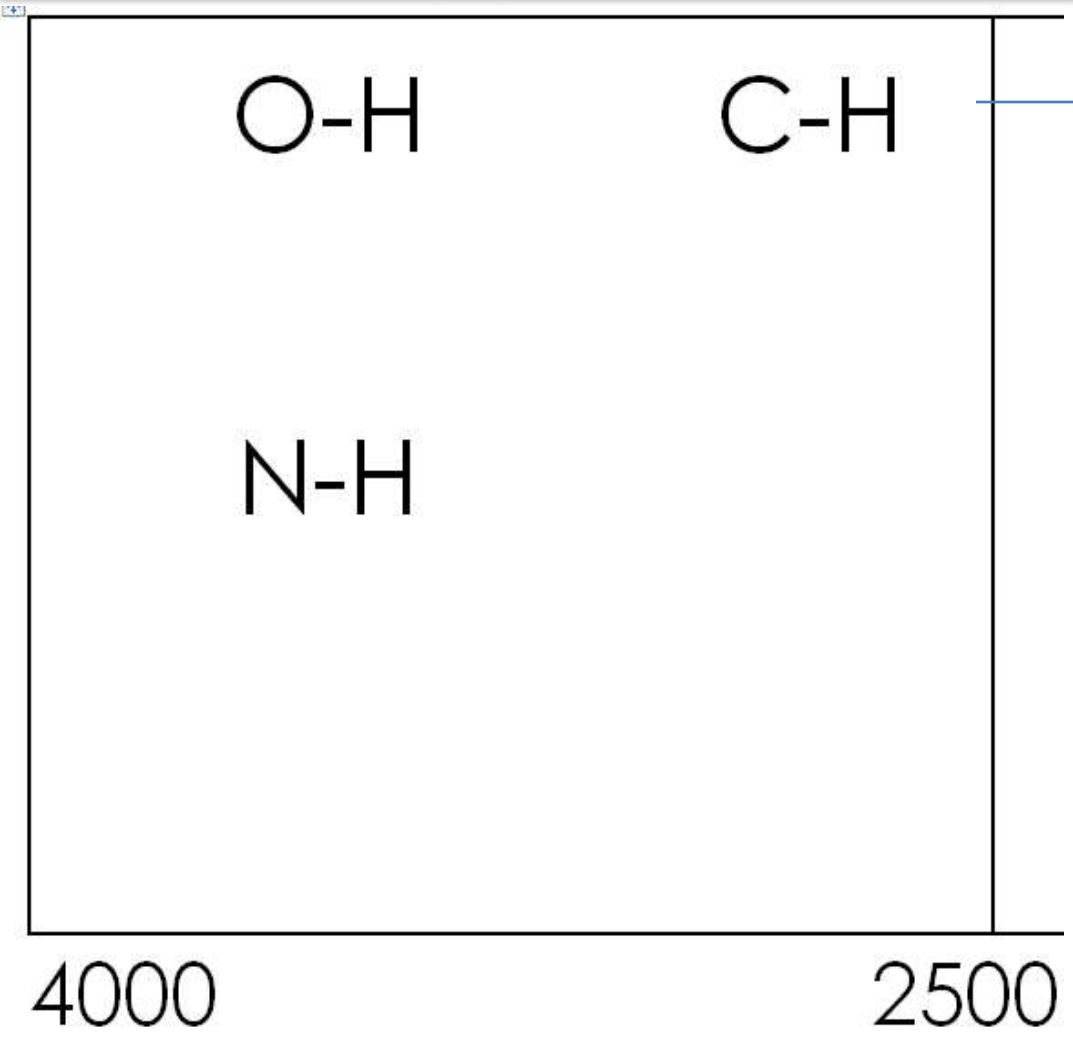
3-ALKANE
3000-2900 cm^{-1}
Simply to the **RIGHT** of 3000 cm^{-1}

1-Alkyne
2-Alkene and Aromatic
3-Alkane
4-Aldehydic

C-H alkane


To the right of
3000 cm-1
%Transmittance
90
80
70
60
50
40
30
20
10
0
4000
2000
Wavenumbers (cm-1)
2971
2863
1470
1383
728
octane

Region 4000-2500 cm^{-1}



Aldehydic hydrogen

Two peaks

2900-2800 cm^{-1}

AND

2800-2700 cm^{-1}

Note:

First peak is indistinguishable from alkane peaks which is in the same range.

1-Alkyne

2-Alkene and Aromatic

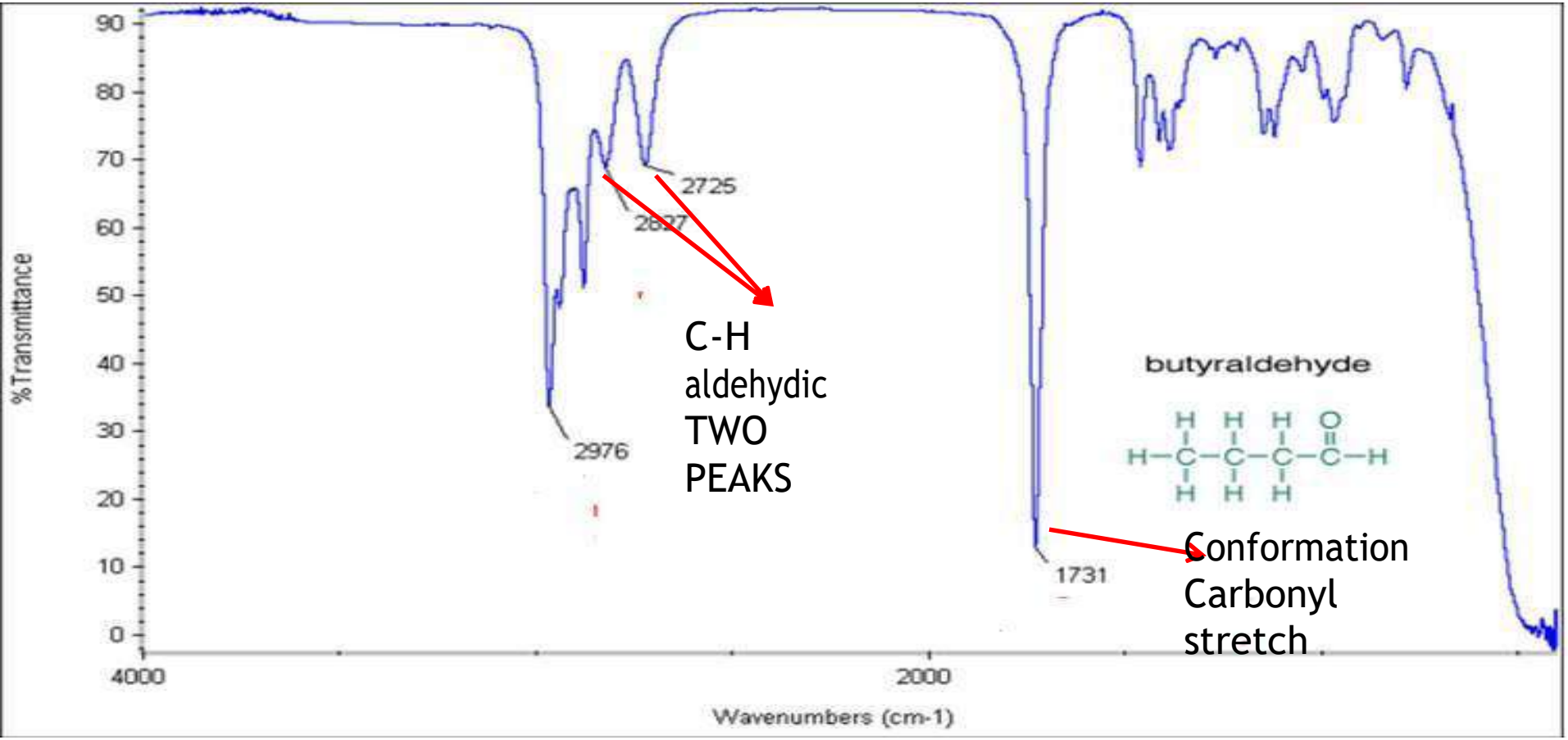
3-Alkane

4-Aldehydic

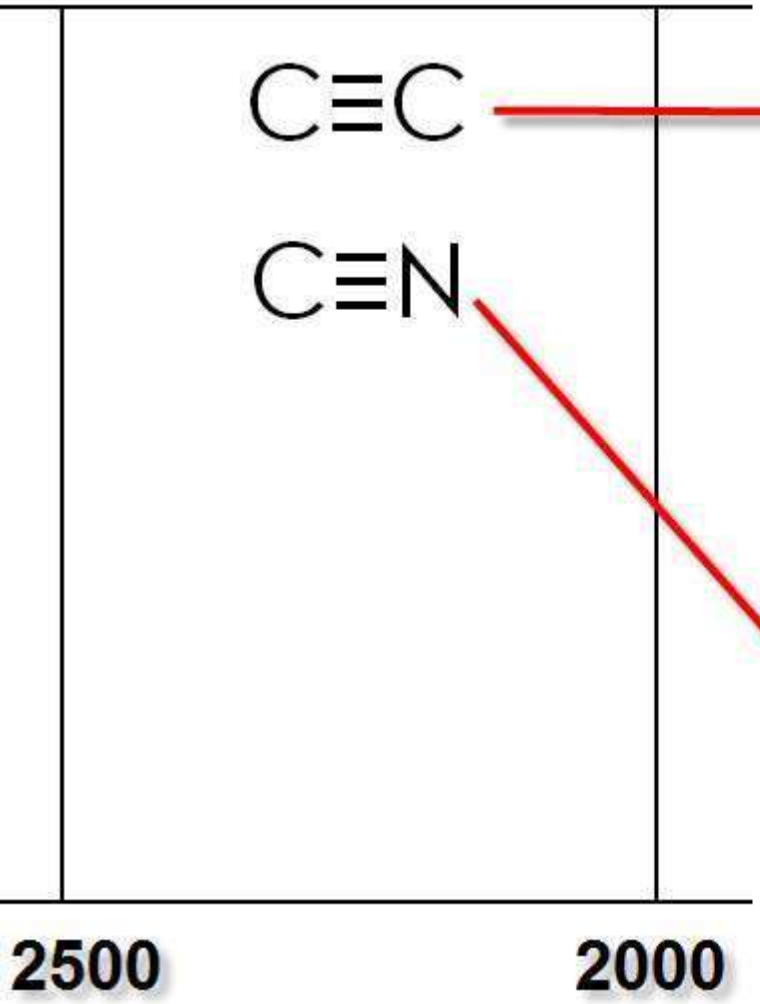
Note :

Second peak appears as a shoulder-type peak just to the right of the alkyl C–H

Aldehydic hydrogen



Region 2500-2000 cm^{-1}

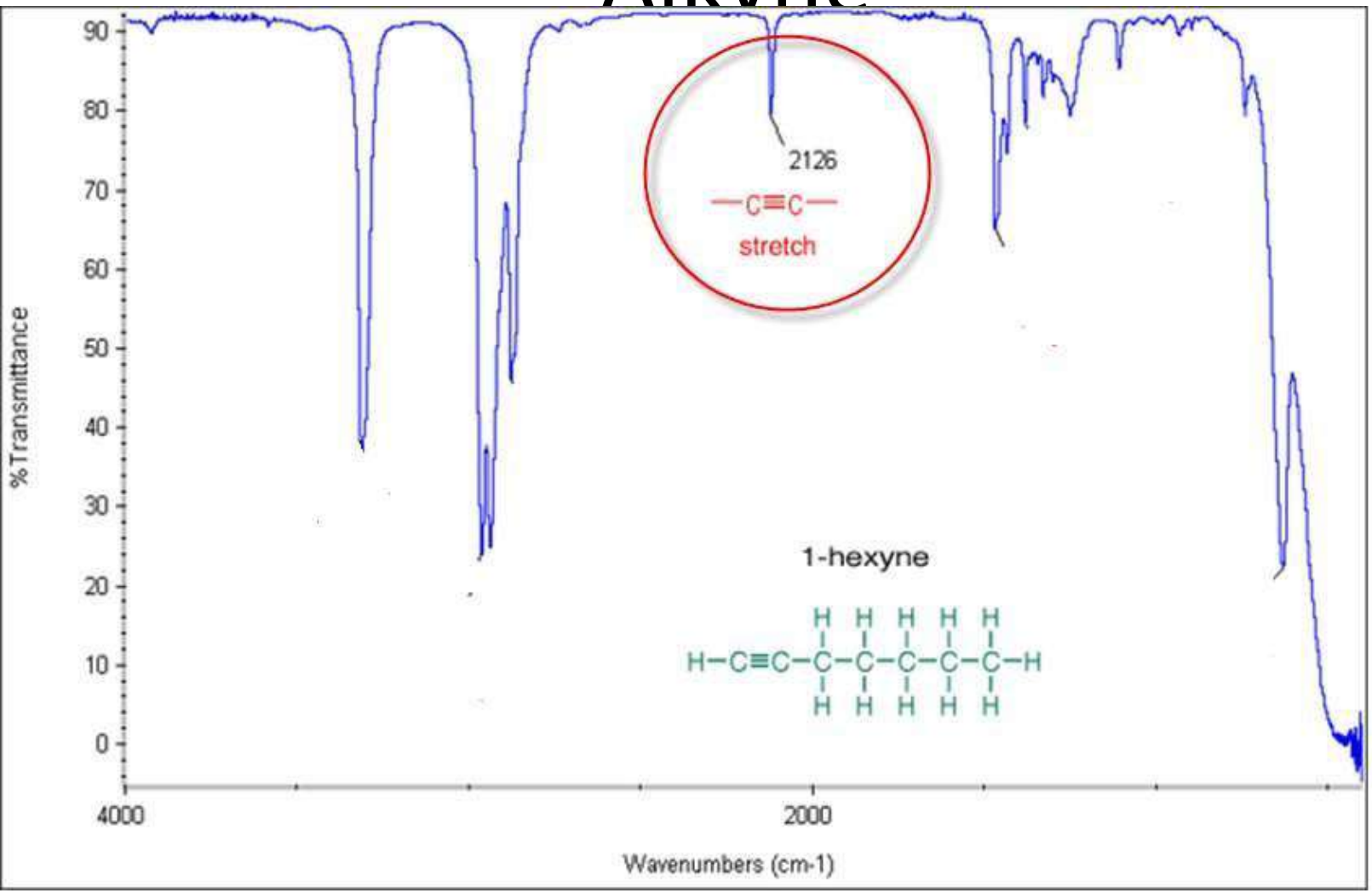


SHARP, medium peak around 2150 cm^{-1} ▪ DoNOT forget to look also for C-H alkyne peak, *although not necessarily present*

- SHARP, medium peak around 2250 cm^{-1}

DoNOT confuse with C≡C *(alkyne)*

Alkyne


%Transmittance
90
80
70
60
50
40
30
20
10
0
4000
2000
Wavenumbers (cm-1)
2126
—C≡C—
stretch
1-hexyne

Carbonyls

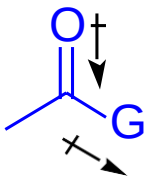
C=O stretch– easily recognized, intense band

• Ketones, aldehydes, acids, esters, lactones, acid halides, anhydrides, amides and lactams all show C=O stretching in the region 1870-1540 cm^{-1}.

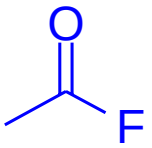
• Position is determined by (1) physical state (2) electronic and mass of neighboring groups (3) conjugation (4) hydrogen bonding (5) ring strain

Ketones

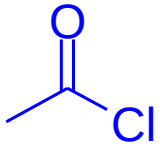
- aliphatic: ‘normal’ position of a neat aliphatic ketone is 1715 cm^{-1}
- competing effects



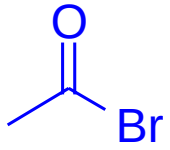
inductive: increases $\bar{\nu}$



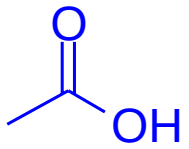
1869



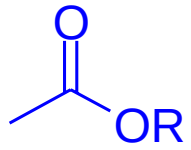
1815–1785



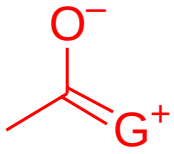
1812



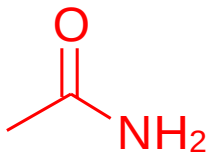
1760 (monomer)



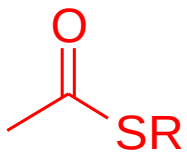
1750-1735



resonance: decreases $\bar{\nu}$



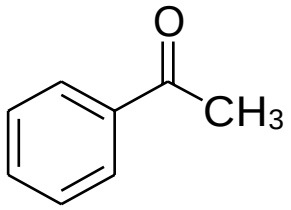
1695-1650



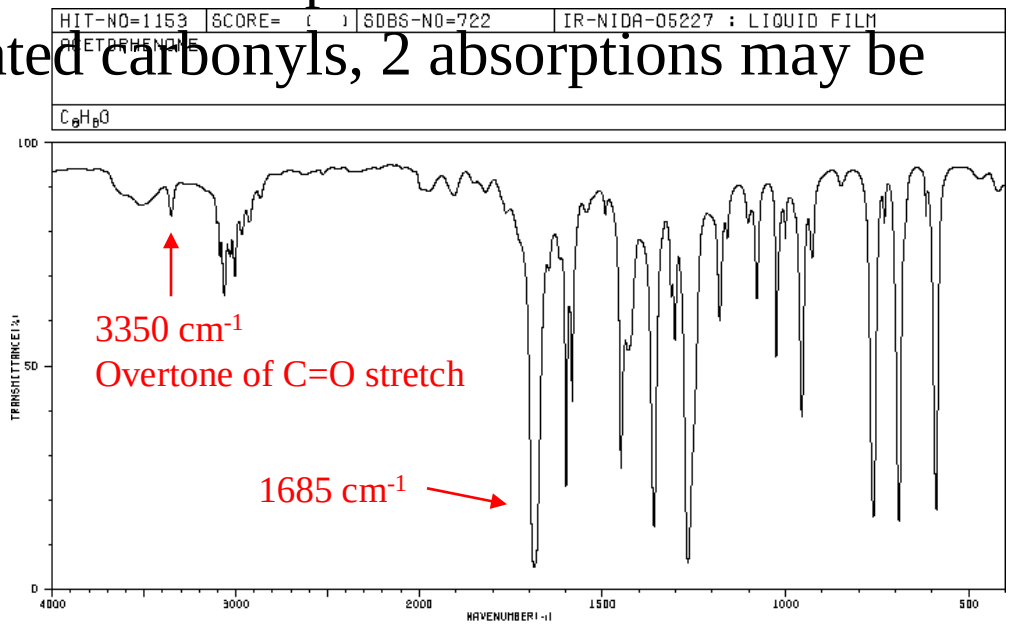
1720-1690

Ketones

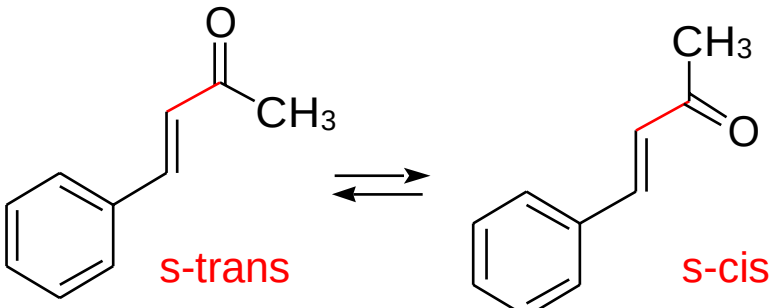
- conjugation: shifts position to lower frequency
  alkene or phenyl group causes absorption in the 1685-1666 cm^{-1} region. For α,β-unsaturated carbonyls, 2 absorptions may be observed



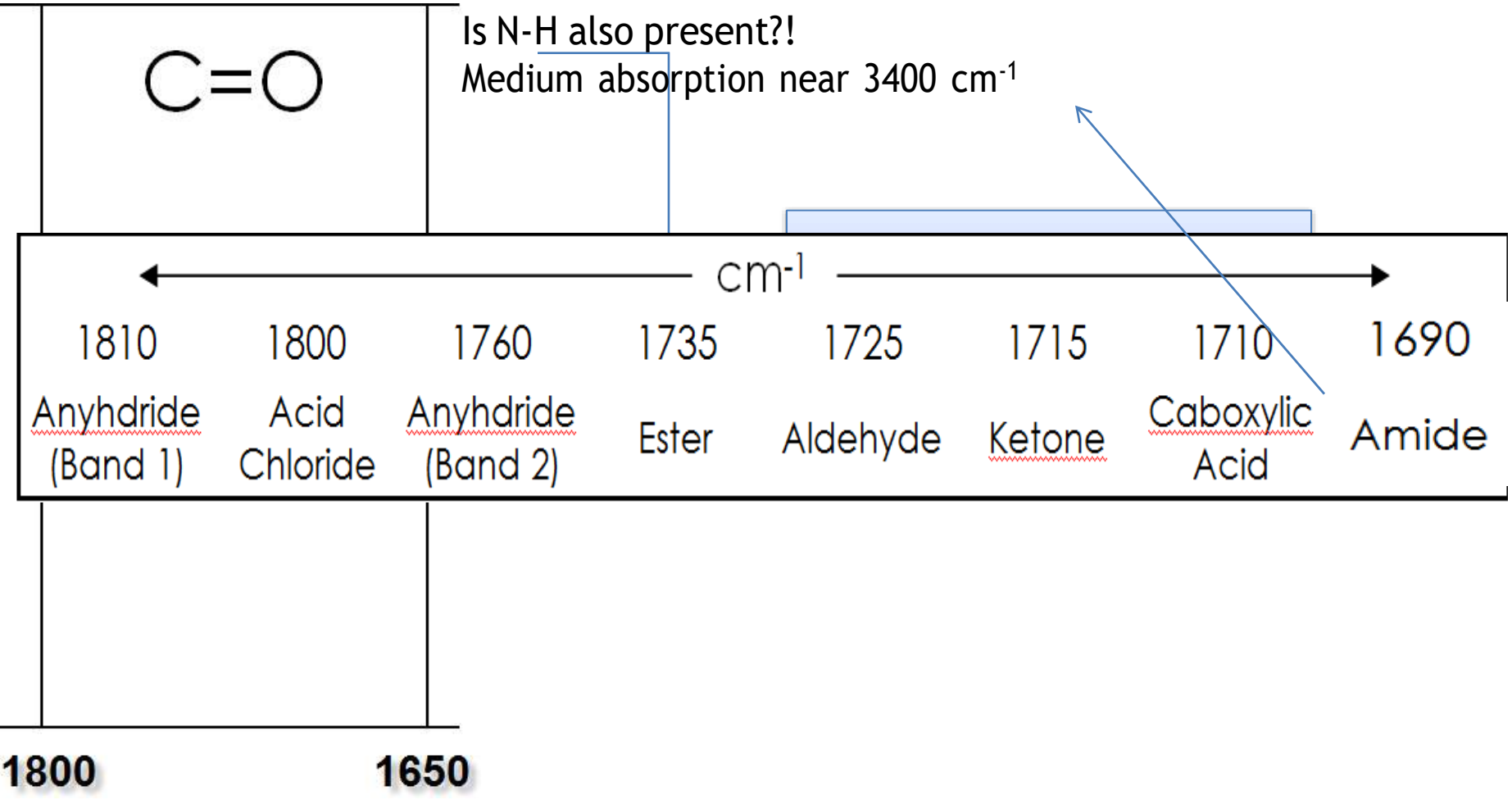
1685 cm^{-1}



- For α,β-unsaturated carbonyls, 2 absorptions may be observed



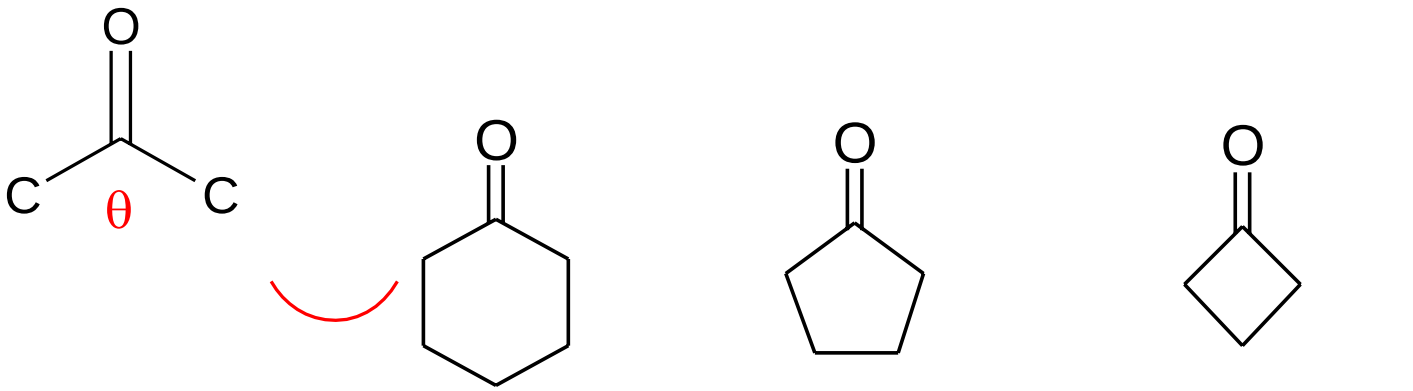
Region 1800-1650 cm^{-1}



Cyclic Ketones

The bond angle influences the absorption frequency of the C=O

In strained rings, interaction with the adjacent
C-C bonds increases the frequency of C=O stretching

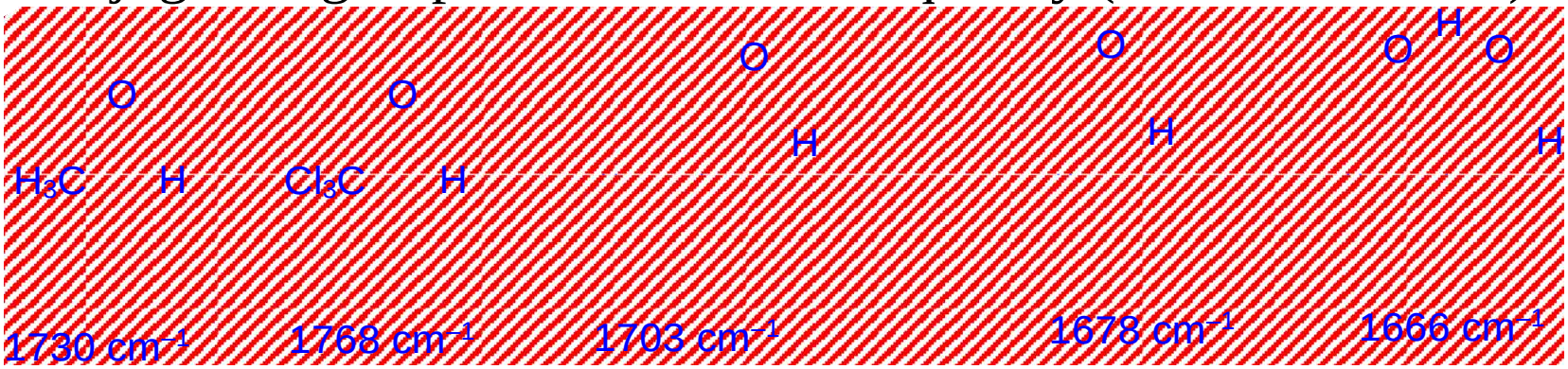


1715 cm^{-1} 1751 cm^{-1} 1775 cm^{-1}

Aldehydes

C=O stretch

- Aliphatic aldehydes: C=O stretch at 1740-1720 cm^{-1}
- Electron withdrawing groups shift to higher frequency
- Conjugative groups shift to lower frequency (1710-1685 cm^{-1})



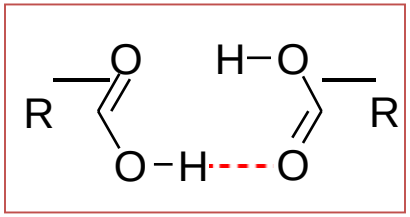
C–H stretch

2830–2695 cm^{-1} Often, two bands are observed (the other is a result of an overtone of the C–H bend of the aldehyde)

Carboxylic Acids

OH stretch

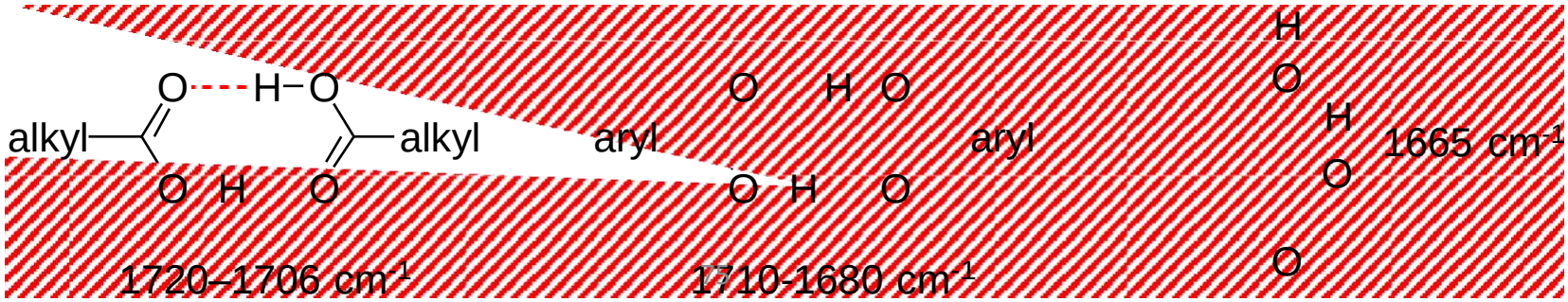
- 'free' OH (ca. 3520 cm^{-1}) is observed only in the vapor phase or in very dilute (<0.01 M) solution in a non-polar solvent
- Otherwise, acids exist as dimers



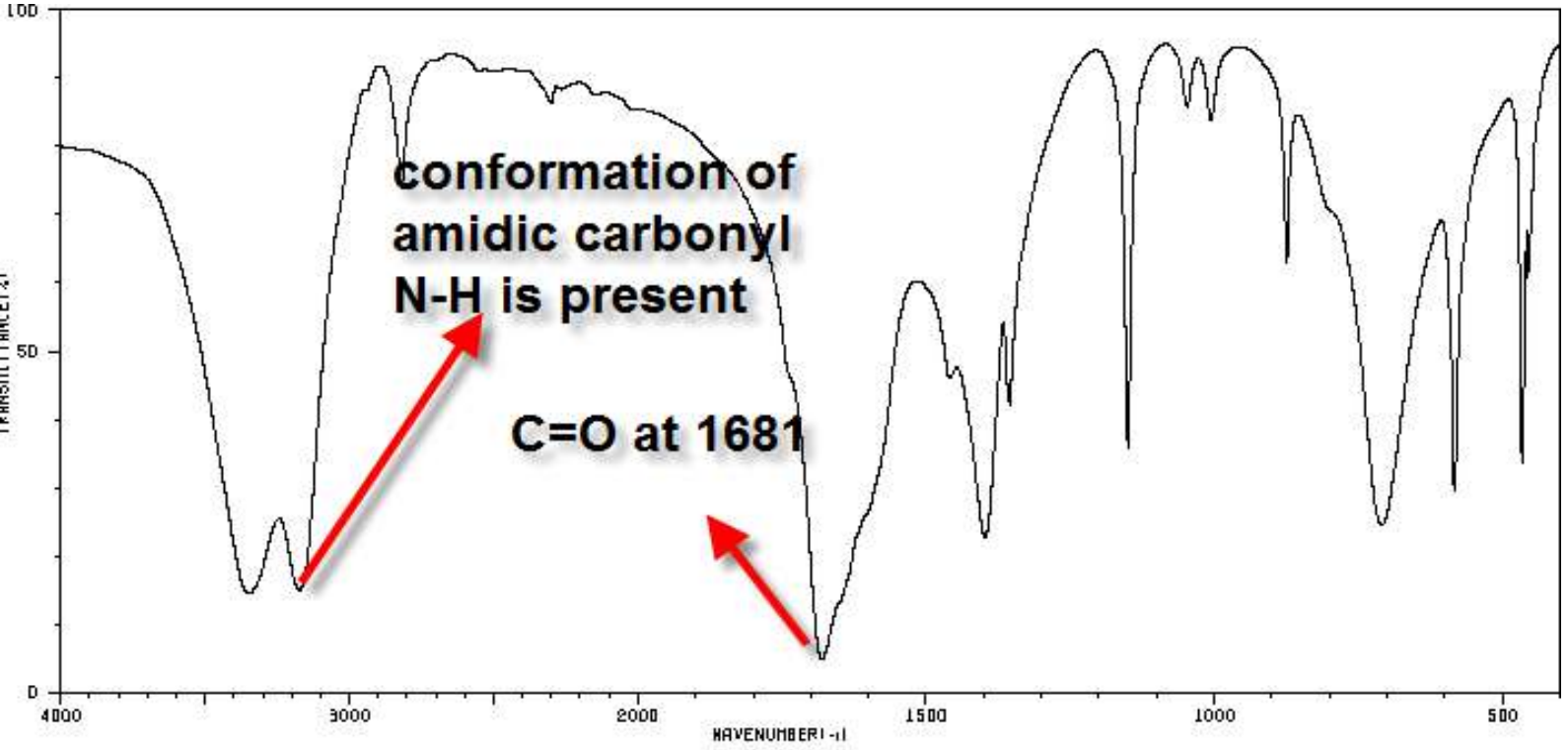
Intense, OH stretching in the 3300–2500 cm^{-1} region centered near 3000 cm^{-1}

C=O stretch

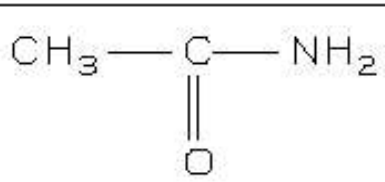
- monomer: 1760 cm^{-1} for aliphatic acids
- dimer: Hydrogen bonding reduces the frequency of the asymmetric C=O stretch, especially when intramolecular



Amide

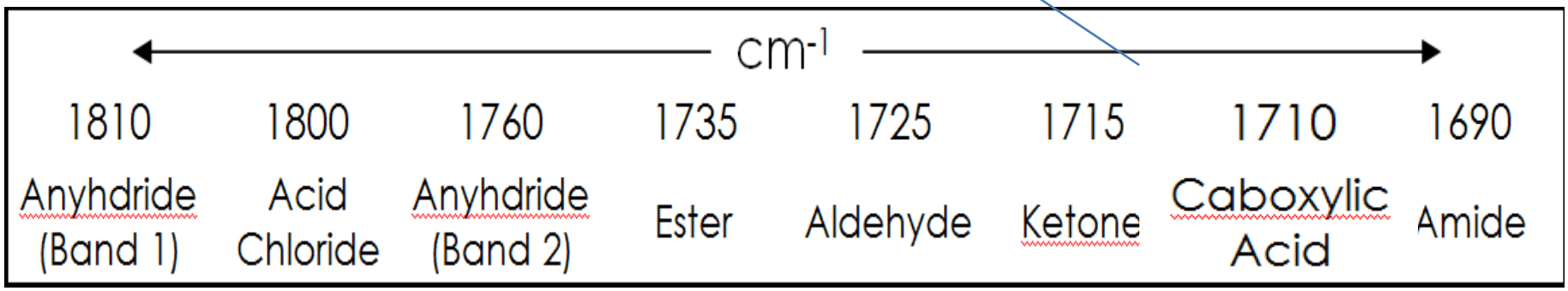


3348	13	1366	41	467	32
3173	14	1151	34	457	58
2819	70	1047	81		
2301	84	1006	81		
1681	4	874	60		
1460	44	710	25		
1398	21	684	28		

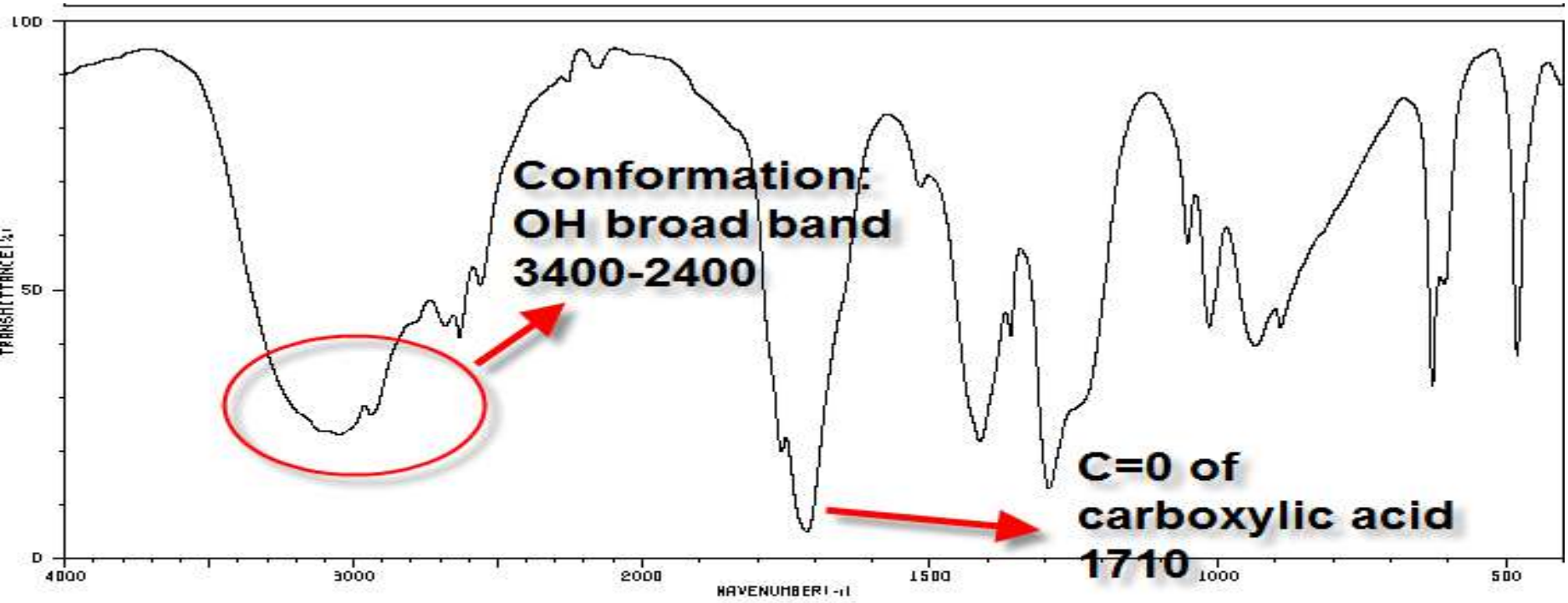


Region 1800-1650 cm^{-1}

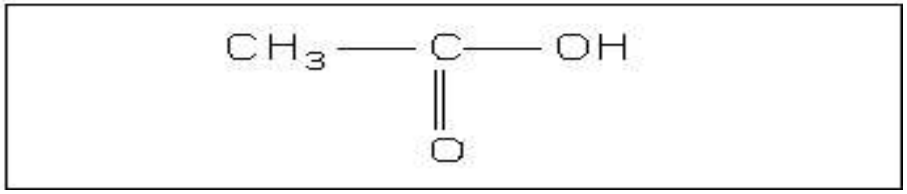
Is O-H also present?!
Broad absorption near 3400-2400cm^{-1} *(usually overlap C-H)*



Carboxylic acid

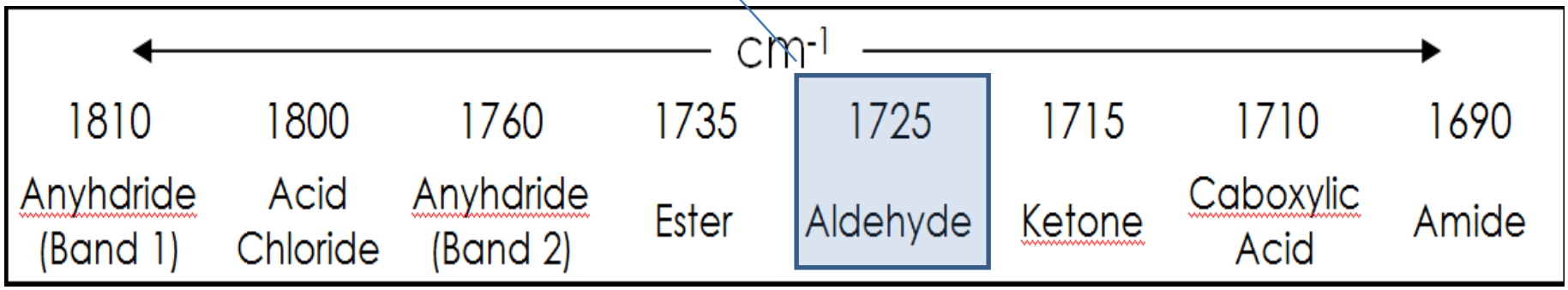


2937	25	1414	20	629	31
2684	41	1360	39	607	49
2631	39	1294	12	481	36
2569	49	1063	57	473	62
1758	19	1016	41		
1714	4	935	37		
1617	66	892	41		

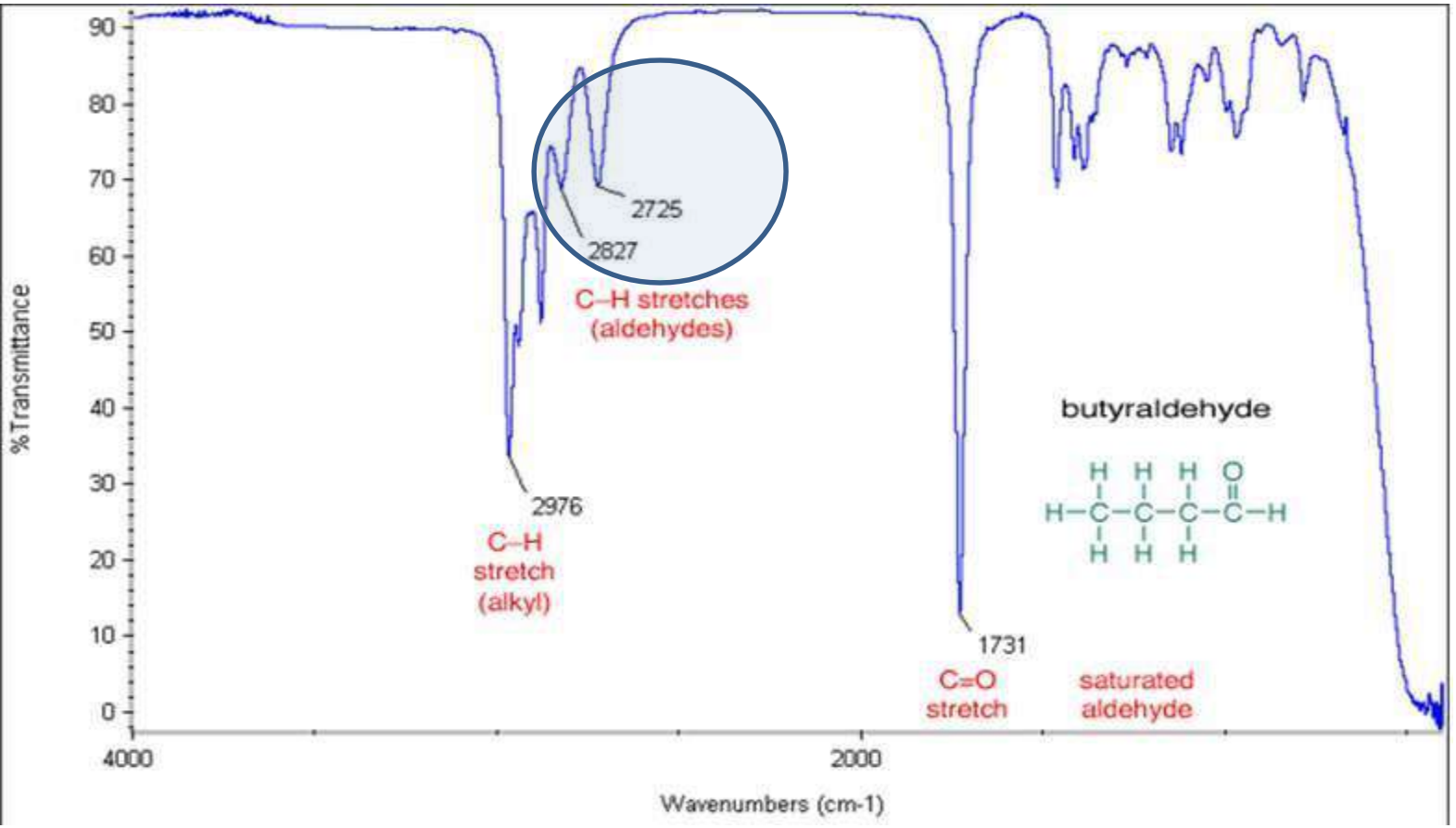


Region 1800-1650 cm^{-1}

Is aldehyde C-H present?!
Two weak absorptions near 2850 and 2750 cm^{-1} on the right side of the aliphatic C-H absorptions

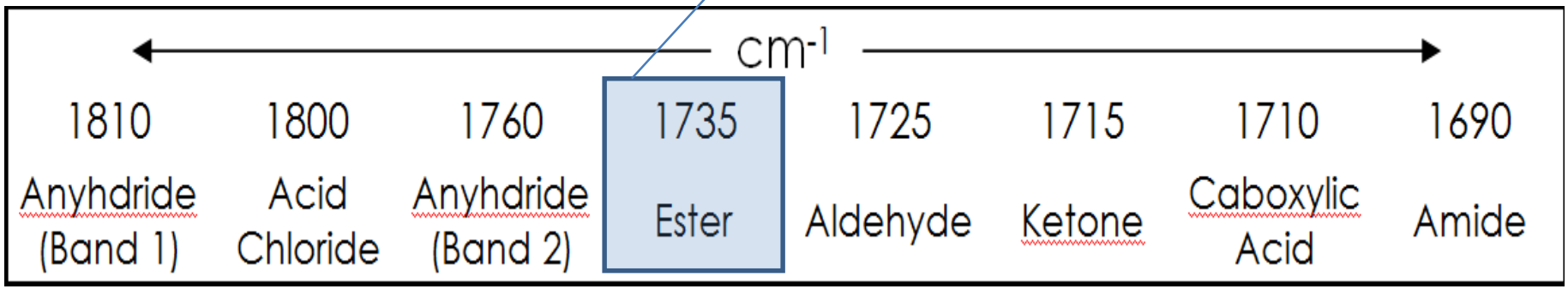


Aldehyde

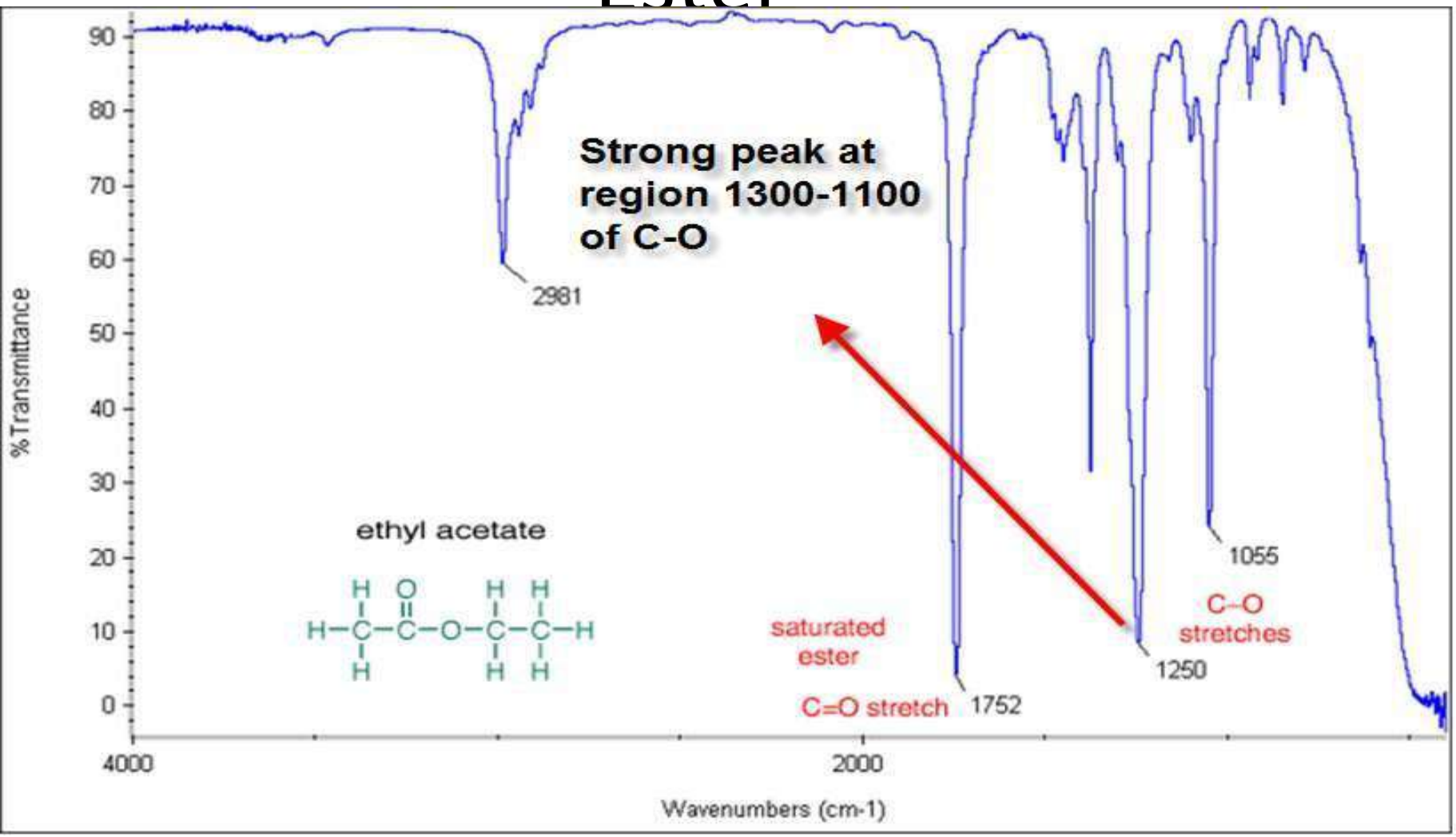

%Transmittance
90
80
70
60
50
40
30
20
10
0
4000
2000
Wavenumbers (cm-1)
2725
2827
C–H stretches
(aldehydes)
2976
C–H
stretch
(alkyl)
1731
C=O
stretch
saturated
aldehyde
butyraldehyde

Region 1800-1650 cm^{-1}

Is C-O also present?!
Strong intensity absorption near 1300-1000 cm^{-1}

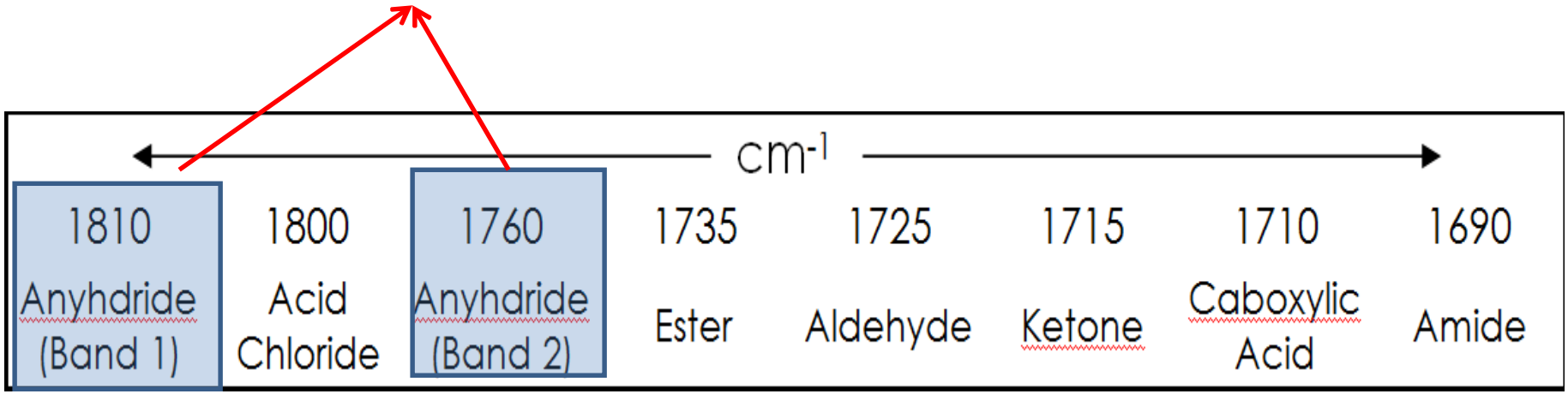


Ester

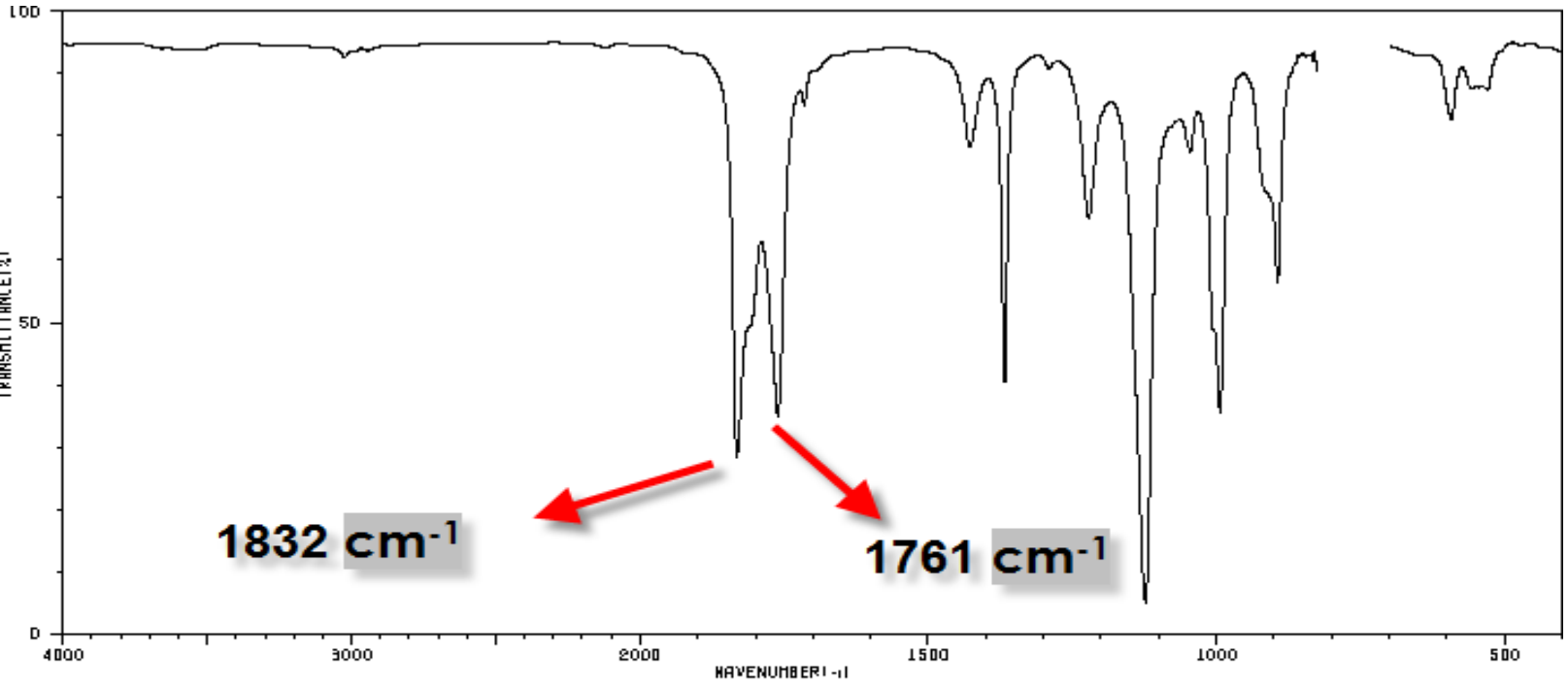

Strong peak at region 1300-1100 of C-O
2981
1055
C-O stretches
1250
saturated ester
C=O stretch
1752
ethyl acetate
%Transmittance
Wavenumbers (cm-1)
4000
2000
90
80
70
60
50
40
30
20
10
0

Region 1800-1650 cm^{-1}

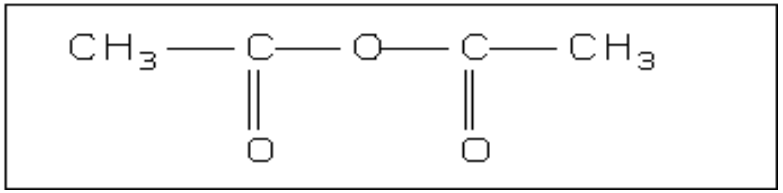
Two C=O absorptions near 1810 and 1760 cm^{-1}



Anhydride

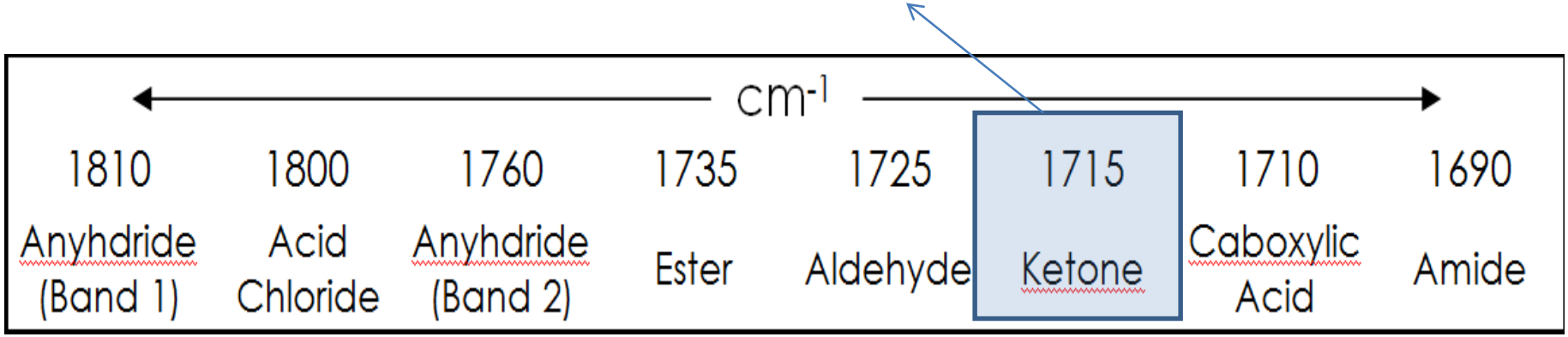


1832	26	1046	74	540	84
1761	33	1005	46		
1715	81	994	34		
1428	74	894	59		
1368	38	603	84		
1222	64	593	79		
1124	4	547	84		

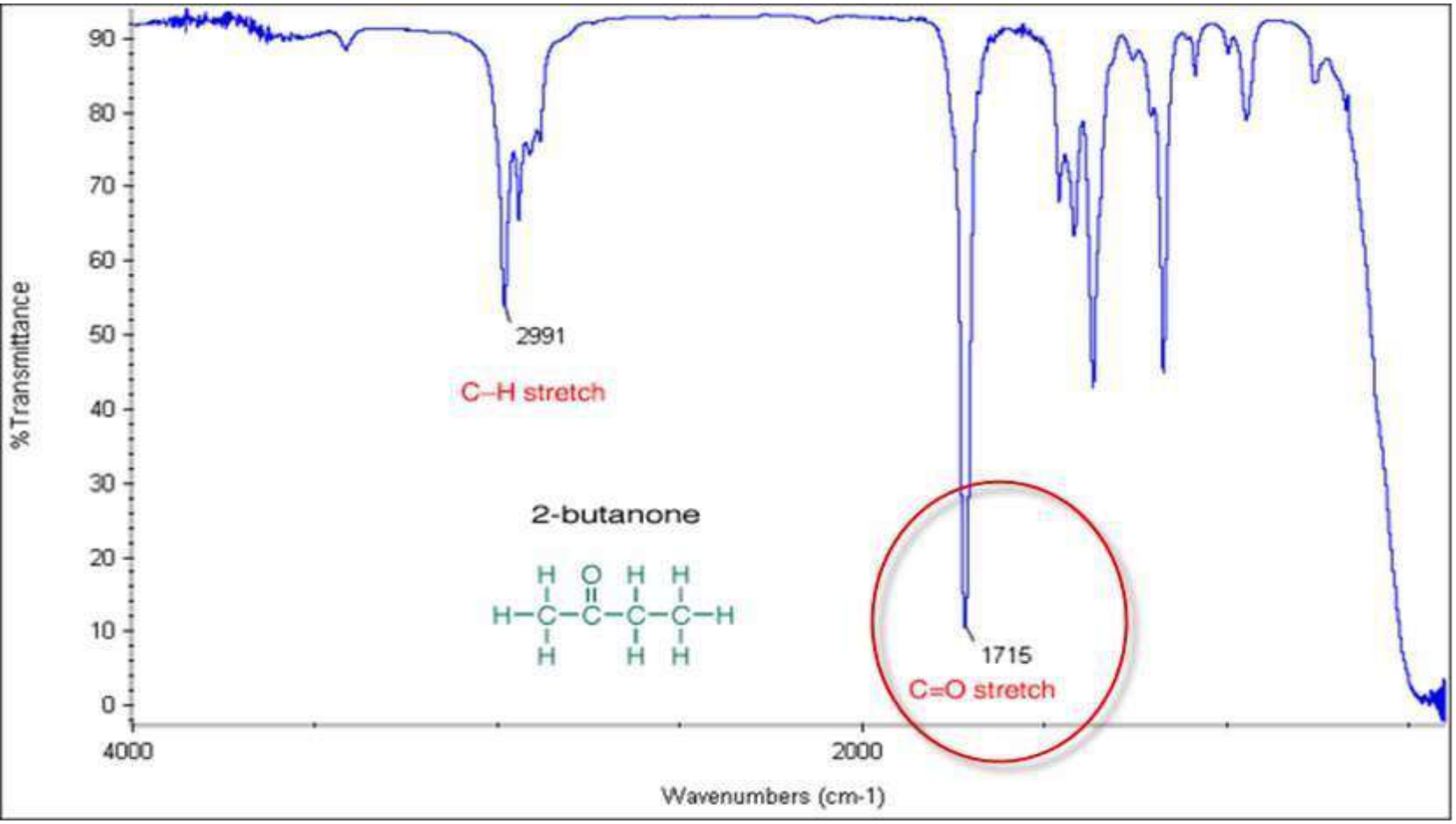


Region 1800-1650 cm^{-1}

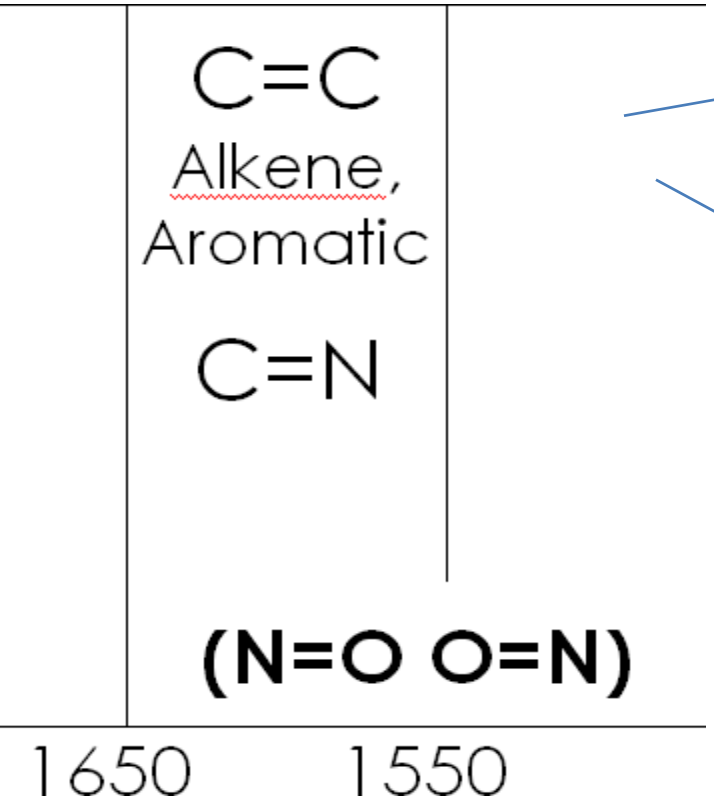
The preceding five choices have been eliminated



Ketone


%Transmittance
90
80
70
60
50
40
30
20
10
0
4000
2000
Wavenumbers (cm-1)
2991
C–H stretch
1715
C=O stretch
2-butanone

Region 1650-1550 cm^{-1}



1680-1600 cm^{-1}

Shows **ONLY ONE PEAK** around 1600cm^{-1}

DoNOT confuse with C=O, which NEVER appears below 1650cm^{-1}

DoNOT confuse with C=C *(aromatic)*..

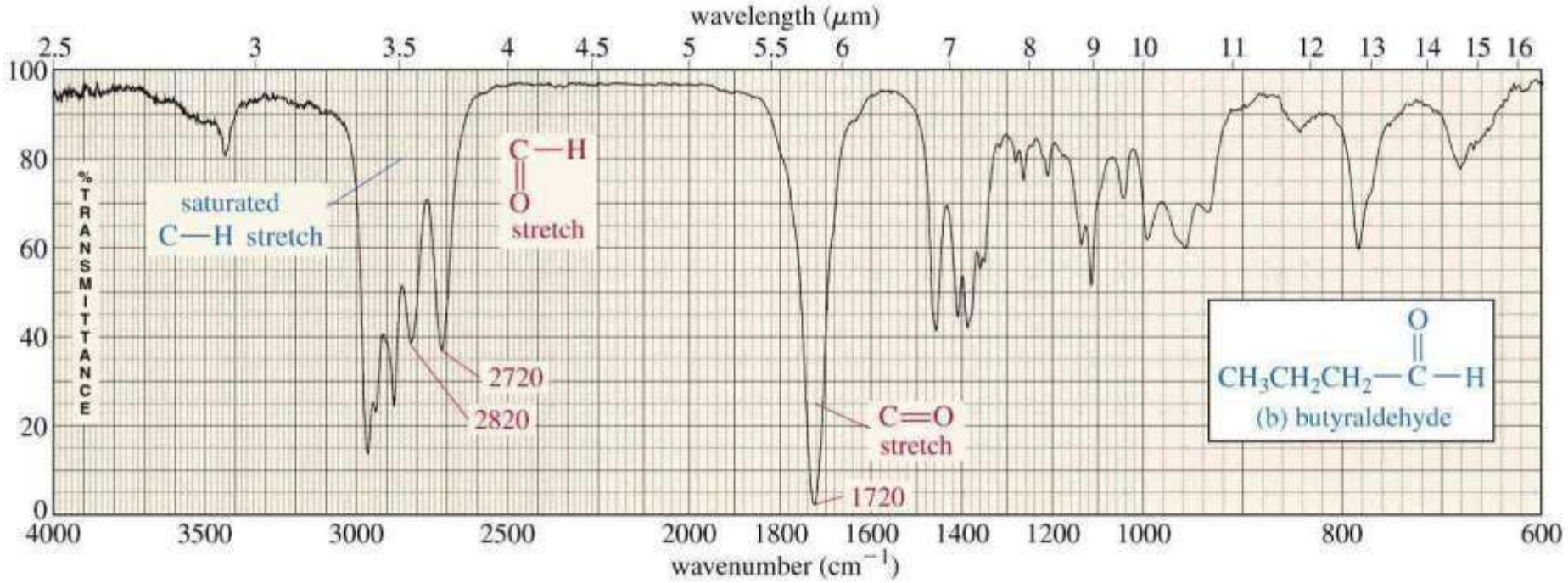
1600 **AND** 1475 cm^{-1}

Shows **TWO PEAKS,**

the first just before 1600cm^{-1},

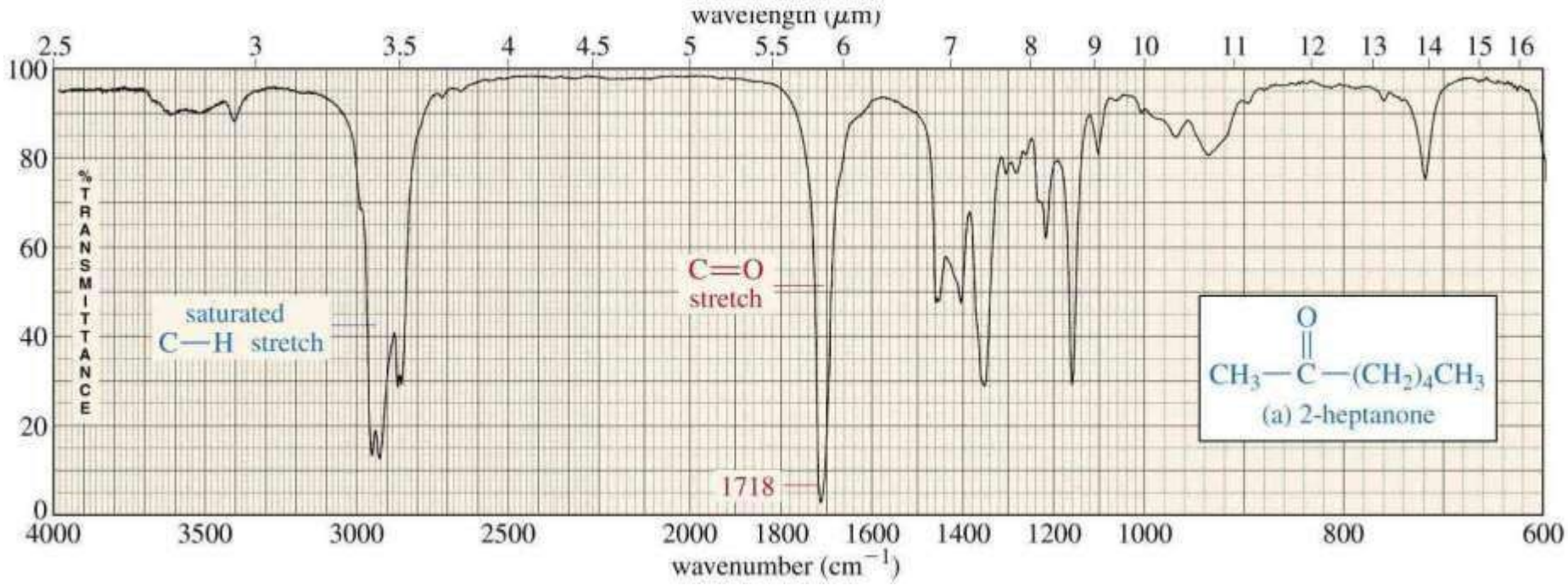
the second just before 1500cm^{-1}

An Aldehyde
IR Spectrum



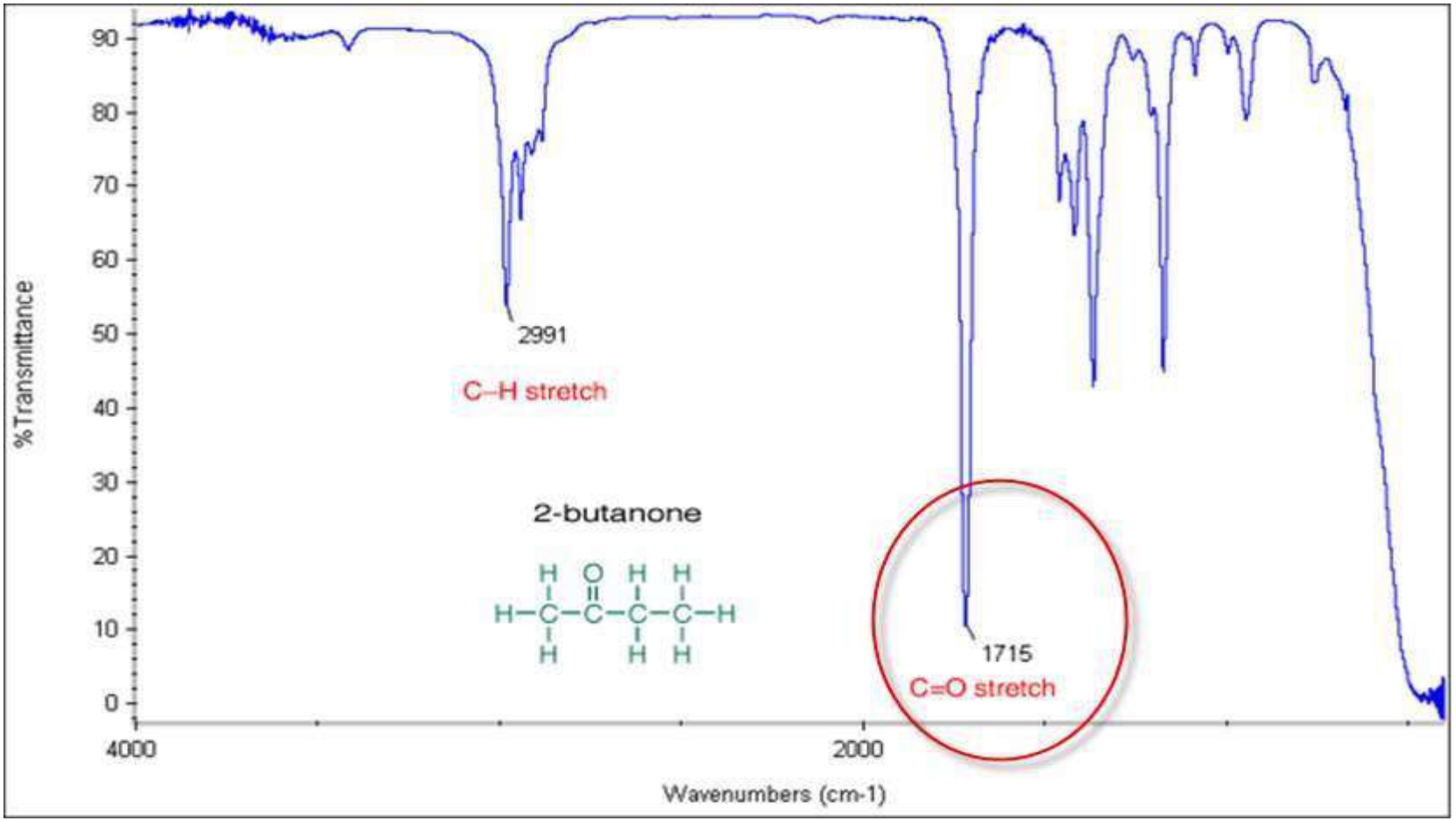
=>

A Ketone
IR Spectrum



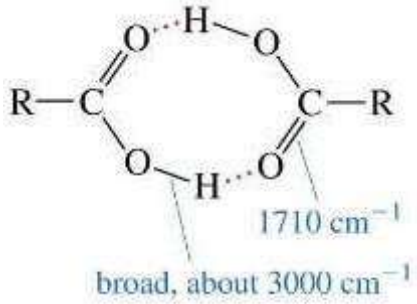
=>

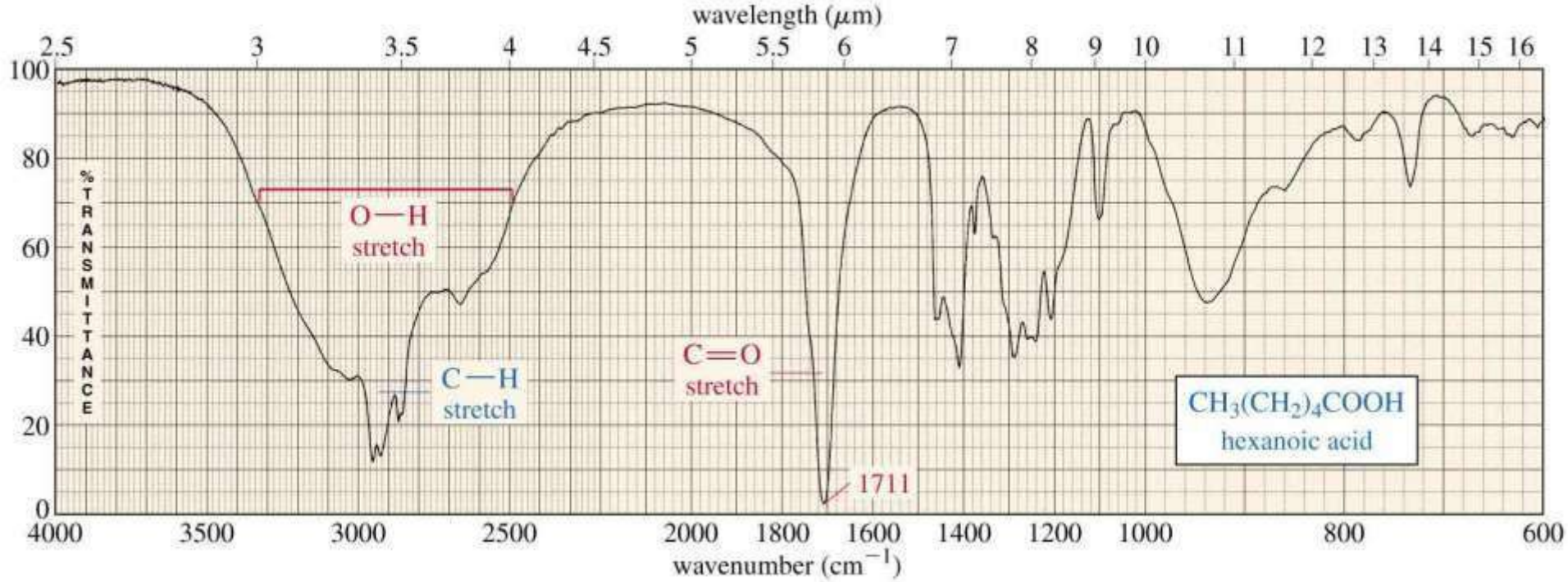
Ketone


%Transmittance
90
80
70
60
50
40
30
20
10
0
4000
2000
Wavenumbers (cm-1)
2991
C–H stretch
1715
C=O stretch
2-butanone

O-H Stretch of a Carboxylic Acid

This O-H absorbs broadly, 2500-3500 cm^{-1}, due to strong hydrogen bonding.





Variations in C=O Absorption

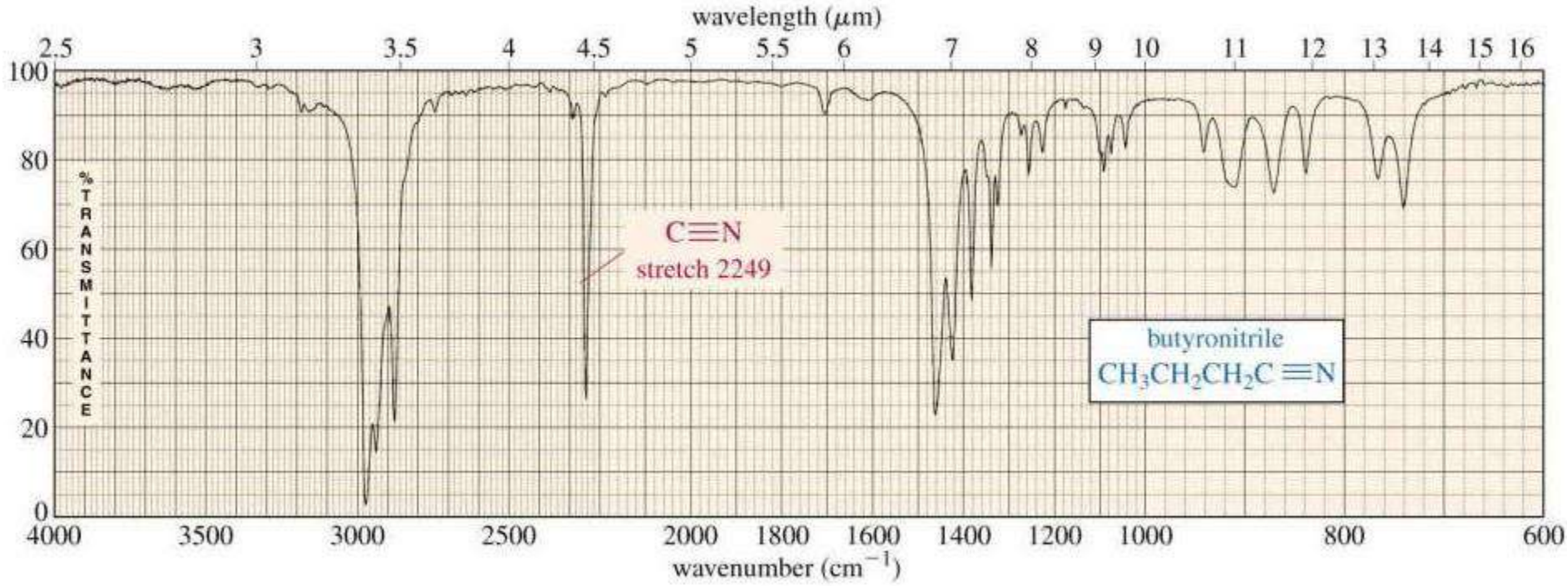
- Conjugation of C=O with C=C lowers the stretching frequency to ~1680 cm^{-1}.
- The C=O group of an amide absorbs at an even lower frequency, 1640-1680 cm^{-1}.
- The C=O of an ester absorbs at a higher frequency, ~1730-1740 cm^{-1}.
- Carbonyl groups in small rings (5 C's or less) absorb at an even higher frequency. =>

Carbon - Nitrogen Stretching

- C - N absorbs around 1200 cm^{-1}.
- C = N absorbs around 1660 cm^{-1} and is much stronger than the C = C absorption in the same region.
- C ≡ N absorbs strongly just *above* 2200 cm^{-1}. The alkyne C ≡ C signal is much weaker and is just *below* 2200 cm^{-1} .

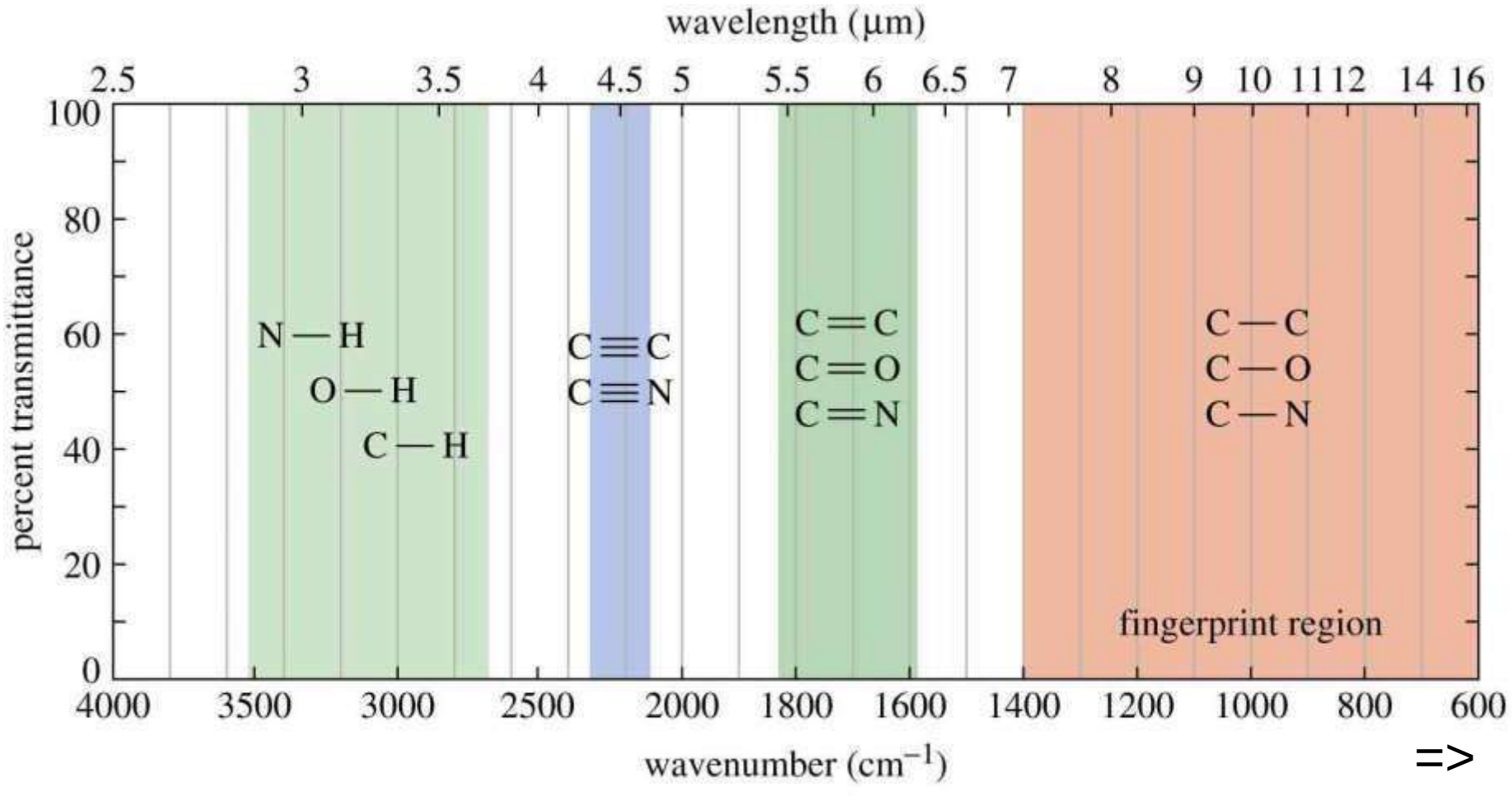
=>

A Nitrile
IR Spectrum



=>

Summary of IR Absorptions



=>


Thank u

ISO 9000

B.BHADRU

Associate Professor

Vikas College of Pharmaceutical Sciences

What is ISO-9000?

- The International Organization for Standardization(ISO)

- Represented by association of 127 countries, give more thrust on quality systems and for stringent quality standards to the manufacturer on quality systems and management.

- ISO has its head quarters in Geneva

- These are not quality standards but, indeed , system standards which ultimately leads to production of desired quality with consistency.

- These standards are globally accepted.

ISO 9000

ISO-9001:- Model for quality assurance in design, development, production, installation and servicing.

ISO-9002:- Model for quality assurance in production , installation and servicing.

ISO-9003:- Used by the supplier as a model for quality assurance in final inspection and testing

ISO-9000:2000 :-Related to Fundamentals and Vocabulary

ISO-9000:2001:- Quality Management system-requirements

ISO-9004:2000:- Guidance for Performance Improvement.

Main characteristics of 2000 version is introduction of the concept of continual improvement and evaluation of process performance.

Principles of ISO-9001:2000

1. Customer focus
2. Leadership
3. Involvement of people
4. Process approach
5. System approach to management
6. Continual improvement
7. Factual approach to decision making
8. Mutually beneficial supplier relationship

ISO 9001:2000 -Requirements

1. Quality management system
2. Management Responsibility
3. Resource Management
4. Product Realization
5. Measurement, Analysis and Improvement

- ISO-9000 series of standards, as a set of guidelines for developing and demonstrating a documented quality system, were introduced by ISO to facilitate international trade in goods and services.
- Then this has been a globally accepted series of uniform quality systems standards.

Quality accreditation is the certification of a company's process. Which will result in attaining certain standards of quality in its output.

It is most effective when awarded by a national controlling authority or international agency.

Third party assessment is widely used for all types of manufacturers and demonstrating the supplier's competence.
The standards are designed to be user friendly.

To Whom it is Applicable

Even a small company having a few employees may opt for ISO-9000 certification. There is no stipulation in this regard as to the size of the company.

1. To all manufacturers who want to export their goods to European common market.

2. To all sub-contractors of an organization that have opted for ISO-9000 series standards.

3. To all regular suppliers to different Government agencies.

4. To all individuals opting for better systems in the company.

Why do we need ISO-9000 Certification

1. **Competitive Advantage:** 72.3% of corporations with ISO-9000:2000 , report a major competitive advantage.

   This means they are winning more business.

2. **Improves Customer Demand:** 33.4% of corporations with ISO-9000 report improved customer demand.

3. **Protect Your Business:** 69.3% of surveyed business that do not have ISO-9000 say have lost a customer bid.

4. **Increased Credibility:** You can generate a significant increase in business , as many corporations will deal only with ISO-9000 registered companies.

Why do we need ISO-9000 Certification

5. Your competitors are doing it : In many industries 35-40% of your competitors are ISO registered. The longer yo wait to start, the greater is their competitive advantage.

6. Easy and Cost effective: Getting ISO 9000 is no longer the costly , time consuming proposition that it once was. It is very simple and inexpensive.

7. Qualify for Government Contracts : The departments of Defense, Food and Drug Administration, NASA, Department of Energy and the General Services Administration have all approved the use of ISO-9000 in contracts and bids.

Why do we need ISO-9000 Certification

8. Develop New Customers:

Once you are Registered you will be published in the ISO 9001:2000 registered company Directory. Used as a purchasing resource book by the thousands of U.S and International companies.

9. Don’t be left Behind: On average it takes 100to 200 hours to complete all the required ISO9001:2000 documents

What steps do we need to take to achieve Certification?

1. **Documentation:** Preparing your Policy and Procedures Manual

2. **Implementation:** ISO 9001:2000 is basically an instructions manual for your business. Implementing ISO means putting it into practice within your company . It typically takes 3-9months.

3. **Inspection Audit:** When your documents are ready and you have implemented them throughout the organization, it's time to call the Auditing Organization, known as Registrar. They will inspect your documents, do an on-site inspection and make a report to Registrar.

4. **Registration:** When you pass the audit, your company will be registered as an ISO-Certified organization and published in the Registered Company Directory.

Advantages of ISO Certification

1. Better product design.

2. Improved product quality . It helps the company achieve consistent levels of quality.

3. Market share can go up with improved credibility.

4. The company's management has greater confidence in its own internal systems.

5. Exports can increase – Standards are globally accepted in more than 60 countries.

6. The company's process become cost-effective as the system facilitates reduction in waste and rejections.

Advantages of ISO Certification

7. Efficient utilization of men, machine and materials, resulting in higher productivity.

8. Elimination of bottlenecks in production and tension free work environments, leading to good human relations.

9. Creation of quality awareness and greater job satisfaction among employees, improving the company's quality culture.

10. It improves confidence in existing/potential customers that the company is capable of delivering quality goods and service.

11. Improvement of company's image and credibility in International markets which is essential for success in the export business.

Route to ISO-9000 Registration

Evaluation of business competitive needs

↓

Management commitment → Resource allocation

↓

Set up team, Tasks, Goals

↓

Training to staff

↓

Review of current procedure

↓

Quality action plan

↓

Quality manual and other procedure manuals

↓

Pre-assessment or internal audits

↓

Audit

↓

Registration

↓

On going monitoring

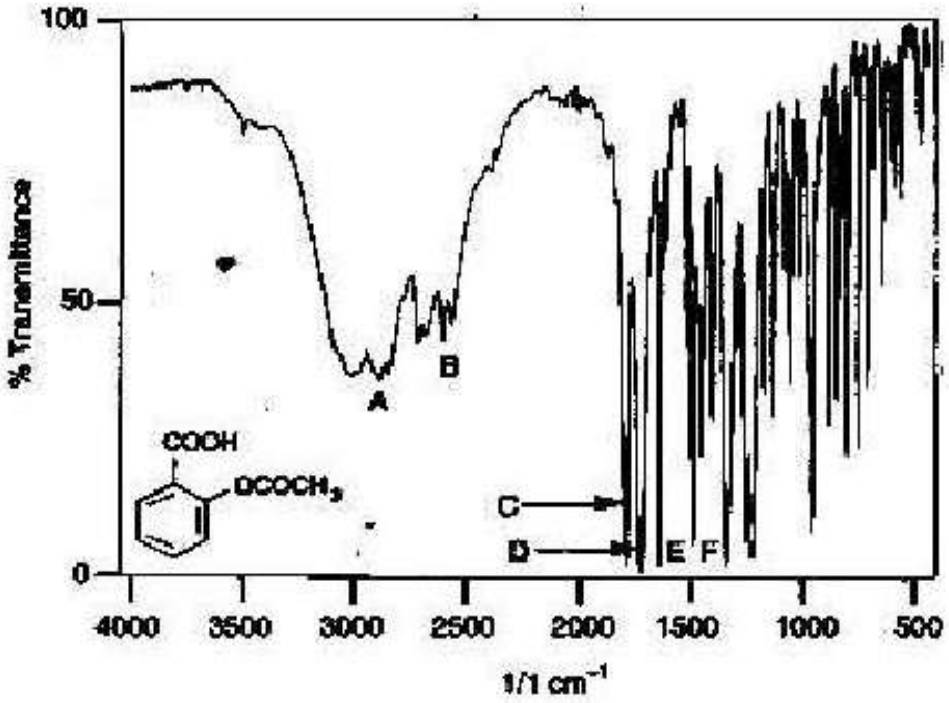
THANK YOU

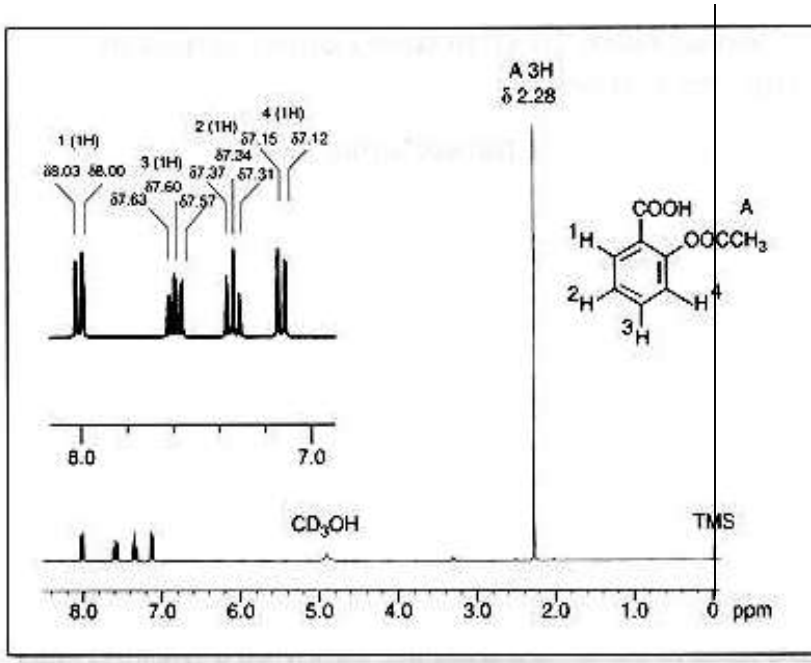
Nuclear Magnetic Resonance Spectroscopy

P.Venkateswarlu

VIKAS COLLEGE OF PHARMACEUTICAL SCIENCES

IR vs. NMR


100
50
0
% Transmittance
A
B
C
D
E
F
COOH
OCOCH3
4000
3500
3000
2500
2000
1500
1000
500
1/1 cm-1


A 3H
δ 2.28
1 (1H)
δ8.03 δ8.00
3 (1H)
δ7.63 δ7.60 δ7.57
2 (1H)
δ7.37 δ7.34
4 (1H)
δ7.31 δ7.15 δ7.12
8.0
7.0
CD3OH
TMS
COOH
A
OCOCH3
1H
2H
3H
4H
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0 ppm

NMR vs. IR

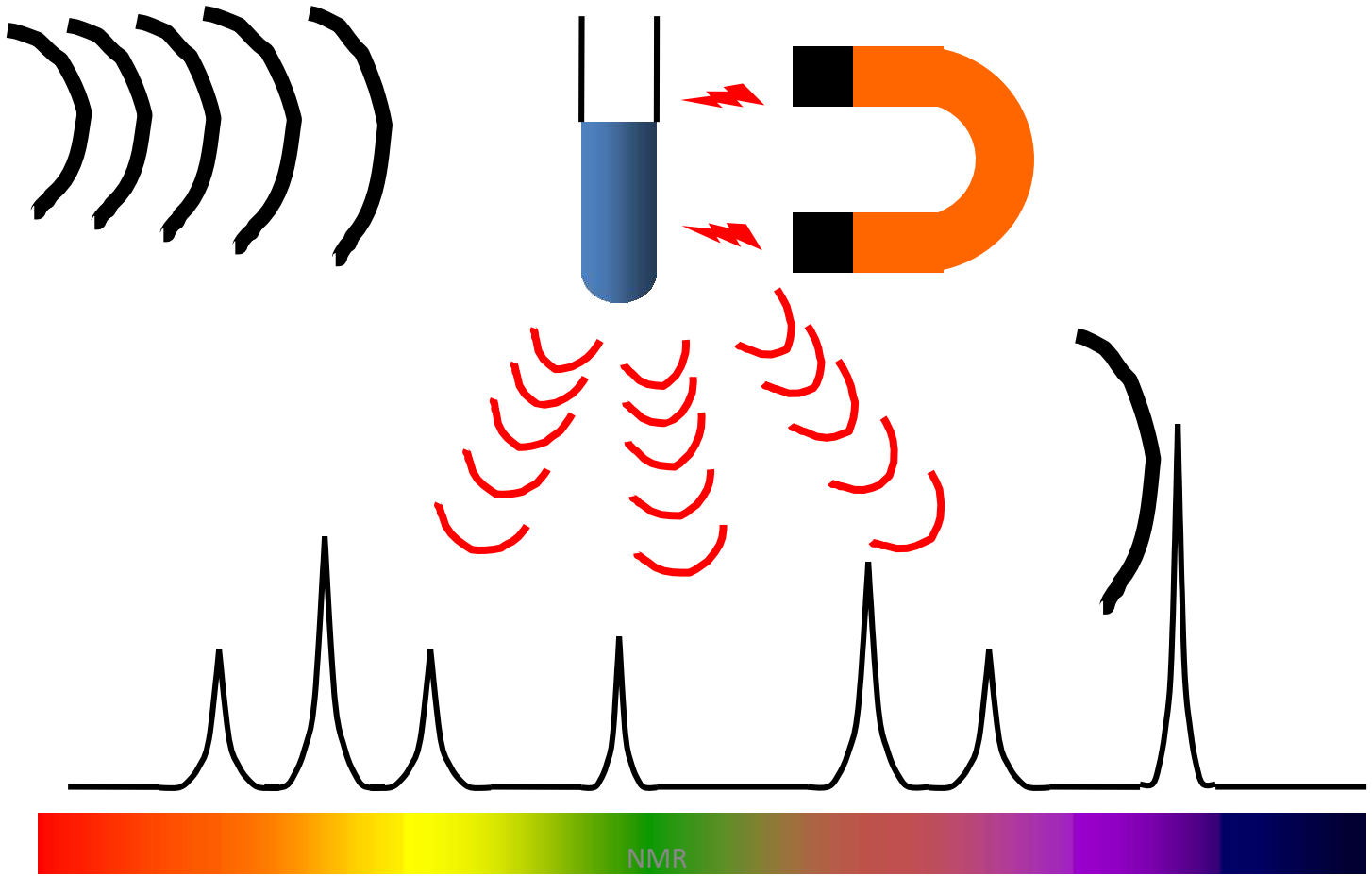
- NMR has narrower peaks relative to IR
- NMR yields far more information than IR
- NMR allows you to collect data on solids & liquids but not gases
- NMR samples are easier to prepare

Introduction

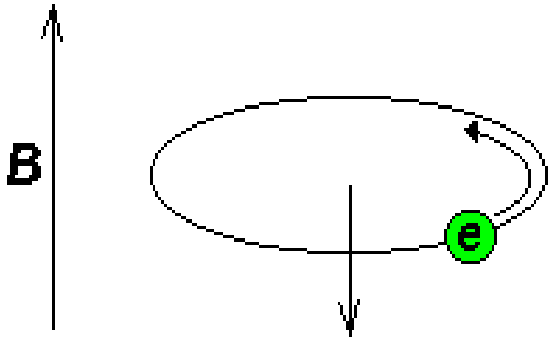
- NMR is the most powerful tool available for organic structure determination.
- It is used to study a wide variety of nuclei:
  - ^{1}H
  - ^{13}C
  - ^{15}N
  - ^{19}F
  - ^{31}P
  - 23Na
  - 25Mg
  - 27Al
  - 29Si
  - 31P
  - 33S
  - 109Ag

I	Atomic Mass	Atomic Number	Example (I)
Half-integer	Odd	Odd or even	${}^{1}_{1}\mathrm{H}(\frac{1}{2})$, ${}^{17}_{8}\mathrm{O}(\frac{5}{2})$, ${}^{15}_{7}\mathrm{N}(\frac{1}{2})$
Integer	Even	Odd	${}^{2}_{1}\mathrm{H}(1)$, ${}^{14}_{7}\mathrm{N}(1)$, ${}^{10}_{5}\mathrm{B}(3)$
Zero	Even	Even	${}^{12}_{6}\mathrm{C}(0)$, ${}^{16}_{8}\mathrm{O}(0)$, ${}^{34}_{16}\mathrm{S}(0)$

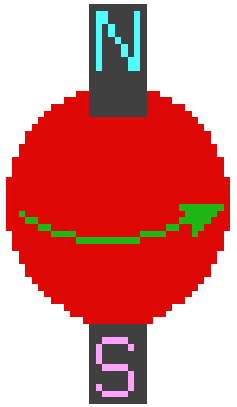
Explaining NMR



Explanation of spinning properties of nuclei



Magnetic field produced
by circulating electron

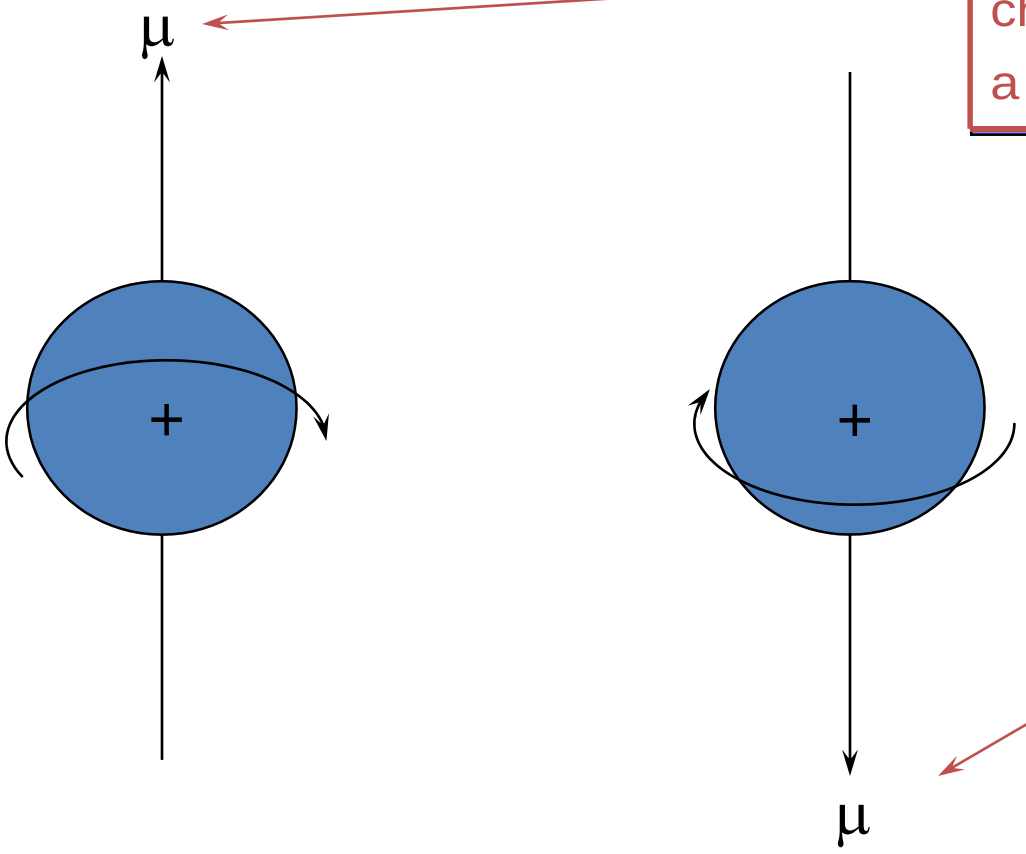




Spin Energy States

Nuclear spin states - hydrogen nucleus

The spin of the positively charged nucleus generates a magnetic moment vector, μ.

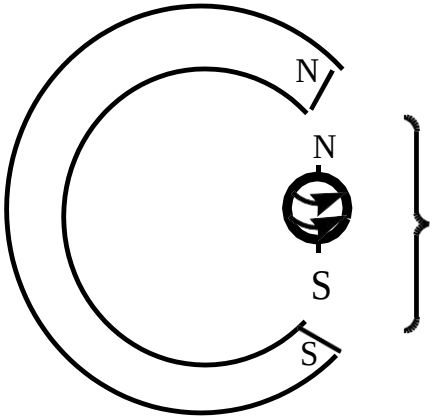


+ 1/2

- 1/2

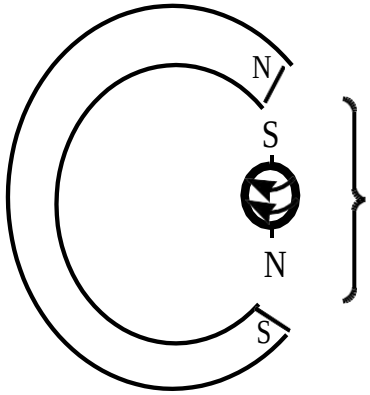
TWO SPIN STATES

The two states are equivalent in energy in the absence of a magnetic or an electric field.



α- spin state, favorable, lower energy

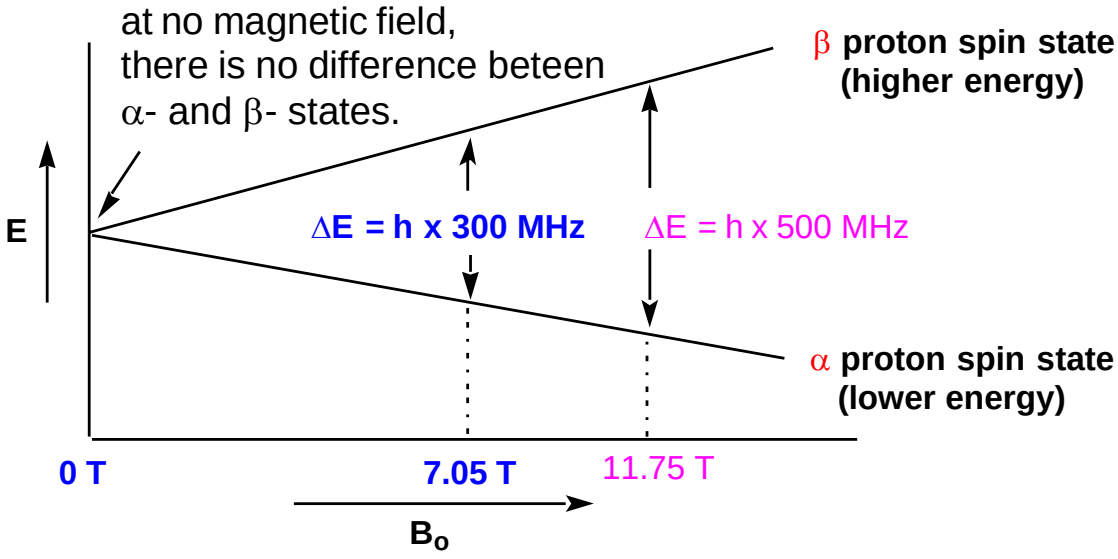
A spinning nucleus with it's magnetic field aligned **with** the magnetic field of a magnet



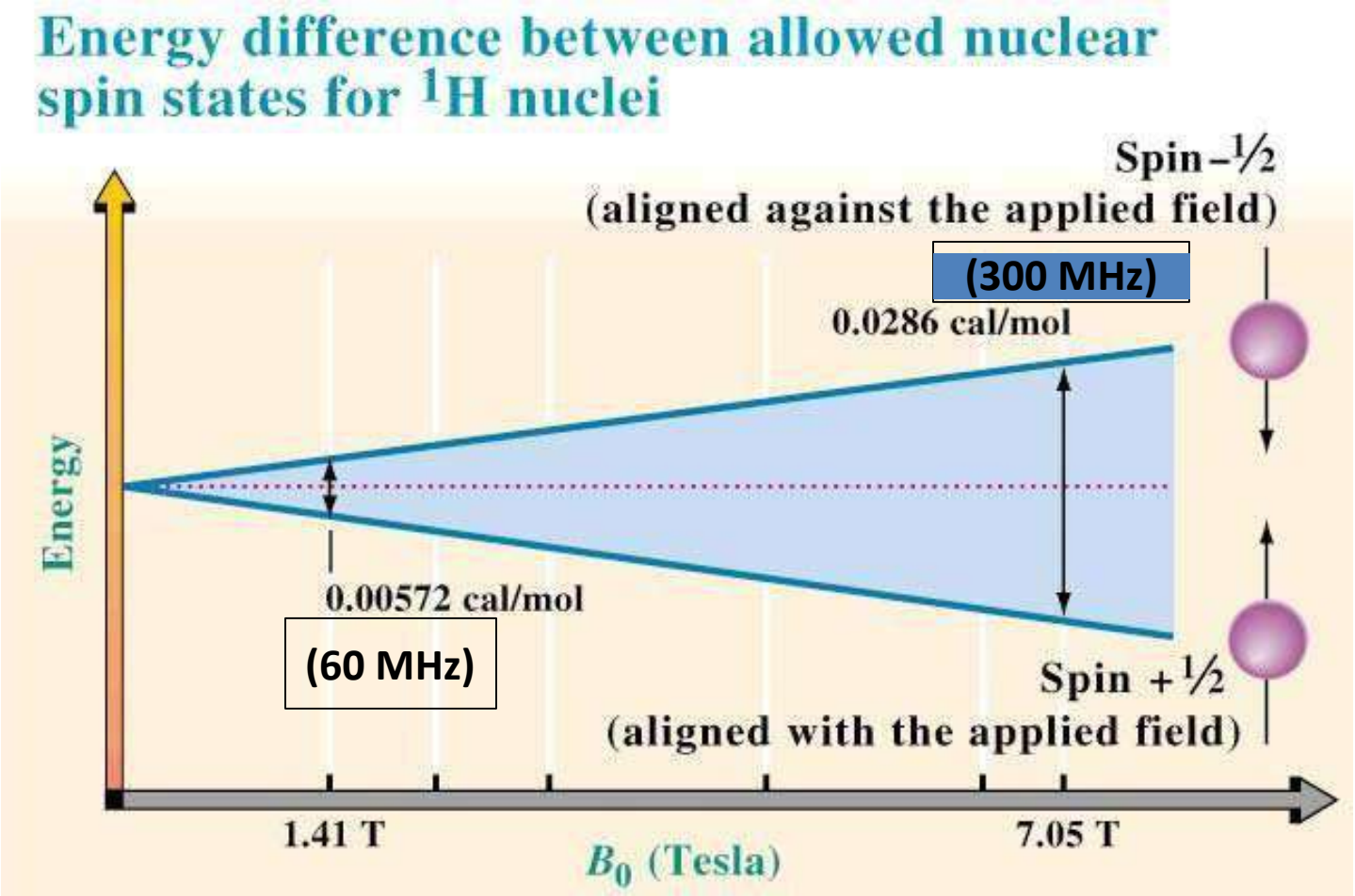
β- spin state, unfavorable, higher energy

A spinning nucleus with it's magnetic field aligned **against** the magnetic field of a magnet

Graphical relationship between magnetic field (B_o) and frequency (ν) for ^{1}H NMR absorptions



Influence of external magnetic field



Resonance

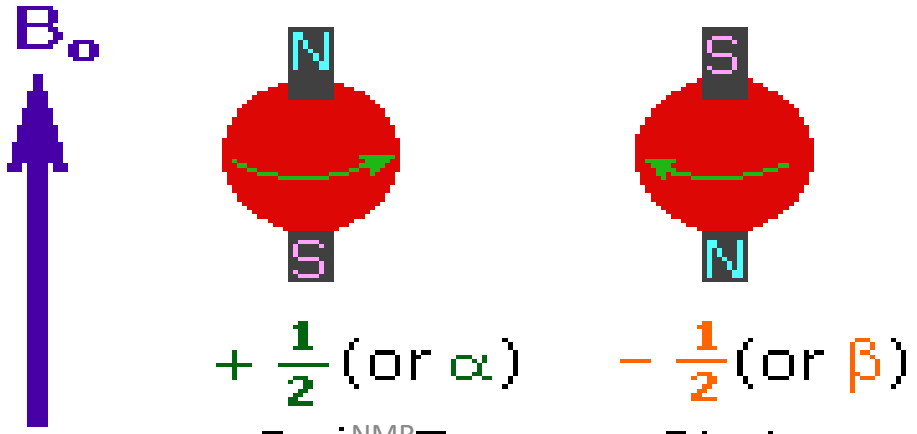
- Resonance: Matching of natural frequency with the applied frequency.
- In NMR the applied radio frequency has to be match with the precessional frequency of nuclei (which is the consequences of applied external magnetic field)
- Absorption of radio frequency occur by nuclei after resonance is achieved.

Precessional frequency (ν)

The number of revolution per second made by magnetic moment vector of the nucleus around the external magnetic field B_0

OR

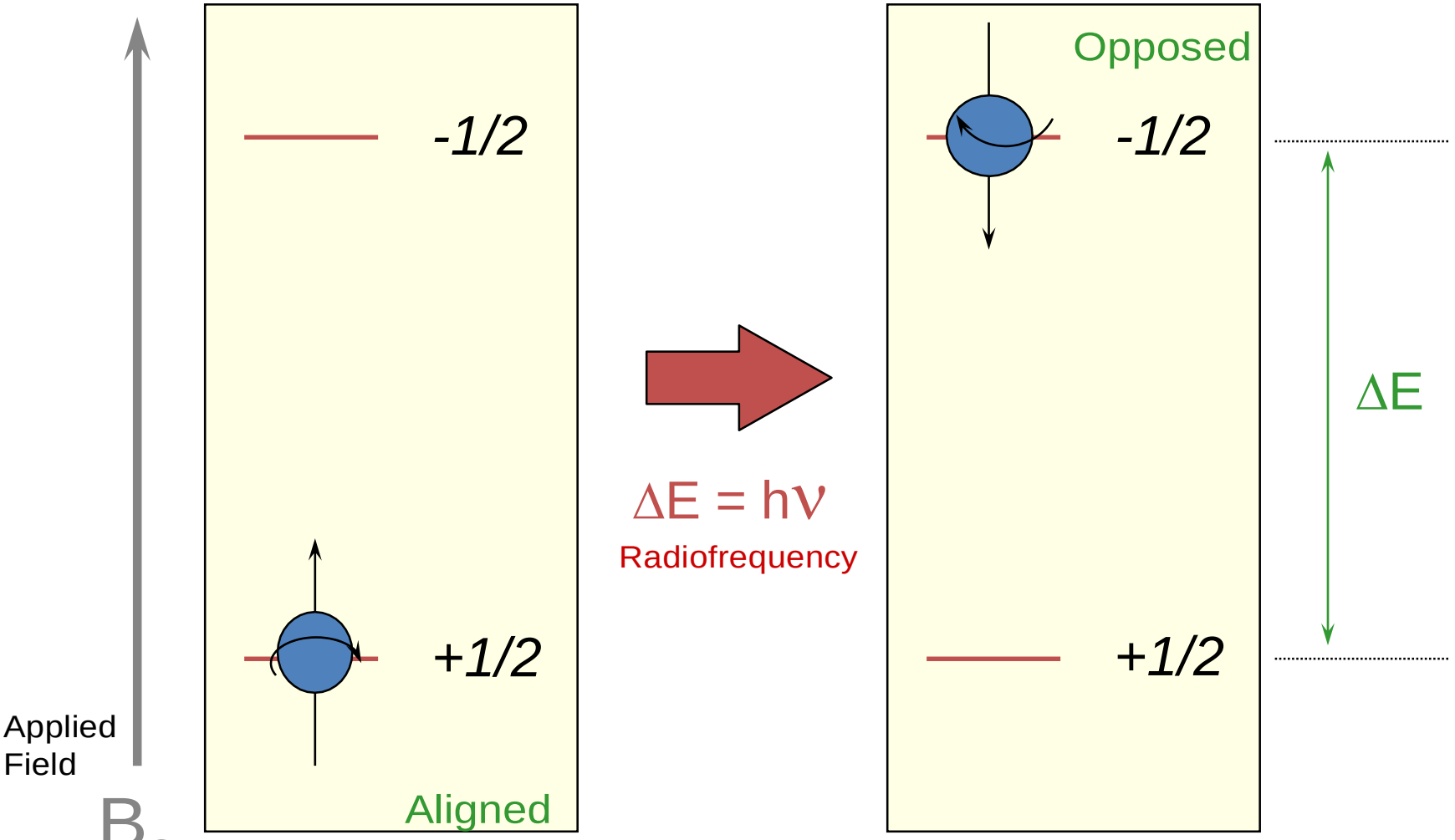
Precessional frequency is equal to the frequency of electromagnetic radiation in MHz (Mega cycles/sec) necessary to induce a transition from one spin state to other.



Spin Energy States

Absorption of Energy

quantized



Mathematical Expression.

$$\omega = \gamma B_0$$

ω = Angular Precessional velocity

B_0 = Applied field in gauss

γ = Gyromagnetic ratio

ν = Precessional frequency

But,

$$\omega = 2\pi\nu$$

So,

$$\gamma B_0 = 2\pi\nu$$

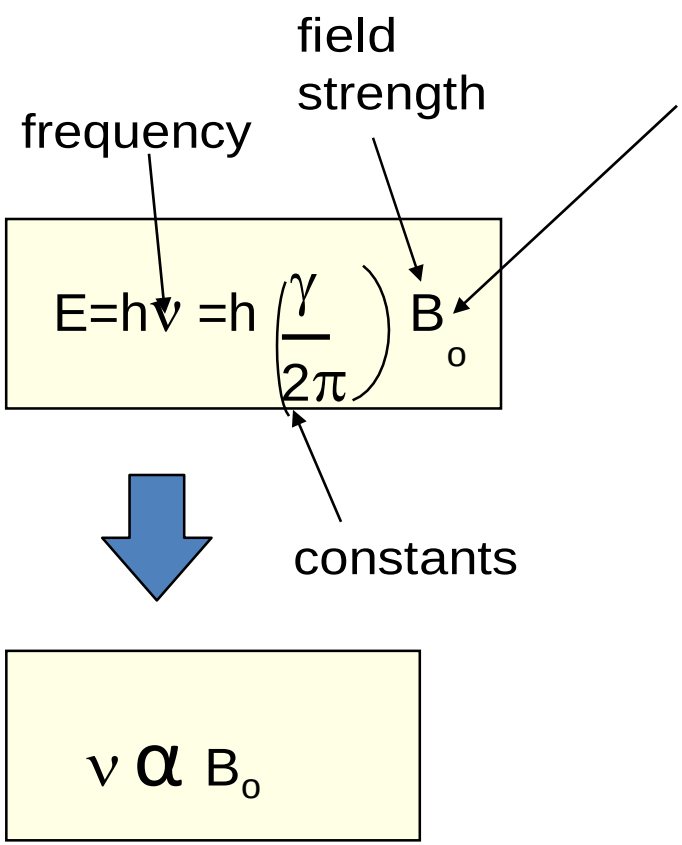
$$\nu = \gamma B_0 / 2\pi$$

$$\nu = \frac{2\pi\mu}{}$$

$$1\ \mathrm{T} = 10^4\ \mathrm{G}$$

$$\Delta E = \frac{h\gamma}{2\pi} \boldsymbol{B}_0$$

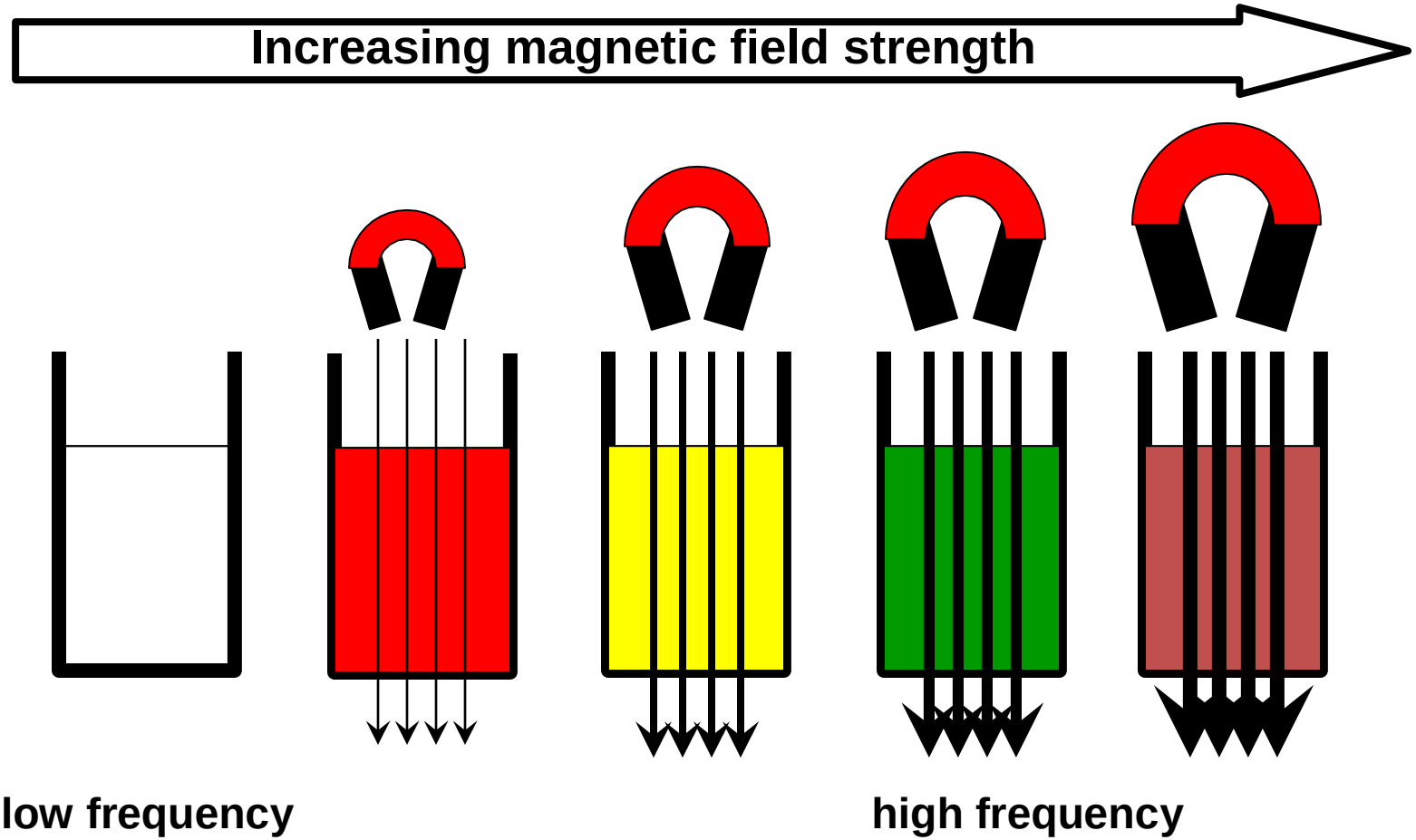
The Larmor Equation



Stronger magnetic fields (B_o) cause the instrument to operate at higher frequencies (ν).

NMR Field Strength	^{1}H Operating Frequency
1.41 T	60 MHz
2.35 T	100 MHz
7.05 T	300 MHz

Isotope	Natural % Abundance	Spin (I)	Magnetic Moment (μ)	Magnetogyric Ratio (γ)
^{1}H	99.9844	1/2	2.7927	26.753
^{2}H	0.0156	1	0.8574	4,107
^{13}C	1.108	1/2	0.7022	6,728
^{19}F	100.0	1/2	2.6273	25,179
^{31}P	100.0	1/2	1.1305	10,840

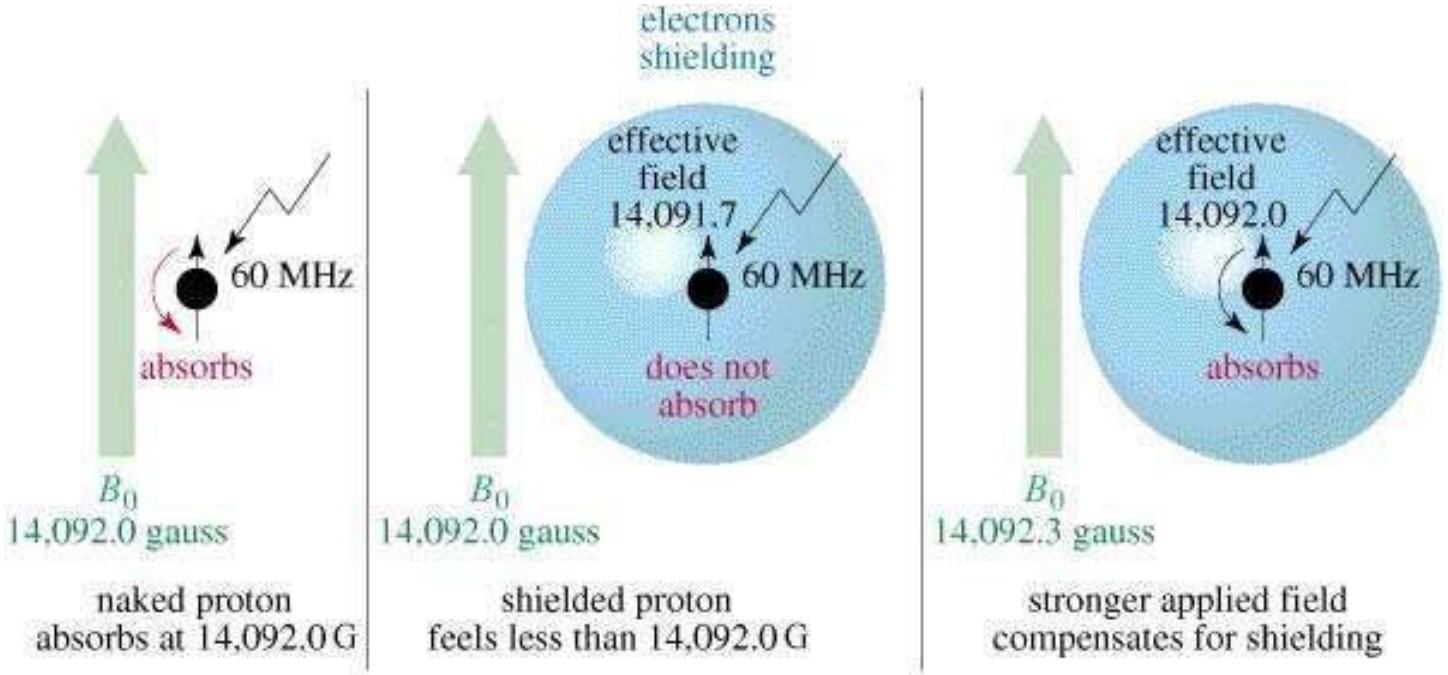

Increasing magnetic field strength
low frequency
high frequency

Magnetic Shielding

- If all protons absorbed the same amount of energy in a given magnetic field, not much information could be obtained.
- But protons are surrounded by electrons that shield them from the external field.
- Circulating electrons create an induced magnetic field that opposes the external magnetic field.

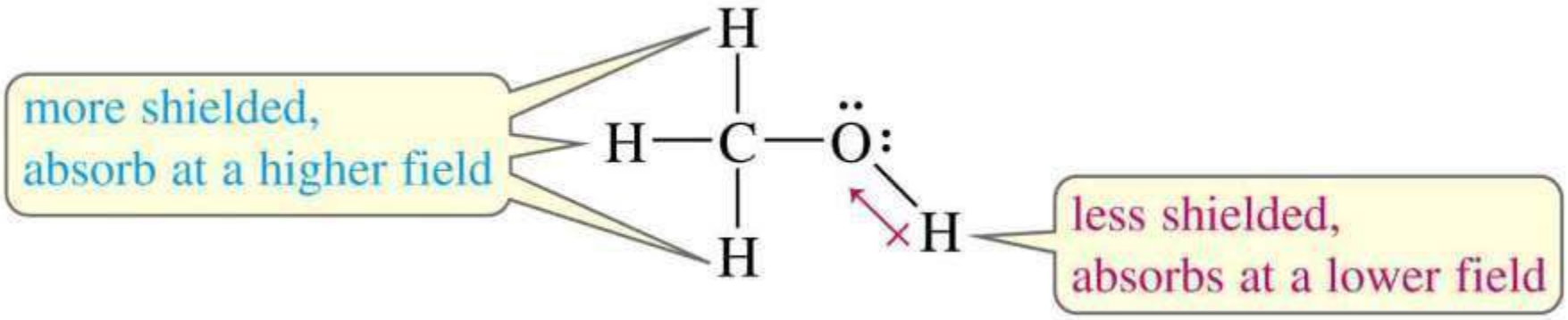
Shielded Protons

Magnetic field strength must be increased for a shielded proton to flip at the same frequency.



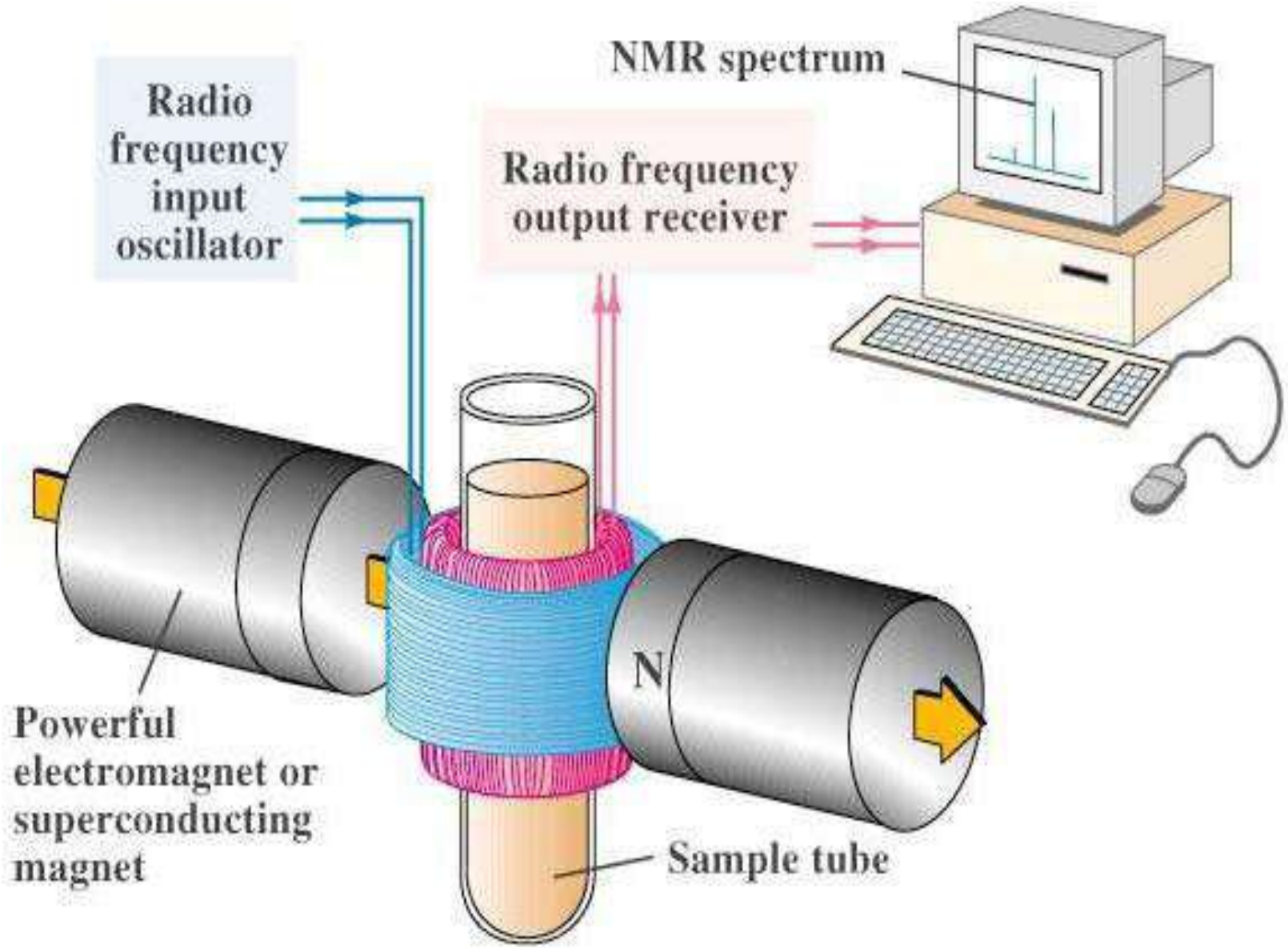
Protons in a Molecule

Depending on their chemical environment, protons in a molecule are shielded by different amounts.



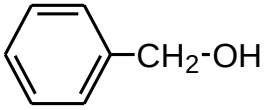
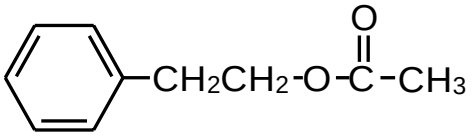
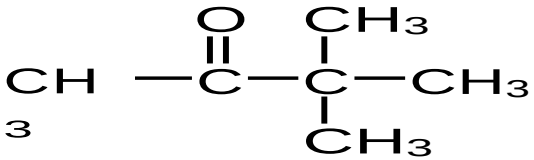
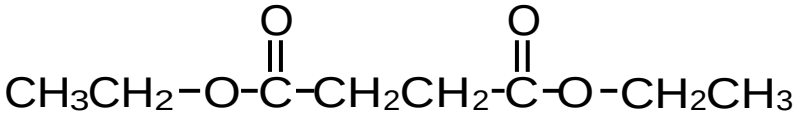
NMR Signals

- The ***number*** of signals shows how many different kinds of protons are present.
- The ***location*** of the signals shows how shielded or deshielded the proton is.
- The ***intensity*** of the signal shows the number of protons of that type.
- Signal ***splitting*** shows the number of protons on adjacent atoms.


NMR spectrum
Radio frequency input oscillator
Radio frequency output receiver
N
Powerful electromagnet or superconducting magnet
Sample tube

Number of signal

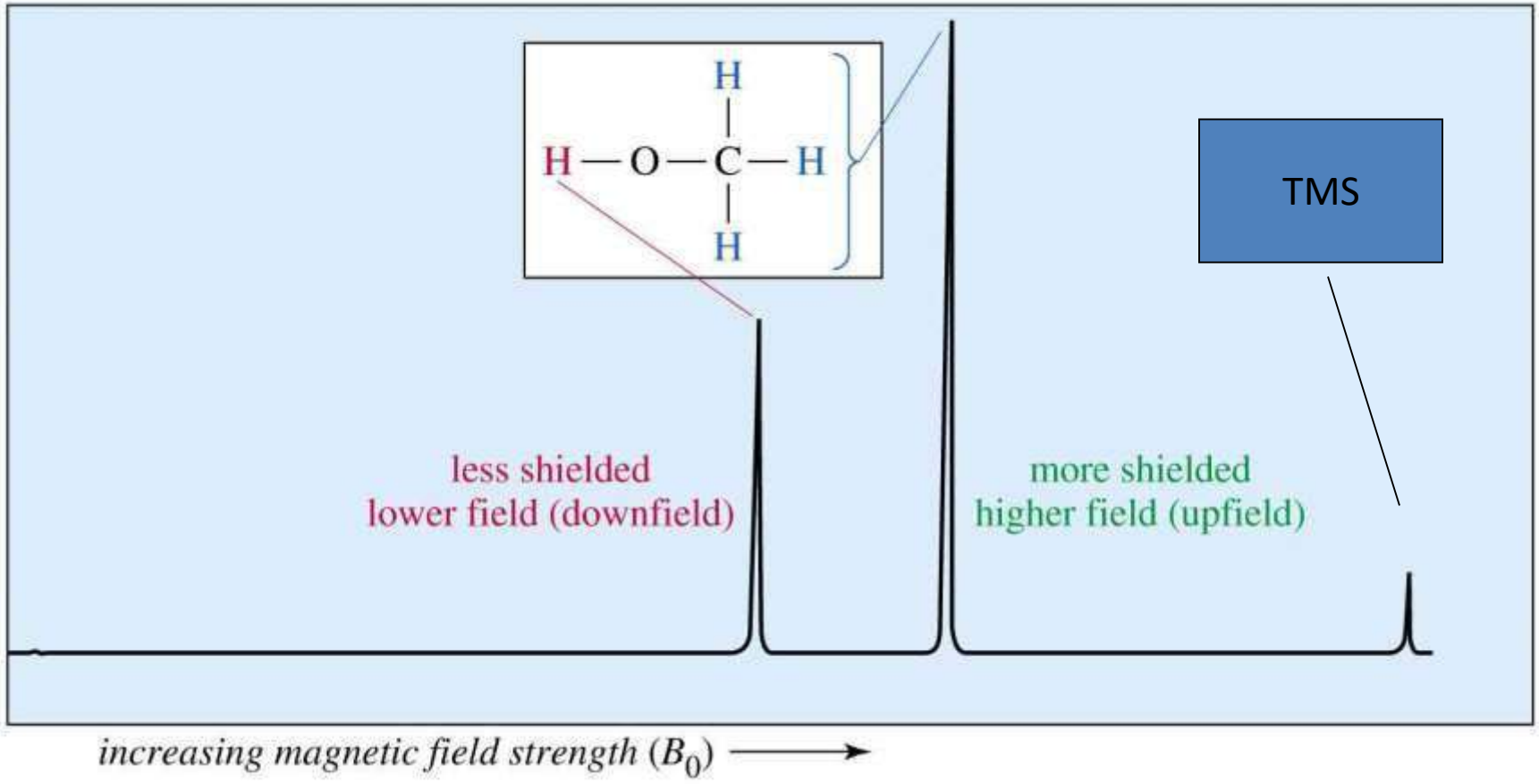
Number of signal α number of magnetically equivalent proton

Compound	No.of signal
	3
	3
	4
	2
	3

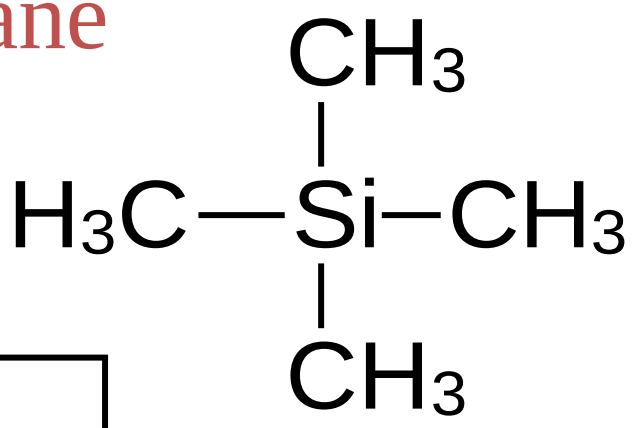
The NMR Graph

Downfield: **the shift of an NMR signal to the left on the chart paper**

Upfield: **the shift of an NMR signal to the right on the chart paper**



Tetramethylsilane



Chemically inert

Highly shielded

Miscibility large range of solvent

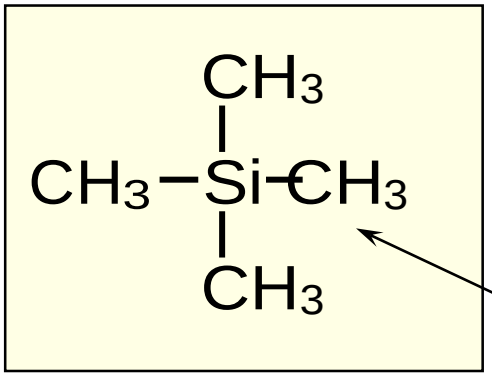
Highly volatile

Does not take part in intermolecular association

For water soluble compound Sodium salt of 3-(trimethyl silyl)propane sulphonate is used as internal standard

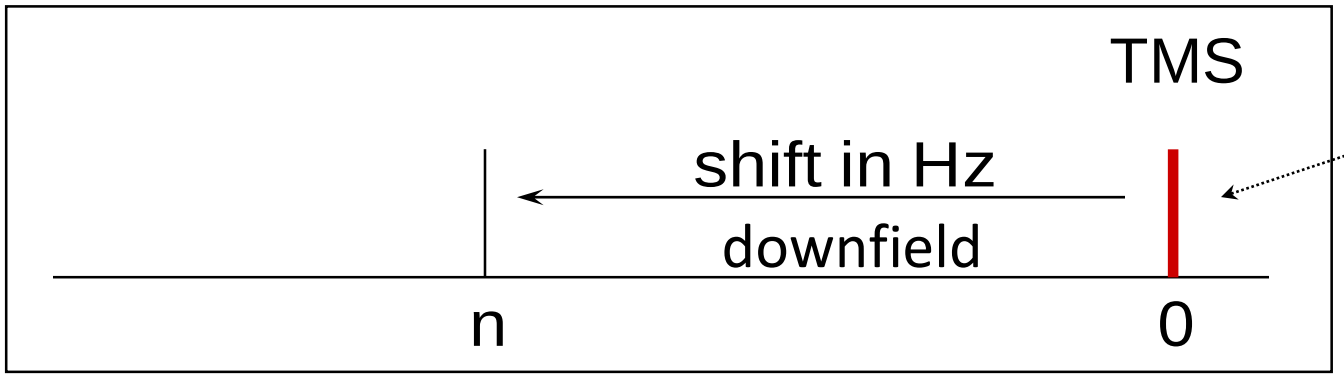
Peaks are measured relative to TMS

Rather than measure the exact resonance position of a peak, we measure how far downfield it is shifted from TMS.



reference compound
tetramethylsilane
"TMS"

Highly shielded protons appear way upfield.



no other compound would come at a higher field than TMS.

The chemical shift

The shifts from TMS in Hz are bigger in higher field instruments (300 MHz, 500 MHz) than they are in the lower field instruments (100 MHz, 60 MHz).

parts per million

$$\text{chemical shift} = \delta = \frac{\text{shift in Hz (sample- Ref)}}{\text{spectrometer frequency in MHz}} = \text{ppm}$$

This division gives a number independent of the instrument used.

The Chemical Shift independent upon applied magnetic field strength

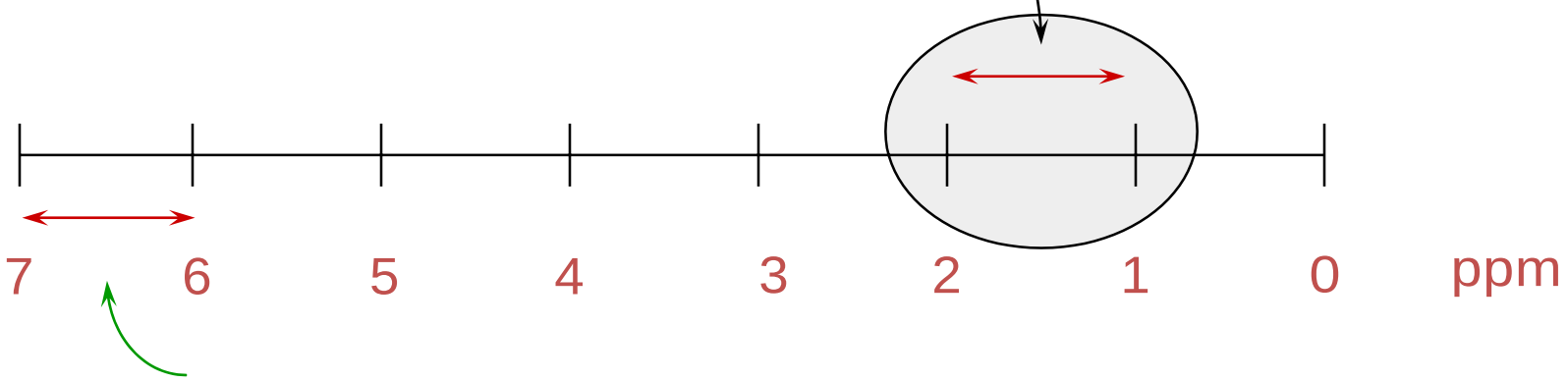
Imagine that we have a magnet where our standard absorbs at 300,000,000 Hz (300 megahertz), and our sample absorbs at 300,000,300 Hz. The difference is 300 Hz, so we take 300/300,000,000 = 1/1,000,000 and call that 1 part per million (or 1 PPM). Now lets examine the same sample in a stronger magnetic field where the reference comes at 500,000,000 Hz, or 500 megahertz. The frequency of our sample will increase proportionally, and will come at 500,000,500 Hz. The difference is now 500 Hz, but we divide by 500,000,000 (500/500,000,000 = 1/1,000,000, = 1 PPM).

Hertz Equivalence Of 1 ppm

^{1}H Operating Frequency	Hz Equivalent of 1 ppm
60 Mhz	60 Hz
100 MHz	100 Hz
300 MHz	300 Hz

1 part per million of n MHz is n Hz

$$n\ \text{MHz}\left(\frac{1}{10^6}\right) = n\ \text{Hz}$$

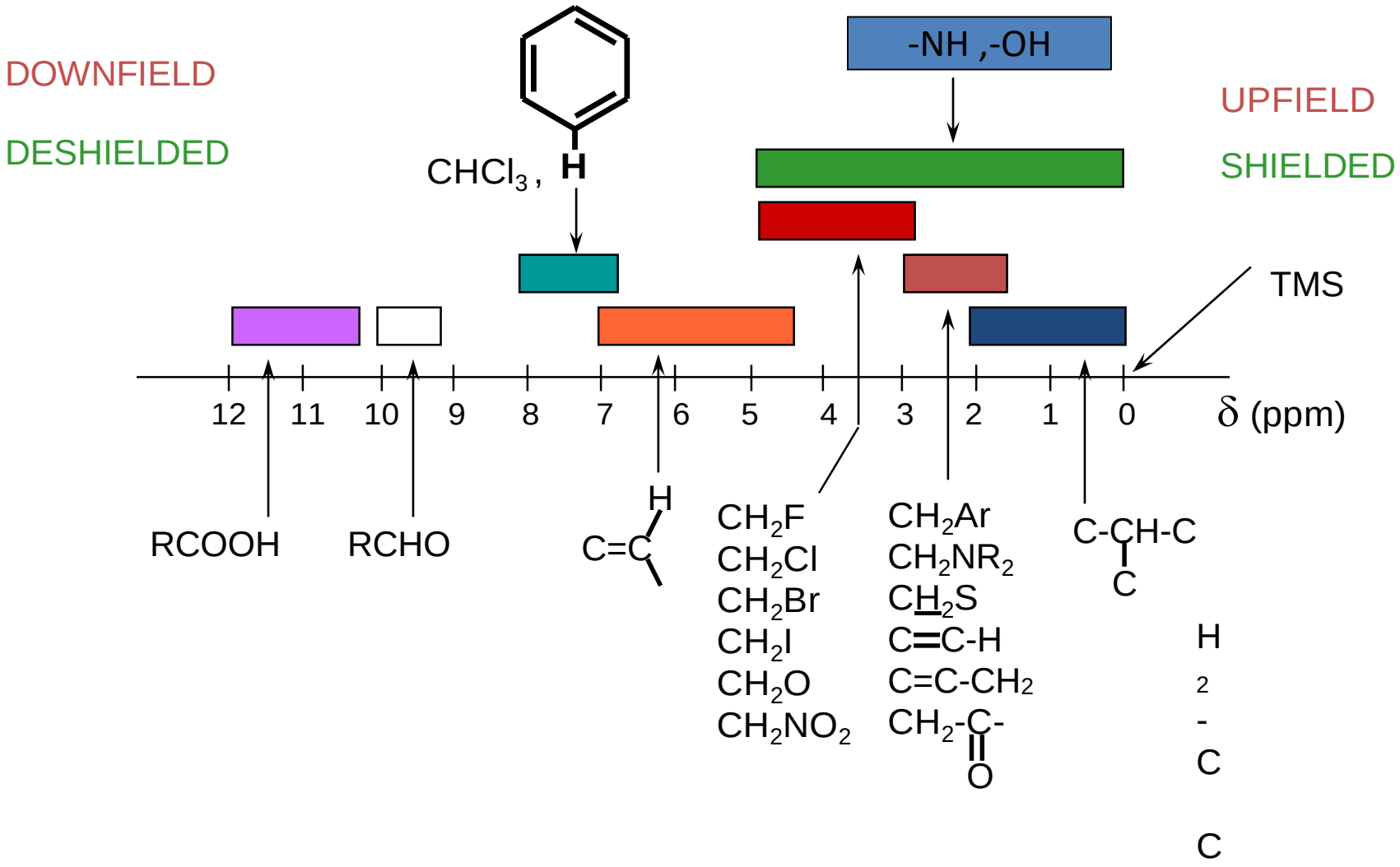


Each ppm unit represents either a 1 ppm change in B_o (magnetic field strength, Tesla) or a 1 ppm change in the precessional frequency (MHz).

Approximate Chemical Shift(δ) Ranges (ppm) For Selected Types Of Protons

Cyclopropane	0.2	Ethers	3.3-4.0
1^0hydrogen	0.9	Esters	2.0-2.2
Vinylic	4.6-5.8	Acids	2.0-2.5
Acetylinic	2.0-3.5	Aldehydic	9.0-10
Aromatic	6.0-9.0	Hydroxy	1.0-5.5
Fluorides	4.0-4.5	Phenolic	4.0-12
Chlorides	3.0-4.0	Carboxylic	10.5-12
Alcohols	3.4-4.0		

NMR Correlation Chart



$-CH_3$

Factors affecting chemical shift

- Inductive effect
- Vanderwaal's deshielding
- Anisotropic/space effect
- Hydrogen bonding

Substitution Effects on Chemical Shift (inductive effect)

Dependence of the Chemical Shift of CH_3X on the Element X

Compound CH_3X	CH_3F	CH_3OH	CH_3Cl	CH_3Br	CH_3I	CH_4	$(CH_3)_4Si$
Element X	F	O	Cl	Br	I	H	Si
Electronegativity of X	4.0	3.5	3.1	2.8	2.5	2.1	1.8
Chemical shift δ	4.26	3.40	3.05	2.68	2.16	0.23	0

most deshielded

TMS

deshielding increases with the electronegativity of atom X

Electronegativity Dependence of Chemical Shift

most deshielded

$CHCl_3$	CH_2Cl_2	CH_3Cl	
7.27	5.30	3.05	ppm

The effect increases with greater numbers of electronegative atoms.

most deshielded

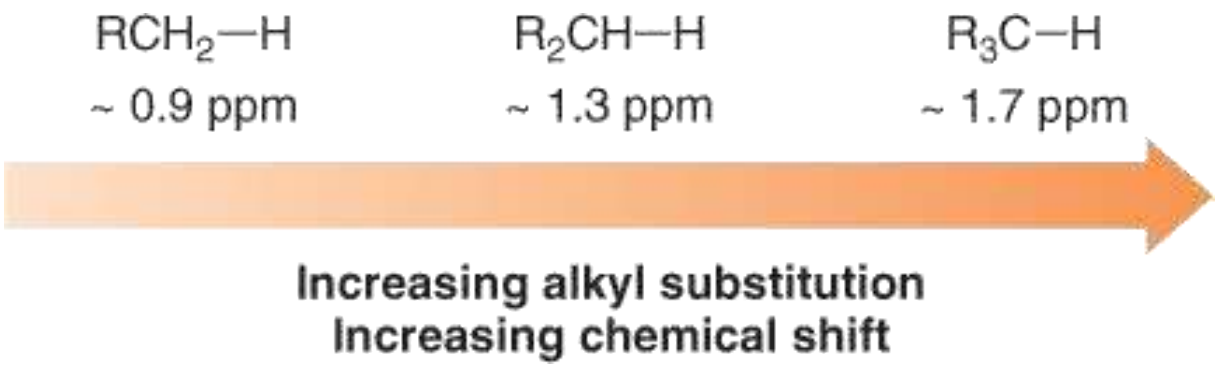
$-CH_2-Br$	$-CH_2-CH_2Br$	$-CH_2-CH_2CH_2Br$	
3.30	1.69	1.25	ppm

The effect decreases with incresing distance.

Nuclear Magnetic Resonance Spectroscopy

^{1}H NMR—Chemical Shift Values

- The chemical shift of a C—H bond increases with increasing alkyl substitution.



Nuclear Magnetic Resonance Spectroscopy

Calculating ^{1}H NMR—Chemical Shift Values

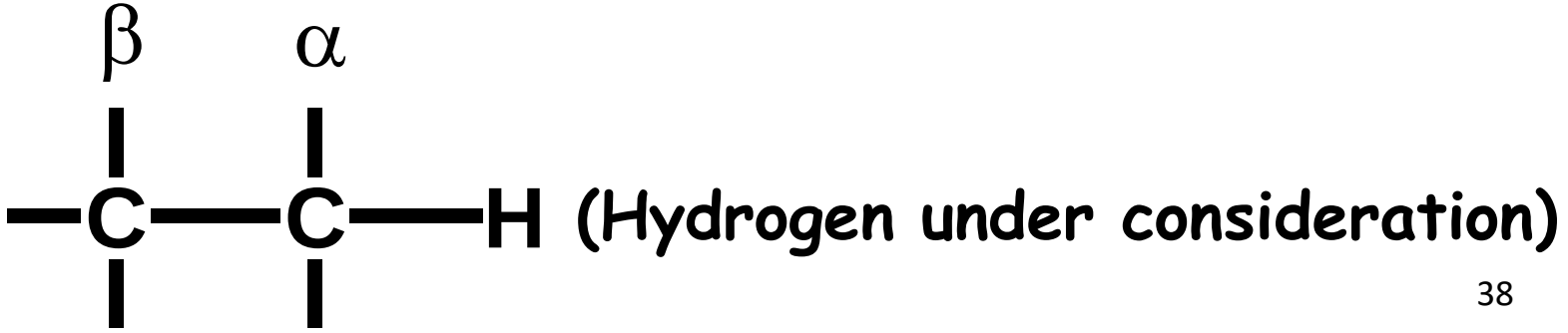
- The chemical shift of a C—H can be calculated with a high degree of precision if a chemical shift additivity table is used.
- The additivity tables starts with a base chemical shift value depending on the structural type of hydrogen under consideration:

		Methylene	Methine
	CH_3—	—C— H_2	—C— H
Base Chemical Shift	**0.87 ppm**	**1.20 ppm**	**1.20 ppm**

Nuclear Magnetic Resonance Spectroscopy

Calculating ^{1}H NMR—Chemical Shift Values

- The presence of nearby atoms or groups will effect the base chemical shift by a specific amount:
  - The carbon atom bonded to the hydrogen(s) under consideration are described as alpha (α) carbons.
  - Atoms or groups bonded to the same carbon as the hydrogen(s) under consideration are described as alpha (α) substituents.
  - Atoms or groups on carbons one bond removed from the a carbon are called beta (β) carbons.
  - Atoms or groups bonded to the β carbon are described as alpha (β) substituents.

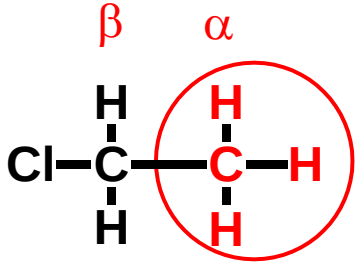


Nuclear Magnetic Resonance Spectroscopy

Calculating ^1H NMR—Chemical Shift Values

Added Chemical Shifts

Substituent	Type of Hydrogen	α-Shift	β-Shift
C=C—	CH_3	0.78	—
	CH_2	0.75	-0.10
	CH	—	—
Y RC—C=C— [Y = C or O]	CH_3	1.08	—
Aryl-	CH_3	1.40	0.95
	CH_2	1.45	0.53
	CH	1.93	—
Cl-	CH_3	2.43	0.63
	CH_2	2.90	0.53
	CH	2.55	0.03
Br-	CH_3	1.80	0.83
	CH_2	2.18	0.60
	CH	2.68	0.25
I-	CH_3	1.28	1.23
	CH_2	1.95	0.58
	CH	2.75	0.00
OH-	CH_3	2.50	0.93
	CH_2	2.90	0.13
	CH	2.20	—
RO- (R is saturated)	CH_3	2.43	0.93
	CH_2	2.95	0.15
	CH	2.00	—
O R-CO— or ArC—	CH_3	2.88	0.98
	CH_2	2.98	0.43
	CH	9.43 (ester only)	—
O R-C—	CH_3	1.23	0.18
where R is alkyl, aryl, OH, OR', H, CO, or N	CH_2	1.05	0.31
	CH	1.05	—



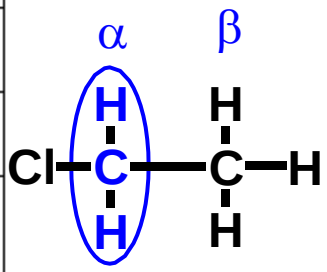
(Hydrogen under consideration)

Base Chemical Shift = 0.87 ppm

no α substituents = 0.00

one β -Cl (CH_3) = 0.63

TOTAL = 1.50 ppm



(Hydrogen under consideration)

Base Chemical Shift = 1.20 ppm

one α -Cl (CH_2) = 2.30

no β substituents = 0.00

TOTAL = 3.50 ppm

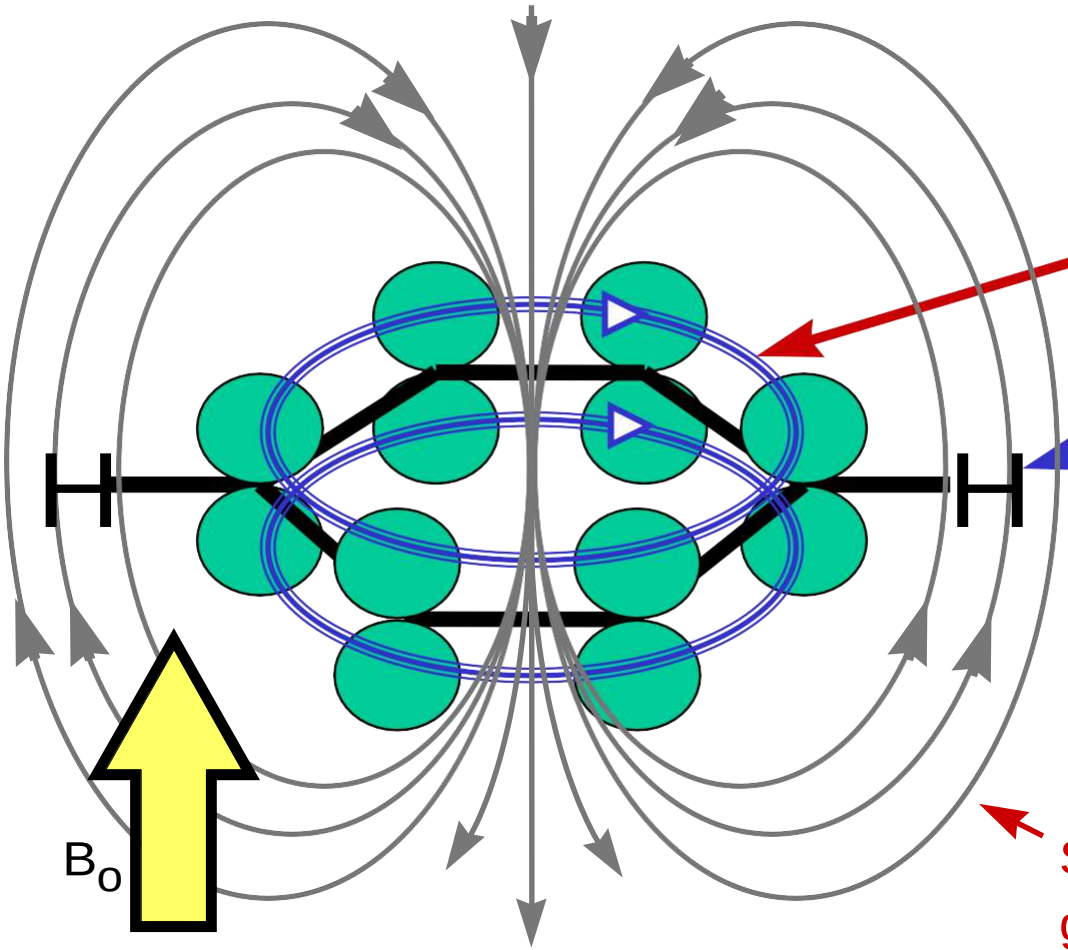
Anisotropic/Space effect

Due To The Presence Of Pi Bonds

The presence of a nearby pi bond or pi system greatly affects the chemical shift.

Benzene rings have the greatest effect.

Ring Current in Benzene



δ =7-8

Circulating π electrons

Paramagnetic

fields add together

Secondary magnetic field generated by circulating π electrons deshields aromatic protons

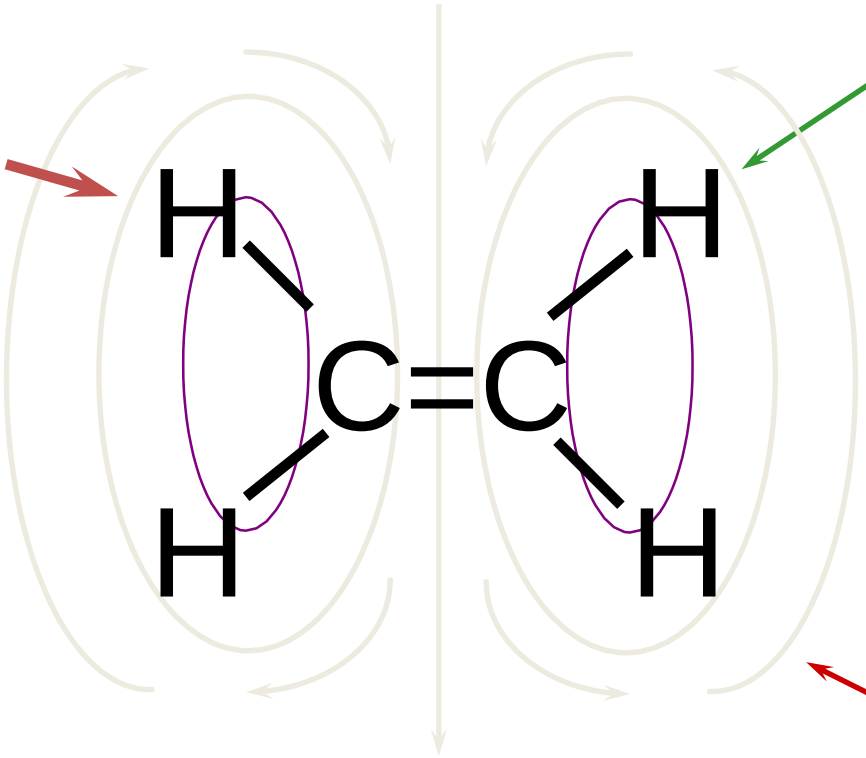
Anisotropic field in alkene

δ= 5 - 6

protons are deshielded

Paramagnetic

fields add



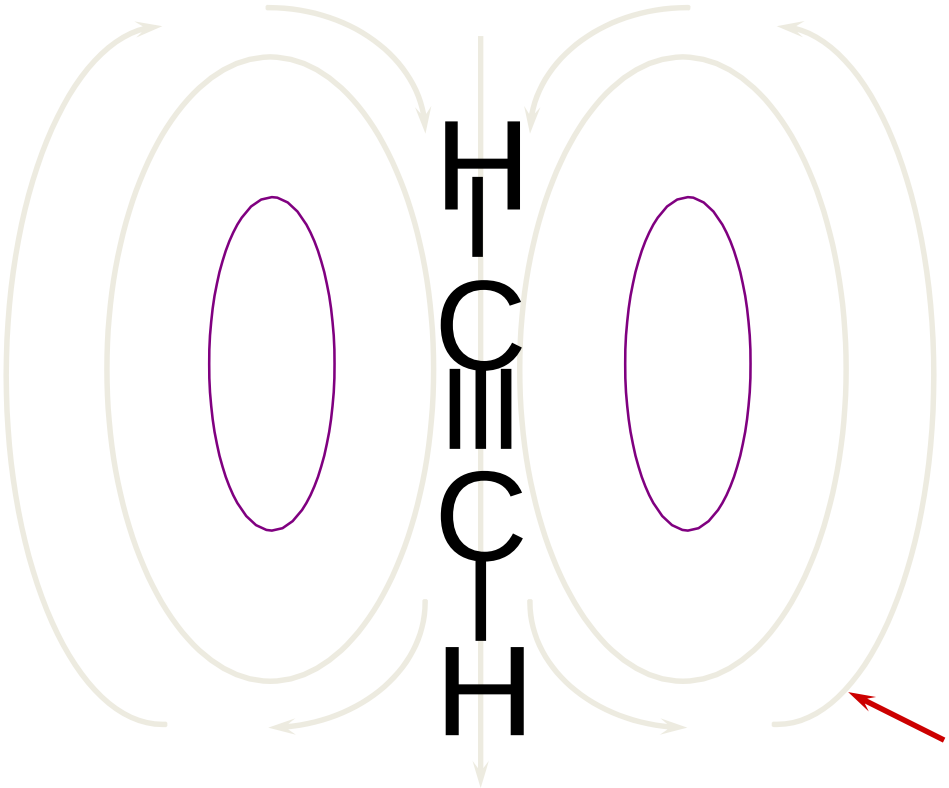
shifted downfield

B_o

secondary magnetic (anisotropic) field lines

Anisotropic field for acetylene

δ=2.5

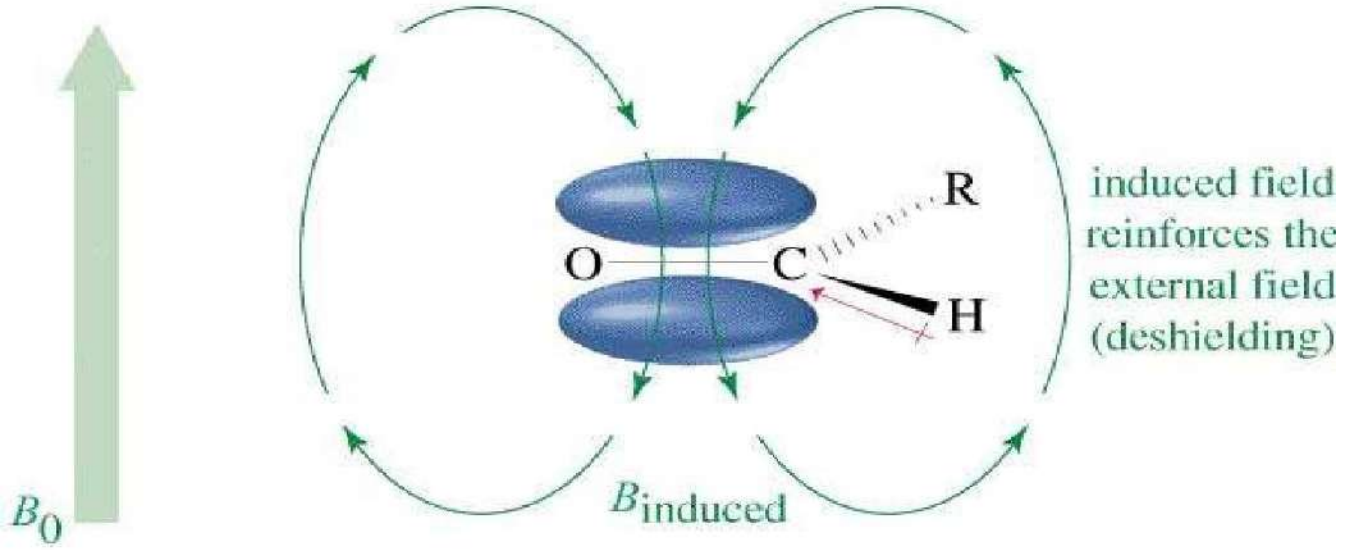


B_o

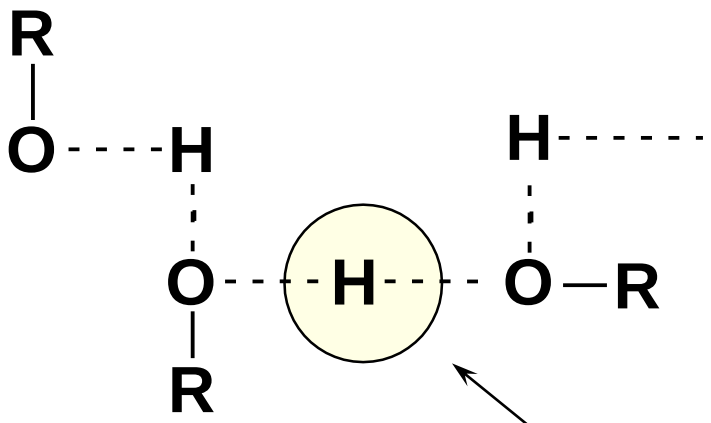
secondary magnetic (anisotropic) field

Anisotropic field for aldehyde

δ= 9-10

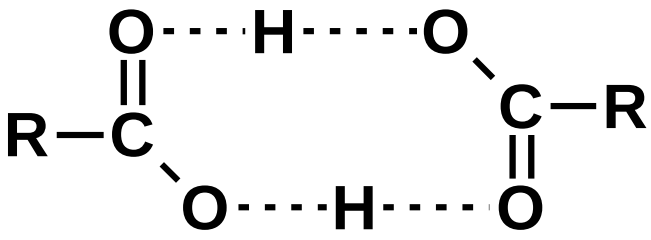


Hydrogen bonding deshields protons



Hydrogen bonding lengthens the O-H bond and reduces the valence electron density around the proton - it is deshielded and shifted downfield in the NMR spectrum.

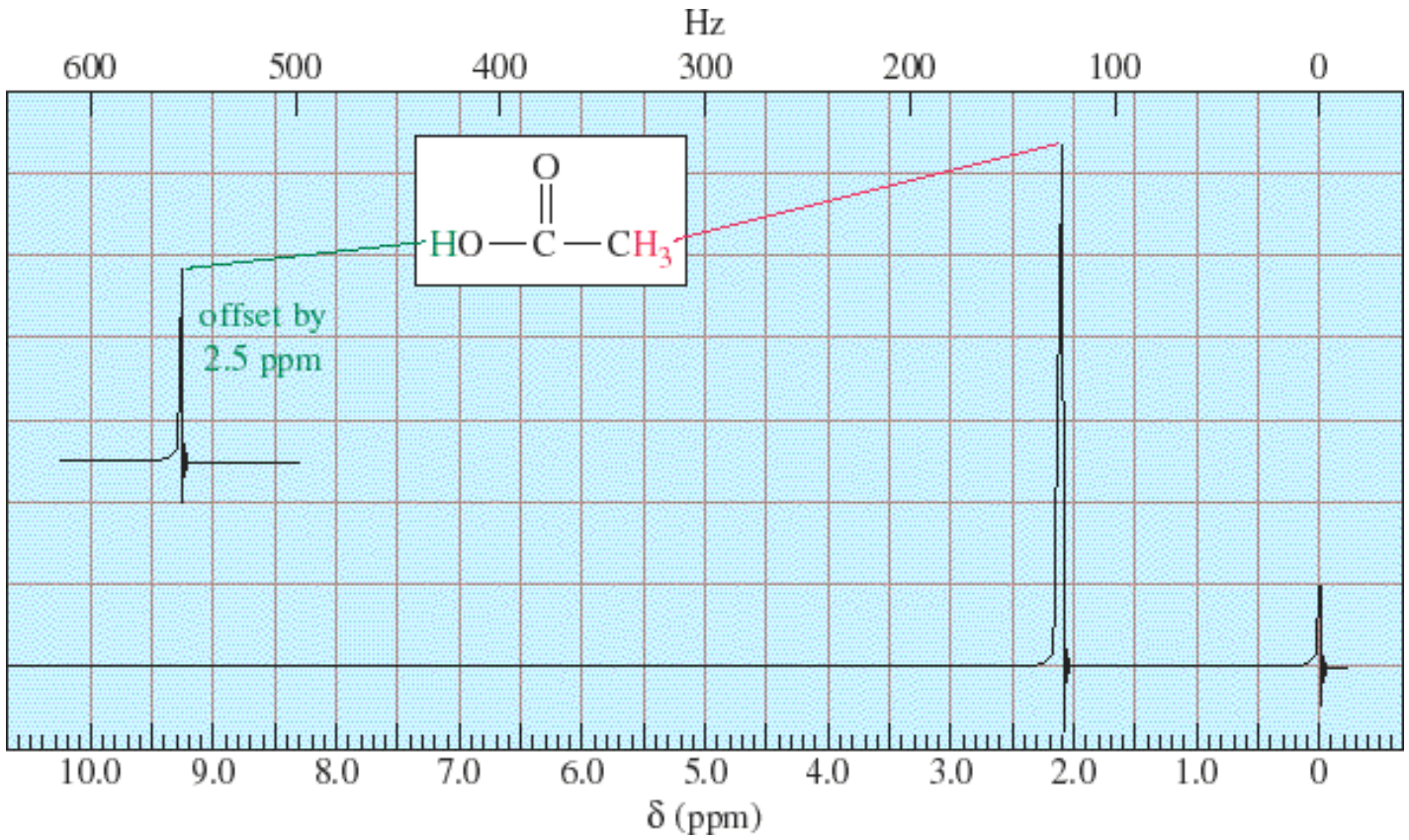
Hydrogen bonding in carboxylic acids



Carboxylic acids have strong hydrogen bonding – they form dimers.

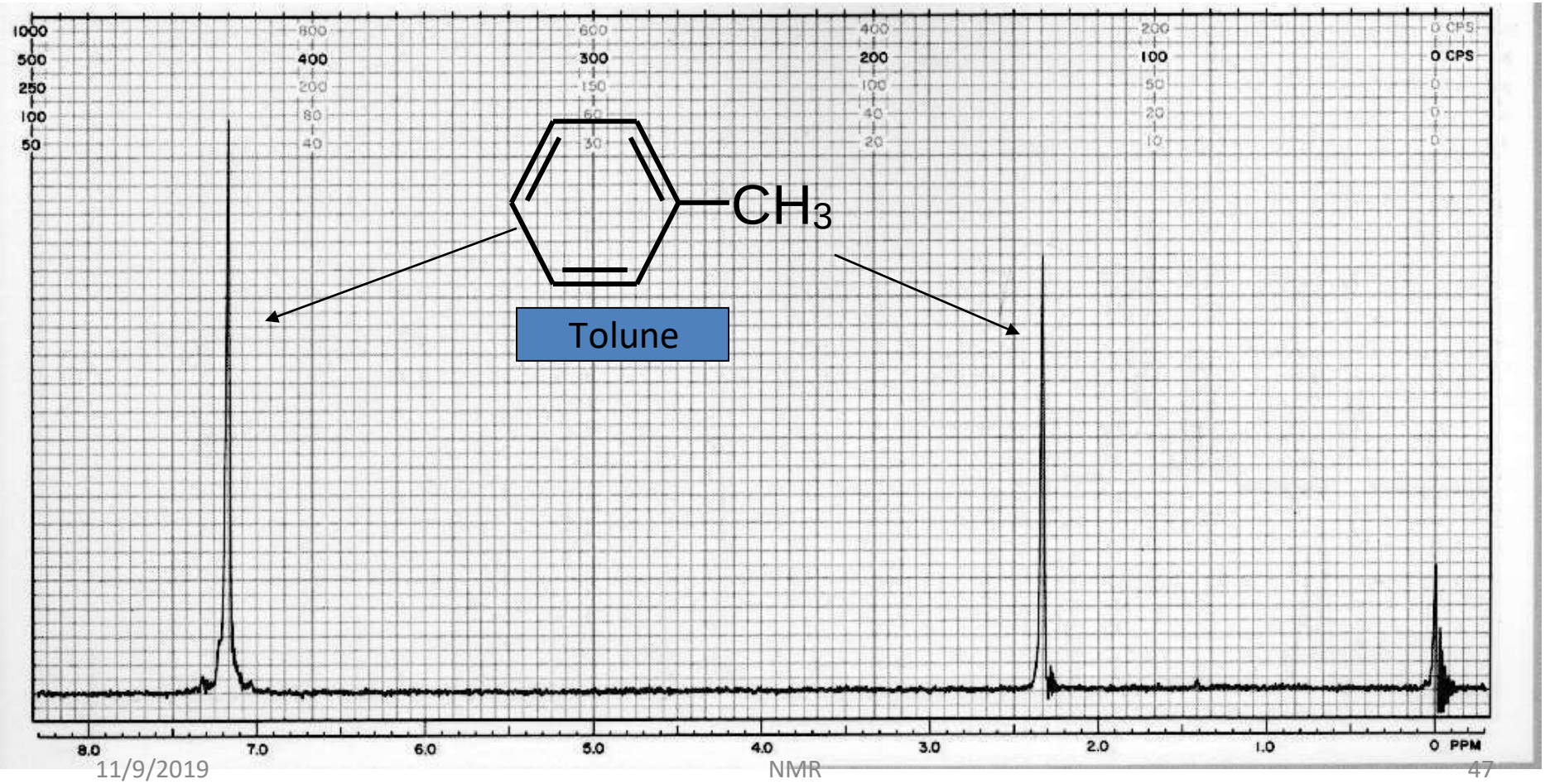
With carboxylic acids the O-H absorptions are found between 10 and 12 ppm.

Carboxylic Acid Proton, δ10+



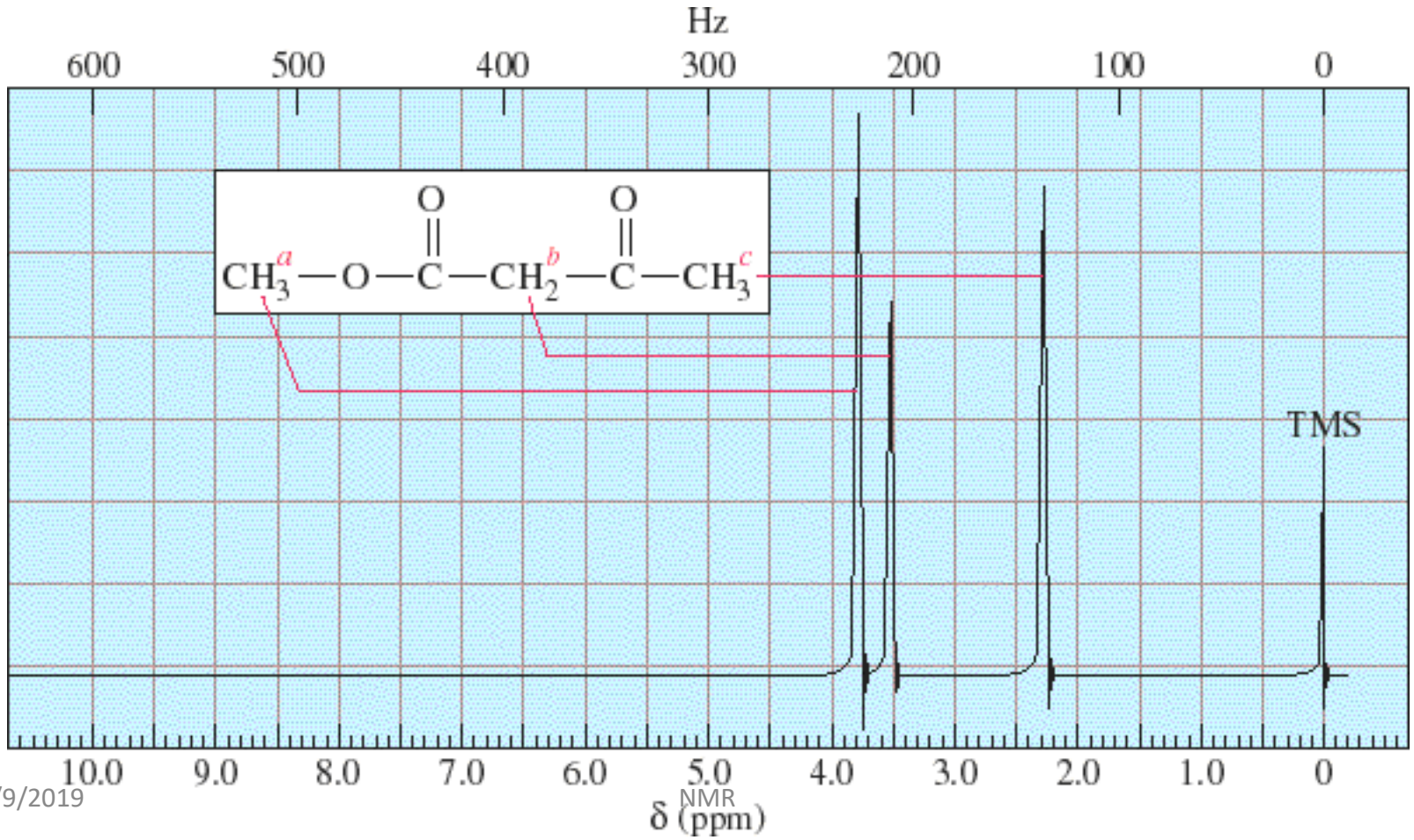
Peak area and proton counting

- Number of proton α peak area



Number of Signals

Equivalent hydrogens have the same chemical shift.



Spin-Spin Splitting in ^{1}H NMR Spectra

- Peaks are often split into multiple peaks due to ***magnetic interactions*** between nonequivalent protons on adjacent carbons, The process is called **spin-spin splitting**
- The splitting is into one more peak than the number of H's on the adjacent carbon(s), This is the **"n+1 rule"**
- The relative intensities are in proportion of a binomial distribution given by Pascal's Triangle
- The set of peaks is a **multiplet** (2 = doublet, 3 = triplet, 4 = quartet, 5=pentet, 6=hextet, 7=heptet…..)

The Origin of ^{1}H NMR—Spin-Spin Splitting

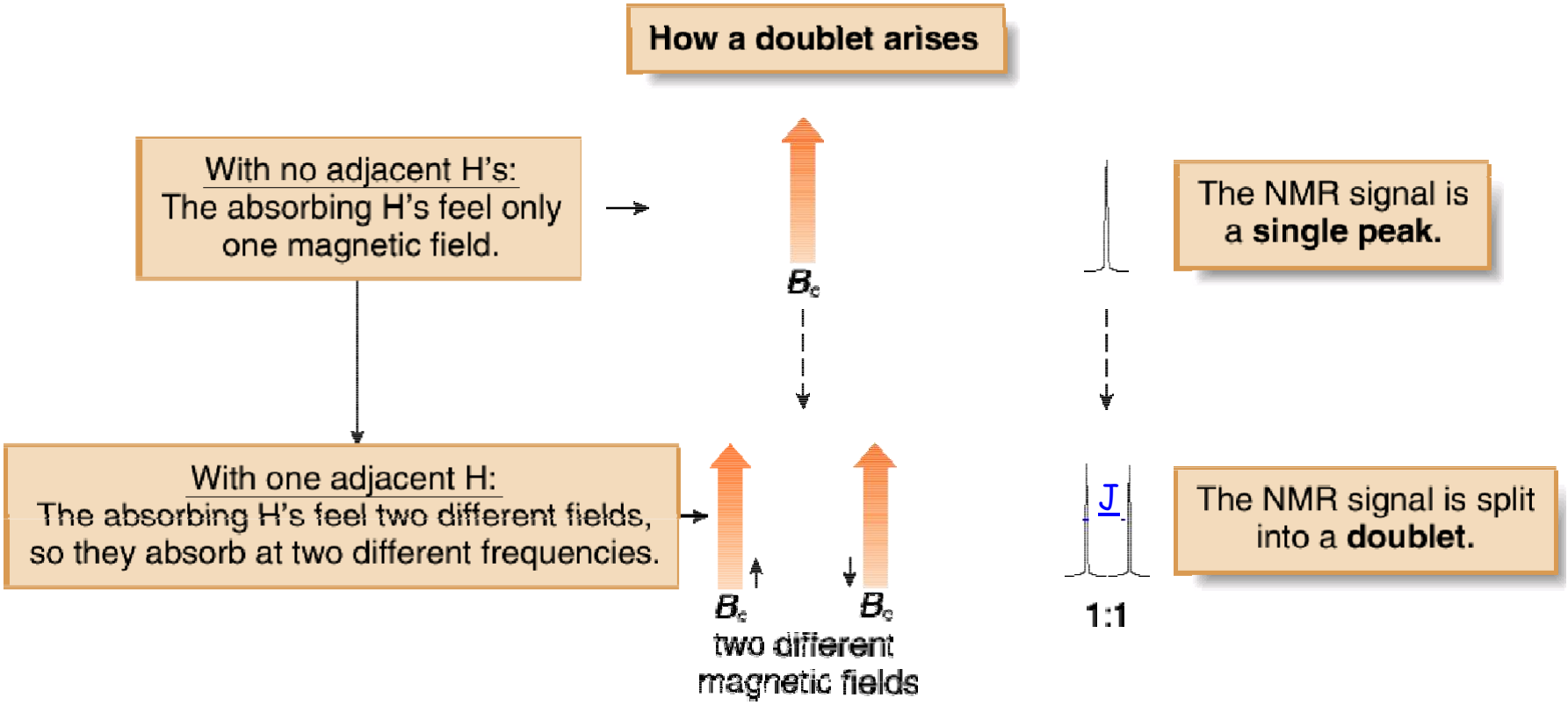
- Spin-spin splitting occurs only between nonequivalent protons on the same carbon or adjacent carbons.

Let us consider how the doublet due to the CH_2 group on $BrCH_2CHBr_2$ occurs:

- When placed in an applied field, (B_0), the adjacent proton ($CHBr_2$) can be aligned with (↑) or against (↓) B_0. The likelihood of either case is about 50% (i.e., 1,000,006↑ vs 1,000,000↓).
- Thus, the absorbing CH_2 protons feel two slightly different magnetic fields—one slightly larger than B_0, and one slightly smaller than B_0.
- Since the absorbing protons feel two different magnetic fields, they absorb at two different frequencies in the NMR spectrum, thus splitting a single absorption into a doublet, where the two peaks of the doublet have *equal* intensity.

The Origin of ^{1}H NMR—Spin-Spin Splitting

The frequency difference, measured in Hz, between two peaks of the doublet is called the coupling constant, *J*.



- One adjacent proton splits an NMR signal into a doublet.

The Origin of ^{1}H NMR—Spin-Spin Splitting

Let us now consider how a triplet arises:

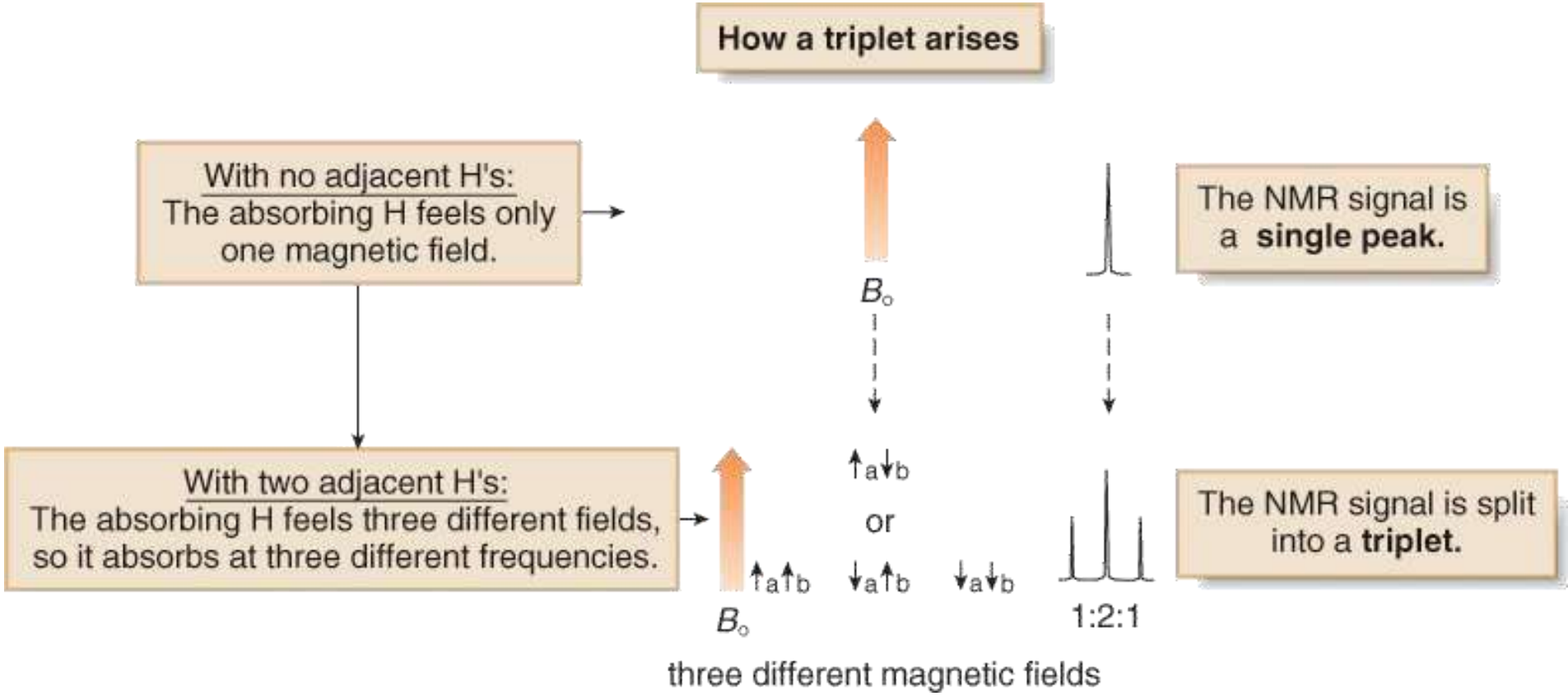


- When placed in an applied magnetic field (B_0), the adjacent protons H_a and H_b can each be aligned with (↑) or against (↓) B_0.
- Thus, the absorbing proton feels three slightly different magnetic fields—one slightly larger than $B_0(\uparrow_a\uparrow_b)$. one slightly smaller than $B_0(\downarrow_a\downarrow_b)$ and one the same strength as B_0 $(\uparrow_a\downarrow_b)$.

The Origin of ^{1}H NMR—Spin-Spin Splitting

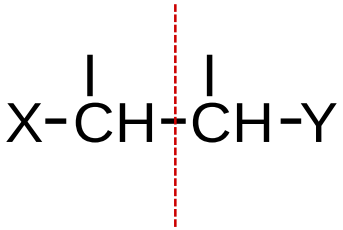
- Because the absorbing proton feels three different magnetic fields, it absorbs at three different frequencies in the NMR spectrum, thus splitting a single absorption into a triplet.
- Because there are two different ways to align one proton with B_0, and one proton against B_0—that is, $\uparrow_a\downarrow_b$ and $\downarrow_a\uparrow_b$—the middle peak of the triplet is twice as intense as the two outer peaks, making the ratio of the areas under the three peaks 1:2:1.
- Two adjacent protons split an NMR signal into a triplet.
- When two protons split each other, they are said to be coupled.
- The spacing between peaks in a split NMR signal, measured by the *J* value, is equal for coupled protons.

The Origin of ^{1}H NMR—Spin-Spin Splitting

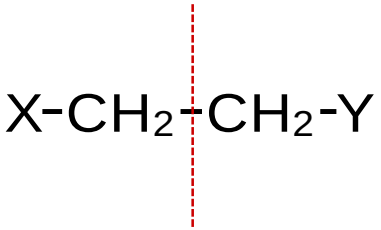


EXCEPTIONS TO THE N+1 RULE

1) Protons that are <u>equivalent by symmetry</u> usually do not split one another

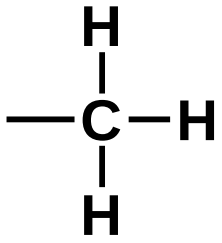


no splitting if x=y

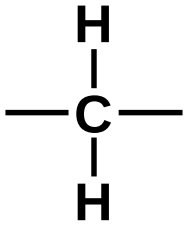


no splitting if x=y

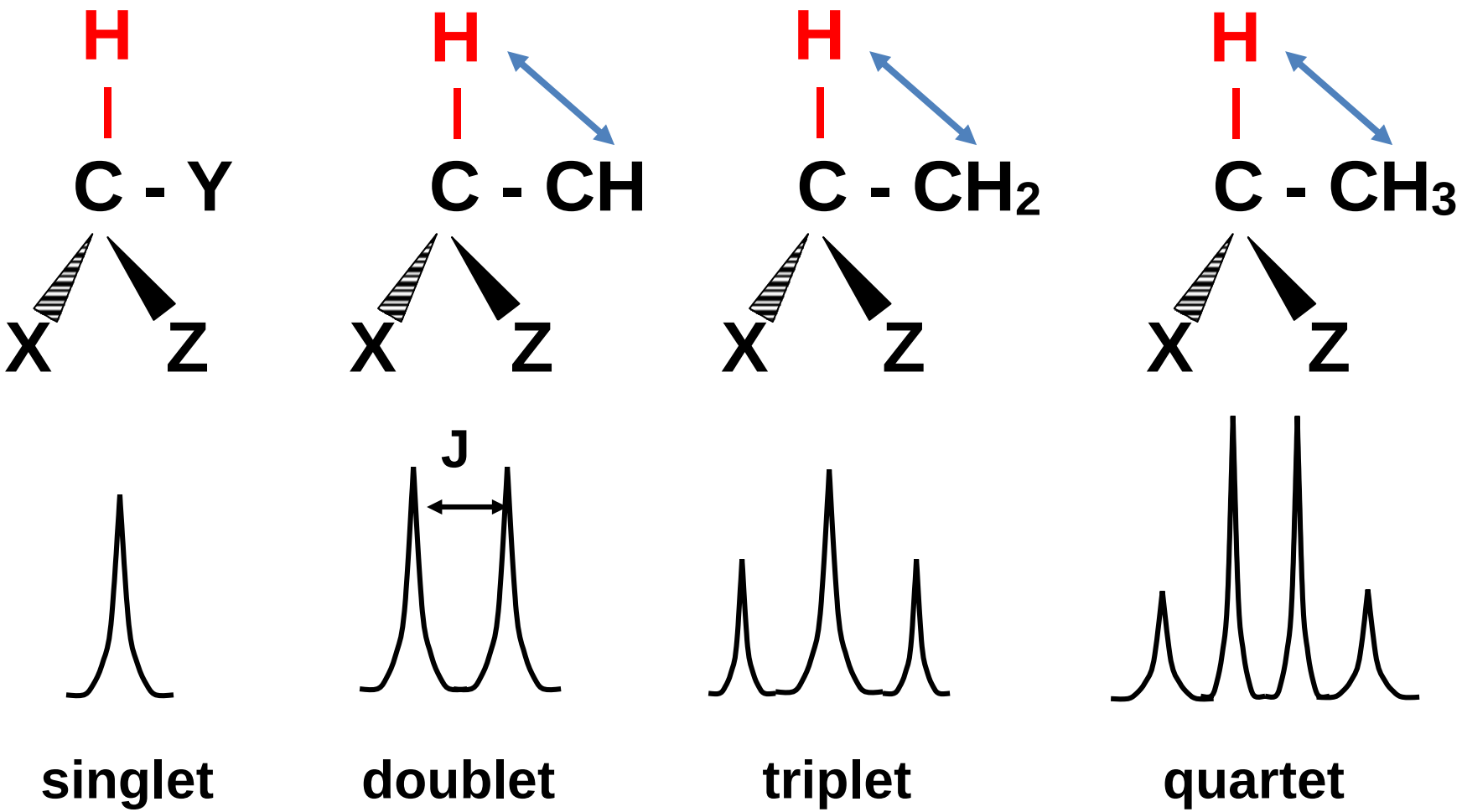
2) Protons in the <u>same group</u> usually do not split one another



or



Examples


H
C - Y
X
Z
H
C - CH
X
Z
H
C - CH2
X
Z
H
C - CH3
X
Z
J
singlet
doublet
triplet
quartet

Intensities

1

1 1

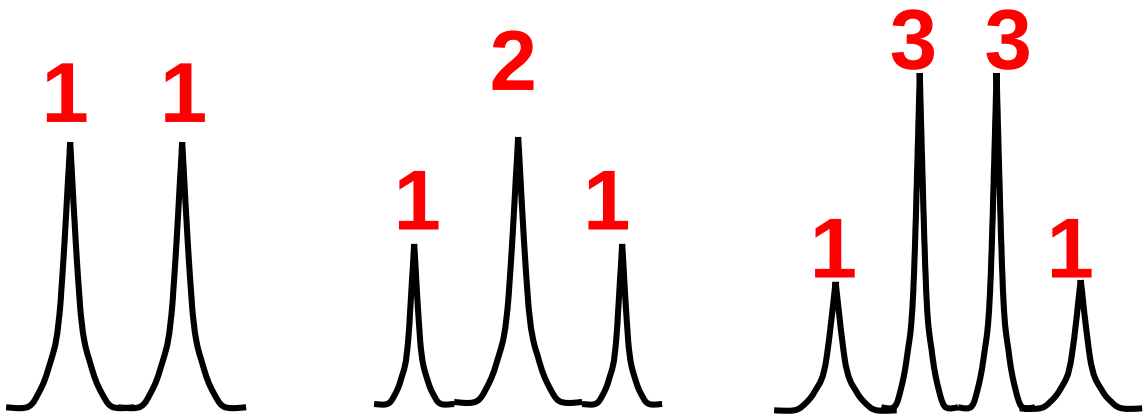
1 2 1

1 3 3 1

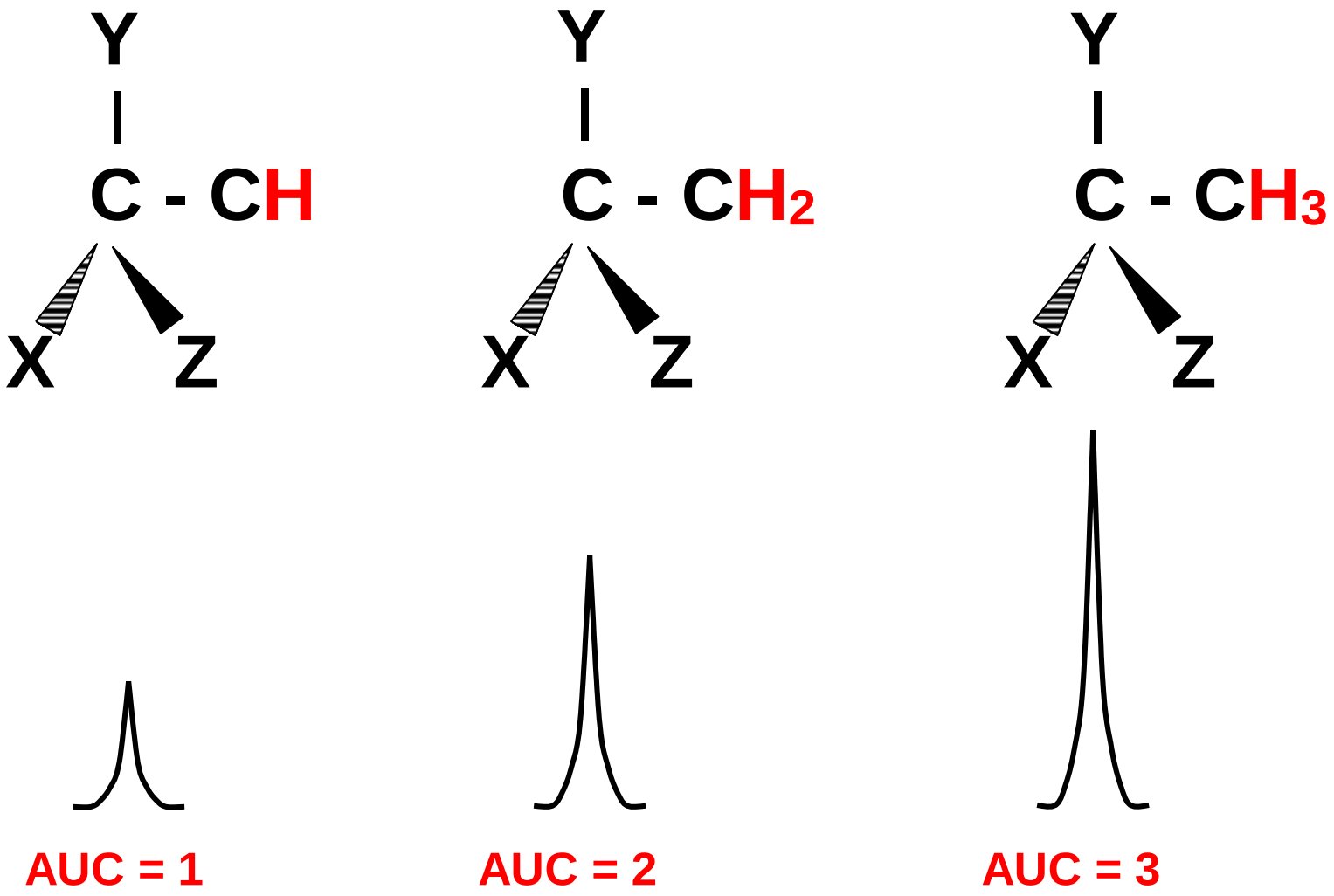
1 4 6 4 1

1 5 10 10 5 1

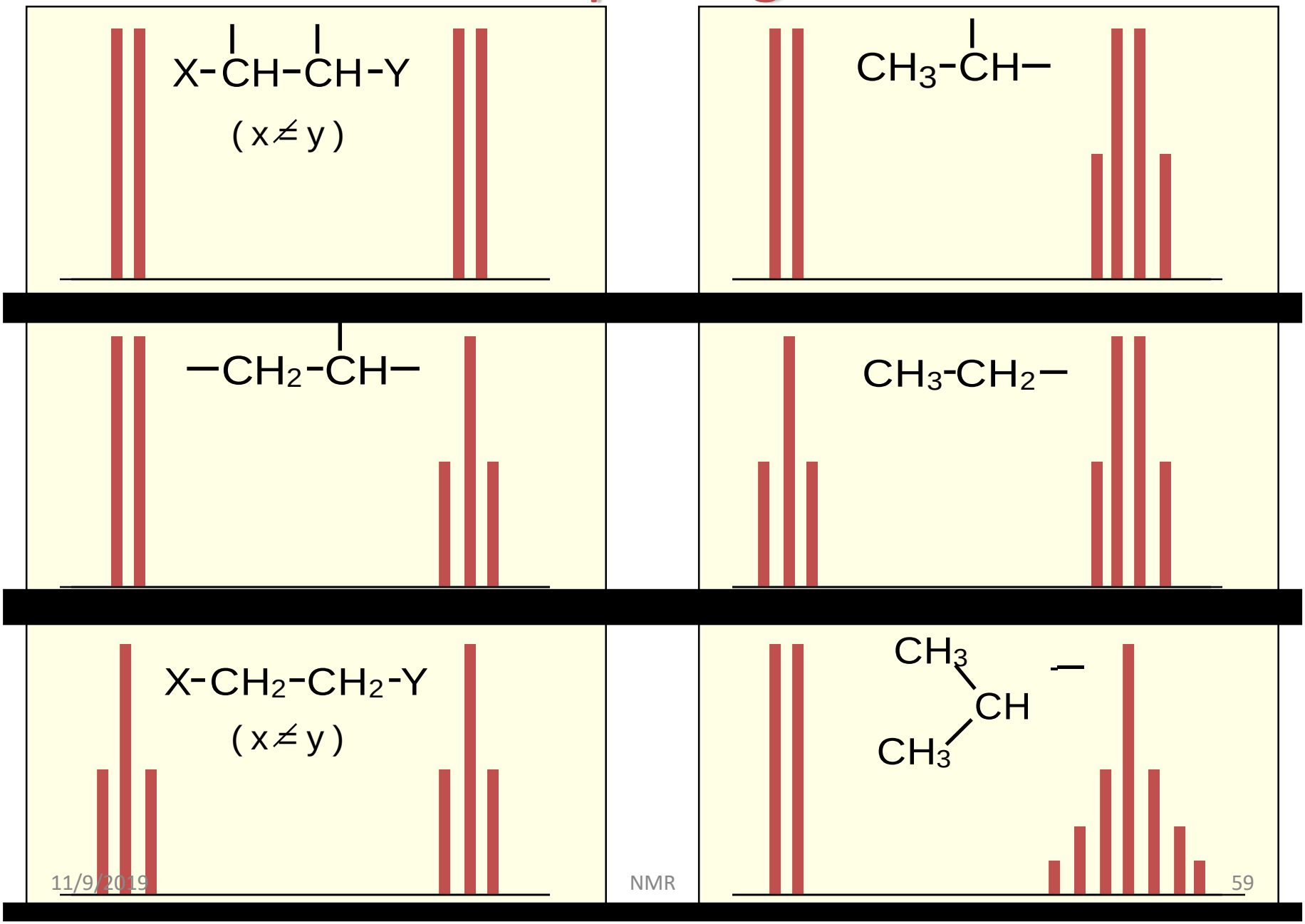
Pascal's Triangle



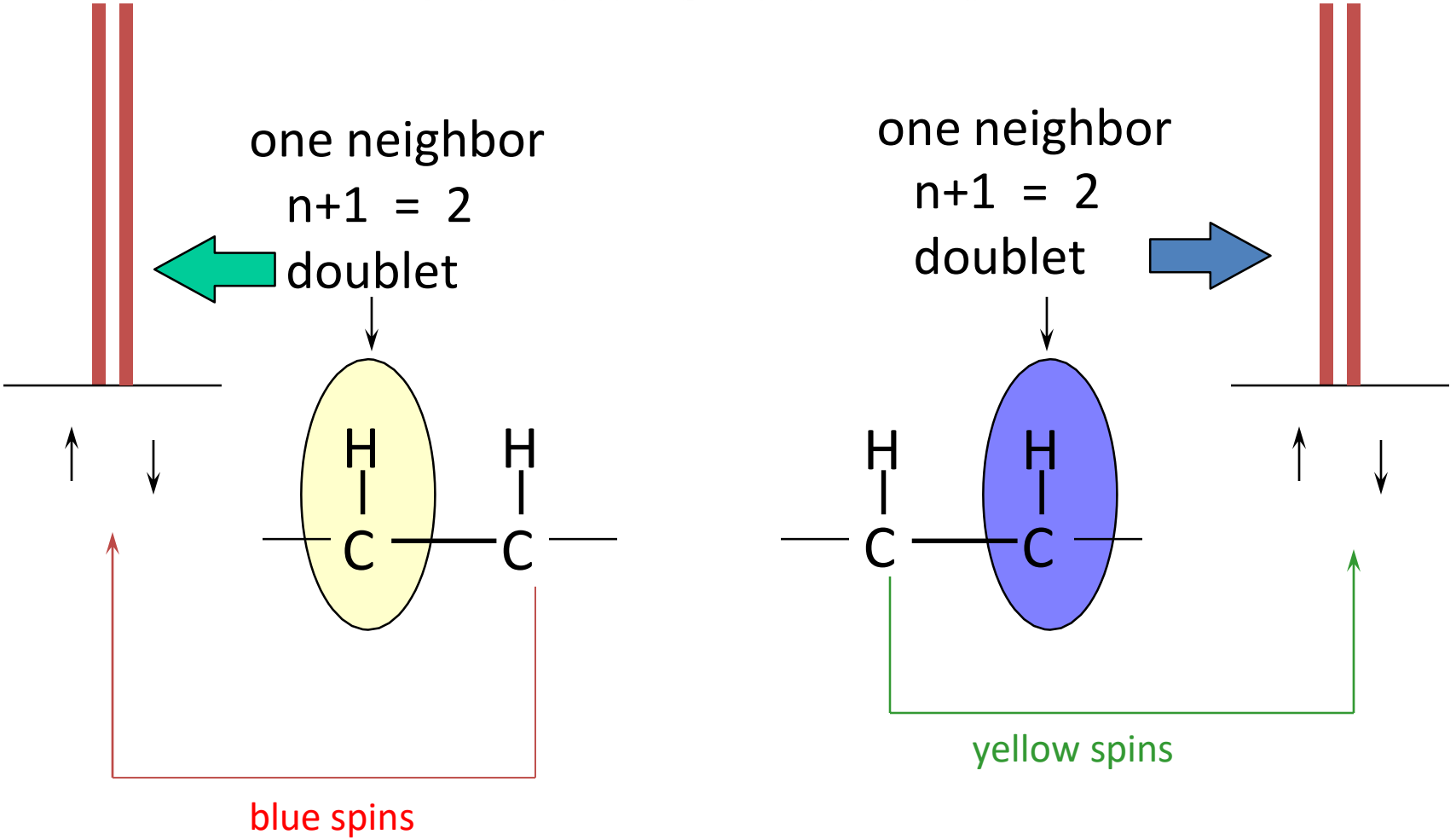
NMR Peak Intensities



Some Common Splitting Patterns

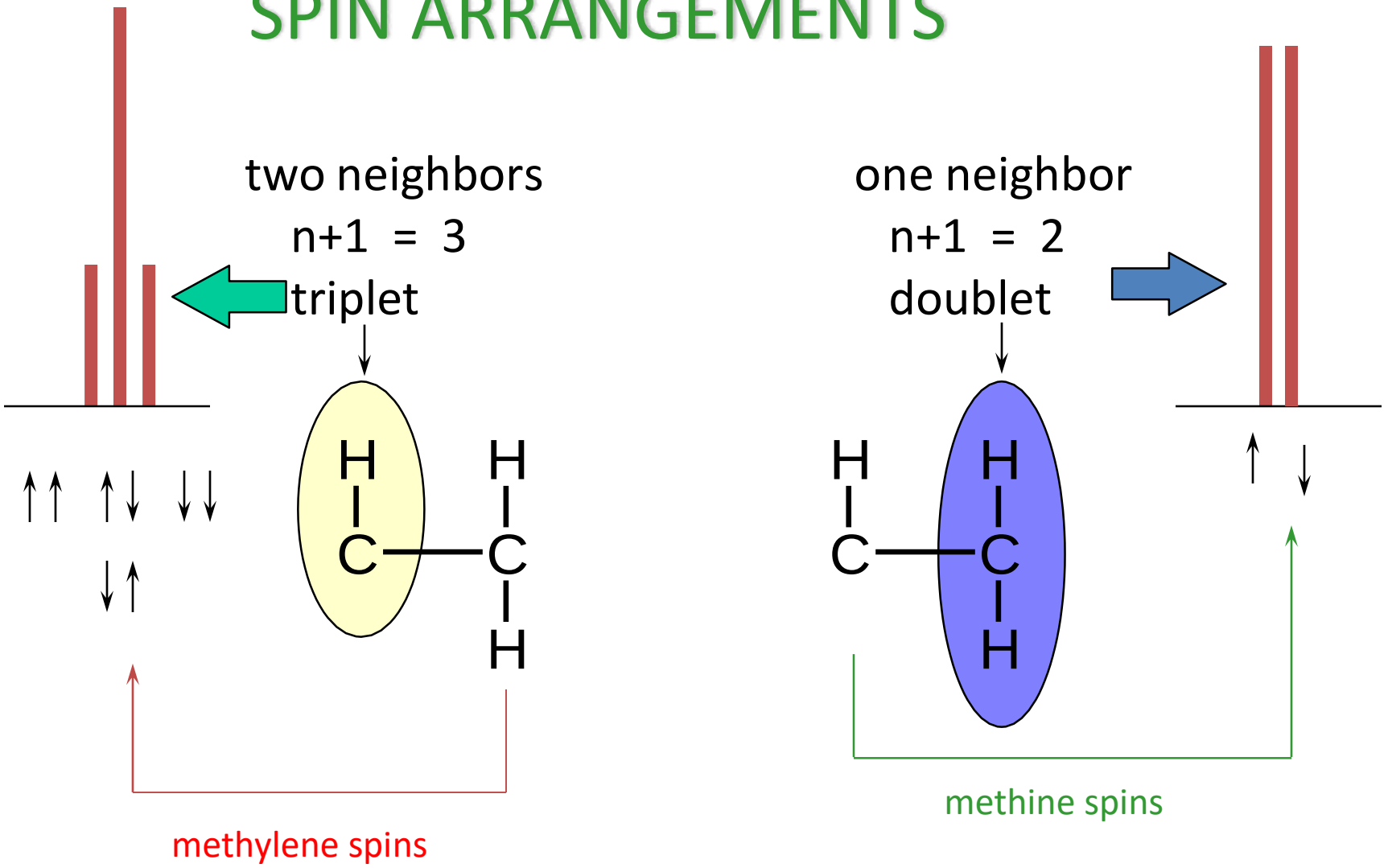


SPIN ARRANGEMENTS

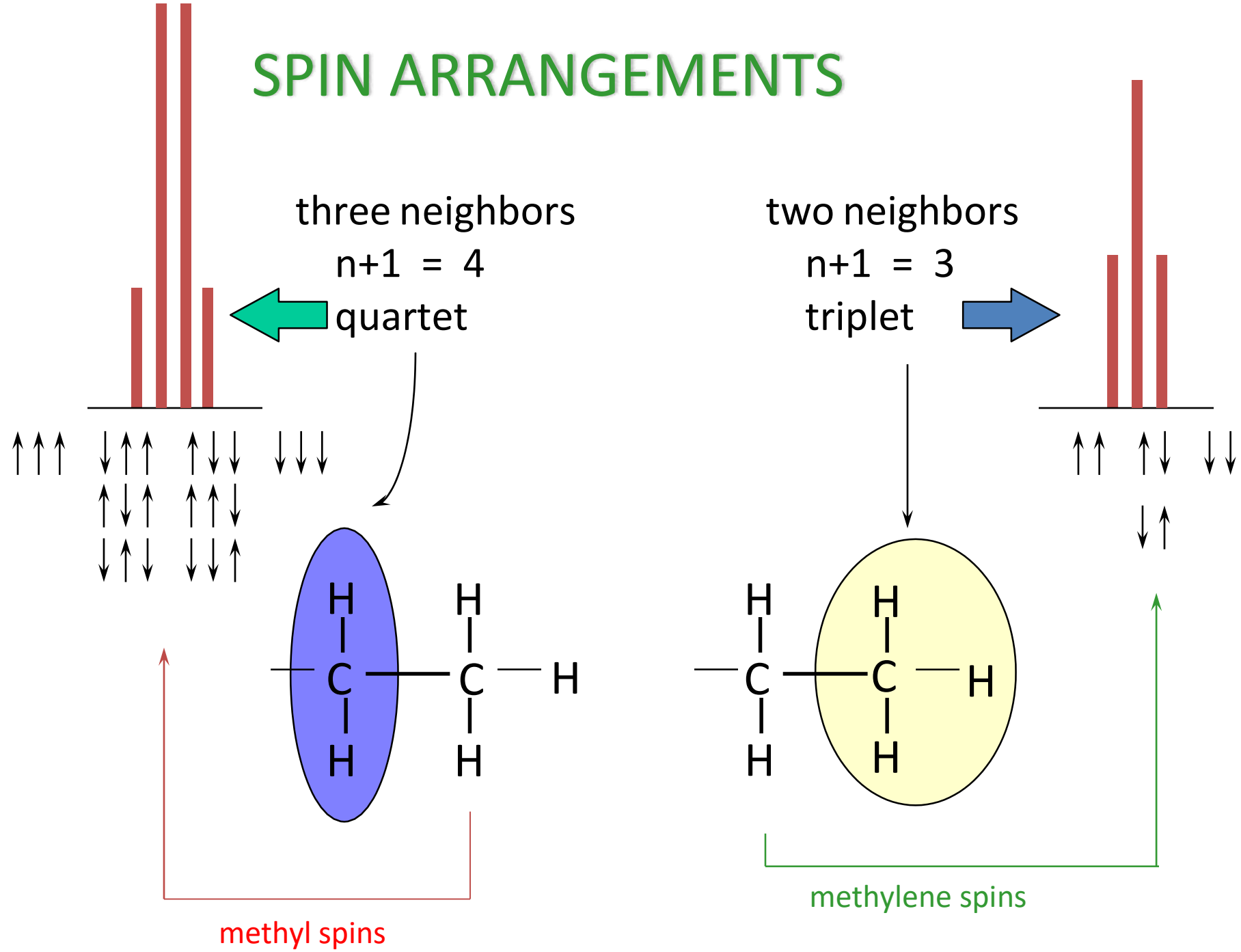


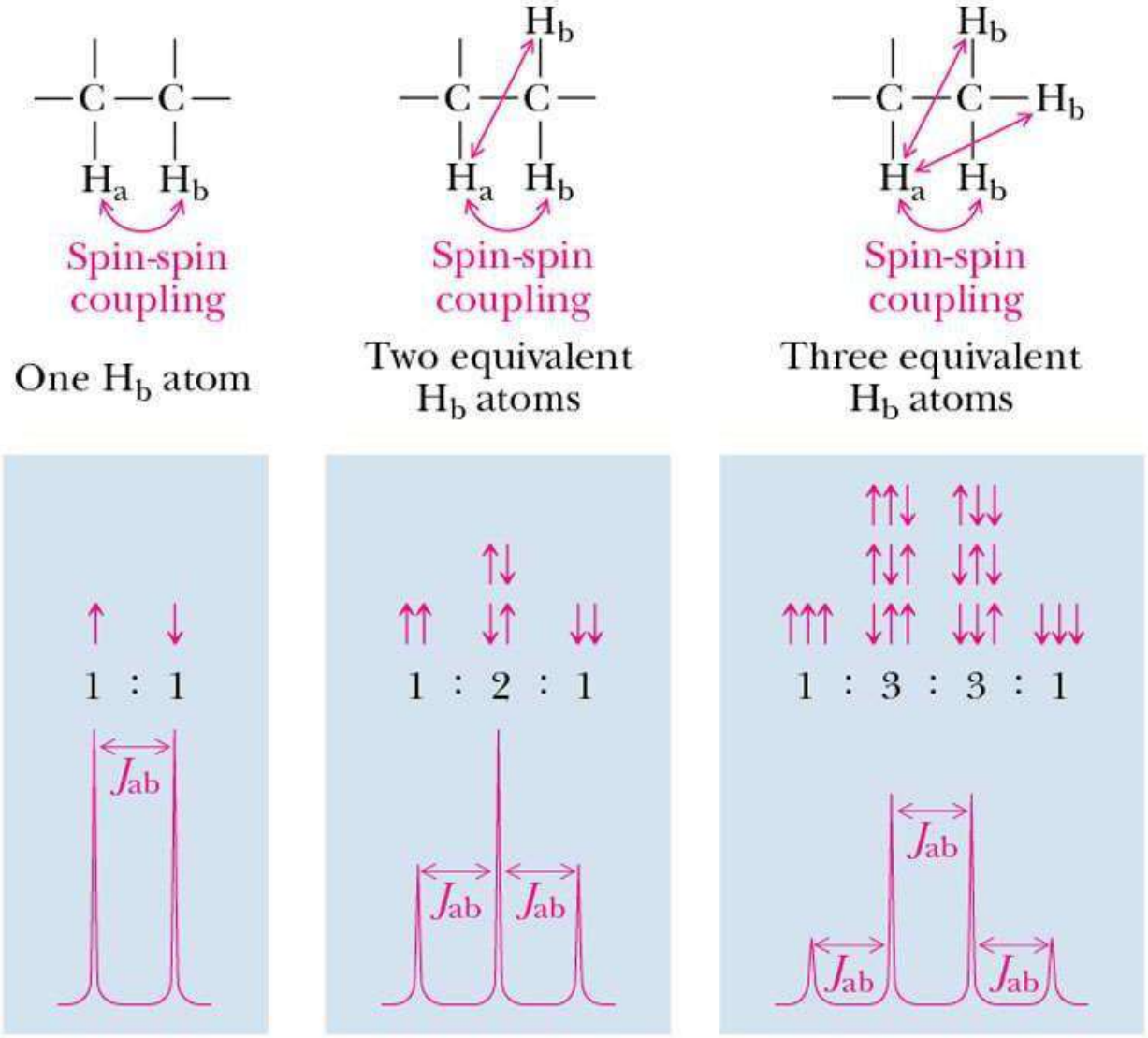
The resonance positions (splitting) of a given hydrogen is affected by the possible spins of its neighbor.

SPIN ARRANGEMENTS



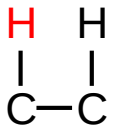
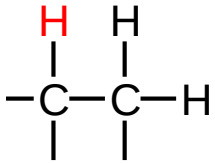
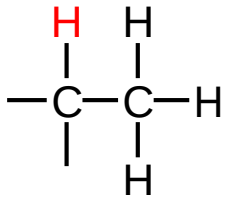
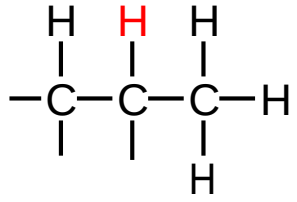
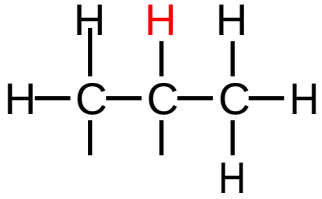
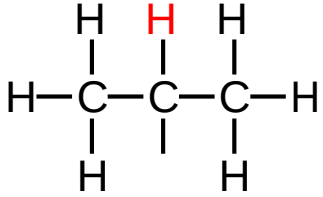
SPIN ARRANGEMENTS





Observed splitting in signal of H_a

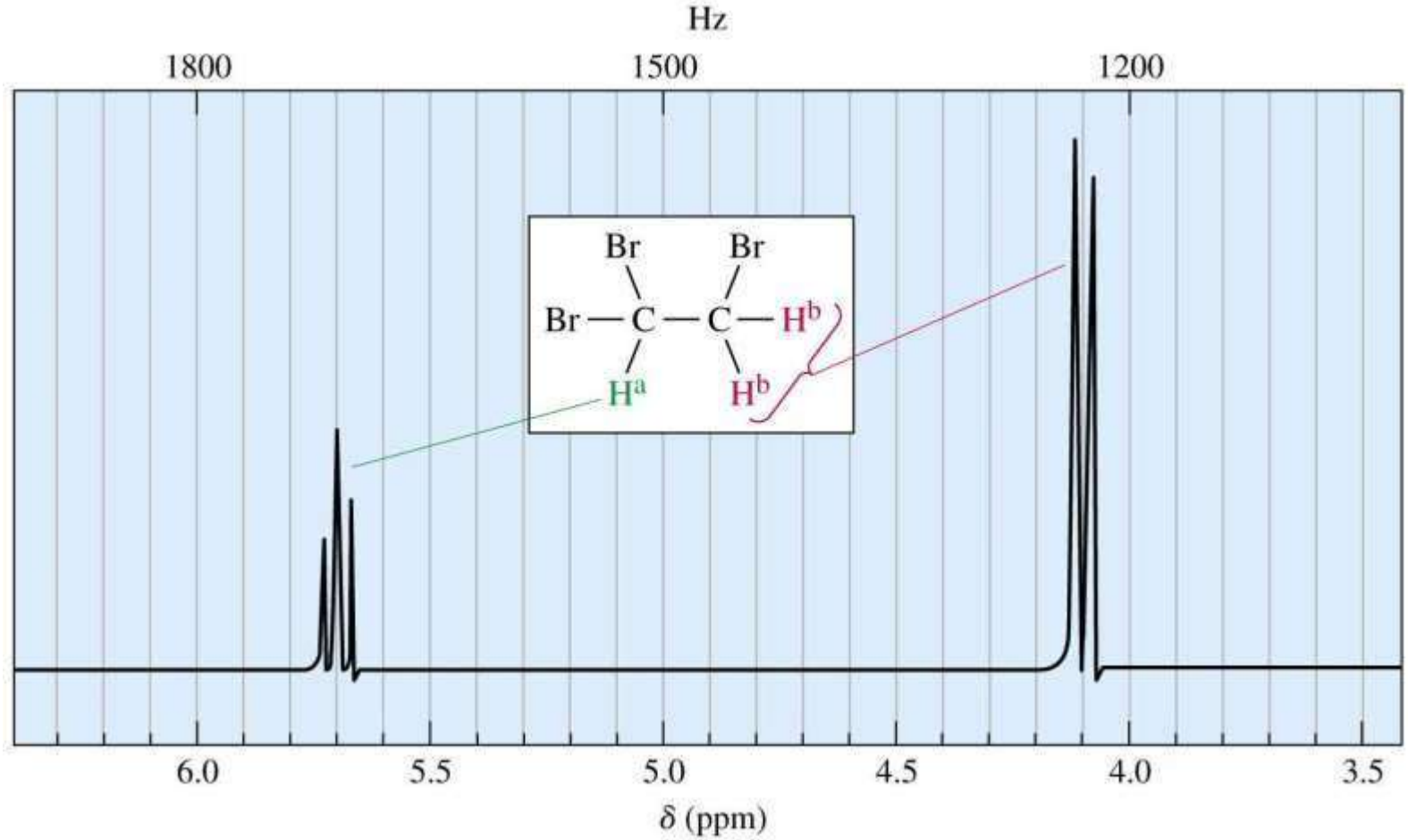
© 2006 Brooks/Cole - Thomson

no. of neighbors	relative intensities	pattern	example
0	1	singlet (s)	
1	1 1	doublet (d)	
2	1 2 1	triplet (t)	
3	1 3 3 1	quartet (q)	
4	1 4 6 4 1	pentet	
5	1 5 10 10 5 1	sextet	
6	1 6 15 20 15 6 1	septet	

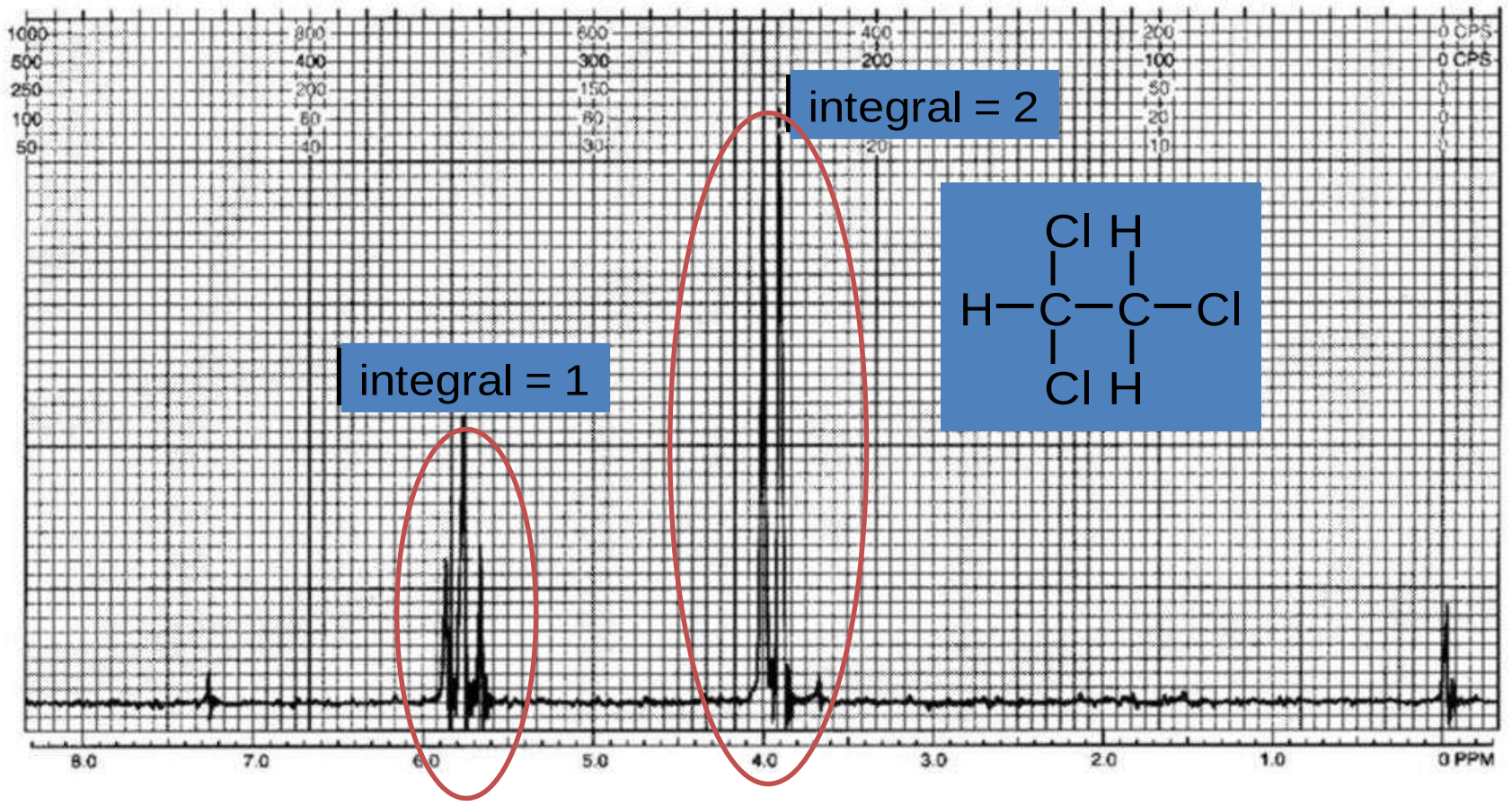
Relative Peak Intensities of Symmetric Multiplets

Number of Equivalent Protons Causing Splitting	*Number of Peaks (multiplicity)*	*Area Ratios (Pascal's triangle)*
0	1 (singlet)	1
1	2(doublet)	1 1
2	3 (triplet)	1 2 1
3	4 (quartet)	1 3 3 1
4	5 (quintet)	1 4 6 4 1
5	6 (sextet)	1 5 10 10 5 1
6	7 (septet)	1 6 15 20 15 6 1

1,1,2-Tribromoethane



1,1,2-Trichloroethane

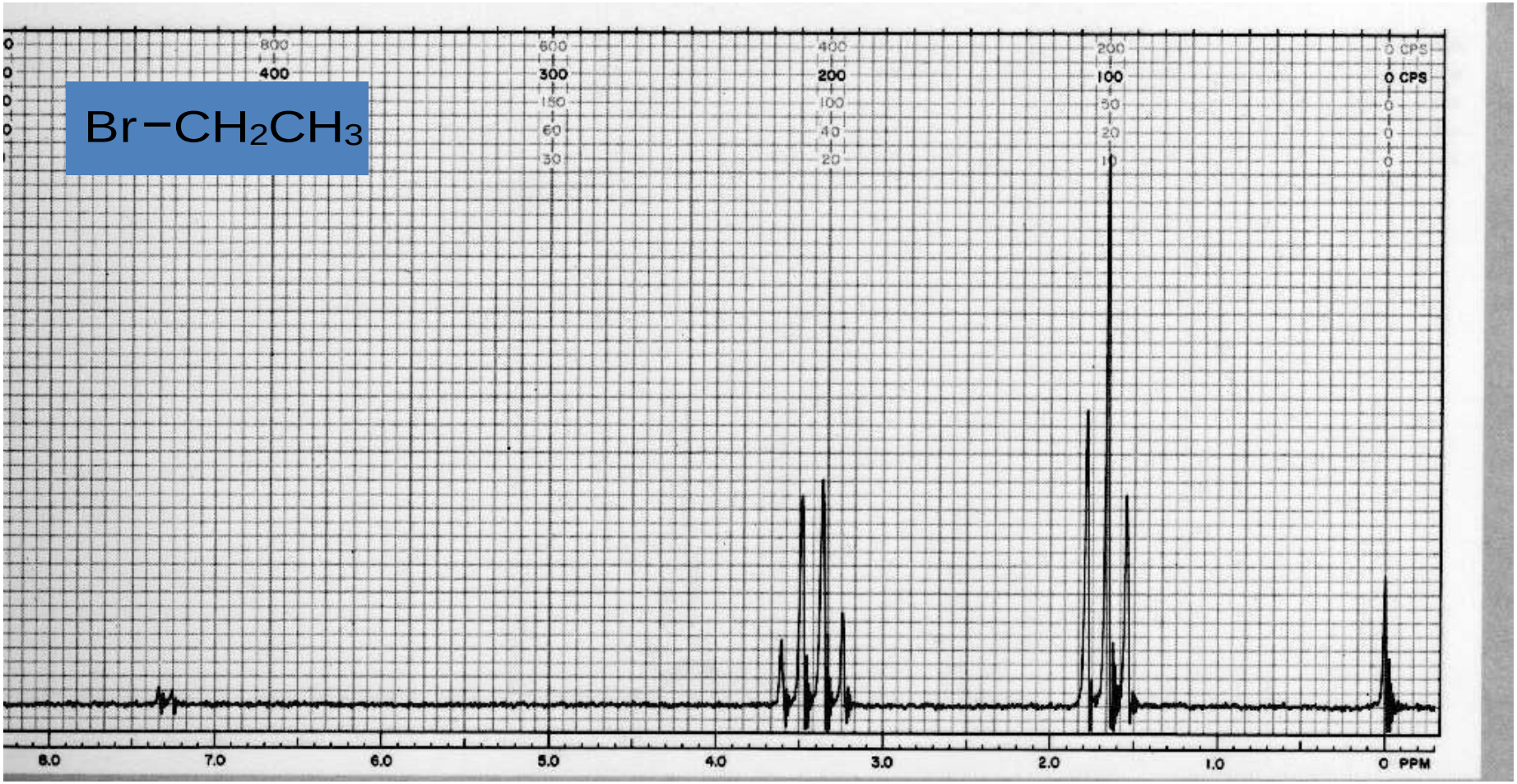


triplet

doublet

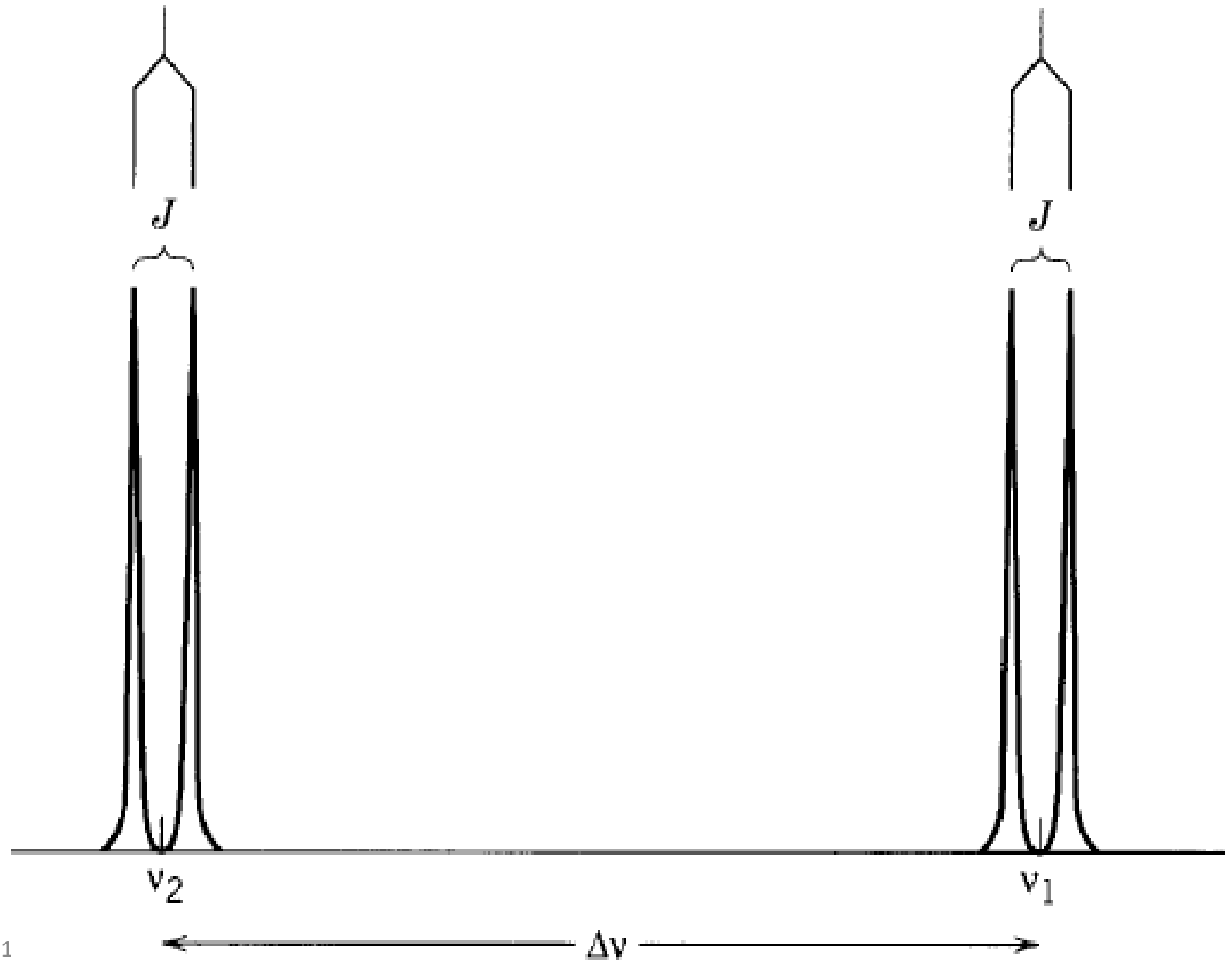
The subpeaks are due to spin-spin splitting and are predicted by the n+1 rule.

NMR Spectrum of Bromoethane



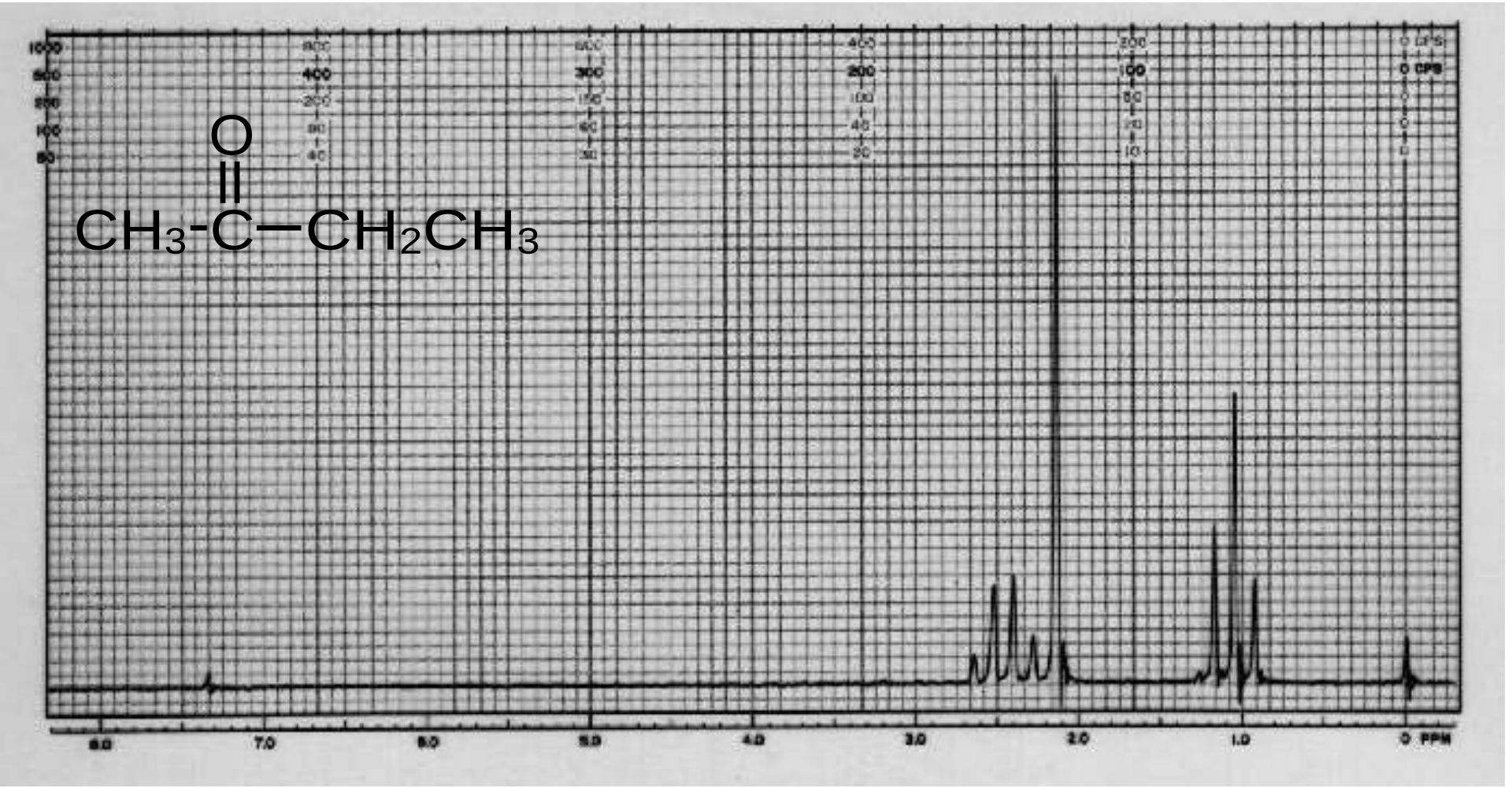
Coupling Constant(J)

- Distance between the peaks of multiplet
- Measured in Hz (0-20 Hz for PMR)
- Not dependent on strength of the external field
- For symmetrical peaks $\Delta\nu / J > 8$

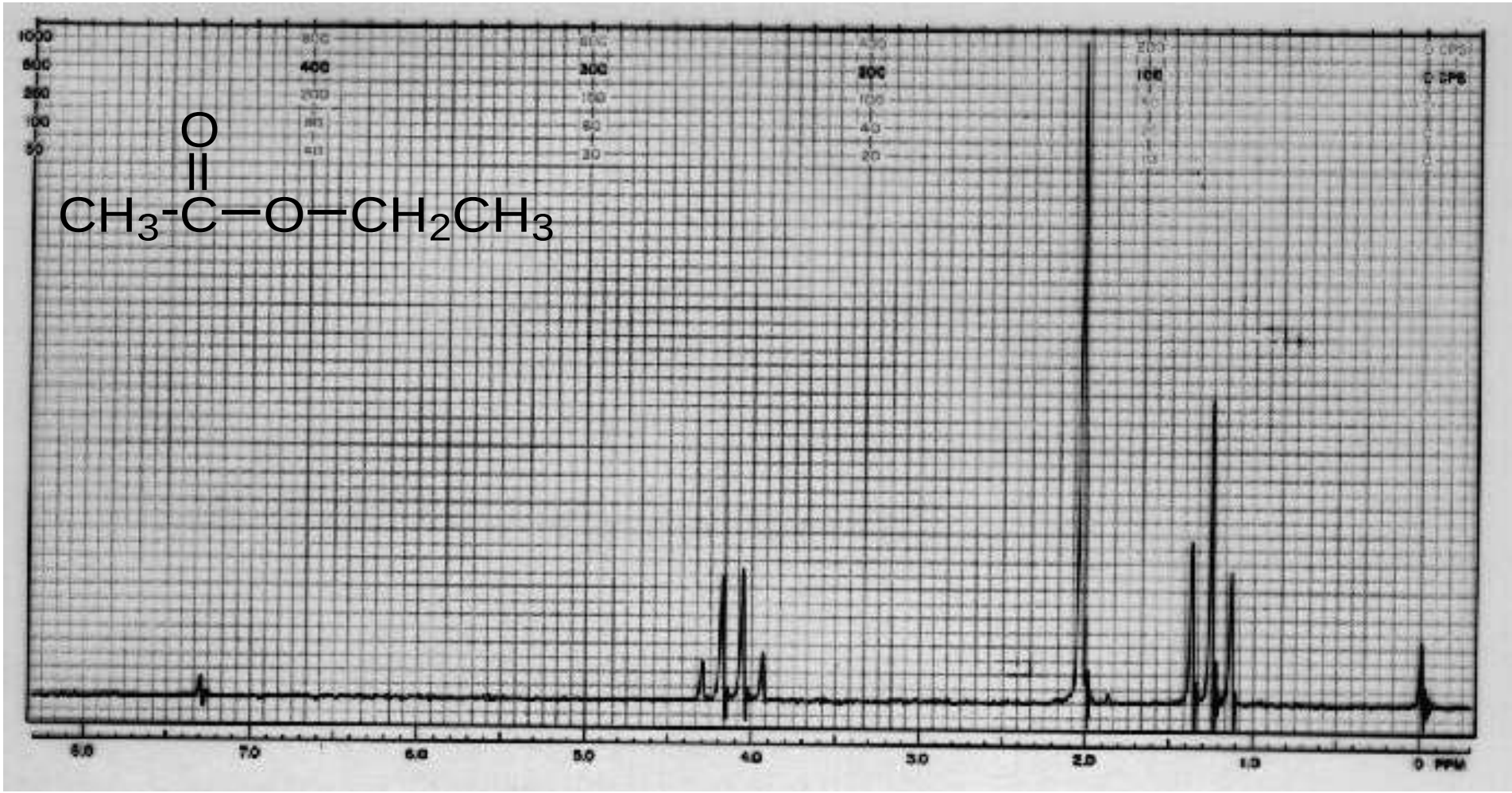

J
J
ν2
ν1
Δν

Example spectra for discussion

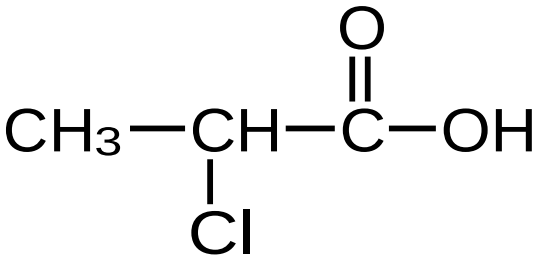
Methyl Ethyl Ketone

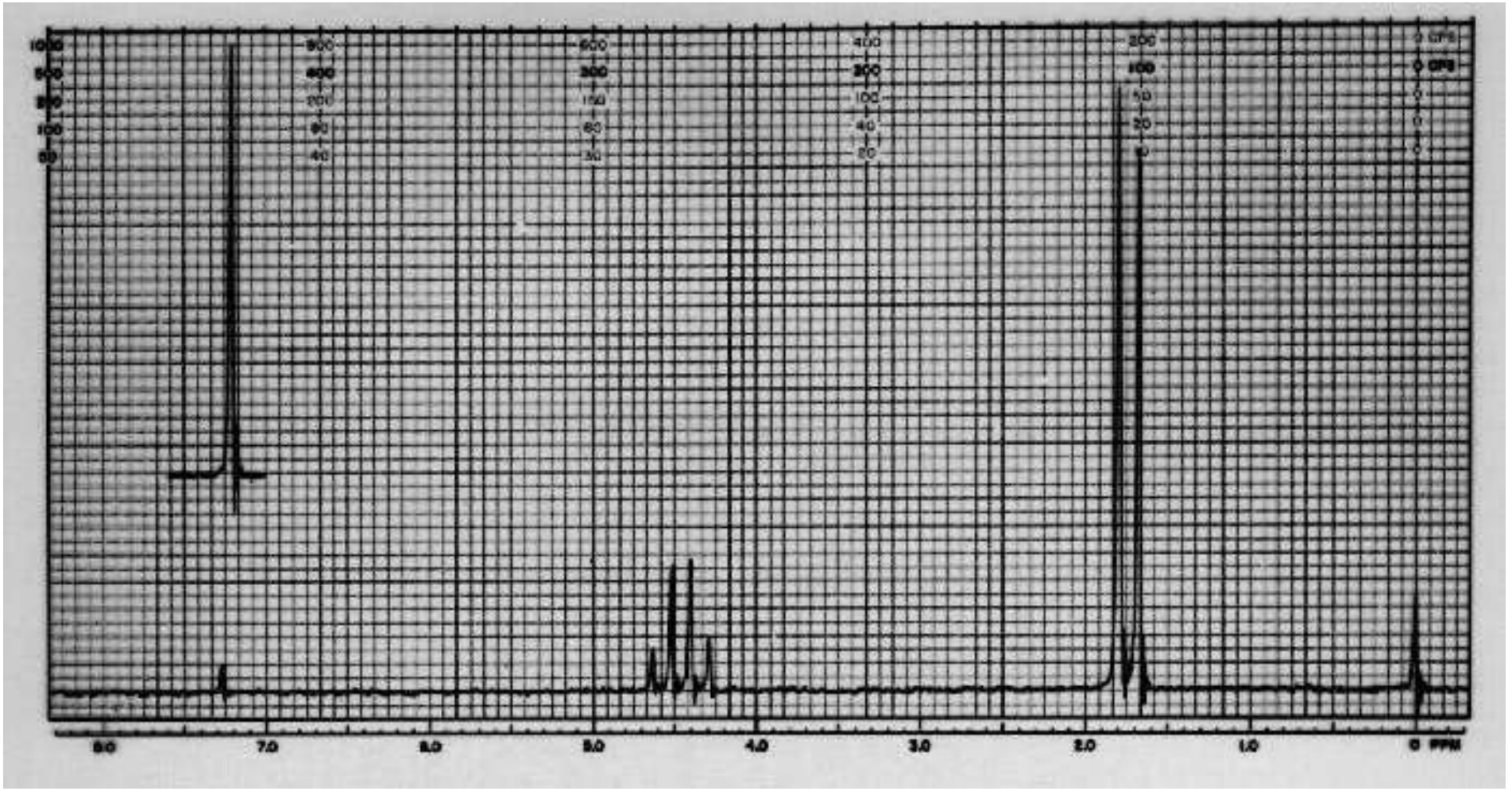


Ethyl Acetate

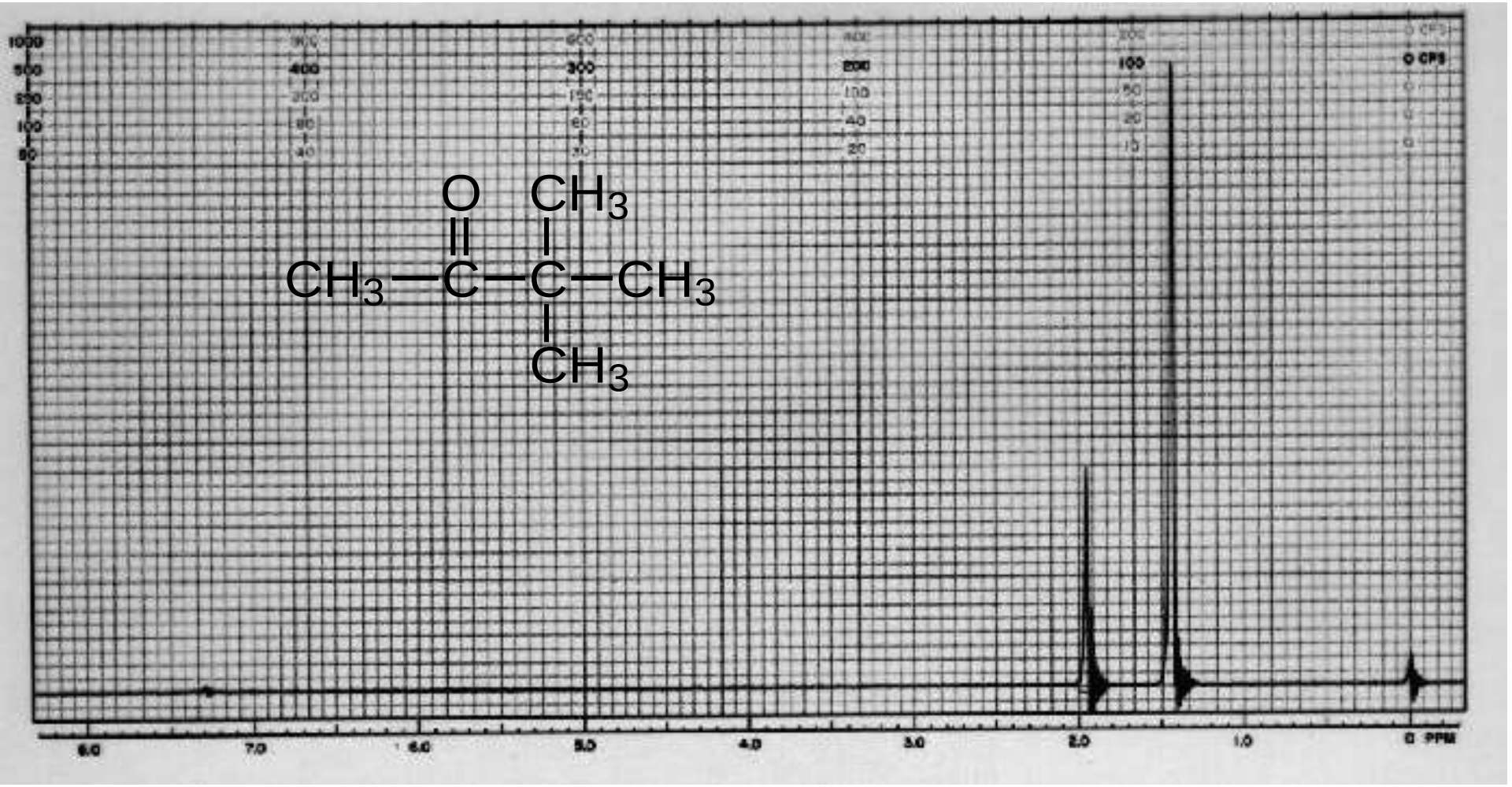


α-Chloropropionic Acid

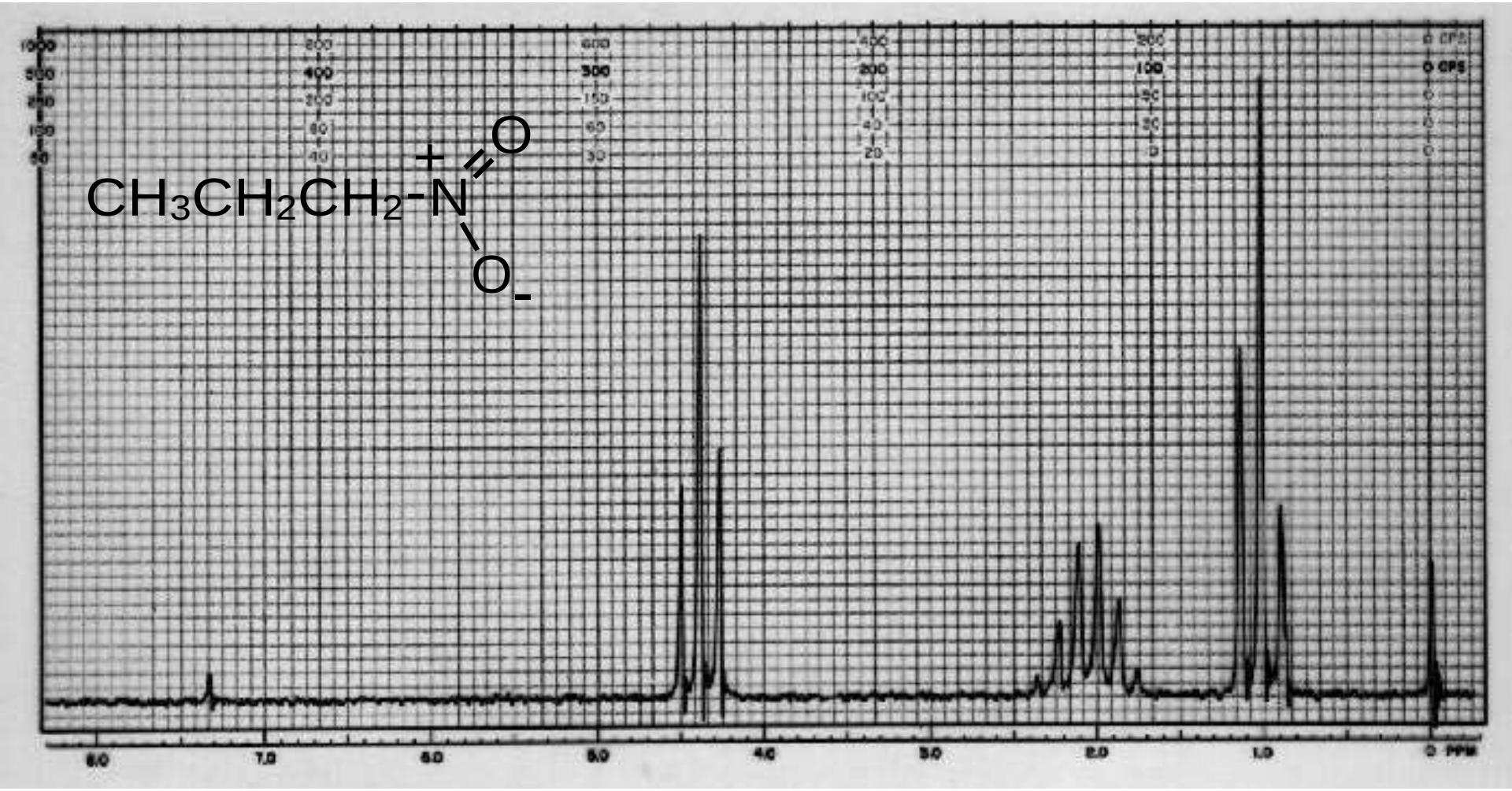




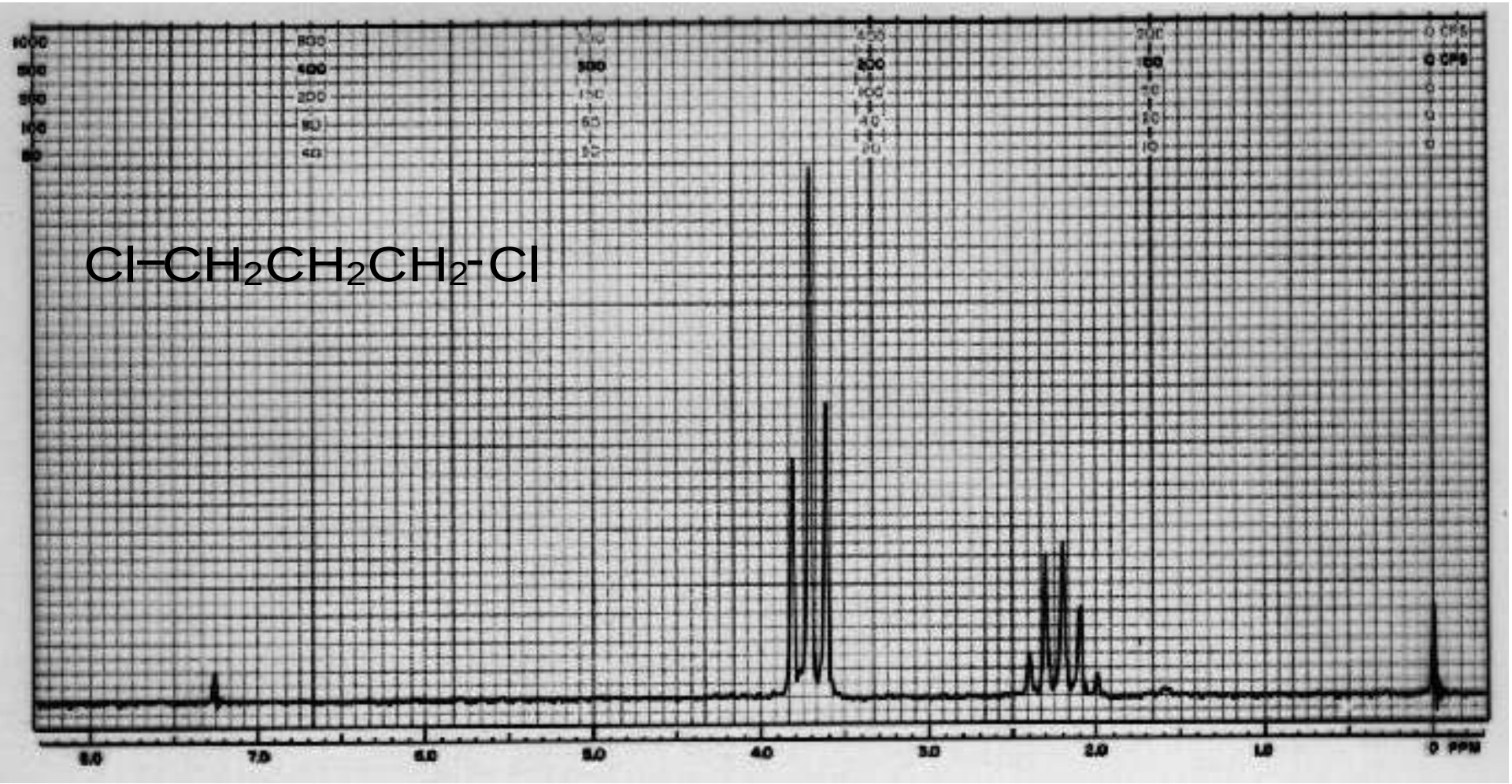
t-Butyl Methyl Ketone
(3,3-dimethyl-2-butanone)



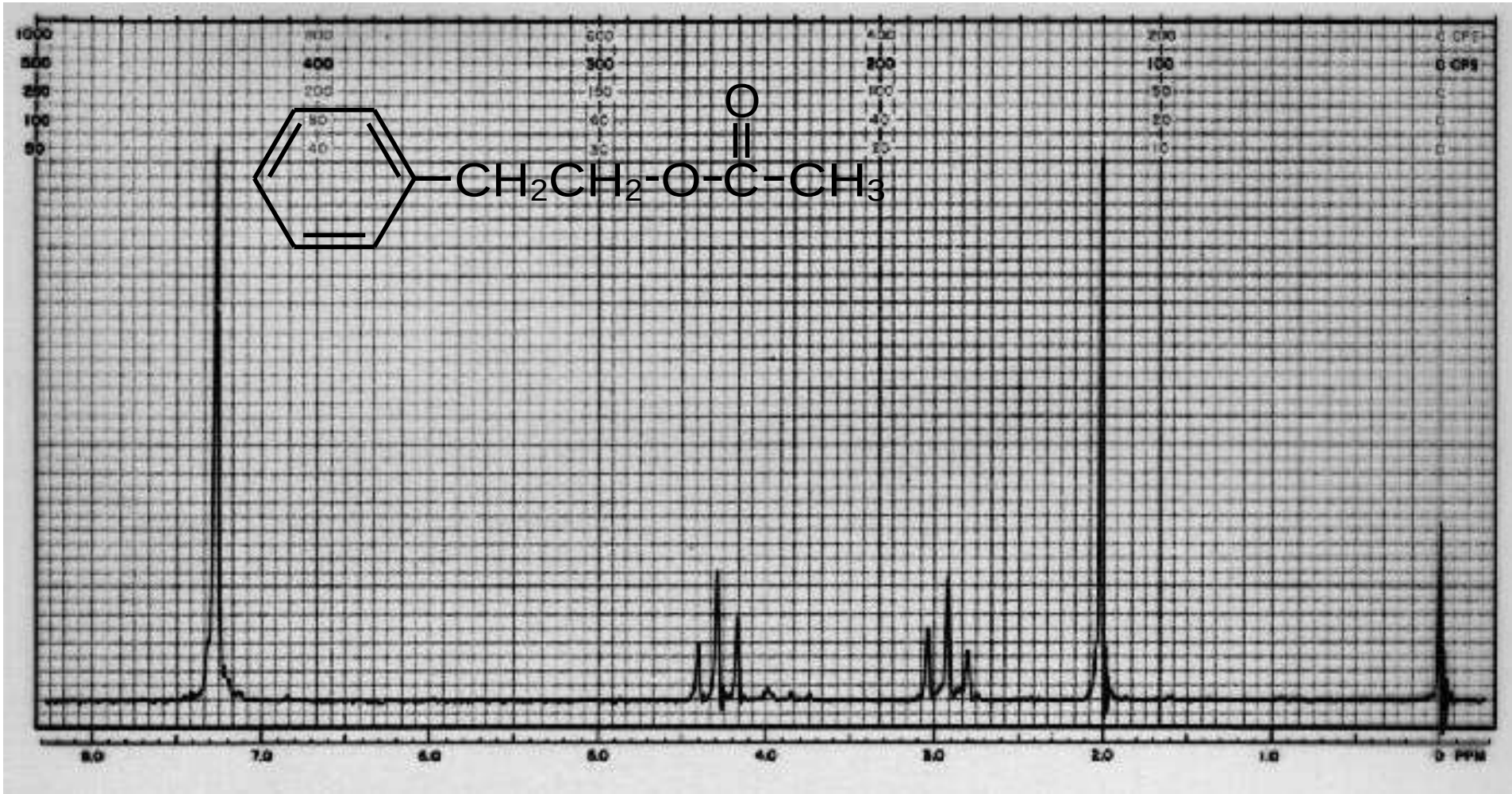
1-Nitropropane



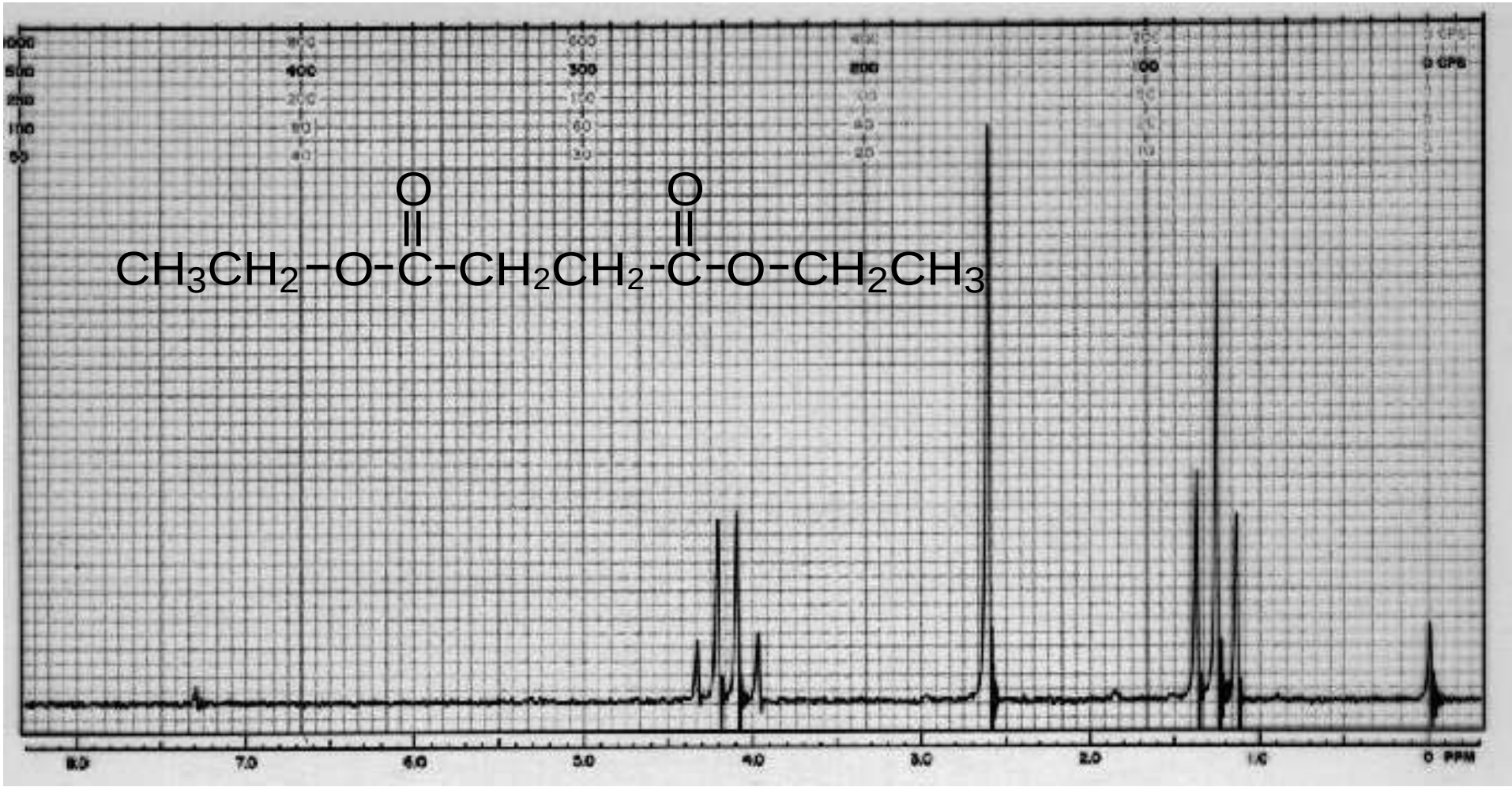
1,3-Dichloropropane



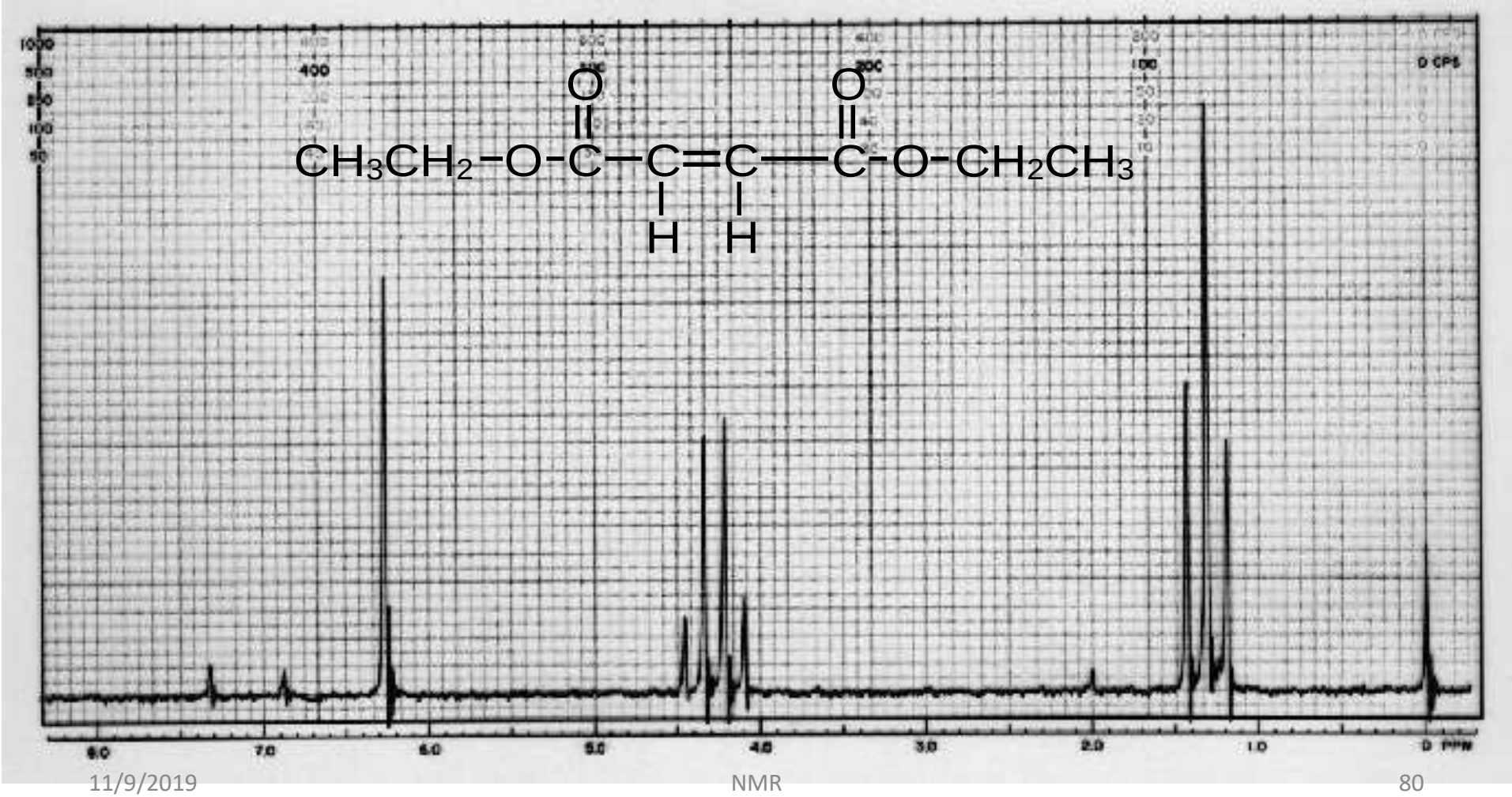
Phenylethyl Acetate



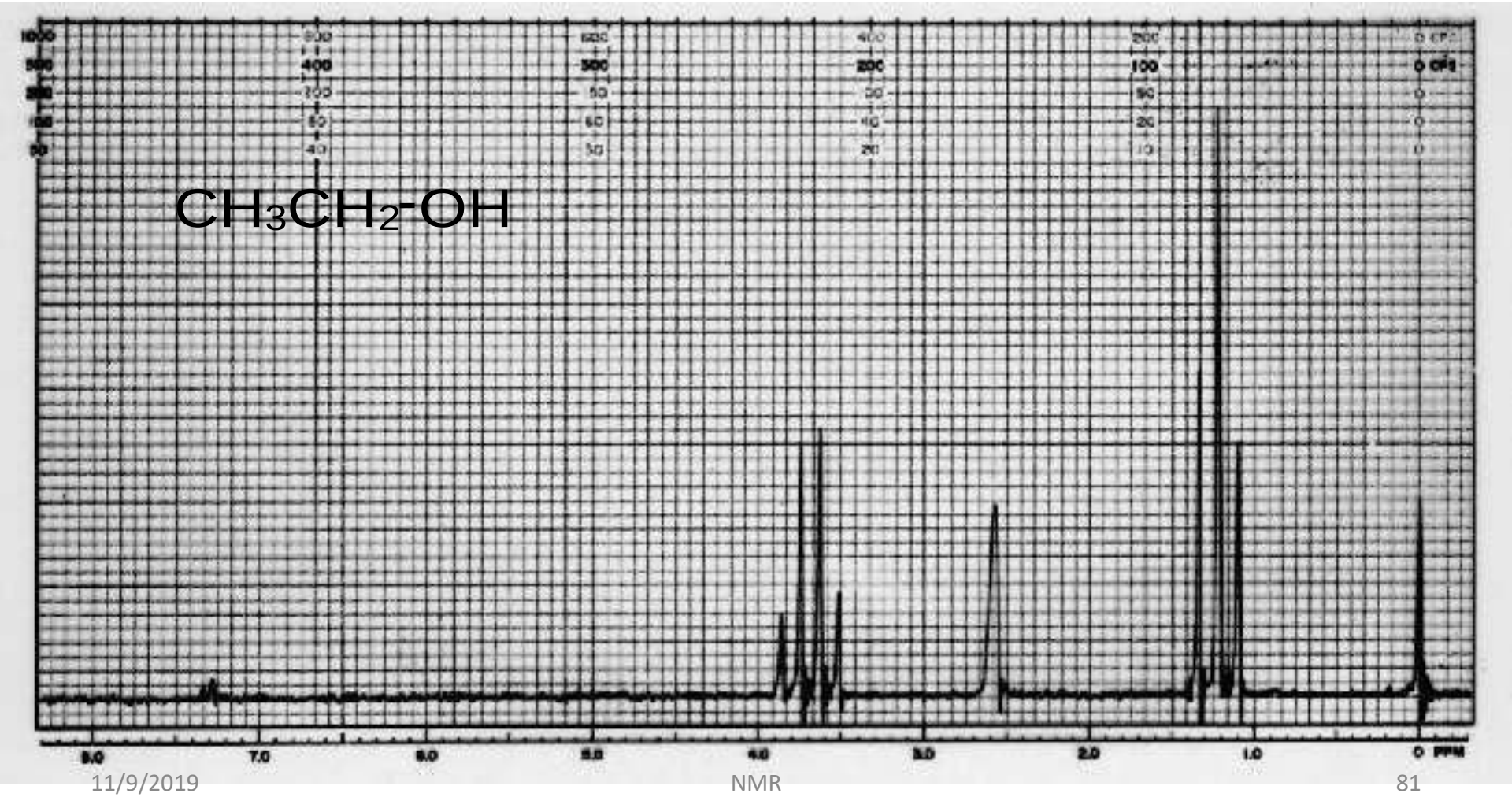
Ethyl Succinate



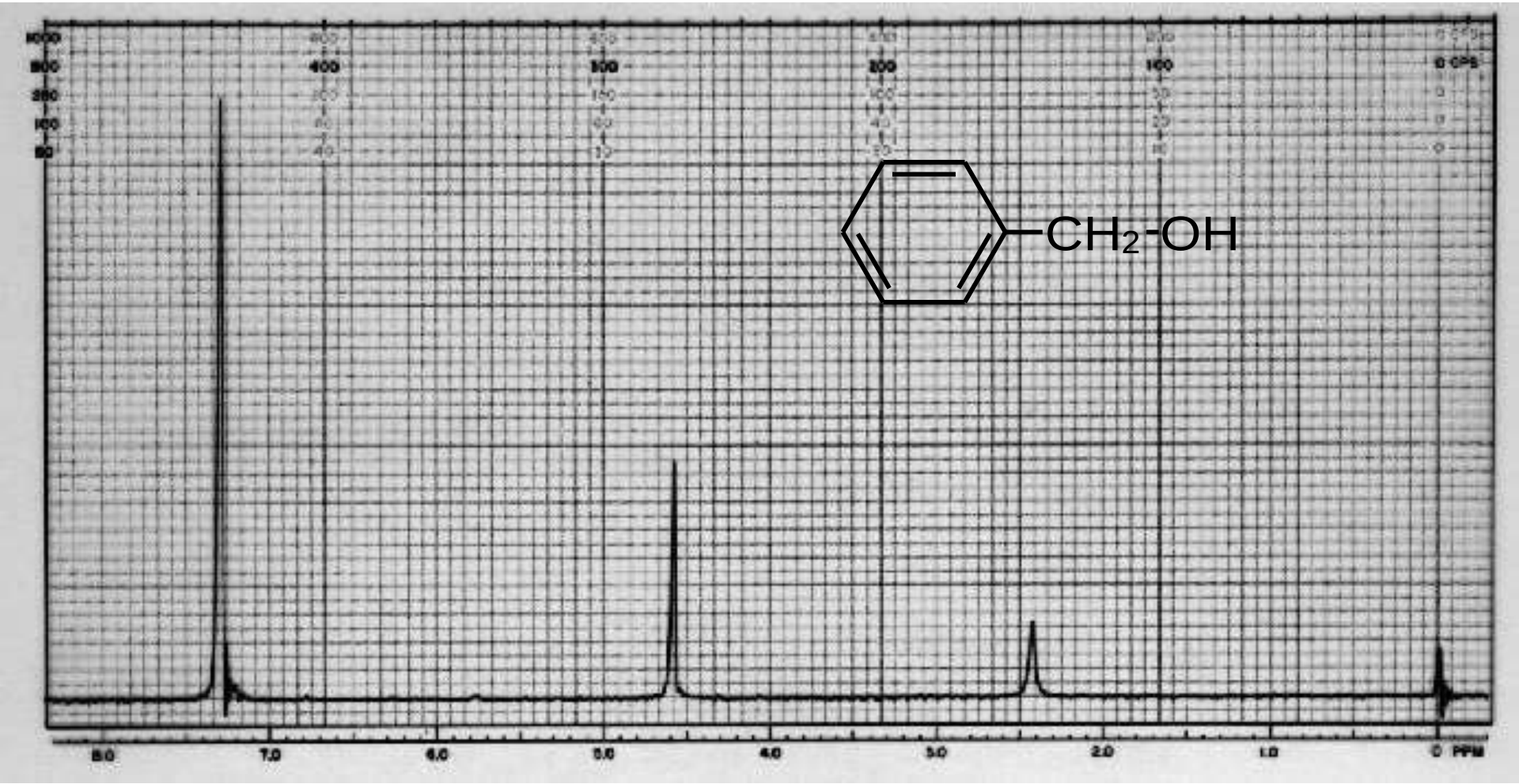
Diethyl Maleate



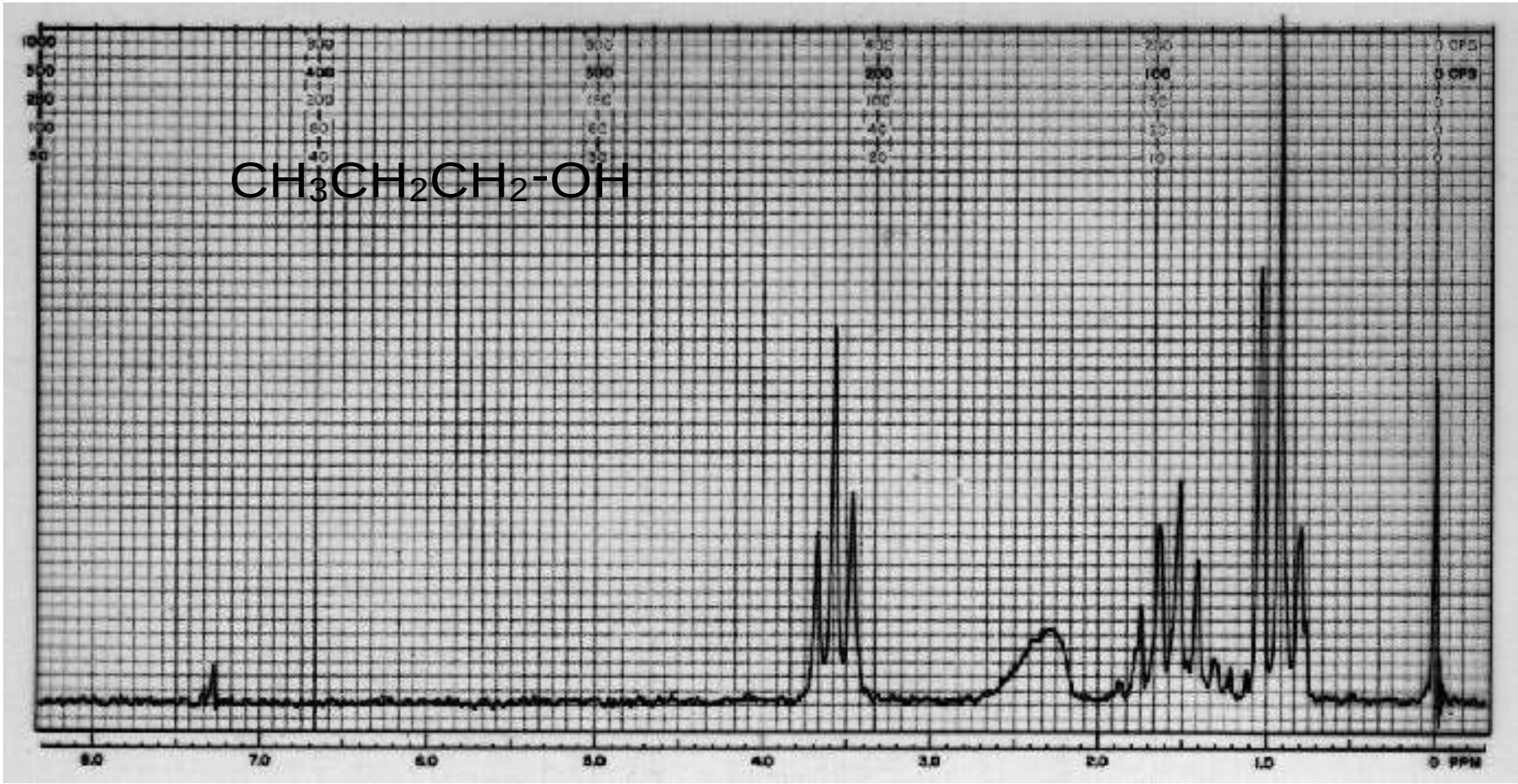
Ethanol



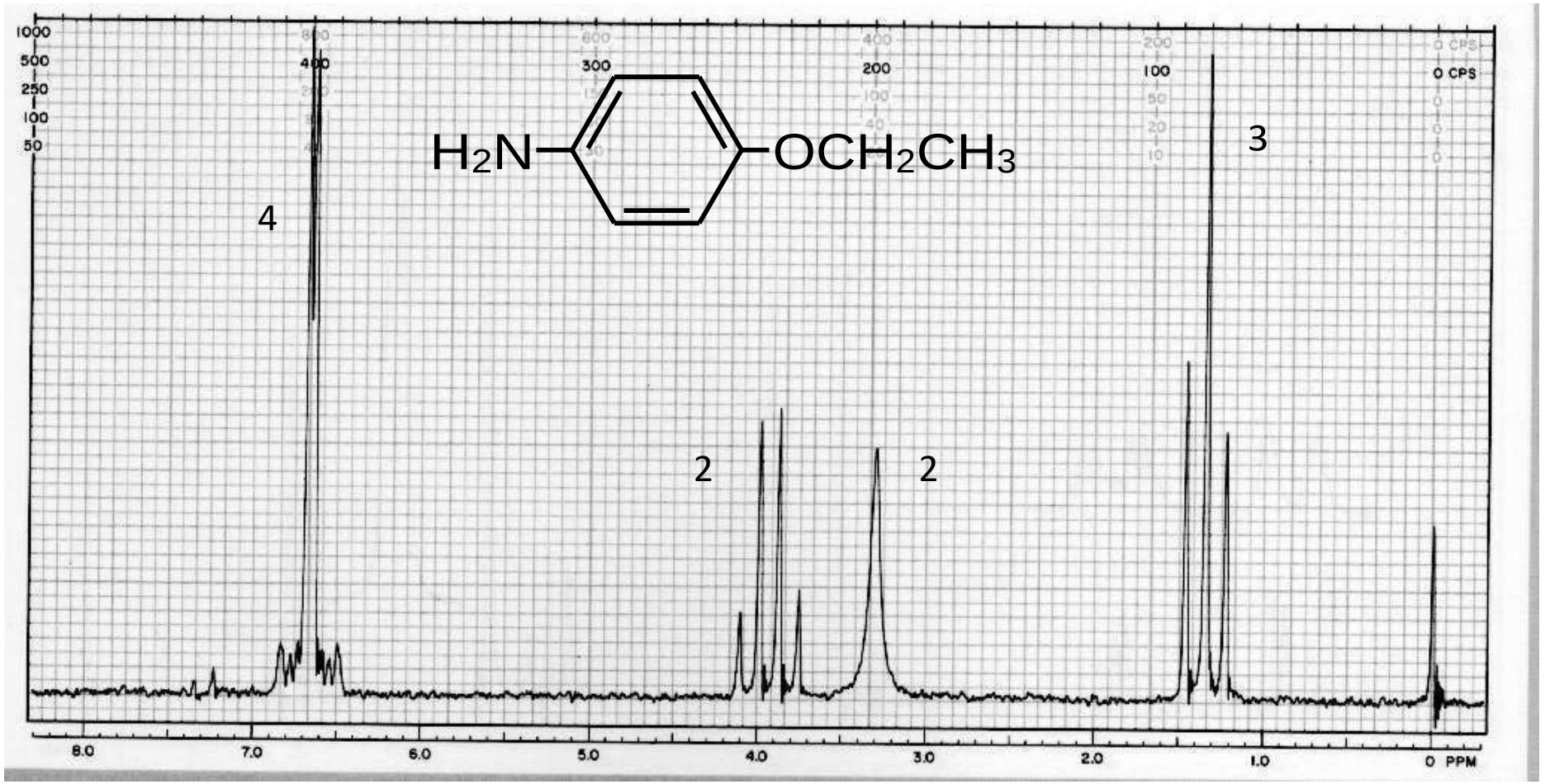
Benzyl Alcohol



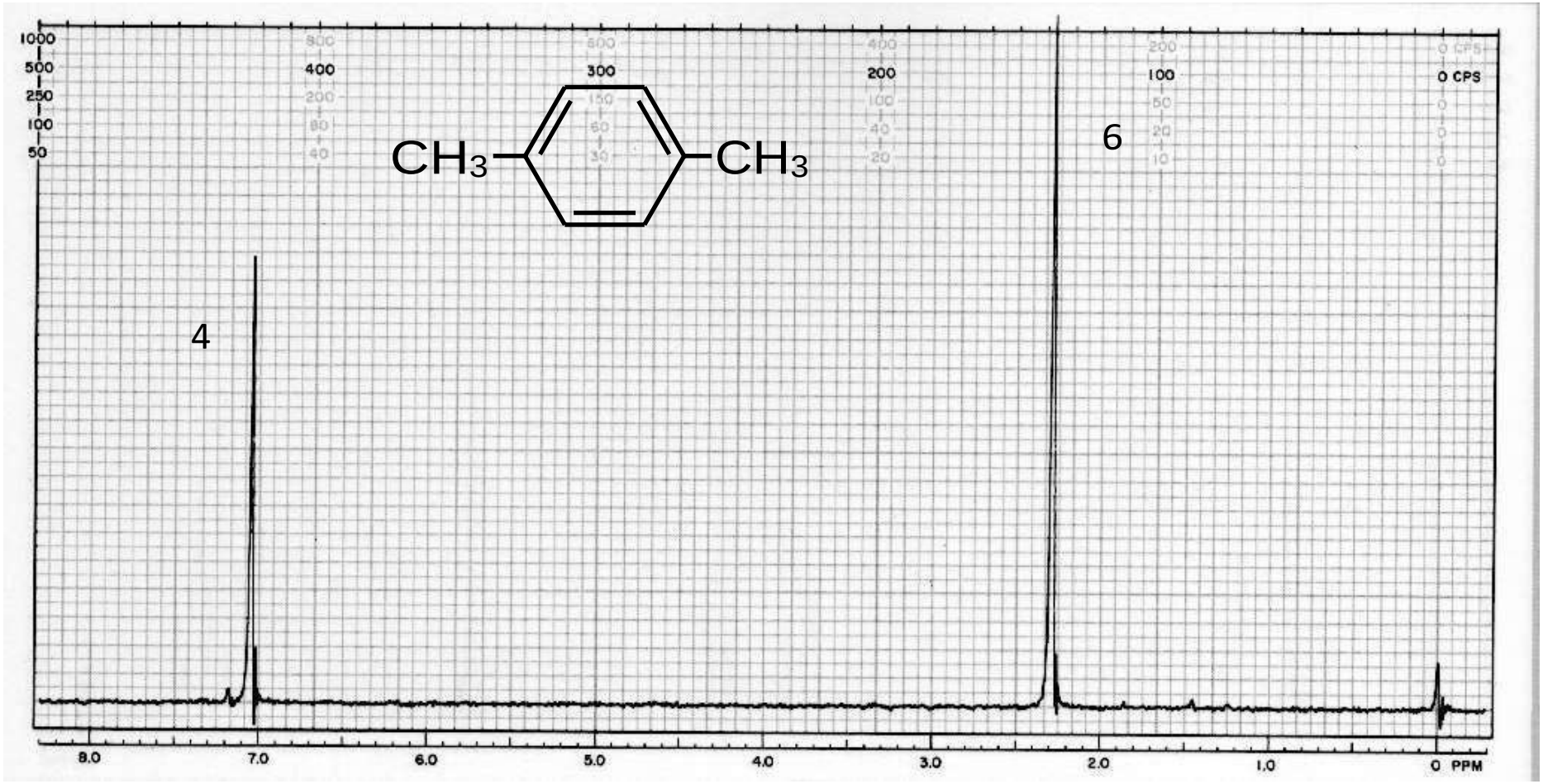
n-Propyl Alcohol



NMR Spectrum of 1-amino-4-ethoxybenzene

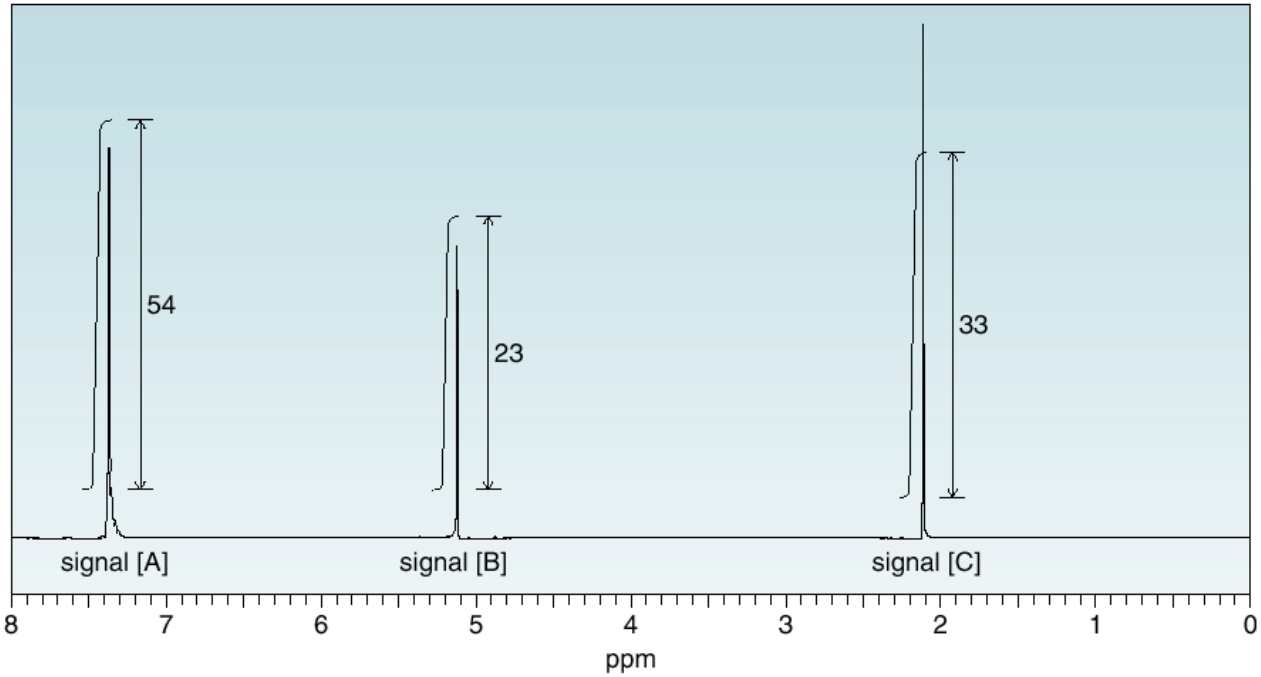


NMR Spectrum of *p*-Xylene (1,4-dimethylbenzene)



How To — Determine the Number of Protons Giving Rise to an NMR Signal

Example A compound of molecular formula $C_9H_{10}O_2$ gives the following integrated ^1H NMR spectrum. How many protons give rise to each signal?



Step [1] **Determine the number of integration units per proton by dividing the total number of integration units by the total number of protons.**

- Total number of integration units: 54 + 23 + 33 = 110 units
- Total number of protons = 10
- Divide: 110 units/10 protons = **11 units per proton**

Step [2] **Determine the number of protons giving rise to each signal.**

- To determine the number of H atoms giving rise to each signal, divide each integration value by the answer of Step [1] and round to the nearest whole number.

Signal [A]:				Signal [B]:				Signal [C]:		
Answer: $\frac{54}{11}$	= 4.9 ≈	**5 H**		$\frac{23}{11}$	= 2.1 ≈	**2 H**		$\frac{33}{11}$	=	**3 H**

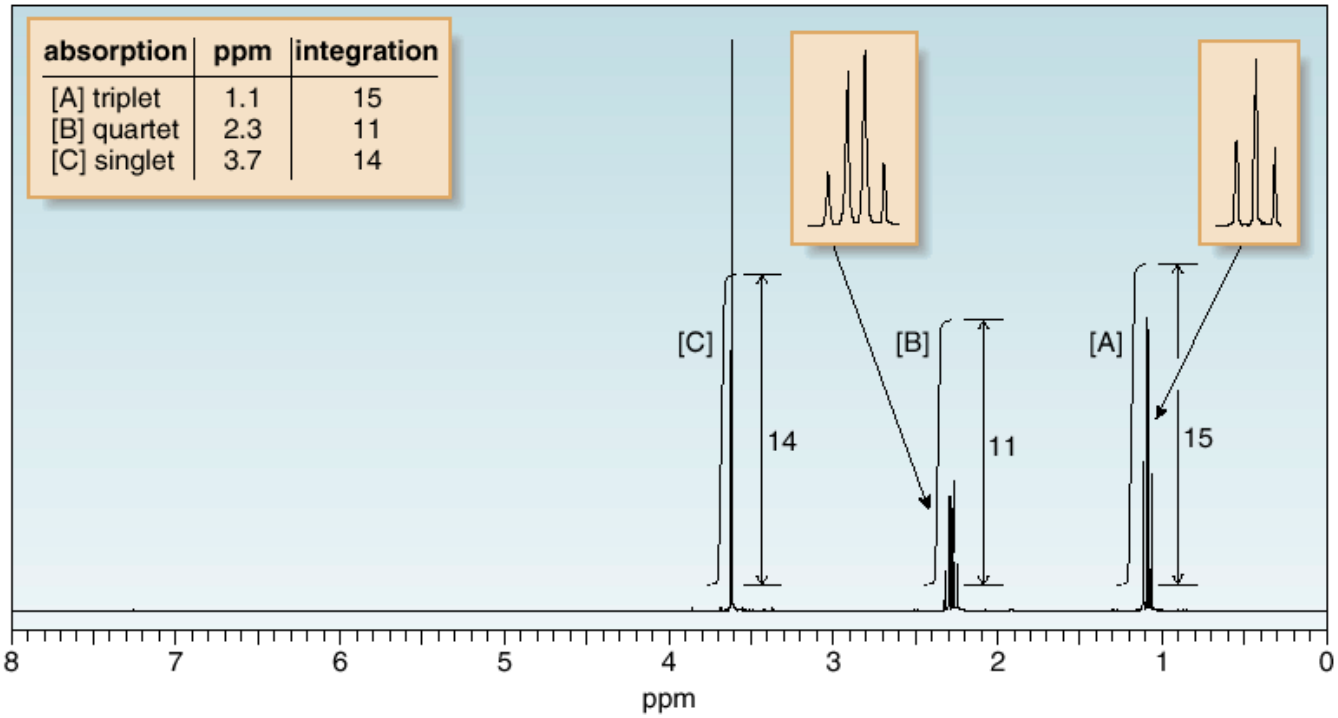
Nuclear Magnetic Resonance Spectroscopy

^{1}H NMR—Structure Determination

How To **Use ^{1}H NMR Data to Determine a Structure**

Example Using its ^{1}H NMR spectrum, determine the structure of an unknown compound **X** that has molecular formula $C_4H_8O_2$ and contains a C=O absorption in its IR spectrum.

absorption	ppm	integration
[A] triplet	1.1	15
[B] quartet	2.3	11
[C] singlet	3.7	14



Step [1] **Determine the number of different kinds of protons.**

- The number of NMR signals equals the number of different types of protons.
- This molecule has three NMR signals ([A], [B], and [C]) and therefore **three** types of protons (H_a, H_b, and H_c).

Nuclear Magnetic Resonance Spectroscopy

^{1}H NMR—Structure Determination

How To, continued . . .

Step [2] **Use the integration data to determine the number of H atoms giving rise to each signal (Section 14.5).**

- Total number of integration units: 14 + 11 + 15 = 40 units
- Total number of protons = 8
- Divide: 40 units/8 protons = **5 units per proton**
- Then, divide each integration value by this answer (5 units per proton) and round to the nearest whole number.

$\frac{15}{5}$ = 3 H_a protons — signal [A] → Three equivalent H's usually means a **CH_3** group.

$\frac{11}{5}$ = 2.2 ≈ 2 H_b protons — signal [B] → Two equivalent H's usually means a **CH_2** group.

$\frac{14}{5}$ = 2.8 ≈ 3 H_c protons — signal [C] → Three equivalent H's usually means a **CH_3** group.

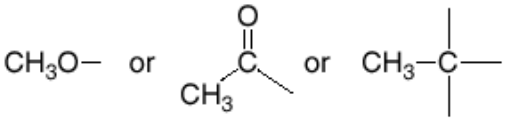
Nuclear Magnetic Resonance Spectroscopy

^{1}H NMR—Structure Determination

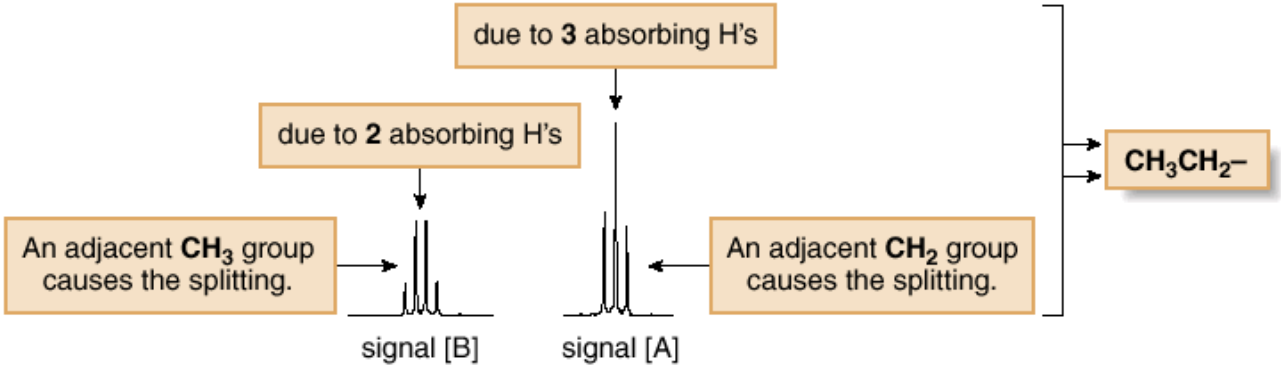
How To, continued . . .

Step [3] **Use individual splitting patterns to determine what carbon atoms are bonded to each other.**

- Start with the singlets. Signal [C] is due to a CH_3 group with no adjacent nonequivalent H atoms. Possible structures include:



- Because signal [A] is a **triplet,** there must be **2 H's** (CH_2 group) on the adjacent carbon.
- Because signal [B] is a **quartet,** there must be **3 H's** (CH_3 group) on the adjacent carbon.
- This information suggests that **X** has an **ethyl** group ⟶ CH_3CH_2-.



To summarize, **X** contains CH_3-, CH_3CH_2-, and C=O (from the IR). Comparing these atoms with the molecular formula shows that one O atom is missing. Because O atoms do not absorb in a ^{1}H NMR spectrum, their presence can only be inferred by examining the chemical shift of protons near them. O atoms are more electronegative than C, thus deshielding nearby protons, and shifting their absorption downfield.

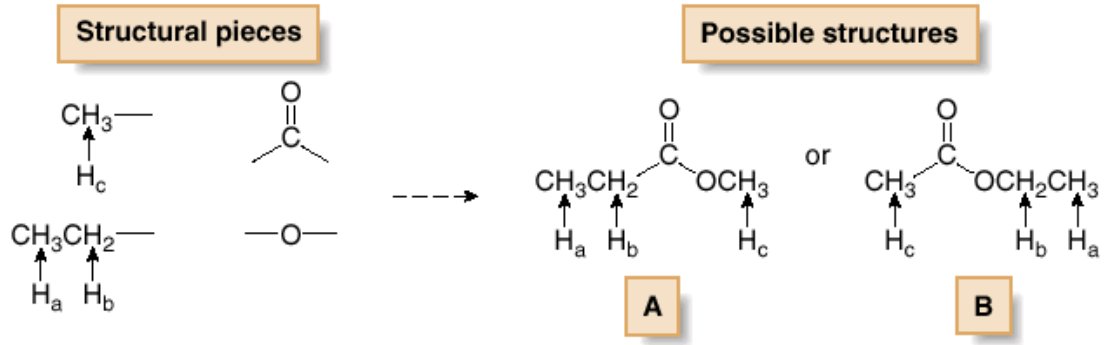
Nuclear Magnetic Resonance Spectroscopy

^{1}H NMR—Structure Determination

How To, continued . . .

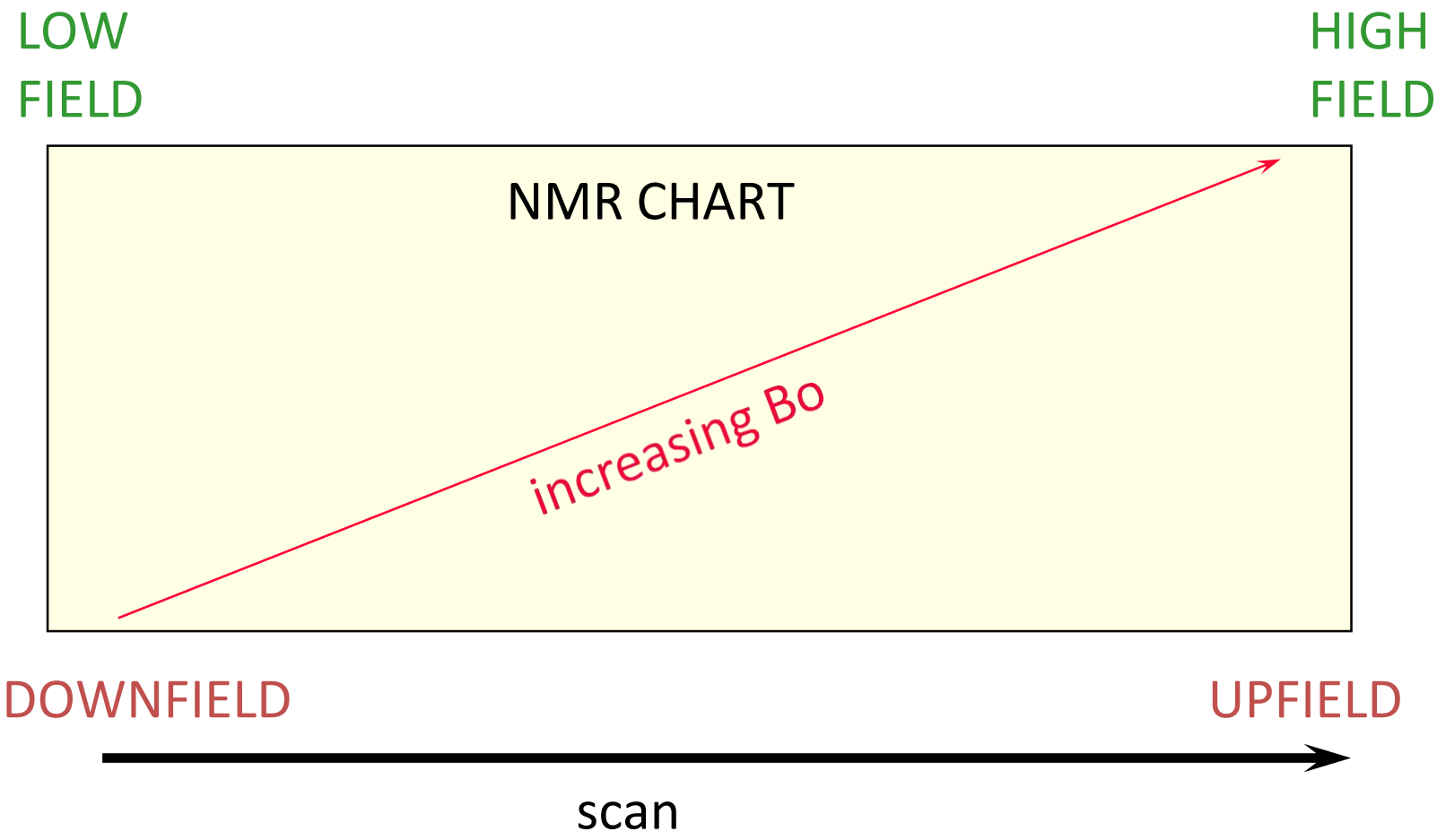
Step [4] **Use chemical shift data to complete the structure.**

- Put the structure together in a manner that preserves the splitting data and is consistent with the reported chemical shifts.
- In this example, two isomeric structures (**A** and **B**) are possible for **X** considering the splitting data only:



- Chemical shift information distinguishes the two possibilities. The electronegative O atom deshields adjacent H's, shifting them downfield between 3 and 4 ppm. If **A** is the correct structure, the singlet due to the CH_3 group (H_c) should occur downfield, whereas if **B** is the correct structure, the quartet due to the CH_2 group (H_b) should occur downfield.
- Because the NMR of **X** has a singlet (not a quartet) at 3.7, **A is the correct structure.**

IN THE CLASSICAL NMR EXPERIMENT THE INSTRUMENT SCANS FROM "LOW FIELD" TO "HIGH FIELD"



Solvents used in PMR

- Carbon tetrachloride
- Carbon Disulphide
- Deuterochloroform
- Hexachloroacetone

Characteristics of solvents:

- It should be chemically inert
- It should devoid of hydrogen atom
- It should dissolve sample to reasonable extent (at least 10%)

Proton Exchange Reaction

- In a molecule, if a proton shuttles between two magnetic environments at a rate which is much faster in comparison with nmr coupling times , then the resonance observed for that proton will be simply that of average effective field in the two environments.
- Thus, only one resonance will be observed, although proton will shuttle between two different magnetic environments
- -OH proton in water has different chemical shift as compared to that –OH proton in acetic acid.

But one signal is seen in nmr spectrum for acetic acid in water at an average position according to following formula

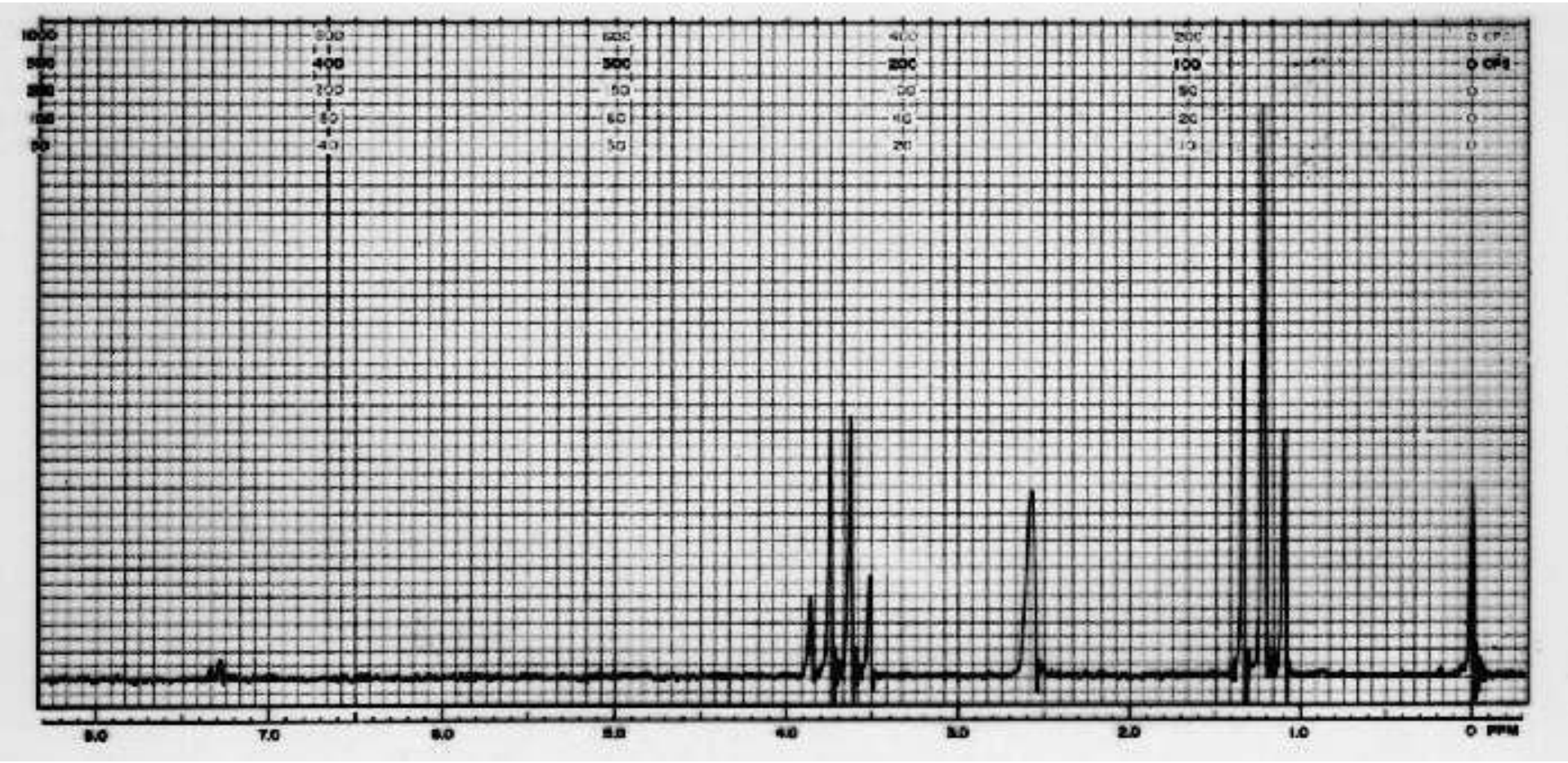
$$N_a\delta_a + N_b\delta_b$$

N_a, N_b= mole fraction of proton a,b respectively

δ_a , δ_b= Chemical shift for unexchanged proton a,b.

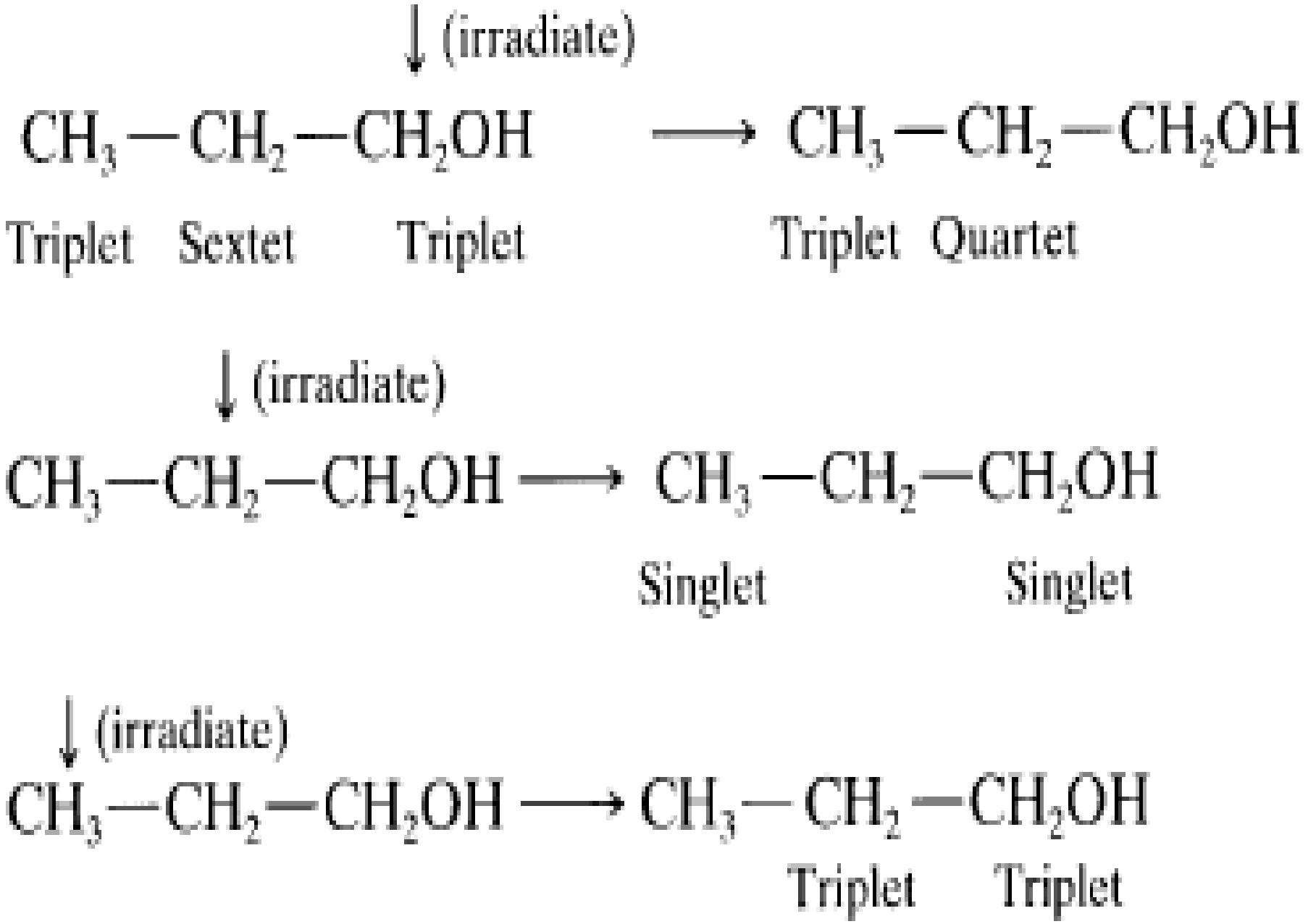
Example : Ethanol vs. anhydrous Ethanol

ROH*+HOH=R-OH+HOH*



Spin decoupling by double resonance

- Powerful tool for simplification of spectra in complex molecule
- This techniques involves the irradiation of a proton or a group of equivalent proton with sufficiently intense radio frequency energy to eliminate completely the observed coupling due to the neighbouring protons.
- The rate of transition between energy states (spin state) becomes much faster, so that the life time of nucleus in any one spin state will be too short to resolve coupling with neighbouring proton


↓ (irradiate)
CH3—CH2—CH2OH
Triplet Sextet Triplet
CH3—CH2—CH2OH
Triplet Quartet
↓ (irradiate)
CH3—CH2—CH2OH
CH3—CH2—CH2OH
Singlet Singlet
↓ (irradiate)
CH3—CH2—CH2OH
CH3—CH2—CH2OH
Triplet Triplet

Deuterium Exchange Reaction

If a few drop of deuterium oxide are added in the sample, the D2O exchanges with labile proton such as –OH,-NH,-SH.

$$ROH+D_2O=R\text{-}OD+H\text{-}OD$$

The signal for –OH proton normally observed in ROH will be missing and instead ,a signal for proton in H-OD will appear.

$$RCOOH+D_2O=RCOOD+H\text{-}OD$$

This technique is employed for detecting the presence of -OH, -NH group etc.

Application of NMR spectroscopy

1. Identification of structural isomers
2. Detection of hydrogen bonding
3. Detection of aromaticity
4. Distinction between cis and trans isomer
5. Detection of electronegative atom/group
6. Detection of some double bond character due to resonance
7. Importance in quantitative analysis

$$\text{Amt. of drug} = \frac{\text{Area signal for drug protons}}{\text{Area signal for int. std. protons}} \times \text{mass of int. std. added} \times \frac{\text{MW drug}}{\text{MW int.std.}} \times \frac{\text{No. protons from int. std.}}{\text{No. protons from drug}}$$

Types of information from the NMR spectrum

1. Each different type of hydrogen gives a peak or group of peaks (multiplet).
2. The chemical shift (δ, **in ppm**) gives a clue as to the type of hydrogen generating the peak (alkane, alkene, benzene, aldehyde, etc.)
3. The integral gives the relative numbers of each type of hydrogen.
4. Spin-spin splitting gives the number of hydrogens on adjacent carbons.
5. The coupling constant J also gives information about the arrangement of the atoms involved.

References:

- *Spectrometric identification of Organic Compounds, Robert. M. Silverstein, Basseler,Morril (John Wiley and Sons. N.Y).*
- *Spectroscopy of Organic Compounds by P. S. Kalsi.*
- *Principles of Instrumental Analysis, Donglas A. Skoog, James, J. Leary, 4th edition.*
- *Organic Spectroscopy,William Kemp, 3rd Edition, Palgrave*
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QUALITY REVIEW

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QUALITY REIVEW

QUALITY REIVEW is the prime Moto of any Pharmaceutical industry, as a vital segment of health care system, should be of producing a product of good quality in terms of safety, purity and efficacy.

Necessity for Quality product:

- As all the countries are marching towards globalization, and this globalization in turn forces the companies to produce a product which meets the quality specifications set by the respective countries.

- Because of increasing complexity of modern Pharmaceutical manufacturing arising from a wide variety of unique drugs and dosage forms.

The Pharmaceutical company has set a department called Quality Assurance (QA) in order to inspect the quality aspects in each and every product.

It is the responsibility of the QA to instill all the quality aspects of a product in each and every product with the help of other departments like production, quality control, stores and maintenance.

It does its duty by reviewing various steps involved in manufacturing of products.

Quality means purity, safety and efficacy

Review means counter checking

As a whole, quality review in a Pharmaceutical company, represents counter checking each and every step starting from acquiring raw material to releasing finished products, including market complaints.

QUALITY REIVEW TEAM

A systematic and effective review team includes knowledgeable, professional and experienced persons form each department.

Team Leader- Generally President or Vice-president

Quality Assurance – 1 person

Production - 1 person

Quality Control - 1 person

Regulatory Affairs - 1 person

Supply Chain Management - 1 person

Objective of QRT

- ✓ To minimize the errors those arise during various stages involved in production
- ✓ To minimize the market complaints
- ✓ To instill safety, purity and efficacy in each and every product.

Responsibilities of QRT

1. Raw material review
2. Production records review
3. Packaging and labeling review
4. Finished product record review

1. **Raw material review**

Quality review team will take decisions for the approval of quality of raw materials from a vendor by auditing the manufacturing premises of vendor and documenting the auditing reports and then the reports will be sent to QRT leader for final approval of vendor to supply the raw material.

2. Production records review

a) **Dispensing** : In dispensing, each and everything has to be documented like raw material name, batch no. quantity, approval signature etc.

b) **Inprocess checks** : The number of units assayed at the end of the process in not likely to be representative of more than a small portion of the actual portion and so as to minimize batch to batch and within batch variation,

It is important to ensure that finished products have uniform purity and quality within batch and between batches.

Conti……

This is accomplished by identifying critical steps involved in manufacturing process like checking parameters of labels (hardness, weight, thickness, friability and disintegration) and pH adjustments in case of parenterals.

Each and every thins during in process checks has to be documented for further reviewing.

3. # Packaging and labeling review

After manufacturing a product, QA member will check that correct labels have been used for correct products and see that no mix-ups had occurred, and the approved labels should be attached to the Batch Manufacturing Records.

4. Finished product record review

Final testing of finished product is done in quality control dept.

The finished product is tested for compliance with predetermined standards prior to release of product for packaging and subsequent distribution.

All the tests and results should be documented.

QRT will review the documents before approving for market release.

Conti…..

This finished product testing along with the process checking assures that each and every unit contains the amount of drug claimed on the label,

that the entire drug in each unit is available for absorption ,

that the drug is stable in the formulation in its specific final container, and

that dosage units themselves contain no toxic foreign substances.

FREQUENCY OF QUALITY REVIEW:

It varies from company to company starting from once in a month to quarterly reviewing , in some instances emergency reviewing (during market complaints).

COMPLIANCE TO QUALITY REVIEW

Compliance with respect to quality review department can be achieved only by following Standard Operational Procedures by concerned officials of respective departments i.e. they should document each and every thing they do and do as per given in SOP.

RESPONSIBILITY OF QUALITY REVIEW DOCUMENTS

Quality Assurance dept. will take the responsibility of all the quality documents concerning quality aspects of products.

THANK YOU

X-Ray Spectroscopy

B.BHADRU

VIKAS COLLEGE OF PHARMACEUTICAL SCINCES

Introduction

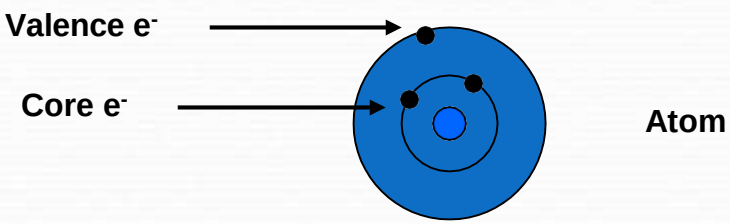
- X-ray spectroscopy is based on the measurement of emission, absorption, scattering, fluorescence and diffraction of EMR
- X-rays are short wavelength EMR produced by the deceleration of high energy electrons or by electronic transition of electrons in the inner orbitals of atoms.
- The wavelength range of X-rays is from about 10^{-5} A^0 to 100 A^0. conventional spectroscopy confined to the region of about 0.1 A^0 to 25 A^0. i.e. 0.01 nm to 2.5 nm (1 A^0 = 0.1 nm)

Method of X-ray analysis

1) X-ray Emission Spectroscopy (XES)
2) Auger Emission Spectroscopy (AES)
3) X-ray Fluorescence Spectroscopy (XFS)
4) Electron Spectroscopy for Chemical Analysis (ESCA)
5) X-ray Absorption Spectroscopy (XAS)
6) X-ray Diffraction Spectroscopy (XRDS)

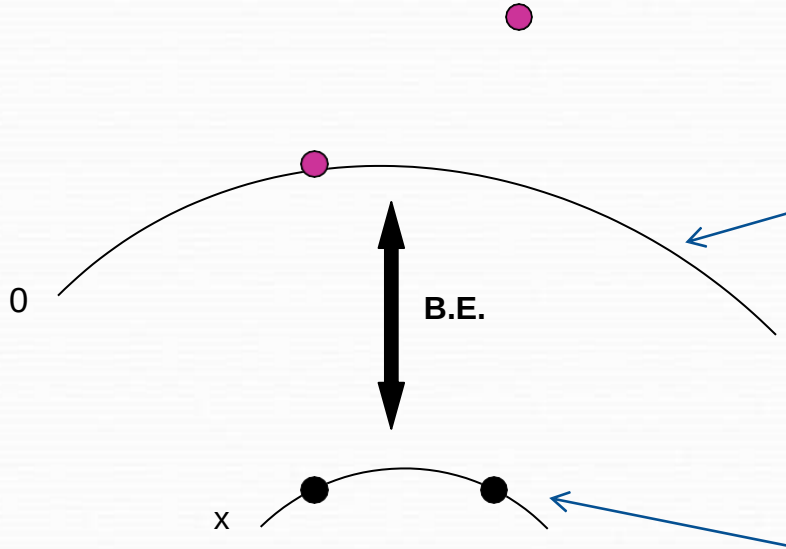
Why the Core Electrons?

- An electron near the Fermi level is far from the nucleus, moving in different directions all over the place, and will not carry information about any single atom.
  - Fermi level is the highest energy level occupied by an electron in a neutral solid at absolute 0 temperature.
  - Electron binding energy (BE) is calculated with respect to the Fermi level.
- The core e^-s are local close to the nucleus and have binding energies characteristic of their particular element.
- The core e^-s have a higher probability of matching the energies of AlKα and MgKα.



Binding Energy (BE)

The Binding Energy (BE) is characteristic of the core electrons for each element. The BE is determined by the attraction of the electrons to the nucleus. If an electron with energy x is pulled away from the nucleus, the attraction between the electron and the nucleus decreases and the BE decreases. Eventually, there will be a point when the electron will be free of the nucleus.



energy x

- **This is the point with 0 energy of attraction between the electron and the nucleus. At this point the electron is free from the atom.**
- **These electrons are attracted to the proton with certain binding**

X-ray Emission Spectroscopy (XES)

- When atom is excited by removal of an electron from inner level, it relax by transfering an electron from some outer level to the vacant inner level with emission of X-ray photon.

- If X-rays emitted, their

  λ ……charecteristic of element

  Intensity α no. of excited atoms

- Here the atom is excited by applying primary electron beam which ejects electron from inner energy level

Auger Emission Spectroscopy (AES)

- When the core electron leaves a vacancy an electron of higher energy will move down to occupy the vacancy while releasing energy by:
  - photons
  - Auger electrons
- Each Auger electron carries a characteristic energy that can be measured.

X-ray Fluorescence Spectroscopy (XFS)

- Atoms are excited by irradiation of the material with X-rays of shorter wavelength (higher energy)

- The primary beam of X-rays ejects electrons from inner energy level. Then X-rays are emitted as electrons from outer levels to vacant inner level

Electron Spectroscopy for Chemical Analysis (ESCA)

- Primary X-rays eject electrons from inner energy levels, the energy of the emitted electrons are measured

X-ray Absorption Spectroscopy (XAS)

- A beam of X-ray is allowed to pass through the sample, and the fraction or attenuation of X-ray photons absorbed is considered to be a measure of the concentration of absorbing substance.

X-ray Diffraction Spectroscopy (XRDS)

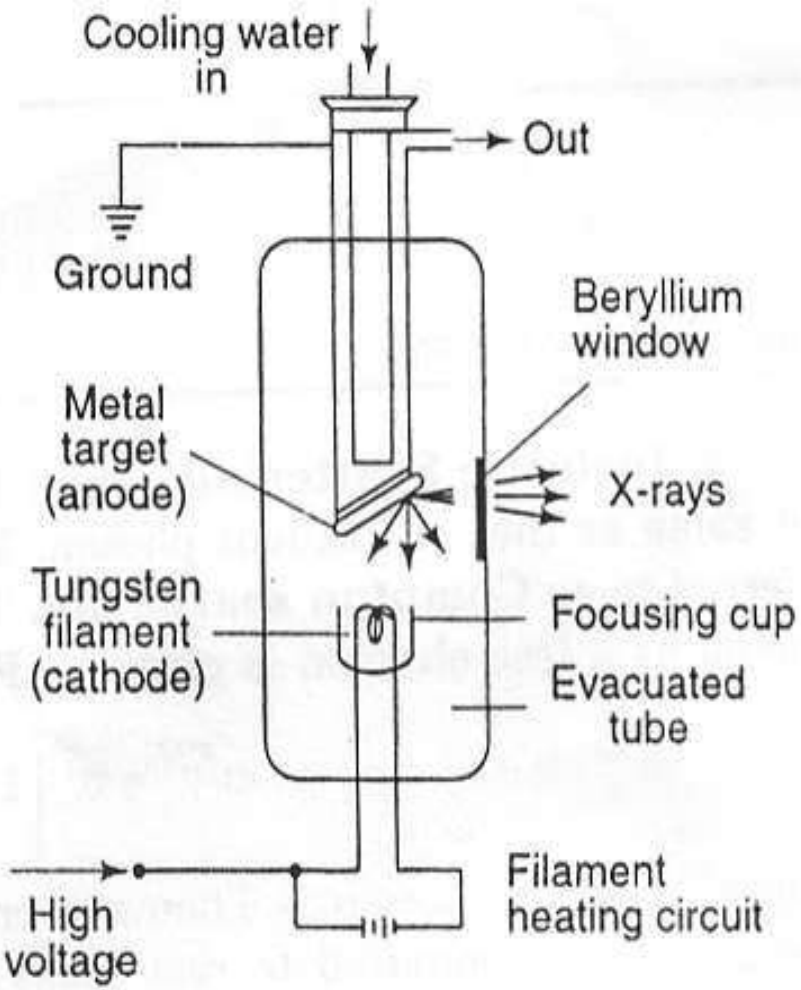
- Diffraction of X-rays from the planes of crystal is observed when a beam of X-rays is allowed to pass through crystal

- This method depends on the wave character of the X-rays and the regular spacing of planes in a crystal

Generation of X- rays

- for analytical purposes, X-rays are obtained in four ways…

1. By bombardment of a metal target with a beam of higher energy electron.
2. By exposure of a substance to a primary beam of X-rays inorder to to generate a secondary beam of X-ray fluorescence.
3. By use of a radioactive source whose decay results in X-ray emission
4. From a synchrotron radiation source



coolidge tube.

Spectra from different sources

- Electron beam sources produce
  - Continuum spectra
  - Line spectra
- Fluorescent source produce
  - line spectra
- radioactive source produces
  - line spectra

X-ray Diffraction (XRD)

A. When X-rays interact with a single particle, it scatters the incident beam uniformly in all directions.

B. When X-rays interact with a solid material the scattered beams can add together in a few directions and reinforce each other to yield diffraction. The regularity of the material is responsible for the diffraction of the beams.

For example if you look at a CD when exposed to white light you can see it diffracted into varies wavelengths of color. The pits (or grooves) in the CD are the regularity of the material that causes the diffraction.

- Diffraction can occur when any electromagnetic radiation interacts with a periodic structure. The repeat distance of the periodic structure must be about the same wavelength of the radiation. For example, light can be diffracted by a grating having scribed lines arranged on the order of the wavelength of light.
- X-rays have wavelengths on the order of a few angstroms (1 Angstrom = 0.1 nm). This is the typical inter-atomic distance in crystalline solids, making X-rays the correct order of magnitude for diffraction of atoms of crystalline materials.

How are Diffractions Patterns Made?

When X-rays are scattered from a crystalline solid they can constructively interfere, producing a diffracted beam. What does this mean?

Constructive vs. Destructive Interference

Interference occurs among the waves scattered by the atoms when crystalline solids are exposed to X-rays. There are two types of interference depending on how the waves overlap one another.

- Constructive interference occurs when the waves are moving in phase with each other. Destructive interference occurs when the waves are out of phase.

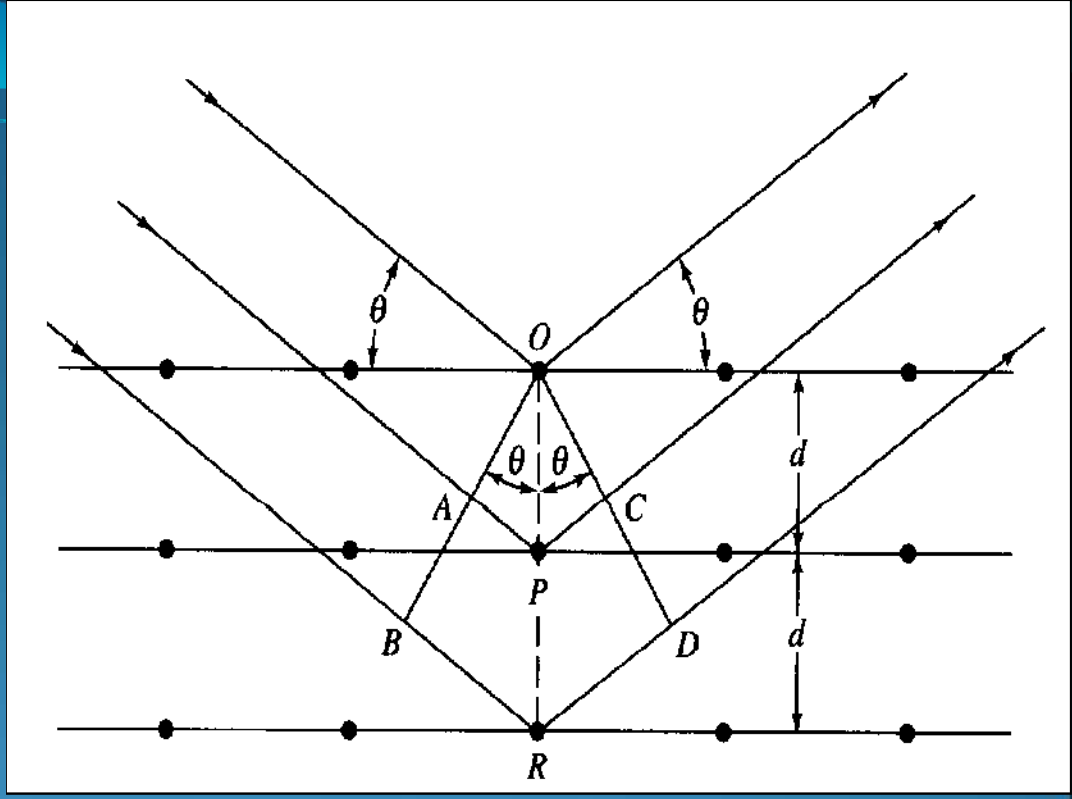
This constructive interference results in diffraction patterns.

$$AP + PC = n\lambda$$

$$AP = PC = d\sin\theta$$

$$n\lambda = 2d\sin\theta$$

$$\sin\theta = \frac{n\lambda}{2d}$$

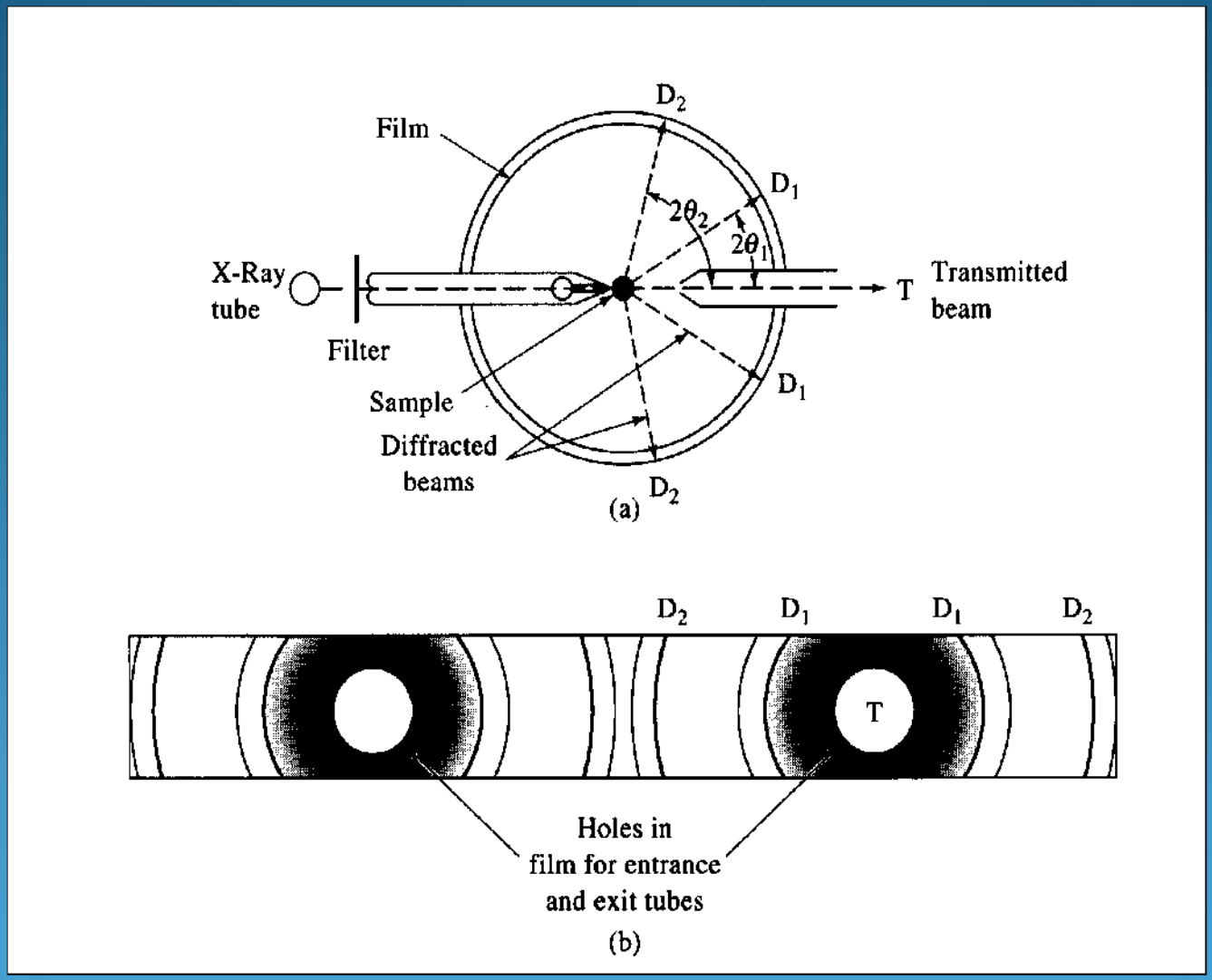


Constructive interference only at angles proportional to l and d!

If l is known and q can be measured then you can calculate d!

If d is known and q can be measured then you can calculate l!

Debye-Scherrer Powder Diffractometer (Camera)



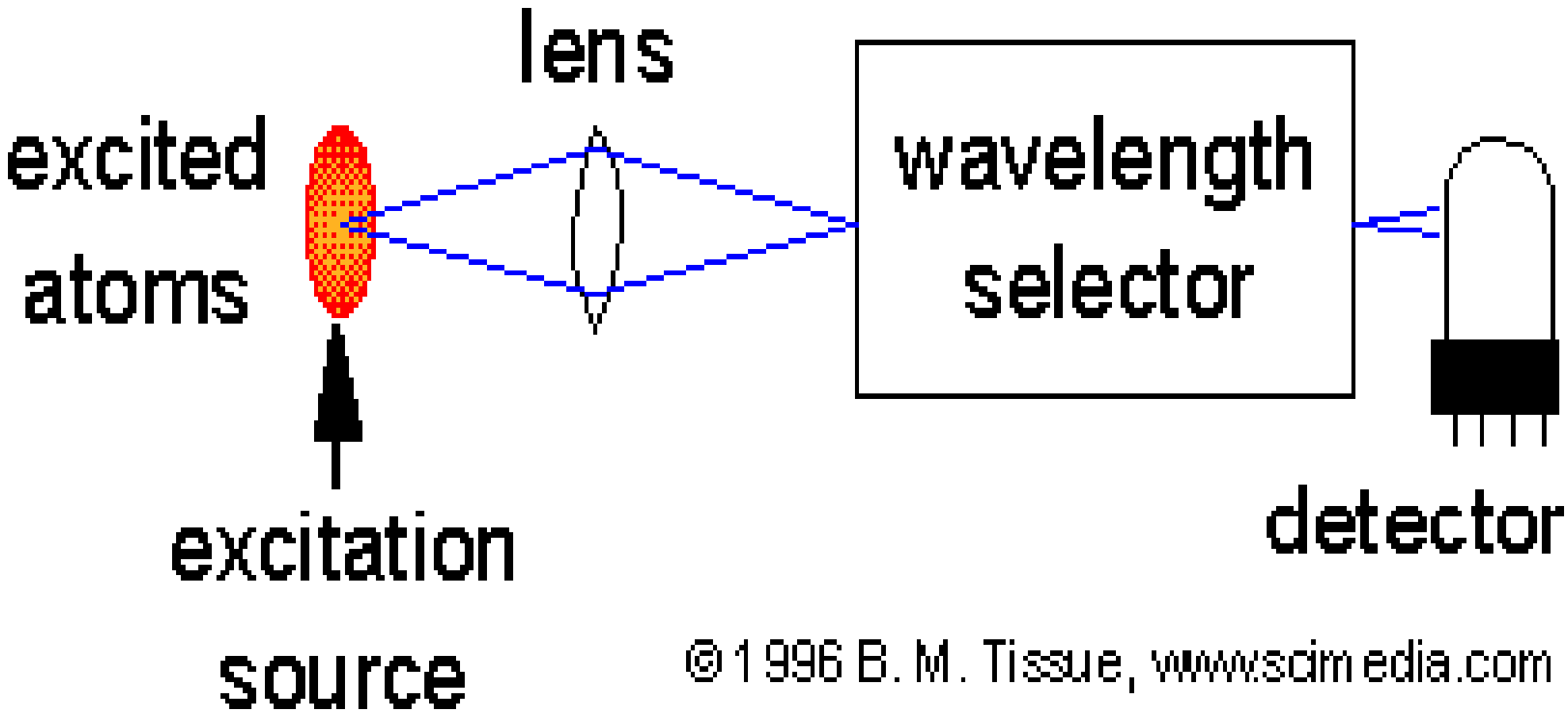
ATOMIC EMISSION SPECTROSCOPY

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INTRODUCTION

- Emission spectroscopy is concerned with the characteristic radiation produced when atoms or molecules are introduced into thermal or electrical sources
- These sources excite the atoms to levels above the ground state
- When they return to lower energy states, the characteristic radiation is emitted in the form of discrete wavelengths of light, called *'spectral lines'*
- The wavelength of the spectral line is inversely proportional to energy difference between initial and final energy levels

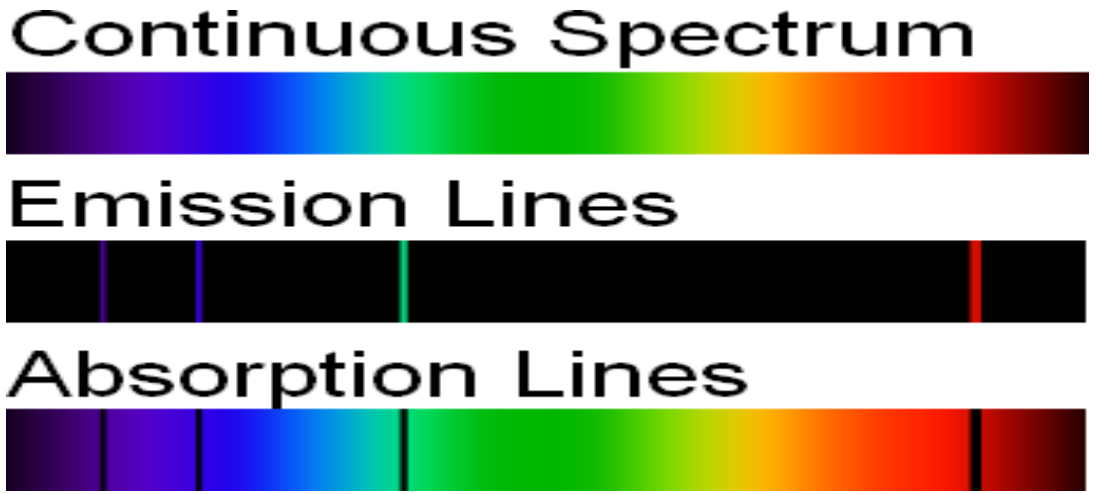
An emission spectrograph



Types of Spectra

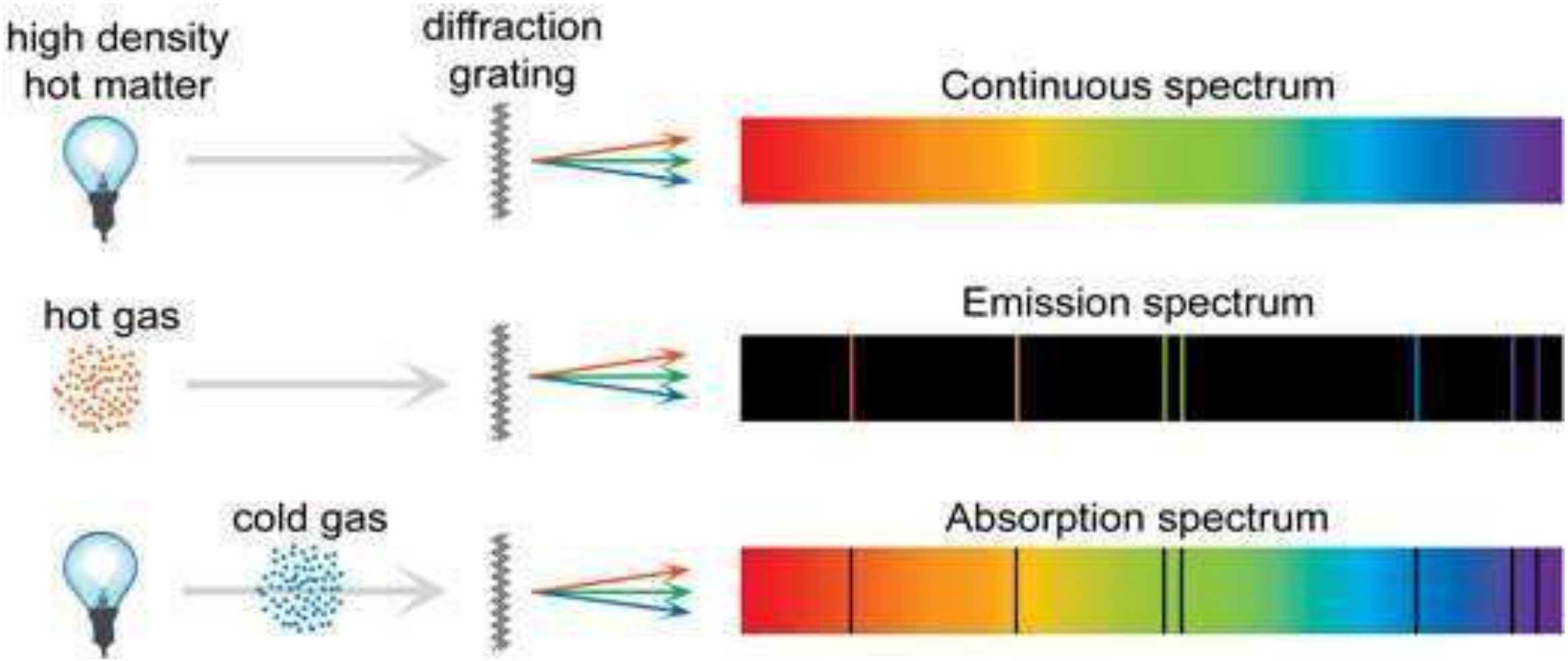
a) Emission spectra

b) Absorption spectra



a) Emission spectra: When a solid is heated to a high temperature or a discharge of electricity is passed through a gas, light is emitted.

On examining this light by a spectroscope, a spectrum has been found to be produced which is known as *'emission spectra'*


high density
hot matter
diffraction
grating
Continuous spectrum
hot gas
Emission spectrum
cold gas
Absorption spectrum

b) Absorption spectra: When the light from the source emitting a continuous spectrum and then absorbed through a spectroscope, it will be observed that certain lines are missing in the spectrum which leaves dark lines or bands at their places

As the light of wavelength corresponding to black bands is absorbed by the substance through which the light is passed, the spectrum so obtained is known as *'absorption spectrum'.*

Types of Emission spectra

a) Continuous spectra:

- It is characterized by uninterrupted emission over a considerable wavelength region and by the absence of sharp lines or discrete bands.
- It is obtained whenever matter in the bulk is heated

  Eg: Incandescent solids like Iron or Carbon

b) Band spectra:

- It consists of group of lines so close together
- We are not interested in band spectra. This can be avoided by providing sufficient energy to molecules so that they be broken up into individual atoms

c) Line spectra:

- A line spectrum consists of discrete irregularly lines
- This type of spectrum is obtained when the light emitting substance is in atomic state

Excitation-Energy Requirements

- For any element to emit a single spectral line, energy equivalent to the '*excitation potential;* of the element must be absorbed
- The complete spectrum is only possible when the energy equivalent to the *'ionization potential'* must be absorbed
- By using an enough energetic source, all the elements in the periodic table could be excited to produce emission spectra
- But most of the spectral lines fall in the vaccum-UV region which cannot be studied easily
- So it is mainly limited to metals and metalloids not for non-metals

INSTRUMENTATION

The sample is excited by thermal or electric method and thus becomes the source of radiation

Then the radiation is passed through a monochromator to select the desired wavelength

Then passed into the detector which measures the radiation for qualitative analysis

The Instrumentation of Emission spectroscopy will contain the following components

1) Excitation source
2) Electrodes
3) Sample holders
4) Monochromators
5) Slits
6) Detectors

1) Excitation source

It must accomplish the following process:

- ✓ The sample must be vapourised
- ✓ It must be dissociated into atoms
- ✓ The electrons in the atoms must be excited to higher energy levels above the ground state
- ✓ It should provide sufficient line intensity so as to detect these lines within detection limit
- ✓ It should provide reproducible excitation conditions from sample to sample

The various sources of excitation are:

a) Flames:

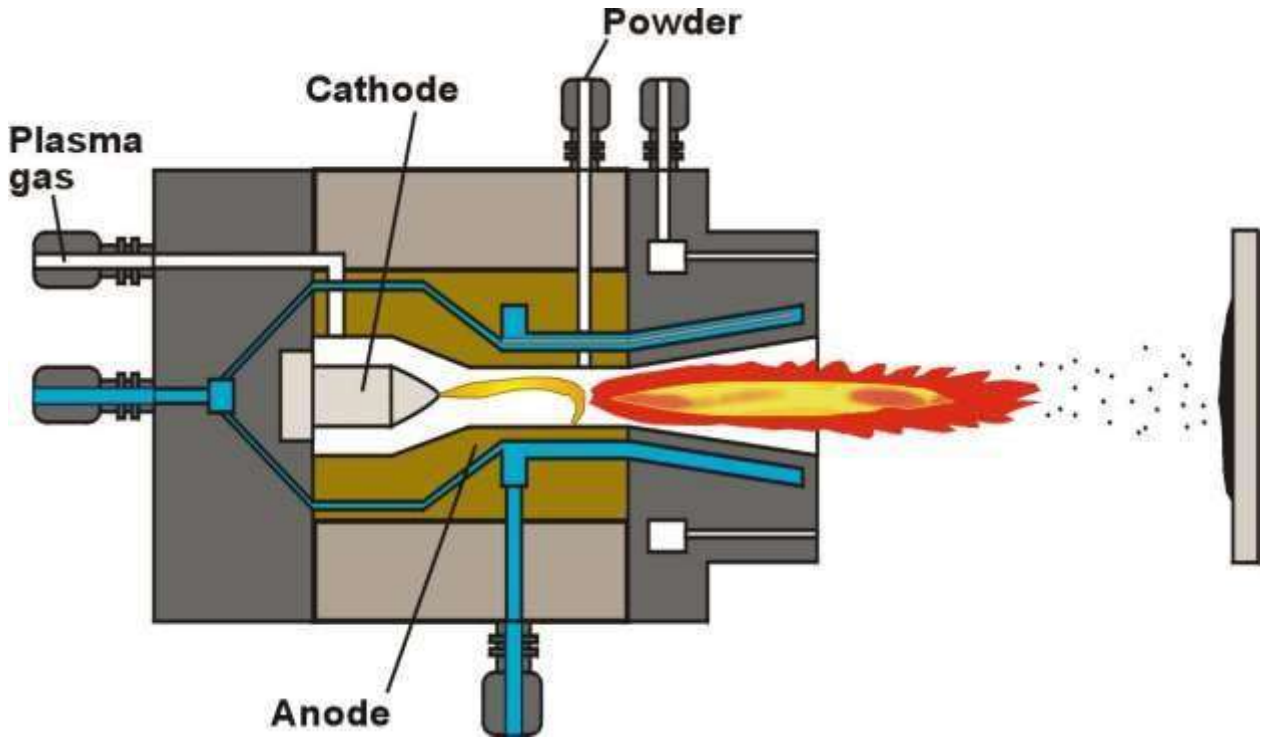
It is used for those molecules which do not require very high temperatures for excitation & dissociation into atoms

The temp of the flame is determined by following factors:

- Type of fuel & oxidant
- Fuel to oxidant ratio
- Type of burner
- Region in the flame that is focused into the entrance slit of the spectral isolation unit

b) Direct current arc:

- The DC source is usually a regulated power supply that furnishes 110 to 220V at 3 to 30A.
- The current is made to flow across the arc gap in series with a variable resistor R and an inductance coil L



- The sample in the form of a solid or liquid is kept on the lower electrode in the arc gap
- Once the current starts flowing, the temp in the gap rises rapidly & then the electrodes are separated byabout 20mm to 1cm

- Immediately, the electric arc will be established across the gap
- By this arc, emission species are neutral atoms rather than ions

"The excitation energy provided by this arc is not electrical but mainly thermal and is thus sufficient for exciting all the elements"

c) Alternating current arc:

- A high voltage of 2000-5000V is maintained by a transformer
- This voltage helps the arc to jump the gap
- Current is alternating at a frequency of 60Hz
- As the arc picks out the new surface area after each cycle, the whole surface of the sample is arched & excited
- The stop and start nature of this source is that the gap temperature is much less than with the direct current arc, with a lower sensitivity

d) Alternating current spark:

- Most spark sources employ a pair of spark gaps arranged in series
- The spark is produced by connecting a high voltage transformer across two electrodes
- The current may be increased by connecting a condenser in parallel with spark gap
- The sample is dissociated into atoms and ions, then volatilizes in a spark

2) Electrodes:

a) Self electrode: When the material under investigation is a good conductor and can withstand high temp, the material as such is used for the electrodes called self-electrode

b) Graphite electrode: When the material under investigation is not a good conductor and cannot withstand high temp, it is placed in a small cavity of the lower graphite electrode whereas the upper electrode is also made up of graphite which is brought to a point in a pencil sharpener

3) Sample Holders:

- The function of the sample holder is to introduce the sample into the electrical discharge

a. Solid Sample Holder:

- Many samples are handled by being first reduced to a powder and then loaded into a carbon sample holder.
- After it is loaded, the sample holder is placed in a position as one of the electrodes used in the discharge
- During discharge, from top surface of electrode the sample is vapourised into the plasmaof the discharge and spectrographic emission takes place

b. Liquid Sample Holders:

1. Porous cup container:

- It permits the liquid sample to percolate through it at a steady rate into the electrical discharge
- The sample slowly runs into the discharge, which can be sustained until the base of the holder is burned away

2. Rotating disk electrode:

- It rotates through the sample, which wets the surface of the disk
- It carries the sample into the discharge at steady rate, emitting the radiation from the excited sample in the discharge

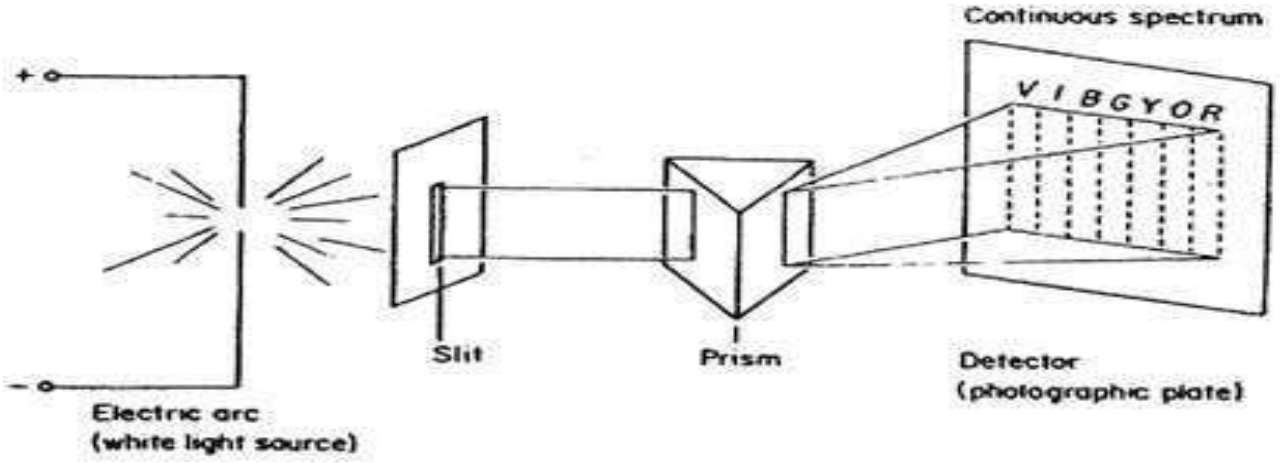
4) Monochromators:

- The function of the monochromator is to separate the various lines of a sample's emission spectra
- Both '*prism*' and '*grating*' monochromators are used

Prism monochromator:

- When one light of one wavelength is passed through the quartz prism, it emerges from the prism as two lines
- This causes the loss of half of the light's intensity
- This can be overcome by using two half prisms

- The first half splits the light into two beams whereas the second half recombines them to a single beam (cornu type prism)



Disadvantage of prism:

- The dispersion of a prism is not constant over a wide wavelength range

Grating monochromator:

- It consists of a large no of parallel, wqually spaced lines ruled upon a glass surface or metal coating on a glass
- The spectrum produced by a grating is dispersed nearly linear with respect to wavelengths
- It replaced the prism as the dispersing element
- By employing gratings the identification of lines become very simple

Disadvantage:

- Higher-order wavelengths overlap but this can be overcome by using filters or by using detectors which are not sensitive to the higher orders

5) Slits:

- All spectrographs are fitted with entrance and exit slits
- **'Entrance slits'** keep out stray light and permit only the light from the sample to enter the optical path
- **'Exit slits'** placed after the monochromator stop all but the desired wavelength range from reaching the detector
- A narrow slit is necessary to obain resolution of two lines at similar wavelengths
- If the slit is too wide, both lines pass through & reach the detector without being resolved
- If the slit is too narrow, there is loss in the intensity of radiation

6) Detectors:

- Two types of detectors are widely used

  a) Photo-multipliers

  b) Photographic plates

Photographic detection:

- The intensity of spectral lines is registered on a photographic emulsion
- A beam of light is passed through a clear portion of the film and then the intensity is measured of the transmitted beam by a phototube fitted in the densitometer

- Again a beam of light is passed through the blackened portion of the film and the intensity is measured
- Now the logarithm of the ratio of the intensity of light passing through the clear film and through the blackened film is computed
- Then it is plotted against the log of the exposure

Photomultiplier detection:

- Direct-reading emission spectrographs involve photomultiplier tubes for detection instead of a photographic plate
- A direct-reading instrument is a grating with an opaque barrier pierced by 12 or more slits
- Behind each slit is mounted a photomultiplier tube and the output of each tube is recorded automatically

Advantages of using photographic plates over photo-multipliers

- A large no of spectral lines can be recorded simultaneously
- The photographic plate provides a permanent record if the spectrum
- The emission intensity can be integrated by a photographic emulsion over a period of time
- Photographic emulsions are preferred because of their high sensitivity through out UV and Visible regions
- The cost of an emission spectrograph having photographic detector is much less than that having photo-multiplier detector

Advantages of emission spectroscopy

- Excellent method of elemental trace analysis as the parts per million level, *i.e,* a low a conc level as 0.0001%
- Used for all metals & metalloids
- Usually no chemical separations or concentration steps are required
- A small sample is sufficient for analysis
- The method can be used for routine quality control work
- It is an ideal method for geological samples, metals & alloys, plants, forensic exhibits, soils and environmental and biological samples

Disadvantages of emission spectroscopy

- The cost of emission spectrometer is very high
- The sample gets destroyed in the process of analysis
- This method cannot be used for concentrated solutions, it is difficult to gethigh precision if the constituent desired is present in a conc much above 30%
- The method is limited to the analysis of elements

Applications:

- It is used for elemental qualitative and quantitative analysis

1. Qualitative Analysis:

Comparision of Spectra and Lines – Obtained by comparision of the sample spectrum with the spectrum produced by pure known standard samples

Last lines, RU lines – As the conc of metallic compound made successively more and more dilute, the number & intensities of the lines in the resulting spectra will vary

- Upon dilution the weaker lines disappear and the stronger lines become less intense
- Ultimately only the strongest lines remain
- These last lines to remain upto successive dilutions are referred to as **raies ultima** or **RU** lines

2.Quantitative Analysis:

Comparision sample method:

The spectra of the unknown sample and various standards are photographed alternately on the same plate under the same set of conditions

After developing the plate, one can calculate the conc of the desired constituent by comparing the blackening of the lines of this constituent with the samelines on the standards

Internal standard method:

- The internal standard is added in known amount
- When the sample having internal standard is exited in the electrical discharge, the sample as well as internal standard emit spectral lines
- These are photographed on photographic plate

Measurements are:

✓ Intensity of the sample emission line is measured

✓ Intensity of the internal standard emission line is measured

We know the conc of IS and the ratio of the two lines

From these data, we can calculate the conc of the element which is being determined

3) Specific applications:

- Ceramics for both traces and major constituents
- Metals, for trace metal constituents as well as oxygen and nitrogen
- Aluminium, for traces of cobalt
- Graphite, for traces fo cobalt, nickel, molybdenum & vanadium
- Spent nuclear fuels, for rare earths
- Blood, for trace amounts of Ca, Cu & Zn
- Pancreas tissue, for Zn
- High purity acids and other analytical reagents, for a variety of trace metal impurities



thankyou

Calibration of Analytical Instruments- UV-VIS Spectrophotometer and HPLC

B.Bhadru

Associate Professor

Vikas College of Pharmaceutical Sciences

Calibration

- What is Calibration?

  Calibration is an operation which is performed to assure that the instrument readings are accurate with reference to the established standards.
- The aim of the calibration program is to ensure that all measuring and testing equipment included in the program are calibrated within the manufacturers accuracy specifications or the tolerance required for the application.

Calibration

Calibration may be called for:

- A new instrument.
- After an instrument has been repaired or modified.
- When a specified time period has elapsed.
- Before and/or after a critical measurement.
- After an event, for example
  - After an instrument has had a shock, vibration, or has been exposed to an adverse condition which potentially may have put it out of calibration or damage it.
  - Sudden changes in weather.
- Whenever observations appear questionable or instrument indications do not match the output of surrogate instruments.
- As specified by a requirement, e.g., customer specification, instrument manufacturer recommendation.

Calibration of UV-VIS Spectrophotometer



- Calibration of UV-VIS spectrophotometer involves following parameters:
  - ***Calibration for wavelength accuracy.***
  - ***Calibration for absorbance measurement.***
  - ***Gratings performance or stray light test.***
  - ***Resolution power.***

Calibration of UV-VIS Spectrophotometer

Calibration for wavelength accuracy:

- Take two empty cuvettes, into one cuvette add two drops of benzene and drain it.
- Place the cuvette in sample cell holder with benzene vapors occupied in it.
- Place the empty cuvette in reference cell holder.
- Scan it in between 240-270nm.
- The maximum absorbance should be at 253.9 ± 0.51 nm.
- Absorbance should be below 1 (one).

Calibration of UV-VIS Spectrophotometer

Calibration for absorbance measurement:

- Prepare 60ppm of potassium dichromate by using 0.01M sulfuric acid as solvent.
- Place the solution in sample cell holder.
- Place blank 0.01M sulfuric acid in reference cell holder.
- Measure the absorbance values at different λmax values.

λmax	**Absorbance**
257	0.864 ± 0.01
235	0.747 ± 0.01
313	0.292 ± 0.01
350	0.639 ± 0.01

Calibration of UV-VIS Spectrophotometer

- ***Gratings performance or stray light test:***
  - Prepare 1.2% potassium chloride solution in distilled water.
  - Distilled water is taken as reference.
  - Place the potassium chloride solution in sample cell holder.
  - Absorbance is measured at 195-220nm.
  - Absorbance is must be greater than 2 at 198nm.

Calibration of UV-VIS Spectrophotometer

- ***Resolution power:***
  - When prescribed in a monograph, record the spectrum of a 0.02% v/v solution of toluene in hexane .
  - The ratio of the maximum absorbance at about 269 nm to that at the minimum absorbance at about 266 nm should not be less than 1.5 unless otherwise specified in the monograph.

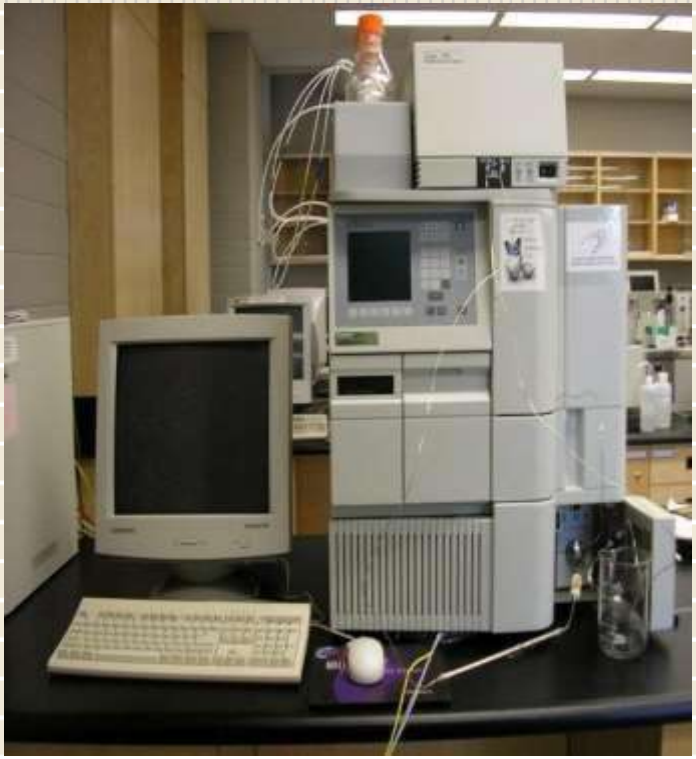
CALIBRATION REPORT

Name of the institution:			
Name of the Instrument: UV-Visible Spectrophotometer			
Make:	Serial No.:	Date of Calibration:	Calibration Due Date:
Signature:			
	Calibrated By	Person Responsible	Approved By

Calibration of HPLC

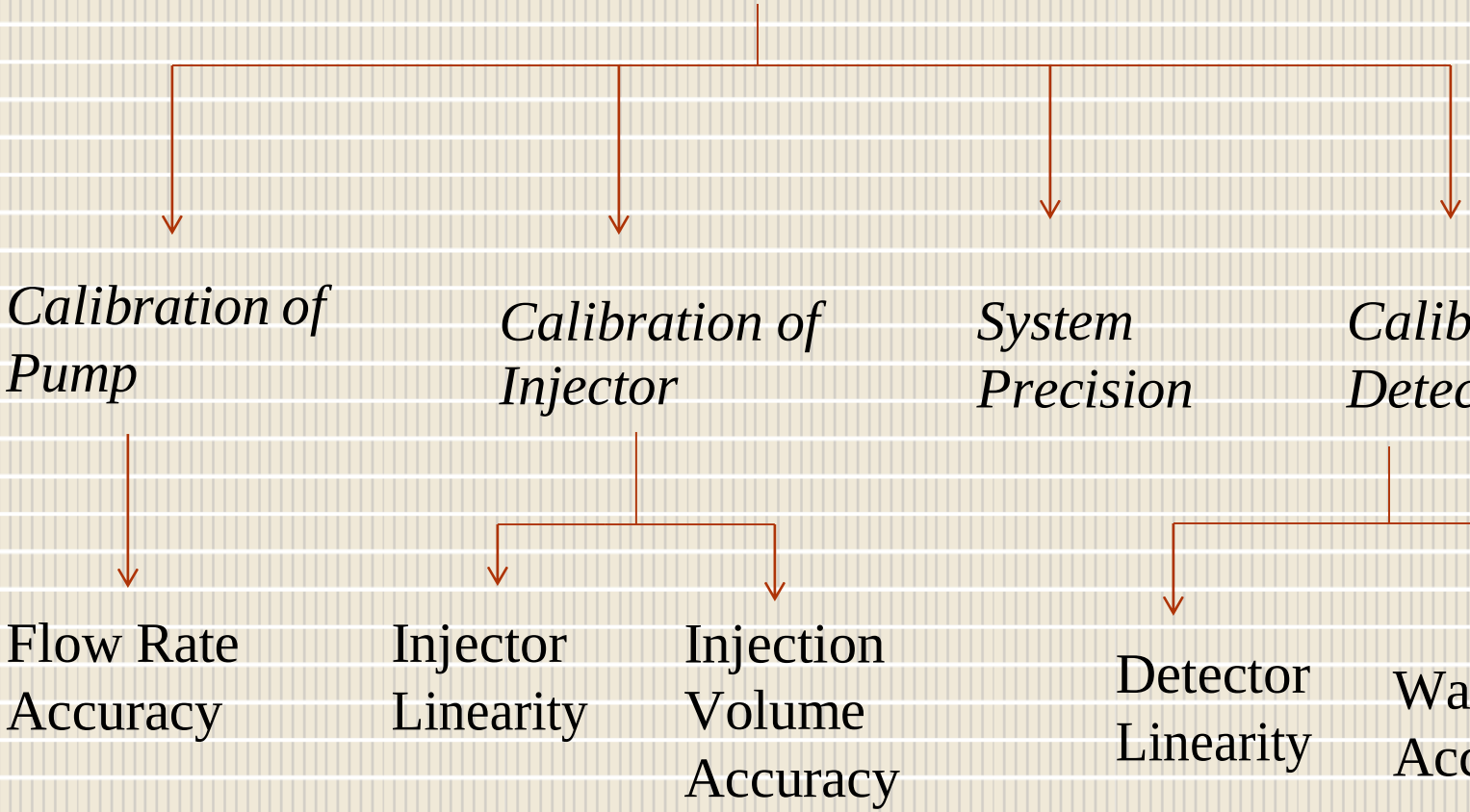
- When it is performed?
  - Most HPLC systems in pharmaceutical laboratories are calibrated every 6 to 12 months.
  - Periods longer than 12 months are not recommended, while periods shorter than 3 months are deemed un-necessary.
  - It also required after annual maintainance (or) major repairs though only effected modules, and not the entire system need to be calibrated.

Calibration of HPLC



- Calibration of HPLC involves following parameters:
  - ***Calibration of Pump***
  - ***Calibration of Injector***
  - ***System Precision***
  - ***Calibration of Detector***

Calibration of HPLC



Calibration of HPLC

Calibration of Pump

Flow Rate Accuracy:

- Prime all the solvent lines with HPLC grade water.
- Set the flow rate to 0.5 ml/min.
- Wait for about 15min to stabilize the system and ensure that the pressure is stable.
- Insert the outlet tubing into a 10 ml volumetric flask and start the stop watch simultaneously.
- Stop the stopwatch when the lower meniscus reaches the 10 ml mark on the flask.
- Record the elapsed time.
- Similarly check the flow for 1.0 ml/m and 2.0 ml/m.
- The time taken to collect the water should be with in ± 2.0% of the actual value.

Calibration of HPLC

Calibration of Injector

Injector linearity

- Duplicate injections of 10µg/ml caffeine solution are injected by setting 5µl, 10µl, 15µl and 20µl respectively one after one as injection volumes.
- The mean peak areas for the above injected volumes are noted.
- Calculate the correlation coefficient by using linearity curve and the value should be with in the acceptance criteria i.e., NLT 0.99.

Calibration of HPLC

Calibration of Injector

Injection volume Accuracy

- HPLC vial is filled with water (HPLC grade) and weighed, it's weight is recorded as W_1
- The vial is placed in the injection tray, then inject six times.
- After completion of six injections remove the vial and weigh, it's weight is recorded as W_2
- The injection volume is calculated using the formula,

$$Injection\ volume(\mu l) = \frac{W_1 - W_2}{0.99602 \times 6}$$

- **Acceptance criteria:** The mean injected should be 50.0±1.0 µl.

Calibration of HPLC

System Precision

- **Standard Preparation**: Accurately weigh and transfer about 60mg of Caffeine into a 100ml volumetric flask. Dissolve and dilute to the volume with mobile phase. Transfer 10ml of this solution into a 100ml volumetric flask and dilute to the volume with mobile phase.
- **Procedure:** Inject blank, followed by standard preparation in 6 replicates. Note down the areas and retention times. Now calculate the %RSD of retention time and peak areas for 6 replicates injections.
- **Acceptance criteria:** The %RSD of retention time & peak area should be <1.0%.

Calibration of HPLC

Calibration of Detector

Detector Linearity

- Prepare a standard stock solution of caffeine (1000µg/ml).
- From the stock solution prepare solutions of concentration 1, 5, 10, 50, 100µg/ml respectively.
- Duplicates of each concentration are injected.
- The mean peak areas of all the concentrations are recorded.
- Calculate the correlation coefficient value by using linearity curve and it should be within the acceptance criteria .i.e., 0.99.

Calibration of HPLC

Calibration of Detector

Wavelength Accuracy

- The wavelength of detector is adjusted to 266nm.
- Then inject duplicates of 10µg/ml solution of caffeine and then the peak area response is recorded.
- The wavelength was increased successively at an increment of +1nm (267, 268, 269……, 276nm) and the peak area is recorded at all the wavelengths.
- The maximum peak area response has to be obtained at 273±2nm (acceptance criteria).

CALIBRATION REPORT

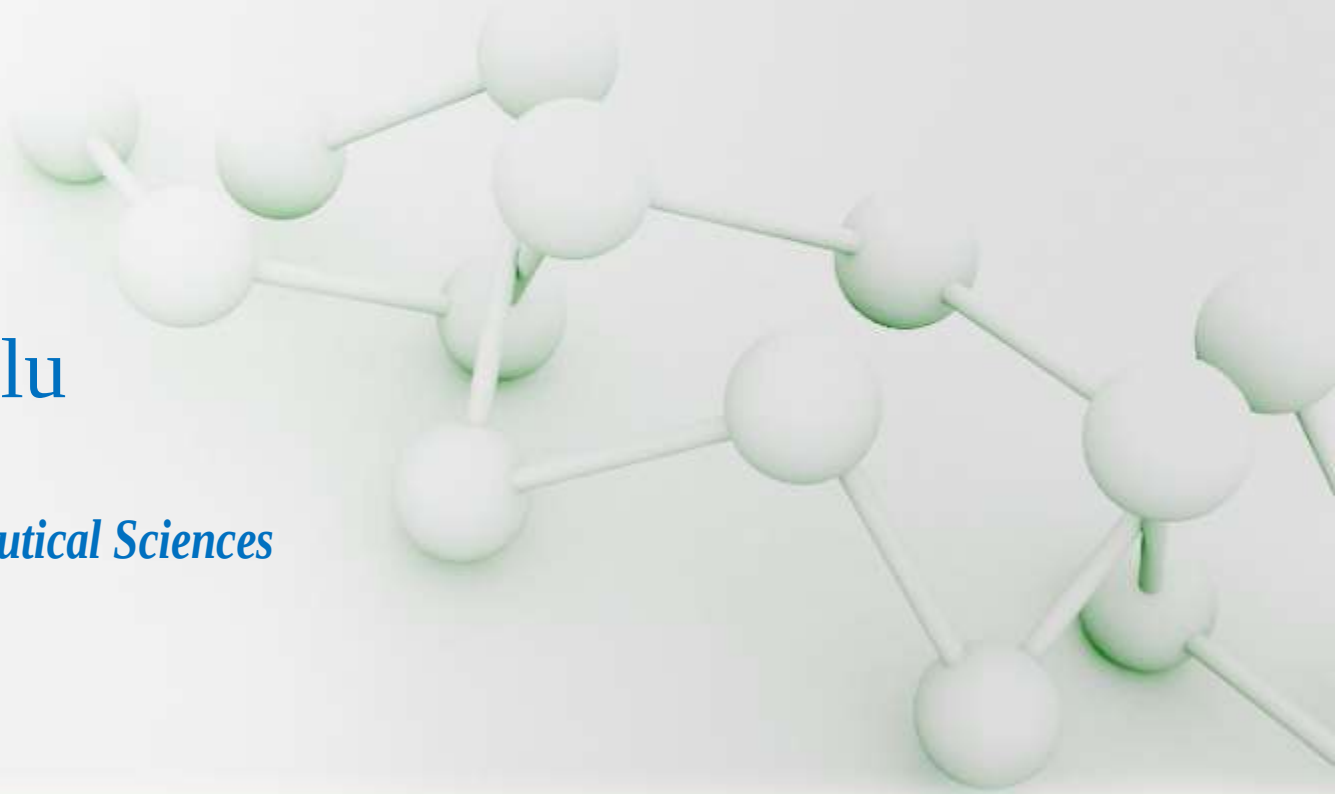
Name of the institution:			
Name of the Instrument: HPLC			
Make:	Serial No.:	Date of Calibration:	Calibration Due Date:
Signature:			
	Calibrated By	Person Responsible	Approved By

References:

- Validation standard operating procedures, 2nd edition, by Syed Imtiaz Haider, pg no.:117 - 125.
- http://www.pharmaguideline.com/2010/05/calibration-of-uv-visible.html
- http://qualityassurancepharma.blogspot.in/2010/12/calibration-of-ultraviolet.html
- http://qualityassurancepharma.blogspot.in/2010/12/operation-calibration-of-hplc-lc.html
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- http://www.pharmaguideline.com/2011/01/hplc-caibration.html


Thank You

ELECTROPHORESIS



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Vikas College of Pharmaceutical Sciences

Introduction:

- Electrophoresis is comparatively a newer technique which can be applied to the migration of individual ions as well as to colloidal aggregates. This technique utilizes electrical properties of compounds.
- This separation technique was first developed by Swedish chemist Arne Tiseliu's in the 1930's.
- Electrophoresis has been applied to analytical separation of different compounds like anions, cations, aminoacids, vitamins, nucleic acids etc…

Definition:

Electrophoresis is a separation method based on the differential rate of migration of charged species in a buffer solution across which has been applied a dc electric current

Procedure:

- An electrophoretic separation is performed by injecting a small band of the sample into an aqueous buffer solution that is contained in a narrow tube or on a flat porous support medium such as paper or semisolid gel.
- A high dc potential is applied across the length of buffer by means of a pair of electrodes located at either end of buffer.
- This potential causes ions of the sample to migrate toward one or the other of the electrodes.
- The rate of migration of species depends upon its charge and also upon its size.
- Separation are then based upon differences in charge to size ratio for the various analytes in the sample
- The larger this ratio, the faster an ion migration in the electric field

Basics of electrophoretic separations:

- Separation of analytes achieved by differences in their velocity in an electric field
- The migration velocity v of an ion in centimeters per second is

$$v = \mu_e E \longrightarrow 1$$

μ_e = electrophoretic mobility

E = applied electric field

- The eletrophoretic mobility is inturn proportional to the ionic charge on the analyte and inversely proportional to the frictional retarding process
- The ion mobility is given by the relationship shown below

$$\mu_e = \frac{\text{Electric Force } (F_E)}{\text{Fractional drag } (F_F)}$$

$F_E = q\,E$

where, q = charge on the ion

E= applied electric field

- The greater the charge on ion the more rapidly it migrates in a particular electric field

For spherical ion : $F_F = -6\pi\eta r\, v$

- When fractional drag and electric field experienced by ion are equal

$$\mathbf{qE = -6\pi\eta r\, v}$$

Substituting this expression in equation 1

$$\mathbf{\mu_e = q / 6\pi\eta r}$$

q = charge on ion
η = viscosity
r = ion radius

- For more irregular shaped solutes

$$\boldsymbol{\mu_e = \varepsilon\zeta / 6\pi\eta}$$

ε=dielectric constant
ζ= zeta-potential

- If two species differ either in charge or in frictional forces they experience in moving through the buffer, they will be separated from each other.
  i. *Greater the charge on ion, higher its mobility.*
  ii. *Smaller the ion, greater its mobility.*
- For ions of same charge , the smaller the ion, the smaller the frictional forces and faster rate of migration.
- The electric field acts only on ions

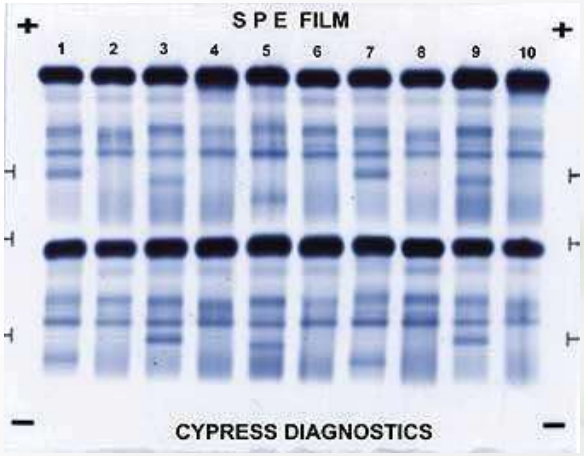
Types of electrophoresis

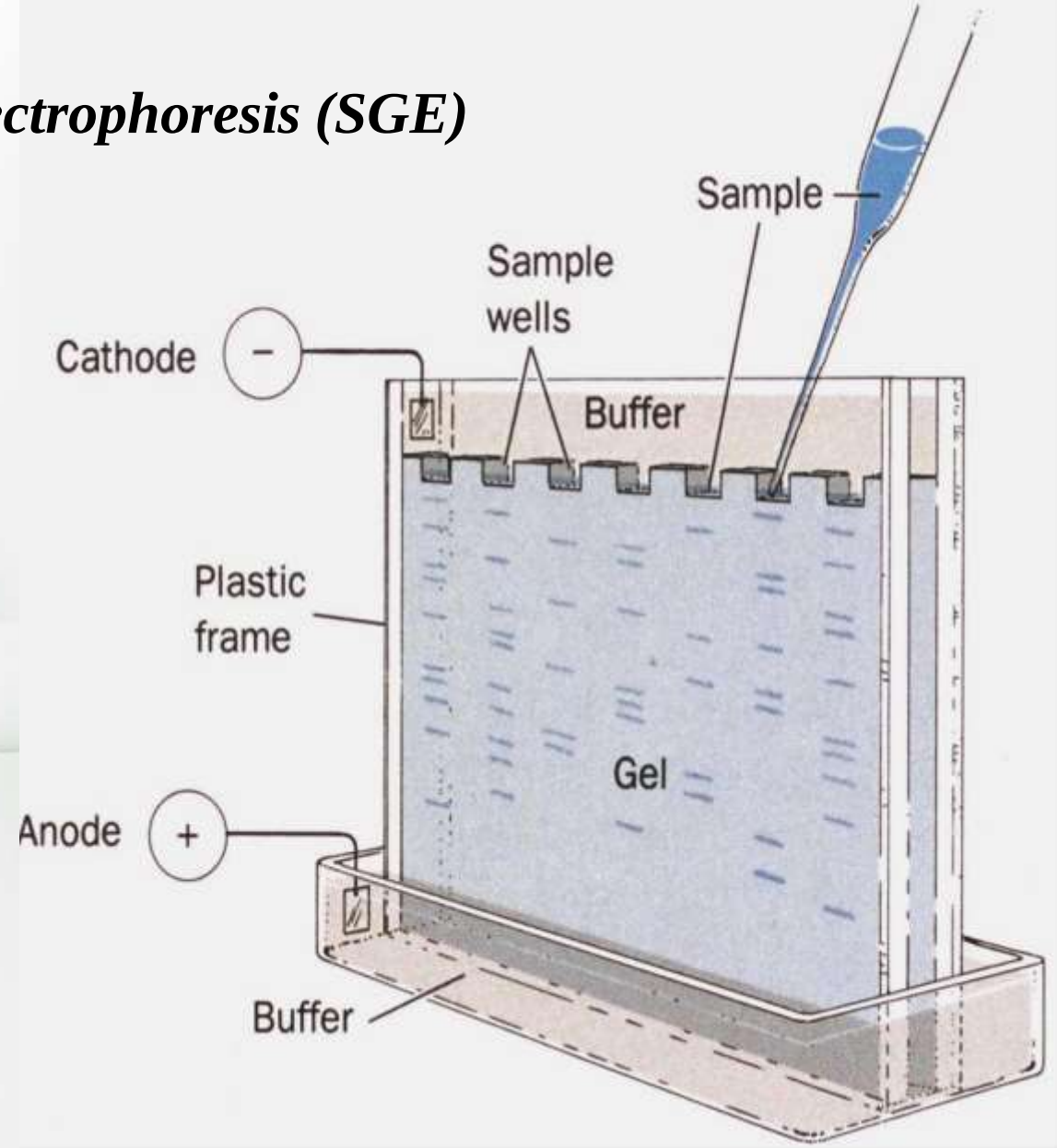
- ***Capillary***
- ***Slab***
- Native Polyacrylimide Gel Electrophoresis (PAGE)
- SDS-PAGE
- Paper

Slab electrophoresis:

a. This is the classic method that has been used for many years to separate complex , high molecular weight species of biological and biochemical interest.
b. Slab separations are carried out on a thin flat layer or slab of a porous semisolid gel containing an aqueous buffer solution within its pores.
c. Ordinarily this slab has dimensions of few centimeters on a side and like a chromatographic thin layer plate, is capable of separating several samples simultaneously.
d. Samples are introduced as spots or bands on the slab and a dc potential is applied across the slab for a fixed period.
e. When the separations are judged complete, the current is discontinued and the separated species are visualized by staining.

Slab Gel Electrophoresis (SGE)





Advantages:

- Allowing for two dimensional analysis, and of running multiple samples simultaneously in the same gel
- For laboratories performing routine nucleic acid analysis and those employing antigenic controls, slab gels have become standard

Disadvantages:

- Does not yield very precise quantitative information
- Labor intensive
- Difficult to automate

Capillary electrophoresis

- Capillary electrophoresis is the most rapidly expanding separation technique in pharmaceutical analysis and is a rival to HPLC in its general applicability
- A separation technique carried out in capillaries based solely on the differences in electrophoretic mobilities of charged species either in aqueous or non aqueous background electrolyte solutions

Advantages :

- Faster results and provides high resolution separation
- Offers new selectivity an alternative to HPLC
- Easy and predictable selectivity
- Small sample sizes
- Fast separations
- Quantitation
- Easily coupled to MS
- Different modes

Strengths :

- Potentially many times more efficient than HPLC in its separating power.
- Shorter analysis times than HPLC
- Cheaper columns than HPLC
- Negligible solvent consumption

Migration rates and plate heights in capillary elelctrophoresis:

•An ions migration velocity v depends upon the electric field strength

•The electric field in turn is determined by the magnitude of the applied potential (v in volts) and the length L over which it is applied. Thus velocity of an ion in an electric field

$$v = \mu e\, E$$

$$E = V/L$$

$$v = \mu e\, V/L$$

•This relationship indicates that high applied potentials are desirable to achieve rapid ionic migration and a rapid separation.

Plate heights in capillary eletrophoresis

- Van-deemter equation

$$H=A+B/\mu+C_{\mu}$$

- In capillary electrophoresis, very narrow capillary tube is used.
- No eddy diffusion (A) as tube is open multipath is not observed.
- As single phase is involved for electrophoresis (there is no stationary phase) there is no mass transfer (C).
- So for electrophoresis only a longitudinal diffusion need be considered

$$H=B/\mu$$

- Generally number of theoretical pates is given by

$$\mathbf{N = L/H}$$

Substitute $H = B/v = 2D/v$

$$v = \mu_e E = \mu_e V/L$$

$$N = L/B/v$$

$$N = L/2D \times \mu e\ V/L$$

- For electrophoresis that the plate count is given by

$$\mathbf{N = \mu e\ V/2D}$$

Where,

N = No of theoretical plates

μe = Electrophoretic mobility

V = Applied potential

D = Diffusion coefficient of solute

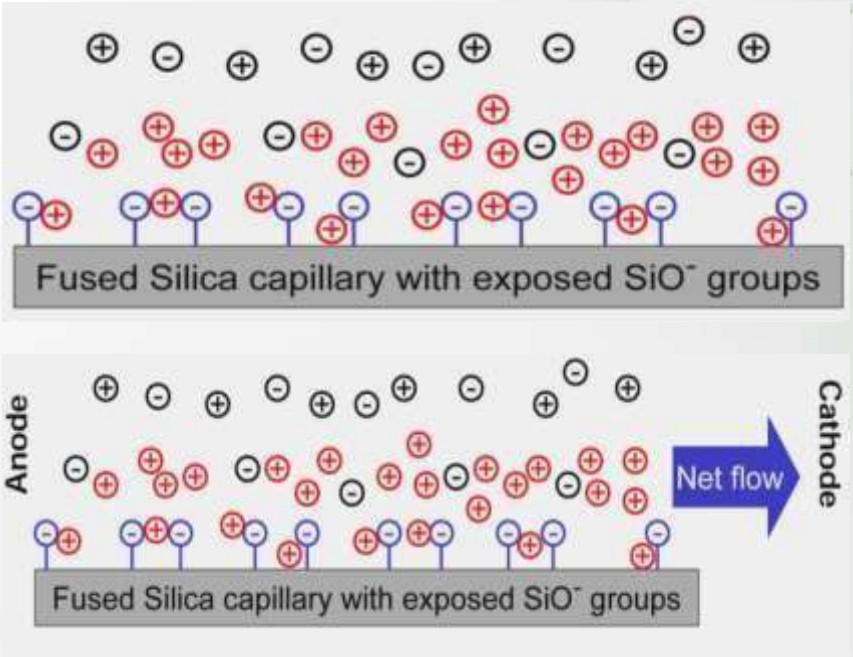
v = Migration velocity

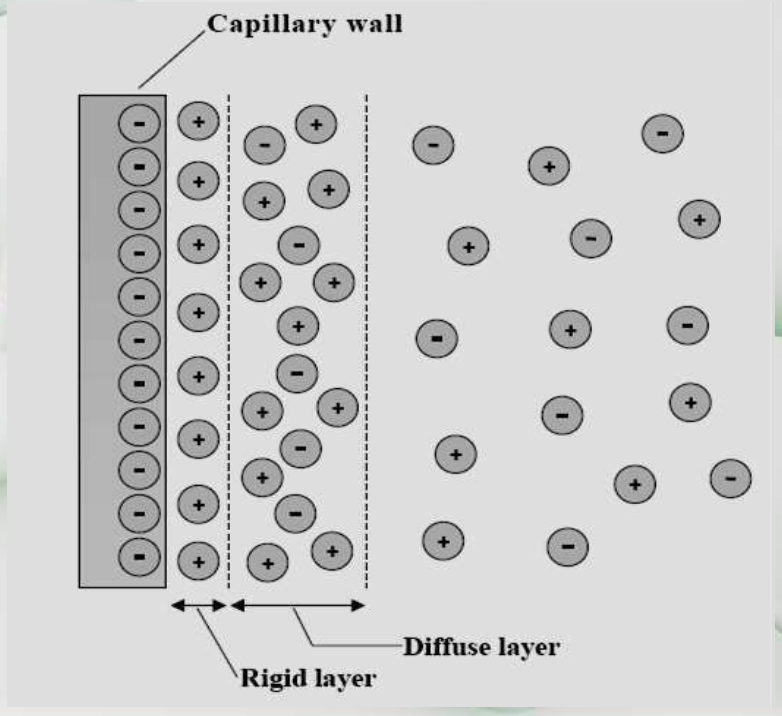
- Because resolution increases as the plate count increases, it is desirable to use high applied potential in order to achieve high resolution separations

Electro osmotic Flow (EOF)

•Relative movement of a liquid to a fixed charged surface caused by an electric field.

•When a high potential is applied across a capillary tube containing buffer solution ,electro osmotic flow usually occurs, in which the solvent migrates toward cathode or anode





- The inside wall of the capillary is covered by silanol groups (SiOH) that are deprotonated (SiO^-) at pH > 2
- SiO^- attracts cations to the inside wall of the capillary
- The distribution of charge at the surface is described by the Stern double-layer model and results in the zeta potential
- It would seem that CE separations would start in the middle and separate ions in two linear directions
- Excess cations in the diffuse Stern double-layer flow towards the cathode, exceeding the opposite flow towards the anode
- Net flow occurs as solvated cations drag along the solution

Electro osmotic mobility:

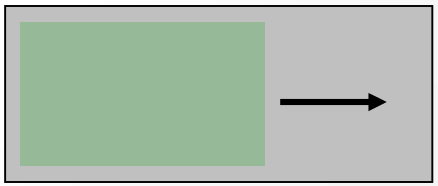
$$v = \mu_{eo} E = \left(- \frac{\varepsilon_0 \varepsilon \zeta}{4\pi\eta} \right) E$$

$$\mu_{eo} = \frac{\varepsilon_0 \varepsilon \zeta}{4\pi\eta}$$

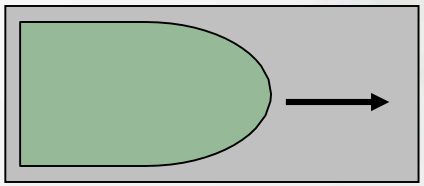
Where:
v = Electro osmotic mobility
εo= Dielectric constant of a vacuum
ε = Dielectric constant of the buffer
ζ = Zeta potential
E = Electric field

- Key factors that affect electro osmotic mobility:
  i. *dielectric constant and*
  ii. *viscosity of buffer (controls double-layer compression)*
- EOF can be quenched by protection of silanols or low pH

Electro osmotic Flow Profile

Anode  Cathode

Electro osmotic flow profile

High Pressure  Low Pressure

Hydrodynamic flow profile

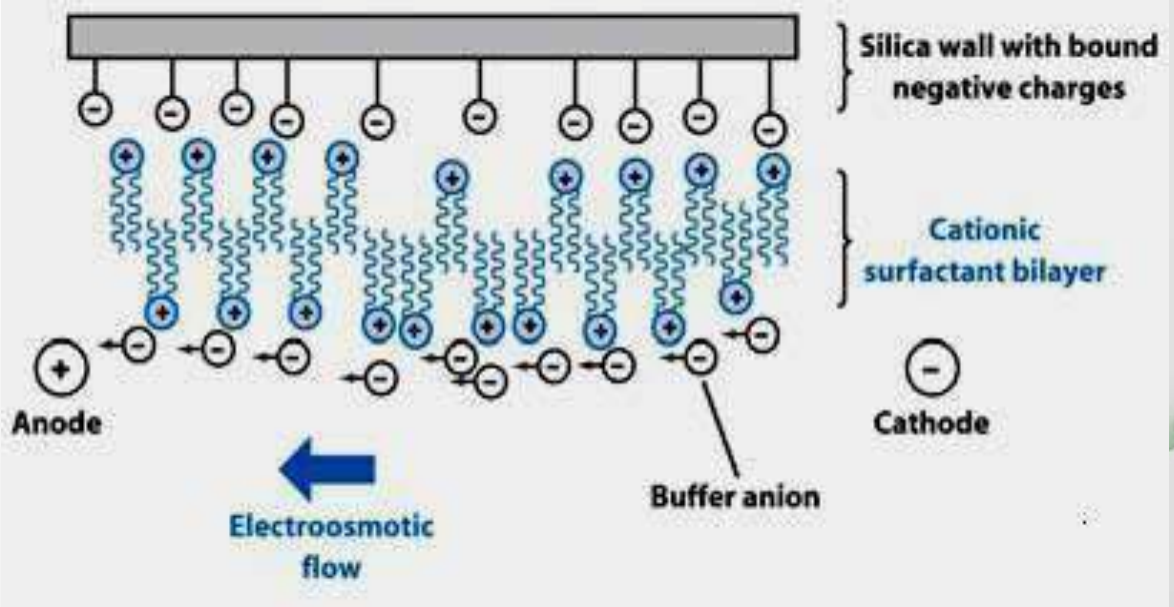
- •Driving force (charge along capillary wall)
- •No pressure drop is encountered
- •Flow velocity is uniform across the capillary
- •Electro driven flow **flat** flow profile

✓Frictional forces at the column walls cause a pressure drop across the column

✓Pressure driven flow (laminar flow) **Parabolic** flow

- ***Result:*** Electro osmotic flow does not contribute significantly to band broadening like pressure-driven flow in LC and related techniques

- It is possible to reverse the direction of the normal electro osmotic flow by adding a cationic surfactant to the buffer. The surfactant adsorbs on the capillary wall and makes the wall positively charged.



Electro osmotic flow can be eliminated by coating the inside capillary wall with a reagent like trimethyl chlorosilane to eliminate the surface silanol groups.

Control of EOF

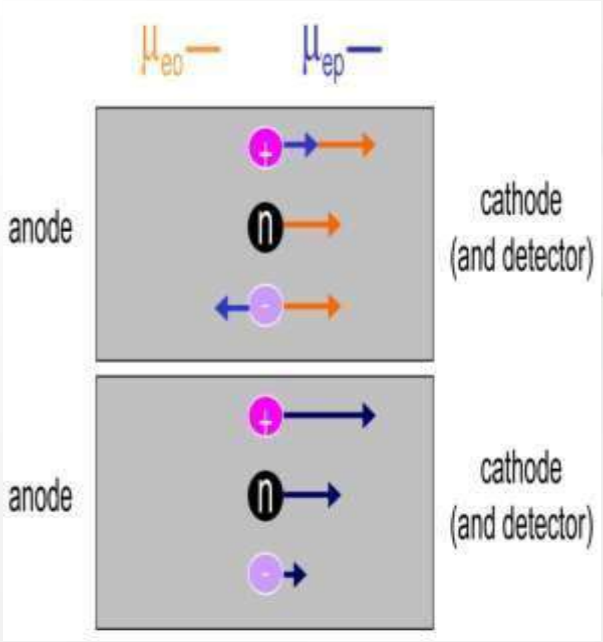
- **Electric field** — EOF proportional change
- **Buffer pH** — $\downarrow_{se}$ at low pH, $\uparrow_{se}$ at high pH

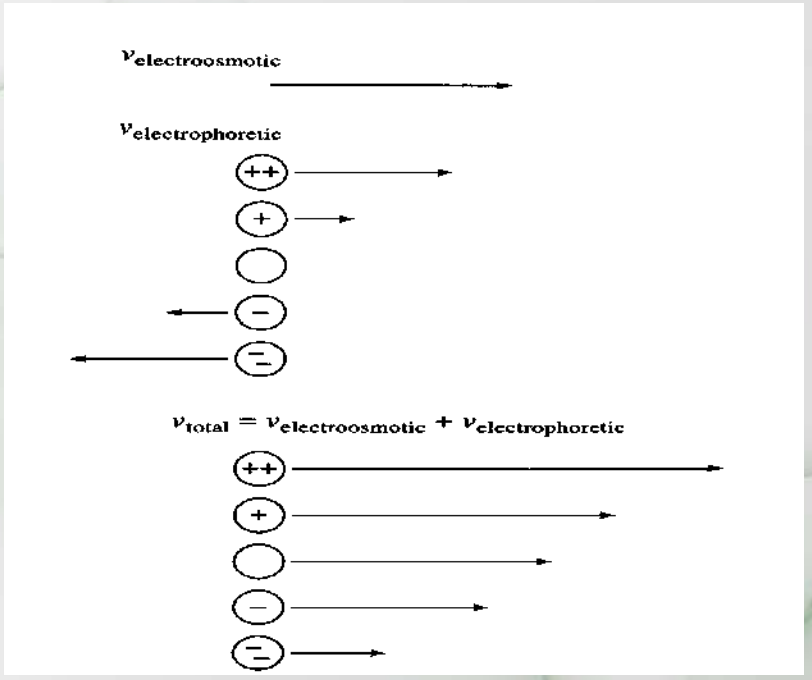
- **Ionic strength and buffer concentration** — Decrease ζ-potential and EOF
- **Temperature** — Changes η 3% per °C
- **Organic modifier** — Decrease ζ-potential and η
- **Surfactant** — Adsorbs to capillary wall
- **Neutral hydrophilic polymers** — Adsorbs to capillary wall
- **Covalent coating** — Stability

Electrophoresis and Electro osmosis

- Combining the two effects for migration velocity of an ion (also applies to neutrals, but with $\mu_{ep} = 0$):

$$\nu = \left(\mu_{ep} + \mu_{eo}\right)E = \left(\mu_{ep} + \mu_{eo}\right)\frac{V}{L}$$





A pictorial representation of the combined effect in a capillary

Instrumentation

1. **Sample Introduction**

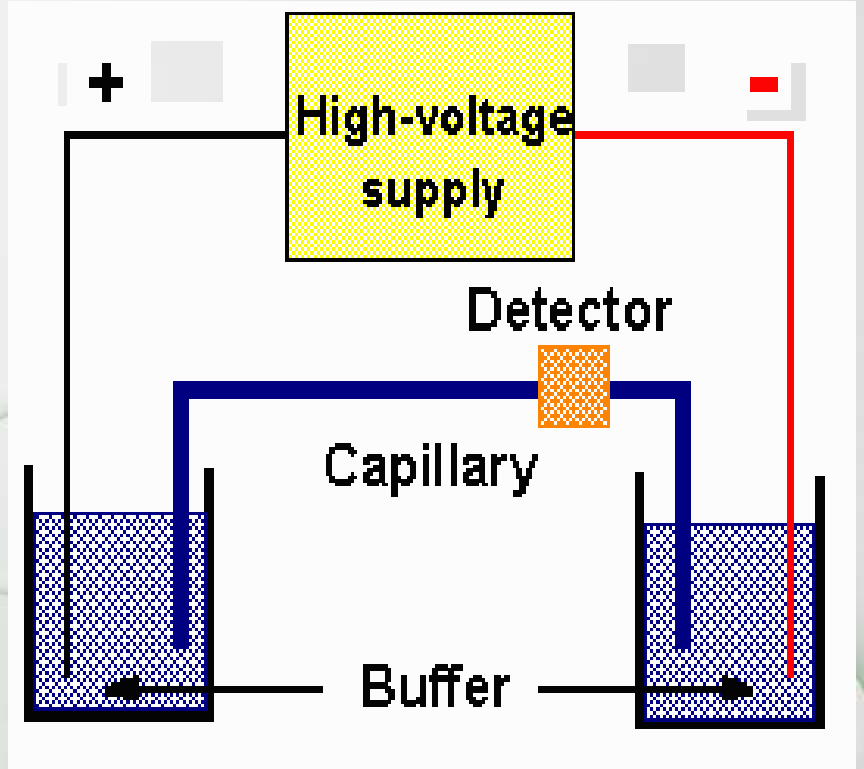
   Electro kinetic Injection

   Hydrodynamic injection

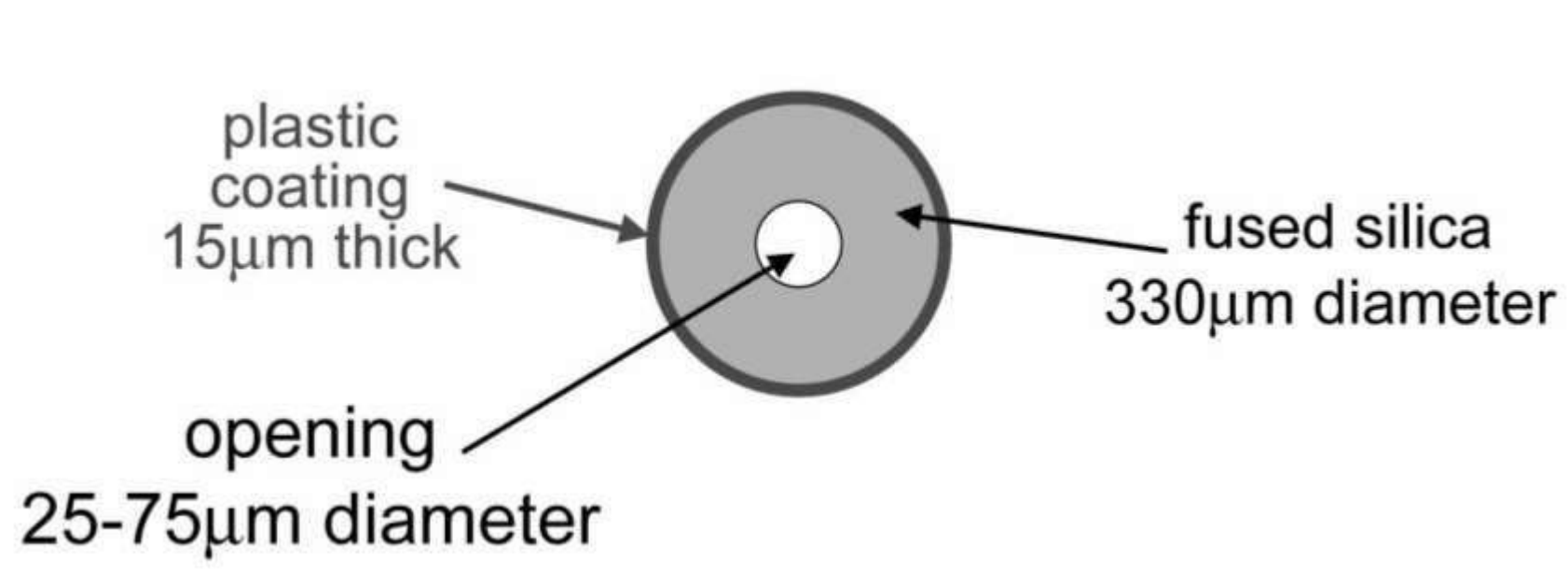
2. **Detection**

   Absorbance Methods

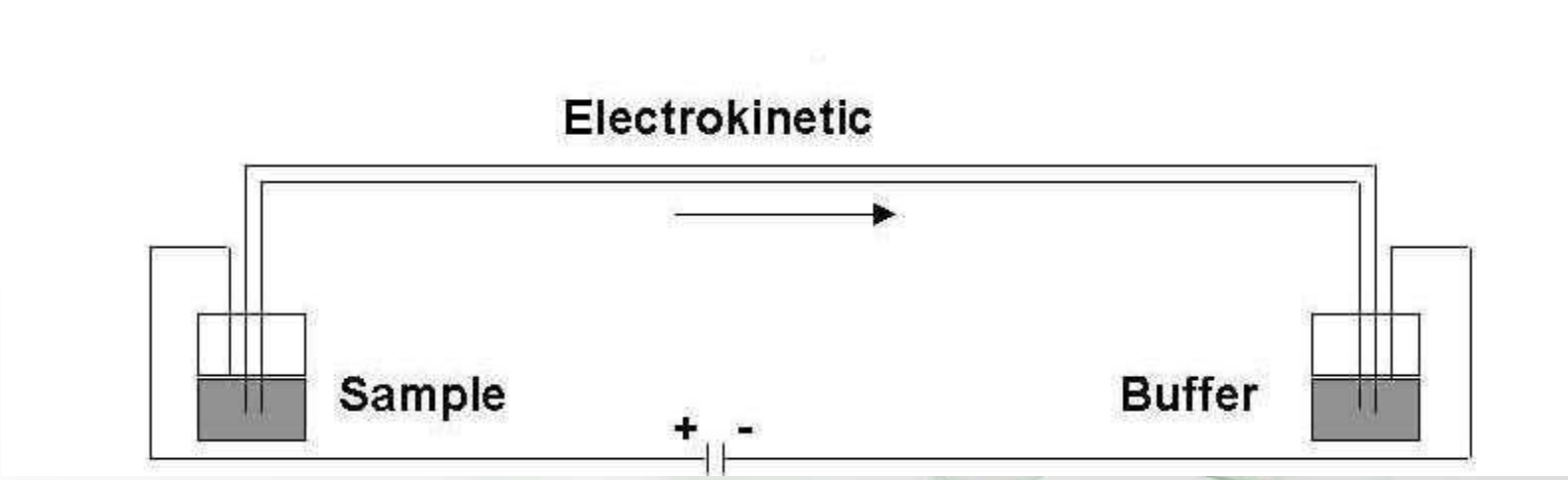
   - Indirect Detection
   - Fluorescence Detection
   - Electrochemical Detection
   - Mass Spectrometric Detection

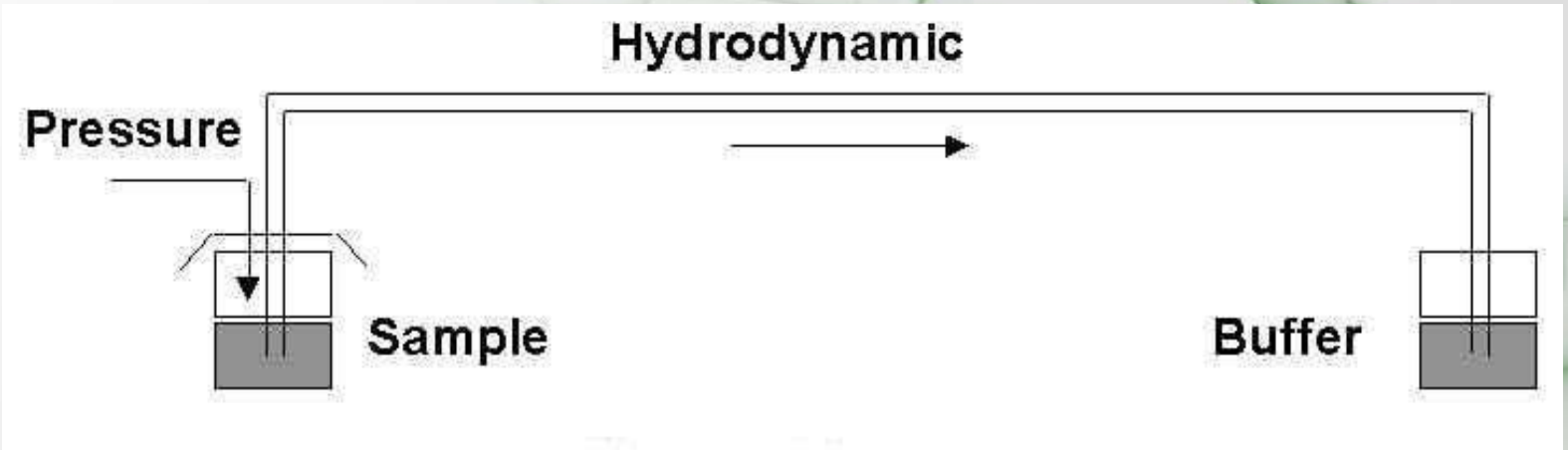


Cross-section of a capillary:



Sample introduction

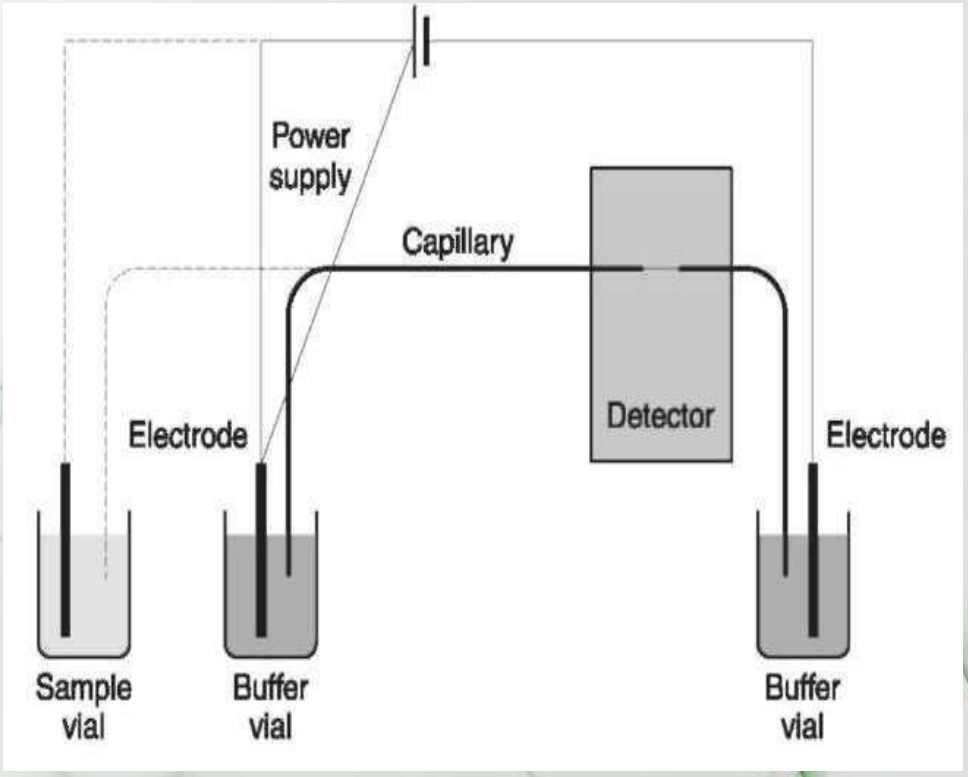




•Elelctrokinetic injection technique discriminates by injecting larger amounts of the more mobile ions relative to the slower moving ions.

•Pressure injection does not discriminate due to ion mobility, but can be used in gel-filled capillaries.

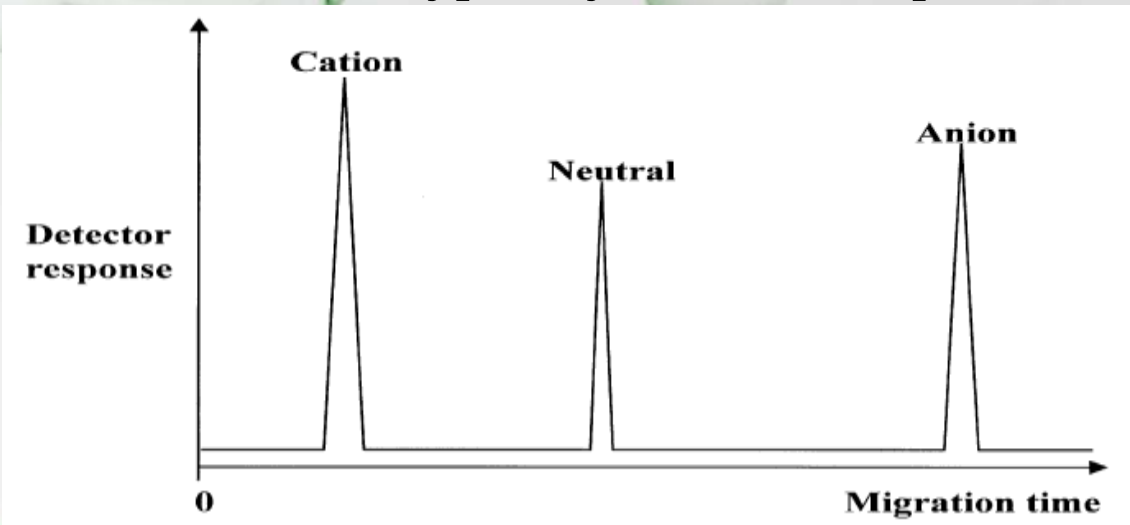
•For both electrokinetic injection and pressure injection the volume injected is controlled by the duration of the injection.



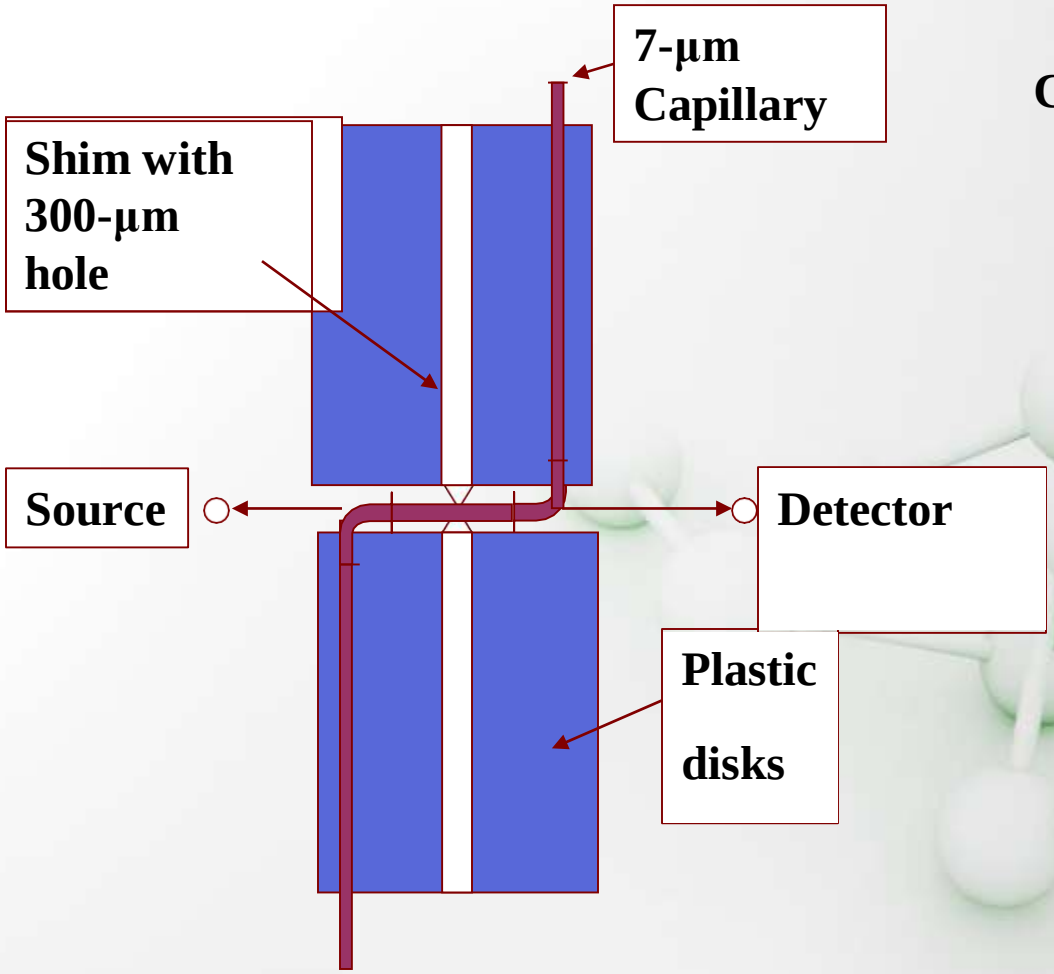
Detectors:

- Detectors are similar in design and function to those described for HPLC.
- In capillary electrophoresis analyte bands pass through the detector at different rates, results in that peak areas are somewhat dependent upon retention times.
- Detectors are placed at the cathode since under common conditions, all species are driven in this direction by EOF.
- Detectors similar to those used in LC, typically UV absorption, fluorescence, and MS.

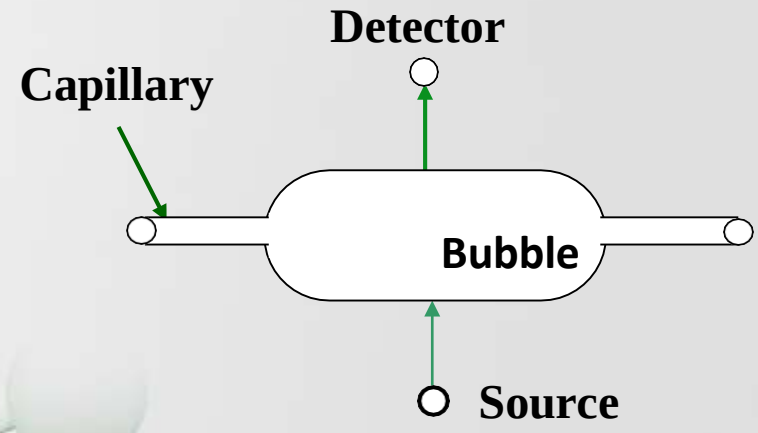
The general layout of an electropherogram



- Detection is performed **on-column**
- A section of the protective polyamide coating is removed from the exterior of the capillary by burning, dissolution, or scrapping.
- In these systems, this section of the capillary itself is used as the detection cell.
- The pathlength of the detection cell in capillary electrophoresis (~ 50 µm) is far less than that of a traditional UV cell (~ 1 cm).
- According to the **Beer-Lambert law**, the sensitivity of the detector is proportional to the path length of the cell.
- In order to improve the sensitivity of absorbance measurements, several techniques have been suggested for increasing the pathlength of the measurements.



The 3-mm z cell



The 150µm bubble cell



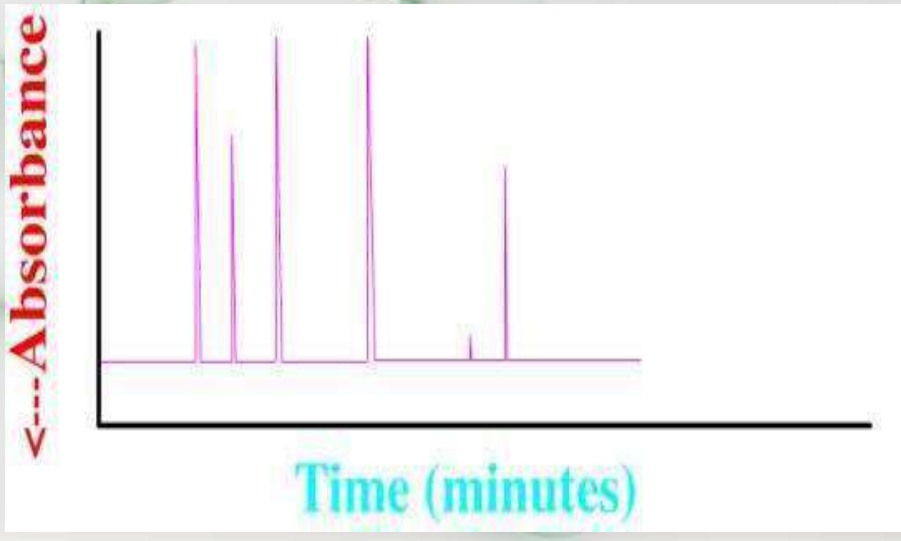
The multireflection cell

Indirect detection:

- Indirect absorbance detection has been used for detection of species that are difficult to detect because of low absoptivities.
- In this method a UV absorbing species is added to the buffer, producing a constant UV signal.
- Arrival of the poorly absorbing analyte yields a drop in absorbance.
- The analyte is then determined from the decrease in absorbance.

CE electropherogram

- Like a chromatogram, it is a plot of the **time from injection** on the x axis vs. **the detector signal** on the y axis.



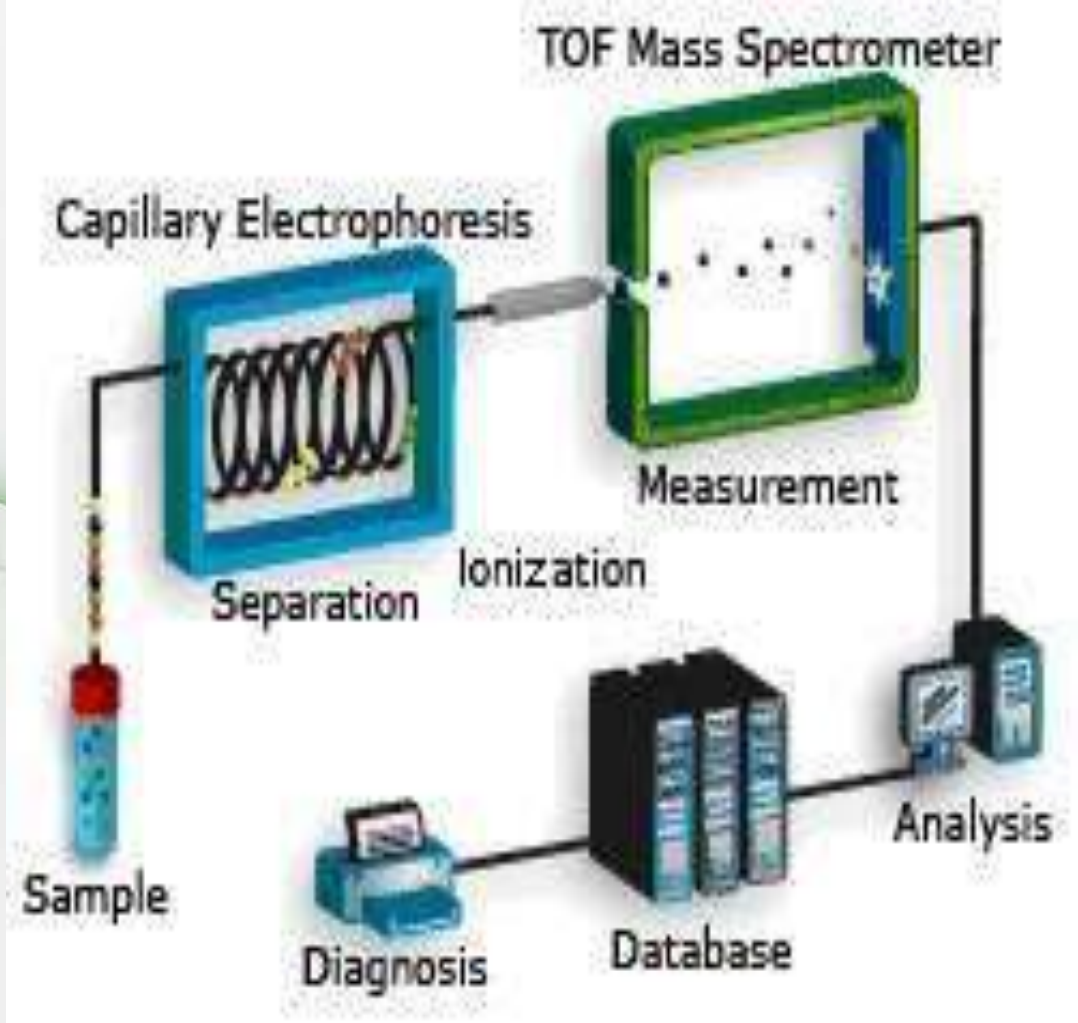
Fluorescence detection:

- Fluorescence detection can also be used in capillary electrophoresis for samples that naturally fluoresce or are chemically modified to contain fluorescent tags.
- This mode of detection offers high sensitivity and improved selectivity for these samples, but cannot be utilized for samples that do not fluoresce.
- Laser -based instrumentation is preferred in order to focus the excitation radiation on the small capillary.
- Laser -induced fluorescence has been used in CE systems with detection limits as low as 10−18 to 10−21 mol.
- The sensitivity of the technique is attributed to the high intensity of the incident light and the ability to accurately focus the light on the capillary

Mass spectrometric detection

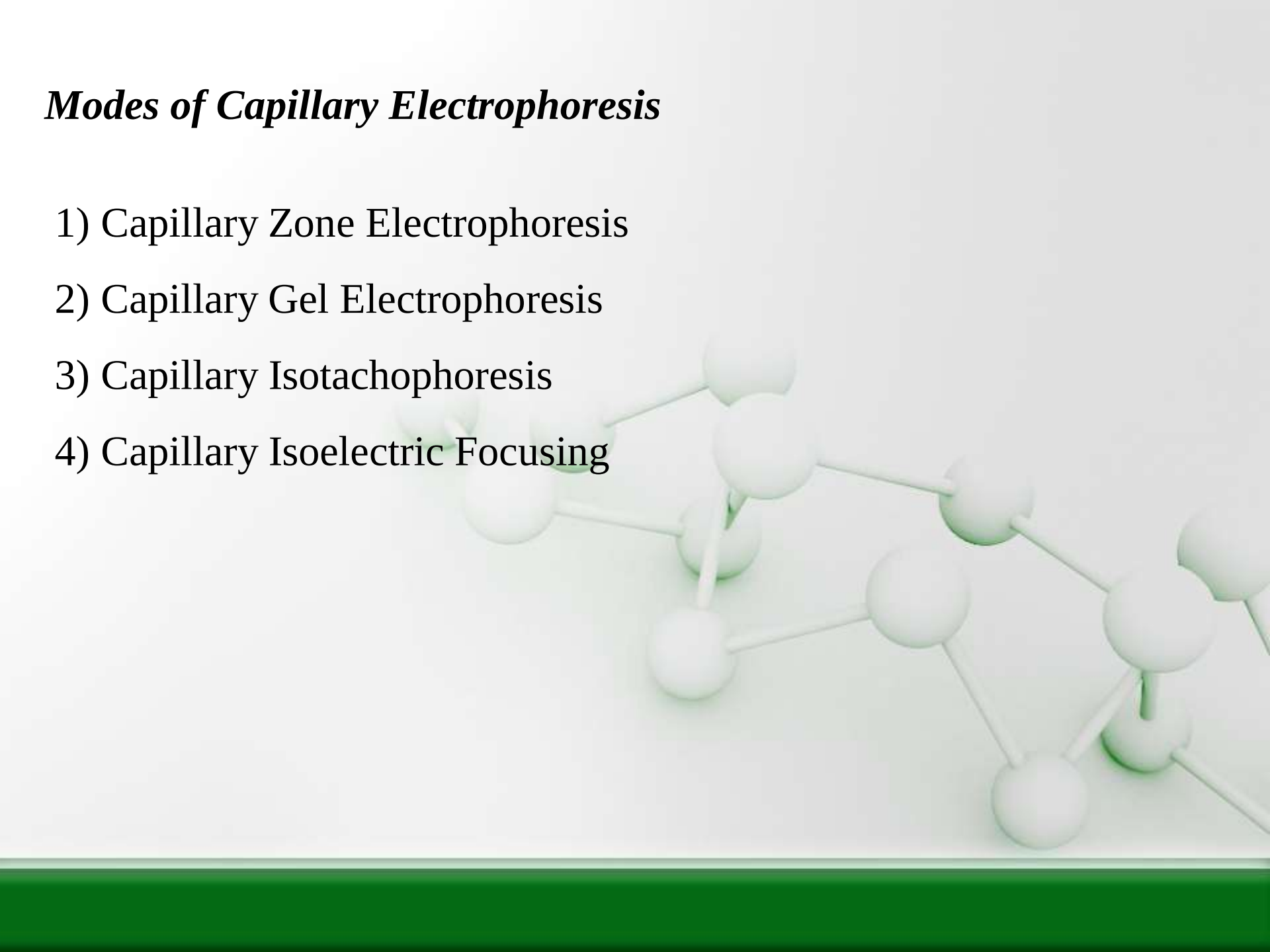
•In order to obtain the identity of sample components capillary electrophoresis can be directly coupled with mass spectrometers.

•Used for determination of large molecules such as DNA fragments, proteins peptides and etc…



Modes of Capillary Electrophoresis

1) Capillary Zone Electrophoresis
2) Capillary Gel Electrophoresis
3) Capillary Isotachophoresis
4) Capillary Isoelectric Focusing

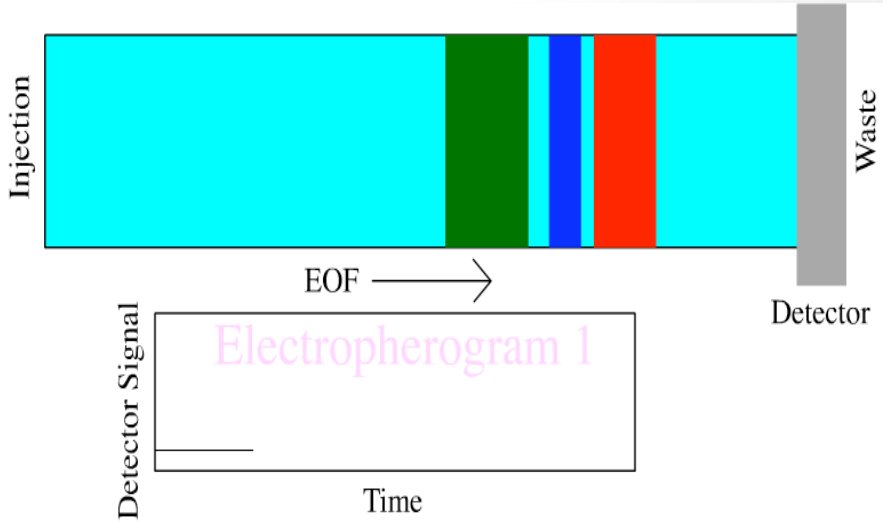


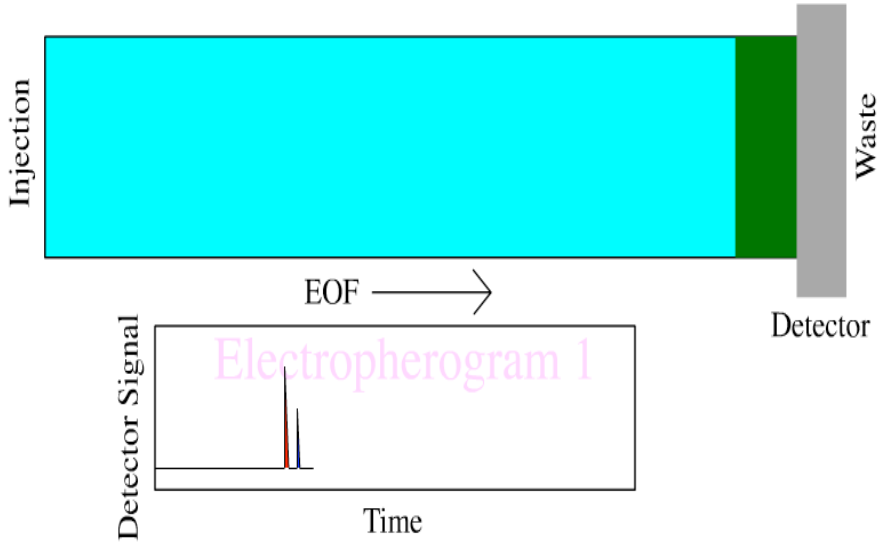
Capillary zone electrophoresis

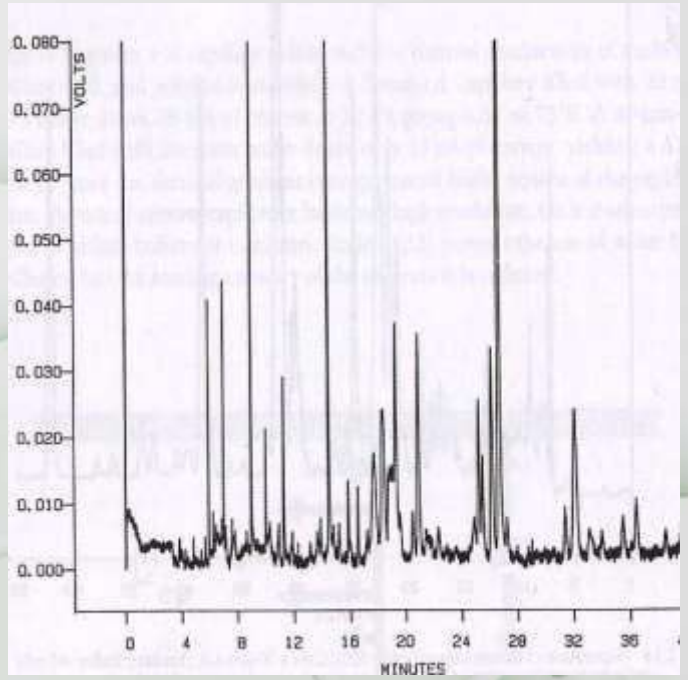
- **CZE** also known as free-solution CE (FSCE), is the simplest form of CE .
- Fundamental to CZE are homogeneity of the buffer solution and constant field strength throughout the length of the capillary.
- The separation mechanism is based on difference in the charge-to-mass ratio of the analytes.
- CZE analytes move in the EOF but separate into bands because of differences in their electrophoretic mobilities, μ.

Applications:

- A variety of small synthetic herbicides ,pesticides ,and pharmaceuticals that are ionic have been separated and analyzed by CZE.
- Proteins ,amino acids, and carbohydrates have all been separated in minimum times by CZE.


Injection
Waste
EOF
Detector
Detector Signal
Electropherogram 1
Time


Injection
Waste
EOF
Detector
Detector Signal
Electropherogram 1
Time


0.080
0.070
0.060
0.050
0.040
0.030
0.020
0.010
0.000
VOLTS
0
4
8
12
16
20
24
28
32
36
MINUTES

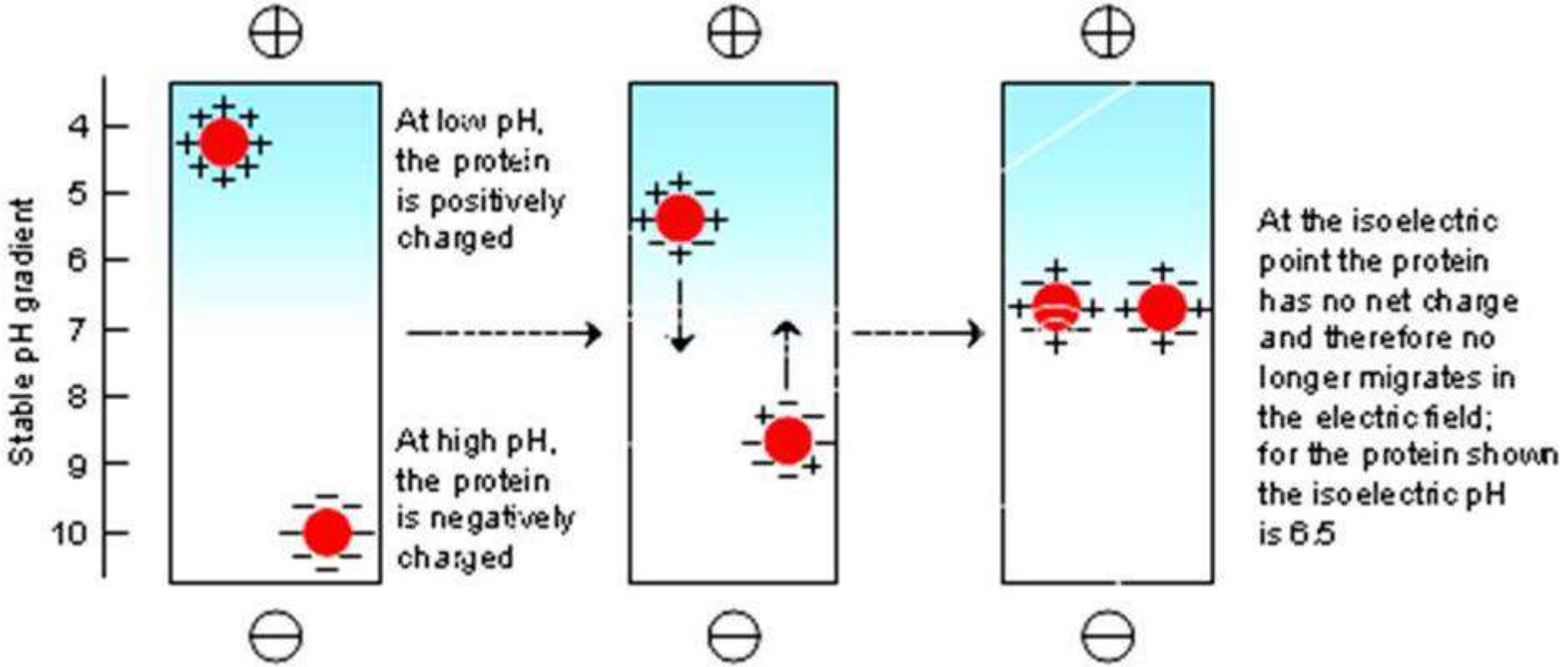
Capillary Gel Electrophoresis (CGE)

- **Capillary Gel Electrophoresis (CGE)** is the adaptation of traditional gel electrophoresis into the capillary using polymers in solution to create a molecular sieve also known as replaceable physical gel.
- This allows analytes having similar charge-to-mass ratios to also be resolved by size.
- This technique is commonly employed in SDS-Gel molecular weight analysis of proteins and in applications of DNA sequencing and genotyping.

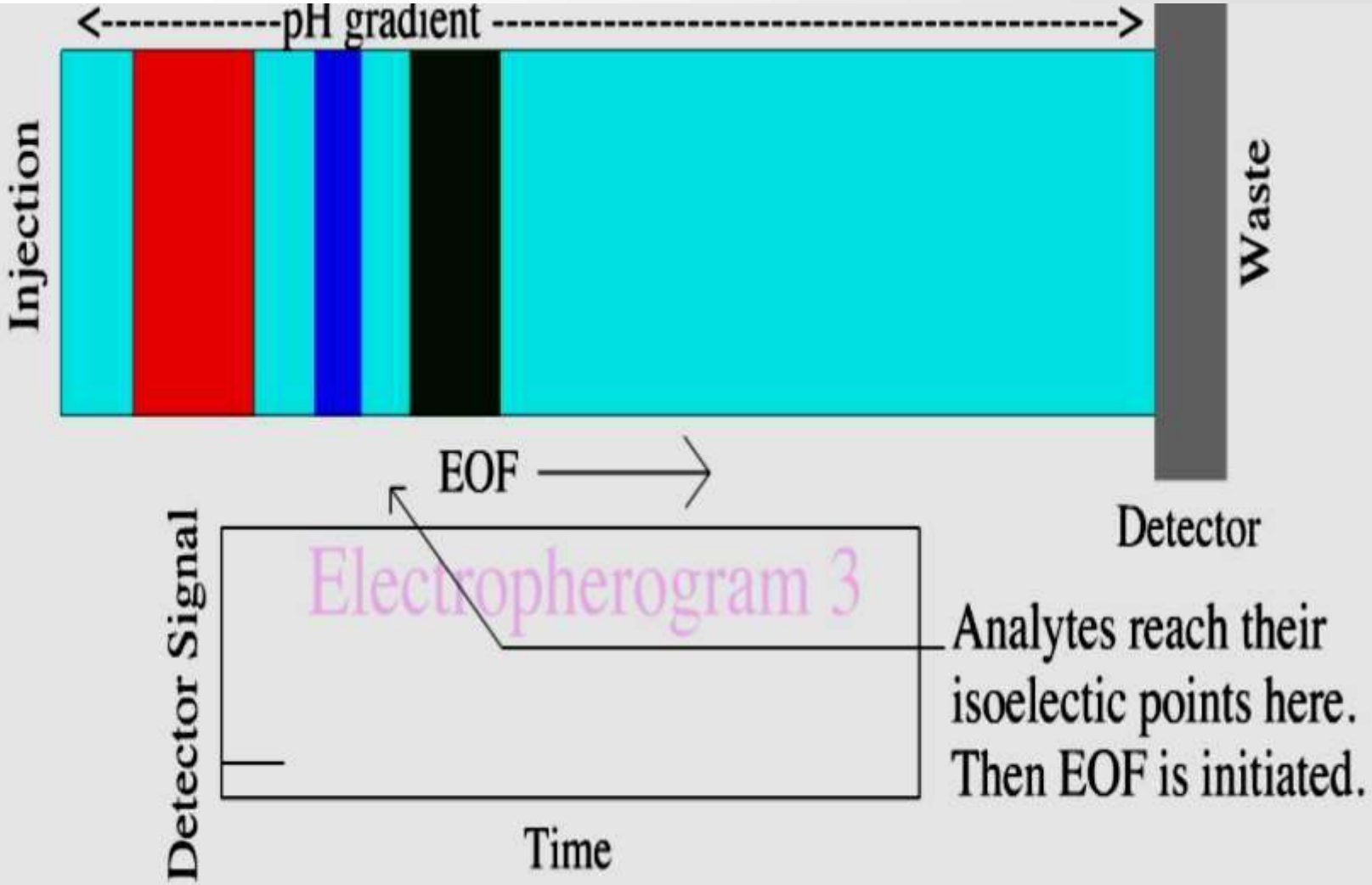
Capillary Isoelectric Focusing (CIEF)

- **Capillary Isoelectric Focusing (CIEF)** allows amphoteric molecules, such as proteins, to be separated by electrophoresis in a pH gradient generated between the cathode and anode.
- A solute will migrate to a point where its net charge is zero. At the solute's isoelectric point (pI), migration stops and the sample is focused into a tight zone.
- In CIEF, once a solute has focused at its pI, the zone is mobilized past the detector by either pressure or chemical means. This technique is commonly employed in protein characterization as a mechanism to determine a protein's isoelectric point.

Separation of protein molecules by isoelectric focussing



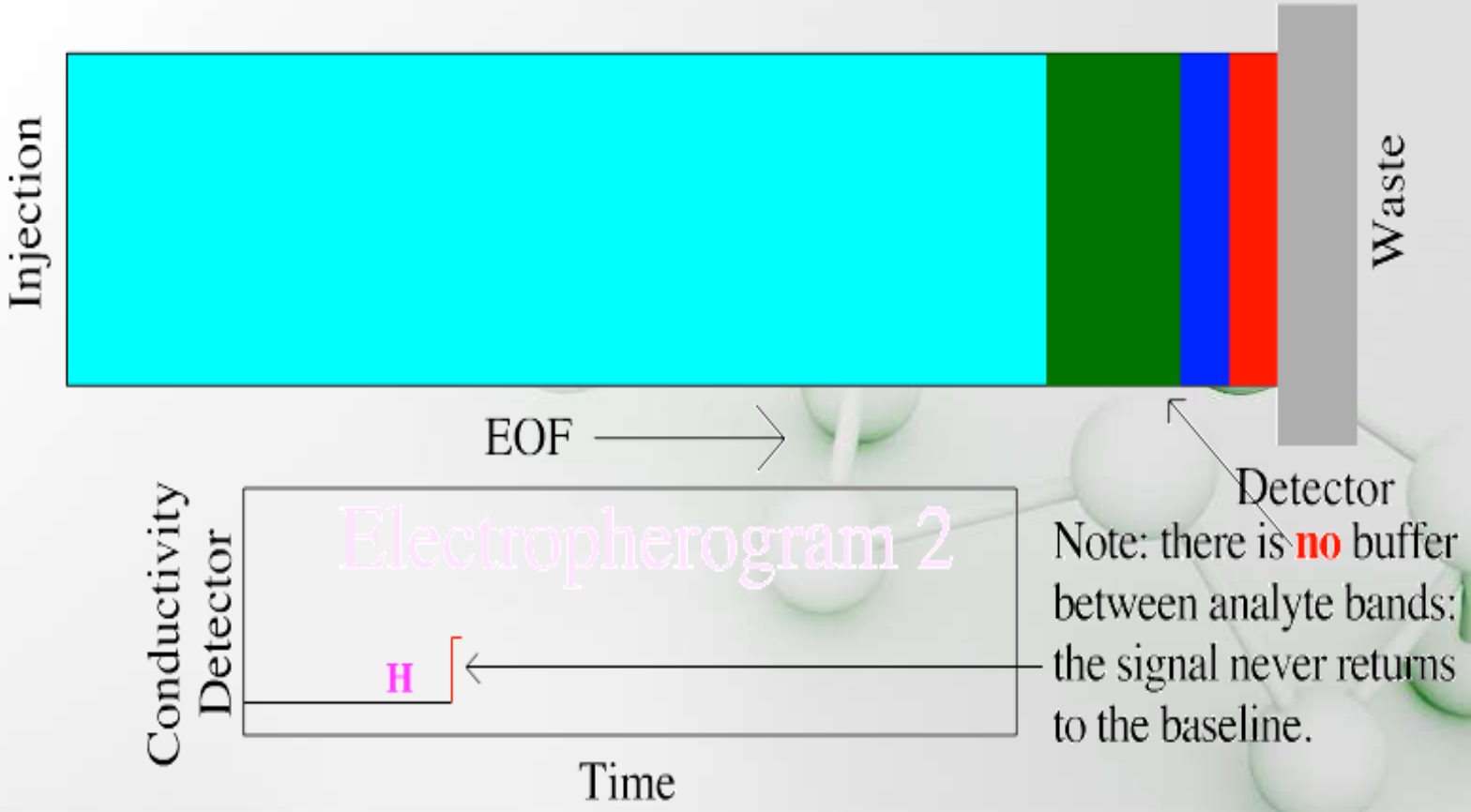
Capillary Isoelectric Focusing (CIEF)



Capillary Isotachophoresis (CITP)

- Capillary Isotachophoresis is a focusing technique based on the migration of the sample components between leading and terminating electrolytes.
- Solutes having mobilities intermediate to those of the leading and terminating electrolytes stack into sharp, focused zones.
- Although it is used as a mode of separation, transient ITP has been used primarily as a sample concentration technique.
- To concentrate the trace compounds in the dilute sample

Capillary Isotachophoresis (CITP)



Applications

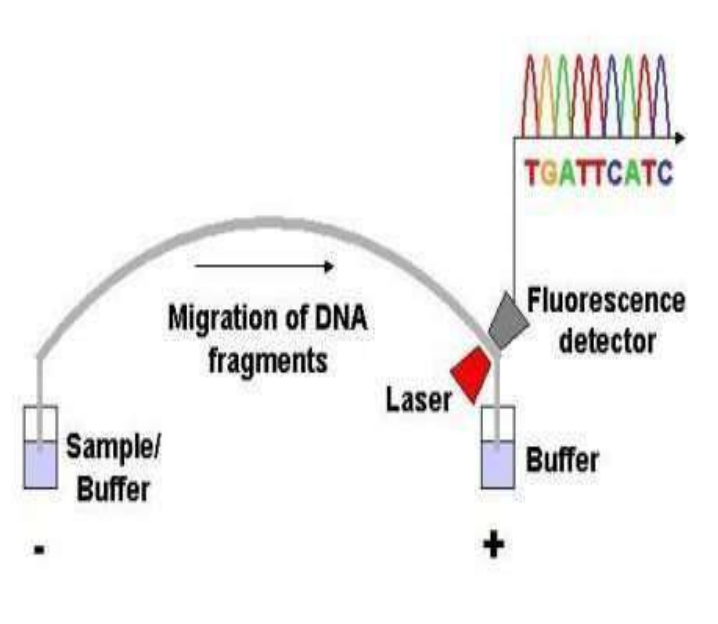
- **Pharmaceutical:** Reaction intermediates, purity validation, stability, final product testing, ion analysis, counter-ions (includes low MW, charged and neutrals, chirals)
- **Bioscience:** Peptides, proteins, DNA, carbohydrates
- **Foods:** Inorganic cations/anions, organic acids, amino acids, carbohydrates
- **Chemical:** Pesticides, PAHs, inorganic ions, transition metals, surfactants, dyes, polymers
- **Forensic:** Drugs of abuse, explosive residue, gun powder residue

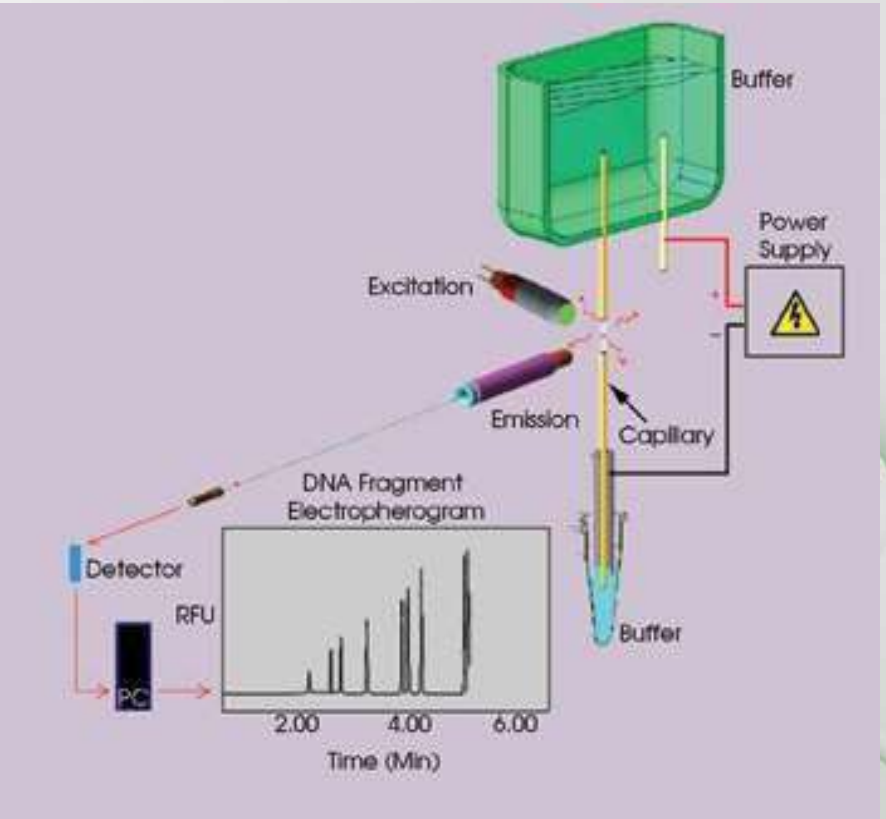
Applications of CE in pharmaceutical analysis

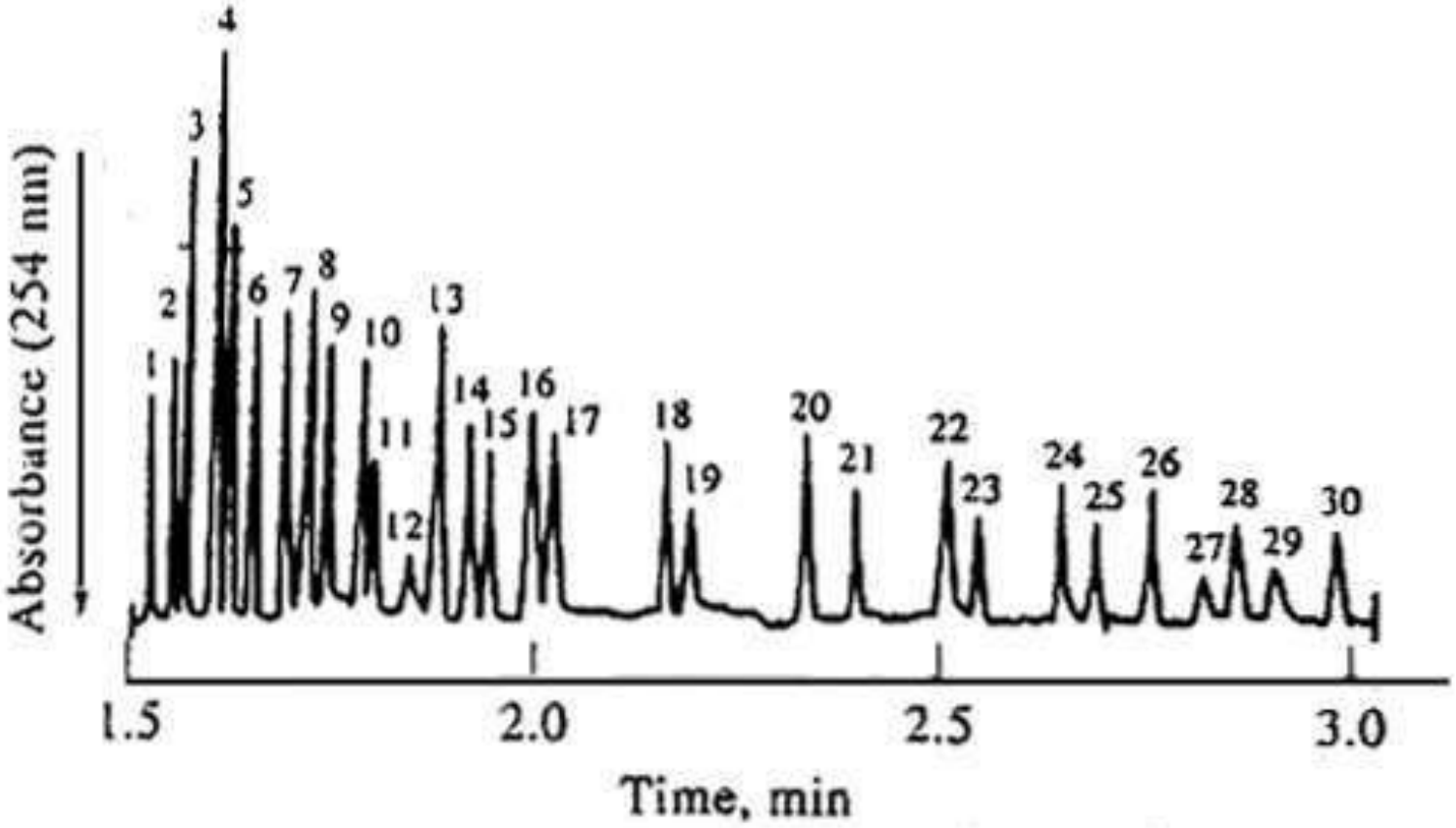
- CE has been heavily studied within the pharmaceutical industry as an alternative to LC in various situations
- Separation of atenolol and related impurities predominantly on the basis of charge
- Separation predominantly on the basis of ionic radius
- Analysis of non-steroidal anti-inflammatory drugs by CE and separation of anions on the basis of ionic radius
- Separation of peptides
- The areas of pharmaceutical analysis covered are enantiomer separation, analysis of small molecules such as amino acids or drug counter-ions, pharmaceutical assay,, and physiochemical measurements such as log P and pKa of compounds.

- The vast applications of electrophoresis are most evident in the health or medical industry, including antibiotic and vaccine analysis.
- Protein and DNA analysis are also important electrophoresis applications.
- Then through electrophoresis, the amount of proteins in your blood or in your urine is measured and compared to established normal values---lower or higher than the normal levels usually indicates a disease.
- There are several vaccines that have been purified, processed and analyzed through electrophoresis, such as the influenza vaccine, hepatitis vaccine and polio vaccine.
- With electrophoresis, experts are not only able to synthesize new antibiotics but are also able to analyze which types of bacteria are antibiotic-resistant.
- Through electrophoresis, specific DNA sequences can be analyzed, isolated and cloned.
- The analyzed DNA may be used in forensic investigations and paternity tests

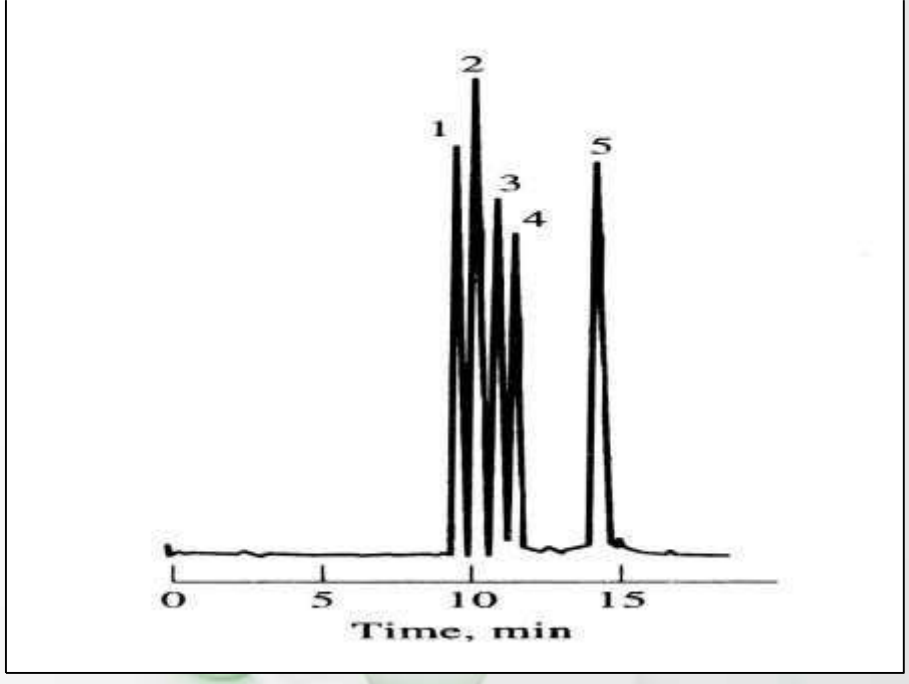
- During capillary electrophoresis, the products of the cycle sequencing reaction are injected electro kinetically into capillaries filled with polymer. High voltage is applied so that the negatively charged DNA fragments move through the polymer in the capillaries toward the positive electrode.





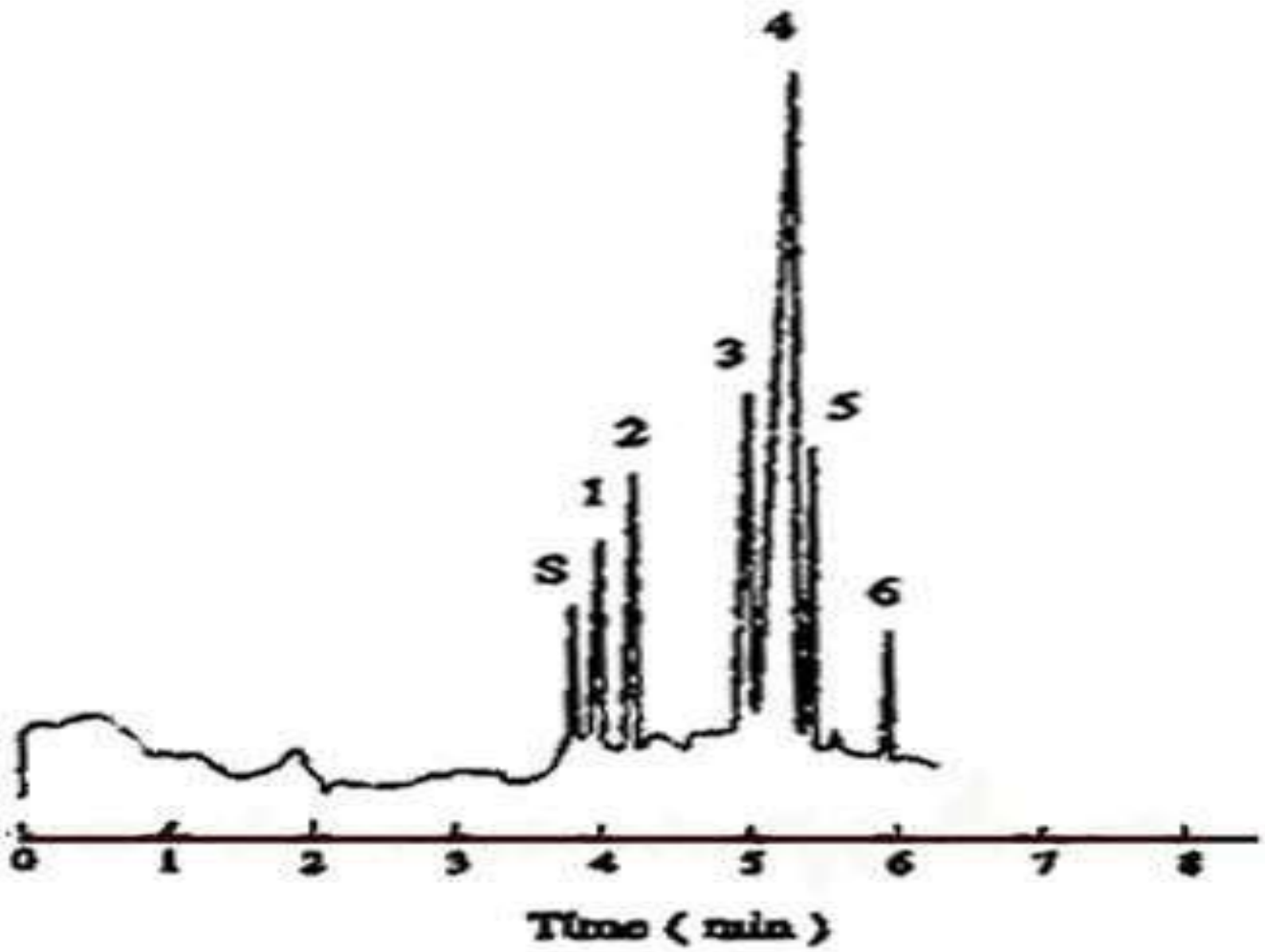


Electropherogram showing the separation of 30 anions. Capillary internal diameter: 50µm(fused silica). Detecton: indirect UV, 254nm.



Model Proteins separated at pH 2.7

Peak No.	*Proteins*	*mol. Wt.*	*Isolelectric Point, PI*
1	Cytochrome c	12,400	10.7
2	Lysozyme	14,000	10.1
3	Trypsin	24,000	10.1
4	Trypsinogen	23,700	8.7
5	Trypsin inhibitor	20,100	4.5



S: system peak; 1: ammonium; 2: potassium; 3: calcium; 4: sodium; 5: magnesium; 6: zinc

Conclusion:

- The capillary electrophoretic methods have been recognized: lower equipment costs, smaller sample size requirements, much greater speed, high resolution, precision, accuracy and sensitivity.
- Compared to conventional HPLC methods, the capillary electrophoresis methods have better resolution, less consumption of buffer solution and the absent organic solvents in the analysis.

References:

1. Pharmaceutical analysis: a textbook for pharmacy students and pharmaceutical chemists, David G. Watson, pg.no:333-353.
2. Principles of instrumental analysis, 5th edition ,Skoog. Holler.Nieman pg no:779-795.
3. en.wikipedia.org
4. www.ncbi.nlm.nih.gov
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Thank u

GAS CHROMATOGRAPHY

M.Kishore

Associate Professor

Vikas College of Pharmaceutical Sciences

GAS CHROMATOGRAPHY

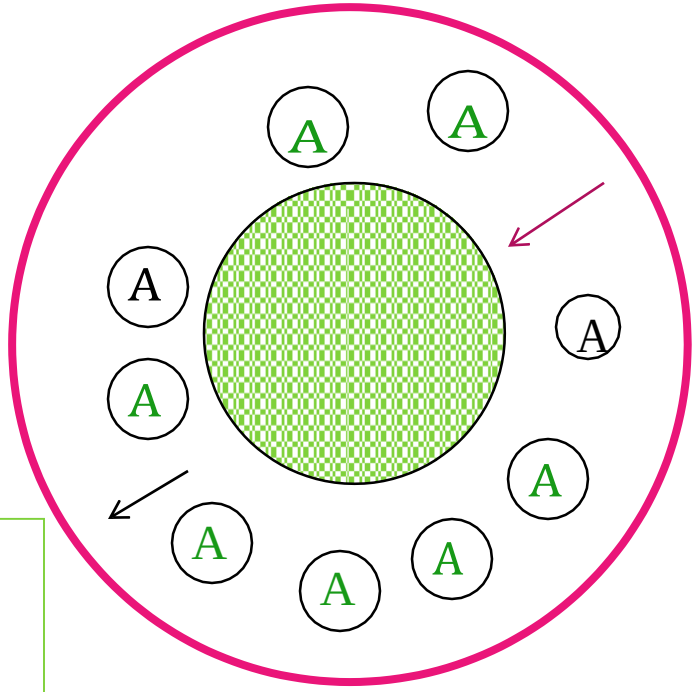
Chromatography is based on a physical equilibrium that results when a solute is transferred between the mobile phase and a stationary phase.

K = distribution coefficient or partition ratio

$$K = \frac{C_S}{C_M}$$

Where C_S is the molar concentration of the solute in the stationary phase and

C_M is the molar concentration of solute in the mobile phase.

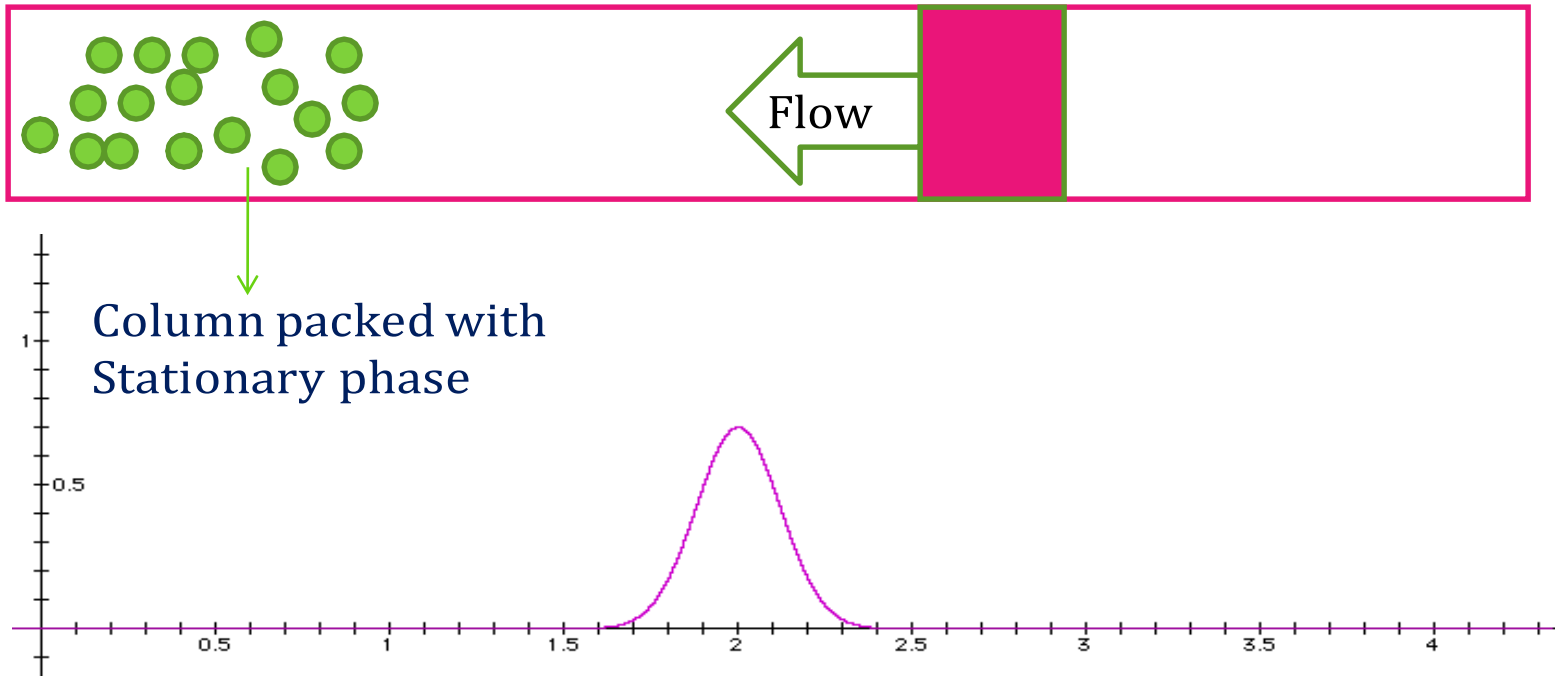


Cross Section of Equilibrium in a column.

"A" are adsorbed to the stationary phase.

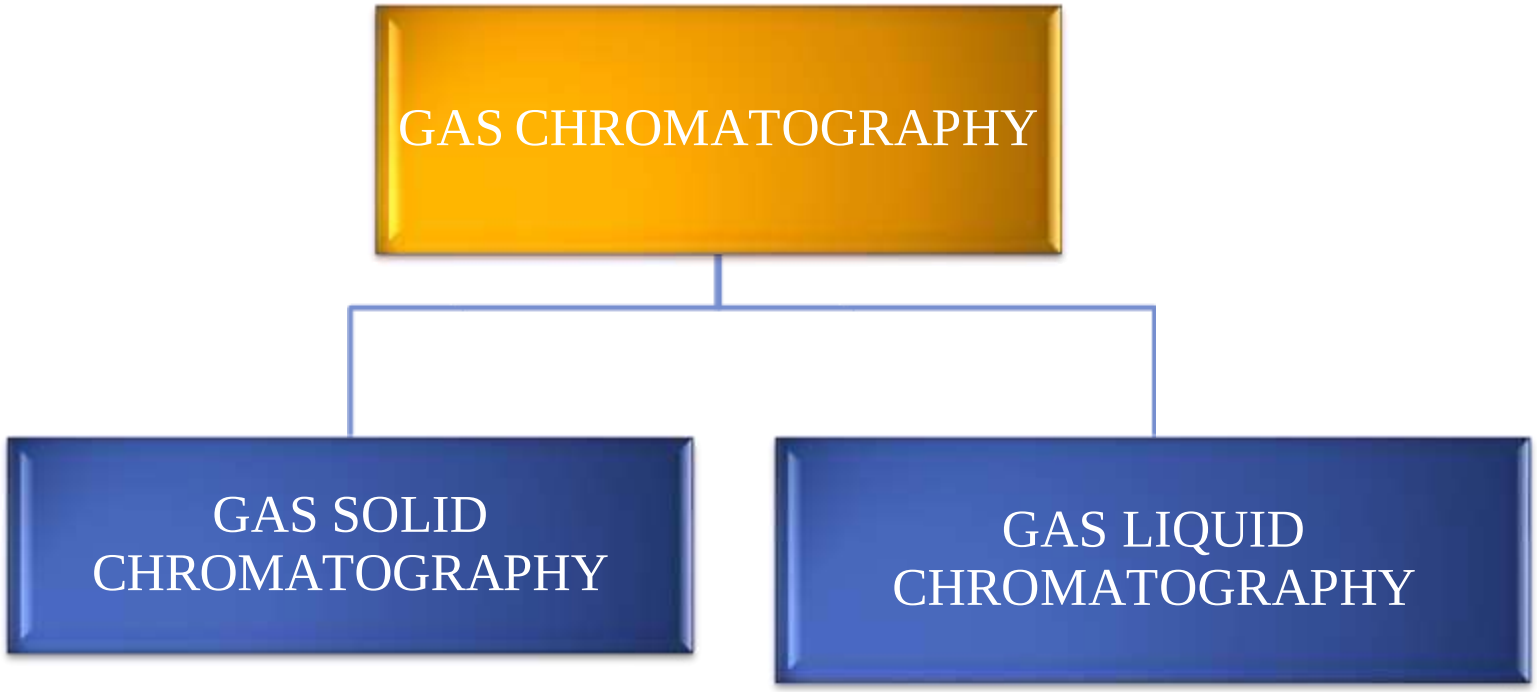
"A" are traveling in the mobile phase.

In a chromatography column, flowing gas or liquid continuously replaces saturated stationary phase and results in movement of sample through the column.



As a material travels through the column, it assumes a Gaussian concentration profile as it distributes between the stationary packing phase and the flowing mobile gas or liquid carrier phase.

Gas chromatography is probably the most utilized of the chromatographic techniques.



In both types, gas is used as mobile phase and either solid or liquid is used as stationary phase.

GAS SOLID CHROMATOGRAPHY

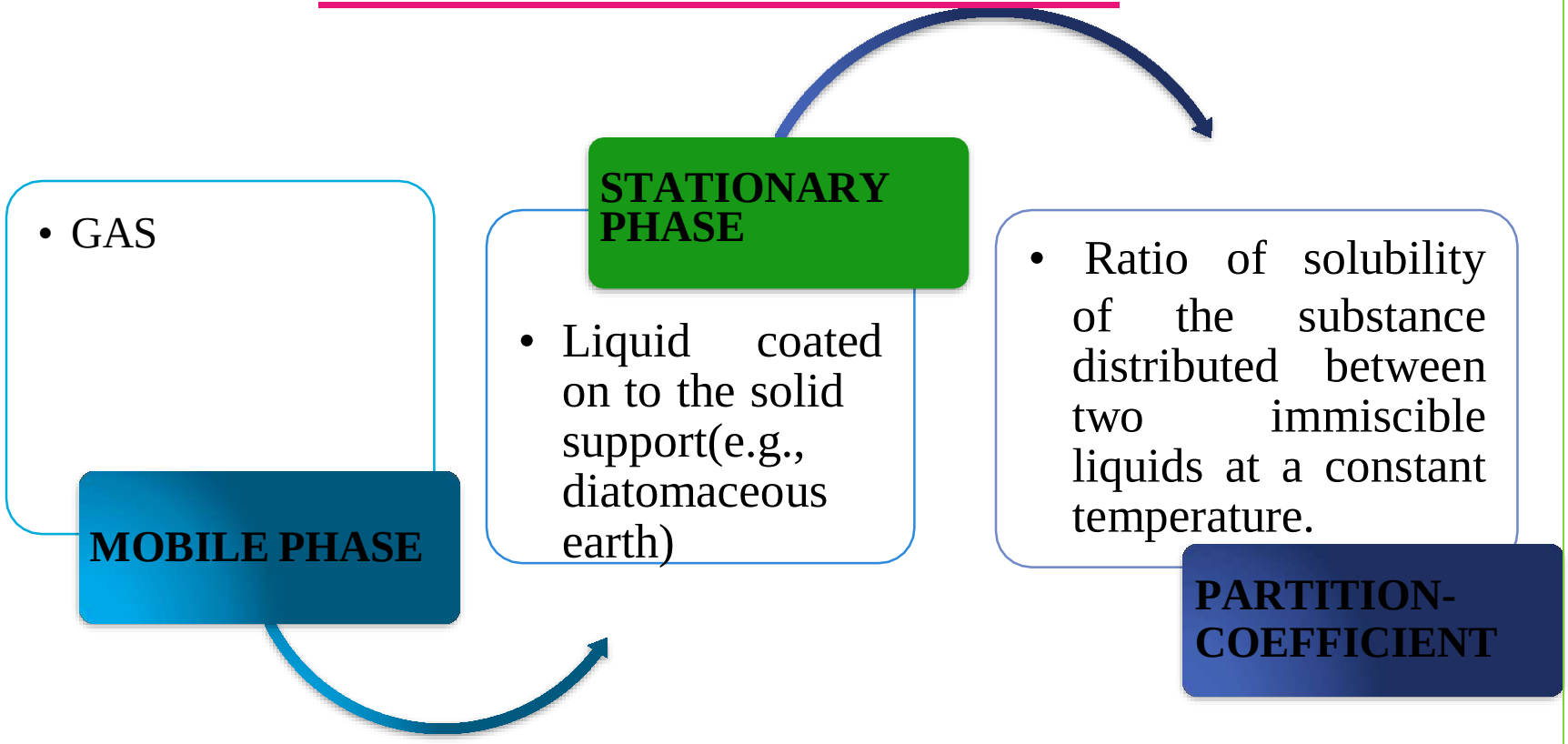
- Based upon the solid stationary Phase in which retention of analytes is a consequence of Physical adsorption.
- Limited application owing to semi permanent retention of active or polar molecules and severe tailing of elution peaks.
- Thus this technique has not found wide application except for the separation of certain low-molecular-weight gaseous species.

First enunciated in 1941 by Martin and Synge.

GAS LIQUID CHROMATOGRAPHY

- Based on the partition of the analyte between a gaseous mobile phase and a liquid phase immobilized on the surface of an inert solid.
- It is applicable to substances or their derivatives which are volatilized under the temperatures employed.
- GC is based on mechanisms of adsorption, mass distribution or size exclusion

PRINCIPLE OF SEPARATION



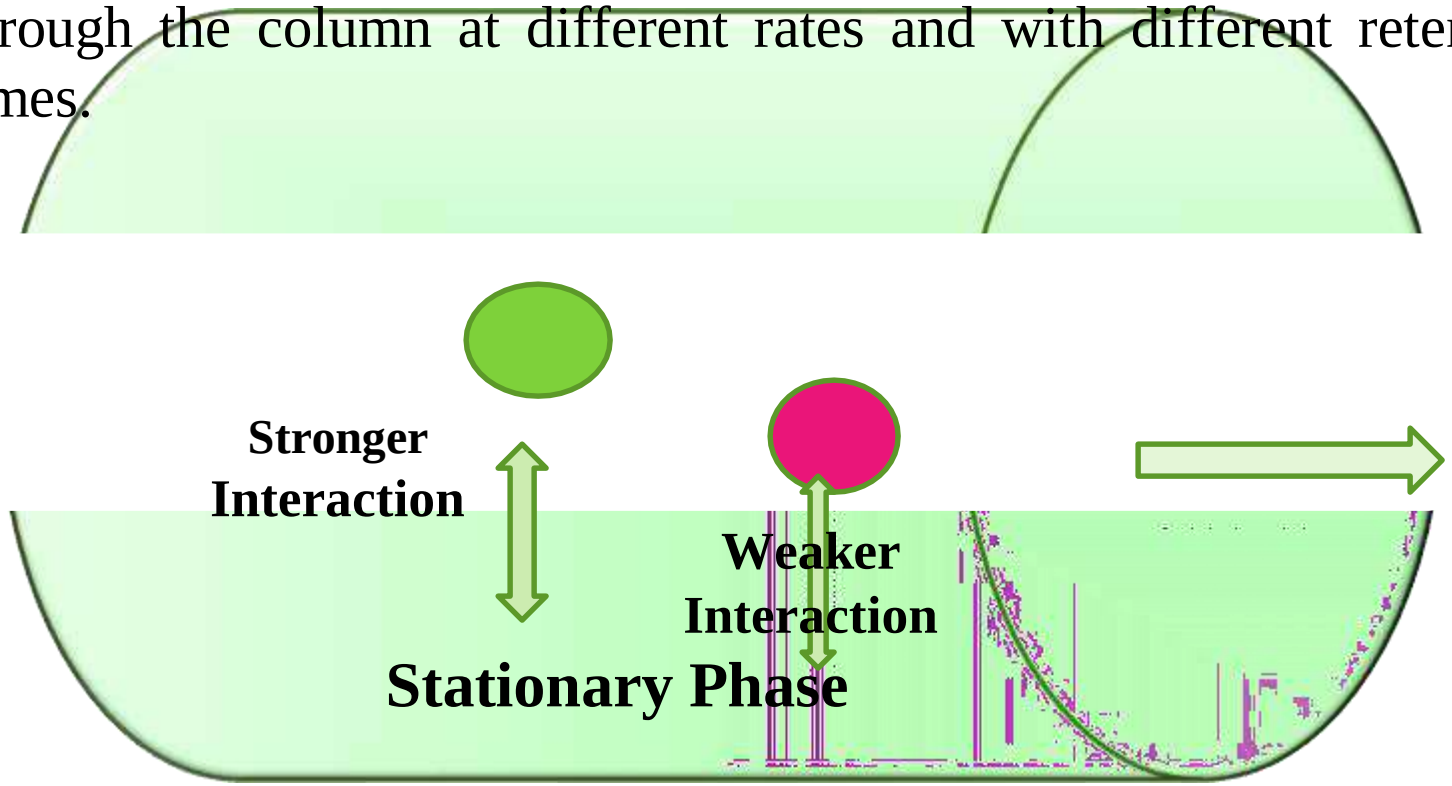
Typically the stationary phase has a sufficient low pressure (mm) at the column temperature so that it can be considered as a non-volatile.

$$\text{Partition - coefficient} = \frac{\text{Amount of solute in the stationary phase}}{\text{Amount of solute in the mobile phase}}$$

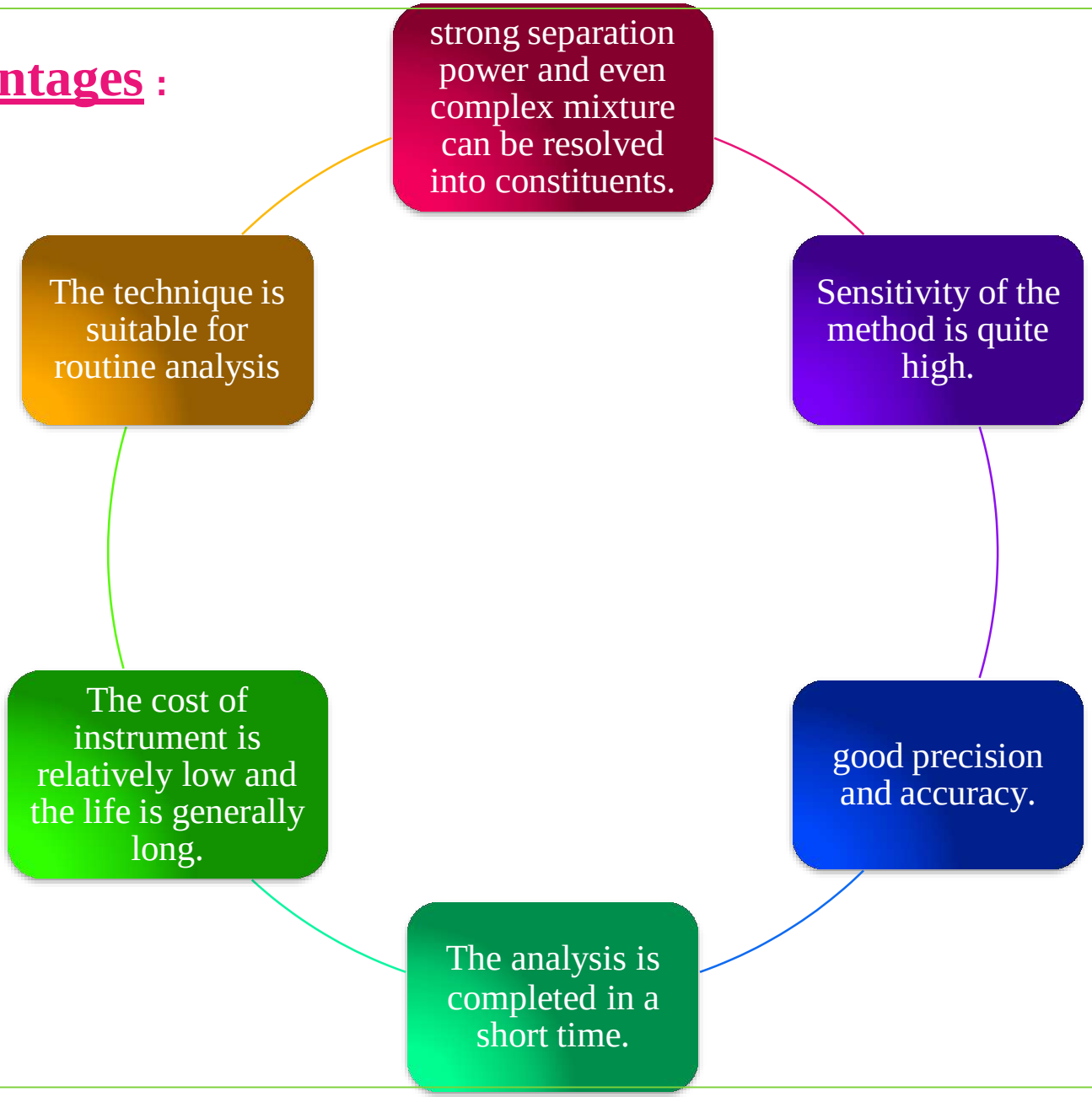
Schematic representation of Separation

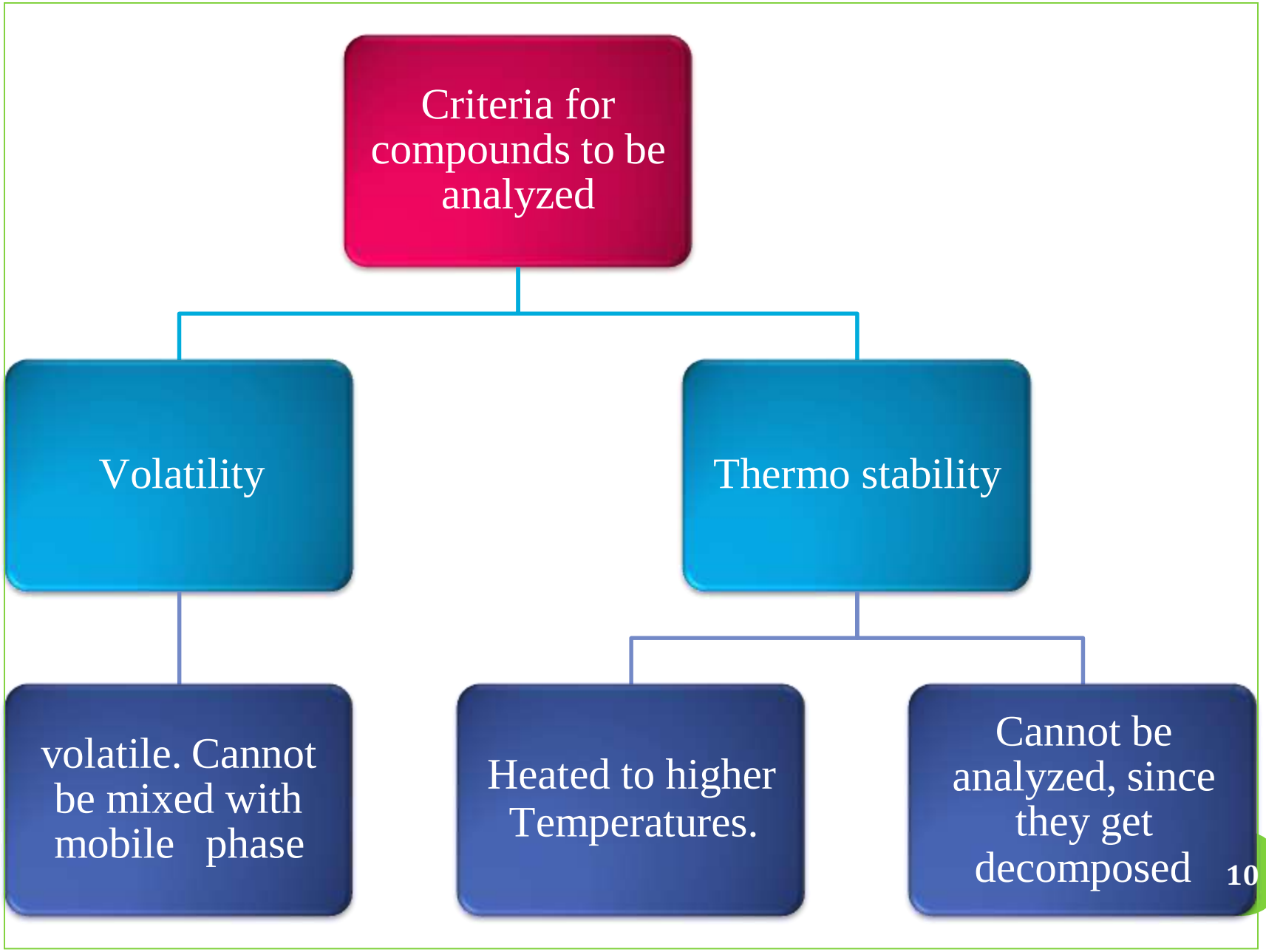
The sample mixture in gaseous form is run through the column with a carrier gas.

Separation can be achieved by the differences in the distribution ratios of the components of the sample between the mobile (gaseous) and stationary (liquid) phases causing them to move through the column at different rates and with different retention times.



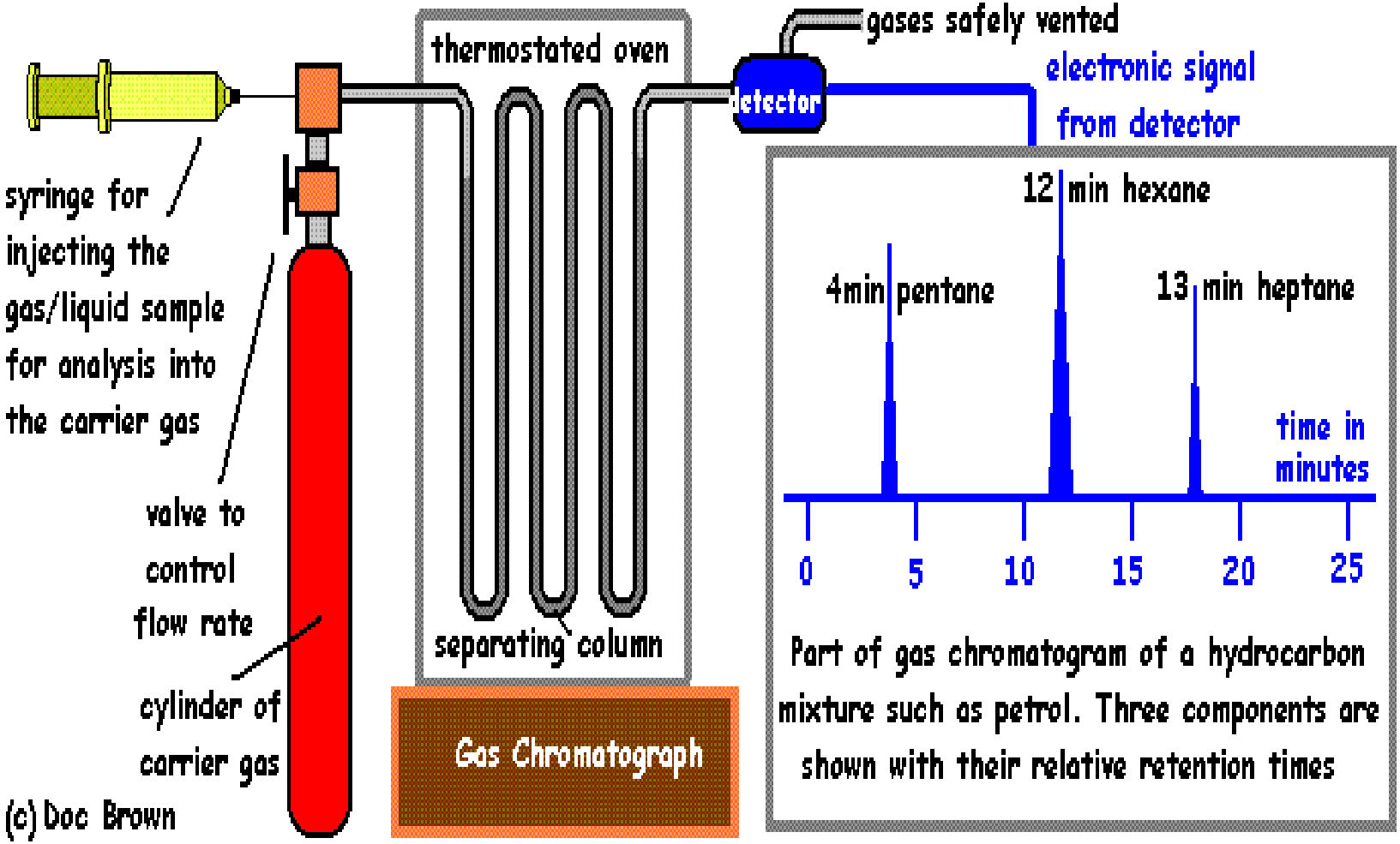
Advantages :

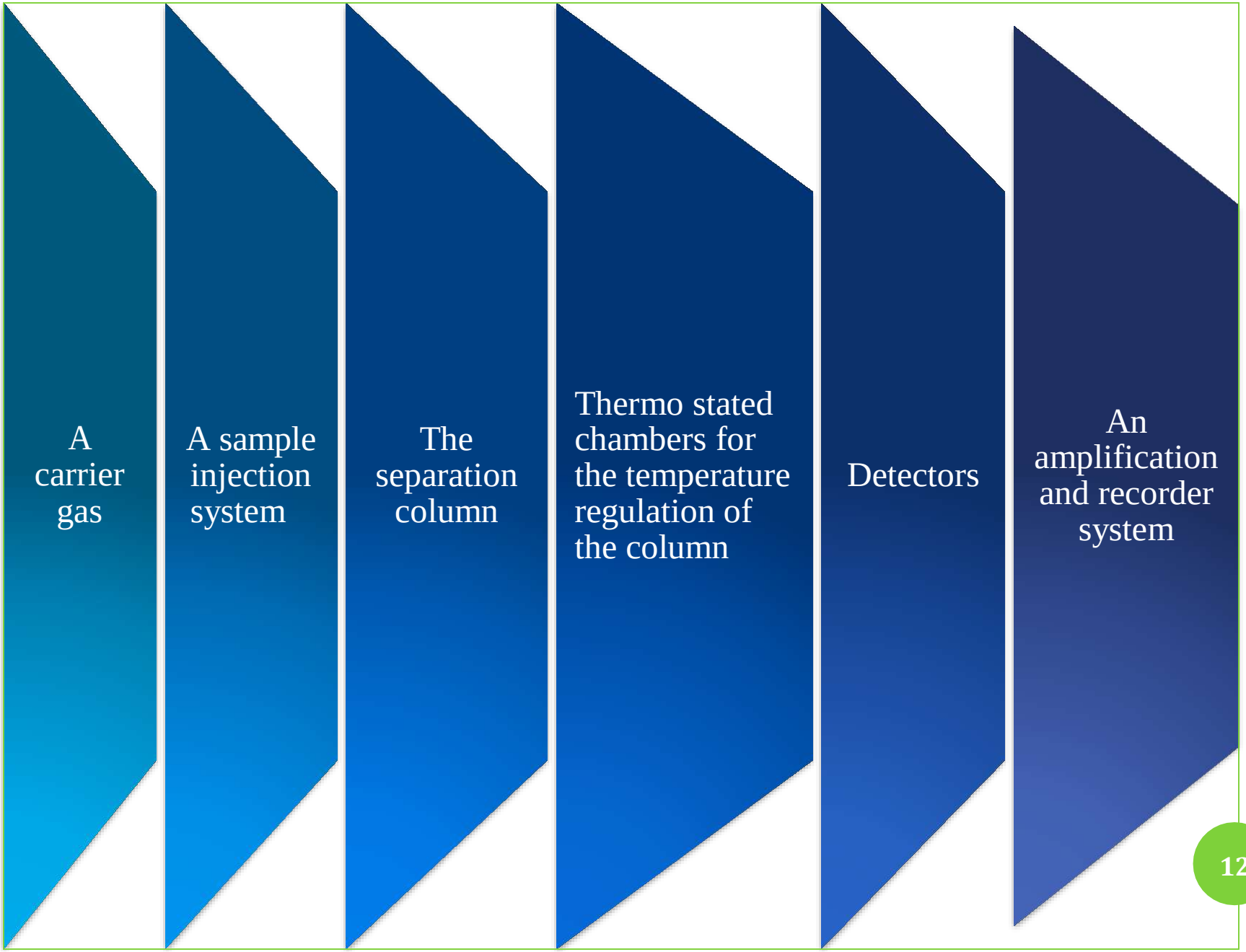

strong separation power and even complex mixture can be resolved into constituents.
Sensitivity of the method is quite high.
good precision and accuracy.
The analysis is completed in a short time.
The cost of instrument is relatively low and the life is generally long.
The technique is suitable for routine analysis


Criteria for compounds to be analyzed
Volatility
Thermo stability
volatile. Cannot be mixed with mobile phase
Heated to higher Temperatures.
Cannot be analyzed, since they get decomposed

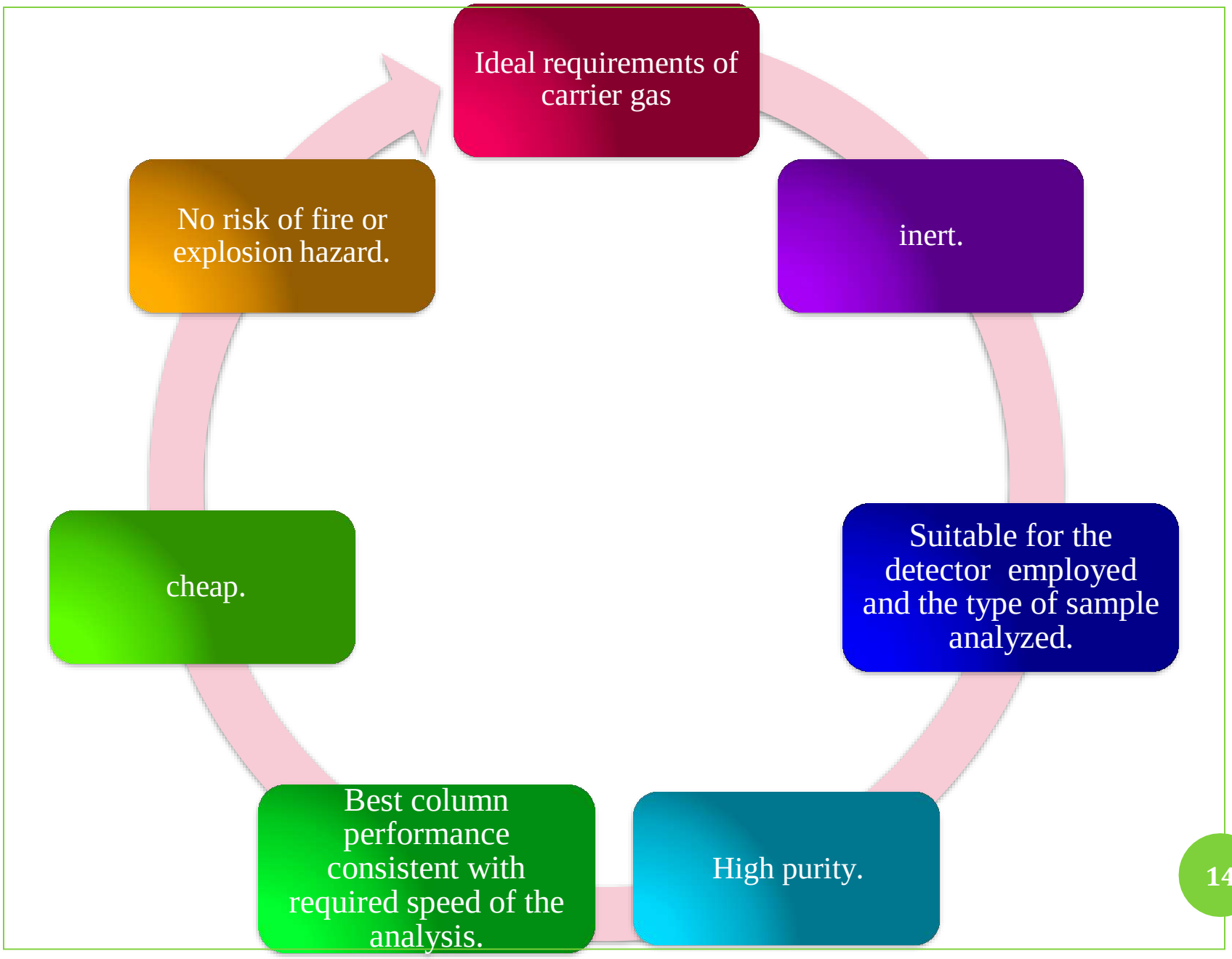
Instrumentation

Essential components of the apparatus used for the Gas Chromatography :




A carrier gas
A sample injection system
The separation column
Thermo stated chambers for the temperature regulation of the column
Detectors
An amplification and recorder system

Hydrogen	• Better thermal conductivity , lower density and greater flow rates. But is it highly Inflammable.
Helium	• Excellent thermal conductivity, inertness, low density and it allows greater flow rates. But it is very expensive.
Nitrogen	• Inexpensive but gives reduced sensitivity.
Air	when the oxygen in the air is useful to the detector or separation.
Oxygen	• Avoided since it oxidizes the stationary phase.


Ideal requirements of carrier gas
inert.
Suitable for the detector employed and the type of sample analyzed.
High purity.
Best column performance consistent with required speed of the analysis.
cheap.
No risk of fire or explosion hazard.

Pyrolysis Gas Chromatography is employed for substances that are not volatile and cannot be easily derivatized to volatile forms.

The solid is heated in a controlled way to break it into smaller, more volatile pieces separated by the column to form a pyrogram, which can be matched to known standards.

The sample is placed directly into a small coil of platinum wire so that it can be heated to several hundred degrees in few seconds while the carrier gas is flowing over it.

Better temperature control can be achieved by the use of a heater of nickel or other magnetic material operated at its Curie point.

Sample Introduction System

SOLIDS

Must be dissolved in volatile liquids for introduction directly if they can be liquefied.

GASES

Require special gas sampling valves for introduction into the carrier gas stream.

LIQUIDS

Hypodermic syringe through a self-sealing rubber septum into small inlet chamber, which may be heated to cause flash evaporation.

The temperature must not be so high that it decomposes the sample.

FLOW METERS & FLOW REGULATORS

FLOW REGULATORS

Deliver the gas with uniform pressure of flow rate

FLOW METERS

Measure the flow rate of carrier gas.

Eg : ROTAMETER ,Soap Bubble meter

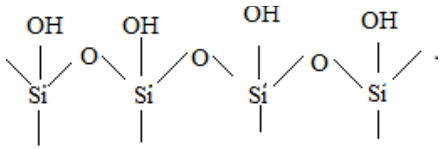
The Stationary Phase:

- Low volatility (ideally, the boiling point of the liquid should be at 100^0 C higher than the maximum operating temperature for the column)
- Thermally stable
- Chemical inert towards the sample

Stationary Phase	Maximum Temperature
Polydimethyl siloxane	350
Poly (biphenyl dimethyl) siloxane	350
Polyalkalene glycol	250
Polyethylene glycol	250
Poly (dicyanoallyldimethyl) Siloxane	240

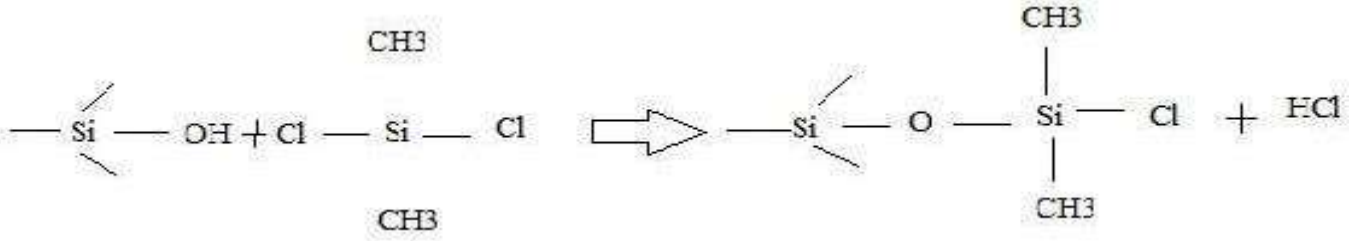
Adsorption on column Packing or Capillary walls

- A problem that has overwhelmed gas chromatography from its beginning has been the physical adsorption of polar or Polarizable analyte species, such as alcohols or hydrocarbons, on the silicate surfaces of the column packings or capillary walls.
- Adsorption results in distorted peaks, which are broadened and often exhibit a tail.
- It has been established that adsorption is the consequence of silanol groups that form on the surface of silicates by reaction with moisture.
- Thus, a fully hydrolyzed silicate surface has the structure



The SiOH groups on the support surface have a strong affinity for polar organic molecules and tend to retain them by adsorption

Support materials can be deactivated by silanization with dimethylchlorosilane. The reaction is



Silanized surfaces of column packing may still show a residual adsorption, which apparently arise from metal oxide impurities in the diatomaceous earth. Acid washing prior to silanization removes these impurities.

DERIVATIZATION :

Derivatization prior to gas chromatography is often desirable to improve the process of separation by column or detection by detector.

There are two types of Derivatization based upon its need:

Pre Column Derivatization

- Improve the thermal stability of compounds, particularly compounds that contain polar functional groups
- Change the separation properties of compounds by the purposeful adjustment of their volatility.

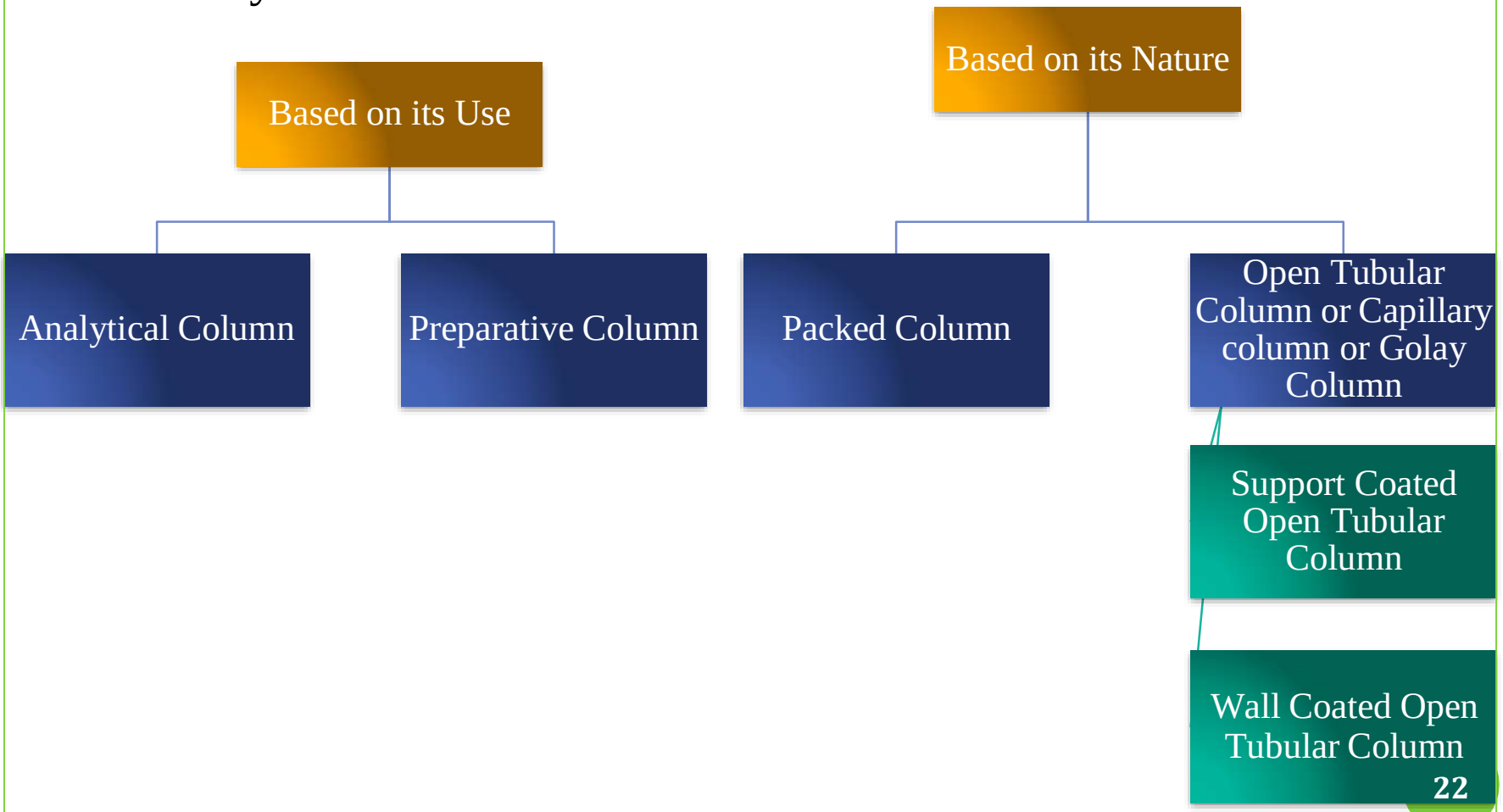
  silylating agents, such as N,O-bis(trimethylsilyl)acetamide convert one active hydrogen in polar groups such as -OH, -COOH, $-NH_2$, =NH , and -SH to an -O- $Si(CH_3)_3$ group.

Post Column Derivatization

- Improve the response shown by the detector.
- The components are converted in such a way that their ionization or affinity towards electrons is increased.

COLUMNS

Column is one of the important part which decides the separation efficiency.



Columns:

The column can be constructed of glass or metal tubing .

length from a few centimeters to over a hundred meters and can be coiled, bent or straight.

Packed Column:

Packed columns are prepared by packing metal or glass tubing's with granular stationary phase.

For Gas Solid Chromatography the columns are packed with **size graded adsorbent or porous polymer,** whereas for

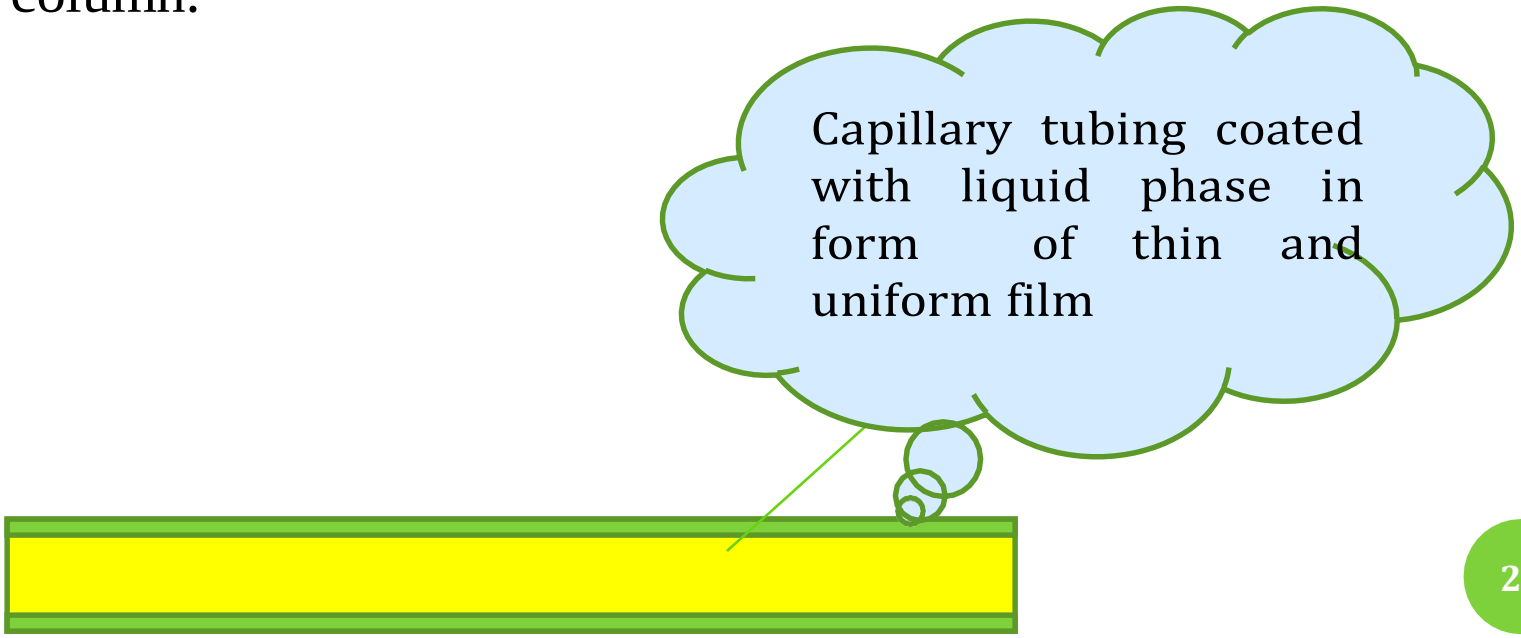
Gas Liquid Chromatography the packing is prepared by **coating the liquid phase over a size graded inert solid support.**

The advantages of porous polymer as packing material :

- Porous polymer beads are mechanically strong and can be easily packed on columns.
- There is no column bleed.
- Most of the porous polymer are stable at 250^0C and cause no base line drift and therefore allows a use of highly sensitive detector.
- There is no adsorption of polar compounds such as water, alcohols or acids and they are eluted rapidly as sharp symmetrical peaks.
- Thus it is quite useful in preparative and trace work analysis because Sample over load recovery is rapid and tailing is not seen.
- Retention data are highly reproducible.

Wall-Coated Open tubular Columns:

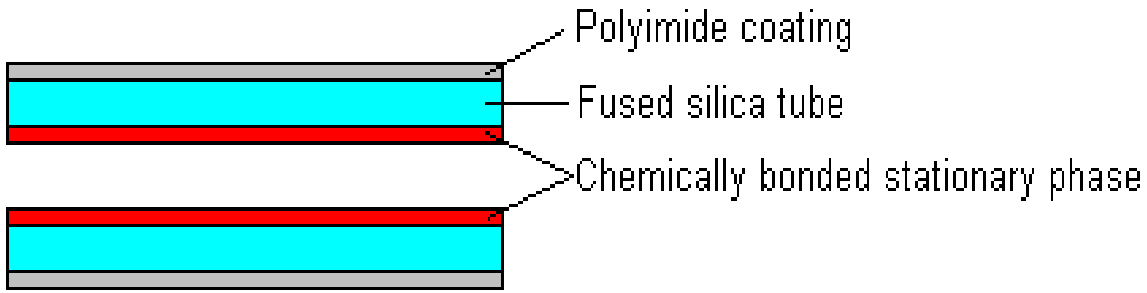
- Long capillary tubing (30-90 meters)
- Uniform and narrow internal diameter (0.025-0.075 cm).
- Made of stainless steel, copper, nylon, or glass etc., the stainless steel being the most popular.
- Carrier gas flow faces least resistance because there is no packing in the column.



Fused-Silica Open Tubular Column:

- Fused-silica capillaries are drawn from specially purified silica that contains minimal amounts of metal oxides.
- These capillaries have much thinner walls than their glass counterparts.
- The tubes are given added strength by an outside protective polyimide coating, which is applied as the capillary tubing.
- The resulting columns are quite flexible and can be bent into coils having diameters of few inches.

Cross section of a Fused Silica Open Tubular Column

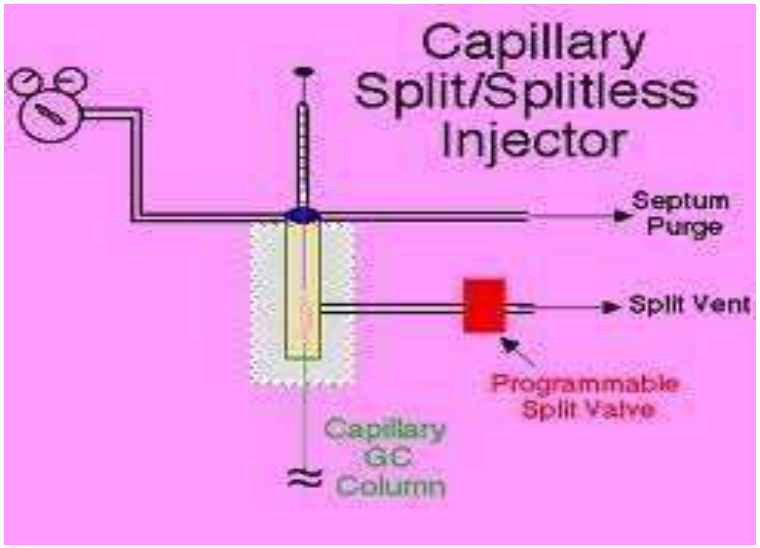


Adantages:

- Offer several important advantages such as physical strength, lower reactivity and flexibility towards sample Components

Disadvantages

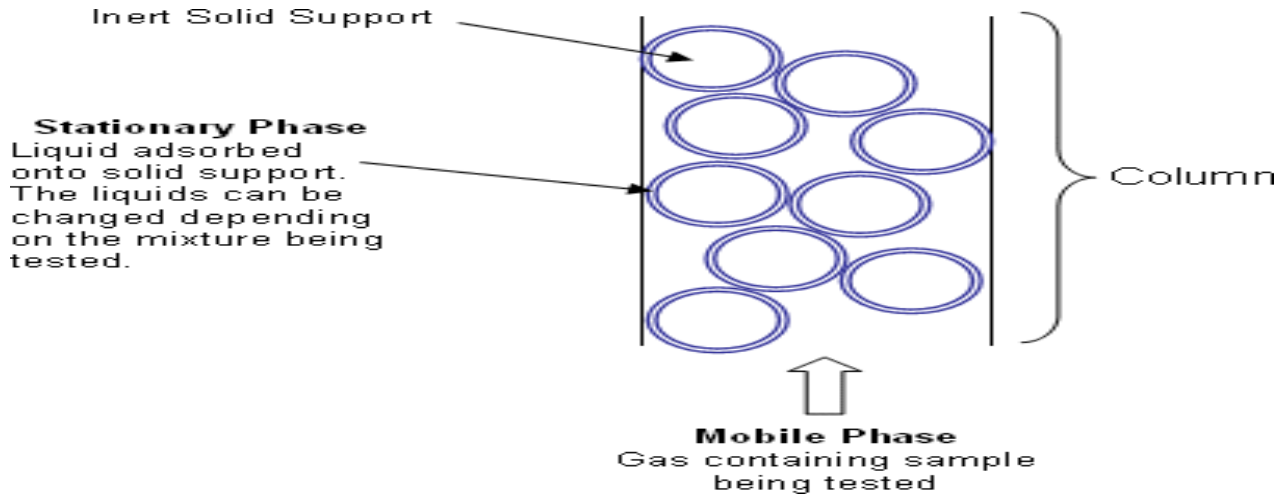
- Troublesome to use and are more demanding of the injection and detection systems.
- Thus, a sample splitter must be used to reduce the size of the sample injected onto the column and a more sensitive detector system with a rapid response time is required.



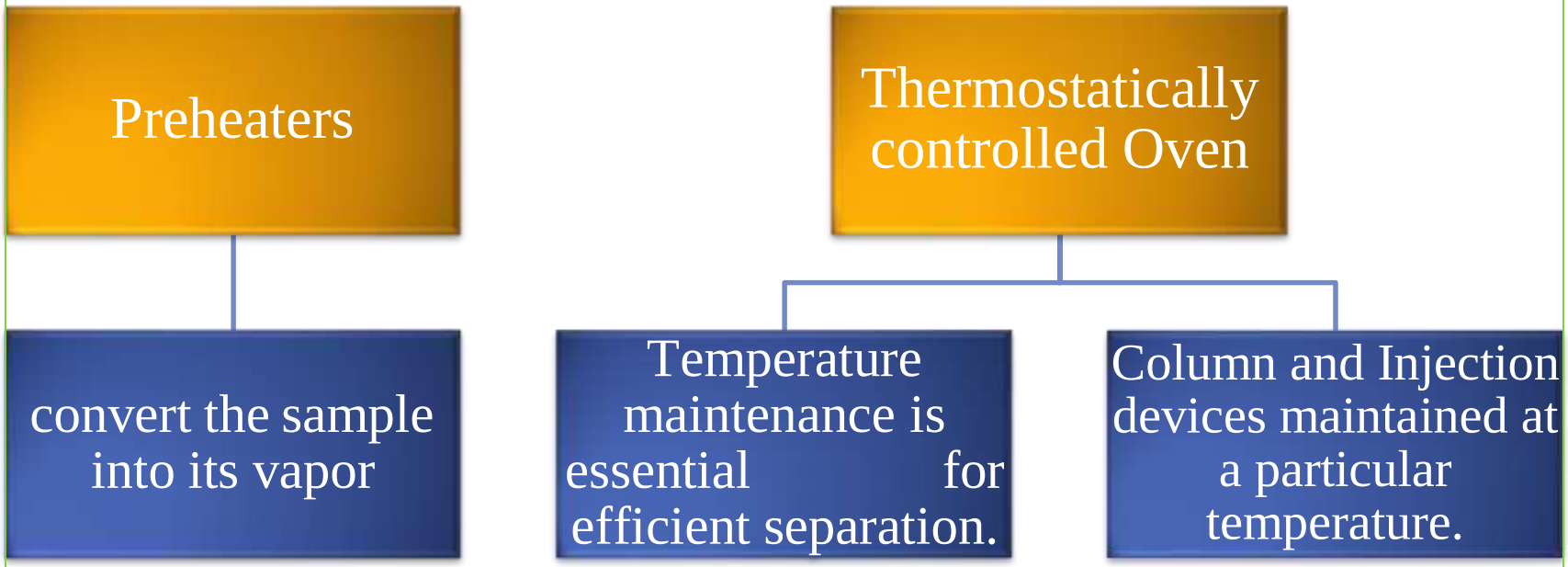
Support Coated open Tubular columns

- prepared by depositing a micron size porous layer of support material on the inside wall of a capillary column and then coated with a thin film of liquid phase.
- These columns have more sample capacity and an inlet splitter may not be required.
- Support Coated Open Tubular is preferred for trace analysis.

Cross section of column with micron size porous polymer



TEMPERATURE CONTROL DEVICES



Types of operation performed

Isothermal Programming

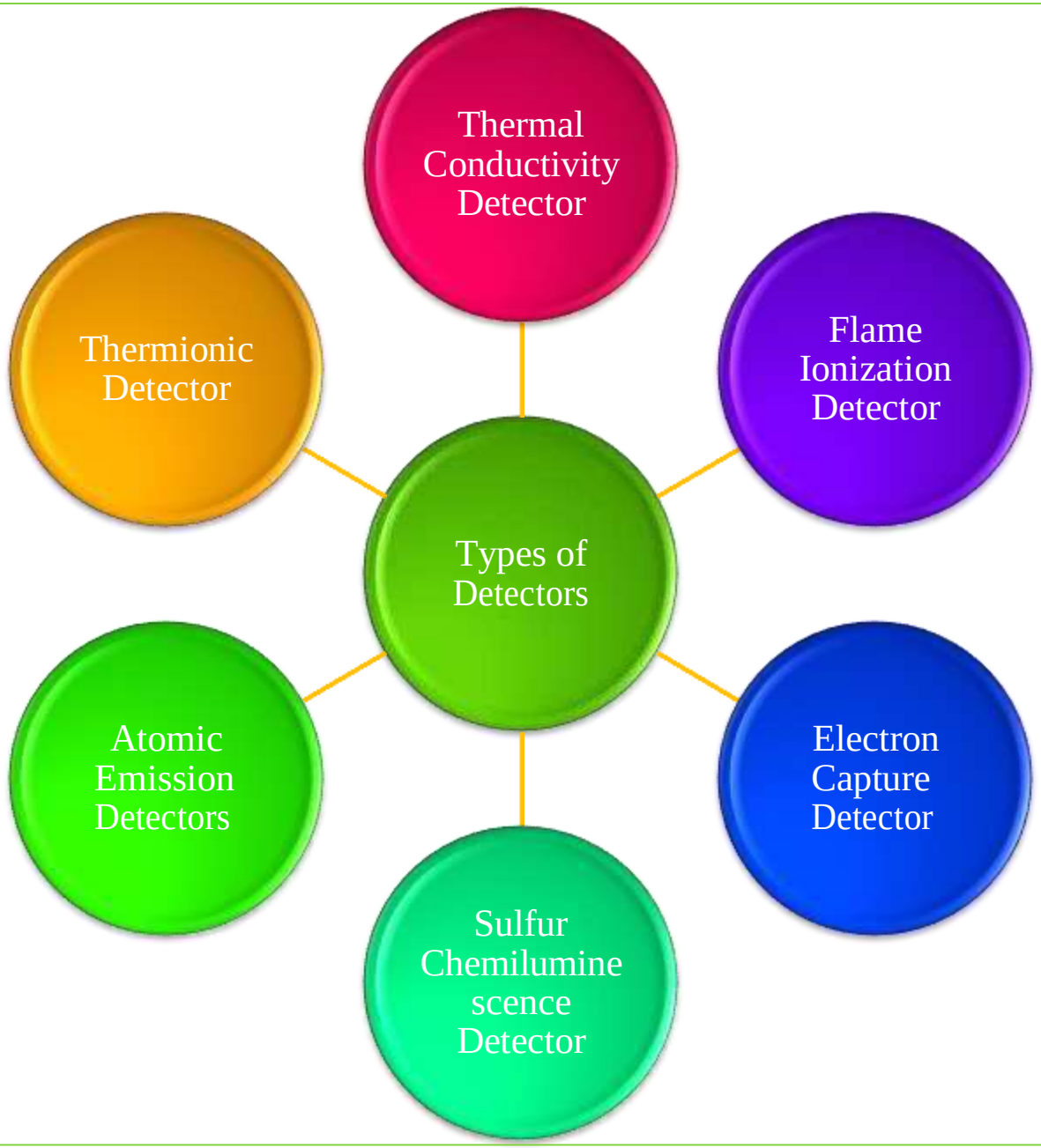
same temperature is maintained throughout the process of separation.

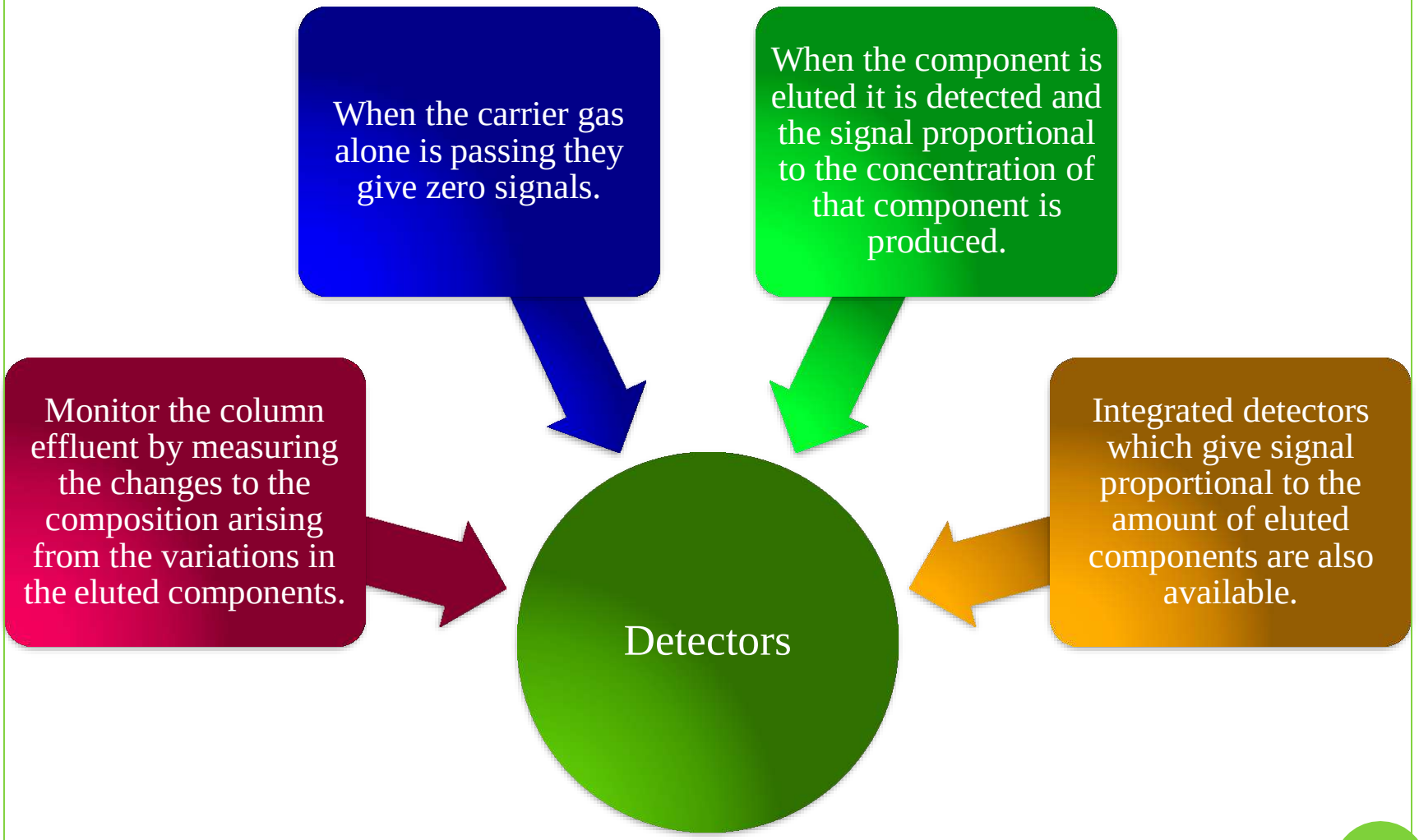
Linear Programming

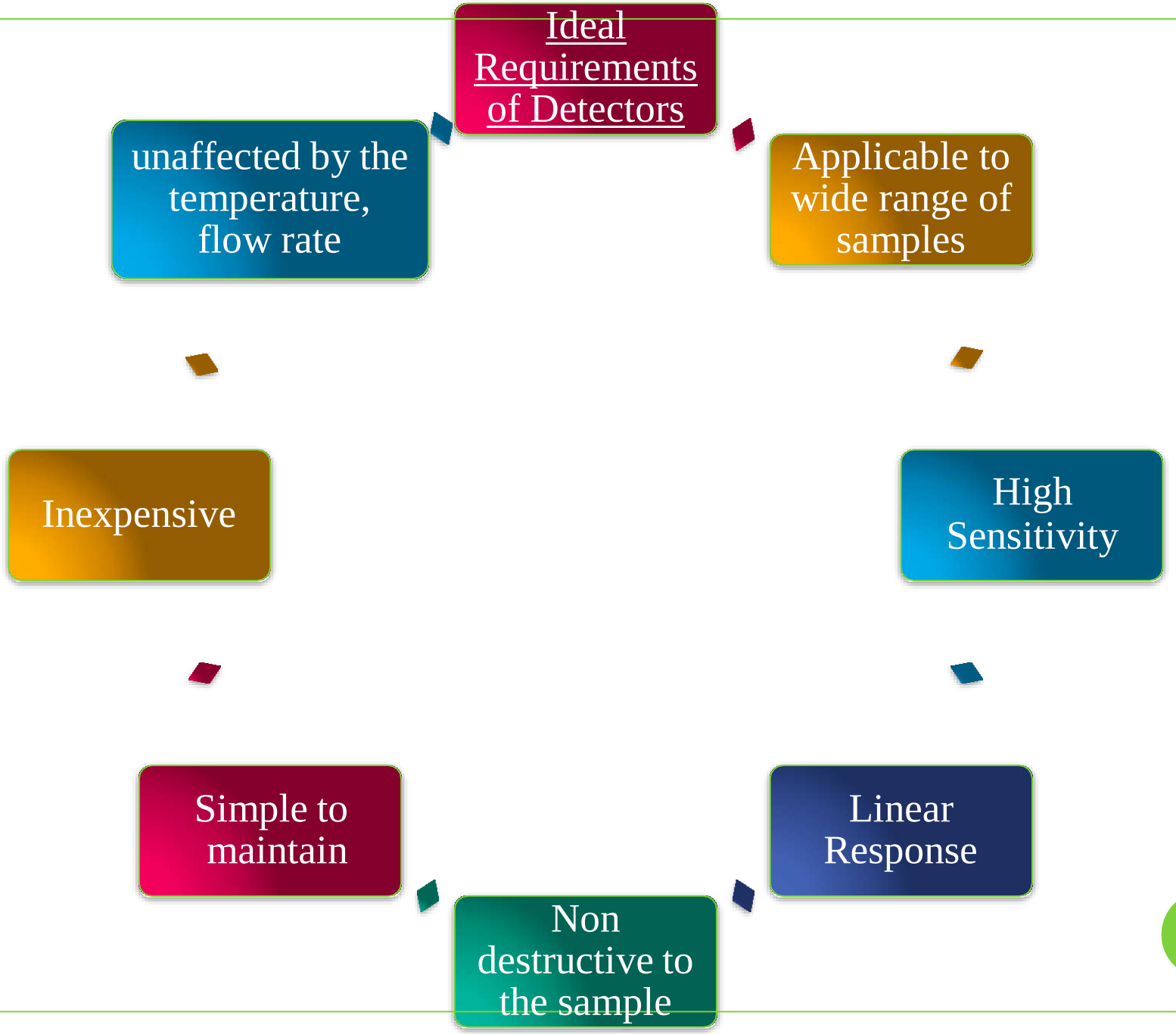
oven is heated over a period of time.

Required when a sample has a mixture of low boiling and high boiling point compounds.

Efficient for separation of complex mixtures


Thermal Conductivity Detector
Thermionic Detector
Flame Ionization Detector
Types of Detectors
Atomic Emission Detectors
Electron Capture Detector
Sulfur Chemilumine scence Detector


When the carrier gas alone is passing they give zero signals.
When the component is eluted it is detected and the signal proportional to the concentration of that component is produced.
Monitor the column effluent by measuring the changes to the composition arising from the variations in the eluted components.
Detectors
Integrated detectors which give signal proportional to the amount of eluted components are also available.


Ideal Requirements of Detectors
unaffected by the temperature, flow rate
Applicable to wide range of samples
Inexpensive
High Sensitivity
Simple to maintain
Linear Response
Non destructive to the sample

Differential Thermal Conductivity Detector or Katharometer

Principle:

- Based upon the thermal conductivity difference between carrier gas and that of the component in the sample.
- Hydrogen and helium have higher thermal conductivity and they are the best carrier gases for katharometer.

Merits:

- Responds to all types of organic and Inorganic compounds
- Linearity is good
- Non-destruction of the sample and hence used in preparative analysis.

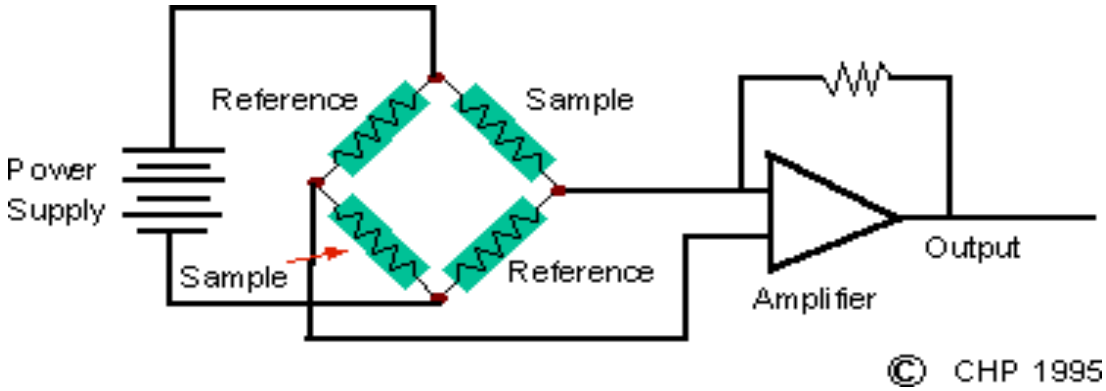
Demerits:

- Low sensitivity
- Biological samples are not analyzed
- Affected by fluctuations in temperature and flow rate.

Procedure:

- Has two platinum wires of uniform dimensions that form part of Wheatstone bridge.
- Carrier gas always passes through one of them and effluents of the column passes through the other column.
- The two wires are heated electrically and hence assume equilibrium conditions of temperature and electrical resistance.
- When the component emerges, it alters the thermal conductivity and the resistance of the wire.
- Hence this produces a difference in resistance and so the conductivity between two wires, is amplified and recorded as a signal.

Schematic Representation of Thermal conductivity Detector



Flame Ionization Detector

Principle :

- The ionization detectors are based on the electrical conductivity of gases.

Advantages:

- Extremely sensitive
- Linearity is excellent
- Insensitive to small changes in water vapor and flow rate of carrier gas.

Drawbacks :

- Different response to different substances and to the fact that the sample is destroyed.
- Responds to all organic compounds except formic acid, the response being greatest for hydrocarbons.

Procedure:

At normal temperature and pressure gases act as insulators but will become conductive of ions if electrons are present.

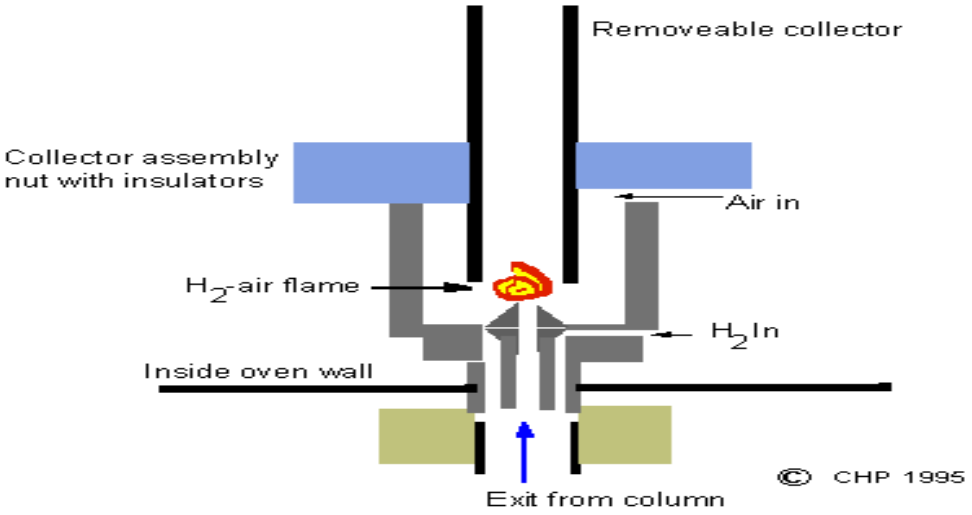
The carrier gas used with this detector is hydrogen.

The mixture is burnt in air (or oxygen) in the detector. The platinum jet serves as one electrode of the cell and the anode is a silver gauge placed above the burner tip.

When the component emerges from the column, the number of ions are produced because of the ionization by the thermal energy of the flame,.

This produces potential difference and causes a flow of current which is amplified and recorded as signal.

Schematic Representation of Flame Ionization Detector



Electron Capture Detector

Principle:

The electron affinity of different substances can be used as the basis for an ionization detector.

Advantages:

- Electron Capture Detector is extremely sensitive to certain compounds, e.g., chlorinated pesticides can be determined down to sub picogram levels.

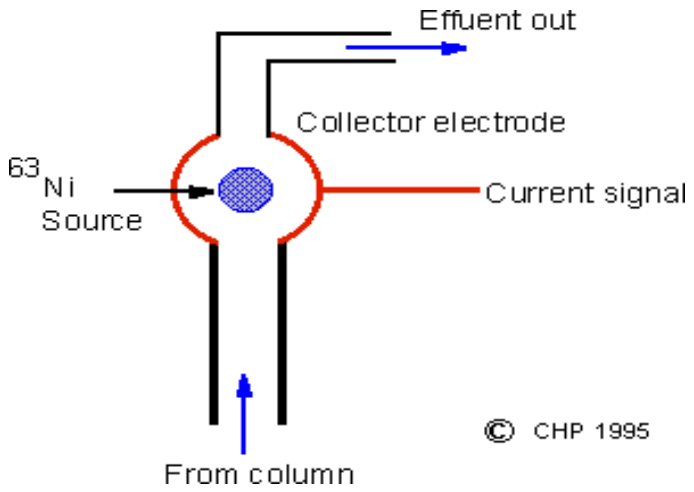
Disadvantages:

- It responds to only those compounds, whose molecules have affinity for electrons. E.g., chlorinated compounds, alkyl leads etc.

- It has high sensitivity for halogenated compounds and is therefore used for the detection of herbicides, pesticides, SF6 traces in fuel gases, organometallics (e.g. lead tetraethyl), poly nuclear aromatic carcinogenic NO_2 and SO_2 in chimney stack gases.

Procedure:

- It has two electrodes, with the column effluent passing between them.
- One is treated with radioactive isotope, which emits electrons as it decays.
- These emitted electrons produce secondary electrons which are collected by the anode, when a potential of 20 V are applied between them.
- Effluent molecules which have affinity for electrons capture these electrons when they pass through the electrodes. Hence the amount of steady state current is reduced. The difference is amplified and recorded as output signal.
- The carrier gas used depends upon the electron affinity of the compounds to be analyzed.



Recorders and Integrators

Recorders

- Used to measure the response obtained from detectors after amplification
- Record the baseline and all the peaks with respect to time.

Integrators

- Improved version of recorders with some data processing capabilities.
- Records the individual peaks, with retention time and width of the peak, peak area .

Evaluation Parameters:

A theoretical plate is hypothetical or imaginary unit of a column where distribution of a solute between stationary phase and mobile phase has attained equilibrium.

The efficiency of the column is measured (or) expressed by the Number of Theoretical Plates (N) in the column or by the Height Equivalent of the Theoretical Plate (HETP).

A theoretical plate is the distance on the column in which equilibrium is attained between the solute in the gas phase and the solute in the liquid phase.

The larger the number of theoretical plates or the smaller the HETP, more efficient is the column for separations.

It is calculated using the following formula:

$$\text{HETP} = \frac{\text{length of the column}}{\text{Number of Theoretical Plates}}$$

Factors effecting the Efficiency of the column

Particle size and surface area

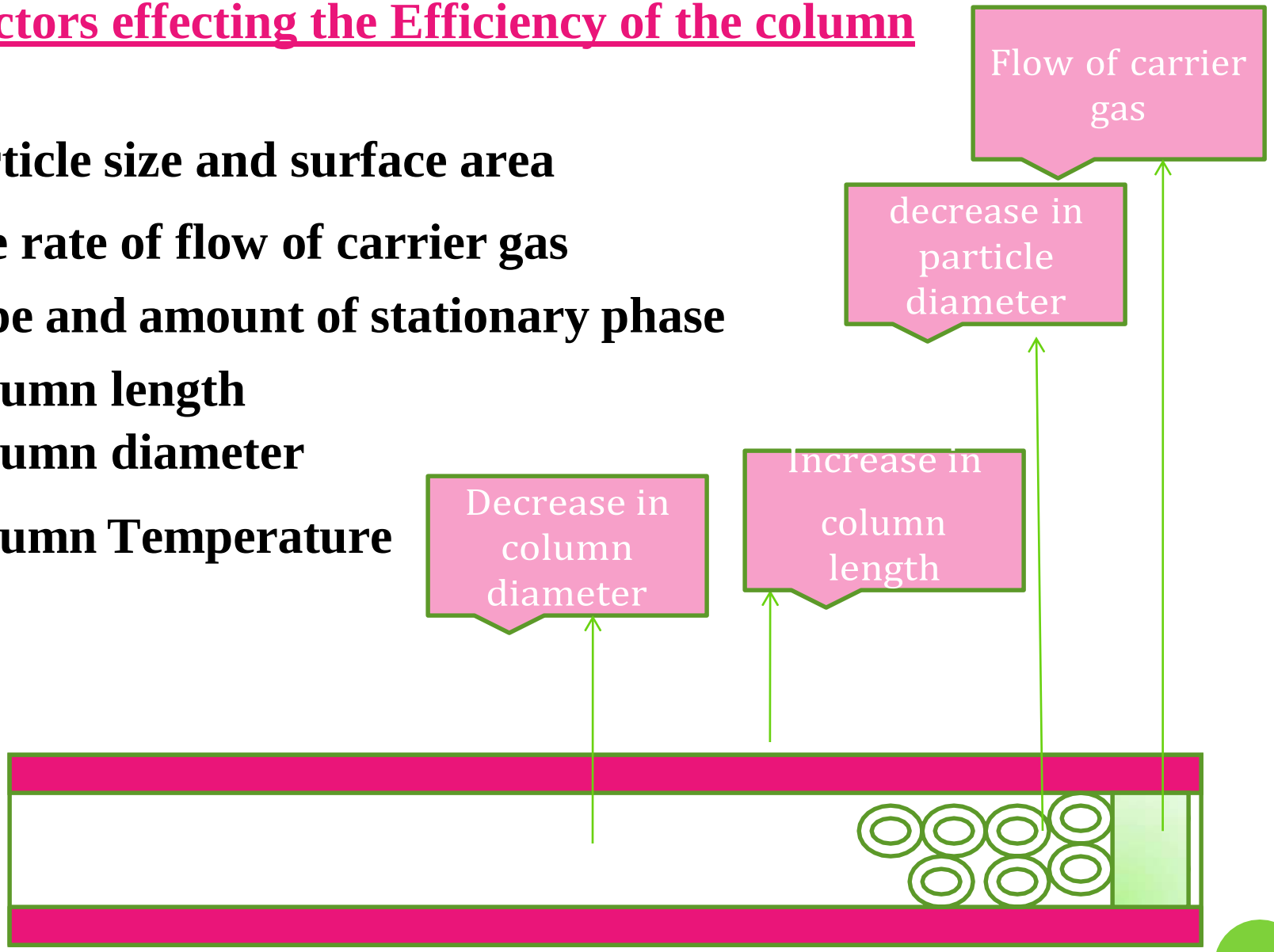
The rate of flow of carrier gas

Type and amount of stationary phase

Column length

Column diameter

Column Temperature

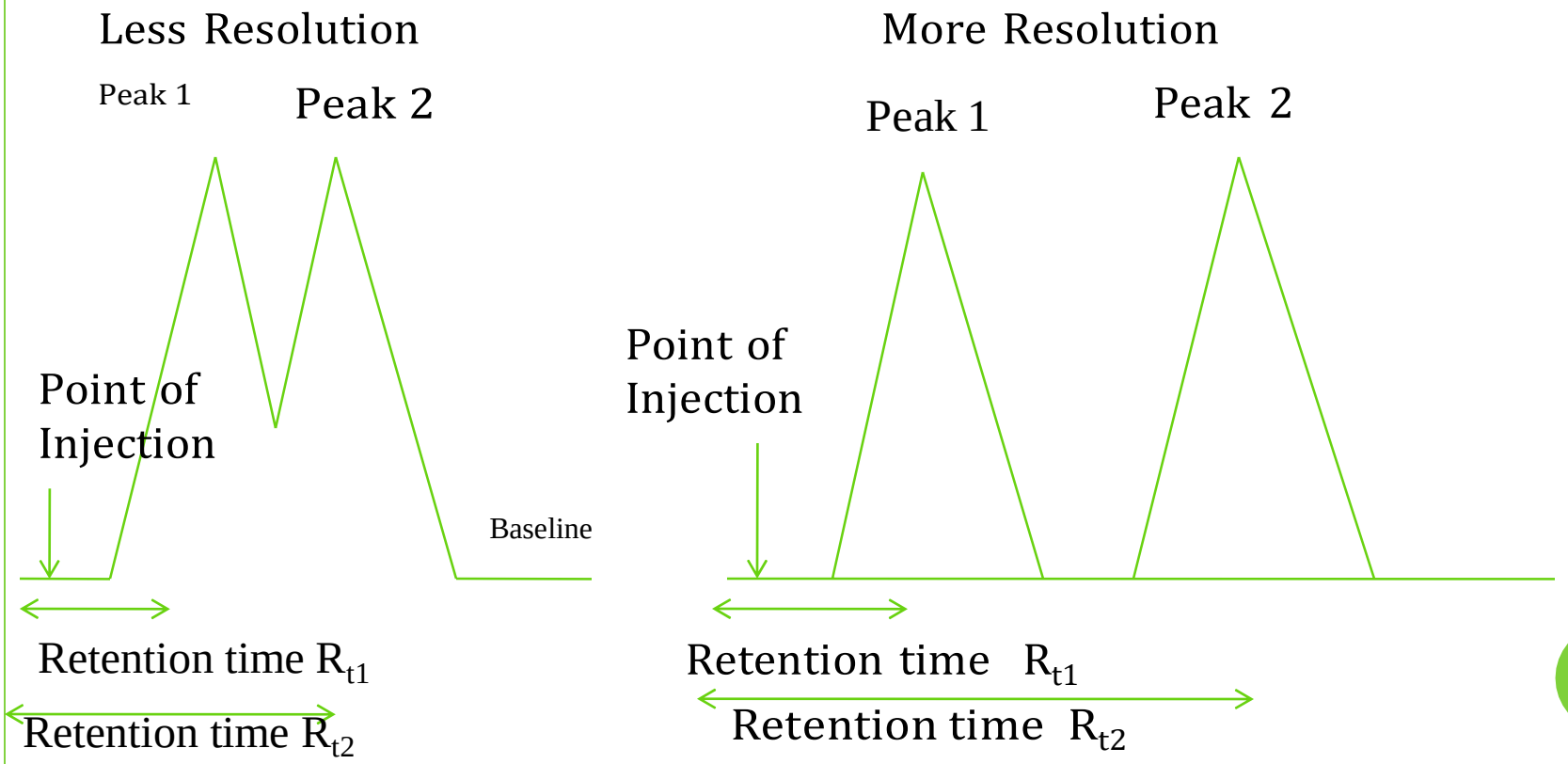


Resolution

The Resolution is a measure of extent of separation of two components and the baseline separation achieved.

It is determined by using the following formula:

$$R = \frac{2(R_{t1} - R_{t2})}{W_2 + W_1}$$



Retention time(R_t):

Retention is the difference in time between the point of Injection and the appearance of peak maxima.

Retention is the time required for 50% of a component to be eluted from a column.

Retention time is measured in minutes or seconds and is also proportional to the distance which can me measured in terms of cm or mm.

Retention volume(V_r):

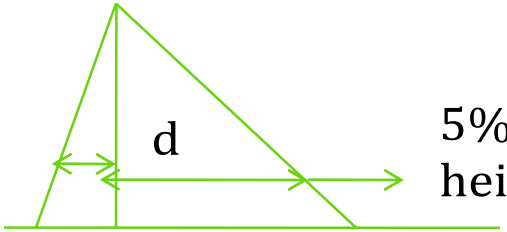
Retention volume is the volume of the carrier gas required to elute 50% of the component from the column.

It is the product of retention time and flow rate

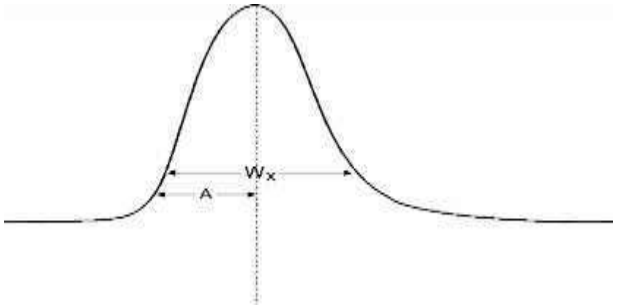
Retention volume = Retention time × flow rate.

Asymmetry factor :

A chromatographic peak should be symmetrical about its centre and said to follow Gaussian distribution



5% of the peak height



The symmetry factor or tailing factor of a peak is calculated from the expression:

$$A_s = \frac{W_{0.05}}{2d}$$

$W_{0.05}$ = width of the peak calculated at the one-twentieth of peak height

d = Distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at one-twentieth of the peak height.

A value of 1.0 signifies complete or ideal symmetry.

Column efficiency

The efficiency of the column is expressed by the number of Theoretical plates.

$$N = 5.54(t_r / W_h)$$

N = Number of Theoretical Plates.

t_r = retention time or

W_h = width of the peak at half height.

If the number of theoretical plates is high , the column is said to be highly efficient.

If the number of theoretical plates is low, the column is said to less efficient.

Separation factor:

It is the ratio of partition-coefficient of the two components to be separated.

It is usually expressed and determined by using the following equation:

$$S = \frac{K_b}{K_a} = \frac{K'_a}{K'_b} = \frac{t_b - t_0}{(t_a - t_0)}$$

S = Depends on the liquid phase and column temperature.

If there is more difference in the partition- coefficient between the two compounds, the peaks are far apart and the separation factor is more.

If the partition coefficient is similar, then the peaks are closer and the separation factor is less.

Applications

- The Gas liquid chromatography is mainly used as tool for performing separations and for providing means of completion of an analysis.
- When the technique is used for providing means for completion of an analysis,
- qualitative identification is determined by retention times or retention volumes, and whereas
- Quantitative identification is determined by peak heights and peak areas.

Qualitative analysis:

- Some qualitative identification can be obtained from the observation of the relative sensitivity of various column liquids and various detectors.
- Further identification can only be gained from an observation of retention time, which is constant, for a given column, flow rate and temperature.
- Retention data should be useful for identification of component mixtures
- It is widely used for studying the purity of the organic compounds by analyzing the gas chromatograms. Contaminants if present, will give additional peaks.
- The areas under these peaks give rough estimates of contamination.
- This technique is also useful for evaluating the efficiency of purification techniques.

QUANTITATIVE ANALYSIS

- Based either on peak height or on peak Ares and hence for known substances, quantitative determinations are generally performed by comparing their peak areas.
- Peak height is often a better analytical parameter than peak area for solutes with low retention time, although both parameters are dependent upon various experiment variables.
- The peaks for solutes with low retention time are narrow and tall and hence very difficult to determine their areas accurately.

Calibration curve method

- Standards of various concentrations are used to determine their peak areas

Other Miscellaneous applications:

- Chromatography is most widely used chemical techniques to separate particles and contaminates in chemical plants.
- For example, in the chemical industries, pesticides and insecticides like DDT in the groundwater and PCBs (Polychlorinated biphenyls) are removed by the process of chromatography.
- As a major testing tool, chromatography is used by government agencies to separate toxic materials from the drinking water and also to monitor air quality.
- Chromatography is used by pharmaceutical companies to prepare large amounts of pure materials that are further required in making medicines.

- In the field of organic chemistry and pharmacy, chiral compounds are very close to each other in terms of atomic or molecular weight, element composition, and the physical properties.
- However, they exist in two different forms, called the enantiomers and optical isomers.
- Both these compounds though may appear to be same, have very different chemical properties.
- So, in pharmacy, chromatography becomes crucial to analyze the exact chiral compound so that correct medicines can be manufactured.
- For instance, a compound called thalidomide has two optical isomers and one of the isomers can cause birth defect if a pregnant women consumes it in early stages of pregnancy. So, it is important to carefully separate the isomers.
- Chromatography is used as a technique to separate the additives, vitamins, preservatives, proteins and amino acids.
- Some other uses are in the detection of drugs or medications in the urine and the separation of traces of chemicals in the case of fire in houses or buildings.

References:

- Principles of Instrumental Analysis by Douglas A. Skoog and Donald M. West.
- Instrumental methods of chemical analysis by Gurdeep R. Chatwal and Sham K. Anand.
- Instrumental Methods of Analysis by Willard.
- Chromatography B.K Sharma
- Indian Pharmacopeia

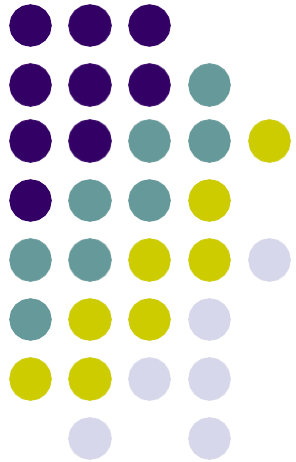
- I Appreciate your Patience and valuable Time
- Thank you

HPLC

B.Bhadru

Associate Professor

Vikas college of Pharmaceutical Sciences





Concept of Chromatography



- Chromatography is an analytical method that the compounds are physically separated prior to measurement
- The main purpose of chromatography is to **separate** and **quantify** the target sample in the matrix
- Column Chromatography was introduced by **Mikhail T Svet**
- HPLC was introduced by **Joseph J Kirkland**

HPLC

High Pressure Liquid Chromatography

- High pressure to be able to use small particle size to allow proper separation at reasonable flow rates

High Performance Liquid Chromatography

- High performance due to its reproducibility

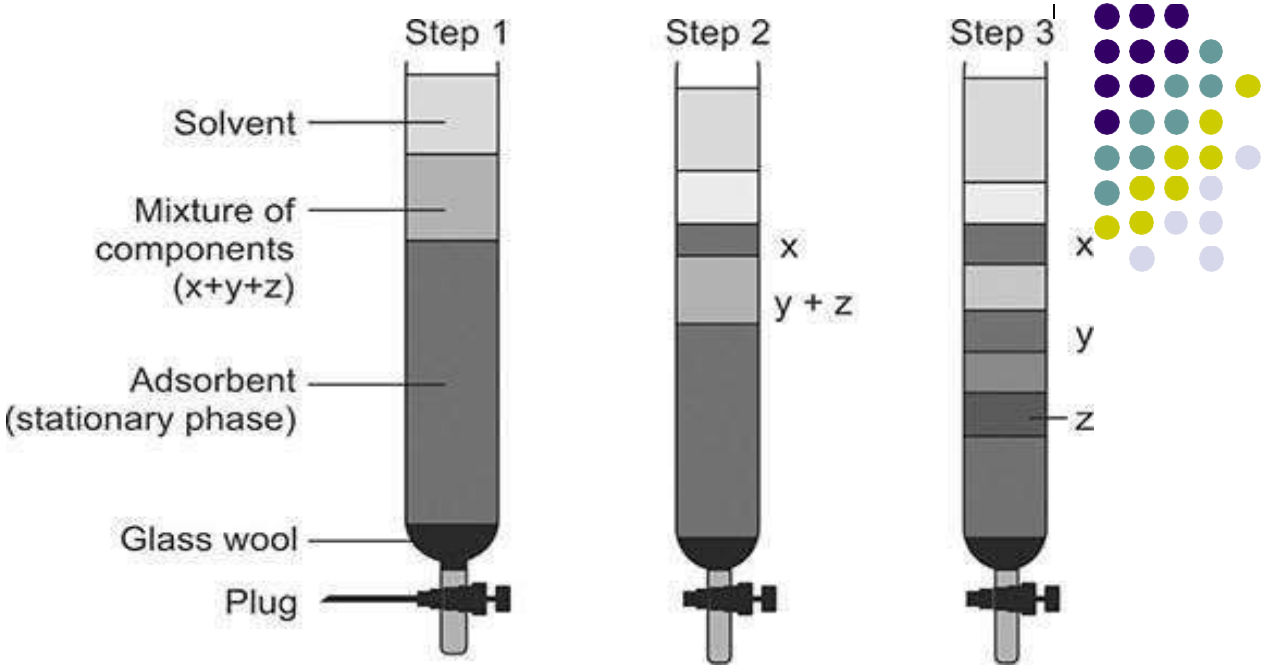
Currently Refers to:

High Precision Liquid Chromatography

Why use HPLC?

- Simultaneous Analysis
- High Resolution
- High Sensitivity (ppm-ppb)
- Good Repeatability
- Small Sample Size
- Moderate Analysis Condition
- No Need to Vaporize the Sample Like GC
- Easy to Fractionate the Sample and Purify
- Non Destructive for Many Detectors

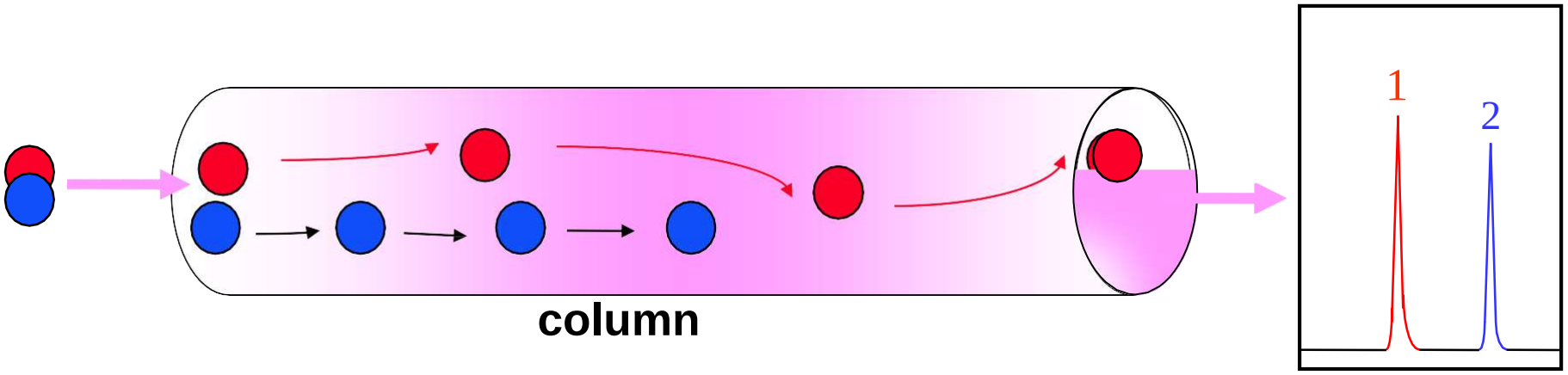
Introduction



- Compounds are separated by injecting a sample mixture onto the column. The different component in the mixture pass through the column at different rates due to differences in their partition/ adsorption behavior between the mobile phase and the stationary phase.

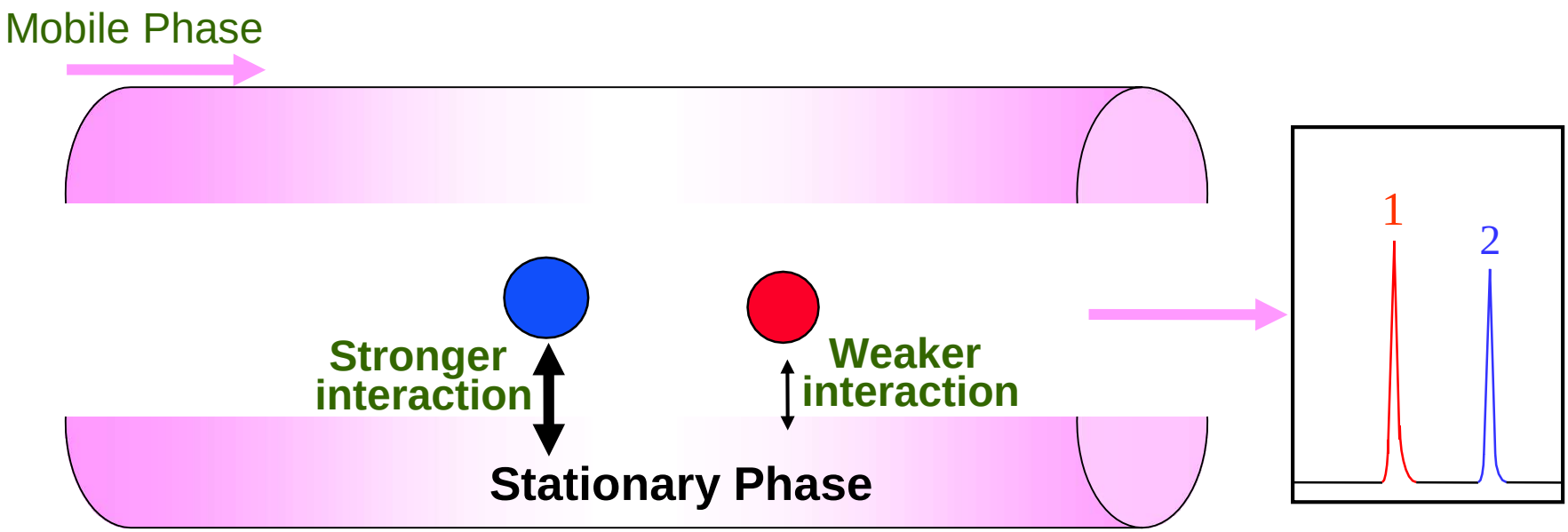
Separation Mechanism

Compounds are separated because the molecules move at different rates in the column.



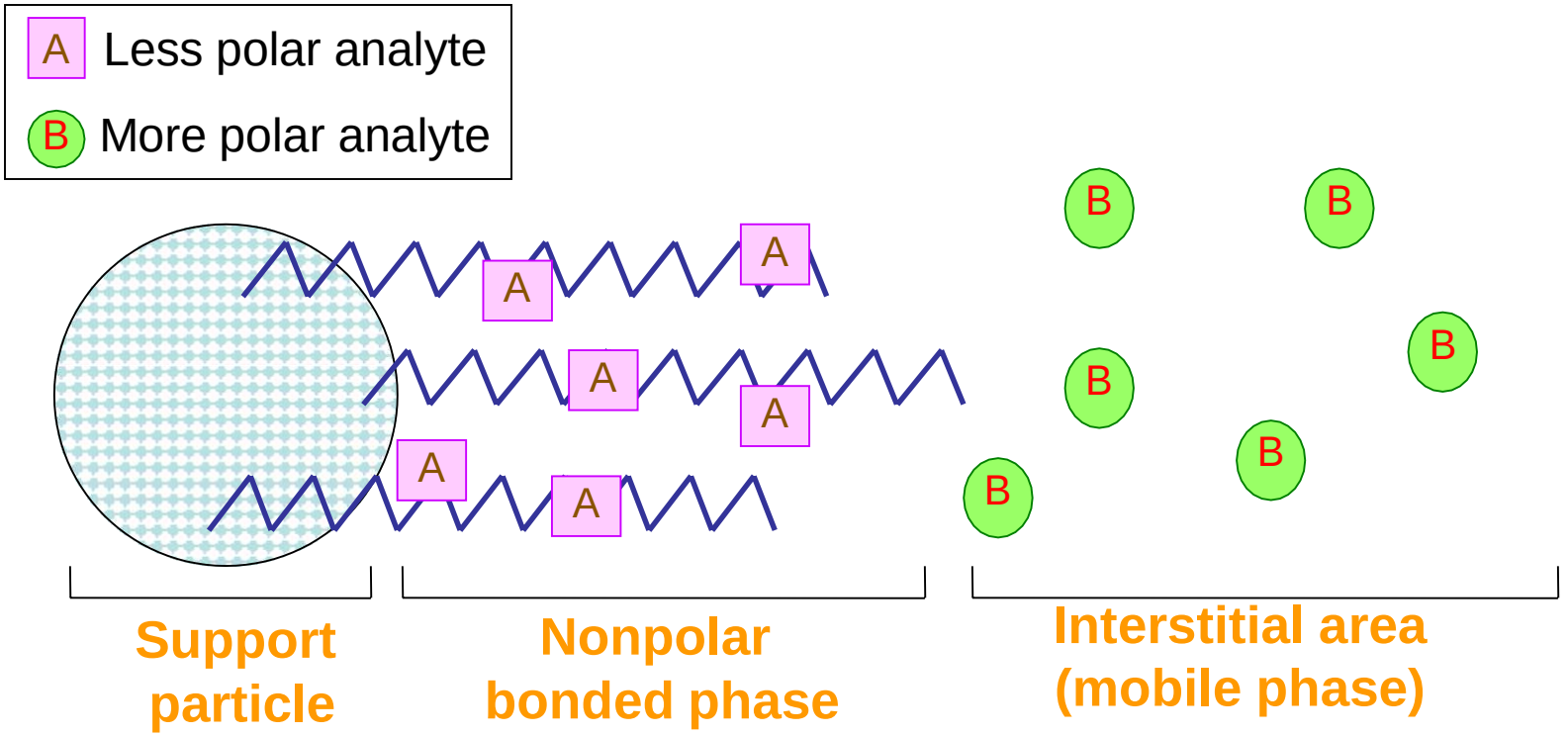
Separation Mechanism

Due to different interaction between stationary phase and different sample, the molecules move at different rate, therefore separation can be done.



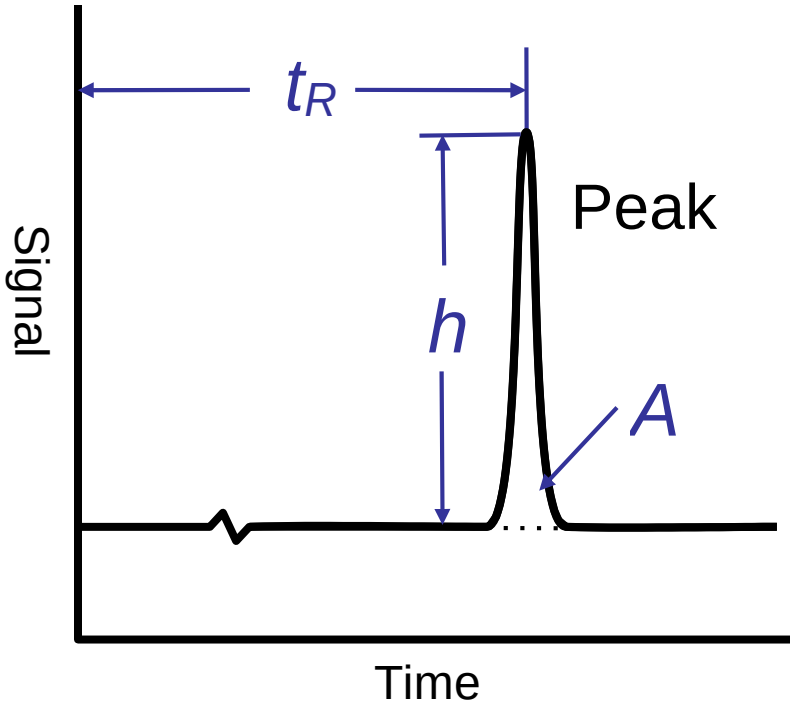
What Is the Interaction?

Hydrophobic Interaction



Less polar (more hydrophobic) analytes are more attracted and spend more time associated with the hydrophobic bonded phase, therefore, they are eluted last.

Chromatogram



t_R : Retention time

A : Area

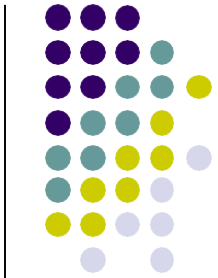
h : Height

Some Important Terms



- **Chromatogram:** A plot of detector signal output versus time or elution volume.
- **Mobile phase:** The liquid that moves the solute through the column.
- **Stationary phase:** The packing material of the column, which is the immobile phase involved in the chromatographic process.
- **Peak:** The visual representation on the chromatogram based on the detector's electrical response due to the presence of a sample component inside the flow cell.
- **Retention time:** The time taken by the analyte peak to reach the detector after sample injection.
- **Qualitation:** An analysis process which is designed to identify the components of a substance or mixture.
- **Quantitation:** An analysis process which is designed to determine the amounts or proportion of the components of a substance.

Types of HPLC Techniques



Based on modes of Chromatography

- Normal Phase mode
- Reverse Phase mode

Based on principle of separation

- Adsorption – solid-liquid, Silica, Alumina
- Partition – Liquid-liquid, PEG coated on silica

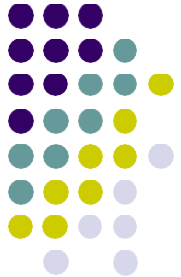
Based on elution technique

- Isocratic separation – Same Composition
- Gradient Separation – Different Composition

Dis-adv : **Time taking for reconditioning for next run**
Base line disturbance because of different solvents

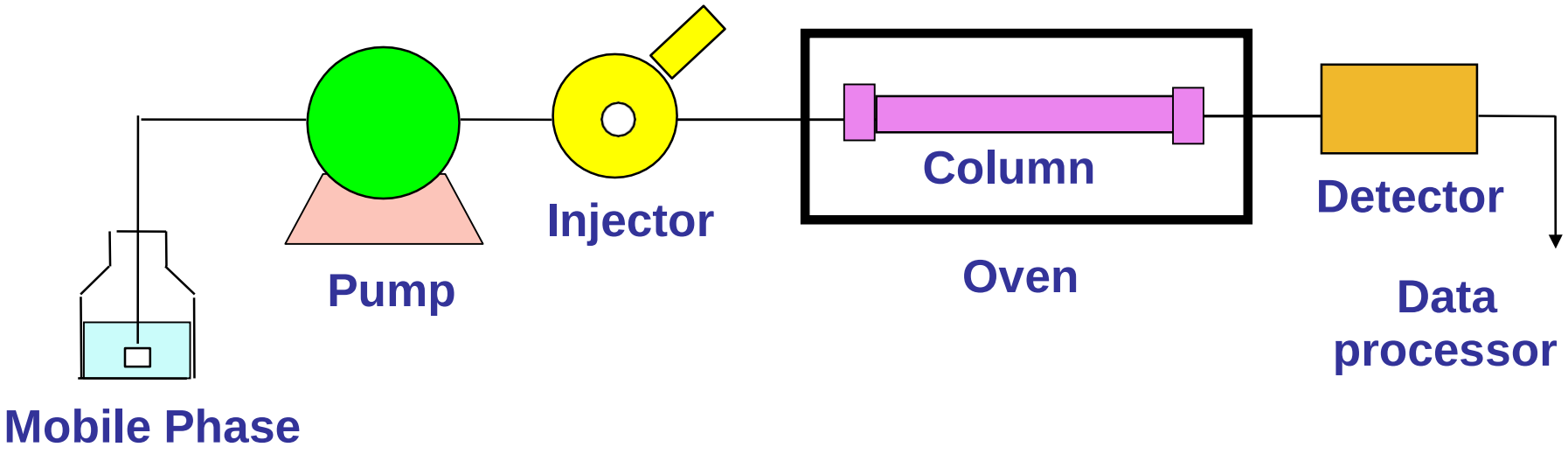
Based on Scale of operation

- Analytical HPLC
- Preparative HPLC

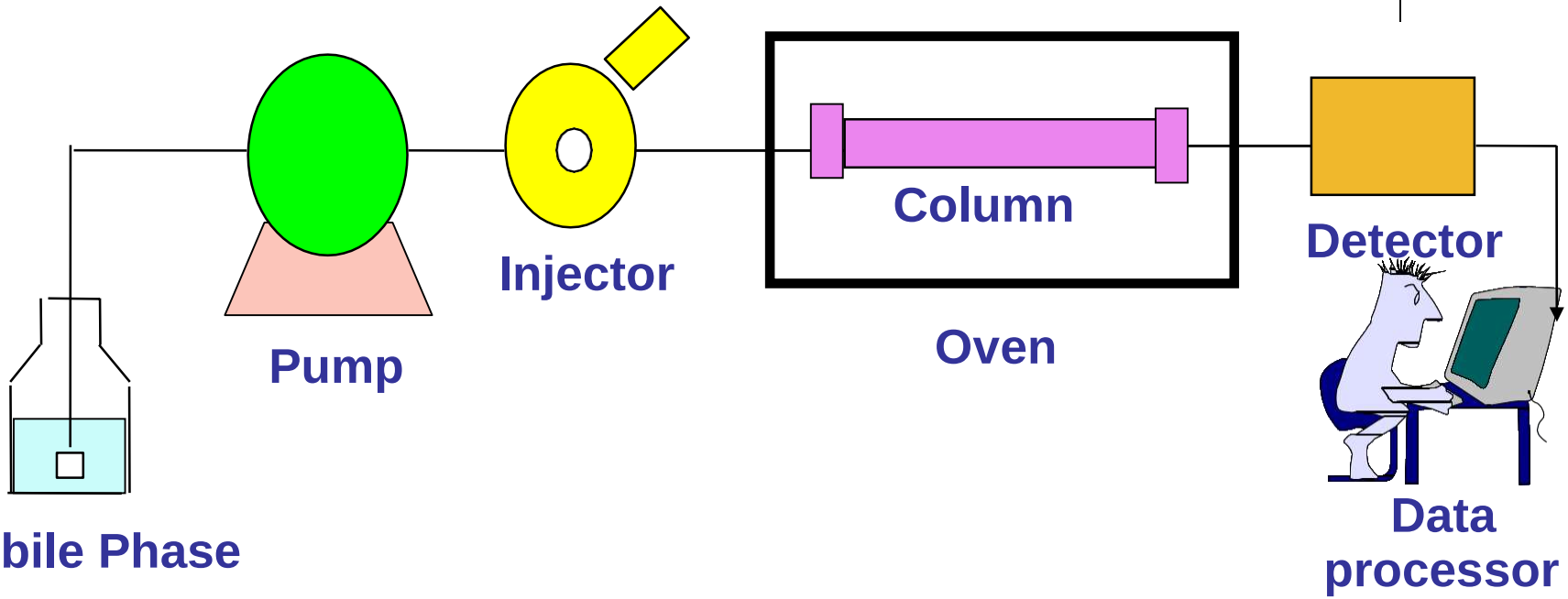


INSTRUMENTATION

Flow Diagram of HPLC



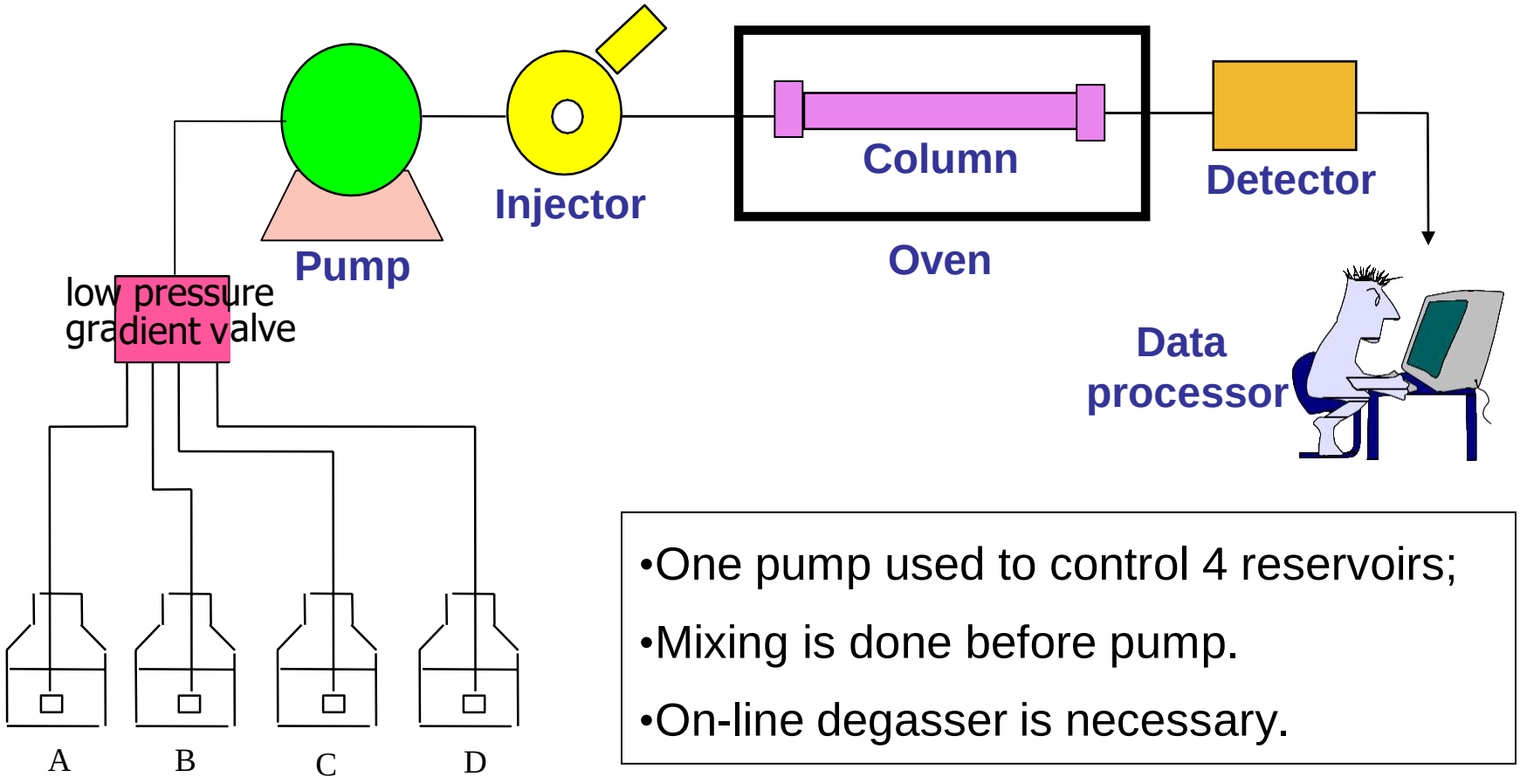
Isocratic System



Simple system with one pump and one solvent reservoir.

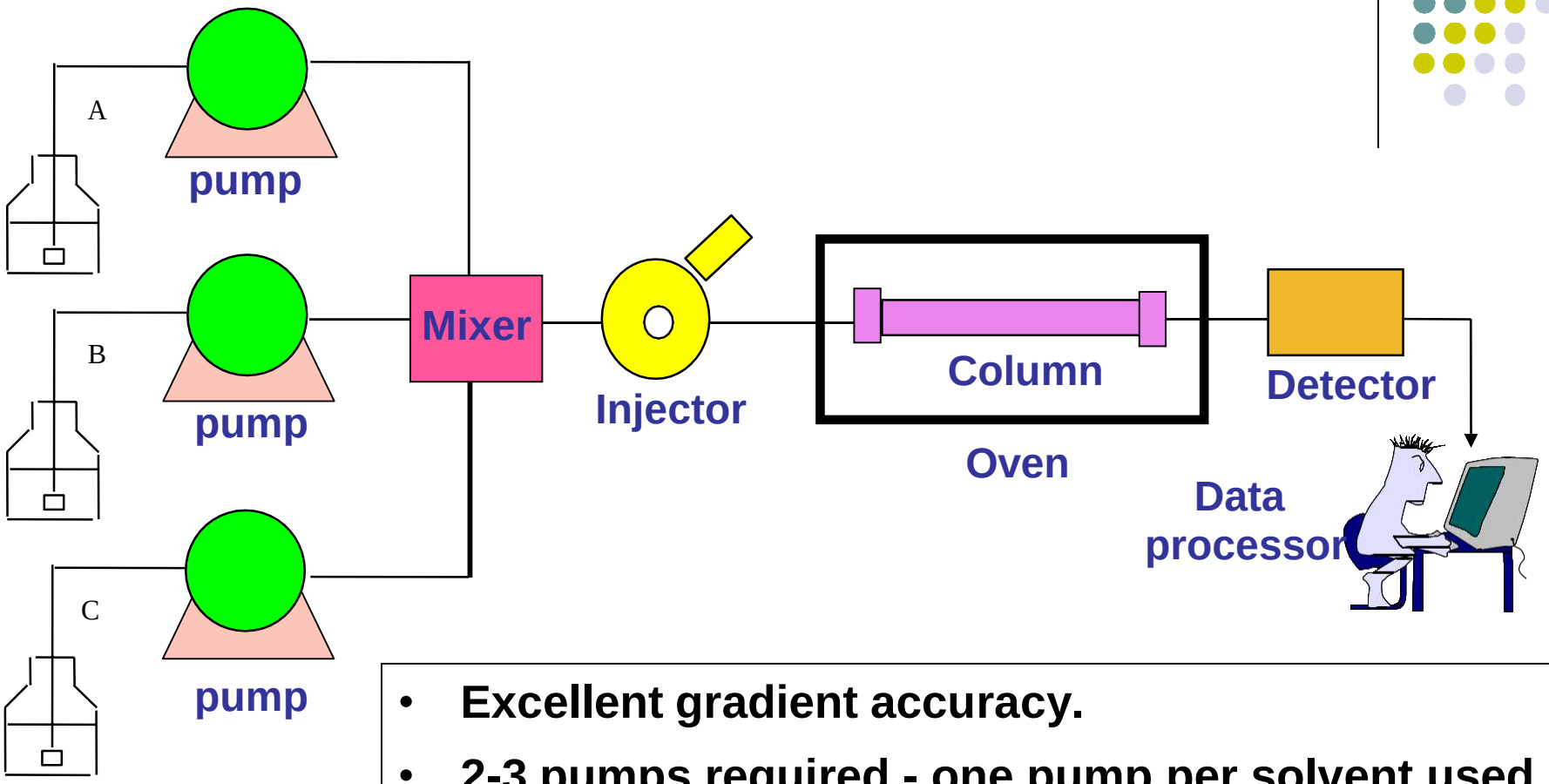
If more than one solvent is used, solvents should be premixed.

Low-pressure Gradient System



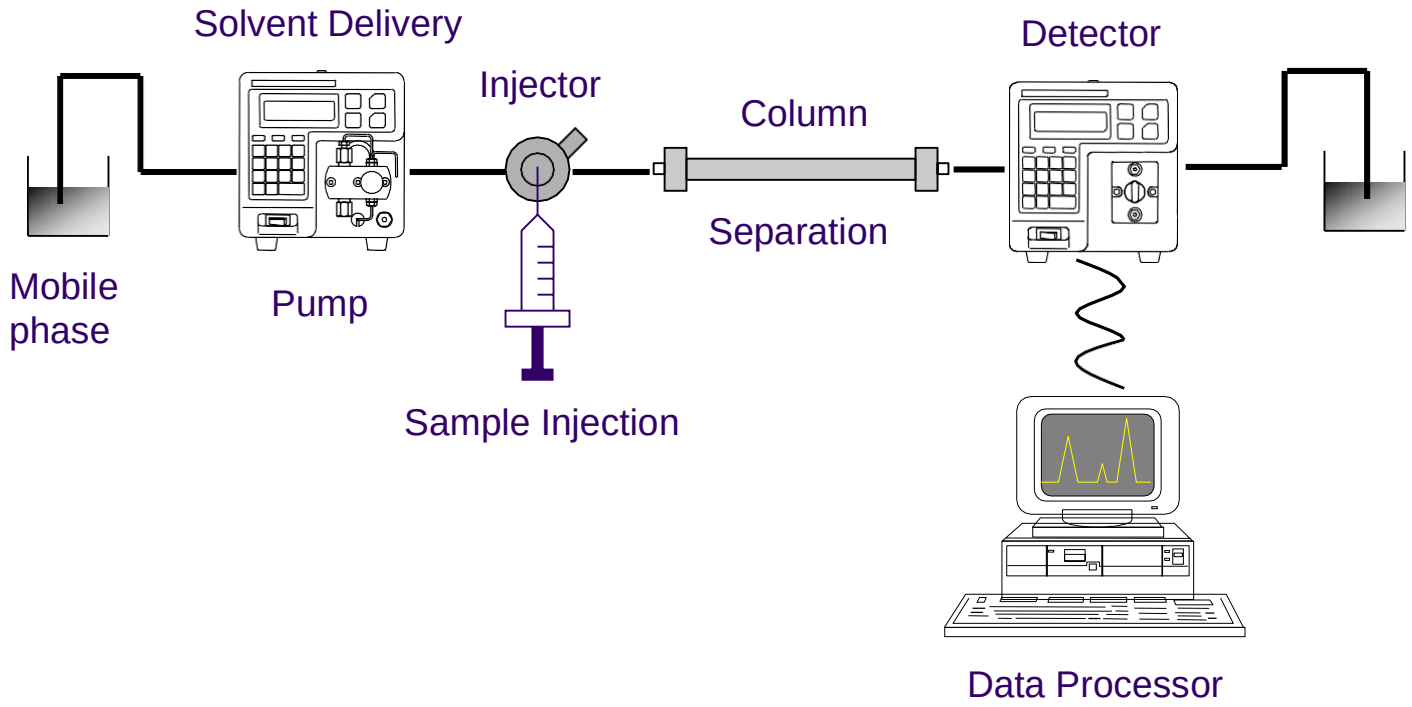
- One pump used to control 4 reservoirs;
- Mixing is done before pump.
- On-line degasser is necessary.

High-pressure Gradient System



- **Excellent gradient accuracy.**
- **2-3 pumps required - one pump per solvent used.**
- **On-line degassing may not be critical.**
- **The sum of the flow rates delivered by each pump is maintained constant**

HPLC Basic Instrumentation



Components of A Liquid Chromatography System

- Mobile Phase / Solvent Reservoir
- Degasser
- Solvent Delivery System (Pump)
- Injector
- Precolumn
- Column
- Temperature Control
- Detectors
- Recorder (Data Collection)

The Mobile Phase in HPLC



- **Must do the following:**
  - **solvate the analyte molecules and the solvent they are in**
  - **be suitable for the analyte to transfer "back and forth" between during the separation process**

- **Must be:**
  - **Compatible with the instrument (pumps, seals, fittings, detector, etc)**
  - **Compatible with the stationary phase**
  - **Readily available (often use liters/day)**
  - **Adequate purity**
  - **Free of gases (which cause compressability problems)**
  - **Low viscous – methanol than ethanol**

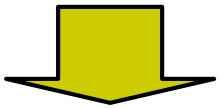
Mobile Phase for Reversed Phase HPLC

- Water / buffer + Organic solvent
  - **Organic solvents:**
  - Methanol
  - Acetonitrile
  - THF
  - **Buffer:**
  - Phosphate buffer
  - Acetate buffer
  - Ammonia buffer
- Ratio of aqueous and organic solvents is important



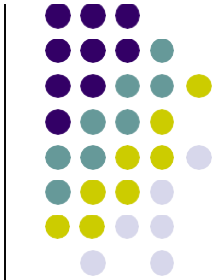
Degasser

- **Problems caused by dissolved air(O_2, N_2)in mobile phase (Deaerated mobile phase)**
  - Unstable delivery in pump
  - Bigger noise and large baseline-drift in detector cell



- **In order to avoid causing the problems, mobile phase should be degassed.**
  - vacuum pumping systems
  - distillation system
  - a system for heating and stirring the solvents
  - sparging system – Passing an inert gas of low solubility through the solvent

PUMPS



Four basic types of LC Pumps are:

- Pneumatic pumps – Preparative purpose only
- Motor driven syringe type pumps
- Reciprocating pumps
- Hydraulic amplifier pumps – not in use

Motor driven syringe type pumps

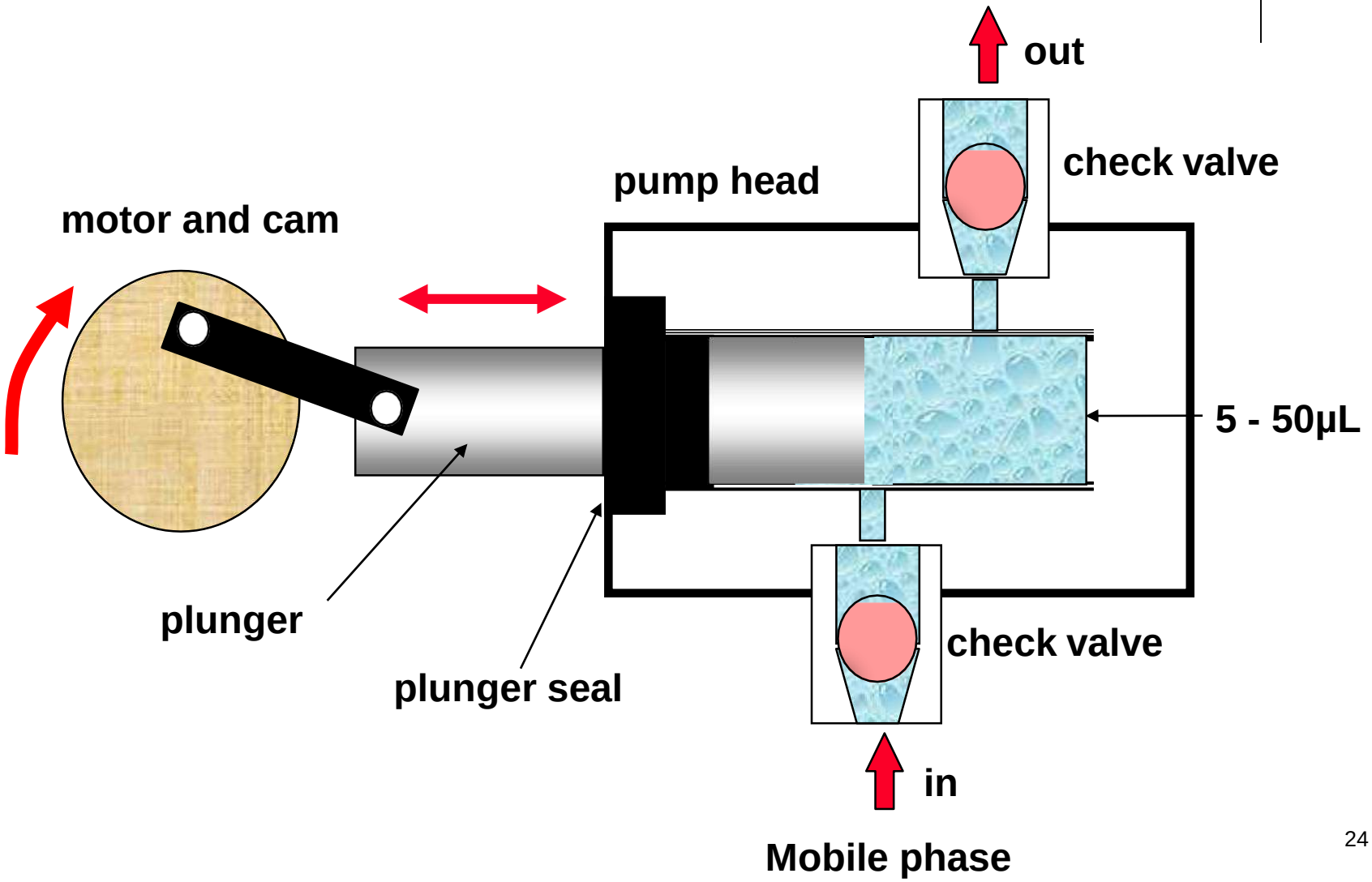
Works on the principle of positive solvent displacement
Double syringe pumps are available:

one for column

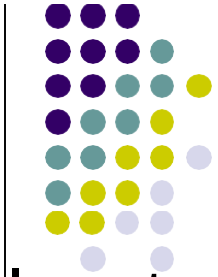
one from reservoir

- **Advantages:**
  - Simple
  - Inexpensive
  - Pulse free
  - Stable flow rate
  - Lowest dead volume
- **Disadvantages**
  - Limited capacity
  - Not suitable for gradient elution

Plunger Reciprocating Pump



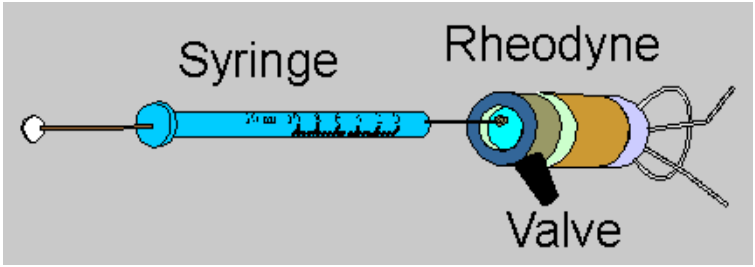
Plunger Reciprocating Pump



- Consists of a small chamber in which the solvent is pumped by the back and forth motion of a motor-driven piston
- Advantage
  - Low pressure fluctuation
  - Very easy to replace other solvent
- Disadvantage
  - Change the plunger seal
  - Flow variation
  - Small volume of solvent delivery is possible

Sample Injection Systems

- Convenient to use
- For injecting the solvent through the column
- Minimize possible flow disturbances
- Volumes must be small
- .1-500 μL
- Sampling loops
  - interchangeable loops (5-500 μL at pressures up to 7000 psi)
  - Chemically inert
  - Reproducible



Different ways of sample injection



- **Fixed volume valve injection**
  - First HPLC sample injection system
  - Valve loop is filled with sample
  - Reproducible sample amounts can be injected
- **Variable injection valve injection**
  - Flow restrictors are used b/w pump-column
- **On column injection**
  - Injected by means of syringe through septum
  - Simple method of injection
  - Dis-adv :Leaching effect of the mobile phase in contact with septum , leads to a ghost peak




1.5
1.0
0.5ml
1.5
1.0
0.5ml
1.5
1.0
0.5ml



Peak broadening depends on

- Type of injection system used
- Connection between injector and column
- Injection volume and time taken for injection

- Commonly used Injectors

  Syringe injection - Trouble is more

  - Peak broadening

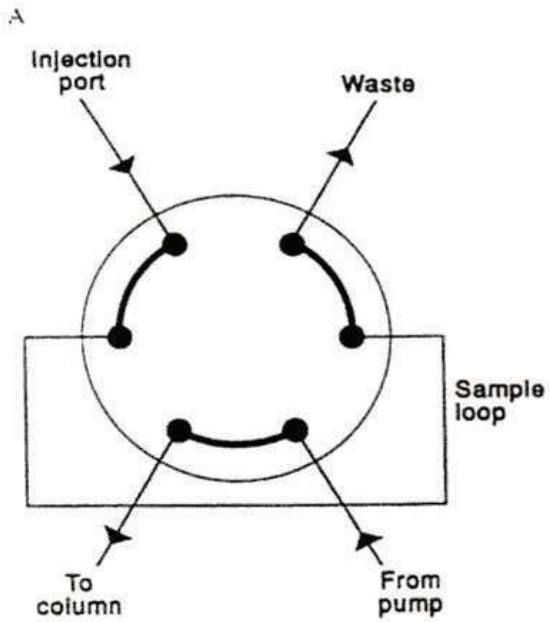
  Valve injection – Automation is possible

  -- Highly précised one

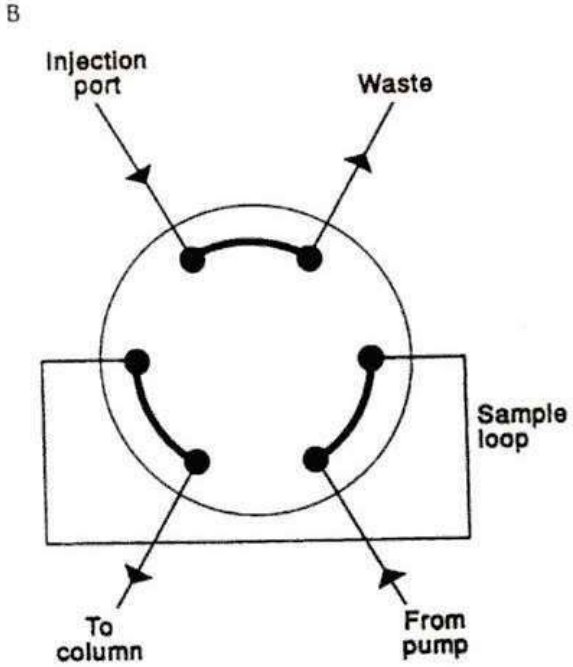
  -- Constant pressure is maintained

Manual injector

- **Valve injectors**

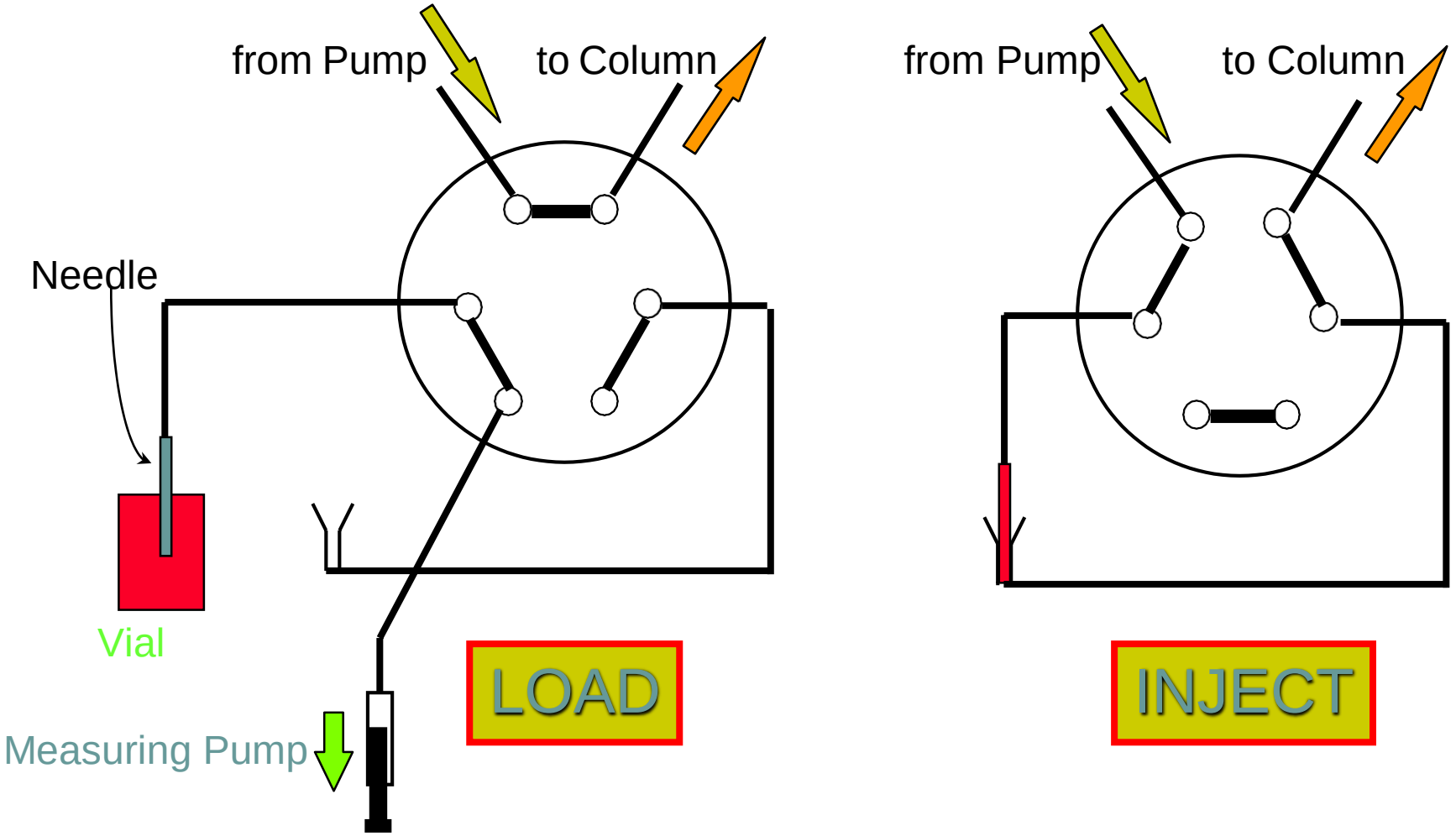


(a) Isolated from the pump eluent Stream (LOAD position)



(b) Positioned in it (INJECT position)

Direct Injection Auto Sampler



Guard column

- Protection device, often included just prior to the analytical column to chemically remove components of the sample that would foul the main column
- Guard column filled with removable protective cartridge

Troubleshooting: if pressure in system high check the cartridge in the guard column

Guard - Protects the analytical column.

- Particles
- Interferences
- Prolongs the life of the analytical column

Columns and Stationary Phases.

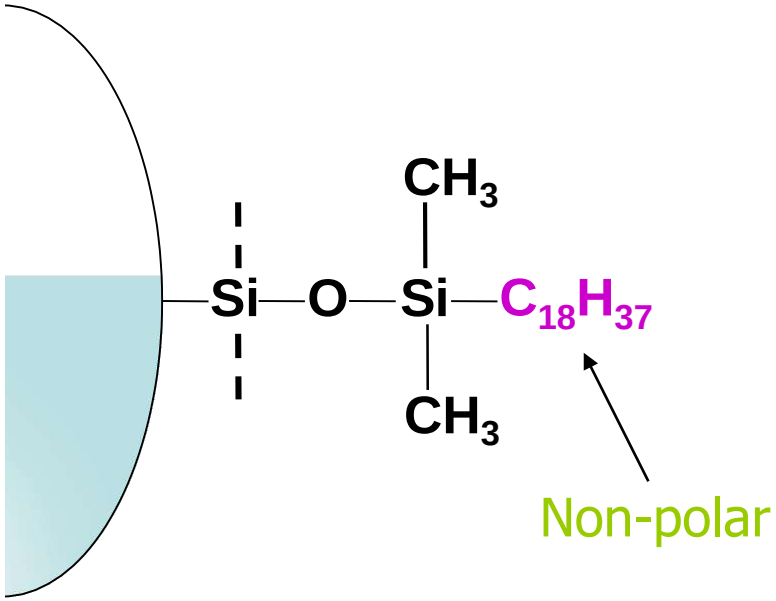


- **HPLC is largely the domain of packed columns some research into microbore/capillary columns is going on.**

- **Stationary phases are particles which are usually about 1 to 20 μm in average diameter (often irregularly shaped)**
- **In Adsorption chromatography, there is no additional phase on the stationary phase particles (silica, alumina, Fluorosil).**
- **In Partition chromatography, the stationary phase is coated on to (often bonded) a solid support (silica, alumina, divinyl benzene resin)**

Stationary Phase in Reversed Phase Column

- C18 (ODS) type
- C8 (octyl) type
- C4 (butyl) type
- Phenyl type
- TMS type
- Cyano type

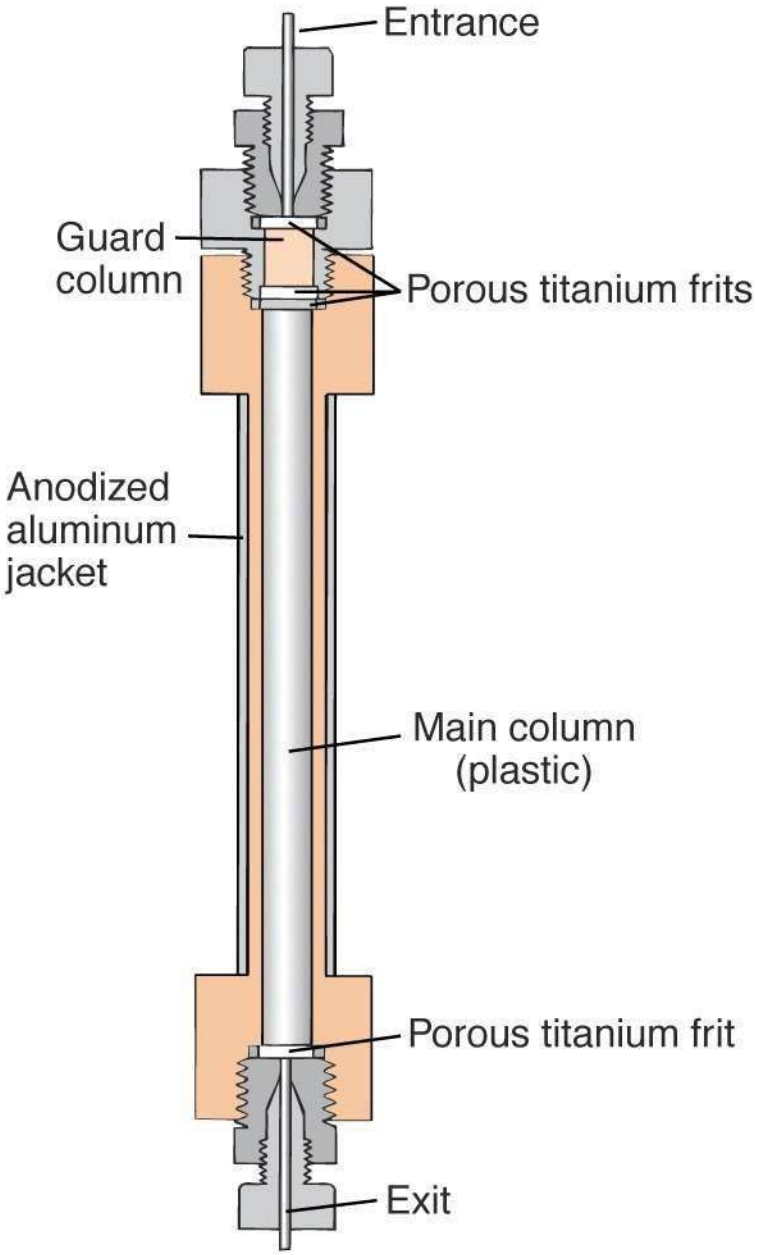


Stationary Phases

- **Polar (“Normal” Phase):**
  - **Silica, alumina**
  - **Cyano, amino or diol terminations on the bonded phase**

- **Non-Polar (“Reversed Phase”)**
  - **C18 to about C8 terminations on the bonded phase**
  - **Phenyl and cyano terminations on the bonded phase**

- **Mixtures of functional groups can be used!!**


Entrance
Guard column
Porous titanium frits
Anodized aluminum jacket
Main column (plastic)
Porous titanium frit
Exit

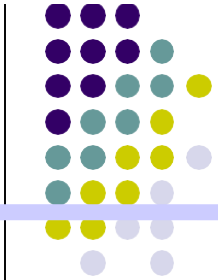


Properties of Detector



- Adequate sensitivity
- Stability and reproducibility
- Short response time
- Minimum volume for reducing zone broadening
- High reliability and ease of use
- Similarity in response toward all analytes
- Non-destructive

Detectors for HPLC



- UV-VIS Ultraviolet / Visible detector
- PDA Photodiode Array detector
- RF Fluorescence detector
- CDD Conductivity detector
- RID Refractive Index detector
- ECD Electrochemical detector
- ELSD Evaporative light scattering detector
- MS Mass spectrophotometer detector

Ultraviolet / Visible Detector



Advantage:

- **Sensitivity is high**
- **Relative robust to temperature and flow rate change**
- **Compatible with gradient elution**

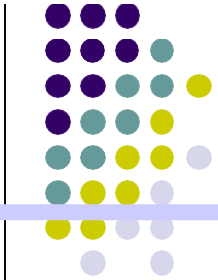
Disadvantage:

- **Only compounds with UV or visible absorption could be detected.**

Additional Functions

- **Dual Wavelength mode**
- **Wavelength Time Program mode**
- **Wavelength Scan mode**

PDA Detector



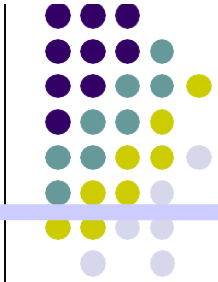
Advantages:

- **PDA Detector could analyze a sample simultaneously at many different wavelengths.**
- **UV Visible spectra are useful for compound identification, checking peak purity, as well as finding the optimum absorbance for the compounds.**
- **UV Visible spectra of many compounds could be stored in the spectrum libraries, which are useful for compound identification.**
- **Relatively robust to temperature and flow rate fluctuations**
- **Compatible with gradient elution.**

Disadvantages:

- **Slightly less sensitive than UV-Visible detector.**

Refractive Index Detector



Advantage

Responds to nearly all solutes

Unaffected by flow rate

Disadvantage

Not as sensitive as most other types of detectors

Could not be used with gradient elution

Selection of Detectors



Detectors	Type of compounds can be detected
UV-Vis & PDA	Compounds with chromophores, such as aromatic rings or multiple alternating double bonds.
RF	Fluorescent compounds, usually with fused rings or highly conjugated planar system.
CDD	Charged compounds, such as inorganic ions and organic acid.
ECD	For easily oxidized compounds like quinones or amines.
RID & ELSD	For compounds that do not show characteristics usable by the other detectors, eg. polymers, sacharides.

Parameters used in HPLC



CAPACITY FACTOR
RESOLUTION
ASYMMETRY FACTOR (TAILING FACTOR)
EFFICIENCY

Retention : When a component in a sample interacts with the stationary phase in the column and a delay in elution occurs.

Column efficiency : Goodness of a column

Parameters used in HPLC

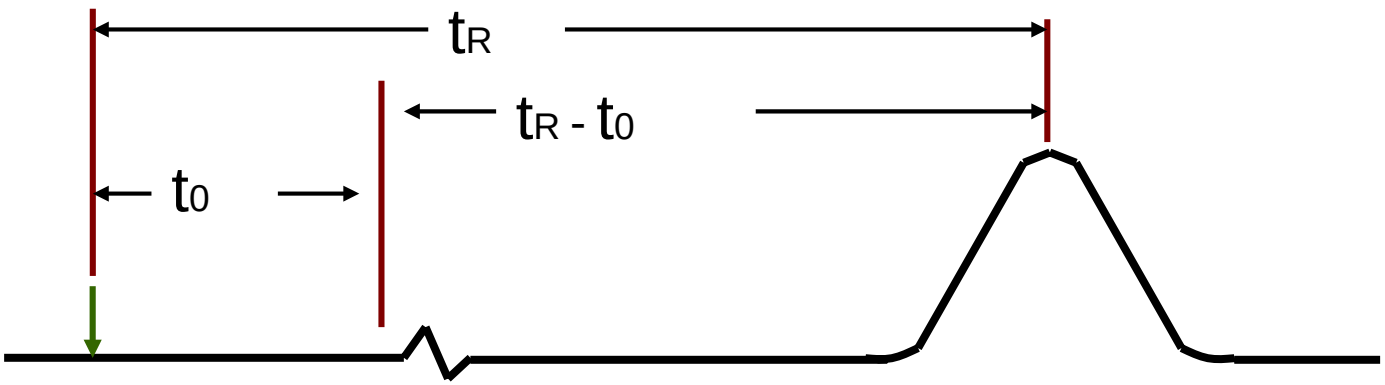
Retention parameters

Rt : Retention Time (the time between the injection point and the maximum detector response for correspondent compound)

vR: retention volume (tR x eluent flow rate)

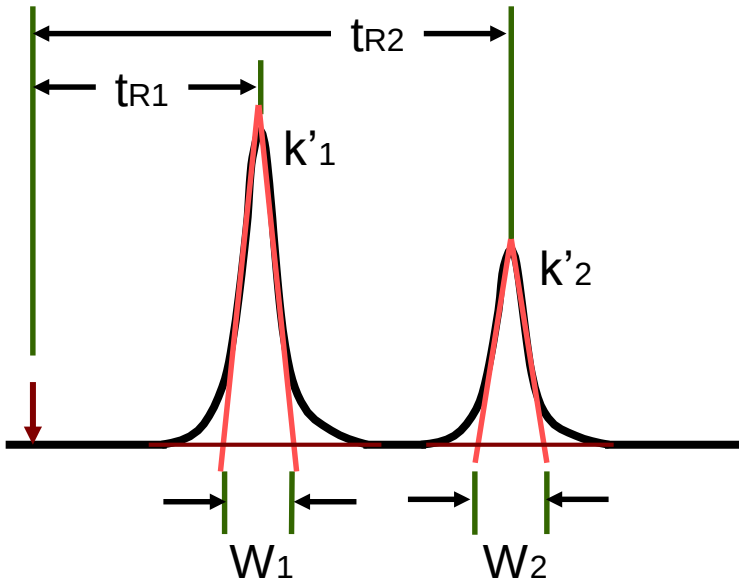
k’ : capacity factor

t0 : the time required for the component not retained by the column to pass through the column



Resolution

The resolution of two bands is a function of both their relative Retentions and peak width.



Resolution : $R_s = 2 \times \frac{t_{R2} - t_{R1}}{W_1 + W_2}$

Separation factor : $\alpha = \frac{k'_2}{k'_1}$

Number of theoretical plates (N): **compare efficiencies of a system for solutes that have different retention times**

$$N = (t_R/\sigma)^2$$

or for a Gaussian shaped peak

$$N = 16\,(t_R/W_b)^2$$

$$N = 5.54\,(t_R/W_h)^2$$

The larger the value of N is for a column, the better the column will be able to separate two compounds.

- **the better the ability to resolve solutes that have small differences in retention**
- **N is independent of solute retention**
- **N is dependent on the length of the column**

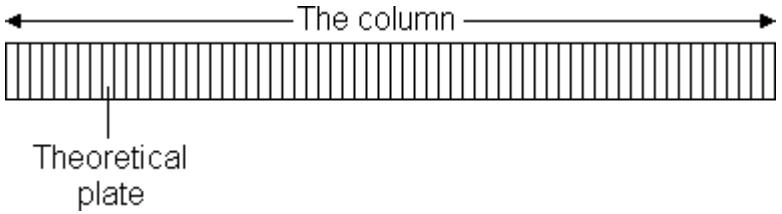


Plate height or height equivalent of a theoretical plate (H or HETP): compare efficiencies of columns with different lengths:

$$H = L/N$$

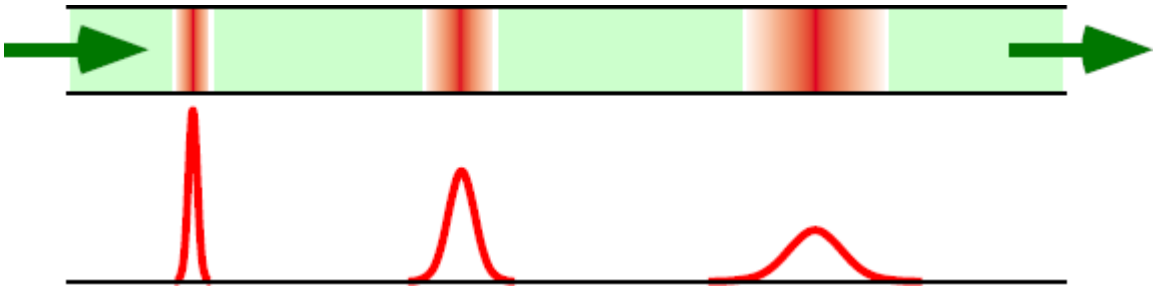
where: L = column length
N = number of theoretical plates for the column

Note: H simply gives the length of the column that corresponds to one theoretical plate

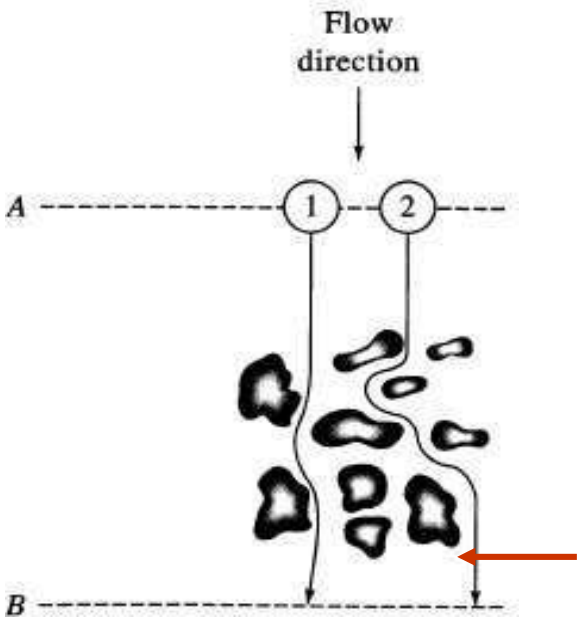
H can be also used to relate various chromatographic parameters (e.g., flow rate, particle size, etc.) to the kinetic processes that give rise to peak broadening:

Why Do Bands Spread?

a. Eddy diffusion
b. Mobile phase mass transfer
c. Stagnant mobile phase mass transfer
d. Stationary phase mass transfer
e. Longitudinal diffusion

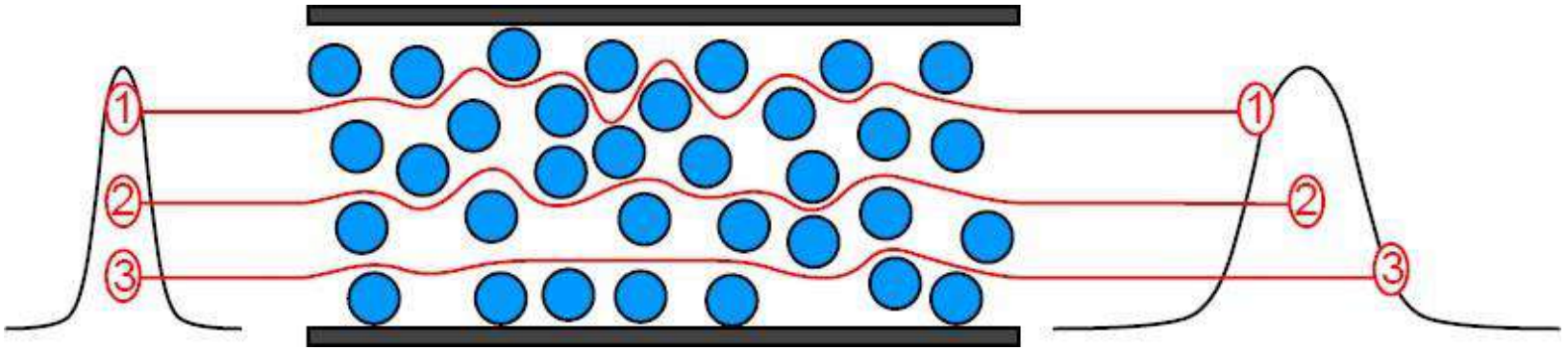


a.) *Eddy diffusion* – a process that leads to peak (band) broadening due to the presence of multiple flow paths through a <u>packed</u> column.

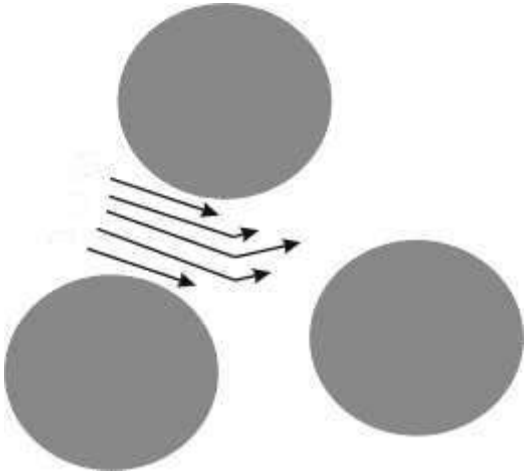


As solute molecules travel through the column, some arrive at the end sooner then others simply due to the different path traveled around the support particles in the column that result in different travel distances.

Longer path arrives at end of column after (1).



b.) Mobile phase mass transfer – a process of peak broadening caused by the presence of different flow profile within channels or between particles of the support in the column.



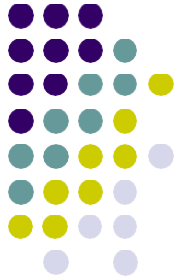
A solute in the center of the channel moves more quickly than solute at the edges, it will tend to reach the end of the channel first leading to band-broadening

The degree of band-broadening due to eddy diffusion and mobile phase mass transfer depends mainly on:

1) the size of the packing material
2) the diffusion rate of the solute

Applications of HPLC

Field	Typical mixtures
Pharmaceuticals	Antibiotics, sedatives, steroids, Amino analgesics, crude drugs, cosmetics
Biochemical	acids, proteins, peptides, carbohydrates, lipids, enzymes, medicines, hormone
Food products	Mycotoxins, additives, saccharides, amino acids, vitamins, fatty acid, coloring agents, antibacterials
Industrial chemicals	Condensed aromatics, surfactants, propellants, dyes, polymers, plasticizers
Forensic chemistry	Drugs, poisons, blood alcohol, narcotics
Environmental field	Inorganic ions, organic acids, agricultural chemicals, pesticides, herbicides, phenols,
Clinical medicine	Bile acids, drug metabolites, urine extracts, estrogens



THANK YOU

Hormone Replacement Therapy

Dr.S.Shiva Prasad
VCPS

What is Menopause?

- Cessation of menstruation as a result of the aging process of or surgical removal of the ovaries; change from fertility to infertility.
- Physiological changes that take place within women between the ages of 30 to 60.
- Hormone levels change in female body; practically the production of estrogen.
- Perimenopause and menopause can last 10 plus years.
- Menopause itself begins 12 months after a woman's final period.

Stages of Menopause

- **Perimenopause**
  - Irregular, short menstrual periods
  - Uncomfortable symptoms include hot flashes, insomnia, irritability, and backaches
  - May last four to five years or longer
- **Menopause**
  - 12 months after the last menstrual period
  - Production of progesterone and eggs stops
  - Normally happens between the ages of 45 and 55
- **Postmenopause**

Symptoms

- Irregular menstrual periods
- Hot flashes
- Irritability, depression, anxiety
- Insomnia, poor sleep quality
- Palpitations, night sweats
- Forgetfulness (in some women)
- Vaginal dryness
- Headaches, vaginal infections, joint aches and pains

Treatment of Menopause

- Hormonal Replacement Therapy
- Non-hormonal therapy
  - SERM
  - Bisphosphonate

Hormonal Replacement Therapy

- Benefit of HRT
  - Relief of Symptoms
  - Preventive Therapy

Hormonal Replacement Therapy

- Benefit of HRT for Relief of Symptoms
  - Hot flashes
    - Highly effective
    - Usually required for a relatively short period of time, e.g. 1 to 3 years
  - Mood disturbance
    - Improve irritability and anxiety in many menopausal women
    - Relieve mild depressive symptoms
    - Mechanism not sure, ? direct effect vs secondary to alleviation of physical symptoms

Hormonal Replacement Therapy

- Benefit of HRT for Relief of Symptoms
  - Urogenital symptoms
    - Improve urogenital atrophy, thinning, dryness and loss of elasticity
    - Relieve symptoms of dyspareunia
    - Improve sexual functioning and sexuality
    - Contradictory data on incontinence
      - Some studies indicate that HRT may relieve symptoms of urinary urgency, urge incontinence, stress incontinence, frequency and dysuria, but some data showed negative effects

Hormonal Replacement Therapy

- Benefit of HRT for Preventive Therapy
  - Coronary disease
    - Primary and Secondary Prevention of IHD was previously demonstrated by nonrandomized, observational studies
    - Until June 2002, there is no randomized, observational studies on primary prevention of IHD
    - However, new data concerning prevention of IHD (primary and secondary) come up recently …

Hormonal Replacement Therapy

- HERS Study
  - Heart and Estrogen/Progesterone Replacement Study
  - Currently the only randomized, placebo-controlled trial of the HRT for secondary prevention
  - 2763 post-menopausal women with IHD, average age 67 years, received HRT (estrogen + progesterone) was followed up for 4.1 years.
  - Risk of MI increased during the first year, although risk seemed to decreased during the remainder of the study.

JAMA. 1998;280:605-613

Hormonal Replacement Therapy

- HERS II Study
  - Follow-up open-label study of HERS, lasting for 2.7 years
  - Designed to evaluate the effects of longer-duration of HRT
  - Initial trend from HERS suggesting a reduced risk of MI with longer duration of HRT did not persist with additional follow up period
- Combining HERS and HERS II Studies
  - There was no risk reduction from HRT during almost 7 years.

JAMA. 2002;288(1):49-57,58-66,99-101

Hormonal Replacement Therapy

- Anything even newer ?
- Read the newspaper or journals recently ?
- To be continued …

Hormonal Replacement Therapy

- Benefit of HRT for Preventive Therapy
  - Coronary disease
    - Current evidence is not sufficient to recommend HRT for cardiovascular indication for most women at risk of IHD
    - Instead, aggressive risk factor modification is recommended.
    - Also, there is no evidence that IHD or the presence of cardiac risk factors is a contraindication to HRT

Hormonal Replacement Therapy

- Benefit of HRT for Preventive Therapy
  - Osteoporosis
    - Prevention and treatment of osteoporosis are well documented and widely accepted use of HRT
    - Beneficial effect of HRT on bone mineral density is well proven
    - Trials
      - No randomized prospective trial for hip fracture
      - Large case-controlled studies showed HRT can prevent vertebral fracture
      - A multicenter clinical trial sponsored by the Women's Health Initiative, focusing on the effect of HRT on fracture risk, is currently underway and data will be available by 2006.

Hormonal Replacement Therapy

- Benefit of HRT for Preventive Therapy
  - Alzheimer's Disease and Cognitive Functioning
    - HRT may improve some aspects of cognitive functioning
    - Long term HRT may : -
      - Reduce the risk of Alzheimer's disease
      - Slow the progression and improve cognitive functioning and mood in elderly women with established Alzheimer's disease.

Hormonal Replacement Therapy

- Benefit of HRT for Preventive Therapy
  - Colon Cancer
    - The protective benefit is suggestive but not proven
    - Long-term use solely for this purpose is NOT recommended
  - Skin / Wound Healing
    - Beneficial effect on collagen metabolism, improve skin tone and wound healing
  - Tooth loss
    - Reduce maxillary and mandibular osteoporosis and prevent resulting tooth loss
  - Macular degeneration
    - Recent date suggests a decreased incidence of macular degeneration in women on HRT

Risk of HRT

- Breast Cancer
  - Real controversy !!!
  - Among the 55 studies published between 1974-1996, 90% failed to demonstrate an increased risk.
  - Meta-analysis in 1997 by the Oxford Group
    - The findings of increased breast cancer in HRT user may not be conclusive and may be open to questions of statistical inaccuracy.

Risk of HRT

- Breast Cancer - some acceptable findings
  - Small increase (up to 1.3x) in breast cancer risk after 5-15 years or more of HRT (i.e. use HRT < 5 years is safe)
  - Breast cancer motality does not increased with HRT because the cancer tends to be less advance, lower rate of node positivity, better differentiated and more favorable histological type.
  - Risk of breast cancer is increase in CURRENT USER only. Previous use of HRT carry no increase risk.

Risk of HRT

- Endometrial Cancer
  - Increased only in women taking unopposed estrogen
  - Post-hysterectomy patient can take unopposed estrogen without any increase in risk.
- Gallbladder Disease
  - The risk of gallbladder disease continues at higher, premenopausal level in women taking HRT
- Venous Thrombosis
  - Risk increased up to 3-folds in CURRENT user only
  - Absolute risk of still relatively low, i.e. increase from approx 10 cases per 100,000 women in general population to approx 30 cases per 100,000 women on HRT.
- Ovarian cancer
  - May be a week association but not proven at this time.

Contraindication

- Absolute contraindication
  - Prior history or existing breast cancer
  - Prior history or existing endometrial cancer
  - Prior history of venous thrombosis
  - Undiagnosed abnormal vaginal bleeding
  - Severe, active liver disease with abnormal LFT
- Relative contraindication
  - Family history of breast cancer
  - Hyper-TG
  - Gallstone and gallbladder disease

Contraindication

- The following are currently NOT contraindicated
  - HT
  - Smoking
  - Obesity
  - Migraine headache
  - Uterine fibroid
  - Endometriosis
  - Fibrocystic breast change

Pre-treatment Assessment

- History
  - General health
  - Perimenopausal symptoms
  - Gynecological history (e.g. endometrial cancer)
  - Risk of osteoporosis and CVS disease
- Physical Examination
  - Complete physical examination
  - Pelvic examination
  - Pap smear

Pre-treatment Assessment

- Optional investigation
  - FSH - if symptoms of menopause is atypical
  - Mammogram - if patient is at risk of CA breast
  - Bone densitometry (e.g. DEXA scan)
  - Pelvic USG / Abdominal USG
  - Lipid profile and FBS
  - LFT
  - Endometrial aspiration

Follow-up Plan

- Follow up at 3rd, 6th and 12th month for
  - Symptom control
  - Compliance, side-effects and bleeding pattern
  - Urine multistix
  - BP measurement
- Other investigation
  - Yearly physical examination + cervical smear
  - 2-yearly mammogram
  - Blood test, endometrial aspiration and bone densitometry when indicated

HRT Regimen

- Patient with Hysterectomy done
  - First Line
    - Unopposed estrogen therapy
    - Examples
      - conjugated estrogen 0.625 mg Daily
      - estradial 2 mg Daily
  - Second Line
    - Non-oral estrogen
      - Transdermal patch (e.g. Estraderm)

HRT Regimen

- Intact uterus & amenorrhoea < 2 year
  - First Line
    - Sequential combined therapy
      - Estrogen is given continuously with sequential addition of progesterone for 10 to 14 days
      - Example 1 -
        - 14 conjugated estrogen 0.625 mg
        - 14 - conjugated estrogen 0.625 mg + medroxyprogesteron 5 mg
      - Example 2 -
        - 12 - estradiol 2 mg
        - 10 - estradiol 2 mg + norethindrone 1 mg
        - 6 - estradiol 1 mg

HRT Regimen

- Intact uterus & amenorrhoea < 2 year
  - Second Line
    - Cyclic estrogen + cyclic progesterone
      - Prempak (28-day cycle)
        - Day 5 to 25 - conjugated estrogen 0.625 mg Daily
        - Day 12 to 21 - add medrogesteron 5 mg Daily
        - Day 26 to 4 - pill free
        - In the absence of menstruation, administration is started arbitrarily.
  - Predictable monthly withdrawal bleeding is expected, and some women may achieve amenorrhoea eventually.

HRT Regimen

- Intact uterus & amenorrhoea > 2 year
  - First Line
    - Continuous combined therapy
      - Very little endometrial stimulation, therefore no withdrawal bleeding in most women (but 5-15% women may have unpredictable spotting indefinitely)
      - Example 1 -
        - conjugated estrogen 0.625 mg
        - medroxyprogesteron 2.5 mg
      - Example 2 -
        - estradiol 2 mg
        - norethisteron 1 mg

HRT Regimen

- Intact uterus & amenorrhoea > 2 year
  - Second Line
    - Tibolone 2.5 mg daily
      - C-19 steroid
      - Estrogenic + progesteogenic + weak androgenic properties
      - No withdrawal bleeding
      - Beneficial effects
        - symptoms + prevent bone loss
        - improve lipid profile
        - libido stimulation
      - An alternative in women who have relative contraindication to estrogen
      - Substantial risk of breakthrough bleeding, therefore recommended to start therapy not earlier than one year after menopause to minimize breakthrough bleeding

Non-hormonal Therapies

- Selective Estrogen Receptor Modulators (SERMs)
  - SERMs bind to all estrogen receptors but have different effects in various tissues
  - Raloxifene
    - First SERM to be approved by the FDA (now the only one)
    - Bind to estrogen receptor in bone and therefore improve bone mineral density, biochemical markers of bone turnover.
    - Also improve serum lipid profiles and can possibly prevent IHD (NOT PROVEN)

Non-hormonal Therapies

- On the other hand, Raloxifen DOES NOT
  - Increase risk of breast cancer (may even protect)
  - Treat hot flashes (may make them worse)
  - Relieve symptoms of vaginal atrophy
  - Appear to stimulate the endometrium
- Current indication – prevent and treatment of osteoporosis
- Contraindications
  - Premenopausal or perimenopausal (worsen symptoms)
  - History of thromboembolism
- Possible Side Effects
  - Hot flashes and leg cramp
  - NO breast pain or breast enlargement
- Dosage - Raloxife 60mg daily (any time of the day, with or w/o meal)

Non-hormonal Therapies

- Bisphosphonates
  - FDA-approved for prevention and treatment of osteoporosis
  - Prevention
    - Alendronate 5 mg daily
    - Residronate 5 mg daily
  - Treatment
    - Alendronate 10 mg daily (or 70mg once weekly preparation)
    - Residronate 5 mg dailyl
  - Side Effect – Esophagitis
    - Women with pre-existing esophageal disease may not tolerate
    - Special precaution in drug intake (alendronate)

Summary

- Women with menopausal symptoms
  - Exclude contraindication
  - Taking the new studies (HERS and WHI) into consideration, short-term use still has risk of coronary heart disease and thromboembolic disease.
  - Discuss with patient and balance the risk against the severity of symptoms.
  - Consider to start HRT for 1 to 5 years and stop.

Summary

- Women with increased risk of osteoporosis
  - Exclude contraindication of HRT $\longrightarrow$ consider HRT
  - Also explain other treatment options available (e.g. SERM, alendronate, etc)
  - Duration of Treatment
    - HRT – may be continued indefinitely, bone loss recur once HRT was stopped
    - Alendronate – therapeutic efficacy has been deomonstrated for 7 years. Safety and efficacy beyond 7 years have not yet been established. No accelerated bone loss observed after discontinuation.
    - Risedronate – therapeutic efficacy and safety had been demonstrated for a 3-year period only.
    - SERM – Efficacy and safety have ben demonstrated for up to 40 months.

Summary

- Women who start HRT for preventive therapy
  - Think twice !
  - May experts (including those from WHI) advise primary care doctor to STOP prescribing HRT for this purpose.

Ischemic Heart Disease

DR.ADEPU RAMESH
VCPS

Terminology/Definition

- **Ischemic heart disease (IHD)**, also known as **coronary artery disease (CAD)**, is defined as: **a lack of oxygen "ischemia" and decreased or no blood flow to the myocardium resulting from coronary artery narrowing or obstruction**

- **Common clinical manifestations of IHD are stable angina and acute coronary syndrome**

Epidemiology

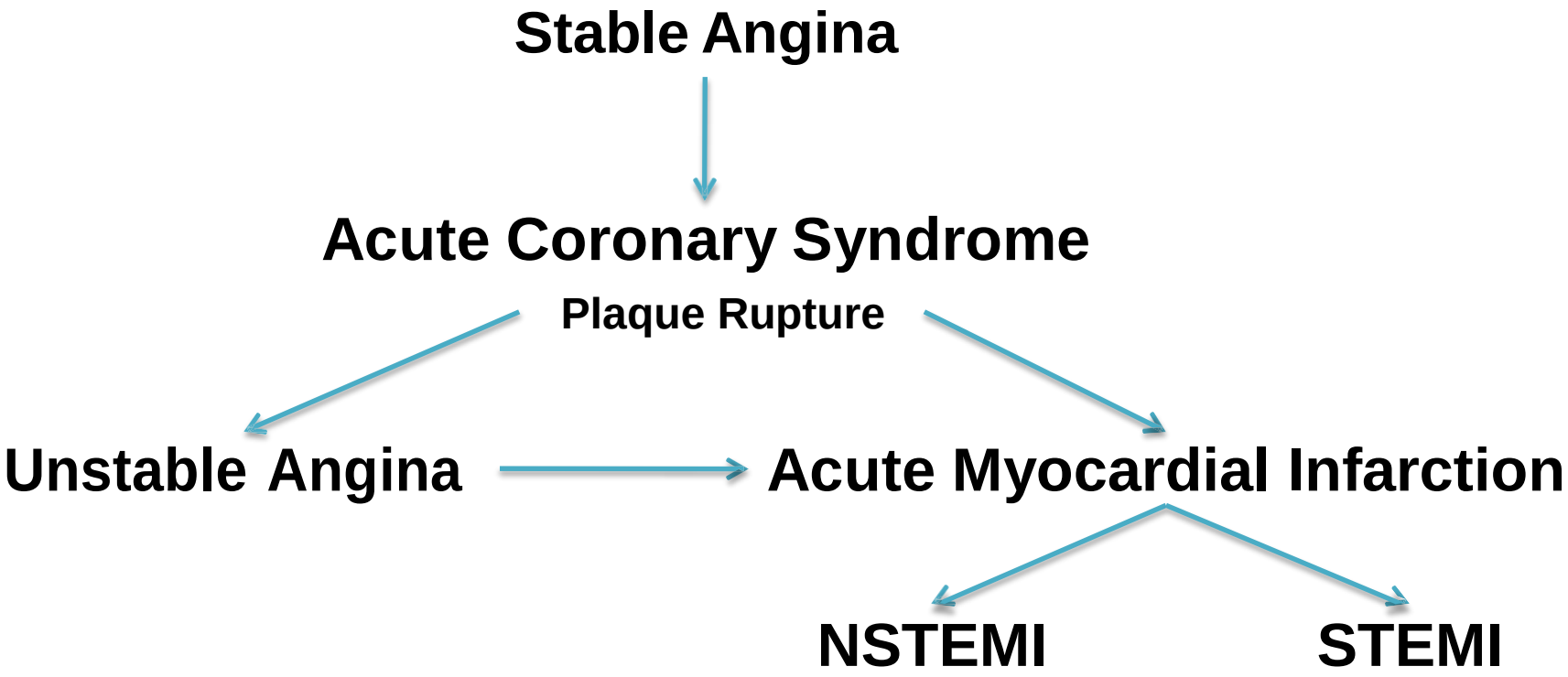
- In India CHD led to 17% of total deaths and 26% of adult deaths in 2001-2003, which increased to 23% of total and 32% of adult deaths in 2010-2013.

- **60 to 79 year old** :~ 23% of men and 15% of women have prevalent IHD, and these figures rise to 33% and 22% among men and women **80 years of age**.

- Although **IHD is widely known** to be the **number 1 cause** of **death in men**, this is **also the case for women**, among whom this condition accounts for 27% of deaths.

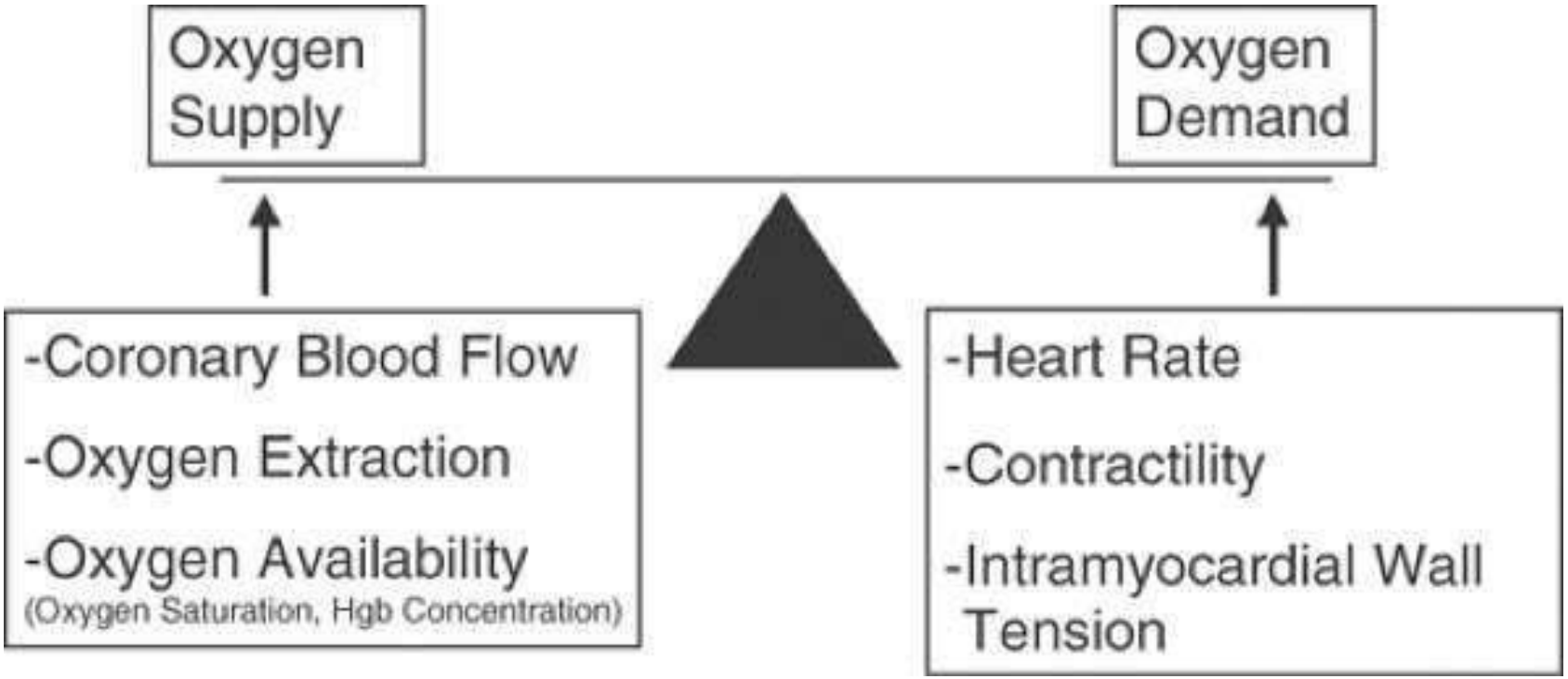
- **IHD** accounts for the **vast majority** of the **mortality and morbidity of cardiac disease.**

Cardiovascular Event Pathway



Pathophysiology

Determinants of myocardial oxygen supply & demand



As the consequences of IHD are a result of increased demand in the face of a fixed supply of oxygen in most situations, alterations in MVO_2 are critically important in producing ischemia and for interventions intended to alleviate ischemia

Pathophysiology

- **Reductions** in **coronary** blood **flow** (secondary to **atherosclerotic plaques, vasospasm**, or **thrombus formation)** and **arterial oxygen content** (secondary to **hypoxia**) decrease **myocardial oxygen supply.**

- Because the coronary arteries fill during diastole, decreases in diastolic filling time (e.g**., tachycardia**) can also **reduce coronary perfusion** and myocardial oxygen supply.

- **In chronic stable angina**, ***atherosclerotic plaques*** are the most common cause of coronary artery **narrowing** and reductions in coronary blood flow. **In contrast, in ACS**, ***disruption of an atherosclerotic plaque*** with subsequent **thrombus** (**blood clot**) formation causes **abrupt reductions** in **coronary blood flow** and **oxygen supply.**

Pathophysiology

- **Conditions** that can **reduce the oxygen-carrying capacity** of blood:
  - **Anemia**
  - **Carbon monoxide poisoning**, and
  - **Cyanotic congenital heart**

→ potentially causing **ischemia** in the face of **adequate coronary perfusion.**

- **Coronary heart disease (CHD)** refers to the failure of coronary circulation to supply adequate circulation to cardiac muscle and surrounding tissue.
  - Common manifestations of coronary heart disease (CHD) include:
    - Angina pectoris, whether stable (SA) or unstable (USA)
    - myocardial infarction (MI),
    - heart failure (HF),
    - arrhythmias,
    - Sudden cardiac death (SCD).

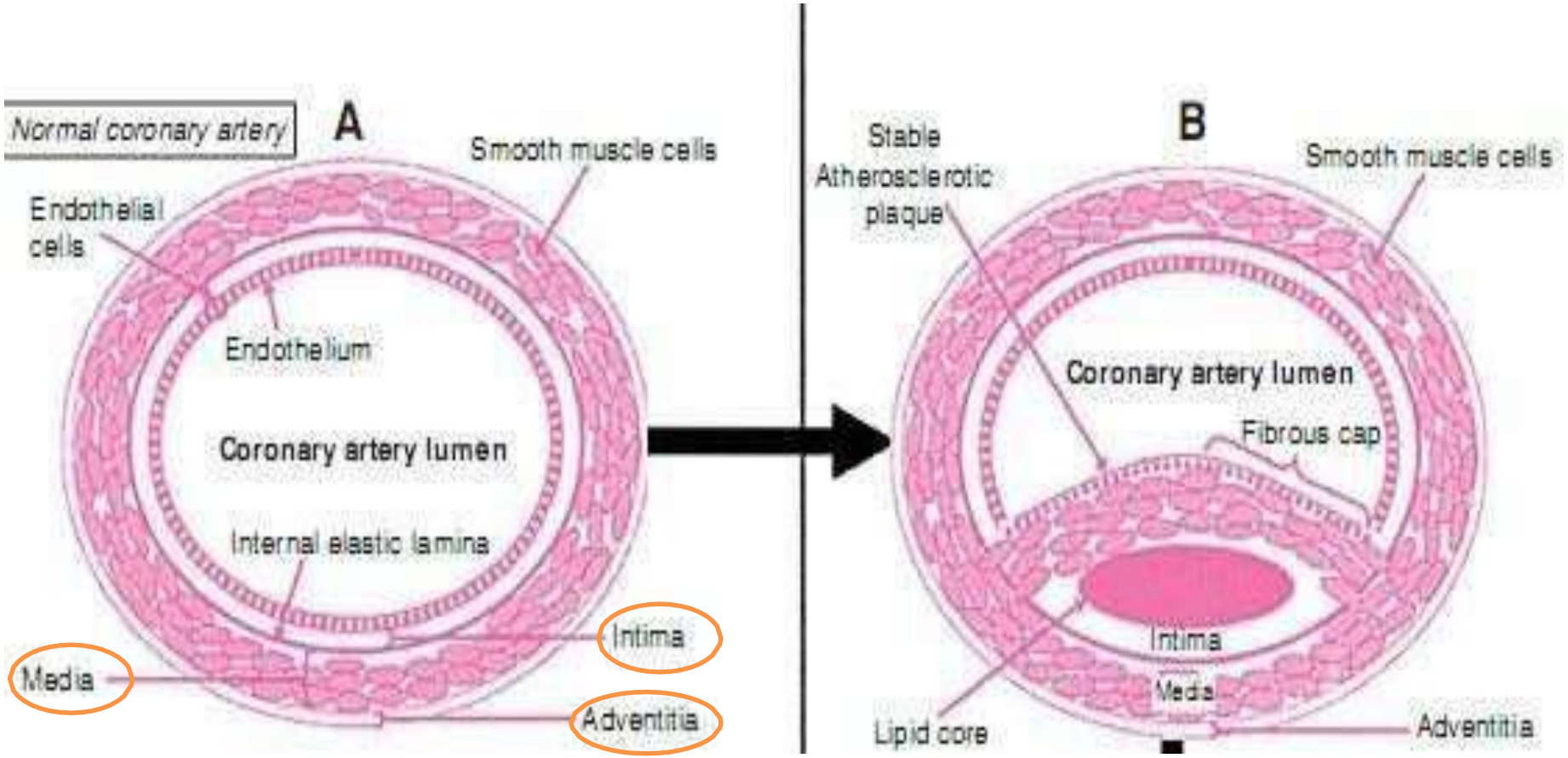
- **Ischemic heart disease (IHD),** or myocardial ischemia, is a disease characterized by reduced blood supply to the heart muscle, usually due to **coronary artery disease (CAD)** (**atherosclerosis of the coronary arteries**).

- **Acute coronary syndrome (ACS)** is a set of signs and symptoms (syndrome) related to the heart.
  - The sub-types of acute coronary syndrome include:
    - **unstable angina** (UA, **not** associated with heart **muscle damage**),
    - two forms of myocardial infarction (**MI, heart attack**), in which heart muscle is damaged.
      - **non-ST segment** elevation myocardial infarction (NSTEMI)
      - **ST segment elevation** myocardial infarction (STEMI).

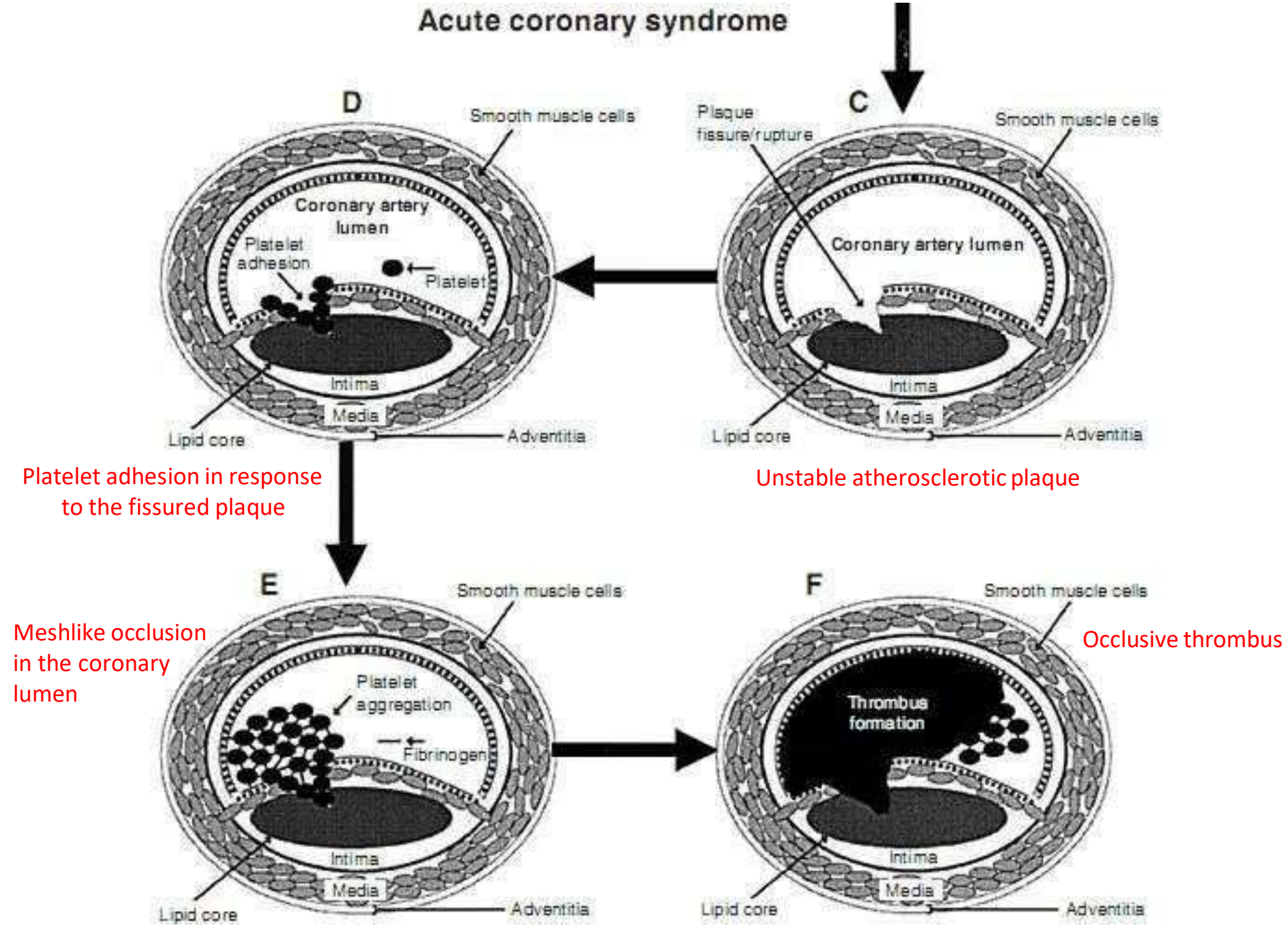
Terminology/Definitions

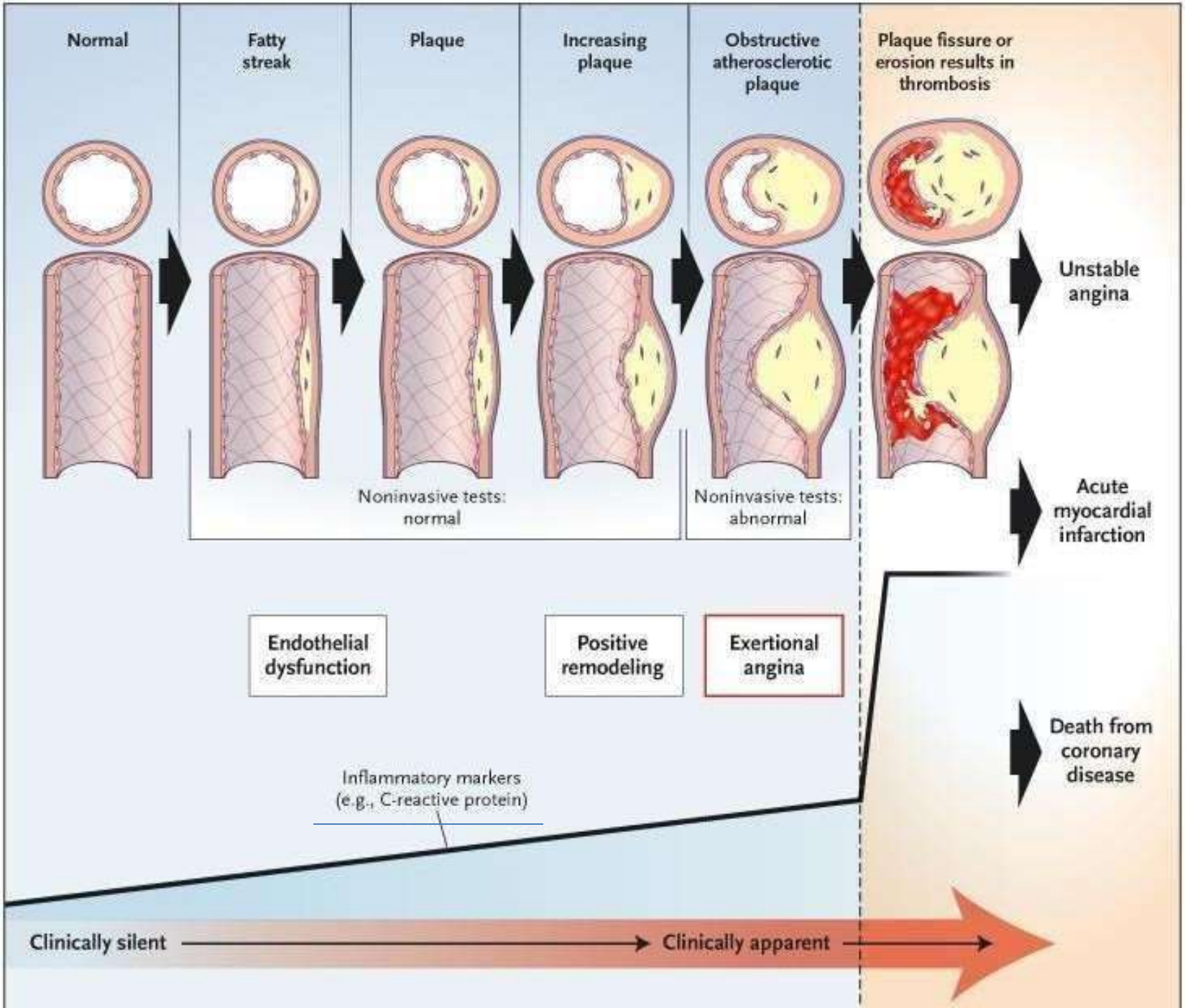
- **Ischemic Heart Disease (IHD)** may present as:
  - **Myocardial ischemia** without clinical symptoms,
  - **Chronic stable exertional angina pectoris (SA)**,
  -
  - **An acute coronary syndrome (ACS)**
    - **Unstable angina (USA)**
    - **Non–ST-segment elevation myocardial infarction (NSTEMI)**
    - **ST-segment elevation myocardial infarction (STEMI)**
  - Coronary artery vasospasm (**variant or Prinzmetal angina**) produces similar symptoms but is **not caused by atherosclerosis**.

Pathophysiology of chronic stable angina:



Panel A depicts the cross-section of a normal coronary artery.
Panel B depicts the cross-section of a coronary artery with a stable atherosclerotic plaque. Note that the lipid core is relatively small in size and the fibrous cap is made up of several layers of smooth muscle cells.


Acute coronary syndrome
D
Smooth muscle cells
Coronary artery lumen
Platelet adhesion
Platelet
Intima
Media
Lipid core
Adventitia
C
Plaque fissure/rupture
Smooth muscle cells
Coronary artery lumen
Intima
Media
Lipid core
Adventitia
Platelet adhesion in response to the fissured plaque
Unstable atherosclerotic plaque
E
Smooth muscle cells
Platelet aggregation
Fibrinogen
Intima
Media
Lipid core
Adventitia
F
Smooth muscle cells
Thrombus formation
Intima
Media
Lipid core
Adventitia
Meshlike occlusion in the coronary lumen
Occlusive thrombus


Normal
Fatty streak
Plaque
Increasing plaque
Obstructive atherosclerotic plaque
Plaque fissure or erosion results in thrombosis
Unstable angina
Noninvasive tests: normal
Noninvasive tests: abnormal
Acute myocardial infarction
Endothelial dysfunction
Positive remodeling
Exertional angina
Death from coronary disease
Inflammatory markers (e.g., C-reactive protein)
Clinically silent
Clinically apparent

Types of Angina

1. Stable Angina

   exertional angina, **typical** or **classic** angina, angina **of effort**, **atherosclerotic angina**

2. Unstable Angina

   Pre-infarction angina**, crescendo** angina, angina **at rest**

3. **Prinzmetal's** Angina

   vasospastic angina**, variant** angina

4. Silent Ischemia

1- Stable Angina

- The patient has occasional periods of anginal symptoms, which are usually **predictable and related to the amount of work the heart is doing (MVO_2).**

- Underlying pathology is **usually atherosclerosis**

- Anginal episodes can be precipitated by **exercise, cold, stress, emotion, or eating**

- Often a **chronic condition**
  - Characterized by the need for **chronic and/or prophylaxis medication to** prevent **chest pain/discomfort**

2- Unstable Angina

- Caused by recurrent episodes of small platelet clots at the site of a ruptured atherosclerotic plaque which can also precipitate local vasospasm

- Associated with **a change** in the **character, frequency**, **and duration of angina** in patients with **stable angina** and when there are **prolonged episodes of angina at rest**

- **Urgent medical** condition
  - Requires **aggressive medical management** to **prevent MI**

- **part of the clinical spectrum of** acute **coronary syndrome**

3- Prinzmetal's Angina

- Results from **transient vasospasm of the coronary vessels**
- **May be associated** with **underlying atheromas**
- **Patients with this angina** may be **relatively young** and **have few or even no cardiac risk factors**
- **The chest pain** is **often unpredictable** and cyclical in nature, sometimes **reverting spontaneously into remission**.
  - More likely to experience **pain at rest** and in **the early morning** hours. **Pain is not usually brought on by exertion** or e**motional stress nor relieved by rest**; the **ECG pattern** is that of current injury **with ST-segment elevation rather than depression.**

4- Silent Ischemia

- **Transient episodes** of **myocardial ischemia** not associated **with angina** or other symptoms **despite ECG changes consistent with ischemic heart disease**

- Silent ischemia is very common
  - As demonstrated by continuous Holter monitor recordings

Clinical presentation

- **CSA** should be distinguished from **UA** since the latter is **associated** with **a greater risk for MI and death** and requires **more aggressive treatment**.

- Because the pathophysiology of CSA is due primarily to increases in **oxygen demand**→ symptoms are **typically reproducible.**
  - patient will generally experience a **similar pattern of discomfort** (i.e., same quality, location, and accompanying symptoms) with **a similar level of exertion with** each **angina attack.**

- The **exception** maybe a patient with **coronary artery vasospas**m, in whom symptoms **maybe more variable and unpredictable.**

Clinical presentation

- Patients with **CSA** will generally be in **no acute distress**. In patients presenting in **acute distress**, the clinician should be **suspicious of ACS.**

- The **five components** commonly used to characterize chest pain are quality, location, and duration of pain; factors that provoke pain; and factors that relieve pain:

  - **Typical pain:** sensation of **pressure**, **heaviness**, or **squeezing** in the **anterior chest area**. **Sharp pain is not a typical symptom of IHD**.
  - may radiate to the **neck, jaw, shoulder, back, or arm.**
  - may be accompanied by **dyspnea, nausea, vomiting, or diaphoresis.**

Clinical presentation

- Symptoms are often provoked by **exertion** (e.g., walking, climbing stairs, and doing yard-or housework) or emotional **stress** and relieved within **minutes by rest or sublingual nitroglycerin.**

- Other precipitating factors include exposure to **cold temperatures** and **heavy meals**. **Pain that occurs at rest (without provocation) or that is prolonged and unrelieved by sublingual nitroglycerin is indicative of an ACS**.
- Some patients, most commonly **women** and patients with **diabetes**, may present with ***atypical symptoms*** including indigestion, gastric fullness, and shortness of breath.

- Patients with **diabetes** may experience associated symptoms, such as **dyspnea and diaphoresis**, without having any of the classic chest pain symptom!!
- **In some cases**, **ischemia may not produce any symptoms** and is termed **"silent ischemia."**

Conditions Provoking or Exacerbating Ischemia

Increased Oxygen Demand

Non-Cardiac

- Hyperthermia
- Hyperthyroidism
- Sympathomimetic toxicity (cocaine use)
- Hypertension
- Anxiety
- Arteriovenous fistula

Cardiac

- Hypertrophic cardiomyopathy
- Aortic stenosis
- Dilated cardiomyopathy
- Tachycardia
  - ventricular supraventricular

Decreased Oxygen Supply

Non-Cardiac

- Anemia
- Hypoxemia
  - pneumonia, asthma, COPD, pulmonary hypertension, interstitial pulmonary fibrosis, obstructive sleep apnea
- Sickle-cell disease
- Sympathomimetic toxicity (cocaine use)
- Hyperviscosity
  - polycythemia, leukemia, thrombocytosis, hypergammaglobulinemia

Cardiac

- Aortic stenosis
- Hypertrophic cardiomyopathy

Symptoms

Sensation of pressure or heavy weight on chest alone or with pain

Pain described variably as feeling of tightness, burning, crushing, squeezing, vicelike, aching, or "deep"

Gradual increase in intensity followed by gradual fading away (distinguished from esophageal spasm)a

SOB with feeling constriction about the larynx of upper trachea

Location of Pain or Discomfort

Over the sternum or very near to it

Anywhere between epigastrium and pharynx

Occasionally limited to left shoulder and left arm

Rarely limited to right arm

Lower cervical or upper thoracic spine

Left interscapular or suprascapular area

Radiation of Pain

Medial aspect of left arm

Left shoulder

Jaw

Occasionally, right arm

Duration of Symptoms

–30 min

Electrocardiogram

ST-segment depression >2 mm

T-wave inversion

Precipitating Factors

Mild, moderate, or heavy exercise, depending on patient

Effort that involves use of arms above the head

Cold environment

Walking against the wind

Walking after a large meal

Emotions: fright, anger, or anxiety

Coitus

Nitroglycerin Relief

Relief of pain occurring within 45 sec to 5 min of taking nitroglycerin

Symptoms

- The **severity of symptoms** does **not correlate** with the **severity of CAD**, but should be assessed to facilitate diagnosis and treatment decisions

- **The Canadian Cardiovascular Society (CCS) classification of angina** is a widely used mechanism for characterizing angina based on an assessment of **disability resulting** from **the anginal symptoms.**

- **Statistically significant correlation between** the **CCS angina class** and the **severity of CAD** as determined by **coronary angiography.**

The Canadian Cardiovascular Society (CCS) classification of angina

Angina Classification	Accompanying Symptoms
Class I	**Normal physical activity (e.g., climbing stairs or walking) does not cause anginal symptoms**; angina occurs primarily with **strenuous, extended, or rapid physical a**ctivity or recreation
Class II	**Angina poses a slight limitation on ordinary activity** and typically occurs with the following types of activities: •**Quickly walking or climbing** stairs •**Uphill walking** •**Walking/stair climbing after meals** •**Windy or cold weather** •**Emotional stress** •Within the first few hours after waking •**Walking more than two blocks** or **climbing more than one flight** of stairs at a **normal pace (under** normal conditions)
Class III	**Anginal symptoms impose marked limitation on physical activity**; angina occurs upon walking one to two blocks or climbing one flight of stairs at a normal pace (under normal conditions)
Class IV	**Symptoms of angina may be present at rest**; physical activity cannot be carried out without discomfort

Diagnosis and evaluation

- A **thorough medical history, physical exam, and laboratory** analysis are necessary to ascertain cardiovascular risk factors and to **exclude nonischemic** and **noncardiac** conditions that could cause **angina-like symptoms.**

- Laboratory analyses should assess **for glycemic control** (i.e., fasting glucose, glycosylated hemoglobin), **fasting lipids, hemoglobin, and organ function** (i.e., blood urea nitrogen, creatinine, liver function tests, thyroid function tests). Additionally, serial measurements of **cardiac enzymes** (usually three measurements within 24 hours) are used to *exclude the diagnosis of MI*. (CK, CK-MB, Troponin I and II usually normal in CSA and UA and elevated in MI)

Assessment of Risk Factors

- Age / gender
  - M > 45yrs, F > 55yrs*
- Family history of premature IHD
  - M < 55yrs, F < 65yrs
- Hypertension
  - BP > 140/90 or on anti- hypertensive therapy
- Smoking
- Hyperlipidemia
- HDL < 40 mg/dL
  - > 60 (subtract one risk factor)
- Diabetes
- Obesity
  - BMI >30 kg/m^2
- Sedentary lifestyle
- CVD
- PVD

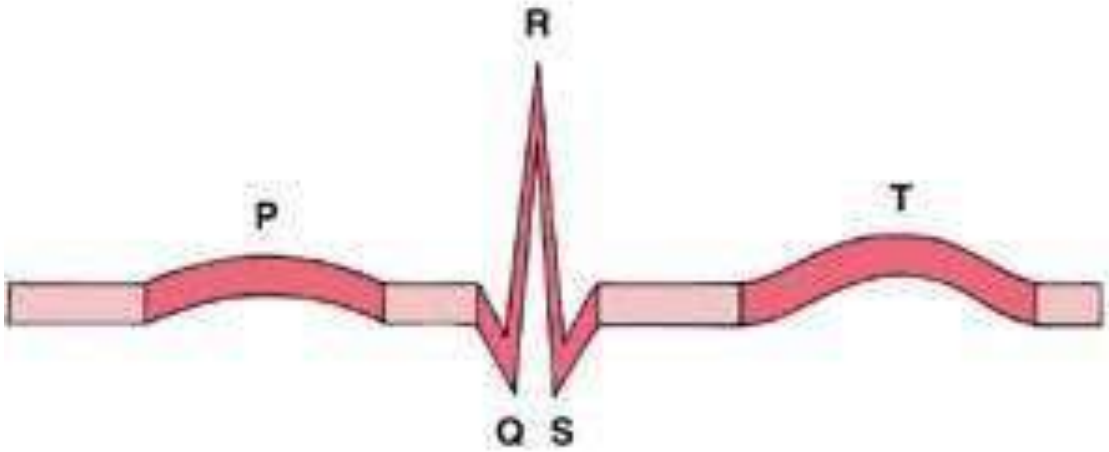
* or post-menopausal, whichever occurs first

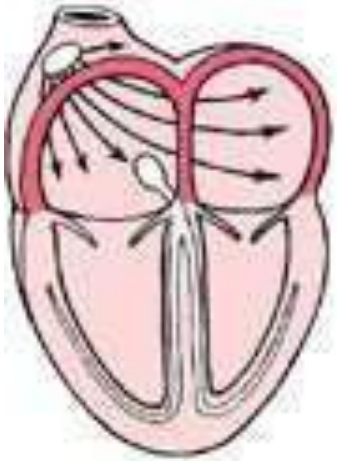
Diagnosis and Evaluation

- **Objective**
  - Physical examination
  - Electrocardiogram (ECG)
  - Laboratory data
  - Radiographic evaluation
    - Echocardiogram
    - Radionuclide scanning
    - Coronary angiography
  - Cardiac stress testing (functional testing)
    - Exercise
    - Pharmacologic : either to **increase myocardial work and oxygen demand (dobutamine)** or to **induce vasodilation-elicited heterogeneity in induced coronary flow (vasodilators)**

Diagnostic tests

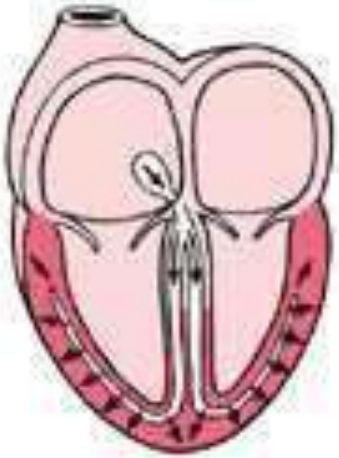
- **A 12-lead ECG recorded during rest** is often normal in patients with **chronic stable angina** in the **absence of active** ischemia.
  - should **be done within 10 minutes** of presentation to the emergency department in patients with **symptoms of ischemia.**
- **Cardiac stress test**
  - **Physical exercise (treadmill),**
  - **Pharmacologic stress** :**Dobutamine is commonly used with stress echocardiography**, whereas **adenosine or dipyridamole are used for nuclear imaging studies**
- **Treadmill or bicycle exercise ECG,** commonly referred to as **a "stress test,"** is considered **positive for IHD** if the ECG shows **at least a 1 mm** deviation of the ST-segment (depression or elevation).
- **Pharmacologic stress test: Dobutamine** (beta1 agonist) is a pharmacologic stressor used in patients who are unable to exercise and is **commonly used with echo**cardiography→ to identify stress-induced wall motion abnormalities indicative of coronary disease.





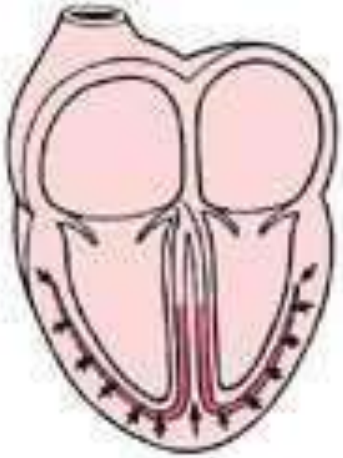
P Wave

Activation of the atria



QRS Complex

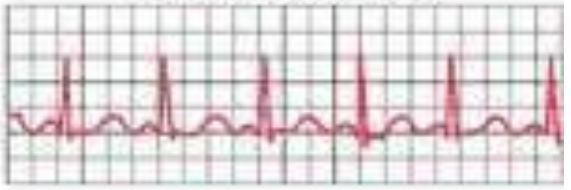
Activation of the ventricles



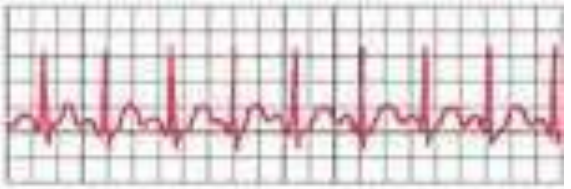
T Wave

Recovery wave

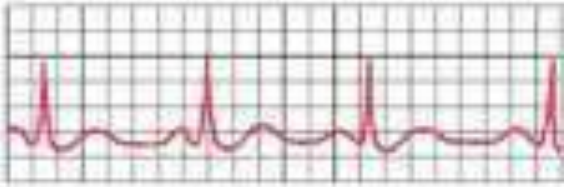
Normal Heartbeat



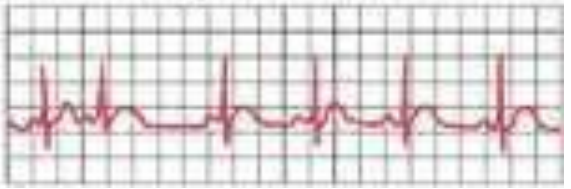
Fast Heartbeat



Slow Heartbeat



Irregular Heartbeat



Diagnosis (cont'd)

- **Radionuclides myocardial perfusion imaging (rMPI)** with the gamma-emitting **radionuclides technetium-99m sestamibi** or **thallium-201** allows for the identification of **multivessel disease** and assessment of **myocardial viability**. (exercise, pharmacological (vasodilator or dobutamine)
  - An IV radioactive tracer is used to detect areas of the heart that receive less blood after adenosine or dipyridamole infusion, indicating a **myocardial perfusion defect and coronary disease**

  - **Coronary artery calcium scoring via CT**, also known as electron beam CT (**EBCT) or "ultra-fast CT**," may be performed as a **noninvasive means to assess for IH**D.
    - **Calcium deposits** within the coronary arteries which are indicative **of IHD** are detected **on CT**. A **calcium score is calculated**, and the **risk for IHD-related events** is estimated.

Diagnostic tests (cont'd)

- **Coronary angiography (invasive)** detects the **location and degree of coronary atherosclerosis** and is used to evaluate the potential benefit from **revascularization procedures**.
  - **Stenosis o**f at **least 70%** of the diameter of **at least one of the major epicardial arteries** on coronary angiography is indicative of **significant IHD**

- **Is considered the gold standard for the diagnosis of IHD when**:
  - **stress testing** results **are abnormal**
  - or symptoms of angina are **poorly controlled.**

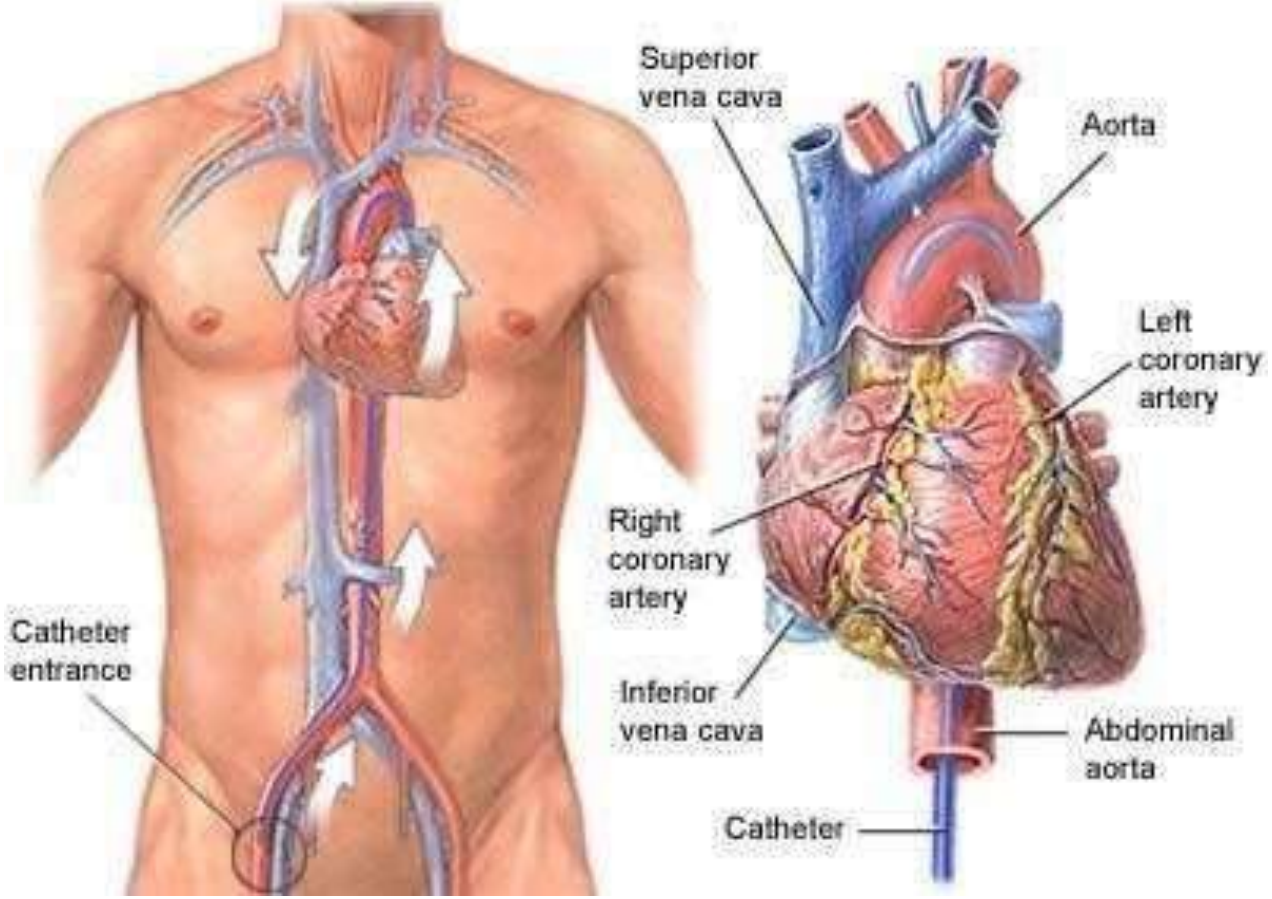
  - **Contrast medium** must be used cautiously **with adequate hydration** in patients with **pre-existing renal disease** (especially in **those with diabetes**) to **avoid contrast-induced nephropathy**

Diagnosis and Evaluation

- **Cardiac catheterization and coronary angiography** used in patients with suspected CAD to document the presence and severity of disease as well as for prognostic purposes.

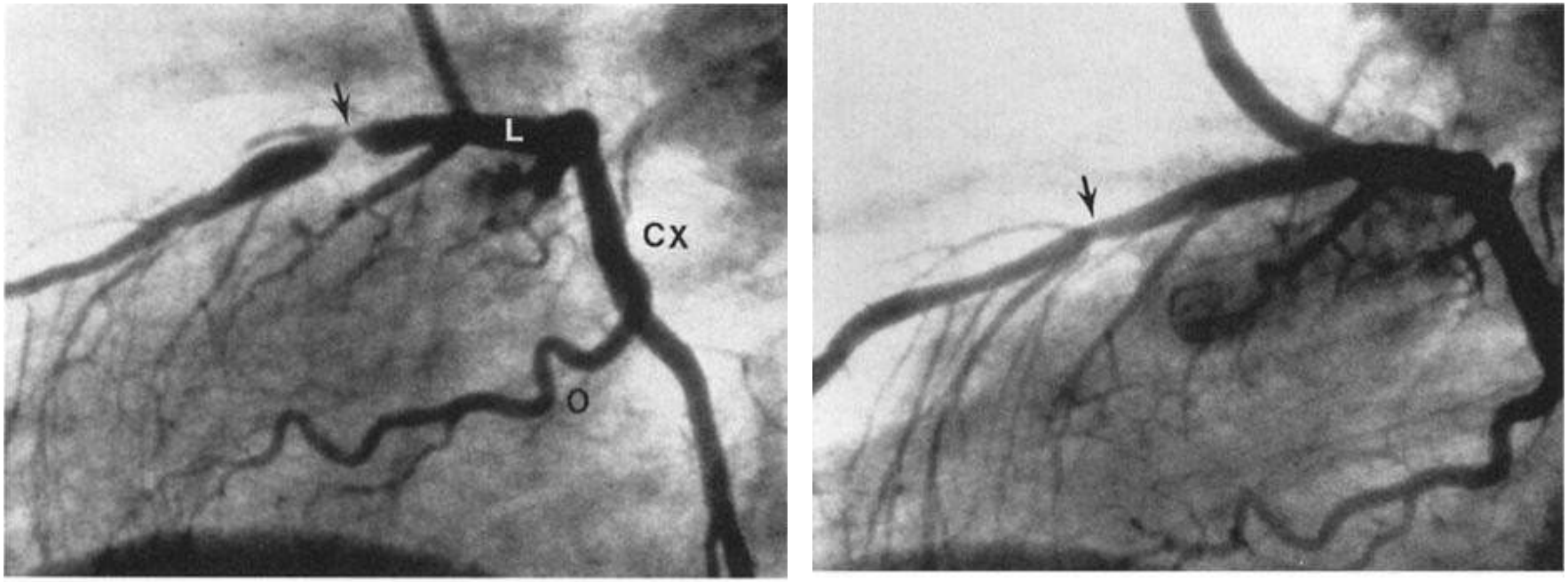
- **Interventional catheterization** used for **thrombolytic therapy** in patients with **acute MI** and for managing patients **with significant CAD to relieve obstruction** through **percutaneous transluminal coronary angioplasty** (PTCA), **atherectomy**, laser **treatment, or stent placement.**

Cardiac Catheterization



Cardiac catheterization is used to study the various functions of the heart. Using different techniques, the **coronary arteries can be viewed by injecting dye or opened using balloon angioplasty.** The oxygen concentration can be measured across the valves and walls (septa) of the heart and pressures within each chamber of the heart and across the valves can be measured. The technique can even be performed in small, newborn infants.

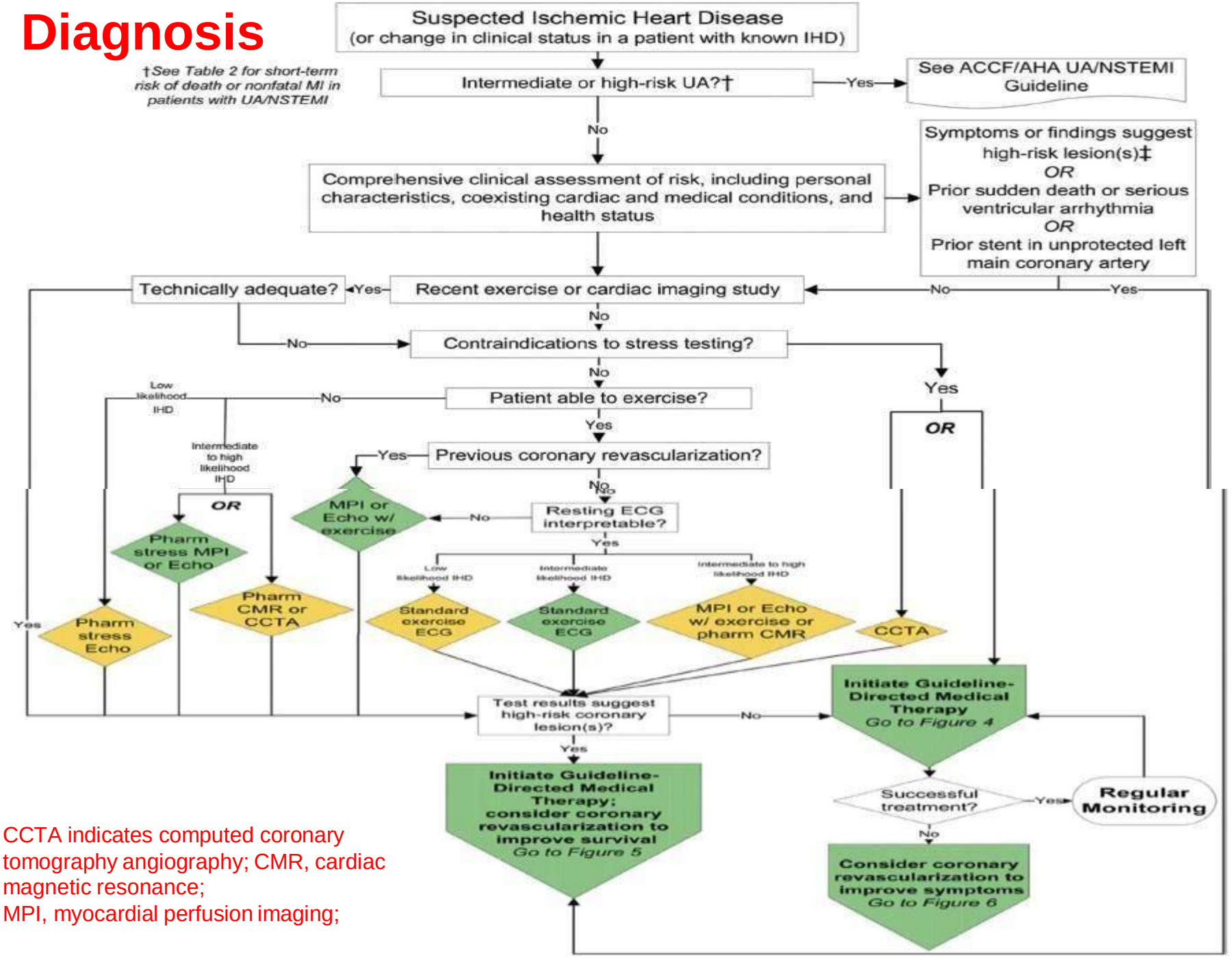
Coronary Angiography



A. Coronary arteriogram. Images were obtained from the left lateral projection with contrast injection into the left main coronary artery. The left anterior descending (L), left circumflex (CX), and first obtuse marginal (O) branches are visualized. Severe stenosis is seen in the midportion of the left anterior descending artery (*arrow*) in this patient, who had unstable angina pectoris.

B. Coronary arteriogram, same projection and patient as in *A*, obtained 1 day later. The stenosis in the left anterior descending coronary artery (*arrow*) has been reduced after percutaneous balloon angioplasty.

Diagnosis



CCTA indicates computed coronary tomography angiography; CMR, cardiac magnetic resonance;
MPI, myocardial perfusion imaging;

Test	Recommended	Not Recommended
Exercise ECG testing (using the Bruce protocol and Duke treadmill score)	Symptomatic patients with intermediate-to-high probability of CAD who are able to exercise and not taking digoxin (LOE: B) After significant change in anginal pattern (LOE: C)	Baseline ECG abnormalities (Wolff-Parkinson-White syndrome, electronically paced ventricular rhythm, > 1 mm ST depression at rest, LVH, or complete LBBB) (LOE: B) Established diagnosis of CAD (prior MI, angiography) (LOE: B)
Cardiac stress imaging (able to exercise) -- exercise MPI or exercise echocardiography	Patients with intermediate pretest probability of CAD with abnormal results on resting ECG or are using digoxin (LOE: B)	Patients with LBBB or cardiac pacing device (LOE: B)
Cardiac stress imaging (unable to exercise) -- adenosine or dipyridamole MPI or dobutamine echocardiography	Patients with an intermediate pretest probability of CAD (LOE: B) or in patients with prior revascularization (LOE: B), but do not have LBBB or a cardiac pacing device	Dobutamine echocardiography not recommended in patients with LBBB or cardiac pacing device (LOE: B)
Cardiac **stress imaging -- adenosine or dipyridamole** MPI	**Patients with LBBB or with cardiac pacing device (regardless of ability to exercise**) (LOE: B)	

CAD = coronary artery disease; ECG = electrocardiographic; LBBB = left bundle branch block; LOE = level of evidence; LVH = left ventricular hypertrophy; MPI = myocardial perfusion imaging

Risk Stratification

Low Risk	High Risk
✓ Stable angina	✓ Unstable angina
✓ Absence of CHF symptoms	✓ CHF symptoms
✓ Normal resting ECG	✓ Resting ECG with Q-waves or ST-T wave changes
✓ Normal LVF	✓ Markedly depressed LVF

Treatment of Ischemic Heart Disease

Treatment of Ischemic Heart Disease

- Establish Goals of Therapy
- **Risk Factor Modification**
- Lifestyle Modification
- Pharmacotherapy
  - **antianginal/anti-ischemic therapies** (i.e., **beta-blocker**s, **calcium channel antagonists**, **and nitrates**)
  - **vasculoprotective agents** (i.e., **lipid-lowering** therapies and **antiplatelet agents**)
  - **ranolazine** was approved in **January 2006** by the **FDA** as a **novel treatment option for CS**A in patients who **have not achieved adequate** control **of angina symptoms with standard therapies.**
- **Interventional therapies**

Goals of Therapy

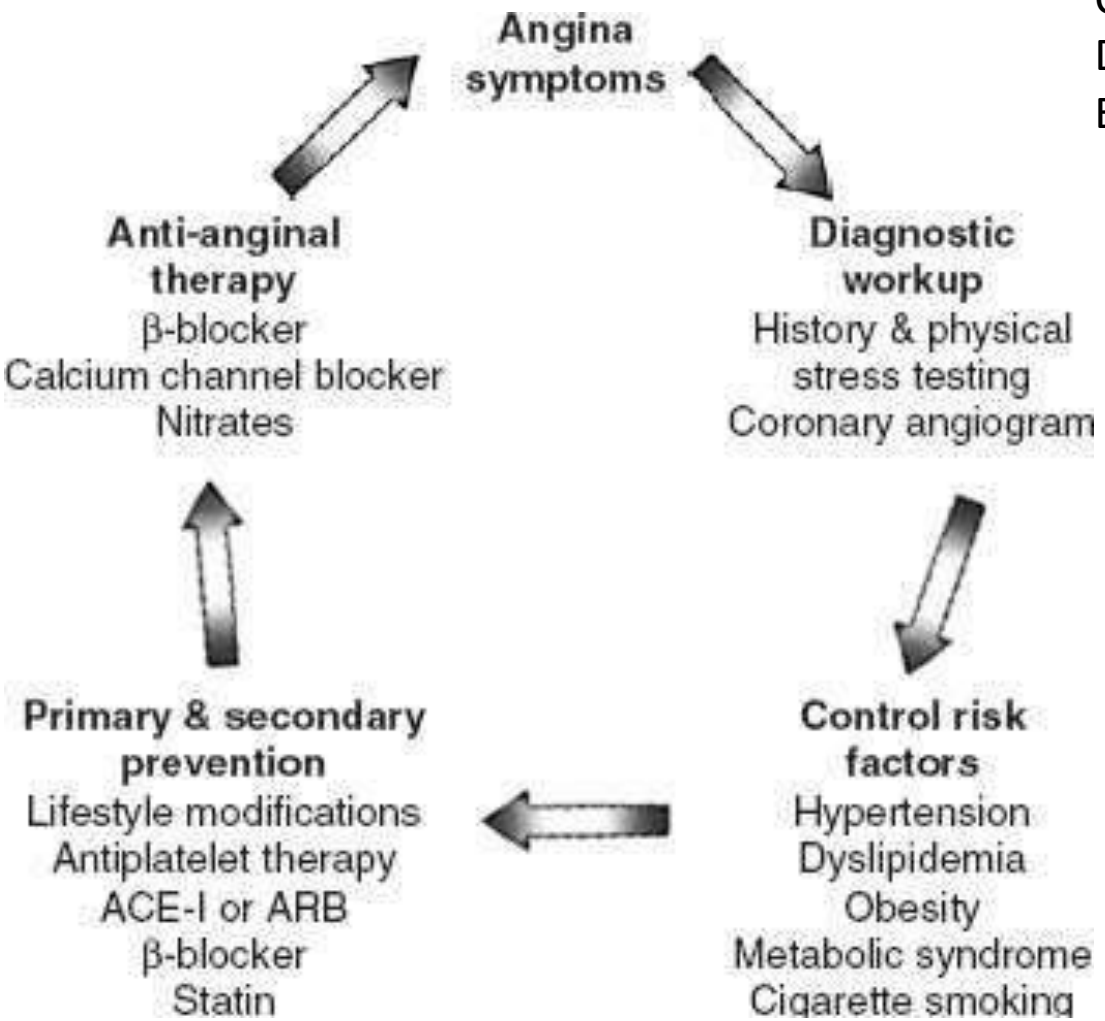
- **Short-Term**
  - **Stabilize chest pain/discomfort** and reduce or prevent anginal symptoms
  - **Prevent ischemia and subsequent infarction**
  - Improve **exercise tolerance** and quality of life

- **Long-Term**
  - **Alter or modify underlying process of ischemia**
    - Risk factor modification and optimization of medical management
  - **Prevent primary or secondary CV** events **MI, HF, ARR**
  - **Stabilize the pattern** of **chest pain**
  - **Decrease overall CV mortality and morbidity**

General treatment strategies

Angina Treatment Mnemonic

A – aspirin, anti-anginals
B – β-blockers and blood pressure
C – cholesterol and cigarettes
D – diet and diabetes
E – education and exercise



ACC/AHA Guideline Recommendations for CV Risk Reduction in Patients With Stable CAD

Risk Factor	Recommendations
Smoking	Complete cessation; no exposure to environmental tobacco smoke
Blood pressure control	< 140/90 mm Hg ***or < 130/80 mm Hg if patient has diabetes or chronic kidney disease??JNC8***
Lipid management **2013 AHA/ACC dyslipidemia***	**There is no evidence to support continued use of specific LDL-C and/or non–high-density lipoprotein cholesterol (non–HDL-C) treatment targets** **The appropriate intensity of statin therapy should be used to reduce risk in those most likely to benefit. (4 groups)** **High-intensity statin therapy is defined as a daily dose that lowers LDL-C by ≥50%** and moderate-intensity by 30% to <50%. All patients with ASCVD (including stable angina) who are age ≤75 years, as well as patients >75 years, should **receive high-intensity statin** therapy; or **if not a candidate** for high-intensity, should receive **moderate-intensity statin therapy.**

Risk Factor	Recommendations
Physical activity	- **30-60 m**in, 7 **days/wk (minimum, 5 days/wk)** - **Moderate-intensity aerobic activity** should be **encouraged on all days of the** week, supplemented by an increase in daily activities
Weight management	Body mass **index: 18.5-24.9 kg/m²** **Waist circumference:** - Men: < 40 in (102 cm) - Women: < 35 in (88 cm)
Diabetes management	HbA1c < 7% (rosiglitazone???)
Antiplatelet agents*	Aspirin should be started **at 75-162 mg/day** and continued **indefinitely in all** patients unless contraindicated *Adding warfarin and/or clopidogrel increase the risk of bleeding→ monitor closely (Harm)

ACE inhibitors	**ACE inhibitors should be started and continued indefinitely in all patients with left ventricular ejection fraction ≤ 40% and in those with hypertension, diabetes, or chronic kidney disease, unless contraindicated**
ARBs	Use in patients who **are intolerant of ACE inhibitors** and **have heart failure** or have had a **myocardial infarction with left ventricular ejection fraction ≤ 40%**
Aldosterone blockade	Use in patients post MI, without significant renal dysfunction or hyperkalemia, who are already receiving therapeutic doses of an ACE inhibitor and beta-blocker, have a left ventricular ejection fraction ≤ 40%, and have either diabetes or heart failure
Beta Blockers	They should be started and continued for 3 years in all patients who have had MI, acute coronary syndrome, or left ventricular dysfunction (EF ≤40%) with or without heart failure symptoms (prior MI), unless contraindicated (2012 guidelines for SIHD)
Influenza vaccination	An annual influenza vaccination is recommended for patients with cardiovascular disease

Risk Factor Modification

- **Primary prevention** through **early recognition** and **modification of risk factors** is considered "optimal management"

- **Secondary prevention** is more prevalent and is an important and effective means **of reducing subsequent morbidity and mortality**

- Risk factors are **considered additive** and are classified as either **alterable or unalterable**

Risk Factor Modification

- **Alterable (Modifiable)**
  - Smoking (A, P)
  - Hypertension
  - Hyperlipidemia
  - Obesity
  - Diabetes
  - Sedentary lifestyle
  - Excessive alcohol use
  - Psychosocial factors
  - -- Type A personality(?)
  - Others
- **Unalterable (Non-modifiable)**
  - Gender

    Male > Female
  - Age

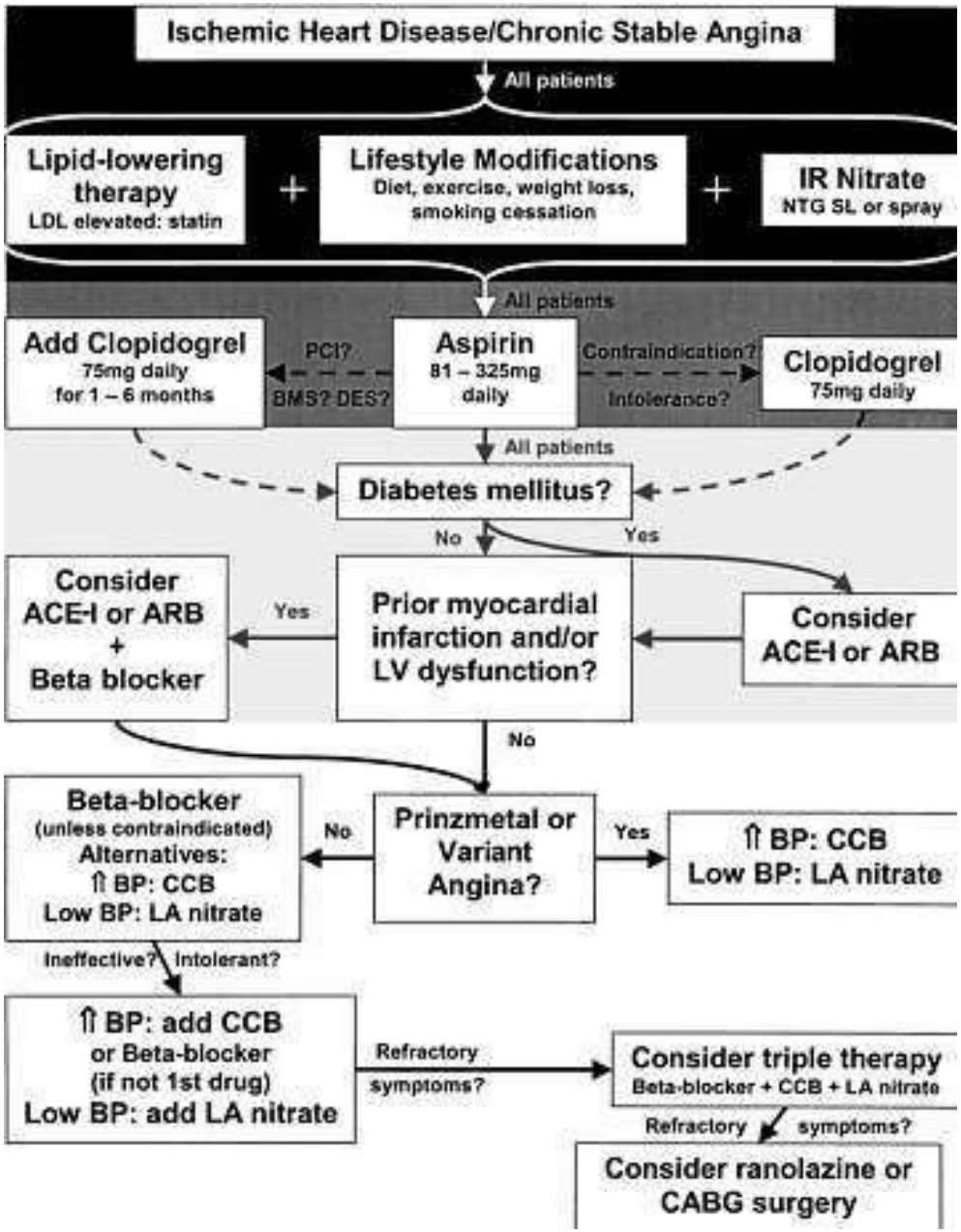
    Male > 45yrs

    Female > 55yrs
  - Family History

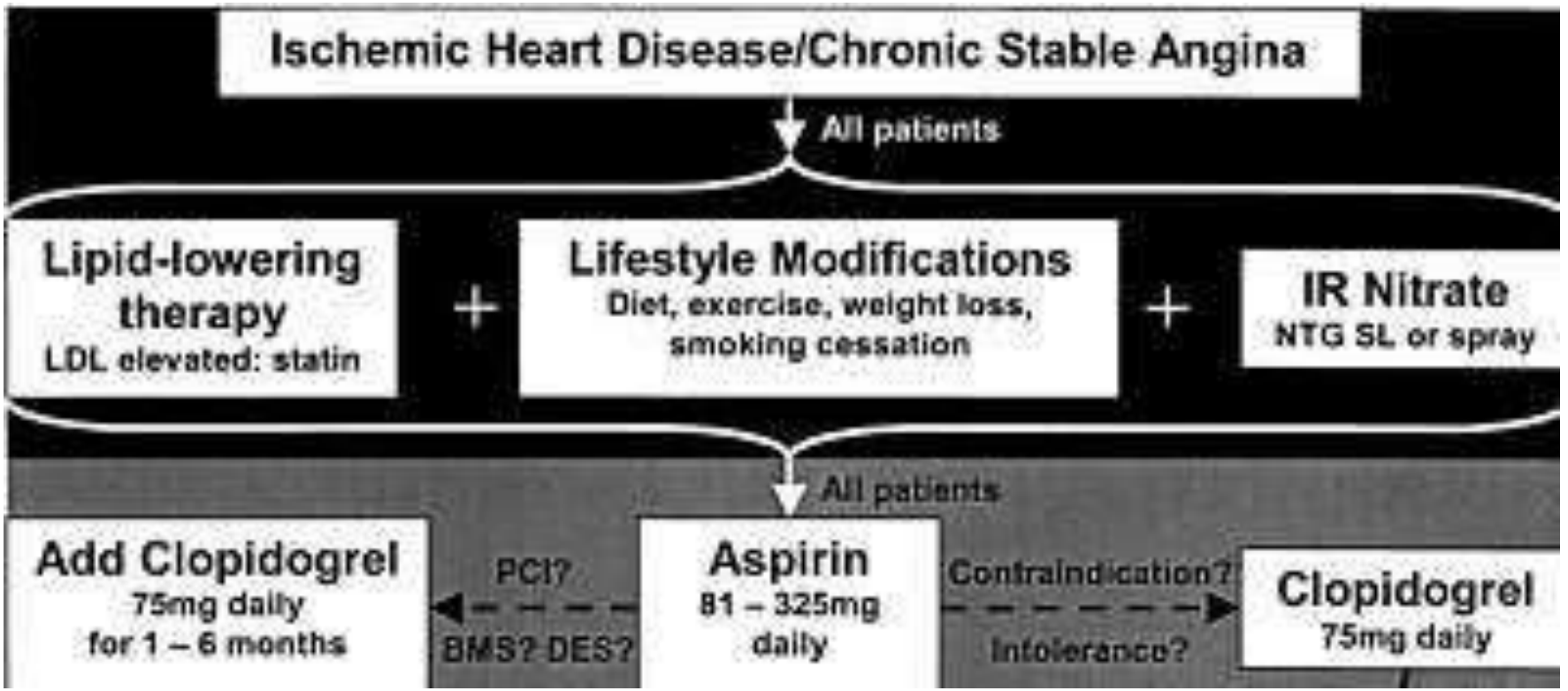
    Male < 55yrs

    Female < 65yrs
  - Genetic composition
  - Environmental factors
  - Diabetes (to some extent)

The treatment algorithm for ischemic heart disease



Treatment (cont'd)

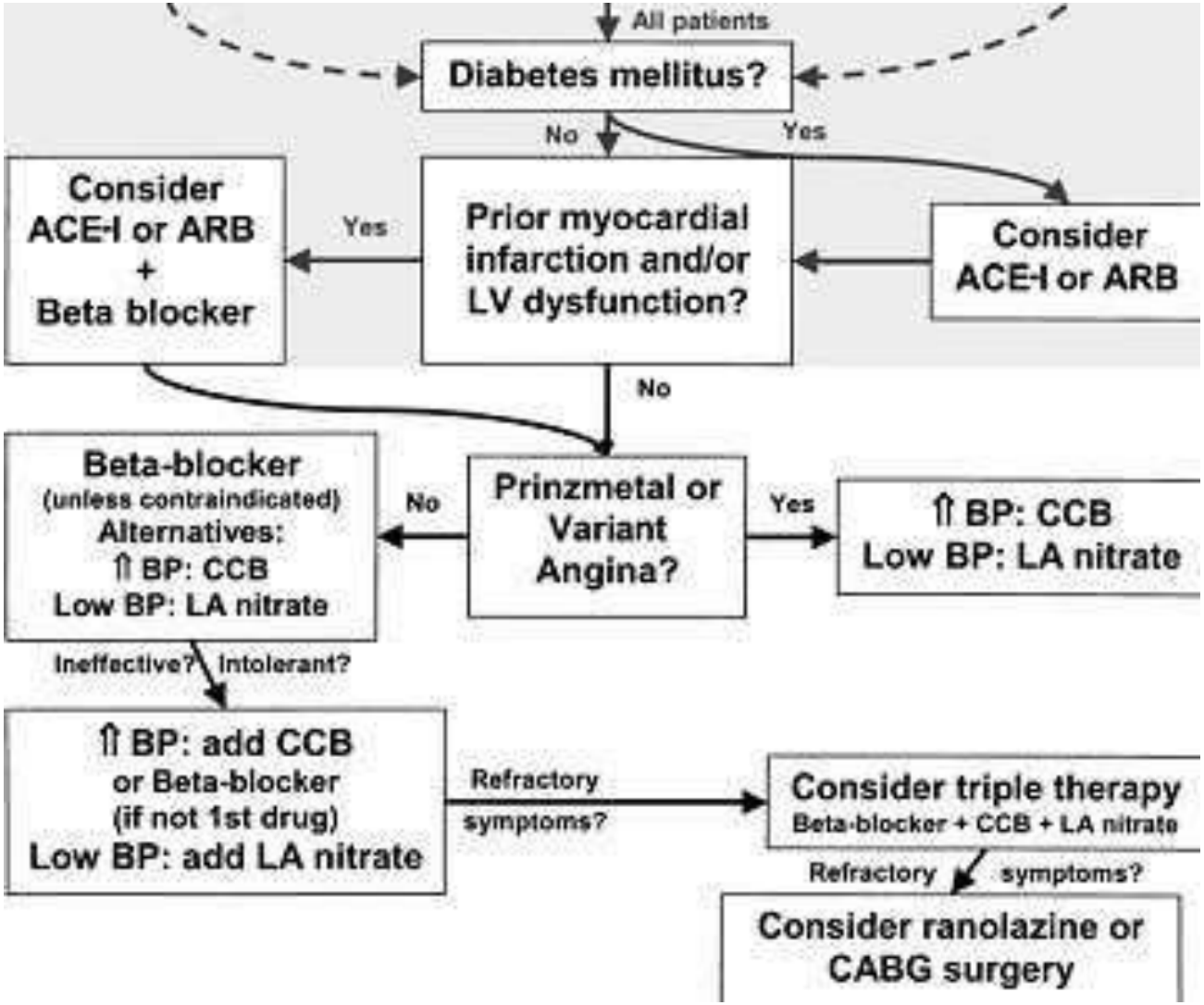


It begins at the top (**black section**), which suggests risk factor modifications as the first treatment modality.

Moving down to the **dark gray section**, **appropriate antiplatelet** therapy is selected.

BMS, bare metal stent; **DES**, drug-eluting stent; **PCI**, percutaneous coronary intervention;

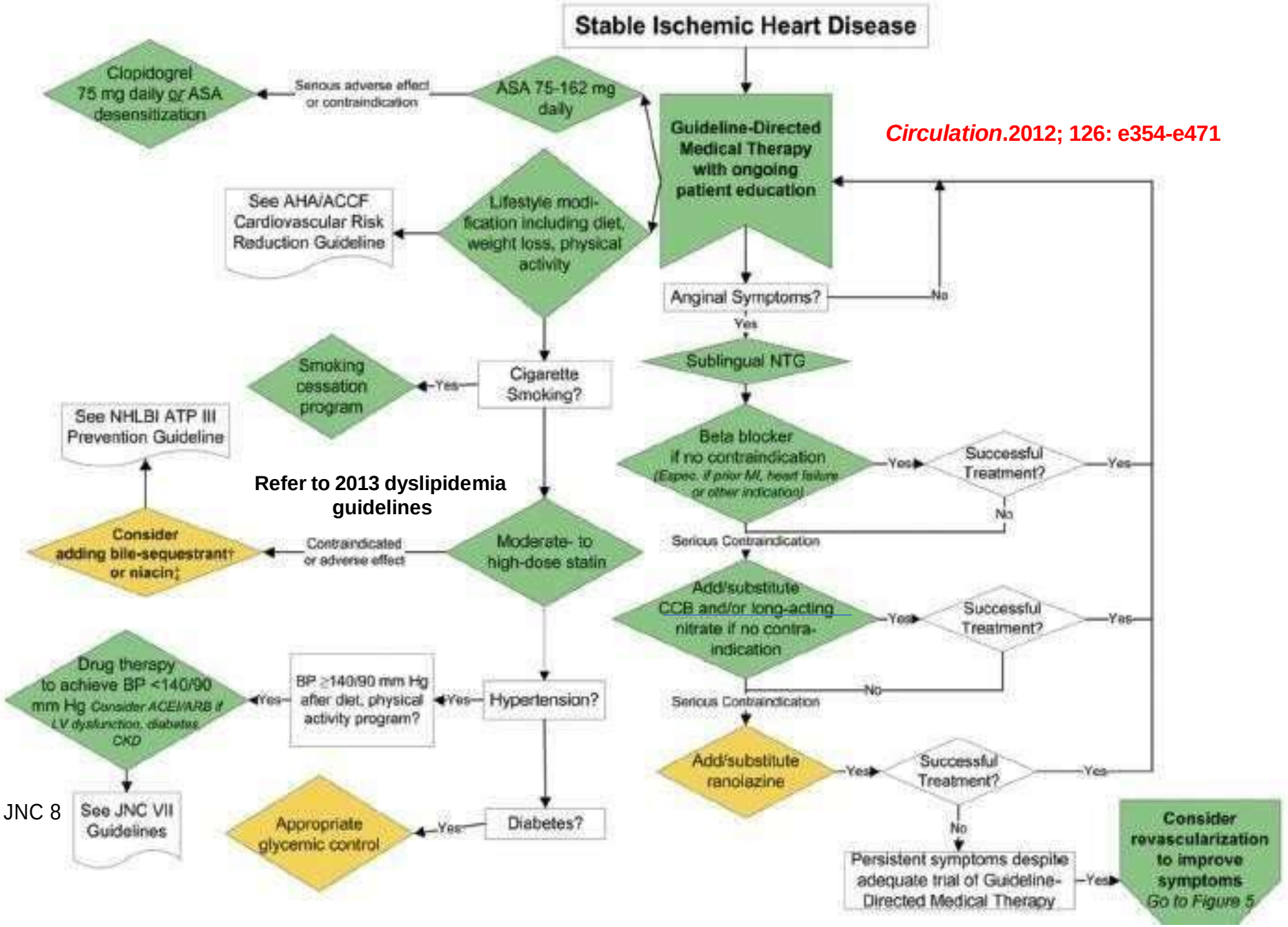
Treatment (cont'd)



The **light gray section** identifies patients at high-risk for major adverse cardiac events and <u>suggests appropriate drug therapy to decrease cardiovascular risk.</u>

The **white section** recommends appropriate anti-anginal therapy.

CABG, coronary artery bypass graft;


Stable Ischemic Heart Disease
Guideline-Directed Medical Therapy with ongoing patient education
Circulation.2012; 126: e354-e471
ASA 75-162 mg daily
Serious adverse effect or contraindication
Clopidogrel 75 mg daily or ASA desensitization
Lifestyle modification including diet, weight loss, physical activity
See AHA/ACCF Cardiovascular Risk Reduction Guideline
Anginal Symptoms?
No
Yes
Sublingual NTG
Cigarette Smoking?
Yes
Smoking cessation program
See NHLBI ATP III Prevention Guideline
Refer to 2013 dyslipidemia guidelines
Beta blocker if no contraindication (Espec. if prior MI, heart failure or other indication)
Yes
Successful Treatment?
Yes
No
Serious Contraindication
Moderate- to high-dose statin
Contraindicated or adverse effect
Consider adding bile-sequestrant† or niacin‡
Add/substitute CCB and/or long-acting nitrate if no contra-indication
Yes
Successful Treatment?
Yes
No
Serious Contraindication
Hypertension?
Yes
BP ≥140/90 mm Hg after diet, physical activity program?
Yes
Drug therapy to achieve BP <140/90 mm Hg Consider ACEI/ARB if LV dysfunction, diabetes, CKD
Yes
See JNC VII Guidelines
JNC 8
Add/substitute ranolazine
Yes
Successful Treatment?
Yes
No
Diabetes?
Yes
Appropriate glycemic control
Persistent symptoms despite adequate trial of Guideline-Directed Medical Therapy
Yes
Consider revascularization to improve symptoms
Go to Figure 5

Lifestyle Modification

- **Diet**
  - **No large clinical** data available in stable angina
  - **Lyon Heart Study**
    - Patients with **h/o MI** were randomly assigned to **Mediterranean-style diet** or "prudent" Western-type diet
      - Mediterranean diet –high in polyunsaturated omega-3 FA
    - Patients following **Mediterranean-style diet had a 50-70% lower risk** of recurrent heart disease
      - Benefit was determined early and remainder continuous throughout 46 months of follow up
  - **Specific dietary recommendations for patients with IHD should include the following:**
    - Limit **fat** intake to less than 30% of total caloric consumption.
    - Limit **cholesterol** intake to less than 200 mg per day.
    - Limit consumption of **saturated fat** found in fatty meats, full-fat dairy products, and hydrogenated vegetable oils to less than 7% of total calories.
    - Consume at least two servings of **fish** per week.
    - Consume at least six servings of **grains**, five servings of **fruits and vegetables**, and two servings of **non-fat or low-fat dairy products** per day.
    - Limit **daily sodium intake** to 2.4 grams (6 grams of salt) for blood pressure control.

Lifestyle Modification

- **Weight Reduction**
  - Obesity is associated with and contributes to other coronary disease risk factors

- **Exercise**
  - ↓ **frequency of anginal symptoms,** functional capacity, and **improves endothelial function**
  - Initiate at low-levels for 20-30 minutes, and as tolerated
  - **Compared** to **revascularization at one-year**, daily exercise resulted in fewer CV major events and **improved exercise tolerance.**

- **Smoking**
  - **No randomized trials** in **chronic stable angina**
  - Smoking CV disease mortality by 50%
  - **Incidence of coronary** events ↓ **by 15-25% within 2 years** of smoking cessation
  - **Primary prevention studies** – **smoking cessation** resulted in a **7 to 47% reduction CV events**

Pharmacotherapy

- **Nitroglycerin to relieve acute symptoms**
- **Pharmacotherapy to prevent recurrent ischemic symptoms**
  - *Beta-blockers*
  - *Calcium channel blockers*
  - *Long-acting nitrates*
  - *Ranolazine*
- **Pharmacotherapy to prevent acute coronary syndromes and death (vasoprotective agents)**
  - *Antiplatelet agents*
  - *Statins*
  - *ACE inhibitors and angiotensin receptor blockers*
  - *Control of risk factors*
- **Pharmacotherapy with no benefit or potentially harmful effects**
  - *Hormone replacement therapy*
  - *Antioxidants*
  - *Folic acid*
  - *Herbal supplements*

Pharmacotherapy to prevent recurrent ischemic symptoms (anti-ischemic medications)

ß-blockers, calcium channel blockers, and nitrates:

•exert their anti-anginal effects by improving the balance between myocardial oxygen supply and demand, with specific effects listed in the table below.

• **reduce from the frequency of angina and delay the onset of angina during exercise.**

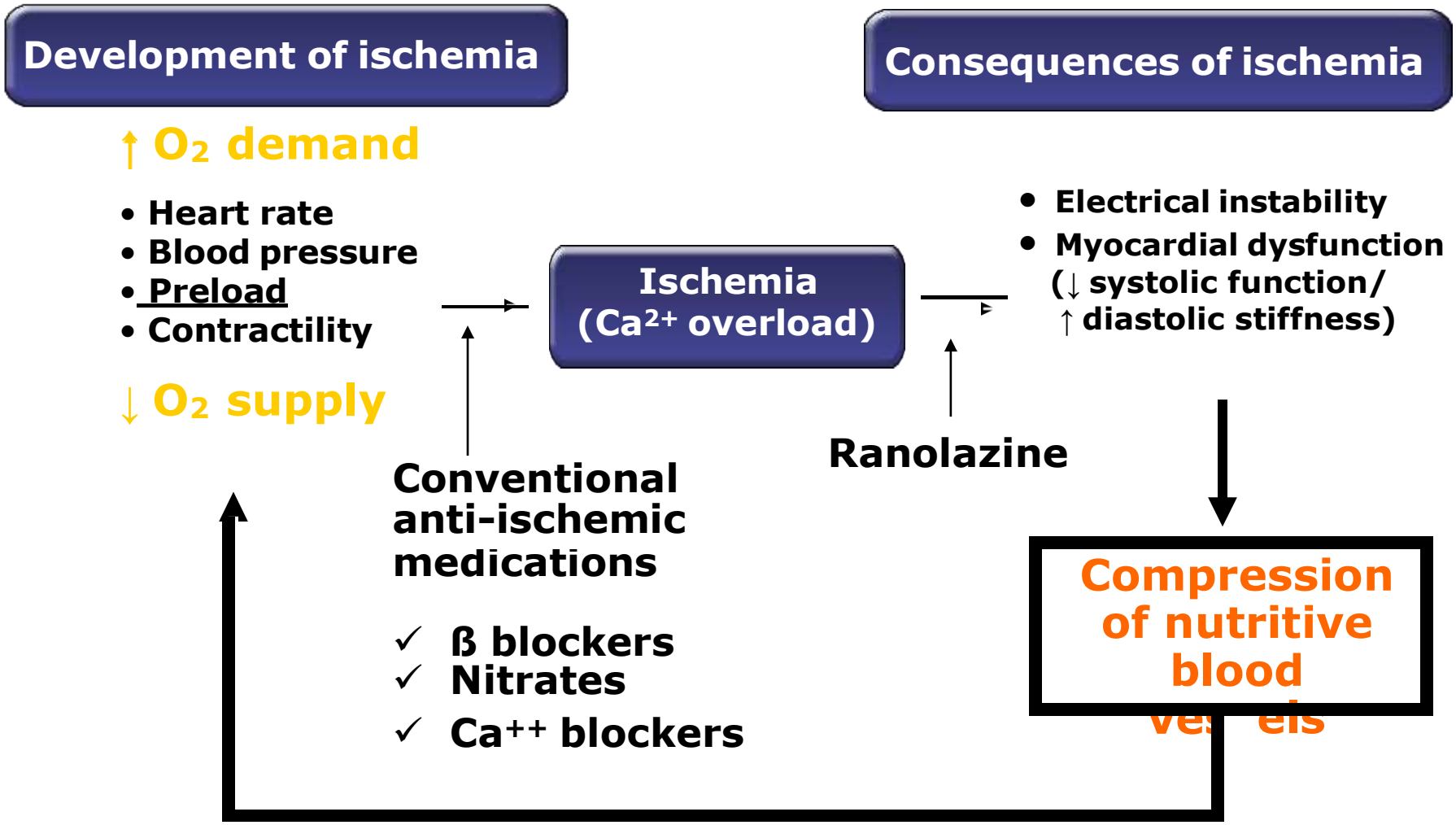
•there is **no** evidence that any of these agents prevent ACS or improve survival in patients with chronic stable angina.

Ranolazine: newly approved for the treatment of chronic stable angina in patients **unresponsive to traditional anti-anginal medications**. **Combination therapy with two or three anti-anginal drugs is often needed.**

	Oxygen Demand			
Anti-anginal Agent	Heart Rate	Wall Tension	Cardiac Contractility	Oxygen Supply
β-Blockers	↓	↔ or ↑	↓	↔
Calcium channel blockers				
Verapamil, diltiazem	↓	↓	↓	↑
Dihydropyridines	↔ or ↑	↓	↓	↑
Nitrates	↑	↓	↔	↑

↓, decreases; ↔, no change; ↑, increases.

Sites of action of anti-ischemia medication

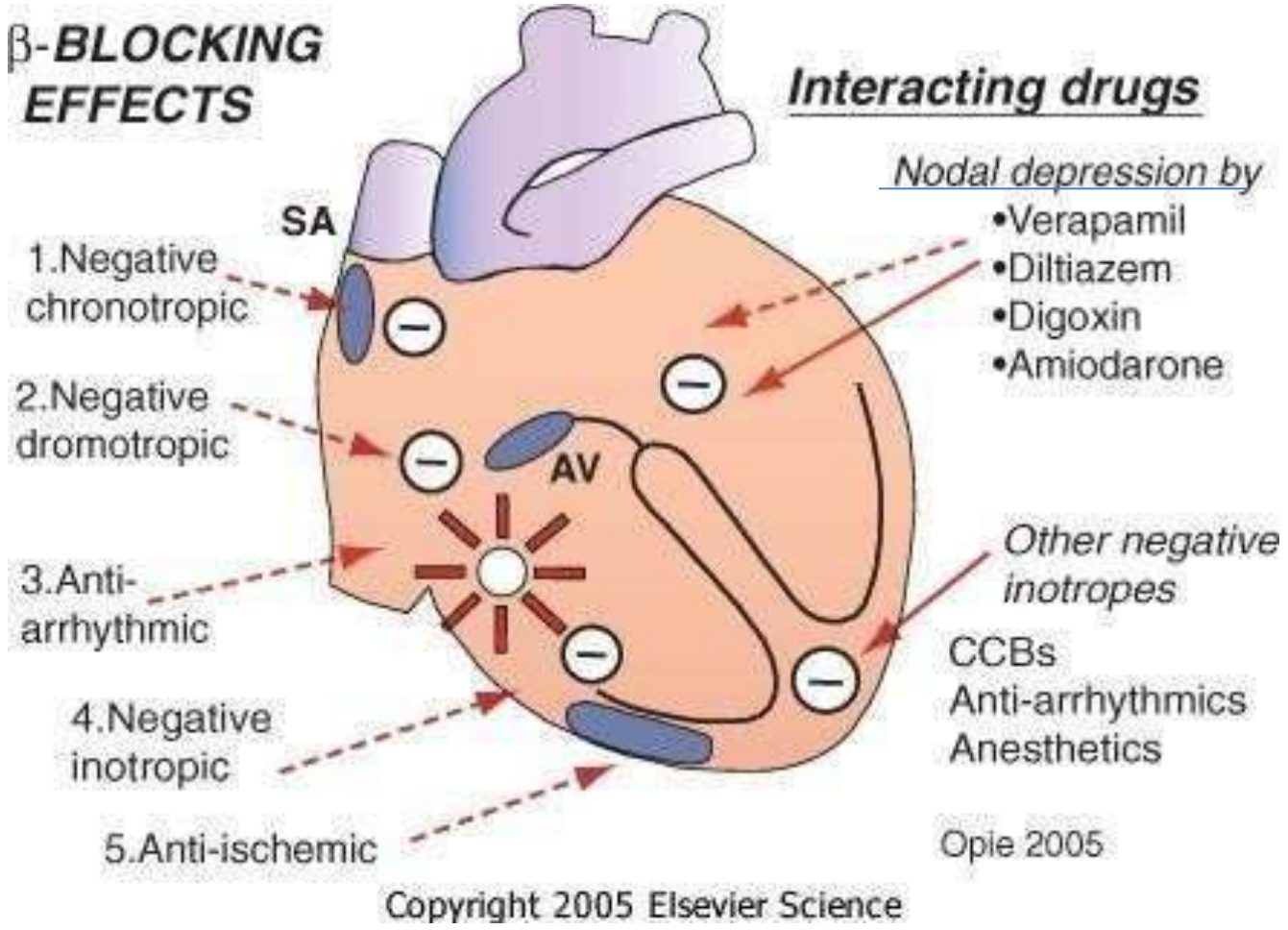


Antianginal agents - BBs

Drug Class	Beta-blockers
Mechanism of Action	Decreased myocardial oxygen demand, resulting from a reduction in heart rate, myocardial contractility, and blood pressure
Place in Therapy	**First-line in all patients without contraindications**
Specific Agents (Dosing)	Atenolol: 25-200 mg qd; Metoprolol tartrate: 100-450 mg/day in 2 to 3 divided doses; Propranolol: 80-320 mg/day in divided doses (2 to 4 times/day); Long-acting formulation: 80-320 mg qd
Side Effects	Bradycardia, hypotension, glucose intolerance, fatigue, exercise intolerance, worsening claudication
Contraindications	**Absolute**: severe bradycardia, sick sinus syndrome, AV block, unstable heart failure **Relative:** asthma, depression, peripheral vascular disease

Drug	Receptor Affinity	Intrinsic Sympathomimetic Activity	Usual Dose Range
Acebutolol	β_1-Selective	Yes	100–400 mg twice daily
Atenolol	β_1-Selective	No	25–100 mg once daily
Betaxolol	β_1-Selective	No	5–20 mg once daily
Bisoprolol	β_1-Selective	No	2.5–10 mg once daily
Carvedilol	α_1, β_1, and β_2	No	6.25–25 mg twice daily
Labetalol	α_1, β_1, and β_2	Yes, at β_2-receptors	100–400 mg twice daily
Metoprolol	β_1-Selective	No	50–100 mg twice daily (once daily for extended-release)
Nadolol	β_1 and β_2	No	40–120 mg once daily
Penbutolol	β_1 and β_2	Yes	10–40 mg once daily
Pindolol	β_1 and β_2	Yes	10–40 mg twice daily
Propranolol	β_1 and β_2	No	20–80 mg twice daily (60–180 mg once daily for long-acting formulation)
Timolol	β_1 and β_2	No	10–20 mg twice daily

Cardiac effects of β-adrenergic blocking drugs at the levels of the SA node, AV node, conduction system, and myocardium



Antianginal agents - CCBs

Drug Class	Calcium channel blockers
Mechanism of Action	Dilation of systemic and coronary **arteries**, leading to an increase in coronary blood flow and decreasing myocardial oxygen consumption
Place in Therapy	First-line in patients **with contraindications to beta-blockers** and in patients with **unacceptable adverse events or inadequate symptom control** on monotherapy with beta-blockers
Specific Agents (Dosing)	**Amlodipine**: 5-10 mg**/day** (**5 mg/day** in **elderly** patients) **Diltiazem**: ER capsule: 120-180 mg **qd** (max dose 480 mg/day); ER tablet: 180-360 **mg/day;** IR tablet: 180-360 mg/day in divided doses **Felodipine: 2.5-10 mg qd** **Nifedipine**: IR capsule: 10-30 mg tid; **SR tablet: 30-60 mg qd** V**erapamil**: 240-480 mg/day in 3 to 4 divided doses (**40 mg tid in elderly patients** and **those with small frames**)
Side Effects DD: digoxin	Bradycardia, hypotension, constipation, worsening of heart failure, peripheral edema, agent specific events, prolongation of QT-interval (bepridil), **AV-block (verapamil, diltiazem**), **excessive heart rate elevation (nifedipine)**
Contraindications	DHP: h**ypersensitivit**y. Non-DHP: **acute MI with pulmonary congestion**, **Wolf Parkinson-White syndrome**, **AV block**, hypersensitivity, **symptomatic hypotension**, **sick sinus syndrome**, **cardiogenic shock**, **heart failure (verapamil)**

Antianginal agents - Nitrates

Drug Class	Nitrates
Mechanism of Action	Vasodilation of peripheral veins and arteries, thus decreasing myocardial oxygen demand through a **reduction in preload**
Place in Therapy	**Treatment of angina in patients without compelling indications for other antianginal therapies, or as adjunct therapy**
Specific Agents (Dosing)	***Isosorbide dinitrate*** **Oral (IR)**: 5-40 mg 4 times/day; **Oral (SR)**: 40 mg every 8-12 h; **Sublingual**: **2.5-5 mg every 5-10 min** (max **3 doses in 15-30** min), can also be used as **prophylaxis** 15 min prior to activities that provoke angina **Isosorbide mononitrate**: **Regular tablet**: **5-20 mg bid**, with **at least 12 h separating doses**; **ER tablet**: initiate therapy **at 30-60 mg as a single dose** in the morning, then titrate upward **every 3 days** as needed to **max daily single dose of 240 mg** **Translingual spray**: 0.4 mg/spray, q5 min (max 3 doses in 15 min)
Side Effects	Headache, dizziness, hypotension
Contraindications	Hypersensitivity to nitrates, **concurrent use of PDEs (sildenafil, tadalafil, vardenafil)**, increased intracranial pressure, symptomatic hypotension

TABLE 4–8. Nitrate Formulations and Dosing for Chronic Use

Formulation	Dose
Oral	
Nitroglycerin extended-release capsules	2.5 mg 3 times daily initially, with up-titration according to symptoms and tolerance; allow a 10–12 hour nitrate-free interval
Isosorbide dinitrate tablets	5–20 mg 2–3 times daily, with a daily nitrate-free interval of at least 14 hours
Isosorbide dinitrate slow-release capsules	40 mg 1–2 times daily, with a daily nitrate-free interval of at least 18 hours
Isosorbide mononitrate tablets	20 mg 2 times daily, with doses 7 hours apart
Isosorbide mononitrate extended-release tablets	30–120 mg once daily
Transdermal	
Nitroglycerin extended-release film	0.2–0.8 mg/hour, on for 12–14 hours, off for 10–12 hours

Glyceryl trinitrate

TABLE 6-12 Nitrate Products

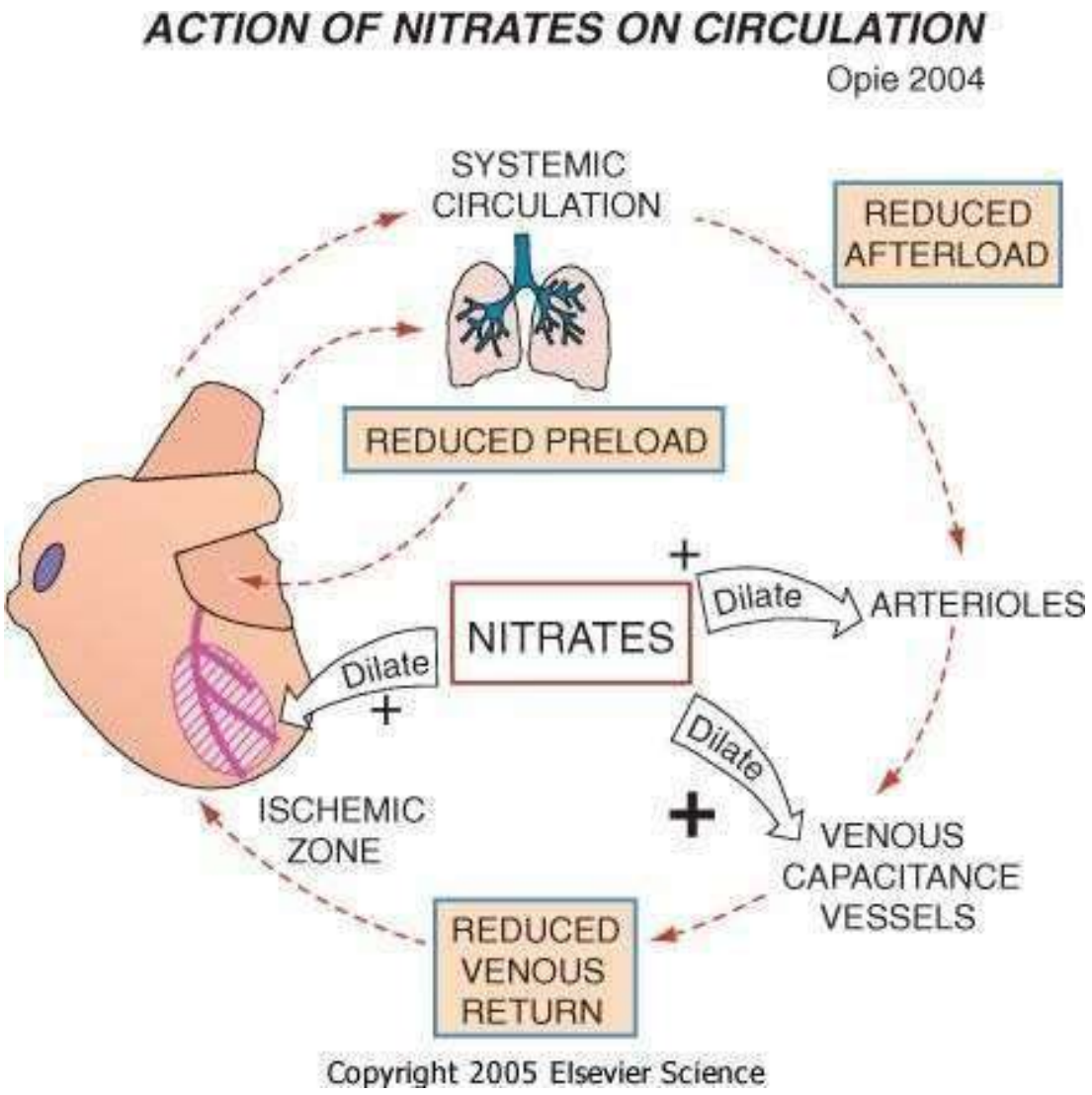
Product	Onset (minutes)	Duration	Initial Dose
Nitroglycerin			
IV	1–2	3–5 minutes	5 mcg/min
Sublingual/lingual	1–3	30–60 minutes	0.3 mg
Oral	40	3–6 hours	2.5–9 mg three times a day
Ointment	20–60	2–8 hours	0.5–1 in
Patch	40–60	>8 hours	1 patch
Erythritol tetranitrate	5–30	4–6 hours	5–10 mg three times a day
Pentaerythritol tetranitrate	30	4–8 hours	10–20 mg three times a day
Isosorbide dinitrate			
Sublingual/chewable	2–5	1–2 hours	2.5–5 mg three times a day
Oral	20–40	4–6 hours	5–20 mg three times a day
Isosorbide mononitrate	30–60	6–8 hours	20 mg daily, twice a day[a]

[a]Product dependent.

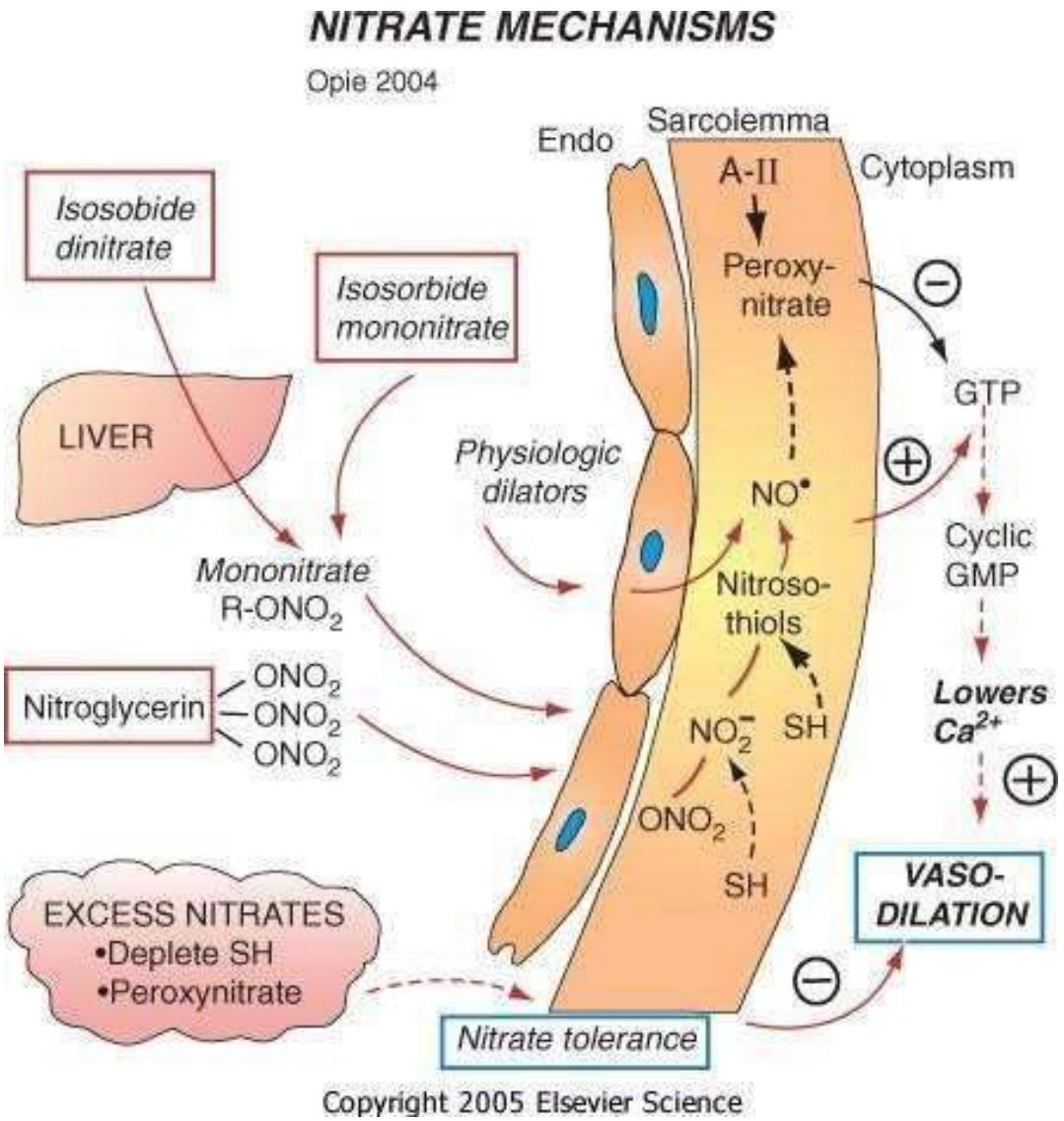
Nitrates

- Nitrate therapy should be the first step in ***managing acute attacks*** for patients with chronic stable angina if the attacks are infrequent (i.e., a few times per month) or for ***prophylaxis of symptoms*** when undertaking activities known to precipitate attacks.
- **Tolerance**
  - "**Monday disease"** – munitions workers
  - **Chronic tolerance** and **withdrawal death**
  - Proposed mechanism(s) for tolerance
    - ↓cGMP production,
    - ↓ cGMP phosphodiesterase, ↓sulfhydrl groups (cysteine) by peroxynitrate
    - Activation of neurohormonal systems
    - intravascular volume (vent. filling pressures)
  - Solution
    - **Avoid Around-the-clock (ATC)** dosing – **need 8 to 12 hr nitrate** free interval
      - Concern **for silent ischemia (rebound ischemia**) in the **nitrate free interval**
    - **acetylcysteine (sulfhydrl donor**) (**NAC has been thought to reverse nitrate tolerance** by replenishing depleted intracellular sulfhydryl groups)
    - Concomitant use of **ACE inhibitors, carvedilol or hydralazine**

Schematic diagram of effects of nitrate on the circulation

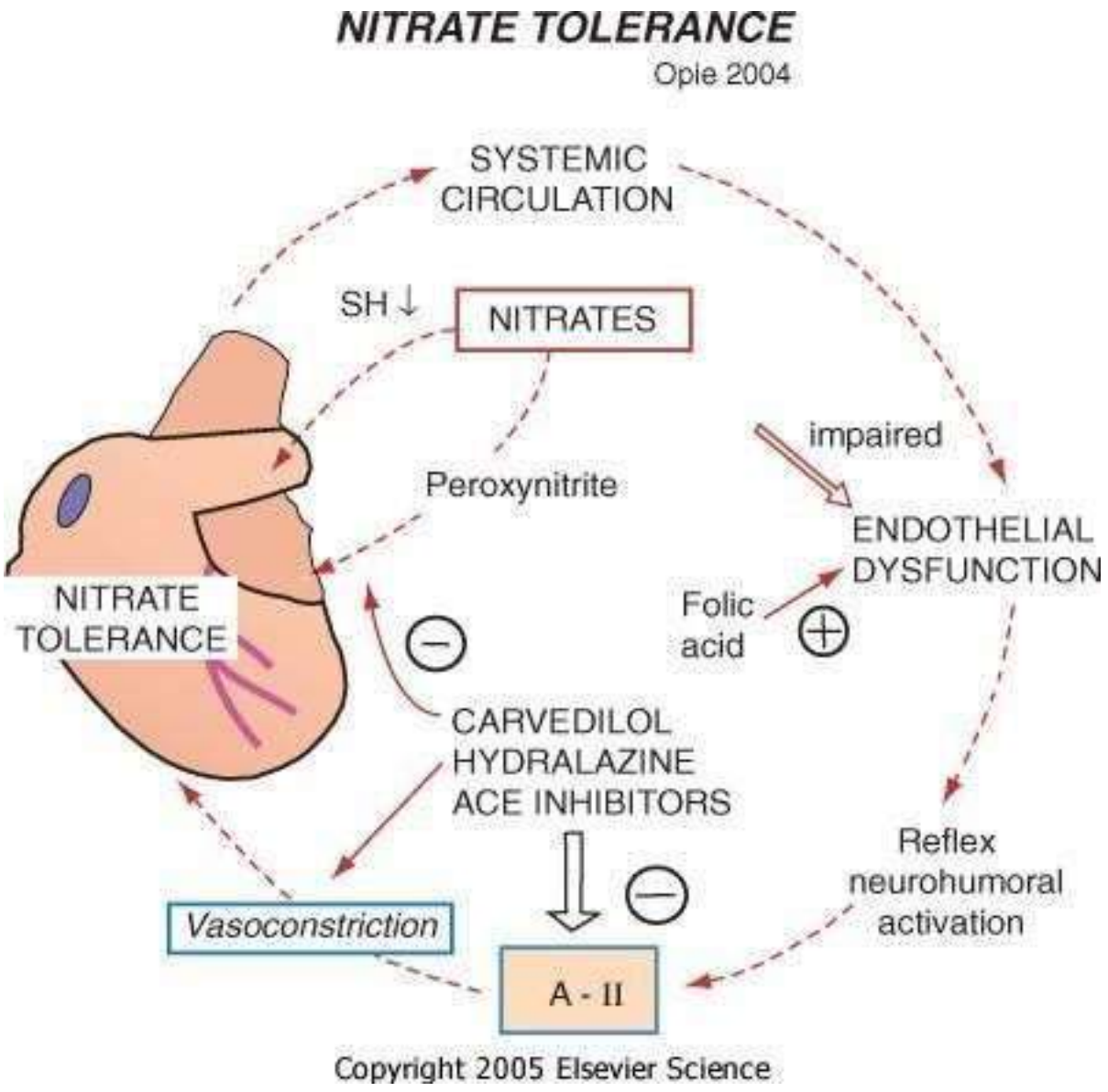


Effects of nitrates in generating NO• and stimulating guanylate cyclase to cause vasodilation



Copyright 2005 Elsevier Science

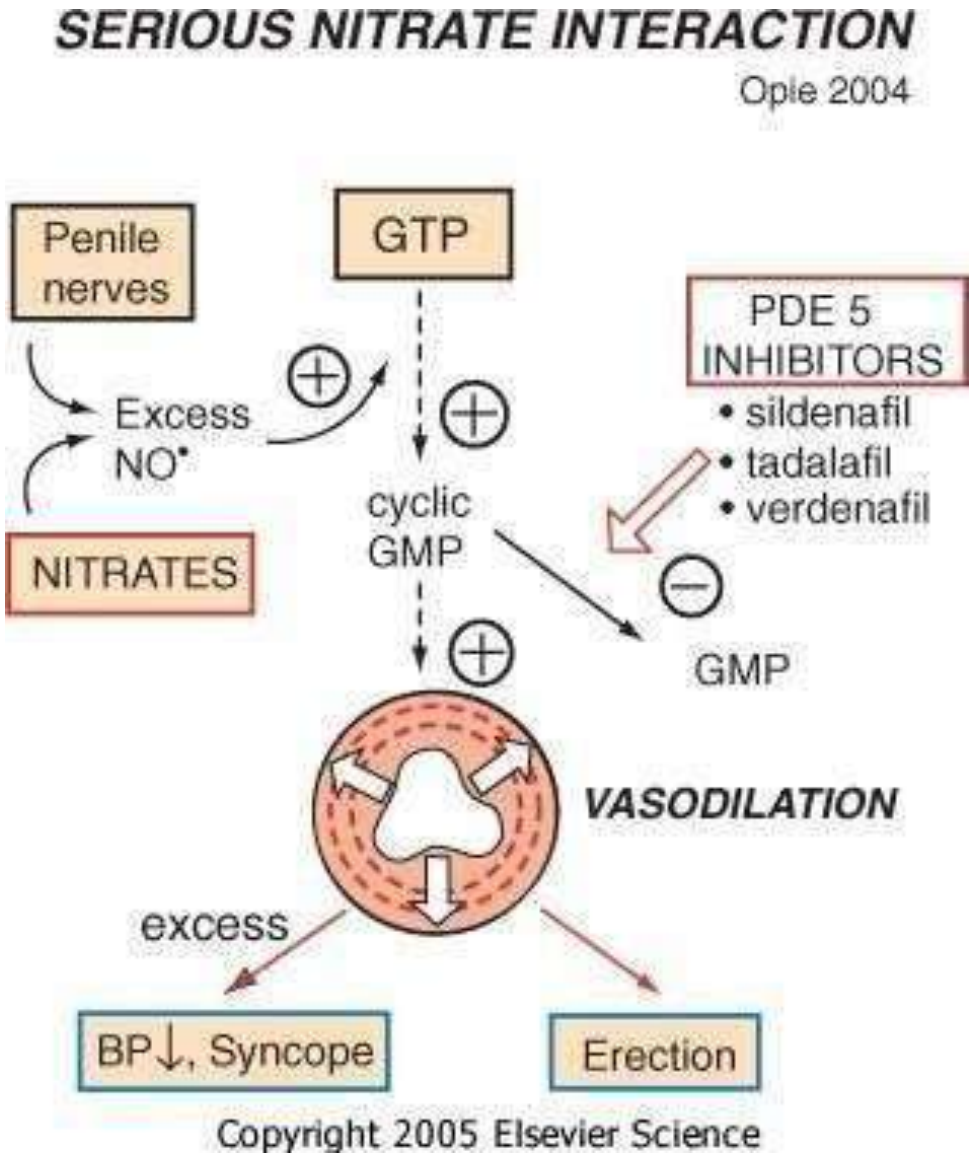
Current proposals for therapy of nitrate tolerance.



Nitrates

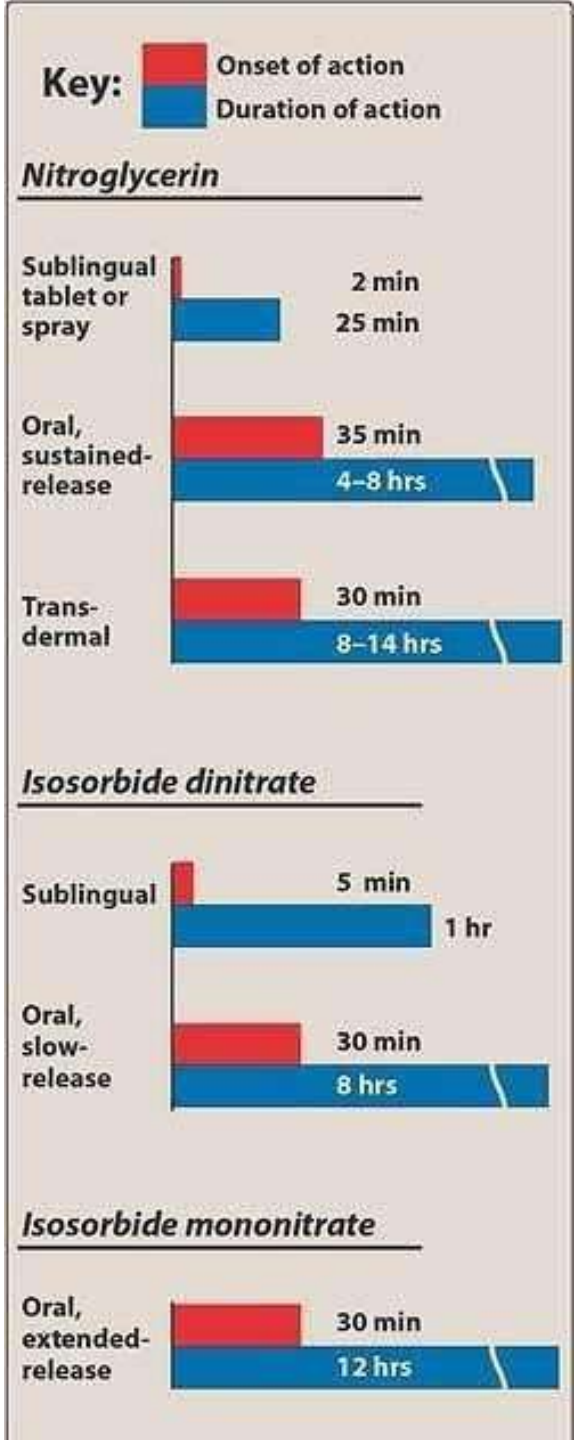
- **Patient related variables**
  - SBP < 90mmHg, HR < 50 bpm–use caution
- **Toxicity**
  - **Headache, flushing, hypotension, reflex tachycardia** and **syncope**
  - Rare: m**ethemoglobinemi**a-Due to **ability of nitrate ions to oxidize hemoglobin**
- **Patient information**
  - Concept of tolerance, side-effects, proper storage
- **Significant Drug Interactions**
  - Phosphodiesterase-5 inhibitors
    - Sildenafil, vardenafil, tadalafil – prevent breakdown of cGMP
      - Sildenafil and vardenafil (24hr washout)
      - Tadalafil (48 hr washout)
  - Heparin
    - Heparin resistance at NTG IV doses >350 mcg/min

A serious nitrate drug interaction



Nitrate preparations

- **Three groups of nitrates:**
  - Glyceryl trinitrate
  - Isosorbide dinitrate (metabolized to the mononitrate)
  - Isosorbide mononitrate



Nitrate preparations

- **IV infusions**

- **Slow-release tablets and capsules** (for majority of pts with stable angina) - cheap, administered 2-3 times D, permits nitrate free period. **Extensive first pass metabolic effect.**

- **Transdermal patches** - expensive, no flexible dosing rate and may not permit a nitrate free period.

- **Ointments** - messy!

- **Adhesive buccal tablets** - expensive, no real therapeutic advantage in regular therapy.

- **Sublingual tablets and sprays** - **rapid onset of action**, and **bypasses the live**r. For the prevention or relieve of acute attacks of pain.

Nitrate preparations

- **Main side effects**
  - Hypotension with dizziness and fainting
  - Throbbing headache

- **Advice patient to**
  - **Sit down (rather than lie or stand)**
  - For **short-acting nitrates - Spit out** or **swallow** the **tablet once angina** is **relieved**
  - **Sublingual glyceryl trinitrate** have very s**hort shelf life - s**tore **carefully and replace frequently.**

Treatment of Angina – acute attack

- All patients with a history of angina should have sublingual nitroglycerin tablets or spray to relieve acute ischemic symptoms.

- **During an Acute Angina attack –**
  - ***GNT Sublingual tablet*,**
    300–600 micrograms (half to 1 tablet) repeated every 3–4 minutes until pain is resolved, to a ***maximum of 2 or 3 tablets over 15 minutes.***

  - ***Sublingual spray*,**
    400–800 micrograms (1–2 sprays).

- **Sublingual nitroglycerin** can also be used **to prevent effort-induced angina** (i.e., angina that occurs **with exertion).** In this case, the patient should use sublingual nitroglycerin **2 to 5 minutes** prior to an **activity known to cause** angina, with the effects **persisting for approximately 30 minutes**.

- **Isosorbide dinitrate**, also available in a sublingual form, has **a longer half-life** with anti-anginal effects lasting **up to 2 hours.**

Counseling pts on sublingual tablets

- **Advice pt to:**
  - **Sit or lie down before use** as it may cause dizziness.
  - Place half to one tablet **under your tongue; do not swallow it;** after the pain has been relieved, you may spit out or swallow what is left of the tablet to avoid adverse effects such as headache.
  - **Call an ambulance if 2 or 3 tablets over 15 minutes do not relieve pain.**
  - It is important **to store these tablets properly** or they may not work as well. Keep them in the original glass bottle away from moisture, heat and light. Do not carry them close to your body. **Write the date** on the bottle when you open it and discard any unused **tablets 3 months later.**
  - **Never to take sildenafil, tadalafil or vardenafil** (contraindicated)
  - **Common adverse effects** include headache, flushing, palpitations, orthostatic hypotension, fainting, peripheral edema

Counseling pts on Sublingual spray

- **Advice pt to :**
  - **Prime the spray before using it** for the first time by pressing the nozzle 5 times, spraying it into the air. Prime it with 1 spray if it hasn't been used for 7 days. Prime it with 5 sprays if it hasn't been used for more than 4 months.
  - When ready to use, **aim the spray under the tongue** and press the nozzle once; do not inhale the spray.
  - **Call an ambulance** if **2 sprays over 15 minutes** do **not relieve pain.**



Case study

- DD came back to your pharmacy later on with a script for *Imtrate SR* (Isosorbide Mononitrate).

- **What would you counsel the patient on?**



Counseling on Isosorbide Mononitrate

- Initially 30–60 mg **once daily**, increased up to 120 mg once daily if necessary.
  - **twice daily dosing with isosorbide mononitrate is *not* recommended**, as there will be no nitrate-free interval and tolerance will be more likely to develop

- Swallow whole; do not crush or chew the tablet.

- **Take at the time of day when angina is most frequent**, e.g. at night for nocturnal angina or in the morning for daytime angina.

- **Isosorbide mononitrate** is ***not*** **recommended for treatment of acute episodes of angina** because of **its slow onset of action**

- Do not cut Imtrate® in half (there is insufficient evidence to show that a halved tablet delivers half the dose of one full tablet (the other products can be halved)

Antianginal agents - Ranolazine

Drug Class	Ranolazine
Mechanism of Action	Mechanism of action not clear; possible **shift in the production of ATP away from oxidation of fatty acids**, thus favoring the **more oxygen-efficient oxidation** of carbohydrates.* **Altering the trans-cellular late sodium current**
Place in Therapy	**Combination therapy** with amlodipine, nitrates, or beta-blockers in patients **not well controlled with monotherapy on these agents**
Specific Agents (Dosing)	Initiate at **500 mg bid**; titrate up to **max dose of 1,000 mg bid**
Side Effects	Dizziness, headache, constipation, nausea, Torsades de pointes
Contraindications	Dizziness, headache, constipation, nausea. **Severe hepatic dysfunction**, **concurrent use of QT-interval-prolonging agents or preexisting QT interval prolongation at baseline, concurrent therapy with CYP3A inhibitors**

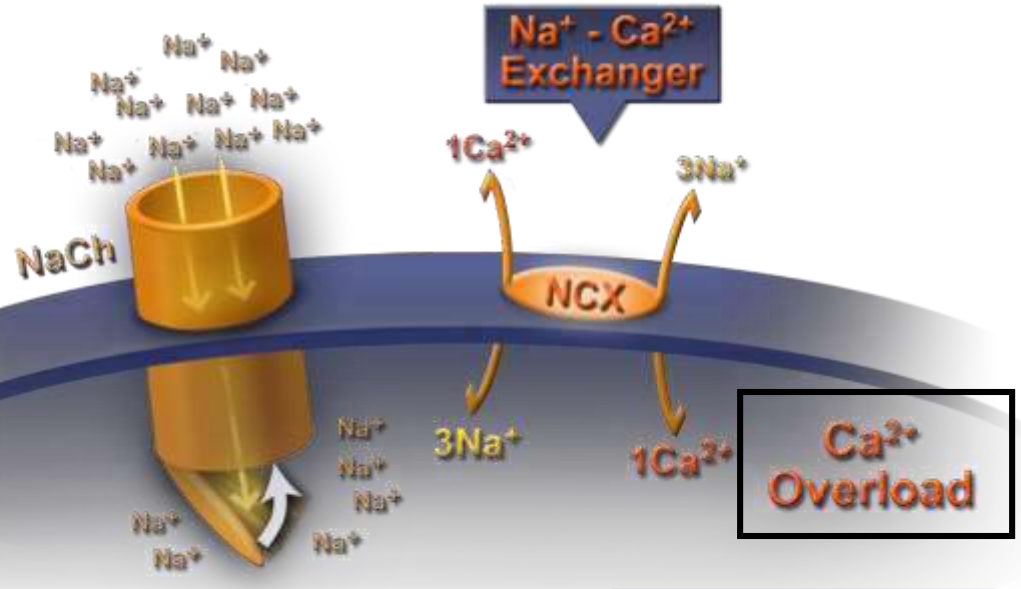
*trimetazidine

Consequences associated with dysfunction of late sodium current

- **Diseases** (eg, ischemia, heart failure)
- **Pathological milieu** (reactive O_2 species, ischemic metabolites)
- **Toxins and drugs** (eg, ATX-II, etc.)

Na^+ channel

(Gating mechanism malfunction)



Mechanical dysfunction

Abnormal contraction and relaxation

↑ diastolic tension (↑LV wall stiffness)

Oxygen supply and demand

Increase ATP consumption

Decrease ATP formation

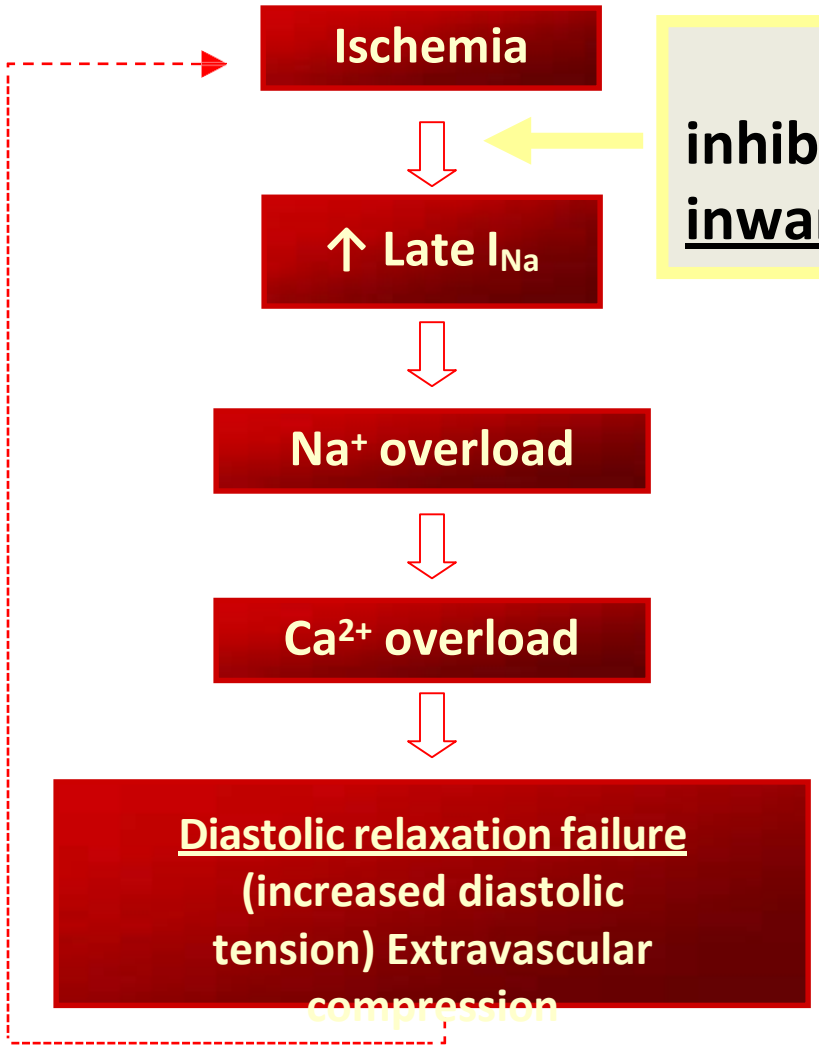
Electrical instability

Early after potentials

Beat-to-beat ΔAPD

Arrhythmias (VT)

Ranolazine: Mechanism of action



Ranolazine inhibits the late inward Na current

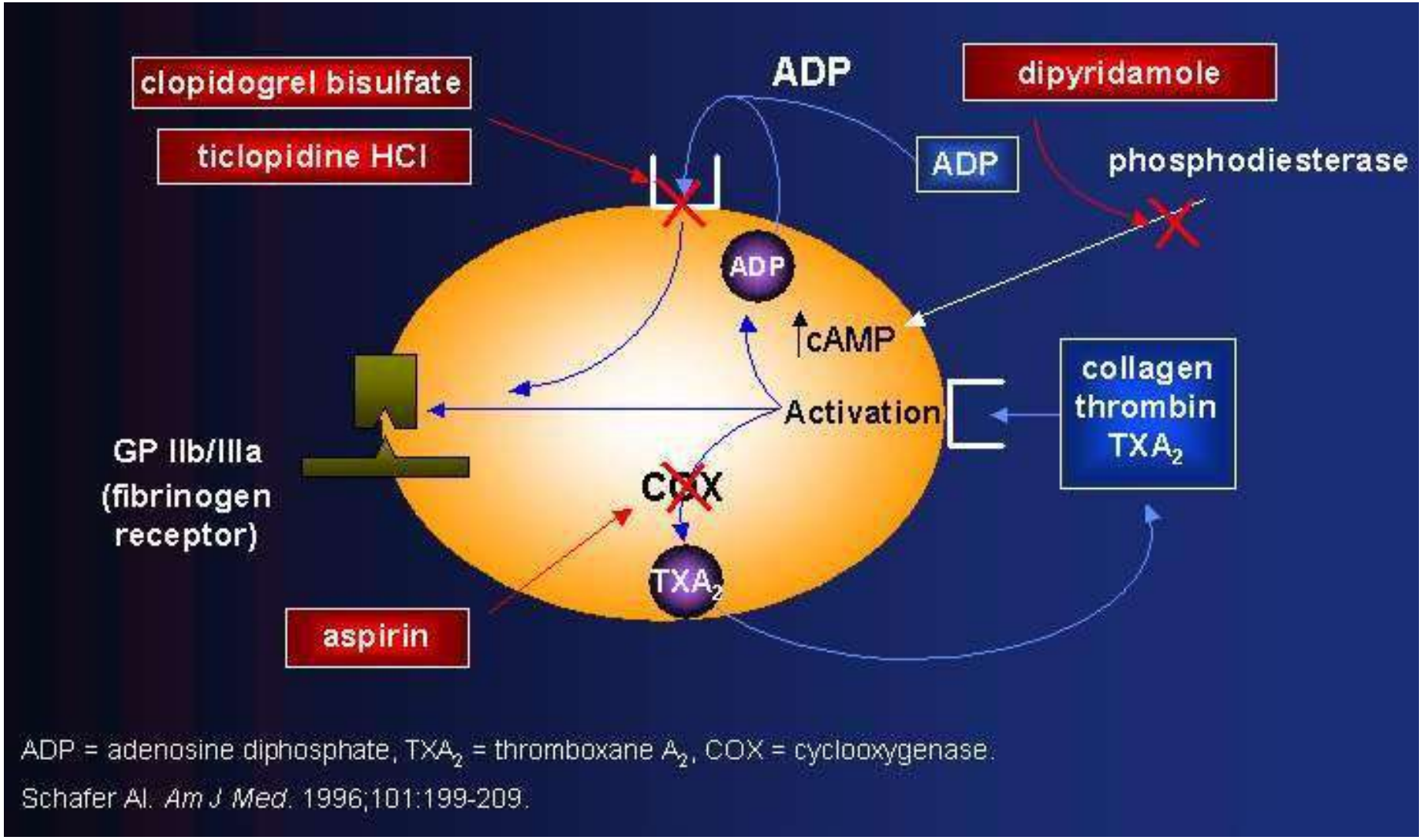
Pharmacotherapy to prevent acute coronary syndromes and death

Antiplatelet Agents

Platelet cascade in thrombus formation:

- **Platelet adhesion**
  - Exposure to subendothelial matrix
- **Platelet activation** and **secretion**
  - changes in the shape of platelets, **activation of the glycoprotein IIb/IIIa receptor**, and induction of platelet coagulant activity
  - **thromboxane A2 (activation)**, thrombin, norepinephrine, collagen, and **adenosine diphosphate (aggregation)**
- Platelet **aggregation--fibrinogen**
  - mediated by the binding of **adhesive proteins** to the **new ligand-receptive** form of the **glycoprotein IIb/IIIa receptor**
- Platelet **integration** with **coagulation factors**

Oral antiplatelet agents



ADP = adenosine diphosphate, TXA_2 = thromboxane A_2, COX = cyclooxygenase.

Schafer AI. *Am J Med*. 1996;101:199-209.

Antiplatelet Agents

- In patients with stable or unstable angina, aspirin has been consistently shown to reduce the risk of major adverse cardiac events, particularly MI.
- Antiplatelet therapy with aspirin should be considered for all patients without contraindications, particularly in patients with a history of myocardial infarction.

- **Aspirin** doses of **75 to 162 mg** daily have been shown to be **cardioprotective.**
- If **aspirin** is contraindicated (e.g., **aspirin allergy**, **active peptic ulcer disease**, or **active internal bleeding**) or is not tolerated by the patient, other antiplatelet agents such as **clopidogrel** should be considered.
- Recent studies have suggested that combination antiplatelet therapy may be synergistic in reducing the risk of IHD-related events.
  - In patients **with ACS**, the **combination of aspirin and clopidogrel 75 mg daily** for up to **9 months** was more effective than aspirin alone in decreasing the risk of death, MI, and stroke.
  - This combination also **prevents complications following PCI**

Statins

- To control risk factors and prevent major adverse cardiac events**, statin therapy should be considered in all patients with ischemic heart disease**, particularly in those with elevated low-density lipoprotein cholesterol.

- Statins are **potent lipid-lowering agents**, possess **non–lipid-lowering effects** that may provide additional benefit to patients with IHD, and have been shown to reduce morbidity and mortality in patients with IHD. Based on these benefits, statins are generally considered the **drugs of choice in patients with dyslipidemias.**

- Moreover, based on evidence that statins improve outcomes in patients with IHD and “normal” LDL cholesterol concentration, statins should be considered in all patients with IHD at high risk of major adverse cardiac events, **regardless of baseline LDL cholesterol.**

ACE-I's and ARB's

- In the absence of contraindications, ACE inhibitors should be considered in ischemic heart disease patients who also have
  - **I. diabetes mellitus,**
  - **II. left ventricular dysfunction,**
  - **III.** history of **myocardial infarction,**
  - or any combination of these.

- In addition, they should also be considered in all patients with IHD and in patients at high risk for developing IHD

- **Angiotensin receptor blockers** may be used in patients who cannot tolerate ACE inhibitors due to side effects (e.g., **chronic cough**).

- There are far more data supporting the use of ACE inhibitors in IHD. Therefore, ACE inhibitors should remain first-line in patients with a history of MI, diabetes, or left ventricular dysfunction.

TABLE 4–5. Doses of ACE inhibitors and Angiotensin Receptor Blockers Indicated in Ischemic Heart Disease (IHD).

Drug	Indications	Usual Dosage in IHD
Angiotensin-Converting Enzyme Inhibitors		
Captopril	HTN, HF, post-MI, diabetic nephropathy	6.25–50 mg 3 times daily
Enalapril	HTN, HF	2.5–40 mg daily in 1–2 divided doses
Fosinopril	HTN, HF	10–80 mg daily in 1–2 divided doses
Lisinopril	HTN, HF, post-MI	2.5–40 mg daily
Perindopril	HTN, IHD	4–8 mg daily
Quinapril	HTN, HF, post-MI	5–20 mg twice daily
Ramipril	HTN, high-risk for IHD, HF, post-MI	2.5–10 mg daily in 1–2 divided doses
Trandolapril	HTN, HF, post-MI	1–4 mg daily
Angiotensin Receptor Blockers		
Candesartan	HTN, HF	4–32 mg daily
Valsartan	HTN, HF, post-MI	80–320 mg daily in 1–2 divided doses

HF, heart failure; HTN, hypertension; MI, myocardial infarction.

Alternative Therapies

- Most have **not shown to provide any clinical** benefit in persons with **chronic stable** angina, and in **some cases** may pose **greater CV risk**
  - Fish oils
  - Garlic
  - Folic acid (lower homocysteine levels ?)
  - Antioxidant vitamins (E and C)
  - Hormone/estrogen replacement therapy
  - Chelation therapy

Mechanical Revascularization

- **Many patients** with **chronic stable angina** can be **successfully managed** by **pharmacologic therapy alone**.

- However, for those with **refractory symptoms** or **in certain high-risk subgroups**, revascularization procedures, including **CABG** surgery and percutaneous transluminal coronary angioplasty (**PTCA**), are recommended.

Interventional Therapies

- PCI vs. CABG
  - Decision multifactorial – risk factors, LVF, # of diseased vessels and % of occlusions

- PCI
  - Several catheter-based interventions may be used during PCI, including:
    - Percutaneous transluminal coronary angioplasty (**PTCA)**;
    - Intracoronary **bare metal stent** placement;
    - Intracoronary **drug-eluting stent** placement;
    - Rotational **atherectomy**
  - Tremendous advances over the past 5-10 years, especially in regards to stent technology

- CABG
  - **Left main coronary artery disease** or **severe proximal LAD disease**
  - **>3 vessel involvement**

LAD: left anterior descending artery

Coronary Artery Bypass Graft Surgery

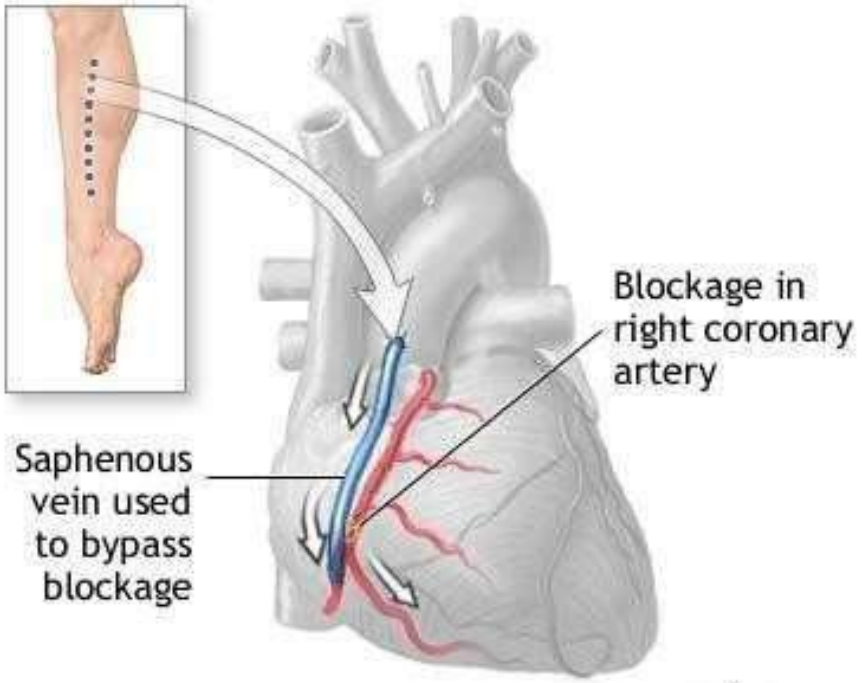
- **Coronary artery bypass surgery involves the placement of grafts to bypass stenosed native coronary arteries**

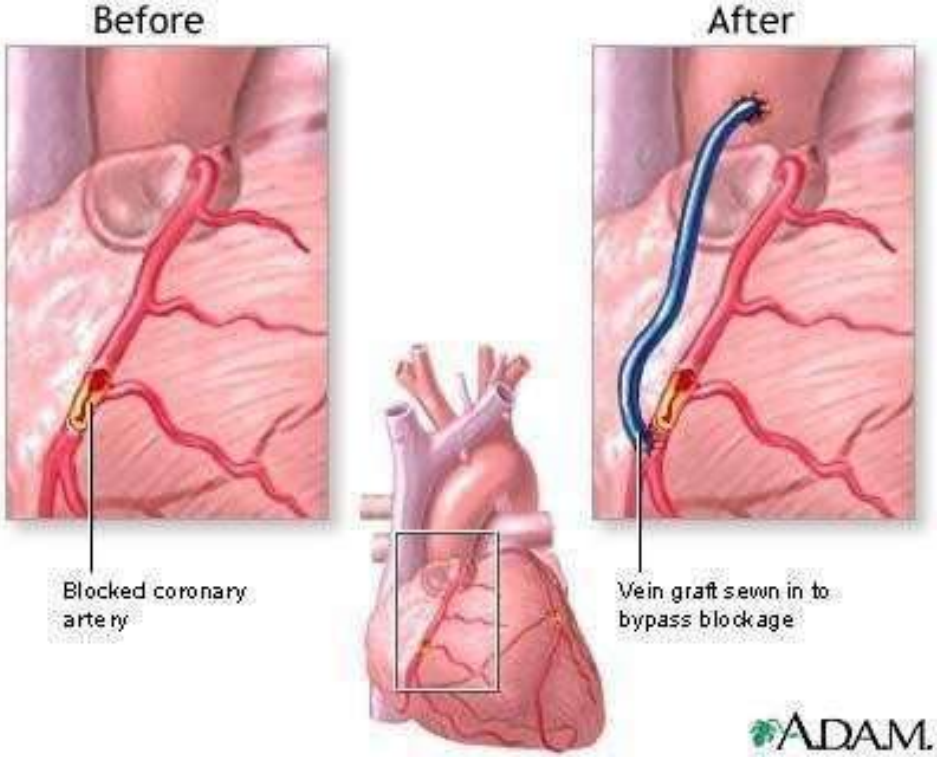
- The grafts are usually **saphenous veins or arteries** (sometimes the left internal mammary artery).

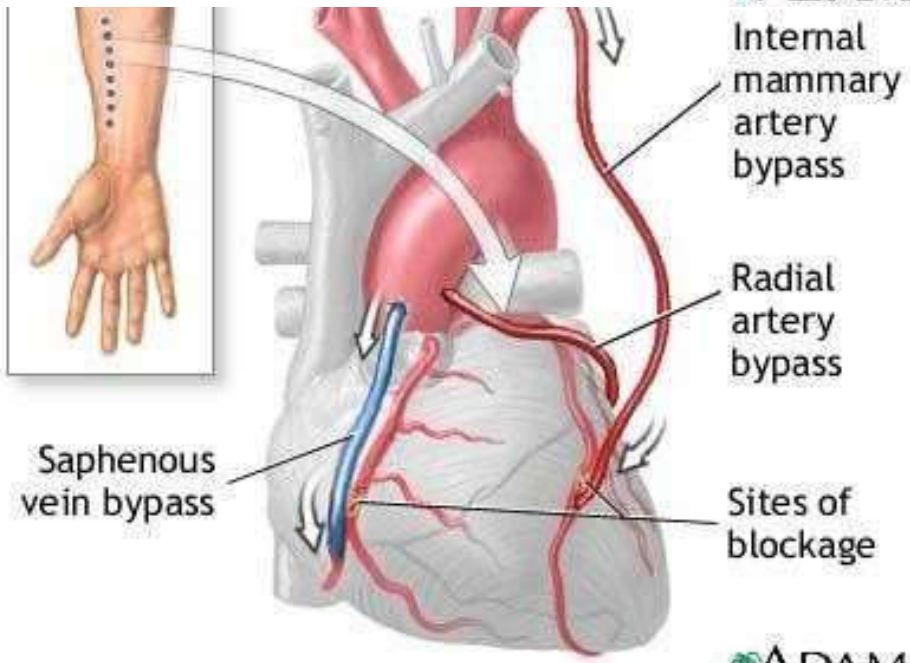
- suturing segments of saphenous vein between the ascending aorta and to the coronary arteries distal to their stenotic narrowings

- Antiplatelet therapy with aspirin has been shown to improve long-term graft patency rates,

- Aggressive lipid-lowering to achieve an LDL cholesterol < 100 mg/dl has been shown to slow the development of atherosclerosis within bypass grafts


Blockage in right coronary artery
Saphenous vein used to bypass blockage


Before
After
Blocked coronary artery
Vein graft sewn in to bypass blockage


Internal mammary artery bypass
Radial artery bypass
Saphenous vein bypass
Sites of blockage

Percutaneous Transluminal Coronary Angioplasty and Intracoronary Stents.

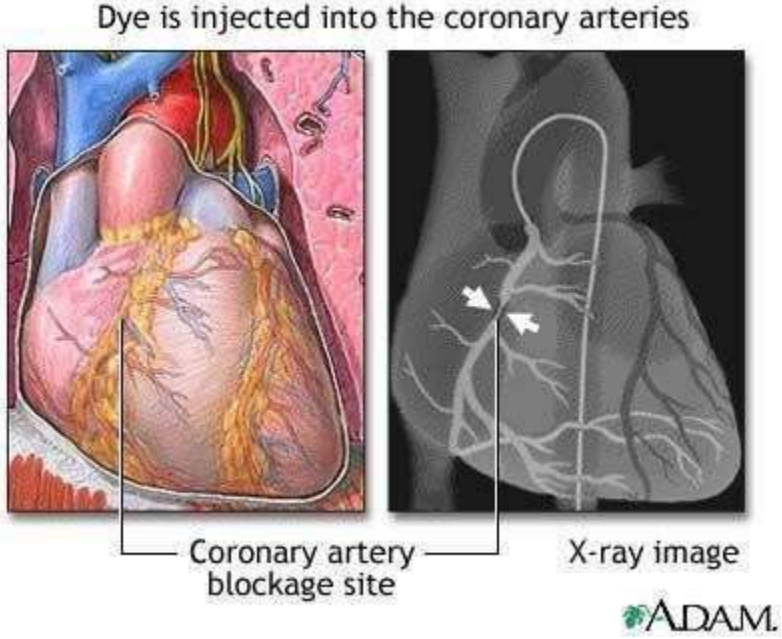
- PTCA is now widely successful in relieving ischemia in patients with one- and two-vessel lesions

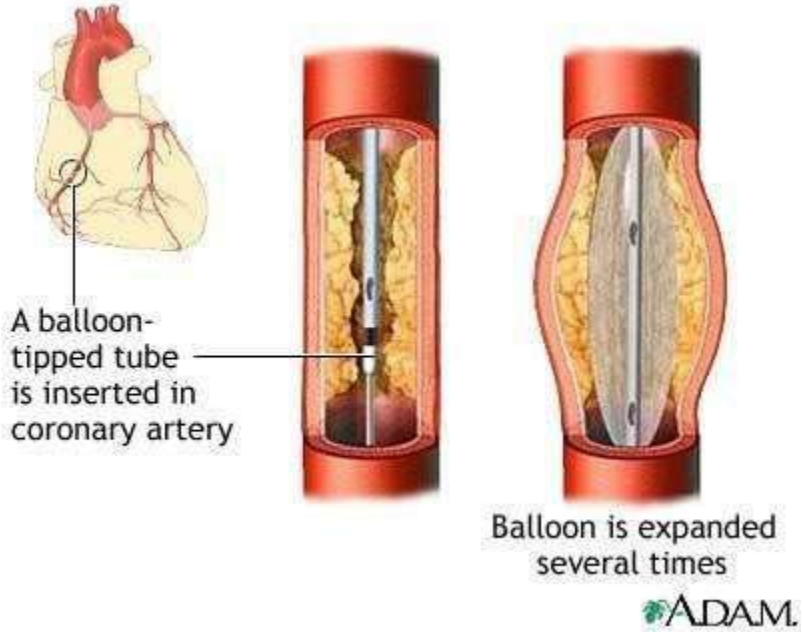
- In more than 60% of angioplasty procedures today, metal tubular stents are permanently deployed in the vessel, leaving no, or minimal, residual stenosis and a much improved rate of occlusion or restenosis, **using antiplatelet protocols that include aspirin plus clopidogril.**

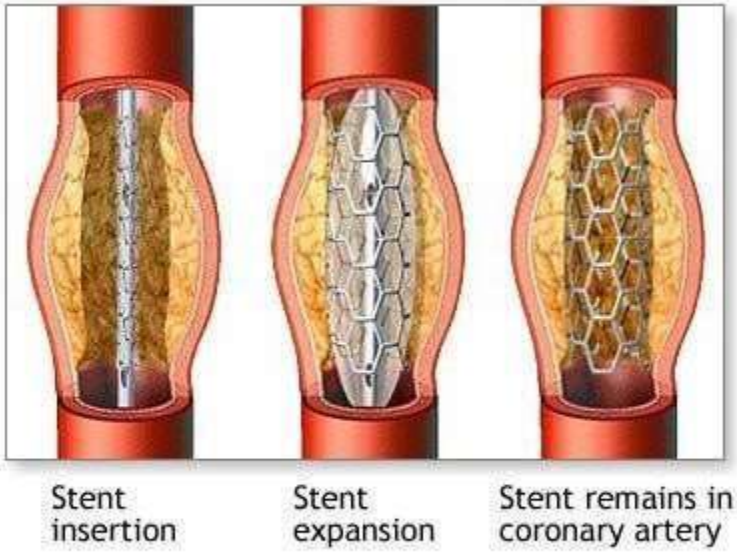
- can be easily repeated. The mortality is low (**0.2%**), and the **most serious late complication** is **restenosis.**

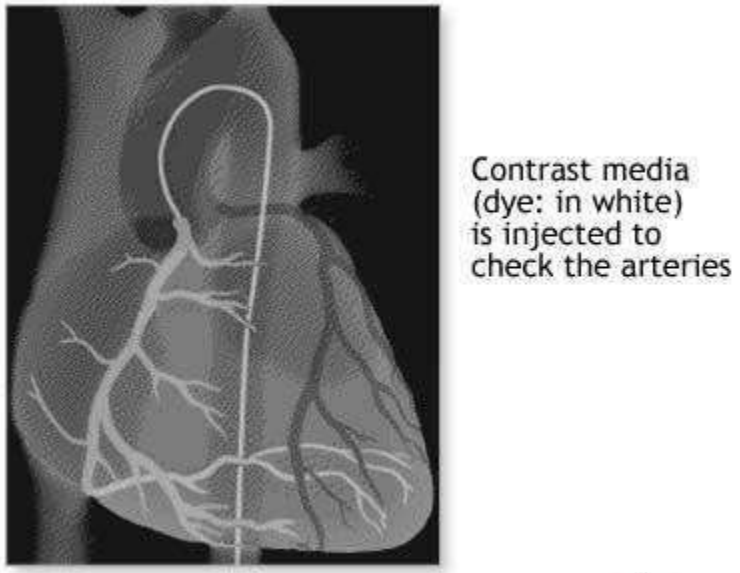
- Drug eluting stents are impregnated with low concentrations of **antiproliferative medications**, such as **paclitaxel or sirolimus,** which are slowly released locally within the coronary artery → **inhibit restenosis**

Interventional catheterization

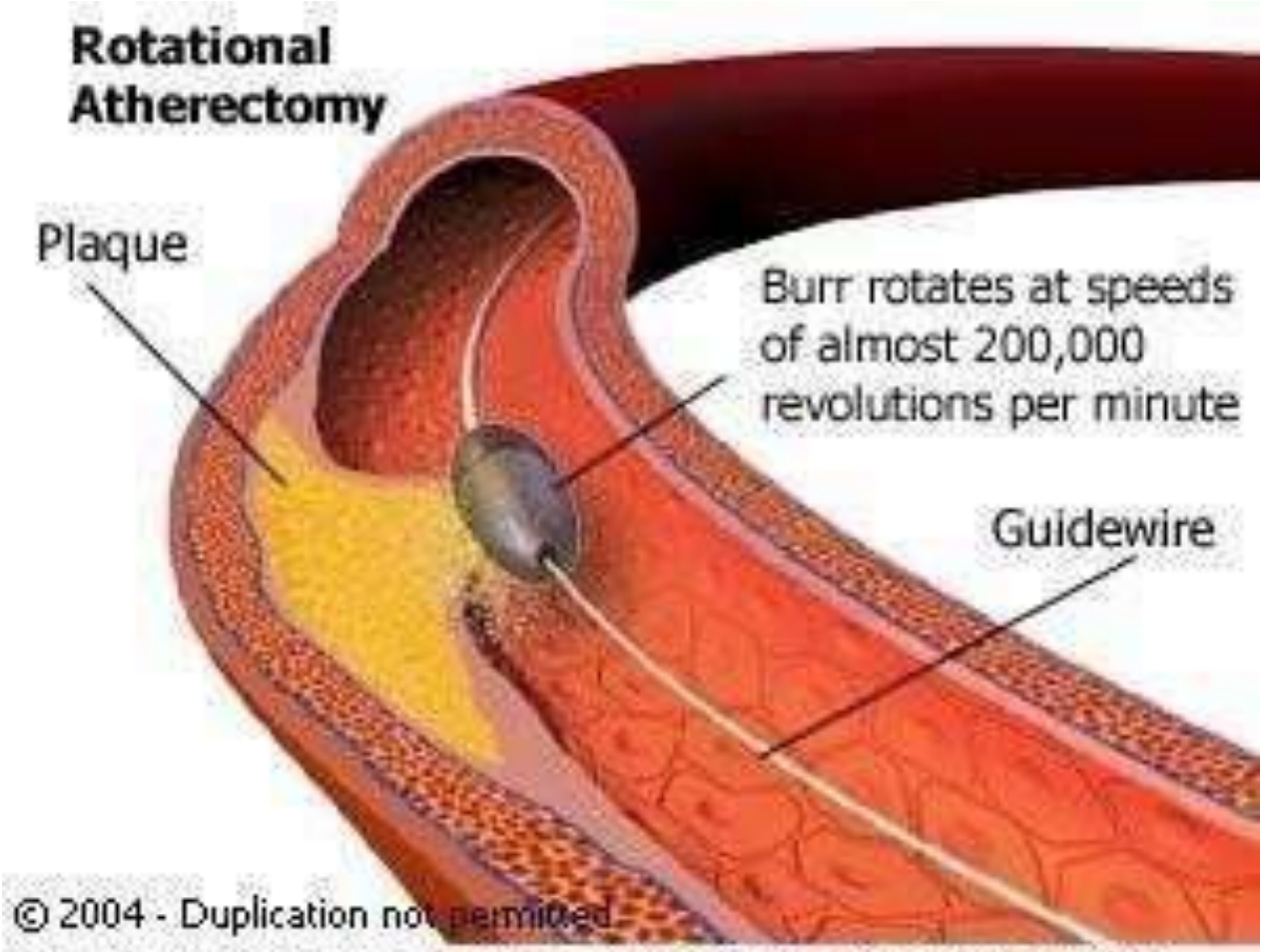








Rotational atherectomy



A catheter equipped with a rotablator is guided to the blockage. Using a diamond-studded tip, the rotablator pulverizes calcified plaque. The small particles can move safely through the circulatory system and out of the body.

Revascularization

Left Main CAD Revascularization

Class I

1. CABG to improve survival is recommended for patients with significant (≥50% diameter stenosis) left main coronary artery stenosis.[328–334] (*Level of Evidence: B*)

Non-Left Main CAD Revascularization

Class I

1. CABG to improve survival is beneficial in patients with significant (≥70% diameter) stenoses in 3 major coronary arteries (with or without involvement of the proximal LAD artery) or in the proximal LAD artery plus 1 other major coronary artery.[125,330,334,358–360] (*Level of Evidence: B*)
2. CABG or PCI to improve survival is beneficial in survivors of sudden cardiac death with presumed ischemia-mediated ventricular tachycardia caused by significant (≥70% diameter) stenosis in a major coronary artery. (*CABG Level of Evidence: B*[122,378,379]; *PCI Level of Evidence: C*[378])

Recommendations for Revascularization With PCI (Percutaneous Coronary Intervention) and CABG in Patients With Stable Angina

1. CABG for patients with significant left main coronary disease. **(>70% stenosis)**
2. CABG for patients with 3-vessel disease. The survival benefit is greater in patients with abnormal LV function (ejection fraction less than 50%).
3. CABG for patients with 2-vessel disease with significant proximal left anterior descending CAD **(>70%) and either**

   abnormal LV function (ejection fraction < 50%) ***l*or**

   demonstrable ischemia on noninvasive test
4. PCI for patients with 2 or 3-vessel disease with significant proximal LAD CAD, who have anatomy suitable for catheter-based therapy and normal LV function and who do ***not*** have treated diabetes (IB)
5. PCI or CABG for patients with 1- or 2-vessel CAD without significant proximal left anterior descending CAD
6. In patients with prior PCI, CABG or PCI for recurrent stenosis associated with a large area of viable myocardium or high-risk criteria on noninvasive testing (IB)
7. PCI or CABG for patients who have not been successfully treated by medical therapy and can undergo revascularization with acceptable risk.(IB)

Management of angina

-Stable exertional angina

-Variant angina

-Silent Ischemia

Stable Angina

- All patients should receive the following unless a contraindication is present:
  - Aspirin
  - Beta-blockers
  - ACE inhibitor (if indication present)
  - LDL-lowering therapy
  - SL NTG
  - Calcium channel blockers or nitrates
    - For contraindication or intolerance to β-blockers, lack of efficacy with β-blocker in controlling symptoms.

Treatment Strategies for Chronic Stable Angina

- Angina Treatment Mnemonic
  - A– aspirin, anti-anginals
  - B – B-blockers and blood pressure
  - C – cholesterol and cigarettes
  - D– diet and diabetes
  - E –education and exercise

Stable Angina

- β-**blocker**s may be **most effective** in those persons who have a **high resting heart rate** and those with a **relatively fixed anginal threshold**

- **Nitrate therapy** for **immediate relief of symptoms** and as **prophylaxis** for symptoms **during exertion**
  - SL taken 5 minutes prior to activity provides for an adequate response in most patients for a duration of ~ 30 minutes
  - Should **not be first-line therapy unless beta-blockers and CCB** are **contraindicated or not tolerated**
  - Tolerance decreases efficacy

- Calcium channel blockers – potential for improving supply and demand, and may be used in place of beta-blockers as initial therapy
  - Useful in **variable threshold** for **exertion angina**

Vasospastic Angina

- Patients are typically younger and have fewer cardiovascular risk factors. Higher rate of smoking
  - Assessment of risk factors and aggressive lifestyle modifications
- Acute attack
  - Nitrates
- Chronic therapy
  - Nitrates and calcium channel blockers
  - Avoid B-blockers

Silent Ischemia

- **Occurrence**
  - As common as 80% with CAD – associated with extent of disease
- **Classification**
  - Class I: Do **not experience angina** at any time
  - Class II: Have **both symptomatic and asymptomatic angina**
- **Treatment**
  - Beta-blockers are first-line
  - CCB are acceptable substitute –but do not confer as great of a benefit with diurnal surge
  - **Combination therapy often provides superior efficacy to monotherapy with any agent**

Coexisting Conditions

Condition	**Use recommended**	**Caution or avoid**
HTN	BB, CCB	
CHF / LVSD	Nitrate, BB*	CCB (nondihydropyridine)
Post-MI	BB (w\o ISA)	CCB (nondihydropyridine)
Hyperlipidemia		BB
SVT, V-arrhythmia	BB CCB (nondihydropyridine)	
COPD / asthma		BB
Raynaud's Disease		BB

* metoprolol SR, carvedilol or bisoprolol

Evaluation of Outcomes

- Symptoms of angina
  - Symptomatic improvement in exercise capacity
  - Fewer symptoms at same level of exertion
  - Questionnaires
  - Effects of optimized therapy
    - Symptom improvement over 2 to 4 weeks and remain stable unless disease progression occurs

- Cardiac performance
  - Assessment only if medical management is failing or in very high risk patients

- Risk factors
  - Assess and reassess

Monitoring Therapy

- **Monitoring Issues (B-blockers, CCBs, Nitrates)**

  (Assess for drug effectiveness and safety)

  – With all agents monitor
    - Blood pressure, clinical signs and symptoms of hypotension (mental status, syncope, etc.)
    - *ECG (*for *HR, intervals, rhythm etc.) -not nitrates*
    - *Edema (CCBs)*

  – **Side effects** which may be important to pursue include: potential **metabolic complications** with **B-blockers (lipids, glucose)**

Major modifications in ACC/AHA guidelines

- For hypertensive patients with well established coronary artery disease, it is useful to add blood pressure medication as tolerated, treating initially with beta blockers and/or ACE inhibitors, with addition of other drugs as needed to achieve target blood pressure.

- Adding **plant stanol/sterols (2 g per day) and/or viscous fiber** (greater than 10 g per day) is **reasonable to lower LDL-C.**

- Reduction of LDL-C to **less than 70 mg/dL** or high-dose statin therapy is **reasonable.**

- Aspirin should be started at 75 to 162 mg per day and continued indefinitely in all patients unless contraindicated.

- ACE inhibitors should be started and continued indefinitely in all patients with left ventricular ejection fraction less than or equal to 40% and in those with hypertension, diabetes, or chronic kidney disease unless contraindicated.

Major modifications in ACC/AHA guidelines

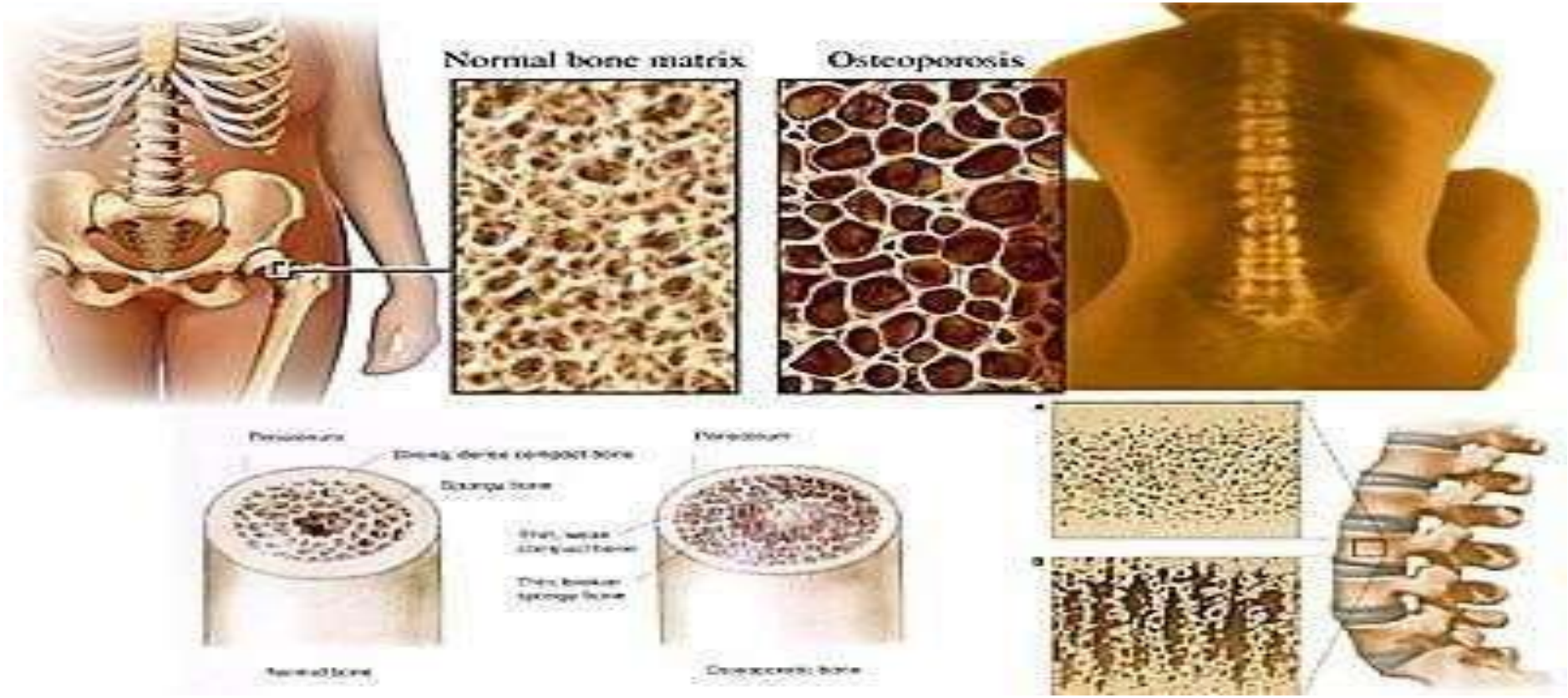
- Angiotensin receptor blockers are recommended for patients who have hypertension, have indications for but are intolerant of ACE inhibitors, have heart failure, or have had a myocardial infarction with left ventricular ejection fraction less than or equal to 40%.
- **Angiotensin receptor blockers** may be considered in **combination with ACE inhibitors for heart failure due** to left **ventricular systolic dysfunction.**

- Aldosterone blockade is recommended for use in post-MI patients without significant renal dysfunction or hyperkalemia who are already receiving therapeutic doses of an ACE inhibitor and a beta blocker, have a left ventricular ejection fraction less than or equal to 40%, and have either diabetes or heart failure.

- It is **beneficial to start and continue beta-blocker** therapy **indefinitely in all patients who have had** MI, **acute coronary syndrome**, **or left ventricular dysfunction with or without heart failure symptoms, unless contraindicated.**
- An annual influenza vaccination is recommended for patients with cardiovascular disease.

Distinctive features of ESC guidelines

- **ASA dose** should be **75 mg**
- Statin therapy for all CAD pts
- **High-dose statins** for high risk pts with CAD
- **ACE-I's for all CAD patients**
- **Beta-blockers post MI or in HF**



OSTEOPOROSIS

Dr.Rasheduddin

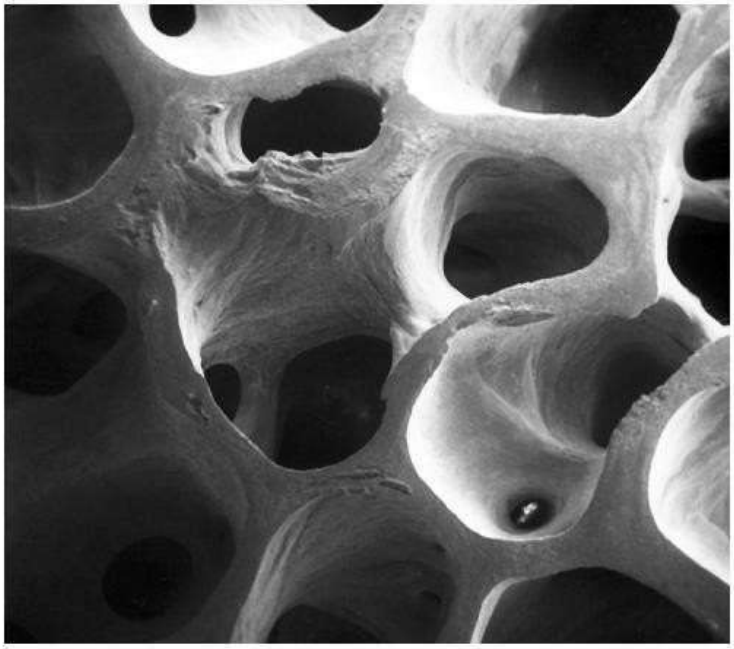
Definition

- **Osteoporosis** is a chronic, progressive disease characterized by:
  - low bone mass **(Osteopenia)**,
  - microarchitectural deterioration of bone tissue and decreased bone strength,
  - bone fragility
  - consequent increase in fracture risk;

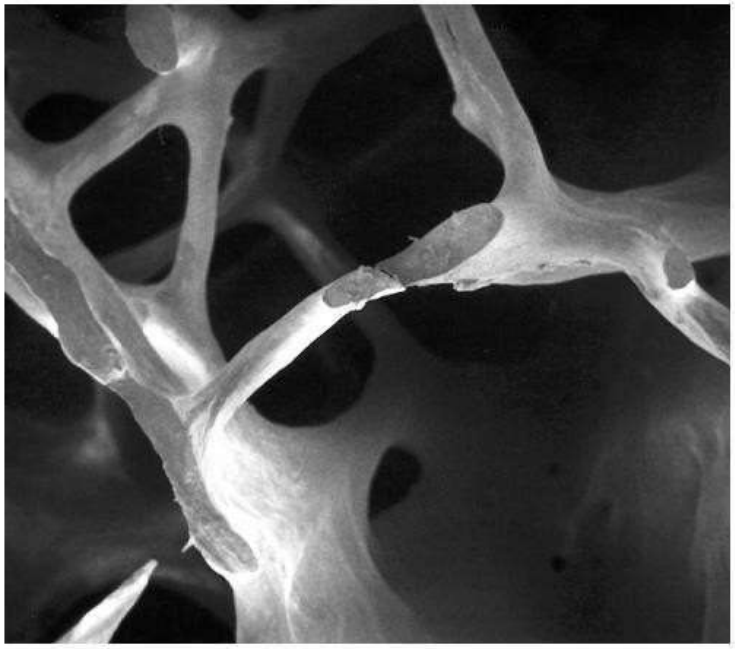
  According to the WHO diagnostic classification, osteoporosis is defined by bone mineral density (BMD) at the hip or spine that is less than or equal to 2.5 standard deviations below the young normal mean reference population (T-score at or below -2.5).

- Figure shows the changes within cancellous bone as a consequence of bone loss. Individual trabecular plates of bone are lost, leaving an architecturally weakened structure with significantly reduced mass.

Normal bone

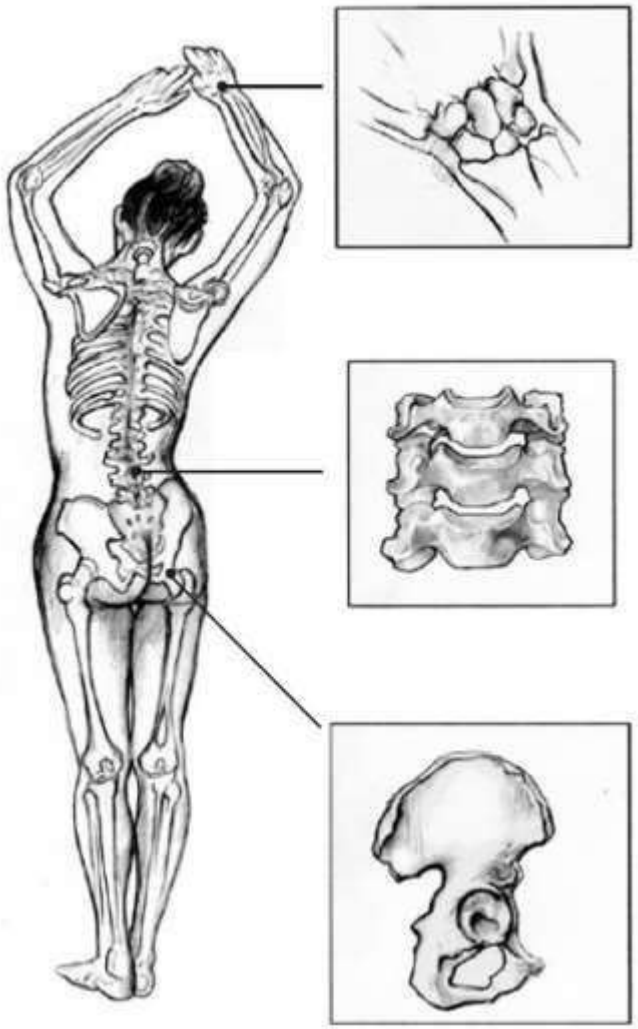


Osteoporotic bone



Medical impact

- Osteoporosis is a silent disease until it is complicated by fracture.
- Fractures can occur following minimal trauma.
- Osteoporosis can be prevented and can be diagnosed and treated before any fracture occurs.
- The most common fractures are those of the
  - Vertebrae (spine)
  - Proximal femur (hip)
  - Distal forearm (wrist).



Epidemiology

- Osteoporosis affects 200 million women worldwide. In fact, approximately 10% of 60 year old women have osteoporosis. This increases to two-thirds of women by the time women get to the age of 90 years.
- Fragility or low trauma wrist and vertebral fractures are common throughout adulthood, whereas hip fractures are more common in seniors.

Epidemiology

- Osteoporosis affects an enormous number of people, of both sexes and all races, and its prevalence will increase as the population ages.
- Based on data from the National Health and Nutrition Examination Survey III (NHANES III), NOF has estimated that more than 9.9 million Americans have osteoporosis and an additional 43.1 million have low bone
- Osteoporosis is less frequent inAfricanAmericans,those with osteoporosis have the same elevated fracture risk as Caucasians.
- About one out of every two Caucasian women will experience an osteoporosis-related fracture at some point in her lifetime, as will approximately one in five men.

Epidemiology

It is estimated that 50% of women over age 50 will develop a fracture in their remaining lifetime and the annualized risk increases with age. Twenty-five percent of women over age 50 will experience an osteoporotic vertebral fracture, so that by age 75 more than one in three women have sustained at least one vertebral fracture.

Relative Risk of Fracture at Various Sites in the Presence of a Radiographic Vertebral Compression Deformity

Site of Subsequent Fracture	Relative Risk (95% CI)
Vertebral	5.4 (4.4, 6.6)
Hip	2.8 (2.3, 3.4)
Any non-vertebral site	1.9 (1.7, 2.1)

Etiology

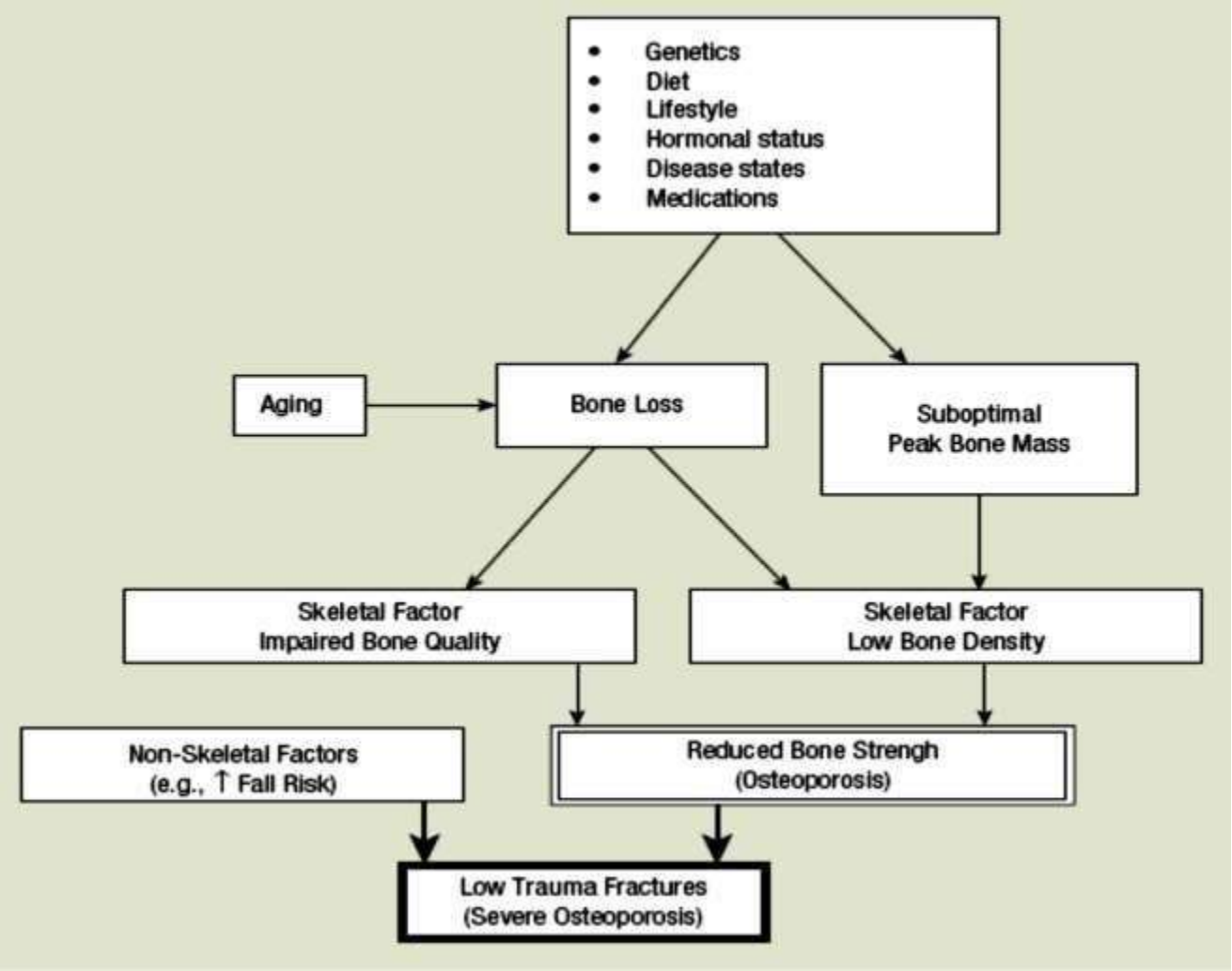
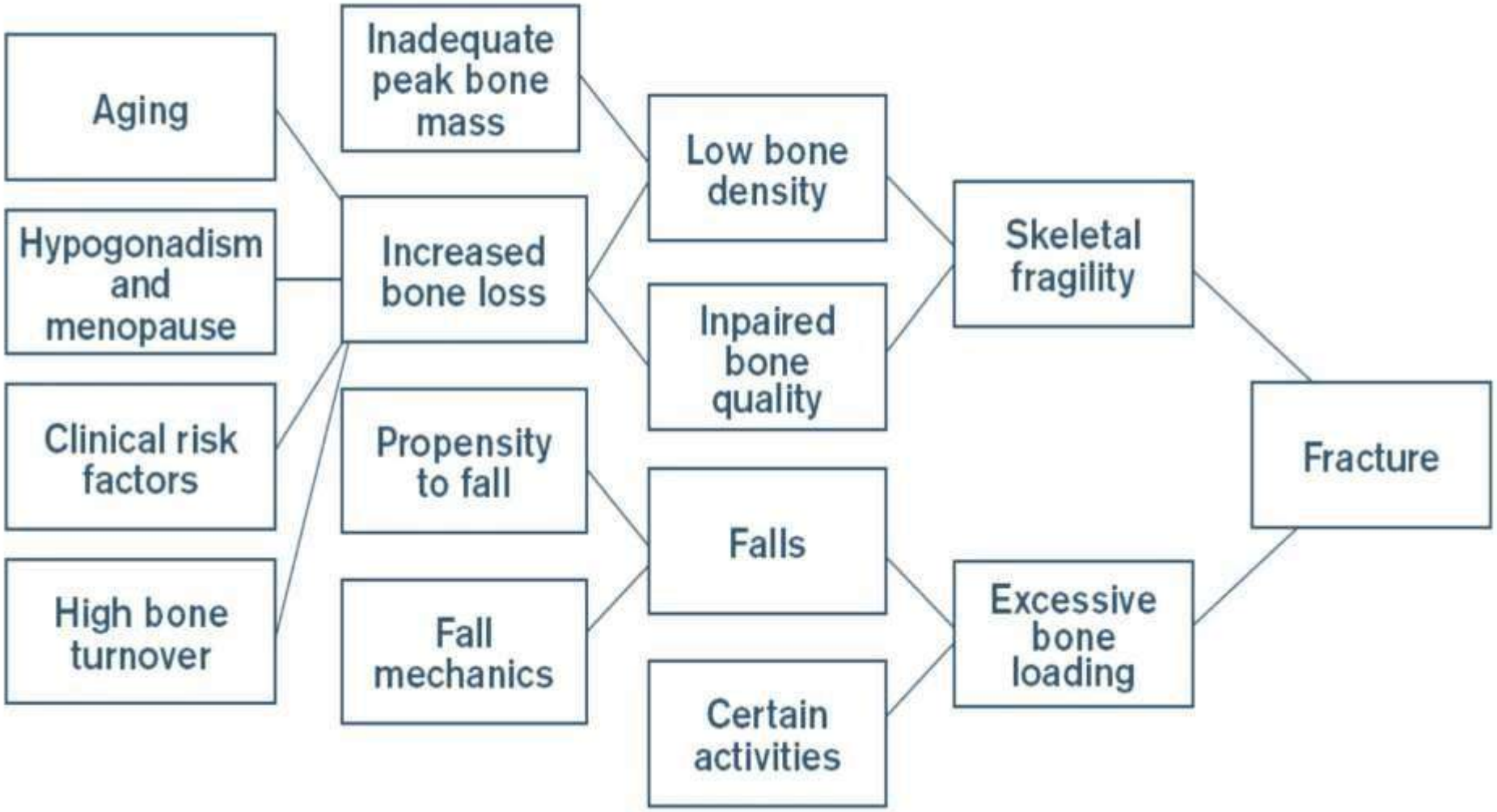

Genetics
Diet
Lifestyle
Hormonal status
Disease states
Medications
Aging
Bone Loss
Suboptimal
Peak Bone Mass
Skeletal Factor
Impaired Bone Quality
Skeletal Factor
Low Bone Density
Non-Skeletal Factors
(e.g., ↑ Fall Risk)
Reduced Bone Strengh
(Osteoporosis)
Low Trauma Fractures
(Severe Osteoporosis)

FIGURE 2. Pathogenesis of Osteoporosis-Related Fractures



Protective Estrogen role in bone remodeling

- Estrogen has many positive effects on the bone remodeling process.
- Most of its actions help to maintain a normal bone resorption rate:
  - suppresses proliferation & differentiation of osteoclasts & ↑ osteoclast apoptosis
  - ↓ production of several cytokines that are potent stimulators of osteoclasts including ILs 1 & 6,&TNF factor-α.
  - ↓ production of RANKL & ↑ production of OPG; both of which ↓ osteoclastogenesis
- Note:
  - mature osteoclasts (bone resorbing cells)
  - osteoblast (bone-forming cells)
  - Receptor Activator of Nuclear Factor Kappa B Ligand(RANKL)
  - osteoprotegerin (OPG)

VITAMIN D, PTH, & Ca++

- Vitamin D and PTH maintain calcium homeostasis
- The most abundant source of vitamin D is the endogenous production from exposure to ultraviolet B light
- Dietary vitamin D sources include **cholecalciferol** & **ergocalciferol** (vitamin D2)
- conversion of cholecalciferol & ergocalciferol to 25-hydroxyvitamin D [25(OH) D] (**calcidiol**) occurs in the liver & then PTH stimulates conversion of 25(OH) D via 25(OH) D-1α-hydroxylase to its final active form, 1α,25-dihydroxyvitamin D (**calcitriol**), in the kidney
- Calcitriol binds to the intestinal vitamin D receptor → ↑ calcium binding protein → ↑ calcium & phosphorous intestinal absorption

VITAMIN D, PTH, & Ca++ (cont'd)

- Calcium absorption under normal conditions is ~ 30-40%
- ↓ to 10% to 15% with low vitamin D concentrations
- ↑ PTH concentrations secondary to hypocalcemia ↑ kidney calcitriol production & calcium reabsorption by the kidney
- PTH concentrations also ↑ when vitamin D concentration falls < ~30 ng/mL, the minimum normal therapeutic vitamin D concentration
- Sometimes increased fractional calcium absorption is insufficient → bone resorption is needed
- Together, PTH & calcitriol increase osteoclast activity → releasing calcium from bone to restore calcium homeostasis

Osteoporosis Categories

1. Postmenopausal Osteoporosis.
2. Male Osteoporosis
3. Age Related Osteoporosis.
4. Secondary Osteoporosis (disease or medication)
   - Hyperthyroidism
   - Renal disease.
   - Chronic obstructive pulmonary disease.

PATHOPHYSIOLOGY

POSTMENOPAUSAL OSTEOPOROSIS

- **Accelerated bone loss** during perimenopause & postmenopause due to ↑ resorption mainly as a result of the **loss in ovarian estrogen production**
- →↑proliferation, differentiation, & activation of new osteoclasts & prolongation of survival of mature osteoclasts
- Number of remodeling sites ↑ & resorption pits are deeper & inadequately filled by normal osteoblastic function
- Significant bone density is lost & bone architecture is compromised
- Trabecular bone is most susceptible leading to vertebral & wrist fractures

MALE OSTEOPOROSIS

- Men are at a lower risk for developing osteoporosis &osteoporotic fractures because of larger bone size, greater peak bone mass, & fewer falls.
- However, men have a higher mortality rate after fractures
- The etiology of male osteoporosis tends to be multifactorial with secondary causes & aging being the most common contributing factors
- In young & middle-age men, hypogonadism is the most common

AGE-RELATED OSTEOPOROSIS

- Occurs in seniors mainly as a result of hormone,Ca++,& vitamin D deficiencies →↑ bone turnover rate in combination with ↓osteoblast bone formation

- Hip fracture risk ↑dramatically as a consequence of the cumulative loss of cortical & trabecular bone & ↑risk for falls

Consequences of osteoporosis

- The most common osteoporosis-related fractures are those of the **vertebrae, proximal femur, & distal radius** (wrist or Colles fracture)
- Depression is common
- Symptomatic vertebral fractures can cause significant pain & physical deformity
- Patients with severe kyphosis can experience respiratory problems as a result of compression of the thoracic region & GI complications
- After a hip fracture, only 33-40% of patients regain their ability to perform basic activities of daily living,
- 14% to 36% will die within 1 year after a hip fracture

Risk Assessment

- All postmenopausal women and men age 50 and older should be evaluated clinically for osteoporosis risk in order to determine the need for BMD testing and/or vertebral imaging

Conditions, Diseases & Medications That Cause or Contribute to Osteoporosis & Fractures (NOF, 2014)

Lifestyle factors		
Low calcium intake	Vitamin D insufficiency	Excess vitamin A
High caffeine intake	High salt intake	Aluminum (in antacids)
Alcohol (3 or more drinks/d)	Inadequate physical activity	Immobilization
Smoking (active or passive)	Falling	Thinness

Genetic factors		
Cystic fibrosis	Homocystinuria	Osteogenesis imperfecta
Ehlers-Danlos	Hypophosphatasia	Parental history of hip fracture
Gaucher's disease	Idiopathic hypercalciuria	Porphyria
Glycogen storage diseases	Marfan syndrome	Riley-Day syndrome
Hemochromatosis	Menkes steely hair syndrome	
Hypogonadal states		
Androgen insensitivity	Hyperprolactinemia	Premature ovarian failure
Anorexia nervosa and bulimia	Premature menopause	Athletic amenorrhea

Endocrine disorders		
Adrenal insufficiency	Cushing's syndrome	Central Adiposity
Diabetes mellitus (Types 1 & 2)	Hyperparathyroidism	Thyrotoxicosis
Gastrointestinal disorders		
Celiac disease	Inflammatory bowel disease	Primary biliary cirrhosis
Gastric bypass	Malabsorption	
GI surgery	Pancreatic disease	
Hematologic disorders		
Multiple myeloma	Monoclonal gammopathies	Sickle cell disease
Hemophilia	Leukemia and lymphomas	Systemic mastocytosis
Thalassemia		
Rheumatologic and autoimmune diseases		
Ankylosing spondylitis	Lupus	Rheumatoid arthritis
Other rheumatic and autoimmune diseases		
Central nervous system disorders		
Epilepsy	Parkinson's disease	Stroke
Multiple sclerosis	Spinal cord injury	
Miscellaneous conditions and diseases		
AIDS/HIV	Congestive heart failure	Muscular dystrophy
Alcoholism	Depression	Post-transplant bone disease
Amyloidosis	End stage renal disease	Sarcoidosis
Chronic metabolic acidosis	Hypercalciuria	Weight loss
Chronic obstructive lung disease	Idiopathic scoliosis	

Medications		
Aluminum (in antacids)	Cyclosporine A and tacrolimus	Proton pump inhibitors
Anticoagulants (heparin)	Depo-medroxyprogesterone (premenopausal contraception)	Selective serotonin reuptake inhibitors
Anticonvulsants	Glucocorticoids (≥ 5 mg/d prednisone or equivalent for ≥ 3 months)	Tamoxifen® (premenopausal use)
Aromatase inhibitors	GnRH (Gonadotropin releasing hormone) antagonists and agonists	Thiazolidinediones (such as Actos® and Avandia®)
Barbiturates	Lithium	Thyroid hormones (in excess)
Cancer chemotherapeutic drugs	Methotrexate	Parenteral nutrition

Risk Factors for Falls

Since the majority of osteoporosis-related fractures result from falls, it is also important to evaluate risk factors for falling

Environmental risk factors
Lack of assistive devices in bathrooms
Loose throw rugs
Low level lighting
Obstacles in the walking path
Slippery outdoor conditions

Risk Factors for Falls (cont'd)

Medical risk factors	
Age	Medications causing oversedation (narcotic analgesics, anticonvulsants, psychotropics)
Anxiety and agitation	Orthostatic hypotension
Arrhythmias	Poor vision and use of bifocals
Dehydration	Previous fall
Depression	Reduced problem solving or mental acuity and diminished cognitive skills
Female gender	Urgent urinary incontinence
Impaired transfer and mobility	Vitamin D insufficiency [serum 25-hydroxyvitamin D (25(OH)D) < 30 ng/ml (75 nmol/L)]
Malnutrition	

Neurological and musculoskeletal risk factors	
Kyphosis	Reduced proprioception
Poor balance	Weak muscles/sarcopenia
Impaired transfer and mobility	Deconditioning
Diseases listed in Table 1	

Risk factors included in the WHO 10-year fracture risk assessment model

TABLE 3: Risk Factors Included in the WHO Fracture Risk Assessment Model

Clinical Risk Factors Included in the FRAX Tool	
• Current age	• Rheumatoid arthritis
• Gender	• Secondary osteoporosis: Type1 (insulin dependent) diabetes, osteogenesis imperfecta in adults, untreated long-standing hyperthyroidism, hypogonadism or premature menopause (<45 years), chronic malnutrition or malabsorption and chronic liver disease
• A prior osteoporotic fracture (including clinical and asymptomatic vertebral fractures)	• Parental history of hip fracture
• Femoral neck BMD	• Current smoking
• Low body mass index (BMI, kg/m^2)	• Alcohol intake (3 or more drinks/d)
• Oral glucocorticoids ≥5 mg/d of prednisone for≥3 months (ever)	

this set of risk factors increases risk independently of BMD and can be combined with BMD measurements and used to assess an individual patient's risk of future fracture.

Diagnosis

- The diagnosis of osteoporosis is established by:
- measurement of BMD or
- occurrence of adulthood hip or vertebral fracture in the absence of major trauma (such as a motor vehicle accident or multiple story fall).
  - BMD
  - Vertebral imaging
  - Biochemical markers of bone turnover

Central Dual-Energy X-Ray Absorptiometry (hip, spine)

- can be used to **assess fracture risk**, **establish the diagnosis & severity of osteoporosis**
- sometimes confirm osteoporosis as causative for low-trauma fractures
- BMD is expressed in absolute terms of grams of mineral per square centimeter scanned (g/cm^2)
- is considered the **gold standard** for measuring BMD because of its high precision, short scan times, low radiation dose (comparable to the average daily dose from natural background), & stable calibration
- **measurement of both lumbar spine & proximal femur or total hip BMD are recommended with the lowest BMD value used for diagnosis**

DXA



DXA

Name: Express Scans, 2
Patient ID:
DOB: August 24, 1944

Sex: Female
Ethnicity: White

Height: 65.0 in
Weight: 150.0 lb
Age: 61

Referring Physician:

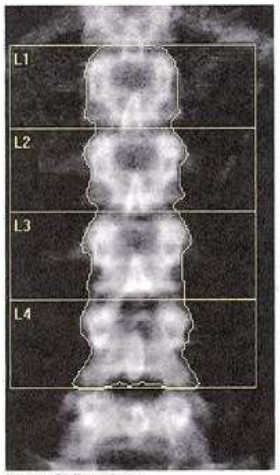


Image not for diagnostic use
k = 1.138, d0 = 48.0
116 x 149

Scan Information:

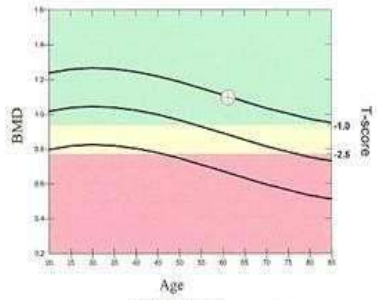
Scan Date: November 12, 2005 ID: A11120501
Scan Type: x Lumbar Spine
Analysis: November 12, 2005 09:48 Version 12.4:3
Lumbar Spine
Operator:
Model: Discovery C (S/N 81202)
Comment:

DXA Results Summary:

Region	Area (cm^2)	BMC (g)	BMD (g/cm^2)	T-score	PR (%)	Z-score	AM (%)
L1	14.41	14.44	1.002	0.7	108	2.0	129
L2	15.27	16.33	1.069	0.4	104	1.8	123
L3	16.99	19.69	1.159	0.7	107	2.2	127
L4	18.74	21.27	1.135	0.2	102	1.8	121
Total	**65.41**	**71.72**	**1.096**	**0.4**	**105**	**1.9**	**124**

Total BMD CV 1.0%, ACF = 1.000, BCF = 1.000, TH = 3.855
WHO Classification: Normal
Fracture Risk: Not Increased

Total



Fracture Risk
Not Increased Increased High
T-score vs. White Female; Z-score vs. White Female Source:Hologic

Physician's Comment:

HOLOGIC®



k = 1.141, d0 = 46.9
103 x 117
NECK: 49 x 15
DAP: 1.5 cGy*cm²

Scan Information:

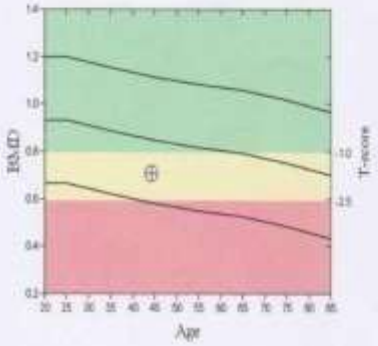
Scan Date: 22 January 2015 ID: A01221507
Scan Type: x Left Hip
Analysis: 22 January 2015 12:17 Version 13.4.2:3
Hip
Comment:

DXA Results Summary:

Region	Area (cm^2)	BMC (g)	BMD (g/cm^2)	T-score	PR (%)	Z-score	AM (%)
Neck	5.64	3.99	0.707	-1.6	76	-1.0	83
Troch	11.98	8.04	0.671	-0.8	86	-0.6	90
Inter	24.80	27.90	1.125	-0.4	94	-0.3	96
Total	42.42	39.92	0.941	-0.6	91	-0.4	94
Ward's	1.16	0.60	0.521	-1.9	66	-0.9	81

Total BMD CV 1.0%, ACF = 1.048, BCF = 1.021, TH = 6.024
WHO Classification: Osteopenia

Neck



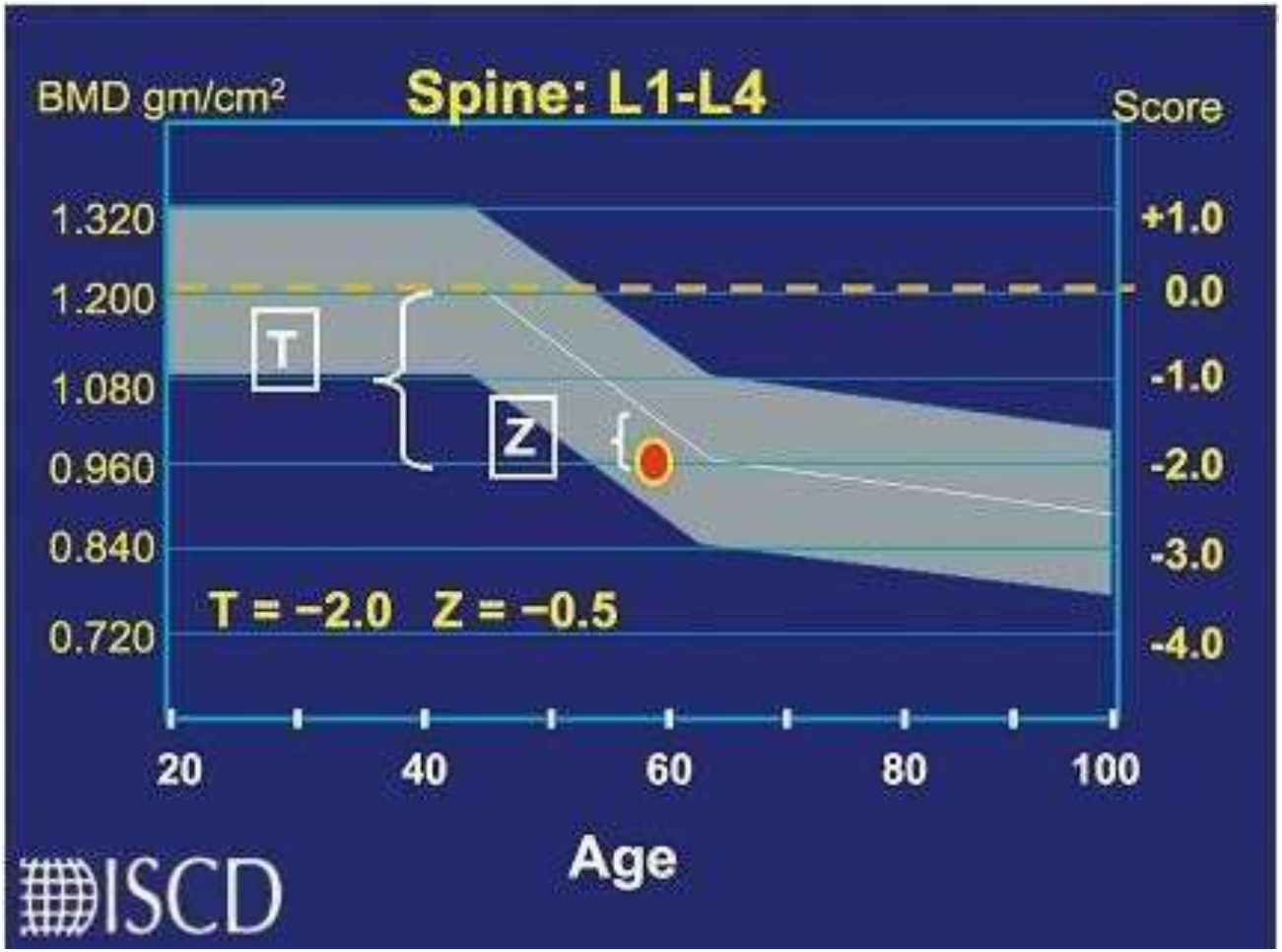
Comment:

Central DXA (cont'd)

- The **T-score** is a comparison of a person's bone density with that of a healthy 30-year-old of the same sex.
- The T-score is the number of standard deviations from the mean of the reference population
- The **Z-score** compares the patient's BMD to the mean BMD for sex, age and ethnicity-matched reference population & is usually low when secondary causes of osteoporosis are present
- The difference between the patient's BMD and the mean BMD of the reference population, divided by the standard deviation (SD) of the reference population, is used to calculate T-scores and Z-scores.

Z- and T-Score

Areal BMD is expressed in absolute terms of grams of mineral per square centimeter scanned (g/cm^2)



Defining Osteoporosis by BMD

WHO Definition of Osteoporosis Based on BMD		
Classification	**BMD**	**T-score**
Normal	Within 1 SD of a young-adult reference population	T-score at -1.0 and above
Low Bone Mass (Osteopenia)	Between 1.0 and 2.5 SD below that of a young-adult reference population	T-score between -1.0 and -2.5
Osteoporosis	2.5 SD or more below that of a young- adult reference population	T-score at or below -2.5
Severe or Established Osteoporosis	2.5 SD or more below that of a young- adult reference population	T-score at or below -2.5 with one or more fractures

Note: Although these definitions are necessary to establish the presence of osteoporosis, they should not be used as the sole determinant of treatment decisions.

In premenopausal women, men less than 50 years of age and children, the WHO BMD diagnostic classification should not be applied.

Indications for BMD Testing – NOF 2014

Table 7: Indications for BMD Testing

Consider BMD testing in the following individuals:
• Women age 65 and older and men age 70 and older, regardless of clinical risk factors
• Younger postmenopausal women, women in the menopausal transition and men age 50 to 69 with clinical risk factors for fracture
• Adults who have a fracture after age 50
• Adults with a condition (e.g., rheumatoid arthritis) or taking a medication (e.g., glucocorticoids in a daily dose ≥ 5 mg prednisone or equivalent for ≥ three months) associated with low bone mass or bone loss

Vertebral imaging

- Independent of BMD, age and other clinical risk factors, radiographically confirmed vertebral fractures (even if completely asymptomatic) are a sign of impaired bone quality and strength, and a strong predictor of new vertebral and other fractures.
- The presence **of a single vertebral fracture** increases the risk of subsequent fractures 5-fold and the risk of hip and other fractures 2- to 3- fold.
- Vertebral imaging can be performed using a lateral thoracic and lumbar spine x-ray or lateral vertebral fracture assessment (VFA), available on most modern DXA machines.
- VFA can be conveniently performed at the time of BMD assessment, while conventional x-ray may require referral to a standard x-ray facility.

Vertebral imaging (NOF 2014)

- A vertebral fracture is consistent with a diagnosis of osteoporosis, ***even*** in the absence of a bone density diagnosis, and is an indication for pharmacologic treatment with osteoporosis medication to reduce subsequent fracture risk.
- Most vertebral fractures are asymptomatic when they first occur and often are undiagnosed for many years.
- Proactive vertebral imaging is the only way to diagnose these fractures.The finding of a previously unrecognized vertebral fracture may change the diagnostic classification, alter future fracture risk calculations and affect treatment decisions.

Indications for vertebral imaging

Table 7: Indications for Vertebral Imaging

Consider vertebral imaging tests for the following individuals:***
• All women age 70 and older and all men age 80 and older if BMD T-score at the spine, total hip or femoral neck is ≤ -1.0.
• Women age 65 to 69 and men age 70 to 79 if BMD T-score at the spine, total hip or femoral neck is ≤ -1.5
• Postmenopausal women and men age 50 and older with specific risk factors: ▪ Low trauma fracture during adulthood (age 50) ▪ Historical height loss of 1.5 inches or more (4 cm)* ▪ Prospective height loss of 0.8 inches or more (2 cm)** ▪ Recent or ongoing long term glucocorticoid treatment

* Current height compared to peak height during young adulthood

** Cumulative height loss measured during interval medical assessment

*** If bone density testing is not available, vertebral imaging may be considered based on age alone

Bone biomarkers

- resorption markers: serum C-telopeptide (CTX) and urinary N-telopeptide (NTX)
- formation markers:serum bone specific alkaline phosphatase (BSAP), osteocalcin (OC) and aminoterminal propeptide of type I procollagen (PINP)] are best collected in the morning while patients are fasting.
- **Biochemical markers of bone turnover may:**
- • Predict risk of fracture independently of bone density in untreated patients.
- • Predict rapidity of bone loss in untreated patients.
- • Predict extent of fracture risk reduction when repeated after 3-6 months of treatment with FDA-approved therapies.
- • Predict magnitude of BMD increases with FDA-approved therapies.
- • Help determine adequacy of patient compliance and persistence with osteoporosis therapy.
- • Help determine duration of 'drug holiday' and when and if medication should be restarted (Data are quite limited to support this use, but studies are underway).

Clinical Presentation of Osteoporosis

General

- Many patients only present after fracture
- Fractures can occur after bending,lifting,or falling,or independent of any activity

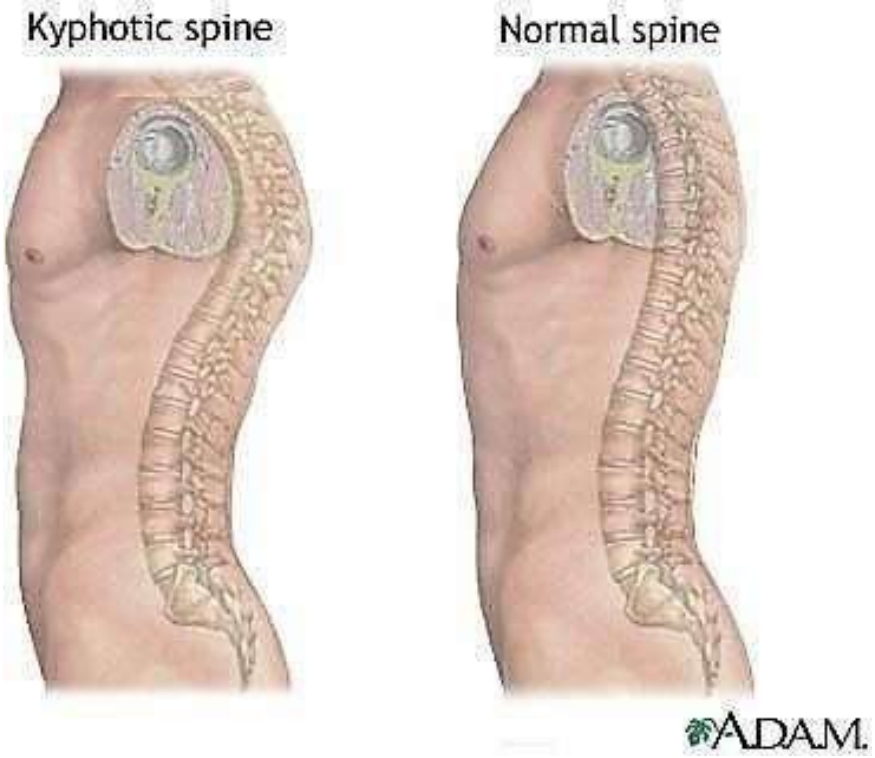
Symptoms

- Pain
- Immobility
- Depression, fear,& low self-esteem from physical limitations & deformities
- 2/3 of vertebral fractures are asymptomatic

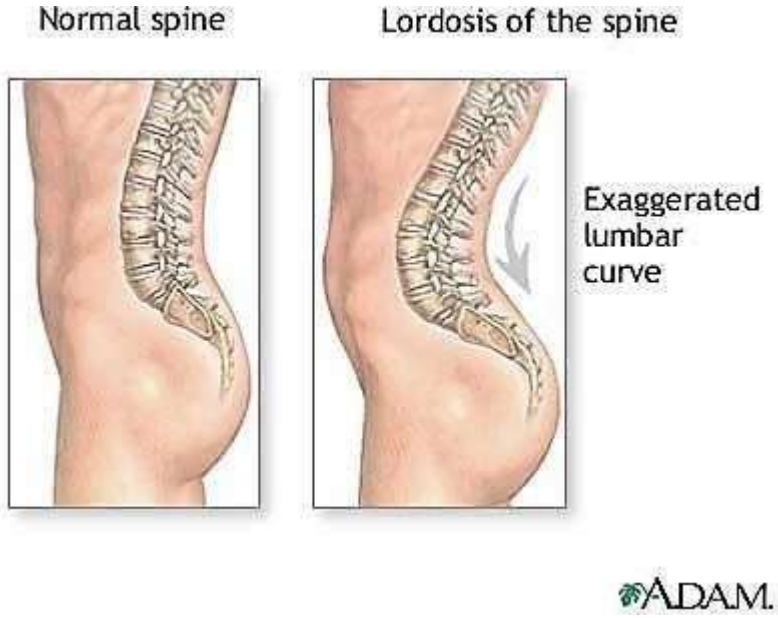
Signs

- Shortened stature (>1.5" loss),kyphosis,or lordosis
- Vertebral,hip,wrist,or forearm fracture
- Low bone density on radiography

Kyphosis & lordosis of the spine



Kyphotic curves refer to the outward curve of the thoracic spine



Lordotic curves refer to the inward curve of the lumbar spine

Clinical Presentation of Osteoporosis (cont'd)

Laboratory tests

- Routine tests to detect a possible secondary cause: CBC, LFT, KFT, Ca++, Ph, AlkPh, albumin, TSH, free testosterone, 25(OH)vitamin D, & 24-hour urine concentrations of Ca++ & Ph
- Urine or serum biomarkers (e.g., (NTX), osteocalcin) are sometimes used, especially to determine if high bone turnover exists

Other diagnostic tests

- Spine & hip bone-density measurement using DXA
- Radiograph to confirm vertebral fracture

DXA, dual-energy x-ray absorptiometry;

NTX, N-terminal crosslinking telopeptide of type 1 collagen

PATIENT ASSESSMENT

- **Bone pain,postural changes** (i.e.,kyphosis),& **loss of height** are simple useful physical examination findings
- Height loss >1.5 inches from the tallest mature height is considered significant & warrants further investigation
- A **spine radiograph** can confirm **vertebral fractures**
- **Low bone density or osteopenia** reported on routine radiographs requires an evaluation for osteoporosis
- Patients can be assessed with **risk factor assessment**, **osteoporosis questionnaires, peripheral & central DXA, & biomarkers**

Risk factor assessment

Major risk factors by the NOF:

- Current smoker
- low body weight (<127 lb (~50Kg) in postmenopausal women)
- history of osteoporotic fracture in a first degree relative
- personal history of low-trauma fracture as an adult

Other identified independent risk factors:

- age
- high bone turnover
- low body mass index (<19 kg/m^2)
- rheumatoid arthritis
- glucocorticoid use

DIAGNOSIS OF OSTEOPOROSIS

- The diagnosis of osteoporosis is based on a low-trauma fracture or central hip and/or spine DXA usingWHOT-score thresholds.
  - Osteopenia or low bone mass is a T-score of -1 to -2.4
  - Osteoporosis is a T-score at or below –2.5.

- Although these definitions are based on data from postmenopausal white women, they are applied to other racial/ethnic groups and senior men.

- The International Society for Clinical Densitometry recommends the presence of risk factors in addition to a low T-score before the diagnosis of osteoporosis can be made in <u>men ages 50 to 65 years.</u>

PREVENTION & TREATMENT

DESIRED OUTCOMES

- **The primary goal should be prevention** - optimizing skeletal development & peak bone mass accrual in childhood, adolescence, & early adulthood

- **once osteopenia or osteoporosis develops**, the objective is to stabilize or improve bone mass & strength & prevent fractures

- **in pts. who have already suffered osteoporotic fractures,** ↓ future falls & fractures, improvingfunctional capacity, ↓ pain & deformity,& improving quality of life

Guidelines for treatment of osteoporosis NOF-2014

Obtain a detailed patient history pertaining to clinical risk factors for osteoporosis-related fracture.
Perform physical examination to evaluate for signs of osteoporosis and its secondary causes.
Modify diet/supplements and other clinical risk factors for fracture.
Estimate patient's 10-year probability of hip and any major osteoporosis-related fracture using the US-adapted WHO algorithm.
Decisions on whom to treat and how to treat should be based on clinical judgment using this Guide and all available clinical information.

Consider FDA-approved medical therapies based on the following:

- A vertebral or hip fracture
- A DXA hip (femoral neck) or spine T-score ≤ -2.5
- Low bone mass and a US-adapted WHO 10-year probability of a hip fracture ≥ 3% or 10-year probability of any major osteoporosis-related fracture ≥ 20%
- Patient preferences may indicate treatment for people with 10-year fracture probabilities above or below these levels

Consider non-medical therapeutic interventions:

- Modify risk factors related to falling
- Consider physical and occupational therapy including walking aids and hip pad protectors
- Weight-bearing activities daily

Patients not requiring medical therapies at the time of initial evaluation should be clinically re-evaluated when medically appropriate.

Patients taking FDA-approved medications should have laboratory and bone density re-evaluation after two years or more frequently when medically appropriate.

Nonpharmacologic Therapy (bone-healthy lifestyle changes)

- includes diet,smoking cessation,exercise,fall prevention,& hip protectors

Diet

- balanced in nutrients & minerals
- ↓ caffeine (2-4 servings/d),
- ↓ alcohol (1 drink/d for women & 2 drinks/d for men)
- ↓ sodium (<2.4 g/day) - why?
- ↓ cola & other carbonated beverages (caffeine & phosphoric acid content of cola beverages might cause bone loss by altering calcium balance)

Calcium

- Adequate intake is an essential component of all osteoporosis ***prevention*** & ***treatment*** strategies
- Achieving daily Ca++ requirements from Ca++-containing foods
- Some food sources are absorbed well but have low elemental Ca++ content (e.g., broccoli), or contain oxalic acid (e.g., spinach) or phytic acid (e.g., wheat bran) that bind Ca++ within the food & ↓ its absorption
- **lactose intolerance**:incidence inAsian (80%) & AfricanAmerican (50%) populations being much higher than in whites (10%) → lactose-reduced milk, lactose-free milk, yogurt with active cultures or Lactaid, along with other nondairy calcium-fortified products (e.g., orange juice, breakfast cereals) can berecommended

Calcium

- NOF supports Institute of Medicine (IOM) recommendations:
- men age 50-70 consume **1,000 mg per day of calcium**
- women age 51 and older and men age 71 and older **consume 1,200 mg per day of calcium**.
- There is no evidence that calcium intake in excess of these amounts confers additional bone strength. Intakes in excess of 1,200 to 1,500 mg per day may increase the risk of developing kidney stones, cardiovascular disease and stroke.The scientific literature is highly controversial in this area.

TABLE 93-4 Calcium and Vitamin D Recommendations

	Institute of Medicine Adequate Intake	
Group and Ages	***Elemental Calcium (mg)***[a]	***Vitamin D (units)***[a,b]
Infants		
Birth to 6 months	210	200
6–12 months	270	200
Children		
1–3 years	500	200
4–8 years	800	200
9–13 years	1,300	200
Adolescents/young adults		
14–18 years	1,300	200
Adults		
19–30 years	1,000	200
31–50 years	1,000	200
51–70 years	1,200	400
>70 years	1,200	600

[a]U.S. Institute of Medicine of the National Academy of Sciences recommends no more than 2500 mg/day elemental calcium and 2000 units/day vitamin D.

[b]Most experts believe the recommended Adequate Intakes for Vitamin D are too low.

TABLE 93-5 Elemental Calcium Content of Selected Foods and Beverages

Foods/Beverages	Elemental Calcium Content (mg)[a]
Milk (skim, low-fat, whole), 1 cup	276–309
Calcium-fortified soy milk, 1 cup	80–300
Calcium-fortified orange juice, 1 cup	300
Calcium-fortified cranberry juice, 1 cup	100
7UP Plus, 1 cup	100
Low-fat fruit yogurt, 1 cup	345
Frozen yogurt, 1 cup	180–240
Vanilla ice cream, 1 cup	176–200
Soft-serve vanilla ice cream, 1 cup	236
Swiss cheese, 1.5 oz.	336
Cheddar, mozzarella, or provolone cheese, 1.5 oz.	307–311
Ricotta cheese, ½ cup	255–335
Cottage cheese, 4 oz.	78–100
Fortified breakfast cereals	236–1,043
Fortified instant oatmeal	99–110
Figs, dried, 10	270
Collard greens, cooked, ½ cup	178
Broccoli, cooked, 1 cup	100–180
Soybeans, cooked, ½ cup	88–130
Okra, cooked, ½ cup	88
Bok choy, raw, 1 cup	160–250
Tofu, firm, ½ cup	253
Almonds, 1 oz.	75
Salmon, canned with bones, 3 oz.	170–210

[a]Food labels are based on a RDA of 1,000 mg/day; multiply percentage on package by 10 (e.g., product containing 30% calcium = 300 mg).

Data from www.health.gov/dietaryguidelines/dga2005/document/html/appendixB.htm.

Calcium

Estimating daily dietary calcium intake

STEP 1: Estimate calcium intake from calcium-rich foods*

Product	Servings/d		Estimated calcium/ serving, in mg		Calcium, in mg
Milk (8 oz.)	_____	x	300	=	_____
Yogurt (6 oz.)	_____	x	300	=	_____
Cheese (1 oz. or 1 cubic in.)	_____	x	200	=	_____
Fortified foods or juices	_____	x	80 to 1,000**	=	_____

STEP 2: Total from above + 250 mg for nondairy sources = total dietary calcium

Calcium, in mg

_____

* About 75 to 80 percent of the calcium consumed in American diets is from dairy products.
** Calcium content of fortified foods varies.

Vitamin D

- NOF guidelines recommend **800-1,000** International Units (IU) vitamin D daily for *adults age 50 years & > in seniors 2,000 may be needed*
- Institute of Medicine Dietary Reference Intakes for vitamin D are **600 IU per day until age 70 and 800 IU per day for adults age 71 years and older.**
- 3 main sources of vitamin D are:**sunlight, diet,& supplements**
- Because few foods are naturally high or fortified with vitamin D, most people, especially seniors, require supplementation

TABLE 93-6 Vitamin D Content of Selected Foods and Beverages

Foods/Beverages	Vitamin D (international units)[a]
Salmon, 3.5 oz	360
Mackerel, 3.5 oz	345
Tuna fish, canned in oil, 3 oz	200
Sardines, canned in oil, 1.75 oz	250
Cow's milk (all forms), 1 cup	100
Vitamin D fortified orange juice	100
Ready-to-eat-cereal (fortified), 1 cup	40
Margarine, 1 tablespoon	60
Egg, 1 whole (or egg yolk)	20
Liver, beef, cooked, 3.5 oz	15

[a]Food labels are based on a RDA of 400 units/day; multiply percentage on package by 4 (e.g., product f
containing 20% vitamin D = 80 units).

Data from http://dietary-supplements.info.nih.gov/factsheets/vitamind.asp.

Treatment **of** Vitamin D**Deficiency**

- Adults who are vitamin D deficient may be treated with 50,000 IU of vitamin D2 or vitamin D3 once a week
- or the equivalent daily dose (7,000 IU vitamin D2 or vitamin D3) for 8-12 wks to achieve a 25(OH)D blood level of approximately 30 ng/ml.

- This regimen should be followed by maintenance therapy of 1,500–2,000 IU/d or whatever dose is needed to maintain the target blood level.

Protein

- low protein intakes ↑ osteoporosis risk & higher protein intakes are protective against bone loss & fractures

Dietary Soy

- Isoflavone phytoestrogens are plant-derived compounds that possess weak estrogenic agonist & antagonist effects
- The most common source for isoflavone is dietary soy products.
- The evidence supporting a positive bone benefit from soy protein intake is conflicting, with some positive data with larger isoflavone intakes

Smoking Cessation

- Cigarette smoking is associated with up to an 80% ↑ relative risk for hip fracture
- Negative bone effects are due to:
  - ↓ in sex hormone concentrations,
  - ↓ intestinal Ca++ absorption
  - direct toxic effect on osteoblasts
- detrimental effects on neurovascular function

Exercise (weight bearing and muscle strengthening)

- can ↓ risk of falls & fractures by improving muscle strength coordination, balance,& mobility
- lack of exercise during growth can lead to:
  - suboptimal loading/straining,
  - ↓ stimulation of bone deposition,
  - ↓ peak bone mass
- All patients who are medically fit should be encouraged to perform a moderate-intensity **weight bearing activity** (e.g., walking,jogging,stair climbing) for at least 30 min.most days of the week & a **resistance activity** (e.g., weight machines, free weights, or elastic bands) at least twice per week for 20-30 min.

Pharmacological trearment

- Current FDA-approved pharmacological options for osteoporosis prevention &/or treatment are:
- Antiresorptive therapy:
  - Calcium and vitamin D supplements
  - bisphosphonates (alendronate, ibandronate,risedronate & zoledronic acid),
  - calcitonin,
  - estrogens &/or hormone therapy,
  - estrogen agonist/antagonist (raloxifene).
  - Denosumab.
- Anabolic Drugs:Teriparatide

Who Should Be Considered for Treatment?

- Postmenopausal women and men age 50 and older presenting with the following should be considered for treatment:
- A hip or vertebral fracture (clinically apparent or found on vertebral imaging).
- In patients with a hip or spine fracture, the T-score is not as important as the fracture itself in predicting future risk of fracture and anti-fracture efficacy from treatment.
- •T-score ≤ -2.5 at the femoral neck, total hip or lumbar spine.
- Low bone mass (T-score between -1.0 and -2.5 at the femoral neck or lumbar spine) and a 10-year probability of a hip fracture ≥3 % or a 10-year probability of a major osteoporosis-related fracture ≥20 %

PHARMACOLOGIC THERAPY

Drug Treatments of First Choice

- Bisphosphonates are the prescription DOC
- Teriparatide, raloxifene,& calcitonin are alternative agents

- Duration of bisphosphonate therapy has not been defined,but safety data exist for periods of 7–10 years

- Short-term (18-24 months) teriparatide is used for severe osteoporosis & then followed by bisphosphonate therapy.

Calcium Supplementation

- Adequate calcium intake is considered the minimal standard for osteoporosis prevention and treatment and should be combined with vitamin D and osteoporosis medications when needed.
- Supplemental calcium intake will be needed in the majority of people with or at risk for osteoporosis

- ***Efficacy:*** ↑ BMD, but fracture prevention is minimal
  - Calcium's BMD effects are less than other antiresorptive & osteoporosis medications

- ***Non-bone benefits***: ↓ blood pressure, cholesterol, & colorectal cancer risk, the last being controversial

Ca supplementation (cont'd)

- ***Adverse Events/Precautions.***
  - most common: constipation →↑ water intake, dietaryfiber, & exercise

    if still unresolved, smaller & > frequent administration or lower total daily dose can be tried
  - calcium carbonate can create gas & cause stomach upset → calcium citrate has fewer GI side effects
  - Ca++ rarely causes kidney stones →↑ fluid intake & ↓ salt intake

Ca supplementation (cont'd)

Administration.

- Because fractional Ca++ absorption is dose-limited,maximum single doses of 600 mg or < of elemental Ca++ are recommended.
- Calcium carbonate is the salt of choice as it contains the highest amount of elemental Ca++ & is the least expensive
- Calcium carbonate tablets should be taken with meals to ↑ absorption
- **Calcium citrate** absorption is acid-independent & need not be administered with meals.

Vitamin D Supplementation

- Vitamin D intake is critical for the prevention and treatment of osteoporosis because it maximizes intestinal calcium absorption.

- ***Efficacy***: While several studies have demonstrated a beneficial effect of vitamin D on **fractures and falls**, not all studies have demonstrated a beneficial effect (due to differences in vitamin D dosing,concomitant calcium administration,adherence,etc.)

Vitamin D Supplementation

Administration.

- Seniors & patients being treated for osteoporosis: min. 800 units through food & supplementation
- **Cholecalciferol (vitamin D3**) is > efficient than **ergocalciferol (vitamin D2)** & is the preferred form
- In patients. with measured insufficient 25(OH) D concentrations, higher daily intakes of vitamin D may be needed
- half-life of vitamin D is about 1 month → ~ 3 months of therapy are required before a new steady state is achieved & repeat 25(OH) D concentration can be obtained
- In pts. with severe hepatic or renal disease, the **activated form of vitamin D (calcitriol)** might be more appropriate
- Some experts believe that the upper tolerable limit for vitamin D should be raised and that the recommended daily allowance for vitamin D should be 2,000 units per day

Bisphosphonates

- mimic pyrophosphate, an endogenous bone resorption inhibitor
- all bisphosphonates become incorporated into bone, giving them long biologic half-lives of up to 10 years.
- **alendronate**, **risedronate**, **oral ibandronate,** and **zoledronic acid** are currently FDA indicated for the prevention & treatment of postmenopausal osteoporosis
- **IV ibandronate is** indicated only for treatment of postmenopausal women
- **zoledronic acid, risedronate & alendronate** are also FDA indicated in male & glucocorticoid-induced osteoporosis

Bisphosphonates (Bis) (cont'd)

***Administration*.**

- bioavailability is very poor + GI side effects → each oral dose should be taken with at least 6 ounces of **plain tap water** (~300 ml) (not coffee, juice, mineral water, or milk) **at least 30 (60 for ibandronate) min.** before consuming any food, supplement (including Ca++ & vit. D), or medication.
- The tablets should be swallowed whole without chewing or sucking.
- Administration should be with water only and not combined with other fluids.
- weekly, raspberry flavored, oral solution only needs to be taken with 2 ounces of water & can be used for pts. with swallowing difficulties
- pt. should also **remain upright** (i.e., either sitting or standing) **for at least 30 min. after alendronate & risedronate &1 hour after ibandronate**
- **Before IV Bis are used, the pt.'s serum Ca++ level must be normal**
- The quarterly ibandronate injection is given IV over 15-30 sec .
- Once-yearly administration of zoledronic acid should be infused over at least 15 min.

Alendronate

- This bisphosphonate, marketed as Fosamax®, is approved as an oral tablet in both the United States and Canada for postmenopausal

- For osteoporosis prevention (5 mg/d or 35 mg/wk) and **treatment** (10 mg/d or 70 mg/wk).

- Alendronate is also available in a single weekly oral tablet of 70 mg with 5,600 IU of vitamin D (Fosamax® Plus D; Fosavance®).

Risedronate

This bisphosphonate, marketed asActonel®,is approved in the United States and Canada for the **prevention and treatment** of postmenopausal osteoporosis in oral tablet doses of 5 mg/day, 35 mg/week, 75 mg on 2 consecutive days once a month, and 150 mg/month.

Ibandronate

- Oral Ibandronate,marketed as Boniva®,is approved as a 2.5-mg tablet once a day, as well as a 150-mg tablet once a month for the prevention and treatment of postmenopausal osteoporosis.

- It is also approved in an IV formulation at a 3-mg dose every 3 months (administered by a healthcare professional) for the **treatment of postmenopausal osteoporosis.**

Zoledronic acid (Most Potent Bisphosphonate)

- The bisphosphonate zoledronic acid, marketed as Reclast® in the United States and Aclasta® in Canada.
- FDA-approved for:
  - **osteoporosis treatment and prevention** in postmenopausal women.
  - to improve bone mass in men with osteoporosis,
  - prevention and treatment of osteoporosis in men and women expected to be on glucocorticoid therapy for at least 12 months.
  - prevention of new clinical fractures in patients (both women and men) who have recently had a low-trauma (osteoporosis-related) hip fracture.

Zoledronic acid

- The annual 5-mg IV infusion is administered by a healthcare professional over a period of no less than 15 minutes.

- An infusion administered once every 2 years is now approved in the United States for **prevention** of osteoporosis in postmenopausal women.

Bisphosphonates (cont'd)

Efficacy.

- Of the antiresorptive agents, provide the **greatest fracture risk reductions & BMD ↑**
- BMD ↑ with bisphosphonates are dose dependent & greatest in the first 6-12 months of therapy
- because of a lack of fracture data in men, bisphosphonates are only FDA indicated to ↑ BMD, not to ↓ fracture risk in men

Long term efficacy of bisphosphonates

- While current treatment guidelines offer recommendations on when to start bisphosphonate therapy, the optimal length of bisphosphonate therapy remains unclear.

- Several clinical studies have shown that up to **ten years** of alendronate therapy is associated with sustained increases in BMD and appear to cause no harm.

Bisphosphonates (cont'd)

- In the only study to compare continuing a bisphosphonate compared with stopping it,the **F**racture InterventionTrial **L**ong-term **Ex**tension (FLEX) trial found that taking alendronate longer than five years does not provide much additional protection against fractures.

- In this trial, women who discontinued alendronate **after five years had a moderate decline in bone mineral density (BMD) and a gradual increase in serum markers of bone turnover compared with women who continued taking alendronate for an additional five years**. However, the mean BMD levels among patients who discontinued therapy remained at or above baseline levels which were measured ten years earlier.

Bisphosphonates (cont'd)

- Similar sustained beneficial effects of long-term (up to five years) treatment with risedronate have been demonstrated.

- However, there is no information regarding the other oral bisphosphonates to support the sustained duration of action after discontinuation.

Bisphosphonates (cont'd)

Adverse Events/Precautions.

- GI ADRs are minimal if the medication is taken correctly
  - weekly & monthly therapies have similar common but less-serious GI effects (perforation,ulceration,GI bleeding) than daily therapy.
  - GI event rates were **not** ↑ with concomitant NSAID use
  - IV ibandronate & zoledronic acid can be used for patients with c/i or intolerances to oral Bisphosphonates
- the most commonADRs of IV Bisphosphonates :fever,flu-like symptoms, & local injection-site reactions
- rare ADR:osteonecrosis of the jaw
  - routine dental care & good oral hygiene should be encouraged in anyone beginning Bisphosphonates therapy.
  - major dental work probably should be completed prior to beginning Bisphosphonates if possible

Osteonecrosis of the jaw

- Osteonecrosis of the jaw is a rare but serious disorder. Jaw osteonecrosis is very difficult to treat, often requiring multiple surgeries and courses of antibiotics.
- The surgeries often cause disfigurement, and areas of necrosis may not heal. Before its association with BPs, osteonecrosisof the jaw was most often seen in cancer patients who had radiation to the head and neck as part of their treatment.
- The exact mechanism is not fully understood. Patients who receive high-dose IV BPs for greater than two years are most at risk for developing osteonecrosis of the jaw.The estimated osteonecrosis risk ranges from 0.8% to 20% in these patients.
- It seems that the higher the BP dose and the longer the exposure time, the more likely the chance that osteonecrosis of the jaw will develop.

Bisphosphonates and Increased Fracture Risk

- BP inhibit an enzyme necessary for formation of the cytoskeleton in osteoclasts thereby strongly inhibiting bone resorption.The estimated half-life of BP is greater than 10 years once they are incorporated into mineralized bone.
- It is postulated that when bone containing BP is resorbed, some of the BP released is ***recirculated and binds again to bone surfaces to inhibit bone resorption***. However, long-term suppression on bone remodeling can be detrimental. One purpose of bone remodeling is to refresh the bone and to repair the microscopic damage that accumulates within any structure.There is increasing evidence to suggest that long-term BP use ***may overly suppress bone metabolism***, limiting repair of micro damage and potentially increasing the risk for fractures in some patients.
- **In a report conducted by the American Society for Bone and Mineral Research in 2010,286 cases of atypical fractures in patients taking BP therapy for osteoporosis were identified. Investigators determined that atypical fractures of the subtrochanteric and femoral shaft more often occurred in patients treated with long-term (1.3 to 17 years, median seven years)BP.**

Comments on the risk of atypical fractures with bisphosphonates:

- Although there appears to be an association of fractures, it is **not known** if bisphosphonates are the cause of the fractures.
- In general, patients with osteoporosis are more likely to fracture bones. Furthermore, other studies have found no association between bisphosphonates and atypical fractures. In addition, the incidence ofatypical fractures is miniscule in comparison to the incidence of hip fractures due to osteoporosis.

Esophageal Cancer and Bisphosphonates

- Oral bisphosphonates can cause esophageal irritation and injury.
- Risk is reduced if **patients drink water and remain upright after administration**. Case reports of esophageal cancer after bisphosphonate use triggered additional study of cancer risk.

- For now, there is not enough information to link bisphosphonates and esophageal cancer.

Mixed Estrogen Agonists/Antagonists (EAAs)

- **Raloxifene**, a second generation (EAA)
- approved for prevention & treatment of postmenopausal osteoporosis
- estrogen agonist on bone but antagonist on the breast & uterus

Efficacy.

- ↓vertebral fractures & ↑ spine & hip BMD, but to a lesser extent than bisphosphonates
- after discontinuation, the effect is lost
- has an FDA-approved indication for invasive breast cancer risk reduction → **dual osteoporosis & breast cancer prevention**
- some positive lipid effects (↓ total & LDL, neutral effect on HDL, slightly ↑ TGs);
- but no ↓ in cardiovascular effects was demonstrated

EAAs (cont'd)

Adverse Events/Precautions.

- hot flushes
- rarely endometrial bleeding
- contraindicated in active or past history of venous thromboembolic event
- should be stopped if a patient anticipates extended immobility
- slight ↑ in fatal stroke → women at high risk for a stroke or coronary events & those with known CAD, PVD, AF, or a prior history of CVA might not be good candidates for this medication

Administration.

- adherence & persistence problems exist

Calcitonin

- is released from the thyroid when SrCa is ↑
- **Salmon calcitonin** is > potent & longer lasting than the mammalian FDA indicated for osteoporosis treatment for women who are at least 5 years past menopause
- not approved for men & concomitantly with glucocorticoids
- ***is reserved as 3rd-line treatment***

Efficacy.

- only vertebral fractures ↓ with intranasal calcitonin
- does not consistently affect hip BMD & does not ↓hip fracture risk
- might **provide pain relief** to some pts. with acute vertebral fractures
- should be prescribed for short-term (4 weeks)
- should not be used in place of other > effective & < expensive analgesics or more appropriate osteoporosis therapy

Estrogen Therapy

- FDA indicated for prevention of osteoporosis,
- should **only be used short-term** in women who need estrogen therapy for the management of menopausal symptoms such as hot flushes.
- **risks of estrogen therapy outweigh the bone benefits**
- Estrogen with (HT) or without (ET) progestin therapy significantly ↓ fracture risk
- ↑ in BMD are < than bisphosphonates or teriparatide,but > than raloxifene & calcitonin
- **oral & transdermal** estrogens at equivalent doses & **continuous or cyclic** HT regimens have similar BMD effects
- fracture risk ↓ has not been demonstrated with lower dose therapy
- when ET or HT is discontinued, bone loss accelerates & fractureprotection is lost
- the lowest effective dose should still be used for preventing & controlling menopausal symptoms with use discontinued with symptom abatement

Testosterone

- ↓ testosterone concentrations are seen with certain gonadal diseases,eating disorders,glucocorticoid therapy,oophorectomy, menopause, & andropause
- testosterone replacement is not approved for the prevention or treatment of osteoporosis
- *should not be used solely for the prevention or trea tment of osteoporosis, but might be beneficia l to ↓ bone loss in pa tients needing thera py for hypog ona dal symptoms*
- Patients should be evaluated within 1-2 months of initiation & then every 3-6 months thereafter
- testosterone & methyltestosterone are in **pregnancy category X**
- testosterone products are **schedule III drugs**
- ge9e9lproducts can rub off & be absorbed by the patient's partner

Thiazide Diuretics

- Thiazide diuretics increase urinary calcium reabsorption.
- Observational studies suggest that patients who receive thiazide diuretics have a greater bone mass, lower rates of bone loss, and fewer fractures.
- Two prospective, controlled trials demonstrated small increases in bone mass over placebo.
- **Prescribing thiazide diuretics solely for osteoporosis is not recommended but is a reasonable choice for the patient with osteoporosis who requires a diuretic and for patients on glucocorticoids with greater than 300 mg of calcium excreted in the urine over 24 hours.**

Anabolic Therapies

Teriparatide

- is the only available medication that ↑ **bone formation**
- is a recombinant product representing the first 34 amino acids in human PTH
- both bone mass & architecture are improved
- FDA indicated for postmenopausal women & men who are at high risk for fracture
- Pts. who have a history of osteoporotic fracture, multiple risk factors for fracture, very low bone density **(e.g., T-score < -3.5),** or have failed or are intolerant of previous bisphosphonate therapy are candidates for PTH therapy
- discontinuation of therapy results in ↓ in BMD, although some anti-fracture efficacy appears to be maintained

Teriparatide

- Teriparatide is approved only for duration **of two years.**

- A gradual decrease in bone mass has been noted after discontinuation of teriparatide therapy;however, following therapy with a bisphosphonate has been shown to preserve the benefits

RANK Ligand (RANKL) Inhibitor/Human Monoclonal Antibody

- **Denosumab** is a receptor activator of nuclear factor kappa-B ligand (RANKL) inhibitor approved by the FDA in 2010 for treatment of postmenopausal osteoporosis with a high risk of fracture.
- Denosumab inhibits theformation, function and survival of osteoclasts by binding to RANKL resulting in decreased bone resorption and increased bone mass and strength.
- Denosumab (**Prolia® 60 mg)** is administered SC every six months by a health professional. In addition calcium 1,000 mg and at minimum 400 IU of vitamin D must be taken daily.The reduced frequency and supervised administration of denosumab may help improve patient compliance.
- Pre-existing hypocalcemia must be corrected prior to initiating therapy.

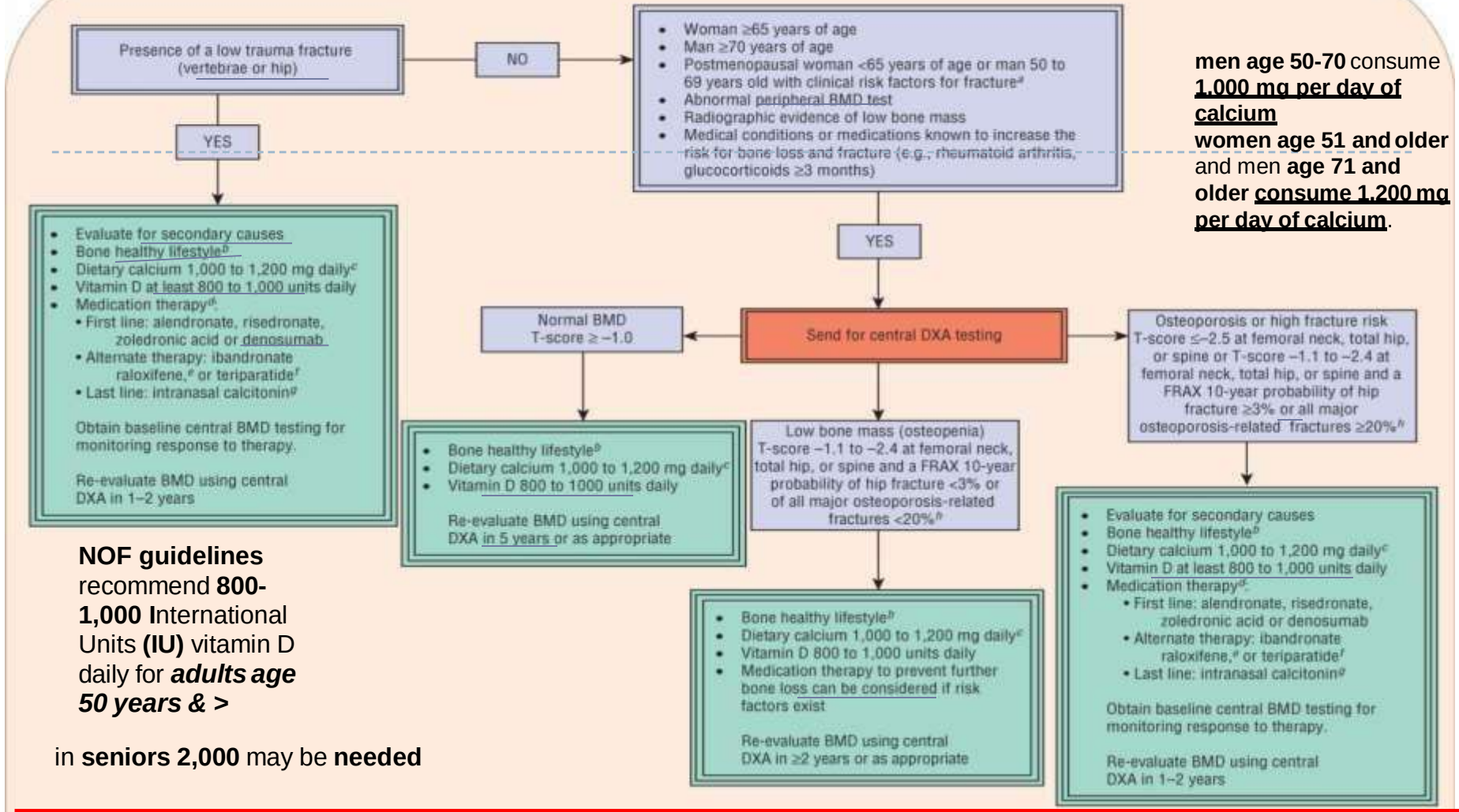
- **Xgeva® 120 mg (hypercalcemia of malignancy)**

Emerging therapies/Sclerostin inhibitors

- **Sclerostin inhibitors** – Sclerostin is produced by osteocytes and **inhibits bone formation**
- inhibition of sclerostin should enhance osteoblast function and improve bone mass
- a phase II trial in postmenopausal women, all doses of a monoclonal anti-sclerostin antibody (**romosozumab**) increased bone density at the LS, total hip, and femoral neck

Combination therapies

Combining potent antiresorptive agents results in small additional increments in bone density. In postmenopausal women (mean age, 61-62 y) with low bone mass,BMD improvements in the spine and hip with combined alendronate and ET were significantly greater (8.3%) than results for either agent alone (6.0%). Combined risedronate and ET/ EPT also has shown favorable, although modest, BMDeffects compared with either agent alone.Whether increases in BMD result in better fracture protection is not known,and the long-term safety of combination therapies has not been evaluated.



men age 50-70 consume **1,000 mg per day of calcium**
women age 51 and older and men **age 71 and older consume 1,200 mg per day of calcium**.

NOF guidelines recommend **800-1,000 I**nternational Units **(IU)** vitamin D daily for ***adults age 50 years & >***

in **seniors 2,000** may be **needed**

[a]Major clinical risk factors for fracture: current smoker, low body weight or body mass index, history of osteoporosis/low trauma fracture in a first-degree relative, personal history of fracture as an adult (after age 50 years), excessive alcohol intake
[b]Bone-healthy lifestyle includes smoking cessation, limit alcohol intake, well-balanced diet with adequate calcium and vitamin D intakes, weight-bearing/resistance exercises, and fall prevention
[c]Dietary calcium preferred. If diet is inadequate, supplement as necessary
[d]Sometimes men with hypogonadism also receive testosterone replacement; sometimes women with menopausal symptoms receive low dose hormone therapy for a short time
[e]Raloxifene can be a good option in women at high risk for breast cancer
[f]Teriparatide can be considered a first-line option in patients with a very high risk of fracture (e.g., T-score <−3.5 or multiple low trauma fractures) or intolerant to other medications
[g]Calitonin is last-line due to limited fracture data and a recent concern over possible slight increased cancer risk.
[h]WHO absolute fracture risk assessment tool (FRAX) for osteoporotic fracture risk estimations

All therapies should be given with calcium and vitamin D supplementation.

men age 50-70 consume **1,000 mg per day of calcium**
women age 51 and older and men **age 71 and older consume 1,200 mg per day of calcium**.

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Source: DiPiro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, Posey LM:
Pharmacotherapy: A Pathophysiologic Approach, Ninth Edition:
www.accesspharmacy.com
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Management

- **Initial therapy** – For most postmenopausal women with osteoporosis,we suggest oral bPs as first-line therapy. (efficacy, favorable cost, and the availability of long-term safety data).
- We suggest alendronate or risedronate as our choice of bPdue to efficacy in reducing vertebral and hip fracture.
- For patients with severe osteoporosis (T-score of -3.5 or below even in the absence of fractures, orT-score of -2.5 or below plus a fragility fracture), initial treatment with teriparatide is an alternative. Because treatment with teriparatide is limited to 24 months, patients with severe osteoporosis who are treated with teriparatide first are typically treated with an antiresorptive agent (eg, bPs) after discontinuing teriparatide.

Contraindications/intolerance to oral bisphosphonates

- Oral bPs should not be used as initial therapy in patients with esophageal disorders, an inability to follow the dosing requirements (e.g., stay upright for at least 30 to 60 minutes),or CKD :[eGFR] rate <30 mL/min
- Oral bPs should also be avoided after certain types of bariatric surgery in which surgical anastomoses are present in the GI tract.
- Alternatives for initial therapy in patients with contraindications or intolerance to oral bPs include IV bPs (except if the contraindication is CKD),denosumab,teriparatide (except in CKD),and selective estrogen receptor modulators (SERMs).
- The choice of initial agent depends upon the nature of the contraindication/intolerance,the severity of the osteoporosis,and subsequent risk for fracture.

Contraindications/intolerance to oral bisphosphonates

- Among IV bPs.:We prefer **IV** zoledronic acid, which has been demonstrated to reduce **vertebral and hip fractures**. IV ibandronate is also available;however,there is as yet no direct fracture prevention data for IV ibandronate.
- Denosumab **is an alternative to IV** zoledronic acid **for women at high risk for fracture (such as older patients) who have difficulty with the dosing requirements of oral bPs**,who prefer to avoid IV bPs due to side effects (eg, acute phase reaction), or who have impaired renal function.
- Teriparatide is rarely used as initial therapy, but it could be prescribed as an alternative to IV zoledronic acid for postmenopausal women with **severe** osteoporosis (T-score of -3.5 or below even in the absence of fractures, or T-score of -2.5 or below plus a fragility fracture) who were treated initially with oral bPs but who are unable to tolerate them.

Response to therapy

- **Decline in BMD**
- When the change in BMD is <5 % and the patient is taking the drug correctly and has no discernible contributing factors, we suggest continuing the same therapy and repeating the BMD two years later.
- •When the decline in BMD is ≥5 %, we usually switch from an oral bP to an IV bP, typically zoledronic acid. If the lack of response is related to poor absorption, switching to an IV preparation should result in a more favorable response. Other alternatives include switching to denosumab or teriparatide.

Response to therapy

- ●**Fracture while taking bPs** – Switching to teriparatide is a good option for patients with severe osteoporosis (T-score <-2.5 and at least one fragility fracture) who continue to fracture after one year of bisphosphonates.Teriparatide is effective in increasing BMD in women previously treated with bisphosphonates, although the improvement may be less than in women not previously exposed to bPs.
- Denosumab is an alternative for patients who are unresponsive to other therapies and in those with impaired renal function. However, if there are no contraindications, it may be beneficial to treat with teriparatide first (maximum of two years), followed by denosumab, to preserve the gains in BMD achieved with teriparatide.

Medication	Indications	Reduction in Fracture Risk[2]	Adverse Drug Reactions[1]	Contraindications
Bisphosphonates				
Alendronate	**TREATMENT** • Postmenopausal osteoporosis • Increase bone mass in men with osteoporosis • Glucocorticoid-induced osteoporosis in men and women **PREVENTION** • Postmenopausal osteoporosis	Vertebral: +++ Non-vertebral: ++ Hip: +++	• Esophagitis, abdominal pain, diarrhea • Jaw osteonecrosis (rare), musculoskeletal pain, dyspepsia, acid regurgitation, esophageal ulcer, dysphagia, flu-like symptoms (rare postmarket experience) • Atypical fracture of the thigh	• Abnormalities of the esophagus that delay esophageal emptying • Inability to stand or sit upright for at least 30 minutes • Hypersensitivity • Uncorrected hypocalcemia • Not recommended for patients with CrCl ≤ 35 ml/min
Ibandronate	**TREATMENT** • Postmenopausal osteoporosis **PREVENTION** • Postmenopausal osteoporosis	Vertebral: +++ Non-vertebral: + Hip: -	• Esophagitis, abdominal pain, diarrhea • Influenza-like illness, jaw osteonecrosis (rare) musculoskeletal pain, dyspepsia, acid regurgitation, esophageal ulcer, dysphagia • Atypical fracture of the thigh	• Uncorrected hypocalcemia • Inability to stand or sit upright at least 60 minutes • Hypersensitivity • Not recommended for patients with CrCl ≤ 30 ml/min

Medication	Indications	Reduction in Fracture Risk[2]	Adverse Drug Reactions[1]	Contraindications
Risedronate	**TREATMENT** • Postmenopausal osteoporosis • Glucocorticoid-induced osteoporosis • Increase bone mass in men with osteoporosis **PREVENTION** • Postmenopausal osteoporosis • Glucocorticoid-induced osteoporosis	Vertebral: +++ Non-vertebral: ++ Hip: +++	• Esophagitis, abdominal pain, diarrhea • Jaw osteonecrosis (rare), musculoskeletal pain, dyspepsia, acid regurgitation, esophageal ulcer, dysphagia • Atypical fracture of the thigh	• Inability to stand or sit upright for at least 30 minutes • Hypersensitivity • Uncorrected hypocalcemia • Not recommended for patients with CrCl ≤ 30 ml/min
Risedronate delayed release	**TREATMENT** • Postmenopausal osteoporosis	Vertebral: +++ Non-vertebral: ++ Hip: +++	• Esophagitis, abdominal pain, diarrhea • Jaw osteonecrosis (rare), musculoskeletal pain, dyspepsia, acid regurgitation, esophageal ulcer, dysphagia • Atypical fracture of the thigh	• Inability to stand or sit upright for at least 30 minutes • Hypersensitivity • Uncorrected hypocalcemia • Not recommended for patients with CrCl ≤ 30 ml/min
Zoledronic acid	**TREATMENT** • Postmenopausal osteoporosis • Paget's disease	Vertebral: +++ Non-vertebral: ++ Hip: ++	• Acute phase reaction: fever, flu-like symptoms, HA, arthralgia/myalgia • Jaw osteonecrosis (rare), transient increase in creatinine, atrial fibrillation, hypocalcemia • Atypical fracture of the thigh	• Hypersensitivity to zoledronic acid or any of its excipients • Uncorrected hypocalcemia • Not recommended in patients with a creatinine clearance less than 35 mL/min

Medication	Indications	Reduction in Fracture Risk[2]	Adverse Drug Reactions[1]	Contraindications
Selective Estrogen Receptor Modulator (SERM)				
Raloxifene (Evista®)*	**TREATMENT** • Postmenopausal osteoporosis **PREVENTION** • Postmenopausal osteoporosis	Vertebral: +++ women without fx ++ women with fx Non-vertebral: - Hip: -	• Hot flashes • Leg cramps • Increased risk of venous thromboembolic events	• Pregnancy • History of venous thromboembolism • Hypersensitivity • Nursing women
Parathyroid Hormone (PTH)				
Teriparatide	**TREATMENT** • Postmenopausal osteoporosis with high risk for fracture (history of osteoporotic fracture, multiple risk factors, failed/intolerant of previous therapy) • Glucocorticoid-induced osteoporosis with high risk of fractures • Increase bone mass in men with primary or hypogonadal osteoporosis who are at high risk of fracture (history of osteoporotic fracture, multiple risk factors, failed/intolerant of previous therapy)	Vertebral: +++ Non-vertebral: +++ Hip: N/A	**BLACK BOX WARNING:** shown to cause an increase in the incidence of osteosarcoma in male and female rats, dependant on dose and duration of treatment. • Orthostatic hypotension • Increase in serum calcium • Increase in urinary calcium • Increase in serum uric acid	• Paget's disease • Any prior therapeutic radiation involving the skeleton • Bone metastases or history of skeletal malignancies • Metabolic bone disease (other than osteoporosis) • Hypercalcemia • Pregnant and nursing women • Unexplained elevated alkaline phosphatase • Hypersensitivity, pediatric populations or young adults with open epiphyses
Calcitonin				
Calcitonin-salmon (Miacalcin® and Fortical® nasal spray)	**TREATMENT** • Postmenopausal osteoporosis in women with at least five years postmenopause with low bone masss relative to healthy premenopausal females	Vertebral: + Non-vertebral: - Hip: -	• Nausea • Flushing • Rhinitis with nasal spray	• Hypersensitivity

Drug Use In Infants & Children

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Pharmacokinetics in Children

1. Absorption

a. ↑ from skin due to thin stratum corneum & better hydration

b. GIT: altered, low acidity, ↑ed gastric emptying time, ↑ed enterohepatic circulation, irregular peristalsis(PCM & phenobarbitone ↓ed & of penicillin G & ampicillin ↑ed)

c. Intramuscular & subcutaneous unpredictable because thin skeletal muscle mass & fat

d. Low bile acid & lipase so less lipophilic drug absorption

e. diarrhoea

2. Drug distribution

a. More in c/o hydrophilic than adult because of higher percentage of body water (65-85%), also 40% is extra cellular in the neonate (aminogycoside)
b. Less fat in pre term (1%) than full term(15%) thus less lipophilic drug concentration in premature
c. Less plasma protein available so more free drug , so more toxicity (diazepam)
d. Competition with bilirubin for albumin may displace it , may cause kernicterus (sulfonamides) & vice versa (phenytoin)

3. Drug metabolism

a. Less liver cytochrome P450 dependent activity , so chances of toxicity high e.g.chloramphenicol causing grey baby syndrome

b. Table shows difference in half life in Neonate & adult p.s.phenobarbital & Phenytoin have low half life during infancy

Drug	Neonatal Age	Neonates $t_{1/2}$ (hours)	Adults $t_{1/2}$ (hours)
Acetaminophen		2.2–5	0.9–2.2
Diazepam		25–100	40–50
Digoxin		60–70	30–60
Phenobarbital	0–5 days	200	64–140
	5–15 days	100	
	1–30 months	50	
Phenytoin	0–2 days	80	12–18
	3–14 days	18	
	14–50 days	6	
Salicylate		4.5–11	10–15
Theophylline	Neonate	13–26	5–10
	Child	3–4	

c. Enzyme induction

Due to mother receiving drugs or child

-enzyme induction may increase interaction

-phenobarbitone is used to treat neonatal jaundice on the same principal

* During toddlerhood metabolite rate of many drugs exceeds adult values .

4. Drug excretion

a. GFR is less , so is tubular secretion, resulting in less renal clearance
b. Normal clearance achieved at the age of 6-12 months
c. Subsequently during toddlerhood it increases than adult value for some (e.g. for digoxin)
d. So appropriate dose adjustment is necessary
e. E.g. penicillin, ampicillin, aminoglycosides

Pharmacodynamic alterations

- Response of drug may be different in children than adults but mechanism remain same
- Due to immature receptors or neurotransmitter system
- e.g. glucocorticoids-premature epiphysial fusion; tetracyclines- malformed bone & teeth also staining of teeth, antihistaminics & barbiturates –excitement, amphetamine- ↓es abnormal hyperactivity

Clinically important

- PDA can be closed with administration of indomethacin
- In case of fallot's tetralogy administration of alprostadil (PGE_1) is life saving

Pediatric Dosage Forms & Compliance

- Use of tablets is inconvenient & injections are rejected by children
- So preferred formulations are liquid e.g. suspension, elixir, drops
- Proper use should be instructed
- Rejection, spitting & vomiting should be considered
- Proper measurement
- Suspensions to be shaken well before use
- Reduce dosing errors
- Encourage children to take medicine themselves

Dose

- Young's rule: dose= Adult dose × Age(years) / Age + 12
- Clark's rule: dose = Adult dose × Weight(kg) / 70
- Determination of drug dosage from surface area

Weight		Approximate Age	Surface Area (m^2)	Percent of Adult Dose
(kg)	(lb)			
3	6.6	Newborn	0.2	12
6	13.2	3 months	0.3	18
10	22	1 year	0.45	28
20	44	5.5 years	0.8	48
30	66	9 years	1	60
40	88	12 years	1.3	78
50	110	14 years	1.5	90
60	132	Adult	1.7	102
70	154	Adult	1.76	103

Drug Use During Pregnancy

Dr.Adepu Ramesh
VCPS

Drug use during Pregnancy

- Every system in the body is affected by pregnancy
- Pharmacokenetics & pharmacodynamics of drugs is affected by pregnancy
- At present these pharmacokinetic factors are beginning to be understood whereas pharmacodynamics information is still incomplete

Pharmacokenetics Of Drugs During Pregnancy

- 1. Absorption: ↑ed
  - Gastric emptying time is delayed & gut motility reduced because of ↑ed progesterone
  - Vasodilation , tissue perfusion ↑ed
- 2. Distribution: ↑ed
  - Total body water increases i.e. hemodilution
  - Plasma albumin conc. ↓ed so ↑ed free drug conc.
  - Increased body fat, reservoir of lipid soluble drug

Pharmacokenetics Of Drugs During Pregnancy

- 3. Metabolism:

➢ hepatic metabolism increases but no altered hepatic blood flow

- 4. Elimination:

➢ Renal blood flow doubles, so rapid elimination of drugs excreted by kidney (e.g.Aminoglycosides)

➢ Dose should be increased in such cases

Factors Affecting Placental Drug Transfer & Subsequent Effect On Fetus

1. Physiochemical properties of the drug
2. Rate at which drug crosses placenta & amount reaching the fetus
3. Duration of exposure to drug
4. Distribution characteristics of drug in different fetal tissues
5. Stage of placental & fetal development at the time of exposure
6. Effects of drugs used in combination

A. Lipid solubility

Lipophilic drugs crosses placenta more

e.g.thiopental may cause apnoea in fetus,

salicylate is ionised but very lipid soluble

B. Molecular size & pH

250-500 easily

500-1000 with difficulty

>1000 poorly

maternal blood pH 7.4 & fetal 7.3 , may lead to ion trapping in c/o pKa >7.4

C. Placental transporters

e.g. P glycoprotein transporter encoded by MDR1 gene pumps back some drugs like cancer drugs (doxorubicin)

and viral protease inhibitors

D. Protein binding

more protein bound drug crosses less

doesn't affect lipid soluble drug that much

differential protein binding – Glyburide

E. Placental & fetal drug metabolism

1) Placenta: aromatic oxidation reactions occur

e.g. Phenobarbital

may also lead to toxic metabolite e.g. ethanol, benzpyrenes

2) Fetus: 40-60% blood enters fetal liver

a proportion of blood is shunted back through placenta

Pharmacodynamics

A. Maternal Drug Actions

- Effect on reproductive tissue may be altered by endocrinal environment due to pregnancy
- Effect on other tissues not changed much
- Altered physiologic context may require treatment

  e.g. cardiac glycosides & diuretics for heart failure , insulin for pregnancy induced diabetes

Pharmacodynamics

B. Therapeutic Drug Actions In The Fetus

emerging area ‘ Fetal Therapeutics’

i. Corticosteroids: e.g. dexamethasone for lung maturation in premature labour
ii. Phenobarbitone: for neonatal jaundice or decrease intra cranial bleeding
iii. Zidovudine or nevirapine: alone or in combination to prevent vertical transmission

Toxic Effects Of Drugs On The Embryo, Fetus, Or Neonate

May vary

- No effect.
- Little
- Serious- fetal toxicity
- Spontaneous abortion
- Death
- Fetal malfunction
- Fetal malformations.

Pharmacodynamics

C. Predictable Toxic Effects

i. Opioids : dependance , neonatal withdrawal syndrome, respiration depression

ii. ACE inhibitors : renal damage

iii. Diethylstilbestrol: adenocarcinoma of vagina after puberty

Pharmacodynamics

D. Teratogenic Drug Actions:

Birth Defects

- Incidence of major structural defects(abnormalities) is about 6% of all pregnancies.
- 3% are caused by drugs or environmental factors/exposure
- 3% have a genetic or unknown causes

- 1/2 of the birth defects are obvious at birth.
- 1/2 of the birth defects aren't discovered until later in life or discovered during an autopsy
- Incidence of minor structural anomalies is not known.
- Incidence of functional abnormalities is not known-growth restrictions, mental retardation, and learning disabilities
- Some abnormalities have multiple causes-genetic factors, environmental factors, chemicals or drugs.

Teratogenic
Teratogenesis

- Teras-”monster”
- Gensis-”producing”
- Birth defects/distortion of gross anatomy.
- Examples- cleft lip/palate, clubfoot, neural tubal defects, missing or malformed limbs/fingers.
- Now also- behavioral and/ or biochemical and/or physiological abnormalities.
- Death of fetus & carcinogenic effect

Mechanism of Teratogenesis

- Direct effect on maternal tissue with secondary effect on fetus
- Indirect-such as interfering with o2 or nutrients.
- Teratogenesis maybe direct-malformations of structures. e.g.

i. Vitamin A analogs (isotretinoin,etretinate)
ii. Deficiency of a critical substance (folic acid causing neural tube defects)
iii. Continues exposure to teratogen may produce cumulative effect (Fetal Alcohol Syndrome)

Proving A Drug Is A Teratogen

3 Criteria must be met:

- 1. Drug must cause a characteristic set of malformations with selectivity for certain organs
- 2. It must act only during a specific window of vulnerability (organogenesis of target organ)
- 3. The incidence of malformations should increase with increased dosage & duration of exposure.

Fetal Effects From Drugs Depend On Several Factors

- Time- when drug is taken in pregnancy.
- Preimplantation/presomite period- conception to 2 week
- High dose- maybe lethal/death/abortions.
- Low dose-maybe nothing.

As per time …..

① 0-2 weeks-period of dividing zygote , implantation

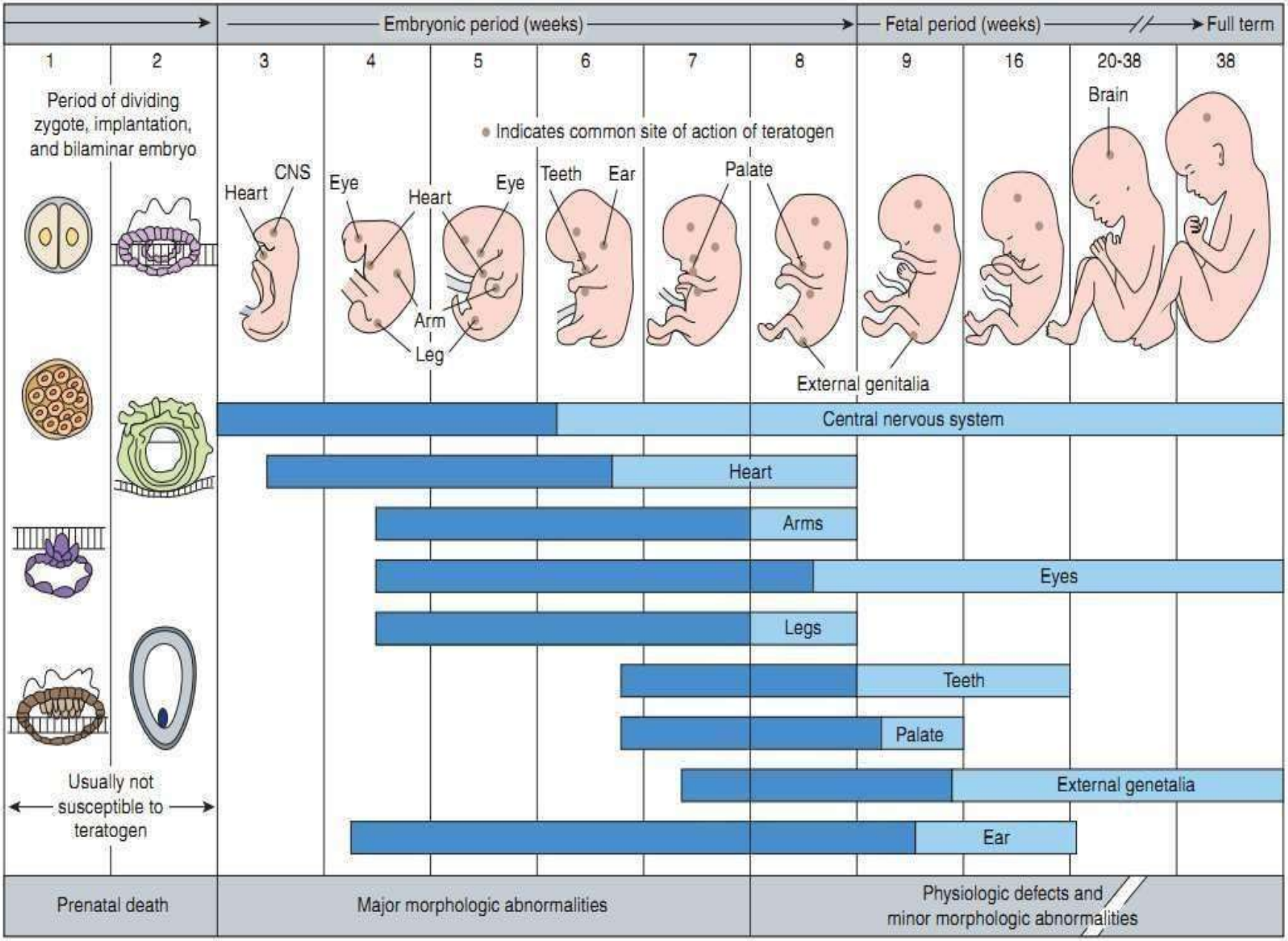
- Prenatal death may occur
- Abortion may occur due to oxytosics e.g. ergot alkaloids, anti metabolites

② Embryonic period-3-8 weeks - First trimester

- Gross malformations
- E.G. One time exposure to thalidomide through 4^{th} to 7^{th} week-causes missing limbs(phocomalia).

③Fetal period-9-40 weeks(term)

- Function problems rather than gross anatomy
- Learning deficits &/or behavioral abnormalities


Embryonic period (weeks)
Fetal period (weeks)
Full term
1
2
3
4
5
6
7
8
9
16
20-38
38
Period of dividing zygote, implantation, and bilaminar embryo
Indicates common site of action of teratogen
Heart
CNS
Eye
Heart
Eye
Teeth
Ear
Palate
Brain
Arm
Leg
External genitalia
Central nervous system
Heart
Arms
Eyes
Legs
Teeth
Palate
External genetalia
Ear
Usually not susceptible to teratogen
Prenatal death
Major morphologic abnormalities
Physiologic defects and minor morphologic abnormalities

Drug	Trimester	Effect
ACE inhibitors	All, especially second and third	Renal damage
Aminopterin	First	Multiple gross anomalies
Amphetamines	All	Suspected abnormal developmental patterns, decreased school performance
Androgens	Second, third	Masculinization of female fetus
Antidepressants, tricyclic	Third	Neonatal withdrawal symptoms have been reported in a few cases with clomipramine, desipramine, and imipramine
Barbiturates	All	Chronic use can lead to neonatal dependence
Busulfan	All	Various congenital malformations; low birth weight
Carbamazepine	First	Neural tube defects
Chlorpropamide	All	Prolonged symptomatic neonatal hypoglycemia
Clomipramine	Third	Neonatal lethargy, hypotonia, cyanosis, hypothermia
Cocaine	All	Increased risk of spontaneous abortion, abruptio placentae, and premature labor; neonatal cerebral infarction, abnormal development, and decreased school performance
Cyclophosphamide	First	Various congenital malformations
Cytarabine	First, second	Various congenital malformations
Diazepam	All	Chronic use may lead to neonatal dependence
Diethylstilbestrol	All	Vaginal adenosis, clear cell vaginal adenocarcinoma
Ethanol	All	Risk of fetal alcohol syndrome and alcohol-related neurodevelopmental defects
Etretinate	All	High risk of multiple congenital malformations
Heroin	All	Chronic use leads to neonatal dependence
Iodide	All	Congenital goiter, hypothyroidism
Isotretinoin	All	Extremely high risk of CNS, face, ear, and other malformations
Lithium	First, third	Ebstein's anomaly, neonatal toxicity after third trimester
Methadone	All	Chronic use may lead to neonatal abstinence

Drug	Trimester	Effect
Methotrexate	First	Multiple congenital malformations
Methylthiouracil	All	Hypothyroidism
Metronidazole	First	May be mutagenic (from animal studies; there is no evidence for mutagenic or teratogenic effects in humans)
Misoprostol	First	Möbius sequence
Mycophenolate mofetil	First	Major malformations of the face, limbs, and other organs
Organic solvents	First	Multiple malformations
Penicillamine	First	Cutis laxa, other congenital malformations
Phencyclidine	All	Abnormal neurologic examination, poor suck reflex and feeding
Phenytoin	All	Fetal hydantoin syndrome
Propylthiouracil	All	Congenital goiter
Smoking (constituents of tobacco smoke)	All	Intrauterine growth retardation; prematurity; sudden infant death syndrome; perinatal complications
Selective serotonin reuptake inhibitors (SSRIs)	Third	Neonatal abstinence syndrome, persistent pulmonary hypertension of the newborn
Tamoxifen	All	Increased risk of spontaneous abortion or fetal damage
Tetracycline	All	Discoloration and defects of teeth and altered bone growth
Thalidomide	First	Phocomelia (shortened or absent long bones of the limbs) and many internal malformations
Trimethadione	All	Multiple congenital anomalies
Valproic acid	All	Neural tube defects, cardiac and limb malformations
Warfarin	First	Hypoplastic nasal bridge, chondrodysplasia
	Second	CNS malformations
	Third	Risk of bleeding. Discontinue use 1 month before delivery.

- ④Late pregnancy

Effect	Likely drug
Masculinization	Sex hormones
Foetal goiter	Antithyroid drugs
Tooth and bone development	Tetracyclines
Growth retardation	Corticosteroids
Early closure of ductus arteriosus	NSAIDs e.g. aspirin, indomethacin
Onset of labour is delayed Impaired CNS development	NSAIDs e.g. aspirin, indomethacin

- ⑤ During labor

Effect	Drug
Respiratory depression	Opioid analgesics
Foetal distress (due to reduced uterine blood Flow)	Sedatives and GA
Prolongation of labour (uterine muscle relaxation)	Sedatives and GA
Foetal circulation	Tocolytic agents (β_2 agonists)
Hypotonia	Benzodiazepines
Floppy baby syndrome	Lithium
Impaired CNS development	Psychotropic drugs used during pregnancy

Others such as

- Thrombocytopenia due to sensitization by thiazide diuretics when given to mother
- Mercury may cause impaired brain development , cerebral palsy, mental retardation etc.
- Other fetal defects due to effect of drug on spermatogenesis are suspected

Why is identification of teratogenic agents sometimes difficult to identify?

- Incidence of congenital anomalies is generally low.
- Animal tests may not be reliable
- Prolonged or increased exposure maybe required.
- Effects maybe delayed or not reconized.
- Behavioral effects are difficult to document.
- Controlled experiments cannot be done on humans.

- Documentation is incomplete
- Only in a limited number of drugs is the teratogenic effects known or proven.
- Lack of proof of teratogenicity does not mean a drug is safe in pregnancy
- May mean there is a lack of research or information.

How Is Data Collected On Drugs Which Cause Problems In Pregnancies?

- No human experimentation
- Systematic collection and analyzing of data on drugs taken by pregnant clients.
- Reporting of information by health professionals.

FDA category of drugs used in pregnancy

Category	Description
A	Controlled studies in women fail to demonstrate a risk to the fetus in the first trimester (and there is no evidence of a risk in late trimesters), and the possibility of fetal harm appears remote.
B	Either animal-reproduction studies have not demonstrated a fetal risk, but there are no controlled studies in pregnant women, or animal-reproduction studies have shown an adverse effect (other than a decrease in fertility) that was not confirmed in controlled studies in women in the first trimester (and there is no evidence of a risk in later trimesters).
C	Either studies in animals have revealed adverse effects on the fetus (teratogenic or embryocidal or other) and there are no controlled studies in women or studies in women and animals are not available. Drugs should be given only if the potential benefit justifies the potential risk to the fetus.
D	There is positive evidence of human fetal risk, but the benefits from use in pregnant women may be acceptable despite the risk (eg, if the drug is needed in a life-threatening situation or for a serious disease for which safer drugs cannot be used or are ineffective).
X	Studies in animals or human beings have demonstrated fetal abnormalities or there is evidence of fetal risk based on human experience or both, and the risk of the use of the drug in pregnant women clearly outweighs any possible benefit. The drug is contraindicated in women who are or may become pregnant.

Precautions While Prescribing During Reproductive Age & Pregnancy

1. In c/o contraception failure – Explain risk of drug already received & to continue pregnancy or abort
2. Similarly, in c/o post coital contraception
3. If a woman is being treated , explain risks to fetus & advise to avoid pregnancy till therapy completes
4. If a woman of reproductive age comes with any ailment pregnancy should be ruled out & then follow guideline as shown next

A. If not pregnant

i) Conception not desired : normal treatment

ii) Conception desired:

- Advise not to take any medication after last menstrual period
- Advise folic acid & iron supplements
- If diabetic start her on insulin
- Avoid self treatment if ill, consult doctor

B. If pregnant

- Advise same as 4.A.ii in last slide
- Avoid use of drug & treat minor problems without drugs
- Strictly avoid proven teratogens i.e. Cat. X & D
- If required prescribe proven safe drug
- Use minimal dose for shortest period of time
- If an unsafe or unproven drug has to be used than do α-fetoproteins at 16 weeks of gestation & high quality ultrasound at 18 weeks to asses anomalies

Education Of Pregnant/Pre-pregnant

- Provide accurate information with rationales
- Information should be current and based on evidence.
- Establish environment conducive to exchange of information - trust.
- Potential harm/risks. Common substances & OTC drugs to avoid in pregnancy- ASA, alcohol, increased doses of multivitamins, caffeine, cigarette smoking, etc.
- Avoid self treatment – OTCs
- Support
- Assist with coping if woman has taken a teratogenic agent..With guilt or fear…associated with drugs taken in pregnancy.

Common Medications Safe In Pregnancy

1. GIT conditions:

a) Nausea & vomiting:

pyridoxine with or without doxylamine,

meclizine, cyclizine, diphenhydramine

Avoid- neuroleptics , metochlorpromide (safe in 3rd trimester)

b) Constipation:

mild purgative , lubricant purgative (less safe)

Avoid- strong purgative as may cause abortion or premature labor pain

c) Heart burn:

milk of magnesia

Avoid- anticholinergics

d) Peptic ulcer:

sucralfate, bismuthsubcitrate & H_2 blockers

2. Hemopoetic

Iron, folic acid

no role of vit.B_{12}

3. Infections

a) Bacterial:

i. UTI

ampicillin, amoxycillin, cephalexin, cefadroxil, cefuroxime etc.

Avoid-fluoroquinolones

ii. Other

β-lactum antibiotics (penicillin G, penicillin V), ampicillin, amoxycillin, cloxacillin) , cephalosporins (cephalexim, cefadroxil, cefuroxime, ceftriaxone) , macrolides(erythromycin avoid estolate salt, azithromycin).

gentamycin & tobramycin only if serious infection

b) Malaria:

chloroquine, quinine (higher dose may induce labor), proguanil, pyrimethamine (with folic acid)

c) Amoebiasis:

metronidazole, diloxanide (avoid single large dose)

d) Worm infestations:

piperazine citrate, pyrantel pamoate, bephenium hydroxynaphthoate, praziquintal (teratogenic in animals not in humans)

e) Fungal infections:

miconazole, clotrimazole & nystatin

f) HIV infection:

none are safe but zidovudine and nevirapine are given to prevent vertical transmission

g) Tuberculosis:

INH and ethambutol, if 3rd needed rifampicin, pyrazinamide only in severe cases like meningitis

4. Endocrine disorders

a) Diabetes mellitus:

always use insulin

b) Hypothyroidism:

thyroxine

c) Thyrotoxicosis:

propylthiouracil

d) Corticosteroid are highly teratanogenic and must be avoided

5. Cardiovascular disorders

a) Hypertension:

α-methyl dopa oral & hydralazine in emergency

β blocker like labetolol (also can be given i.v.), atenolol

b) Thromboembolic disease:

LMWH (note just before labor)

Avoid- warfarin

6. CNS disorders

a) General anesthetics:

thiopentone sodium & nitrous oxide

b) Headache & Inflammation:

paracetamol, codeine preparation

Avoid- other NSAIDS

aspirin can be used in lowest doses upto 2 weeks before EDD

c) Epilepsy:

Avoid-Na valproate, phenytoin

d) Migraine:

paracetamol, propranolol, amitryptyline

e) Sedative :

benzodiazepine; e.g. diazepam

f) Antidepressant:

amitryptyline, imipramine

Avoid- Lithium (fetal cardiac anomalies)

7. Respiratory problems

a) cough:

codeine, dexomethorphan

b) Bronchial asthma:

β_2 agonists (e.g. salbutamol), aminophyline, disodium cromoglycate

8. Others

a) Corticosteroids must be avoided except at term for lung maturation with tocolytics
b) Fat soluble vitamins may cause subaortic stenosis & craniofacial anomalies , so avoid
c) Vaccines: only T.T.; avoid live vaccines
d) avoid indigenous drugs

Drug Use During Lactation

- Most drugs are excreted into breast milk
- But they are less quantity
- Because of large volume of distribution in mother's body compared to small amount of milk
- Lipophilic drugs are readily excreted also non plasma protein bound
- pH of milk = 7.0 , slightly acidic
- Mainly transported by passive diffusion
- Adverse reaction in fetus due to

  i. slower elimination (more accumulation)

  ii. Sensitive & idiosyncratic reaction

- TRH & metochlorpramide ↑es lactation
- Ergotamine & bromocriptine ↓es lactation

  used in loss of fetus or newborn & in hyperprolactinaemia
- Most of the drugs & vaccines are safe
- Drugs which are to be avoided or monitored are discribed next

Chloramphenicol	Significant	Concentrations too low to cause gray baby syndrome; possibility of bone marrow suppression does exist; recommend not taking chloramphenicol while breast-feeding.
Chlorothiazide	Minimal	No adverse effects reported.
Chlorpromazine	Minimal	Appears insignificant.
Codeine	Variable, based on genetic polymorphism	Safe in most cases. Neonatal toxicity described when the mother is an ultra rapid 2D6 metabolizer, producing substantially more morphine from codeine.
Diazepam	Significant	Will cause sedation in breast-fed infants; accumulation can occur in newborns.
Dicumarol	Minimal	No adverse side effects reported; may wish to follow infant's prothrombin time.
Digoxin	Minimal	Insignificant quantities enter breast milk.
Ethanol	Moderate	Moderate ingestion by mother unlikely to produce effects in infant; large amounts consumed by mother can produce alcohol effects in infant.
Heroin	Significant	Enters breast milk and can prolong neonatal narcotic dependence.
Iodine (radioactive)	Significant	Enters milk in quantities sufficient to cause thyroid suppression in infant.
Isoniazid (INH)	Minimal	Milk concentrations equal maternal plasma concentrations. Possibility of pyridoxine deficiency developing in the infant.
Kanamycin	Minimal	No adverse effects reported.
Lithium	Variable	In some cases, large amounts in milk, but not in others.
Methadone	Significant	(See heroin.) Under close physician supervision, breast-feeding can be continued. Signs of opioid withdrawal in the infant may occur if mother stops taking methadone or stops breast-feeding abruptly.
Oral contraceptives	Minimal	May suppress lactation in high doses.
Penicillin	Minimal	Very low concentrations in breast milk.
Phenobarbital	Moderate	Hypnotic doses can cause sedation in the infant.
Phenytoin	Moderate	Amounts entering breast milk are not sufficient to cause adverse effects in infant.
Prednisone	Moderate	Low maternal doses (5 mg/d) probably safe. Doses 2 or more times physiologic amounts (> 15 mg/d) should probably be avoided.
Propranolol	Minimal	Very small amounts enter breast milk.
Propylthiouracil	Variable	Rarely may suppress thyroid function in infant.
Spironolactone	Minimal	Very small amounts enter breast milk.
Tetracycline	Moderate	Possibility of permanent staining of developing teeth in the infant. Should be avoided during

Drugs used during lactation & possible side effects

DRUG INDUCED PULMONARY DISEASES

Dr .Adepu Ramesh
VCPS

DEFINITION

- Drug-induced pulmonary disease is lung disease caused by a bad reaction to a medication.

- Many types of lung injury can result from medications, and it is often impossible to predict who will develop lung disease resulting from a medication or drug.

- The clinical manifestations of Drug Induced Pulmonary diseases are difficult to find because of Non Specific Pathological changes.

- The diagnosis is based on exclusion of all other possible causes.

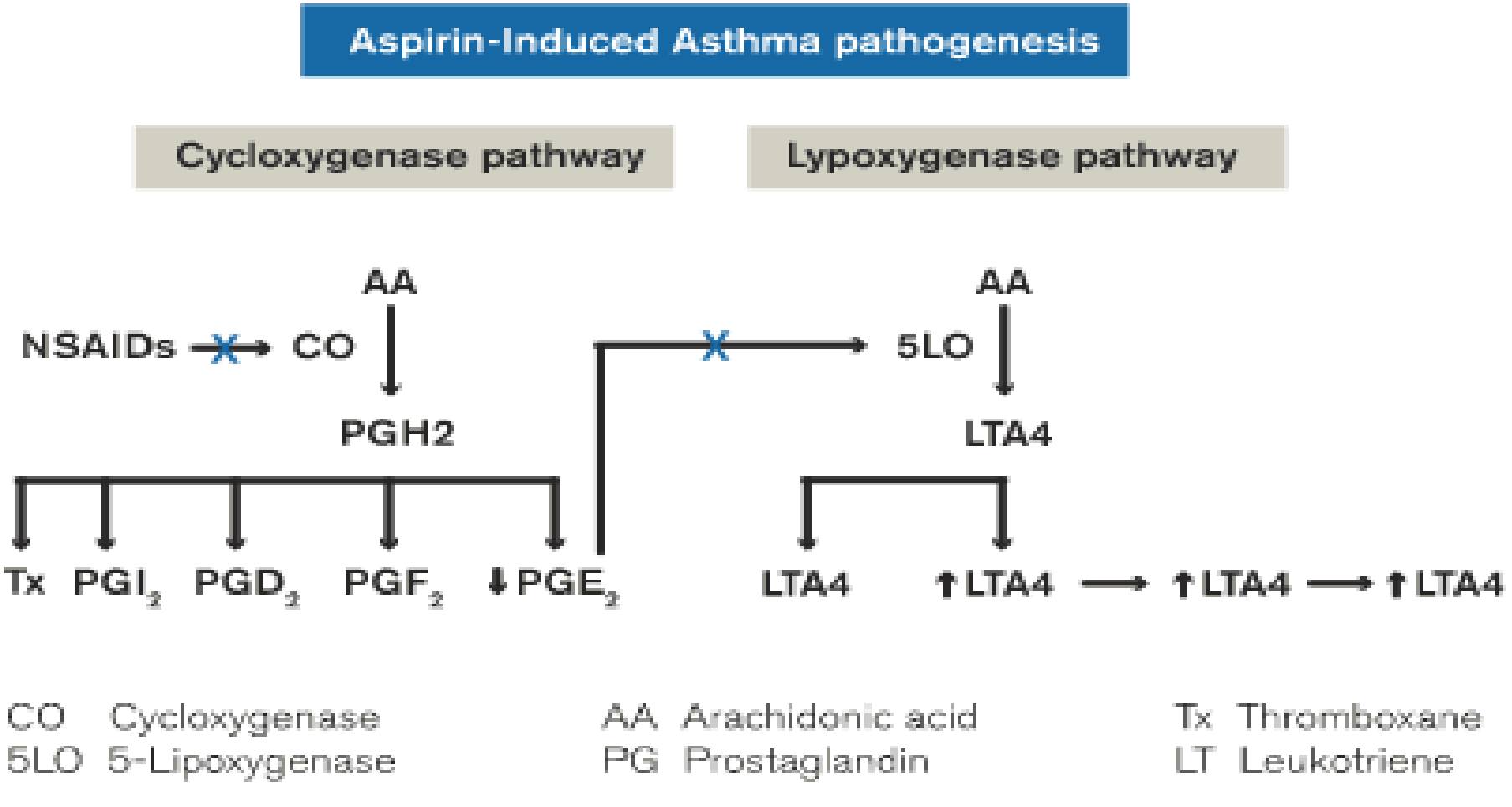
DRUGS THAT INDUCE BRONCHOSPASM

Anaphylaxis (IgE-Mediated)	
Penicillins	F[a]
Sulfonamides	F
Cephalosporins	F
Cimetidine	R
Tetracyclines	I

a=Relative frequency of reactions:
F = frequent;
I = infrequent;
R = rare.

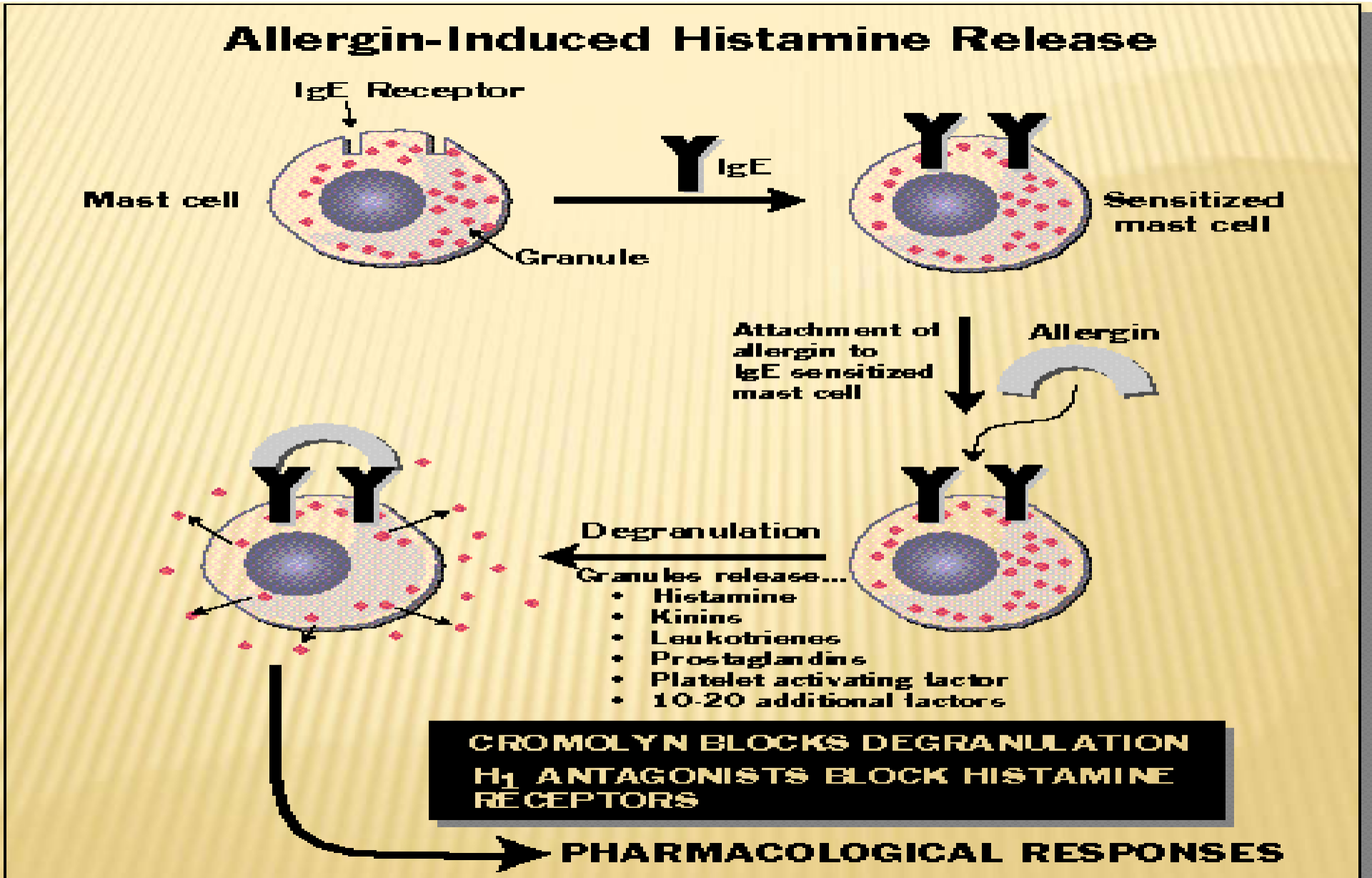
Cyclooxygenase Inhibition

Aspirin	F
Phenylbutazone	I
Acetaminophen	R



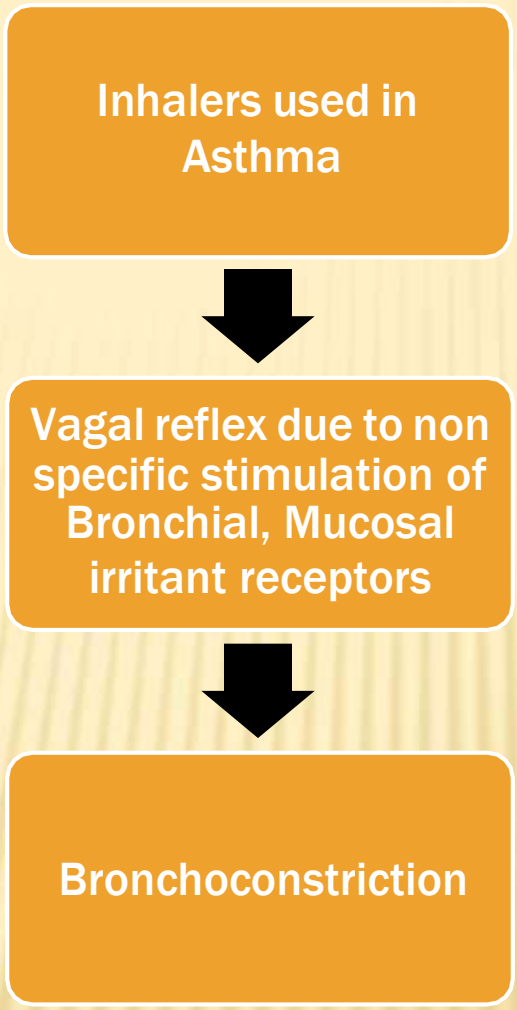
Anaphylactoid Mast-Cell Degranulation

Iodinated-radiocontrast media	F



Direct effect on Smooth Muscle	
Carbachol	
Pilocarpine	
Methacholine	
Inhibit Hydrolysis of Meditor	
Neostigmine	
Physostigmine	
Antagonism at beta receptors	
Propranalol	
Atenalol	

Reflex Bronchoconstriction :



To overcome this effect a combination of Sodium Chromoglicate and Isoprenaline should be given.

SULFITES :



Two mechanisms are involved

Sulfites
E.g. Potassium metabisulfite

Stimulation of afferent parasympathetic irritant receptors

Bronchoconstriction

IGe mediated Sulfite-sensitive anaphylactic reactions

Positive skin test due to reduced hydrolase enzyme

TREATMENT :

- Pretreatment with **Cromolyn, Anticholinergics, Vitamin B_{12}**

- **Vitamin B_{12} catalyses the oxidation of sulfite to sulfate.**

OTHER PRESERVATIVES

- EDTA : Potentiates bronchial responsiveness to Histamine mediated by calcium chelation of EDTA.
- Benzalkonium chloride : Mast cell degranulation and stimulation of irritant –C fibers in airways.
- Treatment : Cromolyn

PULMONARY OEDEMA

- Pulmonary oedema results from failure of Homeoststic mechanisms.

Etiology

- ⇧ in the Hydrostatic pressure due to left ventricular failure.
- Disrupted osmotic and oncotic pressure in vasculature.
- Integrity of alveolar epithelium.
- Interstial lymph flow.

Signs and Symptoms :

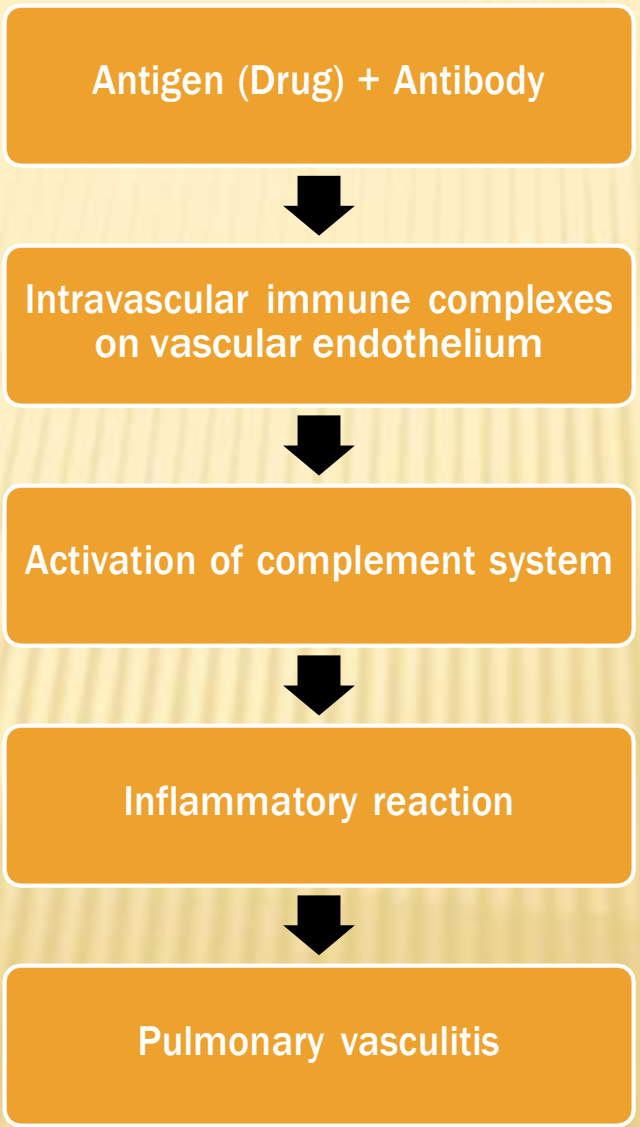
- Persistent cough
- Tachypnea
- Dyspnea
- Tachycardia
- Stiff lungs
- Hypoxemia

DRUGS

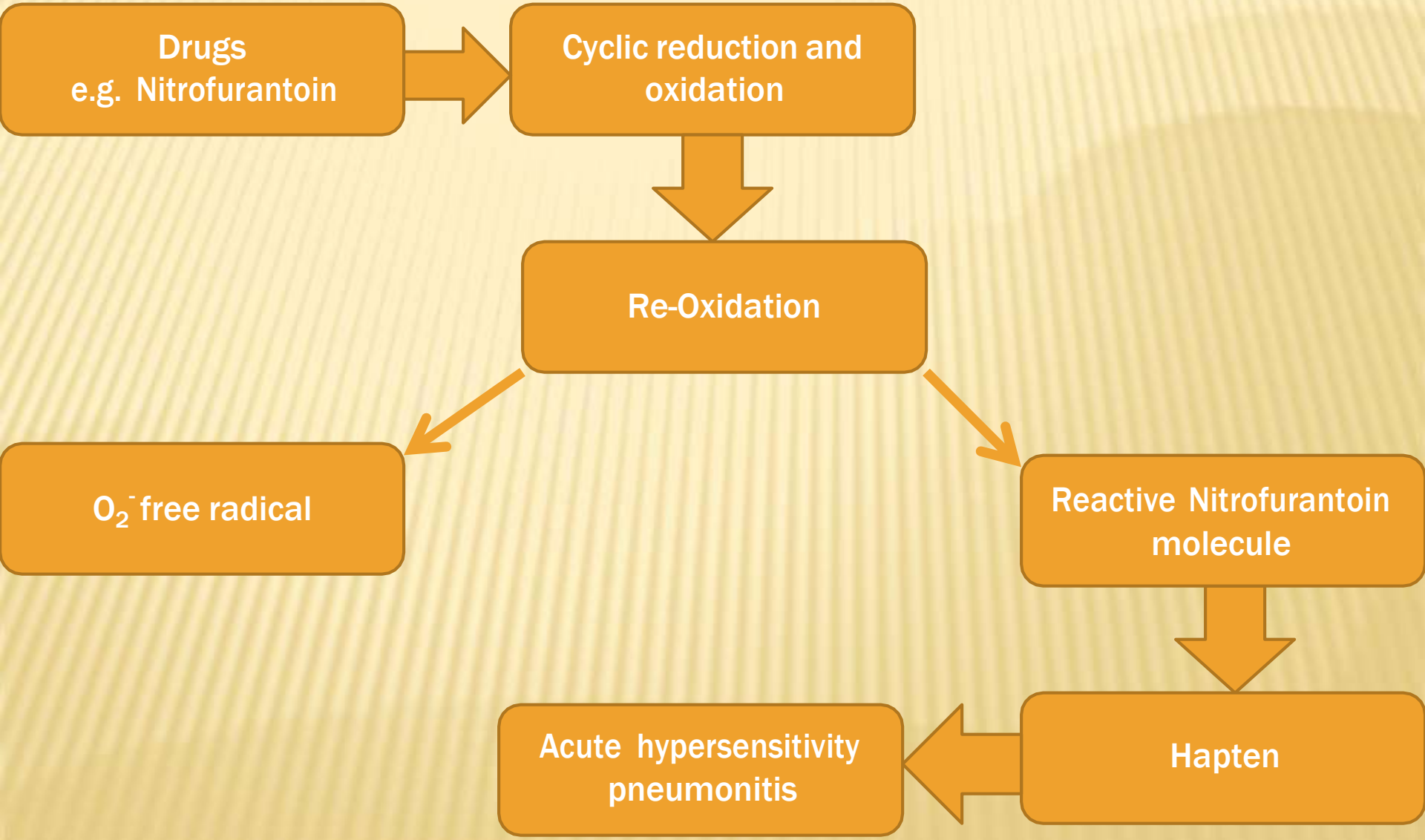
Naloxone,Codeine ⟶ Direct toxicity on alveolar capillary membrane.

PULMONARY VASCULITIS

- Results from a Immune Mechanism

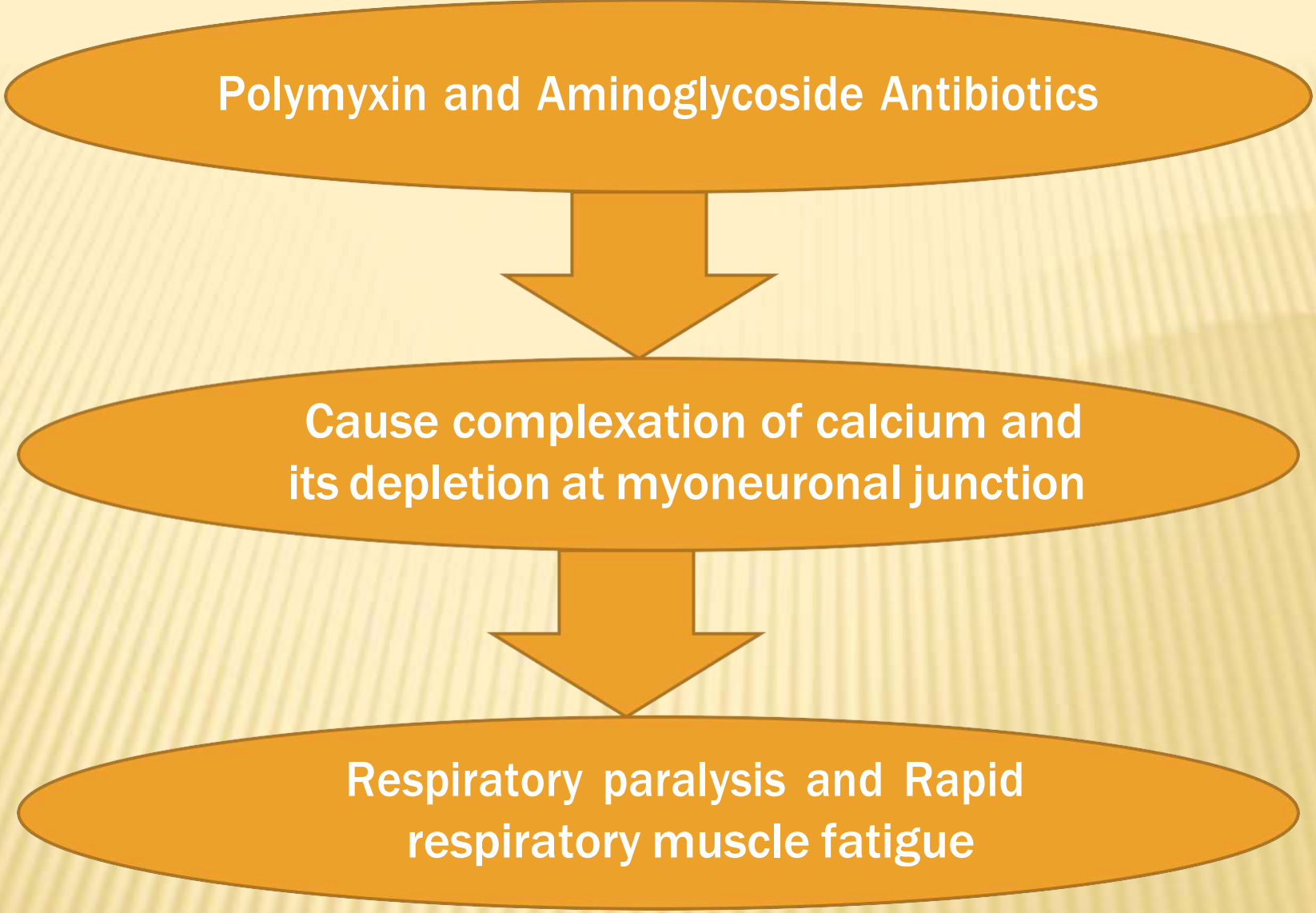


HYPERSENSITIVITY PNEUMONITIS



DRUG INDUCED APNEA

- Patients suffering with COPD, Alveolar hypoventilation, Chronic co_2 retention show exaggerated response to sedatives and narcotic analgesics.
- Class of drugs involved include
  - Benzodiazepines : e.g. midazolam
  - Aminoglycoside Antibiotics: e.g. Streptomycin, Gentamycin



IV Calcium chloride is given to reverse paralysis

INTERSTITIAL LUNG DISEASE

- Types of ILD :

a) Pulmonary Eosinophilia
b) Bronchiolitis Obliterans Organizing Pneumonia
c) Pulmonary Fibrosis
d) Diffuse alveolar damage
e) Oxidant Injury

- **Symptoms :**

  Non productive cough,

  Dyspnea,

  Low grade fever.

- **Management :**

- ✓ Withdrawal of Causative drug.
- ✓ Respiratory failure is treated with high dose of Methyl prednisolone.
- ✓ Respiratory distress treated with low dose of methyl prednisolone.
- ✓ Immunosupperesents

PULMONARY EOSINOPHILIA

- Pulmonary infiltrates with eosinophlia

- Symptoms :
  - ✓ Fever
  - ✓ Non productive cough
  - ✓ Bilateral pulmonary infiltrate
  - ✓ Lung biopsy revels perivasculitis with infiltration of eosinophils, macrophages and proteniaceous fluid in the alveoli.

BRONCHOLITIS OBLITERANS ORGANIZING PNEUMONIA

- It is the inflammation of the lungs characterized by alveolar fibrosis.
- **Symptoms :** Dyspnea, low grade fever, acute pleuretic chest pain.

Amphoterecin-B
Statins
Sulfasalazine
Amiodarone
Acebutalol

DIFFUSE ALVEOLAR HAEMORRHAFE AND DAMAGE

- Characterized by bleeding from capillaries leading to accumulation of RBCs in alveolar spaces.
- Pathogenesis :
  - ✓ Hypersensitivity reactions.
  - ✓ Direct toxicity.
  - ✓ Coagulation defects

- Drugs : Anticoagulants → Pulmonary haemorrhage
- Treatment : Withdrawal of the drug, Corticosteroids
- Chemotherapeutic agents → Direct epithelial injury and damage to alveolar capillary basement membrane.

OXYGEN TOXICITY

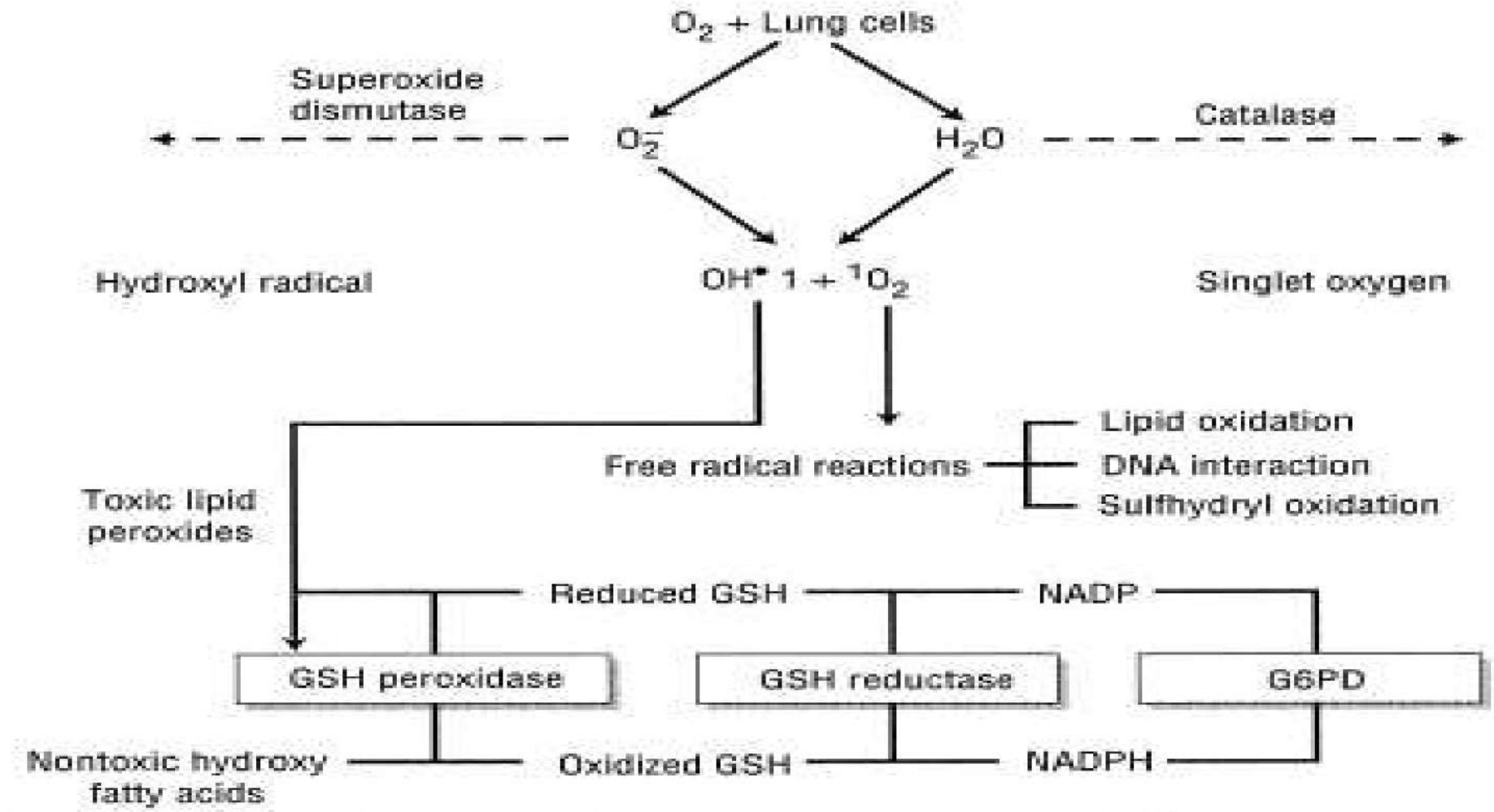


FIGURE 29–1. Schematic of the interaction of oxygen radicals and the antioxidant system. GSH = glutathione; G6PD = glucose-6-phosphate dehydrogenase; NADP = nicotinamide-adenine dinucleotide phosphate; NADPH = reduced NADP.

OXYGEN TOXICITY

- Drugs induce Lung toxicity by

- Production of oxidants

  e.g. of drugs include Bleomycin, Nitrofurantoin,Cyclophosphamide.

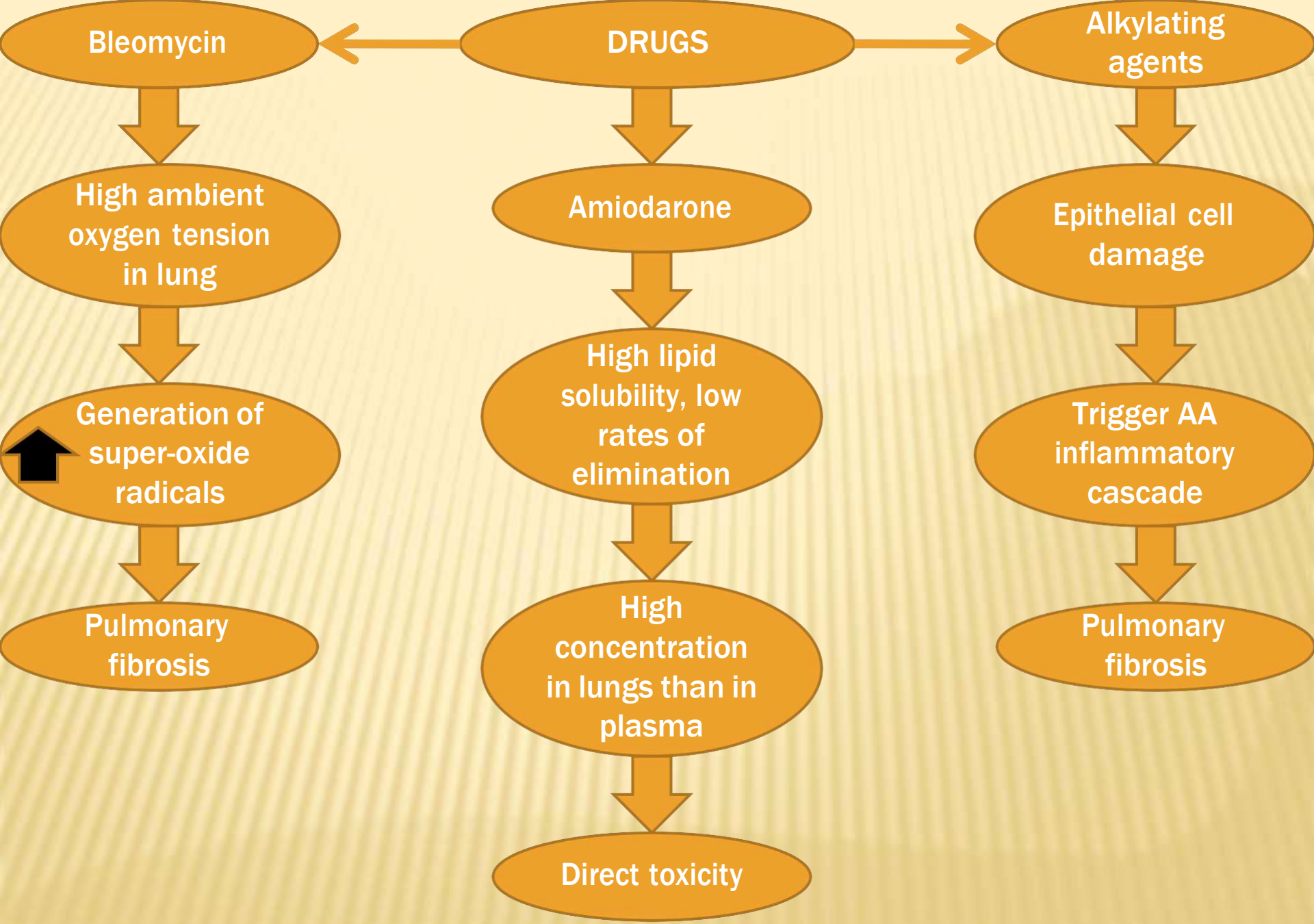
- By inhibiting antioxidant system

  e.g. of drugs include Nitrofurantoin.

PULMONARY FIBROSIS

- Predisposing factors : Cumulative dose, patient's age, Renal dysfunction, previous radiotherapy, Oxygen administration, concurrent cytotoxic therapy .

- Signs and symptoms : Dry cough, Fever, breathlessness developing and progressing over a period of several weeks or months.


DRUGS
Bleomycin
High ambient oxygen tension in lung
Generation of super-oxide radicals
Pulmonary fibrosis
Amiodarone
High lipid solubility, low rates of elimination
High concentration in lungs than in plasma
Direct toxicity
Alkylating agents
Epithelial cell damage
Trigger AA inflammatory cascade
Pulmonary fibrosis

- Amiodarone is an amphiphillic molecule, responsible for phospholipid, storage disorders in the lungs by inhidition of lysosomal phospholipases.

- **Treatment :** Prednisolone, Corticosteroid therapy in severe conditions.

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THANK YOU

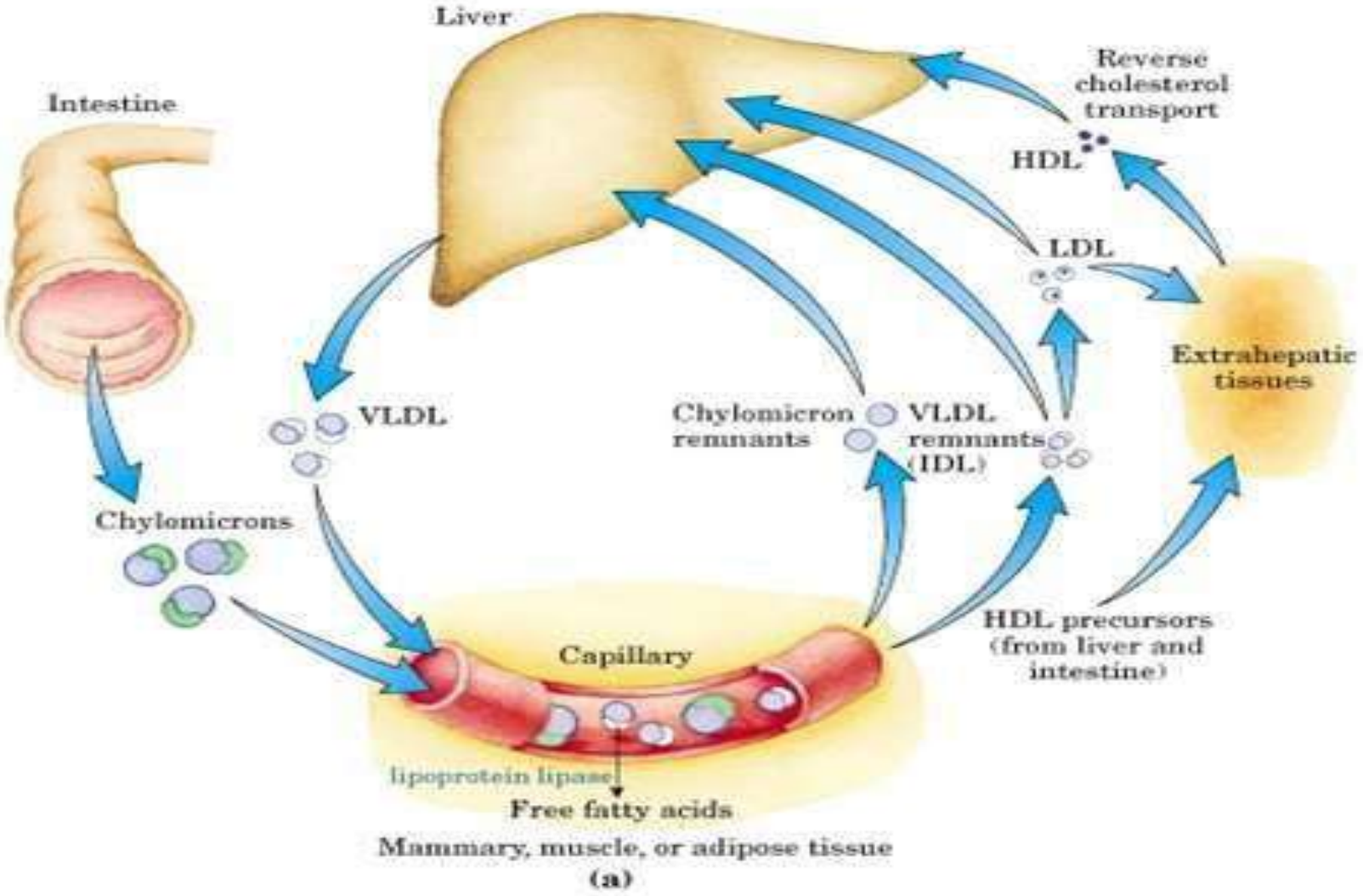
HYPERLIPIDEMIA

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DEFINITION

- Dyslipidemia is defined as **elevated** total cholesterol, LDL cholesterol, or triglycerides; a **low** HDL cholesterol; or a **combination** of these abnormalities.
- Hyperlipoproteinemia describes an increased concentration of the **lipoprotein macromolecules** that transport lipids in the plasma.
- Abnormalities of plasma lipids can result in a *predisposition to coronary, cerebrovascular, and peripheral vascular arterial disease.*

Lipoprotein Cycle:



PATHOPHYSIOLOGY

Pathophysiology:

- Lipids are transported in the bloodstream as **complexes** of lipid and proteins known as lipoproteins.
- Atherosclerosis can result from ***injury to endothelium*** accompanied with or mediated by ***oxidation; infection or immunity; or a combination of those***.
- **Oxidized** LDL provokes an **inflammatory response** mediated by a number of chemoattractants and cytokines.

Pathophysiology

- **Repeated** injury and repair within an atherosclerotic plaque eventually lead to a ***fibrous cap*** protecting the underlying core of lipids, collagen, calcium, and inflammatory cells such as T lymphocytes. **Maintenance** of the fibrous plaque is critical to **prevent plaque rupture** and *subsequent coronary thrombosis*.

Types:

- Dyslipidemia can be primary (Genetic or familial) or secondary to a medication.
- Primary disorders are classified into **six categories**.
- The types and corresponding lipoprotein **elevations** include the following:
- *I (chylomicrons), IIa (LDL), IIb (LDL + very low density lipoprotein, or VLDL), III (intermediate-density lipoprotein), IV (VLDL), and V (VLDL + chylomicrons).*

Secondary:

- Medications like:
  - *progestins, thiazide diuretics, glucocorticoids, β-blockers, isotretinoin, protease inhibitors, cyclosporine, mirtazapine, sirolimus.*

Underlying Etiology:

- The primary defect in familial hypercholesterolemia is the *inability to bind LDL to the LDL receptor* (LDL-R) or, *rarely, a defect of internalizing the LDL-R* complex into the cell after normal binding.
- This leads to **lack of LDL degradation** by cells and unregulated **biosynthesis of cholesterol**, with total cholesterol and LDL cholesterol (LDL-C) **being inversely proportional** to the deficit in LDL-Rs.

TABLE 9-2 Major Risk Factors (Exclusive of LDL Cholesterol) That Modify LDL Goals[a]

Age

Men: ≥45 years

Women: ≥55 years or premature menopause without estrogen-replacement therapy

Family history of premature CHD (definite myocardial infarction or sudden death before 55 years of age in father or other male first-degree relative or before 65 years of age in mother or other female first-degree relative)

Cigarette smoking

Hypertension (≥140/90 mm Hg or on antihypertensive medication)

Low HDL cholesterol (<40 mg/dL)[b]

CLINICAL PRESENTATION

Type I & II:

- Familial **hypercholesterolemia** is characterized by a **selective elevation in LDL** and *deposition of LDL-derived cholesterol in tendons (xanthomas) and arteries (atheromas).*
- Familial **lipoprotein lipase deficiency** is characterized by a **massive accumulation of chylomicrons** and a corresponding ***increase*** in **plasma triglycerides** .

Presentation:

- Presenting manifestations include *repeated attacks of pancreatitis and abdominal pain*, *eruptive cutaneous xanthomatosis, and hepatosplenomegaly beginning in childhood.*
- Symptom **severity** is **proportional** to *dietary fat intake, and consequently to the elevation of chylomicrons.* Accelerated **atherosclerosis** is **not associated** with this disease.

Type III:

- Patients with familial type III hyperlipoproteinemia develop the following clinical features **after age 20:**
  - Xanthoma striata palmaris (yellow discolorations of the palmar and digital creases);
  - Tuberous or tuberoeruptive xanthomas (bulbous cutaneous xanthomas); and
  - Severe atherosclerosis involving the coronary arteries, internal carotids, and abdominal aorta.

Type IV:

- It's common and occurs in adults, primarily in patients who are ***obese, diabetic, and hyperuricemic*** and ***do not have xanthomas***. It may be **secondary** to **alcohol** ingestion and can be aggravated by *stress, progestins, oral contraceptives, thiazides, or β-blockers.*

Type V :

- Type V is characterized by ***abdominal pain, pancreatitis, eruptive xanthomas, and peripheral polyneuropathy***. These patients are commonly **obese, hyperuricemic, and diabetic**; *alcohol intake, exogenous estrogens, and renal insufficiency* tend to be **exacerbating** factors. The risk of atherosclerosis is **increased** with this disorder.

DIAGNOSIS

Tests:

- A fasting lipoprotein profile including total cholesterol, LDL, HDL, and triglycerides should be measured in all adults 20 years of age or older at least once every 5 years.
- Measurement of plasma cholesterol , triglyceride, and HDL levels after a 12-hour or longer fast is **important**, because triglycerides may be **elevated in nonfasted** individuals; **total cholesterol** is only **modestly** affected by fasting.

Diagnostic Parameters:

- **Two determinations**, **1 to 8 weeks apart**, with the patient on a **stable diet and weight**, and in the **absence** of acute illness, are recommended to minimize variability and to obtain a reliable **baseline**.
- If the total cholesterol is **>200 mg/dL**, a **second** determination is recommended, and if the values are **more than 30 mg/dL** apart, the average of **three values should be used.**

TABLE 9-1 Classification of Total, LDL, and HDL Cholesterol and Triglycerides

Total cholesterol	
<200 mg/dL	Desirable
200–239 mg/dL	Borderline high
≥240 mg/dL	High
LDL cholesterol	
<100 mg/dL	Optimal
100–129 mg/dL	Near or above optimal
130–159 mg/dL	Borderline high
160–189 mg/dL	High
≥190 mg/dL	Very high
HDL cholesterol	
<40 mg/dL	Low
≥60 mg/dL	High
Triglycerides	
<150 mg/dL	Normal
150–199 mg/dL	Borderline high
200–499 mg/dL	High
≥500 mg/dL	Very high

Diagnostic Parameters:

- After a lipid abnormality is confirmed, major components of the evaluation are the **history** (including age, gender, and, if female, menstrual and estrogen replacement status), **physical examination, and laboratory investigations.**

Assessment:

- A complete history and physical examination should assess :
  - (1) Presence or absence of *cardiovascular risk factors or definite cardiovascular disease in the individual*;
  - (2) **Family history** of **<u>premature</u>** cardiovascular disease or lipid disorders;

Assessment:

- (3) Presence or absence of **secondary** causes of hyperlipidemia, including **concurrent medications**; and
- (4) Presence or absence of *xanthomas, abdominal pain, or history of pancreatitis, renal or liver disease, peripheral vascular disease, abdominal aortic aneurysm, or cerebral vascular disease (carotid bruits, stroke, or transient ischemic attack).*

Risk Factors:

- *Diabetes mellitus is regarded as a CHD risk **equivalent**.*

  That is, the presence of diabetes in patients without known CHD is associated with the same level of risk as patients without diabetes but having confirmed CHD.

Further Investigations:

- Lipoprotein electrophoresis is useful to determine which class of lipoproteins is affected; if needed.
- If the triglyceride levels are <400 mg/dL and neither type III hyperlipidemia nor chylomicrons are detected by electrophoresis, then one can calculate VLDL and LDL concentrations:
  - VLDL = triglycerides ÷ 5;
  - LDL = total cholesterol – (VLDL + HDL).
- Initial testing uses total cholesterol for case finding, but subsequent management decisions should be based on LDL.

HDL:

- Because total cholesterol is composed of cholesterol derived from ***LDL, VLDL, and HDL***, determination of HDL is useful when total plasma cholesterol is elevated.
- HDL may be ***elevated by moderate alcohol ingestion*** (fewer than two drinks per day), ***physical exercise, smoking cessation, weight loss, oral contraceptives, phenytoin, and terbutaline***.
- HDL may be **lowered** by *smoking, obesity, a sedentary lifestyle, and drugs such as β-blockers.*

DESIRED OUTCOME

- The goals of treatment are to **lower total and LDL cholesterol** in order to **reduce the risk** of first or recurrent events such as myocardial infarction, angina, heart failure, ischemic stroke, or other forms of peripheral arterial disease such as carotid stenosis or abdominal aortic aneurysm.

TREATMENT

TABLE 9-3 LDL Cholesterol Goals and Cutpoints for Therapeutic Lifestyle Changes (TLCs) and Drug Therapy in Different Risk Categories

Risk Category	LDL Goal (mg/dL)	LDL Level at Which to Initiate TLCs (mg/dL)	LDL Level at Which to Consider Drug Therapy (mg/dL)
High risk: CHD or CHD risk equivalents (10-year risk >20%)	<100 (optional goal: <70)	≥100	≥100 (<100: consider drug options)[a]
Moderately high risk: 2+ risk factors (10-year risk 10–20%)	<130	≥130	≥130 (100–129: consider drug options)
Moderate risk: 2+ risk factors (10-year risk <10%)	<130	≥130	≥160
Lower risk: 0–1 risk factor[b]	<160	≥160	≥190 (160–189: LDL-lowering drug optional)

Nonpharmacologic Therapy

Life Style Modifications:

- Therapeutic lifestyle changes are begun on the first visit and include ***dietary therapy, weight reduction, and increased physical activity***.
- Inducing a weight loss of **10% should be discussed** with patients who are overweight.
- In general, physical activity of **moderate intensity 30 minutes a day for most** days of the week should be encouraged.

Life Style Modifications:

- All patients should be counseled to stop smoking and to control hypertension.
- The objectives of dietary therapy are to progressively decrease the intake of total fat, saturated fat, and cholesterol and to achieve a desirable body weight.

Dietary Alternatives:

- Excessive dietary intake of cholesterol and saturated fatty acids leads to decreased hepatic clearance of LDL and deposition of LDL and oxidized LDL in peripheral tissues.
- Increased intake of soluble fiber in the form of oat bran, and whole grain and such; can result in useful adjunctive reductions in total and LDL cholesterol (5% to 20%).

Dietary Alternatives:

- In epidemiologic studies, ingestion of ***large amounts of cold-water oily fish was associated with a reduction in CHD risk***.
- Fish oil supplementation has a *fairly large effect in reducing triglycerides and VLDL cholesterol*, but it ***either has no effect on total and LDL cholesterol or may cause elevations in these fractions***. Other actions of fish oil may account for any cardioprotective effects.

Efficacy:

- If all recommended dietary changes were instituted, the estimated average reduction in LDL would range from 20% to 30%.

PHARMACOLOGIC THERAPY

TABLE 9-5 Effects of Drug Therapy on Lipids and Lipoproteins

Drug	Mechanism of Action	Effects on Lipids	Effects on Lipoproteins
Cholestyramine, colestipol, colesevelam	↑ LDL catabolism ↓ Cholesterol absorption	↓ Cholesterol	↓ LDL ↑ VLDL
Niacin	↓ LDL and VLDL synthesis	↓ Triglyceride ↓ Cholesterol	↓ VLDL, ↓ LDL, ↑ HDL
Gemfibrozil, fenofibrate, clofibrate	↑ VLDL dearance ↓ VLDL synthesis	↓ Triglyceride ↓ Cholesterol	↓ VLDL, ↓ LDL, ↑ HDL
Lovastatin, pravastatin, simvastatin, fluvastatin, atorvastatin, rosuvastatin	↑ LDL catabolism ↓ LDL synthesis	↓ Cholesterol	↓ LDL
Ezetimibe	Blocks cholesterol absorption across the intestinal border	↓ Cholesterol	↓ LDL

TABLE 9-6 Lipoprotein Phenotype and Recommended Drug Treatment

Lipoprotein Type	Drug of Choice	Combination Therapy
I	Not indicated	–
IIa	Statins	Niacin or BARs
	Cholestyramine or colestipol	Statins or niacin
	Niacin	Statins or BARs
		Ezetimibe
IIb	Statins	BARs, fibrates, or niacin
	Fibrates	Statins or niacin or BARs[a]
	Niacin	Statins or fibrates
		Ezetimibe
III	Fibrates	Statins or niacin
	Niacin	Statins or fibrates
		Ezetimibe
IV	Fibrates	Niacin
	Niacin	Fibrates
V	Fibrates	Niacin
	Niacin	Fish oils

Dosages of Selected Lipid-Modulating Drugs

Drug	Initial Dosage	Usual Dosage	Maximal Dosage	Comment
Cholestyramine	4 g before main meal	4 g BID before heaviest meals	8 g BID before heaviest meals	May prescribe 24 g/d, but few patients can tolerate.
Colestipol	5 g of powder or 2 g of tablets every day before main meal	5 g of powder or 4 g of tablets BID before heaviest meals	10 g of powder or 8 g of tablets BID before heaviest meals	May prescribe 30 g of powder per day, but few patients can tolerate.
Colesevelam	6 × 0.63-g tablets/d	Same	7 × 0.63-g tablets/d	Less bulk is associated with less gastrointestinal intolerance.
Niaspan	500 mg QHS	1,000–2,000 mg QHS	2,000 mg QHS	Increase dose by 500 mg daily every 4 weeks.
Atorvastatin	10–40 mg every day	10–40 mg every day	80 mg every day	Administer any time of day.
Fluvastatin	20–40 mg QHS	20–40 mg QHS	40 mg BID 80 mg XL every day	Modified-release form (XL) has similar efficacy but has less bioavailability (and less risk of adverse effects).
Lovastatin	20 mg with dinner	20–40 mg with dinner	40 mg BID	Administration with food increases bioavailability. BID dosing provides greater LDL-C–lowering efficacy than every day.
Pitavastatin	1–2 mg every day	1–2 mg every day	4 mg every day	Administer any time of the day with or without food.
Pravastatin	10–40 mg every day	10–40 mg every day	80 mg every day	Administer with food to reduce dyspepsia.
Rosuvastatin	10–20 mg every day	10–20 mg every day	40 mg every day	Administer any time of the day.
Simvastatin	20–40 mg every PM	20–40 mg every PM	80 mg every PM[b]	Administer with food to reduce dyspepsia.
Gemfibrozil	600 mg BID	Same	Same	
Fenofibrate[a]	67–201 mg every day	Same	201 mg every day	

[a] Multiple formulations are available and doses do vary.

[b] Restricted to patients who have been taking simvastatin 80 mg chronically (e.g., for 12 months or more) without evidence of muscle toxicity. For more information regarding simvastatin, please see http://www.fda.gov/Drugs/DrugSafety/ucm256581.htm.

BID, twice daily; LDL-C, low-density lipoprotein cholesterol; QHS, every evening at bedtime.

HMG-CoA Reductase Inhibitors

- **Agents: (Atorvastatin, Fluvastatin, Lovastatin, Pravastatin, Rosuvastatin, Simvastatin)**
- Statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, interrupting the conversion of HMG-CoA to mevalonate, the rate-limiting step in de novo cholesterol biosynthesis.
- **Reduced** synthesis of LDL and **enhanced** catabolism of LDL mediated through **LDL-Rs** appear to be the principal mechanisms for lipid-lowering effects.

Table 5. High- Moderate- and Low-Intensity Statin Therapy (Used in the RCTs reviewed by the Expert Panel)*

High-Intensity Statin Therapy	Moderate-Intensity Statin Therapy	Low-Intensity Statin Therapy
Daily dose lowers LDL–C on average, by approximately ≥50%	Daily dose lowers LDL–C on average, by approximately 30% to <50%	Daily dose lowers LDL–C on average, by <30%
Atorvastatin (40†)–80 mg **Rosuvastatin 20 *(40)* mg**	**Atorvastatin 10 *(20)* mg** **Rosuvastatin (*5*) 10 mg** **Simvastatin 20–40 mg‡** **Pravastatin 40 *(80)* mg** **Lovastatin 40 mg** *Fluvastatin XL 80 mg* **Fluvastatin 40 mg bid** *Pitavastatin 2–4 mg*	*Simvastatin 10 mg* **Pravastatin 10–20 mg** **Lovastatin 20 mg** *Fluvastatin 20–40 mg* *Pitavastatin 1 mg*

Specific statins and doses are noted in bold that were evaluated in RCTs (17,18,46-48,64-67,69-78) included in CQ1, CQ2 and the CTT 2010 meta-analysis included in CQ3 (20). All of these RCTs demonstrated a reduction in major cardiovascular events. Statins and doses that are approved by the U.S. FDA but were not tested in the RCTs reviewed are listed in *italics*.

*Individual responses to statin therapy varied in the RCTs and should be expected to vary in clinical practice. There might be a biologic basis for a less-than-average response.

†Evidence from 1 RCT only: down-titration if unable to tolerate atorvastatin 80 mg in IDEAL (47).

‡Although simvastatin 80 mg was evaluated in RCTs, initiation of simvastatin 80 mg or titration to 80 mg is not recommended by the FDA due to the increased risk of myopathy, including rhabdomyolysis.

bid indicates twice daily; FDA, Food and Drug Administration; IDEAL, Incremental Decrease through Aggressive Lipid Lowering study; LDL–C, low-density lipoprotein cholesterol; and RCTs, randomized controlled trials.

Formulation, dosing, and common adverse effects of lipid-lowering drugs

Lipid-Lowering Drug	Dosage Forms	Usual Adult Maintenance Dose Range	Adverse Effects
Statins			
Atorvastatin	10, 20, 40, 80 mg tablets	10 to 80 mg once daily (at any time of day	Most frequent side effects are constipation, abdominal pain, diarrhea, dyspepsia, and nausea. Statins should be discontinued promptly if serum transaminase levels (liver function tests) rise to 3 times upper limit of normal, or if patient develops signs or symptoms of myopathy.
Fluvastatin	20, 40 mg capsules; 80 mg extended-release tablets	20–40 mg/day as a single dose (evening) or 40 mg twice daily; 80 mg once daily (evening)	
Lovastatin	10, 20, 40 mg tablets	10 to 80 mg/day as a single dose (with evening meal) or divided twice daily with food	Approximate equivalent doses of HMG-CoA reductase inhibitors are: atorvastatin 10 mg, fluvastatin 80 mg, lovastatin 40 mg, pravastatin 40 mg, simvastatin 20 mg, and rosuvastatin 5 mg.
Lovastatin ER	20, 30, 60 mg tablets	20 to 60 mg/day as a single dose	
Pravastatin	10, 20, 40, 80 mg tablets	10 to 80 mg/day as a single dose at bedtime	
Rosuvastatin	5, 10, 20, 40 mg tablets	5–40 mg/day (at any time of day). 40 mg reserved for those who don't achieve LDL cholesterol goal on 20 mg	
Simvastatin	5, 10, 20, 40, 80 mg tablets	5–80 mg/day as a single dose in the evening, or divided	

Efficacy:

- When used as **monotherapy**, statins are ***the most potent total and LDL cholesterol-lowering agents*** and among the **best tolerated**. Total and LDL cholesterol are reduced in a ***dose-related fashion by 30% or more*** when added to ***dietary*** therapy.

Combinations:

- Combination therapy with a **statin and BAR** is rational because numbers **of LDL-Rs are increased**, leading to **greater degradation of LDL cholesterol**; intracellular **synthesis** of cholesterol is inhibited; and **enterohepatic** recycling of bile acids is **interrupted**.

Combinations:

- Combination therapy with a statin and ezetimibe is also rational because ezetimibe inhibits **cholesterol absorption** across the gut border and adds **12% to 20% further reduction** when combined with a statin or other drugs.

Adverse Reactions:

- **Constipation** occurs in fewer than 10% of patients taking statins. Other adverse effects include **elevated serum aminotransferase levels** (primarily alanine aminotransferase), elevated **creatine kinase** levels, **myopathy, and rarely rhabdomyolysis.**

Bile Acid Resins (BARs):

- Agents: **(Cholestyramine, Colestipol, Colesevelam)**
- The primary action of BARs is to **bind bile acids** in the **intestinal** lumen, with a concurrent interruption of **enterohepatic circulation** of bile acids, which decreases the bile acid pool size and **stimulates hepatic synthesis of bile acids from cholesterol**.

MOA:

- **Depletion** of the hepatic pool of cholesterol results in an **increase in cholesterol biosynthesis** and an increase in the number of **LDL-Rs on the hepatocyte** membrane, which stimulates an enhanced rate of **catabolism** from plasma and lowers LDL levels.
- The increase in hepatic cholesterol biosynthesis may be paralleled by **increased hepatic VLDL production**, and, consequently, BARs may **aggravate hypertriglyceridemia** in patients with **combined** hyperlipidemia.

Indications & SE:

- BARs are useful in treating **primary hypercholesterolemia** (familial hypercholesterolemia, familial combined hyperlipidemia, type IIa hyperlipoproteinemia).
- GI complaints of *constipation, bloating, epigastric fullness, nausea, and flatulence* are most commonly reported. These adverse effects can be **managed by increasing fluid intake**, modifying the diet to **increase bulk, and using stool softeners**.

Administration:

- The gritty texture and bulk may be **minimized by mixing the powder with orange drink or juice.**
- Colestipol may have **better palatability** than cholestyramine because it is **odorless and tasteless**. Tablet forms should help improve **adherence** with this form of therapy.

Adverse effects:

- Impaired absorption of fat-soluble vitamins A, D, E, and K; hypernatremia and hyperchloremia; GI obstruction; and reduced bioavailability of acidic drugs such as *warfarin, nicotinic acid, thyroxine, acetaminophen, hydrocortisone, hydrochlorothiazide, loperamide, and possibly iron*.
- Drug interactions may be avoided by **alternating administration times with an interval of 6 hours or greater** between the BAR and other drugs.

Niacin

- **Niacin** (nicotinic acid) reduces the hepatic synthesis of VLDL, which in turn leads to a ***reduction in the synthesis of LDL.***
- Niacin also **increases HDL** by reducing its catabolism.
- The principal use of niacin is **for mixed hyperlipidemia** or as a ***second-line agent in combination therapy for hypercholesterolemia***.
- It is a ***first-line agent*** *or alternative for the treatment of* ***hypertriglyceridemia and diabetic dyslipidemia***.

Adverse Reactions:

- Niacin has many common adverse drug reactions; most of the symptoms and biochemical abnormalities seen ***do not require discontinuation of therapy.***
- Cutaneous flushing and itching appear to be ***prostaglandin mediated*** and can be **reduced** by taking *aspirin 325 mg shortly before niacin ingestion.*

Adverse Reactions:

- Taking the niacin dose **with meals** and slowly titrating the dose upward may **minimize** these effects. Concomitant ***alcohol and hot drinks may magnify the flushing and pruritus from niacin***, and they should ***be avoided at the time of ingestion***. GI intolerance is also a common problem.
- Potentially important laboratory abnormalities occurring with niacin therapy include *elevated liver function tests, hyperuricemia, and hyperglycemia.*

Adverse Reactions:

- ***Niacin-associated hepatitis*** is more common with ***sustained-release preparations***, and their use should be **restricted** to patients **intolerant** of regular-release products.
- Niacin is **<u>contraindicated</u>** in patients with **active liver disease**, and it may **exacerbate** preexisting **gout and diabetes**.
- Nicotinamide should **<u>not</u>** be used in the treatment of hyperlipidemia because it **does not effectively lower cholesterol or triglyceride levels.**

Fibric Acids

- **Agents: (Gemfibrozil, Fenofibrate, Clofibrate)**
- Fibrate **monotherapy** is effective in **reducing VLDL**, but a reciprocal **rise in LDL may occur** and total **cholesterol** values may remain relatively **unchanged**.
- Plasma HDL concentrations **may rise 10% to 15%** or more with fibrates.

Efficacy:

- Gemfibrozil **reduces** the synthesis of **VLDL** and, to a lesser extent, **apolipoprotein B** with a concurrent **increase** in the rate of **removal of triglyceride-rich lipoproteins** from plasma.
- Clofibrate is **less effective** than gemfibrozil or niacin in reducing VLDL production.

Adverse Reactions:

- GI complaints , rash, dizziness, and transient elevations in transaminase levels and alkaline phosphatase, respectively.
- Clofibrate and, less commonly, gemfibrozil may enhance the formation of **gallstones**.

Adverse Reactions:

- A myositis syndrome of **myalgia**, **weakness, stiffness, malaise**, and **elevations in CK and AST** may occur and seems to be more common in patients with ***renal insufficiency.***
- **Fibrates** may **potentiate** the effects of **oral anticoagulants**, and the INR should be **monitored very closely** with this combination.

Ezetimibe

- **Ezetimibe** interferes with the **absorption** of cholesterol from the brush border of the intestine, a novel mechanism that makes it a good choice for **adjunctive therapy**. It is approved as both **monotherapy and for use with a statin**. The dose is **10 mg once** daily, given **with or without** food. When used alone, it results in an approximate **18% reduction in LDL cholesterol**. When added to a statin, ezetimibe lowers LDL by about an **additional 12% to 20%.**

Uses & SE:

- A combination product containing ezetimibe 10 mg and simvastatin 10, 20, 40, or 80 mg is available.
- Ezetimibe is well tolerated; approximately 4% of patients experience **GI upset**.
- Because cardiovascular outcomes with ezetimibe have **not been evaluated,** it should be reserved for *patients unable to tolerate statin therapy or those who do not achieve satisfactory lipid lowering with a statin alone*.

Fish Oil Supplementation

- Diets high in omega-3 polyunsaturated fatty acids (from fish oil), *reduce cholesterol, triglycerides, LDL, and VLDL and may elevate HDL cholesterol.*
- Fish oil supplementation may be most **useful in patients with hypertriglyceridemia**, but its role in treatment is **not well defined.**

Uses & SE:

- **Lovaza (omega-3-acid ethyl esters)** is a prescription form of concentrated fish oil EPA 465 mg and DEA 375 mg.
- The daily dose is 4 g/day, which can be taken as four 1-g capsules once daily or two 1-g capsules twice daily. This product lowers ***triglycerides by 14% to 30% and raises HDL by about 10%.***
- Complications of fish oil supplementation such as ***thrombocytopenia and bleeding disorders have been noted, especially with high doses*** (EPA, 15 to 30 g/day).

Combination Drug Therapy

- Combination therapy may be considered **after adequate trials of monotherapy** and for patients documented to be **adherent** to the prescribed regimen.
- **Two or three** lipoprotein profiles **at 6-week intervals** should confirm **lack of response** prior to initiation of combination therapy.

Monitoring & Efficacy:

- Contraindications to and drug interactions with combined therapy should **be screened carefully**, and the **extra cost** of drug **product and monitoring** should be **considered**.
- In general, a **statin** plus a **BAR** or **niacin** plus a **BAR** provide the **greatest reduction** in total and LDL cholesterol.

Monitoring & Efficacy:

- Regimens intended to **increase** HDL levels should include either **gemfibrozil** or **niacin**, bearing in mind that **statins** combined **with either** of these drugs may result in a **greater incidence of hepatotoxicity or myositis.**
- Familial combined hyperlipidemia may ***respond better to a fibrate and a statin*** than to a **fibrate and a BAR**.

Treatment Of Hypertriglyceridemia:

- Lipoprotein pattern types I, III, IV, and V are associated with **hypertriglyceridemia**, and these primary lipoprotein disorders should be **excluded prior to implementing therapy.**
- A family history **positive for CHD is important** in identifying patients at risk for **premature atherosclerosis**.
- If a patient with CHD has elevated triglycerides, the associated abnormality is ***probably a contributing factor to CHD and should be treated.***

Treatment Of Hypertriglyceridemia:

- High serum triglycerides should be treated by achieving **desirable body weight**, consumption of **a low saturated fat and cholesterol diet, regular exercise, smoking cessation, and restriction of alcohol** .
- Secondary therapeutic target in persons with high triglycerides is the sum of LDL and VLDL (termed *non-HDL* [total cholesterol – HDL]).

Considerations:

- The goal for non-HDL with high serum **triglycerides is set at 30 mg/d**L higher than that for LDL on the premise that **a VLDL level of 30 mg/dL or less is normal.**
- Drug therapy with **niacin** should be considered in patients with **borderline- high triglycerides <u>but</u>** with *accompanying risk factors of established CHD, family history of premature CHD, concomitant LDL elevation or low HDL, and genetic forms of hypertriglyceridemia associated with CHD.*

Considerations:

- Niacin may be used **cautiously** in persons with **diabetes** ; it can cause a slight increase in glucose and no change in hemoglobin A1C. Alternative therapies include **gemfibrozil, statins**, and **fish oil**.
- The goal of therapy is to **lower triglycerides and VLDL** particles that may be **atherogenic**, **increase HDL, and reduce LDL.**

Considerations:

- Very high triglycerides are associated with **pancreatitis** and other adverse consequences. Management includes *dietary fat restriction (10% to 20% of calories as fat), weight loss, alcohol restriction, and treatment of coexisting disorders* (e.g., diabetes).
- Drug therapy includes **gemfibrozil, niacin**, and higher-potency statins (**atorvastatin, rosuvastatin**, and **simvastatin**).

TREATMENT OF LOW HDL CHOLESTEROL

- Low HDL cholesterol is a **strong independent risk predictor of CHD**. In low HDL, the primary target remains **LDL**, but treatment *emphasis shifts to weight reduction, increased physical activity, smoking cessation, and to* ***fibrates*** *and* ***niacin*** *if drug therapy is required*.

TREATMENT OF DIABETIC DYSLIPIDEMIA

- It's characterized by **hypertriglyceridemia**, **low HDL, and minimally elevated LDL.** Small, dense LDL (pattern B) in diabetes is **more atherogenic** than larger, more buoyant forms of LDL (pattern A).
- Diabetes is a CHD risk equivalent, and the primary target is to **lower the LDL to <100 mg/dL**. When LDL is >130 mg/dL, most patients *require simultaneous therapeutic lifestyle changes and drug therapy.*

Treatment:

- When LDL is between 100 and 129 mg/dL, *intensifying glycemic control, adding drugs* for atherogenic dyslipidemia (**fibrates, niacin**), and *intensifying LDL-lowering therapy* are options. **Statins** are considered by many to be the **drugs of choice** because the primary target is LDL.

GASTRO OESOPHAGEAL REFLUX DISEASE

Dr.Rasheduddin
VCPS

Def:

- Gastro-oesophageal reflux disease is the term used to describe a histopathological alteration resulting from episodes of reflux of acid , pepsin and occasionally bile into the oesophagus from the stomach.

TABLE 34–1 Foods and Medications That May Worsen GERD Symptoms

Decreased lower-esophageal sphincter pressure	
Foods	
Fatty meal	Garlic
Carminatives (peppermint, spearmint)	Onions
Chocolate	Chili peppers
Coffee, cola, tea	
Medications	
Anticholinergics	Ethanol
Barbiturates	Nicotine (smoking)
Caffeine	Nitrates
Dihydropyridine calcium channel blockers	Progesterone
Dopamine	Tetracycline
Estrogen	Theophylline
Direct irritants to the esophageal mucosa	
Foods	
Spicy foods	Tomato juice
Orange juice	Coffee
Medications	
Alendronate	Iron
Aspirin	Quinidine
Nonsteroidal antiinflammatory drugs	Potassium chloride

- Pathogenesis :

- abnormal reflux of gastric contents from the stomach into the esophagus.

- Decreased gastroesophageal sphincter pressures related to spontaneous transient LES relaxations, transient increases in intraabdominal pressure, all of which may lead to the development of gastroesophageal reflux.

- Other factors : mucosal resistance, gastric emptying, epidermal growth factor, and salivary buffering.
- Abnormal oesophageal acid clearence.
- Endoscopy-negative reflux disease : GORD with normal endoscopy.
- Hiatus hernia

- ***LOWER ESOPHAGEAL SPHINCTER PRESSURE***

Esophageal distention, vomiting, belching	straining, bending over, coughing, eating
↓	↓
spontaneous transient LES relaxations	Stress reflux
↓	↓
defective LES pressure not associated with swallowing	increase in intra-abdominal pressure
↓	↓
GERD	GERD

ANATOMIC FACTORS

- The size of a hiatal hernia is proportional to the frequency of transient LES relaxations. Patients with hypotensive LES pressures and large hiatal hernias are more likely to experience gastroesophageal reflux following abrupt increases in intraabdominal pressure compared to patients with a hypotensive LES and no hiatal hernia.

ESOPHAGEAL CLEARANCE

- Common in elderly patients and patients with Sjögren's syndrome or xerostomia.

- Depends on duration of contact of acid with oesophageal mucosa This contact time is, in turn, dependent on the rate at which the oesophageous clears the noxious material, as well as the frequency of reflux.

MUCOSAL RESISTANCE

- defect in the normal mucosal defenses,hydrogen ions diffuse into the mucosa, leading to the cellular acidification and necrosis that ultimately cause esophagitis.

- Intheory, mucosal resistance may be related not only to esophagealmucus, but also to tight epithelial junctions, epithelial cell turnover, mucosal blood flow, tissue prostaglandins

Signs and Symptoms

- Typical symptoms :
  - Heart burn
  - Water brash
  - Regurgitation
  - Belching
- Atypical symptoms :
  - Non-allergic asthma
  - Chronic cough
  - Pharyngitis
  - Chest pain
  - Dental erosions

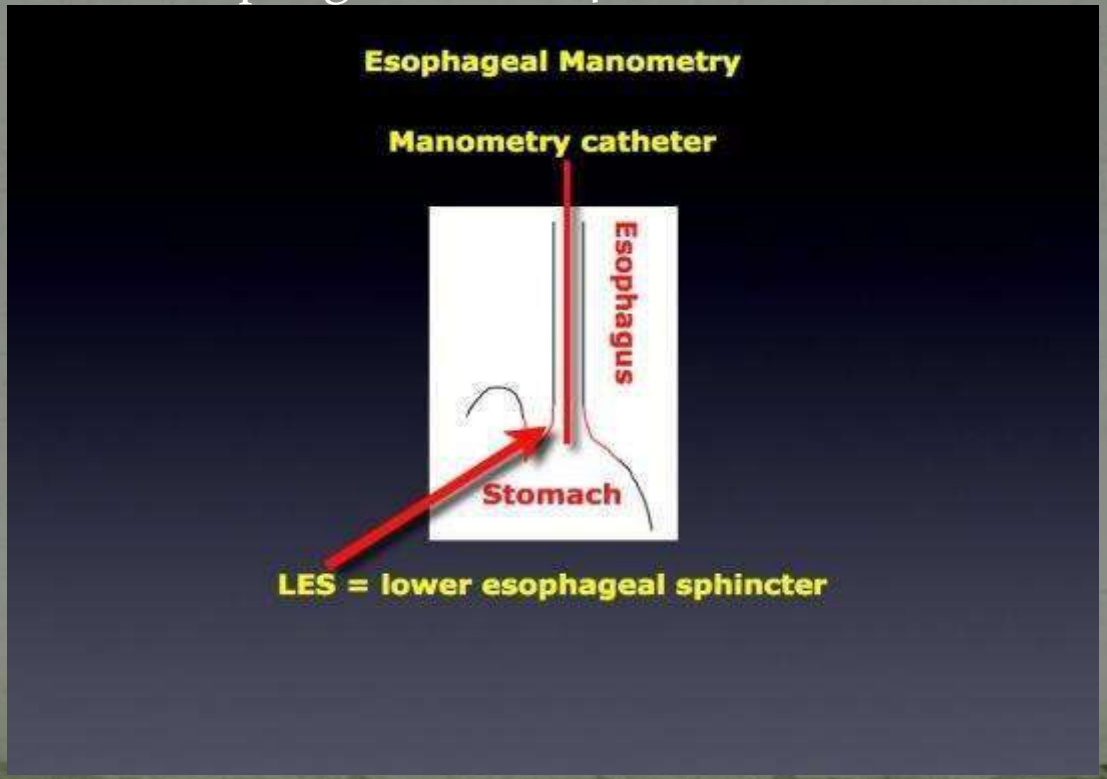
Cont…

- Alarm symptoms :
  - Dysphagia
  - Unexplained weight loss
  - Choking
  - Continual pain
  - Odynophagia

Diagnosis

1. **Ambulatory pH Monitoring :**

A small tube called a catheter, with an acid sensor at its tip, is inserted through the nose and positioned in the esophagus. The other end travels down to the waist after exiting from the nose and then attaching to a recorder. The recorder records every reflux episode in the esophagus and a 24 hour frame of data is analyzed.



- **Acid Perfusion Test or Bernstein Test:**

- **Esophageal Motility Testing:**
  Motility testing of the esophagus determines the working of muscles of the esophagus by passing a catheter through a nostril down the throat into the esophagus. The catheter contains a sensor to detect pressure inside the esophagus and the other end is attached to a recorder. The patient is then permitted to swallow sips of water to record and evaluate the esophageal contraction movements.

Therapeutic goals

- Patients should be assessed for symptoms, such as heartburn and for signs and symptoms of complications (e.g., dysphagia)that require immediate medical attention.

- The goals of GERD treatment are to alleviate symptoms, decrease the frequency of recurrent disease, promote healing of mucosal injury, and prevent complications.

- Many patients with GERD will relapse if medication is withdrawn,so long-term maintenance treatment may be required.A proton pump inhibitor is the drug of choice for maintenance of patients with moderate to severe GERD.

GENERAL APPROACH TO TREATMENT

- Lifestyle modifications and patient-directed therapy
- Pharmacologic intervention with prescription-strength acid suppression therapy
- Interventional therapies (ant reflux surgery or endoscopic therapies

TABLE 34-5 Nonpharmacologic Treatment of GERD with Lifestyle Modifications

- Elevate the head of the bed (increases esophageal clearance). Use 6- to 8-inch blocks under the head of the bed. Sleep on a foam wedge.
- Dietary changes
  - Avoid foods that may decrease lower esophageal sphincter pressure (fats, chocolate, alcohol, peppermint, and spearmint)
  - Avoid foods that have a direct irritant effect on the esophageal mucosa. (spicy foods, orange juice, tomato juice, and coffee)
  - Include protein-rich meals in diet (augments lower esophageal sphincter pressure)
  - Eat small meals and avoid eating immediately prior to sleeping (within 3 hours if possible; decreases gastric volume)
  - Weight reduction (reduces symptoms)
- Stop smoking (decreases spontaneous esophageal sphincter relaxation)
- Avoid alcohol (increases amplitude of the lower esophageal sphincter, peristaltic waves, and frequency of contraction)
- Avoid tight-fitting clothes
- Discontinue, if possible, drugs that may promote reflux (calcium channel blockers, β-blockers, nitrates, theophylline)
- Take drugs that have a direct irritant effect on the esophageal mucosa with plenty of liquid if they cannot be avoided (bisphosphonates, tetracyclines, quinidine, and potassium chloride, iron salts, aspirin, nonsteroidal antiinflammatory drugs)

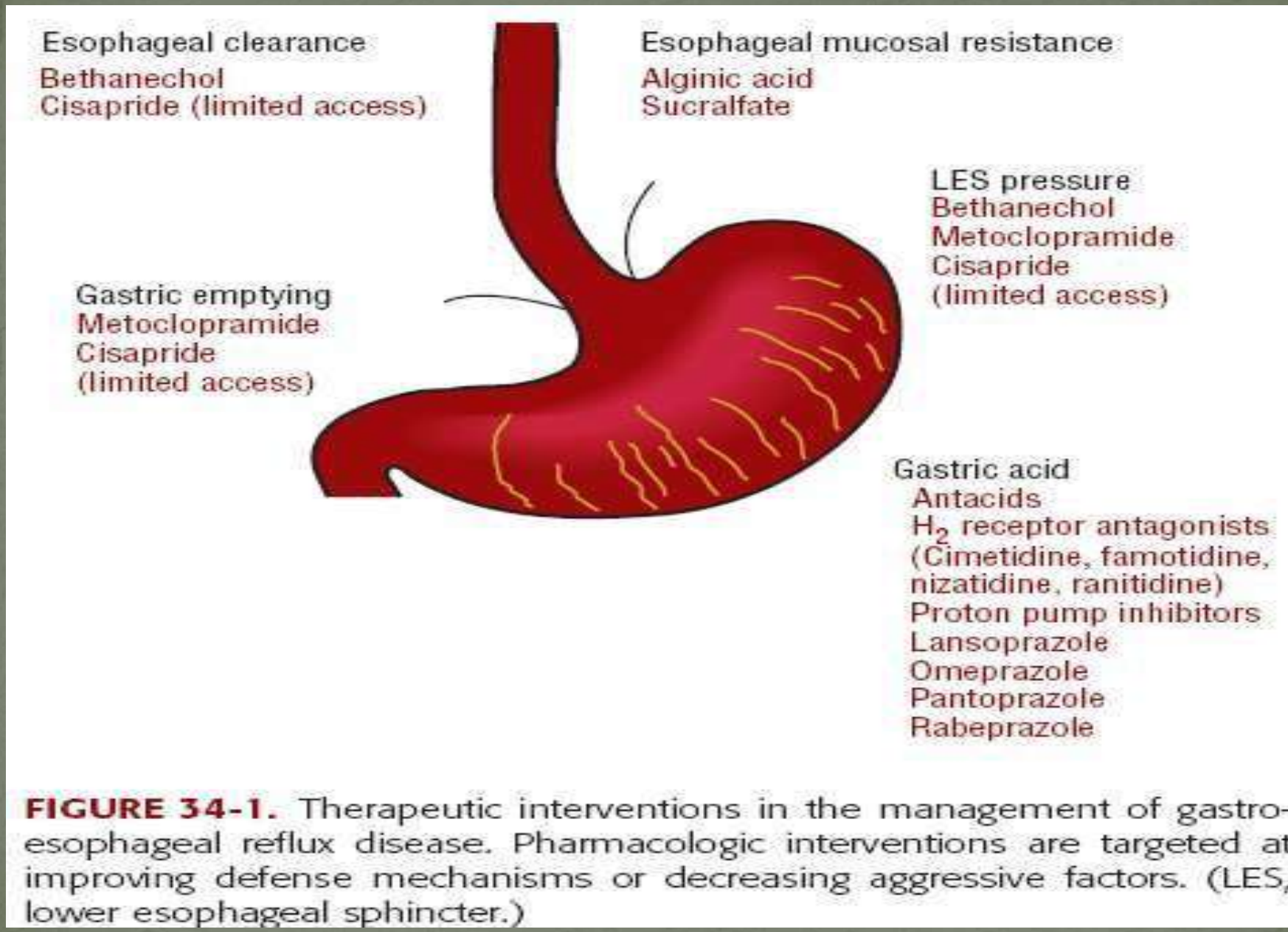


FIGURE 34-1. Therapeutic interventions in the management of gastroesophageal reflux disease. Pharmacologic interventions are targeted at improving defense mechanisms or decreasing aggressive factors. (LES, lower esophageal sphincter.)

TABLE 34-4 Therapeutic Approach to GERD in Adults

Patient Presentation	Recommended Treatment Regimen	Comments
Intermittent, mild heartburn	**Lifestyle modifications** **plus** **patient-directed therapy** Antacids • Maalox or Mylanta 30 mL as needed or after meals and at bedtime • Gaviscon 2 tabs after meals and at bedtime • Calcium carbonate 500 mg, 2–4 tablets as needed **and/or** Nonprescription H_2-receptor antagonists (taken up to twice daily) • Cimetidine 200 mg • Famotidine 10 mg • Nizatidine 75 mg • Ranitidine 75 mg **or** Nonprescription proton pump inhibitor (taken once daily) • Omeprazole 20 mg	Lifestyle modifications should be started initially and continued throughout the course of treatment. If symptoms are unrelieved with lifestyle modifications and nonprescription medications after 2 weeks, patient should seek medical attention.

Symptomatic relief of GERD	**Lifestyle modifications** **plus** **prescription-strength acid suppression therapy**	For typical symptoms, treat empirically with prescription-strength acid-suppression therapy. If symptoms recur, consider maintenance therapy (MT). Note: Most patients will require standard doses for MT.
	H_2-receptor antagonists (for 6–12 weeks) • Cimetidine 400 mg twice daily • Famotidine 20 mg twice daily • Nizatidine 150 mg twice daily • Ranitidine 150 mg twice daily	Mild GERD can usually be treated effectively with H_2-receptor antagonists.
	or	
	Proton pump inhibitors (for 4–8 weeks); all are given once daily • Esomeprazole 20 mg • Lansoprazole 15 mg • Omeprazole 20 mg • Pantoprazole 40 mg • Rabeprazole 20 mg	Patients with moderate to severe symptoms should receive a proton pump inhibitor as initial therapy.

Healing of erosive esophagitis or treatment of patients presenting with moderate to severe symptoms or complications	**Lifestyle modifications** **plus** Proton pump inhibitors for 4–16 weeks (up to twice daily) • Esomeprazole 20–40 mg daily • Lansoprazole 30 mg daily • Omeprazole 20 mg daily • Rabeprazole 20 mg daily • Pantoprazole 40 mg daily **or** High-dose H_2-receptor antagonist (for 8–12 weeks) • Cimetidine 400 mg four times daily or 800 mg twice daily • Famotidine 40 mg twice daily • Nizatidine 150 mg four times daily • Ranitidine 150 mg four times daily	For atypical or alarm symptoms, obtain endoscopy (if possible) to evaluate mucosa. Give a trial of a proton pump inhibitor. If symptoms are relieved, consider MT. Proton pump inhibitors are the most effective maintenance therapy in patients with atypical symptoms, complications, and erosive disease. Patients not responding to pharmacologic therapy, including those with persistent atypical symptoms, should be evaluated via ambulatory reflux monitoring to confirm the diagnosis of GERD (if possible).
Interventional therapies	Antireflux surgery or endoscopic therapies	

Adverse Effects

- Bethanechol and Metoclopramide :
  - ✓ Extra pyramidal effects
  - ✓ Sedation
  - ✓ Irritability
  - ✓ cardiac arrhythmias

- Metoclopramide contraindicated in Parkinson's disease,mechanical obstruction, concomitant use of other dopamine antagonists or anticholinergic agents, and pheochromocytoma.

PATIENT COUNSELING

- Weight loss
- elevation of the head of the bed
- consumption of smaller meals and not eating 3 hours prior to sleeping
- smoking cessation
- Avoid alcohol
- Avoid Spicy foods, orange juice, tomato juice, and coffee
- Avoid fats,chocolate, , peppermint, and spearmint

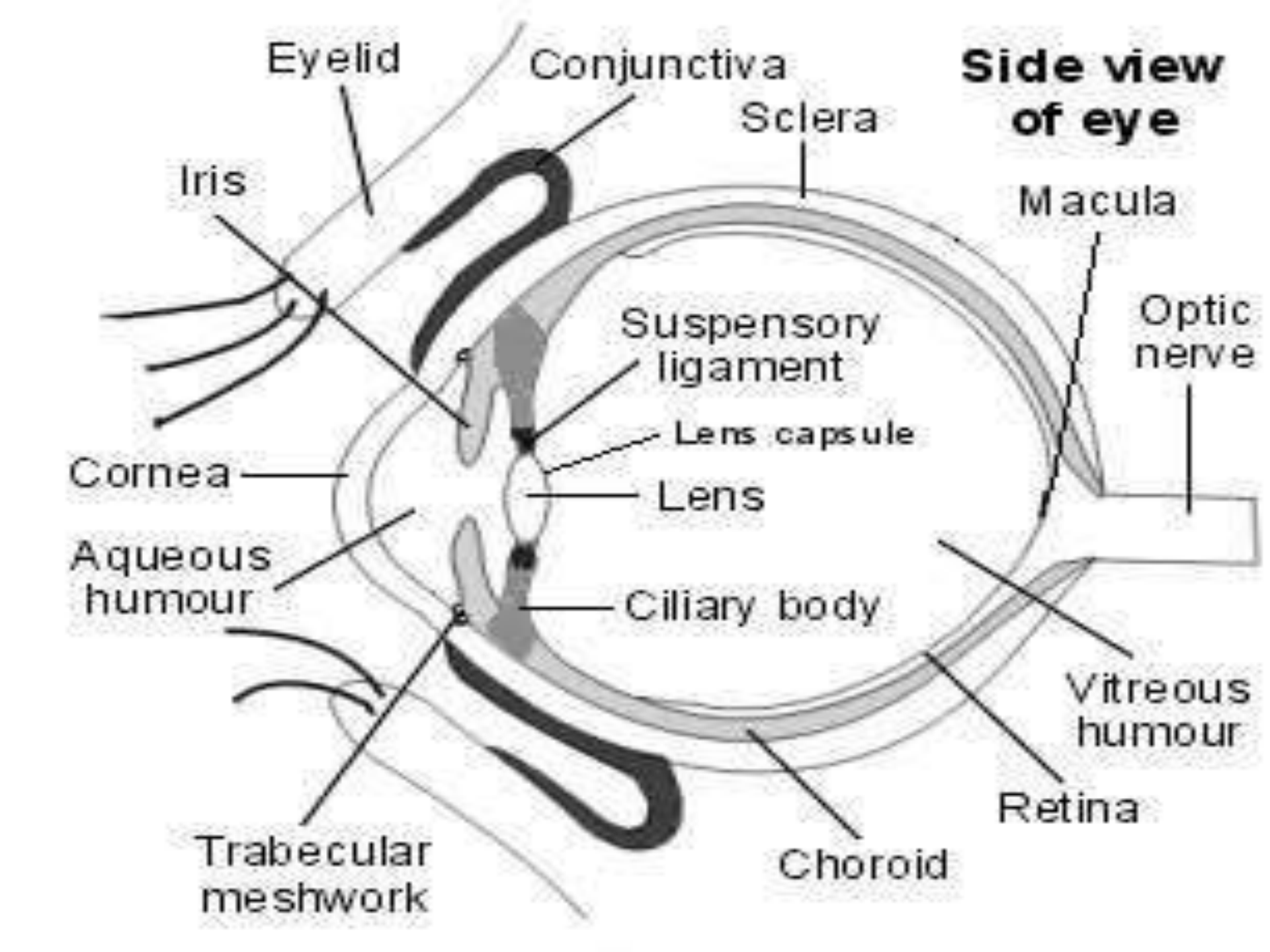
References

- Pharmacotherapy by Dipiro.
- http://www.aafp.org/afp/990301ap/1161.html
- http://www.omnimedicalsearch.com/conditions-diseases/gerd-diagnosis.html

THANK YOU

Glaucoma

Dr.K.Saisree
VCPS


Side view of eye
Eyelid
Conjunctiva
Sclera
Iris
Macula
Optic nerve
Suspensory ligament
Lens capsule
Cornea
Lens
Aqueous humour
Ciliary body
Vitreous humour
Retina
Choroid
Trabecular meshwork

Introduction

- Glaucoma are ocular disorder characterized by changes in the optic nerve head (optic disc) and by loss of visual sensitivity and field.

- It may be generally defined as those conditions in which the intraocular pressure (IOP) is too high for the normal functioning of the optic nerve head.

Classification of glaucoma

- Primary glaucoma
  - Open angle
  - Angle closure
- Secondary glaucoma
  - Open angle
  - Angle closure
- Congenital glaucoma

ETIOLOGY

- The factors that determine the level of IOP are

- The rate of aqueous humour production

- The resistance encountered in the outflow channels

fine balance between these is necessary to keep the pressure within the eye in the range of 16-21mmHg.

Rate of aqueous humour production

- Production of aqueous humour occurs in the ciliary epithelium by two mechanisms:

  Secretion due to an active metabolic process

  Ultrafilteration influenced by the level of blood pressure in the ciliary capillaries

OUT FLOW OF AQUOUS HUMOR

- 80% through the trabecular meshwork into the canal of schelemm and into the venous circulation via the aqueous veins.

- 20% through the clilary body into the suprachoroidal space to be drained into the ciliary body ,choroid sclera via the venous circulation (uveosclera pathway)

PRIMARY OPEN – ANGLE GLAUCOMA

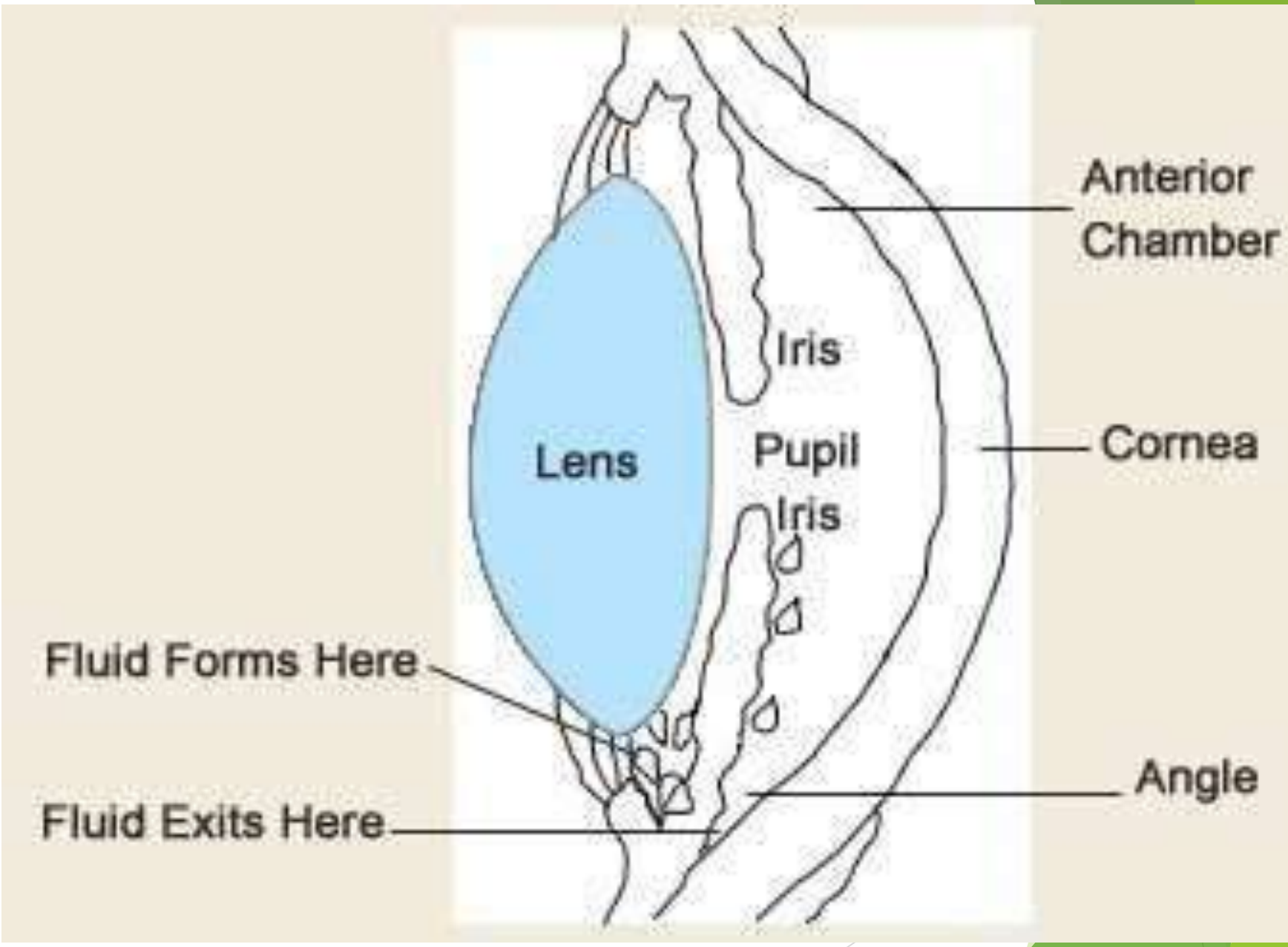
- Referred to as chronic simple glaucoma
- Associated with a relative obstruction to aqueous outflow through the trabecular meshwork
- Is a chronic progressive disease of insidious onset
- Affecting both the eyes .

PREVELANCE

- It is most common type of glaucoma and affects approximately 1in 200 of the population over the age of 40 years.
- POAG is responsible for about 20% of all cases of blindness in the UK
- Affects both sexes equally
- frequently an inherited condition; with approximately 10% of first degree relative of POAG suffers eventually developing the disease.

PATHOPHYSIOLOGY-POAG

- In POAG increased resistance within the drainage channels causes the rise in IOP
- Resistance to aqueous outflow lies in the dense juxtacanalicular trabecular meshwork or the endothelium lining wall of schlemm's canal
- Additional contributing factor include increased suseptibiity of the optic nerve to ischemia, reduced or dysregulated blood flow excitotoxicity autoimmune reaction and other abnormal physiological processes.


Anterior
Chamber
Iris
Lens
Pupil
Iris
Cornea
Fluid Forms Here
Angle
Fluid Exits Here

PRIMARY ANGLE CLOSURE GLAUCOMA

- Is a condition in which closure of the angle by peripheral iris results in a reduction in aqueous outflow .

- The disease affects 1 in 1000 adults over the age of 40 years and occurs in four times as many females as males



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Pathophysiology-PACG

- In PACG the rise in IOP is caused by a decreased outflow of aqueous humor, due to closure of the chamber angle by the peripheral iris

SECONDARY OPEN ANGLE GLAUCOMA

- Decreased outflow of aqueous results from other CAUSES

Psedoexfoliative glaucoma

Pigmentary glaucoma

Topical Corticosteroids

Phacolytic glaucoma

- PAEDIATRIC GLAUCOMA CAN BE CONGENITAL OR DEVELOP DURING INFANCY OR LATER CHILDHOOD.

CLINICAL PRESENTATION

- Open –angle glaucoma is slowly progressive and is usually asymptomatic until the onset of substantial visual field loss.
- <u>Visual field defects include</u>

  - General peripheral visual field constriction
  - Isolated scotomas or blind spots
  - Nasal step
  - Enlargement of blind spot
  - Arc like scotomas
  - Reduced contrast sensitivity
  - Reduced peripheral acuity
  - Altered colour vision

In closed angle glaucoma, patients typically experience intermittent prodromal symptoms

- Blurred vision
- Hazy vision with halos around lights
- Headache

Acute episodes produce symptoms associated with a cloudy. Edematous corna ocular pain nausea vomiting and abdominal pain and diaphoresis

- **Diagnosis of Glaucoma**

- 1. Intraocular pressure (IOP) and its measurement. (tonometry)
- 2. Optic disc examination.
- 3 Visual Field examination (perimetry)

TREATMENT OF OCULAR HYPERTENSION AND OPEN ANGLE GLAUCOMA

- Treatment is indicated for ccular hypertension if the patient has a significant risk factors
- IOP greator than 25mmHg
- vertical cup disc ratio greator than 0.5
- central corneal thickness less than 555µm.
- family history of glaucoma
- sever myopia,and presence of only one eye.

Treatment is indicated for all patients with elevated IOP and characteristic optic disc changes or visual field defects

B adrenergic blocking agents

- Reduces aqueous production by ciliary body
- Betaxolol 0.5% 1 drop bid
  0.25% 1 drop bid
- Cartelol 1% 1 drop bid
- Levobunolol 0.25 , 0.5% 1 drop bid
- Metipranolol 0.3% 1 drop bid
- Timolol o.25, 0.5% 1 drop qd or bid

- Non specific adrenergic agonist
  increases aqueous humor outflow

Epinephrine	0.5%	1 drop bid
Dipivefrin	o.1%	1 drop bid

- Alpha adrenergic agonist
- Reduces aqueous humor production
- Increase uveoscleral outflow

Apraclonidine	0.5%	1 drop tid
Brimonidine	0.15%	1 drop bid

- Prostaglandin analogues
- increased aqueous uveoscleral outflow and to a lesser extent , trabecular outflow

Latanoprost	0.0005%	1 drop q h s	bimatoprost	0.035 1 drop q h s
Travoprost	0.004%	1 drop q h s		

- Carbonic anhydrase inhibitors
- reduces aqueous production by ciliary body

Brinzolamide	1%	bid or tid
Drozolamide	2%	bid or tid

- Systemic carbonic anhydrase inhibitor

Acetazolamide	125 to 250 mg	2to 4 times a day
Dichlorphenamide	25to 50 mg	1to 3 times a day
Methazolamide	25 to 50 mg	2 to 3 tmes a day

- Cholinergic agonist
- increased aqueous humor outflow through trabecular
- Carbachol 0.75, 1.5,2.25, 3% 1 drop bid or tid
- pilocarpine 0.25, 0.5,1,2,4,6,8,10% 1 drop bid or qid.

TREATMENT OF CLOSED ANGLE GLAUCOMA

- Requires rapid reduction of IOP .
- Iredectomy is the definitive treatment , which produces a hole in the iris that permits aqueous flow to move directly from posterior to anterior chamber
- Drug therapy of an acute attack typically consist of an osmotic agent and secretory inhibitors (eg. Beta blockers , alpha 2 agonist latanoprost, or CAI)with or without pilocarpine

TREATMENT OF CLOSED ANGLE GLAUCOMA

- Osmotic agent are used because they rapidly decrease IOP

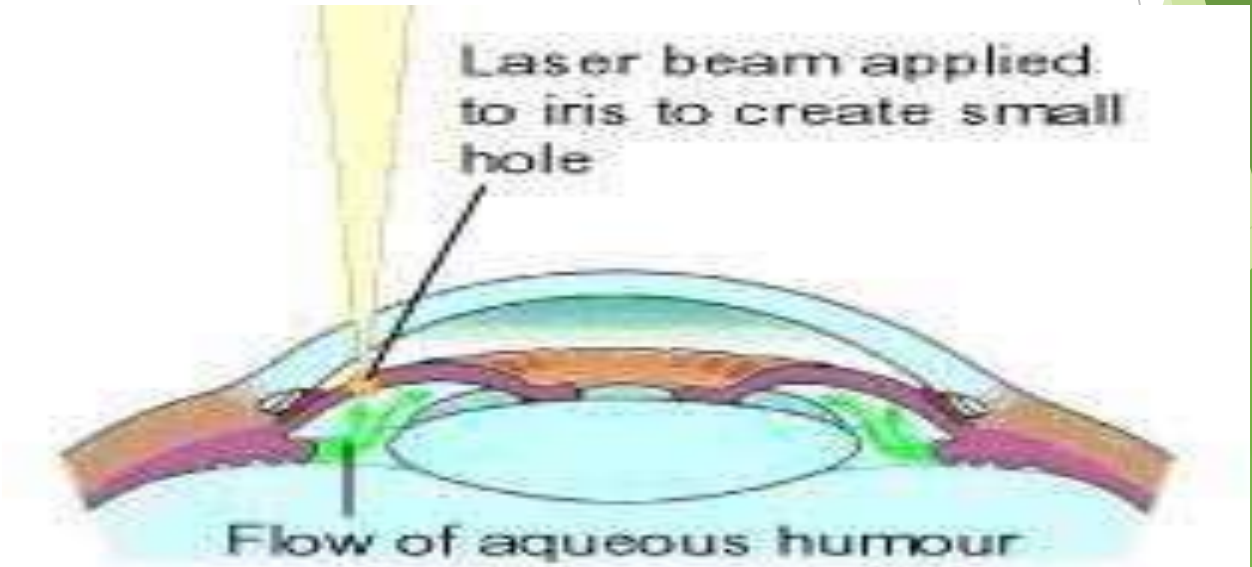
Eg: GLYCERINE 1to 2 g /kg orally

Mannitol 1to 2g/kg iv

Topical corticosteroids can be used to reduce ocular inflammation

- **Argon laser trabeculoplasty**

- This treatment involves the application of laser energy (usually argon green) to the trabecular meshwork, thereby improving the rate of outflow of aqueous humour.



Source: International Glaucoma Association (www.glaucoma-association.com)

- Surgical Procedures for Glaucoma

Trabeculectomy (with or without antimetabolites

- Cataracts may progress rapidly after trabeculectomy.


thank you

Substitution and Elimination Reactions

P.Mamatha

Contents

- Nucleophilic Aliphatic Substitution
  - **S_N1** and **S_N2**
- Electrophilic Aromatic Substitution
- Elimination Reactions
  - **E1**, **E2** and **E1CB**

Substitution Reactions-Definition

- Reactions which involve the **replacement or substitution** of one or more atoms or groups of a compound by other atoms or groups are known as ***SUBSTITUTION REACTIONS.***

Classification

- Based on the ***nature of substituents*** involved:

1. **Free Radical Substitution**

$$A{:}B + Q\cdot \longrightarrow A{:}Q + B\cdot$$

2. **Electrophilic Substitution**

$$A{:}B + Q^{-} \longrightarrow A{:}Q + B^{-}$$

3. **Nucleophilic Substitution**

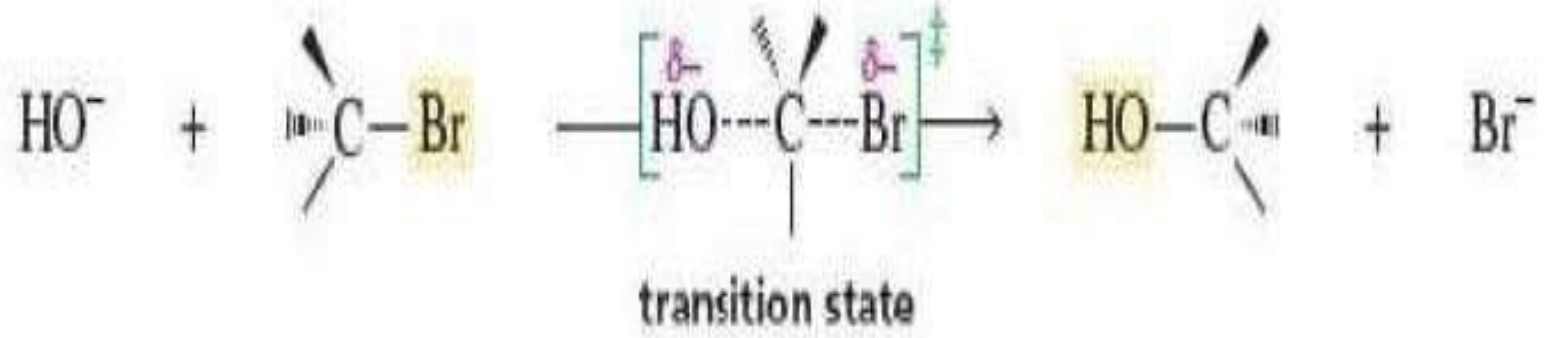
$$A{:}B + Q^{+} \longrightarrow A{:}Q + B^{+}$$

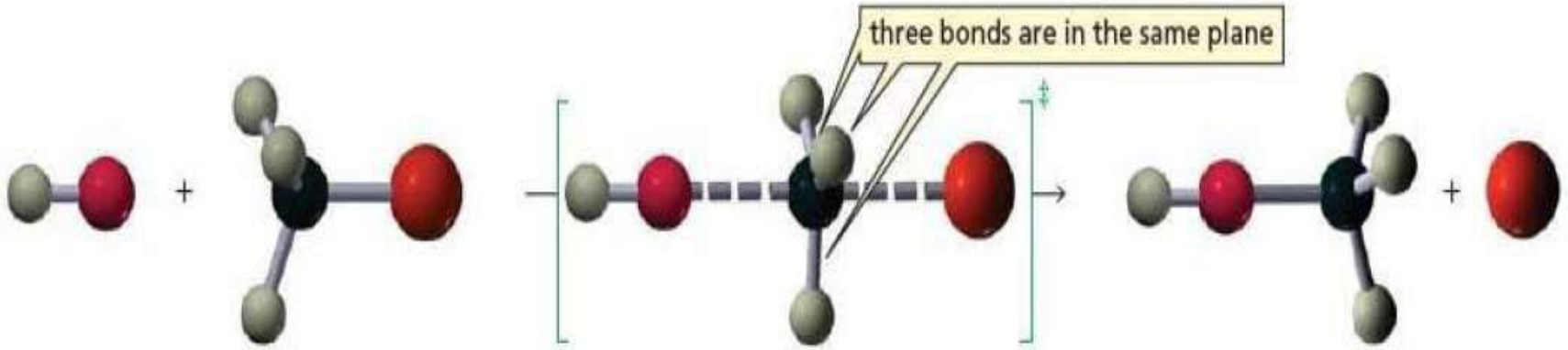
Aliphatic Nucleophilic substitution

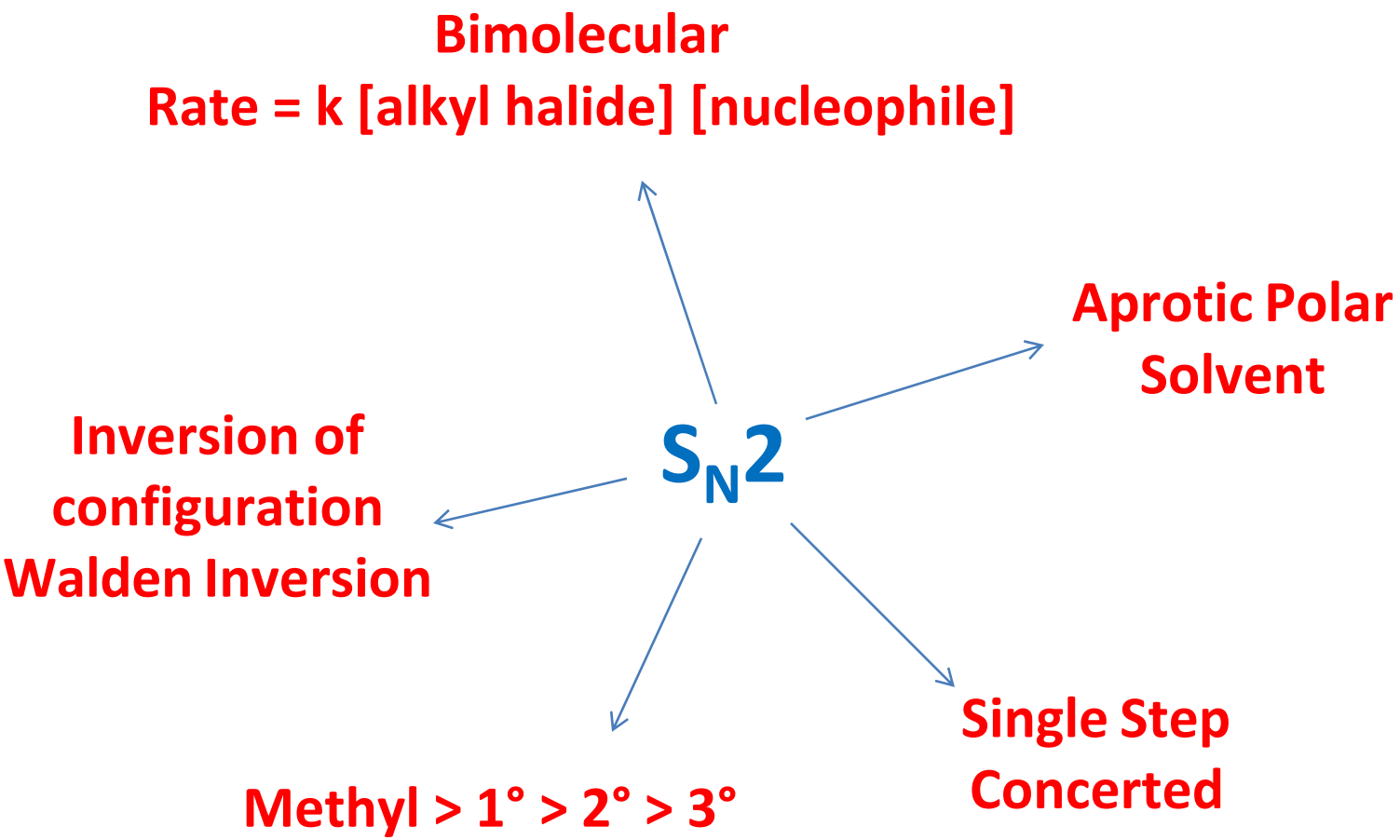
- In 1935, **Edward D. Hughes** and **Sir Christopher Ingold** studied nucleophilic substitution reactions of alkyl halides and related compounds.
- They proposed two main mechanisms—

  the **S_N1 reaction** and the **S_N2 reaction.**
- S stands for chemical substitution, N stands for nucleophilic, and the number represents the kinetic order of the reaction.

S_N2 Mechanism

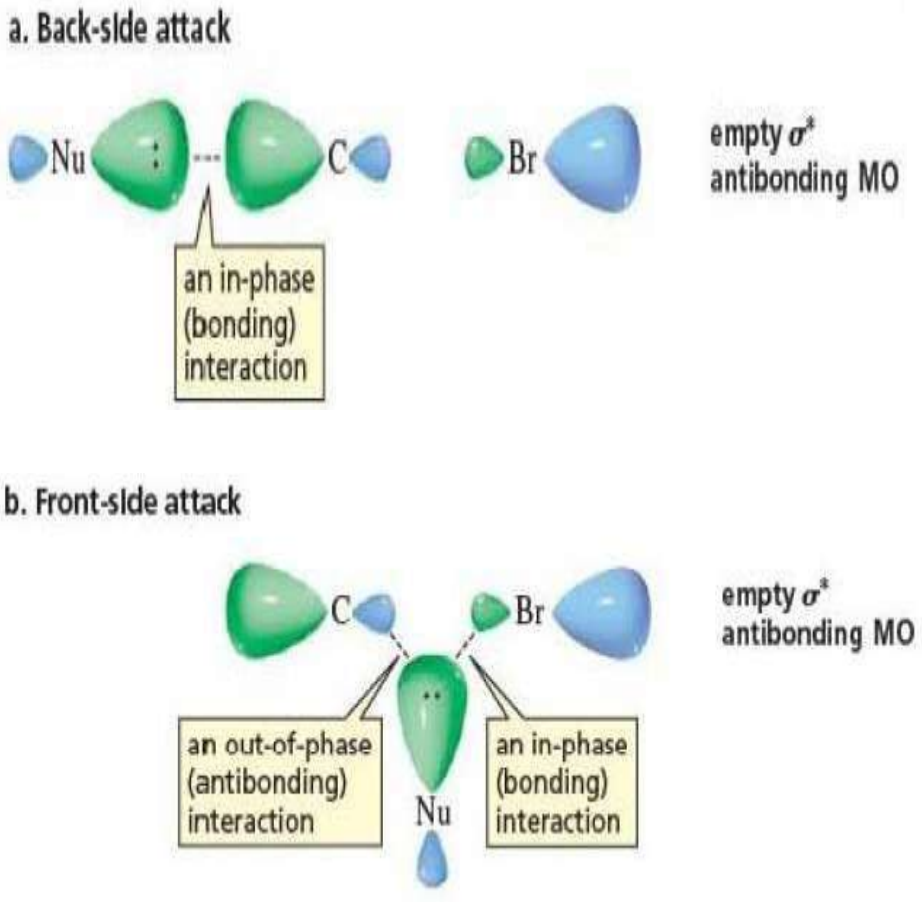





Bimolecular
Rate = k [alkyl halide] [nucleophile]
Aprotic Polar Solvent
Inversion of configuration
Walden Inversion
S_N2
Single Step
Concerted
Methyl > 1° > 2° > 3°

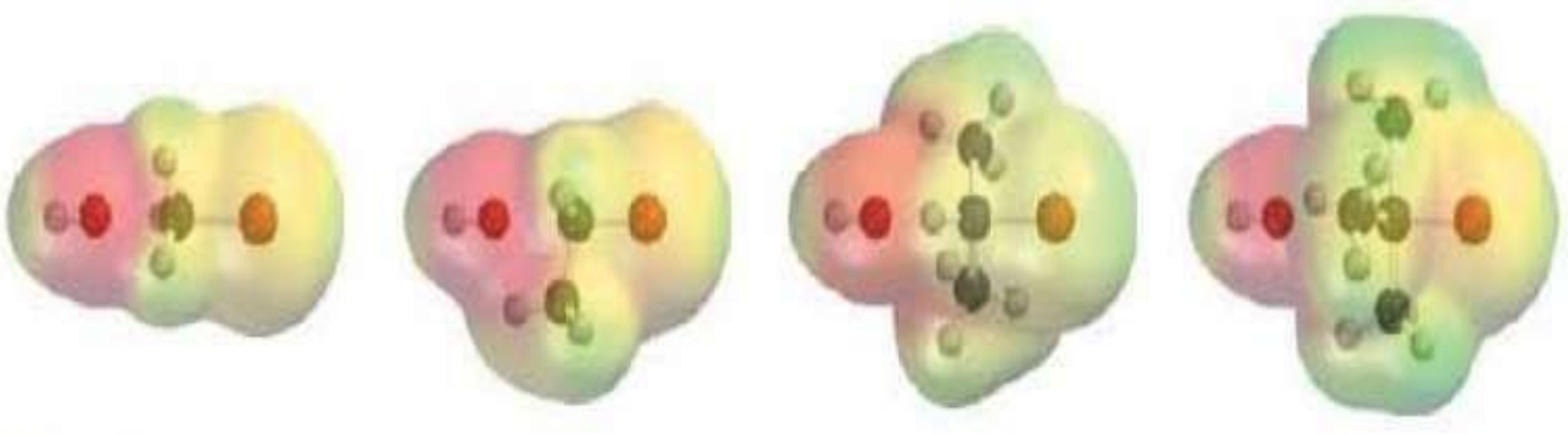
Attack of Nucleophile

- Nucleophile attacks the carbon from the side opposite to the leaving group because of blockade by leaving group.
- Also front side attack results in formation of a bonding and anti-bonding interaction that cancel each other

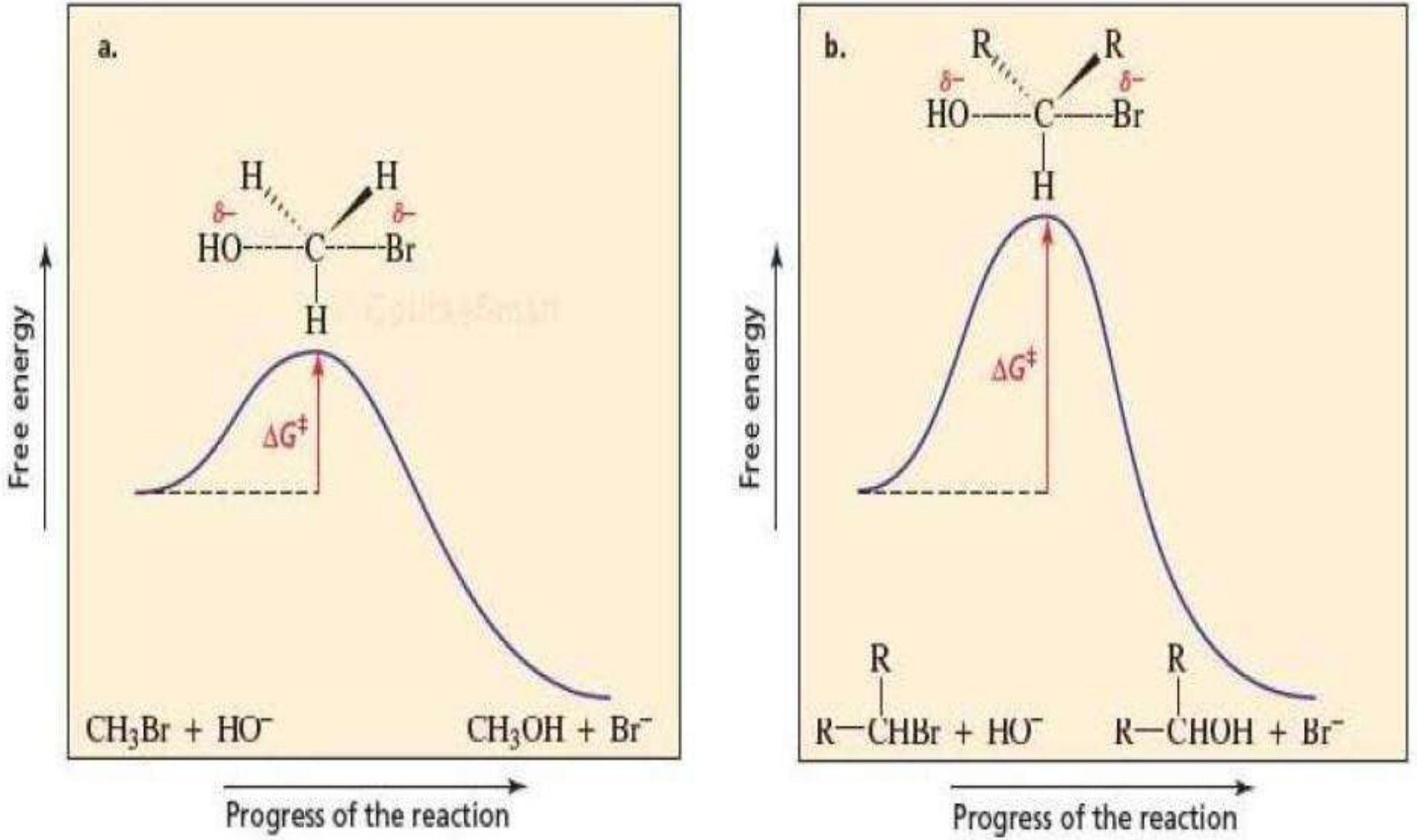


Relative Reactivities of different halides to S_N2 reaction

- **Methyl Halide> 1°Halide> 2°Halide> 3°Halide**
- **Reason : Steric Hindrance**



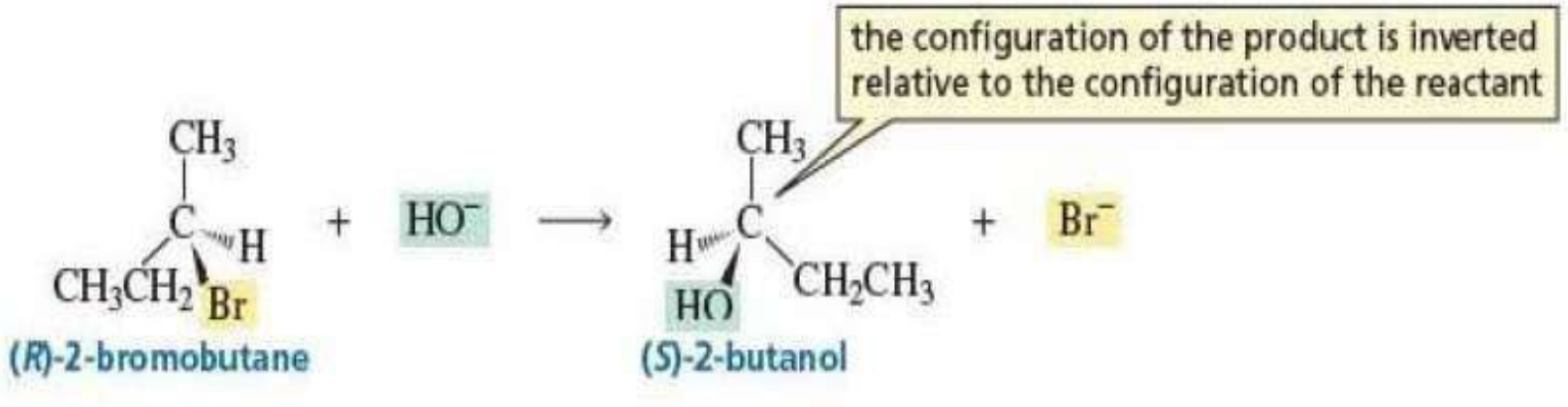
The approach of HO- to a methyl halide, a primary alkyl halide, a secondary alkyl halide, and a tertiary alkyl halide



Reaction coordinate diagrams for
(a) the SN2 reaction of bromomethane with hydroxide Ion;
(bl an SN2 reaction of a sterically hindered secondary alkyl bromide with hydroxide Ion.

Walden Inversion

- Inverted product relative to reactant is formed in S_N2.
- Because of Back-side attack of the nucleophile.
- Example:



Factors affecting S_N2 reaction

- Leaving group:

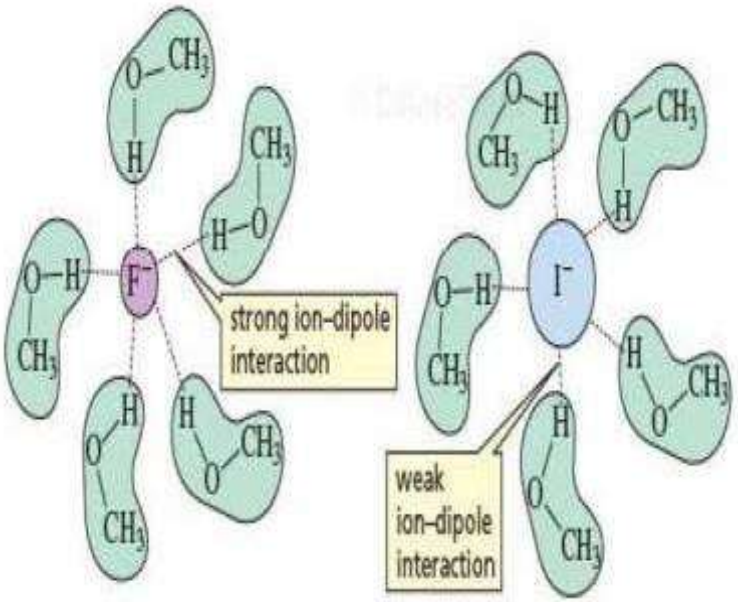
  The weaker the basicity of a group, the better is its leaving propensity.

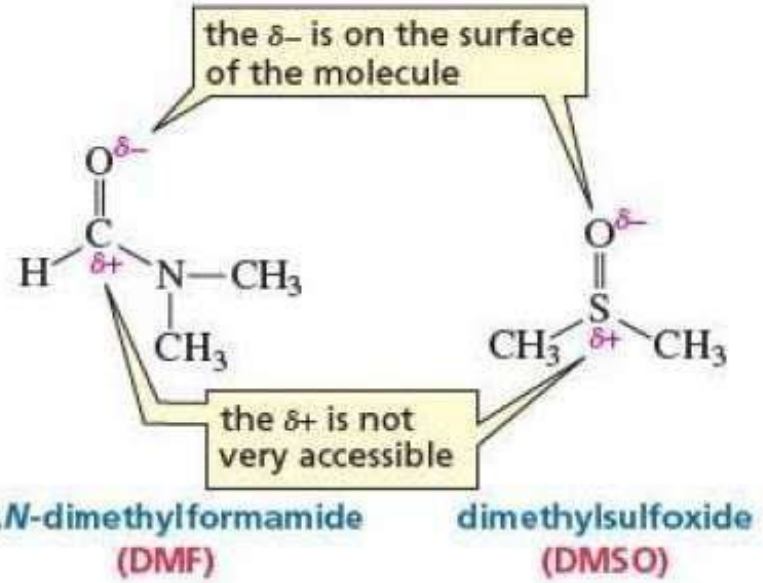
  I>Br>Cl>F

- Attacking Nucleophile:
  - Nucleophilicity
  - Size of the nucleophile
  - Solvent employed (aprotic polar solvent)
  - Steric factors of the nucleophile

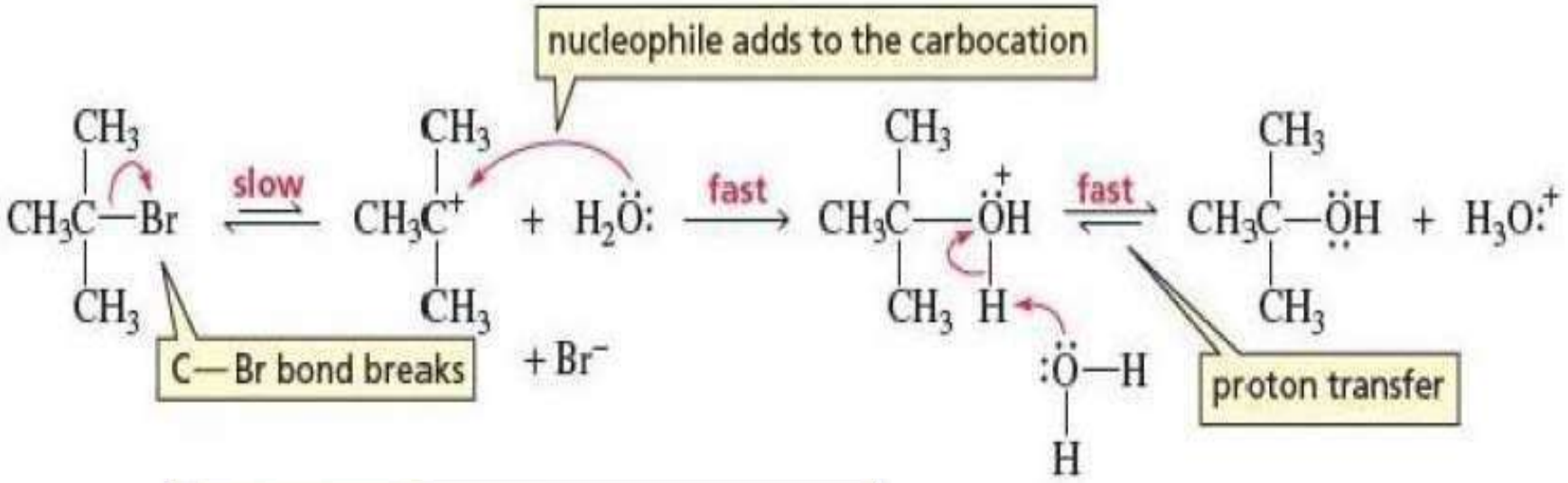
Role of solvent in S_N2

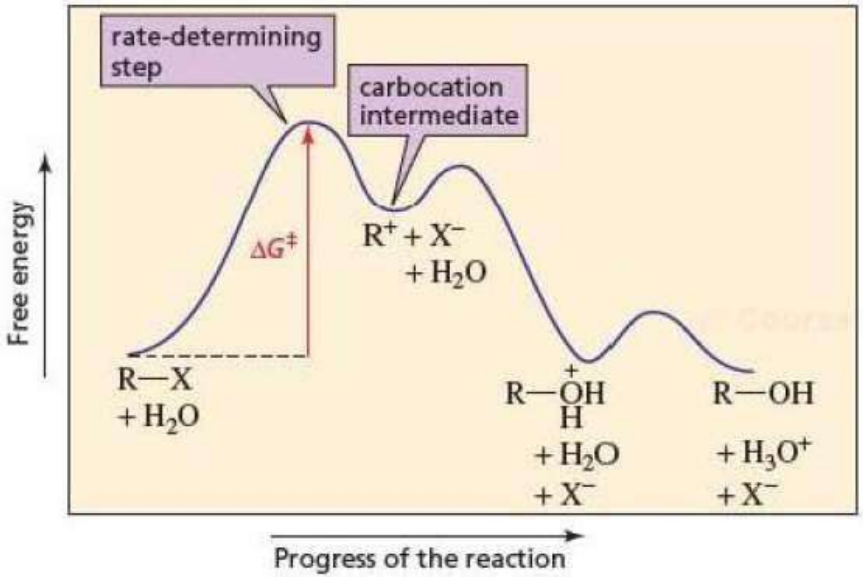
- Non-polar solvents: Ionic compounds are usually insoluble and hence not used.
- Protic polar solvents: Aprotic Polar solvents:

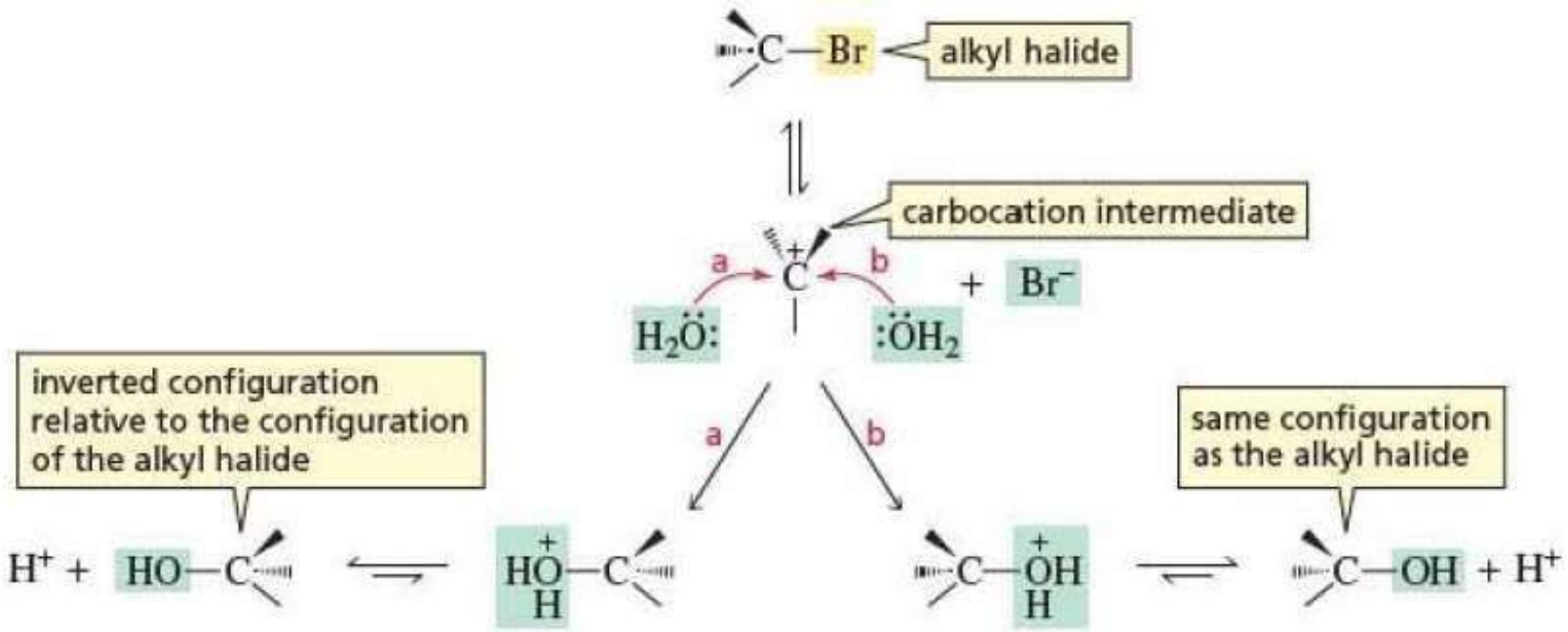




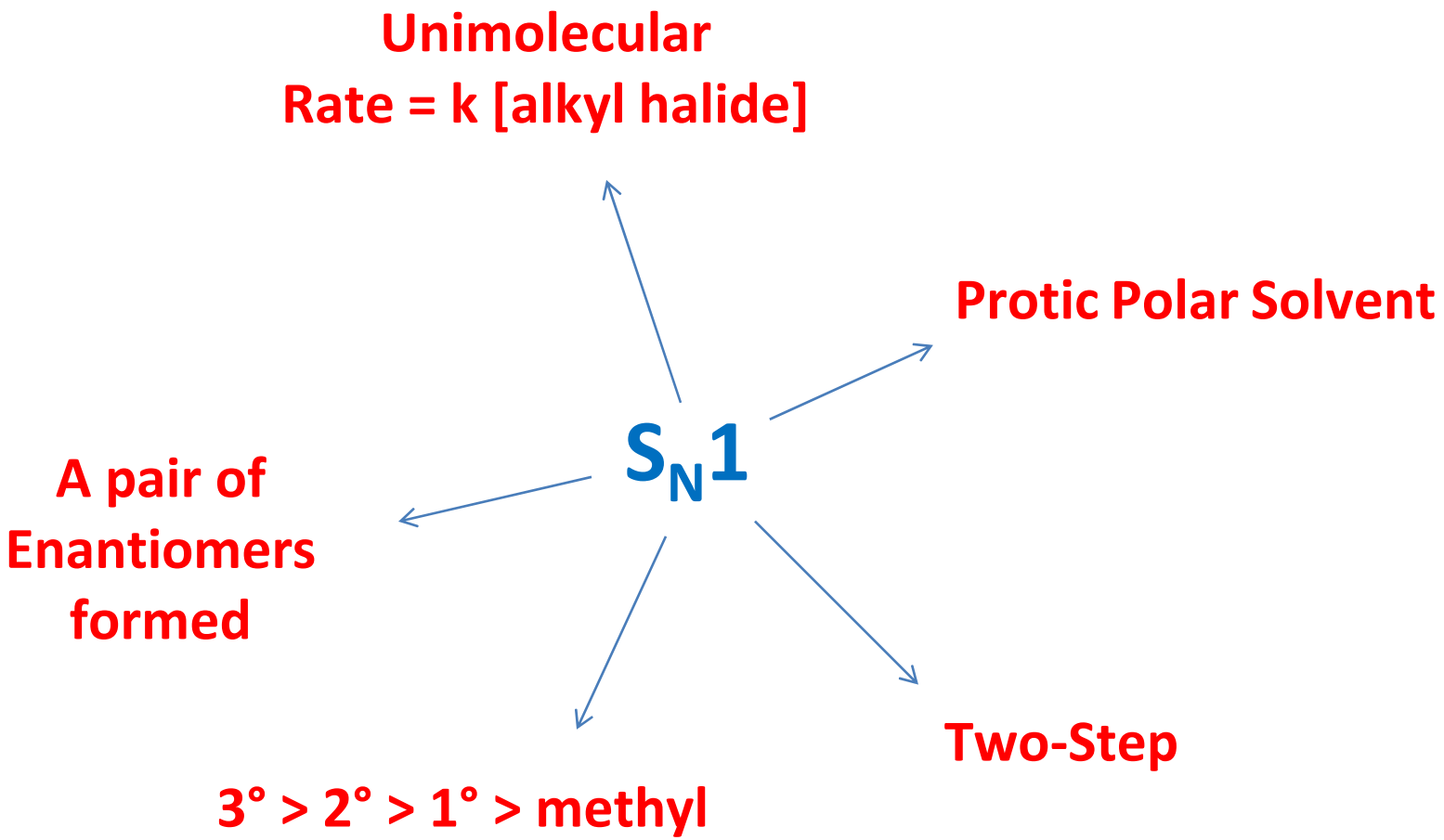
S_N1 Mechanism







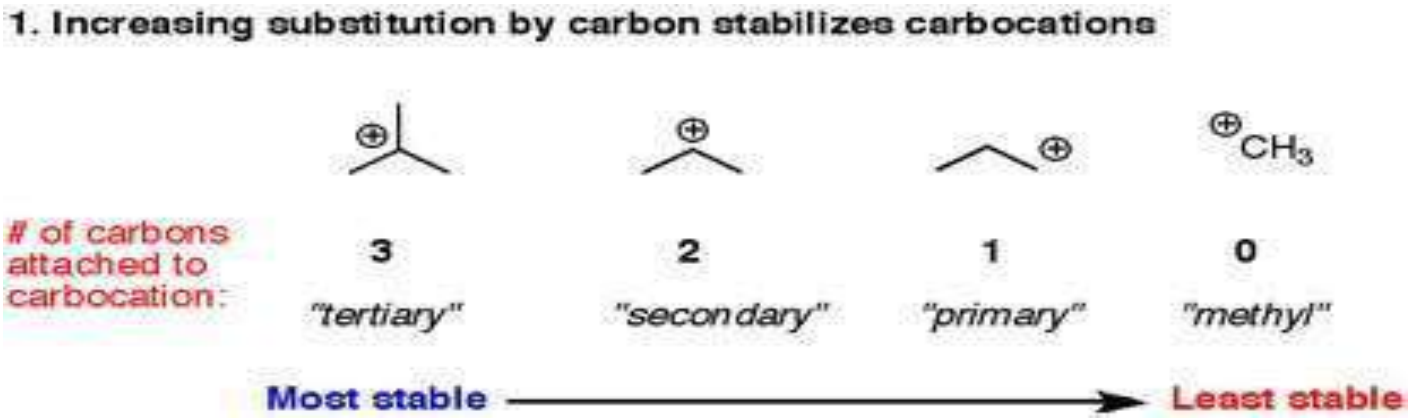
Generally more of inverted product is formed.


Unimolecular
Rate = k [alkyl halide]
Protic Polar Solvent
S_N1
A pair of
Enantiomers
formed
Two-Step
3° > 2° > 1° > methyl

Relative Reactivities of different halides to S_N1 reaction

- **3° Halide> 2°Halide> 1°Halide> Methyl Halide**
- Reactivity depends on stability of carbocation formed in step 1.
- Carbocation stability: **3° > 2°> 1°**

Stability of Carbocations



Reasons:

•Electron release by adjacent carbons by inductive effect i.e a carbon (electronegativity 2.5) connected to hydrogen (electronegativity 2.2) will be electron rich, and can donate some of those electrons to the neighboring carbocation.

•Hyper conjugation invokes stabilization through donation of the electrons in C-H sigma bonds to the empty p orbital of the carbocation.

Factors affecting S_N1 reaction

- Leaving group:

  Similar to S_N2 reaction, *The weaker the basicity of a group, the better is its leaving propensity.*
- Stability of Carbocation
- Solvent (protic polar solvent)
- Nucleophile:

  No effect as it has no part to play in rate-determining step.

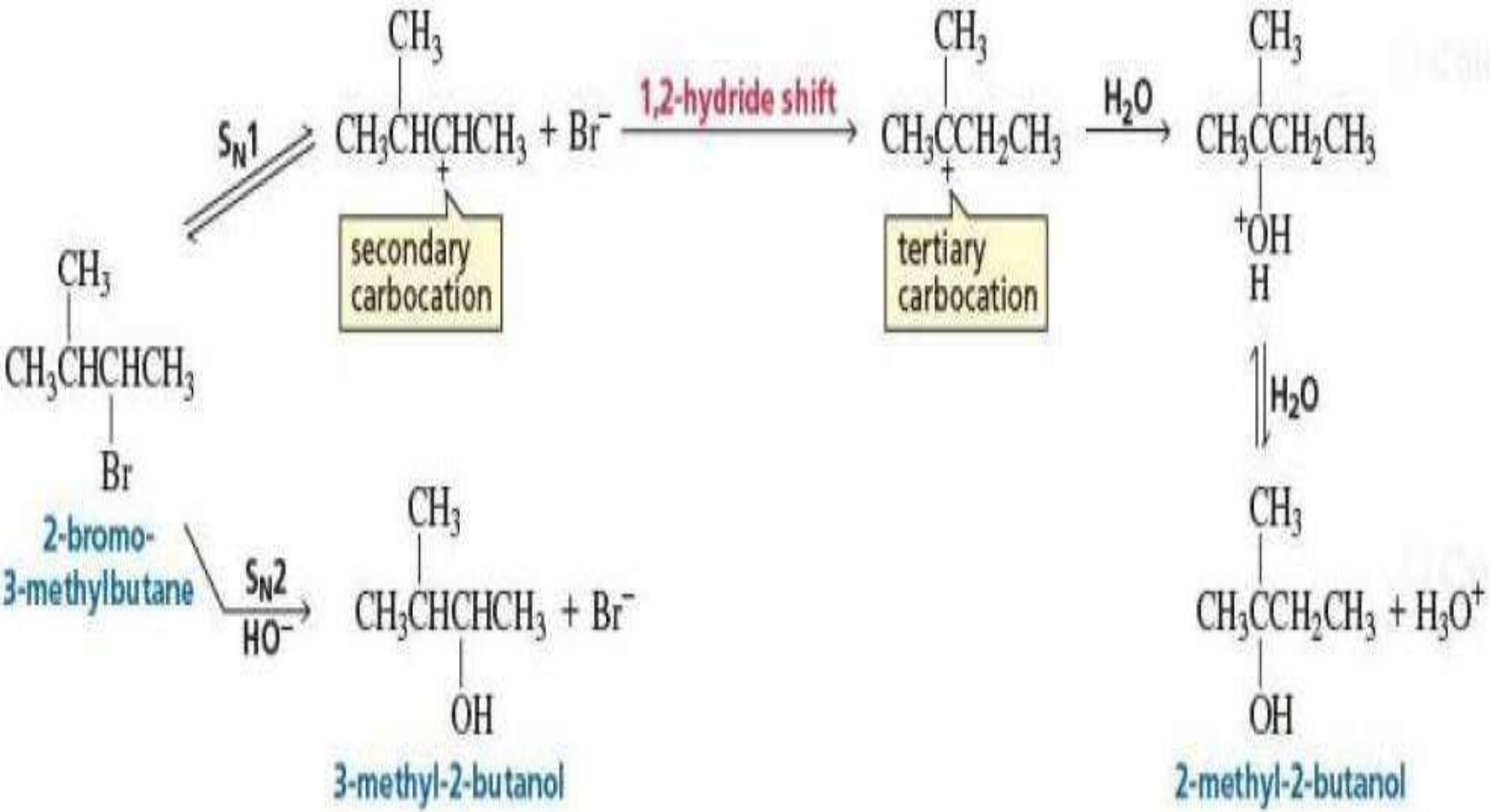
Role of solvent in S_N1

- Protic polar solvents: proton reacts with the leaving group and eliminates its influence.
- Generally solvent is the nucleophile.
- Thus solvolysis is the reaction.
- For example, water acts as both nucleophile and solvent.

Factor	S_N1	S_N2
Kinetics	Rate = k[RX]	Rate = k[RX][Nuc]
No. of steps	Two step	One step
Primary alkyl	Never	Good
Secondary alkyl	Moderate	Moderate
Tertiary alkyl	Excellent	Never
Leaving group	Less Basic	Less Basic
Nucleophilicity	Unimportant	Important
Preferred Solvent	Polar protic	Polar aprotic
Stereochemistry	Racemisation(more inversion possible)	Walden Inversion
Rearrangements	Common	Rare

Carbocation Rearrangements

- In S_N1, a carbocation will rearrange if it becomes more stable in the process.
- If a secondary carbocation formed initially undergoes a 1,2-hydride shift to form a more stable tertiary carbocation; this changes the carbon skeleton of the reactant.
- SN I and SN2 reactions of the same alkyl halide can produce different constitutional isomers as products. since a carbocation is not formed in an SN2 reaction and therefore the carbon skeleton cannotrearrange.


S_N1
$CH_3CHCHCH_3 + Br^-$
1,2-hydride shift
$CH_3CCH_2CH_3$
H_2O
secondary carbocation
tertiary carbocation
$CH_3CHCHCH_3$
Br
2-bromo-3-methylbutane
S_N2
HO^-
$CH_3CHCHCH_3 + Br^-$
OH
3-methyl-2-butanol
$CH_3CCH_2CH_3$
^+OH
H
H_2O
$CH_3CCH_2CH_3 + H_3O^+$
OH
2-methyl-2-butanol

Examples

- *Organic reductions* with hydrides, for example

  $R\text{-}X \rightarrow R\text{-}H$ using $LiAlH_4$ (S_N2)

- *Hydrolysis* reactions such as

  $R\text{-}Br + OH^- \rightarrow R\text{-}OH + Br^-$ (S_N2) or

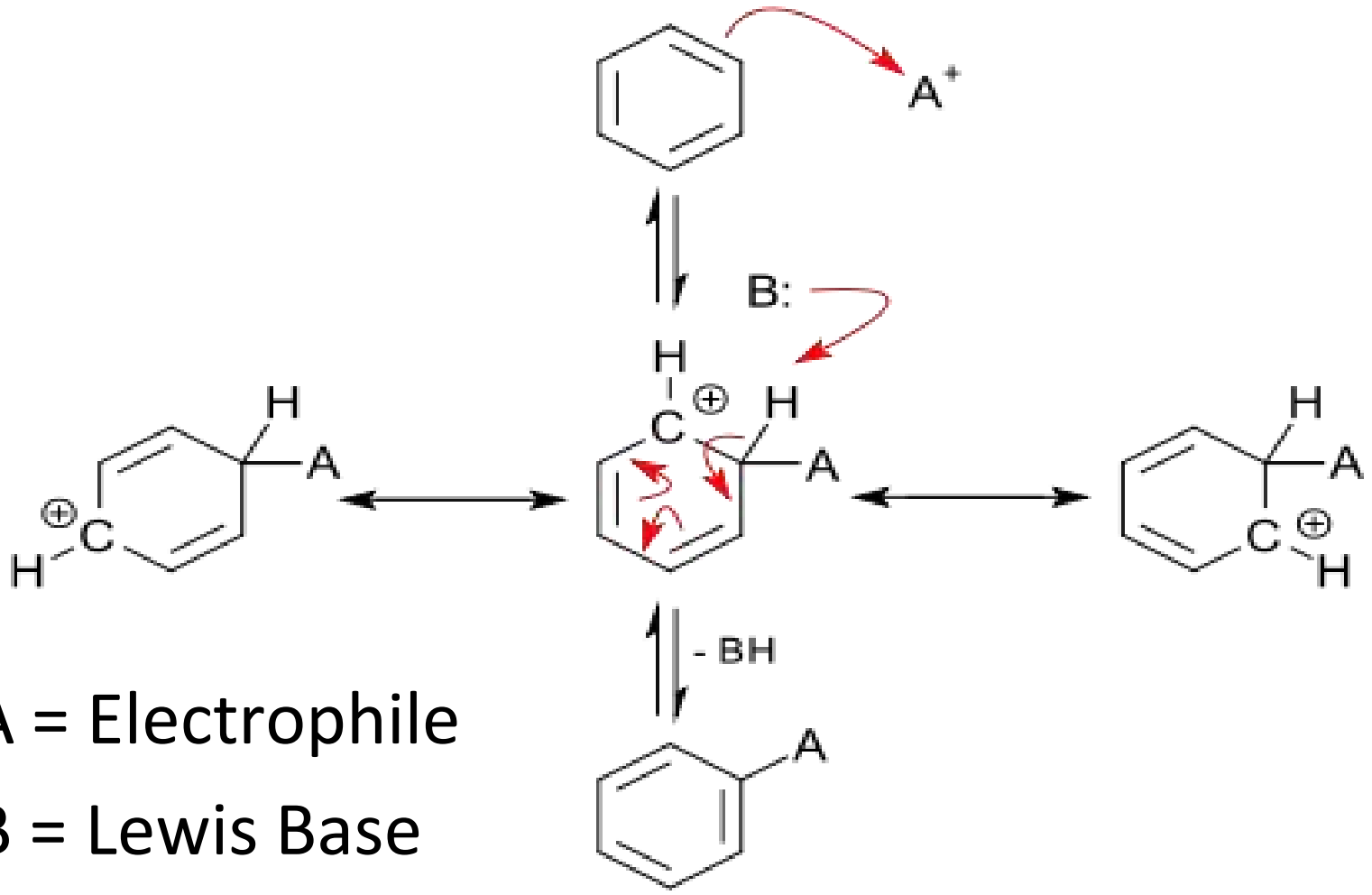
  $R\text{-}Br + H_2O \rightarrow R\text{-}OH + HBr$ (S_N1)

- *Williamson ether synthesis*

  $R\text{-}Br + OR'^- \rightarrow R\text{-}OR' + Br^-$ (S_N2)

- The *Wenker synthesis*, a ring-closing reaction of aminoalcohols.
- The *Finkelstein reaction*, a halide exchange reaction
- The *Kolbe nitrile synthesis*, the reaction of alkyl halides with cyanides.

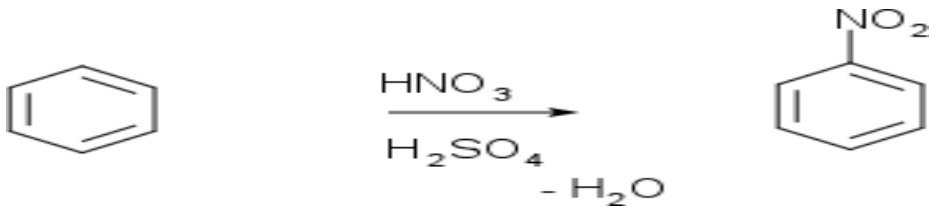
Electrophilic Aromatic Substitution



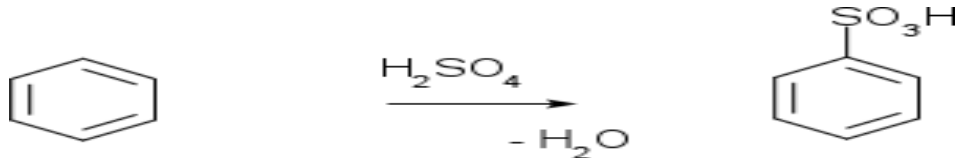
- A = Electrophile
- B = Lewis Base

Examples

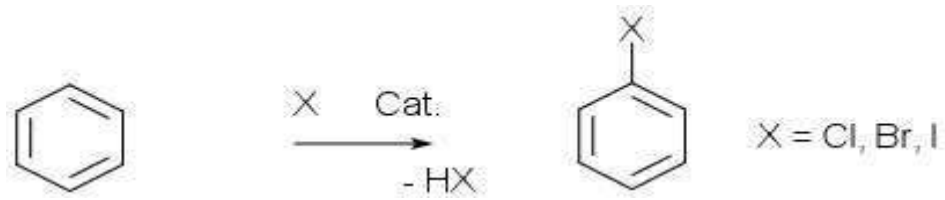
- **Nitration:**



- **Sulphonation:**

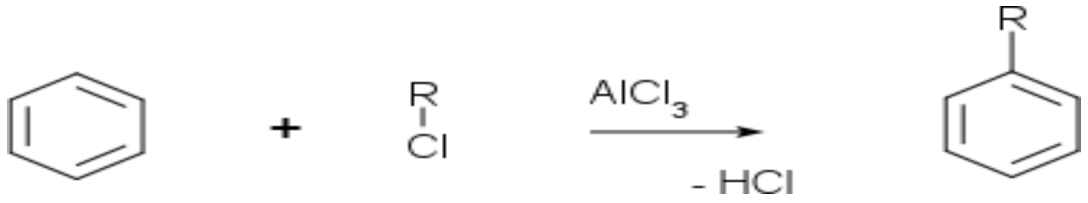


- **Halogenation:**

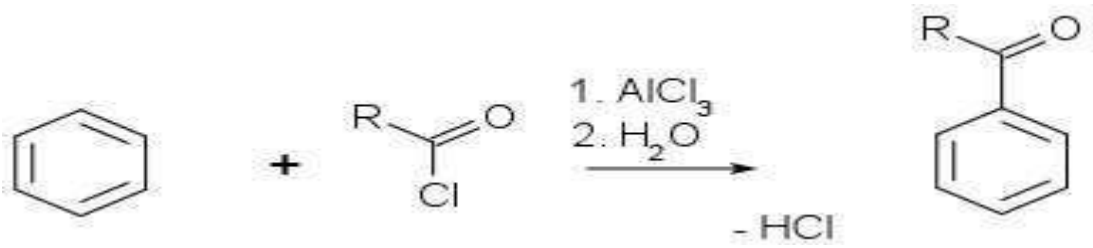


Examples

- **Friedel-Craft's alkylation:**



- **Friedel-Craft's acylation:**



Effect of Substituents

- Any substituents, if present, affect both the **regioselectivity** and **speed** of the reaction.
- In terms of regioselectivity, the substituents may be **ortho-para directing** or **meta directing.**
- In terms of kinetics, substituents may **increase (activating)** or **decrease (deactivating)** the rate of reaction.

Activating Substituents:

- They ***stabilize*** the cationic intermediate formed during the substitution by *donating* electrons into the ring system, by either inductive effect or resonance effects.

Examples are *toluene, aniline and phenol*

Deactivating Substituents:

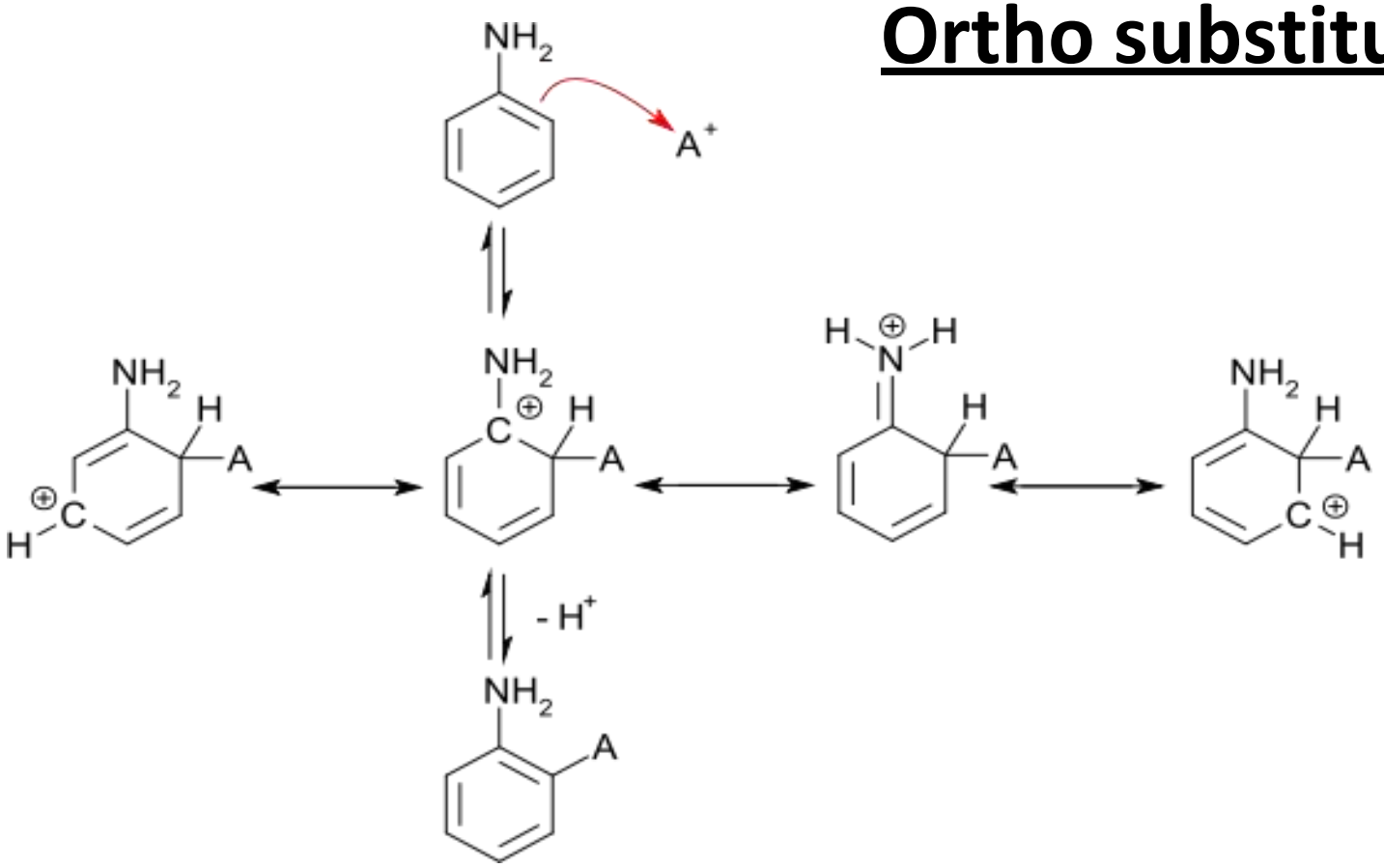
- These ***destabilize*** the intermediate cation and thus decrease the reaction rate by *withdrawing* electron density from the aromatic ring.

Examples are *nitrobenzene, benzaldehyde* and *trifluoromethylbenzene*

ACTIVATING GROUPS ELECTRON RELEASING GROUPS	Strongly activating groups	-OH, -OR, -NH_2, NHR
	Moderately activating groups	-NHCOR, -OCOR
	Weak activating groups	-R, -Ar, -CH=CHR
DEACTIVATING GROUPS ELECTRON WITHDRAWING GROUPS	Weak deactivating groups	-F, -cl, -Br, -I
	Moderate deactivating groups	-CHO, -COR, -COOR, -COOH, -COcl
	Strong deactivating groups	-SO_3H, -NH_2R,-NR_3, -NO_2, -NHR_2, -NH_3, -CN

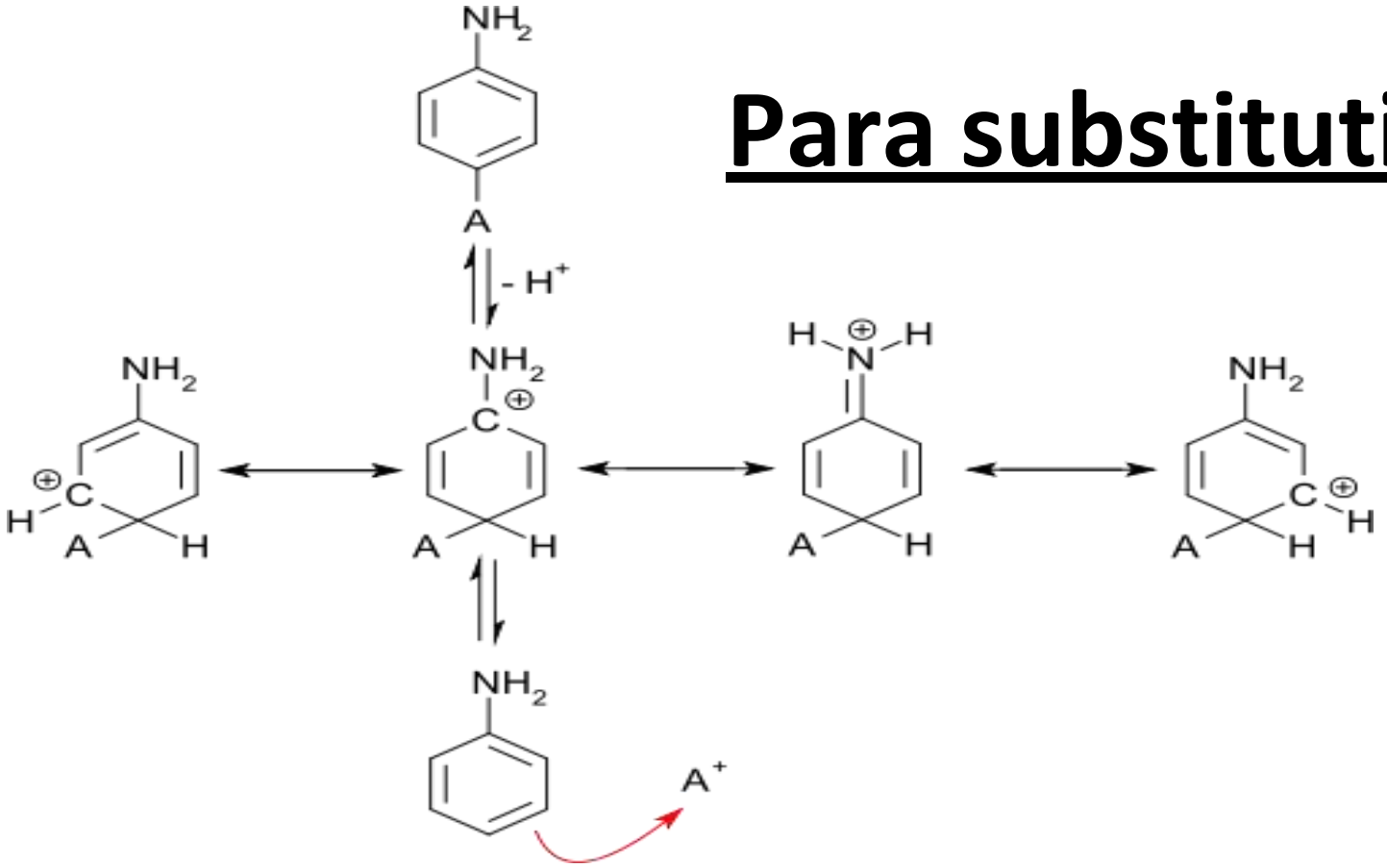
Ortho/para directors

Ortho substitution



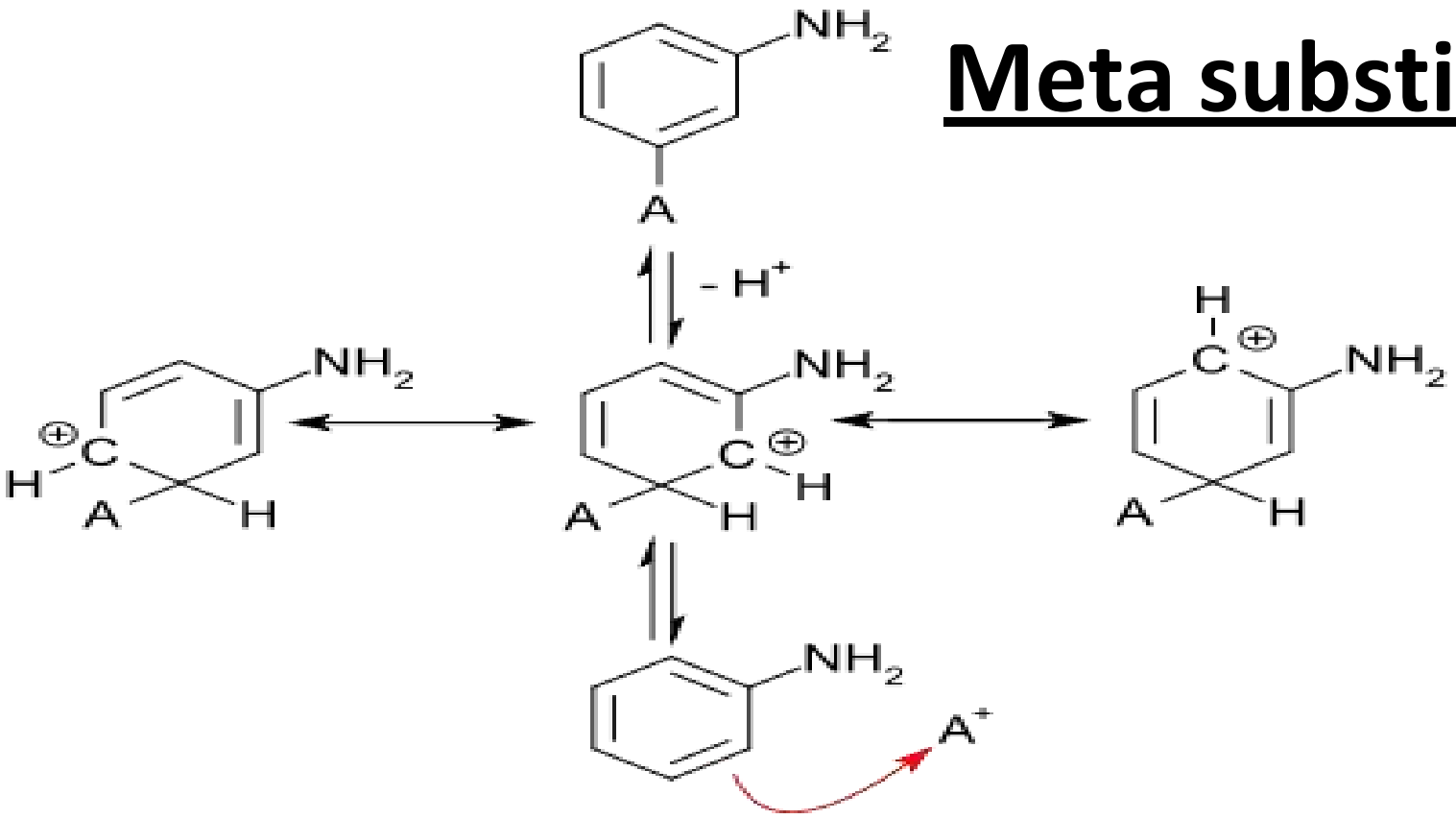
Ortho/para directors

Para substitution



Ortho/para directors

Meta substitution



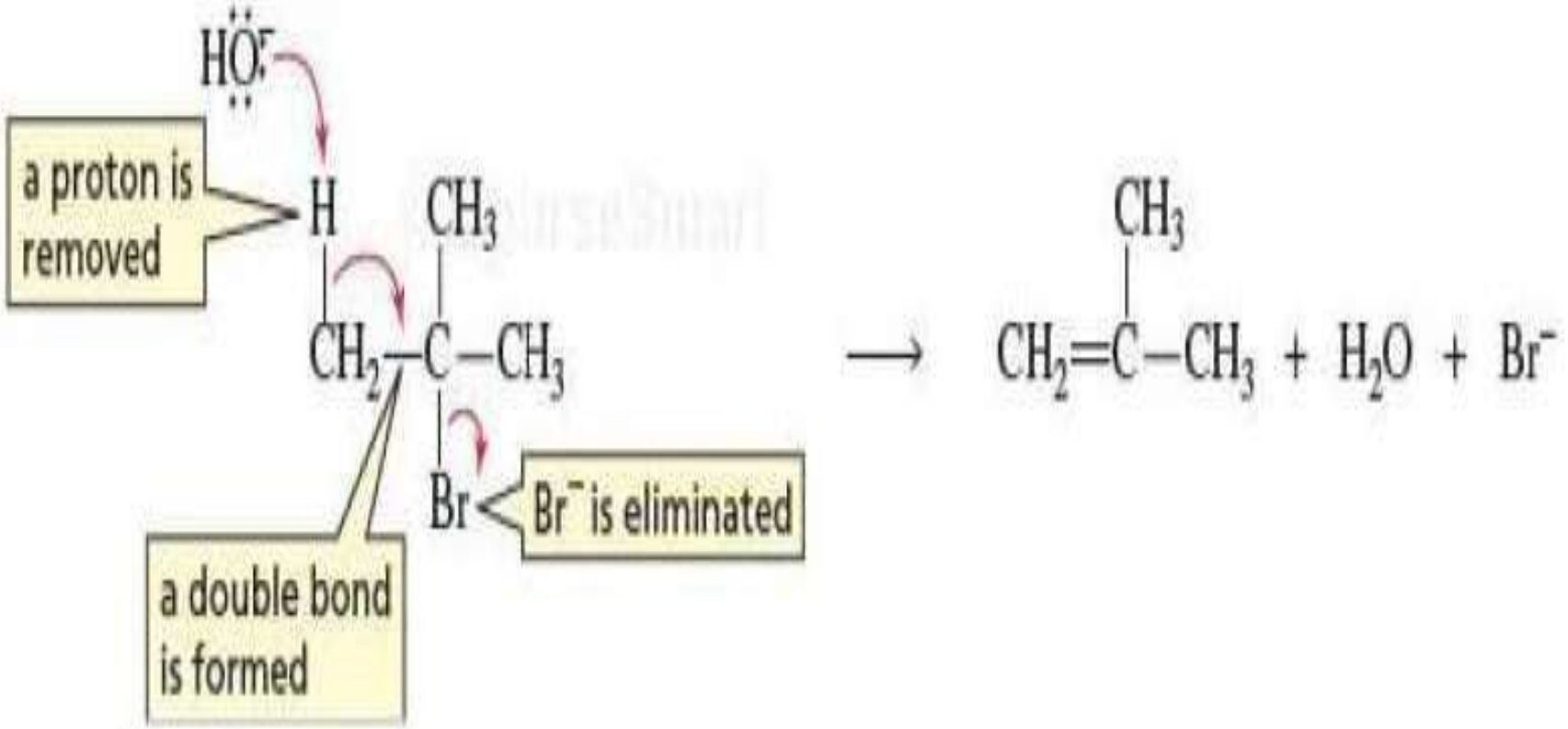
Meta directors

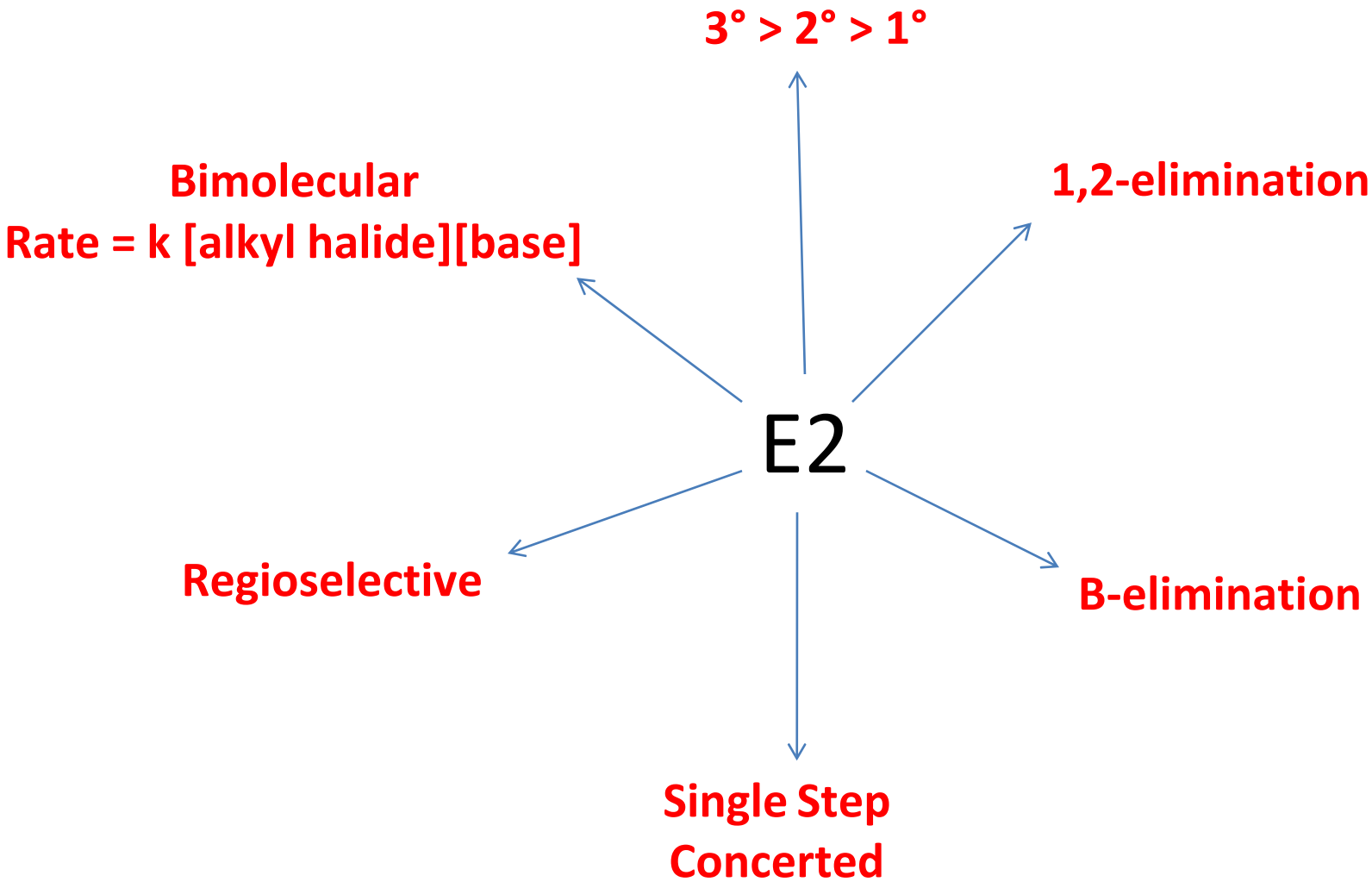
- Non-halogen groups with atoms that are more electronegative than carbon, such as a carboxylic acid group (CO_2H) draw substantial electron density from the *pi* system.
- These groups are strongly deactivating groups.
- Additionally, since the substituted carbon is already electron-poor, the resonance contributor with a positive charge on this carbon (produced by *ortho/para* attack) is less stable than the others.
- Therefore, these electron-withdrawing groups are *meta* directing

Elimination Reactions

- In an elimination reaction, atoms or groups are removed from a reactant.
- For example, when an alkyl halide undergoes an elimination reaction, the halogen (X) is removed from one carbon and a hydrogen is removed from an adjacent carbon and a double bond is formed between the two carbons from which the atoms are eliminated.
- The product of an elimination reaction is an alkene.
- Two important elimination reactions: E1 and E2.

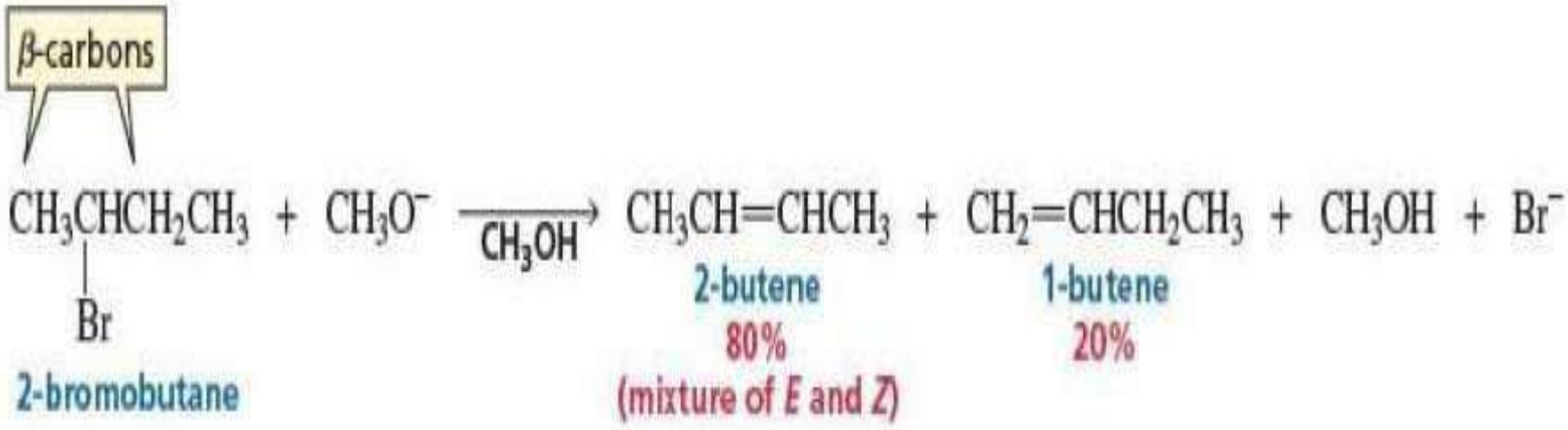
The E2 Reaction

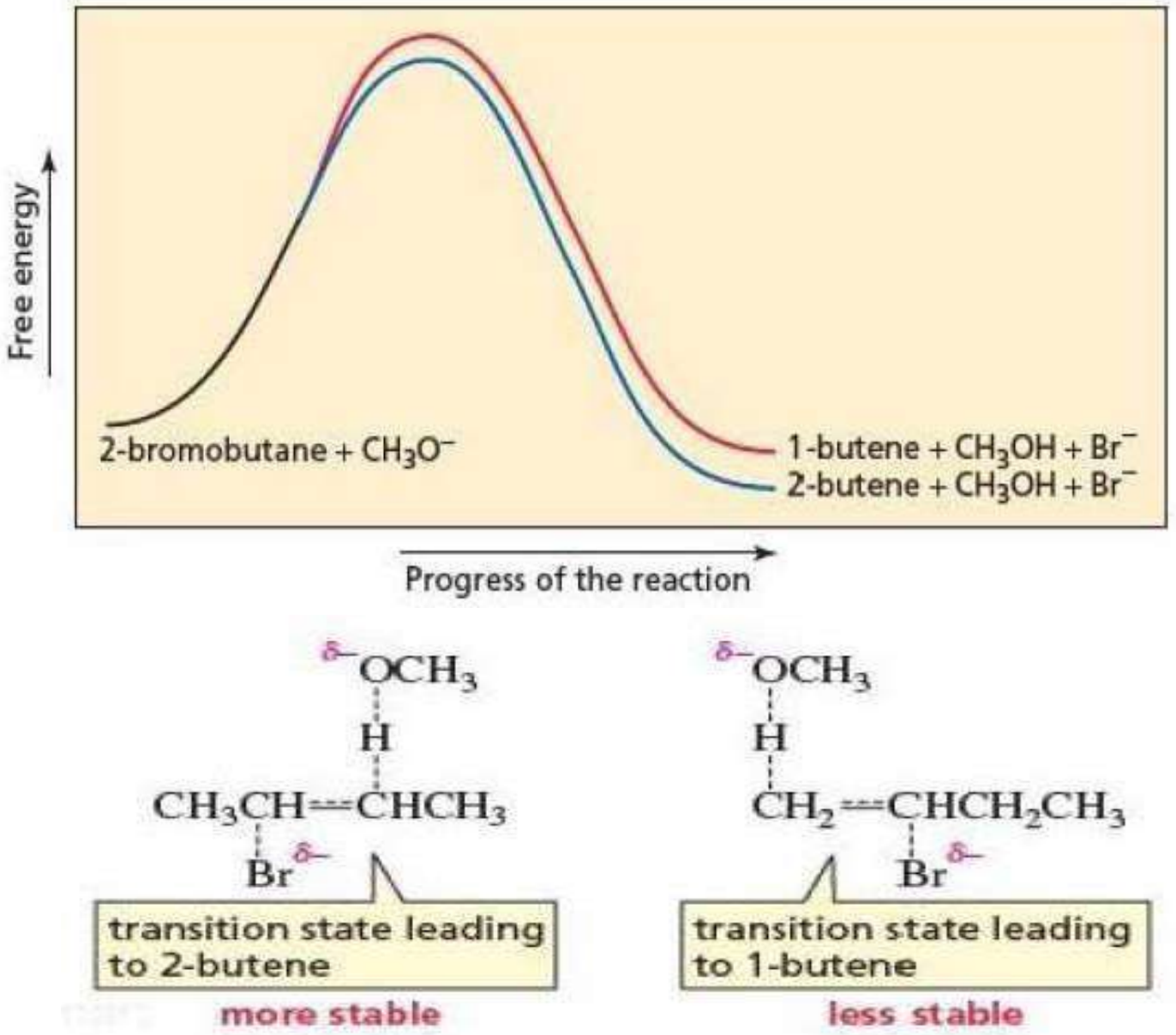



3° > 2° > 1°
Bimolecular
Rate = k [alkyl halide][base]
1,2-elimination
E2
Regioselective
B-elimination
Single Step
Concerted

Regioselectivity

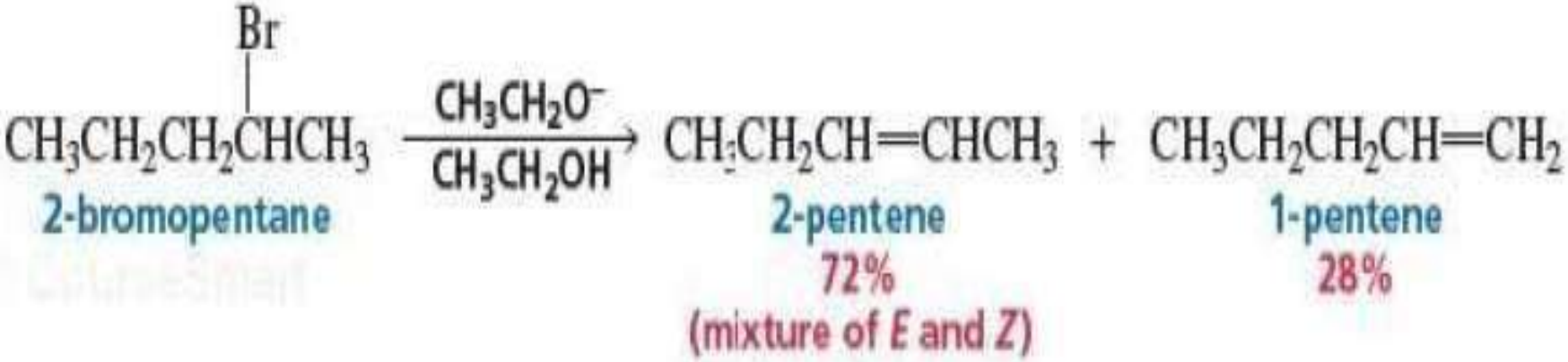
- When two structurally different β-carbons are available from which hydrogen can be eliminated, more stable alkene is formed as major product.

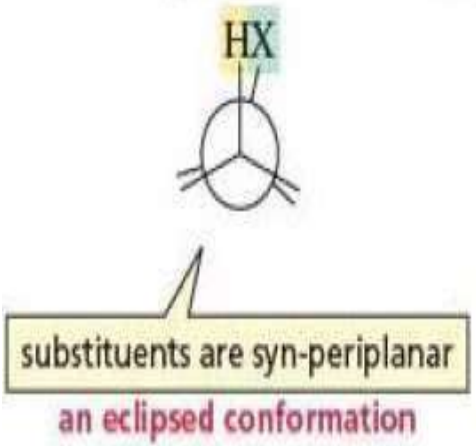


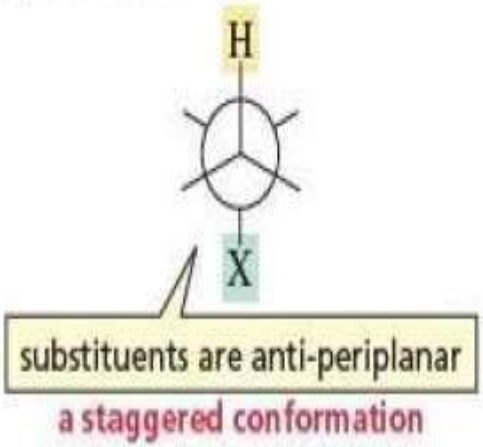


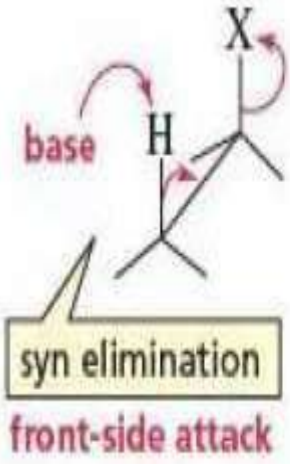
Greater the number of alkyl substituents on alkene,more stable it is.

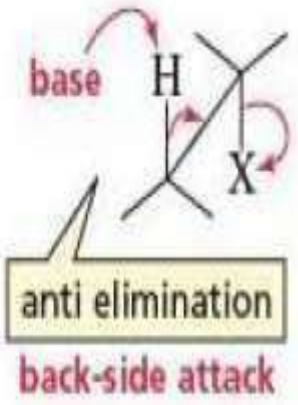
Stereoselectivity of E2 (Anti-elimination)



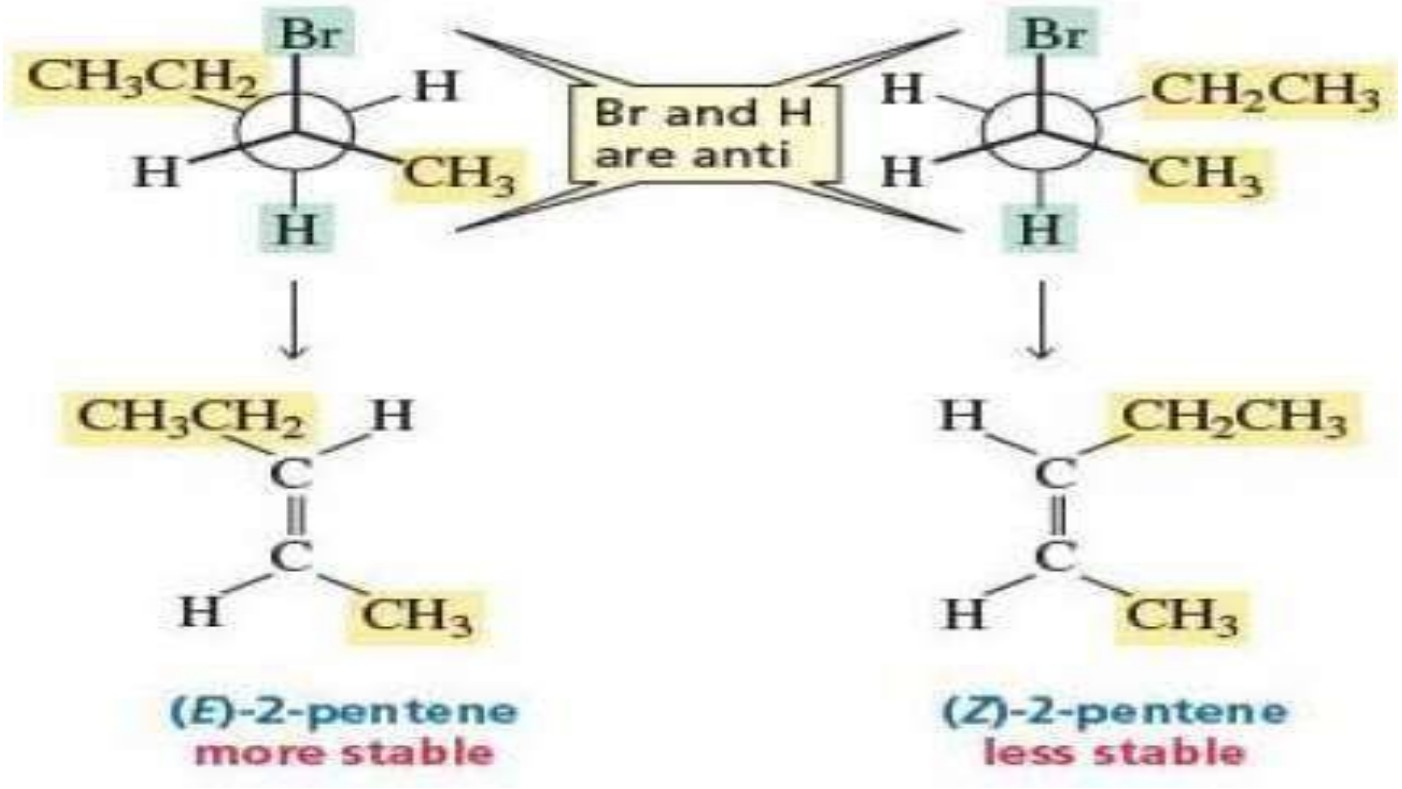






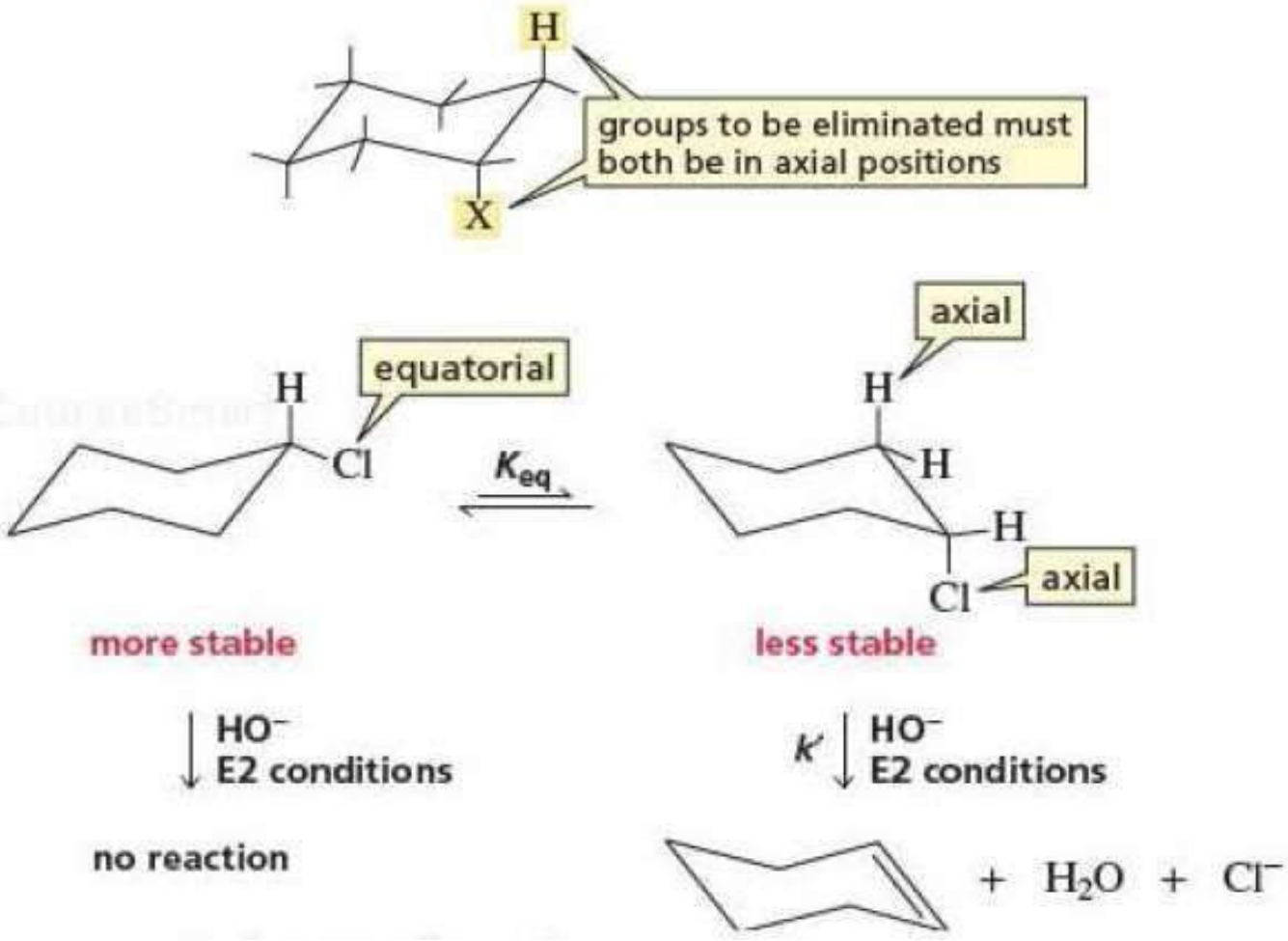


If the reactant has *two hydrogens bonded to the carbon from which a hydrogen is to* be removed both the *E and Z products will be formed, because the reactant has two conform*ers in which the groups to be eliminated are anti.



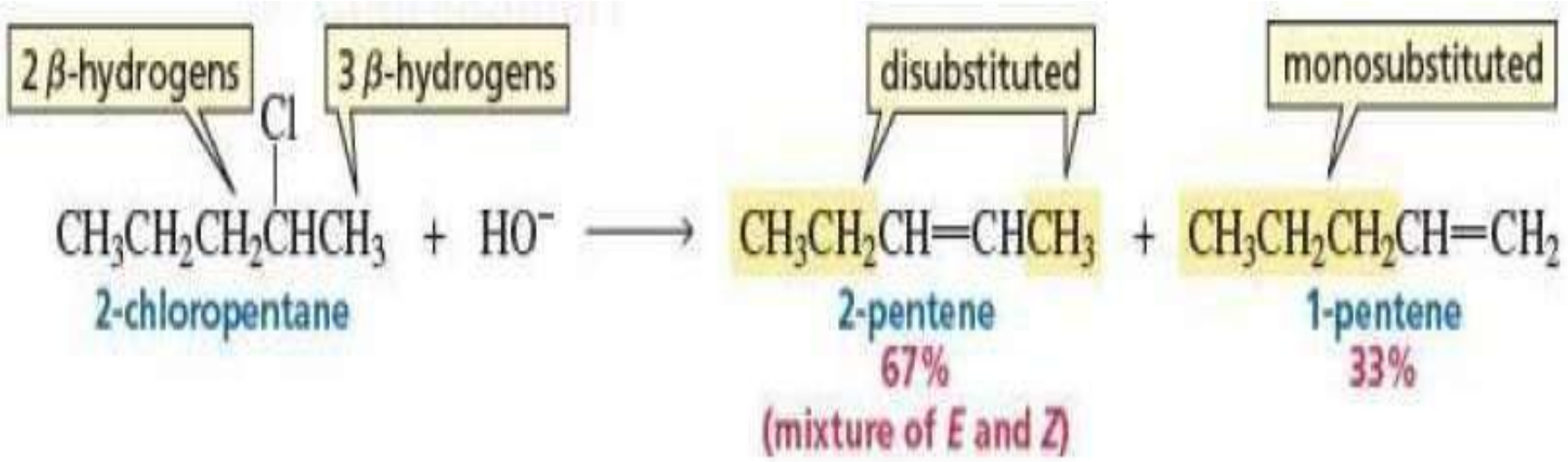
The alkene with the *bulkiest groups on opposite sides of the double bond will be formed* in greater yield because it is the more stable alkene, so it will have the more stable transition state leading to its formation.

E2 reaction of substituted cyclohexanes



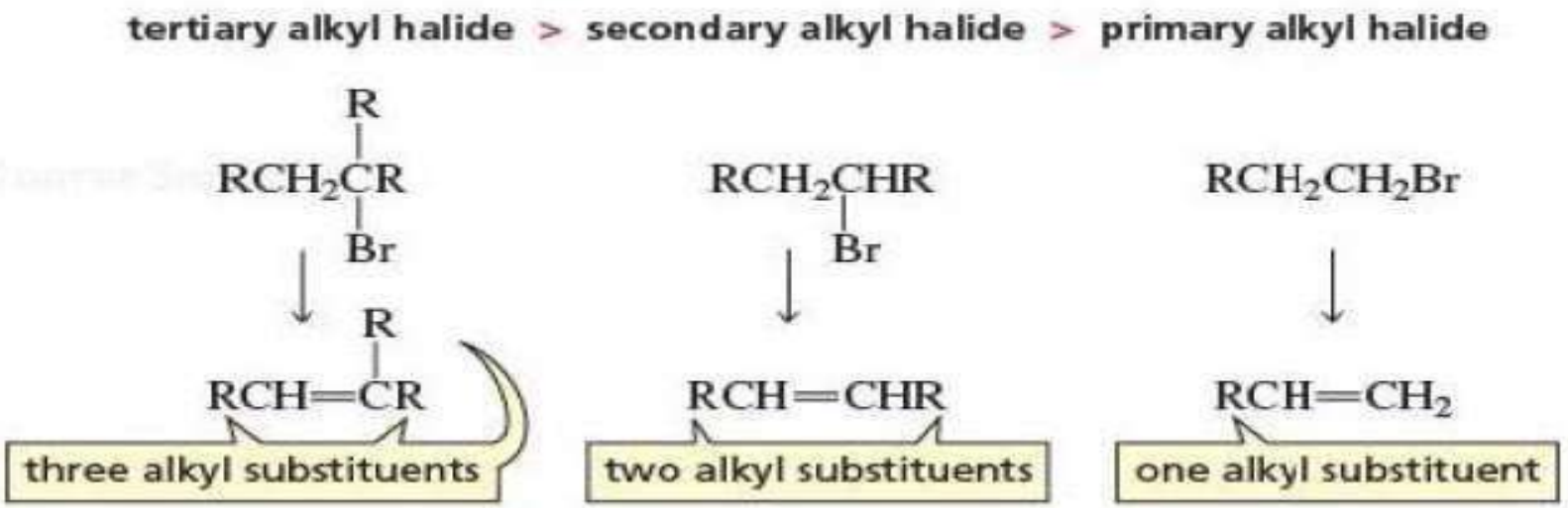
Saytzeff's Rule

- The more substituted alkene is obtained when hydrogen is removed from the β-carbon that is bonded to the fewest hydrogens.



- Hence as per saytzeff rule,

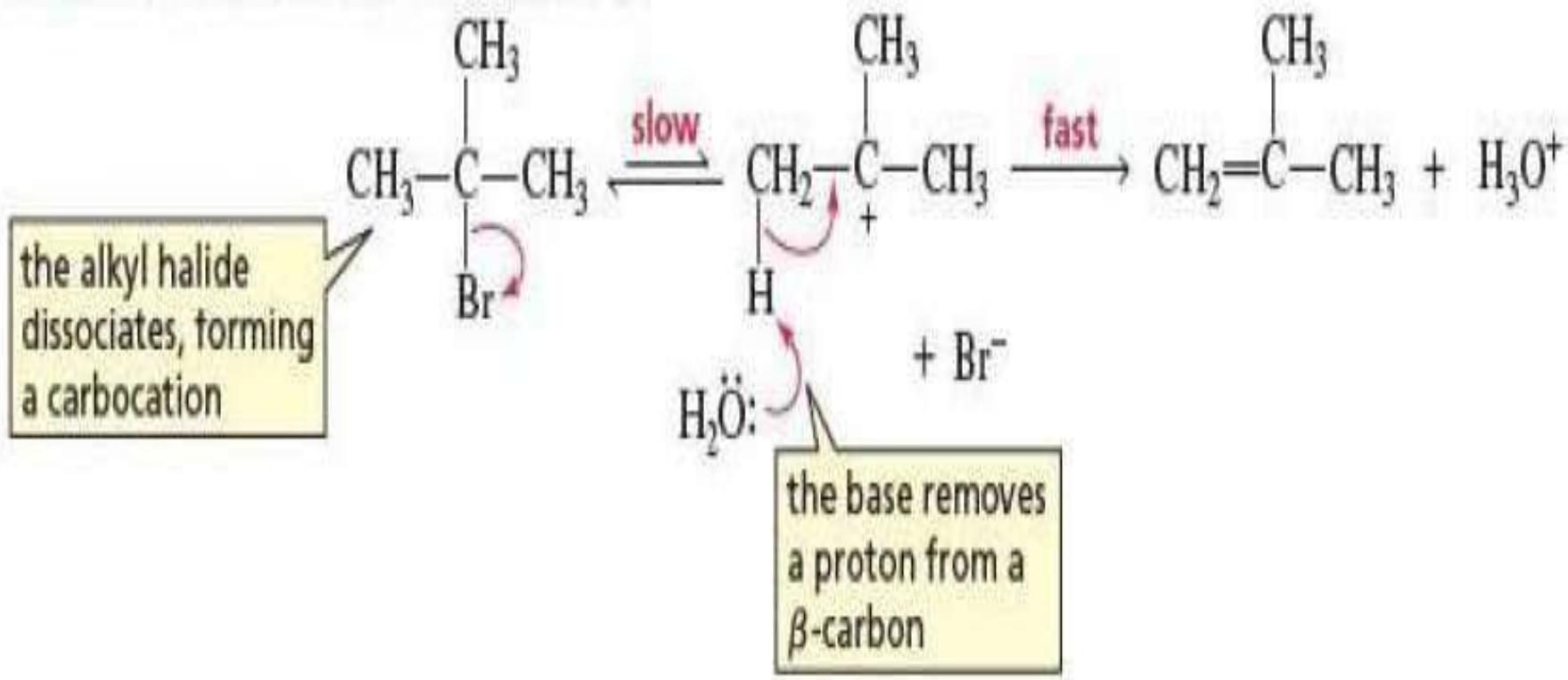
  elimination from a tertiary alkyl halide leads to a more substituted product than secondary alkyl halide, and a secondary alkyl halide more than a primary alkyl halide.
- Thus, relative reactivities in an E2 reaction are:

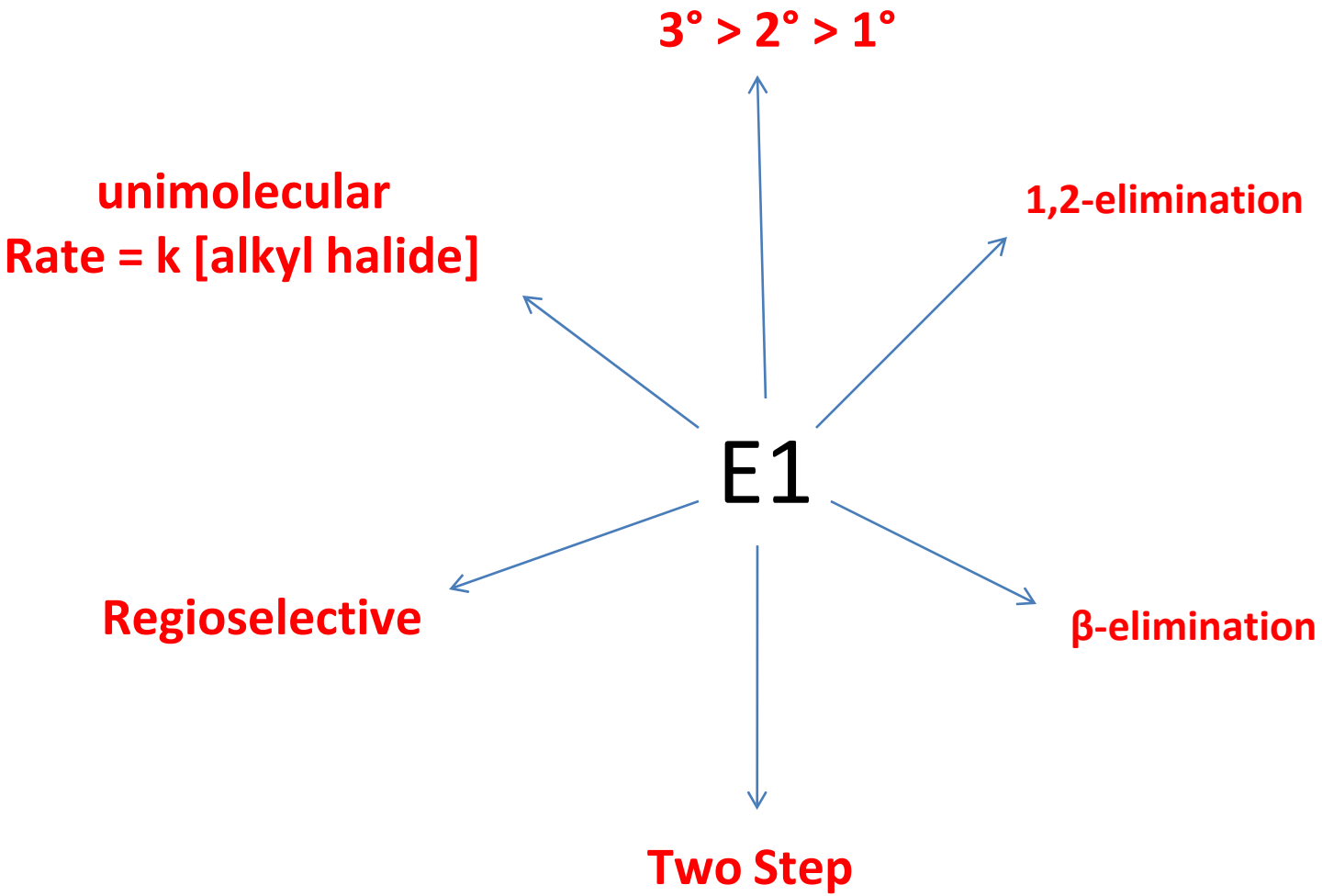


Deviations from Saytzeff rule

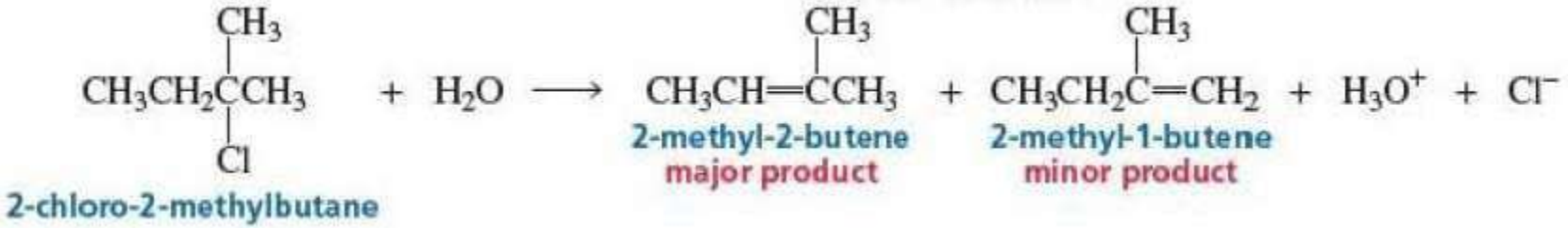
- When fluoride ion is the leaving group,
- When base is sterically hindered,
- When the product of elimination may lead to formation of a conjugated diene, even though the product is a less substituted one.

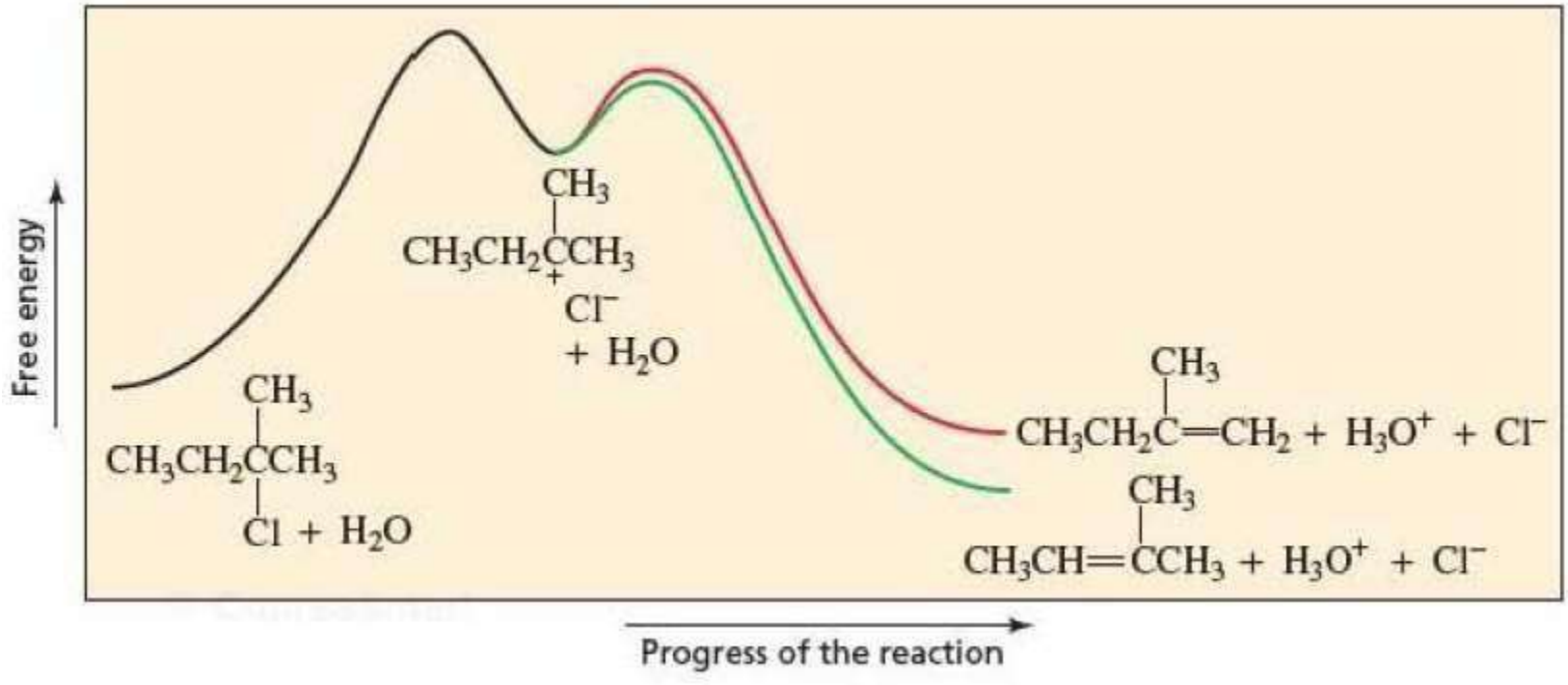
The E1 reaction




3° > 2° > 1°
unimolecular
Rate = k [alkyl halide]
1,2-elimination
E1
Regioselective
β-elimination
Two Step

- Similar to E2, more substituted product is formed as major product in accordance with Saytzeff rule.





- Relative reactivity of alkyl halide in E1 depends on stability of carbocation formed in the 1st step. Thus,

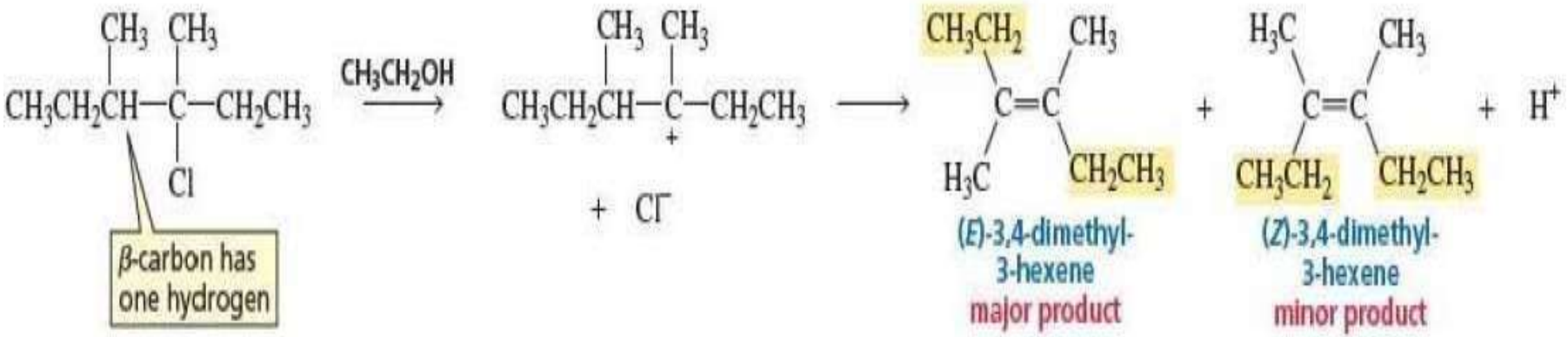
  3° benzylic ≈ 3°allylic > 2° benzylic ≈ 2°allylic ≈ 3° > 1° benzylic ≈ 1°allylic ≈ 2° > 1° > vinyl

- Since weak bases are better leaving groups,

  RI > RBr > Rcl > RF

Factor	E1	E2
Kinetics	Rate = k[RX]	Rate = k[RX][Base]
No. of steps	Two step	One step
Primary alkyl	Never	Good
Secondary alkyl	Moderate	Moderate
Tertiary alkyl	Excellent	May happen
Leaving group	Less Basic	Less Basic
Basicity of the attacking base	weak	strong

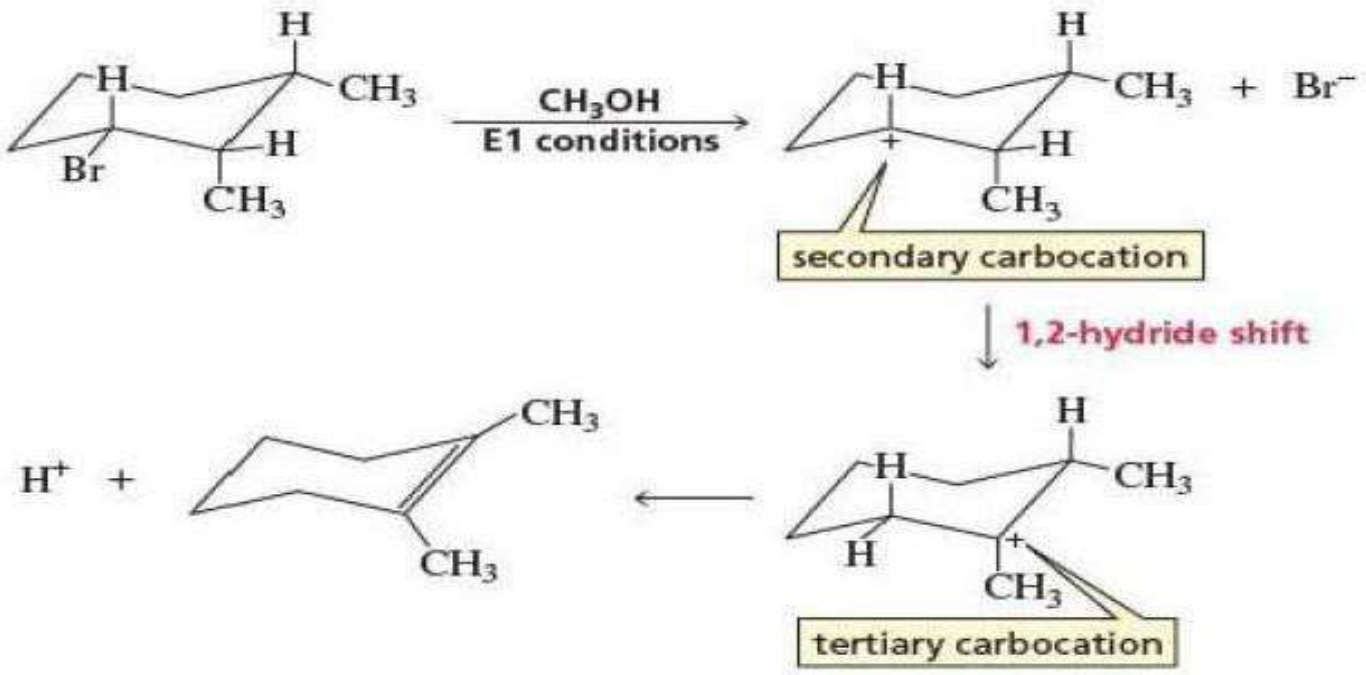
Stereoselectivity of E1



- The carbocation created in the first step is planar. so the electrons from a departing proton can move toward the pos itively charged carbon from *either side. Therefore, both syn and anti* elimination can occur.
- The major product is the one with the bulkiest groups on opposite sides of the double bond. because that is the more stable alkene .

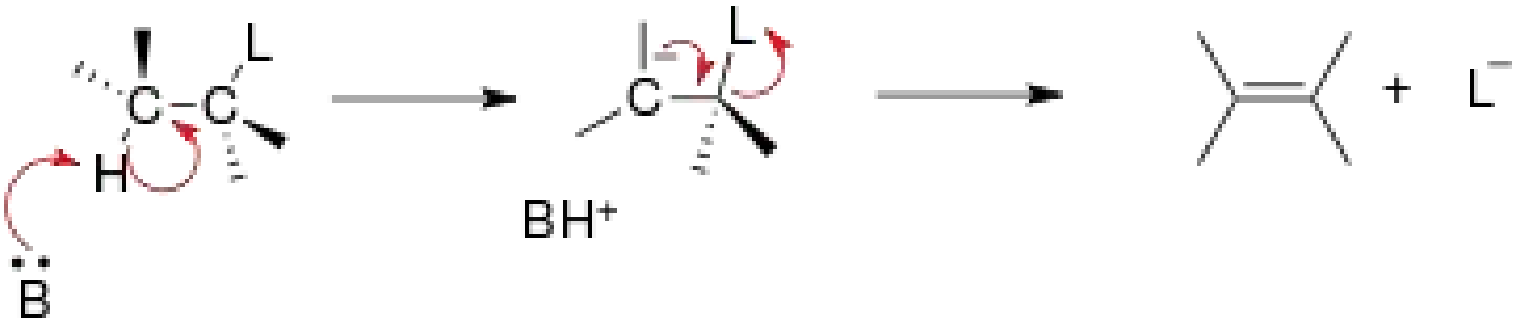
E1 reaction of substituted cyclohexanes

- The two groups that are eliminated do not both have to be in axial positions because the elimination reaction is not concerted.
- Saytzeff rule is followed



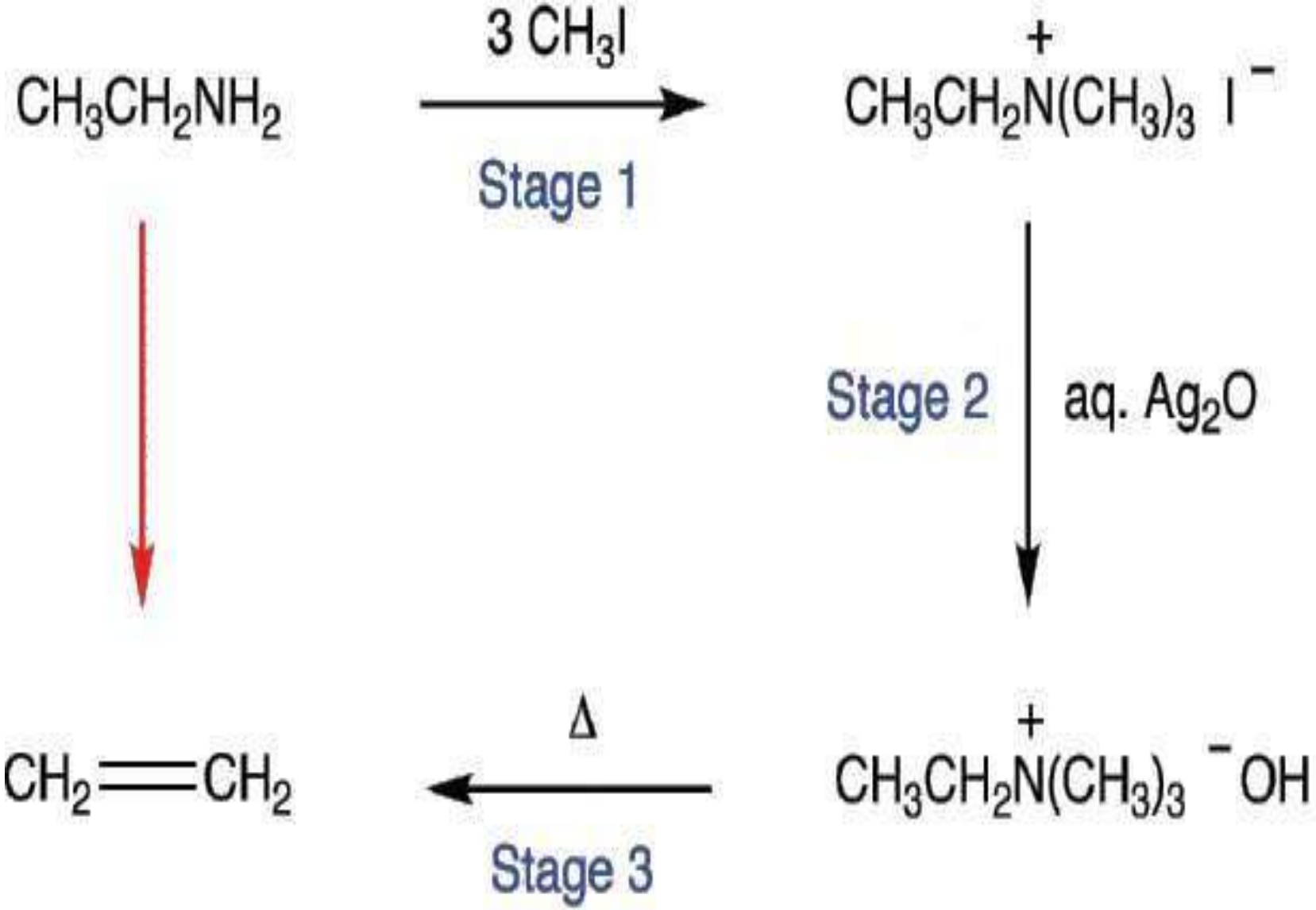
E1cB-elimination reaction

- **E**limination **U**nimolecular **c**onjugate **B**ase
- The reaction takes place around a sp^3 - sp^3 carbon to carbon covalent bond with an α-acidic hydrogen atom substituent and a β-leaving group.
- Carbanion intermediate is formed.



Hofmann Elimination

- When a primary amine bearing one or more beta hydrogens is treated with methyl iodide, followed by aqueous silver oxide, followed by heat, the primary amine is converted to an alkene.
- Occurs in 3 steps and the product is usually anti-saytzeff because of the E1CB reaction in the 3rd step.


3 CH3I
CH3CH2NH2
+
CH3CH2N(CH3)3 I−
Stage 1
Stage 2
aq. Ag2O
Δ
CH2=CH2
+
CH3CH2N(CH3)3 −OH
Stage 3

Substitution	Elimination
Less sterically hindered nucleophile	Sterically hindered nucleophile
Lewis base acts as nucleophile attacking the carbon	Lewis base abstracts proton from β carbon
Weak base	Strong base
Increase in temperature increases rate of reation but is not favourable	Increase in temperature significantly increases rate of reaction
Lowest practical temperature with considerable reaction rates	Higher temperatures

REFERENCES

- Organic Chemistry by P.Bruice; 6th edition
- Organic Chemistry by Carey; 8th edition
- Wikipedia


Thank You

TETRACYCLINES

Dr. P.Venkateswra Rao
VCPS

Introduction:

- Tetracyclines are introduced 50 years ago as potent broad spectrum antibiotics.
- They are biosynthesized from acetic acid and propionic acid units in microorganisms.
- Tetracyclines possess a wide specturm of acitivty i.e. gram+ve and gram-ve bacteria.
- They are mainly designed for oral route but parenteral and topical forms are available.
- **Historical background:**
- In 1945 Chlortetracycline (prototype) of tetracyclines was discovered by Dr. Benjamin, M. Duggar under the guidance of Yellapragada Subba Rao.
- He was born in a poor Telugu Niyogi Brahmin family in Bhimavaram in West Godavari district, Andhra Pradesh.
- He was an employee of Lederle Laboratories in U.S.A.



- Dr. Duggar produced Chlortetracycline (Aureomycin) form golden – colored soil bacterium called Streptomyces aureofaciens by fermentation technology.

- He discovered many life saving drugs.
- Vitamin B-12 (Cyanocobalamine).
- Folic acid vitamin : A remedy medicine to cure “sprue”
- Aureomycine (world’s first Tetracycline and cure cholera, typhoid, plague & dysentry).
- He was the first to discover Gramicidine (polypeptide antibiotic).
- Methotrexate (To prevent blood cancer in children).
- Hetrazin (elephantiasis & isnophelia, filariasis).
- This **Fisco Subbarao method** got recognition amongst the world famous
- scientists.
- Isonicotinic acid Hydrazide (INH) (To cureTuberculosis).
- Discovered the role of ATP which are the sources of energy in human body.

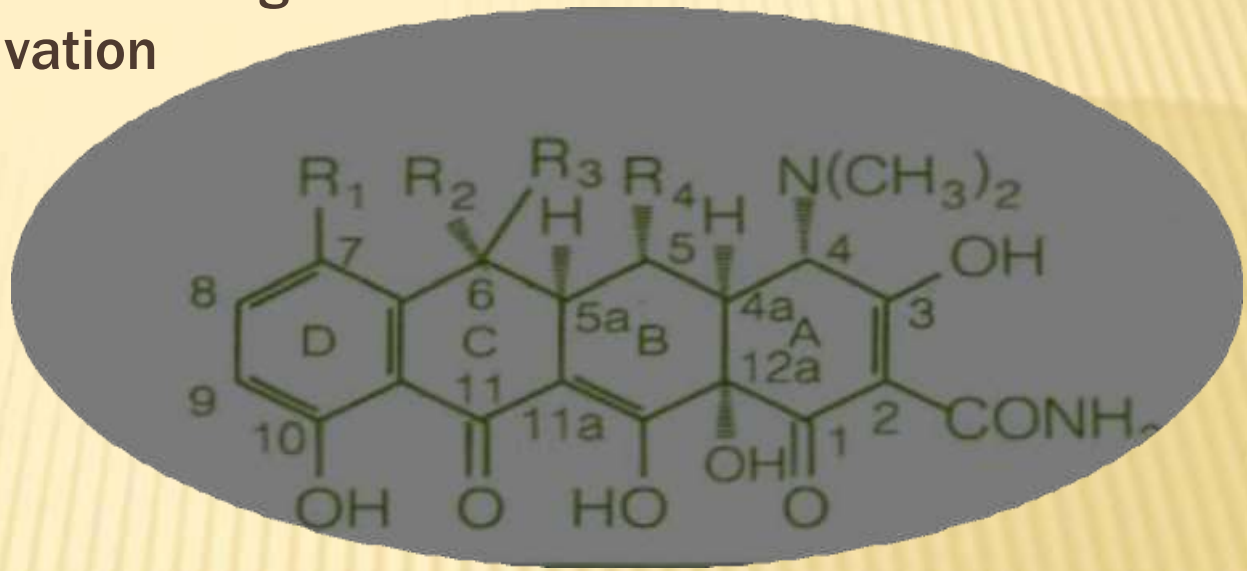
TETRACYCLINES

Tetra means = Four

Cycl means = Hyrocarbon ring

Ine means = Derivation

Definition :



General sructure

Tetracyclines are octahydro napthacene derivatives which are bacteriostatic and broad spectrum antibiotics that kills certain infection - causing microorganisms and are used to treat wide variety of infections.

Sources :

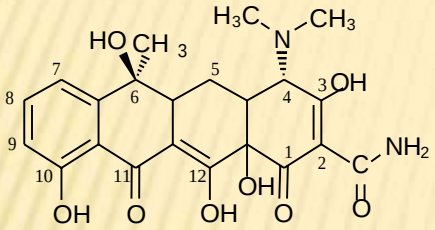
Tetracyclines are obtained from various species of Streptomyces bacteria by fermentation technology

- Chlortetracycline(aureomycin) from *Streptomyces aureofaciens.*
- Oxytetracycline (Terramycin) from *Streptomyces rimosus.*

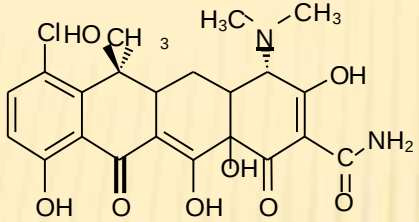
Tetracyclines were divided into different categories i.e., based on soucre, and duration of action .

According to source:

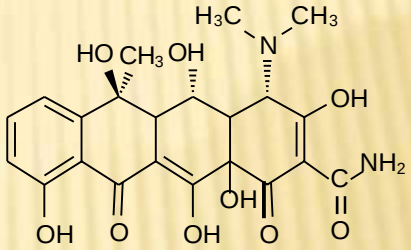
1. Naturally occurring compounds:



TETRACYCLINE
Achrommycin, Sumycin,
Panmycin, Teracap, Tetracyn, Tetralan

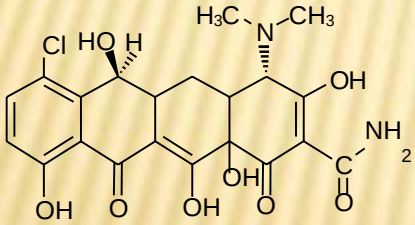


CHLORTETRACYCLINE
Aureomycin

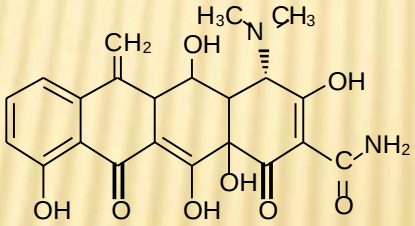


OXYTETRACYCLINE
Terramycin, (Urobiotic)

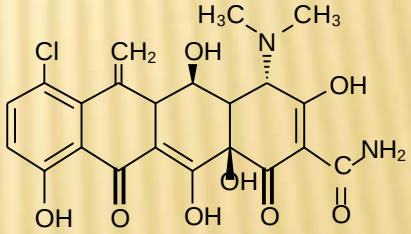
2. Semi- synthetic compounds:



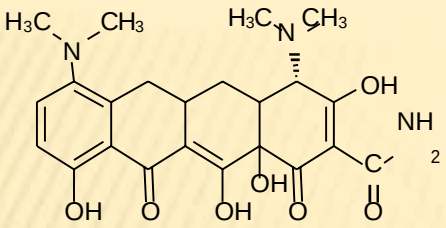
DEMECLOTETRACYCLINE
Declomycin



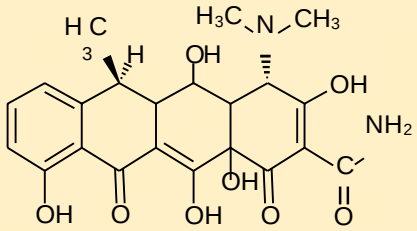
METHACYCLINE
[Rondomycin]



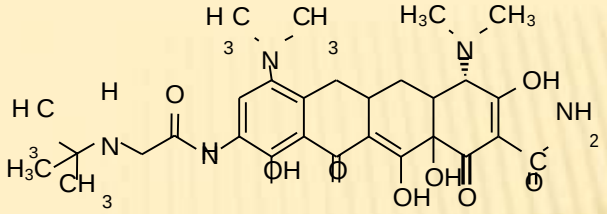
MECLOCYCLINE
Meclan



MINOCYCLINE
Arestin, Dynacin,
Vectrin, Minocin



DOXYCYCLINE
Vibramycin, Vibra–Tabs
Doryx, Doxy



TIGECYCLINE
Tygacil™

Classification:

According to duration of action:

1. **Short-acting (Half-life is 6-8 hrs)**

Tetracycline Chlortetracycline Oxytetracycline

2. **Intermediate-acting (Half-life is ~12hrs)**

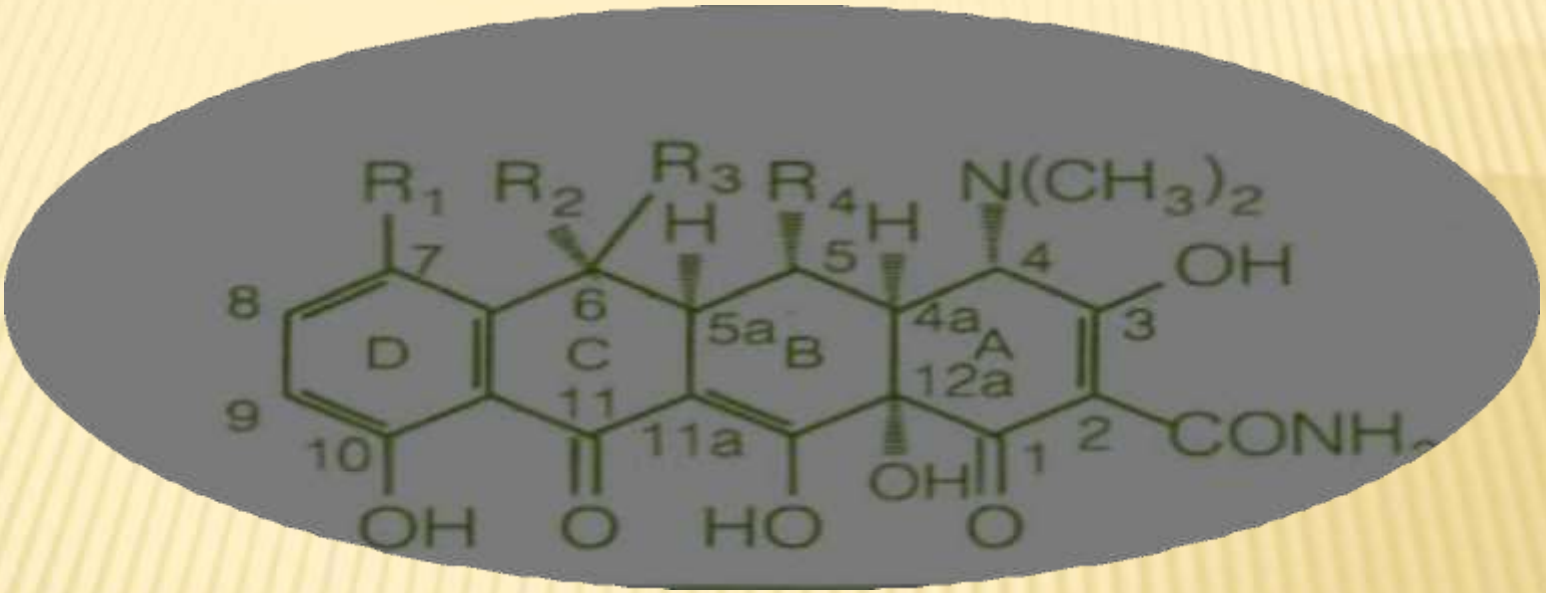
Demeclocycline Methacycline

3. **Long-acting (Half-life is 16 hrs or more)**

Doxycycline Minocycline Tigecycline

Stereochemistry of tetracyclines:

It is verycomplex



4,4a,5,5a,6,12a

Methacycline , Oxytetracycline, Meclocycline, Doxycycline possess 5-hydroxy Substituent have 6 chiral carbonatoms.

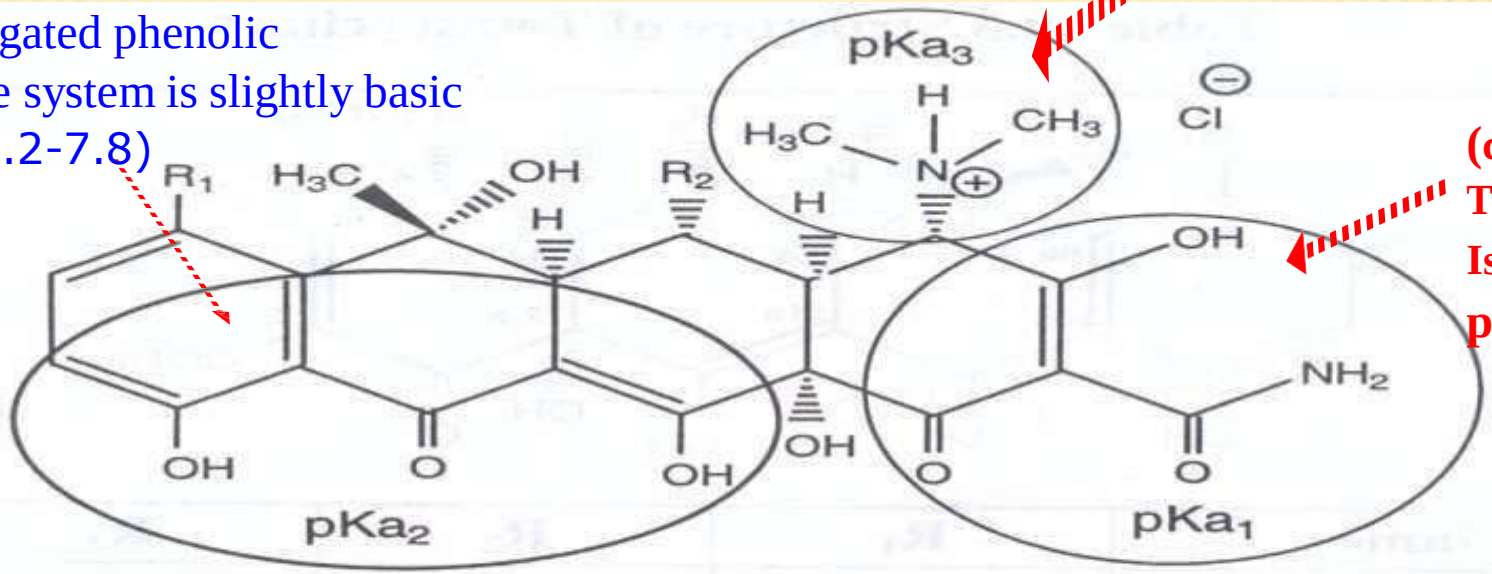
Others have only 5 chiral carbonatoms.

Important structural units and the three acidity constants in the tetracycline molecule.

Strong alkaline Pka3 (9.1-9.7)

(Conjugated phenolic
Enone system is slightly basic
Pka (7.2-7.8)

(conjugated
Trione system
Is acidic nature)
pka1 (2.8-3.4)



pKa values of Tetracyclines Hydrochlorides in Aqueous Solution at 25°C

S.No	Name	Pka_1	pKa_2	pKa_3
1.	Tetracycline	3.3	7.7	9.5
2.	Chlortetracycline	3.3	7.4	9.3
3.	Demeclocycline	3.3	7.2	9.3
4.	Oxytetracycline	3.3	7.3	9.1
5.	Doxycycline	3.4	7.7	9.7
6.	Minocycline	2.8	7.8	9.3

Nature of tetracyclines (Amphoteric):

- Tetracyclines are amphoteric compounds.
- Amphoteric = form salts with both strong acids and bases.

- Three structrual units of tetracyclines representing 3pka values.

- Pka1--- **Conjugated trione system** extending from **C_1 to C_3**

  of ring A is acidic nature of **Pka 2.8-3.4.**

- Pka2--- **Conjugated phenolic enone system** from C_{10}-C_{12} is

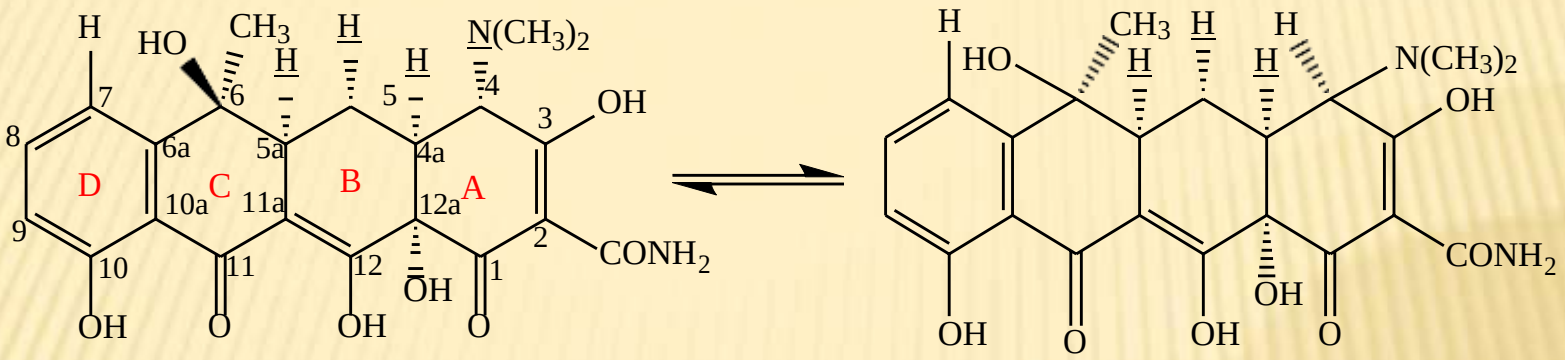
  - associated with weak basic Pka values ranging from **7.2-7.8.**

- Pka3-- **C4** atom and its substituents exhibits Pka3 ranging from

  - **9.1 to 9.7**. which represents strong alkaline nature.

Because of the amphoteric nature tetracyclines forms water soluble salts with strong acids such as HCl and strong bases such NaOH, KOH.

Epimerization:



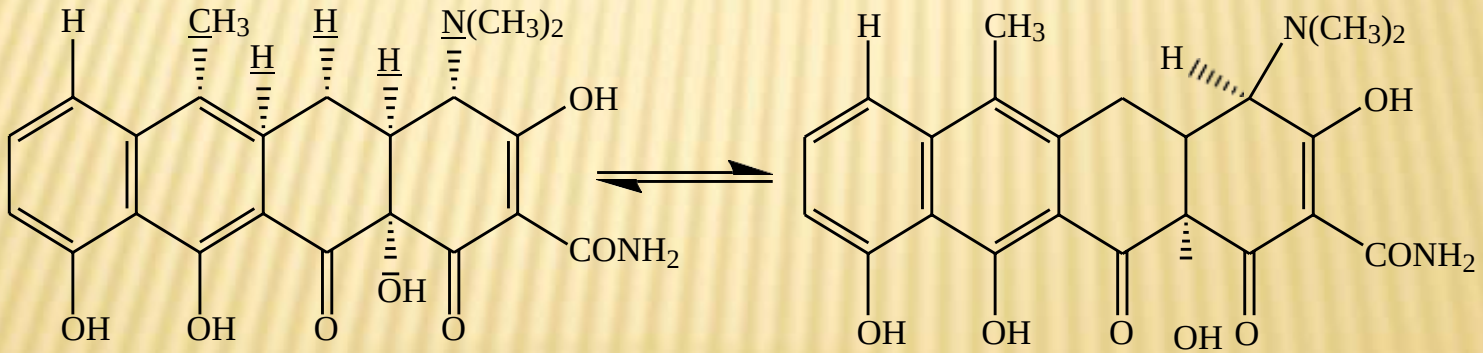
Tetracycline
(Active)

4-Epi tetracycline
(Inactive)

Strong acids and bases Attack those tetracyclines Which possess an –OH group at C-6 and form inactive anhydrotetracyclines

H^+

$-H_2O$

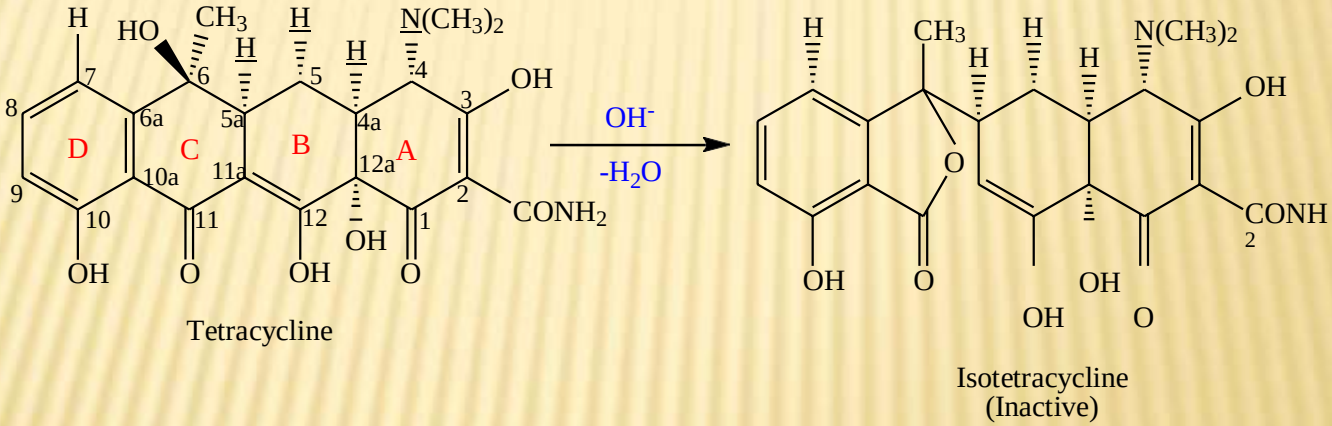


Anhydrous Tetracycline
(Inactive)

4-Epianhydro tetracycline
(Inactive)

Base-catalyzed instability of tracylines:

Under **alkaline** condition, OH at **C_6** change into oxygen anion and then attacks the C-11 (ketone group) lead to **intramolecular nuclear reaction, by electron transfer**, C ring rupture to generate inactive isotetracycline lactone.



Tetracycline

Isotetracycline
(Inactive)

CHELATION:



- This insolubility is not only inconvenient for the preparation of solutions but also interferes with blood levels on oral administration.
- The tetracycline's are incompatible with co-administered, multivalent ion-rich antacids and with hematinics and concomitant consumption of daily products rich in calcium ion also is contraindicated.

SPECTRUM OF ACTIVITY :

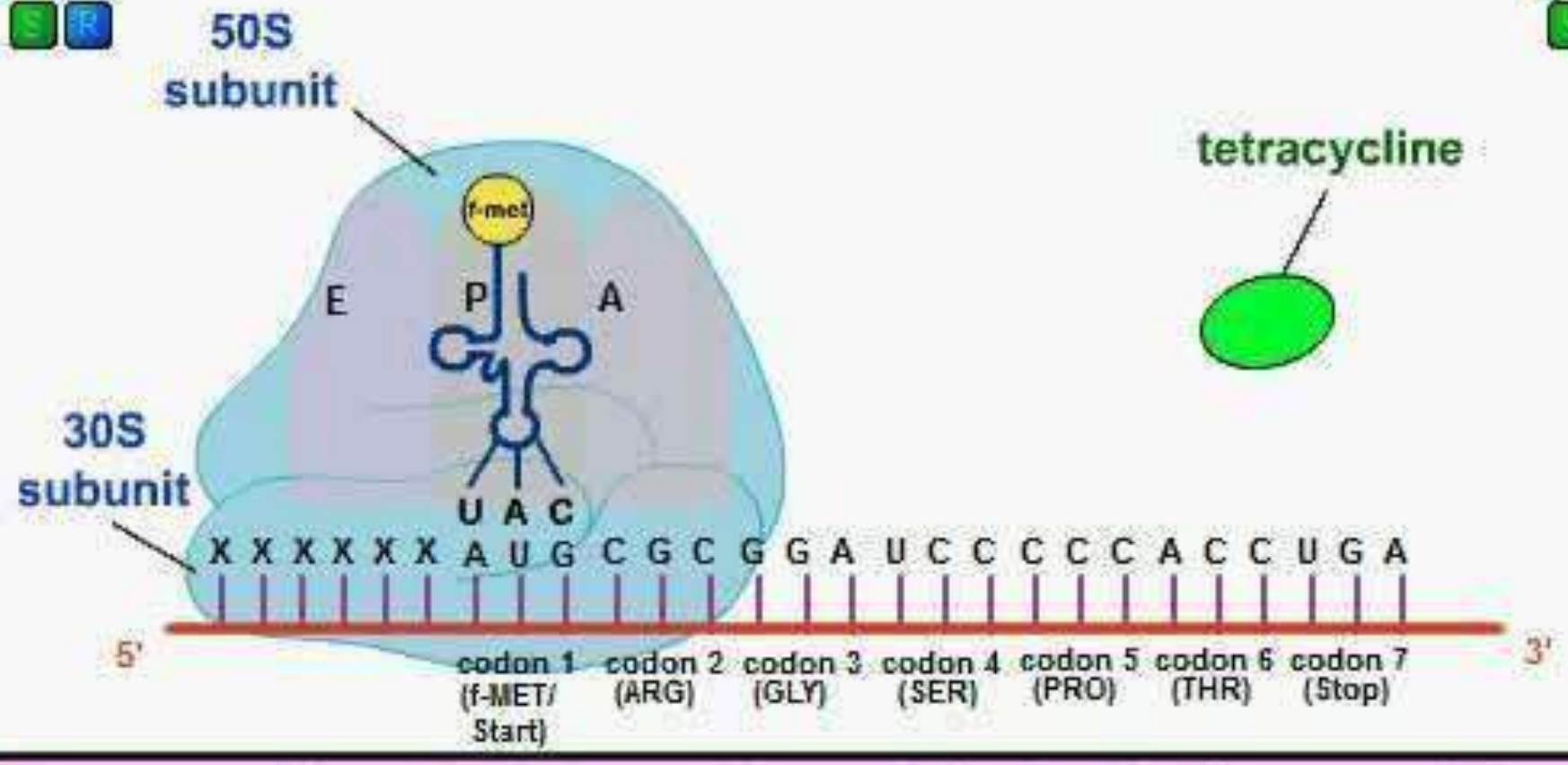
TETRACYCLINES ARE BROAD SPECTRUM ANTIBIOTICS.

THEY ARE ACTIVE AGAINST FOLLOWING MICRO ORGANISMS:

- ✓ Gram +ve & -ve bacteria
- ✓ Spirochetes
- ✓ Mycoplasms
- ✓ Rickettsiae
- ✓ Candida Albicans
- ✓ Mycoplasma Pneumoniae
- ✓ Chlamydia Trachomatis
- ✓ Borrelia Recurrentis
- ✓ Yersinia Pestis
- ✓ Vibrio Cholerae
- ✓ Campylavacter Fetus
- ✓ Brucella Specie
- ✓ Neisseerie Gonorrhoeae

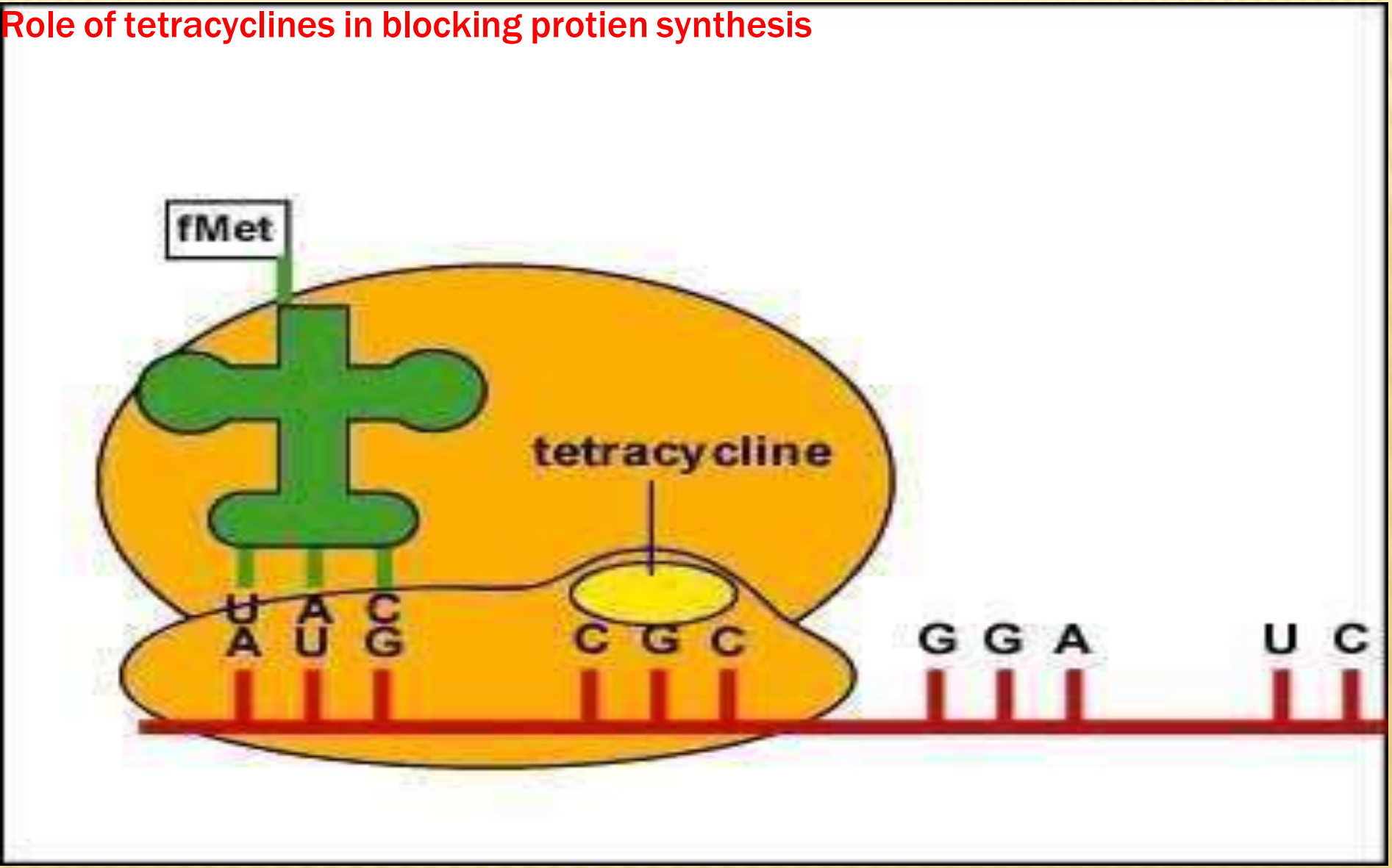
MECHANISM OF ACTION OF TETRACYCLINES:

- Tetracyclines **inhibit protein synthesis** by binding to the bacterial ribosome involved in the translation(protein synthesis) process and making them bacteriostatic.
- The bacterial ribosome is a 70s particle **made up of 30s subunit and 50s subunit**.
- The 30s subunit binds mRNA and initiates the proteinsynthesis.
- The **50s subunit combines with the 30s subunit-mRNA complex to form a ribisome** then binds aminoacyl tRNA and catalyses the building of the protein chain..
- There are two main binding sites for the tRNAmolecule.
- The peptidyl(p-site) binds the tRNA bearing the peptidechain
- The acceptor aminoacyl site (A-site)
- ***Tetracyclines reversibly bind to the 30S subunit at the A-site to prevent attachment of the amino acyl tRNA, terminating the translation process..***

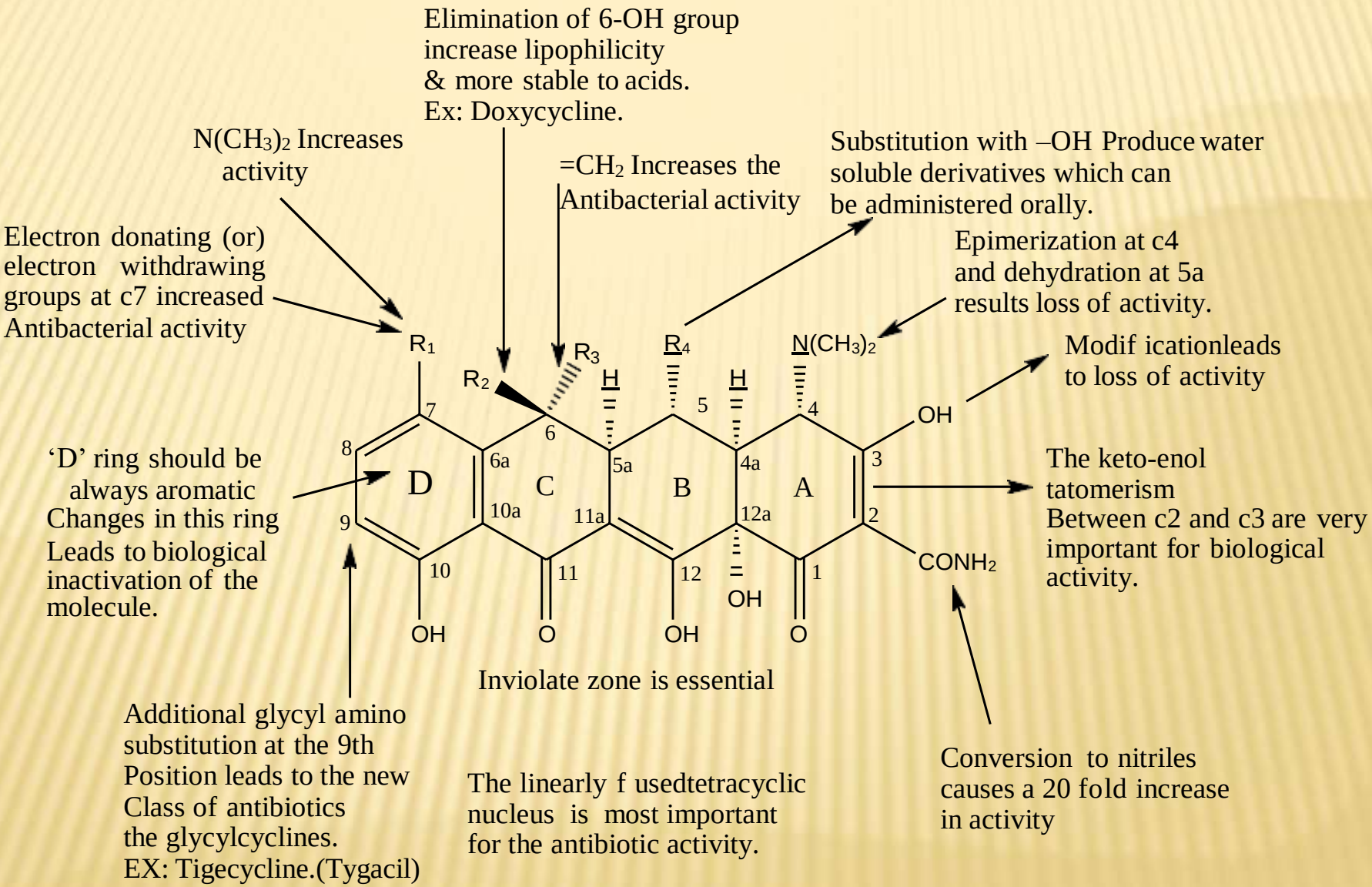


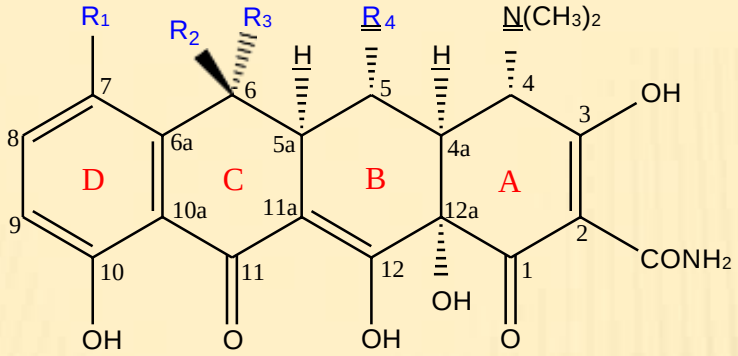
The tetracyclines block bacterial translation by binding reversibly to the 30S ribosomal subunit. This prevents the binding of the aminoacyl tRNAs (charged tRNAs) to the A-site of the ribosome.

Role of tetracyclines in blocking protien synthesis



Structural Importance: Structural Activity Relationship





General structure of tetracyclines

S.NO	Structural Modifications	Effects
1.	Any modification	No bacterial activity
2.	Acetyl group only	Slightly activity retained
3.	Any modification	No bacterial activity
4.	α dimethylamino group	$NHCH_3$ retains more activity
5a.	Loss of H	Inactive degradation product
6.	Remove OH,CH_3 or both	More stable compound
7.	Cl,Br,NO_2,$(CH3)_2N$-	Activity retained
8.	Little information available	_
9.	Cl and CH_3	Decreased activity
10,11,11a,12.	“Inviolate zone” including C-1	Diminished activity

- Uses of tetracyclines :
- Tetracyclines are called "broad-spectrum" antibiotics, because they can be used to treat a wide variety of infections.
- Physicians may prescribe these drugs to treat eye infections.
- Tetracyclines are generally a low-cost alternative among antibiotics.
- Interestingly, a form of tetracycline has recently been used in prevention of cancer recurrence.
- Tetracyclines may be used in the treatment of infections of the respiratory tract, sinuses, middle ear, intestines.
- Gonorrhoea

Contraindications of Tetracycline Antibiotics :

- Can stain developing teeth (even when taken by the mother during pregnancy)
- Inactivated by Ca2+ ion, not to be taken with milk, yogurt, and other dairy products.
- Skin photosensitivity; exposure to the Sun or intense light is not recommended
- Drug-induced lupus, and hepatitis
- Can induce microvesicular fatty liver.
- May interfere with methotrexate by displacing it from the various protein binding sites

Tetracyclines should therefore be avoided in pregnant or lactating women.



They can cause skin reactions



Tetracycline might cause stains to developing adult teeth, which cannot be easily removed with conventional tooth whitening



Side effects :

- Mild nausea, vomiting, diarrhea.
- White patches or sores inside your mouth or on your lips .
- Swollen tongue, trouble swallowing.
- Vaginal itching or discharge.
- Loss of appetite, jaundice (yellowing of the skin or eyes).

Some of drugs eefects tetracyclines:

- Cholesterol-lowering medications such as cholestyramine (Prevalite, Questran) .
- Isotretinoin (Accutane).
- Tretinoin (Renova, Retin-A, Vesanoid) .
- A blood thinner such as warfarin (Coumadin).
- A penicillin antibiotic such as amoxicillin (Amoxil, Trimox, others).
- Penicillin (BeePen-VK, Pen-Vee K, Veetids, others).
- Dicloxacillin (Dynapen)

Some of indications to use tetracyclines:

- Finish the prescription.
- Takeon emptystomach.
- Takewith plenty of water.
- Shake well.
- Do not take with milk, antacids, or iron
- Avoid exposure to sun.

- Do not use this medication if you are pregnant..
- Tetracycline passes into breast milk and may affect bone and tooth development in a nursing baby.
- Do not give tetracycline to a child younger than 8 years old.
- Avoid exposure to sunlight or artificial UV rays.
- Do not take iron supplements, multivitamins, calcium supplements, antacids.
- Throw away any unused tetracycline when it expires or when it is no longer needed.

Thank you ....

GMP AND cGMP CONSIDERATIONS

Dr. P.Venkateswra rao
VCPS

- cGMP refers to the Current Good Manufacturing Practice regulations enforced by the US Food and Drug Administration (FDA).
- cGMP provide for systems that assure proper design, monitoring, and control of manufacturing processes and facilities. Adherence to the cGMP regulations assures the identity, strength, quality, and purity of drug products by requiring that manufacturers of medications adequately control manufacturing operations.
- This includes establishing strong quality management systems, obtaining appropriate quality raw materials, establishing robust operating procedures, detecting and investigating product quality deviations, and maintaining reliable testing laboratories.
- This formal system of controls at a pharmaceutical company, if adequately put into practice, helps to prevent instances of contamination, mix-ups, deviations, failures, and errors.
- This assures that drug products meet their quality standards.

➢ *TRAGEDIES PREECEDING CGMP REGULATIONS*

- 1902 - Development of the Biologic Control Act
- 1906 - Development of the Pure Food and Drug Act
- 1938 - Federal Food, Drug and Cosmetic Act
- 1941 - Initiation of GMP
- 1944 - Development of Public Health Services Act
- 1962 - Kefauver-Harris Drug Amendments released (It introduced a requirement for drug manufacturers to provide proof of the effectiveness and safety of their drugs before approval, required drug advertising to disclose accurate information about side effects, and stopped cheap generic drugs being marketed as expensive drugs under new trade names as new "breakthrough medications)
- 1963 - Establishment of GMPs for Drugs
- 1975 - cGMP for Blood and Components Final Rule
- 1976 - Medical Device Amendments
- 1978 - cGMP for Drugs and Medical Devices

- 1979 - GLPs Final Rule
- 1980 - Infant Formula Act is passed
- Sulfathiazole tablets contaminated with phenobarbital
- 1941 - 300 people died/injured
- FDA to enforce and revise manufacturing and quality control requirements
- 1941 - GMP is born
- Thalidomide tragedy
- Thousands of children born with birth defects due to adverse drug reactions of morning sickness pill taken by mothers
- Strengthen FDA’s regulations regarding experimentation on humans and proposed new way how drugs are approved
- and regulated
- “Proof of efficacy” law

What are cGMPs

- Regulations are established by the Food and Drug Administration **(FDA) to ensure that minimum standards are met for drug product quality in the United States.**

OR

- Rules set up by the FDA that drug manufacturers needs to follow in order to ensure that a **safe and effective product is manufactured**

➢ *COMPONENTS OF cGMP*

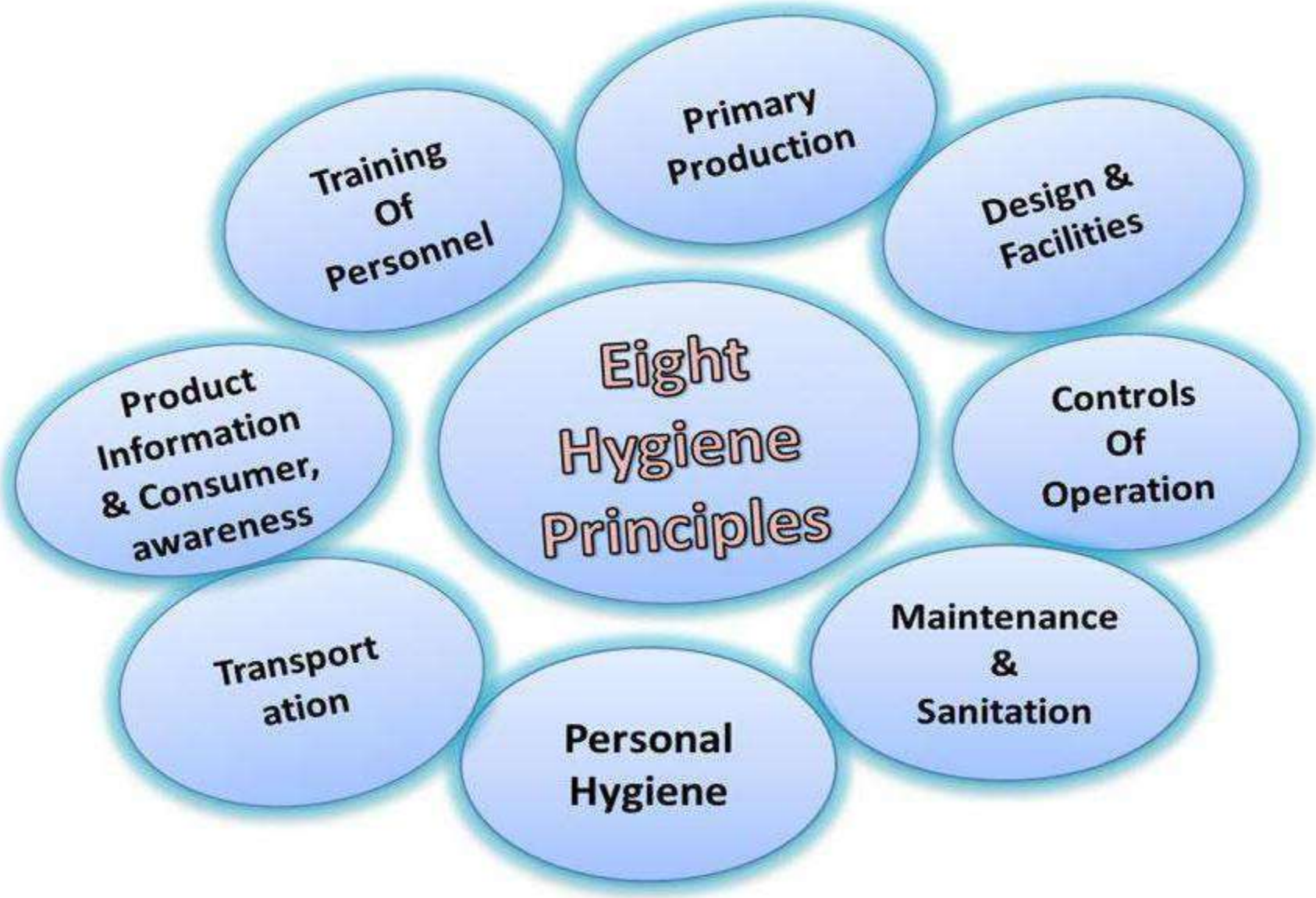
- a) GMP is a part of Q.A
- b) GMP’s main function is to produce quality products consistently.
- c) GMP must meet legal requirements of country.
- d) GMP must meet both production and Q.C. related issues.
- e) WHO further comments that the main function of GMP is to avoid mix-ups and contamination risks.

- ***cGMP COVERS***
- a) General considerations
- b) Personnel
- c) Premises
- d) Equipment
- e) Sanitation
- f) SOP’s
- g) Raw Materials
- h) Self Inspection And Audit
- i) Master Formula Records
- j) Batch Manufacturing Records
- k) Warehousing Area
- l) Labels And Other Printed Materials
- m) QA

cGMP For Finished Pharmaceuticals

- **General Provision**
- **Organization & Personnel**
- **Building & Facilities**
- **Equipment**
- **Control of Components & Drug**
- **Product Containers & Closures**
- **Production & Process Control**
- **Packaging & Labeling Control**
- **Handling & Distribution**
- **Laboratory Control**
- **Records & Reports**
- **Returned & Salvaged Drugs**

GMP


Eight Hygiene Principles
Primary Production
Design & Facilities
Controls Of Operation
Maintenance & Sanitation
Personal Hygiene
Transport ation
Product Information & Consumer, awareness
Training Of Personnel

GMP



CURRENT GOOD MANUFACTURING PRACTICE FOR FINISHED PHARMACEUTICALS

The GMP Institute was established Ten Principles of cGMP

- **Principle1:** Writing detailed step-by-step procedures that provide a roadmap for controlled and consistent performance
- **Principle2 :**Carefully following written procedures to prevent contamination, mix-ups and errors.

Importance of Written Procedures:
Procedures should be Clear , Concise and Logical

Importance of Written Procedures
Taking shortcuts may save time or make the task easier, but you **should never deviate from a written procedure without the approval of a supervisor or Quality Department**

- **Principle3 :** Promptly and accurately documenting work for compliance and traceability
- **Principle 4:** Proving that systems do what they are designed to do by validating

Validate and Document Work:
To prove that our equipment and process consistently do what they are supposed to do

Principle 5 : Develop a good design for the facility and the equipment from the beginning

Principle 6 : Properly maintaining facilities and equipment

Design , construction and maintenance of the facility and equipment

A Logical and well planning layout will improve productivity.

Remove unnecessary traffic in the production area

Segregate materials , products, and their components to minimize the confusion and potential mix-ups and errors

It is important to control :

Air, Water, Lighting, Ventilation, Temperature and RH

- **Principle7 :** Clearly defining, developing and demonstrating job competence

GMP makes for Competent Employees

Training: include basic training on the theory and practice of GMP as well as specific training relative to their role .

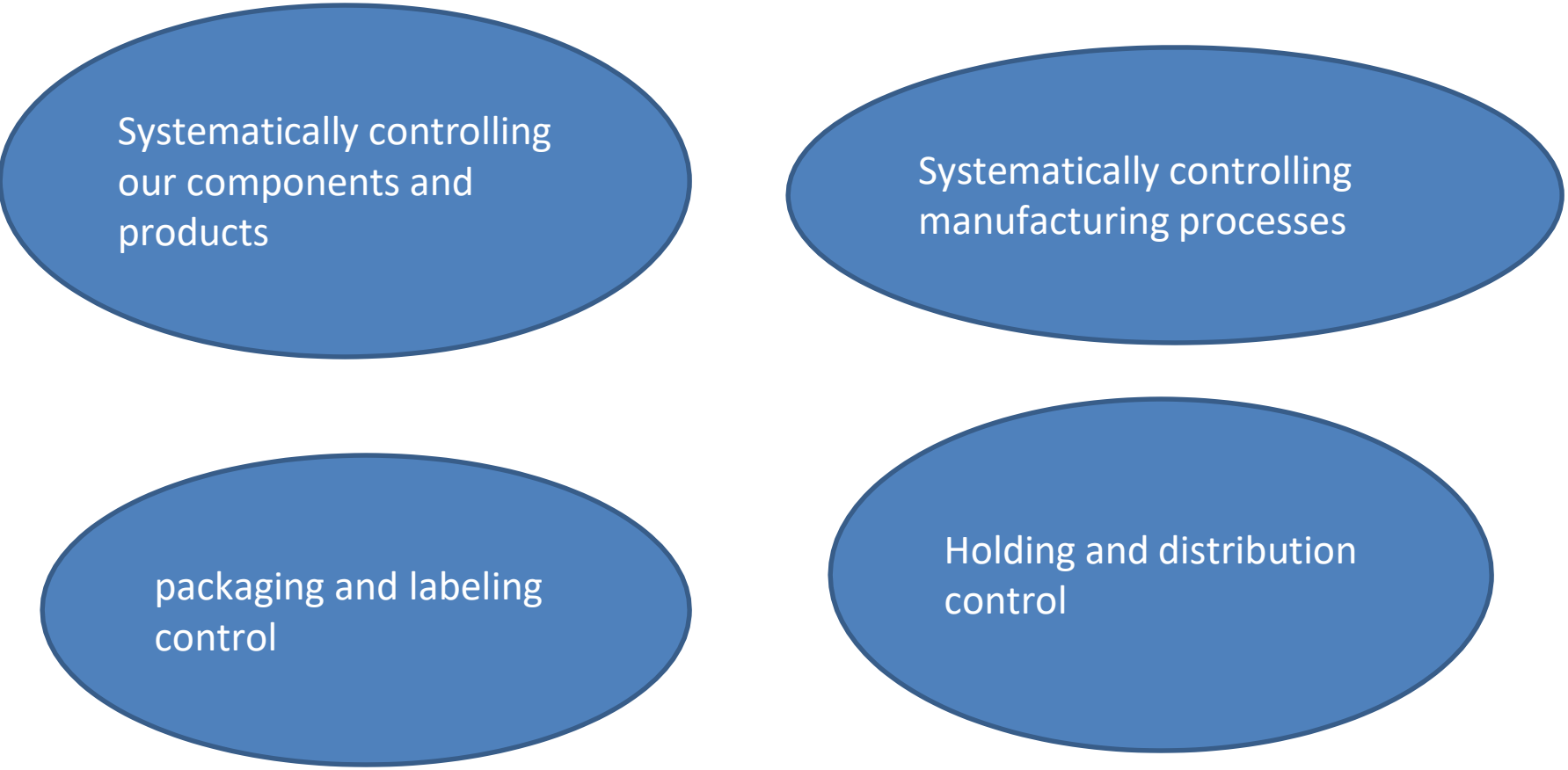
Companies need people who know to do the job right the first time , every time

Principle 8 : Protecting products against contamination by making cleanliness a continual habit.

Practice good Hygiene

- Health examinations
- *Written procedures and instructions - to wash hands before entering production areas*
- Direct contact between product, raw materials and operator *Should be avoided*
- Protection of product from contamination:
  - *Clean clothes appropriate to personnel activities*
  - *Including hair covering (e.g. caps)*
- Check change rooms/changing facilities
- Smoking, eating and drinking not allowed in production areas, laboratories and storage areas
- No chewing (e.g. gum), or keeping food or drinks allowed
- No plants kept inside these areas
- Rest and refreshment areas should be separate from manufacturing and control areas

Principle 9: Building quality into products by.



Principle 10 : Conducting planned and periodic audits for compliance and performance**.**

- **CURRENT GOOD MANUFACTURING PRACTICE FOR FINISHED PHARMACEUTICALS**
- **Subpart A--**General Provisions
- **Subpart B--**Organization and Personnel
- **Subpart C--**Buildings and Facilities
- **Subpart D—**Equipment
- **Subpart E--**Control of Components and Drug Product Containers and Closures
- **Subpart F--**Production and Process Controls

- **Subpart G--**Packaging and Labeling Control
- **Subpart H--**Holding and Distribution
- **Subpart I--**Laboratory Controls
- **Subpart J--**Records and Reports
- **Subpart K--**Returned and Salvaged Drug Products

Active ingredient or active pharmaceutical ingredient (API): Any component that is intended to furnish pharmacologic activity or other direct effect in the diagnosis, cure, mitigation, treatment or prevention of disease, or to affect the structure or function of the body of man or other animals.

- **Batch:** A specific quantity of a drug of uniform specified quality produced according to a single manufacturing order during the same cycle of manufacture
- **Lot:** A batch or any portion of a batch having uniform specified quality and a distinctive identifying lot number.
- **Lot number, control number, or batch number:** Any distinctive combination of letters, numbers, or symbols from which the complete history of the manufacture, processing, packaging, holding, and distribution of a batch or lot of a drug product may be determined.

- **Certification:** Documented testimony by qualified authorities that a system qualification, calibration, validation, or revalidation has been performed appropriately and that the results are acceptable.
- **Compliance:** Determination through inspection of the extent to which a manufacturer is acting in accordance with prescribed regulations, standards, and practices.
- **Component:** Any ingredient used in the manufacture of a drug product, including those that may not be present in the finished product.
- **Inactive ingredient:** Any component other than the active ingredients in a drug product
- **Drug product:** A finished form that contains an active drug and inactive ingredients. The term may also include a form that does not contain an active ingredient, such as a placebo.

- **Master record:** Record containing the formulation, specifications, manufacturing procedures, quality assurance requirements, and labeling of a finished product .

- **Quality assurance:** Provision to all concerned the evidence needed to establish confidence that the activities relating to quality are being performed adequately.
- **Quality audit:** A documented activity performed in accordance with established procedures on a planned and periodic basis to verify compliance with the procedures to ensure quality.

- **Quality control:** The regulatory process through which industry measures actual quality performance, compares it with standards, and acts on the difference.

- **Quality control unit:** An organizational element designated by a firm to be responsible for the duties relating to quality control

- **Quarantine:** An area that is marked, designated, or set aside for the holding of incoming components prior to acceptance testing and qualification for use.
- **Reprocessing:** The activity whereby the finished product or any of its components is recycled through all or part of the manufacturing process.
- **Strength:** The concentration of the drug substance per unit dose or volume

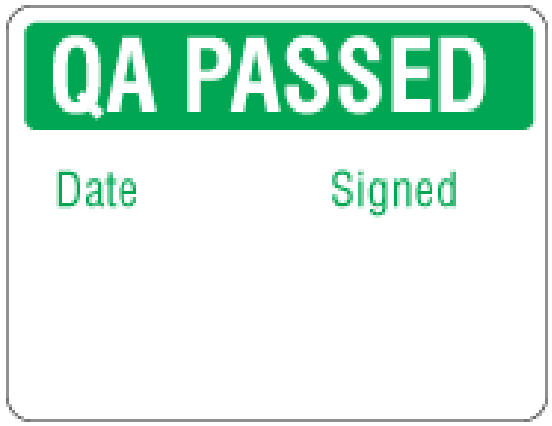
Subpart A--General Provisions

- The regulations in this part contain the minimum current good manufacturing practice for preparation of drug products (excluding positron emission tomography drugs) for administration to humans or animals.

Subpart B--Organization and Personnel

- Responsibilities of quality control unit.
- Personnel qualifications.
- Personnel responsibilities.
- Consultants.









Responsibilities of quality control unit.

(a) There shall be a quality control unit that shall have the responsibility and authority to approve or reject all components, drug product containers, closures, in-process materials, packaging material, labeling, and drug products, and the authority to review production records to assure that no errors have occurred

(b) Adequate laboratory facilities for the testing and approval (or rejection) of components, drug product containers, closures, packaging materials, in-process materials, and drug products shall be available to the quality control unit.

(c) The quality control unit shall have the responsibility for approving or rejecting all procedures or specifications impacting on the identity, strength, quality, and purity of the drug product.

- **Personnel qualifications.**

(a) Each person engaged in the manufacture, processing, packing, or holding of a drug product shall have education, training, and experience, or any combination thereof, to enable that person to perform the assigned functions.

(b) There shall be an adequate number of qualified personnel to perform and supervise the manufacture, processing, packing, or holding of each drug product.

Personnel responsibilities.

(a) Personnel engaged in the manufacture, processing, packing, or holding of a drug product shall wear clean clothing appropriate for the duties they perform. Protective apparel, such as head, face, hand, and arm coverings, shall be worn as necessary to protect drug products from contamination.

(b) Personnel shall practice good sanitation and health habits.

(c) Only personnel authorized by supervisory personnel shall enter those areas of the buildings and facilities designated as limited-access areas.

- **Consultants.**

  Consultants advising on the manufacture, processing, packing, or holding of drug products shall have sufficient education, training, and experience, or any combination thereof, to advise on the subject for which they are retained

- **Subpart C--**Buildings and Facilities:
- Design and construction features.
- Any building or buildings used in the manufacture, processing, packing, or holding of a drug product
- shall be of suitable size, construction and location to facilitate cleaning, maintenance, and proper operations.
- shall have adequate space

➢ Lighting.
➢ Ventilation, air filtration, air heating and cooling.
➢ Plumbing.
➢ Sewage and refuse.
➢ Washing and toilet facilities.
➢ Sanitation.
➢ Maintenance.

- **Subpart D—Equipment - Equipment design, size, and location. - Equipment construction.** Equipment shall be constructed so that surfaces that contact components, in-process materials, or drug products shall not be reactive, additive, or absorptive so as to alter the safety, identity, strength, quality, or purity of the drug product

Equipment cleaning and maintenance. Equipment and utensils shall be cleaned, maintained, and, as appropriate for the nature of the drug, sanitized and/or sterilized at appropriate intervals to prevent malfunctions or contamination that would alter the safety, identity, strength, quality, or purity of the drug product - **Automatic, mechanical, and electronic equipment.**

Filters.

Filters for liquid filtration used in the manufacture, processing, or packing of injectable drug products intended for human use shall not release fibers into such products.

- **Subpart E--Control of Components and Drug:**

-Receipt and storage of untested components, drug product containers, and closures.

-Testing and approval or rejection of components, drug product containers, and closures.

- Use of approved components, drug product containers, and closures.
- Retesting of approved components, drug product containers, and closures.
- Rejected components, drug product containers, and closures.
- Drug product containers and closures.

- The component is assigned a control number that identifies both the component and the intended product.

- **Subpart F--Production and Process Controls**

- Written procedures; deviations. - Charge-in of components. - Calculation of yield. - Equipment identification. - Sampling and testing of in-process materials and drug products. - Time limitations on production. - Control of microbiological contamination. - Reprocessing.

- **Subpart G--Packaging and Labelling Control**

-Materials examination and usage criteria. - Labelling issuance. - Packaging and labeling operations. - Tamper-evident packaging requirements for over-the-counter (OTC) human drug products. - Drug product inspection. - Expiration dating.

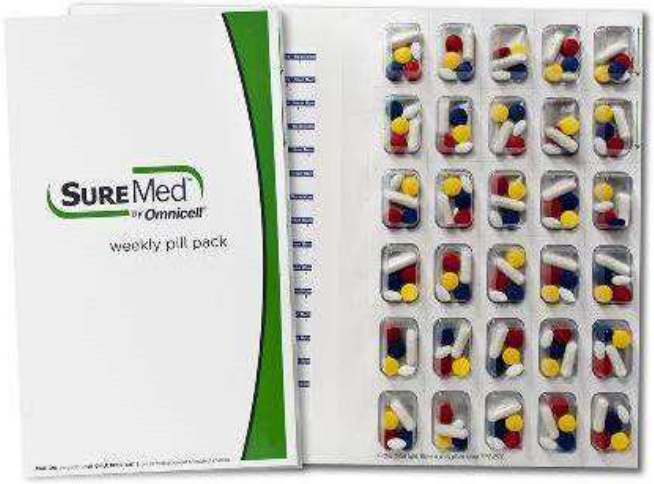







TYLENOL
EXTRA




SUREMed
by Omnicell
weekly pill pack

- **Subpart H--Holding and Distribution**
- - Warehousing procedures. - Distribution procedures.

- **Subpart I--Laboratory Controls**
- - General requirements.
- - Testing and release for distribution
- - Stability testing.
- - Special testing requirements.
- - Reserve samples.
- - Laboratory animals.
- - Penicillin contamination.

Subpart J--Records and Reports

- General requirements.
- Equipment cleaning and use log.
- Component, drug product container, closure, and labeling records.
- Master production and control records.
- Batch production and control records.
- Production record review.
- Laboratory records.
- Distribution records.
- Complaint files.

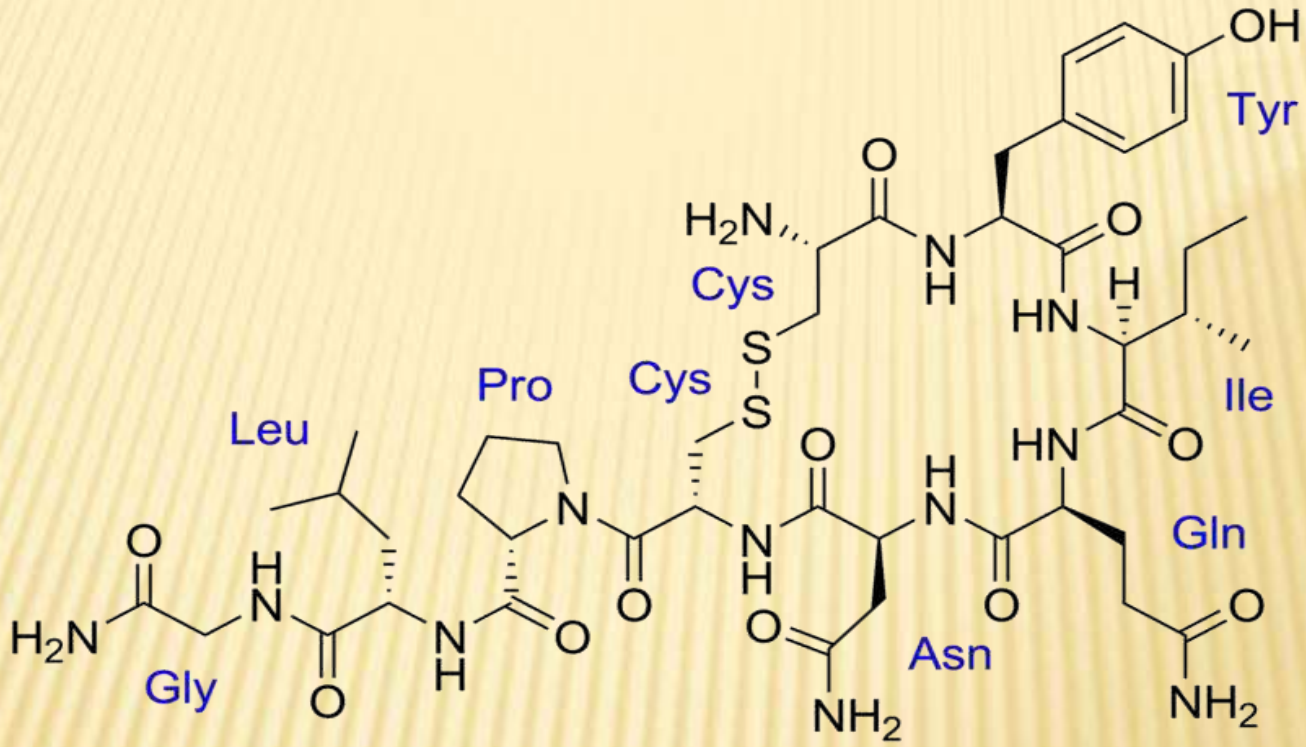
Subpart K--Returned and Salvaged Drug Products

- Returned drug products. - Drug product salvaging.

Complete master production and control records for each batch must be kept and must include the following:

- Name and strength of the product
- Dosage form
- Quantitative amounts of components and dosage units
- Complete manufacturing and control procedures
- Specifications
- Special notations
- Equipment used
- In-process controls
- Sampling and laboratory methods and assay results
- Calibration of instruments
- Distribution records
- Dated and employee-identified records

CHEMISTRY OF OXYTOCIN



Dr.P.Naresh
VCPS

BIOSYNTHESIS:

- The oxytocin peptide is synthesized as an inactive precursor protein from the *OXT* gene.
- This precursor protein also includes the oxytocin carrier protein neurophysin I.
- The inactive precursor protein is progressively hydrolyzed into smaller fragments (one of which is neurophysin I) via a series of enzymes. The last hydrolysis that releases the active oxytocin nonapeptide is catalyzed by peptidylglycine alpha-amidating monooxygenase (PAM).
- The activity of the PAM enzyme system is dependent upon vitamin C (ascorbate), which is a necessary vitamin cofactor.
- By chance, sodium ascorbate by itself was found to stimulate the production of oxytocin from ovarian tissue over a range of concentrations in a dose-dependent manner.
- Many of the same tissues (*e.g.* ovaries, testes, eyes, adrenals, placenta, thymus, pancreas) where PAM (and oxytocin by default) is found are also known to store higher concentrations of vitamin C.

METABOLISM:

- Oxytocin is known to be metabolized by the oxytocinase, leucyl/cystinyl aminopeptidase.
- Other oxytocinases are also known to exist.
- Amastatin, bestatin (ubenimex), leupeptin, and puromycinhave been found to inhibit the enzymatic degradation of oxytocin, though they also inhibit the degradation of various other peptides, such as vasopressin, met-enkephalin, and dynorphin A.

Vertebrate Oxytocin Family		
Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2	Oxytocin (OXT)	Most mammals, ratfish
Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Pro-Gly-NH_2	Prol-Oxytocin	Some New World monkeys, northern tree shrews
Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Ile-Gly-NH_2	Mesotocin	Most marsupials, all birds, reptiles, amphibians, lungfishes, coelacanths
Cys-Tyr-Ile-Gln-Ser-Cys-Pro-Ile-Gly-NH_2	Seritocin	Frogs
Cys-Tyr-Ile-Ser-Asn-Cys-Pro-Ile-Gly-NH_2	Isotocin	Bony fishes
Cys-Tyr-Ile-Ser-Asn-Cys-Pro-Gln-Gly-NH_2	Glumitocin	skates
Cys-Tyr-Ile-Asn/Gln-Asn-Cys-Pro-Leu/Val-Gly-NH_2	Various tocins	Sharks

Vertebrate Vasopressin Family		
Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2	Argipressin (AVP, ADH)	Most mammals
Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Lys-Gly-NH_2	Lypressin (LVP)	Pigs, hippos, warthogs, some marsupials
Cys-Phe-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2	Phenypressin	Some marsupials
Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Arg-Gly-NH_2	Vasotocin†	Non-mammals

Invertebrate VP/OT Superfamily		
Cys-Leu-Ile-Thr-Asn-Cys-Pro-Arg-Gly-NH_2	Inotocin	Locust
Cys-Phe-Val-Arg-Asn-Cys-Pro-Thr-Gly-NH_2	Annetocin	Earthworm
Cys-Phe-Ile-Arg-Asn-Cys-Pro-Lys-Gly-NH_2	Lys-Connopressin	Geography & imperial cone snail, pond snail, sea hare, leech
Cys-Ile-Ile-Arg-Asn-Cys-Pro-Arg-Gly-NH_2	Arg-Connopressin	Striped cone snail
Cys-Tyr-Phe-Arg-Asn-Cys-Pro-Ile-Gly-NH_2	Cephalotocin	Octopus
Cys-Phe-Trp-Thr-Ser-Cys-Pro-Ile-Gly-NH_2	Octopressin	Octopus
†Vasotocin is the evolutionary progenitor of all the vertebrate neurohypophysial hormones.[40]		









CHEMISTRY OF PROTEINS

P.Naveen kumar
VCPS



What are proteins?

- Proteins are necklaces of amino acids – long chains molecules.
- Proteins are the basis of how biology get this done. As enzymes, they are the driving force behind all the biochemical reactions which make biology work.
- As structural elements, they are main constituents of our bones, muscles, hair, skin, and blood vessels.
- As antibodies, they recognize invading elements and allow the immune system to get rid of the unwanted invaders.
- For these reasons, scientists have sequenced the human genome – the blueprint for all of the proteins in biology.

General Characteristics of Proteins

- They are the most complex and most diverse in chemical composition, conferring upon the different tissues.
- Protein molecule contains elements of C, H, O,N, S, and P together with traces of Fe, Cu, I, Mn, and Zn.
- It has a molecular weight of 5,000 to 3,000,000
- They are the most important of the biologic substances being the fundamental constituent of cell cytoplasm.
- They supply not only heat and energy but also material for building and repair.
- Unlike carbohydrates and lipids, only small amounts of protein is temporarily stored in the body, and which can be quickly used up upon demand.

Classificati



Based on Composition:

- Simple proteins – composed of entirely amino acids only.

  Ex. Albumin, Globulin

- Complex or Conjugated proteins – made up of amino acids and other organic compounds. The non-amino acid group is termed as the prosthetic group.

  Ex. Nucleoproteins, lipoproteins,

  glycoproteins, metalloproteins

Classification of Proteins

Based on Axial Ratio:

Axial ratio is the ratio of the length to the breath.

- **Globular proteins** – with axial ratio less than 10 but not below 3 or 4. They are compactly folded and coiled.

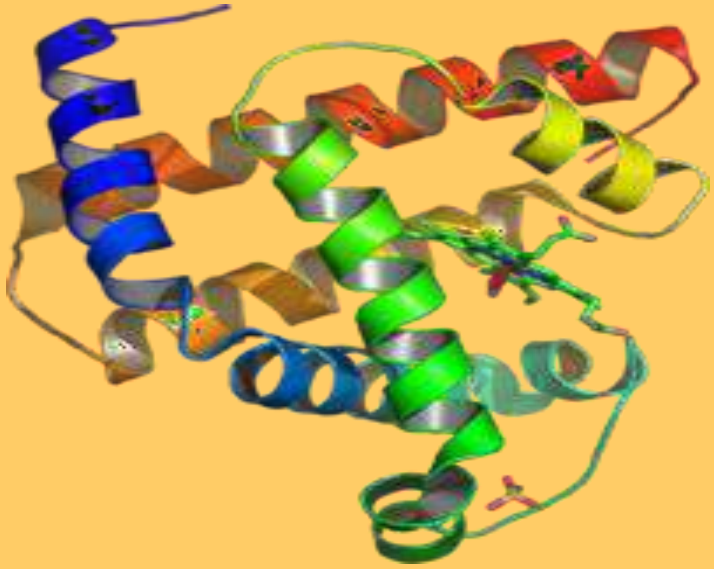
  Ex. Insulin, plasma albumin, globulin,

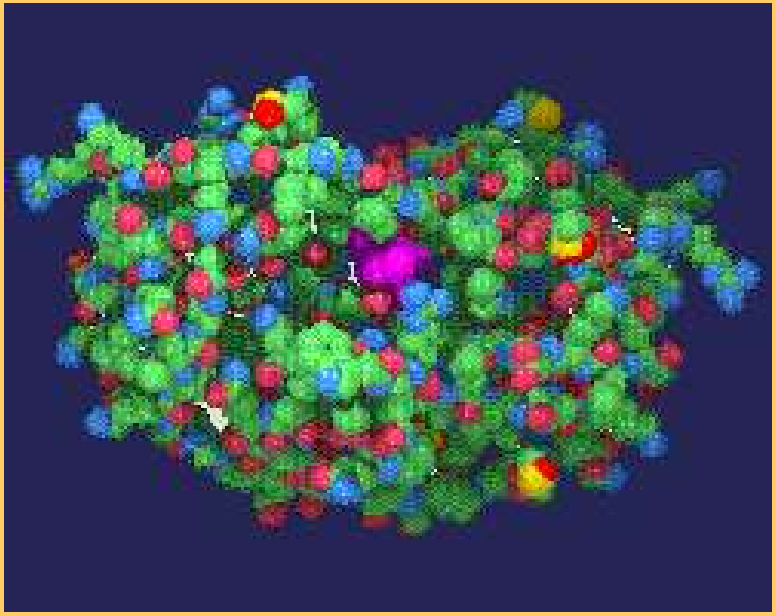
  enzymes

- **Fibrous proteins** – with axial ratio greater than 10. They are spiral and helical and are cross linked by disulfide and hydrogen bonds.

  Ex. Keratin, myosin, elastin, collagen

Globular Proteins

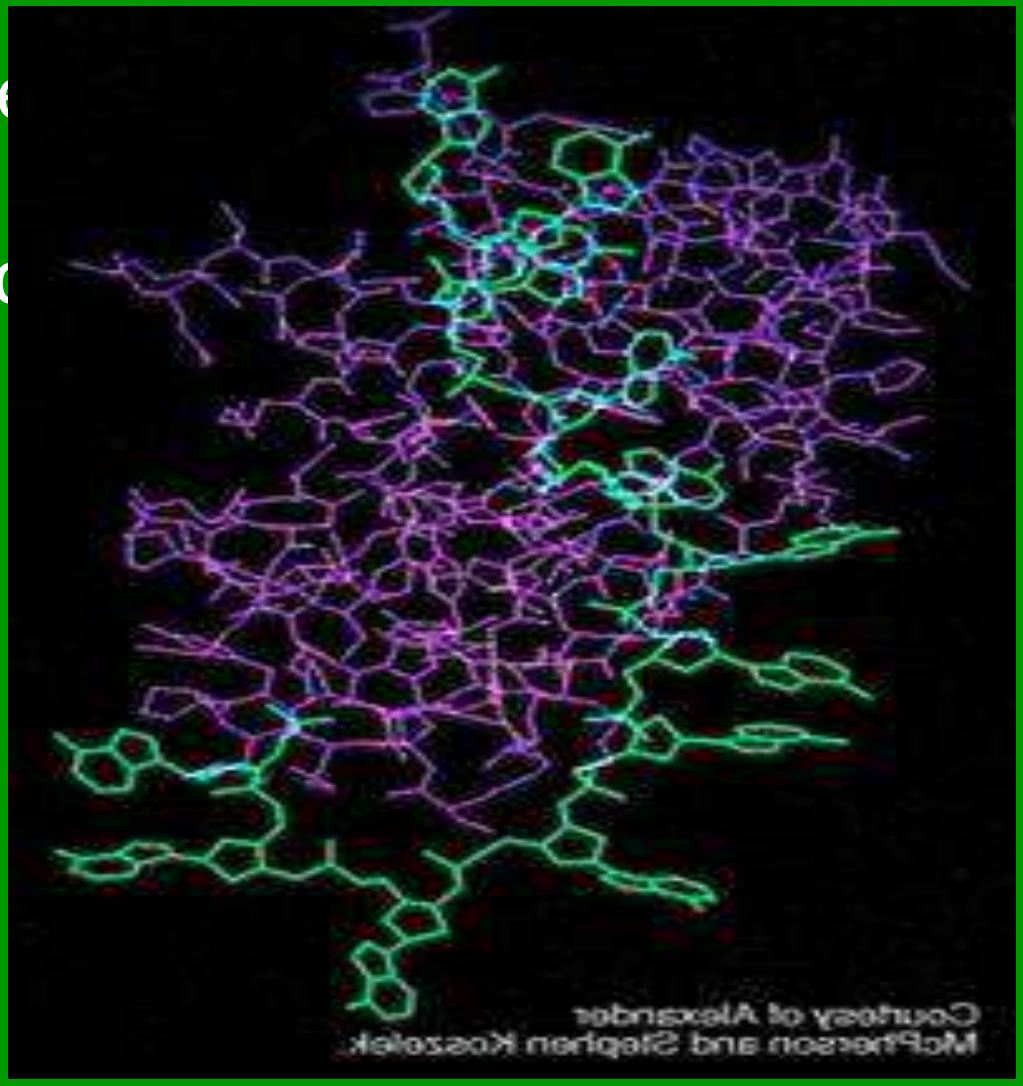




- Globular proteins have their axial ratio less than 10 but not below 3 or 4. They are compactly folded and coiled.
- Examples are insulin, plasma albumin, globulin, enzymes

Fibrous Proteins

- Fibrous proteins are spiral and helical and are cross linked by disulfide and hydrogen bonds
- Examples are keratin, myosin, elastin, collagen



Based on Biologic Functions

- **Structural proteins:** collagen, elastin, keratin, fibroin **of** silk and webs
- **Transport proteins:** hemoglobin, myoglobin, lipoproteins
- **Protective proteins:** immunoglobulins, fibrinogen**,** thrombin, snake venoms, bacterial toxins
- **Contractile proteins:** actin, myosin, tubulin
- **Catalytic proteins:** enzymes
- **Regulatory proteins:** hormones
- **Storage proteins:** ferritin, hemosiderin, gluten, casein, ovalbumin
- **Reception of Stimuli:** rhodopsin, membrane receptor proteins, acethylcholine, insulin
- **Germicidal proteins:** Polymyxin B1, Gramicidin S

Amino Acids

- **Proteins when hydrolyzed either by boiling with acids or through the action of enzymes like trypsin, are broken down into their component amino acids.**
- **The amino acids are of the α-form, that is the amine group (NH_2) is attached to the C atom next to the carboxyl group (-COOH).**
- **Amino acids are classified as:**

1. Neutral amino acids

a) Alipathic amino acids

b) Aromatic amino acids

c) Thionine group

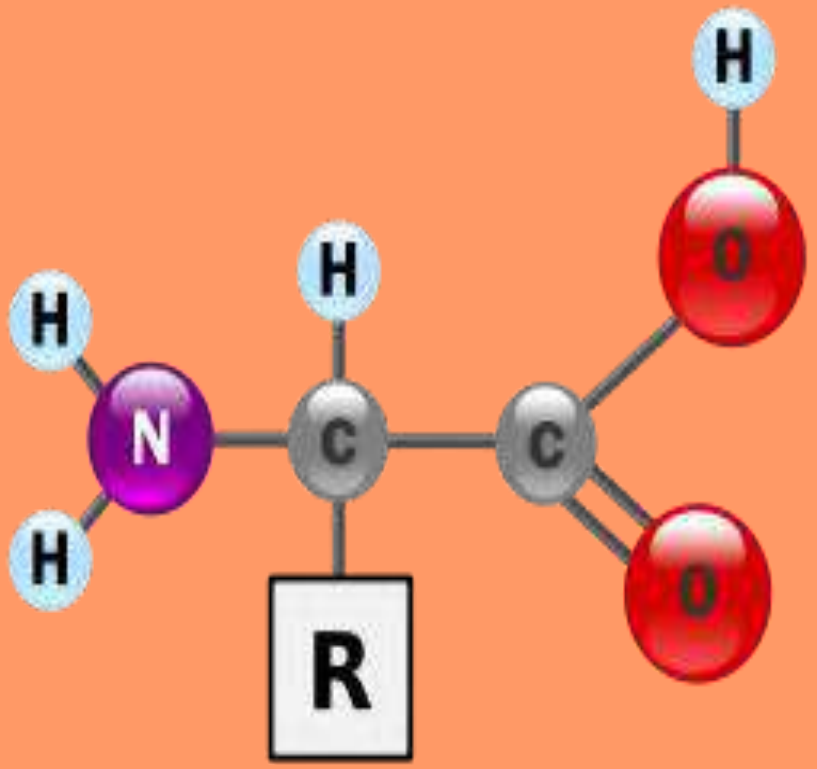
d) Secondary amino acids

2. Acid amino acids

3. Basic amino acids



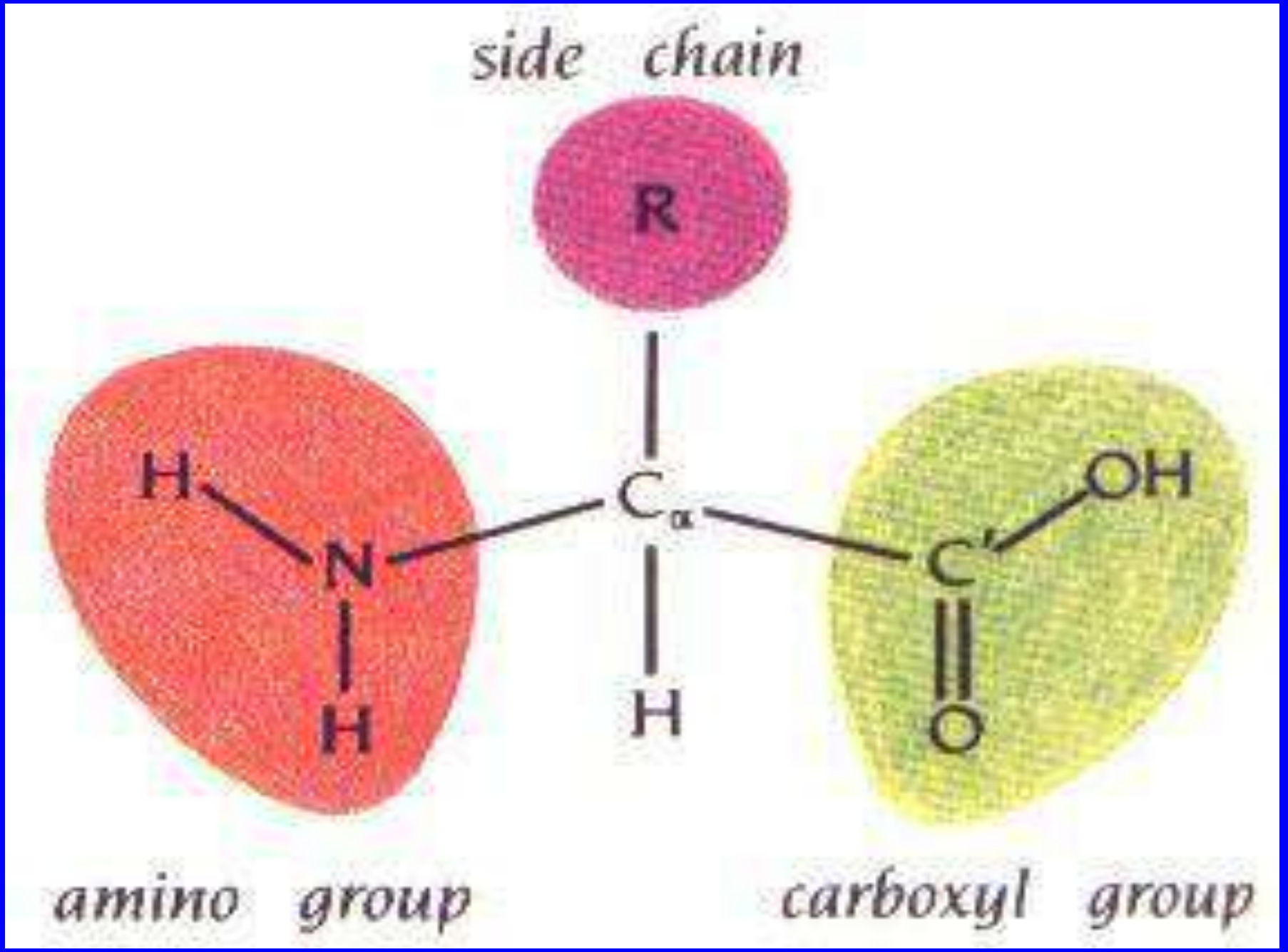
General Structure of Amino Acids



- The amino group is attached to the

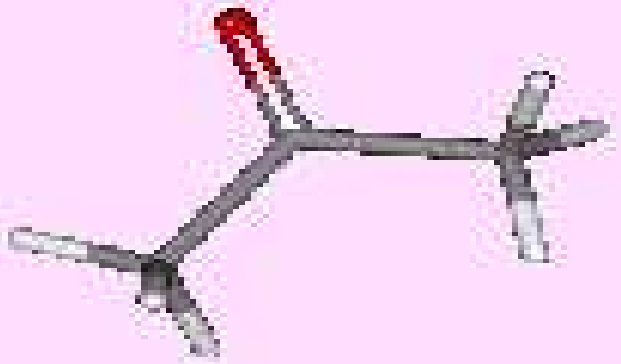
  α- carbon which is next to the carboxyl group; hence the name

  α-amino acid

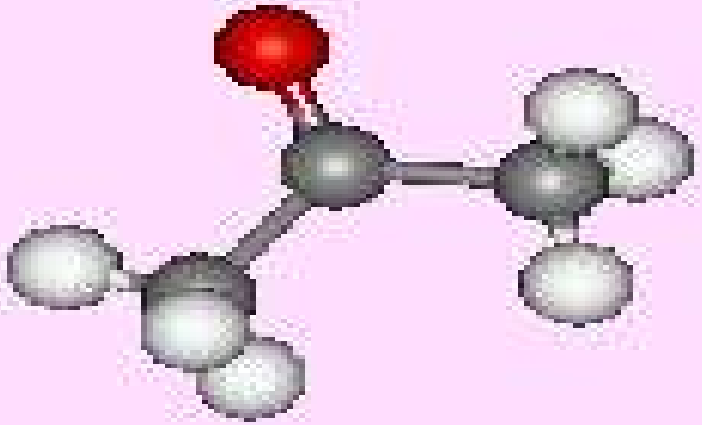

side chain
R
H
N
H
Cα
H
C′
OH
O
amino group
carboxyl group



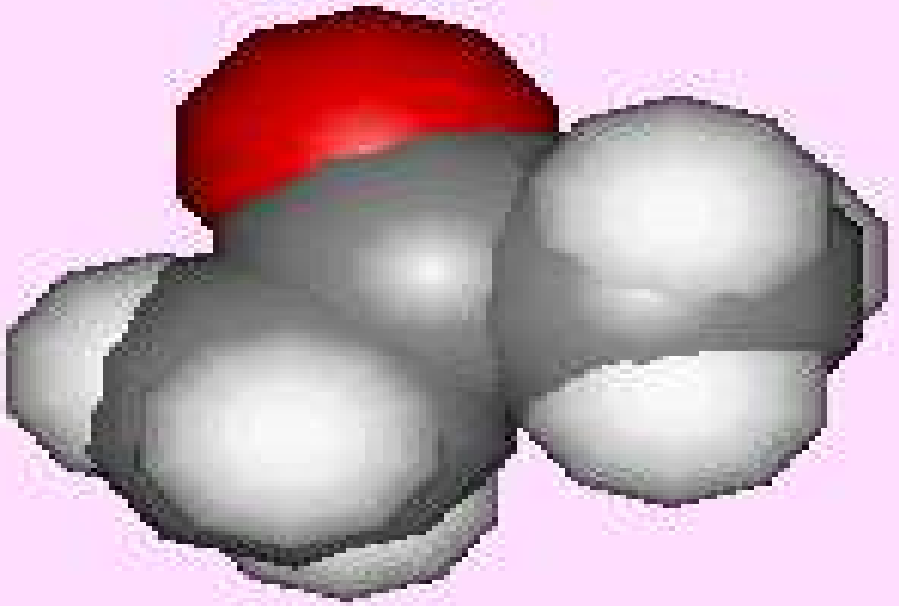
Wireframe



Sticks



Ball&Stick

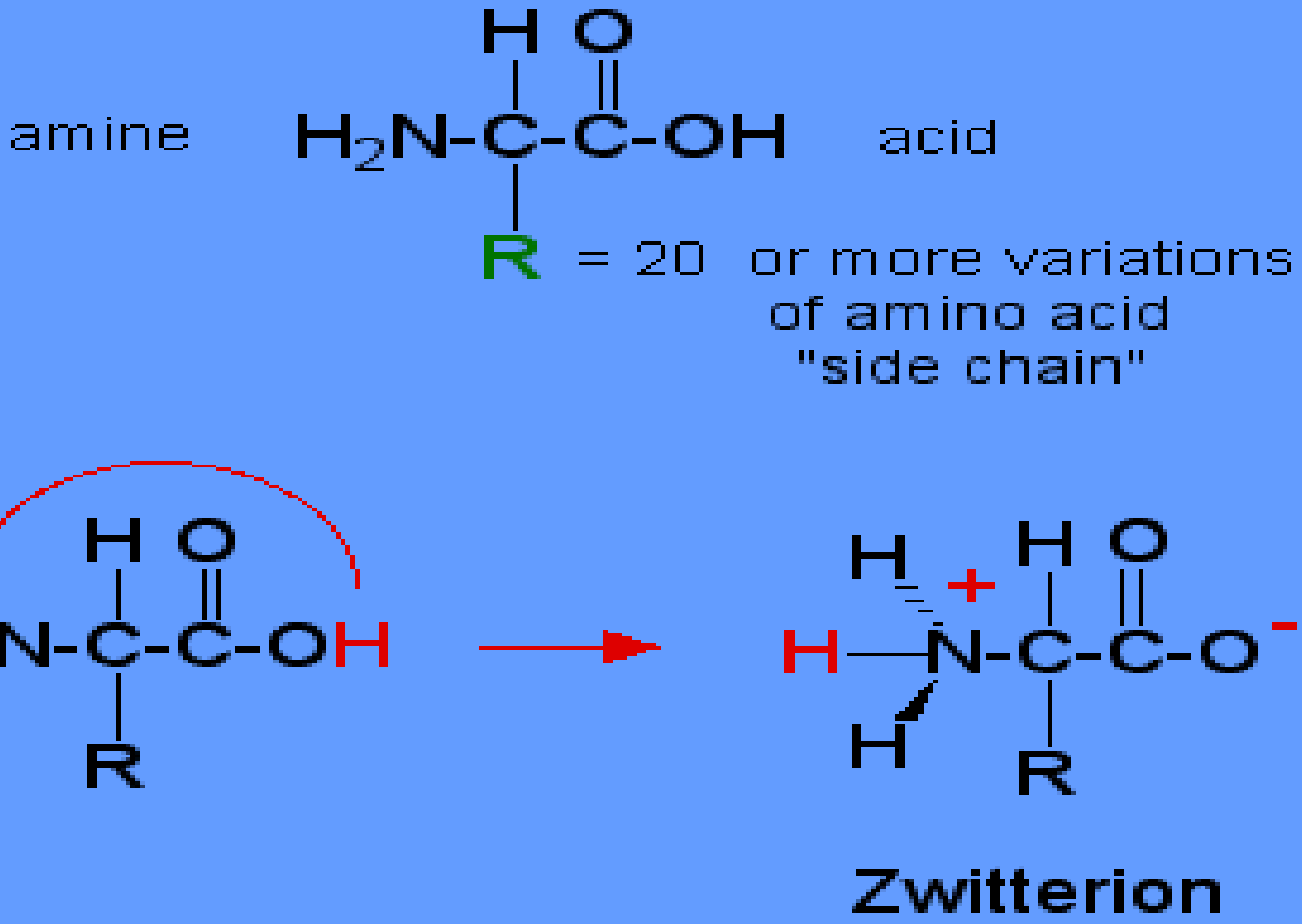


Spacefill

Amino Acids

- Amino acids are the basic structural units of proteins.
- Proteins when hydrolyzed either by boiling with acids or by the action of enzymes like trypsin are broken down into amino acids.
- All amino acids are ampholytes; each contains an amino and carboxyl group.
- H and –COOH groups bound to the alpha C are both acidic
- The basic characteristic of the amino acid is due to the lone pair of electrons of the amino group.
- Naturally occurring amino acids belong to the α-configuration.

Amino Acid Structure



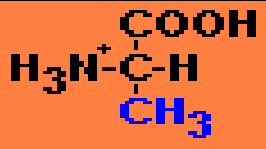
C. Ophardt, c. 2003

Amino Acids found in Proteins

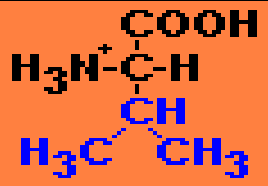
Amino acids	1-Letter Abbreviation	3-letter Abbreviation
Alanine	A	Ala
Arginine	R	Arg
Asparagine	N	Asn
Aspartic acid	D	Asp
Cysteine	C	Cys
Glutamine	Q	Gln
Glutamic acid	E	Glu
Glycine	G	Gly
Histidine	H	His

Amino Acids found in Proteins

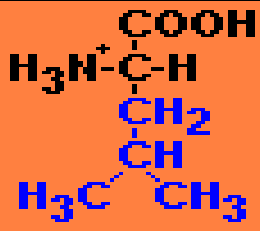
Isoleucine	I	Ile
Leucine	L	Leu
Lysine	K	Lys
Methionine	M	Met
Phenylalanine	F	Phe
Proline	P	Pro
Serine	S	Ser
Threonine	T	Thr
Tryptophan	W	Trp
Tyrosine	Y	Tyr
Valine	V	Val



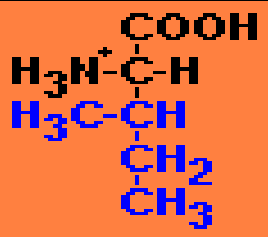
Alanine



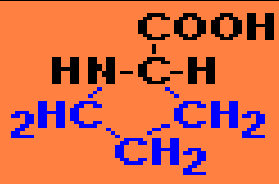
Valine



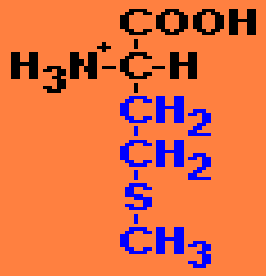
Leucine



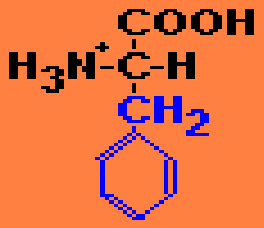
Isoleucine



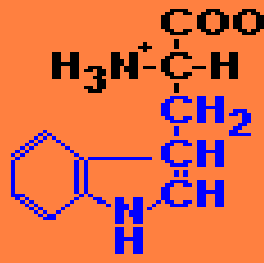
Proline



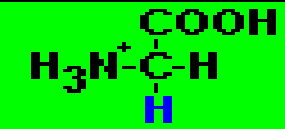
Methionine



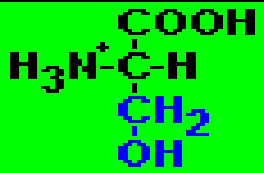
Phenylalanine



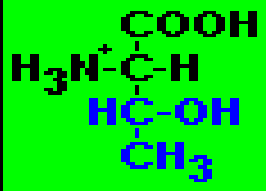
Tryptophan



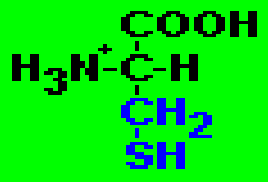
Glycine



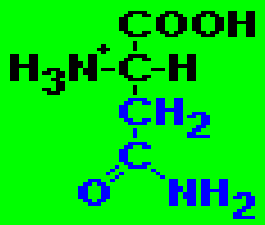
Serine



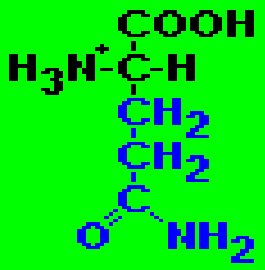
Threonine



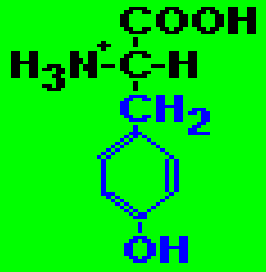
Cysteine



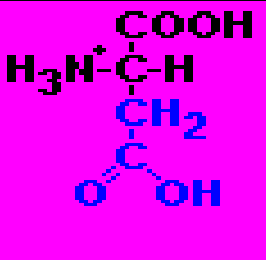
Asparagine



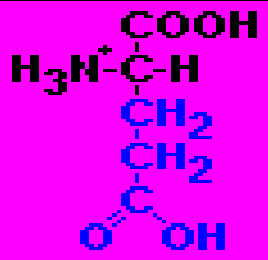
Glutamine



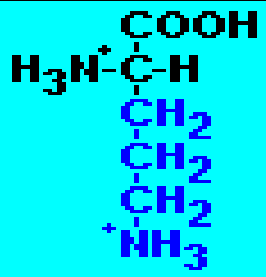
Tyrosine



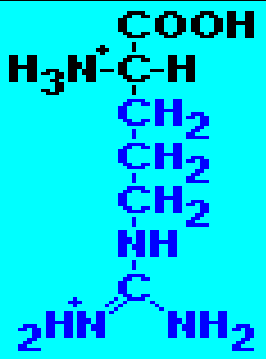
Aspartic Acid



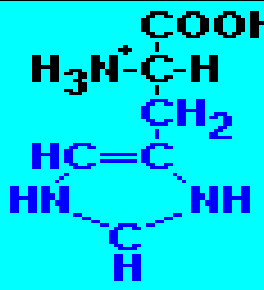
Glutamic Acid



Lysine



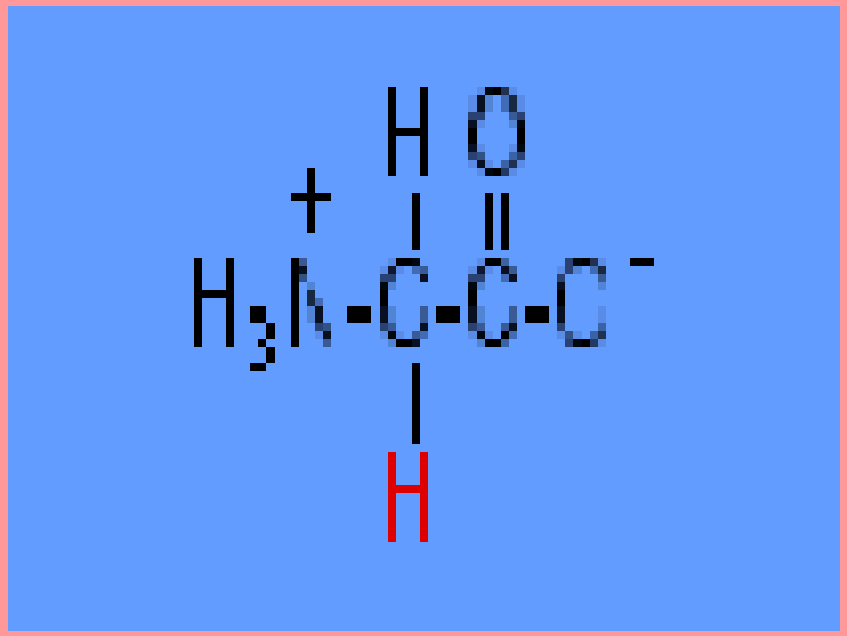
Arginine

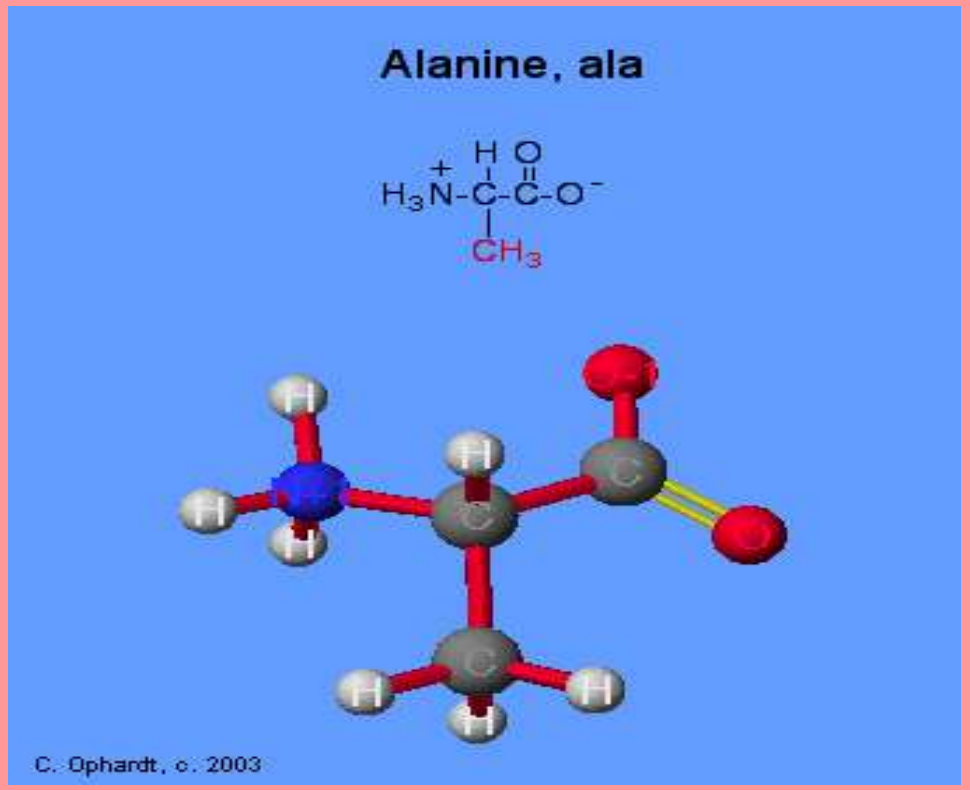


Histidine

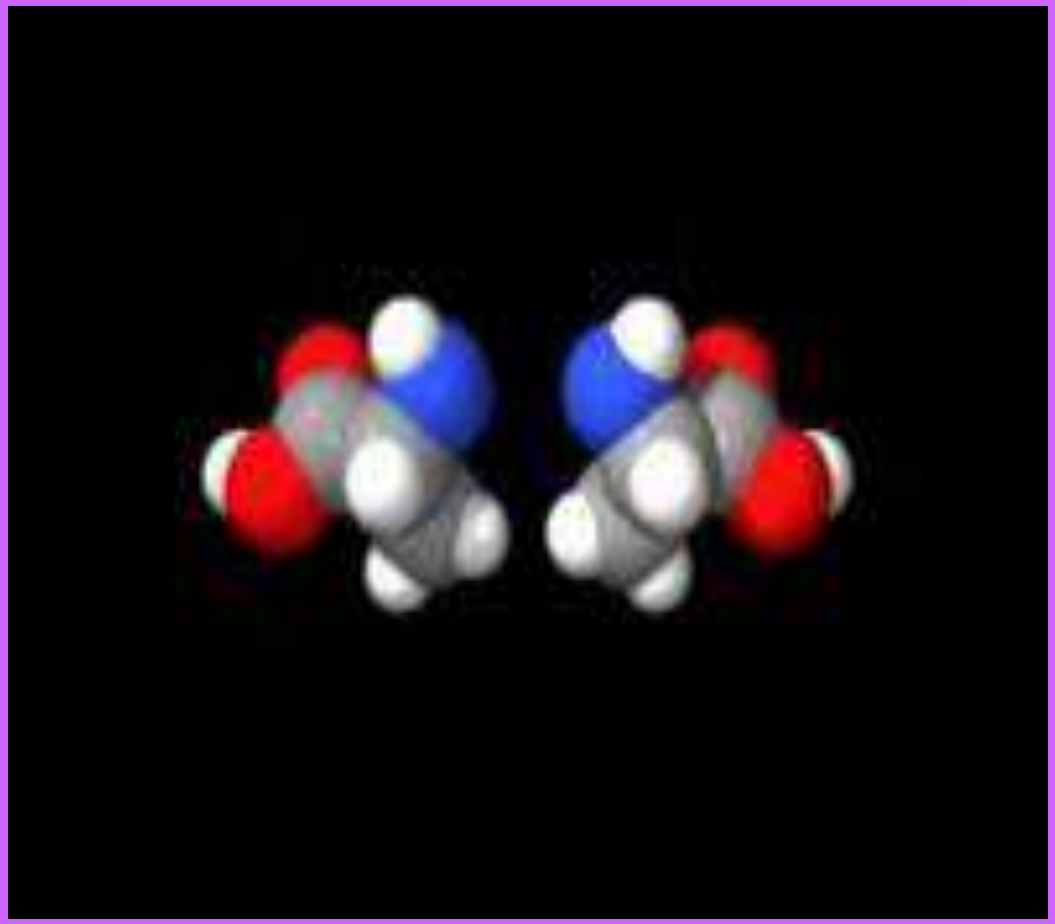
Neutral Amino Acids

- Alipathic Amino acids:
- Glycine, Alanine, Valine, Leucine, Isoleucine



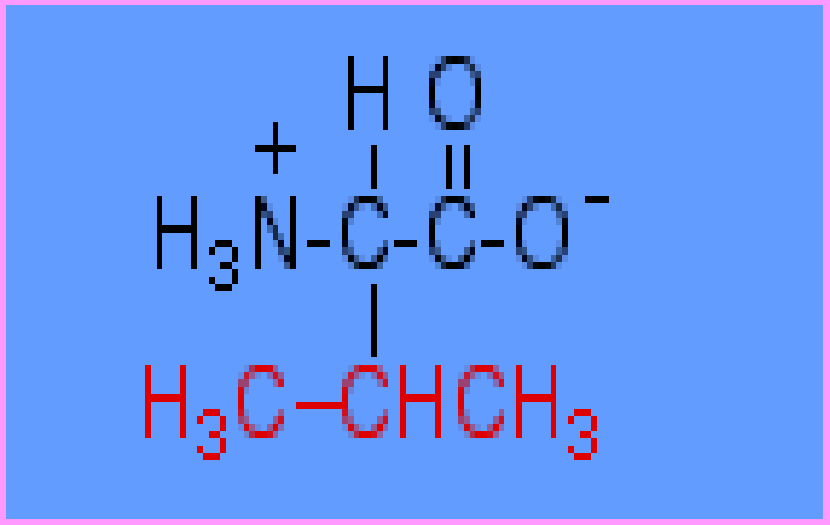


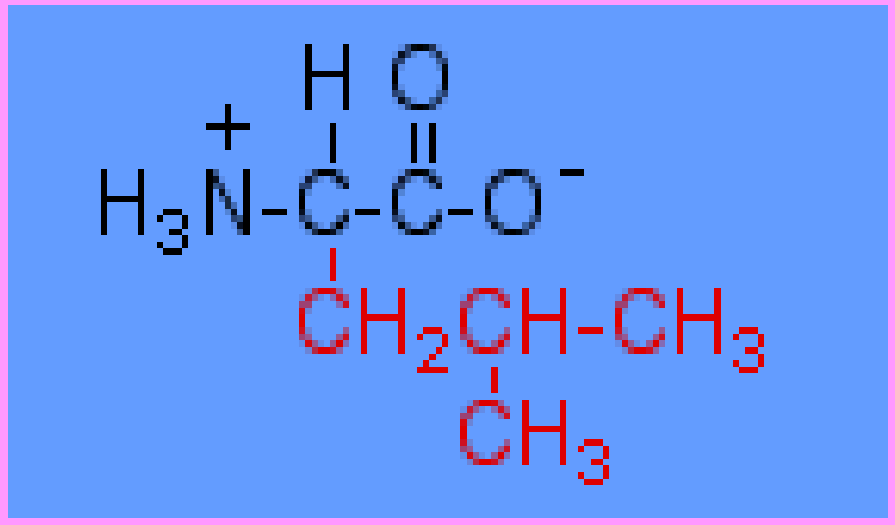
Streoisomers of Alanine

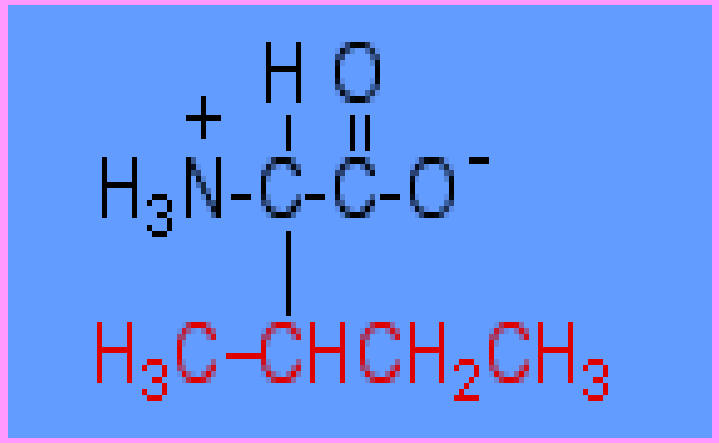


- α-alanine and its enantiomer D-alanine are shown here as mirror images which are not superimposable.
- All α-amino acids except glycine contain a assymmetric α-carbon and thus have D and L enatiomers.
- Only L-enantiomer are found in proteins.

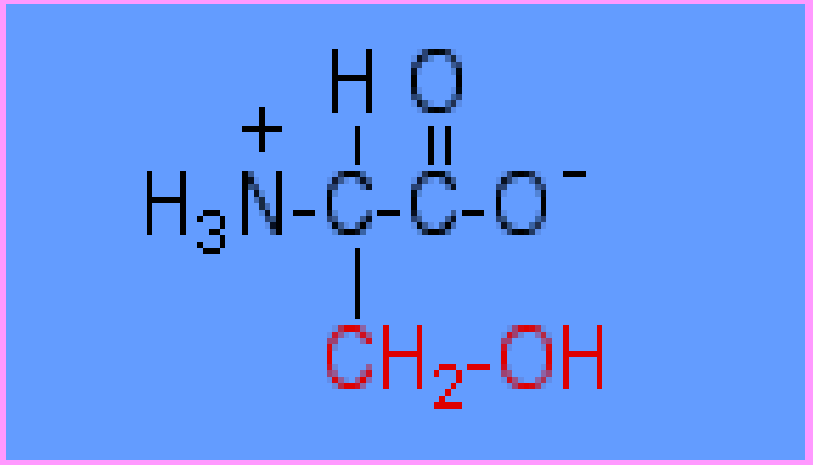
Valine, Leucine, Isoleucine

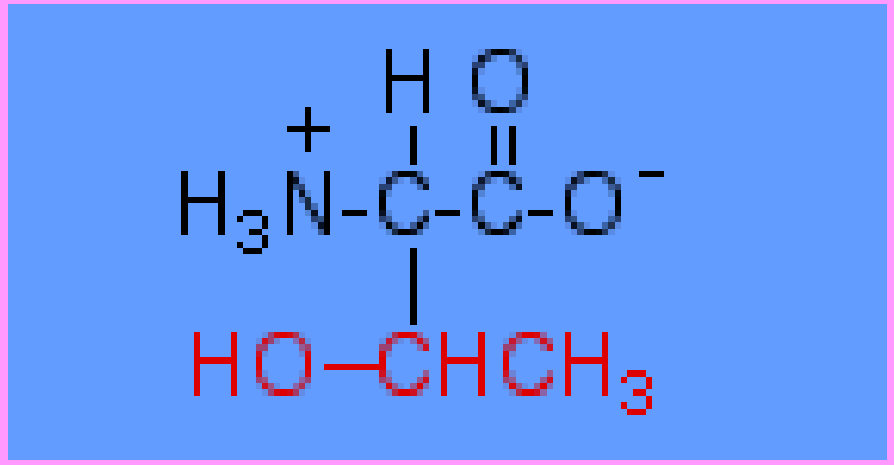






Amino Acids with Hydroxyl





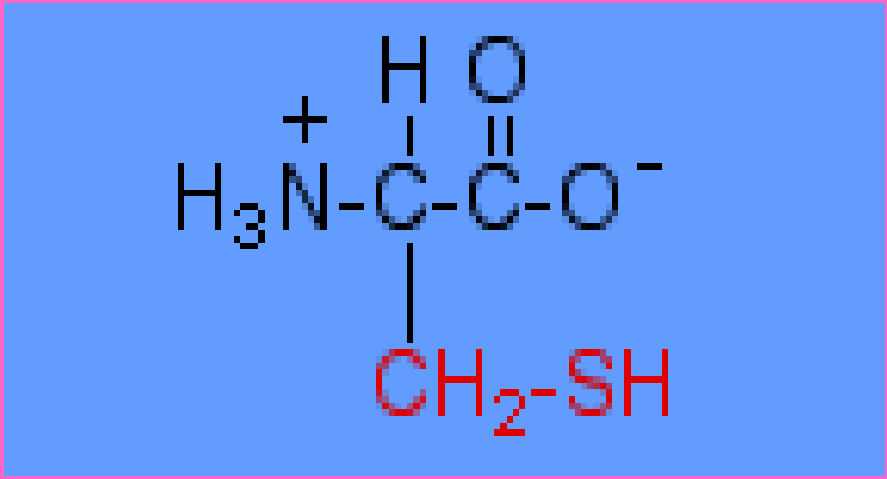
Serine

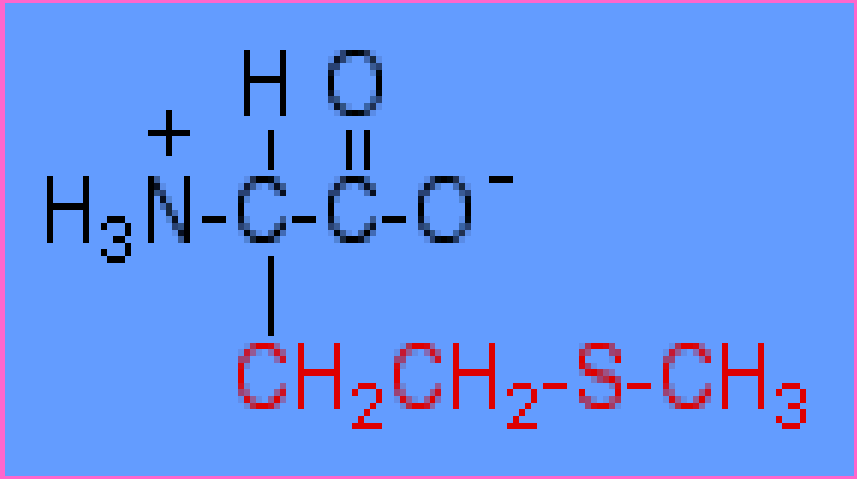
(α-amino β-hydroxy propanoic acid)

Threonine

(α-amino b-y-propionic hydroxy butyric) acid)

Sulfur-containing Amino Acids

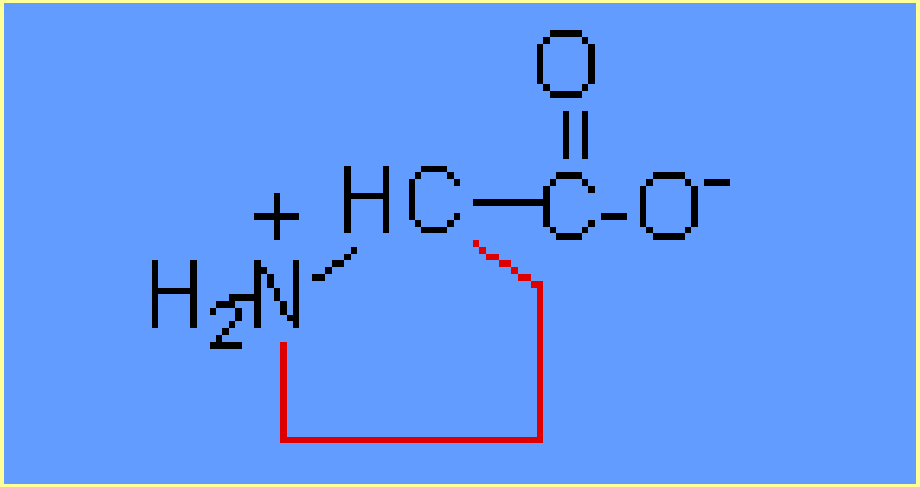




Cysteine
(α-amino β-thio propionic acid)

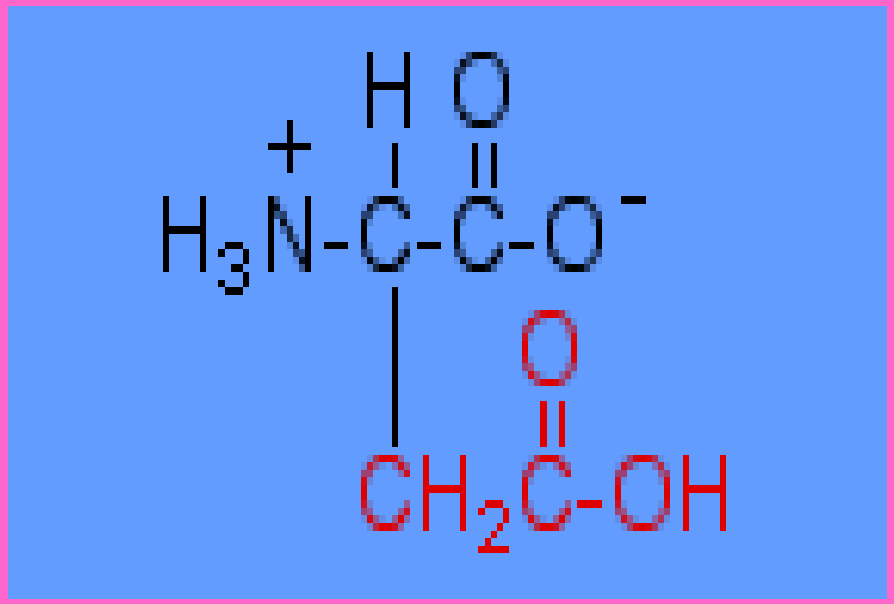
Methionine
(α-amino g-methyl thiobutyric acid)

Secondary Amino Acids



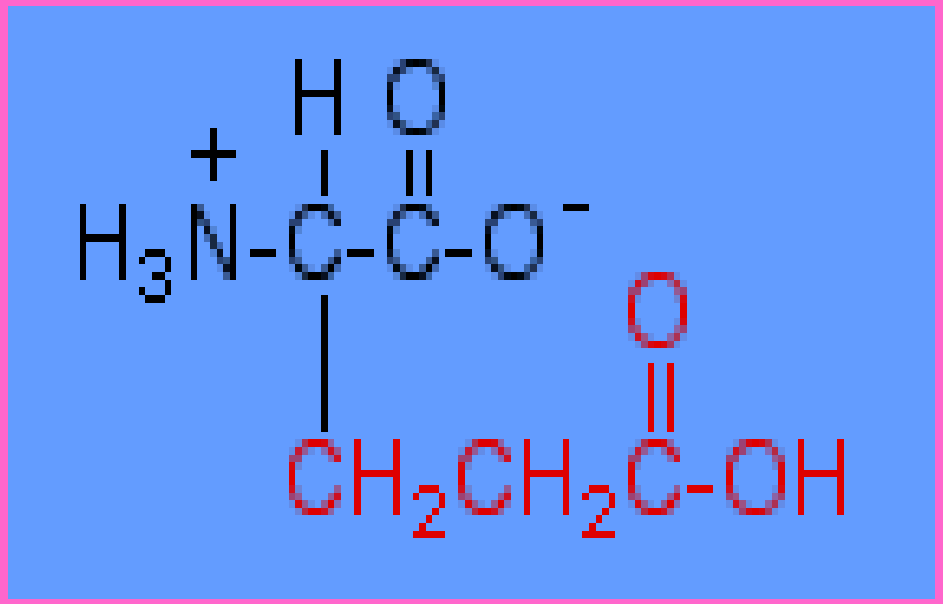
- Proline (pyrrolidine carboxylic acid)

Acid Amino Acids



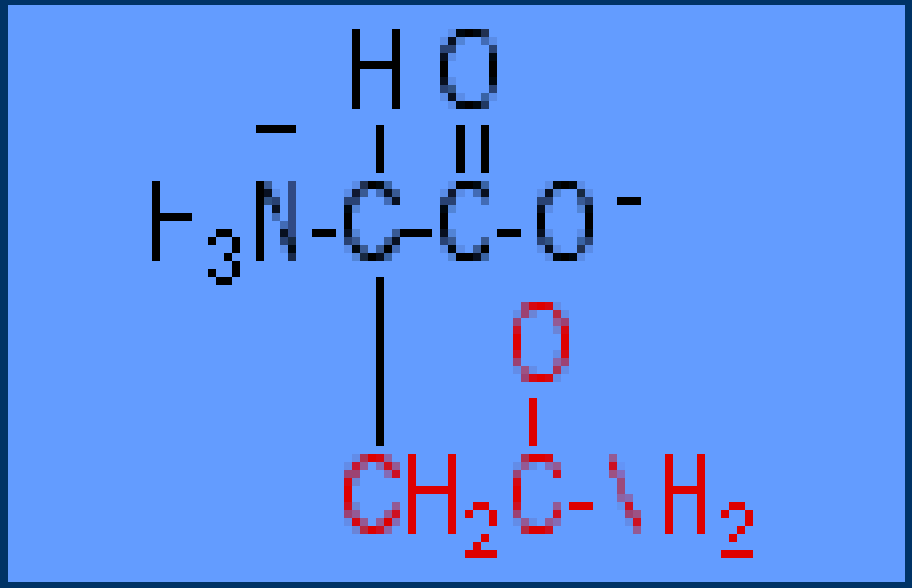
Aspartic Acid

(amino succinic acid)



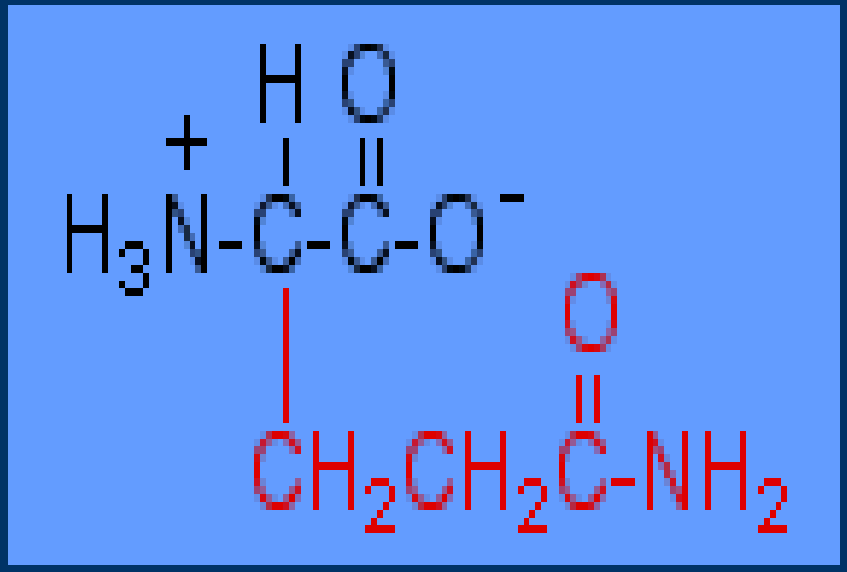
Glutamic Acid

(α-amino glutaric acid)



Asparagine

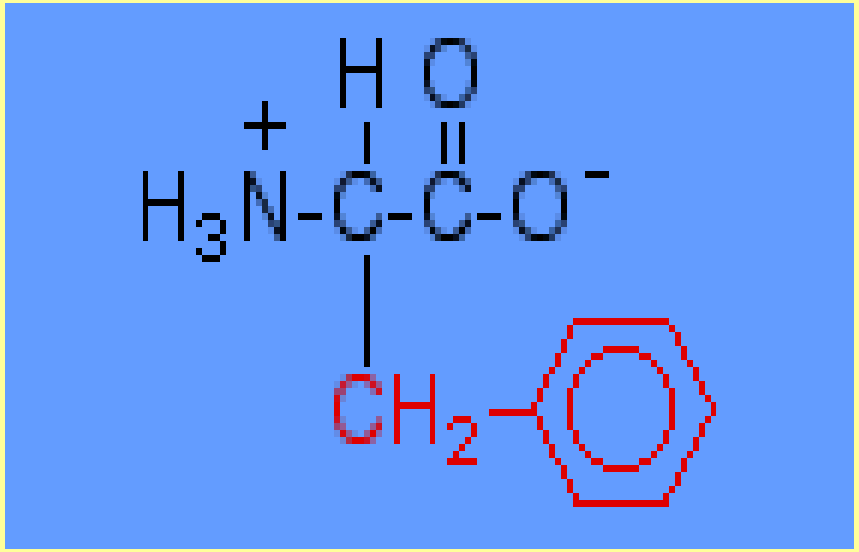
(β-amide of aspartic acid)



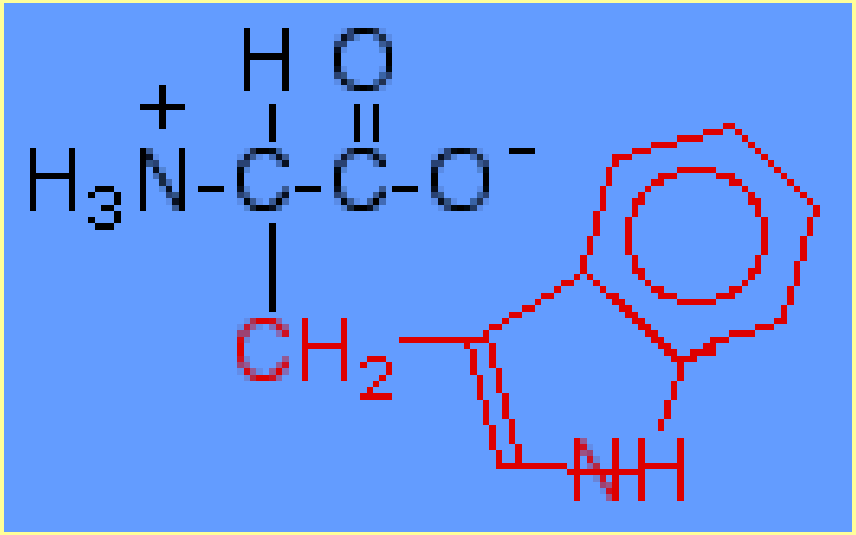
Glutamine

(y-amide of glutamic acid)

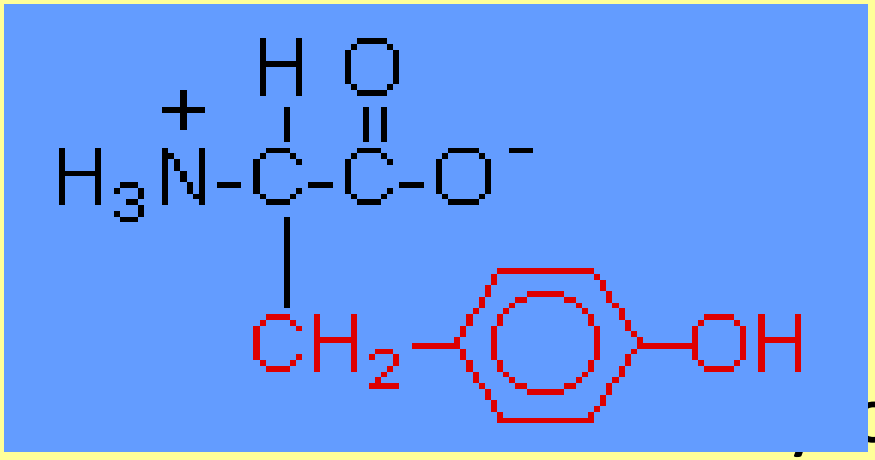
Aromatic Amino Acids



Phenylalanine

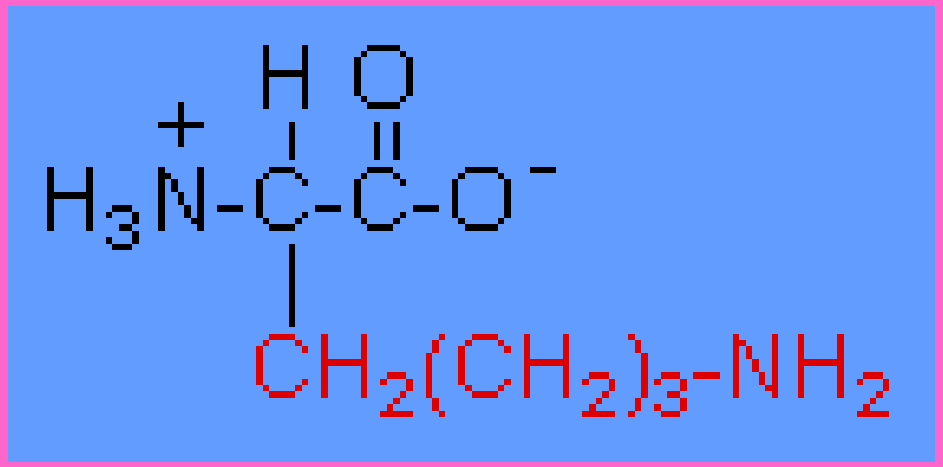


Tryptophan



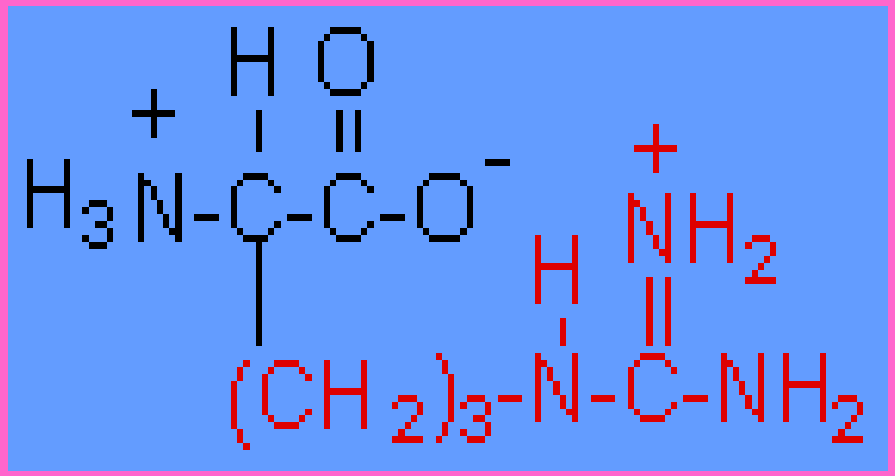
osine

Basic Amino Acids



Lysine

(α-e-diamino caproic acid)



Arginine

(α-amino d-guanido valeric acid)

How many types of proteins are in our body?

- The proteins in our bodies are made of **20 different amino acids** strung together like beads on a string.
- All 20 have a part in common: a central C attached to a carbonyl, an amino and a H atom
- The 20 AA contain, in their 20 different side chains, a remarkable collection of chemical groups.
- It is this diversity of the monomers that allows proteins to exhibit such a great variety of structures and properties.

Biochemical Importance of Amino Acids

Amino Acids	Systematic name	Importance
Glycine	Aminoetha-noic acid	▪Helps trigger the release of oxygen to the energy requiring cell-making process ▪Important in the manufacture of hormones for strong immune system
Alanine	α-amino propanoic acid	▪Important AA as it is an energy source for the liver, muscles, and CNS ▪Strengthens the immune system by producing antibodies ▪Helps in the metabolism of sugars and organic acids

Valine	α-amino 3-methylbutanoic acid	▪Essential AA ▪Promotes mental vigor, muscle coordination and calm emotions
Leucine	α-amino 4-methyl pentanoic acid	▪Essential AA ▪Provides necessary substances for energy production ▪Stimulants to the upper brain and helps to be more alert
Isoleucine	α-amino 3-methylpentanoic acid	▪Essential AA ▪Same functions as leucine

Phenylalanine	α-amino 3-phenylpropanoic acid	▪Essential AA ▪Used by the brain to produce norepinephrine, ▪Reduces hunger pains ▪Functions as antidepressant ▪Helps improve memory
Tyrosine	α-amino 3-(4-hydroxyphenyl)propanoic acid	■ Transmits nerve impulses to the brain; helps overcome depression; improves memory; increases mental alertness; promotes the healthy functioning of the endocrine glands
Tryptophan	α-amino 3-indole propanoic acid	▪Essential AA ▪A natural relaxant, helps alleviate insomia by inducing normal sleep ▪Reduces anxiety and depression ▪Helps in the treatment of migraines and headaches ▪Helps stabilize the immune system ▪Helps reduce risk of artery and heart spasms ▪Works with lysine in reducing cholesterol levels

Methionine	**α-amino 4-methyl thiol butanoic acid**	**▪Essential AA** **▪Principal supplier of sulfur which prevents disorder of the hair, skin and nails** **▪Helps lower cholesterol levels by increasing the liver's production of lecithin** **▪A natural chelating agent for heavy metals** **▪Regulates the formation of ammonia and creates ammonia-free urine which reduces bladder irritation** **▪Influences hair follicles and promotes hair growth**

Cysteine	2-amino 3-mercaptopropanoic acid	▪Functions as an antioxidant and is a powerful aid to the body in protecting against radiation and pollution ▪Helps slow down the aging process, deactivate free radicals, neutralizes toxins ▪Aids in protein synthesis and promotes cellular repair ▪Necessary for skin formation, in the recovery from burns and surgical operations
Serine	2-amino 3 hydroxy propanoic acid	▪A storage source of glucose by the liver and muscles ▪Helps strengthen immune system by providing antibodies ▪Synthesizes fatty acid sheath around nerve fibers
Threonine	2-amino 3 hydroxy butanoic acid	▪Essential AA ▪Important constituent of collagen, ▪Assists metabolism and assimilation ▪Helps the digestive and intestinal tracts functions normally ▪Helps prevents fat build-up in the liver

Histidine	α-amino 3 (1H-imidazol-4-yl) propanoic acid	▪Essential AA ▪Found abundantly in hemoglobin ▪Used in the treatment of rheumatoid arthritis, allergic diseases, ulcers, anemia
Lysine	2,6 diamino hexanoic acid	▪Essential AA ▪Insures adequate absorption of calcium ▪Helps form collagen (which makes up bone and cartilages) ▪Aids in the production of antibodies, hormones and enzymes
Arginine	α-amino 5-guanidino pentanoic acid	▪Helps improve immune responses to bacteria, viruses and tumor cells ▪Promotes wound healing and regeneration of the liver ▪Causes the release of growth hormones ▪Crucial for optimal muscle growth and tissue repair

Aspartic Acid	α-amino butanedioic acid	▪Most easily used as energy source ▪Aids in the expulsion of toxic ammonia from the body ▪Located most closely to the TCA cycle, the site of energy production ▪Found in increased levels in people with epilepsy and in decreased amounts in some cases of depression
Glutamic acid	α-amino pentanedioic acid	▪Considered to be nature's "brain food" by improving mental capacities ▪Helps speed the healing of ulcers; gives a "lift" from fatigue ▪Helps control alcoholism, schizophrenia and the craving of sugar, Parkinson's disease, mental retardation, and muscular dystrophy
Asparagine	α-amino 3 carbamoyl propanoic acid	▪Found in the surfaces of proteins where they can interact with water molecules

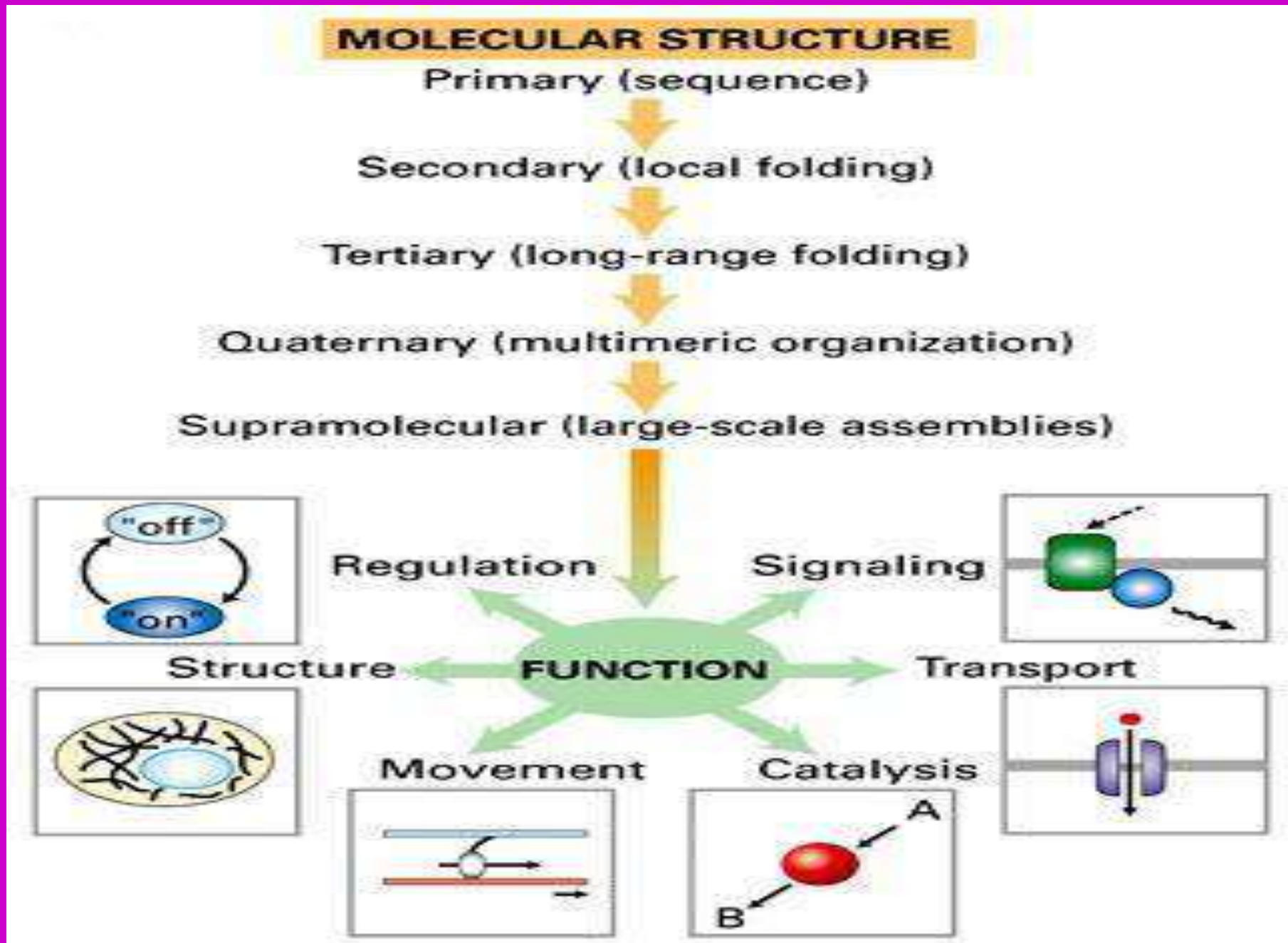
Glutamine	α-amino 4 carbamoyl butanoic acid	▪Found in the surfaces of proteins where they can interact with water molecules ▪The polar amide groups can also form hydrogen bonds with atoms in the side chains of other polar amino acids
Proline	Pyrrolidine-2-carboxylic acid	▪Non-essential AA ▪Important for the proper functioning of joints and tendons ▪Helps maintain and strengthen heart muscles ▪Helps repair processes after cell injury or for any type of wound healing
Hydroxy lysine	Hydroxy hexanoic acid	▪It is a hydroxy derivative of lysine. ▪It is most widely known as a component of collagen

Biologically Important Proteins

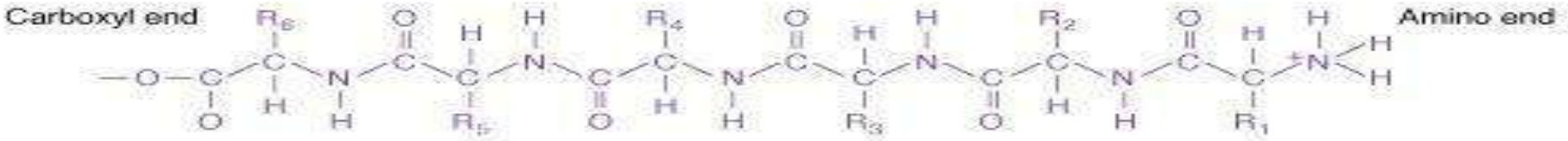
Protein	No. of AA	Function
Insulin	51	Enzyme for sugar metabolism
Cytochrome C	104	Enzyme for cell respiration
Growth hormone	191	Used as anti-aging treatment
Hemoglobin	574	Oxygen transport in blood
Hexokinase	730	Enzyme for glycolysis
Gamma globulin	1320	Part of immune system in blood
Myosin	6100	Muscle action

Blood Proteins

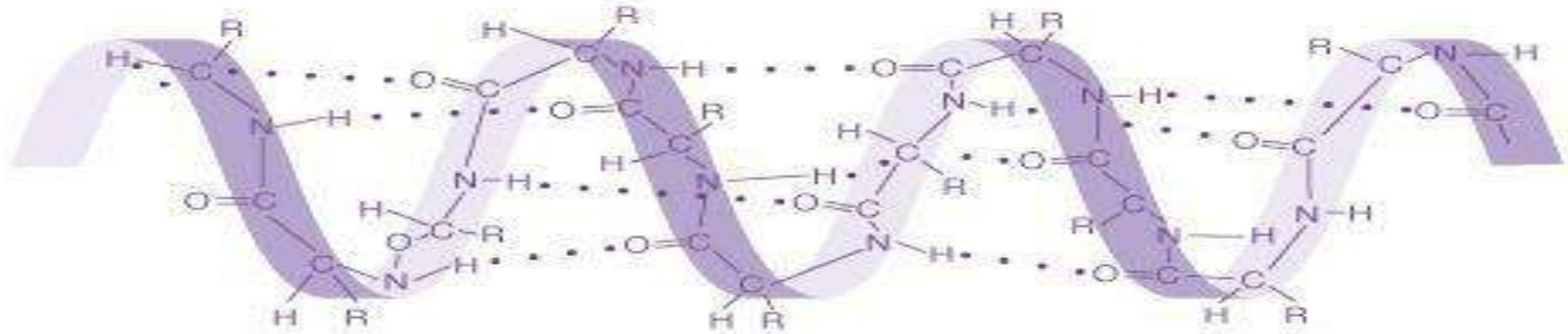
Albumins	Create osmotic pressure and transport other molecules
Immunoglobulins	Participate in immune system
Fibrinogens	Blood coagulation
Alpha-1-Antitrypsin	Neutralize trypsin that has leaked from the digestive system
Regulatory proteins	Regulation of gene expression


MOLECULAR STRUCTURE
Primary (sequence)
Secondary (local folding)
Tertiary (long-range folding)
Quaternary (multimeric organization)
Supramolecular (large-scale assemblies)
"off"
"on"
Regulation
Signaling
Structure
FUNCTION
Transport
Movement
Catalysis
A
B

(a) Primary structure

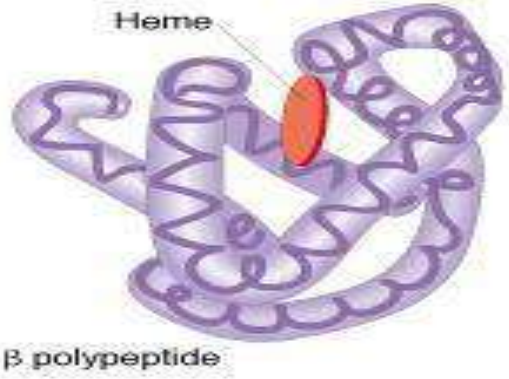


(b) Secondary structure

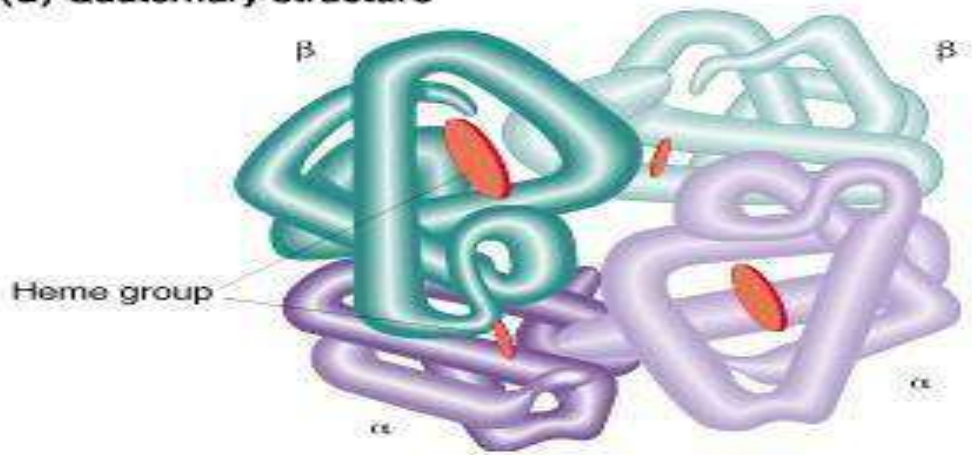


Hydrogen bonds between amino acids at different locations in polypeptide chain

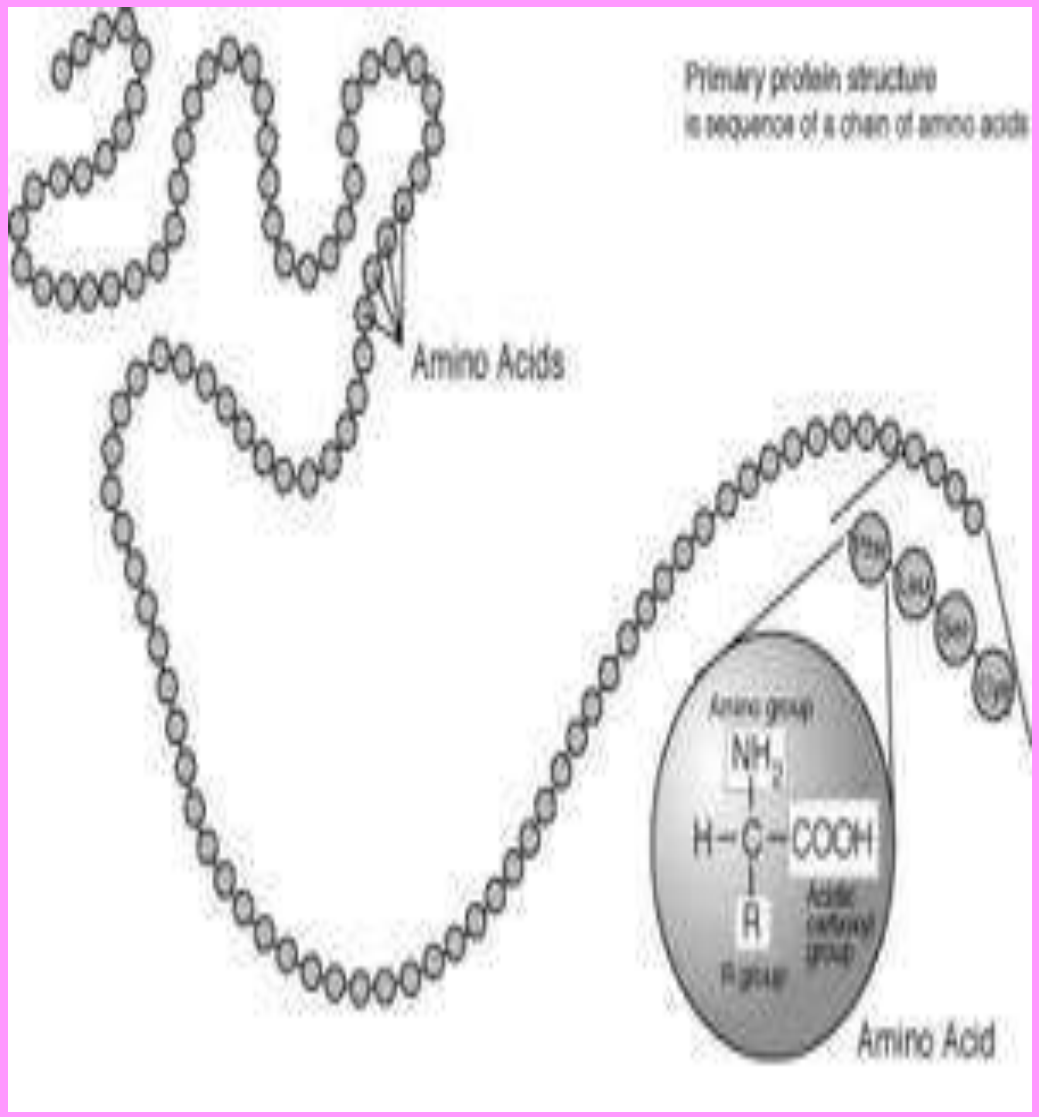
(c) Tertiary structure



(d) Quaternary structure



Four Levels of Architectural Organization of Proteins

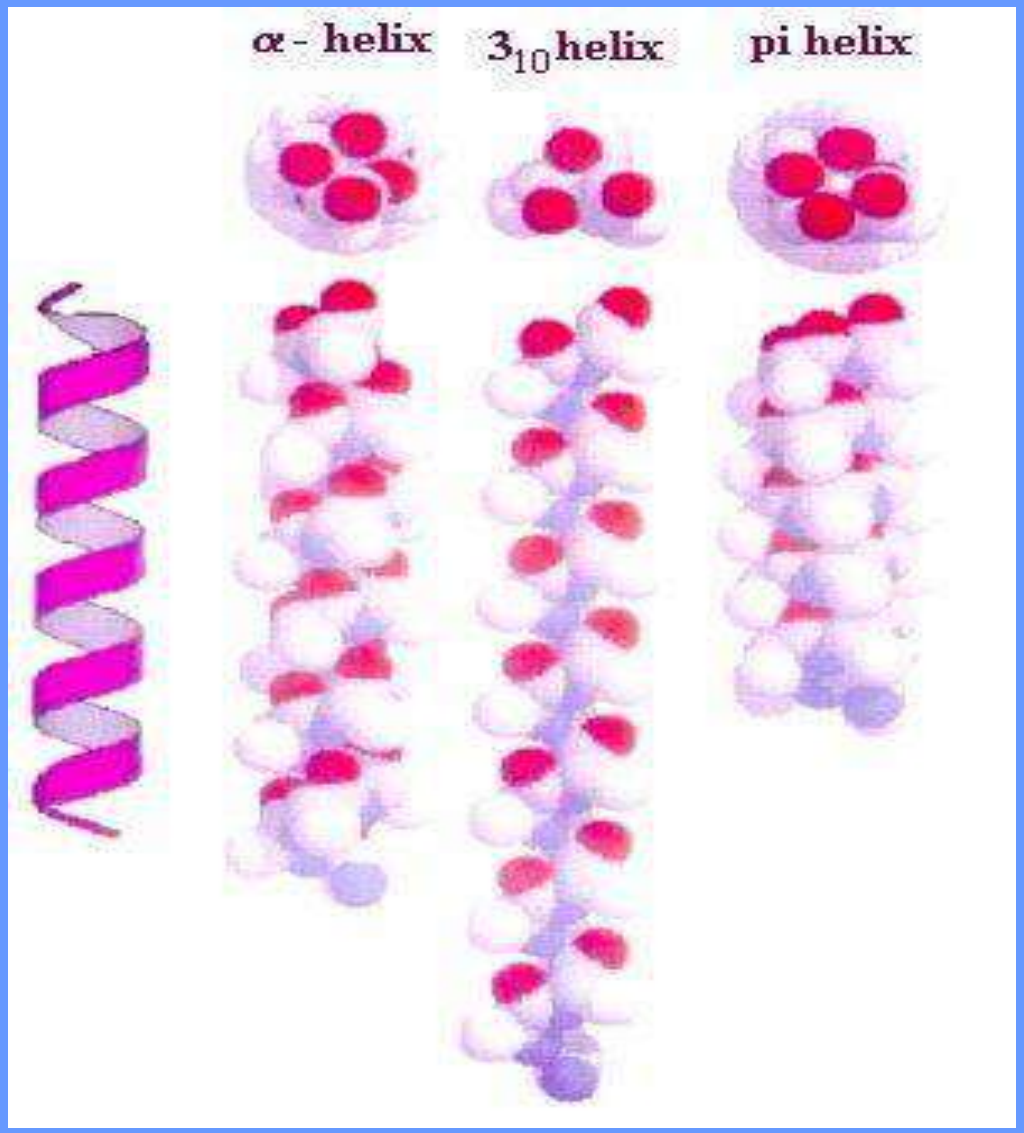


Primary Structure

It is the sequence of amino acids that make up a protein

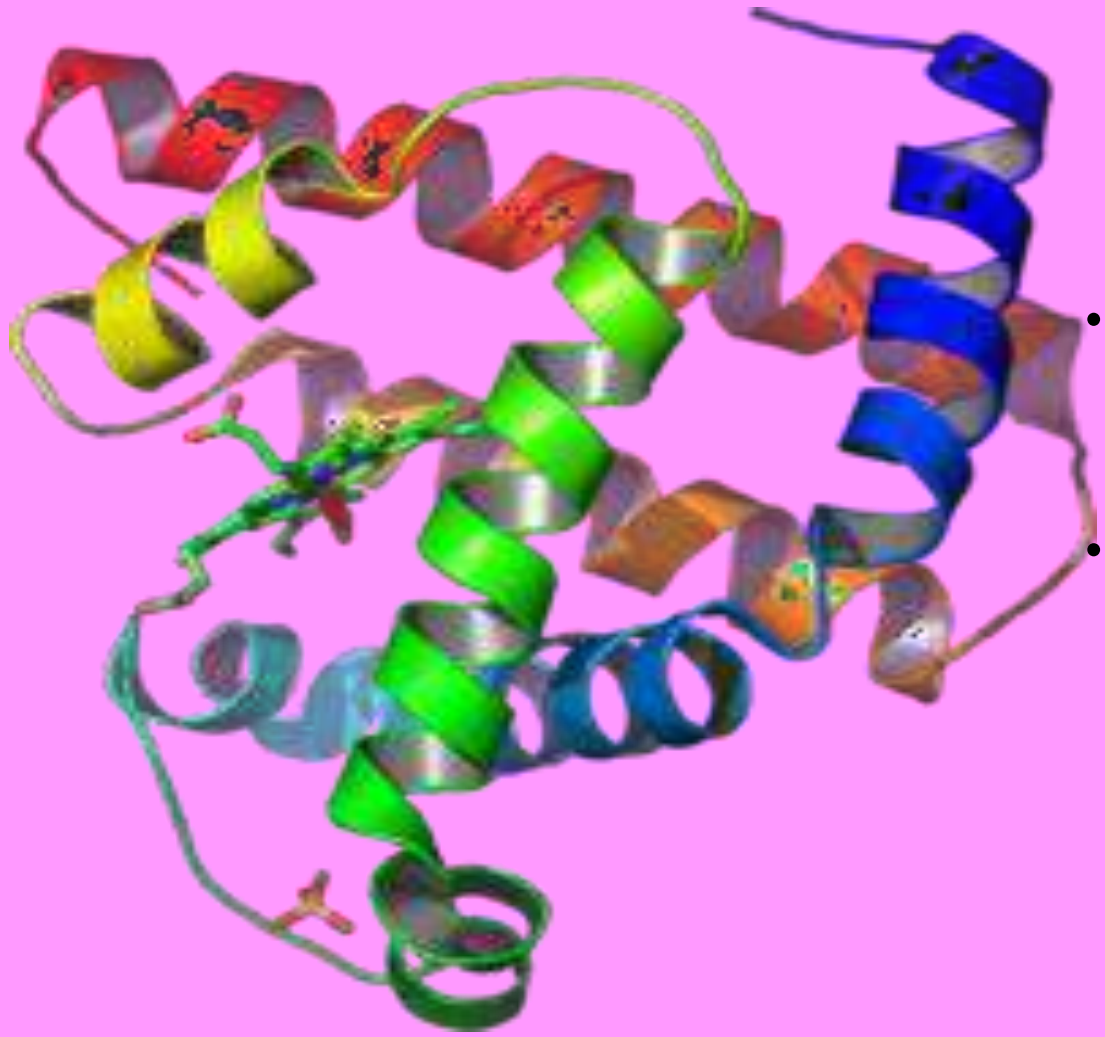
It is stabilized by peptide bonds (non-specific attractive forces that occur between adjacent –COOH and NH_2 groups)

Secondary Structure of Proteins

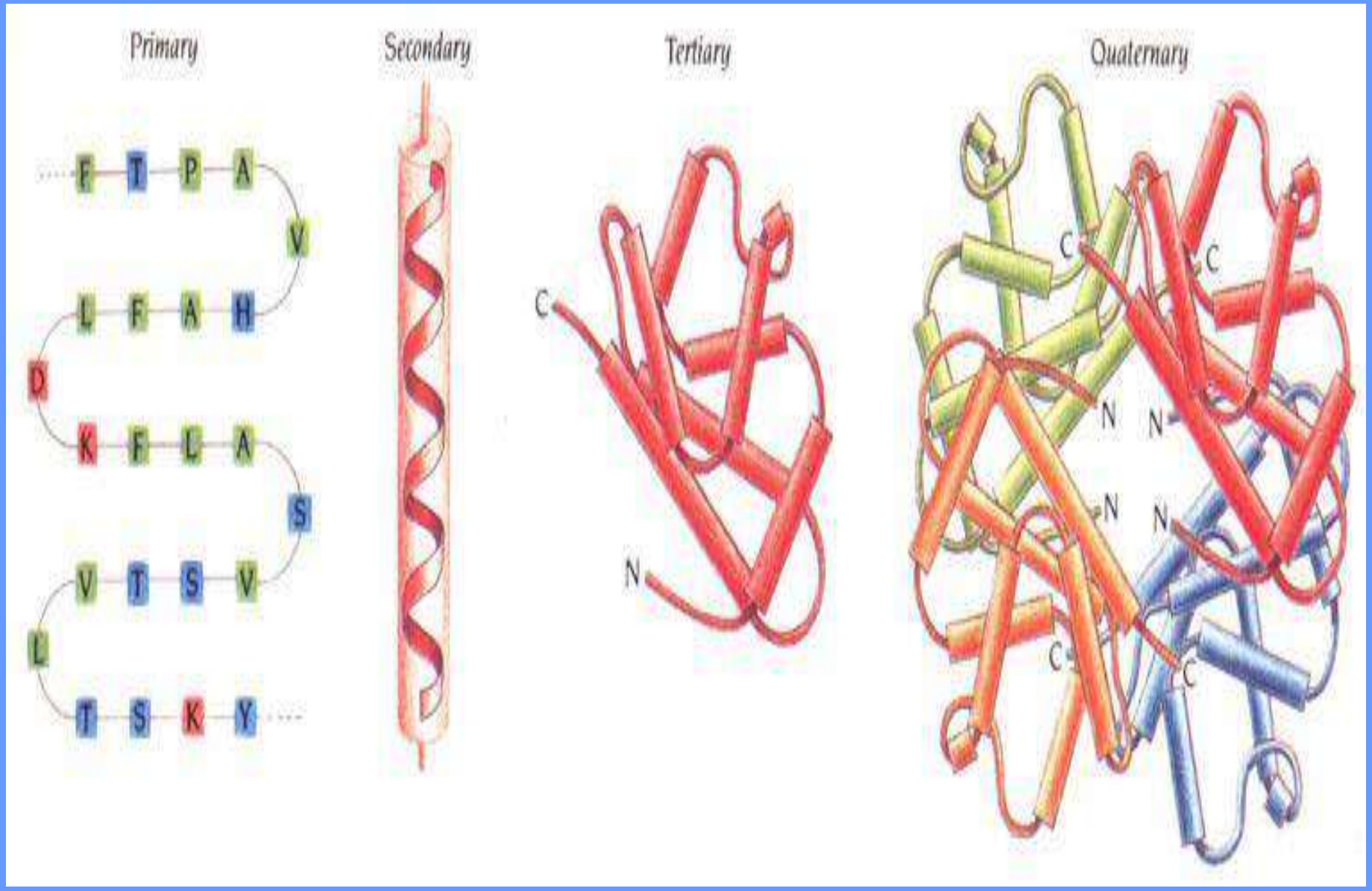


- Refers to the steric relationship of amino acid residues that arte close to one another in a linear sequence.
- It is stabilized by hydrogen bonding between carbonyl group of 1 peptide chain and the amino group of another chain.
- The polypeptide chain can change its orientation because of free rotation around the polypeptide backbone (-N-C-C).
- The hydrogen bonding produces a regular coiled arrangement called helix.

3D Structure of Myoglobin

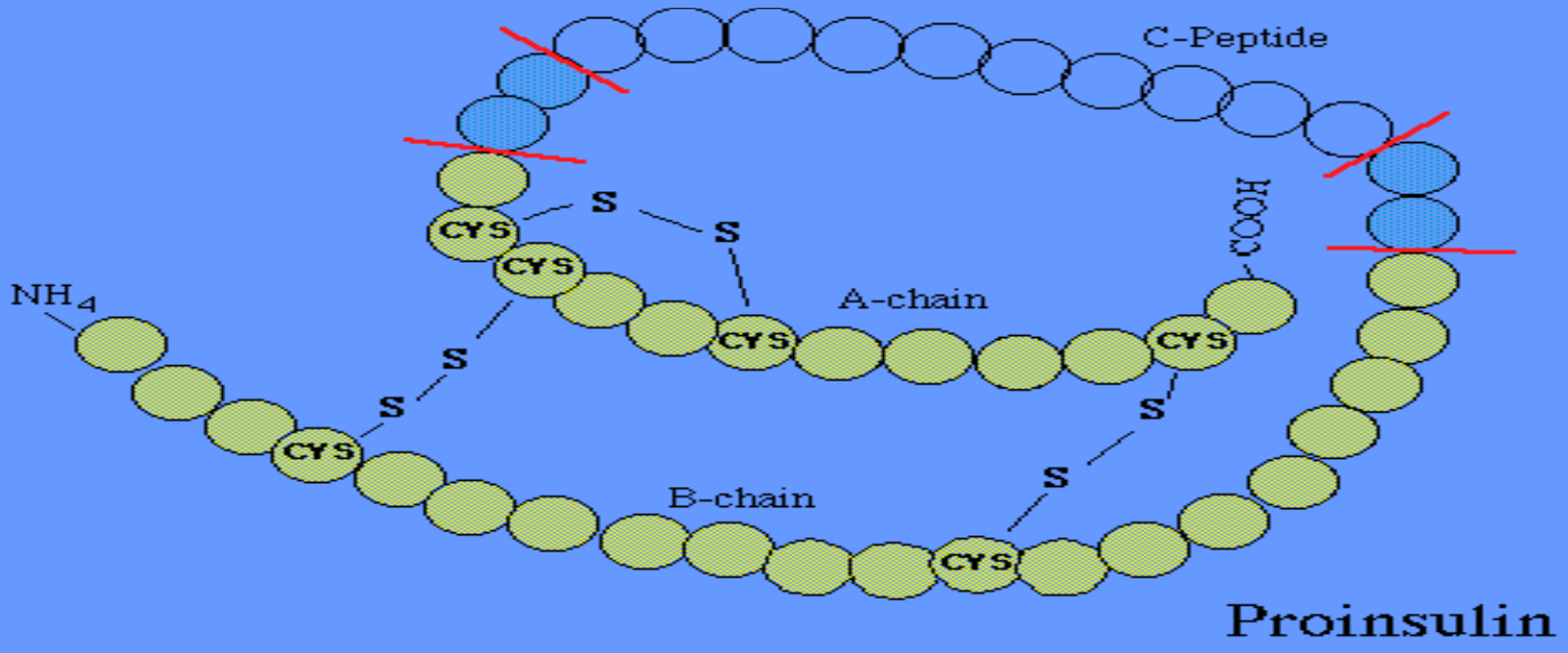


- **Tertiary structure -**
  refers to the steric relationship of amino acid residue that are far apart in the linear sequency.
- **It also refers to how the polypeptide chain is bent or folded in 3 dimensions.**
- **It is stabilized by non-covalent bonds, hydrohobic bonds, electrostatic bons, hydrogen bonding, Van der Waals forces, and covalent disulfide bonds.**

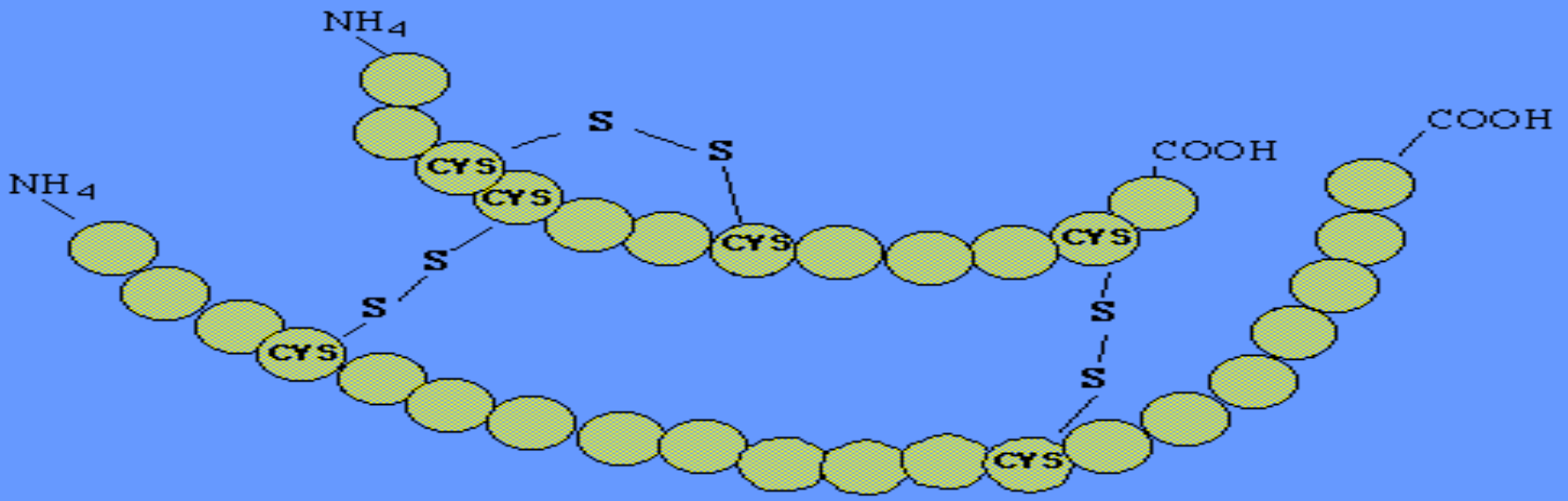

Primary
Secondary
Tertiary
Quaternary
F T P A
V
L F A H
D
K F L A
S
V T S V
L
T S K Y
C
N
C
C
N N
N N
C
C

Quaternary Structure

- Contains more than one polypeptide chains stabilized by the same bonds as in tertiary structure
- Quaternary level donates the way the chains are packed together in a protein.
- Each chain in a protein is called a subunit or domain (protomers).
- Proteins with more than 1 chain are called oligomers.

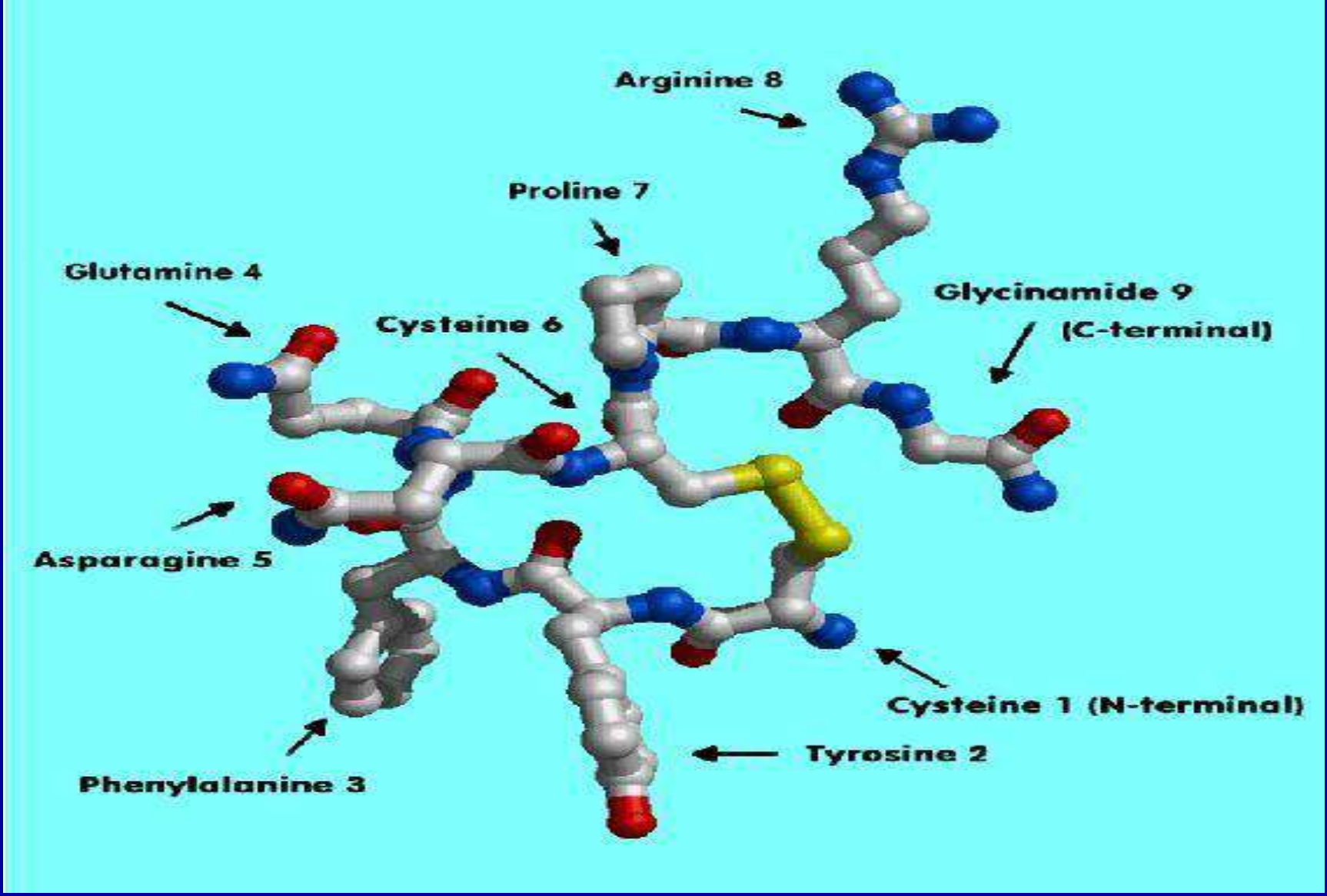


Proinsulin



Insulin

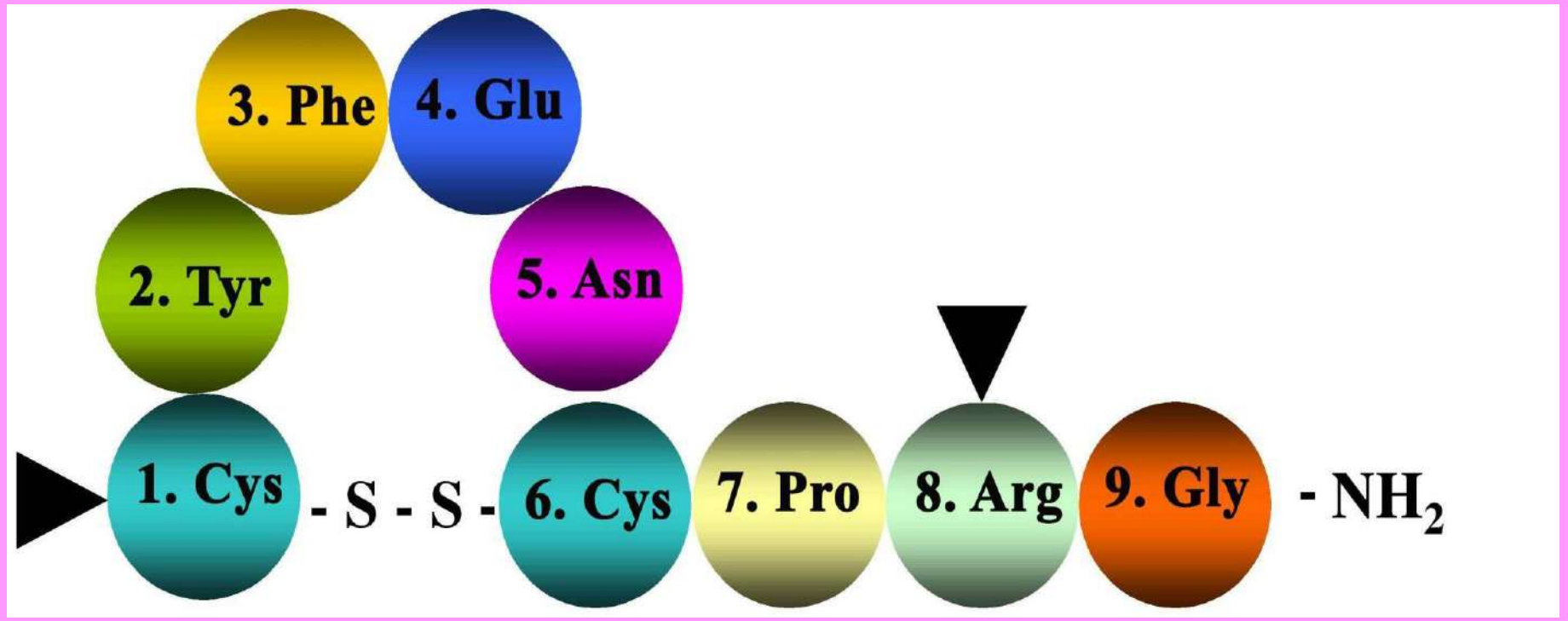
Vasopressin



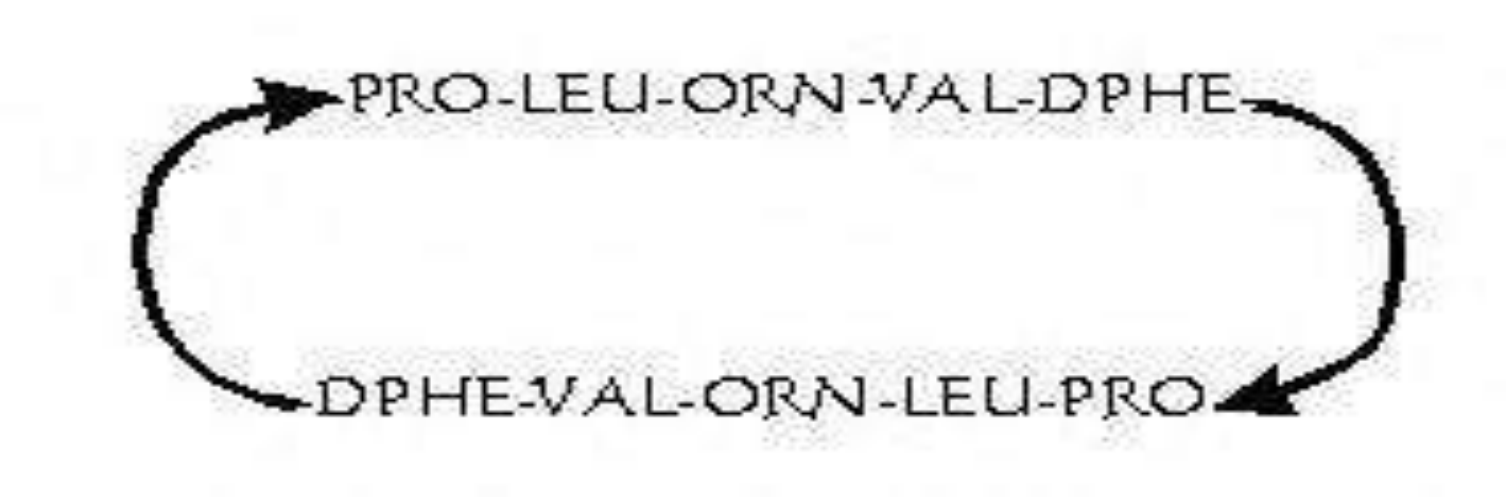
Vasopressin

- Vasopressin is also called ADH (antidiuretic hormone)
- Vasopressin increases blood pressure and inhibits diuresis.
- It constricts blood vessels rising the blood pressure and affecting water and electrolyte balance

Vassopressin

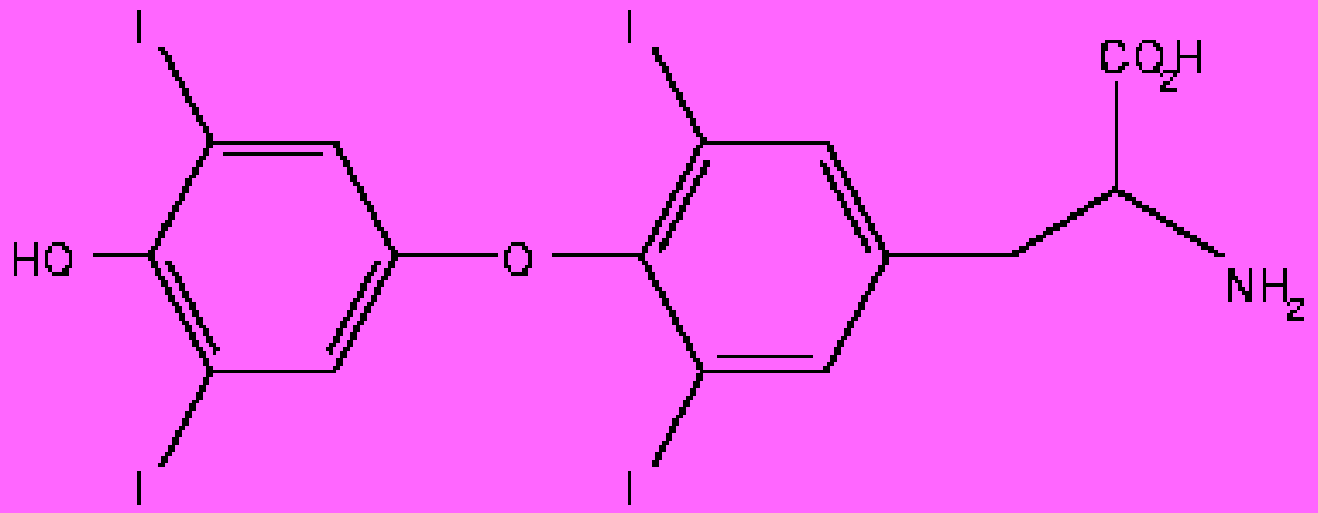


Gramicidin S



Gramicidin S is an antibiotic for topical use and effective for gram-positive bacterial infection.

Thyroxine



- **Thyroxine (T4) and Triiodothyronine (T3) represents 2 iodonated tyrosine residues on the same polypeptide chain.**
- **The synthesis of these hormones involves iodination of the tyrosine ring, which concentrates iodide ion from the blood serum .**

Aspirin





The anti-inflammatory and analgesic properties of aspirin derive from the inhibition of PGHS-2 or prostaglandin H synthetase-2.

Chemical test for Proteins



Separation of Amino Acids and Proteins

1. **Chromatography** – the method of separating amino acids on the basis of differences in absorption, ionic charges, size and solubility of molecules
2. **Electrophoresis** – effects separation in an electric field on the basis of differences in charges carried by amino acids and proteins under specific condition
3. **Ultracentrifugation** – effects separation on the basis of molecular weight when large gravitational forces are applied in the ultracentrifuge.
4. **Precipitation Methods** – salts as sodium sulfate, ammonium sulfate, cadmium nitrate, silver nitrate and mercuric chloride at specific conc. precipitate some proteins while others remain in solution
5. **Dialysis** – is for the removal of small, crystalloidal molecules from protein solution.

Chromatography

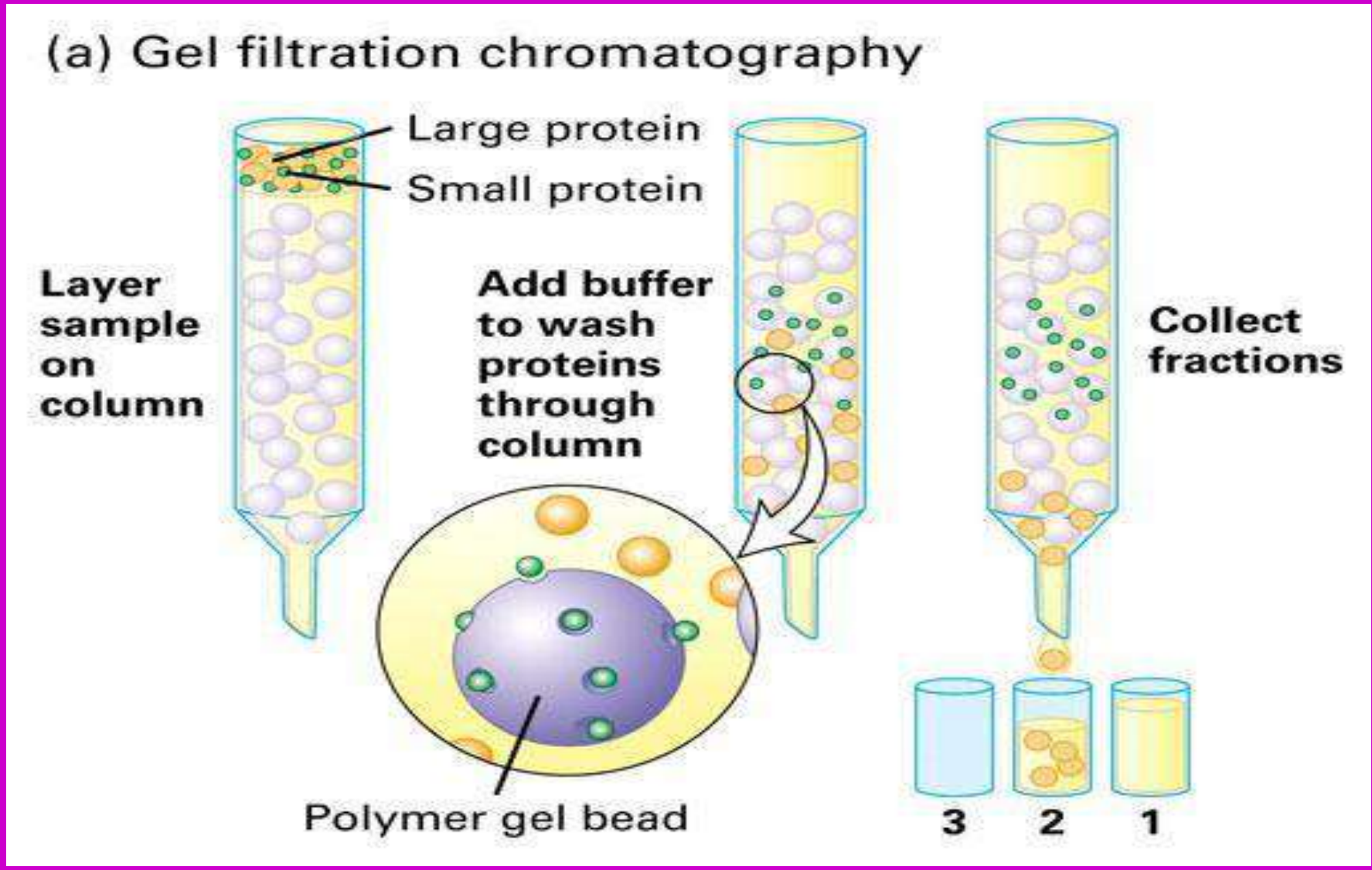
- Much of modern biochemistry depends on the use of column chromatographic methods to separate molecules.
- Chromatographic methods involve passing a solution (the mobile phase) through a medium (the immobile phase) that shows selective solute components.
- The important methods of chromatography are:

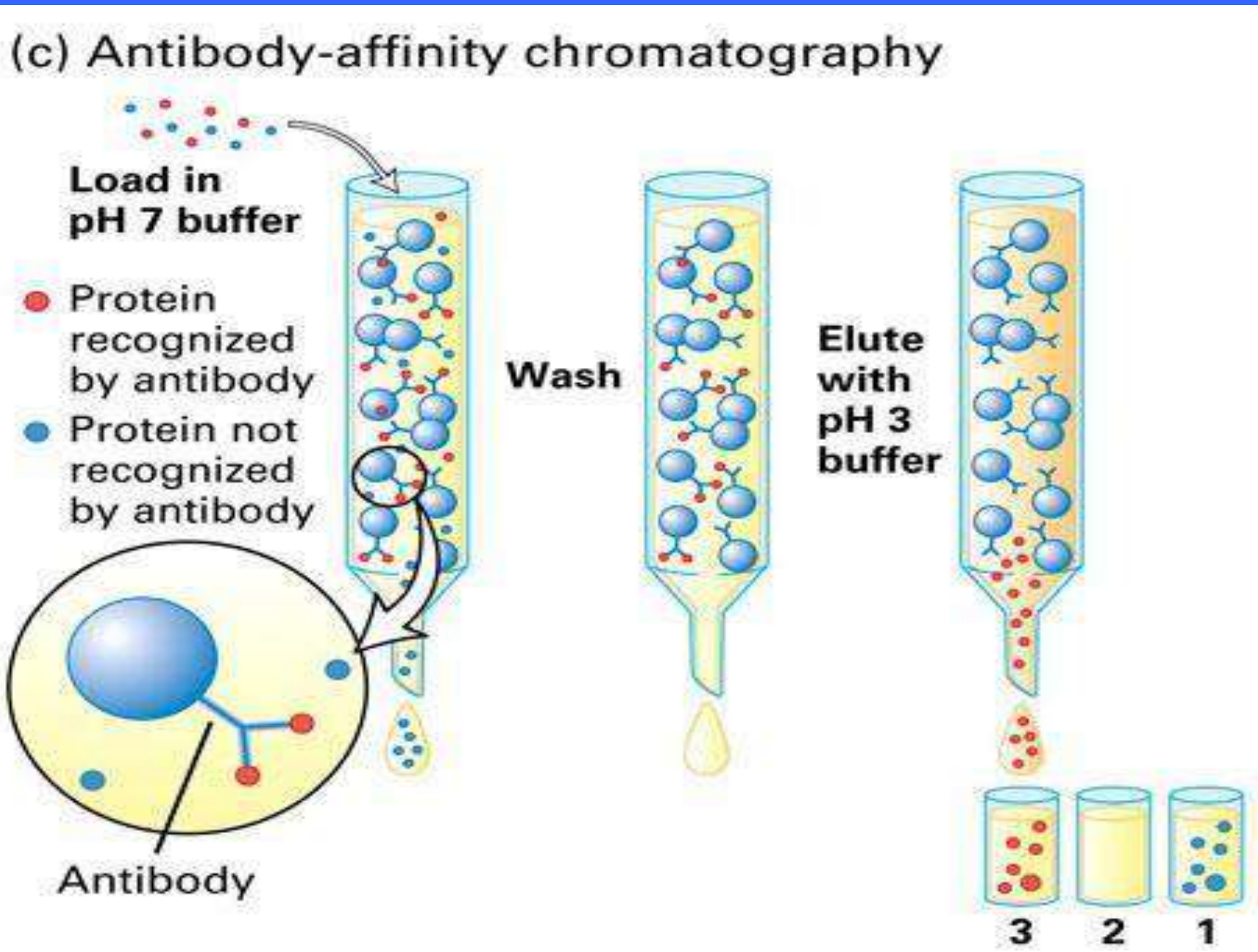
  1. Ion-Exchange Chromatography
  2. Antibody Affinity Chromatography
  3. Gel Filtration Chromatography
  4. HPLC (High Performance Liquid Chromatography)

HPLC



Gel Filtration Chromatography

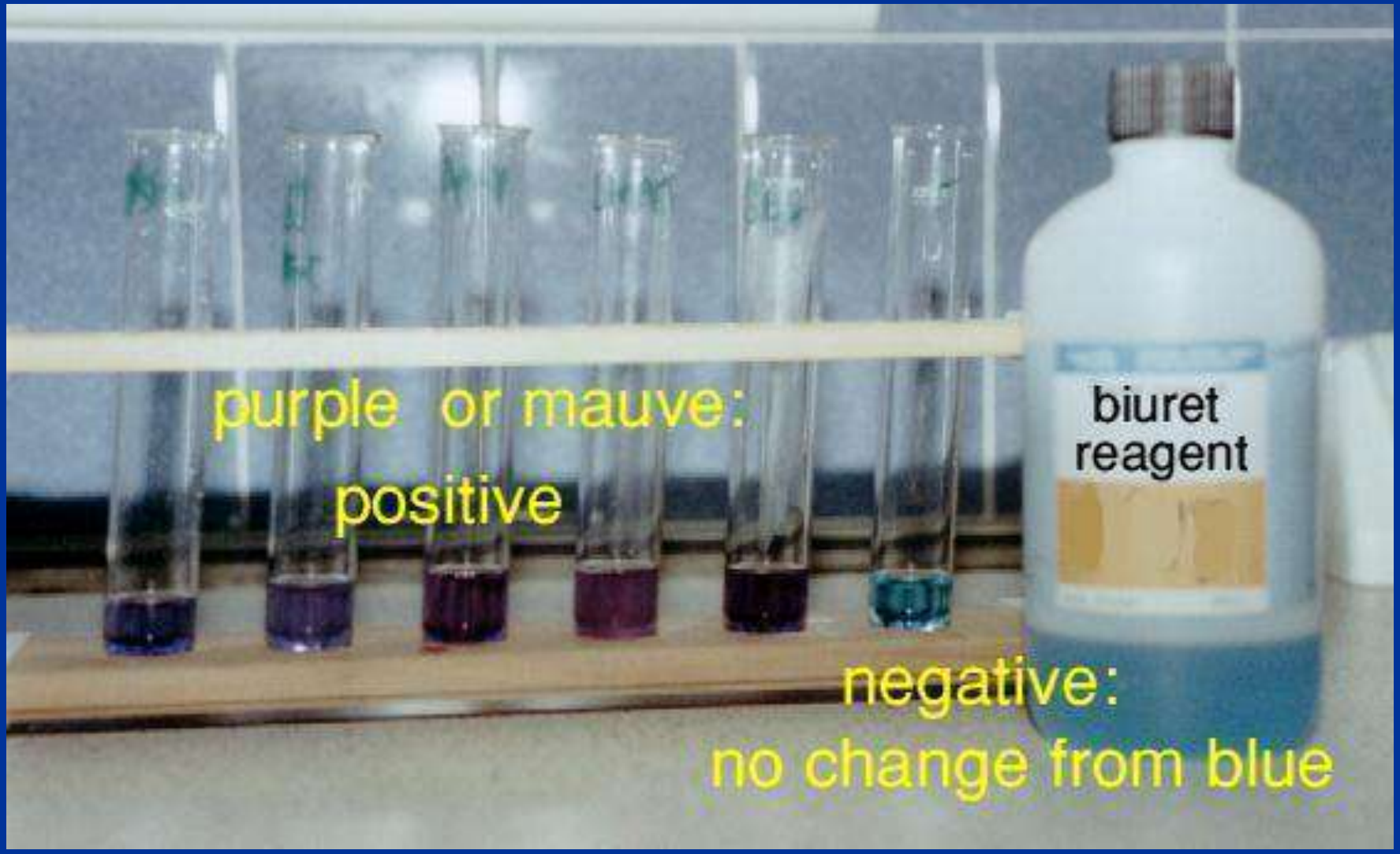



(c) Antibody-affinity chromatography
Load in pH 7 buffer
Protein recognized by antibody
Protein not recognized by antibody
Antibody
Wash
Elute with pH 3 buffer
3
2
1

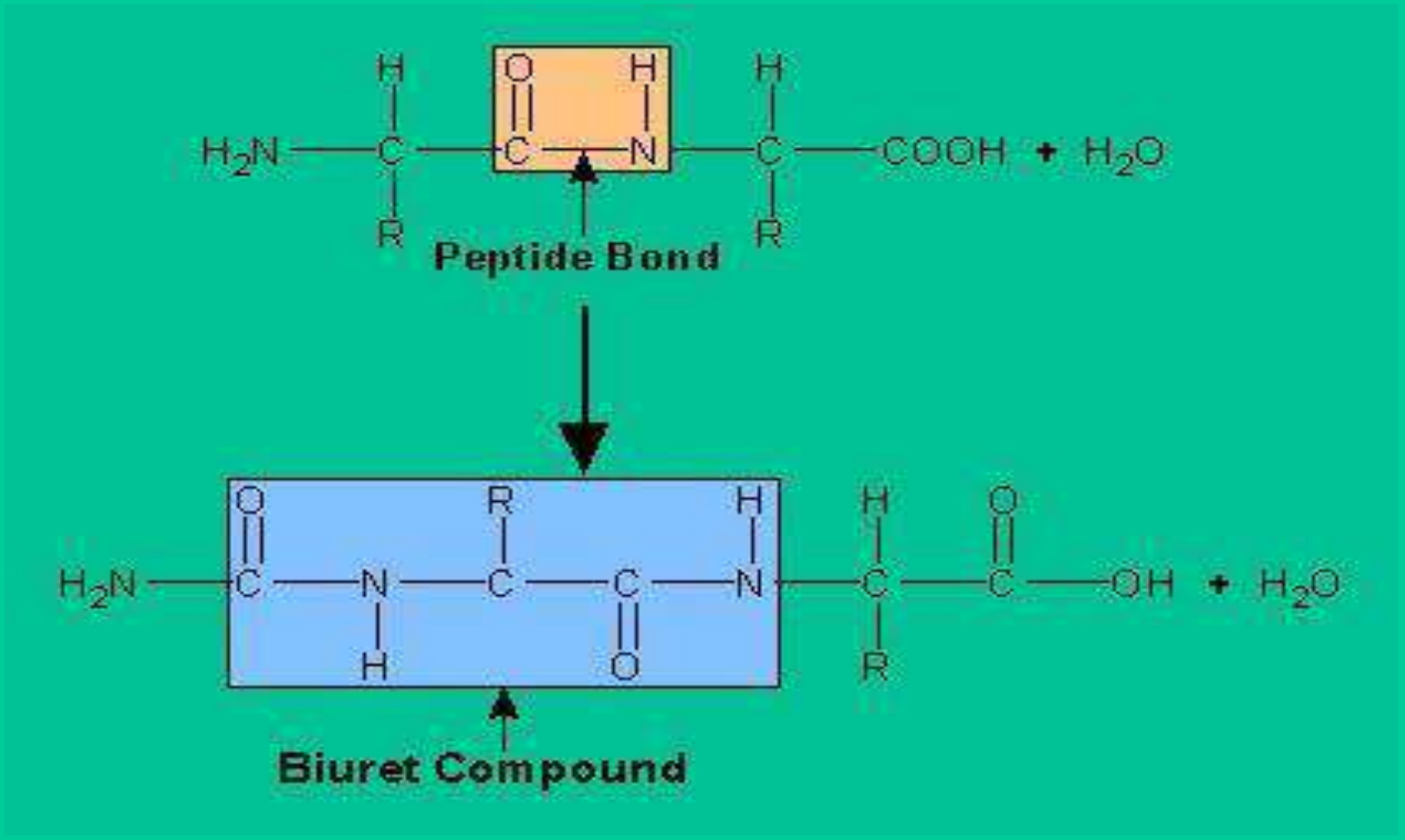
Precipitation of proteins



Biuret Test


purple or mauve:
positive
biuret
reagent
negative:
no change from blue

Chemical Reaction in the Biuret test



Proteomics

- Proteomics is the science of protein expression of all the proteins made by a cell
- Proteome pertain to all proteins being made according to the transcriptome (RNA profile). It is often visualized by a system interaction map as seen in the proteogram.

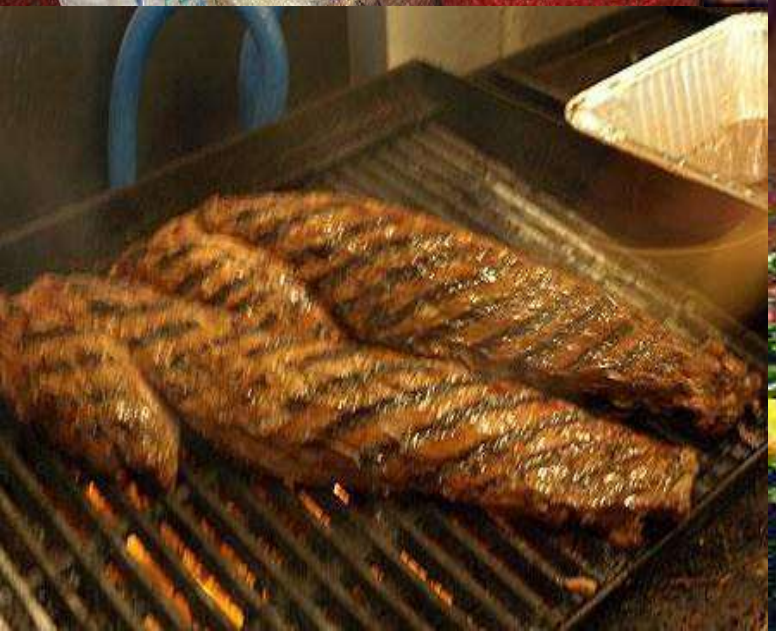
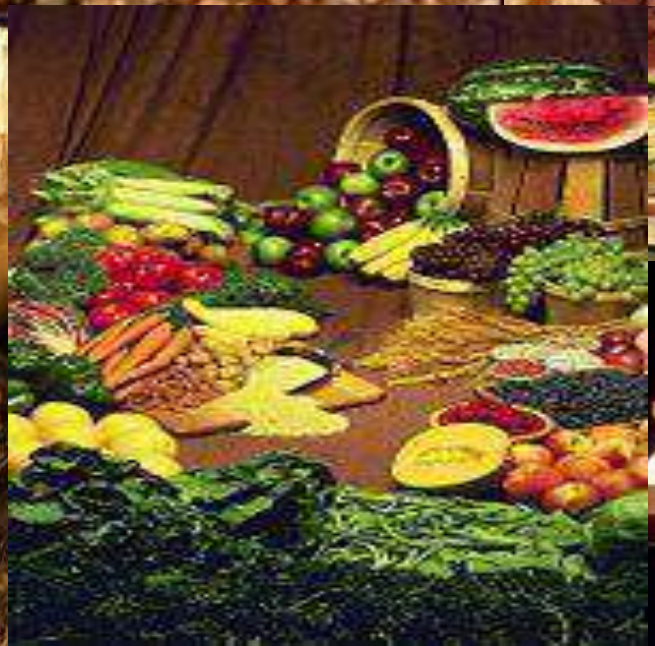
Procedures of the Proteomics

- Commonly used procedures by Proteomics are:
- Mass Spectrophotometry – detects exact mass of small peptides (molecular weight).
- X-ray Crystallography – determines 3D shape of molecules mathematically
- NMR Spectroscopy – magnetic signal indicates distances between atoms

Summary

- Proteins are polymers of α-amino acids. Twenty different amino acids are coded for in genes and incorporated into proteins. Other non-protein amino acids exist, and there are also modifications of amino acids found in proteins.
- The variety of side chains – hydrophilic, hydrophobic, acidic, basic, neutral – allows much functional complexity in proteins.
- Additional variation is made possible by modification of some amino acids after they have been incorporated into proteins. The presence of both positive and negative charges on side chains makes proteins polyampholytes.





Next meeting

- Quiz on Proteins
- Discussion on Chemistry of Nucleoproteins

Green Chemistry

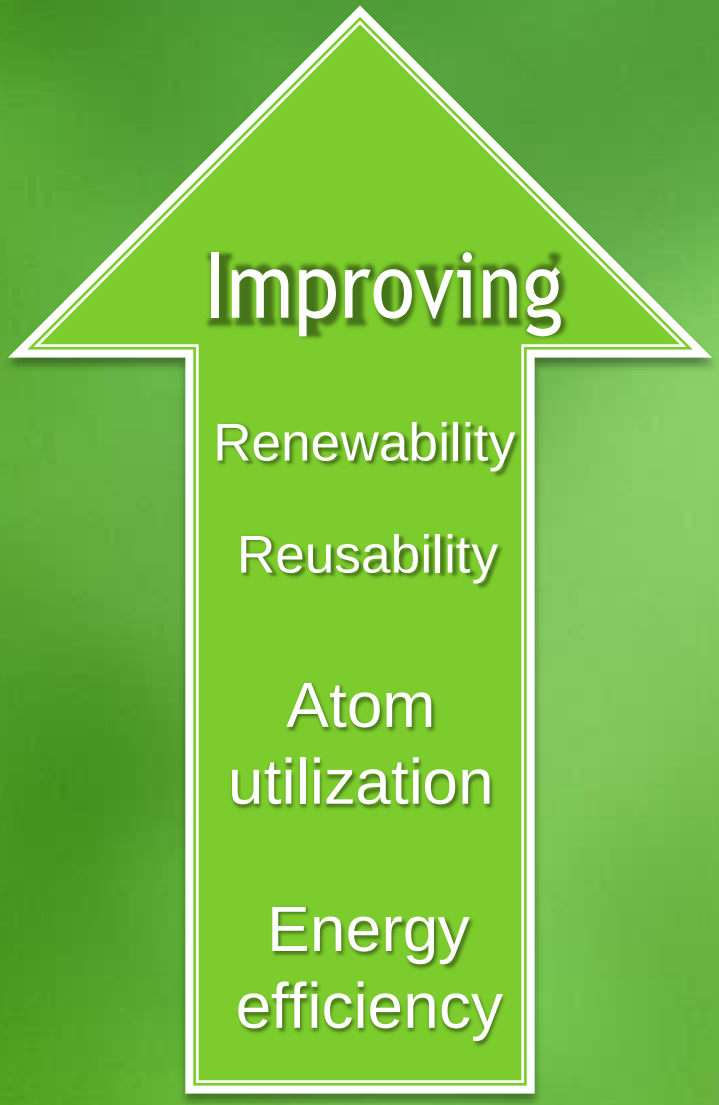
S.JAYANTHIMALA
VCPS

Definition

- **Green chemistry**, also called sustainable chemistry, is a philosophy of chemical research and engineering that encourages the design of products and processes that minimize the use and generation of hazardous substances*.
- The utilization of a set of principles that reduces or eliminates the use or generation of hazardous substances in the design, manufacture and application of chemical products**.
  - *As defined by United States Environmental Protection Agency
  - ** Anastas, P. T.; Warner, J.C. Green Chemistry: Theory and Practice, Oxford University Press,1998

Green chemistry is about :





Principles of Green Chemistry

- 1.PREVENTION

It is better to prevent waste than to treat or clean up waste after it is formed.

Waste and the chemical industry

Industry	Tonnage	Ratio (kg of byproduct/kg of product)
Oil refining	10^6 - 10^8	<0.1
Bulk chemicals	10^4 - 10^6	1-5
Fine chemicals	10^2 - 10^4	5-50
Pharmaceuticals	10 - 10^3	25-100+

Pharmaceuticals and chemical industries though have a lesser waste production compared to other industries like oil refining, have an opportunity of reducing the waste further since the amount of waste per kg of product is relatively higher

2. Atom Economy

Synthetic methods should be designed to maximise the incorporation of all materials used in the process into the final product.

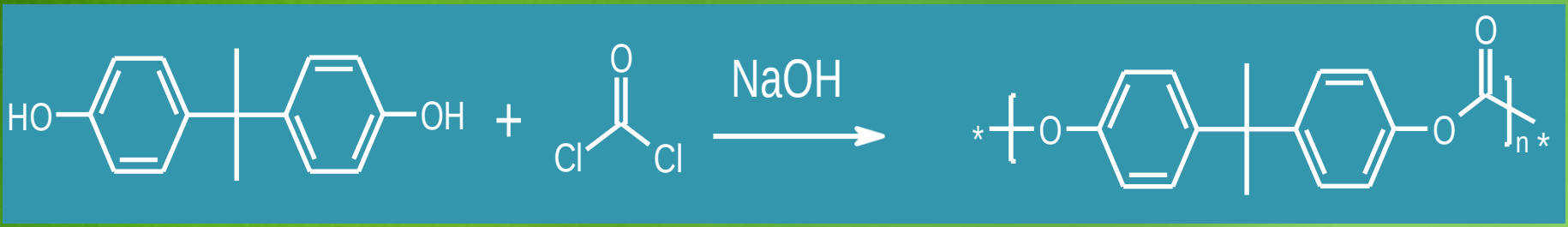
- **Atom economy** (**atom efficiency**) describes the conversion efficiency of a chemical process in terms of all atoms involved (desired products produced).
- In an ideal chemical process, the amount of starting materials or reactants equals the amount of all products generated and no atom is wasted.

% atom economy= (MW of desired product/MW of all reactants) *100

3. Less Hazardous Chemical Synthesis

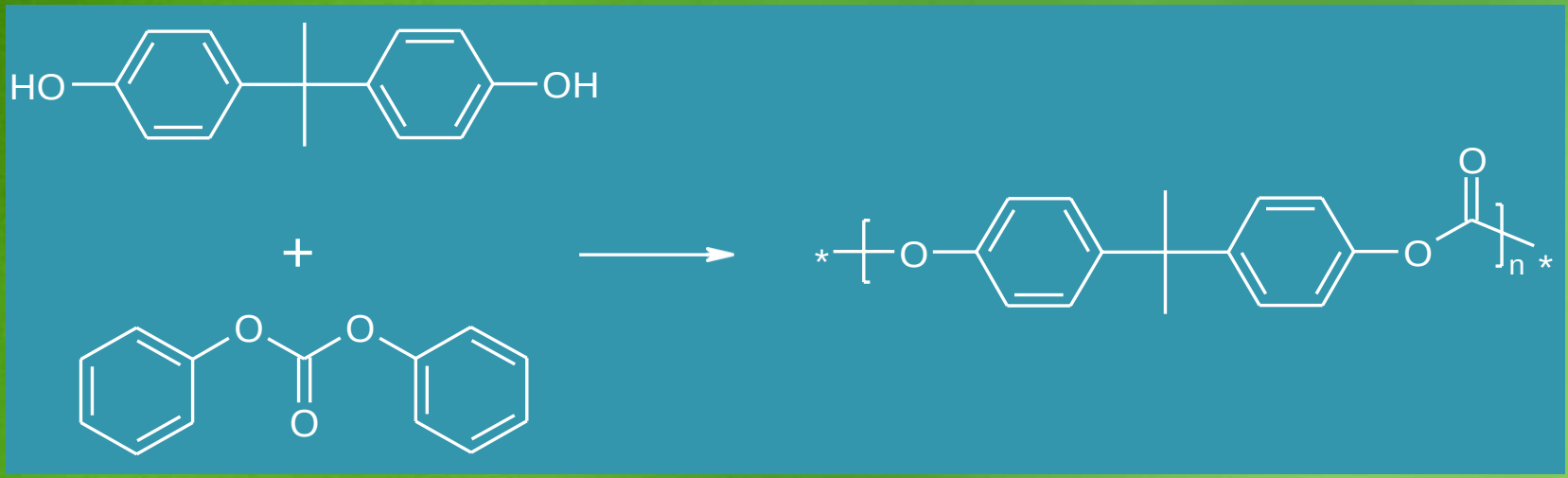
Whenever practicable, synthetic methodologies should be designed to use and generate substances that possess little or no toxicity to human health and the environment.

Polycarbonate Synthesis using Phosgene



- Disadvantages
  - Phosgene is highly toxic and corrosive
  - Requires large amount of CH_2cl_2
  - Polycarbonate gets contaminated with chlorine impurities

Polycarbonate Synthesis using solid state process



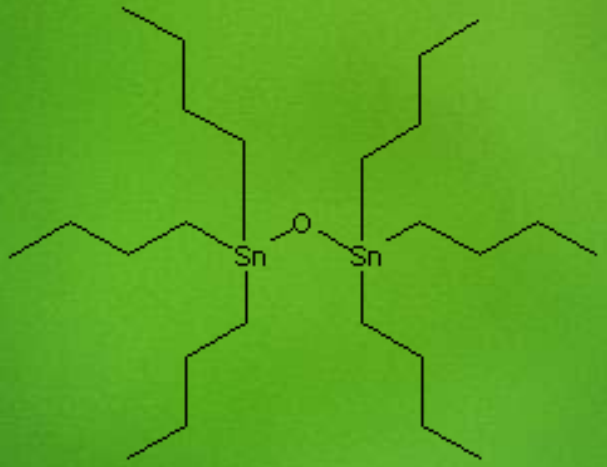
- Advantages
  - diphenylcarbonate synthesized without phosgene
  - Eliminates use of CH_2Cl_2
  - Higher-quality polycarbonates without chlorine impurities.

Komiya *et al.,* Asahi Chemical Industry Co.

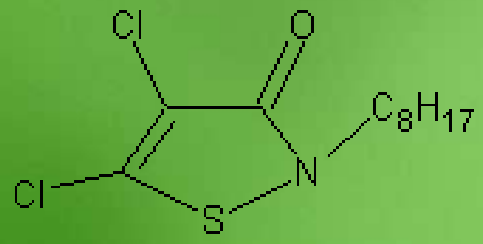
4. Designing Safer Chemicals

Chemical products should be designed to preserve efficacy of the function while reducing toxicity.

- Tributyltin oxide is usually used mixed with paints as an antifoulants for ships. This chemical is usually toxic to marine organisms and also bio-accumulate.
- Sea-Nine® 211, 4,5-dichloro-2-*n*-octyl-4-isothiazolin-3-one (DCOI), is now used which is relatively less toxic.



Tributyltin Oxide



4,5-dichloro-2-n-octyl-4-isothiazolin-3-one

DCOI

http://academic.scranton.edu/faculty/CANNM1/environmentalmodule.html

5. Safer Solvents and Auxiliaries

The use of auxiliary substances (solvents, separation agents, etc.) should be made unnecessary whenever possible and, when used, innocuous.

Preferred

- Water
- Acetone
- Ethanol
- 2-Propanol
- 1-Propanol
- Ethyl acetate
- Isopropyl acetate
- Methanol
- Methyl ethyl ketone
- 1-Butanol
- *t*-Butanol

Useable

- Cyclohexane
- Heptane
- Toluene
- Methylcyclohexane
- Methyl t-butyl ether
- Isooctane
- Acetonitrile
- 2-MethylTHF
- Tetrahydrofuran
- Xylenes
- Dimethyl sulfoxide
- Acetic acid
- Ethylene glycol

Undesirable

- Pentane
- Hexane(s)
- Di-isopropyl ether
- Diethyl ether
- Dichloromethane
- Dichloroethane
- Chloroform
- Dimethyl formamide
- N-Methylpyrrolidinone
- Pyridine
- Dimethyl acetate
- Dioxane
- Dimethoxyethane
- Benzene
- Carbon tetrachloride

Pfizer solvent replacement table

Red solvents	Green solvents
Pentane	Heptane
Hexane(s)	Heptane
Di-isopropyl ether or diethyl ether	2-MeTHF or *tert*-butyl methyl ether
Dioxane or dimethoxyethane	2-MeTHF or *tert*-butyl methyl ether
Chloroform, dichloroethane or carbon tetrachloride	Dichloromethane
Dimethyl formamide, dimethyl acetamide or N-methylpyrrolidinone	Acetonitrile
Pyridine	Et_3N (if pyridine is used as a base)
Dichloromethane (extractions)	EtOAc, MTBE, toluene, 2-MeTHF
Dichloromethane (chromatography)	EtOAc/heptane
Benzene	Toluene

- Ionic solvents:
- Room temperature ionic liquids(RTILs) can be used as substitutes for aromatic solvents in chemical reactions and separation processes.
- RTILs are organic salts with melting points below 100^0 C, often below room temperature, have no vapour pressure and composed of entirely cations and anions.
- Exhibit good solvent properties and often facilitate chemical reactions without being transformed in the process.
- Have negligible vapour pressure and miniscule flammability.

- Exhibit high thermal stability and wide working temperatures.
- Owing to multitude of possible combinations of cation and anion, they are susceptible to numerous permutations that allow various physical and chemical properties to be adjusted at will.
- Eg: 1-butyl-3-methylimidazoliumhexafluorophosphate, and some imidazolium tetrafluoroborates

Philip E.Rakita, Ozark Fluorine Specialities

6. Design for Energy Efficiency

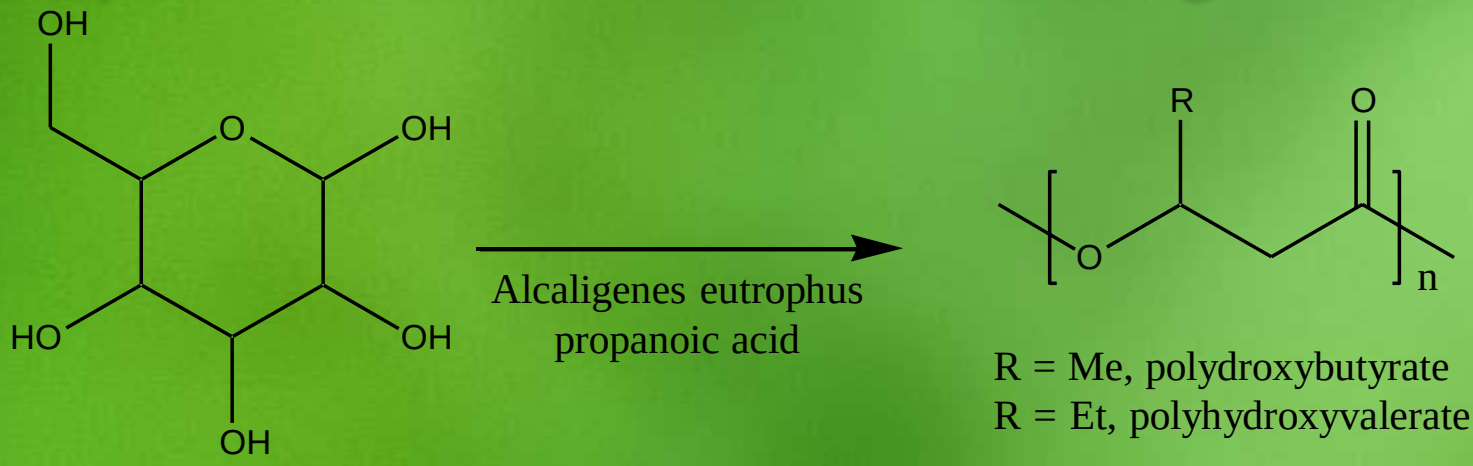
Energy requirements should be recognized for their environmental and economic impacts and should be minimized. Synthetic methods should be conducted at ambient temperature and pressure.

- Use of alternate, recyclable and more efficient energy resources.
- Use of light energy as a source for photochemical reactions.
- Use of microwave chemistry for more efficient energy utilization

7. Use of Renewable Feedstocks

A raw material or feedstock should be renewable rather than depleting whenever technically and economically practical.

- Fermentation of glucose in the presence of bacteria and propanoic acid gives Polyhydroxy alkanoates (PHAs).
- The PHAs are similar to polypropene and polyethene but however are biodegradable.



- PLA (polylactic acid) is another plastic that is being made from renewable feedstocks such as corn and potato waste.

8. Reduce Derivatives

Unnecessary derivatization (blocking group, protection/deprotection, temporary modification of physical/chemical processes) should be avoided whenever possible.

- An increase in the number of synthetic steps would eventually reduce the overall yield and atom economy.
- Protecting groups are generally used because there is no direct way to solve the problem without them.
- Attempts to reduce the number of steps and derivatization is considered important.

9. Catalysis

Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.

- A green catalyst has advantages such as:
  - Readily separated
  - Readily regenerated & recycled
  - Long service life
  - Very high rates of reaction
  - Robust to poisons
  - High selectivity
  - Works under milder conditions

- Certain chemicals are used as green catalysts which reduce the incidence of toxic chemicals formed in a reaction by converting them to less toxic or harmless substances.
- Oxidation catalysts, called Fe-TAML® (tetra-amido macrocyclic ligand) activators, are made from elements found in nature and work with hydrogen peroxide to convert harmful pollutants into less toxic or harmless substances*.

*Terrence J. Collins;Teresa Heinz Professor of Green Chemistry

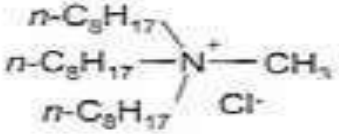
Biocatalysis:

- Enzymes or whole-cell microorganisms are used.
- Benefits include:
  - Fast reactions due to correct orientations
  - Orientation of site gives high stereospecificity
  - Substrate specificity
  - Water soluble
  - Naturally occurring
  - Moderate conditions
  - Possibility for tandem reactions

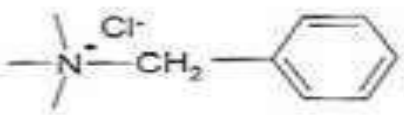
Phase Transfer Catalyst:

- A phase transfer catalyst is a catalyst which facilitates the migration of a reactant in a heterogeneous system from one phase into another phase where reaction can take place.
- Ionic reactants are often soluble in an aqueous phase but are insoluble in an organic phase unless the phase transfer catalyst is present.
- Advantages of PTC
  - Elimination of organic solvents
  - High yields and purity of products
  - Simplicity of the procedure
  - Highly scalable
  - Low energy cosumption and low investment cost
  - Minimization of industrial waste

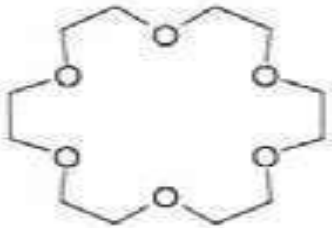
- Quarternary ammonium or phosphonium salts are most widely used PTCs.
  - Eg: methyltrioctyl ammoniumchloride (Aliquat 336 or Adogen 464),
  - Tetra-n-butylammonium bromide (TBAB)
  - Triethylbenzylammonium chloride (TEBA)
  - Cetyltrimethylammonium bromide (cetrimide)
  - benzyltrioctyl ammoniumchloride,
  - polyethylene glycoether,
  - crown ethers



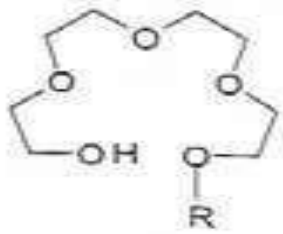
Methyltrioctyl-ammonium chloride (Aliquat 336)



Benzyltrimethyl-ammonium chloride

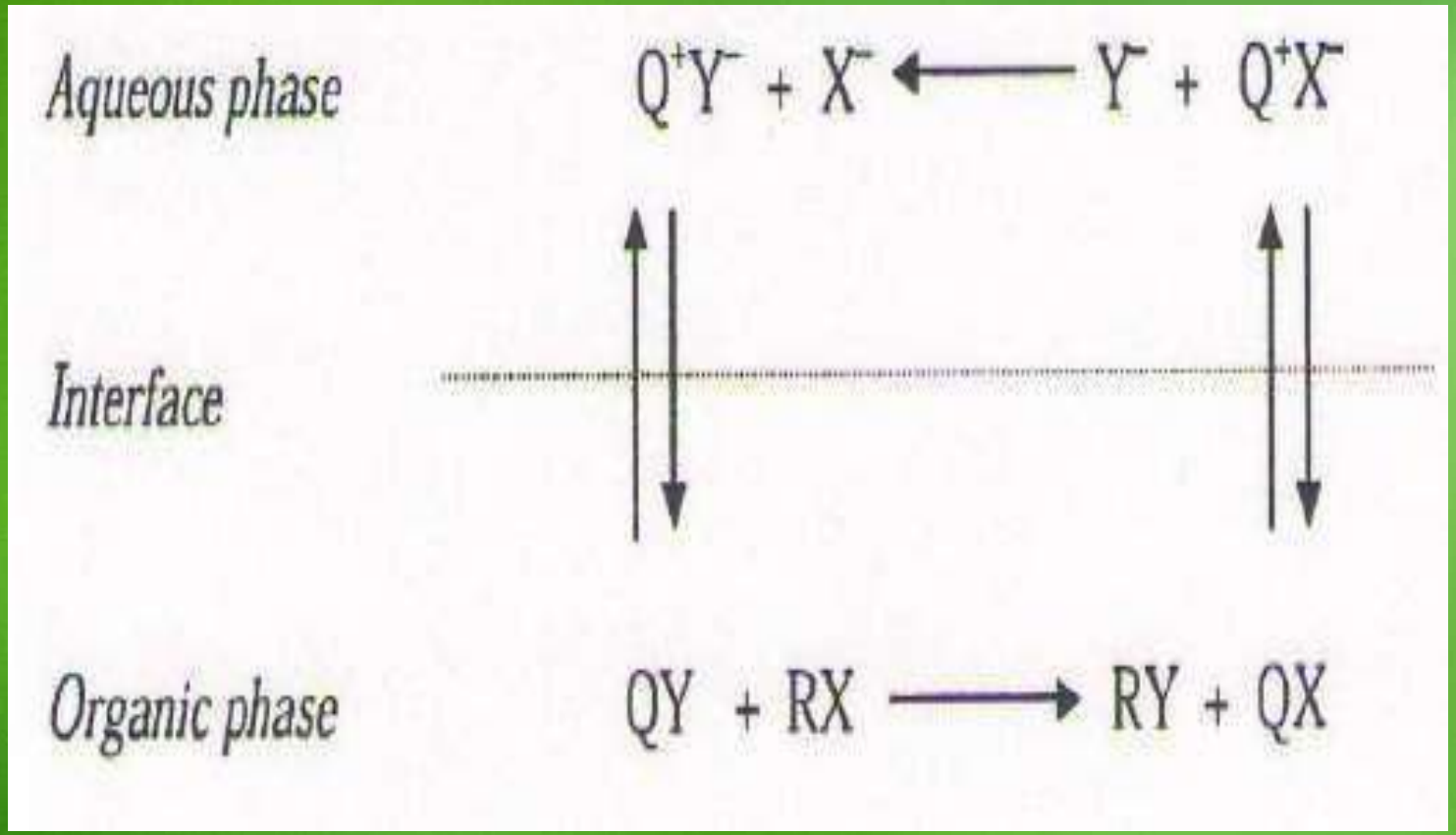


Crown ether (18-crown-6)



Polyetylene glycolether

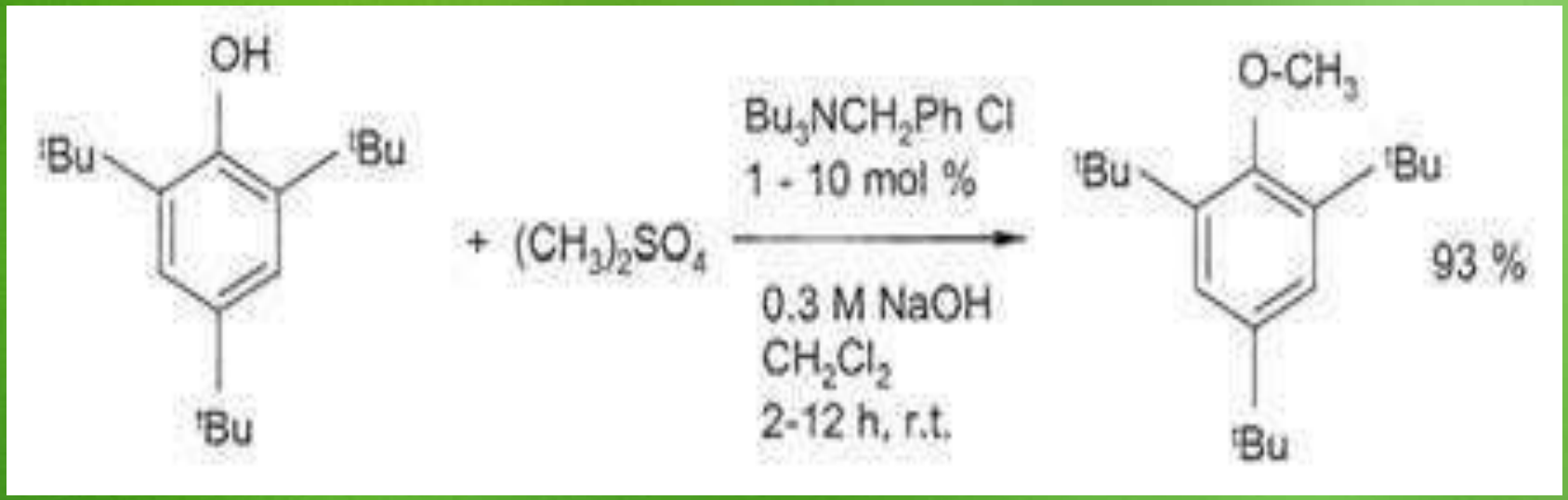
- Mechanism of phase transfer by PTC:



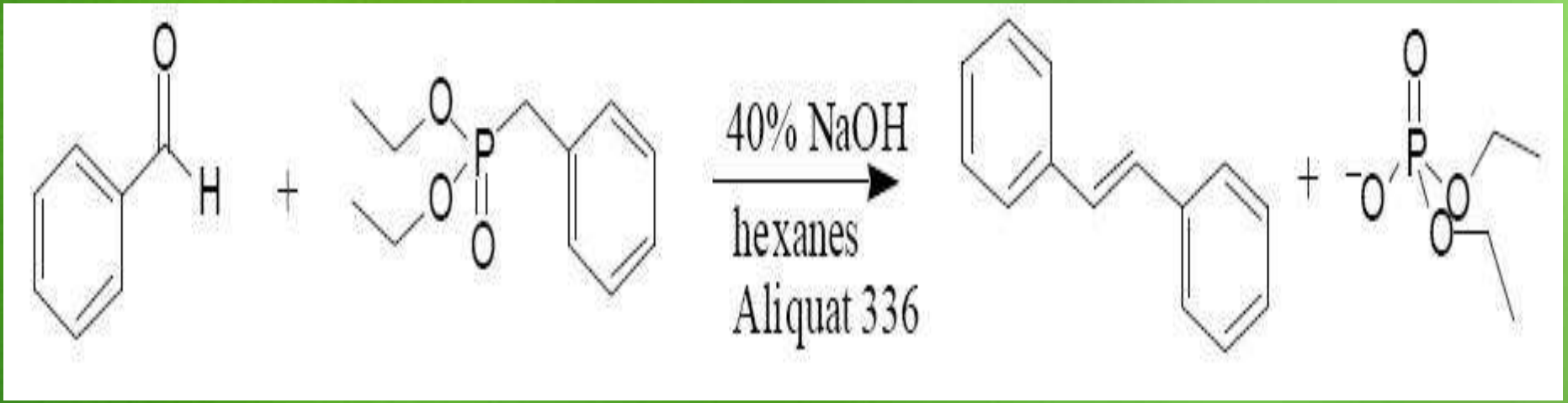
Applications of PTCs:

- In nucleophilic substitution reactions
- Synthesis of fine chemicals
- In perfumery and fragrance industry
- Is synthesis of drugs like dicyclonine, phenoperidine, oxaladine, ritaline etc.
- Provides liberty of use of cheaper and easily available raw materials like potassium carbonate and aqueous sodium hydroxide thereby obviating the need of severe anhydrous conditions, expensive solvents and dangerous bases such as metal hydrides and organometallic reagents.

- **Williamsons ether synthesis by PTC:**
- High-yield etherification
- No need for excess pre-formed alkoxide
- Usually short cycle time and easy workup
- Non-dry mild reaction conditions



- Wittig reaction by PTC
- Aliquat 336 (N-Methyl-N,N-dioctyloctan-1-ammonium chloride) is used as PTC.



10. Design for Degradation

Chemical products should be designed so that at the end of their function they do not persist in the environment and instead break down into innocuous degradation products.

- Plastics, long chain hydrocarbons, CFCs have longer persistence.
- Chemicals such as DDT bioaccumulate.
- Drugs such as antibiotics build up in water streams.
- Design of degradable chemicals is the need of the hour.
- Polylactic acid:
  - Manufactured from renewable resources such as corn or wheat;
  - Uses 20-50% fewer fossil fuels than conventional plastics
  - PLA products can be recycled or composted

11. Real-time Analysis for Pollution Prevention

Analytical methodologies need to be further developed to allow for real-time in-process monitoring and control prior to the formation of hazardous substances.

- In the process of a chemical reaction, analysing when a reaction is exactly complete can save a lot of energy, waste and time.
- Overdoing a reaction may result in energy wastage and under-doing the same may result in material wastage.
- An advanced and sophisticated analytical tool helps reduce this pollution

12. Inherently Safer Chemistry for Accident Prevention

Substance and the form of a substance used in a chemical process should be chosen so as to minimize the potential for chemical accidents, including releases, explosions, and fires.

- Use of potential toxic, hazardous and highly inflammable chemicals may result in accidents which eventually lead to pollution and danger to plant and animal life.
- U.S. Public Interest Research Group Reports (April 2004) find that chemical industry has had more than 25,000 chemical accidents since 1990
- More than 1,800 accidents a year or 5 a day.
- In Bhopal gas tragedy, release of 40 tons of Methyl isocyanate (MIC) took the lives of 15000 people leaving hundreds of thousands seriously affected.

THANK YOU

INTROUDUCTION TO BIOPHARMACEUTICS AND PHARMACOKINETICS

B.Rajeswar
VCPS

Biopharmaceutics:

The study of various factors influencing the rate and amount of drug that reaches the systemic circulation and the use of this information to optimize the therapeutic efficacy of the drug products is known as biopharmaceutics.

Pharmacokinetics:

It is defined as the study of time course of drug ADME and their relationship with its therapeutic and toxic effects of drugs.

Bioavailability:

Rate and extent of drug enter into systemic circulation in unchanged form.

Absorption:

It is defined as the process of movement of drug from its site of administration to the systemic circulation.

Distribution:

The movement of drug between one compartment and the other (generally blood and the extravascular tissues) is referred to as drug distribution.

Elimination:

It is defined as the process that tends to remove the drug from the body and terminates its action

Takes place by two processes

1.Biotransformation

2.Excretion

Biotransformation:

It is defined as conversion from one chemical form to another. The term is used synonymously with metabolism.

Excretion:

It is the process whereby drugs and/or their metabolites are irreversibly transferred from internal to external environment.

Pharmacodynamic studies:

It is the study of relationship between the drug concentration to that of response given by the specific organ or tissue of a body.

Pharmacotherapeutic studies:

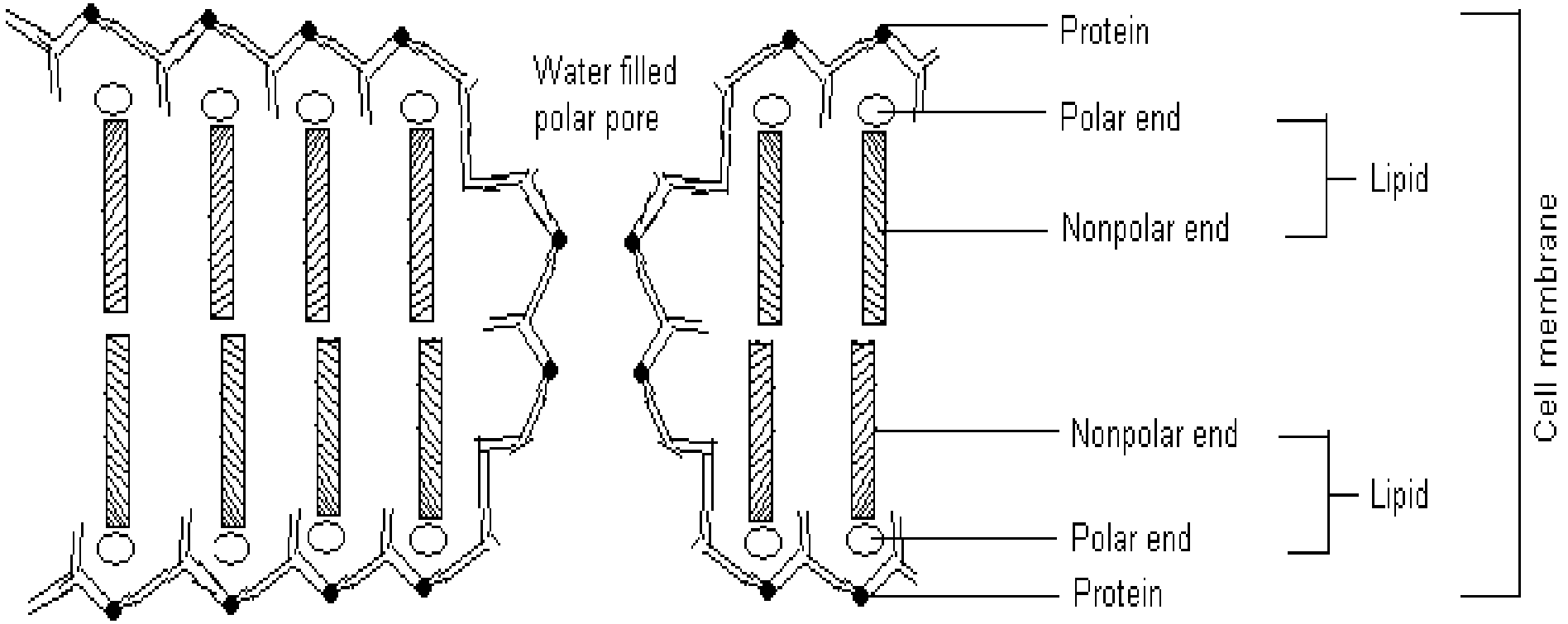
It is the study of relationship between the therapeutic efficacy of the drug to that of the clinical response given by a suitable dosage form.

Dosage regimen:

Frequency of administration of a drug in a particular dose.

ABSORPTION

Basic structure of functional cell membrane



Mechanisms of Drug Absorption

1. Passive Diffusion
2. Pore Transport
3. Facilitated Diffusion
4. Active Transport
5. Ionic or Electrochemical Diffusion
6. Ion-pair Transport
7. Endocytosis

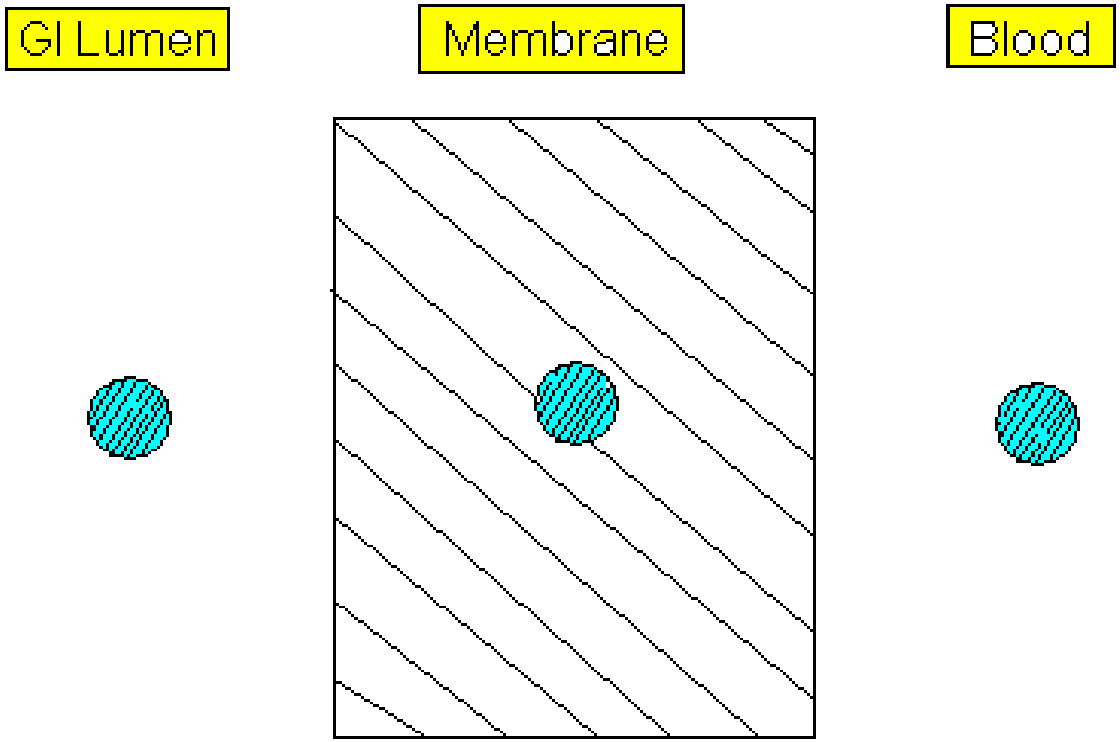
Passive Diffusion

- Also called nonionic diffusion.
- The driving force is concentration or electrochemical gradient.
- Fick's first law of diffusion

$$\frac{dQ}{dt} = \frac{DAK_{m/w}}{h}(C_{GIT} - C)$$

Permeability coefficient, P

$$\frac{dQ}{dt} = P\,C_{GIT}$$

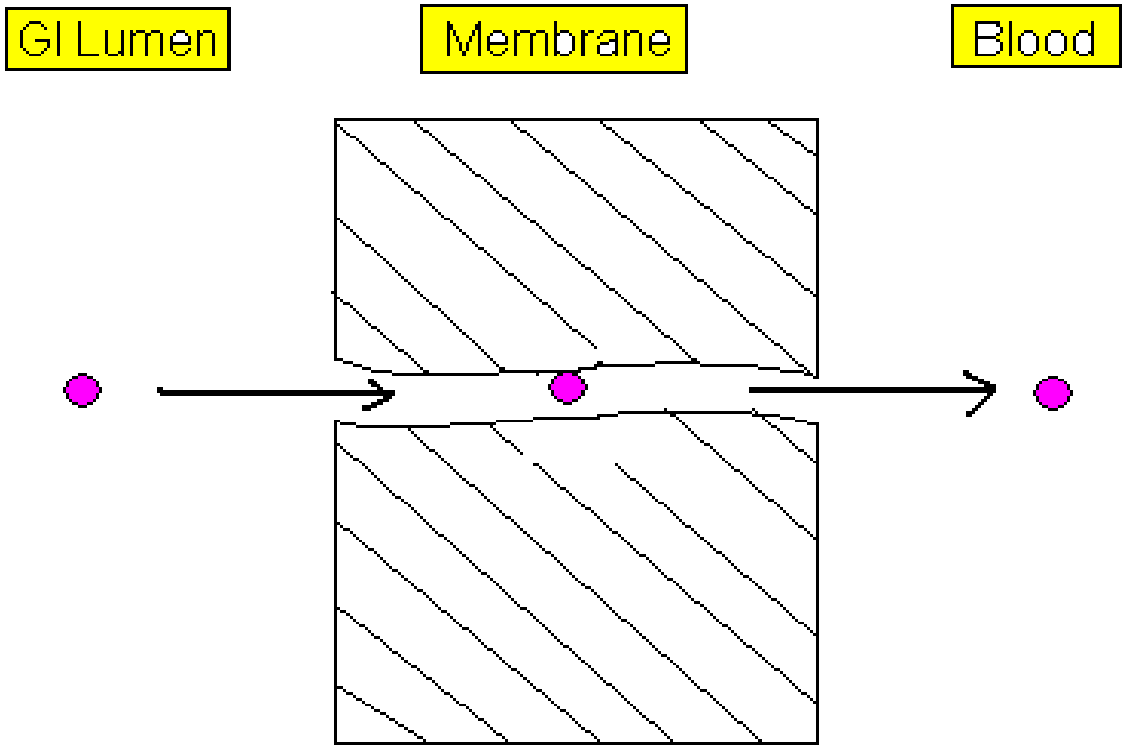


Passive Diffusion

Pore Transport

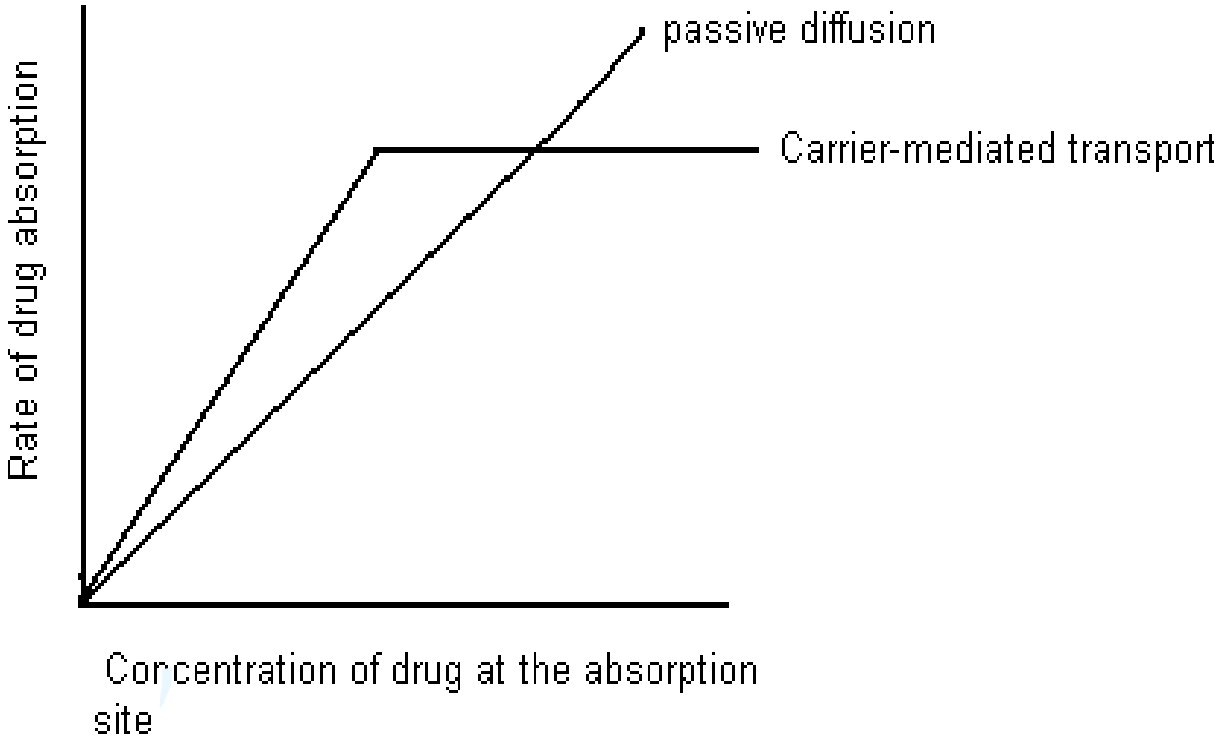
- Also called as convective transport, bulk flow or filtration.
- The driving force is hydrostatic pressure or osmotic pressure difference across the membrane due to which bulk flow of water along with small solid molecules across aqueous channels.
- Water flux that promotes such a transport is called as solvent drag.
- It is important in the absorption of low molecular weight, low molecular size and water-soluble drugs.

  Eg: urea, water and sugars.

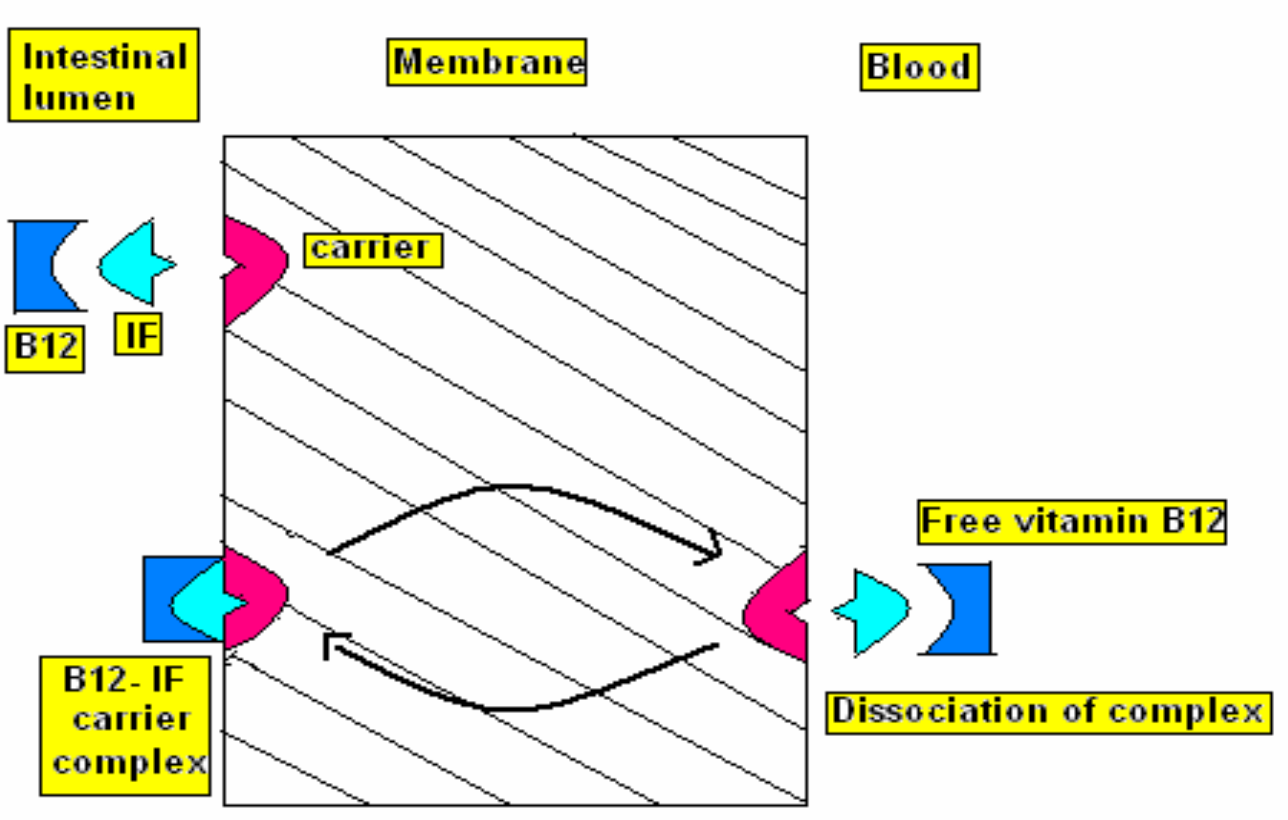


Pore Transport

Carrier-mediated Transport System

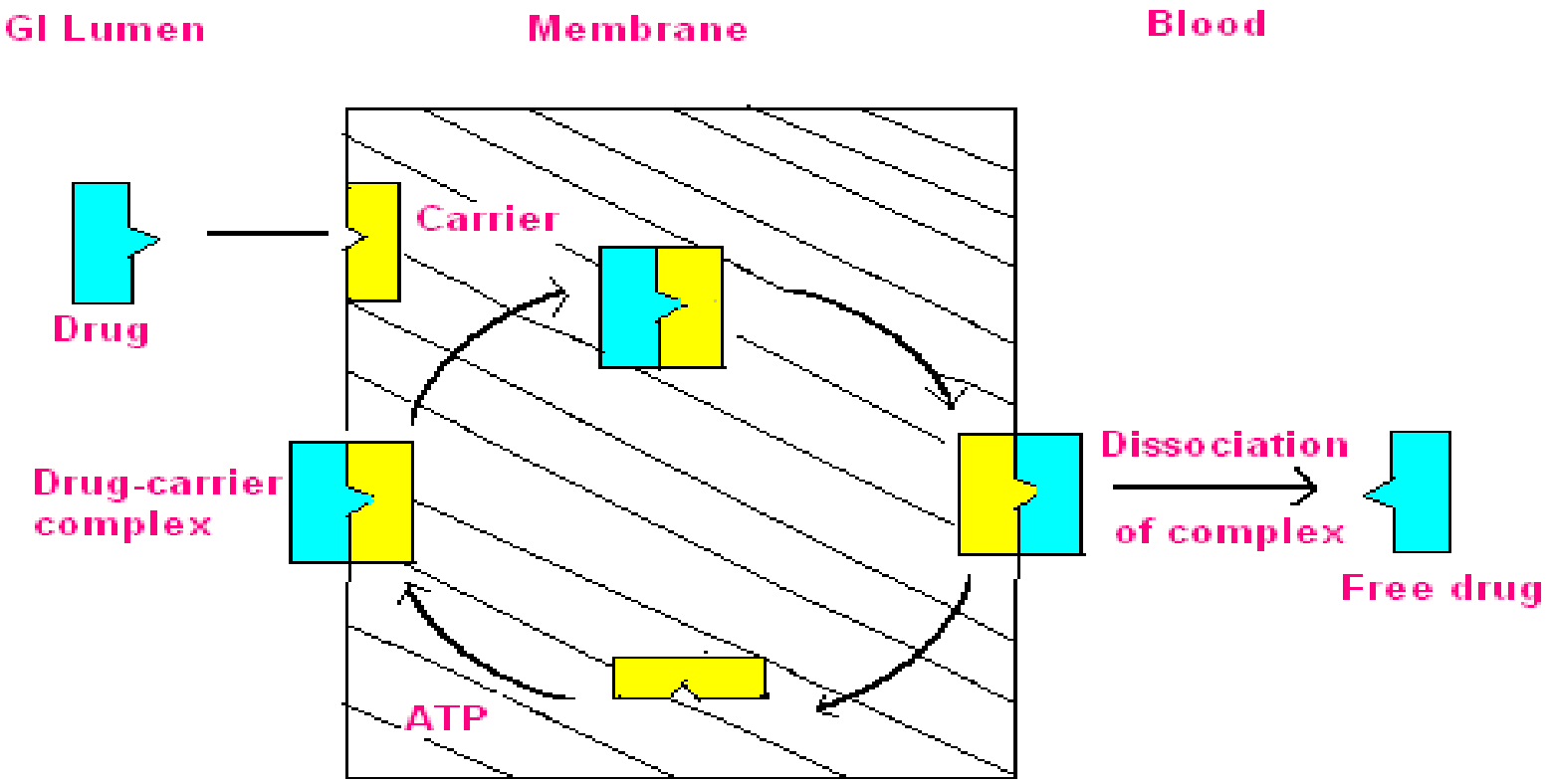


Facilitated Diffusion



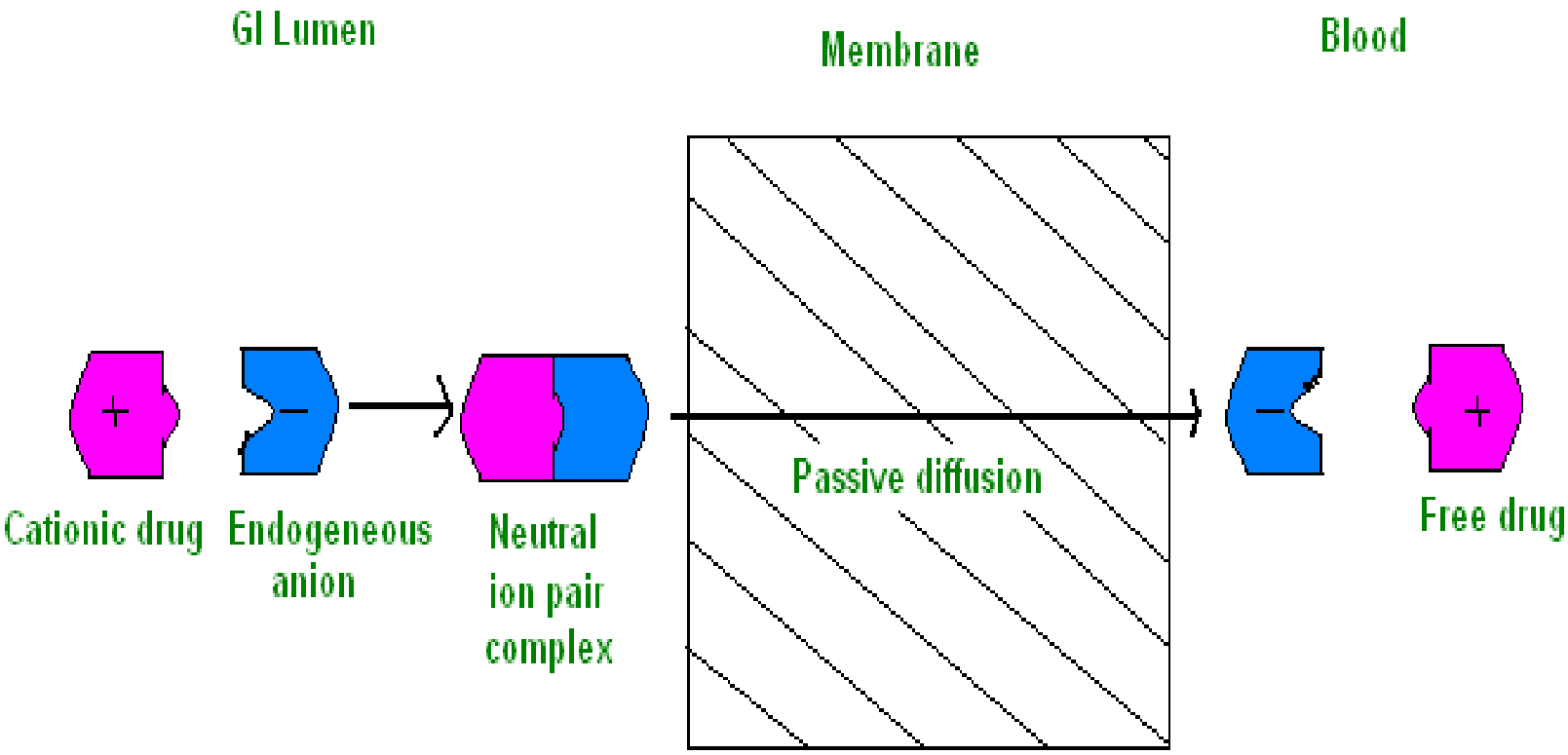
GI absorption of vitamin B_{12}

Active Transport



Absorption of 5-fluorouracil and 5- bromouracil via an pyrimidine transport system, absorption of methyl dopa and levodopa via an L-amino acid transport

Ion or Electrochemical Diffusion



The rate of permeation is in the following order:
Unionized molecules > anions > cations.

Ion-pair Transport

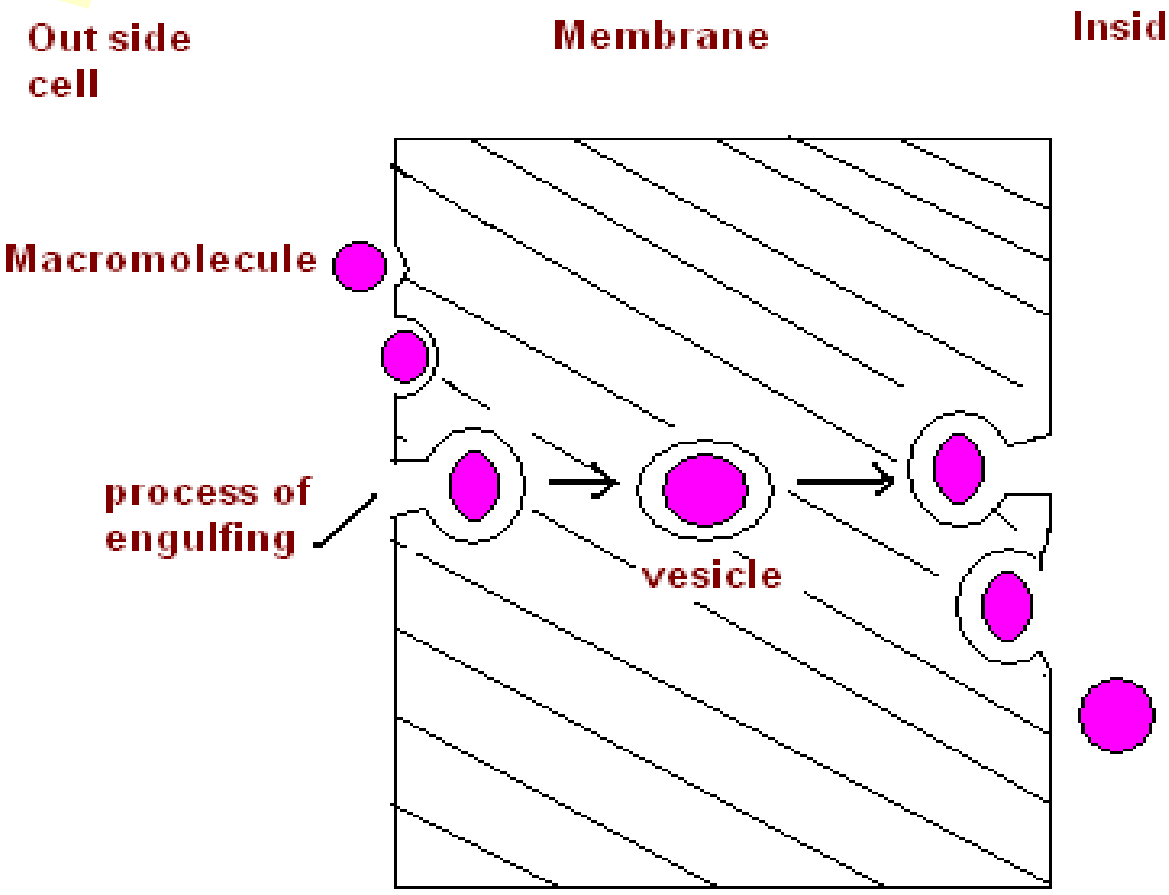
- Quaternary ammonium compounds and sulphonic acids ionize under all p^H conditions. such agents penetrate membrane by forming reversible neutral complexes with endogenous ion like mucin.
- These complexes have required lipophilicity and aqueous solubility.

Endocytosis

- It is responsible for cellular uptake of macromolecular nutrients like fats and starch, oil soluble vitamins like A,D,E,& K and drugs like insulin.

- It includes two types of process:

1. Phagocytosis(cell eating)
2. Pinocytosis (cell drinking)
3. Transcytosis (endocytic vesicle is transferred from one extra cellular compartment to another).



Endocytic uptake of Macromolecules

Biopharmaceutical factors influencing GI absorption of a drug from the dosage form

A. PHARMACEUTICAL FACTORS

I. Physicochemical properties of drug substances

1. Drug solubility and dissolution rate
2. Particle size and effective surface area
3. Polymorphism and amorphism
4. Pseudo polymorphism (hydrates or solvates)
5. Salt form of the drug
6. Lipophylicity of the drug
7. Pka of the drug and Ph
8. Drug stability

II. Dosage form characteristics and pharmaceutic ingredients

1. Disintegration time (tablets or capsules)
2. Dissolution time
3. Manufacturing variables
4. Pharmaceutic ingredients (excipients or adjuvants)
5. Nature and type of dosage form
6. Product age and storage conditions

PHYSICOCHEMICAL PROPERTIES AFFECTING BIOAVAILABILITY

- Drug solubility and dissolution rate
- Particle size and effective surface area
- Polymorphism and Amorphism
- Pseudopolymorphism(hydrates/solvates)
- Salt form of the drug
- Lipophilicity of the drug
- Pka of the drug and pH
- Drug stability

Dissolution:

Definition:

Dissolution is defined as a process in which a solid substance solubilizes in a given solvent i.e. mass transfer from the solid surface to the liquid phase.

Solubility:

Definition:

It is defined as a process in which maximum amount of solute dissolved in a given solvent under standard conditions of temperature, pressure, pH is known as solubility.

Theories of drug dissolution:

a) Diffusion Layer Model/ Film theory.

b) Danckwert Model/ Surface Renewal Theory.

c) Interfacial Barrier Model / Limited solvation theory.

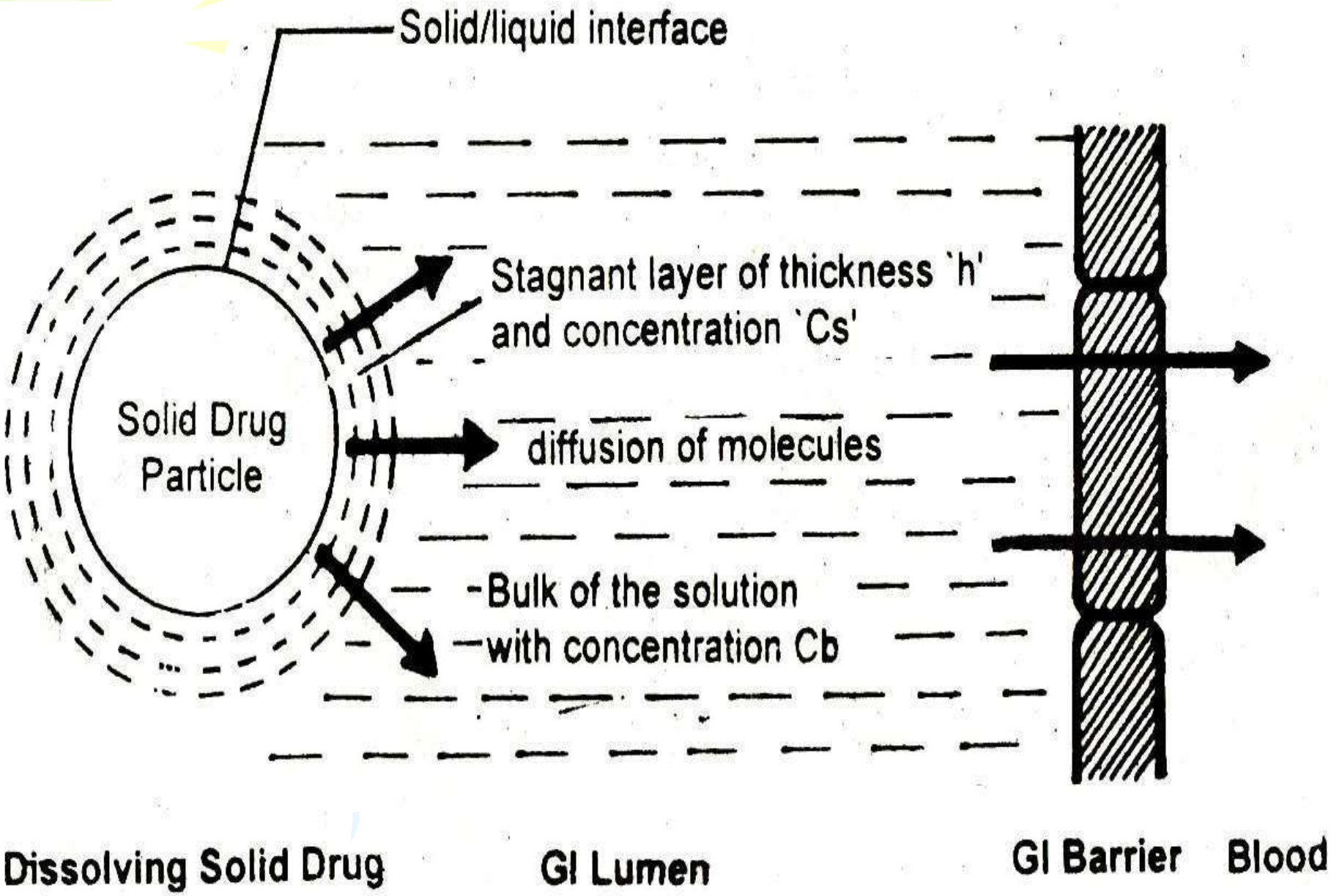


Fig. 2.11 Diffusion layer model for drug dissolution

Diffusion layer model (or)Film theory:

Noyes- whitney equation:

$$\frac{dC}{dT} = k\,(Cs - Cb)$$

dC/ dT = dissolution rate of drug

k = dissolution rate constant (first order)

Cs = Maximum drug solubility/ concentration of drug in stagnant layer

Cb = Concentration of drug in bulk of solution at time 't'

$$\frac{dC}{dT} = \frac{DAKw/o\,(Cs - Cb)}{Vh}$$

where, D = Diffusion coefficient of the drug

A = Surface area of dissolving solid

Kw/o = Water in oil partition coefficient.

Intrinsic dissolution rate constant

V = Volume of dissolution medium

h = Thickness of stagnant layer

(Cs- Cb) = Concentration gradient for diffusion of drug

Sink conditions

Definition:

Sink conditions is a process in which drug concentration in a solution maintained constant at low level is known as sink conditions.

1. The saturated solubility of a drug is a key factor in the Noyes-whitney equation . The driving force for dissolution is the concentration gradient across the boundary layer.

2. The driving force depends upon the thickness of the boundary layer and the concentration of drug dissolved.

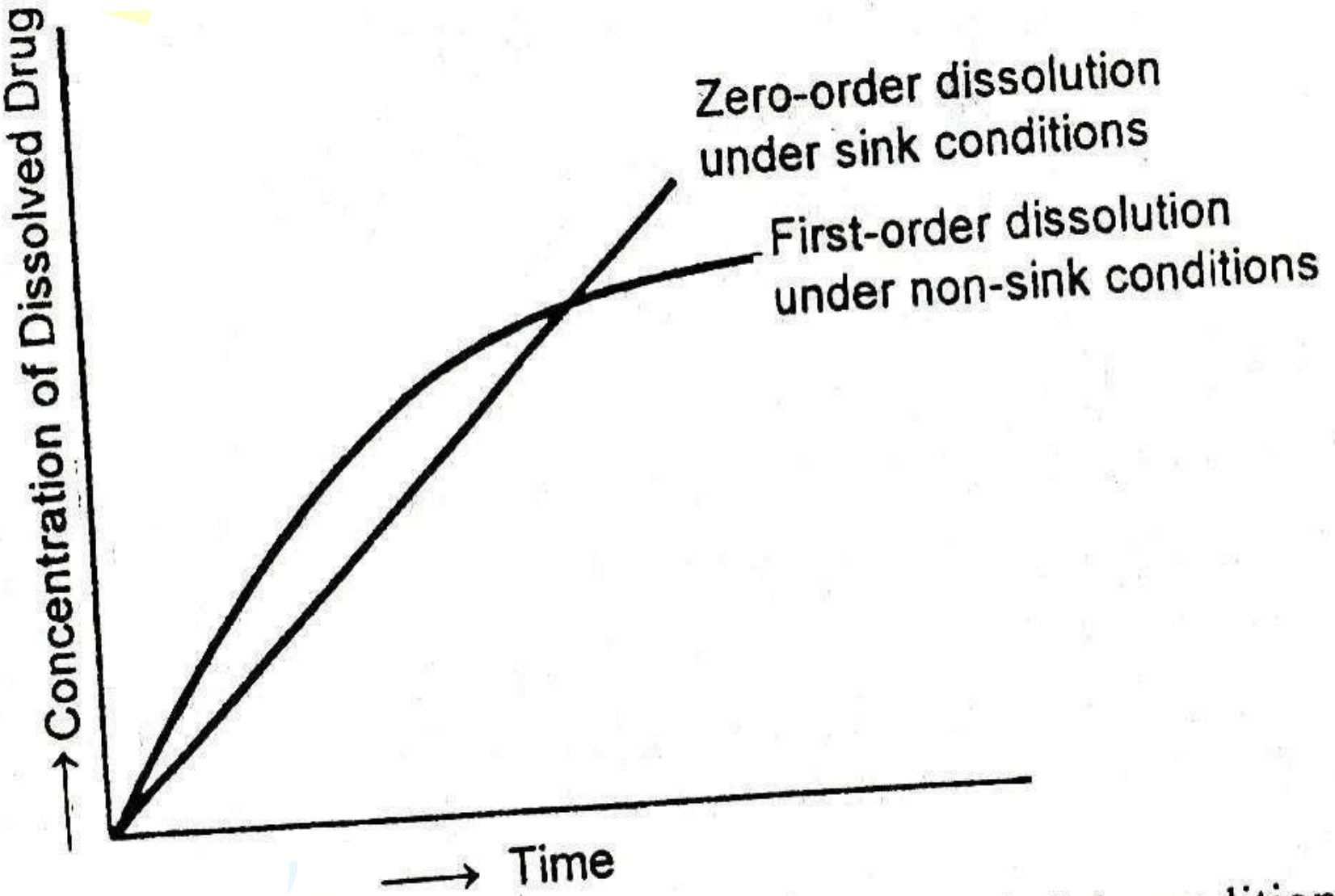


Fig. 2.12 Dissolution rate under nonsink and sink conditions.

Importance of sink conditions :

1. The driving force for dissolution is greatest when the system is under sink conditions.

2. Bathing the dissolving solid in fresh solvent from time to time.

3. Increasing the volume of dissolution fluid.

4. Removing the dissolved drug by partitioning it from the aqueous phase of the dissolution fluid into an organic phase placed either above or below the dissolution fluid .

e.g. : hexane (or) chloroform.

- *Hixson Crowell's cubic root law of* dissolution is used:

$$W_o^{1/3} - W_o^{1/3} = Kt$$

Where,

- Wo = Original mass of drug
- W = mass of drug remaining to dissolve at time t
- K = dissolution rate constant

Danckwert Model (or) Penetration (or) Surface Renewal Theory:

1. Danckwert did not approve of the existence of a stagnant layer and suggested that turbulence in the dissolution medium exists at the solid/liquid interface.

2. As a result the agitated fluid consisting of macroscopic mass of eddies (or) packets reach the solid/liquid interface in a random fashion due to eddy current absorb the solute by diffusion and carry it to the bulk of the solution.

The Danckwert's model is expressed by equation:

$$V\frac{dC}{dt} = \frac{dm}{dt} = A(Cs - Cb).\sqrt{\gamma D} \qquad (2.7)$$

where,

m = mass of solid dissolved, and

γ = rate of surface renewal (or the interfacial tension).

The model is depicted in Fig. 2.13.

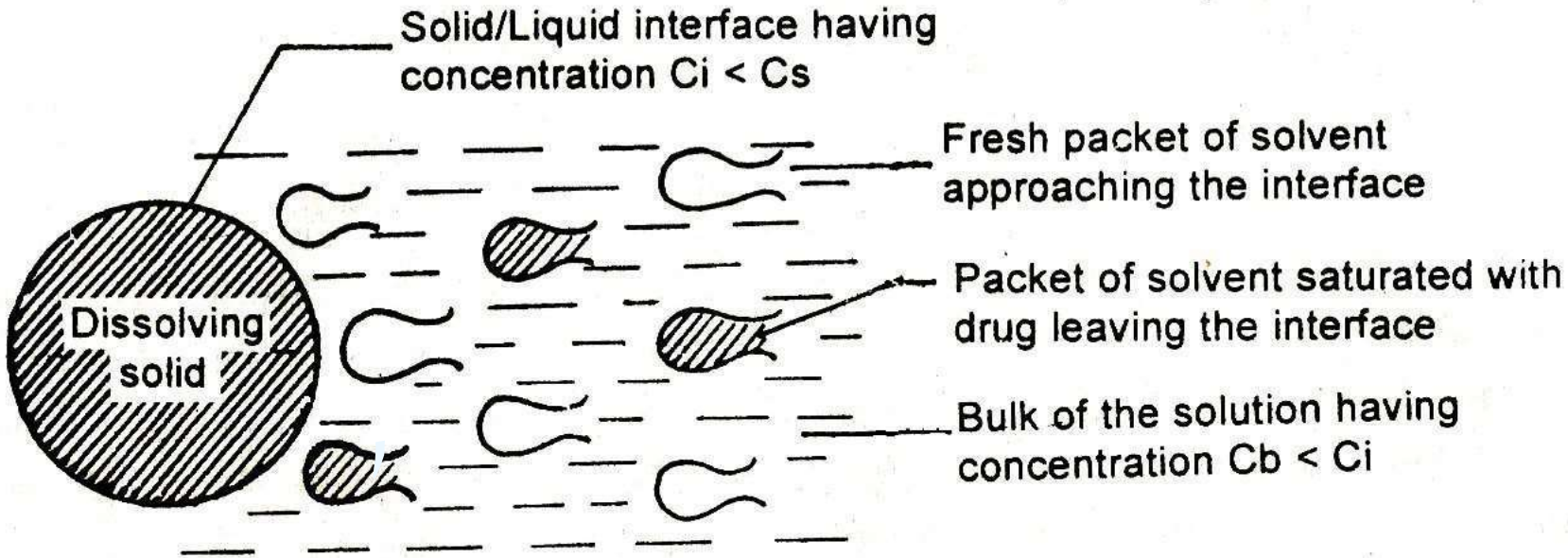


Fig. 2.13 Danckwert's model for drug dissolution

Interfacial barrier (or) Double barrier (or) Limited Solvation theory:

This model is based on the two assumptions.

1. The rate-determining steps the controls the dissolution is the mass transport.

2. Solid solution equilibrium is achieved at the solid / liquid interface.

According to the interfacial barrier model an intermediate concentration can exists at the interface as a result of salvation mechanism and is a function of solubility rather the diffusion.

Compendial methods of Dissolution :

Apparatus :1 Rotating basket apparatus.

Apparatus : 2 Paddle assembly apparatus:

Apparatus :3 Reciprocating cylindrical

Apparatus :4 flow – through cell apparatus.

Apparatus :5 Paddle over disk apparatus.

Apparatus :6 Cylinder apparatus.

Apparatus :7 Reciprocating disc method.

Alternative methods : Diffusion cell

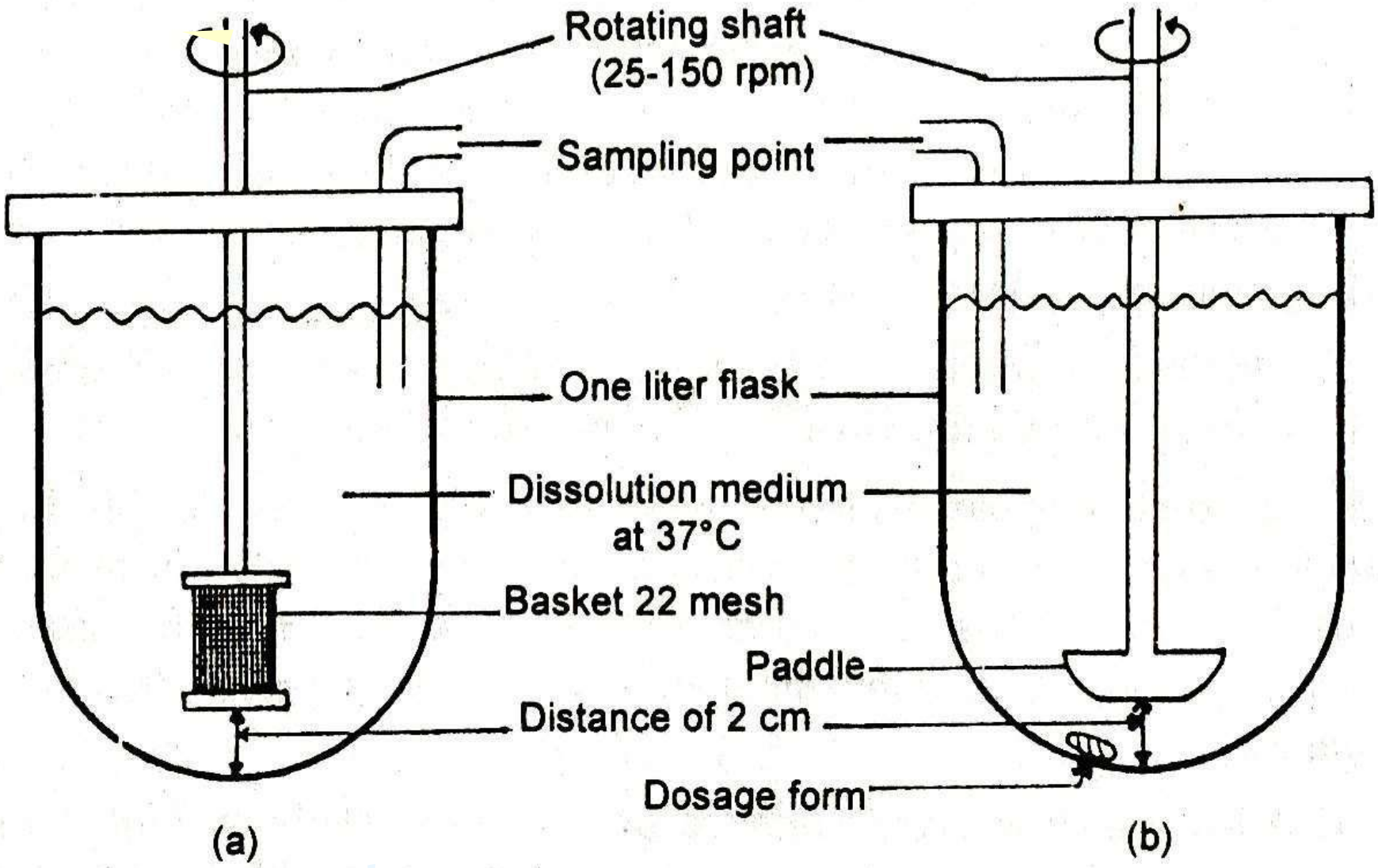


Fig. 12.3 Schematic representation of official dissolution apparatus —forced convection nonsink type (*a*) rotating basket apparatus, and (*b*) rotating paddle apparatus.

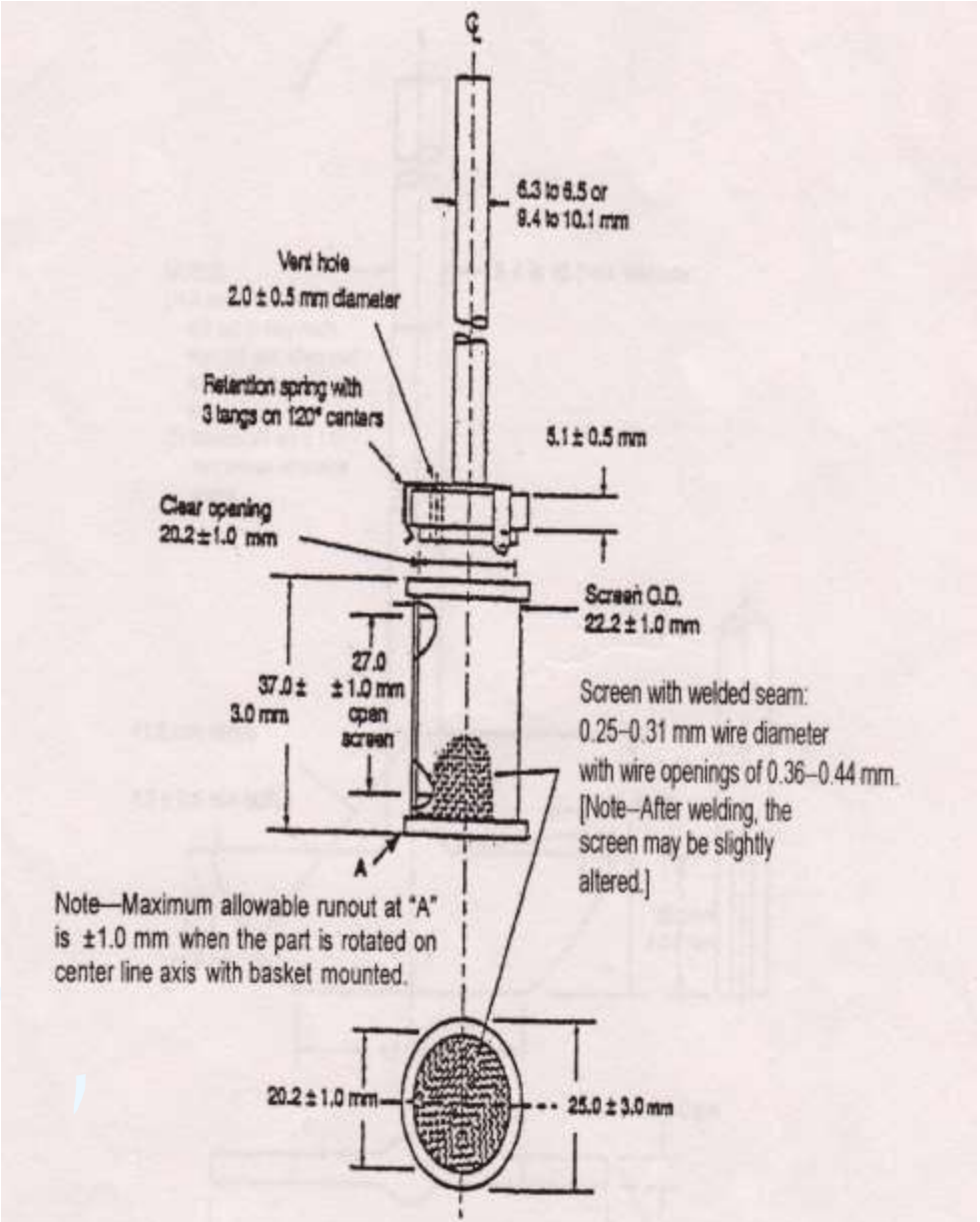


Fig. 1. Basket Stirring Element

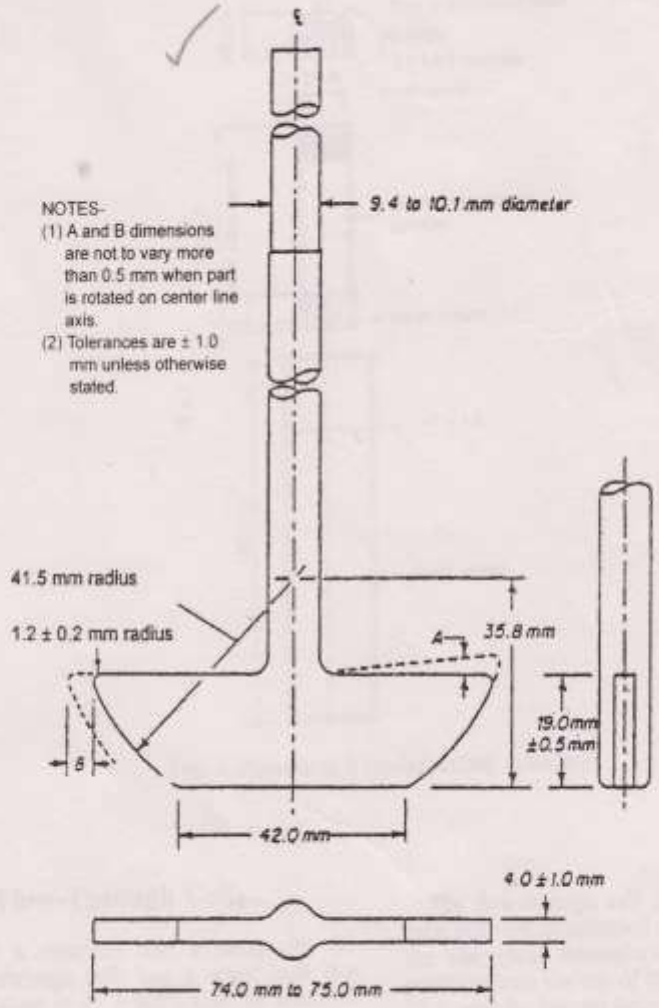


Fig. 2. Paddle Stirring Element

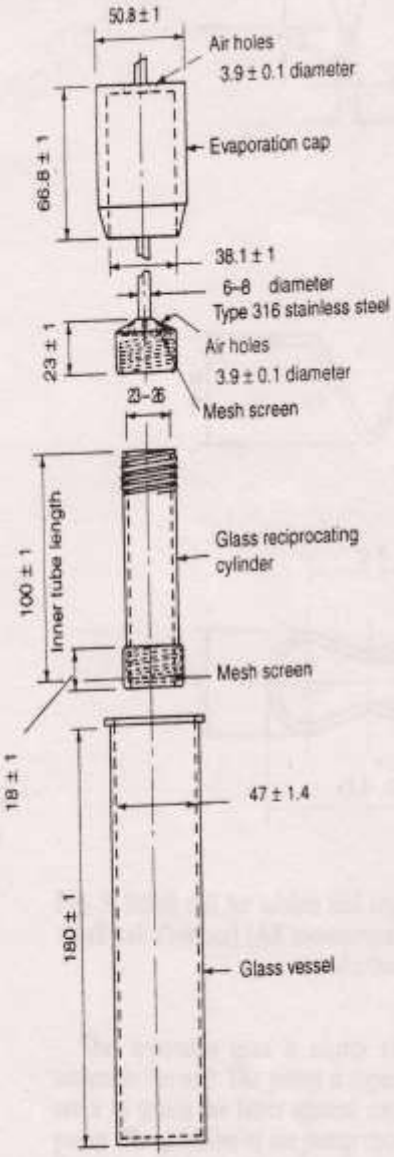
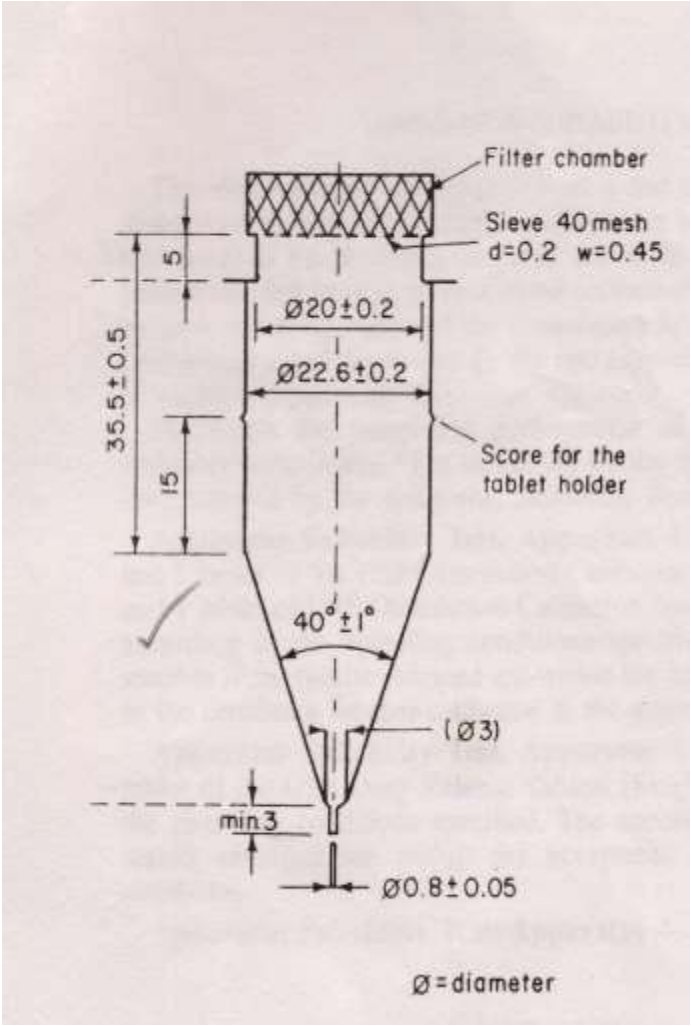
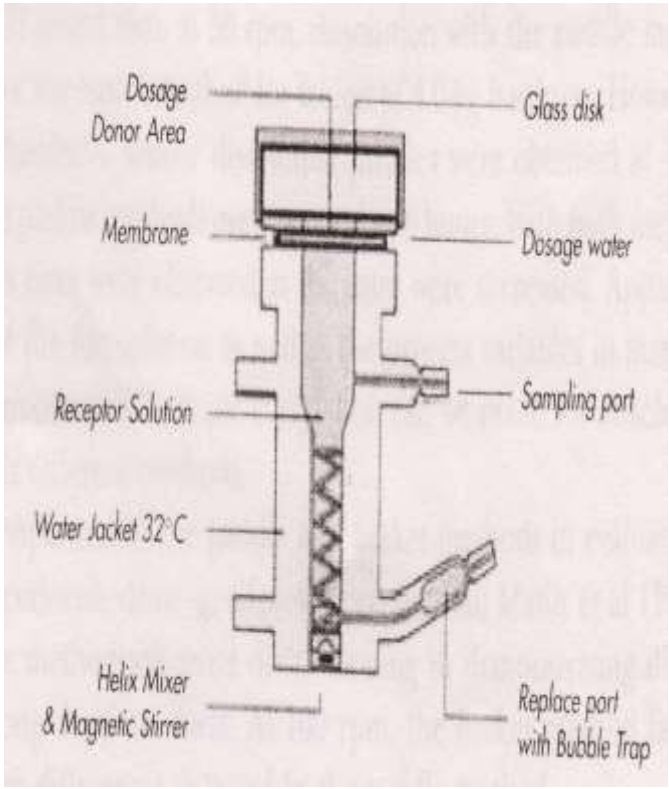


Fig. 3. Apparatus 3 (reciprocating cylinder)


Filter chamber
Sieve 40 mesh
d=0.2 w=0.45
5
Ø20±0.2
Ø22.6±0.2
35.5±0.5
15
Score for the
tablet holder
40°±1°
(Ø3)
min.3
Ø0.8±0.05
Ø=diameter


Dosage
Donor Area
Glass disk
Membrane
Dosage water
Receptor Solution
Sampling port
Water Jacket 32°C
Helix Mixer
& Magnetic Stirrer
Replace port
with Bubble Trap

Acceptance Table 1

Stage	Number Tested	Acceptance Criteria
S_1	6	Each unit is not less than $Q + 5\%$.
S_2	6	Average of 12 units ($S_1 + S_2$) is equal to or greater than Q, and no unit is less than $Q - 15\%$.
S_3	12	Average of 24 units ($S_1 + S_2 + S_3$) is equal to or greater than Q, not more than 2 units are less than $Q - 15\%$, and no unit is less than $Q - 25\%$.

- **Particle size and Effective surface area:**

- Particle size and surface area are inversely related to each other. Two types of surface area can be defined.

- *Absolute surface area*
- *Effective surface area*

- Micronization of poorly aqueous soluble drugs like Griseofulvin, Chloramphenicol and several salts of Tetracycline results in superior dissolution rates in comparision to simple milled form of these drugs

- Micronization also lead to decrease in dose of drugs because of increased absorption efficiency.

- Ex; *Griseofulvin* dose was reduced to half and that of *Spironolactone* was decreased 20 times

→In case of hydrophobic drugs like Aspirin, Phenacetin, Phenobarbital micronization results in decrease in effective surface area of such powders and fall in dissolution rate

→Absolute surface area of hydrophobic drugs can be converted to their effective surface area by

→ Use of surfactant as a wetting agent that decrease interfacial tension and displaces the adsorbed air with the solvent

→Ex; Tween-80 increases the bioavailability of Phenacetin by promoting its wettability

→ Adding Hydrophobic diluents such as *PEG, PVP, Dextrose* etc; which coat the surface of hydrophobic drug particles and renders them hydrophilic

- But the particle size reduction and increase in surface area and dissolution rate is not always advisable especially when the drugs are unstable and degrade in solution form

- *Ex; PenicillinG and Erythromycin* produce undesirable effects (Gastric irritation caused by Nitrofurantoin) or when a sustained effect is desired

- The second mechanism by which a reduction in particle size improves drug dissolution is through an increase in its solubility.

- Molecular dispersion or solid solution where the sparingly soluble drug is molecularly trapped in lattice of hydrophilic agent

Ex; Cyclodextrins

- Solid dispersion where drug dispersed in carrier such as PVP,PEG, Urea

- **Polymorphism and Amorphism**

Enantiotropic polymorph**:** Ex; sulfur

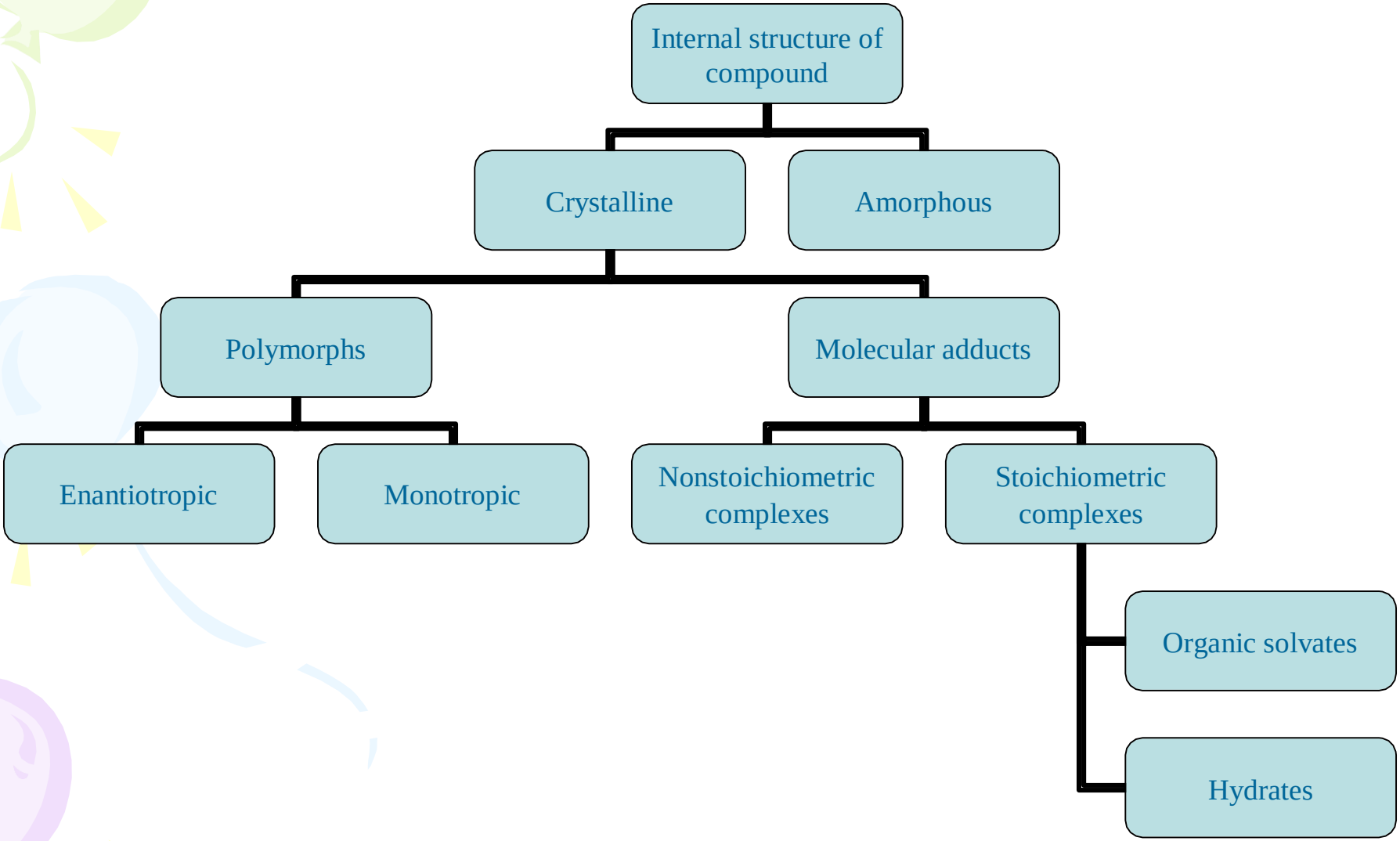
Monotropic polymorph; Ex Glyceryl monostearates

Some drugs can exist in Amorphous forms (having no internal crystal structure)**.**

- Ex Amorphous form of Novobiocin is 10 times more soluble than crystalline form.
- Chloramphenicol Palmitate , Cortisone acetate , Phenobarbital
- Order for dissolution of different solid forms of drugs is

Amorphous> Metastable> Stable

Classification of Internal Structure of a Compound



- **Hydrates / Solvates (Pseudopolymorphism):**

- **Ex;** Anhydrous form of Theophylline and Ampicillin have higher aqueous solubilities, dissolve at faster rate and show better bioavailability in comparision to their monohydrate and trihydrate forms
- Organic solvates have greater aqueous solubility than the nonsolvates
- **Ex;** n-pentanol solvate of fludrocortisone and succinylthiazole and the chloroform solvate of griseofulvin are more water soluble than their nonsolvated forms

Salt Form of Drug:

- Weakly acidic drugs, a strong base salt is prepared
- Ex; Sodium and Potassium salts of barbiturates and sulfonamides
- Weakly basic drugs, a strong acid salt is prepared
- Ex; Hydrochloride salts or sulfate salts of several alkaloidal drugs

Formation of fine precipitate

Insitu salt formation

Aspirin and Penicillin from buffered alkaline tablets.

Selection of suitable salt form

Ex: Choline and Isopropanolamine salts of Theophylline dissolve 3-4 times more rapidly than the Ethylene diamine salt and show better bioavailability

Size of counter ions :

Smaller the size of counter ion , greater the solubility of salt

Ex: Bioavailability of Novobiocin from its Sodium salt , Calcium salt and free acid form was found to be in ratio of 50:25:1

Ex; Pamoates,stearates and Palmitates of weak bases have poor aqueous solubility. These forms are used to prolong the duration of action (Steroidal salts) to overcome bad taste (Chloramphenicol palmitate), to enhance GI stability (Erythromycin estolate), or to decrease the side effects, local/ systemic

- **Drug pKa and Lipophilicity and GI pH-pH Partition Hypothesis:**

- The theory states that for drug compounds of molecular weight greater than 100, which are primarily transported across the biomembrane by passive diffusion, the process of absorption is governed by:

- *The dissociation constant (pKa) of the drug
- *The lipid solubility of unionized drug
- *The pH at the absorption site

Drug pKa and Gastrointestinal pH:

Hendersen-Hasselbalch equations

For weak acids

$$pH = pKa + \log \frac{\text{Ionized drug concentration}}{\text{Unionized drug concentration}}$$

$$\% \text{ Drug ionized} = \frac{10^{pH - pKa}}{1 + 10^{pH - pKa}} \times 100$$

For weak bases

$$pH = pKa + \log \frac{\text{Unionized drug concentration}}{\text{Ionized drug concentration}}$$

$$\% \text{ Drug ionized} = \frac{10^{pKa - pH}}{1 + 10^{pKa - pH}} \times 100$$

For weak acids;

$$Ra = \frac{C_{GIT}}{C_{Plasma}} = \frac{1+10^{\,pH\ GIT\ -\ pKa}}{1+10^{\,pH\ Plasma\ -\ pKa}}$$

For weak bases:

$$Ra = \frac{C_{GIT}}{C_{Plasma}} = \frac{1+10^{\,pKa\ -\ pH\ GIT}}{1+10^{\,pKa\ -\ pH\ Plasma}}$$

Influence of drug pKa and GI pH on drug absorption

Very weak acids *(pKa> 8.0)*

Drugs	pKa	pH/ site of absorption
Pentobarbital	8.1	Unionized at all p H values;
Phenytoin	8.3	absorbed along the entire length of GIT

Moderately weak acids *(pKa 2.5-7.5)*

Cloxacillin	2.7	Unionized in gastric pH, Ionizein
Aspirin	3.5	intestinal pH ;better absorbed from Stomach

Stronger acids *(pKa < 2.5)*

Disodium cromoglycate	2.0	Ionized at all pH values; poorly absorbed from GIT

Very weak bases (*pKa < 5.0)*

Theophylline	0.7	Unionized at all pH values

Moderately weak bases *(pKa 5 to 11.0)*

Codeine	8.2	Ionized at gastric pH ,Unionized
Heroin	7.2	at intestinal pH;better absorbed from Intestine

Stronger bases *(pKa > 11.0)*

Mecamylamine	11.2	Ionized at all pH values;
Guanethidine	11.7	poorly absorbed from GIT

- **Lipophilicity and Drug Absorption:**

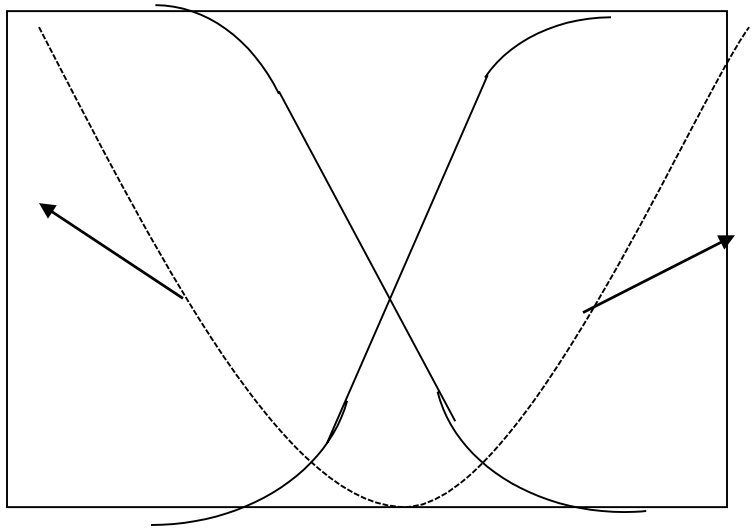
- A perfect hydrophilic-lipophilic balance (HLB) should be there in the structure of drug for optimum bioavailability

- To enhance the bioavailability of a drug, not only its dissolution rate but also its rate of permeability should be considered.

- Rate of dissolution can be increased by altering the physical properties such as particle size or crystalline structure but its permeability can only be promoted by modification of chemical structure

- **Limitations of pH –Partition Hypothesis**
- Presence of virtual membrane pH
- Absorption of ionized drugs
- Influence of GI surface area and residence time of drug
- Presence of aqueous unstirred diffusion layer

- **Presence of Virtual Membrane pH:**



pH absorption curve for acidic and basic drugs . Dotted lines indicate curves predicted by pH partition hypothesis and bold lines indicate the practical curves

- **Absorption of Ionized drugs:**

- Ex: Morphinan derivatives

- **Influence of GI surface area and Residence time of drug**

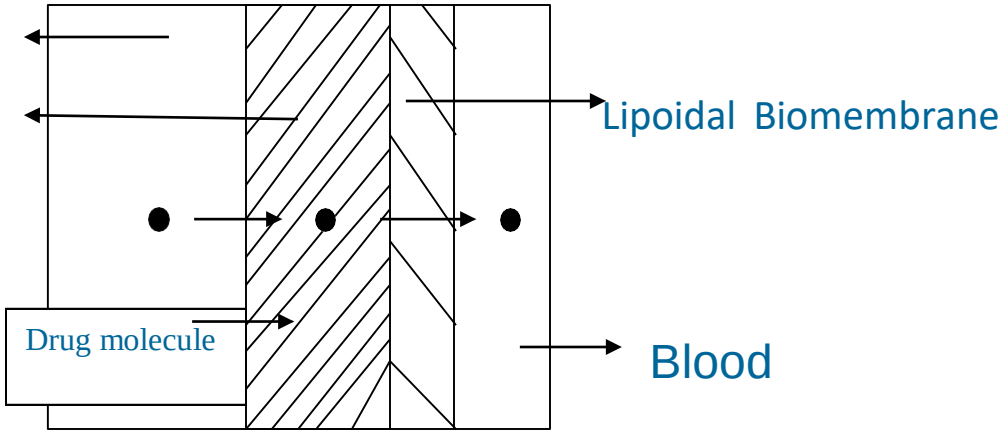
- Both acidic drugs and basic drugs are more rapidly absorbed from the intestine, because of its large surface area and secondly ,because of long residence time of drug in intestine.

Presence of Aqueous unstirred diffusion layer on the membrane surface

Ex*; High molecular weight fatty acids and bile salts*

Aqueous bulk fluid of GIT

Aqueous unstirred diffusion layer



Biopharmaceutical factors influencing GI absorption of a drug from the dosage form

B. PATIENT RELATED FACTORS

1. Age
2. Gastric emptying time
3. Intestinal transit time
4. Gastrointestinal Ph
5. Disease state

- Blood flow through the GIT
- Gastrointestinal contents: Other drugs, food, fluids, other normal GI contents
- Presystemic metabolism by:
  Lumenal enzymes, gutwall enzymes, bacterial enzymes, hepatic enzymes

Physiological factors:

- Age:

Infants: gastric pH – high

intestinal surface, blood flow to GIT – low

Therefore, altered absorption pattern compared to adults.

Elderly persons: impaired drug absorption include

altered gastric emptying, decreased intestinal surface area and GI blood flow, higher incidents of achlorhydria and bacterial over growth in small intestine .

Gastric emptying time:

- Rate limiting step in drug absorption

- Gastric emptying of a drug is delayed by co-administering food because unless the gastric contents are fluid enough or the size of the solid particles is reduced below 2mm, its passage through the pylorus into the intestine is not possible.

- Delay in gastric emptying is recommended where:

- food promotes drug dissolution and absorption(Griseofulvin)
- drugs irritate the gastric mucosa(aspirin, nitrofurantoin)
- Disintegration and dissolution is promoted by gastric fluids

- Gastric emptying is a First order process. Parameters used to quantify gastric emptying:
  - Gastric emptying rate
  - Gastric emptying time
  - Gastric emptying t1/2

- Factors influencing gastric emptying:
  - Volume of meal
  - Composition of meal
  - Physical state and viscosity of meal
  - Temperature of meal
  - Gastrointestinal Ph
  - Electrolytes and osmotic pressure
  - Body posture
  - Emotional state
  - Exercise
  - Disease states
  - drugs

- Intestinal transit:

- Long intestinal transit time is desirable for complete drug absorption
- Delayed intestinal transit is desirable for:
  - Drugs that dissolve or release slowly from their dosage form (sustained release products)
  - Drugs that dissolve only in intestine (enteric coated formulations)
  - Drugs absorbed from specific sites of intestine (several B vitamins)
- Metochlopramide promote gastric emptying and intestinal transit enhance absorption of rapidly soluble drugs.
- Laxatives also promote the rate of intestinal transit

Gastrointestinal pH:

- 10^7 fold difference in the hydrogen ion concentration is observed between the gastric and colon fluids
- GI fluid pH influence drug absorption in several ways:
    - Disintegration
    - Dissolution
    - Absorption
    - Stability

Disease states:

Gastrointestinal diseases:

- Altered GI motility
- Gastrointestinal diseases and infections
- Gastrointestinal surgery
- Cardiovascular diseases
- Hepatic diseases

Blood flow to the GIT:

The GIT is extensively supplied by blood capillary network and the lymphatic system.

since the blood flow rate to the GIT +6is 500-1000times more than the lymph flow, much drugs reach the systemic circulation verses blood.

Food influences blood flow to the git. the perfusion rate increases after and persists per hours but drug absorption is not influenced significantly.

Gastrointestinal contents:

1. Food - drug interactions
2. Fluid volume
3. Interactions of drug with normal GI constituents
4. Drug – Drug interactions in the GIT

Influence of food on drug absorption

Delayed	Decreased	Increased	Unaffected
Aspirin Paracetamol Diclofenac Nitrofurantoin Digoxin	Penicillins Erythromycin Ethanol Tetracyclines Levodopa Iron	Griseofulvin Nitrofurantoin Diazepam Actively absorbed Water soluble vitamins	Methyldopa Propylthiouracil Sulfasomide

Drug-drug interaction in the GIT:

It is two types

a) Physicochemical Drug-drug interaction

b) Physiologic Drug-drug interaction

a) Physicochemical Drug-drug interaction:

- Adsorption
- Complexation
- pH range

b) Physiologic Drug-drug interaction

» Decreased GI transit

» Increased gastric emptying

» Altered GI metabolism

Presystemic metabolism:

- For a drug administered orally 2 main reasons for its decreased bioavailability are:
  - Decreased absorption
  - First pass/presystemic metabolism
- The 4 primary systems which effect presystemic metabolism of a drug are
  - Lumenal enzymes
  - Gutwall enzymes
  - Bacterial enzymes
  - Hepatic enzymes

Dosage related factors

- Disintegration time
- Dissolution time
- Manufacturing variables
- Pharmaceutic ingredients
- Nature and type of dosage form
- Product age and storage condition

❖ Disintegration time

- Disintegration time is of particular importance in case of solid dosage forms like tablets and capsules.

- However if a solid forms does forms conform to the disintegration time, it portends problems, because the subsequent process of dissolution will be much slower and absorption much be insufficient.

❖ Manufacturing variables

- Drug dissolution is the single most important factor in the absorption of drugs, especially for conventional solid dosage forms like tablets and capsules.
- Several manufacturing processes influence drug dissolution from solid dosage forms are
- (a) Method of granulation.
- (b) Compression force.

Method of Granulation:

- The wet granulations process is the conventional technique in the manufacture of tablets.

The limitations of this methods includes:

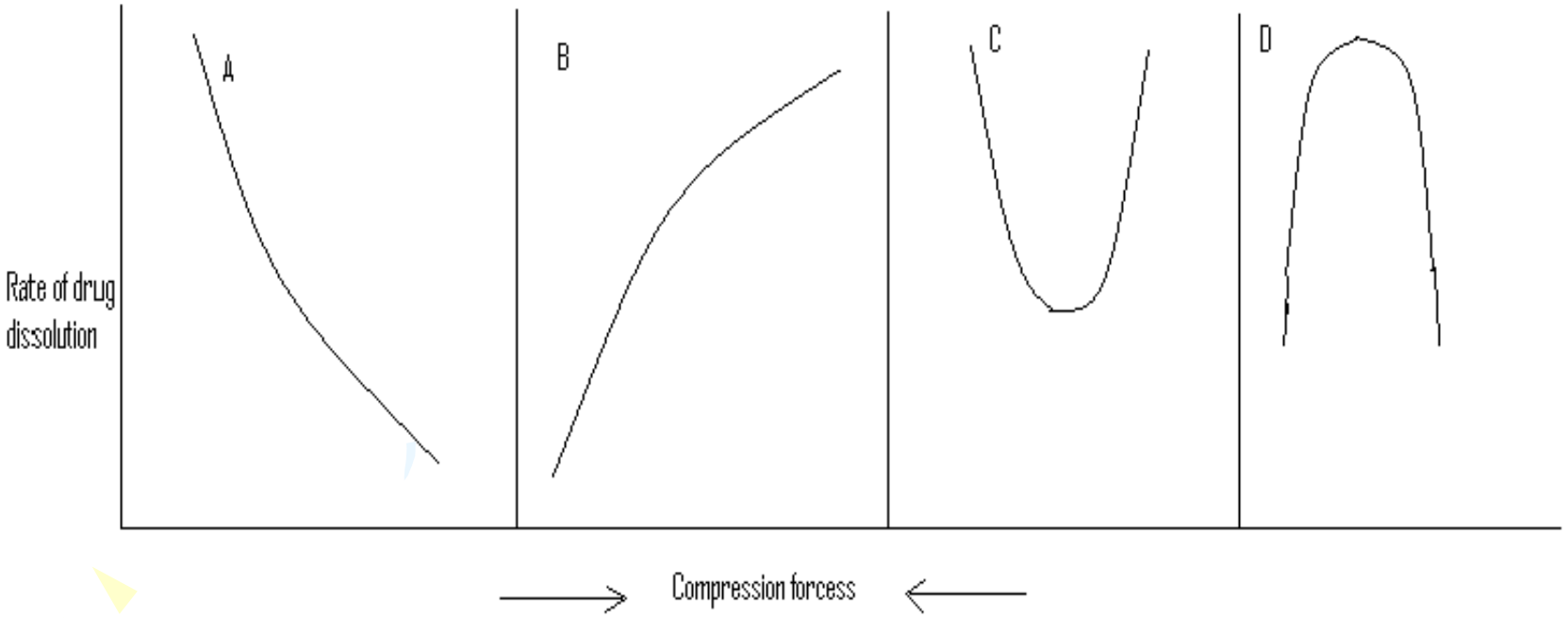
(i) Formation of crystals bridge by the presence of liquid.

(ii) The liquid may acts as a medium for effecting chemical reactions such as hydrolysis.

(iii) The drying step may harm the thermo labile drugs.

Compression Force:

- The compression force employed in tabulating process influence density, porosity, hardness, disintegration, and dissolution of tablets.
- The curve obtained by plotting compression force verses rate of dissolution can take one of the 4 possible shapes.



- *On the one hand, higher compression force increases the density and hardness*

- *of tablet, decreases porosity and hence penetrability of the solvent in to the tablet, retards wet ability by forming a firmer and more effective sealing layer by the lubricant.*

- *On other hand, higher compression forces causes deformation, crushing (or) fracture of drug particle in to smaller ones (or) convert a spherical granules in to a disc shaped particle with a large increases in the effective surface area. This increases in dissolution rate of tablets.*

Intensity of packing of capsule content:

- *Compression force for tablets, packing density in case of capsule dosage form either inhibit (or) promote dissolution.*

- *Diffusion of GI fluids in to the tightly filled capsules resulting in rapid bursting and dissolution of contents.*

❖ *Pharmaceutical Ingredients (or) Excipients:*

- *A convenient dosage form to be administered by suitable route is prepared such a formulation contains no: of excipients.*

- *Excipients are added to ensure acceptability, physico-chemical stability during the shelf life, uniformity of composition and dosage, optimum bioavailability and functionality of the drug products.*

Vehicle:

- *Vehicles is the major component of liquid orals (or) parental.*

- *They are three categories of vehicles in use are aqueous vehicles (water, syrup) non -aqueous water miscible vehicles (propylene glycol, glycerol sorbitol) and non -aqueous water immiscible vehicles (vegetable oils).*

- *Bioavailability of a drug from vehicles depend to a large extent on its miscibility with biological fluid.*

- *Aqueous and water miscible vehicles are miscible with the body fluids and drugs from them are rapidly absorbed.*

Diluents:

- *Diluents are commonly added to tablet formulations if the required dose is inadequate to produce the necessary bulk.*
- *One of the classical example of drug-diluents interaction results in poor bioavailability is that of tetracycline complex and dicalcium phosphate.*

Binders and granulating agents:

- *The materials are used to hold powders together to form granules (or) promote cohesive compacts for directly compressible materials, and to ensure that the tablet remains intact after compression.*

- *Popular binders include polymeric materials like starch, cellulose derivatives, acacia, pvp etc.*

Disintegrants:

- *These agents over come the cohesive strength of tablet and break them up on contact with water which is an important prerequisite to tablet dissolution.*
- *All the disintegrants are hydrophilic in nature. A decrease in the amount of disintegrants can significantly lower bioavailability.*

Lubricants (or) ant frictional agents:

- *These agents are added to tablet formulations to aid the flow of granules, to reduce interparticle friction and sticking (or) adhesion of particles to dies and punches.*

- *The commonly used lubricants are hydrophobic in nature, and to inhibit wet ability, penetration of water in to tablet and their disintegration and dissolution.*

Suspending agents:

- *Suspending agents are hydrophilic polymers like vegetable gums, (acacia, tragcanth) semi synthetic gums (cmc, mc) and synthetic gums which stabilize the solid drug particles by reducing their rate of settling through an increase in the viscosity of the medium.*

Surfactants:

- *Surfactants are widely used in formulations as wetting agents, solubilizers, emulsifiers etc.*

mechanism involved in the increased absorption of drug by use of surfactants includes:

- ✓ *Promotion of wetting and dissolution of drugs':. Tween 80 with phenacetin.*
- ✓ *Better membrane contact of the drug for absorption.*
- ✓ *Enhanced membrane permeability of the drug.*

Buffers:

- *Buffers are useful in creating the right atmosphere for drug dissolution was observed for buffered aspirin tablets.*
- *Buffer systems containing potassium cat ions inhibit the drug absorption as seen in vitamin B2 and sulfonamide.*

Complexing agents:

- *Complex formation has been used to alter the physico chemical and biopharmaceutical properties of a drug.*

- *several examples where complexation has been used to enhance drug bioavailability are :*

- *1.enhanced dissolution through formation of a solute complex.*

  eg: ergotamine tartarate-caffeine complex.

Colorants:

- *Low concentration of water-soluble dye can have an inhibitory effect on dissolution rate of several crystalline drugs.*

- *The dye molecules get absorbed on to the crystal faces and inhibit drug dissolution.*

  e.g.: brilliant blue retards dissolution of sulfathiazole.

Crystal growth inhibitors:

- *Crystal growth inhibitors like PVP and PEG inhibit conversion of a high energy meta stable polymorph in to stable, less soluble polymorph.*

Nature and type of dosage:

- *For a proper selection of a drug ,clinical success depends to a great extent on the proper selection of dosage form of that drug.*

- *For a given drug, 2 to 5 folds (or) perhaps more difference could be observed in the oral bioavailability of a drug depending up on the nature and type of dosage form.*

- *Such a difference is due to the relative rate at which a particular dosage form releases the drug to the biological fluids and the membrane.*

- *The relative rate at which a drug from a dosage form is presented to the body depends up on the complexity of dosage form.*

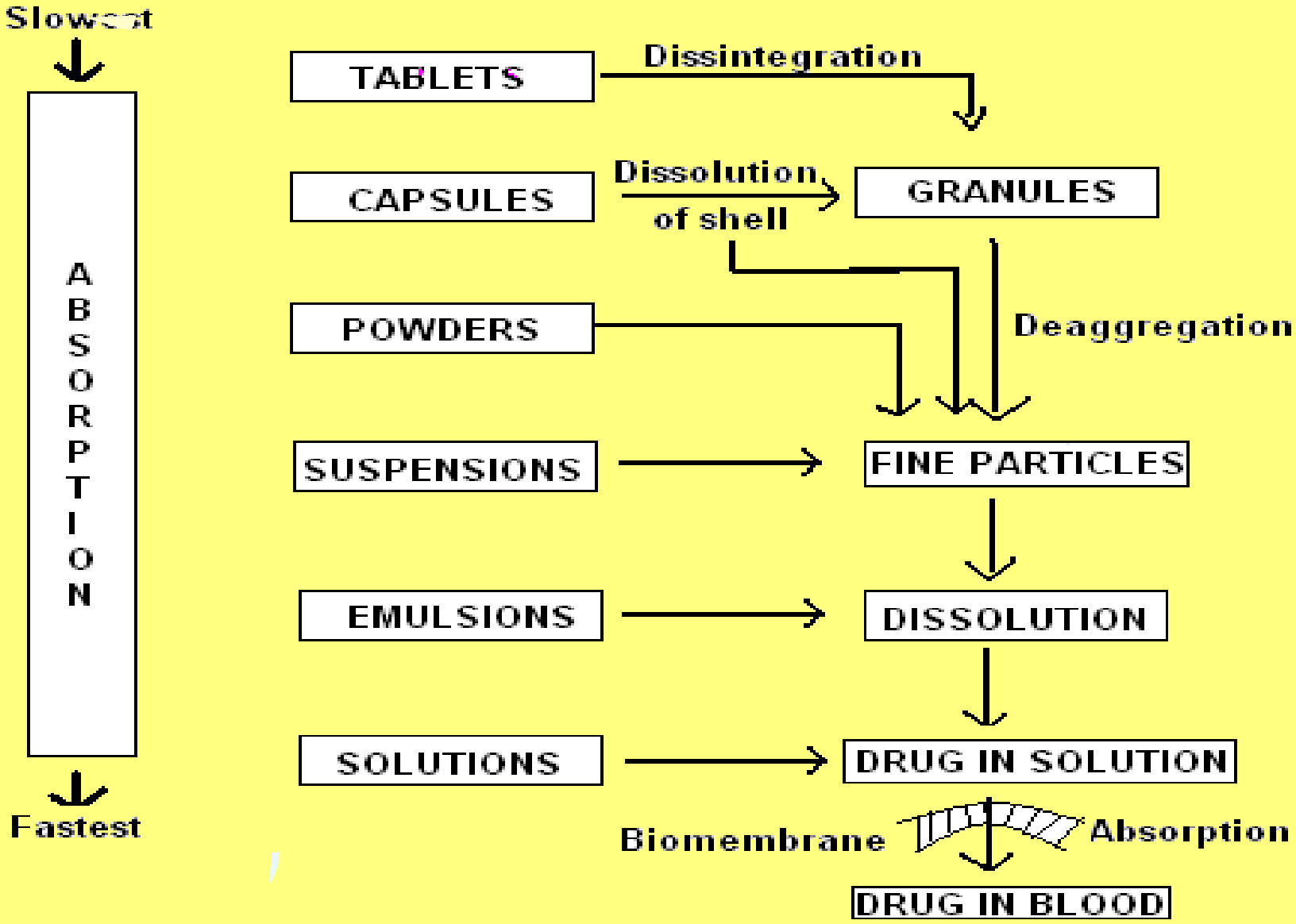
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Course of events that occur following oral administration of various dosage forms

Solutions:

A Drug in solutions (syrups, elixirs) is most rapidly absorbed since the major rate- limiting step, drug dissolution is absent.

- *Factors that influence bioavailability of a drug from solution dosages forms includes the nature of solvent, viscosity, surfactants, solubilizers, stabilizers etc.*

Emulsions:

- *Emulsion dosage forms have been found to be superior to suspensions in administering poorly aqueous soluble lipophilic drugs.*
- *It was observed with indoxole that when it is dissolved in a vegetable oil and emulsified in water, absorption increases 3 fold over its aqueous suspension.*

Suspensions:

- *The major rate- liming step in the absorption of a drug from suspension dosage form is drug dissolution which is generally rapid due to the large surface area of particles.*
- *Important factors in the bioavailability of a drug from suspensions include particle size, polymorphism, wetting agents, viscosity of the medium, suspending agents etc.*

Powders:

- *powders are superior to tablets and capsules, they are not in use now a days due to handling and palatability problems.*
- *Major factor to be considered in the absorption of a drug from powders are particle size, polymorphism, wettability etc.*

Capsules:

- *powders and granules are popularly administered in hard gelatin capsules where as viscous fluids and oils in soft elastic shells.*
- *In case of hard gelatin capsules include drug particle size, density, polymorphism, intensity of packing and influence of diluents and excipients.*

Tablets:

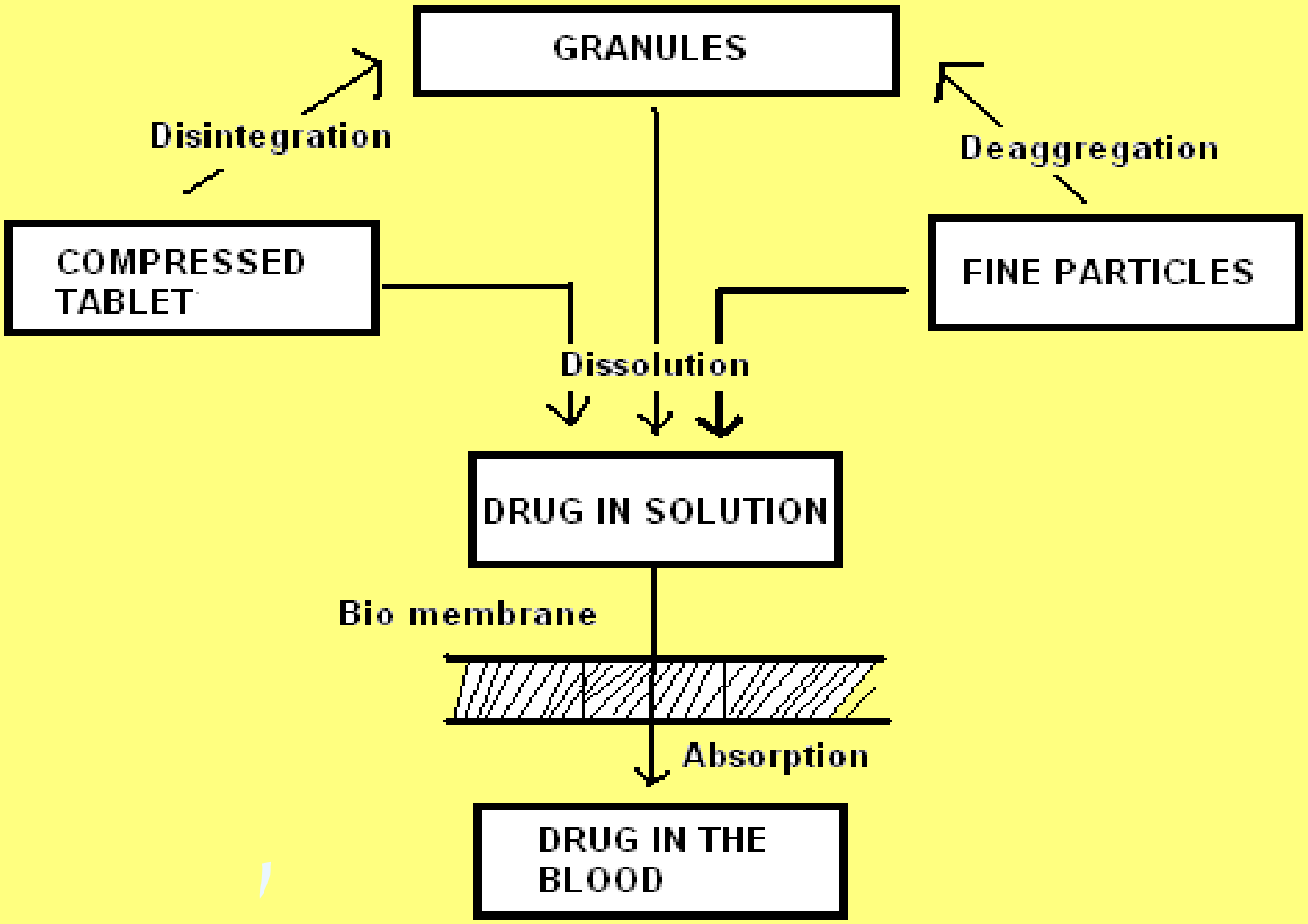
- *Compressed Tablets are the most rapid widely used convenience and effective dosage forms.*
- *The bioavailability problems with tablets arise from the reduction in the effective surface area due to granulation and compression in to a dosage form.*

Coated tablets:

- *The factors that influence drug release from compressed tablets , the coating acts as another barrier which must first dissolve to give way to disintegration and dissolution of tablet.*

two types of coatings are :

- ✓ *Film coating.*
- ✓ *Sugar coating.*



sequence of events in the absorption of a drug from tablet dosage form

Enteric coated tablets:

- *Enteric coatings tablets have great potentials in creating bioavailability problems , because the coat dissolves only in the alkaline Ph of the intestine and it may take as long as 2 to 4 hours for such a tablet to empty from the stomach in to the intestine depending up on the meals and GI motility.*

Product age and storage conditions:

- *A number of changes, especially in the physic-chemical properties of a drug in dosage form, can result due to ageing and alterations n storage conditions which can adversely affect bioavailability.*

- *With solution dosage form, precipitation of drug due to altered solubility, especially due to conversion of metastable in to poorly soluble, stable polymorph can occur during shelf-life of the product.*

- *In case of solid dosage forms, especially tablets, disintegration and dissolution rates are greatly affected due to aging and storage conditions.*

Parenterals:

- *Oral route is the most preferred route for drug administration, however certain situations demand delivery of drugs through parental routes.*

- *For quick restoration of fluid and electrolyte imbalances in patients suffering from severe dehydration (or) electrolyte depletion for a variety of reasons such as severe diarrhea and vomiting electrolyte solutions are administered.*

- *To achieve adequate concentrations of the drug in to diseased antibiotics tissues.*

- *e.g.: Intraventricular injection of amino glycoside antibiotics.*

ABSORPTION OF DRUGS FROM NON PER OS EXTRAVASCULAR ROUTES

ROUTE	ABSORPTION MECHANISMS	DRUGS DELIVERED
Buccal /Sublingual	Passive diffusion, Carrier mediated transport	Nitrites and Nitrates, Antianginals, Nifedipine, Morphine, Fenoterol
Rectal	Passive diffusion	Aspirin, Paracetmol, Theophylline, Few Barbiturates.
Transdermal	Passive diffusion	Nitroglycerine, Lidocaine, Scopolamine, Testosterone, Betamethasone
Intramuscular	Passive diffusion Endocytosis, Pore transport.	Phenytoin, Digoxin, Several Steroids and Antibiotics and many other drugs.

ROUTE	ABSORPTION MECHANISMS	DRUGS DELIVERED
Subcutaneous	Passive diffusion	Insulin, heparin, C.R. Implants.
Inhalation	Passive diffusion, pore transport	Salbuterol, cromolyn, Beclomethasone
Intranasal	Passive diffusion, pore transport.	Phenylpropanolamine, Antihistamines
Intraocular	Passive diffusion	Atropine, pilocarpine, Adrénaline, Antibiotics
Vaginal	Passive diffusion	Steroidal drugs and Contraceptives, Metronidazole

THANK YOU

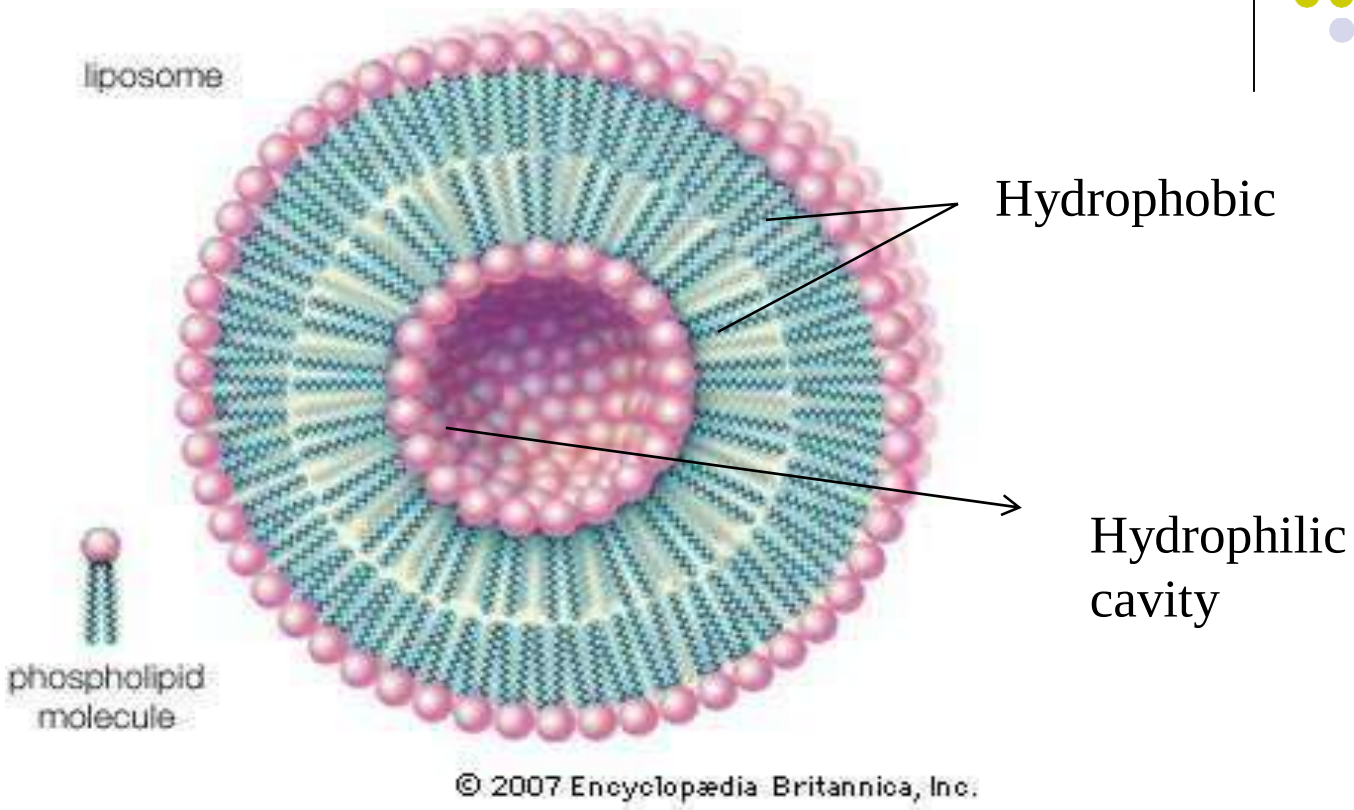
LIPOSOMES

P.Geetha

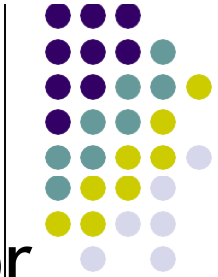
VCPS



- Liposomes are simple microscopic vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid molecule.
- Structurally, liposomes are concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a membraneous lipid bilayer mainly composed of natural or synthetic phospholipids.


liposome
Hydrophobic
Hydrophilic cavity
phospholipid molecule
© 2007 Encyclopædia Britannica, Inc.

ADVANTAGES OF LIPOSOMES



- Provides selective passive targeting to tumor tissues (liposomal doxorubicin)
- Increased efficacy and therapeutic index
- Reduction in toxicity of the encapsulated agent
- Site avoidance effect (avoids non-target tissues)
- Improved pharmacokinetic effects (reduced elimination increased circulation life times)
- Flexibility to couple with site specific ligands to achieve active targeting

DISADVANTAGES

- The development of liposomes at industrial level is difficult due to its physiological and physicochemical instability.
- They aggregate and fuse together upon prolonged storage disturbing the reproducibility.
- They are prone to degradation by oxidation and hydrolysis.

STRUCTURAL COMPONENTS OF LIPOSOMES

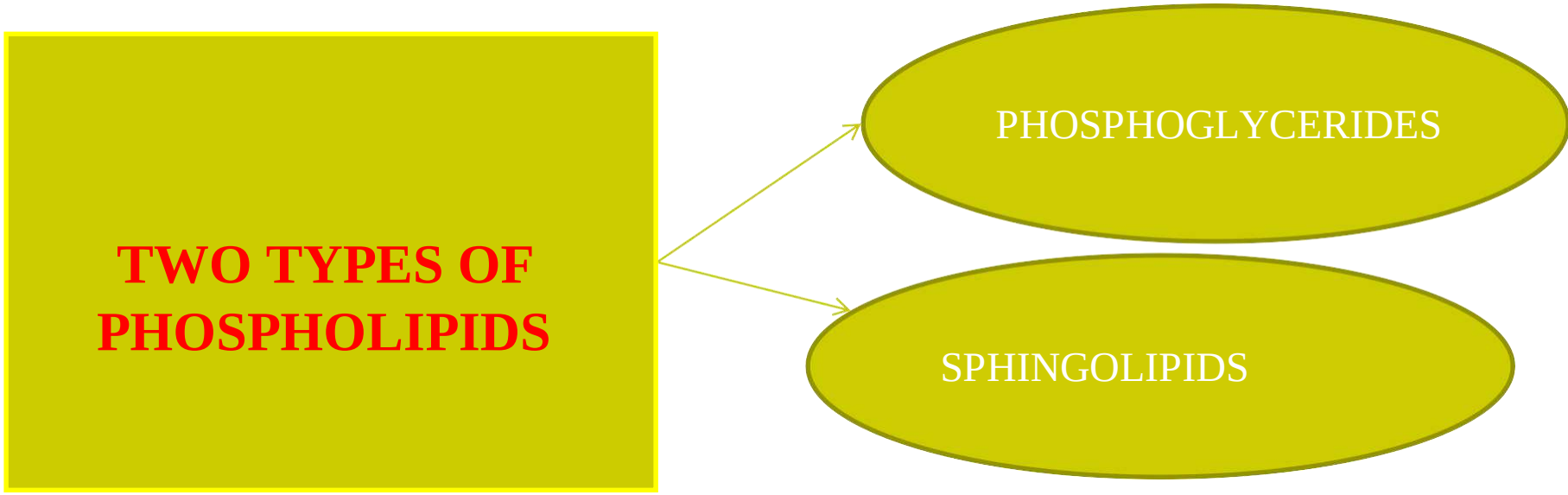


The main components of liposomes are

- PHOSPHOLIPIDS
- CHOLESTEROL

PHOSPHOLIPIDS

- Phospholipids are the major structural component of biological membranes such as the cell membrane.



Some Other Commonly Used Phospholipids



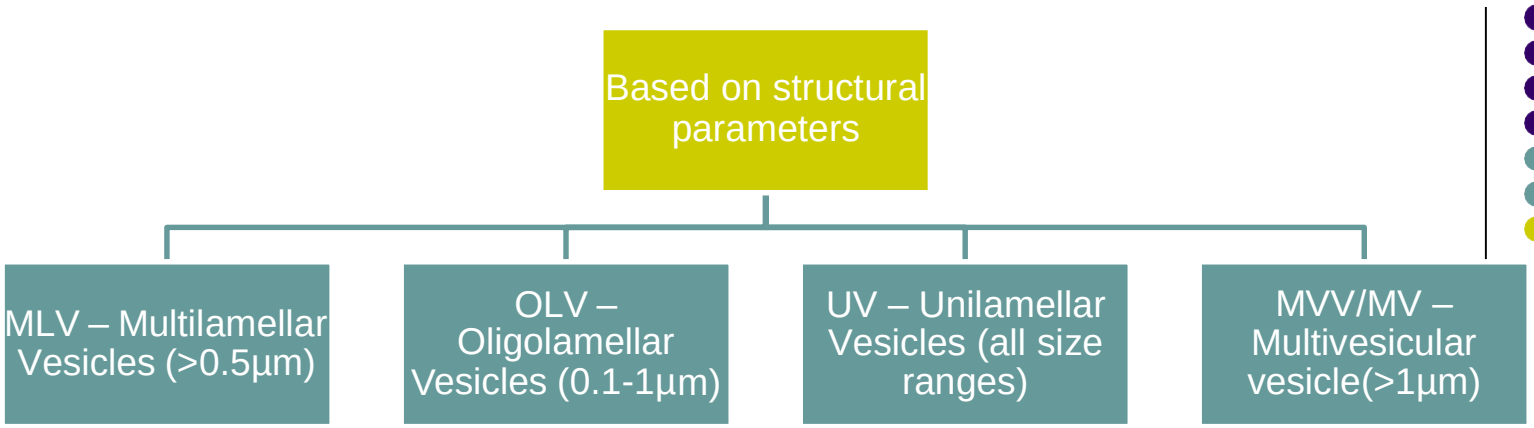
- Naturally occurring phospholipids:
  - -PC : Phosphatidylcholine
  - – PE : Phosphatidylethanolamine
  - – PS : Phosphatidylserine
- Synthetic phospholipids:
  - -DOPC : Dioleoylphosphatidylcholine
- – DSPC : Distearoylphosphatidylcholine

CHOLESTEROL



- Incorporation of sterols in liposome bilayer brings about major changes in the preparation of these membranes.
- Cholesterol by it self does not form a bilayer structure.

However, cholesterol acts as a fluidity buffer, i.e. below the phase transition temperature, it makes the membrane less ordered and slightly more permeable; while above the phase transition temperature it makes the membrane more ordered and stable.


Based on structural parameters
MLV – Multilamellar Vesicles (>0.5µm)
OLV – Oligolamellar Vesicles (0.1-1µm)
UV – Unilamellar Vesicles (all size ranges)
MVV/MV – Multivesicular vesicle(>1µm)

CLASSIFICATION :

- **Based on Structural Parameters:**

  a. **Multi-laminar vesicles (MLV):** made up of series of concentric bi-layer of lipid enclosing a small internal volume with size range > 0.5um.

  b. **Oligolamelar vesicles (OLV):** constitutes 2 to 10 bi layer of lipids surrounding a large internal volume with size range of 0.1 – 1um.

c. Unilamellar vesicle (ULV): single layer of lipids. Based on the size of the single layer they are further divide into the following types with in ULV as

- Small unilaminar vesicle: size of 20 to 40 nm
- Medium unilaminar vesicle: size of 40 to 80 nm
- Large unilaminar vesicle: size of 100 to 1000 nm
- Gaint unilaminar vesicle: size of more than 1000 nm

d. Multivesicular Vesicle (MV): constitutes for multiple vesicles and size range >1um.

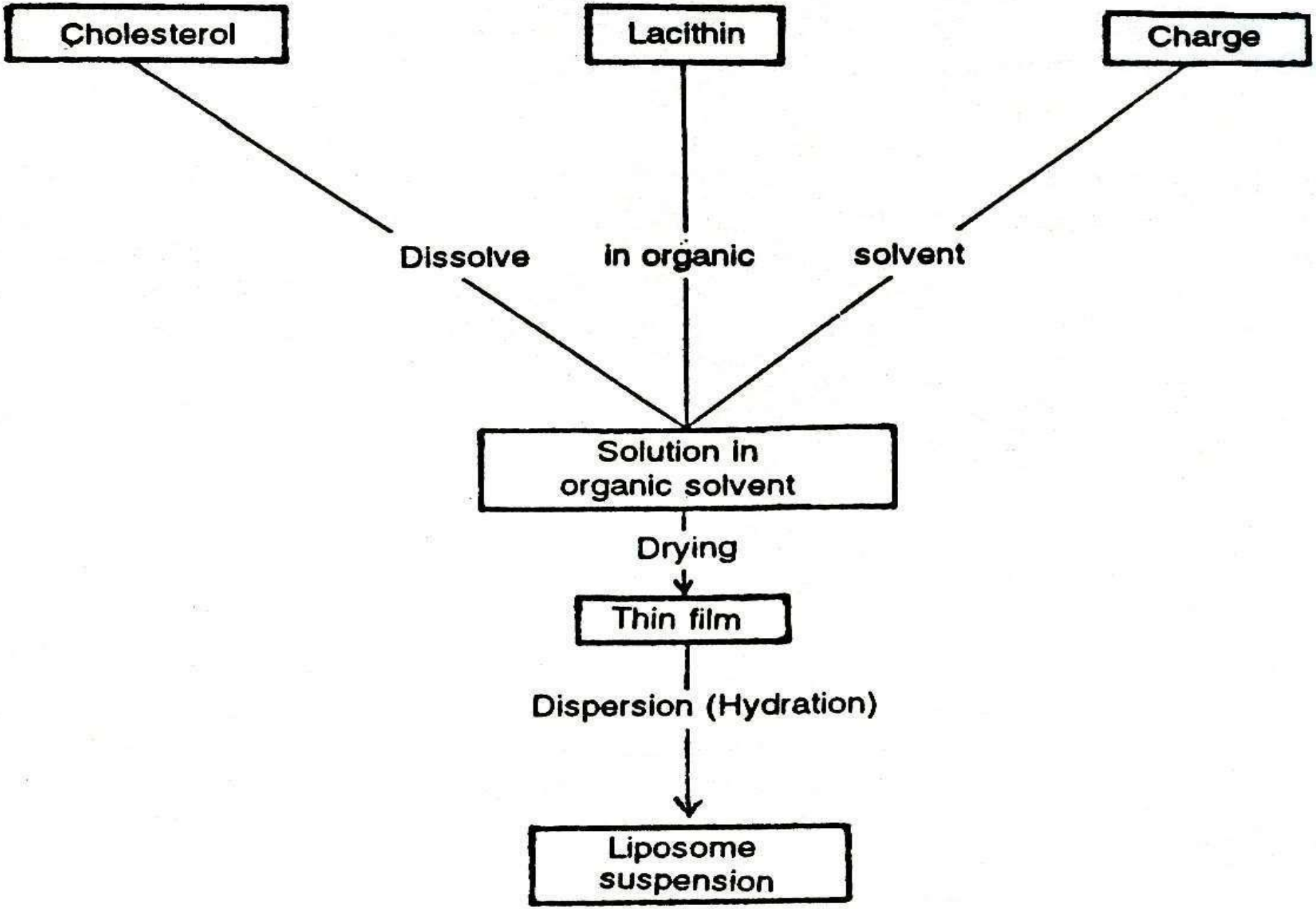


Fig. 15.4. Common stages of all methods of preparation of liposomes.

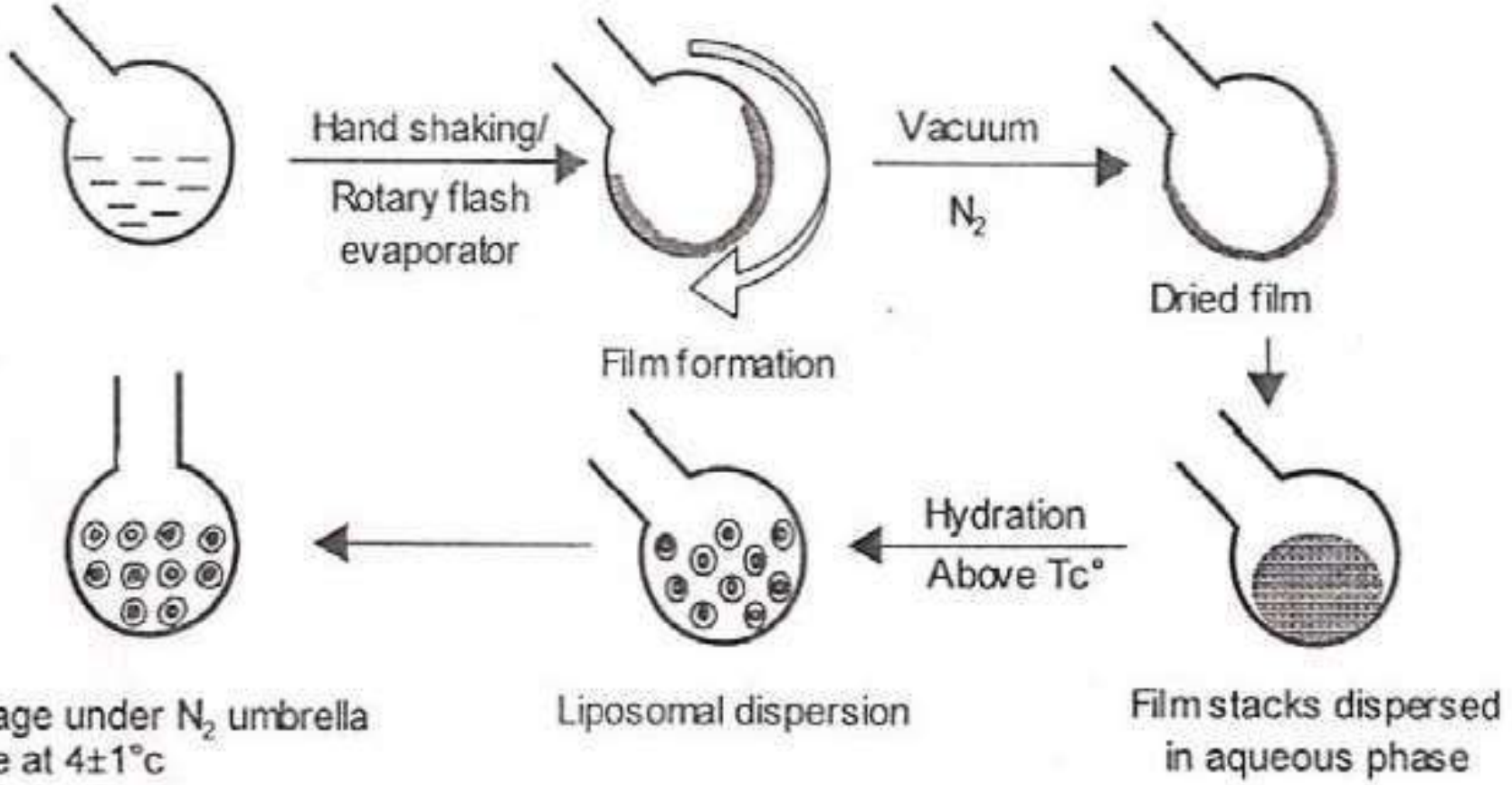
Method of Preparation of Liposomes



Passive Loading Techniques

- **Mechanical dispersion methods**
  - Lipid film hydration by hand shaking,non-hand shaking or freeze drying
  - Microemulsification
  - Sonication
  - French pressure cell
  - Membrane extrusion
  - Dried reconstituted vesicles
  - Freeze-thawed liposomes
- **Solvent dispersion methods**
  - Ethanol injection
  - Ether injection
  - Double emulsion vesicles
  - Reverse phase evaporation vesicles
  - Stable plurilamellar vesicles
- **Detergent removal methods**
  - Dialysis
  - Column chromatography
  - Dilution
  - Reconstituted Sendai Virus enveloped vesicles

HAND SHAKING METHOD:



Multilamellar Vesicles (MLVs) Formed by either Hand Shaking Technique or Using Rotary Flash Evaporator

Micro Emulsification Liposomes (Mel):

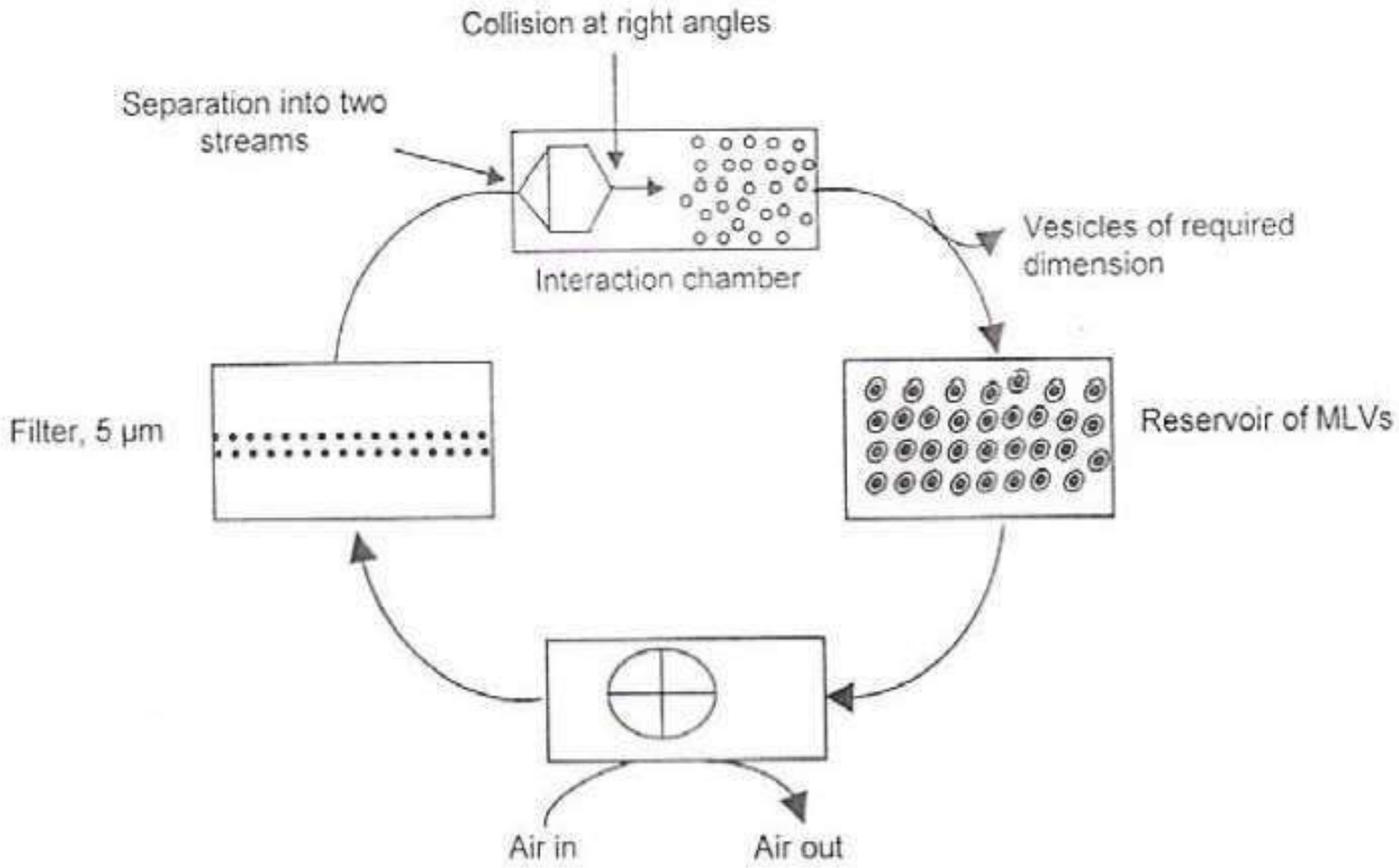


Fig. 5-10. Representation of Use of Micro-Fluidizer to Prepare Small Unilamellar Vesicles (SUVs) From MLVs

Sonicated unilamellar vesicles (SUVs)

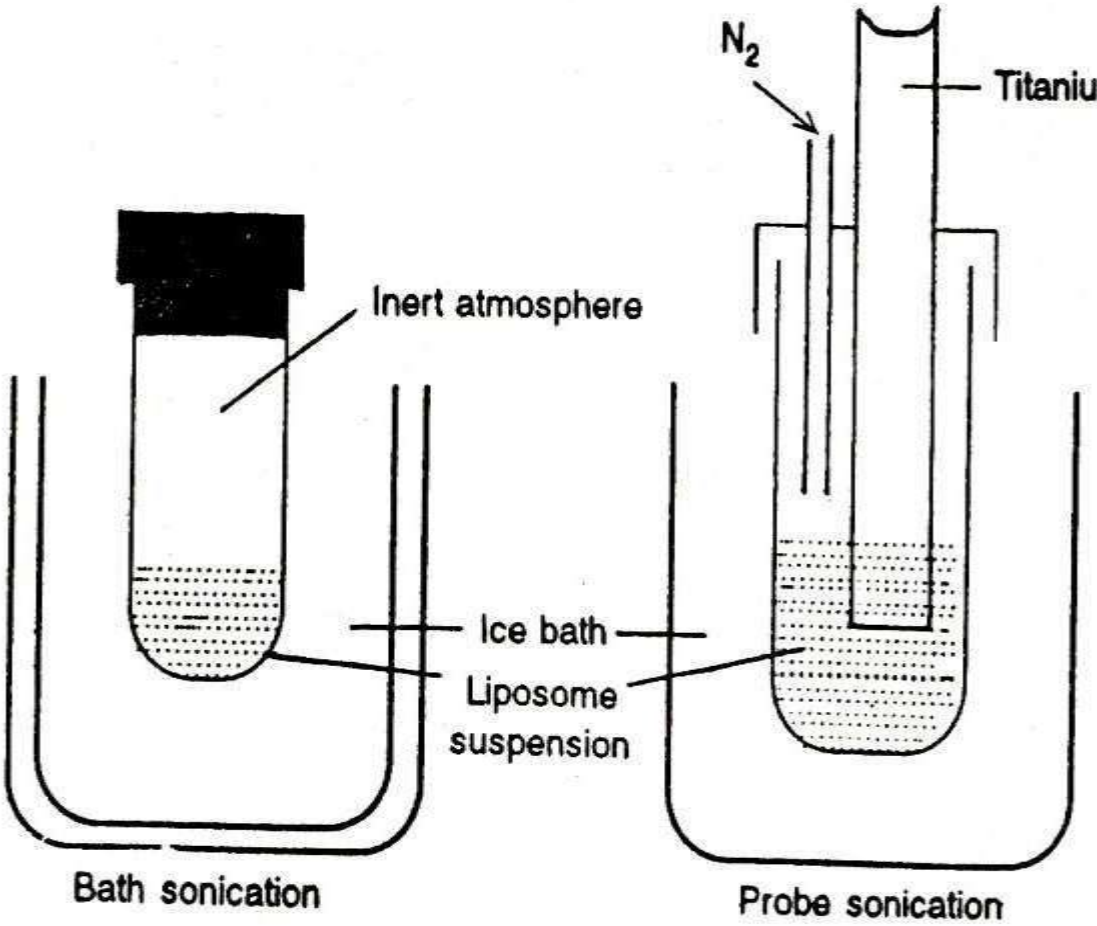


Fig 15.6. Preparation of liposomes by sonication.

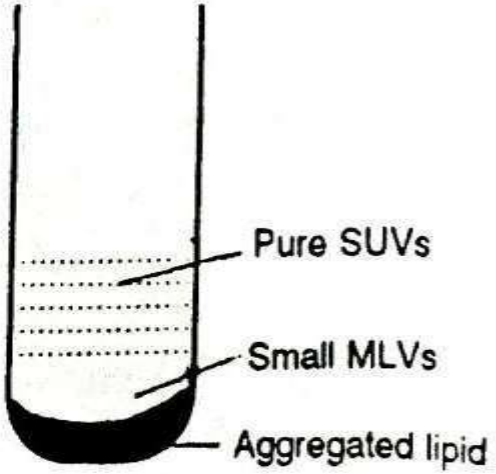


Fig. 15.7. Purification of small unilamellar vesicles.

FRENCH PRESSURE CELL

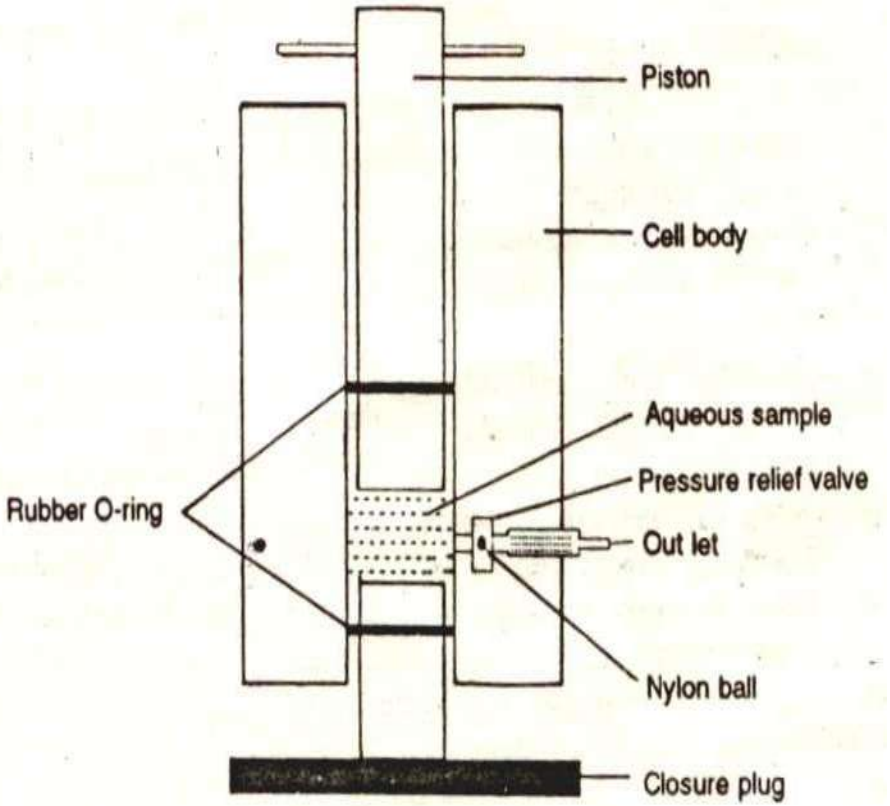


Fig. 15.8. French pressure cell.

Solvent Dispersion methods

- Ethanol injection method
- Ether injection method

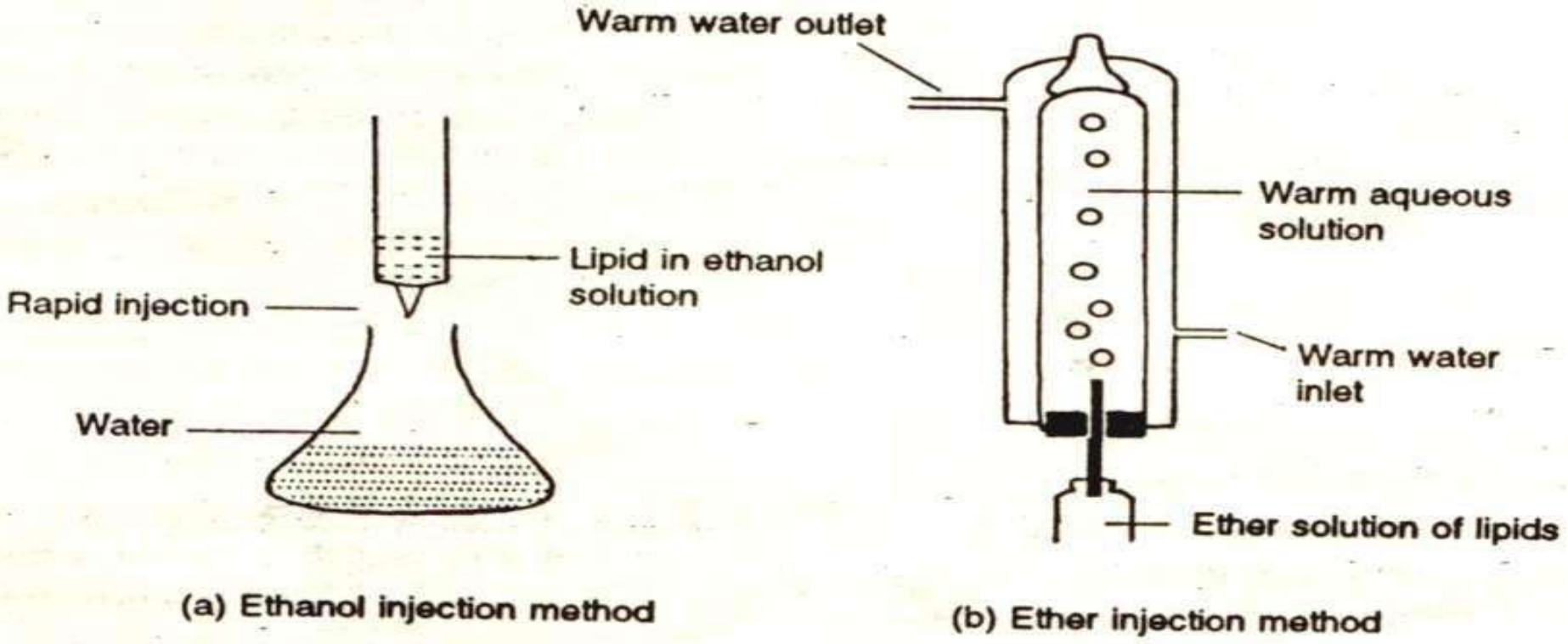


Fig. 15.10. Solvent dispersion techniques for liposome preparation.

CHARACTERISATION OF LIPOSOMES:

	Characterization parameters	Analytical method/Instrument
1.	Vesicle shape and surface morphology	Transmission electron microscopy, Freeze-fracture electron microscopy
2.	Mean vesicle size and size distribution (submicron and micron range)	Dynamic light scattering, zetasizer, Photon correlation spectroscopy, laser light scattering, gel permeation and gel exclusion
3.	Surface charge	Free-flow electrophoresis
4.	Electrical surface potential and surface pH	Zetapotential measurements & pH sensitive probes
5.	Phase behavior	Freeze-fracture electron microscopy, Differential scanning colorimetery
6.	Percent of free drug/ percent capture	Minicolumn centrifugation, ion-exchange chromatography, radiolabelling
7.	Drug release	Diffusion cell/ dialysis

2. CHEMICAL CHARACTERISATION

	Characterization parameters	*Analytical method/Instrument*
1.	Phospholipid concentration	Barlett assay, stewart assay, HPLC
2.	Cholesterol concentration	Cholesterol oxidase assay and HPLC
3.	Phopholipid peroxidation Phospholipid hydrolysis,	UV absorbance, Iodometric and GLC
4.	Cholesterol auto-oxidation.	HPLC and TLC
5.	Osmolarity	Osmometer

3. BIOLOGICAL CHARACTERISATION

	Characterization parameters	*Analytical method/Instrument*
1.	Sterility	Aerobic or anaerobic cultures
2.	Pyrogenicity	Limulus Amebocyte Lysate (LAL) test
3.	Animal toxicity	Monitoring survival rates, histology and pathology

Marketed product	*Drug used*	*Target diseases*	*Company*
Doxil™ or Caelyx™	Doxorubicin	Kaposi's sarcoma	SEQUUS, USA
DaunoXome™	Daunorubicin	Kaposi's sarcoma, breast & lung cancer	NeXstar, USA
Amphotec™	Amphotericin-B	fungal infections, Leishmaniasis	SEQUUS, USA
Fungizone®	Amphotericin-B	fungal infections, Leishmaniasis	Bristol-squibb, Netherland
VENTUS™	Prostaglandin-E_1	Systemic inflammatory diseases	The liposome company, USA
ALEC™	Dry protein free powder of DPPC-PG	Expanding lung diseases in babies	Britannia Pharm, UK
Topex-Br	Terbutaline sulphate	Asthma	Ozone, USA
Depocyt	Cytarabine	Cancer therapy	Skye Pharm, USA
Novasome®	Smallpox vaccine	Smallpox	Novavax, USA
VincaXome	Vincristine	Solid Tumours	NeXstar, USA

Liposomes as carrier of antigens

Antigen	Route of administration & animal species	Major observations
Diptheria toxoid	i.v , s.c mice	-ve liposome's with PA elicited greater absorption. DCP is superior to PA .s.c & i.m administration showed greater absorption.
Herpes simplex virus type 1 antigen	i.p mice	Enhanced immunogenicity with liposomal antigen.Max. absorption with liposome bound antigen containing lipid A. No CMP with liposome preparation.

Liposome's for topical application

DRUG	RESULTS
Benzocain gel	Shows prolonged anesthesia as compared to plain benzocaine cream
Hydrocortisone	High concentration of drug in individual layers of human skin than control ointment
Diclofenac gel	Increase concentration of drug in subcutaneous tissue and increased permeation through the skin

Liposome's for pulmonary delivery

DRUG	RESULTS
Enriroxine	Liposome encapsulated drug was observed to be 10 – 50 times less toxic to tissue culture cells than free drug
Atropine glutathione	Maintained much higher level of entrapped drug in lung than solution form
Tobramycin	Liposome entrapped drug shows pulmonary level 3 times higher than those of free drug
Salbutamol	Dehydrated liposome entrapped drug ensured an effective sustained release system following inhalation

Liposome's for ophthalmic delivery

DRUG	RESULTS
Idoxuridine	Improved efficacy of liposome's encapsulated drug in the treatment of Herpes simplex keratitis
Benzyl penicillin indoxol	Ocular bioavailability enhanced by delivery in liposome's
Penicillin G	Flux was enhanced by positive charged unilamellar liposome's


THANK YOU

MICROENCAPSULATION

G.SAMATHA

VCPS

- Micro encapsulation is the means of applying thin coatings to small particles of solids or droplets of liquids and dispersions. The coating of particles ranging from several tenths of a micron to 5000 microns in size.

- Microencapsulation provides the means of converting the liquids to solids, of altering the colloidal and surface properties, of providing environmental protection,

ADVANTAGES:

- Microencapsulation provides the means of converting liquids to solids.
- Altering colloidal and surface properties.
- Providing environmental protection
- Controlling the release characteristics.

DISADVANTAGES:

- No single microencapsulation process is adaptable to all core material candidates or product applications.
- Difficulties such as incomplete or discontinuous coating
- Inadequate stability or shelf life of sensitive pharmaceuticals.
- Non reproducible and unstable release characteristics of coated products
- Economic limitations often are encountered in the attempt to apply particular microencapsulation method.

➢ Fundamental Considerations:

✓ Core Material:

- The core material, defined as the specific material to be coated , can be liquid or solid in nature.
- The solid core can be a mixture of active constituents , stabilizers , diluents, excipients, and accelerants.

TABLE 13-6. *Properties of Some Microencapsulated Core Materials*

Core Material	*Characteristic Property*	*Purpose of Encapsulation*
Acetaminophen	Slightly water-soluble solid	Taste-masking
Activated charcoal	Adsorbent	Selective sorption
Aspirin	Slightly water-soluble solid	Taste-masking; sustained release; reduced gastric irritation; separation of incompatibles
Islet of Langerhans	Viable cells	Sustained normalization of diabetic condition
Isosorbide dinitrate	Water-soluble solid	Sustained release
Liquid crystals	Liquid	Conversion of liquid to solid; stabilization
Menthol/methyl salicylate camphor mixture	Volatile solution	Reduction of volatility; sustained release
Progesterone	Slightly water-soluble solid	Sustained release
Potassium chloride	Highly water-soluble solid	Reduced gastric irritation
Urease	Water-soluble enzyme	Permselectivity of enzyme, substrate, and reaction produ
Vitamin A palmitate	Nonvolatile liquid	Stabilization to oxidation

Coating Material:

The selection of a specific coating material depends upon that

- What are the specific dosage or product requirements- stabilization, reduced volatility, release characteristics , environmental conditions.
- What coating material will satisfy the product objectives and requirements.
- What microencapsulation method is best suited to accomplish the coated product objectives.

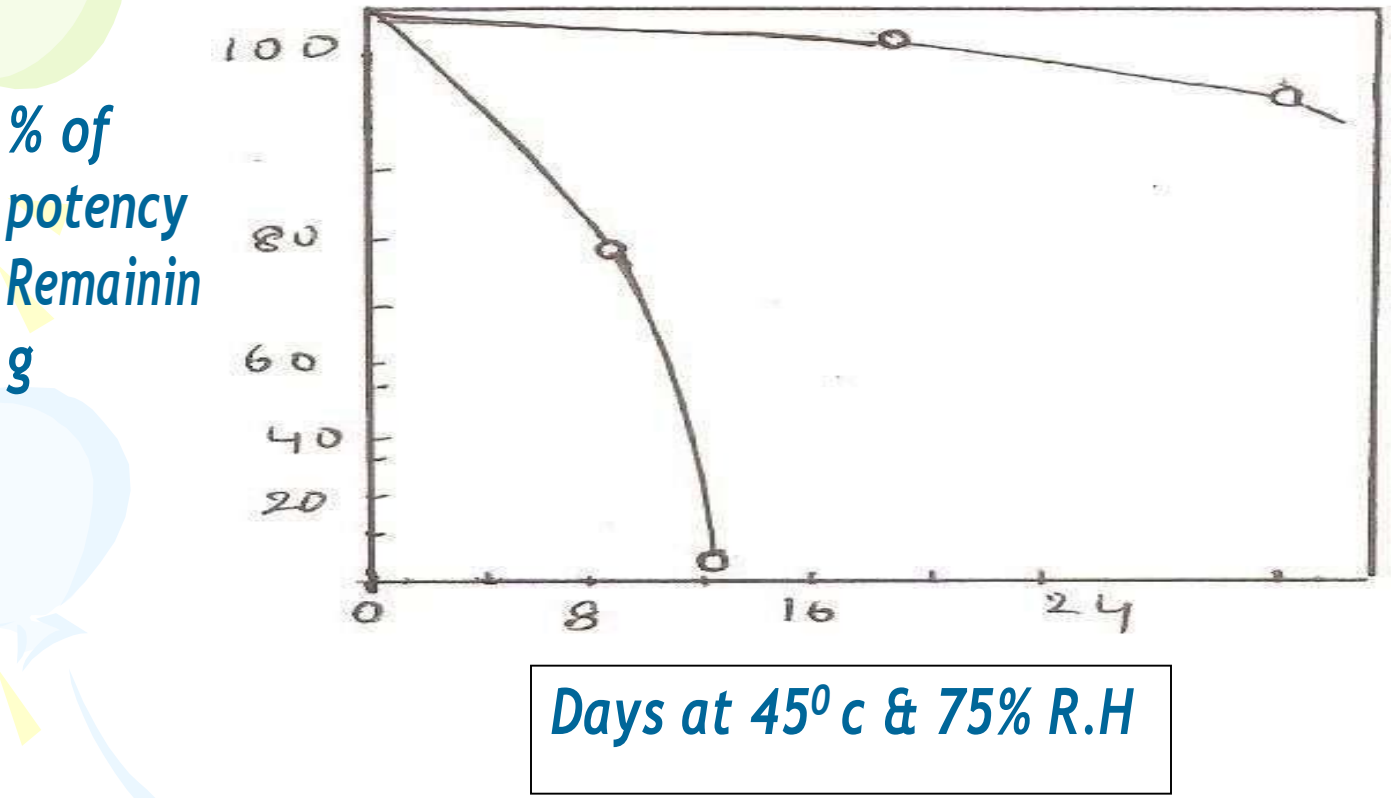
- The coating material should be capable of forming a film that is cohesive with the core material, be chemically compatible and non reactive with the core material and provide a desired coating properties, such as strength, flexibility, impermeability, optical properties, and stability.

- The typical coating properties such as cohesiveness , permeability,moisture sorption, solubility, stability and clarity must be considered in the selection of the proper microcapsule coating material.

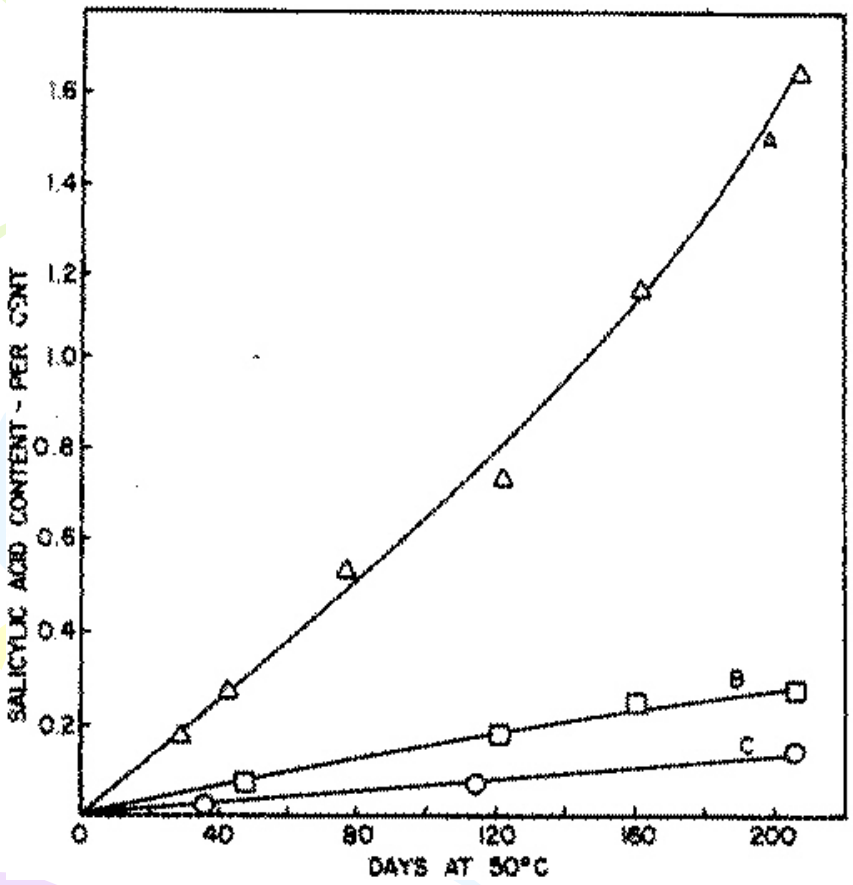
TABLE 13-7. *Representative Coating Materials and Appli*

Coating Materials	*Multiorifice—Centrifugal*	*Phase Separation—Coacervation*
Water-soluble resins		
Gelatin	X	X
Gum arabic		X
Starch		X
Polyvinylpyrrolidone	X	X
Carboxymethylcellulose		X
Hydroxyethylcellulose		X
Methylcellulose		X
Arabinogalactan		X
Polyvinyl alcohol	X	X
Polyacrylic acid		X
Water-insoluble resins		
Ethylcellulose		X
Polyethylene	X	
Polymethacrylate		X
Polyamide (Nylon)		
Poly [Ethylene-Vinyl acetate]	X	X
Cellulose nitrate	X	X
Silicones		
Poly (lactide-co-glycolide)		X
Waxes and lipids		
Paraffin	X	X
Carnauba		
Spermaceti		X
Beeswax		
Stearic acid		
Stearyl alcohol		
Glyceryl stearates		
Enteric resins		
Shellac		X
Cellulose acetate phthalate		X
Zein		X

➢ Selected Stability , Release And Other Properties:



Stability of microencapsulated vitamin A palmitate corn oil prepared by Phase separation or coacervation technique compared with unencapsulated control

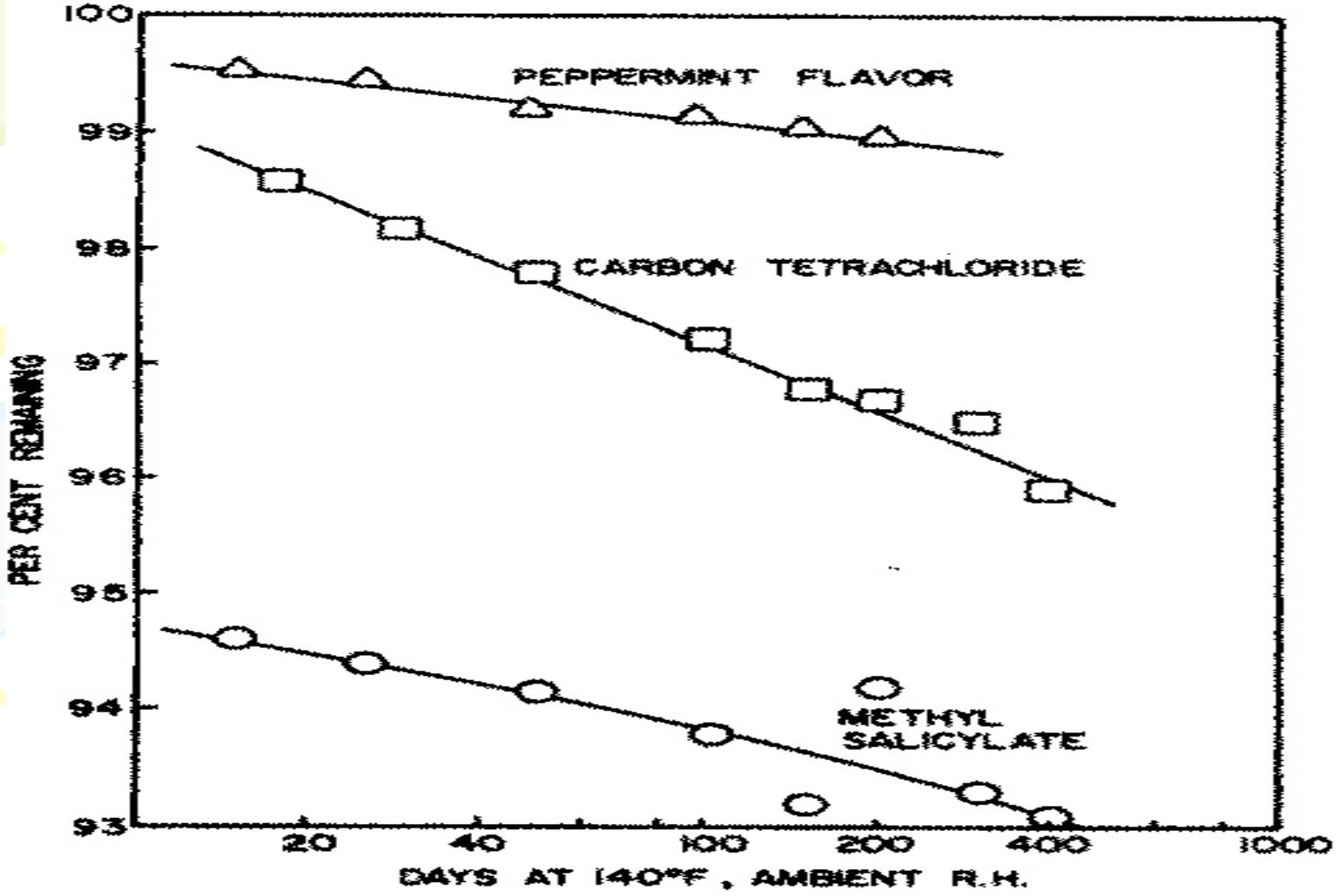


a. Aspirin hydrolysis of chlorpheniramine maleate- aspirin mixture

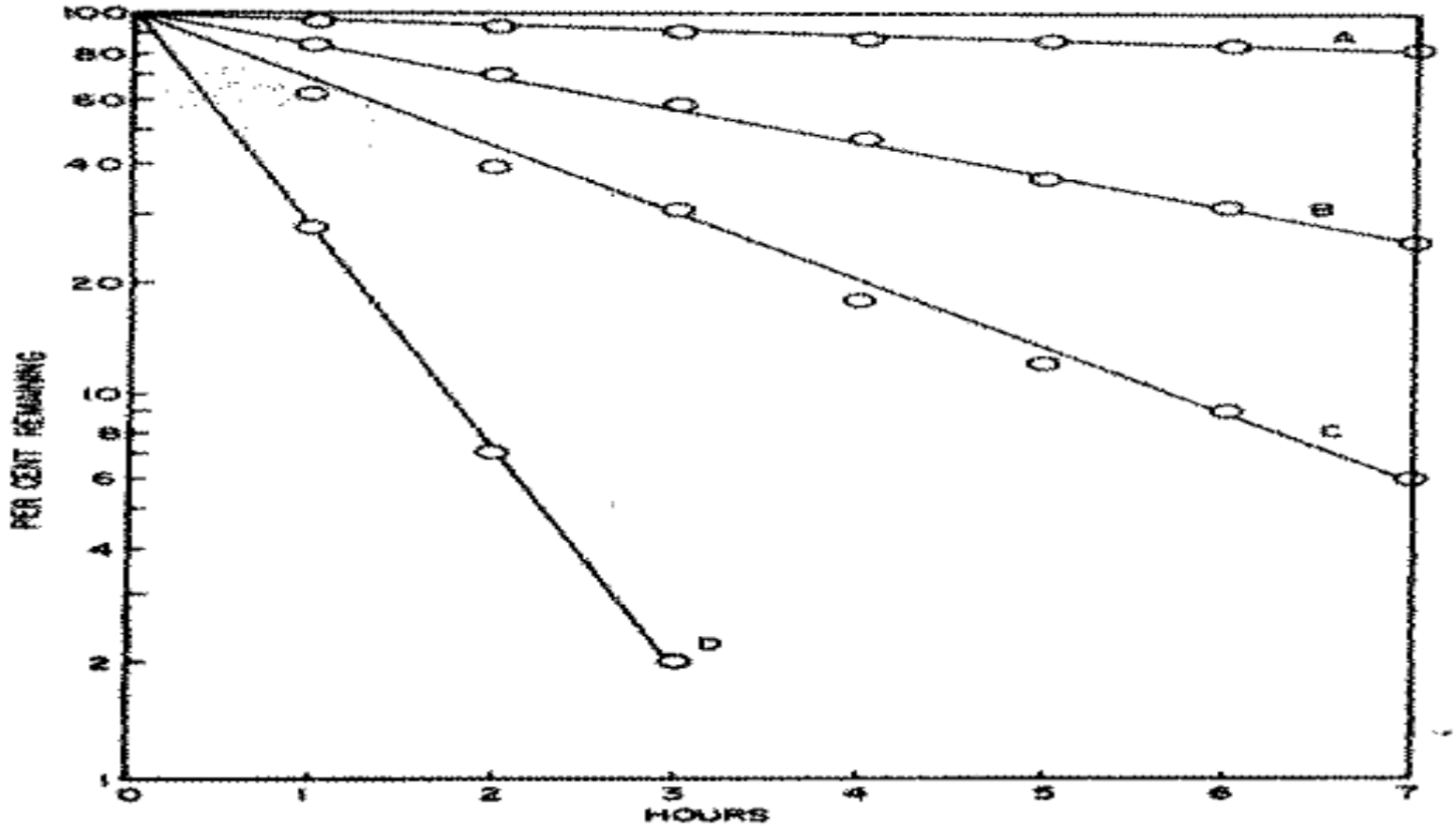
b. Aspirin hydrolysis of microencapsulated mixture.

c. Hydrolysis of aspirin control.

Stability enhancement of incompatible aspirin mixture by microencapsulation



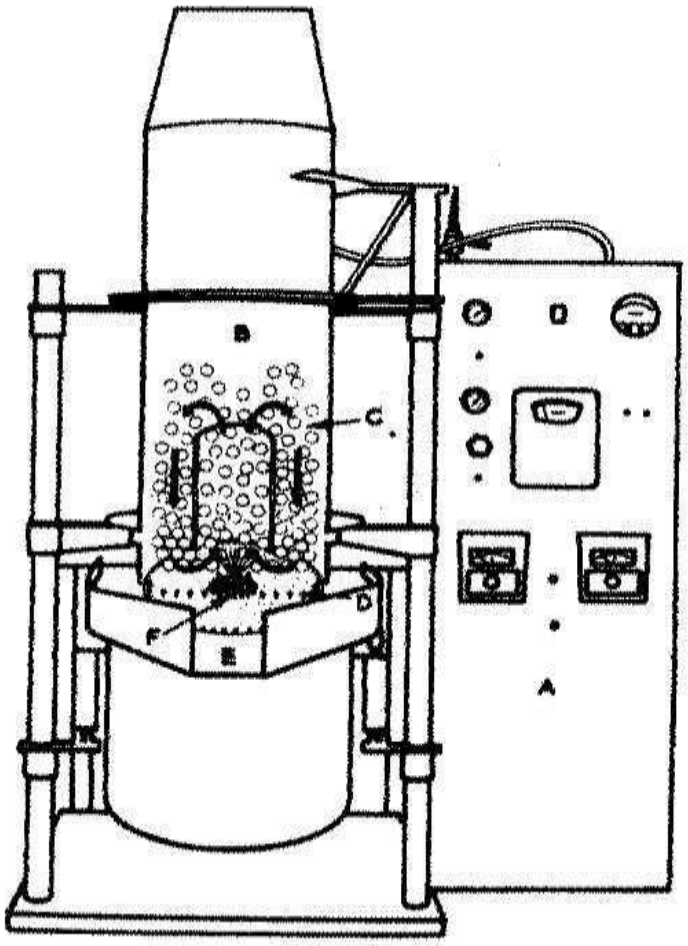
The above graph shows that the stability of microencapsulated Volatile liquids, prepared by coacervation phase separation



Invitro release patterns of crystalline aspirin coated with various amounts of ethyl cellulose using phase separation coacervation techniques.

EQUPMENT AND PROCESSING:

- Air suspension.
- Coacervation phase separation.
- Multi orifice-centrifugal process.
- Spray drying and congealing
- Pan Coating.
- Solvent evaporation techniques.
- Polymerization.



WURSTER AIR SUSPENSION APPARATUS

a. Control panel

b. Coating chamber.

c. Particles being treated.

d. Process air flow.

e. Air distribution plate.

f. Nozzle for applying film coatings.

Processing variables that receive consideration for efficient , effective, encapsulation by air suspension include:

- Density, surface area, melting point, solubility, friability, volatility , crystallinity and flowability to the core material.
- Coating material concentration.
- Coating material application rate.
- Volume of air required to support and fluidize the core material.
- Amount of coating material required.
- Inlet and outlet operating temperatures.

Coacervation Phase Separation:

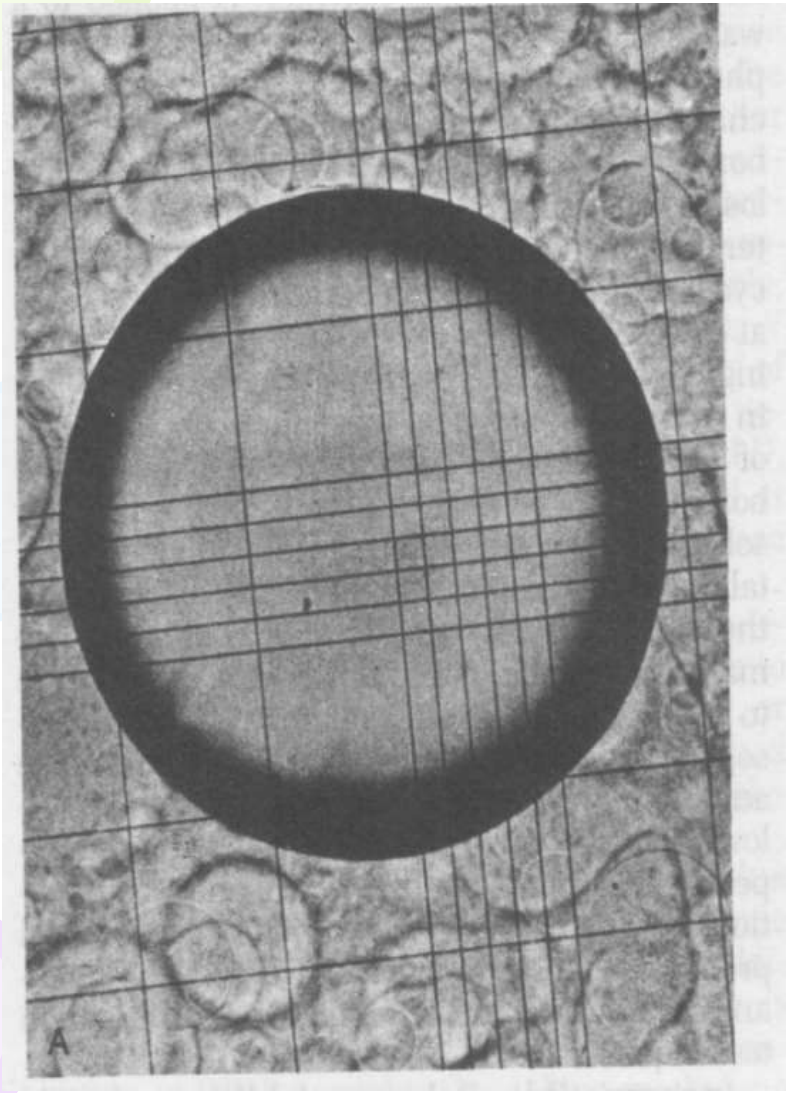
- Microencapsulation by coacervation phase separation is generally attributed to The National Cash Register (NCR) The process consists of three steps

- Formation of three immiscible phases; a liquid manufacturing phase, a core material phase and a coating material phase.

- Deposition of the liquid polymer coating on the core material.

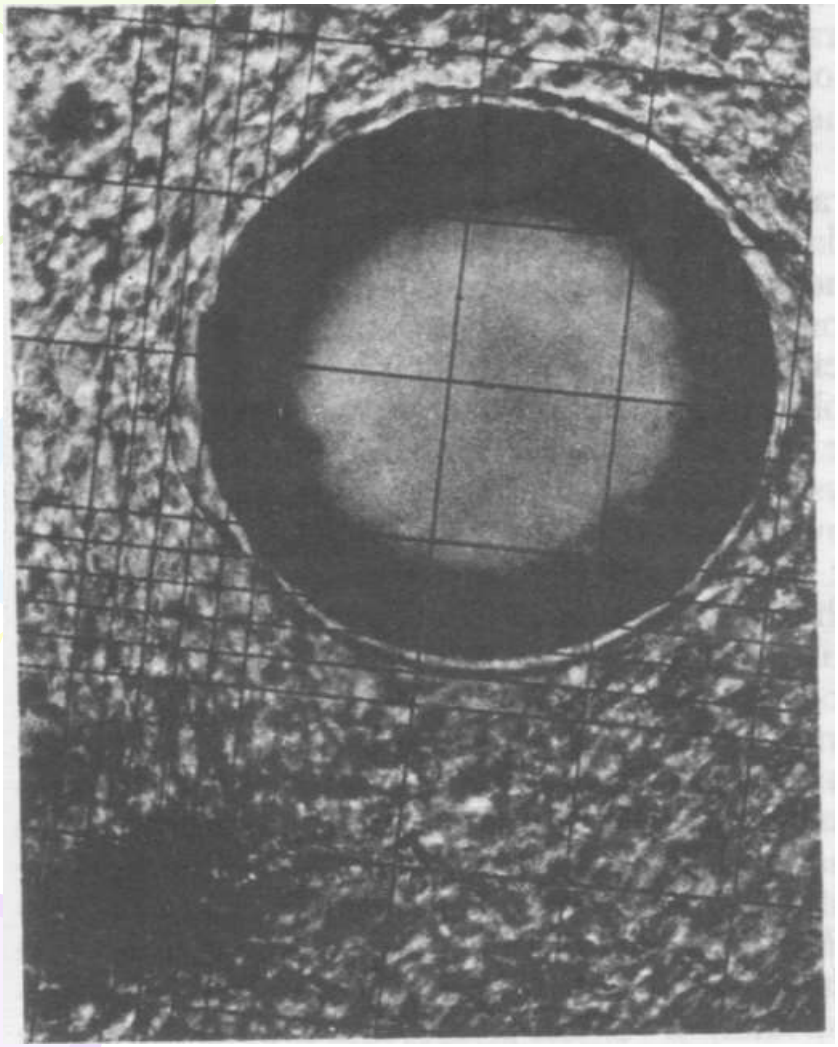
- Rigidizing the coating usually by thermal, cross linking or desolvation techniques to form a microcapsule.

STEP -I



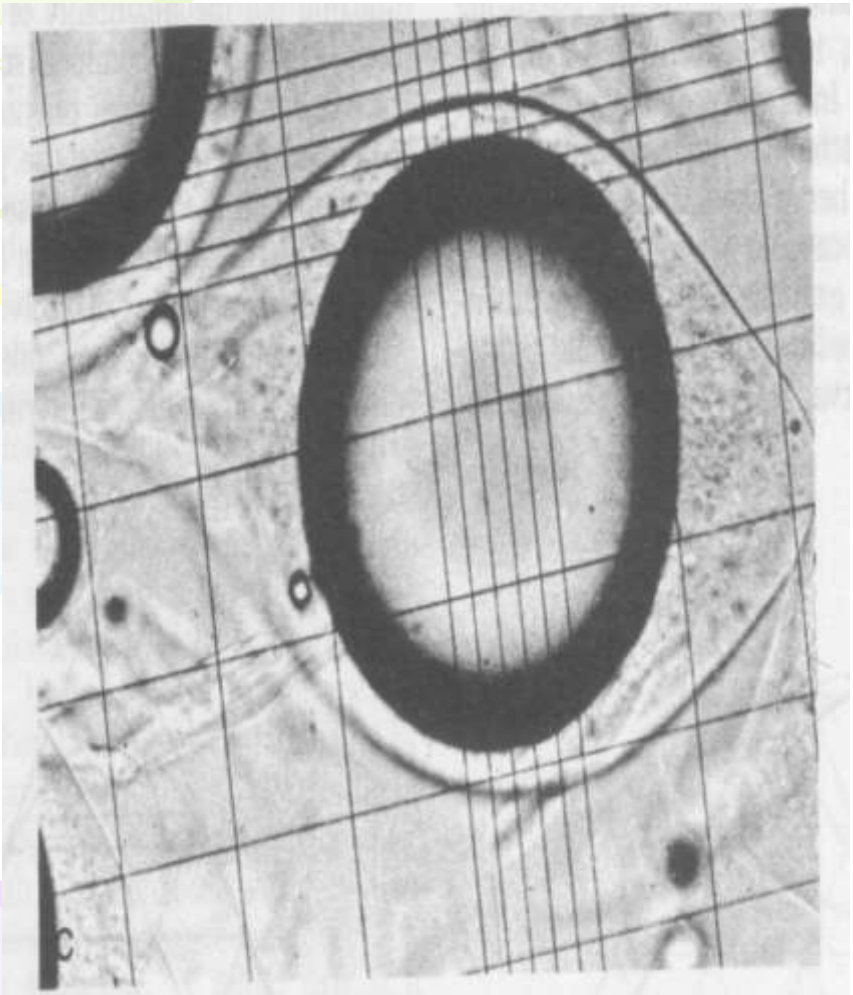
- To form the three phases, the core material is dispersed in a solution of coating polymer, the solvent for the polymer being the liquid manufacturing vehicle phase.
- The coating material phase, an immiscible polymer in a liquid state, is formed by utilizing one of the methods of phase separation coacervation i.e. by changing temperature of polymer solution or by adding the salt, non solvent, or incompatible polymer to the polymer solution.

STEP-II



The deposition of the liquid polymer around the interface formed between the core material and the liquid vehicle phase.

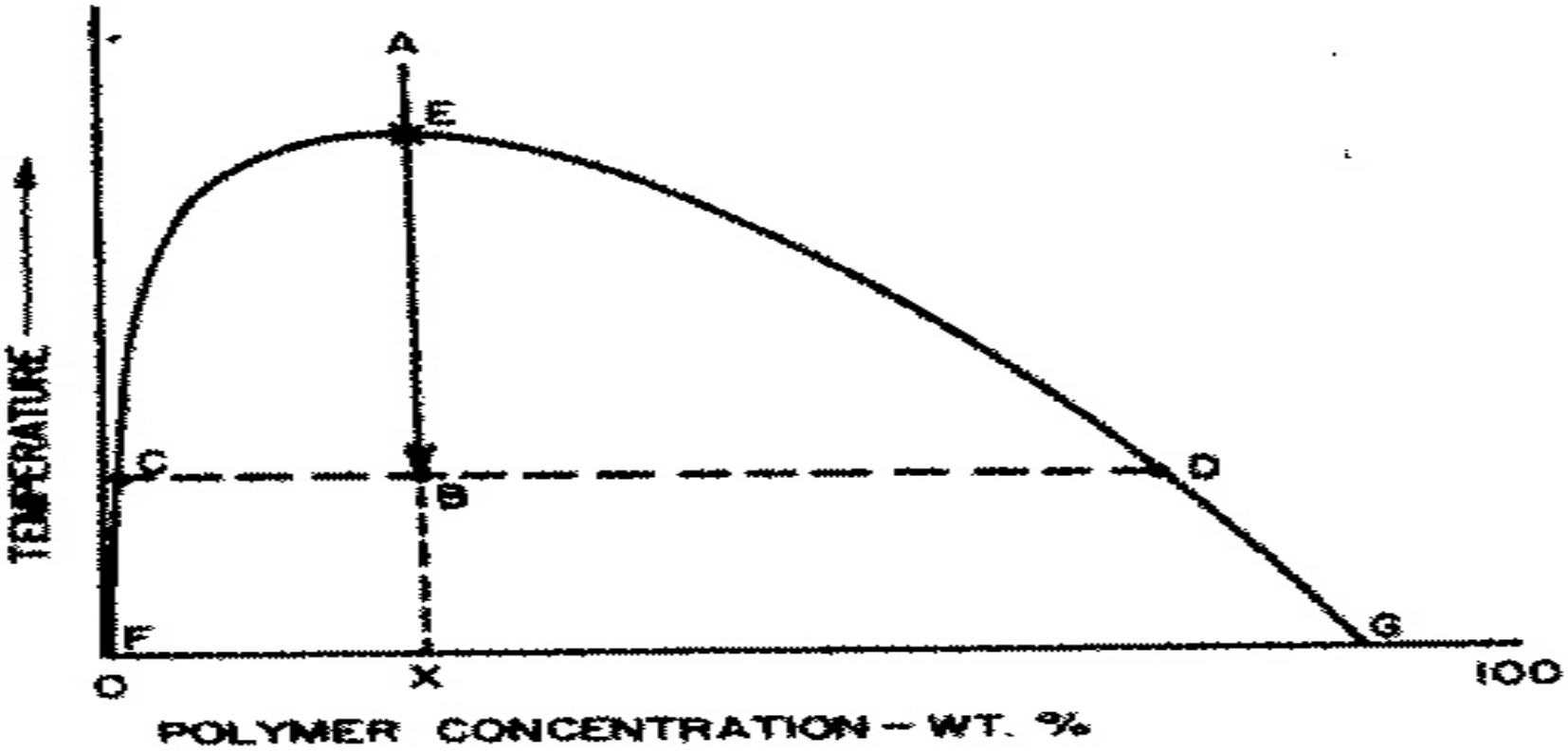
STEP-III



Step 3. Completed Capsules in Manufacturing Vehicle

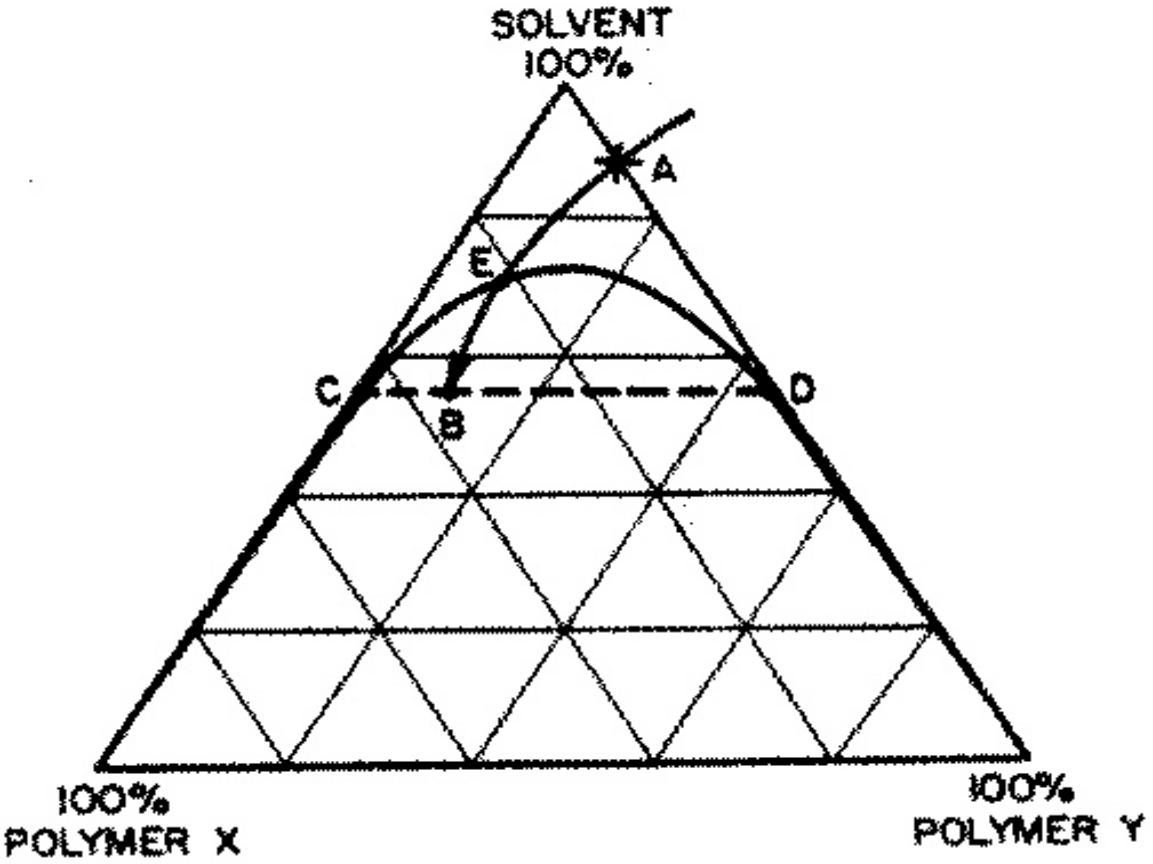
This process involves rigidizing the coating , usually by thermal, cross linking, or desolvation techniques, to form self sustaining microcapsule.

Temperature Changes:



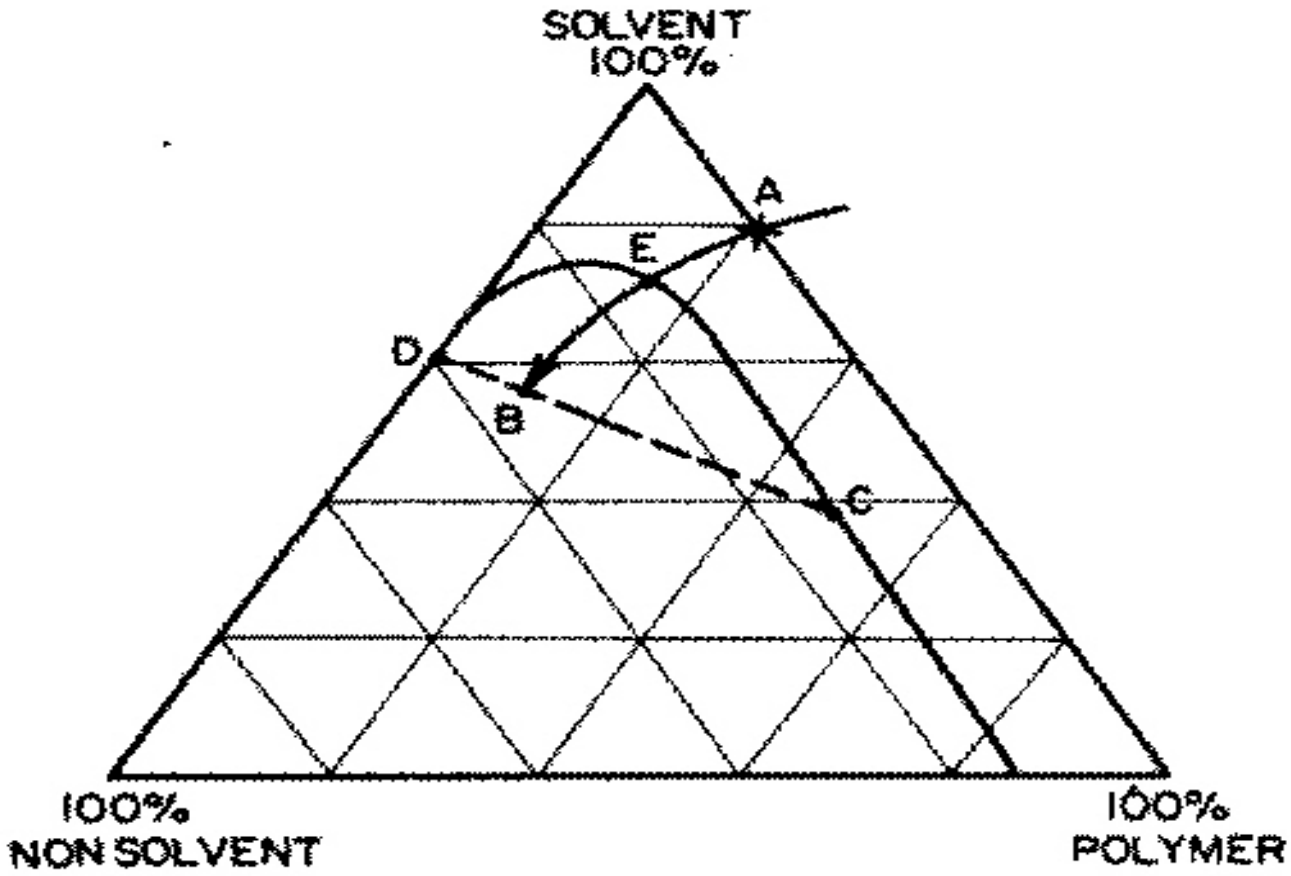
- Ex:N – Acetyl Para Aminophenol coated with EC dissolved in cyclohexane as solvent.

Incompatible Polymer Addition:



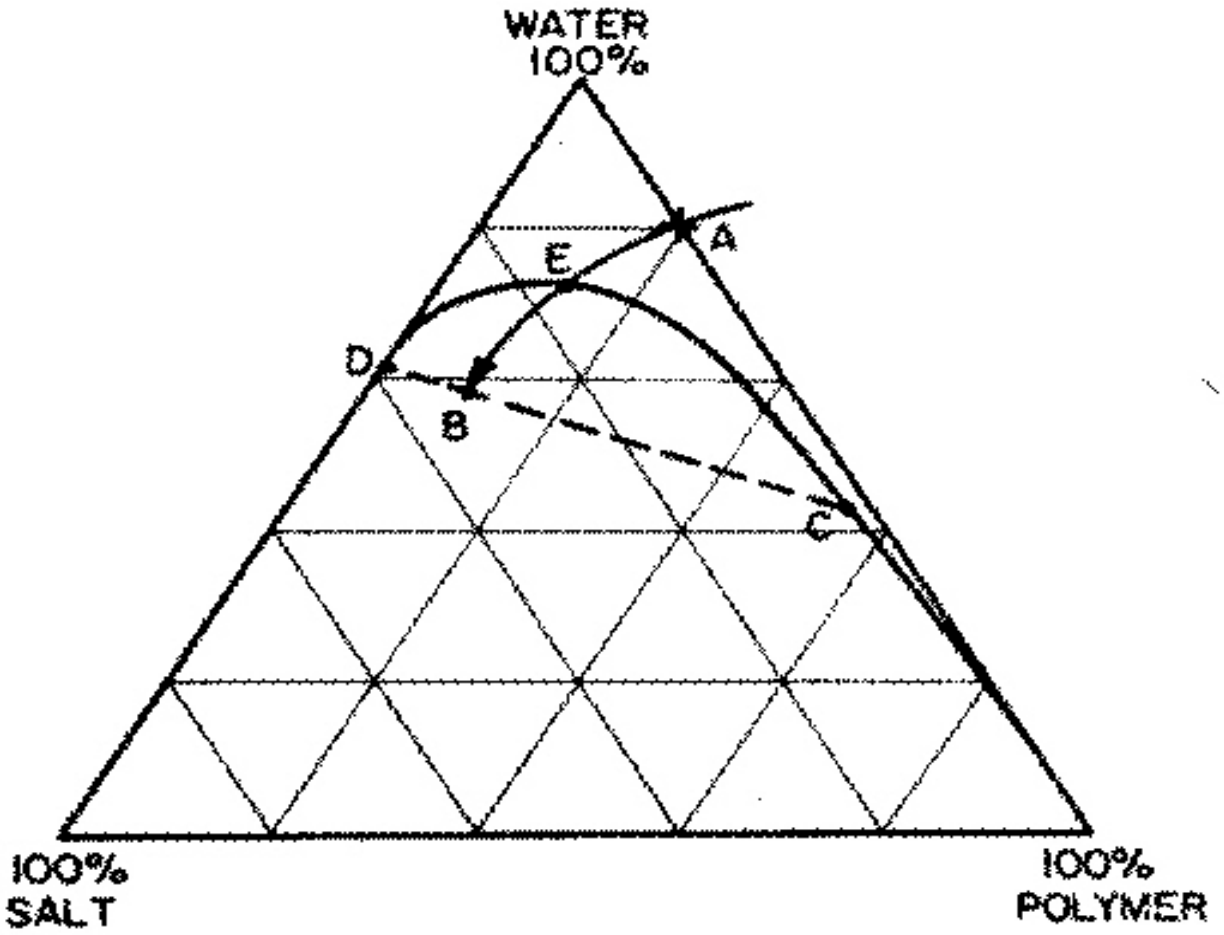
Four Parts of Methylene Blue HCl is coated with two % EC dissolved in toluene is phase separated by the addition of 25 parts of incompatible polymer Polybutadiene

Non Solvent Addition:



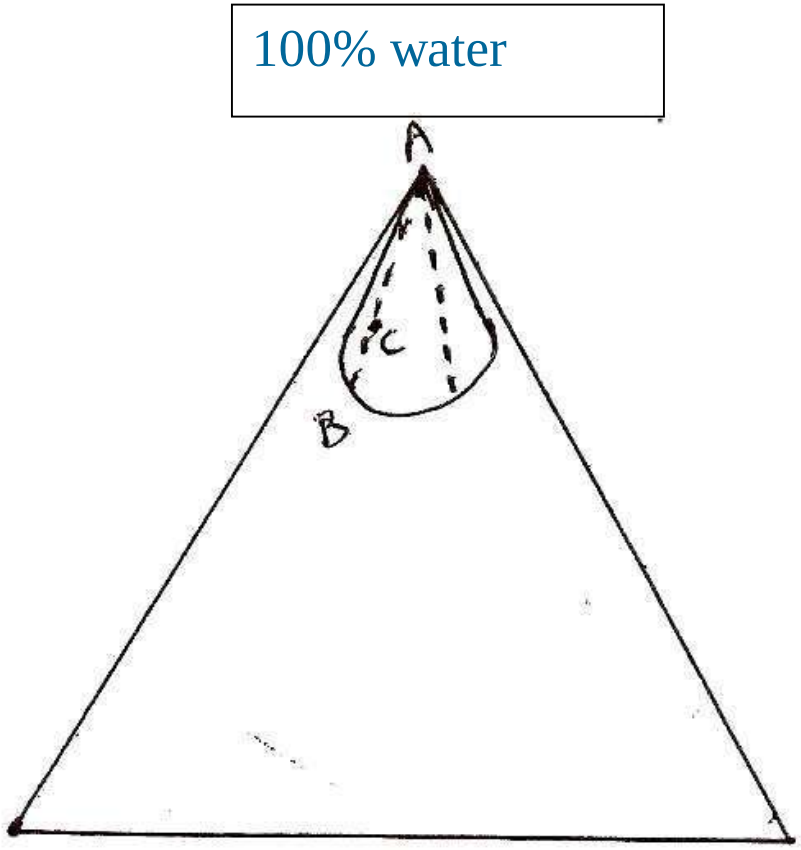
Methyscopolamine Hydrobromide is coated with 5 % W/V of CAB dissolved in methylethyl ketone is phase separated by the addition of 1:2 parts of isopropyl ether as non solvent

Salt Addition:



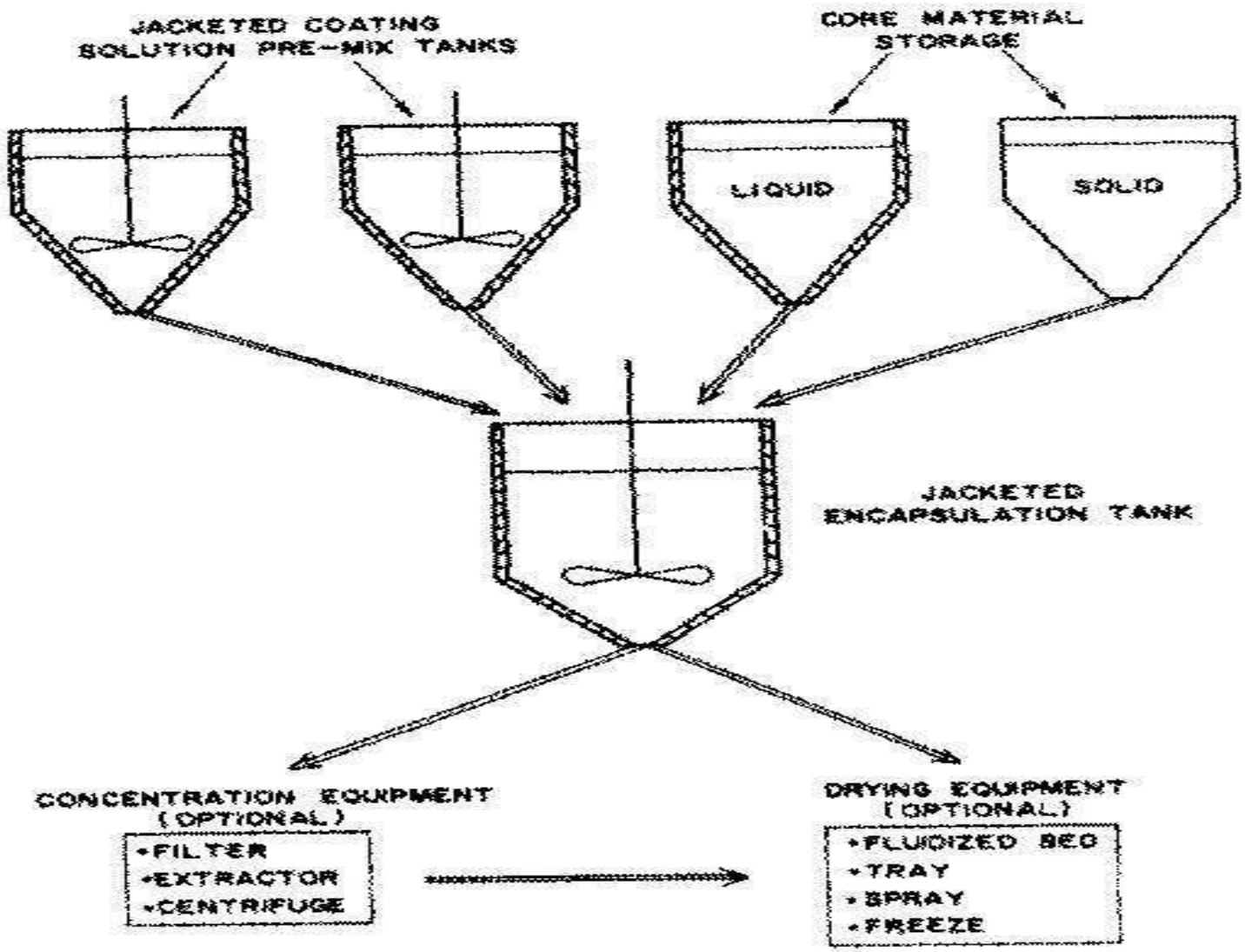
Vitamin A in corn oil is emulsified with 10% Gelatin at 50^0c is microencapsulated by adding 20% Sodium sulphate as salt solution which is further rigidized by transferring them in to 7% sodium sulphate solution at 19^0C

Polymer- Polymer Interaction:

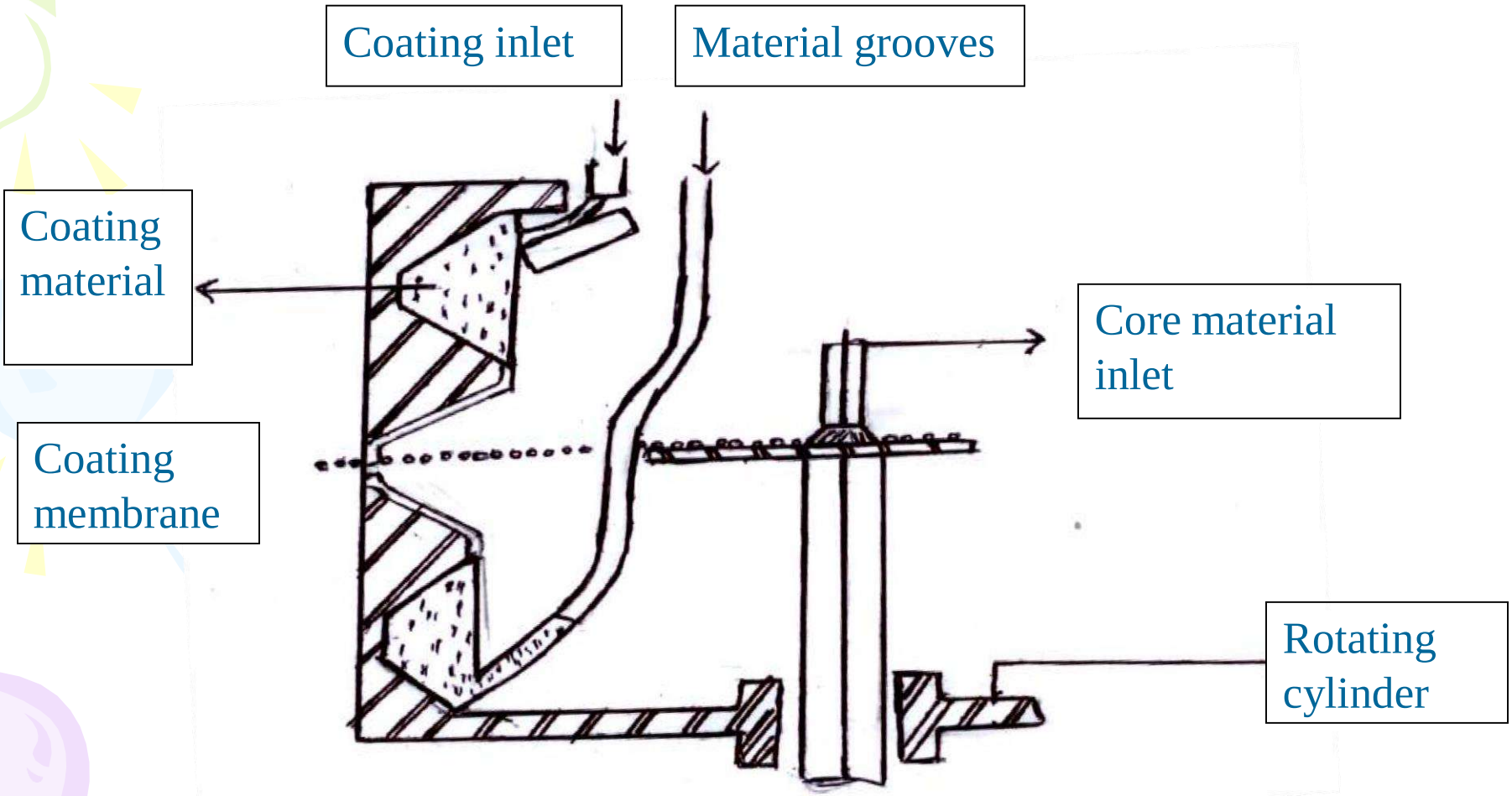


25 % of Methyl Salicylate is emulsified by interacting positively charged 2% Gelatin with negatively charged 2% Gum Arabic at 4.5pH while maintaining the temperature at 40 – 45 ^{0}C. The microcapsules are rigidized by further stirring at 20-25^{0}C.

Flow diagram of typical phase separation /coacervation process



➢ MULTIORIFIC-CENTRIFUGAL PROCESS:



PAN COATING:

- The coating is applied as a solution or as an atomized spray to the desired solid core material in the coating pan. Usually, to remove the coating solvent, warm air is passed over the coated materials as the coatings are being applied in the coating pans. In some cases, final solvent removal is accomplished in drying oven.

SPRAY DRYING AND SPRAY CONGEALING

- Spray drying and spray congealing processes are similar in that both involve dispersing the core material in a liquefied coating substance and spraying or introducing the core coating mixture into some environmental condition, whereby, relatively rapid solidification of the coating is effected.

- The principal difference between the two methods, Coating solidification in the case of spray drying is effected by rapid evaporation of a solvent in which the coating material is dissolved.

- Coating solidification in spray congealing method however is accomplished by thermally congealing a molten coating material or by solidifying a dissolved coating by introducing the coating core material mixture into a nonsolvent. Removal of the nonsolvent or solvent from the coated product is ten accomplished by sorption extraction or evaporation techniques.

- Microencapsulation by spray drying is conducted by dispersing a core material in a coating solution, in which the coating substance is dissolved and in which the core material is insoluble, and then by atomizing the mixture in to an air stream.

- Micro encapsulation by spray congealing can be accomplished with spray drying equipment, when the protective coating is applied as melt.

- Many solidification is accomplished by spraying the hot mixture in to a cool air stream.

- Waxes, fatty acids, alcohols, polymers & sugars which are solid at room temperature but meltable at reasonable temperatures, are applied to spray congealing techniques.

SOLVENT EVAPORATION:

- The process involves dissolving microcapsule coating (polymer) in a volatile solvent which is immiscible with the liquid manufacturing vehicle phase. A core material (drug) to be microencapsulated is dissolved or dispersed in the coating polymer solution.

- With agitation, the core – coating material mixture is dispersed in the liquid manufacturing vehicle phase to obtain appropriate size microcapsules. Agitation of system is continued until the solvent partitions into the aqueous phase and is removed by evaporation.

- Factors that must be considered when preparing microcapsules by solvent evaporation techniques include choice of vehicle phase and solvent for the polymer coating, as these choice greatly influence microcapsule properties as well as the choice of solvent recovery techniques.

POLYMERIZATION:

- The methods involve the reaction of monomeric units located at the interface existing between a core material substance and a continuous phase in which the core material is dispersed.

- Microcapsules having coatings of nylon formed by interfacial polymerization, or colloidan formed by phase separation/coacervation techniques.

APPLICATIONS:

The applications of microencapsulation include:

- Sustained release or prolonged action medications.
- Taste masked chewable tablets, powders and suspensions.
- Single layer tablets containing chemically incompatible ingredients
- New formulations concepts for creams, ointments, aerosols, dressings, plasters, suppositories and injectables.

Physicochemical evaluation characterization:

The physico chemical evaluation include:

- **Morphology Of Microspheres.**
- **Particle Size.**
- **Density Determination**
- **Capture Efficiency.**
- **Angle Of Contact.**

In Vitro Methods :

❖ Beaker Method

- The dosage form in this method is made to adhere at the bottom of the beaker containing the medium and stirred uniformly using over head stirrer.
- Volume of the medium used in the literature for the studies varies from 50- 500 ml and the stirrer speed form 60-300 rpm.

❖ Dissolution Apparatus

- Standard USP or BP dissolution apparatus have been used to study *in vitro* release profiles using both rotating elements, paddle [20, 21, 22 and basket 23, 24].
- Dissolution medium used for the study varied from 100-500 ml and speed of rotation from 50-100 rpm.

Thank you

FORMATTING, ASSEMBLING & SUBMISSION OF NEW DRUG APPLICATION

G.Natesh

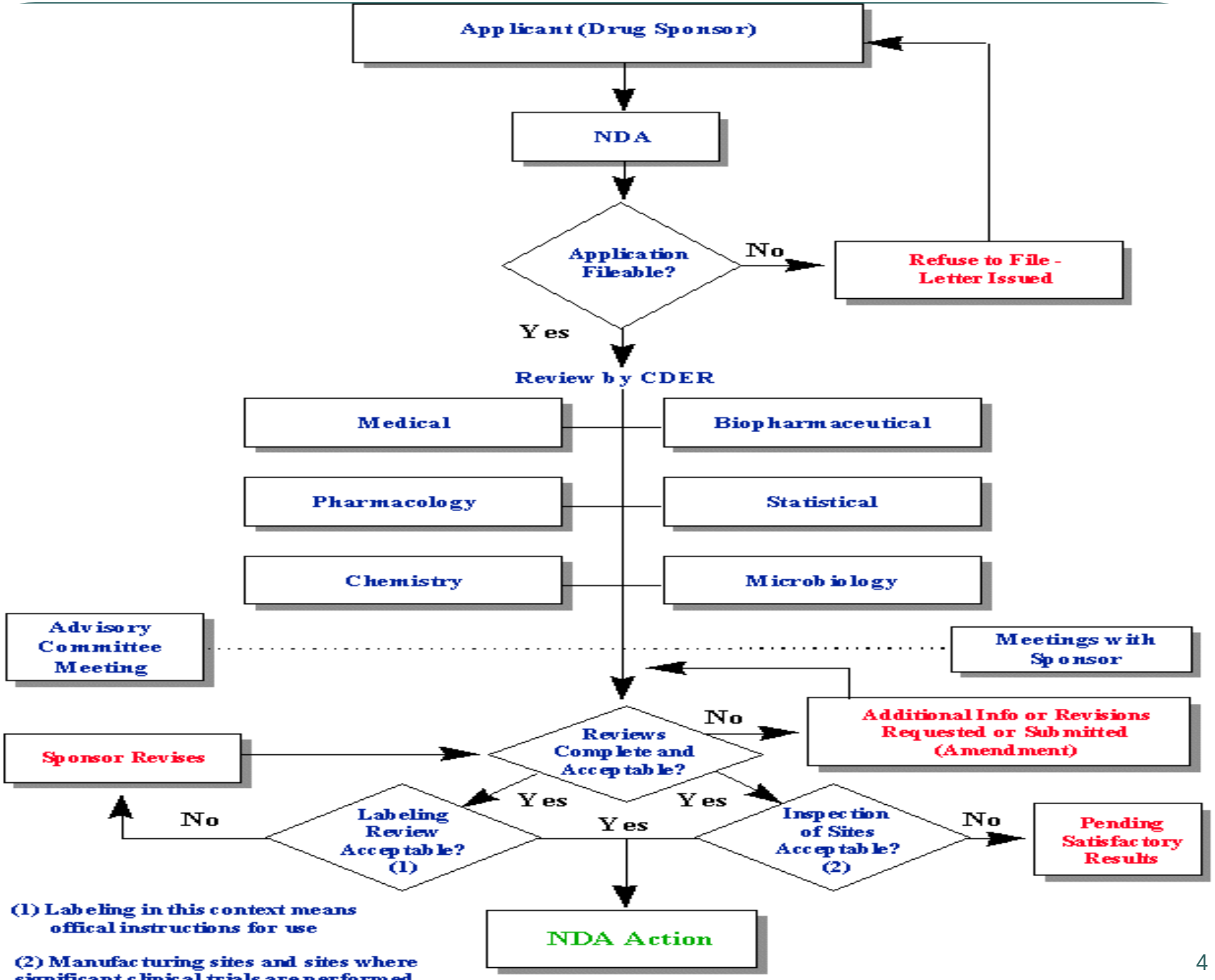
VCPS

- The US Food & drug administration requires drug sponsors to submit an NDA for review before a new pharmaceutical can be approved for marketing and sale in USA

- The NDA contains clinical and non clinical test data, analytical reports, drug chemistry information and description of manufacturing procedures.

NDA Classifications

- New Molecular Entity
- New Salt of Previously Approved Drug (not a new molecular entity)
- New Formulation of Previously Approved Drug (not a new salt OR a new molecular entity)
- New Combination of Two or More Drugs
- Already Marketed Drug Product - Duplication (i.e., new manufacturer)
- New Indication (claim) for Already Marketed Drug (includes switch in marketing status from prescription to OTC)
- Already Marketed Drug Product - No Previously Approved NDA


Applicant (Drug Sponsor)
NDA
Application Fileable?
No
Refuse to File - Letter Issued
Yes
Review by CDER
Medical
Biopharmaceutical
Pharmacology
Statistical
Chemistry
Microbiology
Advisory Committee Meeting
Meetings with Sponsor
Reviews Complete and Acceptable?
No
Additional Info or Revisions Requested or Submitted (Amendment)
Sponsor Revises
Yes
Yes
Yes
Labeling Review Acceptable? (1)
No
Inspection of Sites Acceptable? (2)
No
Pending Satisfactory Results
NDA Action
(1) Labeling in this context means offical instructions for use
(2) Manufacturing sites and sites where significant clinical trials are performed

FORMAT FOR US NDA

Cover letter

1. Index
2. Labeling
3. Application summary

RIEVEW SECTIONS

4. Chemistry
5. Non Clinical Pharmacology & Toxicology
6. Human P.K Bioavailability
7. Clinical Microbiology
8. Clinical data
9. Safety update report
10. Statistical data
11. Case report tabulations
12. Case report forms
13. Patient information
14. Patient certification
15. Establishment description
16. Debarment Certification
17. Field copy certificate
18. User fee cover sheet
19. Financial disclosure
20. Other/ Pediatric

Index:

- It is a comprehensive table of contents that enables reviewers to find specific information in this massive document.

Labeling:

- This section must include all the draft labeling that is intended for use on product container, packings including the proposed package insert.
- NDA must have four copies of graft labeling.

Section 3 - Application summary

- It is abbreviated version of the entire application. The summary consists of 50-200 pages.
- It must include **proposed annotated package insert** and must contain the following sections

1. Description
2. Clinical pharmacology
3. Indications and usage
4. Contraindications
5. Drug abuse and dependence
6. Dosage and administration
7. Clinical data summary
8. Warnings
9. Warnings
10. Precautions
11. Adverse reactions
12. Over dosage
13. Microbiology summary

Section 4: Chemistry, Manufacturing & Controls

This section includes description of drug substance

- Generic name
- Chemical name
- Molecular structure & formula
- Molecular weight
- Appearance, M.P. & B.P.
- Refractive index, viscosity & specific gravity.
- Polymorphs
- Solubility
- Synthesis scheme & description.

Section 5: Non clinical pharmacology & toxicology

- This section should provide individual study reports, including pharmacology, toxicology and ADME studies.

Section 6: Human Pharmacokinetics and Bioavailability

- This section includes data from Phase –I studies.
- The summary should address all bioavailability and pharmacokinetic data.
- This section also includes data on drug information, analytical methods used in *in vivo* biopharmaceutics study.

Section 7 : Microbiology.

- This section is required only for anti infective drug products.
- This section requires following technical information
- Complete description of biochemical basis of drug action on microbial physiology.
- The drugs anti microbial spectrum
- Clinical microbiology laboratory methods.

Section-8: Clinical data

- This section consists of
- Background / overview of clinical investigation.
- Clinical pharmacology
- Controlled clinical trials
- Un controlled clinical trials
- Other studies and information
- Integrated summary of effectiveness data.
- Integrated summary of safety information.
- Drug abuse and over dosage information.
- Integrated summary of benefits and risks of the drug.

Section- 10 : Statistics

- This section includes documentation of the statistical analyses performed to evaluate the controlled clinical trials and other safety information.

Section-11: case report form tabulations

- This include complete tabulations for each patient from phase- II & phase-III efficacy study and from phase-I clinical pharmacology study

Section 12:Case report forms

- It is necessary to include CRF for each patient who died during the study and who were dropped from the study due to adverse events.

ANDA

"A drug product that is comparable to a brand/reference listed drug product in dosage form, strength, route of administration, quality and performance characteristics, and intended use"

It termed "abbreviated" because they generally not required to include preclinical (animal) and clinical (human) data to establish safety and effectiveness.

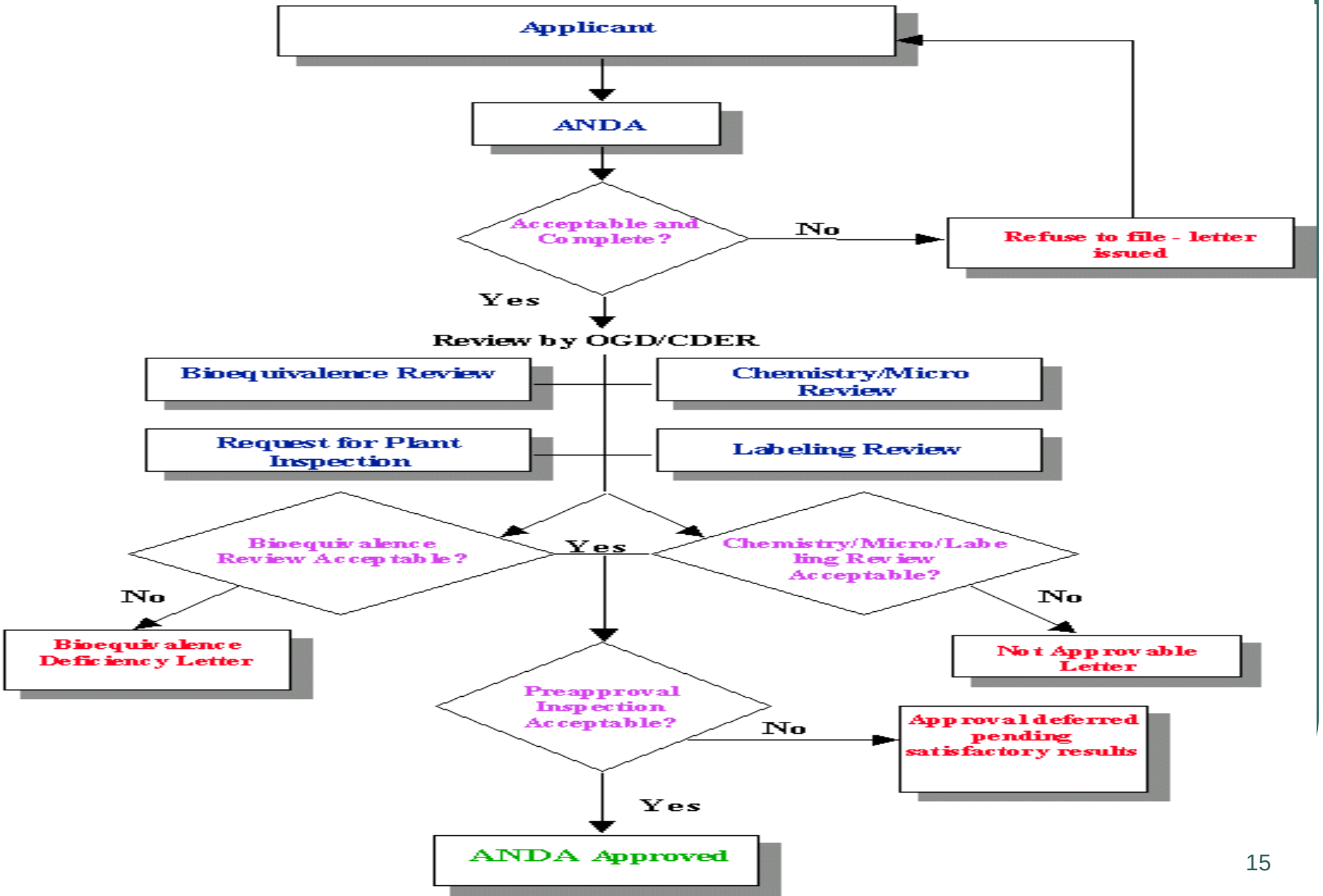
Basic Generic Drug Requirements are:--

- Same active ingredient(s)
- Same route of administration
- Same dosage form
- Same strength
- Same conditions of use
- Inactive ingredients already approved in a similar NDA

Goal of ANDA

- To reduce the price of the drug.
- To reduce the time development.
- Increase the bioavailability of the drug in comparison to references list drug.

ANDA Review process



NDA vs. ANDA Review Process

NDA Requirement	ANDA Requirement
1. Labeling	1. *Labeling*
2. Pharm/Tox	2. Pharm/Tox
3. Chemistry	3. Chemistry
4. Manufacturing	4. Manufacturing
5. Controls	5. Controls
6. Microbiology	6. Microbiology
7. Inspection	7. Inspection
8. Testing	8. Testing
9. Animal Studies	
10. Clinical Studies	9. Bioequivalence
11. Bioavailability	

CONTROLLED-SUSTAINED DRUG DELIVERY SYSTEMS

D.Priyatham

VCPS

An ideal drug delivery system must have two prerequisites:

- It would be a single dose for the duration of treatment, whether it be for days or weeks as with infection or for the lifetime of the patient as in hypertension or diabetes.

- It should deliver the active entity directly to the site of action, thereby minimizing or eliminating side effects. This may necessitate delivery to specific receptors or to localization to cells or to specific areas of the body.

Terminology:

Sustained Release Drug Delivery Systems:

Sustained release constitutes any dosage form that provides medication over an extended time to maintain therapeutic blood or tissue levels of the drug.

Controlled Release Drug Delivery Systems:

controlled release system is able to provide some actual therapeutic control, whether this be of a temporal nature, spatial nature, or both. The system attempts to control drug concentrations in the target issue.

Repeat-Action drug delivery systems:

Repeat-action tablets are an alternative method of sustained release in which multiple doses of drug are contained within a dosage form, and each dose is released at a periodic interval.

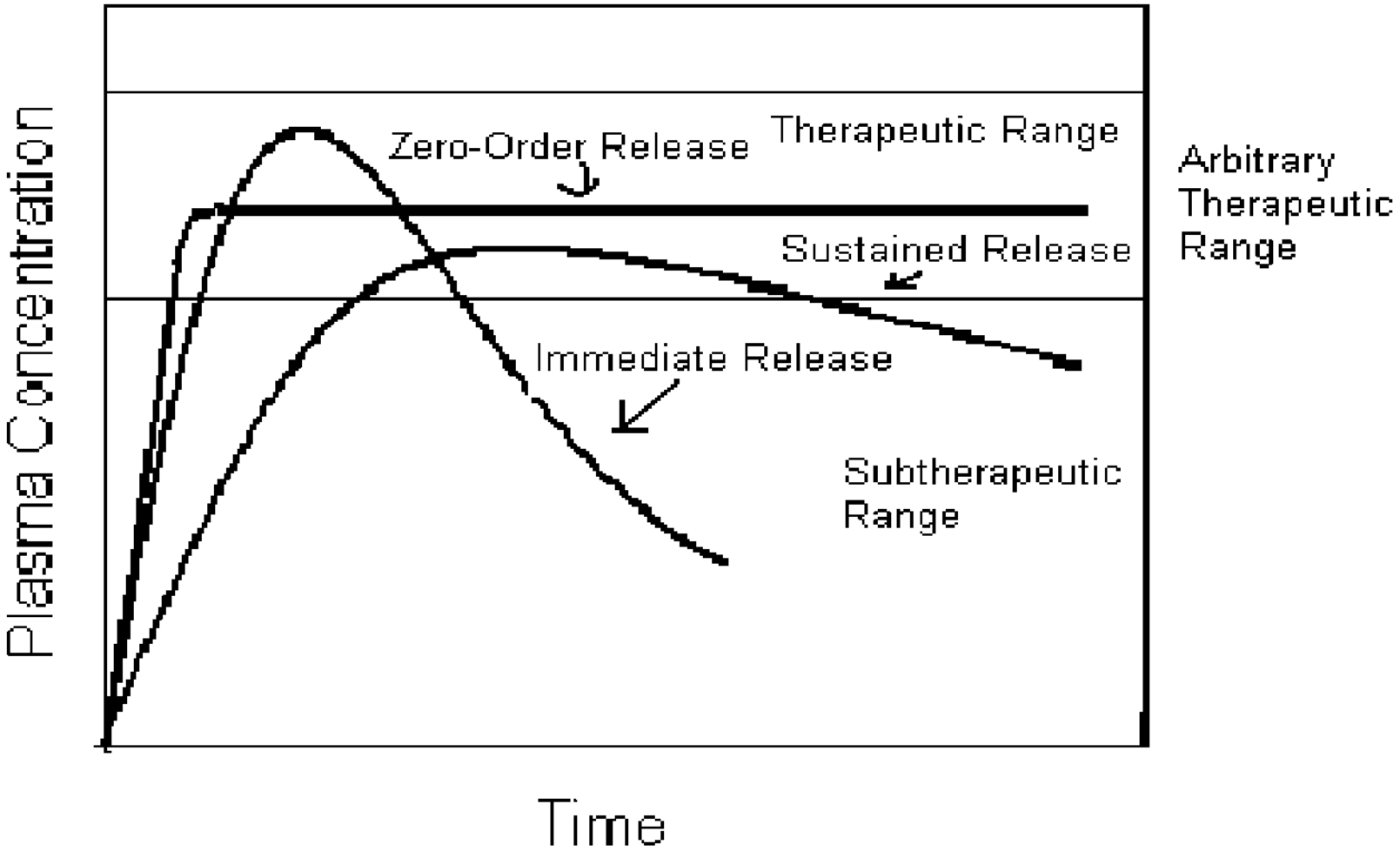
Delayed Release Drug Delivery Systems:

Delayed release systems may not be sustaining but these dosage forms maintains the drug within the dosage form for some time before release.

Targetted Delivery Systems

- *Site-specific systems* and *targeted delivery systems* are those which regulates the release to the specific site or target at a controlled manner so as to improvise the therapeutic efficacy.

- Concept of SR/CRDDS:



Drug level versus time profile showing differences between zero-order controlled release, slow first-order sustained release , and release from a conventional tablet or capsule.

Advantages of Controlled Release Systems:

- Decreased incidence and/or intensity of adverse effects and toxicity.
- Better drug utilization.
- Controlled rate of release.
- More uniform blood concentrations.
- Improved patient compliance.
- Reduced dosing frequency.
- More consistent and prolonged therapeutic effect.
- A great selectivity of pharmacological activity.

Disadvantages of Controlled Release Systems:

- Increased variability among dosage units.
- Stability problems.
- Toxicity due to dose dumping.
- Increased cost.
- More rapid development of tolerance.
- Need for additional patient education and counseling.

Characteristics of Drugs Suitable For Controlled Release:

- Exhibit moderate rates of absorption and excretion.
- Uniform absorption throughout the GIT.
- Administered in relatively small doses.
- Possess a good margin of safety.
- For the treatment of chronic therapy.

Characteristics of Drugs Unsuitable For Controlled Release:

- Not effectively absorbed in lower intestine (Riboflavin).
- Absorbed and excreted rapidly; short biological half lives,<1hr (penicillin G, Furosemide).
- Long biological half lives >12hr (Diazepam, Phenytoin).
- Large doses required, 1g (sulphonamides).
- Drugs with low therapeutic index (phenobarbital, Digoxin)
- Precise dosage titrated to individuals required (Anticoagulants, cardiac glycosides).
- No clear advantage for sustained release formulation (Griseofulvin).

Physicochemical Properties Of A Drug Influencing Drug Product Design and Performance:

- **Aqueous solubility:**

  Tetracycline dissolves to a greater extent in the stomach than in the intestine, although it is best absorbed in the intestine. Hence, poor candidates for controlled release systems, unless the system is capable of retaining the drug in the stomach and gradually releasing it to the small intestine or unless the solubility is made higher and independent of the external environment by encapsulating the drug with an acid.

- **Partition Coefficient and Molecular Size:**

  Drugs with extremely high partition coefficient (very oil soluble) readily penetrate the membranes but are unable to proceed further and vice versa with drugs with very low partition coefficient (excessive aqueous solubility).

- **Drug Stability:**

  Controlled release of Nitroglycerin.

- **Protein Binding:**

  Many drugs bind to plasma proteins. Drug protein binding can serve as depot for the drug producing a prolonged release profiles, especially if a high degree of drug binding occurs.

Biological Factors Influencing Design and Performance of Controlled Release Systems:

- **Absorption:**

  Aminoglycosides such as gentamycin and kanamycin.

  Riboflavin is absorbed by an active transport process in the upper part of the GIT.

- **Distribution:**

  The distribution of drugs into tissues can be one important factor in the overall drug elimination kinetics, since it not only lowers the concentration of circulating drug but it also can be rate-limiting in its equilibration with blood and extracellular fluid.

- **Metabolism:**

  Hydralazine is metabolized by the intestinal wall and/or the liver during absorption, although it is well absorbed.

  In contrast, Bromocriptine is incompletely absorbed, the poor bioavailability of which is further reduced by first pass metabolism in the liver resulting in an absolute bioavailability of only 6%.

- **Duration of Action:**

  For a drug with very short half-life, the desired rate of release will be quite large.

- **Side Effects:**

  A controlled release form of levodopa, lowered the incidence of drug induced dyskinesis and the patients able to tolerate a larger daily dose of the drug.

- **Margin of Safety:**

  Drugs with narrow therapeutic index gives precise drug release patterns so that the plasma concentration achieved is within the therapeutically safe and effective range.

- **Role of Disease State:**

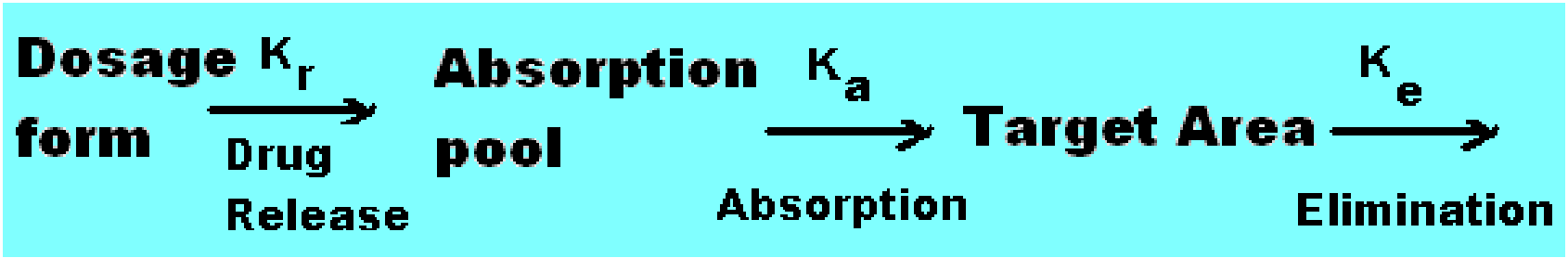
  Controlled release Aspirin tablets in the proper dosage provided and maintained blood levels at therapeutic concentration over 8-10hrs, a duration that was about twice as long as that provided by conventional tablets.

- **Role of Circadian Rhythm:**

  Several biological processes and disease states shown to be influenced by circadian rythms. As a result,the response to certain drugs also follows cicadian rythms.

  Digitalis glycosides, antianginal, diuretics, Psychoactive drugs such as amphetamines, Barbiturates, carbamazepine, ethyl alcohol and chlordiazepoxide.

Design principle of sustained release dosage forms for release rate and dose considerations:





- K_r^o = Rate In = Rate Out = $K_e.C_d.V_d$
- $W = D_i + D_m$
- $W = D_i + K_r^o T_d$
- $W = D_i + K_r^o T_d - K_r^o T_p$
- $W = D_i + (K_e C_d / K_r) V_d$
- $W = D_i + (K_e C_d / K_r) V_d – D_m K_e T_p$

ORAL CONTROLLED RELEASE SYSTEMS:

Controlled Release Systems:

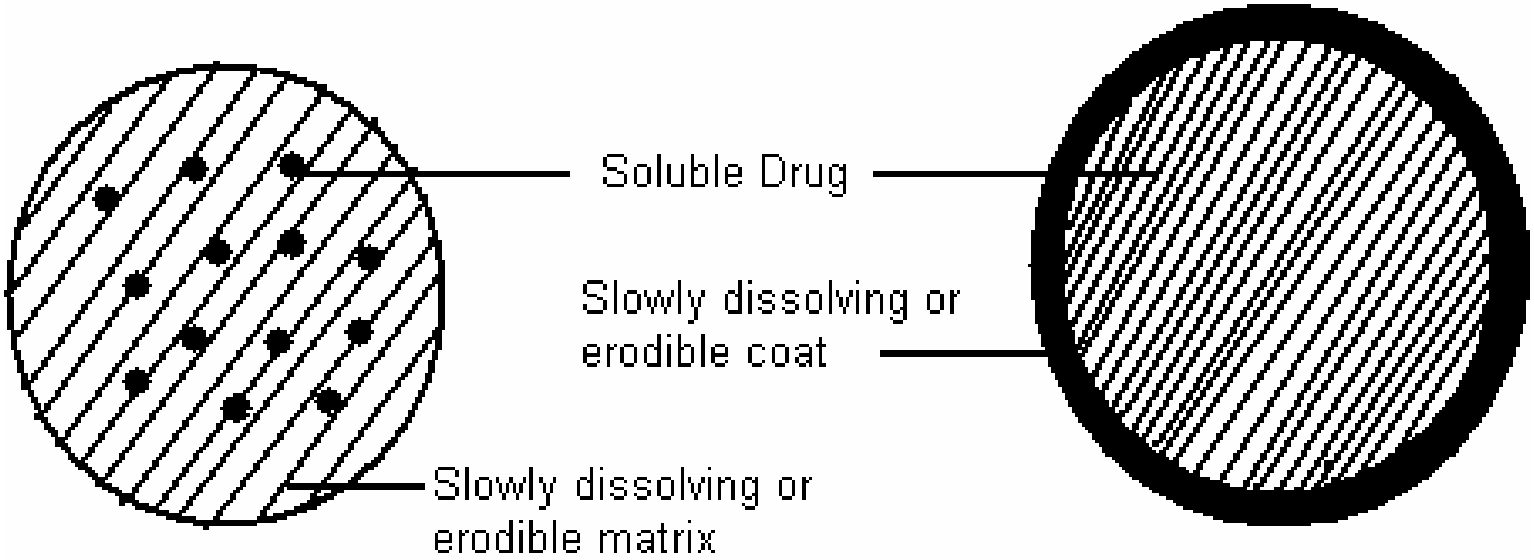
- Dissolution Controlled release systems
- Diffusion Controlled release systems
- Dissolution-Diffusion Controlled release systems
- Ion-exchange resin-drug complexes
- Slow dissolving salts and complexes
- pH-dependent formulations
- Osmotic pressure controlled systems
- Hydrodynamic pressure controlled systems

Delayed Transit and Continuous Release Systems:

- Altered density systems
- Mucoadhesive Systems
- Size-based Systems

Dissolution-Controlled Systems:

Dissolution-controlled systems can be made to be sustaining in several different ways. By altering layers of drug with rate-controlling coats, a pulsed delivery can be achieved.



Schematic representation of dissolution controlled release systems

(a) Matrix Systems, and (b) Coated/encapsulated System

$$dc/dt = K_D A(C_S - C) = D/h\ A(C_S - C)$$

Where,

dc/dt = dissolution rate

K_D = dissolution rate constant

D = diffusion coefficient

C_s = saturation solubility of the solid

C = concentration of solute in the bulk solution

$$W_o^{1/3} - W^{1/3} = K_D t$$

Where,

K_D=Cube-root dissolution rate constant

W_o & W =Initial weight and the weight of the amount remaining at time t, respectively

Diffusional Systems:

Diffusional systems are characterized by the release rate of a drug being dependent on its diffusion through an inert membrane barrier.

Two types of Diffusional systems are recognized:

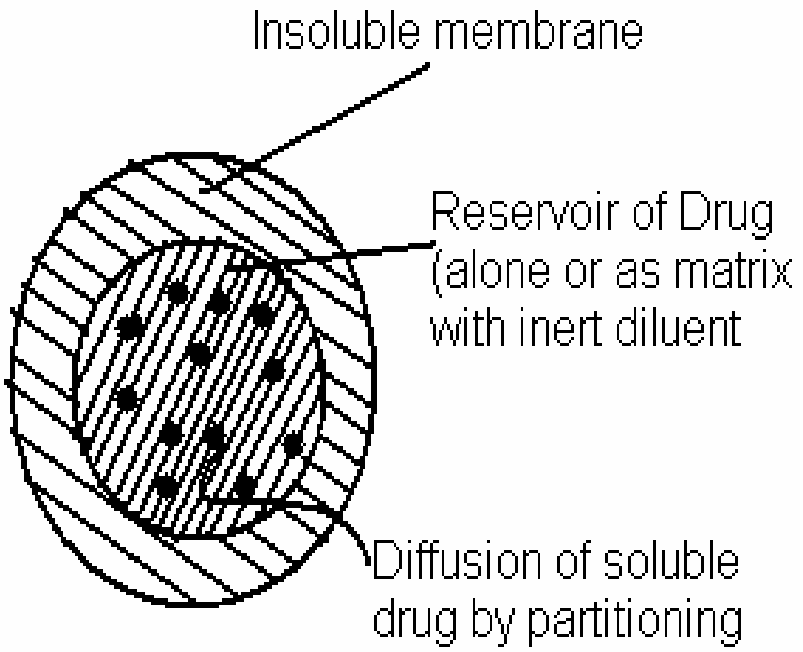
- **Reservoir Devices**
- **Matrix Devices**

Reservoir devices:

- Reservoir devices, as the name implies, are characterized by a core of drug, the reservoir, surrounded by a polymeric membrane.
- The nature of membrane determines the rate of release of drug from the system.
- The process of diffusion is generally described by Fick's law which states that the amount of drug passing across a unit area is proportional to the concentration difference across that plane.

Rate Controlling Step:

Polymeric content in coating,
Thickness of coating,
Hardness of microcapsule



Drug release by Diffusion across the insoluble membrane of the reservoir device

$$J = - D.dC/dX$$

Where,

J = flux (amount /area- time)

D = diffusion coefficient of the drug in the membrane (area/time)

dC/dX = rate of change in the concentration C relative to distance X in the membrane.

NOTE: **D** is a reflection of drug molecule's ability to diffuse through the solvent and is dependent on such factors as molecular size &charge.

$dM_t/dt = ADK\Delta C/d$

where,

M_t = mass of drug released after time t.

dM_t/dt = steady-state release at time t.

A = surface area of the device.

A true Controlled–Release system with a zero-order release rate can be possible only if all of the variables on the right side of the above equation remain constant.

Advantages of Reservoir Diffusional Systems:

- Zero-order delivery is possible.
- Release rate variable with polymer type.

Disadvantages of Reservoir Diffusional Systems:

- System must be removed from implant sites.
- Difficult to deliver high molecular weight compounds.
- Generally increased cost per dosage unit.
- Potential toxicity if system fails

Matrix Devices:

- A matrix device, as the name implies, consist of drug dispersed homogenously through out a polymer matrix.

- The equation which described the rate of release of drugs dispersed in an inert matrix system, have been derived by Higuchi.

- The following equation can be written based on it

$$dM/dh = C_o dh - C_s/2$$

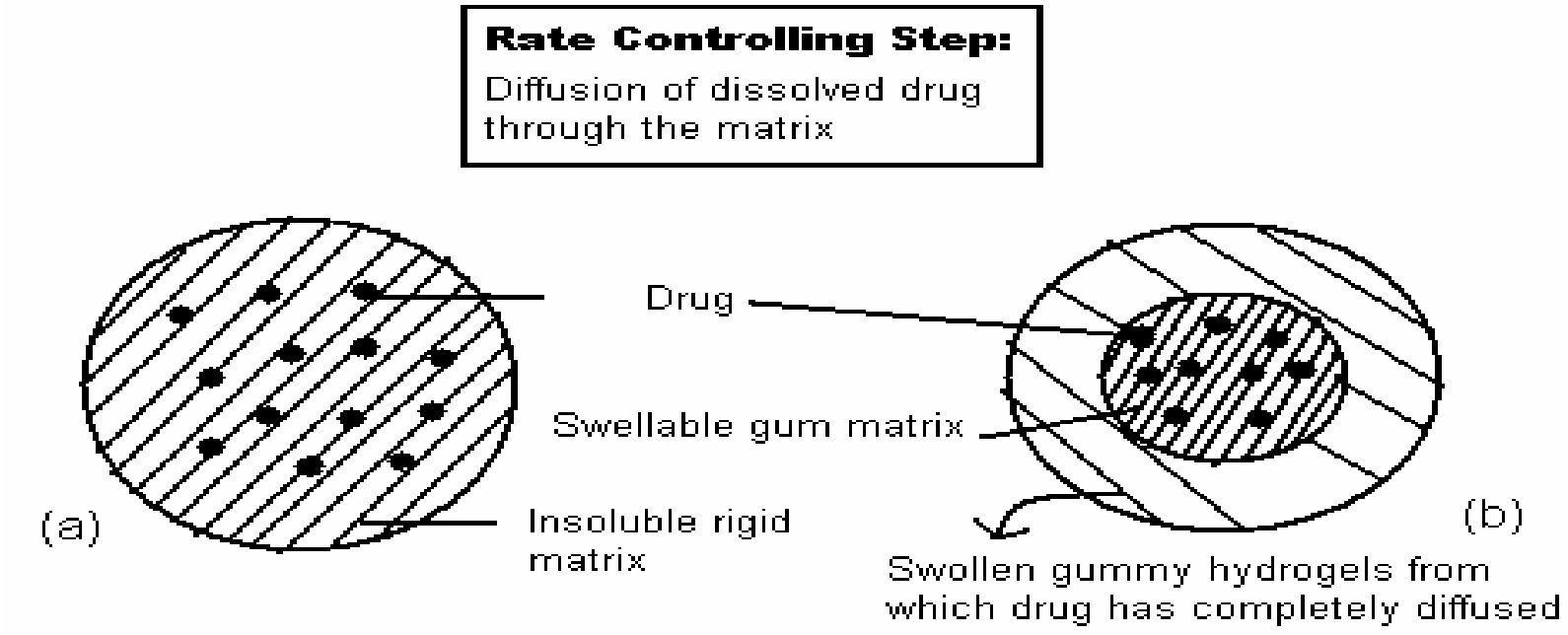
where,

dM = change in the amount of drug released per unit area.

dh = change in the thickness of the zone of matrix that has been depleted of drug .

C_o = total amount of drug in a unit volume of the matrix.

C_s = saturated concentration of drug within the matrix.



Diffusion Controlled Devices

(a) Rigid matrix, and (b) Swellable matrix

- From diffusion theory,

$$dM = D_m C_s / h.dt$$

Where,

D_m= diffusion coefficient in the matrix

$$M = kt^{1/2}$$

Where,

k = constant

Advantages of Matrix Diffusion Systems:

- Easier to produce than reservoir devices.
- Can deliver high molecular weight compounds.

Disadvantages of Matrix Diffusion Systems:

- Cannot obtained zero-order release .
- Removal of remaining matrix is necessary for implanted systems.

Bioerrodible and Combination Diffusion and Dissolution Systems:

$$M_t/M = 1 - (1 - k_o t/C_o a)^n$$

Where,

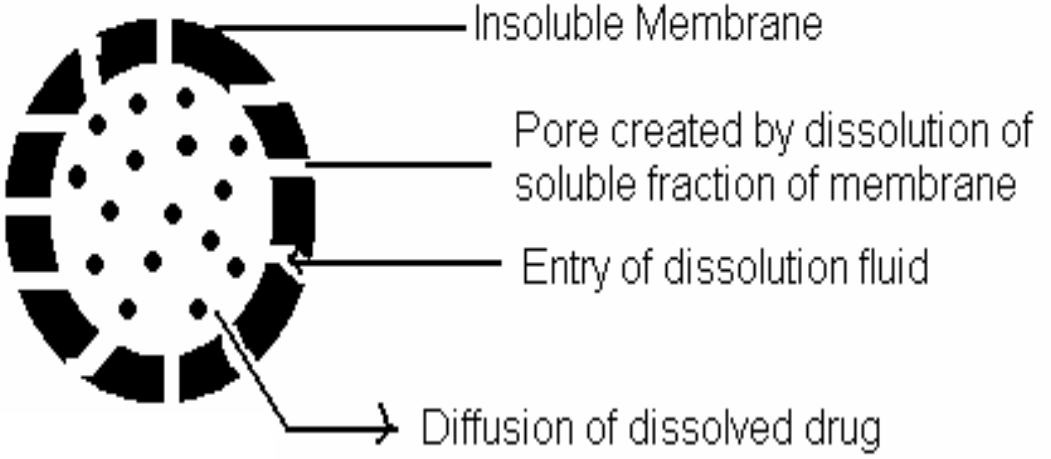
n =1, for a slab

n = 2, for a cylinder

n = 3, for a sphere

Rate Controlling Factor:

Fraction of soluble polymer in the coat



Dissolution and Diffusion Controlled Systems

Advantages of Bioerrodible Matrix Systems:

- Easier to produce than reservoir devices.
- Can deliver high molecular weight compounds.
- Removal from implant sites not necessary.

Disadvantages of Bioerrodible Matrix Systems:

- Difficult to control kinetics owing to multiple processes of release.
- Potential toxicity of degraded polymer must be considered.

Ion-exchange systems:

- Ion-exchange systems generally use resins composed of water-insoluble cross-linked polymers.
- These polymers contain salt-forming functional groups repeating positions on the polymer chain.
- The drug is bound to resin and released by exchanging with appropriately charged ions in contact with the ion-exchange groups.

$$\mathbf{Resin^{+} - drug^{-} + X^{-} \rightarrow resin^{+} - X^{-} + drug^{-}}$$

$$\mathbf{Resin^{-} - drug^{+} + Y^{+} \rightarrow resin^{-} - Y^{+} + drug^{+}}$$

Osmotically Controlled Systems:

In this systems, osmotic pressure provides the driving force to generate controlled release of drug.

The rate of flow, dV/dt, of water into the device can be represented as

$$\mathbf{dV/dt = Ak/h(\Delta\pi - \Delta P)}$$

Where,

k = membrane permeability

A = area of the membrane

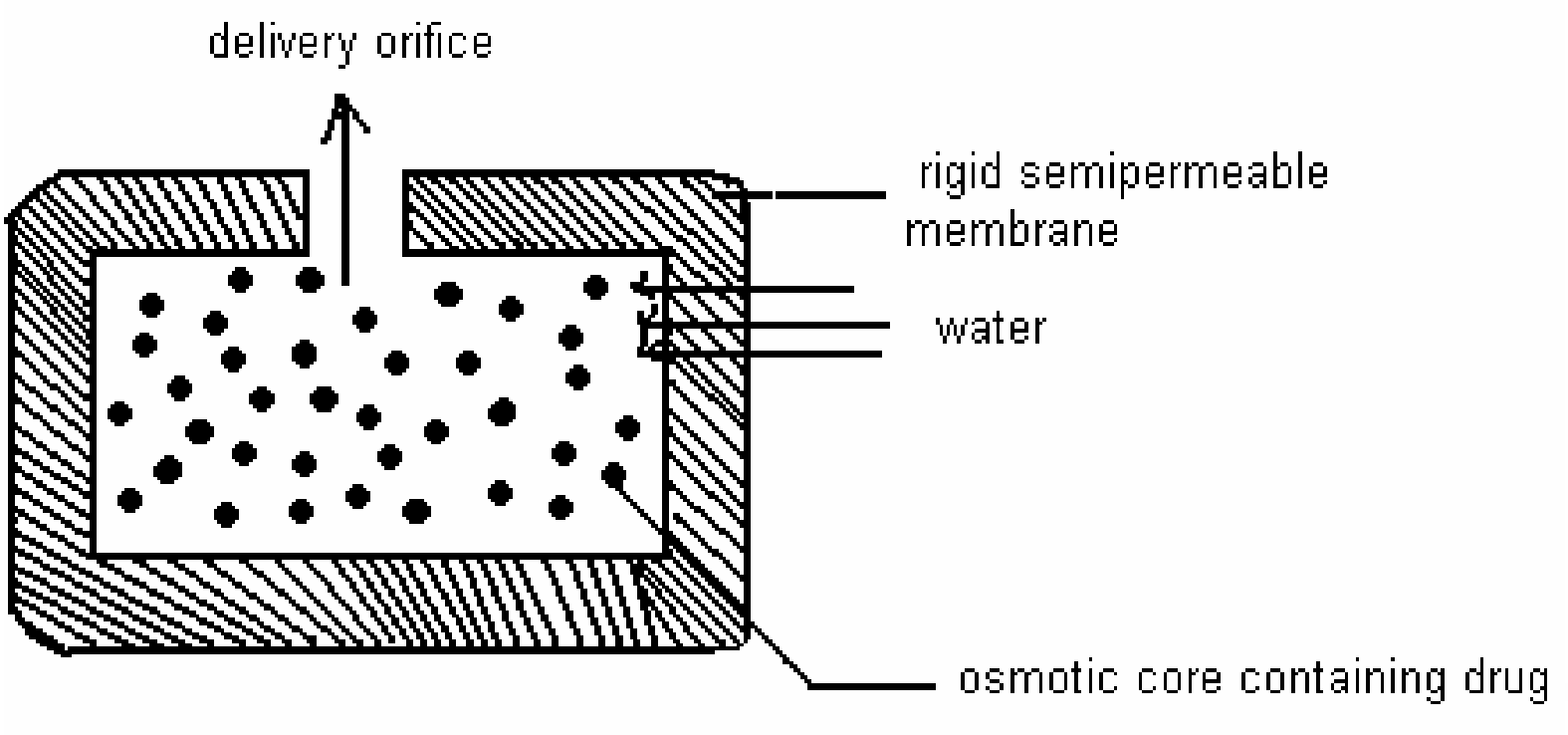
h = membrane thickness

Δπ = osmotic pressure difference

ΔP = hydrostatic pressure difference

Rate Controlling Factors:

Orifice diameter
Membrane area
Membrane thickness
Membrane permeability
Osmotic properties of the core
Drug solubility



Oral Osmotic Pump (oros)

Advantages of osmotically controlled devices:

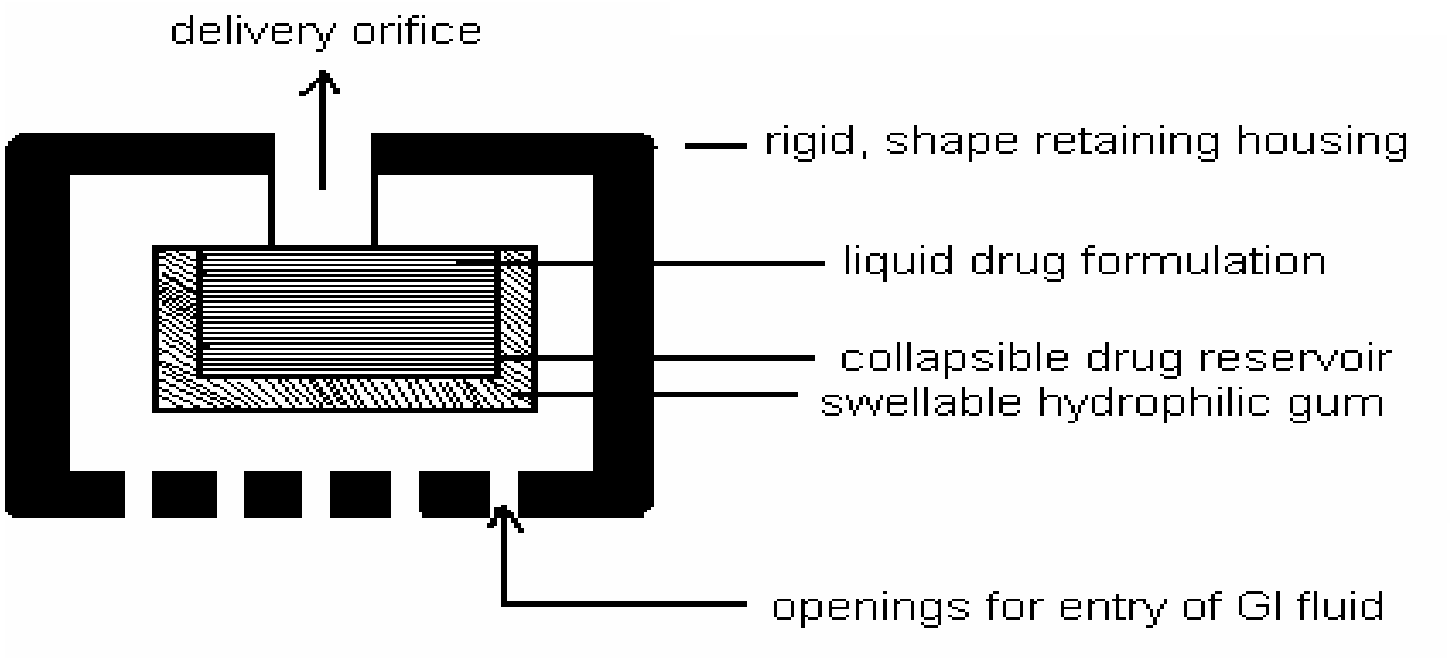
- Zero-order release obtainable.
- Preformulation not required for different drugs.
- Release of drug independent of the environment of the system.

Disadvantages of osmotically controlled devices:

- Systems can be much more than expensive than conventional counterparts.
- Quality control more extensive than most conventional tablets.

Hydrodynamic Pressure Controlled Systems

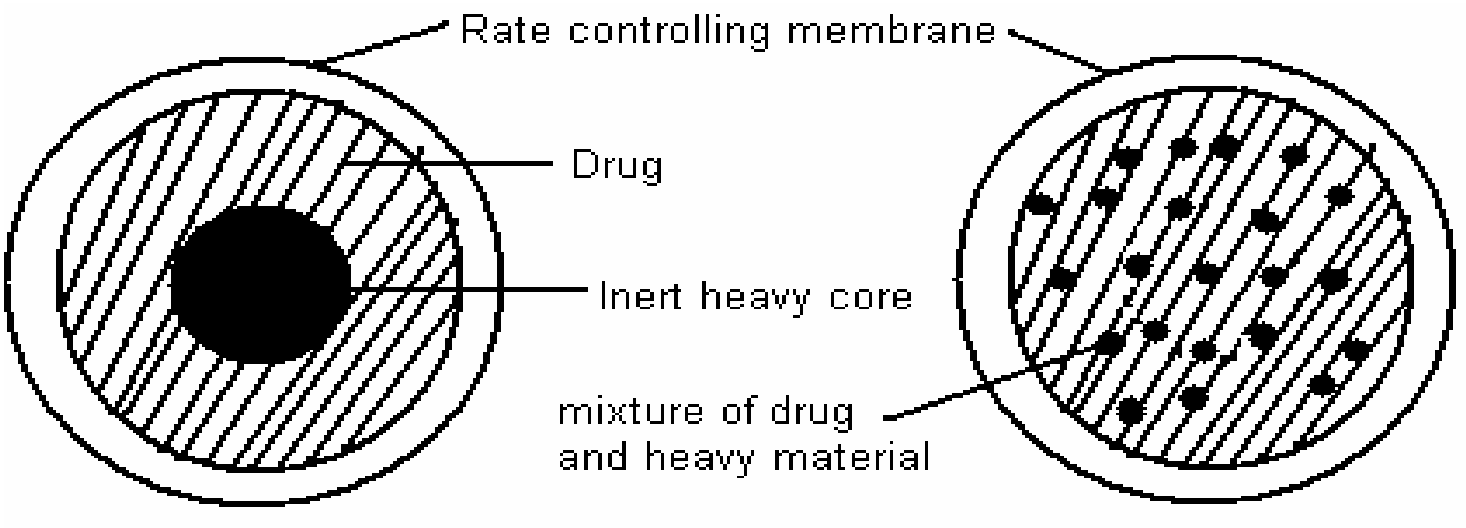
Rate Controlling Factors:
Fluid Permeability
Surface area of wall with openings
Hydrodynamic pressure gradient



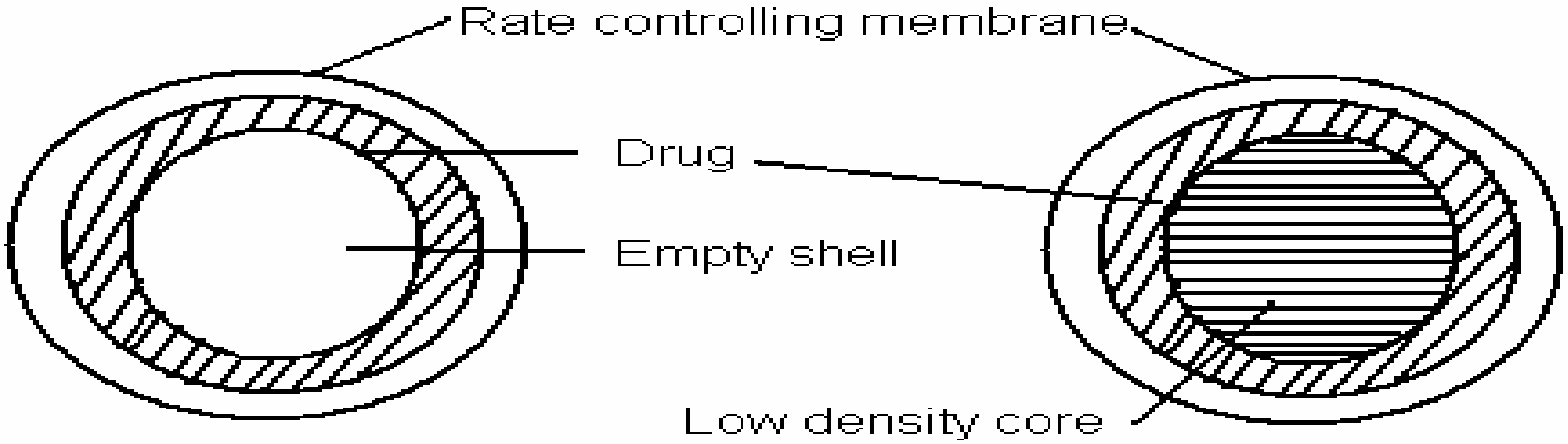
Hydrodynamic Pressure Controlled Systems

(Push-pull Osmotic Pump)

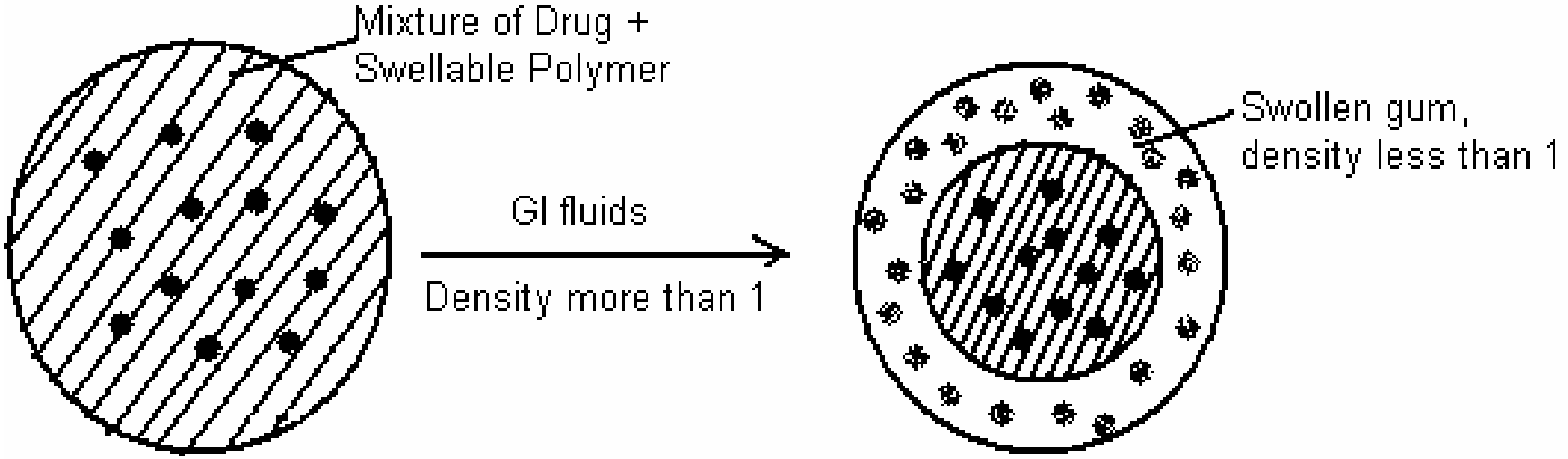
- Altered Density Systems:



High Density Pellets



Low Density Pellets with Hollow Core



Low Density Pellets with Hydrogels

Others

- Slow Dissolving Salts and Complexes
- pH-Independent Formulations
- Floating or Buoyant Tablets/Capsules

Thank you

PARENTERAL CONTROLLED RELEASE DRUG DELIVERY SYSTEMS

D.Mallelatha

VCPS

INDEX

- Introduction.
- Routes of parentral administration.
- Biopharmaceutics of SR/CR parentral products.
- Different approaches.
- Evaluation tests.

- The Parenteral administration route is the most common and efficient for delivery of active drug substances with poor bio-availability and the drugs with a narrow therapeutic index.
- Though parenteral route offers rapid onset of action in results in rapid declines of systemic drug level.
- It requires frequent injection, which ultimately leads to patient discomfort.
- For the sake of effective treatment it is often desirable to maintain systemic drug levels within the therapeutically effective concentration range for as long as treatment calls for.

ADVANTAGES:

- Wide variety of drugs can be formulated as PDDS.
- Most of the PDDS are biocompatible with vascular system
- The possible biotransformation reactions encountered by several drugs after oral administration can be minimized by this approach.
- Targeting of several drugs particularly anti-Neoplastic drugs to specific site and also to maintain desired therapeutic conc. Is achieved.
- Prolonged residence of certain drugs at a specific site can be made possible by using suitable parenteral approach.
- Biotransformation and excretion loss can be minimized so that the dose required for parenteral administration can be reduced.
- Maintaining therapeutic concentrations over a longer period of time.

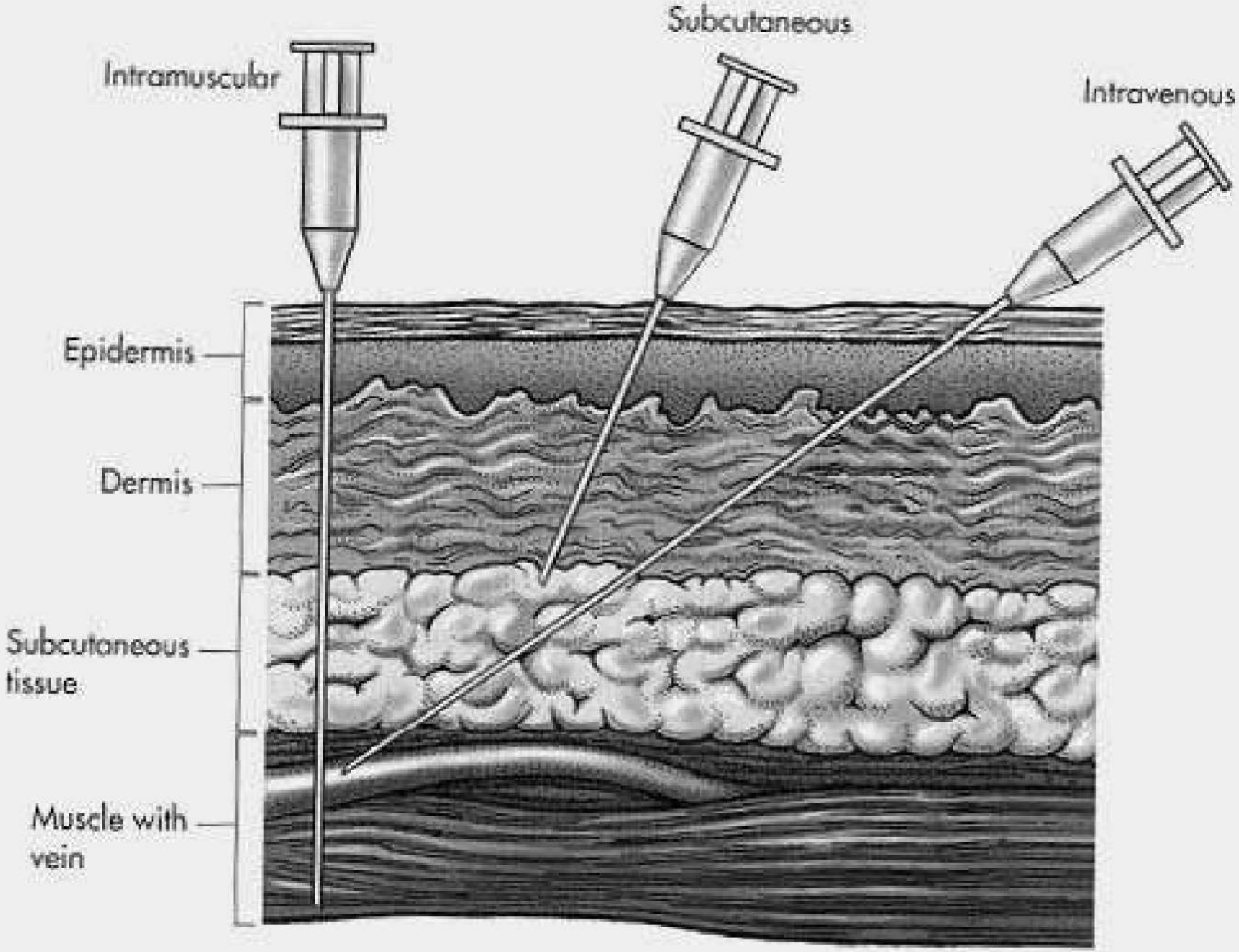
DISADVANTAGES:

- Patient compliance is less.
- Withdrawal of the dose is not possible.
- Self administration is not possible.
- Administration requires strict adherence to aseptic procedures, and some pain on injection.
- The manufacturing and packaging requirements, are more expensive than preparations of given by other routes.

routes of administration

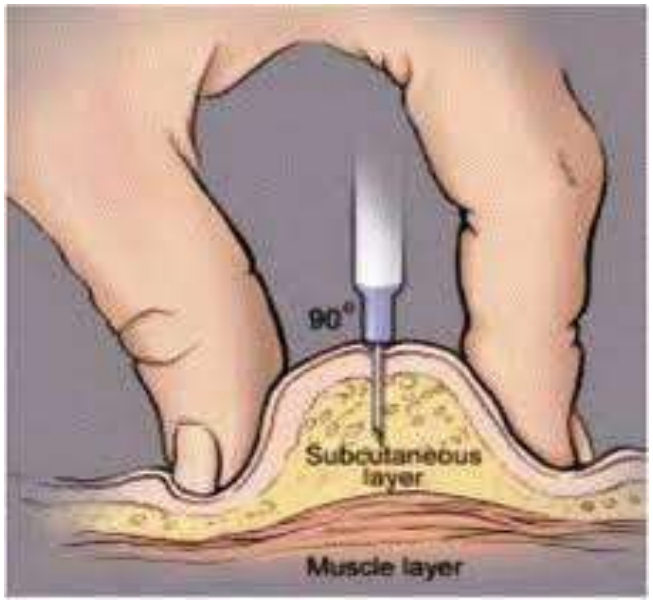
The major routes of parentral administration include :

1. Subcutaneous administration.
2. Intramuscular administration.
3. Intravenous administration.
4. Intraperitoneal administration.


Intramuscular
Subcutaneous
Intravenous
Epidermis
Dermis
Subcutaneous tissue
Muscle with vein

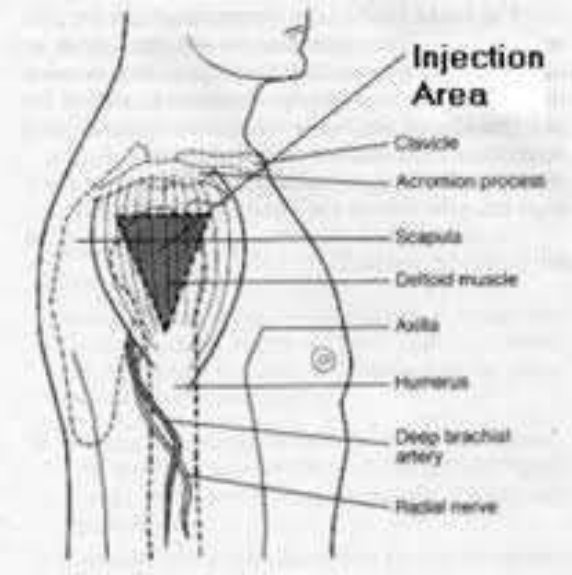
Subcutaneous route:

- Poorly perfused with blood.
- Limited to drugs that are non-irritating , water soluble and well absorbed.
- For chronically administered drugs injection site must be rotated.
- Volume restricted is 0.5 - 1.5ml.



Intramuscular route:

- Gluteal, deltoid and vastus lateralis muscles are the sites.
- Injected deep into muscle and away from nerves and arteries.
- Volume must not be more than 2ml.





Intravenous route:

- Used for SR/CR dosage forms such as Liposomes, nanoparticles, erythrocytes and polypeptides.

Drug particle size (μm)	Target site
> 1	lung
0.1 – 7	liver/spleen
< 0.1	bone marrow

Advantages :

• precise, accurate and immediate onset of action,100% bioavailability.

Disadvantages :

•risk of embolism.
•high concentrations attained rapidly leading to greater risk of adverse effects.

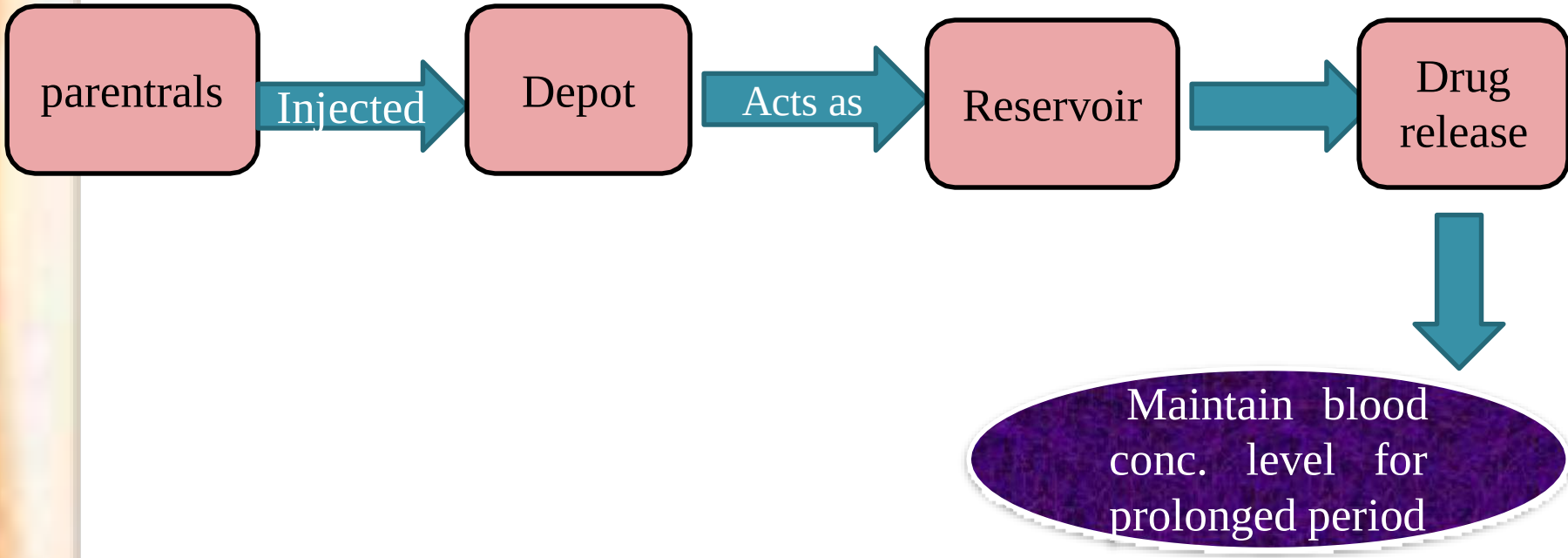
Intraperitoneal route:

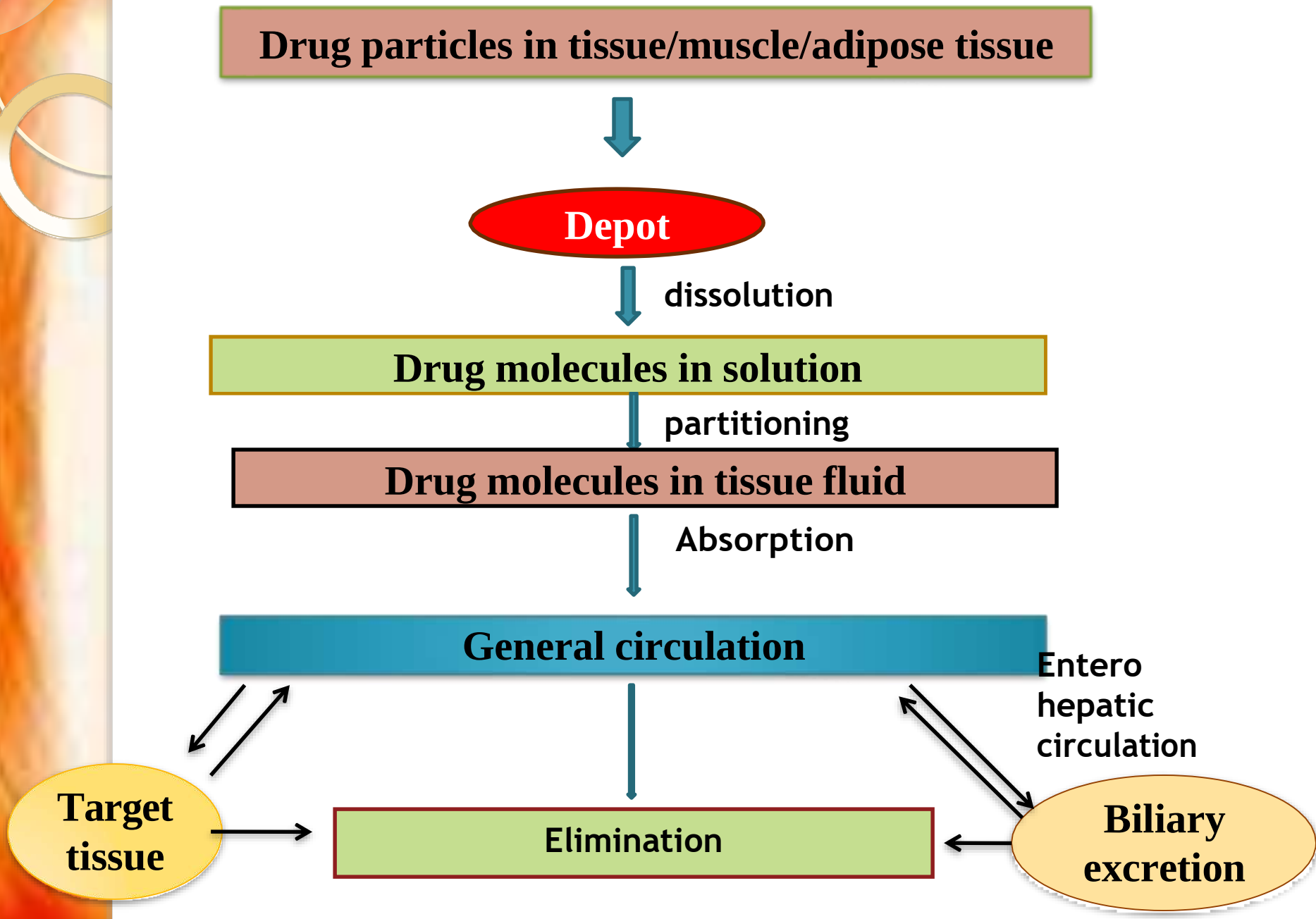
- Macromolecules administered through this route gain direct access into lymphatic system.
- Tumors are metastatized majorly by the route of lymphatic channels.
- Advantageous to target antineoplastic drugs.

S. No	Route Of Administration	Site Of Injection	Injection Volume &Needle Size	Examples
1.	Intra muscular(I.M)	gluteal, deltoid and Vastus lateralis muscles	NMT 2ml 22 size	For less soluble drugs Butorphanol tartrate,contraceptives
2.	Subcutaneous (S.C)	Adipose tissue	0.5-1.5 ml 24-25size	non-irritating, water-soluble drugs eg:insulin,vaccines, vit B12
3.	(Intravenous (I.V) Less common for C.R)	Veins	</=100ml 18-22size	liposomes, nanoparticle, erythrocytes, and polypeptides
4.	Intra peritoneal (I.P)	Lymphatic system	1ml 23 size	Antineoplastic agents into the lymphatic system.

biopharmaceutics

MECHANISM:


parentrals
Injected
Depot
Acts as
Reservoir
Drug release
Maintain blood conc. level for prolonged period


Drug particles in tissue/muscle/adipose tissue
Depot
dissolution
Drug molecules in solution
partitioning
Drug molecules in tissue fluid
Absorption
General circulation
Entero hepatic circulation
Target tissue
Elimination
Biliary excretion





formulation



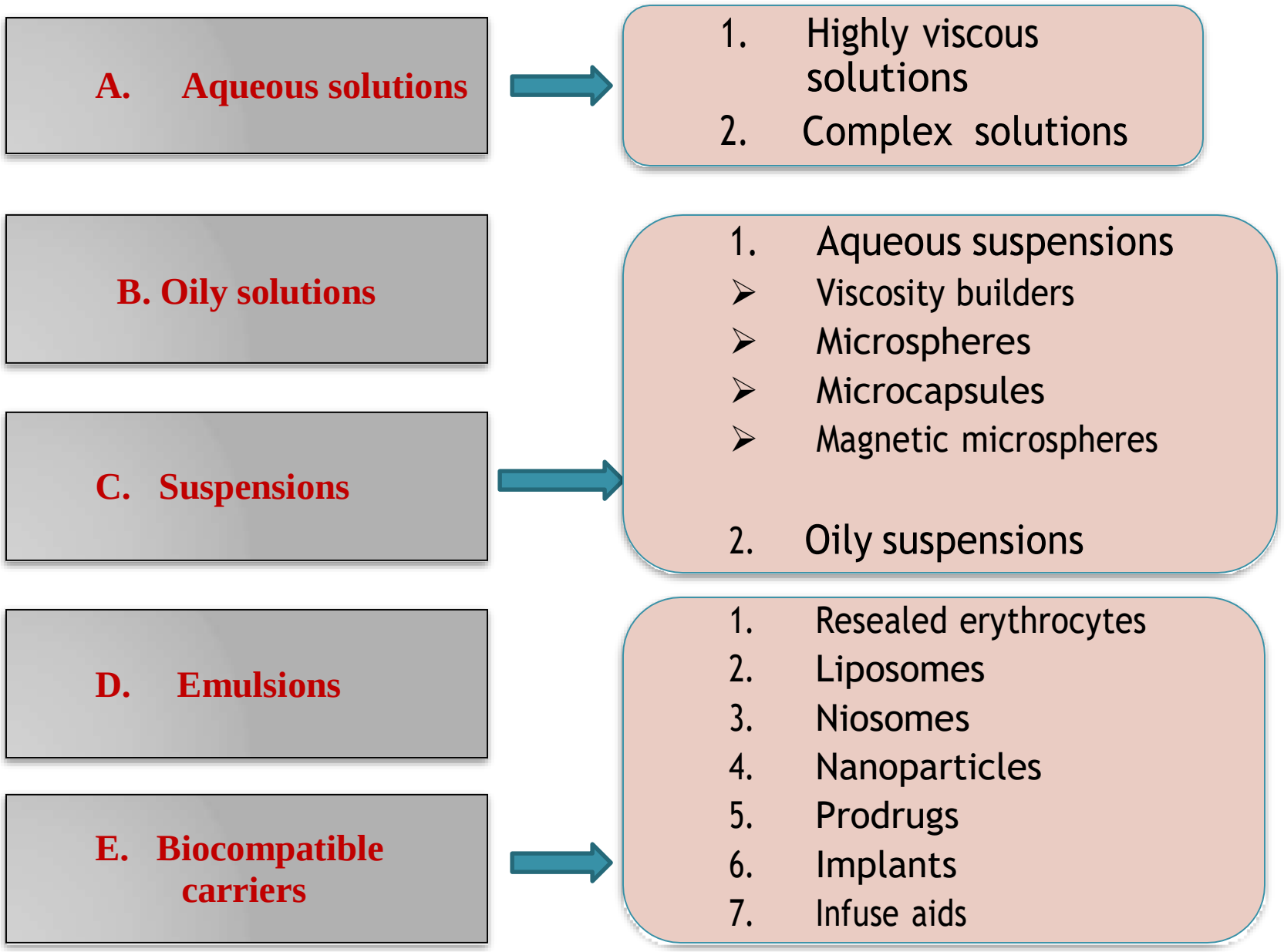
Formulation ingredients:

Excipients	Functions	IV	SC/IM
Surfectant	Particle stabilization	+	+
Buffer	pH adjustment and control	+	+
Polysaccharides	Viscosity enhancement	-	+
Sugars	Tonicity adjuster and/or lyoprotectants	+	+
Additional polymers	Bioadhesives, matrices for sustained release	-	+
Preservatives	Preservatives for multidose products	-	+
Chelating agents	Scavenging of metal ions (depend on drug stability)	+	+

IV - intravenous; SC - subcutaneous; IM - intramuscular

different approaches

CLASSIFICATION OF PCDDS



AQUEOUS SOLUTIONS:

1. HIGH VISCOSITY PRODUCTS

- By increasing the viscosity of the vehicle, the diffusion coefficient of the drug is reduced hence the drug transfer is delayed.

- Methylcellulose, Sodium CMC, PVP are some viscosifying agents.

- Incorporation of gelling agents like aluminum monostearate into oil solutions causes the reduction in absorptive area and the rate of drug transfer is better controlled.

- Diffusivity plays a major role in the release of the drug from viscous reservoir.

- Low mol.wt compounds have high diffusivity.

- Drugs with mol.wt > 750 daltons fails to undergo diffusion.

2. COMPLEX FORMATION

- Principle involved is similar to that of plasma protein binding and tissue binding in prolonged drug action.

- Polyvinylpyrrolidone, methylcellulose, sodium CMC are the macromolecules used for complex formation.

- Delay in the drug absorption occurs if the drug molecules undergo complexation with macromolecules.

- rate of drug release from the complex is expressed as

$$d[c]/dt = -k\, f\, [c]$$

where, k = release rate constant.
f = fraction of drug that is freely available.
c = total conc. of drug in dosage form.

- Another mechanism for delaying the drug release is using caffeine micromolecules which lower the solubility of the drug and achieve the sustained release.

Ex: Acetaminophen forms 1:1 complexes with theophylline and caffeine.

- Long chain molecules having micro molecules can also be used to extend drug release

Ex:

1. Tannic acid forms complex with insulin to give zinc insulin tannate.
2. cyanacobalamine forms complex with tannic acid to give cyanacobalamine zinc tannate.

OILY SOLUTIONS:

- Cotton seed oil, linseed oil etc., are some of the oils used as vehicles.

- Drugs that are soluble in oils are preferred mostly in the formulation of oily solutions.

- Drugs inherent partition coefficient play a major role in the release of the drug

- Utricaria, Allergic dermatitis, lipoidal utricaria are some of the problems encountered and hence usage is minimized.

SUSPENSIONS:

1. AQUEOUS SUSPENSIONS

- Suspension gives a longer duration of action than an aqueous solution when given iv/sc.
- Here the drug is in finely divided soluble state, it has to undergo dissolution and then the action is achieved.
- The dissolution rate of drug can be described by modified Noyes-Whitney equation

$$\frac{dc}{dt} = \frac{DAC}{h}$$

where D = diffusion coefficient of the drug
A = surface area
C = conc. of the drug
h = viscosity of external phase.

❖ Precautions to be followed in the formulation of parentral aqueous suspension:

i. solid particulate component should be restricted to 0.5 – 5% w/v.
ii. size of particulate matter should be 0.1 – 10μ in size.

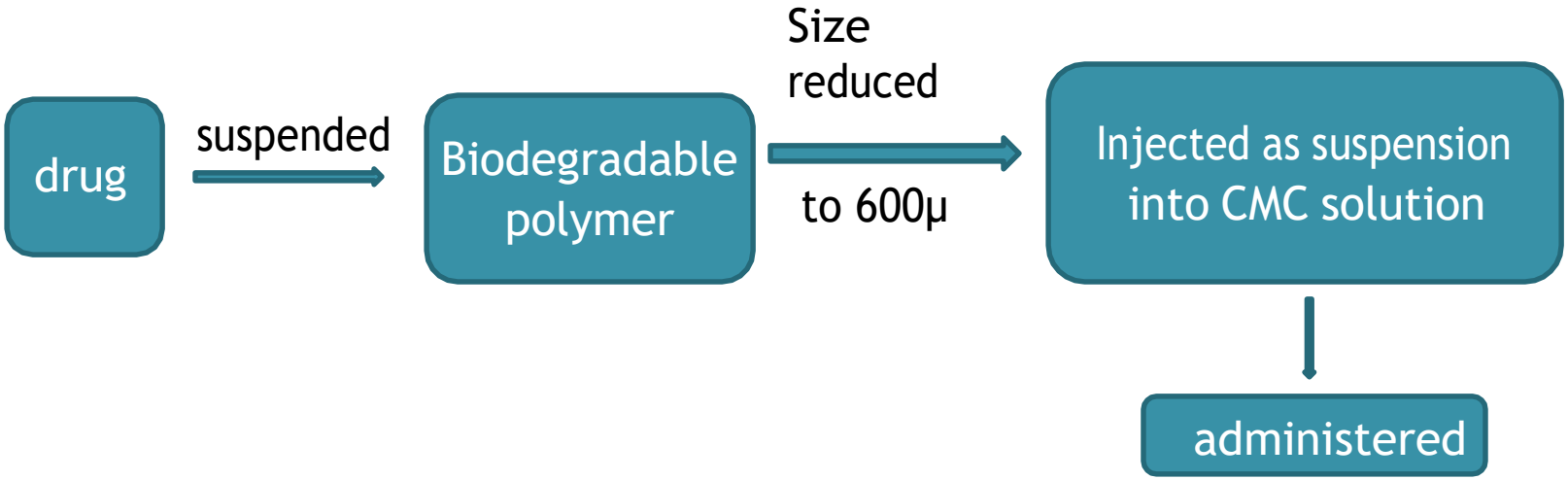
1. USE OF VISCOSITY BUILDER

- Used to increase the vehicle viscosity and reduce the diffusion coefficient of the drug.

- Methyl cellulose, sodium CMC, polyvinylpyrrolidone are some of the viscosity builders.

2. MICROSPHERES :

- Spherical shaped particles containing the drug in solid or liquid state enclosed or embedded into polymeric matrix system.



- Polymers used are polygalactin, polylactic acid, etc.,

❖ Drug release mechanism:

➢ If it is matrix.
➢ If it is continuous polymer sheet.

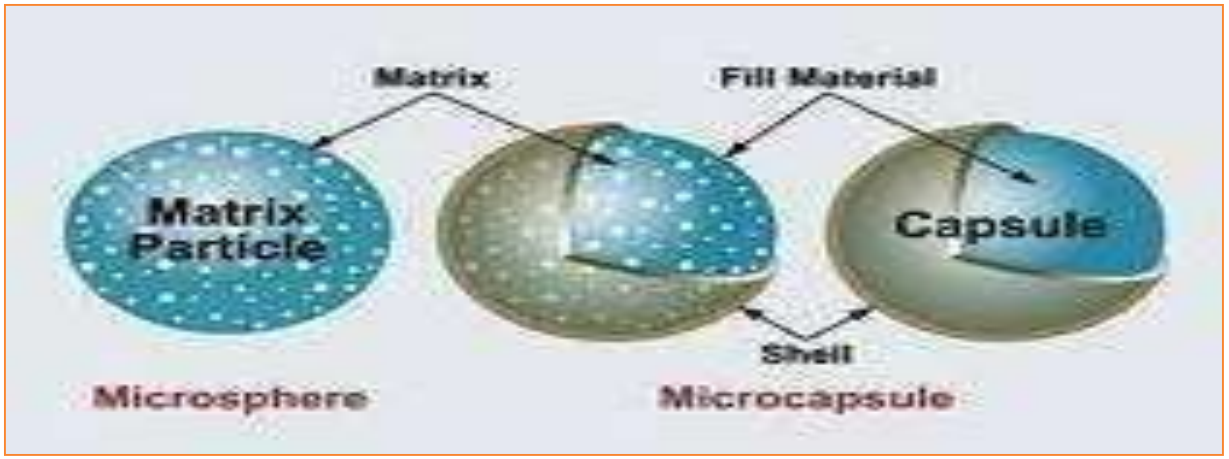
3. MICROCAPSULES :

- Microcapsules are spherical particles containing drug concentrated in the core.

- Microencapsulation method is used to encase particles of gases, liquids or solids.

- Polymers used are nylon, dipolylactic acid, albumin, etc.,

Microspheres and Microcapsules



Microspheres

Drugs: Narcotic antagonists
Antineoplastic agents

Polymers: Polygalactin,
Polylacticacid

Release mechanism:
Diffusion controlled

Microcapsules

Drugs: Antineoplasticagents,
Steroid hormones

Polymers: Nylon,
Dipolylactic acid
Crosslinked starch

Release mechanism: Both dissolution &diffusion

4. MAGNETIC MICROSPHERES

Uses :

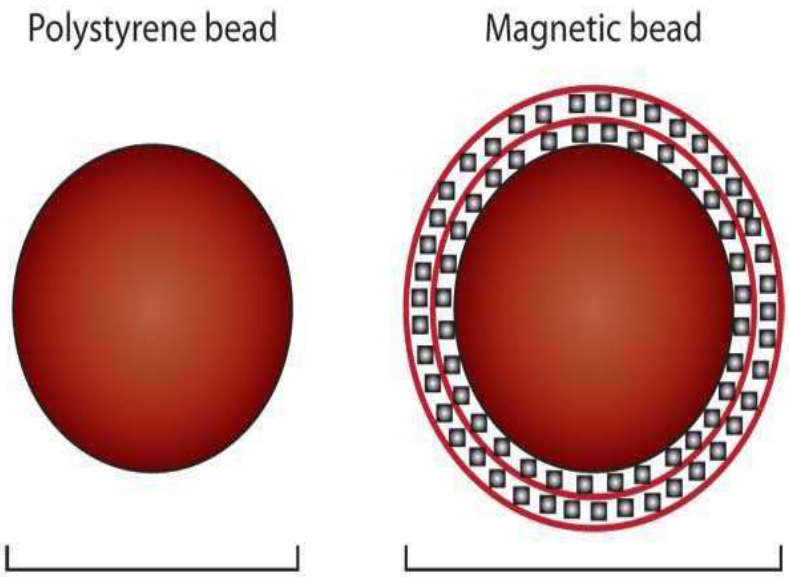
- To increase target specificity
- Used to entrap wide variety of drugs
- Prepared from albumin and magnetite
- Particle size-1μm

Route: **i.v**

Advantages :

nontoxic and non reactive

Eg: Doxorubicin



List of marketed microspheres

Drug	Commercial name	Company
Risperidone	Risperdal® consta®	Janseen®/Alkermes, Inc
Naltrexone	Vivitrol®	Alkermes
Octreotide	Sandostatin®LAR	Novartis
Minocycline	Arestin®	Orapharma

2. OILY SUSPENSIONS:

- Vehicle used is oil phase.

- These are prepared by dissolving drug in aq. phase 1st and then dispersed into oil phase.

Drug release mechanism:

- Involves combined mechanism of oily solution and suspension.

- First the drug is partitioned from aq. Phase to oily phase and diffusion occurs from oily phase.

EMULSIONS

- Emulsions may be classified into:

  o/w type emulsions.

  w/o type emulsions.

  Multiple emulsions(o/w/o, w/o/w)

  Micro emulsions.

1. o/w type emulsions.

- o/w type of emulsions are mostly used for parenterals.
- Prepared by dissolving drug in the oil phase and then dispersed into aq. Phase with the help of emulsifying agent.

Mechanism:

•Partition of drug from oil phase to aqueous phase.

2. w/o type emulsions:

- Prepared by dissolving drug in the aq.phase and then dispersed into oil Phase with the help of emulsifying agent.

Mechanism:

- Partition of drug from aqueous phase to oil phase.

3. o/w/o type emulsions:

Mechanism:

- partitioning of the drug from the oil phase into aq. Phase and from the aq. Phase into oil phase and finally diffusion of the drug takes place.

4. w/o/w type emulsions

BIO COMPATIBLE CARRIERS :

NIOSOMES :

- Niosomes are nonionic surfactant vesicles obtained on hydration of synthetic nonionic surfactants of the alkyl or dialkylpolyglycerol ether class, with or without incorporation of cholesterol or other lipids.

Applications:

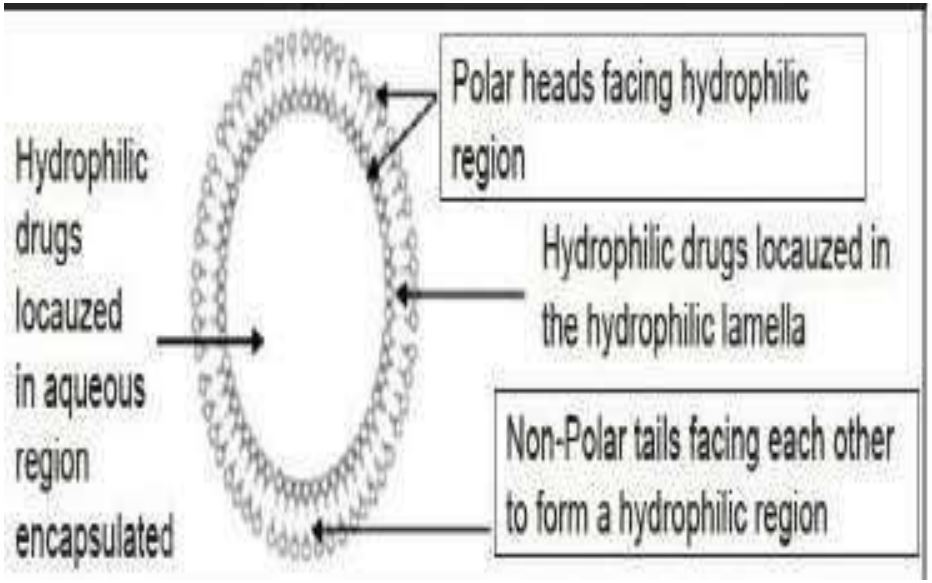
- Anticancer
- Target sites (spleen ,liver)

Drug loading techniques:

1) Passive trapping.
2) Active trapping.

Preparation of niosomes

1. Ether injection method.
2. Hand shaking method.
3. Sonication.



LIPOSOMES :

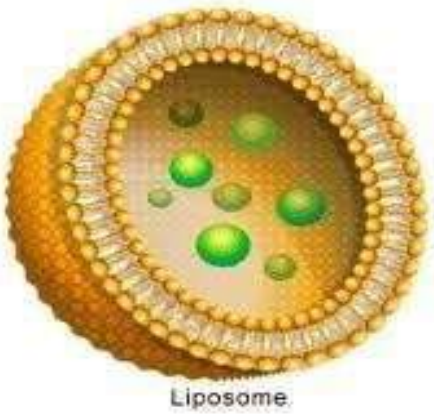
- Vesicles of lipid bilayer enclosing an aqueous compartment.

Routes of administration: I.T, S.C, I.M.

Advantages: versatile , nontoxic

Disadvantages: high production cost, leakage of drug, short half life and low solubility

Brand	Generic	Route	Indication
Ambisome	Amphotericin B	Intravenous	Antifungal
Depocyte	Cytarabine	Intrathecal	Antineoplasti
DaunoXome	Daunorubicin	Intravenous	Antineoplasti
Doxil	Doxorubicin	Intravenous	Antineoplasti

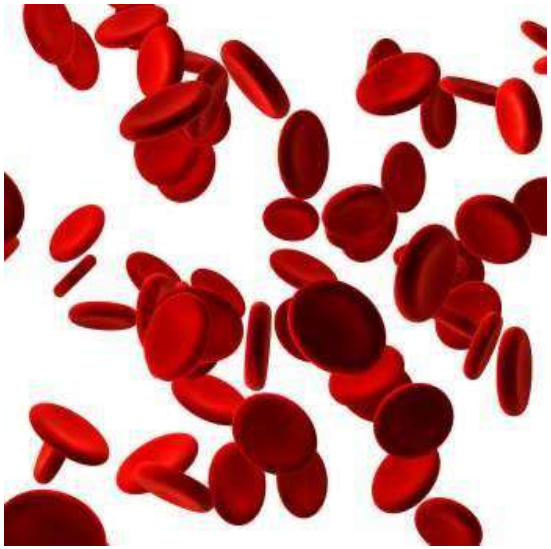

Liposome

RESEALED ERYTHROCYTES :

- Drug is loaded in body's own erythrocytes for controlled release.

➢ Advantages:

- Biocompatible and bio degradable.
- Can load large amounts of drug.
- targeting the drug to the organs.
- No drug exposure to non target cell



➢ Release patterns:

1. Phagocytosis
2. Diffusion through membrane

➢ Applications:

- Treatment of lysosomal diseases like gaucher disease
- Treatment of liver tumors

IMPLANTS :

➢ Implants are the devices which when administered into layers of skin by incision/microsurgery expected to release the drug over prolonged period of time

route of administration : S.C

Drug release: diffusion or dissolution or both mechanisms

Polymers generally employed:

- Polydimethyl siloxane
- Polycaprolactone
- Polylactic acid
- Polyglycolic acid

Biodegradable polymers

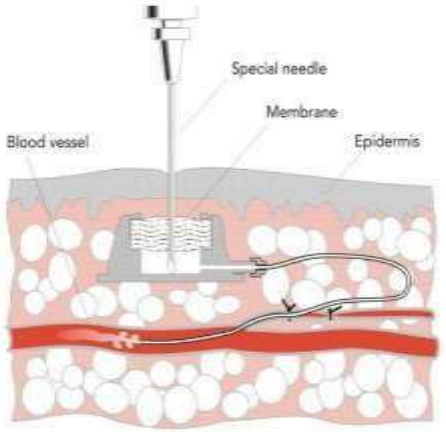


Figure 9-14 Implanted infusion port

NORPLANT

- Polymer: Silicone
- Eg: a subdermal implant to deliver levonorgestrel.

PROGESTRASERT

- Reservoir type system
- Drug reservoir: progesterone
- Polymer: Ethylene vinyl acetate copolymer

OCUSERT

- Membrane controlled system
- Drug reservoir: Pilocarpine alginate
- Treatment : glaucoma.
- Polymer: ethylene vinyl acetate co polymer

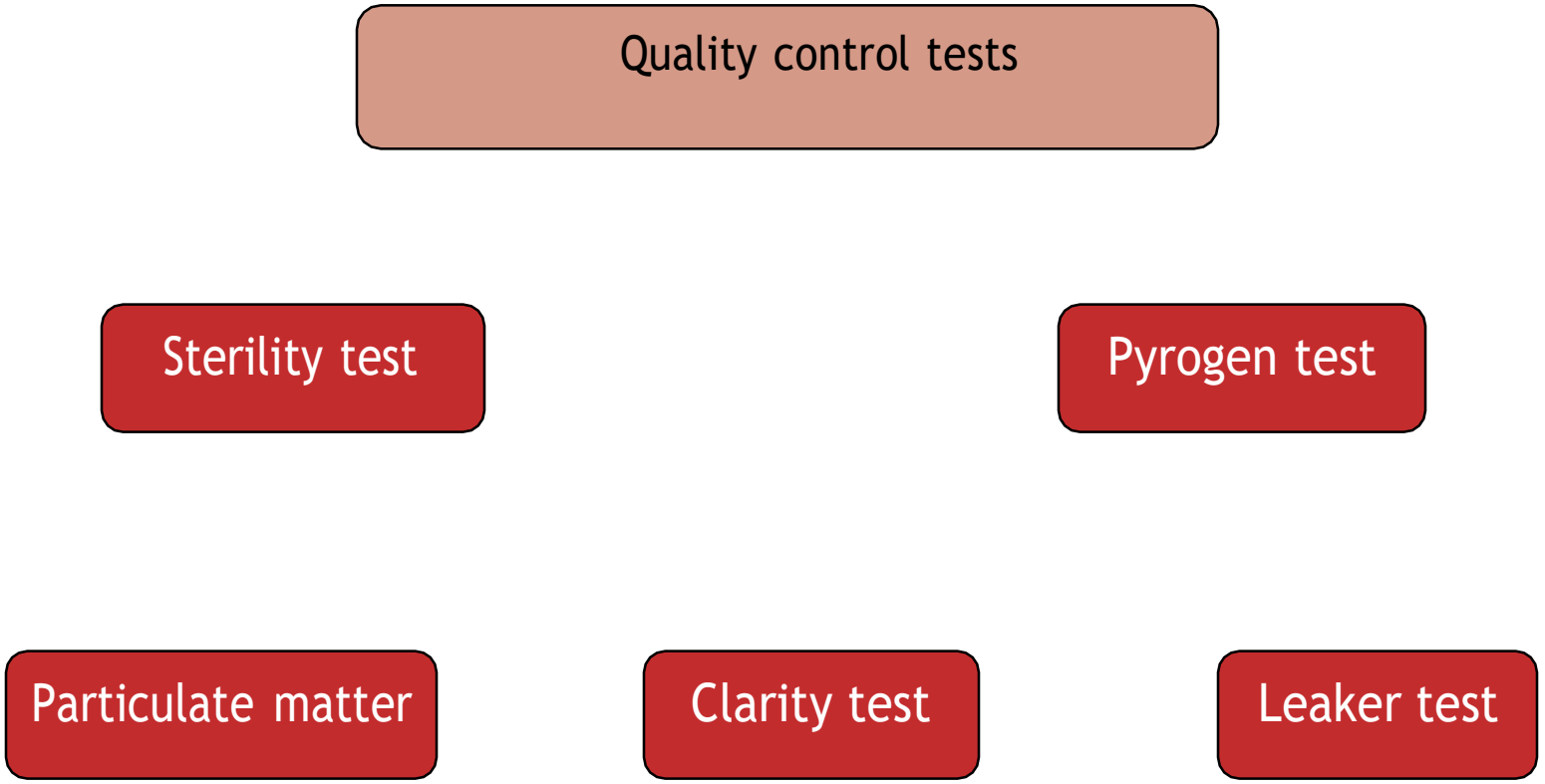
PRODRUGS :

- Prodrug approach is useful to improve bioavailability.
- If poor B.A is due to unfavorable partitioning it can be improved by forming a more lipophilic prodrug.

  Eg: Oxazolidines.
- If poor B.A is due to poor dissolution then it can be improved by forming a more hydrophilic prodrug.

  Eg: Phenytoin.

QUALITY CONTROL TESTS OF PARENTERALS



LEAKER TEST ::



- Intended to detect incompletely sealed ampoules.
- Ampoules are hermetically sealed containers meant for single use.
- Presence of any capillary pores or tiny cracks may contaminate the product and spoil the appearance of the product.
- Ampoule is completely submerged in a deeply colored dye solution(0.5 – 10% methylene blue).
- Bottles are tested for the presence of vacuum by striking the base with hand to produce “water hammer” sound

CLARITY TEST :

- A clean solution having a high polish conveys that the product is of exceptional quality and purity.
- Should be free from particulate visible matter.
- Presence of any particulate visible matter the product should be discarded.
- Acc.To USP for a large volume parenteral :

✓ Limit of 50 particles of 10 µm and larger

✓ 5 particles of 25 µm and larger per millimeter.

PYROGEN TEST :

- Presence of pyrogenic substances is determined by a qualitative biologic test based on fever response of rabbits.
- If a pyrogenic substance is injected into the vein of the rabbit, an elevation in the temp. occurs with in 3hours.
- Elevation in the temp. can be determined by rectal thermometers.
- Recently developed test is LAL test which is 5 – 10 times more sensitive than rabbit test.




Thank You!

PHASES OF CLINICAL TRAILS

G.Natesh

VCPS

Clinical Trails

- Clinical trials are studies performed with human subjects to test new drugs or combinations of drugs, new approaches to surgery or radiotherapy or procedures to improve the diagnosis of disease and the quality of life of the patient.
- clinical trials are generally considered to be biomedical or health-related research studies in human beings that follow a pre-defined protocol.

CLINICAL TRIAL:

- Carefully and ethically-designed experiment, in which participating subjects are assigned to the different modes of intervention simultaneously (in the same period of time), at random and are also supervised in a simultaneous way.

OPEN CLINICAL TRIAL:

- Confusing term, used to indicate that a clinical trial does not have any specific methodological characteristic.
- An open clinical trial is a clinical trial without a control group, as opposed to a controlled clinical trial.
- It can also be a non-blinded clinical trial, as opposed to a single-blind or double-blind clinical trial.

SINGLE-BLIND CLINICAL TRIAL:

- Trial in which the subject, but not the observer, does not know which of the possible treatments he is receiving.

DOUBLE-BLIND CLINICAL TRIAL:

- Trial in which neither the subject nor the observer know which treatment is being administered.

TRIPLE-BLIND CLINICAL TRIAL:

- Clinical trial in which the participating subject, the observer-researcher and the researcher who analyzes the data do not know which treatment is being received.
- This is done when the clinical variables examined are soft, that is, they can be interpreted in different ways.

CROSSOVER CLINICAL TRIAL:

- Clinical trial in which each individual consecutively receives each of the treatments under study.

UNICENTER CLINICAL TRIAL:

- A trial carried out by a single researcher or research team in one hospital or another type of centre.

MULTICENTER CLINICAL TRIAL:

- According to Royal Decree 561/1993, “A trial carried out in two or more centres with the same protocol and a coordinator who is responsible for processing all the data and for analysing the results”.

PARALLEL CLINICAL TRIAL:

- Clinical trial in which each group of patients receives a single treatment simultaneously.

Randomized Controlled Clinical Trial (RCT):

- A prospective, analytical, experimental study using primary data generated in the clinical environment. Individuals similar at the beginning are randomly allocated to two or more treatment groups and the outcomes the groups are compared after sufficient follow-up time. Properly executed, the RCT is the strongest evidence of the clinical efficacy of preventive and therapeutic procedures in the clinical setting.

Randomized Cross-Over Clinical Trial:

- A prospective, analytical, experimental study using primary data generated in the clinical environment. Individuals with a chronic condition are randomly allocated to one of two treatment groups, and, after a sufficient treatment period and often a washout period, are switched to the other treatment for the same period. This design is susceptible to bias if carry over effects from the first treatment occur.

PHASES OF CLINICAL TRAILS

Phase 0

- It is a recent designation for exploratory, first-in-human trials conducted in accordance with the U.S. Food and Drug Administration's (FDA) 2006 Guidance on Exploratory Investigational New Drug (IND) Studies.
- Phase 0 trials are also known as human micro dosing studies and are designed to speed up the development of promising drugs or imaging agents by establishing very early on whether the drug or agent behaves in human subjects as was expected from preclinical studies and pharmacodynamics (how the drug works in the body).

- Distinctive features of Phase 0 trials include the administration of single sub therapeutic doses of the study drug to a small number of subjects (10 to 15) to gather preliminary data on the agent's pharmacokinetics (how the body processes the drug)

- A Phase 0 study gives no data on safety or efficacy, being by definition a dose too low to cause any therapeutic effect.

- **Phase I** - The first studies in humans are Phase I trials. They are performed with small numbers of patients or healthy volunteers and are used to answer questions such as what dose of the drug is likely to be effective and what side effects might occur.

Phase I

- Objectives
  1. To assess a safe & tolerated dose
  2. To see if pharmacokinetics differ much from animal to man
  3. To see if kinetics show proper absorption, bioavailability
  4. To detect effects unrelated to the expected action
  5. To detect any predictable toxicity
- Inclusion criteria
  - Healthy volunteers : Uniformity of subjects: age, sex, nutritional status [Informed consent a must]
  - Exception: Patients only for toxic drugs Eg AntiHIV, Anticancer
- Exclusion criteria
  - Women of child bearing age, children.

- Methods:
  - First in Man : Small number of healthy volunteers
  - First in a small group of 20 to 25
  - Start with a dose of about 1/10 to 1/5 tolerated animal dose
  - Slowly increase the dose to find a safe tolerated dose
  - If safe → in a larger group of up to about 50 –75
  - No blinding
  - Performed by clinical pharmacologists
  - Centre has emergency care & facility for kinetics study
  - Performed in a single centre
  - Takes 3 – 6 months [70% success rate]

- **Phase II** - The trials with larger numbers of patients and focus on how well the treatment or procedure works, perhaps in particular situations or groups of patients.

Phase II

- First in patient [different from healthy volunteer]
- Early phase [20 – 200 patients with relevant disease]
  - Therapeutic benefits & ADRs evaluated
  - Establish a dose range to be used in late phase
  - Single blind [Only patient knows] comparison with standard drug
- Late phase [50 – 500]
  - Double blind
  - Compared with a placebo or standard drug
- Outcomes
  - Assesses efficacy against a defined therapeutic endpoint
  - Detailed P.kinetic & P.dynamic data
  - Establishes a dose & a dosage form for future trials
- Takes 6 months to 2 years [35% success rate]

Phase III

- The trials enroll large numbers of patients and are used to compare the effectiveness and safety of the new treatment with that of the standard existing treatment.

- Information obtained from Phase III trials that demonstrates the benefits a new drug over the existing treatments are presented to regulatory authorities in order to obtain a licensee to market and sell the drug.

Phase III

- Large scale, Randomised, Controlled trials
- Target population: 250 – 1000 patients
- Performed by Clinicians in the hospital
- Minimises errors of phases I and II
- Methods
  - Different patient subgroups Eg: pediatric, geriatric, renal impaired
  - Randomised allocation of test drug /placebo / standard drug
  - Double blinded:
  - Cross over design
  - Vigilant recording of all adverse drug reactions
  - Rigorous statistical evaluation of all clinical data
- Takes a long time: up to 5 years [25% success]

Phase IV trial

- Also known as **Post Marketing Surveillance Trial.**
- Phase IV trials involve the safety surveillance (pharmacovigilance) and ongoing technical support of a drug after it receives permission to be sold.
- Phase IV studies may be required by regulatory authorities or may be undertaken by the sponsoring company for competitive (finding a new market for the drug) or other reasons
- Helps to detect
  - rare ADRs
  - Drug interactions
  - Also new uses for drugs

- *Venlafaxine* a popular antidepressant whose misuse is possibly related to the increase it produces in dopamine neurotransmission mainly at the prefrontal cortex level
- The abuse of *tropicamide*, an ophthalmic anticholinergic compound which, when injected, may be associated with hallucinations, dysphoria, psychomotor agitation, tachycardia and suicidal ideation.
- The abuse potential of the above molecules is not mentioned in the medications' package leaflet/patient information leaflet

Hydromorphone

- The FDA approved this molecule in 2004 for the management of moderate/severe pain Eventually, a sponsor's study identified that, compared to taking hydromorphone alone, when co-administered with alcohol the blood hydromorphone concentrations were significantly higher. FDA's Role in Preventing Prescription Drug Abuse. Eventually, sales and marketing of the drug were suspended.

Documents for the conduct of clinical trial

- The list of documents to be maintained and submitted in the clinical research study are grouped in to three sections according to the stage of the clinical trial.

1. Before the clinical phase of the trial commences.
2. During the conduct of the trial.
3. After completion of the trial.

Before the clinical phase of the trial commences

- Investigators brochure.
- Signed protocol and amendments and sample case report form.
- Information given to the trial subject.
  1. Informed consent form
  2. Advertisement for subject recruitment.
  3. Any other information.
- Financial aspects of the trial.
- Insurance statement.
- Signed agreement between involved parties.
- IRB/IEC composition.
- Regulatory authority approval
- Meical/ laboratory procedures certification.
- Sample of label attached to investigators drug containers.
- Trial initiation monitoring report.

During the conduct of the trial

- Investigator brochure updates
- Any revision of protocol, informed consent form.
- Regulatory authority approvals.
- Cirriculam vitae for new investigators or sub investigators.
- Updates of Meical/ laboratory procedures certification.
- Monitoring visit reports.
- Signed informed consent forms.
- Notification of investigator or sponsor of serious adverse events & reports.
- Notification by sponsor or investigator to authority & IRB /IEC of unexpected serious ADR.
- Subject screning and enrollment log.

After completion of the trial

- Investigational product accountability at site.
- Documentation of investigational product destruction.
- Completed subject identification list.
- Audit certificate.
- Final trial close out monitoring report.
- Final report by investigator to IRB/ IEC where required and applicable to authority.

- India emerged as a global hub for clinical research. According to a report by Mc Kinsey the global Clinical trial outsourcing opportunity in India in the pharmaceutical industry is estimated to be around $2 billion by 2010 and there will be the demand for more than 10,000 investigators trained in Good Clinical Practice (GCP) and 50,000 clinical research professionals.
- According to records collected by the Indian Council of Medical Research and the Drug Controller General's office, between July-December 2007, only 11 trials were registered. The number increased to 137 between Jan-Dec 2008 and then to an all-time high of 546 between Jan-Dec 2009. This year, while January saw 58 trials registered, February recorded 60.

- Top multinational pharmaceutical companies like Pfizer, Glaxo Smith Kline, Aventis, Novartis, Novo Nordisk, Astra Zenica, Eli Lilly are conducting clinical trials in India apart from the Indian companies like Dr. Reddys, Nicholas Piramal, Cipla and Lupin. Currently, there are more than 150 CROs in India, out of which 20 comply with ICH-GCP guidelines.

Post Marketing Surveillance

G.Samatha
VCPS

HISTORY

- In the 1960 at least two serious drugs reactions were observed in many patients. thalidomide causes limb deformities(phocomelia).
- observed in Japan, was the optic nerve damage(subacute myelooptic –neuropathy) due to Clioquinol
- As a result, the joint commission on Prescription Drugs Use was established in 1976,funded largely by the drug industry, with the mandate to design a post-marketing surveillance system to detect, quantify, and describe the anticipated and unanticipated effects of marketed drugs.

SOURCES OF PMS INFORMATION

- The following may be considered as sources of information, some source are proactive and some are reactive.
  - Expert user groups.
  - Customer surveys.
  - Customer complaints and warranty claims.
  - Post CE-market clinical trials.
  - Literature reviews.
  - Device tracking/implant registries.
  - User reaction during training programmers.
  - The media.

Are there benefits to a PMS system

- Detection of manufacturing problems
  - Improvement of medical device quality
  - verification of risk analysis
  - intelligence of long-term performance
  - chronic complications
  - performance trends
  - performance in different user populations

Why do we need Post- Marketing Surveillance

- The primary objective of post-marketing surveillance is to develop information about drug effects under customary condition of drug use.
- Rare adverse events may not be detected in pre-licensure studies because in very large clinical trials have limitation.
- Access to more patient and given data
- Given diversity of data sources, innovative approaches to retrieval of key data may have great potential vs. single unified system.
- Better background rates, comparable “control’’ population.
- Increase in “non-medical’’ data sources

Post- Marketing Surveillance Opportunity

- Access to additional health system data.
- Access to global data: regulatory, inspectional, health system, international surveillance and pharmacovigilence.
- Better analytical tools and methods.

Practical Aspects of PMS

- PMS should be proactive.
- Manufacturer should document compliance.
- Manufacturer`s PMS procedure should discuss the information that will be collected and obtained as a part of system.

ABOUT PMS PROCEDURE

- It should assign departments or position a responsible for performing a particular function.
- Manufacturer may find it helpful to a have report at the end of year, as well as PMS tracking schedule and log.
- This information could constitute feedback received from user .

Methods of Surveillance

- Thus, four types of studies are generally used to identify drugs effects:
  1. Controlled clinical trials,
  2. Spontaneous or voluntary recording
  3. Cohort, studies and
  4. Case control studies

1. **Controlled clinical trials:**

- Controlled clinical trials match treatment and control groups as closely as possible, minimize bias through such methods as randomization and “double-blinding,” and directly monitor patients for the duration of the study.
- Controlled clinical trials are considered the most definitive method for evaluating a drug’s efficacy and safety.
- But they are often costly or impractical in specific situations, for example, when a drug’s effects are rare, or appear only after long-term use or a long latency period.

2. Spontaneous or voluntary reporting

- Voluntary reporting by physicians and other health providers, hospitals, and consumers may act to alert FDA and pharmaceutical firms to possible adverse effects of drugs, so that the inference of an association between a drug and an observed health condition may be further studied by cumulative, careful reporting, and confirmed or disconfirmed by more vigorous methods.
- Underreporting may be a serious deficiency of this method.
- A drug may also be erroneously associated with an adverse effect until the suspected association fails to show up in repeated, statistically validated studies

3. Cohort studies

- Cohort studies follow a defined group of patients (the cohort) for a period of time.
- In this method, patients are not randomly assigned to groups, and there is no blinding. Cohort studies are usually prospective and observe the cohort from the beginning of drug use.
- A group of patients taking the drug of interest is assembled and followed to see, for example, if adverse reactions occur. A second group of patients (the controls) with the same medical condition, who are not taking the drug and who may be receiving alternative treatment, but who are otherwise matched as closely as possible with the cohort, may also be studied in parallel.

- The control group is used to identify the frequency of occurrence of any condition observed in the drug-exposed group which is due to causes other than the drug (i. e., the “background incidence” of the condition).
- In this method, patients can be directly monitored to ensure they take the drug appropriately, and to observe the drug’s effects; or monitoring can be less controlled. With less control, a larger cohort can be followed, but bias is thus increased.

4. Case-control studies :

- Case-control studies identify patients with the adverse effects to be studied (the cases), and compare them with a sample (the controls), drawn from the same cohort that gave rise to the cases.
- Controls are matched as closely as possible with the cases, except with regard to the drug's suspected adverse effect, to examine whether exposure to the drug is the cause.

PREFORMULATION STUDIES

N.Shanthi
VCPS

CONTENTS

- Introduction
- Organoleptic properties
- Purity
- Particle size, shape and surface area
- Solubilisation, Surfactants and its importance
- Temperature, pH, co-solvency, solid dispersion, β-cyclodextrin drug-dispersion system
- Preformulation stability studies
- A consideration of physico-chemical characteristics of new drug molecules with respect to different dosage forms

Preformulation

- Preformulation is branch of Pharmaceutical science that utilizes biopharmaceutical principles in the determination of physicochemical properties of the drug substance.
- Prior to the development of any dosage form new drug , it is essential that certain fundamental physical & chemical properties of drug powder are determined .
- This information may dictate many of subsequent event & approaches in formulation development.
- This first learning phase is called as preformulation.

INTRODUCTION

DEFINITION:-

Investigation of physico-chemical properties of the new drug compound that could affect drug performance and development of an efficacious dosage form".

Preformulation commences when a newly synthesized drug shows a sufficient pharmacologic promise in animal model to warrant evaluation in man.

INTRODUCTION

- The preformulation is the first step in the rational development of a dosage form of a drug substance alone and when combined with excipients.

- Objective :

  To generate useful information to the formulator to design an optimum drug delivery system.

GOALS OF PREFORMULATION

- To establish the necessary physicochemical parameters of new drug substances.
- To determine kinetic rate profile.
- To establish physical characteristics.
- To establish compatibility with common excipients.

PRELIMINARY EVALUATION

a) Compound identity.

b) Formula and molecular weight.

c) Structure.

d) Therapeutic indications:

- Probable human dose.
- Desired dosage form(s)
- Bioavailability model
- Competitive products

PRELIMINARY EVALUATION

e) Potential hazards

f) Initial bulk lots:

- Lot number
- Crystallization solvent(s)
- Particle size range
- Melting point
- % volatiles

g) Analytical methods:

- HPLC assay
- TLC assay
- UV/ Visible spectroscopy

ORGANOLEPTIC PROPERTIES

COLOR	ODOUR	TASTE
OFF-WHITE	PUNGENT	ACIDIC
CREAM-YELLOW	SULFUROUS	BITTER
SHINY	FRUITY	SWEET
	AROMATIC	TASTELESS
	ODOURLESS	TASTELESS

COLOR

- Color is generally a function of a drug's inherent chemical structure relating to a certain level of unsaturation.
- Color intensity relates to the extent of conjugated unsaturation as well as the presence of chromophores.
- Some compound may appear to have color although structurally saturated.

PURITY

- Designed to estimate the levels of all known & significant impurities & contaminates in the drug substance under evaluation.
- Study performed in an analytical research & development group.
- It is another parameter which allows for comparison with subsequent batches.
- Occasionally, an impurity can affect stability.

  e.g.

  - Metal contamination
  - Appearance

PURITY

- The techniques used for characterizing the purity of a drug are the same as those used for other purpose in a preformulation study.
- Thin layer chromatography is a wide ranging applicability & is an excellent tool for characterizing the purity.
- HPLC, paper chromatography & gas chromatography are also useful.
- More quantitative information can be obtained by using quantitative differential scanning colorimetry.

PARTICLE SIZE

- Particle size is characterized using these terms :
  i. Very coarse (#8)
  ii. Coarse (#20)
  iii. Moderately coarse (#40)
  iv. Fine (#60)
  v. Very fine (#80)

PARTICLE SIZE

- Particle size can influence variety of important factors :
  - Dissolution rate
  - Suspendability
  - Uniform distribution
  - Penetrability
  - Lack of grittiness

Methods to Determine Particle Size

- Sieving
- Microscopy
- Sedimentation rate method
- Light energy diffraction
- Laser holography
- Cascade impaction

Methods to Determine Particle Size

1. **Sieving method :**

- Range : 50 – 150 µm
- Simple, inexpensive
- If powder is not dry, the apertures get clogged.

2. **Microscopy :**

- Range : 0.2 – 100 µm
- Particle size can be determined by the use of calibrated grid background.
- Most direct method.
- Slow & tedious method.

Methods to Determine Particle Size

3. Sedimentation method :

- Range : 1 - 200 µm
- Andreasen pipette is used.
- Particle size is calculated by stoke's law :

$$d_{st} = \sqrt{\frac{18\,\eta_0 h}{(\rho_s - \rho_0)\,g t}}$$

Where,

h = distance of fall in time, t

n_o= viscosity of the medium

ρ_s= density of the particles

ρ_0= density of the dispersion medium



Methods to Determine Particle Size

4. Light energy diffraction :

- Range : 0.5 – 500 µm
- Particle size is determined by the reduction in light reaching the sensor as the particle, dispersed in a liquid
- or gas, passes through the sensing zone.

  Quick & fast.

5. Laser holography :

- Range : 1.4 – 100 µm
- A pulsed laser is fired through an aerosolized particle spray & photographed in three dimensional with holographic camera, allowing the particles to be individually imaged & sized.

Methods to Determine Particle Size

6. Cascade impaction :

- The principle that a particle driven by an airstream will hit a surface in its path, provide that its inertia is sufficient to overcome the drug force that tends to keep in it in airstream.

POWDER FLOW PROPERTIES

- Powder flow properties can be affected by change in particle size, shape & density.

- The flow properties depends upon following-

1. Force of friction.
2. Cohesion between one particle to another.

- Fine particle posses poor flow by filling void spaces between larger particles causing packing & densification of particles..
- By using glident we can alter the flow properties.

eg. Starch, Talc

Determination Of Powder Flow Properties

- By determining **Angle Of Repose.**
- A greater angle of repose indicate poor flow.
- It should be less than 30°. & can be determined by following equation.

$$\tan \theta = h/r.$$

where, θ = angle of repose.

h=height of pile.

r= radius.

Angle Of Repose (In degree)	Type Of Flow
<25	Excellent
25-30	Good
30-40	Passable
>40	Very poor

Determination Of Powder Flow Properties

- Measurement of free flowing powder by *compressibility*.
- Also known as *Carr's index*.

$$\text{CARR'S INDEX}(\%) = \frac{(\text{TAPPED DENSITY} - \text{POURED DENSITY})}{\text{TAPPED DENSITY}} \times 100$$

- It is simple, fast & popular method of predicting powder flow characteristics.

Determination Of Powder Flow Properties

- Carr's Index
  - 5-15
    - 12-16
    - 18-21
    - 23-35
    - 33-38
    - >40
- Type of flow
  - Excellent
  - Good Fair To Passable
    - Poor Very Poor
  - Extremely Poor

PARTICLE SHAPE

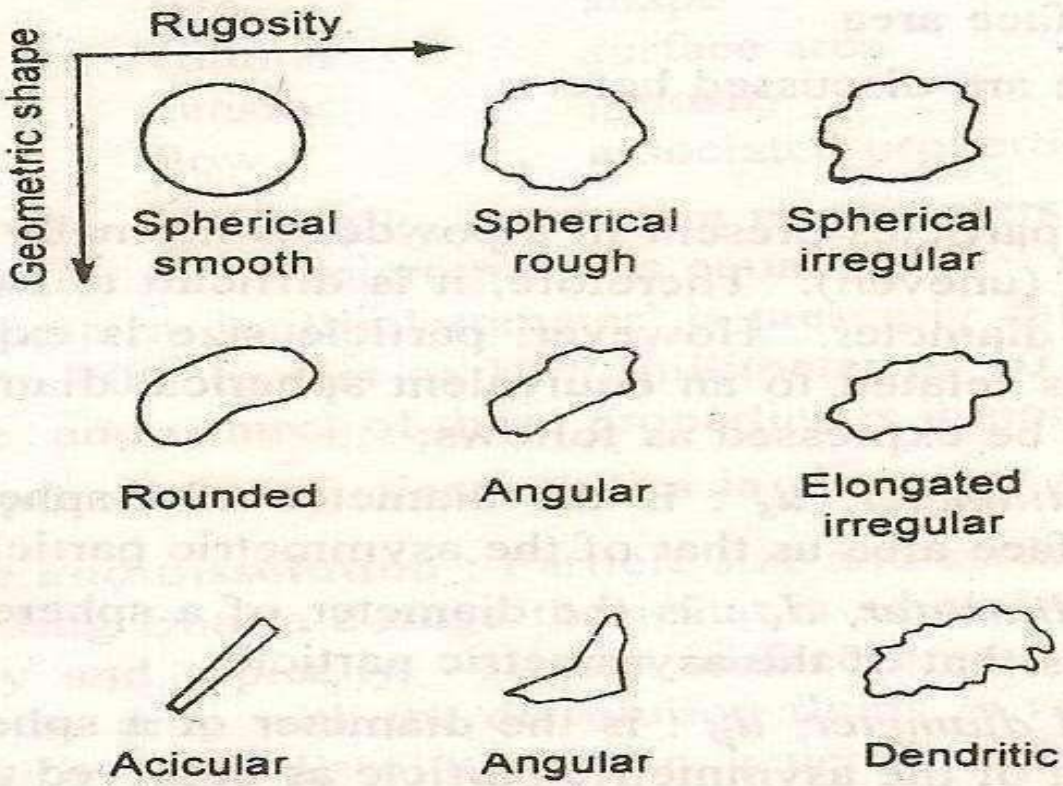


Figure 6-1. Characteristic particle shapes related to geometric shape and surface irregularity.

PARTICLE SHAPE

- Particle shape will influence the surface area, flow of particles, packing & compaction properties of the particles.
- A sphere has minimum surface area per unit volume.
- Therefore, these properties can be compared for spheres & asymmetric particles, in order to decide the shape.
- The following expression can be obtained:

Property	Sphere	particle
surface area	πd_{s_2}	$\alpha_s x\, d_{p2}$
volume	$(1/6)\pi d_{s_3}$	$\alpha_v x\, d_{p_3}$

Cont…

PARTICLE SHAPE

- When particle shape is spherical, the $d_s = d_p$
- Thus, $\alpha_s = \pi = 3.124$ & $\alpha_v = \pi/6 = 0.524$
- Therefore, Shape factor $= \frac{\alpha_s}{\alpha_v} = \frac{3.124}{0.524} = 6$

SURFACE AREA

- Particle size & surface area are inversely related to each other.
- Smaller the drug particle, greater the surface area.

Specific surface is defined as the surface area per unit weight (Sw) or unit volume (Sv) of the material.

SURFACE AREA

Estimation of S_v:

$$S_v = \frac{\text{Surface area of the particles}}{\text{Volume of particles}}$$

$$= \frac{n \alpha_s d^2}{n \alpha_v d^3}$$

$$= \frac{\alpha_s}{\alpha_v d}$$

- According to shape factor,

$$\alpha_s = 6$$

$$\alpha_v$$

- So, $S_v = 6 / d$.

SURFACE AREA

- Estimation of S_w:

$$S_w = \frac{\text{Surface area}}{\text{Weight}} = \frac{\text{Surface area}}{\text{density x volume}}$$

$$= \frac{S_v}{\rho}$$

$$= \frac{6}{\rho.d}$$

Methods for determining surface area

1. Adsorption method :

- Particles with a large specific surface are good adsorbents for the adsorption of gases & of solutes from solution.
- The volume of nitrogen gas, V_m, in cm^3 that 1 g of the powder can adsorb when the monolayer is complete is more accurately given by using the BET equation, however, which can be written as:

$$\frac{P}{V(P_0 - P)} = \frac{1}{V_m b} + \frac{(b-1)}{V_m b} \cdot \frac{P}{P_0}$$

Cont….

Methods for determining surface area

- Where,

V = Volume of gas in cm^3 adsorbed per gram of powder at pressure P.

P = Pressure of the adsorbate, in mmHg.

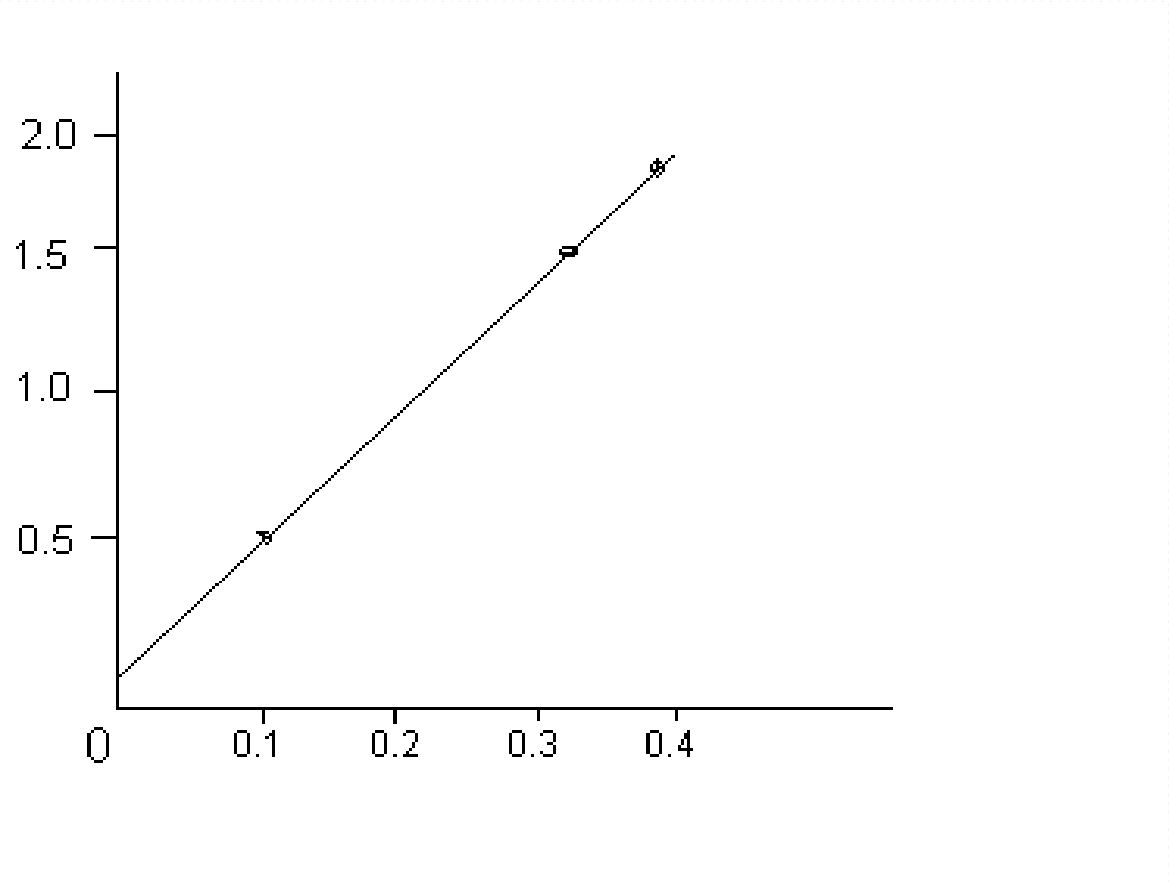
P_o = Saturation vapor pressure (monolayer)

V_m = Amount of vapor adsorbed per unit mass adsorbent, when the surface is covered with monomolecular layer

b = Constant that express the difference between the heat of adsorption & heat of liquefaction of the adsorbate (nitrogen).

Quantasorb QS – 16 instrument

$$\frac{P}{V(P_0 - P)}$$



Air permeability method :

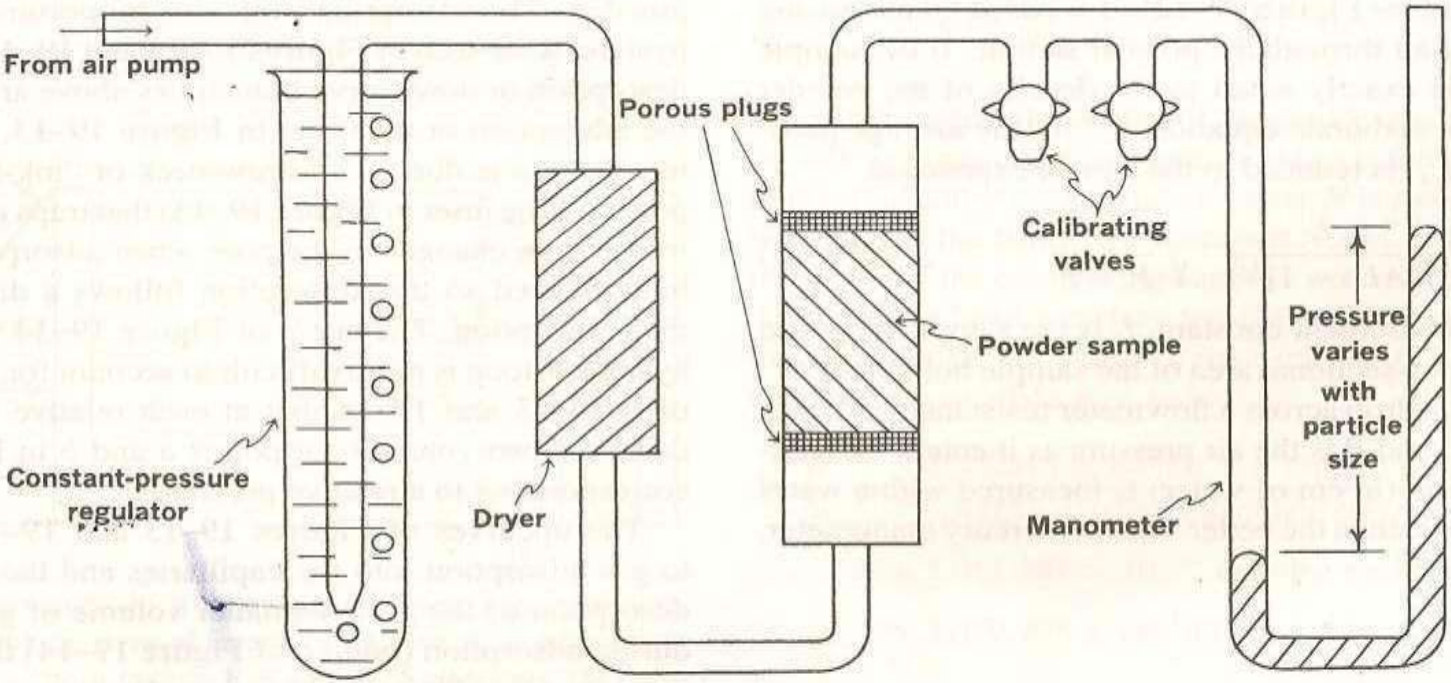


Fig. 19–12. The Fisher subsieve sizer. An air pump generates air pressure to a constant head by means of the pressure regulator. Under this head, the air is dried and conducted to the powder sample packed in the tube. The flow of air through the powder bed is measured by means of a calibrated manometer and is proportional to the surface area or the average particle diameter.

HOWEVER SIZE REDUCTION IS NOT REQUIRED IN FOLLOWING CASES

- WHEN DRUG IS UNSTABLE.
- DEGRADE IN SOLUTION FORM.
- PRODUCE UNDESIRABLE EFFECTS.
- WHEN SUSTAINED EFFECT IS DESIRED.

SOLUBILIZATION

“ Solubilization is defined as the spontaneous passage of poorly water soluble solute molecules into an aqueous solution of a soap or detergent in which a thermodynamically stable solution is formed ”.

SOLUBILIZATION

- It is the process by which apparent solubility of an otherwise sparingly soluble substance is increased by the presence of surfactant micelles .

- ***MICELLES: -***

- The mechanism involves the property of surface active agents to form colloidal aggregates known as micelles .

SOLUBILIZATION

- **When surfactants are added to the liquid at low concentration they tend to orient at the air-liquid interface .**
- **On further addition of surfactant the interface becomes completely occupied and excess molecules are forced into the bulk of liquid.**
- **At very high concentration surfactant molecules in the bulk of liquid begin to form micelles and this concentration is know as *CRITICAL MICELLE CONCENTRATION {CMC}***

SOLUBILIZATION

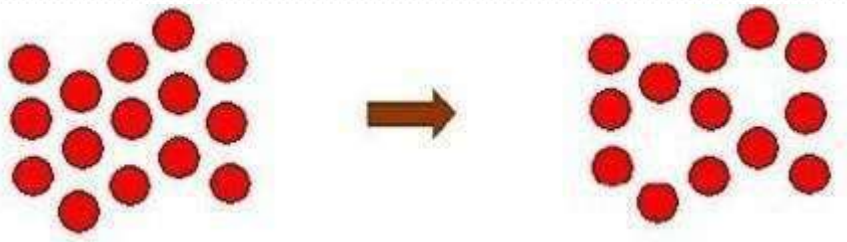
- Solubilization is thought to occur by virtue of the solute dissolving in or being adsorbed onto the micelle.
- Thus the ability of surfactant solution to dissolved or solubilize water insoluble materials starts at the CMC and increase with increase in the concentration of micelles.

- Solubilization of any material in any solvent depends on proper selection of solubilising agents.

Process Of Solubilization

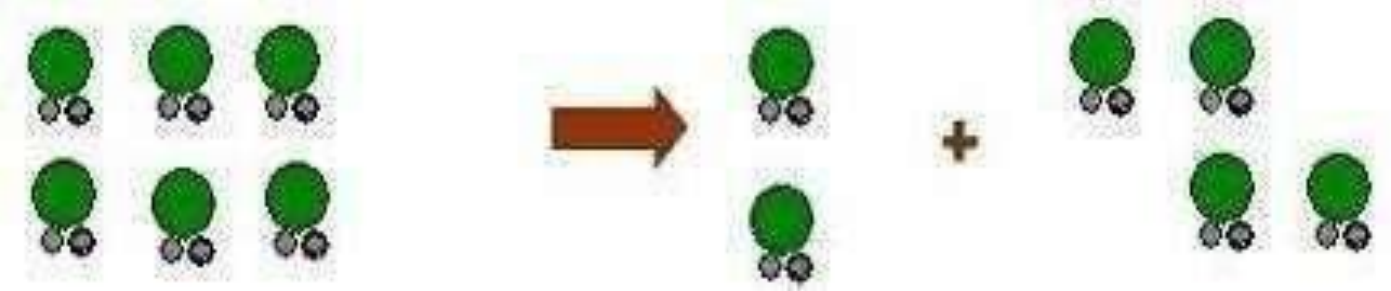
- The process of solubilization involves the breaking of inter-ionic or intermolecular bonds in the solute, the separation of the molecules of the solvent to provide space in the solvent for the solute, interaction between the solvent and the solute molecule or ion.

Step 1: Holes opens in the solvent



Process Of Solubilization

Step2: Molecules of the solid breaks away from the bulk



Step 3: The free solid molecule is intergraded into the hole in the solvent



SOLUBILITY

- **The amount of substance that passes into solution in order to establish equilibrium at constant temperature and pressure to produce a saturated solution.**

SOLUBILITY

- If solubility is <1mg/ml indicates need for salt formation to improve solubility.
- If solubility is <1mg/ml in pH= 1 to 7, preformulation study should be initiated.
- Solubility should ideally be measured at two temperatures: 4°C and 37°C.
  - 4°C to ensure Physical stability.
  - 37°C to support Biopharmaceutical evaluation.

DESCRIPTIVE SOLUBILITIES (I.P.)

Description	Parts of solvent required for one part of solute
Very soluble	< 1
Freely soluble	1 - 10
Soluble	10 - 30
Sparingly soluble	30 - 100
Slightly soluble	100 - 1000
Very slightly soluble	1000 - 10,000
Insoluble	> 10,000

SOLUBILITY ANALYSIS

- **Preformulation solubility studies focus on drug solvent system that could occur during the delivery of drug candidate.**

- **For e.g. A drug for oral administration should be examined for solubility in media having isotonic chloride ion concentration and acidic pH.**

SOLUBILITY ANALYSIS

- **Analytic method that are particularly useful for solubility measurement include HPLC, UV spectroscopy, Fluorescence spectroscopy and Gas chromatography.**

- **Reverse phase HPLC offer accurate and efficient mean of collecting solubility data of drug.**

Ionization constant (pKa)

Can be calculated by Henderson Hasselbach equation-

For acidic drugs….pH= pKa+ log [ionized drug] / [unionized drug]

For basic drugs….pH= pKa+ log [unionized drug] / [ionized drug]

pH Solubility Profile

- **The solubility of acidic or basic drug will show difference in solubility with changes in pH.**

- **pH solubility profile of a drug can be established by running the equilibrium solubility experiment within pH range of 3-4.**

Partition Coefficient

- **It is the ratio of unionized drug distributed between organic and aqueous phase at equilibrium.**

$$\mathbf{P}\ o/w = (\ \mathbf{C}\ oil\ /\ \mathbf{C}\ water\ )equilibrium$$

Effect Of Temperature

- **The heat of solution ΔH_s, represents the heat released or absorbed when a mole of solute is dissolved in large quantity of solvent.**

- **Endothermic reaction**
- **Exothermic reaction**

Determination of solubility

- **The following points should be considered**
- **The solvent & solute must be pure.**
- **A saturated solution must be obtained before any solution is removed for analysis.**
- **The method of separating a sample of saturated solution from undissolved solute must be satisfactory.**
- **The method of analyzing solution must be reliable**
- **Temperature must be adequately controlled .**

Solubility Determination Method

- **Solubility is normally depends on temperature, so temperature is recorded in each solubility measurement.**
- **Plot of solubility against temperature is commonly used for solubility determination.**
- **Two methods are available for determination are as follow.**

I.Analytical method

II.Synthetic method

Analytical method

- **Temperature of equilibrium is fixed and concentration of the solute in the saturated solution is determined at equilibrium by a suitable analytical procedure.**

- **In other words a saturated solution in the presence of an excess of the undissolved solute is prepared at an accurately known temperature. This situation can be achieved by suitable contact b/w solute and solvent.**

Synthetic method

- In this method a weighed amount of solute is placed in the vessel.
- While agitating the system at constant temperature known amount of solvent is added gradually until the solubility limit is reached.
- At equilibrium, temperature and content of the system is recorded.
- This method is carried out at micro scale level by examining the small amount of the system under hot stage microscope.

General Method of Increasing the Solubility

- **Addition of co-solvent**
- **pH change method**
- **Reduction of particle size**
- **Temperature change method**
- **Hydotrophy**
- **Addition of Surfactant**
- **Dielectrical Constant**
- **Complexation**

Addition Of Co-Solvent

- **Weak Electrolyte :- Phenobarbitone**
- **Non polar :- Nitro Cellulose**

These are poorly soluble in given solvent.

For such poorly soluble materials, to enhance their solubility, the water miscible solvents are used in which the drug has good solubility.

This process of improving solubility is known as co-solvency and the solvent used is known as co-solvents.

Addition Of Co-Solvent

e.g. **Phenobarbitone is insoluble in water. A clear solution is obtained by dissolving in mixture of Alcohol, Glycerin, Propylene glycol.**

e.g. Of Cosolvents:-

PG, glycerin, sorbitol, PEG, Glyceryl formal, glycofurol, ethyl carbamate, ethyl lactate and dimethyl acetamide.

pH change Method

- **Weak base:- Alkaloids, Local Anaesthesia**
- **Weak acid:- Sulphonamides, Barbiturates**
- **In aqueous medium they dissociate poorly and undissociated portion is insoluble.**

e.g. **Benzoic acid, Phenobarbitone**

- **So, solubility of the undissociated portion is improved by pH control.**
- **For weak acidic drug:- increase pH, solubility is increase.**
- **For weak base drug:- decrease pH, increase solubility.**

Reduction Of Particle size

- **Reduction in Particle size improve solubility of drug.**

- **Basically reduction in particle size increase contact surface area of the particle, there by ultimately it increase rate of solubility of drug.**

Temperature Change Method

- **In endothermic reaction by increasing temperature solubility is increase.**

- **In exothermic reaction by increasing temperature solubility is decrease.**

e.g. **Methyl Cellulose when mixed with water and temperature is raised, it becomes insoluble. To dissolve it cold water is added.**

HYDROTROPHY

The term Hydotrophy has been used to designate the increase in solubility in water of various substances due to the presences of large amount of additives.

e.g. **Solubilization of Benzoic acid with Sodium benzoate.**

Addition of Surfactant

- **Surfactants are molecules with well defined polar and non-polar region that allow them to aggregate in solution to form micelles. Non polar drugs can partition into micelles and be solubilized.**

e.g. **Surfactant based solution of Taxol, that is solubilized in 50% solution of Cremophor.**

Dielectrical Constant

Dielectrical Constant is the effect that substances has, when it acts as a solvent on the case with which it separates oppositely charged atoms.

e.g. **DEC of Water- 80**

Kerosene- 2

Glycerine- 48

Benzene- 2.2

Complexation

- **For the Complexation occur both drug and ligand molecule should be able to donate or accept electrons.**
- **The solubility of compound is the sum of solubility of the compound and its complex.**

e.g. HgI_2 (Mercuric Iodide) is sparingly soluble in water. Its solubility in water is increased by forming complex with KI.

$$HgI_2 + 2KI \longrightarrow K_2HgI_4 \text{ (water soluble)}$$

Applications of solubilization

- **Drugs with limited aqueous solubility can be solubilized. These include oil-soluble vitamins, steroid hormones and antimicrobial agents etc.**
- **Solubilization of orally administered drugs results in an improved appearance and improves unpleasant taste.**
- **Both oil-soluble and water-soluble compounds can be combined in a single phase system as in case of multivitamin preparations.**

Applications of solubilization

- **Solubilization may lead to enhanced absorption and increased biological activity.**
- **Improves the intestinal absorption of vitamin A.**
- **Drug absorption from ointment bases and suppositories also increased.**
- **Liquid preparations with small quantity of preservative can be prepared by solubilization.**

Applications of solubilization

- **Aqueous concentrates of volatile oils can be prepared by solubilization.**

Example: soaps used for solubilising phenolic compounds for use as disinfectants- Lysol, Roxenol etc.

- **Barbiturates, anticoagulant, alkloidal drugs are dissolved with polysorbate by solubilization.**

SURFACTANT

- ***Surfactants:-***

are wetting agents that lower the surface tension of a liquid, allowing easier spreading, and lower the interfacial tension between two liquids.

- **Classification**

Some commonly encountered surfactants of each type include:

1. **Ionic**
   - **Cationic**
   - **Anionic**
   - **Zwitterionic**
2. **Non ionic**

Cationic Surfactants:-

Quaternary ammonium salts are more preferred because they are less affected by pH.

e.g. **Cetyl Trimethyl Ammonium Bromide (CTAB) Hexadecyl Trimethyl Ammonium Bromide, and other Alkyltrimethyl Ammonium Salts, Cetylpyridinium Chloride (cpc)**

IONIC

?*Anionic Surfactants:-*

They are the most commonly used surfactants, containing Carboxylate, Sulfonate, Sulfate ions.

e.g. **Sodium Dodecyl Sulphate (SDS), Ammonium Lauryl Sulphate and other alkyl sulfate salts, Sodium Laureth Sulphate, also known as Sodium Lauryl Ether Sulphate (SLES).**

IONIC

Zwitterionic:-

When a single surfactant molecule exhibit both anionic and cationic dissociations it is called amphoteric or Zwitterionic.

The anion include carboxylates and phosphate group and the cation include quaternary ammonium group.

e.g. **Dodecly Betamine**

Dodecly Dimethylamine Oxide

NONIONIC

- These are most widely used because they are free from non compatability, stability and potential toxicity and classified as water soluble and water insoluble non ionic surfactants.

- ***e.g.*** Long chain fatty acids, fatty alcohols

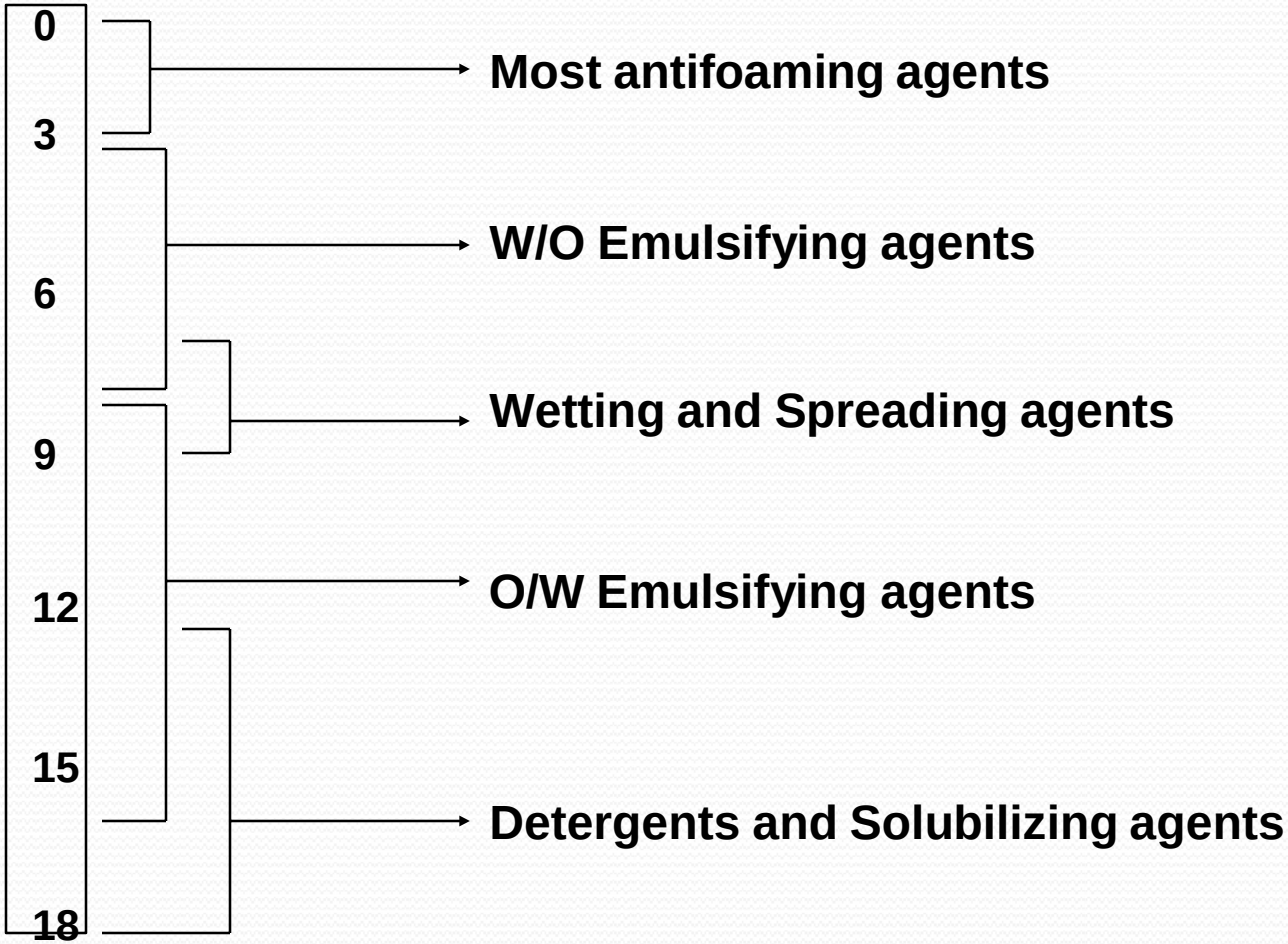
Water solubility of these agents is further increased by addition of polyoxyethylene groups through ether linkage with one of the alcohol group.

e.g. spans

HLB SCALE

- Griffin in 1947 developed the system of the hydrophilic-lipophilic balance [HLB] of surfactant.
- The higher the HLB of the an agent, the more hydrophilic it is.
- Tween, polyoxyethylene derivative of the spans are hydrophilic and have high HLB value (9.6-16.7)
- The lower the HLB of the agent, the more lipophilic it is.
- The sorbitan ester are lipophilic and have low HLB value (1.8-8.6)

HLB SCALE


0
3
6
9
12
15
18
Most antifoaming agents
W/O Emulsifying agents
Wetting and Spreading agents
O/W Emulsifying agents
Detergents and Solubilizing agents

HLB SCALE

- The HLB of non ionic surfactant whose only hydrophilic portion is polyoxyethylene is calculated using the formula

- HLB = E/5

  Where, E = Percentage weight of ethylene oxide

Importance Of Surfactant

- **Surfactants play an important role in many practical applications and products, including:**

- **Detergents**
- **Fabric Softener**
- **Emulsifier**
- **Paints**
- **Adhesive**
- **Inks**
- **Soil remediation**
- **Wetting**

Importance Of Surfactant

- **Ski Wax**
- **Snowboard Wax**
- **Foaming**
- **Defoaming**
- **Laxatives**
- **Agrochemical formulations Herbicides Insecticides**
- **Quantum dot coating**
- **Biocides (Sanitizers)**
- **Hair Conditioners (after shampoo)**
- **Spermicide (Nonoxynol 9)**

TEMPARATURE ,pH, COSOLVANCY, SOLID DISPERSIONS

Effect of Temperature

- The solubility of a solute in a solvent is dependent on temperature, nature of solute and nature of solvent.
- Heat of solution represents the heat released or absorbed when a mole of solute is dissolved in a large quantity of solvent.
- Most of the substances are endothermic, absorbing heat in the process of dissolution.

Effect of Temperature

- For this substances, an increase in temperature results in an increase in solubility.
- Exothermic substances give off heat in the process of dissolution. The solubility of such substances would decrease with increase in temperature.
- Care should be taken as heat may destroy a drug or cause other changes in the solution.

e.g. On excess heating the sucrose solution it can get converted in to the invert sugar.

Effect of Temperature

- Depending on the type of reactions weather it is exothermic or endothermic heat is either released or absorbed.

  e.g. Mixture of chloroform and acetone. The heat produced by the solute-solvent interaction is so much greater than the heat necessary to separate the molecules of acetone and chloroform, which can be detected as a rise in temperature of the liquid.

Effect of Temperature

- Applications:
- Pharmaceutical solutions must be administered at or near room temperature. So, it is more important factor for product storage than the formulation.
- To increase the solubility of sparingly soluble solute.
- To increase the stability by reducing the moisture content.

Effect of pH

- Weak electrolytes undergo ionization and are more soluble when in ionized form. The degree of ionization depends on dissociation constant (pKa) and the pH of the medium.
- Solubility is a function of pH, that is related to its pKa which gives ratio of ionized and unionized forms of the substance.

  This can be shown as:

$$pH = pKa + \log \frac{[A^-]}{[HA]}$$

Effect of pH

- If the substance is brought outside its pKa, i.e. the pH value where half the substance is ionized and half is not, than solubility will be changed because we are introducing new intermolecular forces, mainly ionic attraction.

- e.g. –COOH has pKa value at pH around 4. If pH is increased then –COOH is converted into $–COO^-$. This may interact with the $H^+$of water.

Effect of pH

- The effect of pH on solubility for weak electrolytes can be described by:

$$pHp = pKa + \log \left[\frac{S - S_0}{S_0} \right]$$

- Where,

pHp = pH below which the drug precipitates from solution as the undissociated acid.

S = total solubility.

S_0= molar solubility of the undissociated acid.

Effect of pH

- It is to be ensured that pH change for one single compound should not affect the other requirements of product.

- e.g. the chemical stability of drug may depend on pH, and this pH of optimum stability should not coincide with the pH of other ingredients specially colors, preservatives and flavors.

Cosolvancy

- To enhance the solubility of poorly soluble materials, the water miscible solvents are used in which the drug has good solubility. This process of improving solubility is known as co-solvency.

- Solvents used to increase the solubility are known as co-solvents.

Cosolvancy

- The mechanism for solubility enhancement by co-solvency is not clearly understood. But it is proposed that, solubility is increased may be by reducing the interfacial tension between the solvent and hydrophobic solutes and decreasing dielectric constant of solvent.

Cosolvancy

- The commonly used and acceptable co-solvents in formulation of aqueous liquids for oral solutions are Ethanol, Sorbitol, Glycerin, Several members of PEG series.

- For parenteral products, Dimethylacetamide is widely used. But in case of oral liquids its application is limited, because of its objectionable odour and taste.

Cosolvancy

- Some characteristics of co-solvent, which are used in preparation:

  1. It must be non-toxic. Non-irritating.
  2. It should be able to solubilize the drug in given solvent.
  3. It should be able to cross the membrane.

- Apart from increasing solubility, they are also used to improve the solubility of volatile constituents used to impart a desirable flavour and odour to the product.

Solid – Dispersion System

- Definition :

Solid dispersion is defined as dispersion of one or more active ingredients in an inert carrier or matrix at solid state prepared by the melting, solvent or melting solvent method.

Classification (Based on FastRelease mechanism)

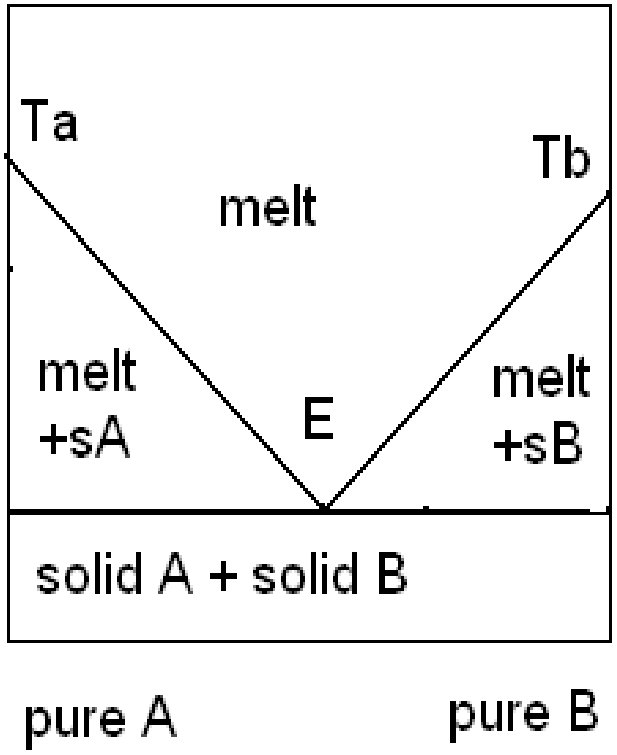
- Simple Eutectic Mixtures
- Solid Solutions
- Glass Solutions and Glass Suspensions
- Amorphous precipitation of drug in crystalline carrier
- Compounds or Complex formation between drug and carrier
- Any combination among the above

A. Eutectic Mixtures

- When two or more substances are mixed together they liquefy due to the lowering of melting point than their individual melting point. Such substances are called as eutectic substances.

  e.g. paracetamol-urea, griseofulvin-urea

A. Eutectic Mixtures



- Simple binary phase diagram showing eutectic point E.
- The eutectic composition at point E of substance A and B represents the melting point.
- T_A and T_B are melting point of pure A and pure B.

A. Eutectic Mixtures

- The following factors may contribute to faster dissolution rate of drug dispersed in the eutectic mixtures:-

  1. Increase in drug solubility.
  2. Solubilization effect by the carrier which completely dissolves in a short time in diffusion layer surrounding drug particles.
  3. Absence of aggregation and agglomeration between fine crystallites of pure hydrophobic drug.

A. Eutectic Mixtures

4. Excellent wettability and dispersibility of a drug as the encircling soluble carrier readily dissolves and causes water to contact as wet drug particles.

5. Crystallization of drug in a metastable form after solidification from fused solution, which has high solubility.

A. Eutectic Mixtures

- Eutectics are easy to prepare and economical with no solvents involved. The method however cannot be applied to:

  - Drugs which fail to crystallize from mixed melt.

  - Thermolabile drugs.

  - Carriers such as succinic acid that decompose at melting point.

B. Solid Solutions

- It is made up of a solid solute dissolved in a solid solvent. It is often called a “mixed crystal” because the two components crystallize together in a homogenous phase system.
- It is prepared by fusion method.
- A solid solution of poorly soluble drug in a rapidly soluble carrier achieves a faster dissolution because particle size of drug is reduced to molecular size.

Classification

- According to extent of miscibility :

1. Continuous (iso-morphous, unlimited, complete) solid solution.
2. Discontinuous (limited, restricted, incomplete) solid solution.

- According to crystalline structure of solid solutions :

1. Substitutional solid solutions.
2. Interstitial solid solutions.

Classification

a) Continuous Solid Solutions :-

The two components are miscible or soluble at solid state in all proportions.

No established solutions of this kind has been shown to exhibit fast release dissolution properties.

The faster dissolution rate would be obtained if the drug is present as a minor compartment.

b) Discontinuous Solid Solutions :-

There is only limited solubility of a solute in a solid solvent in this group of solid solutions.

C. Glass Solutions and Glass Suspensions

- A glass solution is a homogenous, glassy system in which a solute is usually obtained by abrupt quenching of the melt.
- Many compounds have been shown to be able to form glasses readily upon cooling from liquid state.
- These compounds include sucrose, glucose, ethanol and 3- methyl hexane.

C. Glass Solutions and Glass Suspensions

- It is presumably due to their strong hydrogen bonding which may prevent their crystallization.
- Polymers possessing linear, flexible chains can freeze into a glass state to transparency and brittleness.
- The strength of chemical binding in a glass solution is much less compared to that in a solid solution.
- Hence, dissolution rate of drugs in the glass solution is faster than in solid solution.
- e.g. Glass solution of citric acid

D. Amorphous Precipitation of Drug in Crystalline Carrier

- Instead of forming a simple eutectic mixture in which both drug and the carrier crystallize simultaneously from a solvent method of preparation, the drug may also precipitate out in an amorphous form in crystalline carrier.

- It has faster dissolution and absorption rates than crystalline form.
- e.g. Amorphous novobicin has 10 fold higher solubility than its crystalline form.

E. Compound or Complex Formations

- Dissolution and absorption of a drug can occur from a complex or a compound formed between the drug and an inert soluble carrier.
- Complexation also implies that dissolution could be retarded as observed with PEG 4000 - phenobarbital.
- However, the formation of a soluble complex with a low association constant results in increased rates of dissolution and absorption.

F. Combinations and Miscellaneous Mechanisms

- A solid dispersion entirely belongs to any five groups discussed so far, but it can also be made up of combinations of different groups.

- These combinations increase the dissolution and absorption rate.

- The griseofulvin dispersed at high concentrations in PEG may exist as individual molecules and as micro-crystalline particles.

Methods of Preparations

- Melting Method
- Solvent Method
- Melting - Solvent Method
- Hot Melt Extrusion Technique

1. Melting Method or Fusion Method

- The physical mixture of a drug and water soluble carrier is heated until it melts.
- The melt is then cooled and solidified rapidly in an ice bath with vigorous stirring .
- The final solid mass is crushed, pulverized and sieved.
- To facilitate faster solidification, the homogenous melt is poured in the form of a thin layer onto stainless steel plate and cooled by flowing air or water on the opposite side of the plate.

1. Melting Method or Fusion Method

- Advantages :
- Simplicity of method.
- Supersaturation of a solute or a drug in a system can often be obtained by quenching the melt rapidly from high temperature.
- Disadvantage :
- Some drugs or carriers may decompose or evaporate during fusion process at high temperatures .

  e.g. succinic acid used as a carrier for griseofulvin is quite volatile and may also partially decompose by dehydration near its melting point.

2. Solvent Method

- They are prepared by dissolving a physical mixture of two solid components in a common solvent, followed by evaporation of the solvent.

- The method is used to prepare solid dispersions of griseofulvin-polyvinylpyrrolidone, sulphathiazole - pvp.

2. Solvent Method

- Advantage :
  - Thermal decomposition of drugs or carriers can be prevented because of low temperature required for the evaporation of organic solvents.

- Disadvantages :
  - High cost of preparation.
  - Difficulty in completely removing the solvent.
  - Difficulty in producing crystal forms.

3. Melting Solvent Method

- It is prepared by first dissolving the drug in a suitable solvent and then incorporating this solution in a melt of PEG without removing the solvent.

- Advantages :

  Same as above two methods

- Disadvantage :

  From practical stand point, it is only limited to drugs with a low therapeutic dose, e.g. below 50mg.

4. Hot Melt Extrusion Method

- In this method, a blend of active ingredients, polymeric carrier and other processing aids like plasticizers and antioxidants is heated and softened.
- This softened material is called as extrudate.
- When the extrudate is cooled at room temperature, the polymeric thermal binder solidifies and bonds the excipients together to form a matrix.

4. Hot Melt Extrusion Method

- Advantages :
  - There are no concerns with solvent handling or recovery after processing

  -It is simple and continuous process for preparation of tablets and granulations.
  - The process is faster and there were fewer steps than the wet granulation method.

  -Can be used for formulating sustained release granules.

  e.g. Diltiazem granules.

Methods of Determination of Solid Dispersion Systems

- Thermal analysis

  a) Cooling curve method

  b) Thaw-melt method

  c) Thermoscopic method

  d) Differential thermal analysis (DTA)

  e) Zone Melting Method

Methods of Determination of Solid Dispersion Systems

- X-Ray diffraction Method
- Microscopic method
- Spectroscopic method
- Thin layer chromatography
- Solubility determinations

A Thermal Analysis

b) Thaw-melt method

- Here a sample of solidified mixture in a capillary melting point tube is heated gradually till the thaw point.

- The thaw point is referred to as crossing solidus line.

- It is useful in differentiating between a simple eutectic system and a limited solution.

A. Thermal Analysis

c) Thermoscopic method :

- Polarized microscopy is used with hot stage to study phase diagrams of binary systems.
- The physical mixture is gradually heated on a slide until it completely liquefies.
- After cooling, the mixture is heated at rate of 4 degree per minute.
- The thaw and melting points are determined by visual observations.

A. Thermal Analysis

d) Differential thermal analysis (DTA) :

- An effective thermal method for studying phase equilibria of either pure compound or mixture.

- Different effects, associated with physical or chemical changes are automatically recorded as function of time or temperature as the substance is heated in uniform rate.

- In addition; evaporation, sublimation, polymorphic transition, desolvation can be detected.

B X-Ray Diffraction Method

- In this method the intensity of x-ray diffraction or reflection from a sample is measured as a function of diffraction angles.
- Counter and film methods detect diffraction intensity.
- Counter method provides better resolution of diffraction and relative intensity which can be easily compared.
- This method is used to characterize physico-chemical properties of Griseofulvin dispersed in PEG 4000 and 6000.

C Microscopic Method

- It has been used to study polymorphism and morphology of solid dispersion.
- The fine particles of crystallization in glass PVP can be easily detected by polarizing microscope.
- The resolution of electron microscope was used to study dispersed particle size of iopanic acid in PVP.

D. Spectroscopic Method

- In the UV study, the spectra of pure drug and the dispersed drug are scanned.
- e.g. The spectrum of the dispersed beta – carotene resembles that beta–carotene is dissolved in organic solvents but do not indicate the molecular dispersion of drug in polymer.

E. Thin Layer Chromatography

- TLC characteristics of pure and dispersed drugs are studied to test whether the drugs are decomposed by process.

- A single spot with same 'R_f 'value is expected for both the pure and processed samples in thin layer plate.

F. Solubility determinations

- Results from aqueous solubility studies of drug in various concentrations of carrier would indicate interactions between drug and carrier.
- Such studies indicated weak or insignificant interactions between griseofulvin and PEG 6000.
- Increased rate of dissolution due to solubility of the drug by carrier can be predicted by this method.

Pharmaceutical Applications

- To obtain a homogenous distribution of small amount of drugs at solid state.
- To stabilize unstable drugs.
- To dispense liquid or gaseous compounds.
- To formulate a faster release priming dose in a sustained release dosage form.
- To formulate sustained release dosage or prolonged release regimens of soluble drugs by using poorly soluble or insoluble carriers.

β-cyclodextrin drug dispersion system,

techniques for studies of crystals, polymorphism

β-cyclodextrin drug dispersion system

- The poorly dissolution of relatively insoluble drug has for long been a problem in the formulation of oral dosage form.

- This limits the aspect such as
  - Absorption &
  - Bioavailability

β-cyclodextrin drug dispersion system

- Several approach have been followed in improving the solubility of drug, one of them being complexation using cyclodextrin.

- Cyclodextrin is cyclic structure oligomers of glucose which are obtained from the starch digests of the bacteria Bacillus macerans.

Method of preparation of β-cyclodextrin complex

- Physical mixture method
- Kneading method
- Co-evaporation method
- Solid dispersion method
- Spray drying method
- Neutralization method

Physical mixture method

- Here the drug and b-cyclodextrin (1:2) are mixed physically with spatula & then the pulverized powder is passed through 100#.

- Eg. Diclofinac sodium

Kneading method

- Here the b-cyclodextrin is dissolved in small vol. of water-methanol solution(6:4).
- To the above solution required drug is added in small amount.
- The slurry is then kneaded for 45 min. & dried at 45°c.
- The dried mass is pulverized and sieved through 100#.
- Eg. Nimesulide , Omeprazole

Co-evaporation method

- In this method, aq. solution of b-cyclodextrin is added to an alcoholic solution of drug.
- The resulting mix. is stirred for 1 hr. & evaporated at 45^{o}c until it is dried.
- The dried mass is pulverized and sieved through 100#.
- Eg. Steroids & Diclofenac sodium

Solid dispersion method

- Here the drug & molar qty. of b-cyclodextrin is dissolved in methanol.
- The solution is then evaporated in vacuum at 40^{o}c with rotatory evaporator.
- The powder is stored under vacuum in dessicator for 3 days & analysed.
- Eg. Rifampicin

Spray drying method

- In this, the drug & double molar of β-cyclodextrin are dissolved in methanol.

- The solution was then spray dried under foll. conditions –

  Feed rate – 10 ml/min

  Inlet temp. - 95^{o}c

  Outlet temp. - 65^{o}c

  Press. – 5 bar

  Drying air – 35 m^3

Spray drying method

- The powder is then collected & stored under vacuum in dessicator for 3 days & analysed.

- Eg. Naproxene

Neutralization method

- Here the drug & b-cyclodextrin are dissolved in 0.1N HCl & then 0.1N NaOH is added to precipitate the complex at pH-7.5.
- The ppt. is washed with distilled water.
- Then it is pulverized & sieved through 90# and stored in dessicator over fused $CaCl_2$.
- Eg. Ketoconazole

Applications

- To increase aq. solubility
- To increase dissolution rate of drug
- To improve bioavailability of drug
- To increase chemical/physical stability
- To decrease drug irritation

Crystallinity

- Crystal habit & internal structure of drug can affect bulk & physicochemical property of molecule.
- Crystal habit is description of outer appearance of crystal.
- Internal structure is molecular arrangement within the solid.

Crystallinity

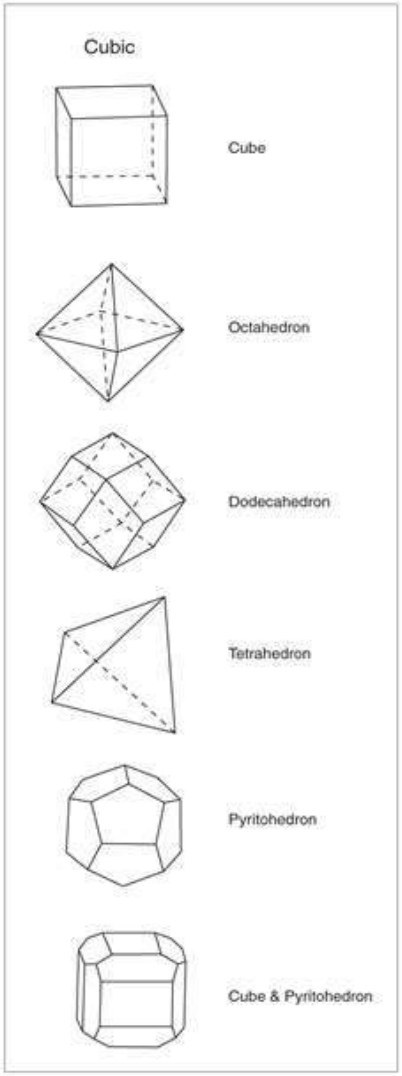
- Change with internal structure usually alters crystal habit.

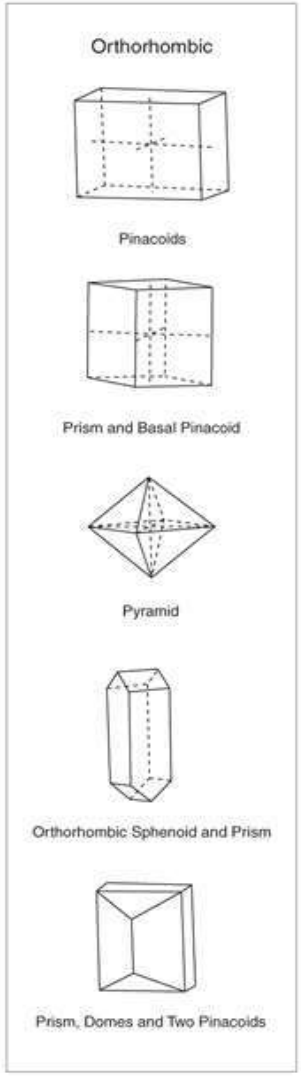
  Eg. Conversion of sodium salt to its free acid form produce both change in internal structure & crystal habit.

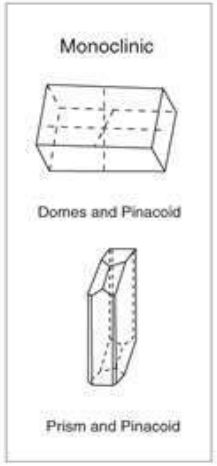
Different shapes of crystals

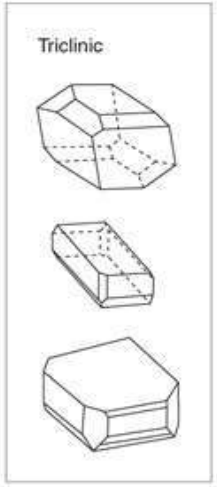
- **Cubic or isometric** - not always cube shaped. Also find as octahedrons (eight faces) and dodecahedrons (10 faces).
- **Tetragonal**- similar to cubic crystals, but longer along one axis than the other, forming double pyramids and prisms.
- **Orthorhombic** - like tetragonal crystals except not square in cross section (when viewing the crystal on end), forming rhombic prisms or dipyramids (two pyramids stuck together).
- **Hexagonal** - six-sided prisms. When you look at the crystal on-end, the cross section is a hexagon.
- **Trigonal** - possess a single 3-fold axis of rotation instead of the 6-fold axis of the hexagonal division.
- **Triclinic** - usually not symmetrical from one side to the other, which can lead to some fairly strange shapes.
- **Monoclinic** - like skewed tetragonal crystals, often forming prisms and double pyramids.

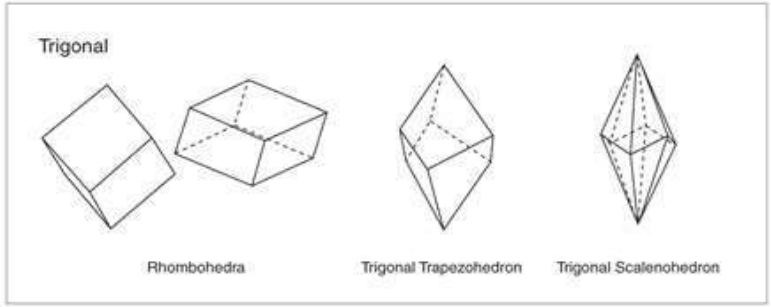
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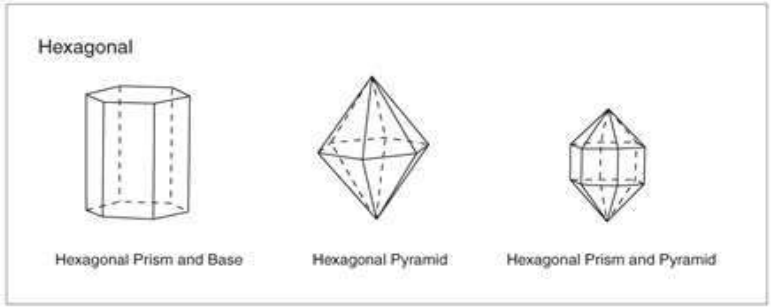


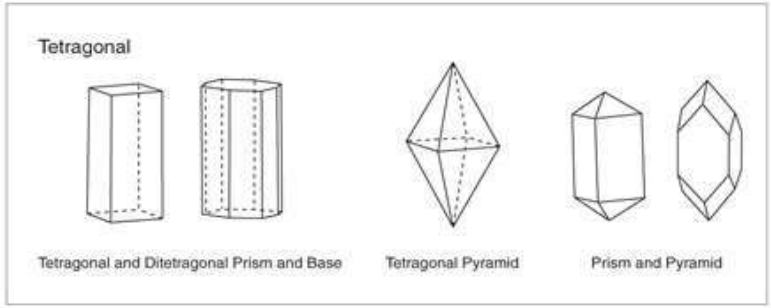












Different shapes of crystals

- Depending on internal structure compounds is classified as
  1. Crystalline
  2. Amorphous
- Crystalline compounds are characterized by repetitious spacing of constituent atom or molecule in three dimensional array.
- In amorphous form atom or molecule are randomly placed.

Different shapes of crystals

- Solubility & dissolution rate are greater for amorphous form then crystalline, as amorphous form has higher thermodynamic energy.

Eg. Amorphous form of Novobiocin is well absorbed whereas crystalline form results in poor absorption.

Polymorphism

- It is the ability of the compound to crystallize as more than one distinct crystalline species with different internal lattice.
- Different crystalline forms are called polymorphs.
- Polymorphs are of 2 types
  1. Enatiotropic
  2. Monotropic

Polymorphism

- The polymorph which can be changed from one form into another by varying temp. or pressure is called as Enantiotropic polymorph.

  Eg. Sulfur.

- One polymorph which is unstable at all temp. & pressure is called as Monotropic polymorph.

  Eg. Glyceryl stearate.

Polymorphism

- Polymorph differ from each other with respect to their physical property such as
  - Solubility
  - Melting point
  - Density
  - Hardness
  - Compression characteristic

Polymorphism

- During preformulation it is important to identify the polymorph that is stable at room temp.

Eg. 1)Chloromphenicol exist in A,B & C forms, of these B form is more stable & most preferable.

2)Riboflavin has I,II & III forms, the III form shows 20 times more water solubility than form I.

Techniques for studies of crystals

- Microscopy
- Hot stage microscopy
- Thermal analysis
- X-ray diffraction

Microscopy

- Material with more than one refractive index are anisotropic & appear bright with brilliant colors against black polarized background.
- The color intensity depends upon crystal thickness.
- Isotropic material have single refractive index and this substance do not transmit light with crossed polarizing filter and appears black.

Microscopy

- Advantage :

  By this method, we can study crystal morphology & difference between polymorphic form.

- Disadvantage :

  This require a well trained optical crystallographer, as there are many possible crystal habit & their appearance at different orientation.

Hot stage microscopy

- The polarizing microscope fitted with hot stage is useful for investigating polymorphism, melting point & transition temp.

- Disadvantage :

  In this technique, the molecules can degrade during the melting process.

Thermal analysis

- Differential scanning calorimetry (DSC) & Differential thermal analysis are (DTA) are particularly useful in the investigation of polymorphism.

- It measures the heat loss or gain resulting from physical or chemical changes within a sample as a function of temp.

Thermal analysis

- For characterizing crystal forms , the heat of fusion can be obtained from the area under DSC- curve for melting endotherms.
- Similarly, heat of transition from one polymorph to another may be calculated.
- A sharp symmetric melting endotherm can indicate relative purity of molecule.

Thermal analysis

- A broad asymmetric curve indicates presence of impurities.

- Disadvantage :

  Degradation during thermal analysis may provide misleading results.

X-ray diffraction

- Working :

  When beam of nonhomogenous X-ray is allow to pass through the crystal, X-ray beam is diffracted & it is recorded by means of photographic plate.

- Diffraction is due to crystal which acts as 3 dimensional diffraction grating toward X-ray.

X-ray diffraction

- Random orientation of crystal lattice in the powder causes the X-ray to scatter in a reproducible pattern of peak intensities.

- The diffraction pattern is characteristic of a specific crystalline lattice for a given compound.

X-ray diffraction

- An amorphous form does not produce a pattern mixture of different crystalline forms.

- Single – Crystal x-ray provide the most complete information about the solid state.

Stability testing….



Why Stability?



- Provide a evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as….. temperature, Humidity and light.

- Establish a re-test period for the drug substance or a shelf life for the drug product and recommended storage conditions.
- Because physical, chemical or microbiological changes might impact the efficiency and security of the final product

Where and Why?



Stability Studies are preformed on ...

- **Drug Substances (DS)** The unformulated drug substance that may subsequently be formulated with excipients to produce the dosage form.

- **Drug Products (DP)** The dosage form in the final immediate packaging intended for marketing…….

- controlled and documented determination of acceptable changes of the drug substance or drug product

What are changes?



- Physical changes
  - Appearance
  - Melting point
  - Clarity and color of solution
  - moisture
  - Crystal modification (Polymorphism)
  - Particle size
- Chemical changes
  - Increase in Degradation
  - Decrease of Assay
- Microbial changes

Forced degradation studies

- Acidic & Basic conditions.
- Dry heat exposure
- UV radiation exposure
- Influence of pH
- Influence of temperature
- Influence of ionic strength

Arrhenius Equation

- $K = Se^{-Ha/RT}$

where..k = specific rate of degradation.

R = gas constant (1.987 calories degree $^{-1}$mole)

T = absolute temperature.

S = frequency factor.

Logarithmically ,

$\ln k = -Ha/ RT + \ln S$

converting to log $_{10}$

$\text{Log } k = -\Delta Ha/2.303\ R\ .1/T + \log S$

log k = specific rate of degradation

S = constant

Arrhenius Equation

- Plot of log K v/s 1/T….yields a slope equal to -ΔHa/2.303 R ….. From which heat of activation (ΔHa) can be calculated.

- Log k2/k1 = ΔHa/2.303 R . (T2 – T1)/ T2.T1

Mean Kinetic Temperature

Clasius – Clapeyron equation

- $\ln = P_2 / P_1 . \Delta H V (T_2 – T_1) / R (T_2 _ T_1)$

where…. P_2 & P_1 = vapour pressure at T_1 & T_2

ΔH =molar (latent) heat of evaporation

Relative humidity

Q =PD / PS . 100

RH is expressed in percentage (%)

Q = Relative humidity

PD = partial pressure of unsaturated air

PS = saturation pressure

Chemical degradation studies

- Hydrolysis
- Oxidation
- Reduction
- Decarboxylation
- Photolysis

Stability studies at different stages

- Stress- and accelerated Testing with drug substances
- Stability on pre-formulation batches
- Stress testing on scale-up Batches
- Accelerated and long term testing for registration
- On-going Stability testing
- Follow-up Stabilities

Testing scope for Solid dosage

Tablet & Capsule



- **Physical-chemical properties**
  - Appearance
  - Elasticity
  - Mean mass
  - Moisture
  - Hardness
  - Disintegration
  - Dissolution
- **Chemical properties**
  - Assay
  - Degradation
- **Microbial properties**
- **Container closure system properties**
  - Functionality tests (e.g. extraction from blister)

Testing scope for Oral liquid form

- Physical-chemical properties
  - pH
  - Color & clarity of solution
  - Viscosity
  - Particle size distribution (for oral suspensions only)
- Chemical properties
  - Assay
  - Degradation products
  - Degradation preservatives
  - Content antioxidants
- Microbial properties
- Container closure system properties
  - Functionality tests

Testingscope for *liquid forms* for inj. And parenterals

Physical-chemical properties

- pH
- Loss on weight
- Color & clarity of solution

Chemical properties

- Assay
- Degradation products
- Degradation preservatives
- Content antioxidants

Microbial properties

-

- Container closure system properties
  - Functionality tests

Testing scope for *SEMI LIQUID FORMS*

- Physical-chemical properties
  - Appearance, odor, homogenesity, consistency
  - Loss on weight, Viscosity
  - Content uniformity (within the container)
- Chemical properties
  - Assay
  - Degradation products & preservatives
  - Content preservatives
  - Degradation– Content antioxidants
- Microbial properties

- Container closure system properties
  - Functionality tests

Climatic Zones / Storage conditions

Climatic Zone Countries	Calculated data			Derived data	
	Temp. °C	MKT °C	humidity % r.h.	Temp °C	humidity % r.h.
Climatic Zone I "Temperate" Japan, United Kingdom, Northern Europe, Canada, Russia, United States	**20**	**20**	**42**	**21**	**45**
Climatic Zone II "Mediterranean, Subtropical" Japan, United States, Southern Europe	**26.4**	**22**	**52**	**25**	**60**

Climatic Zones / Storage conditions

Climatic Zone Countries	Calculated data			Derived data	
	Temp. °C	MKT °C	humidity % r.h.	Temp °C	humidity % r.h.
Climatic Zone **III** "Hot, dry" Iran, Iraq, Sudan	**26,4**	**27,9**	**35**	**30**	**35**
Climatic Zone **IV** "Hot, humid" Brazil, Ghana, Indonesia, Nicaragua, Philippines	**26,7**	**27,4**	**76**	**30**	**70**

What or Who is ICH?

- **ICH** stands for **I**nternational **C**onference on **H**armonization of Technical Requirements for Registration of Pharmaceuticals for Human use

- **Objectives of ICH**
- Harmonization of registration applications within the three regions of the EU, Japan and the United States.
- ICH is a joint initiative involving both regulators and industry as equal partners in the scientific and technical discussions of the testing procedures which are required to ensure and assess
- The quality ,safety and efficasy of the product.

What or Who is ICH?

There are Six Parties directly involved in the decision making process

- **EU: European Commission - European Union**
- **EFPIA: European Federation of Pharmaceutical Industries and Associations**
- **MHLW: Ministry of Health, Labor and Welfare, Japan**
- **JPMA: Japan Pharmaceutical Manufacturers Association**
- **FDA: US Food and Drug Administration**
- **PhRMA: Pharmaceutical Research and Manufacturers of America**

There are additionally observers installed to act as a link with non-ICH countries and regions

- WHO
- The European Free Trade Area (EFTA), represented by Swissmedic Switzerland
- Health Canada
- Global guidelines

ICH Guidelines

- Quality Guidelines "**Q**" (chemical and pharmaceutical QA)
  - details see next slide
- Safety Guidelines "**S**" (in vitro and in vivo pre-clinical studies)
  - covering Carcinogenicity Testing, Genotoxicity Testing, Toxicokinetics and Pharmacokinetics ….. etc.
- Efficacy Guidelines "**E**" (clinical studies in human subject)
  - Covering clinical safety, Dose Response Studies, Good Clinical Practices, Clinical evaluation …. etc.
- Multidisciplinary Guidelines "**M**"
  - Covering Medical Terminology, Electronic Standards for Transmission of Regulatory Information …… etc.
  - Important for Stability !
    - » Guideline **M4**: The Common Technical Document (CTD)

ICH Q-Guidelines (Quality)

-
- Stability Testing in Climatic Zone I and II (Q1A)
- Photostability Testing (Q1B)
- Stability Testing for New Dosage Forms (Q1C)
- Bracketing and Matrixing Designs (Q1D)
- Evaluation of Stability Data (Q1E)
- Stability Testing in Climatic Zones III and IV (Q1F)
- Validation of Analytical Procedures (Q2)
- Impurities (Q3)
- Biotechnological Products (Q5)
- Specifications (Q6)

New Drug Substances & Products

- Stability Testing in Climatic Zone I and II (Q1A)
- Photostability Testing (Q1B)
- Stability Testing for New Dosage Forms (Q1C)
- Bracketing and Matrixing Designs (Q1D)
- Evaluation of Stability Data (Q1E)
- Stability Testing in Climatic Zones III and IV (Q1F)
- Validation of Analytical Procedures (Q2)
- Impurities (Q3)
- Biotechnological Products (Q5)
- Specifications (Q6)

Drug substances - General case

Study	Storage condition	Minimum time period covered by data at submission
Long term	**25°C ± 2°C / 60% ± 5% r.h or 30°C ± 2°C / 65% ± 5% r.h.**	**12 months**
Intermediate	**30°C ± 2°C / 65% ± 5% r.h.**	**6 months**
Accelerated	**40°C ± 2°C / 75% ± 5% r.h.**	**6 months**

Drug substances - intended for storage in a Refrigerator

Study	Storage condition	Minimum time period covered by data at submission
Long term	**5°C ± 3°C**	**12 months**
Accelerated	**25°C ± 2°C / 60% ± 5% r.h.**	**6 months**

Drug substances/Product- intended for storage in Freezer

Study	Storage condition	Minimum time period covered by data at submission
Long term	**-20°C ± 5°C**	**12 months**

Drug products - General case

Study	Storage condition	Minimum time period covered by data at submission
Long term	**25°C ± 2°C / 60% ± 5% r.h. or 30°C ± 2°C / 65% ± 5% r.h.**	**12 months**
Intermediate	**30°C ± 2°C / 65% ± 5% r.h.**	**6 months**
Accelerated	**40°C ± 2°C / 75% ± 5% r.h.**	**6 months**

Drug products - packaged in Semi-permeable containers

Study	Storage condition	Minimum time period covered by data at submission
Long term	**25°C ± 2°C / 40% ± 5% r.h. or 30°C ± 2°C / 35% ± 5% r.h.**	**12 months**
Intermediate	**30°C ± 2°C / 65% ± 5% r.h.**	**6 months**
Accelerated	**30°C ± 2°C / 65% ± 5% r.h.**	**6 months**

Drug products - intended for storage in a Refrigerator

Study	Storage condition	Minimum time period covered by data at submission
Long term	**5°C ± 3°C**	**12 months**
Accelerated	**25°C ± 2°C / 60% ± 5% r.h.**	**6 months**

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3. Textbook of physical pharmaceutics by C.V.S. Subrahmanyam, published by Vallabh prakashan, page no:- 182-208, 222-226.

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Thank You

OFFICIAL QUALITY CONTROL TESTS OF TABLETS

By

Sahera

VCPS

OFFICIAL QUALITY CONTROL TESTS OF TABLETS AS PER I.P

- ***WEIGHT UNIFORMITY***
- ***FRIABILITY***
- ***CONTENT UNIFORMITY***
- ***DISINTEGRATION TEST***
- ***DISSOLUTION TEST***
- ***HARDNESS (Un Official)***

WEIGHT UNIFORMITY

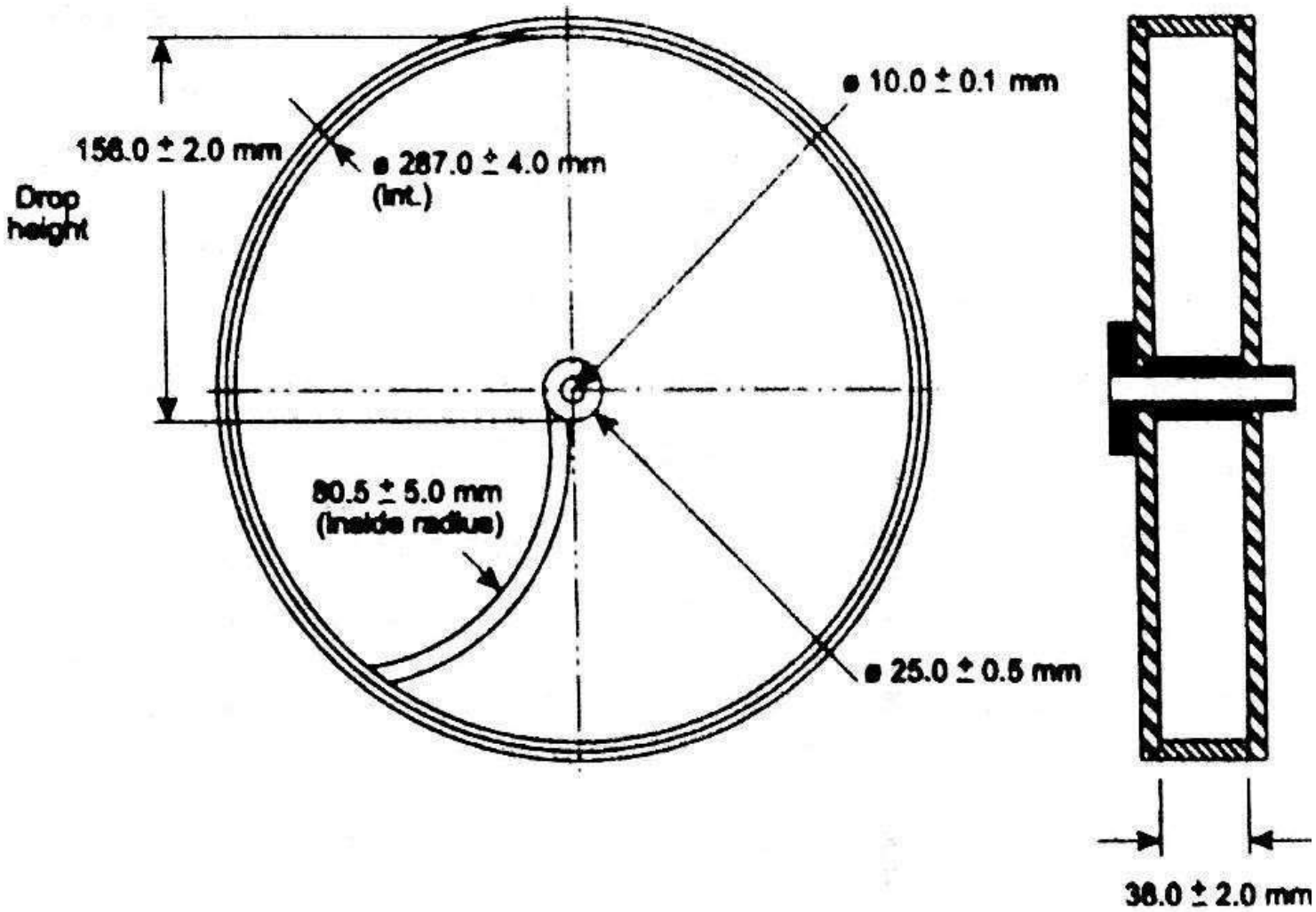
Table

Dosage form	Average weight	Percentage deviation
Uncoated and film-coated tablets	80 mg or less	10
	More than 80 mg but less than 250 mg	7.5
	250 mg or more	5

FRIABILITY



FRIABILITY



Tablet friability apparatus

CONTENT UNIFORMITY

Table

Weight of active ingredients in each tablet	Subtract from lower limit for samples of			Add to the upper limit for samples of		
	15	10	5	15	10	5
0.12 g or less	0.2	0.7	1.6	0.3	0.8	1.8
More than 0.12 g but less than 0.3 g	0.2	0.5	1.2	0.3	0.6	1.5
0.3 g or more	0.1	0.2	0.8	0.2	0.4	1.0

DISINTEGRATION APPARATUS



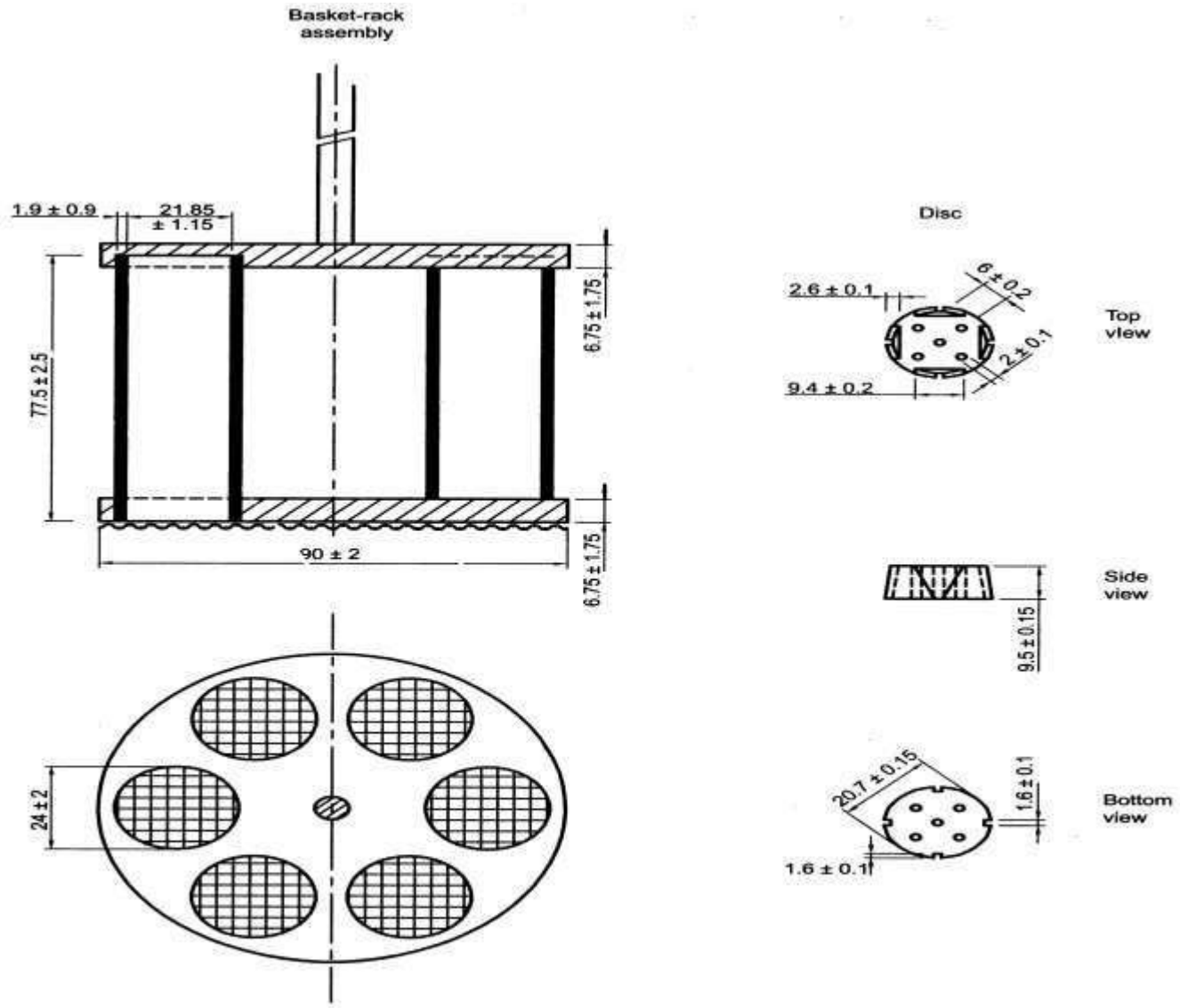


Figure 2.9.1.-1. – *Disintegration apparatus A*

Dimensions in millimetres

A. UNCOATED TABLETS

- No of Tablets : 06
- Disintegration Medium : Distilled Water
- Disintegration Time : 15 minutes
- Temperature : 37^0c

B. COATED TABLETS

- No of Tablets : 06
- Disintegration Medium : Distilled Water/ replaced with 0.1NHCL
- Disintegration Time : 60 minutes
- Temperature : 37^0c

C. DISPERSIBLE TABLETS

UNIFORMITY OF DISPERSION

- No of Tablets : 02
- Temperature : 24^0C to 26^0C
- Disintegration Medium : **100ml** Distilled Water
- Dispersion Test : Pass through Sieve No ≠ **22**

  Disintegration Time : 3minutes

D. EFFERVESCENCE TABLETS

TEST FOR EFFERVESCENCE

- No of Tablets : **06**
- Disintegration Medium : **250ml** Distilled Water
- Disintegrated Time : **5minutes**
- Temperature : **20^0C to 30^0C**

E. ENTERIC – COATED TABLETS

- No of Tablets : 06
- Disintegration Medium : 0.1N HCl for 120min and replace it with 6.8*pH* phosphate buffer for 60min
- Temperature : 37^0c

DISSOLUTION APPARATUS



DISSOLUTION TEST

Apparatus 1

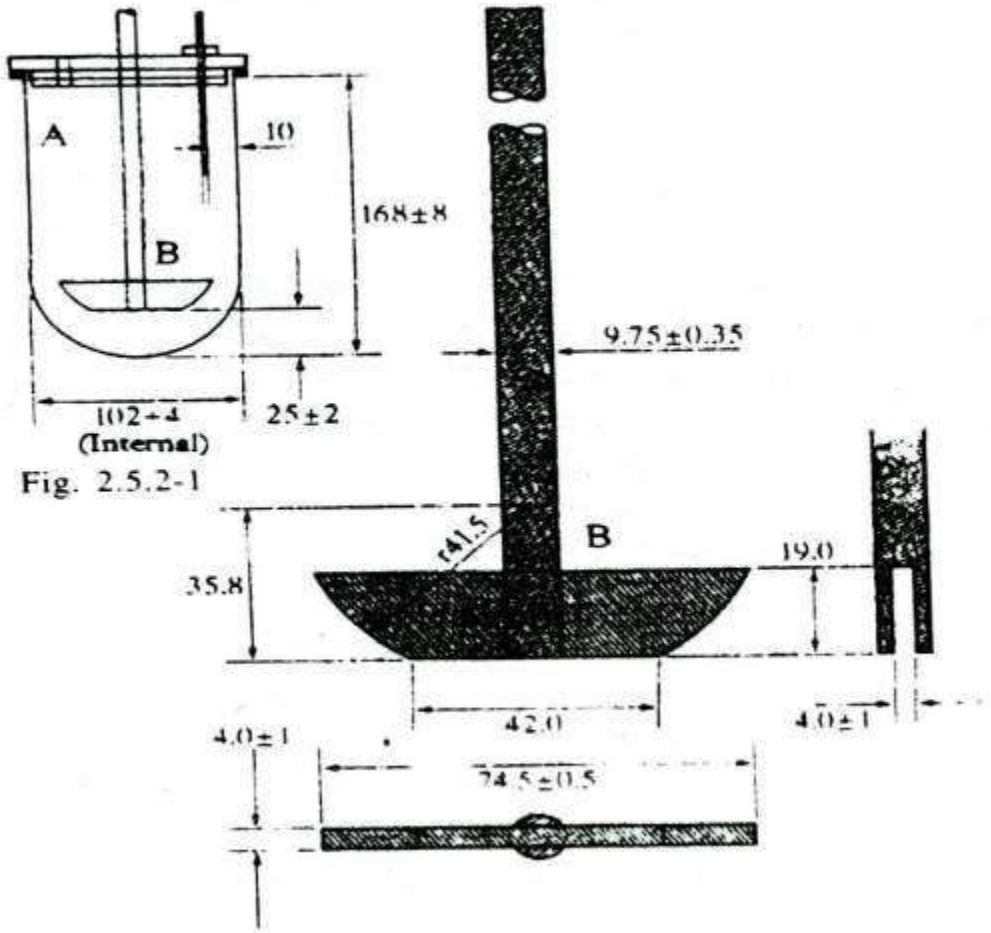


Fig. 2.5.2-1

DISSOLUTION TEST

Apparatus 2

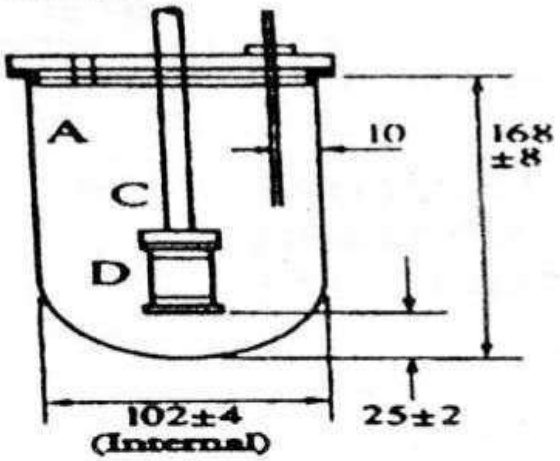
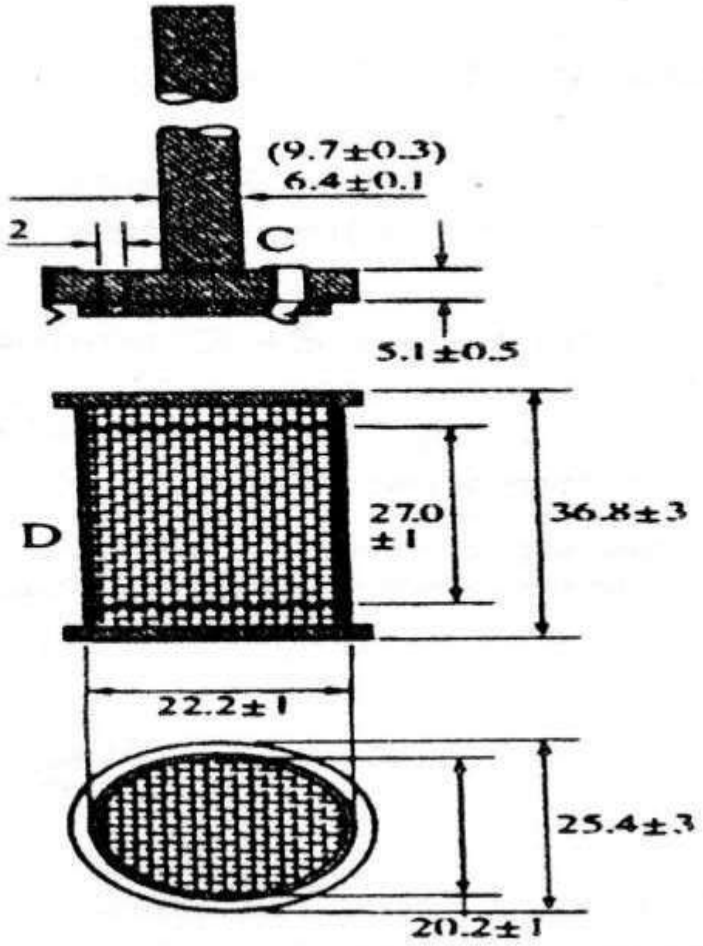


Fig. 2.5.2-3



DISSOLUTION TEST ACCEPTANCE CRITERIA

Table 1

Level	Number tested	Acceptance criteria
S_1	6	Each unit is not less than D* + 5 per cent**.
S_2	6	Average of 12 units (S_1 +S_2) is equal to or greater than D, and no unit is less than D – 15 per cent**.
S_3	12	Average of 24 units (S_1+S_2+S_3) is equal to or greater than D, not, More than 2 units are less than D – 15 per cent** and no unit is less than D – 25 per cent**.

*D is the amount of dissolved active ingredient specified in the individual monograph, expressed as a percentage of the labelled content.

**Percentages of the labelled content.

HARDNESS (UNOFFICIAL)

MONSANTO HARDNESS TESTER



HARDNESS (UNOFFICIAL)

Pfizer HARDNESS TESTER





TABLET COATING

G.Shankar

VCPS.



Contents

- Definition
- Principles
- Advantages
- Disadvantages
- Types of tablet coating
- Equipment used in tablet coating
- Defects of tablet coating
- Advances in tablet coating
- Applications
- References



Tablet coating:

It is the application of a coating composition to a moving bed of tablets with the concurrent use of heated air to facilitate evaporation of the solvent.

The distribution of the coating is accomplished by the movement of tablets either perpendicular or vertical to the application of the coating composition.



Principles of tablet coating:

1. To mask the taste, odour, or colour of the drug.
2. To provide physical and chemical protection for the drug.
3. To control the release of the drug from the tablet.
4. To protect the drug from the gastric environment of the stomach with an acid-resistant enteric coating.
5. To incorporate another drug or formula adjuvant in the coating to avoid chemical incompatibilities or to provide sequential drug release.
6. To improve the pharmaceutical elegance by use of special colours and contrasting printing.



Advantages of tablet coating:

- ✓ Enhance permeability, mask unpleasant taste, odour and colour of the API.
- ✓ Increase the stability.
- ✓ Increase the mechanical integrity.
- ✓ Enhance the elegance.
- ✓ Modify the drug release profile.
- ✓ Avoid the side effects.
- ✓ Reduces friction and increases packaging rate.



Disadvantages of tablet coating:

- ✓ Relatively high cost.
- ✓ Time consuming process.
- ✓ Requires the expertise of highly skilled technician.



Types of tablet coating:

1. Sugar coating
2. Film coating
3. Enteric coating
4. Press coating
5. Specialized coatings

    a) Compressed coating

    b) Electrostatic coating

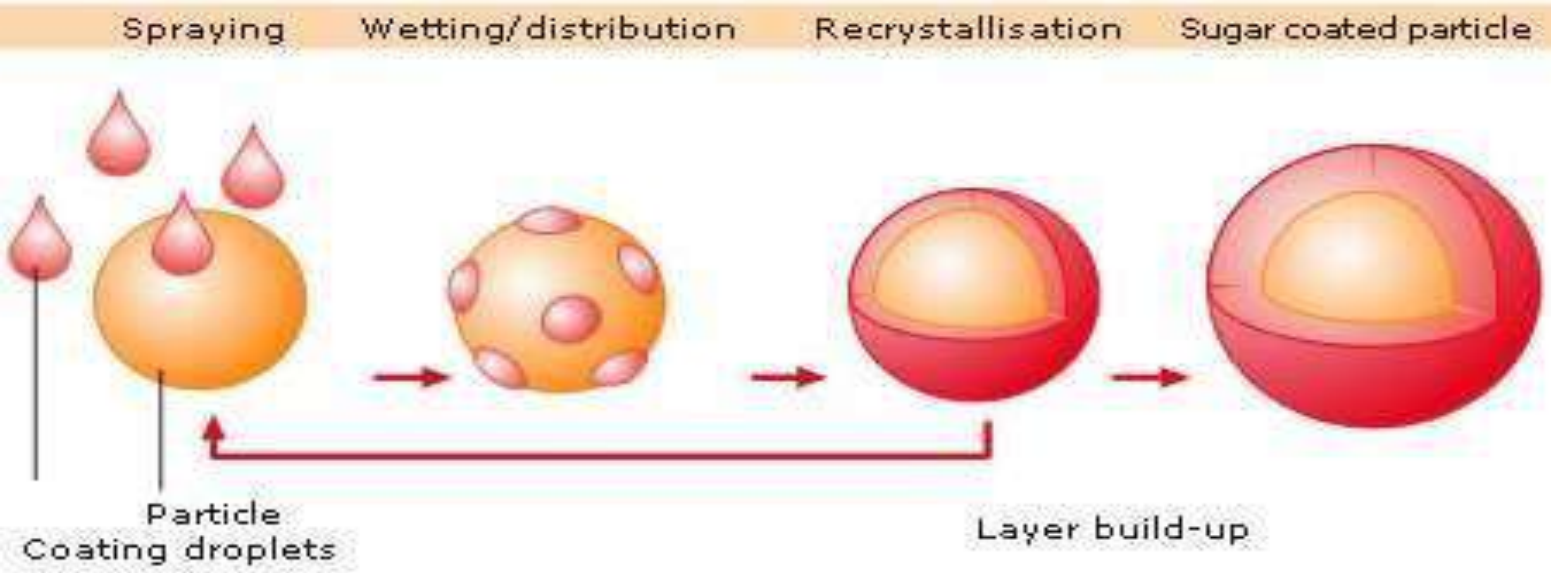
    c) Dip coating

    d) Vacuum film coating

1. SUGAR COATING:

It is a multistep process.

- In suitable sugar–coating equipment, the tablet cores are successively treated with aqueous sucrose solutions.
- Water evaporates from the syrup leaving thick film.
- Sugar coats are often shiny and highly coloured.





Advantages:

✓ Constituent raw materials are widely accepted.

✓ No complex equipment are required.

✓ For high humidity climates, it offers stability advantage over film coated tablets.

Disadvantages:

✓ Time consuming process.

✓ Logo or break lines not possible.

✓ 30-50% weight increase due to coating material.



Sugar coating process involves following steps:

- ✓ Sealing
- ✓ Subcoating
- ✓ Smoothing
- ✓ Colouring
- ✓ Polishing
- ✓ Printing





Sealing:

✓Application of one or more coats of water impermeable polymer such as shellac, cellulose acetate phthalate and polyvinyl acetate phthalate.

✓The main purpose of sealing is to prevent the penetration of moisture in to the tablet core.

✓ The lengthening of tablet disintegration and dissolution times may occur with the usage of shellac as a sealant due to polymerization of shellac.

Subcoating:

✓By adding bulking agents such calcium carbonate or talc in combination with sucrose solution.

✓Used in order to round of the tablet edges.

✓It tyically increases the tablet weight by 20%



Smoothing:

✓ By the application of the thick sucrose syrup, it removes the rough surfaces formed during the sub coating.

✓ It increases the tablet weight by 30%

Colouring:

✓ It usually consists of thin sucrose syrup containing the requisite coloring material (water soluble dyes or water insoluble pigments)

✓ Water insoluble pigments along with the opacifier such as titanium dioxide develop desired colour more rapidly, thus resulting in thinner color coat.



Polishing:

After coloring step, the tablet surfaces are smooth but with dull appearance, to achieve glossy finish, waxes are used.

Ex: Bees wax, Carnauba wax.

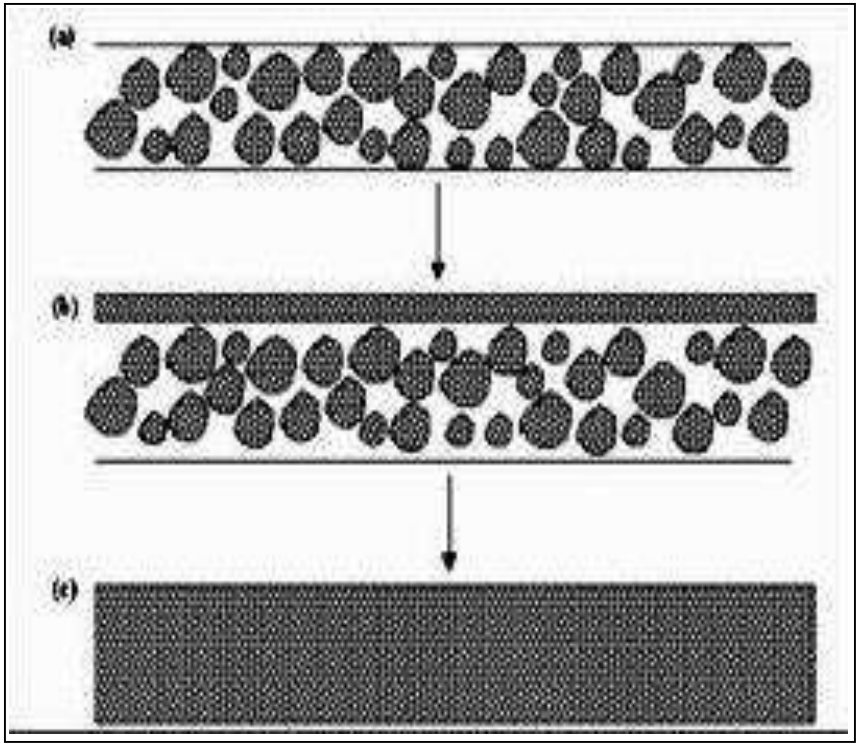
Printing:

For sugar coated tablets, edible ink is used for printing different identification marks such as manufacturer logo, product name, dosage strength etc.



2. ***FILM COATING:***

It involves spraying a solution of polymer along with the plasticizer and pigments on a rotated, mixed tablet bed forms a thin uniform film on the tablet surface.



latex particles dispersed in aqueous phase

formation of thin film with water evaporation through film

continuous film

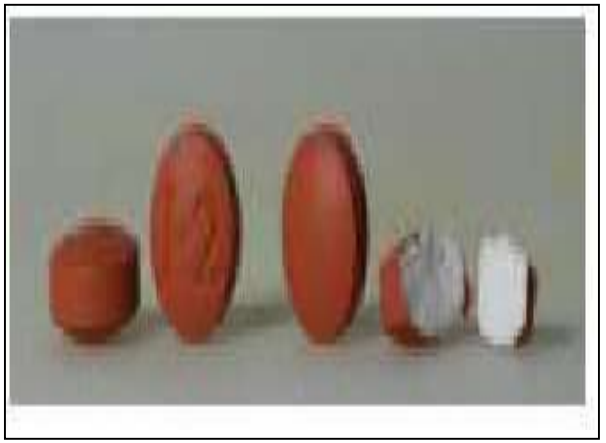


Mechanism of film formation:

In the wet state, the polymer is present as a no. of discrete particles, and these have to come together in close contact, deform, coalesce and ultimately fuse together to form a discrete film. During processing, the substrate surface will be wetted with the diluted dispersion. Under the prevailing processing conditions, water will be lost as water vapour and the polymer particles will increase in proximity to each other, a process which is greatly assisted by the capillary action of the film of water surrounding the particles. Complete coalescence occurs when the adjacent particles are able to mutually diffuse into one another.

Film forming ingredients:

- ✓ Polymers
- ✓ Plasticizers
- ✓ Colorants
- ✓ Solvents
- ✓ Opacifier







Polymers:

A film former is capable of producing smooth thin films reproducible under the prescribed coating conditions.

Ex : Cellulose derivatives - HPMC, MC, HPC

Vinyl derivatives - PVP

Phthalate esters - CAP

Plasticizers:

Affords flexibility and elasticity to the coat and thus provide durability.

Ex: PEG, Diethyl phthalate, Coconut oil



Plasticizers are of two types:

Internal plasticizer:

Chemical modification of the polymer alters;

Degree of substitution

Type of substitution

External plasticizer:

They are non-volatile or other polymers, which when combine with the primary polymeric film former alters;

Flexibility

Tensile strength

Adhesion properties of resulting film



Colorants:

These provide an elegant appearance. They may be soluble in the solvent systems or suspended as insoluble powders.

Ex: Iron oxide pigment, Titanium dioxide.

Opaquant –extenders:

These are fine inorganic powders used in coating solution formulation to increase film coverage.

Ex: Silicates- Aluminium silicate

Carbonates - Magnesium carbonate

Sulfates - Calcium sulfate

Oxides - Magnesium oxide



Solvents:

Volatile organic solvents may be used to allow spreadability of the coat components over the tablets and allow rapid evaporation. But they are expensive, show environmental hazards and solvent residues in the formulation so limited use.

Aqueous vehicles are safer, but slower evaporation which may effect drug stability.

Ex: Water, Methanol, Ethanol, Isopropyl alcohol, Chloroform, Acetone, Methyl ethyl ketone and Methylene chloride.



Comparision of Coating

Film coating

Tablet appearance:

✓Retains shape of original core.

✓Small weight increase of 2-3% due to coating material.

✓logo or 'break lines' possible.

Process:

✓Can be automated.
e.g. Accela Cota

✓Easy training operation

✓Single stage process

✓Easily adaptable for controlled release allows for functional coatings.

Sugar coating

Tablet appearance:

✓Rounded with high degree of polish.

✓Larger weight increase 30-50% due to coating material.

✓Logo or 'break lines' are not possible.

Process:

✓Difficult to be automated.
e.g. Traditional coating pan

✓Considerable training operation required.

✓Multistage process.

✓Not able to be used for controlled release apart from enteric coating.



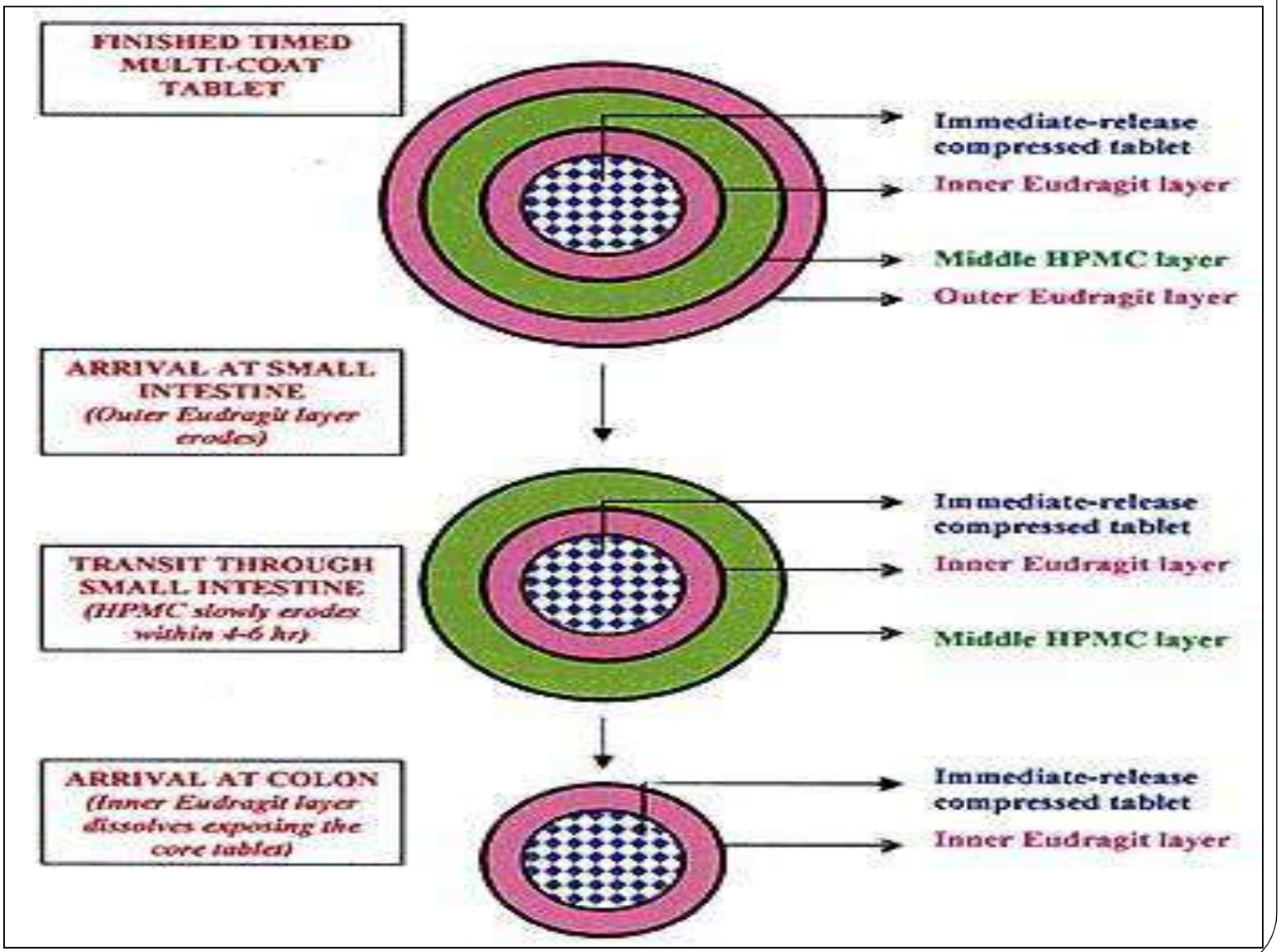
3. ENTERIC COATING:

The technique involved in enteric coating is protection of the tablet core from disintegration in the acidic environment of the stomach by employing pH sensitive polymer, which swell or solubilize in response to an increase in pH to release the drug.

Aim:

- ✓ To mask taste or odour.
- ✓ Protection from local irritation of the stomach mucosa.
- ✓ Release of active ingredient in specific target site.
- ✓ Protection of active ingredients from acidic environment of the stomach.

Enteric Coating

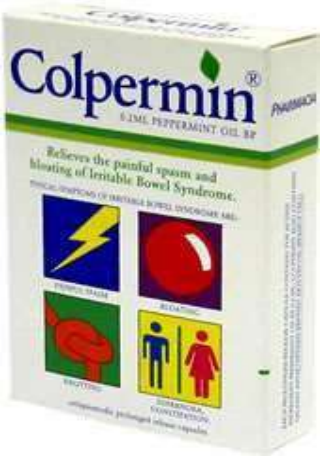


Examples of Enteric coated products:

Enteric Coated Aspirin. Eg. Micropirin 75mg EC tablets.



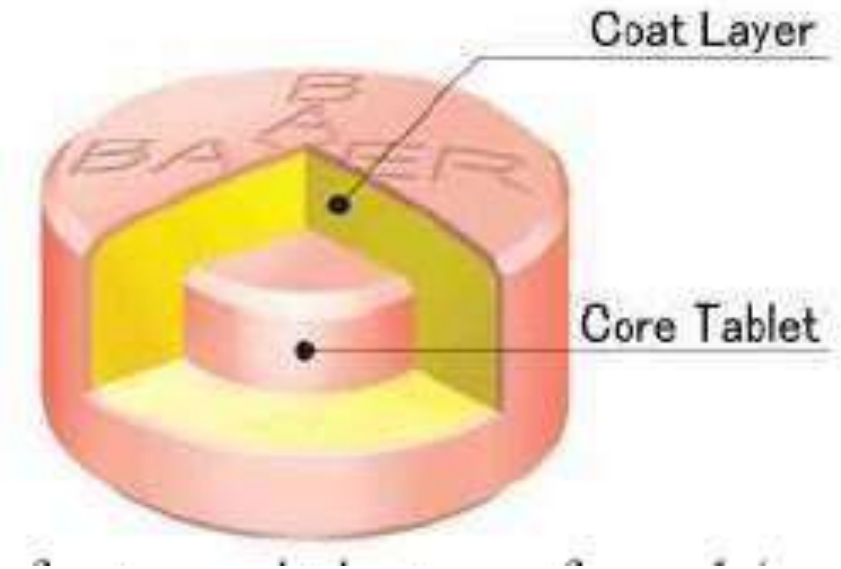
Enteric Coated Peppermint oil. Eg. Colpermin.



4. ***PRESS COATING:***

It involves the compaction of granular materials around preformed tablet core using specially designed tablet equipment. Compression coating is a dry process.

It is mainly used to separate chemically incompatible materials.



Equipment:

Most coating processes use one of the 3 general types of equipment.

1. The Standard coating pan
2. The Perforated coating pan
3. The Fluidized bed coater

1. STANDARD COATING PAN:

It consists of a circular metal pan mounted somewhat angularly on a stand. The pan is 8 to 60 inches in diameter and is rotated on its horizontal axis by a motor. The heated air is directed into the pan and onto the tablet bed surface, and is exhausted by means of ducts positioned through the front of the pan.



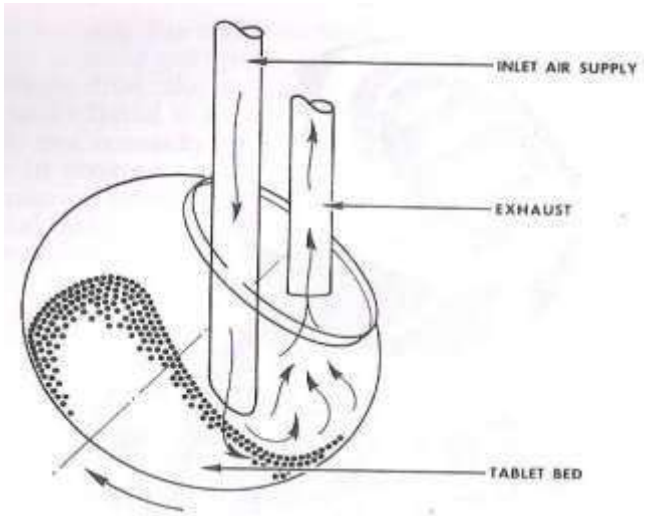
A significant improvement in the **particle movement** & **drying efficiency** of the standard coating pan is achieved by the;

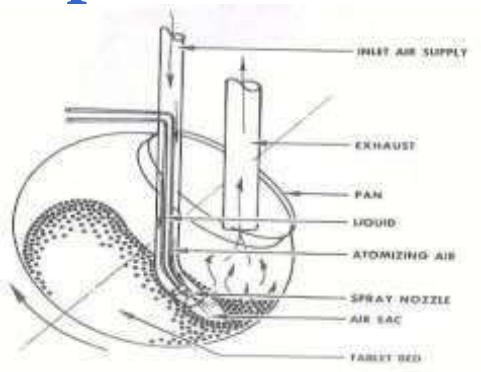
Pellegrini pan

Immersion sword systems

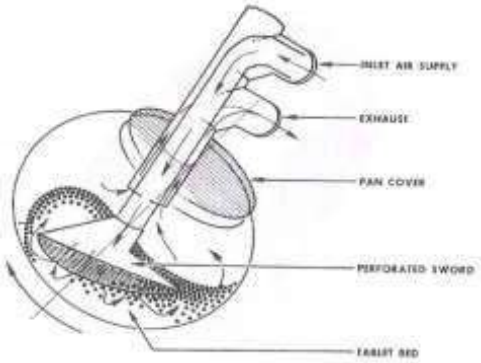
Immersion tube systems

Conventional pans

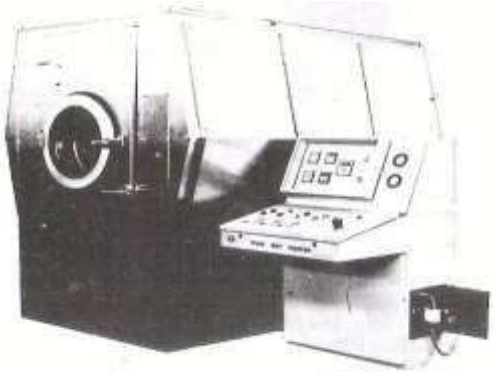




Immersion-tube system



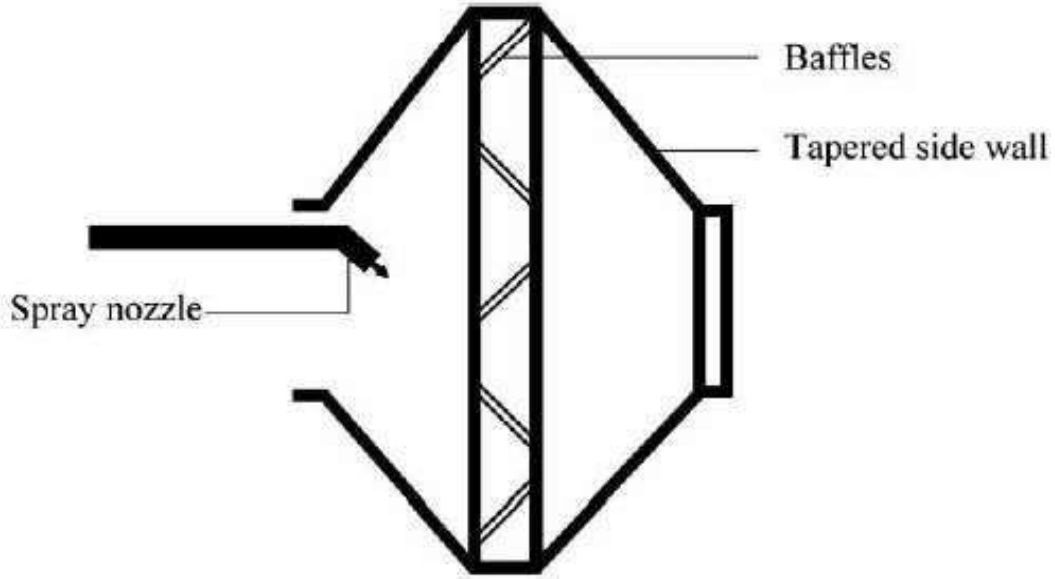
Immersion sword system



Pellegrini pan system

Pellegrini pan:

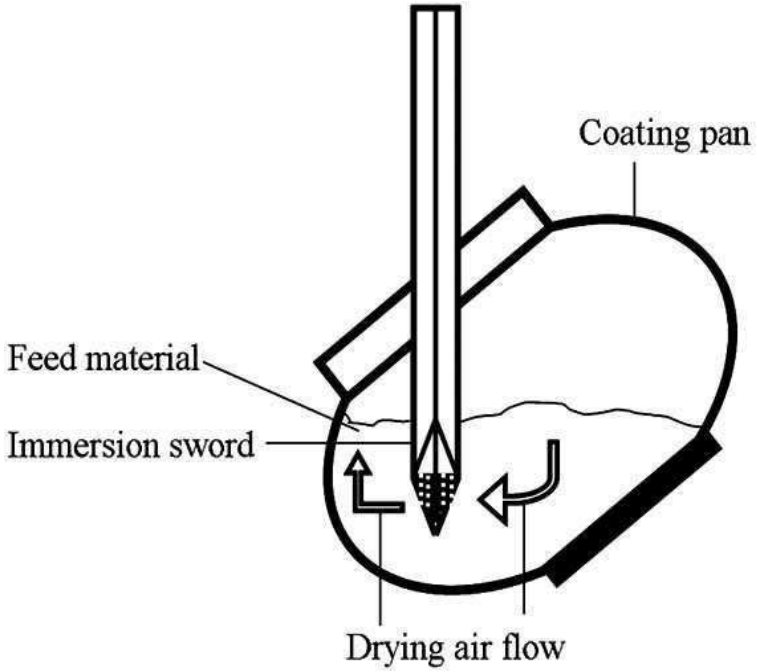
The side walls of this pan are shaped with a pronounced taper, which increases the efficiency of particle movement by forcing the cores into an additional lateral movement. This results in a composite core movement yielding improved exposure of the core to the coating material.



Immersion Sword System:

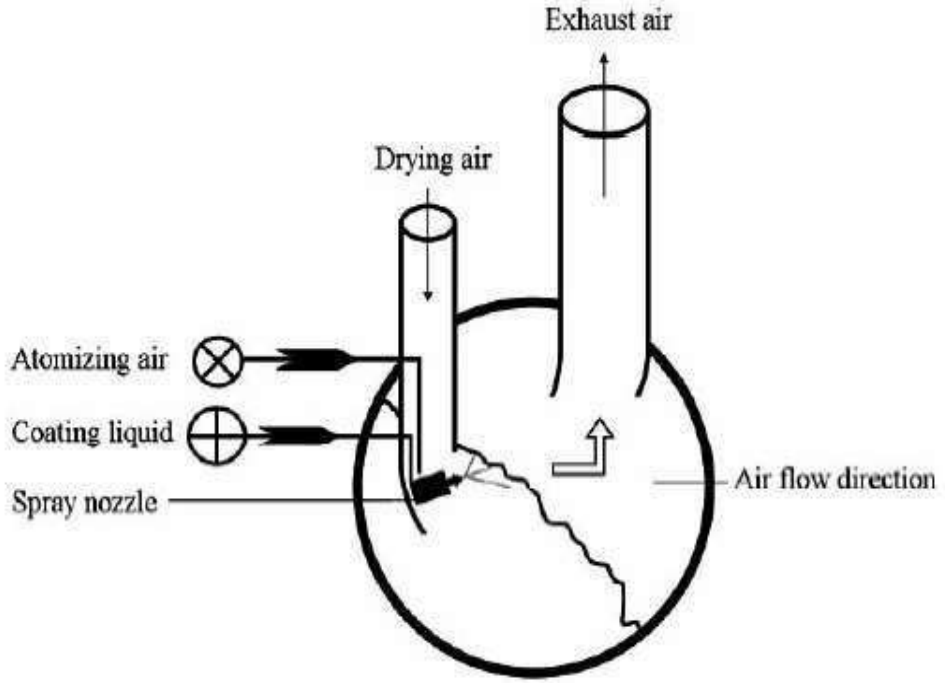
- With the immersion sword system, drying air is introduced through a perforated metal sword device that is immersed in the tablet bed.
- The drying air flows upward from the sword through the bed. Since the air is more intimately mixed with the wetted tablets, a more efficient drying environment is provided.

.



Immersion Tube System

- In this type of system, the immersed tube delivers the heated air and a spray nozzle is built in the tip of the tube. During this operation , the coating solution is applied simultaneously with the heated air from the immersed tube.
- The drying air flows upward through the tablet bed and is exhausted by a conventional duct.





2. PERFORATED PAN COATING:

It consists of a perforated drum that is rotated on its horizontal axis in an enclosed housing.

In Accela-Cota and Hi-Coater systems, drying air is directed into the drum, is passed through the tablet bed, and is exhausted through perforations in the drum.

The Driacoater introduces drying air through hollow perforated ribs located on the inside periphery of the drum. As the coating pan rotates, the ribs dip into the tablet bed, and drying air passes up through and fluidizes the tablet bed. Exhaust is from the back of the pan.



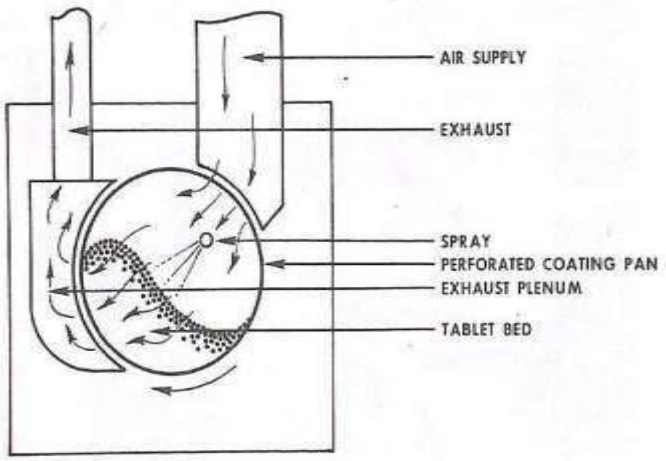
In Glatt coater, drying air can be directed from inside the drum through the tablet bed and out an exhaust duct. Drying air can be directed in the reverse manner up through the drum perforations for partial fluidization of the tablet bed. Several airflow configurations are possible.

In all four of these perforated pan systems, the coating solution is applied to the surface of the rotating bed of tablets through spraying nozzles that are positioned inside the drum.

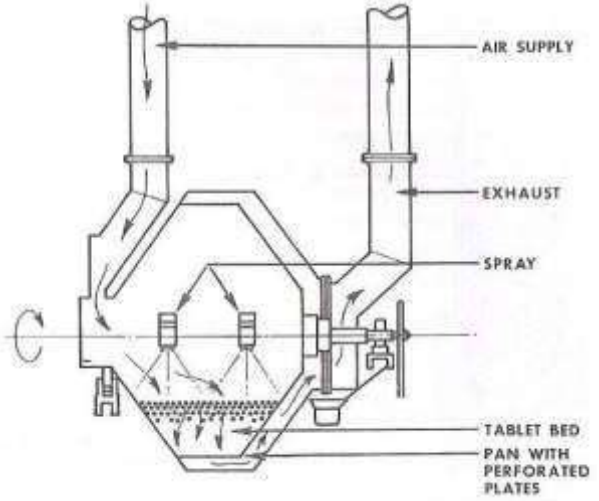
Perforated pan coaters are the efficient drying systems with high coating capacity, and can be completely automated for both sugar coating and film coating processes.



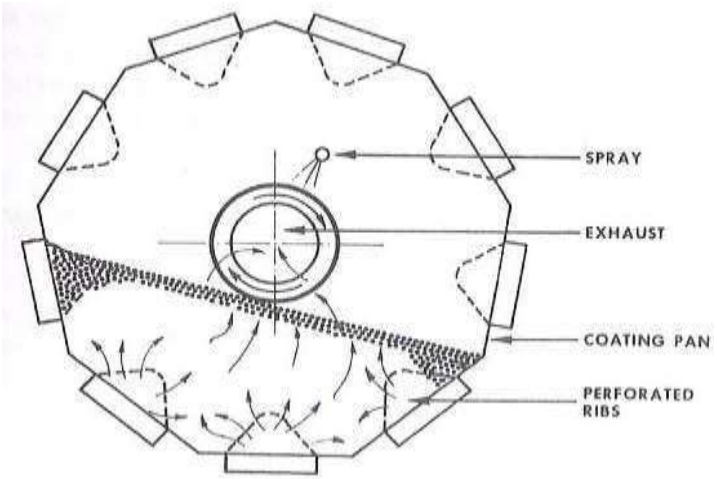
Perforated Coating Pans



Accela cota system



Hi-coater system



Dria Coater Pan

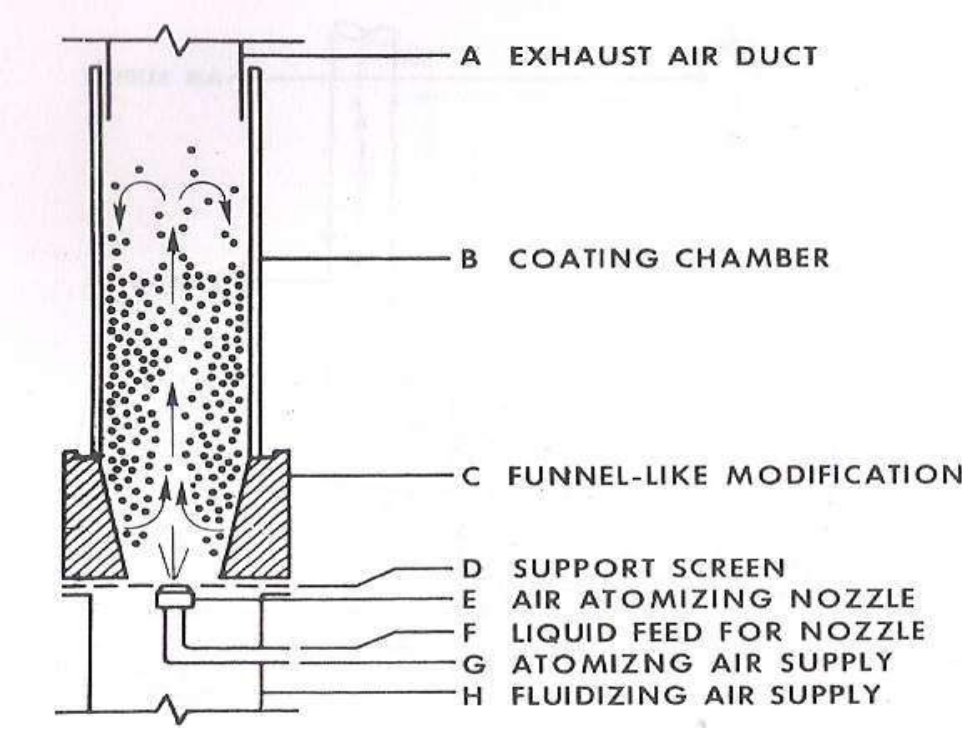


3. FLUIDIZED BED COATER:

These are high efficient drying systems. Fluidization of the tablet mass is achieved in a columnar chamber by the upward flow of drying air. The airflow is controlled so that more air enters the center of the column, causing the tablets to rise in the center. The movement of tablets is upward through the center of the chamber. They then fall towards the chamber wall and move downward to re-enter the air stream at the bottom of the chamber. In some units, a smaller column is used to direct tablet movement within the main column. Coating solutions are continuously applied from a spray nozzle located at the bottom of the chamber or are sprayed onto the top of the cascading tablet bed by nozzles located in the upper region of the chamber.

Principle of operation:

With fluid bed coating, particles are fluidized and the coating fluid is sprayed on and dried. Small droplets and a low viscosity of the spray medium ensure an even product coating.





Process advantages:

✓ Uniform, continuous product coating.

✓ Aqueous or organic coatings can be applied. Coating and drying takes place in one machine.

✓ In terms of Total Containment, the coating process and the filling and emptying of the machine can be carried out in complete isolation and without product spreading into the environment.

✓ When using organic solvents, the process machines can also be made inert and used with a solvent recovery system.

Tablet Coating Defects:



Picking and sticking:

This occurs when the coating removes a piece of the tablet from the core. It is caused by over-wetting the tablets, by under-drying, or by poor tablet quality



Cracking:

This occurs if internal stresses in the film exceeds the tensile strength of the film.

The tensile strength of the film can be increased by using high molecular weight polymers or polymer blends.





Roughness:

This is observed when coating is applied by spray. Some of the tablets may dry too rapidly before reaching the tablet bed and deposits on tablet surface.

Surface roughness also increases with pigment concentration and polymer concentration in the coating solution.



Orange peel effect:

Inadequate spreading of coating solution before drying causes a bumpy or orange peel effect on the tablet.



Specialized coatings

1. Dip coating
2. Laminated coating
3. Vaccum film coating
4. Electrostatic coating



1. ***Dip coating:***

- In this, cores to be coated are held in a suitable device. eg: Baskets.
- Dipped into coating solution and then dried taking care to prevent adherence to one another.
- For obtaining more perfect or heavier coats the dipping and drying steps may be repeated several times one after another.
- The sophisticated devices comprise tiny suction tubes, which hold the individual tablets apart until drying is accomplished.



2. ***Laminated coating:***

- Laminated coating provides multiple layers for incorporation of medicament; for example
- Repeat-action tablet, here a portion of the drug is kept in outer lamella or coating.
- Enteric tablet, here one drug could be made available for gastric absorption while another for release in intestine.
- Buccal-swallow tablet, this could first be administered sublingually, and upon a signal, such as release of flavour from the inner core, the same may be swallowed as a normal peroral tablet.



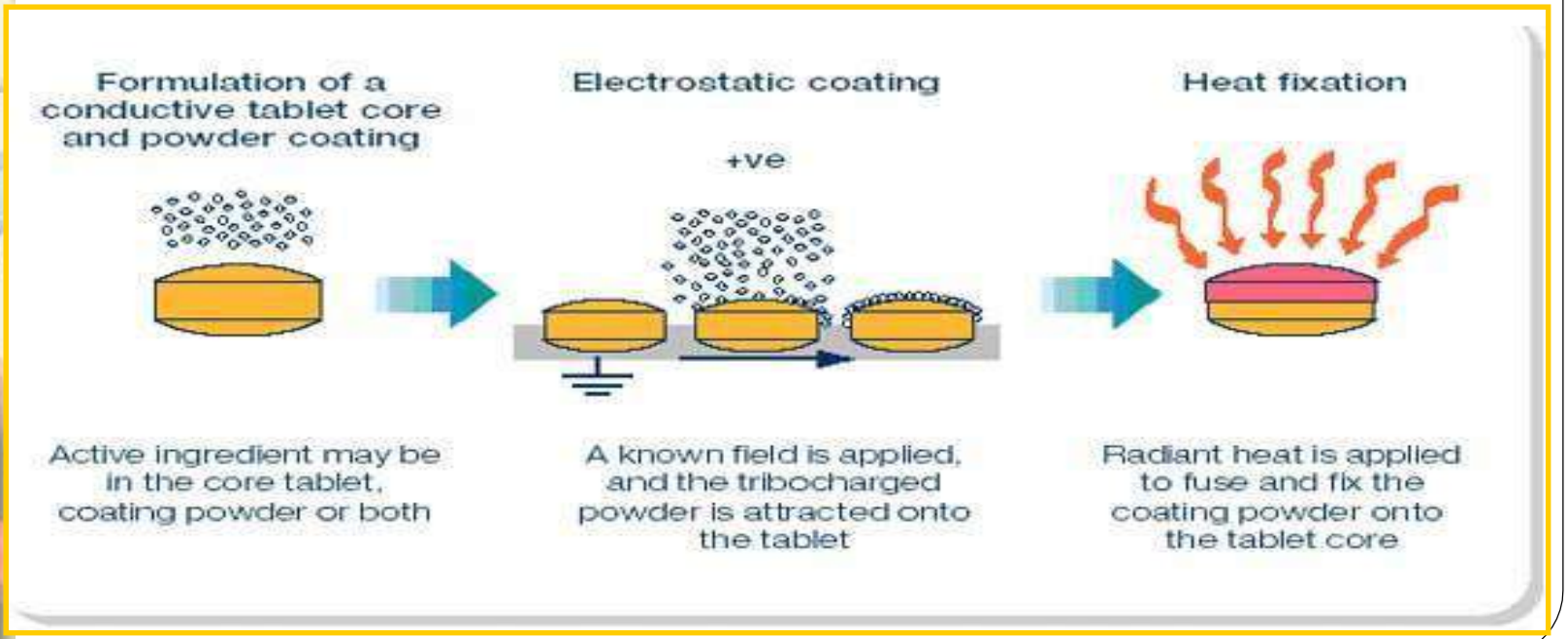
3. *Vaccum film coating:*

- This employs a specially designed baffled pan, which is water-jacketed and could be sealed to achieve vacuum.
- Tablets are placed in the sealed pan, the vacuum is applied and the coating material is introduced through airless hydraulic spray system, since the pan is completely sealed.
- Organic solvents could be effectively used with minimal environmental or safety concern



4. Electrostatic coating:

- Electrostatic coating is employed for applying films of electro-conductive materials.
- In this, an ionic charge is imparted to the core and an opposite charge to the coating material. This technology ensures thin, continuous and electronically perfected film to the surface.





Applications of tablet coating:

In Therapy:

- ✓ Avoid activation of drug in stomach.
- ✓ Avoid irritation of oesophagus and stomach.
- ✓ Avoid bad taste.
- ✓ Improve drug effectiveness.
- ✓ Prolong dosing interval.
- ✓ Improve patient compliance.

In Technology:

- ✓ To reduce the influence of moisture.
- ✓ To avoid dust formation.
- ✓ To improve drug stability.



- ✓ To prolong shelf-life.
- ✓ To reduce friction and increase the production rate in high speed packaging systems.

In Marketing:

- ✓ Improve product identity.
- ✓ Improve appearance and acceptability.

CONCLUSION

- Coating is done to give not only for esthetic appeal and to control bioavailability of the drug but also for various other reasons.
- There are various techniques for applying coating and depend on the need of formulator.
- Film coating is mostly used and has now shifted to aqueous based systems due to environmental and economical reasons.
- An extensive array of equipment is used in modern pharmaceutical coating operations.



References:

1. The Theory and Practice of Industrial Pharmacy- Lachman & Lieberman.
2. Pharmaceutical coating Technology- John Hogen.
3. The science and Practice of Pharmacy- Remington, Volume 1.
4. Pharmaceutical Dosage Forms- Liberman, Volume 3.


Thank you!

TRANSDERMAL DRUG DELIVERY

N.Natesh

CVPS

Definition:

- Self-contained, discrete dosage forms which, when applied to the intact skin, deliver the drug(s), through the skin, at controlled rate to the systemic circulation.

Advantages

- Delivers a steady infusion of a drug over an extended period of time.
- Increase the therapeutic value of drug by avoiding specific problems associated with the drug e.g., gastro-intestinal irritation, low absorption, decomposition due to hepatic "first-pass" effect, formation of metabolites that cause side effects, short half-life necessitating frequent dosing etc.
- An equivalent therapeutic effect can be elicited via transdermal drug input with a lower daily dose of the drug than is necessary e.g., as the drug is given orally.
- Improved patient compliance due to simplified medication regimen and reduced inter and intra –patient variability
- Self administration is possible
- Drug input can be terminated at any point of time by removing transdermal patch

Disadvantages

- The drug must have some desirable physicochemical properties for penetration through stratum corneum. Daily doses of less than 5mg/day are preffered
- Skin irritation or contact dermatitis due to the drug, excipients and percutaneous absorption enhancers.
- The barrier function of skin changes from one site to another on the same person, from person to person and with age.

Kinetics of transdermal permeation

- Permeation of drug involves the following steps:
  i. Sorption by stratum corneum
  ii. Penetration of drug through viable epidermis
  iii. Uptake of the drug by the capillary network in the dermal papillary layer

- Rate of permeation across skin:

$$\frac{dQ}{dt} = P_s (C_d - C_r)$$

Where,

C_d, C_r = concentrations of skin penetrant in donor (side of stratum corneum) and receptor compartment (body) respectively

P_s = permeability coefficient of skin tissues to drug = $\frac{K_{ss} D_{ss}}{h_s}$

K_{ss} = Partition coefficient, D_{ss} = apparent diffusivity for the steady-state diffusion

h_s = overall thickness of skin tissues

Kinetics of transdermal permeation

- Constant rate of drug obtained can be obtained only when $C_d >>> C_r$
  Therefore, $\frac{dQ}{dt} = P_s C_d$
- Rate of skin permeation is constant if C_d is constant throughout the course of skin permeation. To maintain this, rate of drug release (R_r) must be greater than rate of skin uptake (R_a)
- Therefore, maximum rate of skin permeation, $(dQ/dt)m = P_s C_s$
- Thus, skin permeation appears to be stratum corneum-limited

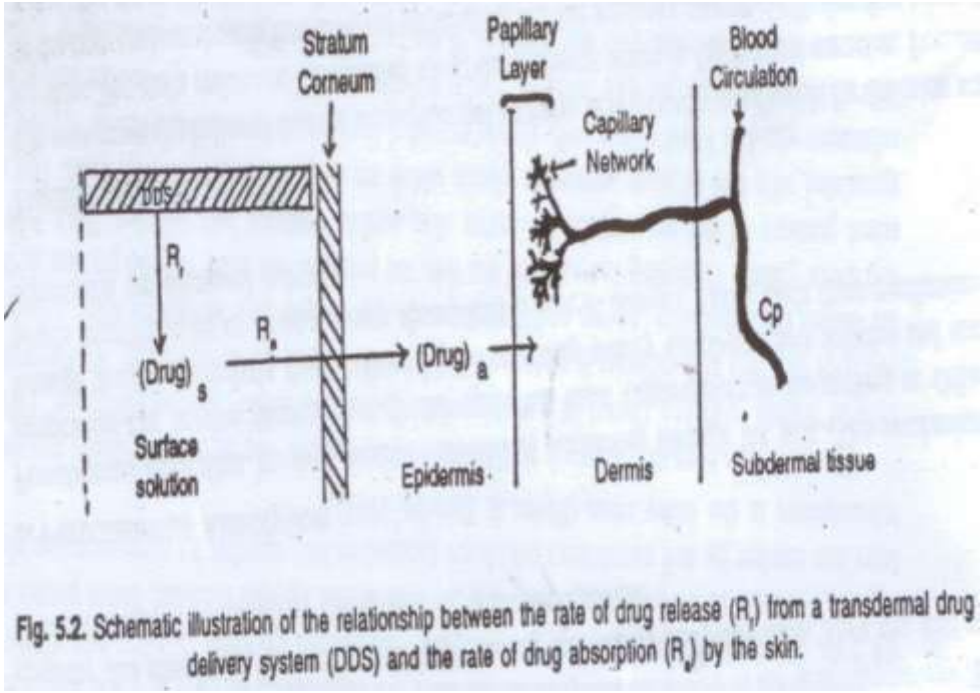


Fig. 5.2. Schematic illustration of the relationship between the rate of drug release (R_r) from a transdermal drug delivery system (DDS) and the rate of drug absorption (R_a) by the skin.

Basic components of TDDS:

1) Polymer matrix or matrices
2) The drug
3) Permeation enhancers
4) Other excipients

Polymer matrix or matrices - controls the release of the drug from the device.

Criteria to be met by a polymer to be used in TDDS:

- Molecular weight, glass transition temperature and chemical functionality of the polymer should be such that the specific drug diffuses properly and gets released through it.
- Should be stable, non-reactive with the drug, easily manufactured and fabricated into the desired product; and inexpensive
- The polymer and its degradation products must be non-toxic and non antagonistic to the host.
- The mechanical properties of polymer should not deteriorate excessively when large amounts of active ahent are incorporated into it.

Polymer matrix or matrices

- Possible useful polymers for transdermal devices:

- **Natural polymers:-**

cellulose derivatives, Zein, waxes, proteins, gums and their derivatives, natural rubber, starch

- **Synthetic elastomers:-**

Polybutadiene, Hydrin rubber, polysiloxane, Nitrile, Acrylonitrile, Styrene, Neoprene etc.

- **Synthetic pulymers:-**

Polyvinyl alcohol, Polyethylene, Polyacrylate, Polyvinylpyrrolidonr, Polymethylmethacrylate, Epoxy etc.

The drug - Its desirable properties for transdermal delivery

Physicochemical properties:

- Molecular weight less than approximately 1000 daltons
- Should have affinity for both lipohilic and hydrophilic phases. Extreme partitioning characteristics are not conductive to successful drug delivery via skin.
- Drug should have low melting point.

Biological properties:

- Should be potent with a daily dose of the order of a few mg/day.
- Half-life of the drug should be short
- Must not induce cutaneous irritant or allergic response.
- Drugs which degrade in the GI tract or are inactivated by hepatic first-pass effect are suitable candidates for transdermal delivery
- Drugs which have to be administered for a long period of time or which cause adbverse effects to non-target tissues can also be formulated for transdermal delivery.

Permeation enhancers

- These are the compounds which promote skin permeability by altering the skin as a barrier to the flux of a desired penetrant

- The flux J across the skin:
  - $$J = D \frac{dc}{dx}$$

Where,

D = diffusion coefficient

C = concentration of diffusing species

X = spatial coordinate

Permeation enhancers - Classification

- **Solvents**

- Increase penetration possibly by swelling the polar pathway and/or by fluidizing lipids.
- e.g., water alcohols (methanol&ethanol), alkyl methyl sulfoxides (dimethyl sulfoxide, dimethyl acetamide,alkyl homologs of methyl sulfoxide), pyrrolidones (laurocapram, 2-pyrrolidone)

- **Surfactants**

- Enhance polar pathway transport, especially of hydrophilic drugs.
- These compounds being irritants, a balance between penetration enhancement and irritation has to be considered.
  Potential for irritation:- cationic > anionic > nonionic
  - **Anionic surfactants**:- Dioctyl sulfosuccinate, sodium lauryl sulphate
  - **Nonionic surfactants**:- Pluronic F127, Pluronic F68 etc.
  - **Bile salts**:- Sodium taurocholate, sodium deoxycholate

Permeation enhancers - Classification

- **Binary systems**
  - These systems apparently open up the heterogenous multilaminate pathway aswell as the continate pathway.
  - e.g., propylene glycol-oleic acid & 1,4-butane diol-linoleic acid

- **Miscellaneous chemicals**
  - Hydrating and keratolytic agent
  - N,N-dimethyl-m-toluamide
  - Recently described:- eucalyptol, di-o-methyl-β-cyclodextrin

Other excipients - Adhesives

- Fastening of the transdermal devices to skin has been done by using a pressure sensitive adhesive

- pressure sensitive adhesive can be positioned on the face of the device or in the back of the device and extending peripherally.

- Both type of adhesive systems should fulfill the following criteria
  - Should not irritate or sensitize the skin
  - Should adhere to skin aggressively during dosing interval without its position disturbed by activities such as bathing, exersise etc.
  - Should be easily removed
  - Should not leave an unwashable residue on the skin
  - Should hav excellent contact with the skin at macroscopic and microscopic level.

Other excipients - Adhesives

- **Criteria for face adhesive system**

- Permeation of drug should not be effected
- Delivery of simple or blended permeation enhancers should not be affected
- e.g., polyisobutylenes, acrylics and silicones

- **Backing membrane**

- Flexible, provide good bond to the drug reservoir, prevent drug from leaving the dosage form through the top
- e.g., metallic plastic laminate, plastic backing with absorbent pad and occlusive base plate (aluminium foil), adhesive foam pad (flexible polyurethane) with occlusive base plate (aluminium foil disc) etc.

Approaches used in development of transdermal drug delivery systems

- Four different approaches have been used

1) Membrane permeation – controlled systems

2) Adhesive dispersion – type systems

3) Matrix diffusion – controlled systems

4) Microreservoir type or microsealed dissolution controlled systems

Membrane permeation - controlled systems

- Constant release rate of drug is the major advantage
- A rare risk-- Accidental breakage of the rate controlling membrane can result in dose dumping or a rapid release of the entire drug content.
- Intrinsic rate of drug release:

$$\frac{dQ}{dt} = \frac{C_R}{1/Pm + 1/Pa}.$$

- Pa = permeability coefficient of adhesive layer
- Pm = permeability coefficient of rate controlling membrane = $\frac{K_{m/r} \cdot D_m}{h_m}$

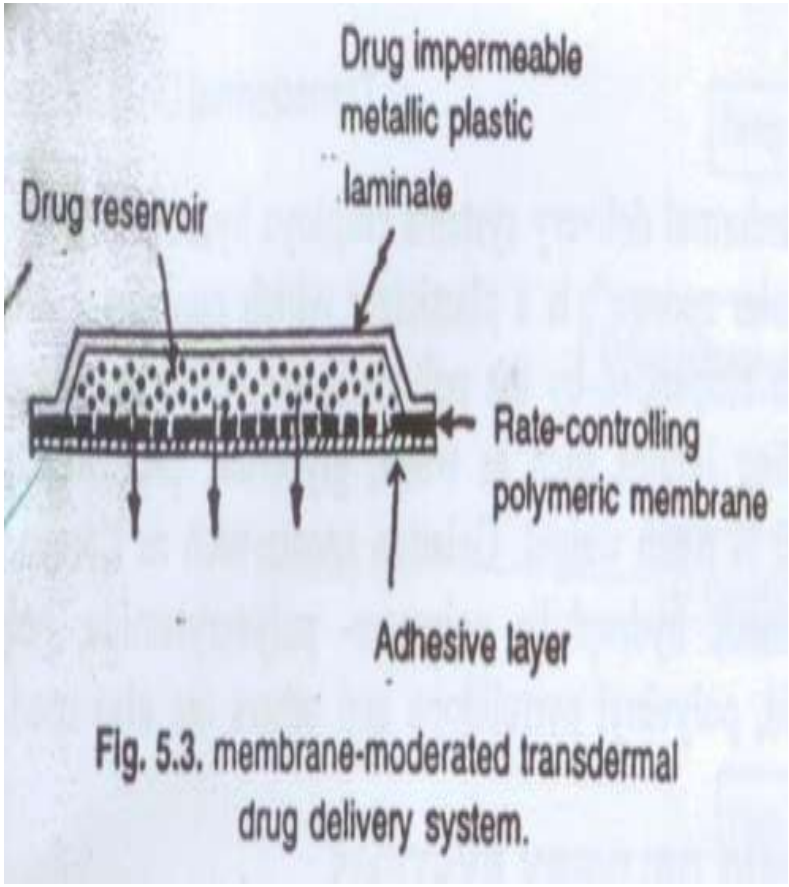


Fig. 5.3. membrane-moderated transdermal drug delivery system.

Membrane permeation - controlled systems

- Nitroglycerine – releasing transdermal system. For once a day medica in angina pectoris
- Scopalamine – releasing transdermal system for 72 hrs. Prophylaximotion sickness
- Clonidine – releasing transdermal system for 7 day therapy hypertension
- Estradiol – releasing transdermal system for treatment of menopausal syndrome for 3 -4 days.

Adhesive dispersion - type systems

- The rate of drug release is defined by:

- $$\frac{dQ}{dt} = \frac{K_{a/r} \cdot D_a}{h_a} \times C_R$$

Where,

$K_{a/r}$ = Partition coefficient for the interfacial partitioning of the drug from the reservoir layer to adhesive layer

- Example:

Isosorbide dinitrite – releasing transdermal therapeutic system

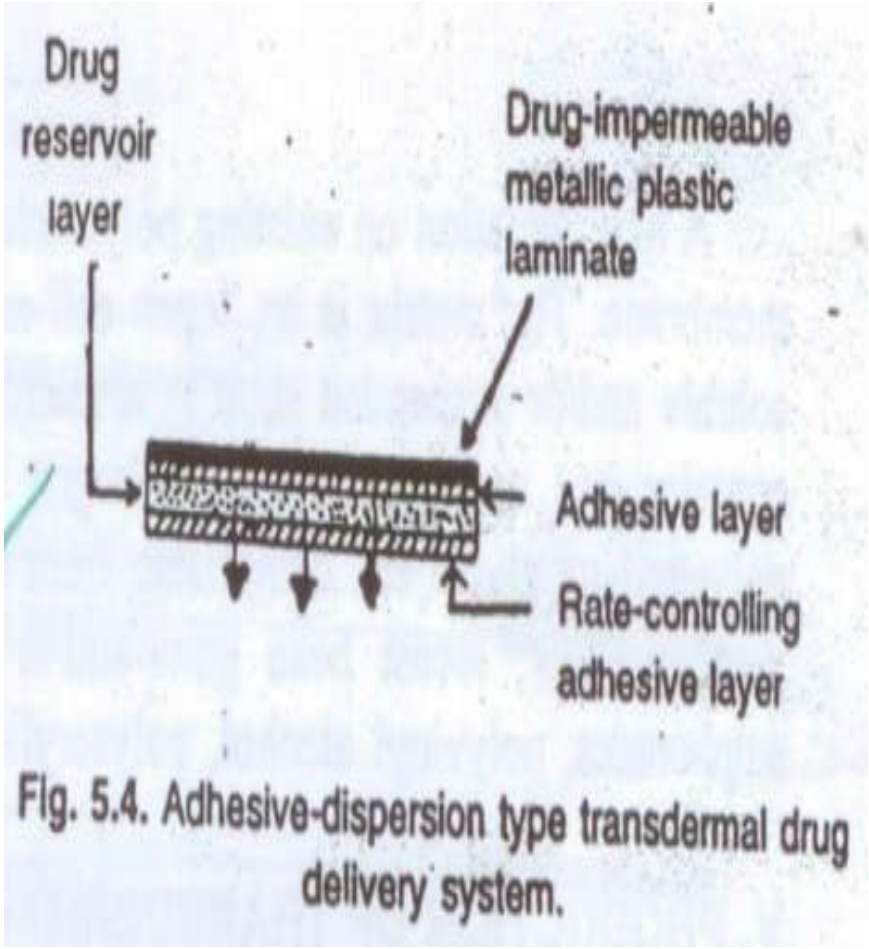


Fig. 5.4. Adhesive-dispersion type transdermal drug delivery system.

Matrix diffusion - controlled systems

- **Example:**

Nitroglycerine – releasing transdermal therapeutic system

- These are designed to be applied to the intact skin to provide a continuous transdermal infusion of nitroglycerine for therapy of angina pectoris
- **Advantage:-** Absence of dose dumping
- The rate of drug release is defined by:

$$\frac{dQ}{dt} = \left[\frac{AC_p\ D_p}{2t}\right]^{1/2}$$

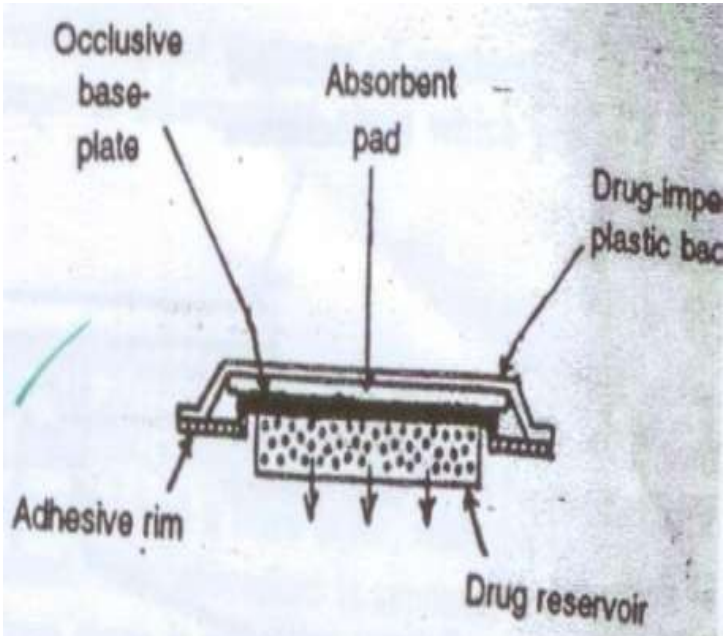


Fig. 5.6. Cross-sectional view of matrix diffusion co transdermal drug delivery system showing maj structural components.

Microreservoir type or microsealed dissolution controlled systems

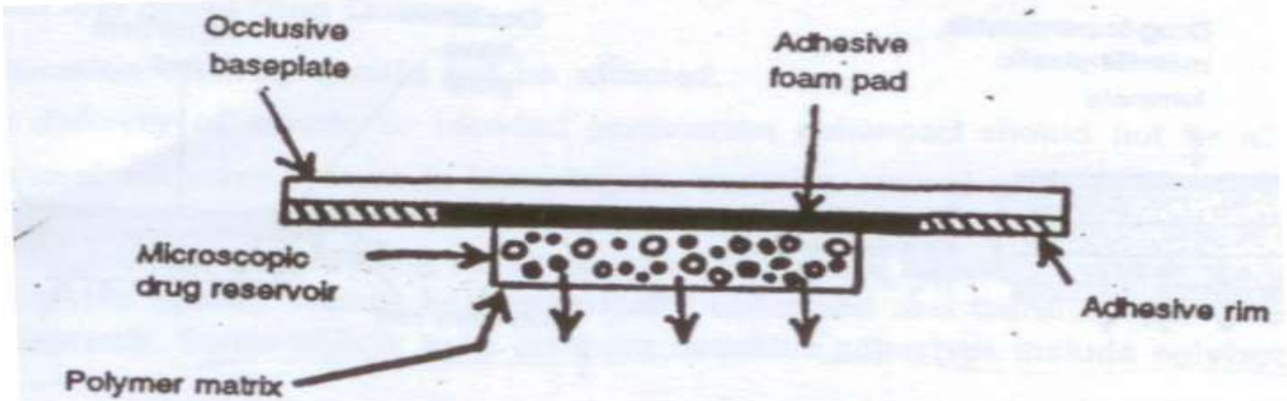


Fig. 5.7. Microreservoir dissolution-controlled transdermal drug delivery system.

ogy has been successfully ...

- **Example:**

Utilised in the preperation of nitrodisc, a nitroglycerine releasing transdermal therapeutic system.

Evaluation of TDDS

1. Evaluation of adhesive

a) Peal adhesion properties

- Peel adhesion is the force required to remove an adhesive coating from a test substrate
- It is affected by molecular weight of adhesive polymer, type and amount of additives, and polymer composition.

Test:

- The test invovles measuring the force required to pull a single coated tape, applied to substrate at a 180^0 angle.
- No residue on substrate indicates "adhesive failure"
- Remnants on substrate indicates "cohesive failure"

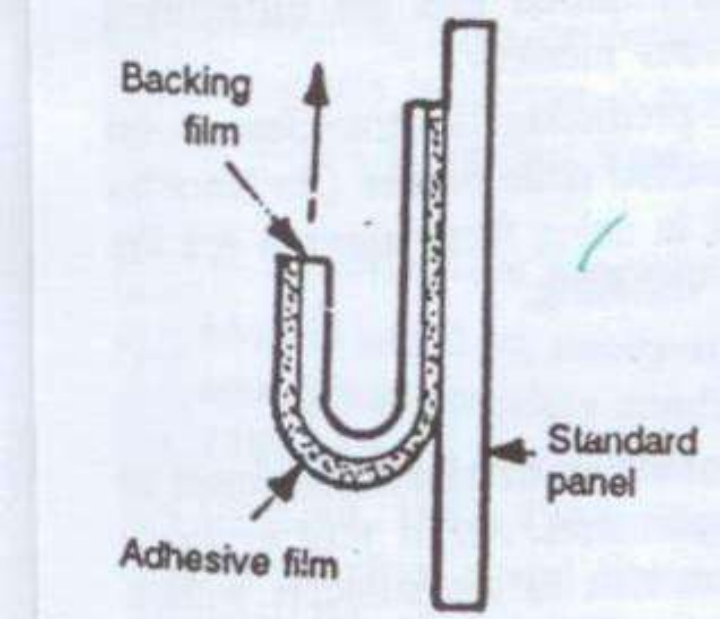


Fig. 5.14. Peel adhesion test for adhesive evaluation.

Evaluation of TDDS

b) Tack properties

- Ability of polymer to adhere to a substrate with little contact pressure
- Affected by molecular weight and compostion of polymer, tackifying resins in the polymer.
- **"Thumb tack test"** – measured by pressing the thumb briefly into the adhesive layer
- **"Rolling ball tack test"** – measurement of distance that a stainless steel ball travels along an upward placed adhesive.
- **"Quick-stick test (peel tack test)"** – pulling the tape away from substrate at 90^0 at a speed of 12inch/min
- **"Probe tack test"** – force required to pull a probe away from adhesive at a fixed rate is recorded as tack



Fig. 5.15. Rolling ball tack test for adhesive evaluation.

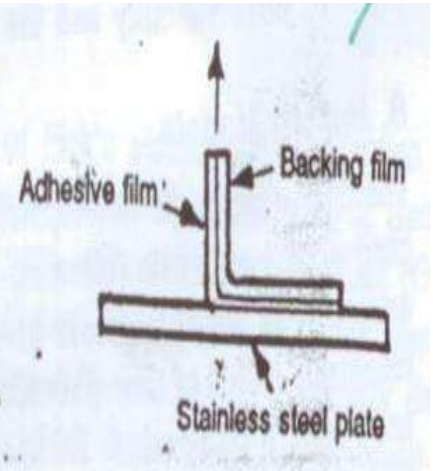


Fig. 5.16. Quick-stick (peel-tack) test for adhesive evaluation.

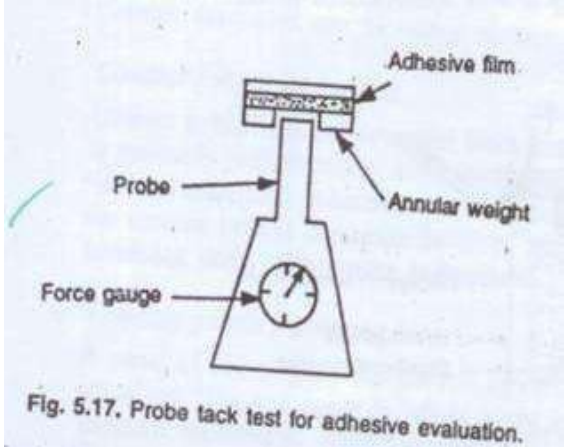


Fig. 5.17. Probe tack test for adhesive evaluation.

Evaluation of TDDS

c) Shear strength properties

- Measurement of the cohesive strenght of an adhesive polymer
- Affected by molecular weight, type & amount of tackifier added .
- The time taken to pull the adhesive coated tape off a stainless steel plate in a direction parallel to the plate, by a specific weight.

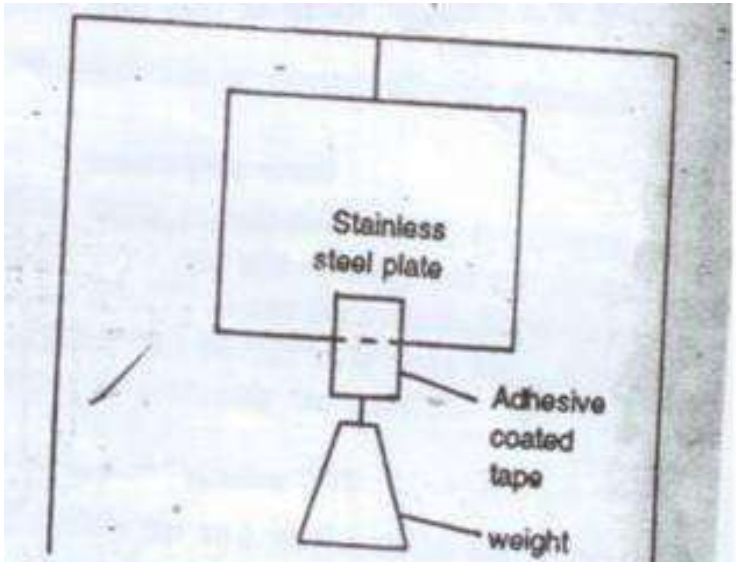


Fig. 5.18. Shear strength test for adhesive evaluation

Evaluation of TDDS

2. Invitro drug release evaluation

- Help in vestigating mechanism of skin permeation before developing a TDDS.
- Studies on skin metabolim can also be performed

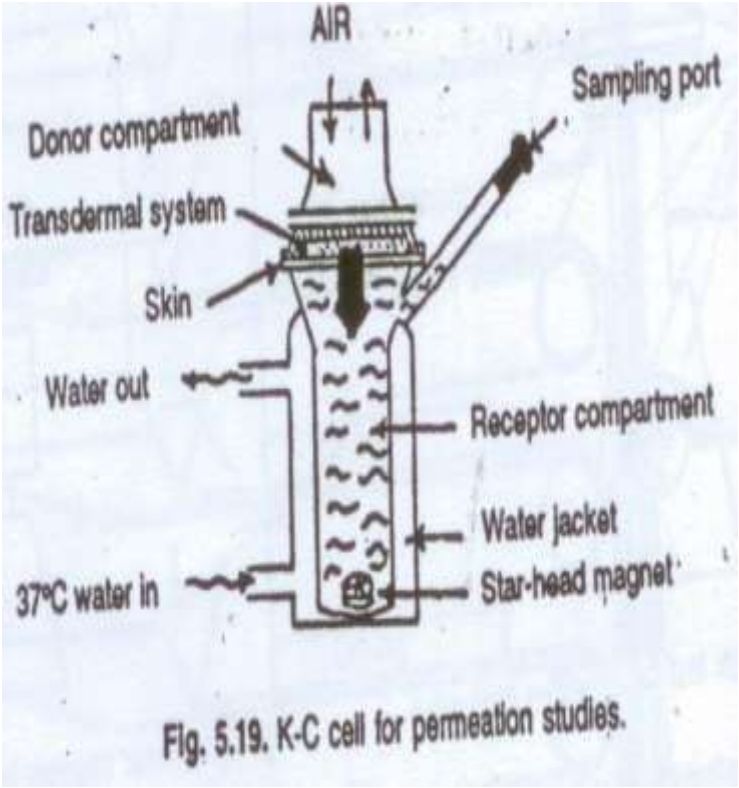


Fig. 5.19. K-C cell for permeation studies.

Advantages:

- Methodology
- Ease of analytical assay since there are no complications arising from disposition of drug in the body
- Better control of experimental conditions than in invivo

Evaluation of TDDS

2. Invivo evaluation

a) Animal models

i. Penetration values obtained with small hairy animals are higher than those seen in man. Preparation of their skin for study, by shaving or depilation lead to changes in resistance.

ii. Rhesus monkey is the most reliable model. Limtation of using this animal:- cost, handling capabilities, difficulty of accessibility and ethical considerations

iii. Other animals used:- weanling pig, human skin grafted-nude mouse

Evaluation of TDDS

2. Invivo evaluation

b) Human models

- Percutaneous absorption determined by indirect method:- measuring radioactivity of excreta following topical application of the labelled drug

$$\% \text{ dose absorbed} = \frac{\text{Total radioactivity excreted after topical administration}}{\text{Total radioactivity excreted after Intravenous administration}} \times 100$$

- "Reservoir technique" :- to overcome the inherent limitations in above process
- Short exposure of skin to the radiolabelled drug followed by removal of stratum corneum.

c) Biophysical models

Evaluation of TDDS

5. Cutaneous toxicological evaluations

a) Contact dermatitis

- Contact irritant dermatitis
  - Ten day primary irritation test
  - Twenty one day irritation test
    - Laser doppler
    - Evaporative water loss measurements
- Contact allergic dermatitis

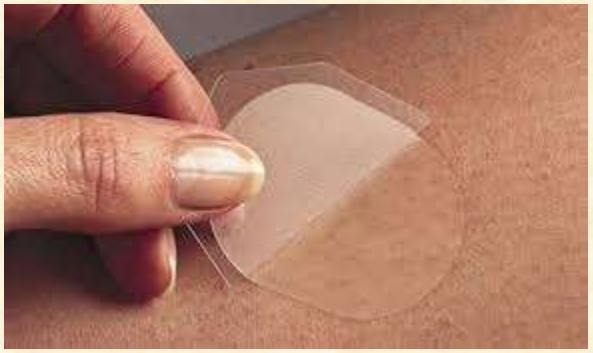
b) Growth of microorganisms

- Localised superficial infections
- Miliaria

THANK YOU

TRANSDERMAL DRUG DELIVERY,

IONOTOPHORESIS
&
SONOPHORESIS



N.Shanthi

Definition:

Self-contained, discrete dosage forms which, when applied to the intact skin, deliver the drug(s), through the skin, at controlled rate to the systemic circulation



Advantages :



- Delivers a steady infusion of a drug over an extended period of time.
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Where,

$\mathbf{C_d}$, $\mathbf{C_r}$ = concentrations of skin penetrant in donor (side of stratum corneum) and receptor compartment (body) respectively

$\mathbf{P_s}$ = permeability coefficient of skin tissues to drug = $\frac{K_{ss} D_{ss}}{h_s}$

$\mathbf{K_{ss}}$ = Partition coefficient, $\mathbf{D_{ss}}$ = apparent diffusivity for the steady-state diffusion

$\mathbf{h_s}$ = overall thickness of skin tissues

- Constant rate of drug obtained can be obtained only when $C_d >>> C_r$

  Therefore, $\frac{dQ}{dt} = P_s C_d$

- Rate of skin permeation is constant if C_d is constant throughout the course of skin permeation. To maintain this, rate of drug release (R_r) must be greater than rate of skin uptake (R_a)

- ❑ Therefore, maximum rate of skin permeation, $(dQ/dt)m = P_s C_s$

- ❑ Thus, skin permeation appears to be stratum corneum-limited

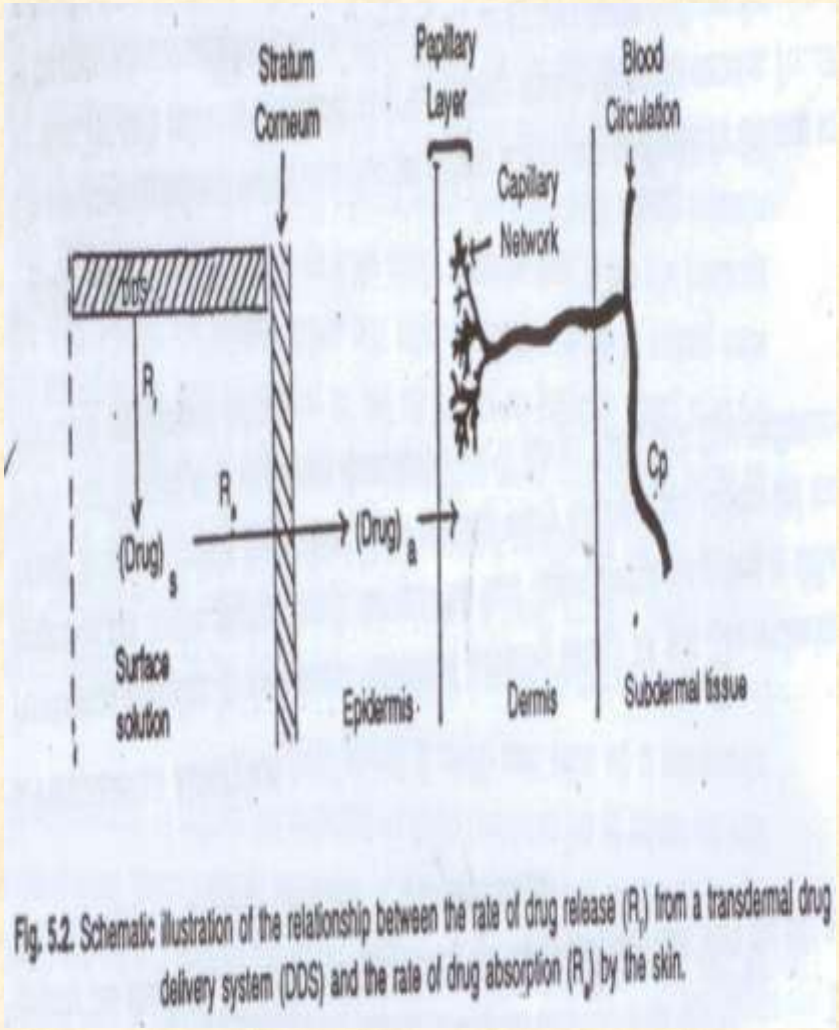


Fig. 5.2. Schematic illustration of the relationship between the rate of drug release (R_r) from a transdermal drug delivery system (DDS) and the rate of drug absorption (R_a) by the skin.

Basic components of TDDS:

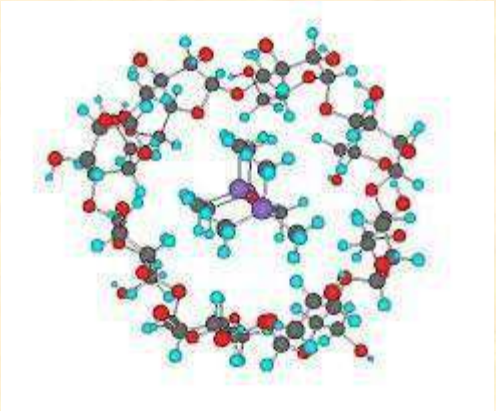
1) Polymer matrix or matrices

1) The drug

2) Permeation enhancers

3) Other excipients

Polymer matrix or matrices



- Possible useful polymers for transdermal devices:

Natural polymers:-

cellulose derivatives, Zein, waxes, proteins, gums and their derivatives, natural rubber, starch

Synthetic elastomers:-

Polybutadiene, Hydrin rubber, polysiloxane, Nitrile, Acrylonitrile, Styrene, Neoprene etc.

Synthetic pulymers:-

Polyvinyl alcohol, Polyethylene, Polyacrylate, Polyvinylpyrrolidonr, Polymethylmethacrylate, Epoxy **etc**.

Drug :



Physicochemical properties:

- Molecular weight less than approximately 1000 daltons
- Should have affinity for both lipohilic and hydrophilic phases. Extreme partitioning characteristics are not conductive to successful drug delivery via skin.
- Drug should have low melting point.

Biological properties:

- Should be potent with a daily dose of the order of a few mg/day.
- Half-life of the drug should be short
- Must not induce cutaneous irritant or allergic response.
- Drugs which degrade in the GI tract or are inactivated by hepatic first-pass effect are suitable candidates for transdermal delivery
- Drugs which have to be administered for a long period of time or which cause adbverse effects to non-target tissues can also be formulated for transdermal delivery.

Permeation enhancers

- These are the compounds which promote skin permeability by altering the skin as a barrier to the flux of a desired penetrant

- The flux J across the skin:

$$J = D \frac{dc}{dx}$$

Where,

D = diffusion coefficient

C = concentration of diffusing species

X = spatial coordinate

Classification

S.NO	TYPE	MECHANISM	EXAMPLES
1.	Solvents	Increase penetration possibly by swelling the polar pathway and/or by fluidizing lipids.	water alcohols, alkyl methyl sulfoxides, pyrrolidones
2.	Surfactants	Enhance polar pathway transport, especially of hydrophilic drugs	**Anionic surfactants**:- Dioctyl sulfosuccinate, sodium lauryl sulphate **Nonionic surfactants**:- Pluronic F127, Pluronic F68 etc. **Bile salts**:- Sodium taurocholate, sodium deoxycholate
3.	Binary systems	These systems apparently open up the heterogenous multilaminate pathway aswell as the continate pathway	propylene glycol-oleic acid & 1,4-butane diol-linoleic acid
4.	Miscellaneous chemicals	Hydrating and keratolytic agent	•N,N-dimethyl-m-toluamide •Recently described:- eucalyptol, di-o-methyl-β-cyclodextrin

Adhesives :-

✓Fastening of the transdermal devices to skin has been done by using a pressure sensitive adhesive

✓pressure sensitive adhesive can be positioned on the face of the device or in the back of the device and extending peripherally.

Criteria for face adhesive system

➢Permeation of drug should not be effected

➢Delivery of simple or blended permeation enhancers should not be affected

➢e.g., polyisobutylenes, acrylics and silicones



Backing membrane:

•Flexible, provide good bond to the drug reservoir, prevent drug from leaving the dosage form through the top

•e.g., metallic plastic laminate, plastic backing with absorbent pad and occlusive base plate (aluminium foil), adhesive foam pad (flexible polyurethane) with occlusive base plate (aluminium foil disc) etc.

Approaches used in development of transdermal drug delivery systems

Four different approaches have been used

1) Membrane permeation – controlled systems

2) Adhesive dispersion – type systems

3) Matrix diffusion – controlled systems

4) Microreservoir type or microsealed dissolution controlled systems

Membrane permeation - controlled systems

- Constant release rate of drug is the major advantage
- A rare risk-- Accidental breakage of the rate controlling membrane can result in dose dumping or a rapid release of the entire drug content.
- Intrinsic rate of drug release:

$$\frac{dQ}{dt} = \frac{C_R}{1/Pm + 1/Pa}.$$

- Pm = permeability coefficient of rate controlling membrane = $\frac{K_{m/r} \cdot D_m}{h_m}$
- Pa = permeability coefficient of adhesive layer

$$= \frac{K_{a/m} \cdot D_m}{h_m}$$

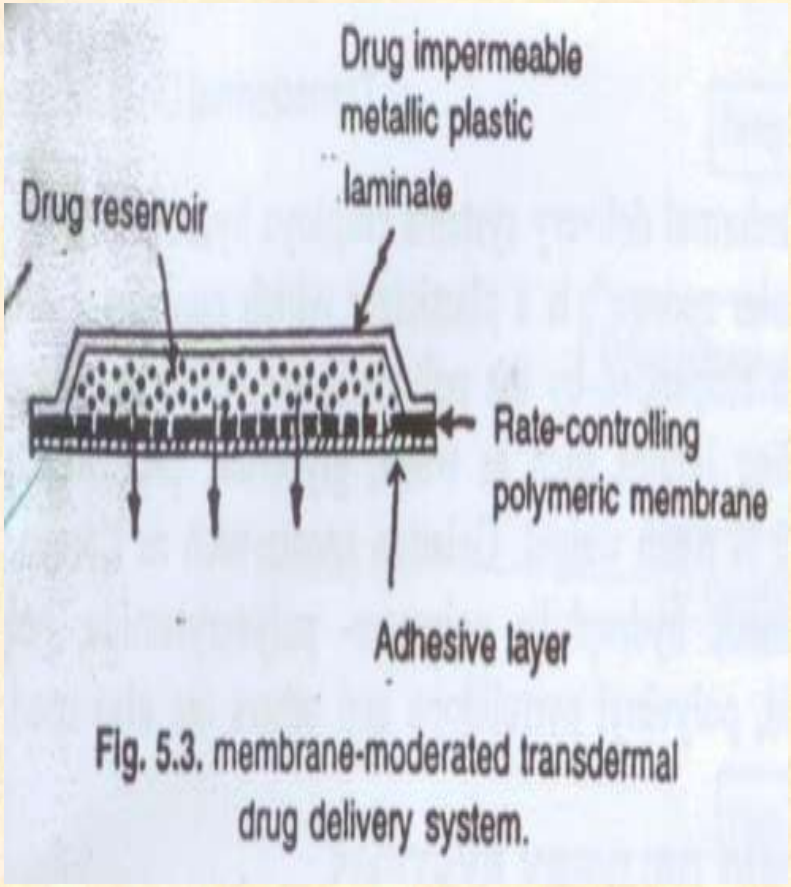


Fig. 5.3. membrane-moderated transdermal drug delivery system.

Adhesive dispersion - type systems

- The rate of drug release is defined by:

- $\frac{dQ}{dt} = \frac{K_{a/r} \cdot D_a}{h_a} \times C_R$

Where,

$K_{a/r}$ = Partition coefficient for the interfacial partitioning of the drug from the reservoir layer to adhesive layer

Example:

Isosorbide dinitrite – releasing transdermal therapeutic system

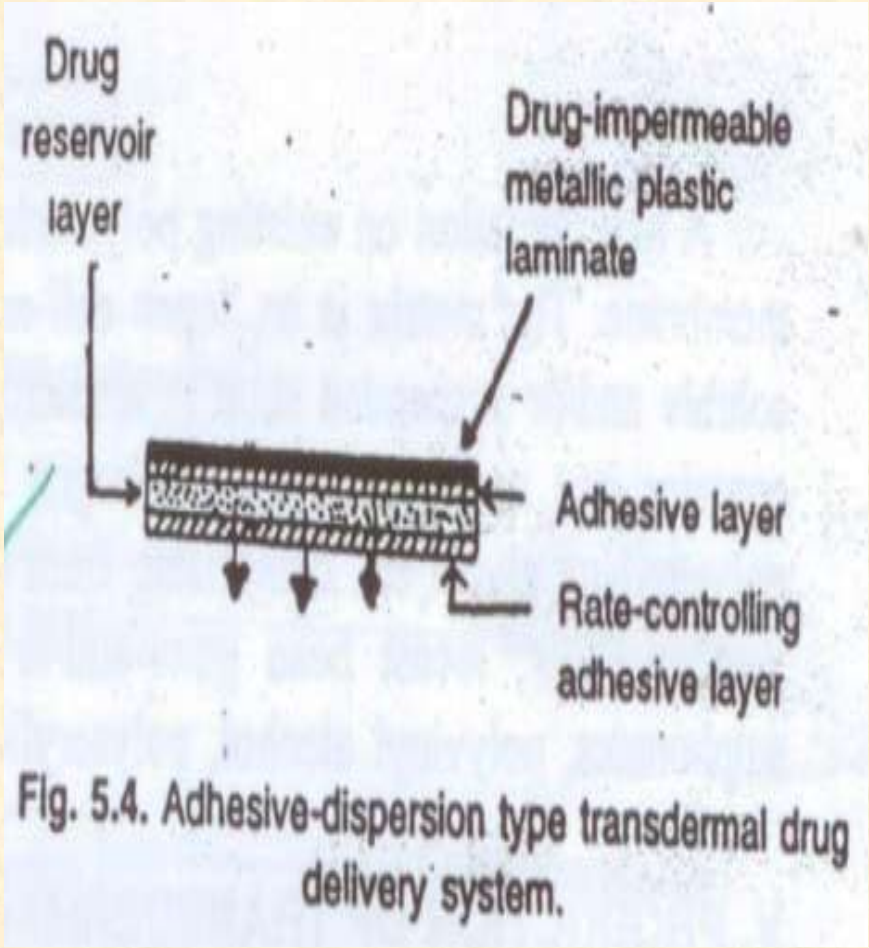


Fig. 5.4. Adhesive-dispersion type transdermal drug delivery system.

Matrix diffusion - controlled systems

Example:

- Nitroglycerine – releasing transdermal therapeutic system

- These are designed to be applied to the intact skin to provide a continuous transdermal infusion of nitroglycerine for therapy of angina pectoris

Advantage:- Absence of dose dumping

- The rate of drug release is defined by:

$$\frac{dQ}{dt} = \left[\frac{AC_p D_p}{2t}\right]^{1/2}$$

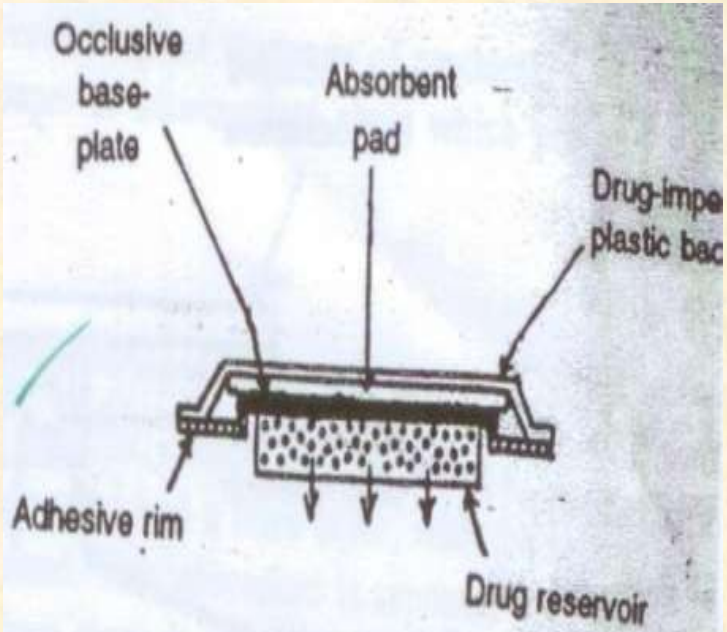


Fig. 5.6. Cross-sectional view of matrix diffusion co transdermal drug delivery system showing maj structural components.

Microreservoir type or microsealed dissolution controlled systems

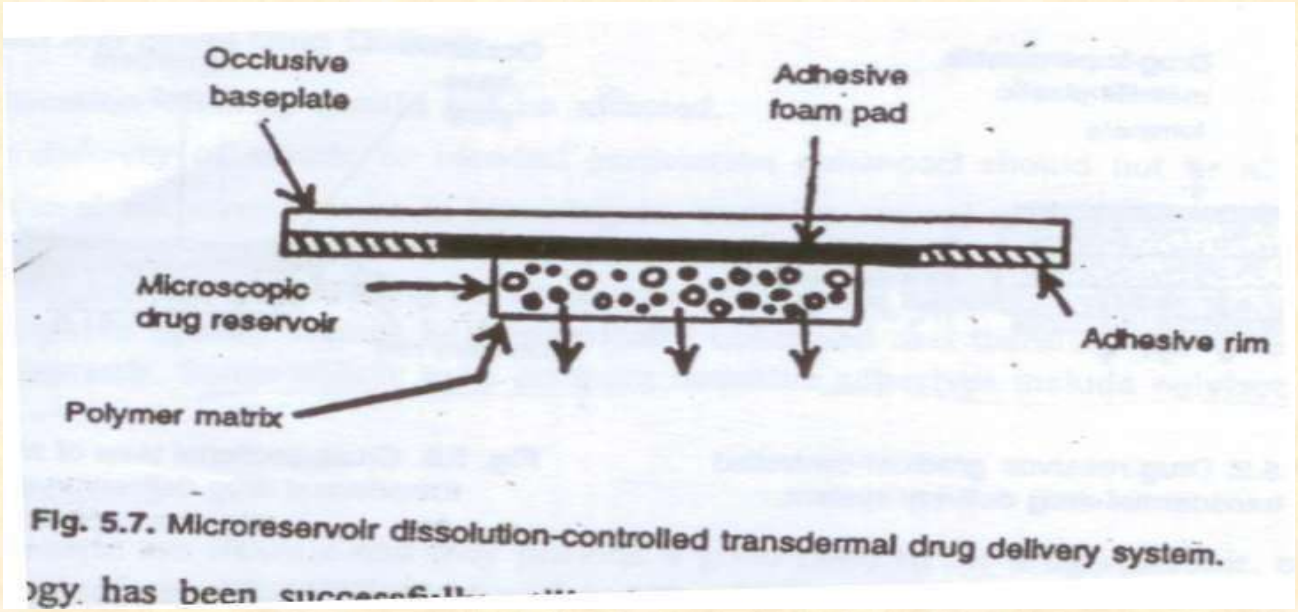


Fig. 5.7. Microreservoir dissolution-controlled transdermal drug delivery system.

ogy has been

Example:

Utilised in the preperation of nitrodisc, a nitroglycerine releasing transdermal therapeutic system.

- The release rate of drug is defined by:

$$\frac{dQ}{dt} = \left[n.S_p \frac{D_1.S_1(1-n)}{h_1} \left\langle \frac{1}{K_1} + \frac{1}{K_m} \right\rangle \right]$$

Where,

m= a/b. **'a'** is the ratio of drug concentration in the bulk of the elution medium over drug solubility in the same medium.

'b' is the ratio of drug concentration at the outer edge of the polymer coating over drug solubility in the same polymer composition.

n = ratio of drug concentration at the inner edge of the interfacial barrier over drug solubility in the polymer matrix

D_1,D_p,D_d = drug diffusivities in the liquid layer surrounding the drug particles, polymer coating membrane surrounding the polymer matrix and the hydrodynamic diffusion layer surrounding the polymer coating with respective thickness of h_1,h_p&h_d

K_1,K_m,K_p = partition coefficients for the interfacial partitioning of the drug from the liquid compartment to the polymer matrix, from the polymer matrix to the polymer coating membrane and from polymer coating to the elution solution.

S_1,S_p = solubilities of the drug in the liquid compartment and in the polymer matrix.

Evaluation of TDDS

1. Evaluation of adhesive:

a) Peal adhesion properties:

- Peel adhesion is the force required to remove an adhesive coating from a test substrate
- It is affected by molecular weight of adhesive polymer, type and amount of additives, and polymer composition.
- Test:
- The test invovles measuring the force required to pull a single coated tape, applied to substrate at a 180^0 angle.
- No residue on substrate indicates “adhesive failure”
- Remnants on substrate indicates “cohesive failure”

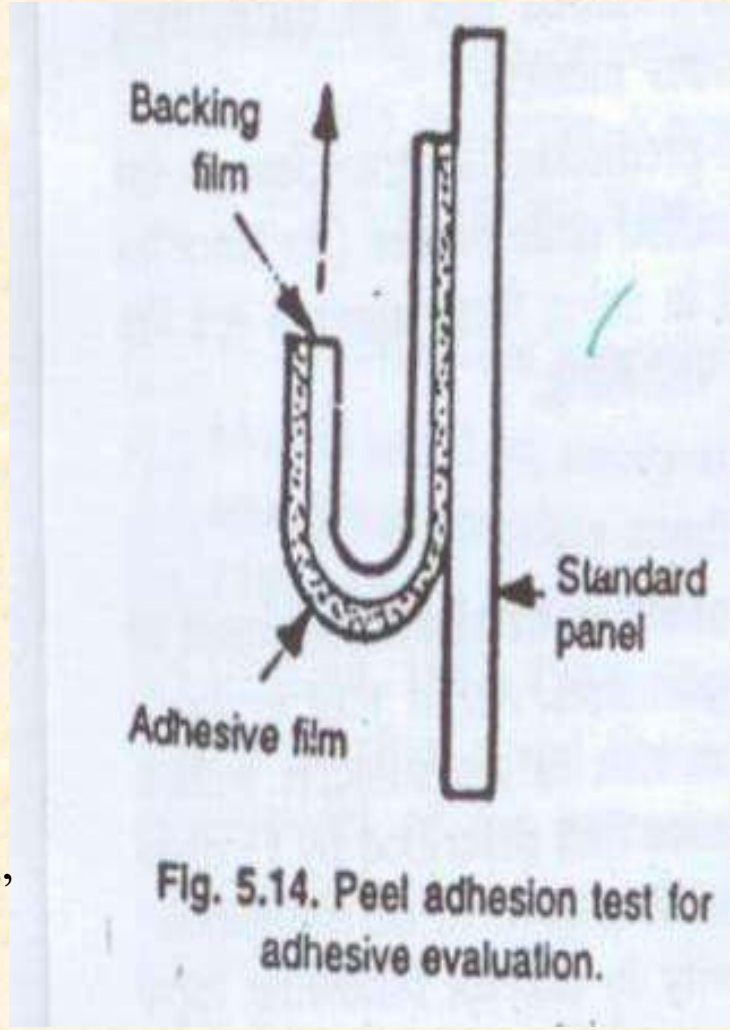


Fig. 5.14. Peel adhesion test for adhesive evaluation.

b) Tack properties:

- Ability of polymer to adhere to a substrate with little contact pressure
- Affected by molecular weight and compostion of polymer, tackifying resins in the polymer.
- **"Thumb tack test"** – measured by pressing the thumb briefly into the adhesive layer
- **"Rolling ball tack test"** – measurement of distance that a stainless steel ball travels along an upward placed adhesive.
- **"Quick-stick test (peel tack test)" –** pulling the tape away from substrate at 90^0 at a speed of 12inch/min
- **"Probe tack test"** – force required to pull a probe away from adhesive at a fixed rate is recorded as tack



Fig. 5.15. Rolling ball tack test for adhesive evaluation.

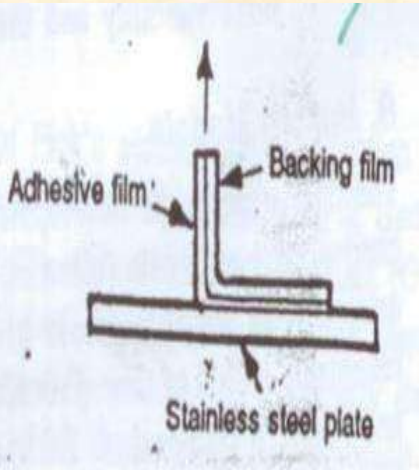


Fig. 5.16. Quick-stick (peel-tack) test for adhesive evaluation.

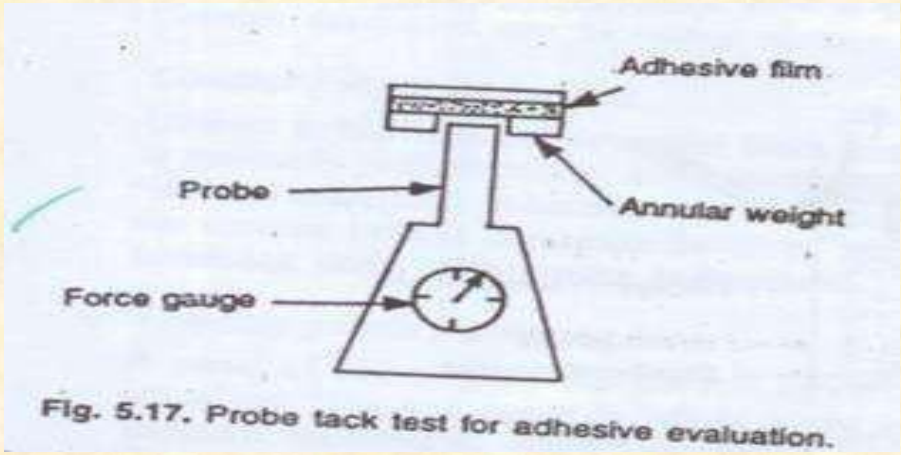


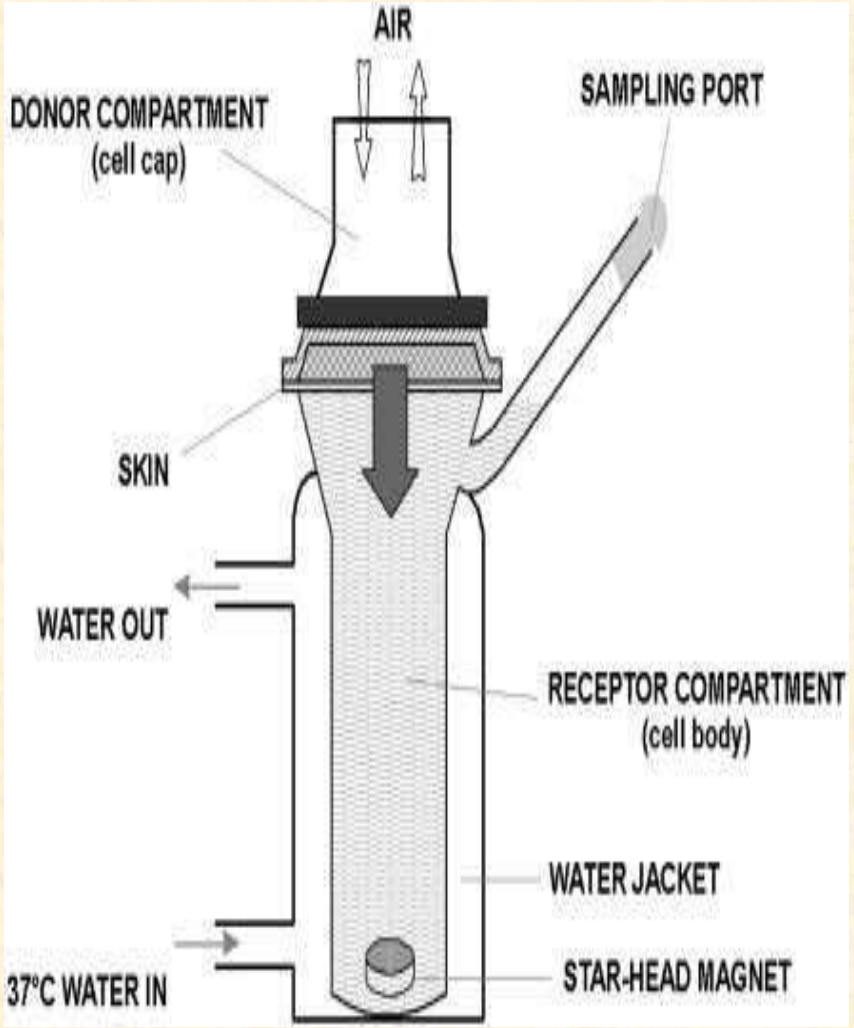
Fig. 5.17. Probe tack test for adhesive evaluation.

2. *Invitro* drug release evaluation

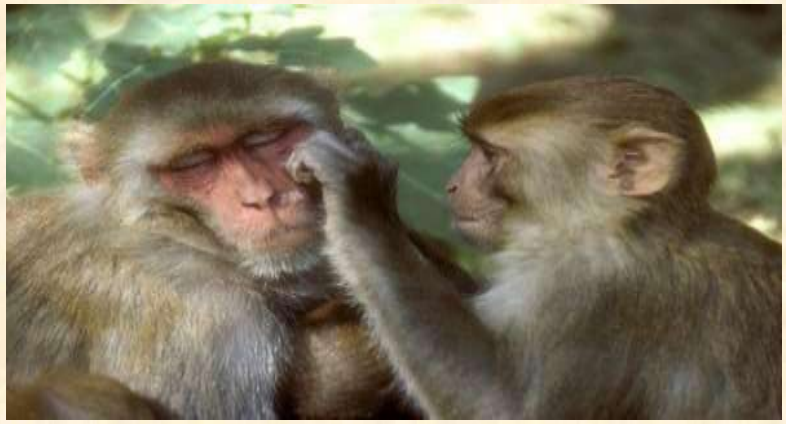
- Help in vestigating mechanism of skin permeation before developing a TDDS.
- Studies on skin metabolim can also be performed

Advantages:

- Methodology
- Ease of analytical assay since there are no complications arising from disposition of drug in the body
- Better control of experimental conditions than in invivo



FRANZ DIFFUSION CELL



3.*Invivo* evaluation:

a) Animal models:

i. Penetration values obtained with small hairy animals are higher than those seen in man. Preparation of their skin for study, by shaving or depilation lead to changes in resistance.

ii. Rhesus monkey is the most reliable model. Limtation of using this animal:- cost, handling capabilities, difficulty of accessibility and ethical considerations

iii. Other animals used:- weanling pig, human skin grafted-nude mouse





b) Human models

- Percutaneous absorption determined by indirect method:- measuring radioactivity of excreta following topical application of the labelled drug

$$\% \text{ dose absorbed} = \frac{\text{Total radioactivity excreted after topical administration}}{\text{Total radioactivity excreted after Intravenous administration}} \times 100$$

- "Reservoir technique" :- to overcome the inherent limitations in above process
- Short exposure of skin to the radiolabelled drug followed by removal of stratum corneum.

c) Biophysical models

5. Cutaneous toxicological evaluations



a) Contact dermatitis

❖ Contact irritant dermatitis

- Ten day primary irritation test
- Twenty one day irritation test
  - ➢ Laser doppler
  - ➢ Evaporative water loss measurements

❖ Contact allergic dermatitis



b) Growth of microorganisms

❖ Localised superficial infections

❖ Miliaria



IONTOPHORESIS

Definition: Iontophoresis can be defined as the permeation of ionized drug molecules across biological membranes under the influence of electric current.



SONOPHORESIS

Definition: Sonophoresis is the enhancement of migration of drug molecules through the skin by ultrasonic energy



Sr no	Sonophoresis	Iontophoresis
1	Sonophoresis is the enhancement of migration of drug molecules through the skin by ultrasonic energy	Iontophoresis is movement of ions of soluble salts across a membrane under an externally applied potential difference
2	Sonophoresis uses acoustic energy (ultrasound) to drive molecules into tissues	Iontophoresis uses electiral current to transport ions into tissues
3	Proper choice of ultrasound parameters including ultrasound energy dose, frequency, intensity, pulse length, and distance of transducer from the skin is critical for efficient sonophoresis.	Proper choice of electricity parameters including Current density, Current profile, Duration of treatment, Electrode material, Polarity of electrodes, is critical for efficient Iontophoresis
4	Sonophoresis usually employs a ultrasound between 20 KHz to 20 MHz	Iontophoresis usually employs a direct current between 0.5 mA to 5.0 mA

5	In sonophoresis drugs mixing with a coupling agent like gel, cream, ointment	In Iontophoresis drug is mix with solvent
6	The main mechanism for transport of drug is “Cavitation”	The main mechanism for transport of drug is “Electroporation”
7	Drug should be in aqueous or non aqueous and ionized or non ionized form	Drug must be in aqueous and must be in ionized form
8	Enhanced partitioning, Lipid bilayer disordering, Keratin denaturation etc. gives the synergetic effect of sonophoresis	Electrophoresis, Lipid bilayer disordering, Electroosmosis etc. gives the synergetic effect of Iontophoresis
9	Ultrasound can be applied in a continuous or pulse mode	Electrical current can be applied only in continuous mode
10	Sonophoresis mostly used for delivery of corticosteroids, local anesthetics and salicylates	Iontophoresis mostly used for hyperhydrosis diagnosis of cystic fibrosis, metallic and non-metallic ions



REFERENCES



- Controlled and Novel Drug Delivery by N.K. Jain, Pg. No : 100 to 127, 191 to 206 and 207 to 233.

- Ionotophoreseis : A novel approach to transdermal drug delivery, Pharma buzz, November 2007, Vol 2/ Isuue 11


Thank You!

FORMATTING, ASSEMBLING & SUBMISSION OF ABBREVIATED NEW DRUG APPLICATION

Dr.Y.Kiran Kumar

Associate professor

VCPS

ANDA

- An Abbreviated New Drug Application (ANDA) contains data which when submitted to FDA's CDER, Office of Generic Drugs, provides for the review and ultimate approval of a generic drug product.

- Once approved, an applicant may manufacture and market the generic drug product to provide a safe, effective, low cost alternative to the public.

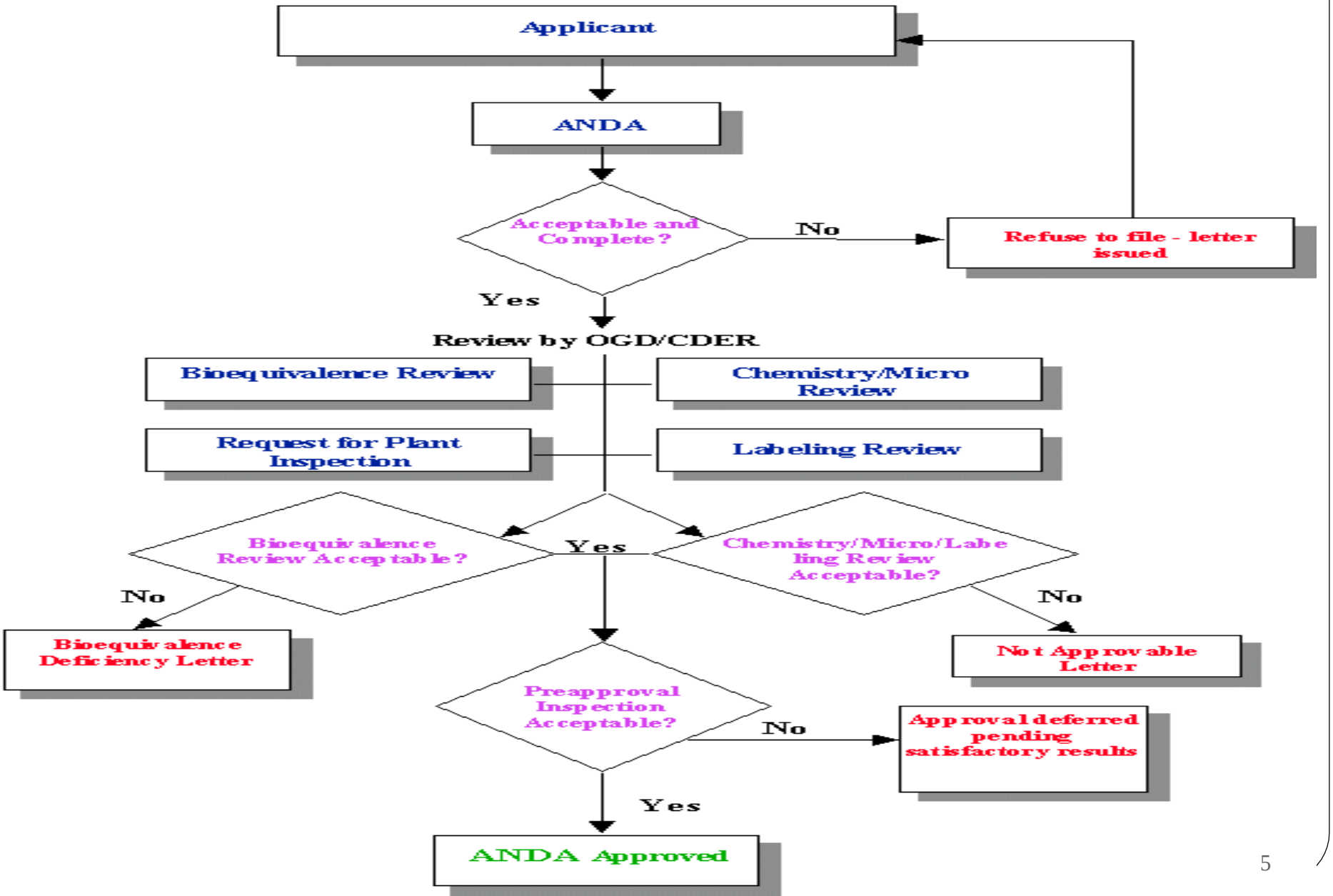
- All approved products, both innovator and generic, are listed in FDA's *Approved Drug Products with Therapeutic Equivalence Evaluations* (*Orange Book*).

- Use of bioequivalence as the base for approving generic drug products was established by the "*Drug Price Competition and Patent Term Restoration Act of 1984*," also known as the WAXMAN-HATCH ACT.
- A generic drug product is one that is comparable to an innovator drug product (also known as the reference listed drug (RLD) product as identified in the FDA's list of ***Approved Drug Products with Therapeutic Equivalence Evaluations***) in dosage form, strength, route of administration, quality, performance characteristics and intended use.

Goal of ANDA

- To reduce the price of the drug.
- To reduce the time development.
- Increase the bioavailability of the drug in comparison to references list drug.

ANDA Review process



NDA vs. ANDA Review Process

NDA Requirement	ANDA Requirement
1. Labeling	1. *Labeling*
2. Pharm/Tox	2. Pharm/Tox
3. Chemistry	3. Chemistry
4. Manufacturing	4. Manufacturing
5. Controls	5. Controls
6. Microbiology	6. Microbiology
7. Inspection	7. Inspection
8. Testing	8. Testing
9. Animal Studies	
10. Clinical Studies	9. Bioequivalence
11. Bioavailability	

ANDA Requirement

1) **Signed FDA form 356h**. Provides information regarding the applicants name & address, name of the drug product, the product strength & route of administration, indication of drug master files cited, proposed indications, a statement regarding whether the product is for prescription or over the counter.

2) **An index** should specify volume & page number for each complete & detailed item.

3) **Information on the basis for which the ANDA is being submitted**.

a) Name of the reference drug, its dosage form & strength.

b) Information on exclusively for the listed drug.

c) If a suitability petition is approved a reference to the FDA number that was assigned to that suitability petition.

4) **Condition for use, including**

a) A statement regarding the condition for which the drug will be used.

b) b) A reference to the annoted labeling for the product & the currently approved labeling for the listed drug product.

5) **A statement that active ingredient** is the same as for that of the reference drug. For the combination product this must be shown for both active ingredient.

6) **Route of administration**, dosage form & strength. This should include a statement that the route of administration, dosage form & strength are same as the reference drug. Bioequivalence. This should include information to demonstrate that the proposed drug is bioequivalent to the listed drug product.

7. Index:

- It is a comprehensive table of contents that enables reviewers to find specific information in this massive document.

8. Labeling:

- This section must include all the draft labeling that is intended for use on product container, packings including the proposed package insert.
- NDA must have four copies of graft labeling.

9. Chemistry, Manufacturing & Controls

This section includes description of drug substance

- Generic name
- Chemical name
- Molecular structure & formula
- Molecular weight
- Appearance, M.P. & B.P.
- Refractive index, viscosity & specific gravity.
- Polymorphs
- Solubility
- Synthesis scheme & description.

10. Microbiology.

- This section is required only for anti infective drug products.
- This section requires following technical information
- Complete description of biochemical basis of drug action on microbial physiology.
- The drugs anti microbial spectrum
- Clinical microbiology laboratory methods.

11. Human Pharmacokinetics & Bioavailability.

- This include information concerning
- The Design
- The Dosing procedure
- The number & frequency of blood & urine collection & Methodology for the assay.

12. Samples

- The sample of the Drug substance & finished product should be provided four individuals units with sufficient quantities in each unit to permit the FDA to perform all the tests included in the specifications at least three times

13. Analytical method for drug substance & drug product.

- This section should consists of the specifications, analytical method, certificates of analysis, method of analysis, method validation & stability indicating data as contained in the chemistry, manufacturing & control part of the application.

Refuse to file letter issued

- If the application is missing one or more essential components, a “Refuse to File” letter is sent to the applicant.
- No further review of the application occurs until the applicant provides the requested data & the application is found acceptable & complete.

Bioequivalence Review:

- The Bioequivalence Review process established that the proposed generic drug is bioequivalent to the reference listed drug, based upon a demonstration that both the rate & extent of absorption of the active ingredient of the generic drug fall within established parameters when compared to that of the reference listed drug.
- Applicants may request a waiver from performing in vivo (testing done in humans) bioequivalence studies for certain drug products where bioavailability may be demonstrated by submitting data such as

1) a formulation comparison for products whose bioavailability is self evident, for example, oral solutions, injectables, or ophthalmic solutions where the formulations are identical.

2) comparative dissolution.

ANDA Approved

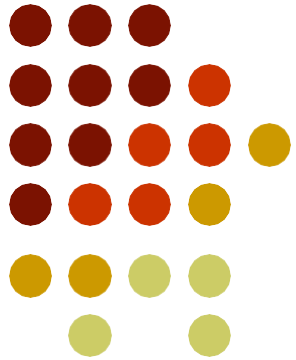
- After all components of the application are found to be acceptable an approval or tentative approval letter is issued to the applicant.
- If the approval occurs prior to the expiration of any patents or exclusivities accorded to the reference listed drug product, a tentative approval letter is issued to the applicant which details the circumstances associated with the tentative approval of the generic drug product & delays approval until all patent/exclusivity issues have expired.
- A tentative approval does not allow the applicant to market the generic drug product.

BIOAVAILABILITY AND BIOEQUIVALENT STUDIES

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M. Pharm,

Vikas College Of Pharmaceutical Sciences



BIOAVAILABILITY :

Bioavailability is the rate and extent of drug that reaches systemic circulation in an unchanged form so as to provide desired therapeutic activity.

OBJECTIVES :



1. **For development of preliminary formulation designing a new drug moiety (investigational pre formulative stage).**

2. **To check influence of various physicochemical factors, biological factors and excepients related factors on the drug in a particular dosage form.**

3. **It is mainly used to develop newer formulation for existing drug moieties.**

Ex: Aspirin is available as ordinary compressed tablet , effervescent aspirin tablet and also as buffered aspirin tablet.

4. **To improve quality control of dosage forms containing a drug moiety due to processing variables, storage problems and stability problems arise with dosage forms.**

VARIOUS CONSIDERATIONS IN ASSESING BIOAVALABILITY



BIOAVAILABILITY –ABSOLUTE (VS) RELATIVE

Absolute bioavailability (F) :- it is the measure of systemic ability of drug moiety in comparison with the intravenously administered dosage form of the same drug.

$$F = \frac{\textbf{(AUC)}\text{oral}.\ \textbf{DOSE}\ \text{IV}}{\textbf{(AUC)}\text{IV}\ .\textbf{DOSE}\ \text{oral}}$$

Sigficance: to characterize a drugs inherent absorption property from the .E.v site.

VARIOUS CONSIDERATIONS IN ASSESING BIOAVALABILITY

Relative bioavailability (Fr) :- it is the measure of systemic availability of a particular drug from a test dosage form is in comparison with the standard dosage form of similar type.

$$F_r = \frac{(AUC)_{test} \cdot DOSE_{std}}{(AUC)_{std} \cdot DOSE_{test}}$$

Significance:

To characterize absorption of a drug from its formulation.

SINGLE DOSE (VS) MULTIPLE DOSE STUDIES



- **Single dose:-**

➢ These are simple.

Note:-

✓ difficult to predict-steady state concentration.

✓ lot of errors.

✓ inter subject variables.

✓ more samples are required.

MULTIPLE DOSE STUDIES



- More accurate.
- Steady state concentration is achieved.
- No. of subjects required is less.
- Inter subject variables are less.

DISADVANEAGES

- Need expertise.
- Costly.
- Greater exposure to test drug. (adverse reactions)

HUMANVOLUNTEERS- healthy subjects (vs.) patients

PATIENTS

ADVANTAGES

- The patient will be benefited.
- Reflects the therapeutic efficacy of the drug.
- Drug absorption pattern in disease state can be evaluated.
- Avoids the ethical quandary of administering of drugs to a healthy subjects.

DISADVANTAGES

- Disease, other drugs, physiological changes may modify the drug absorption pattern.

SELECTION OF HEALTHY HUMANS

- Adult healthy human of age 20-40,with uniform weight should be considered.
- For certain drugs adult human females of age 20-40 can be selected.
- The voluntaries selected should be clinically examined for any diseases.
- The human voluntaries selected should be kept under restricted dietary conditions prior to12hrs before drug administration.
- The voluntaries are advised not to undergo vigorous physical exercise during the testing.

SELECTION OF HEALTHY HUMANS



- voluntaries consent must be obtained before involvement in testing.
- The voluntaries should be insured.
- voluntaries must be advised to abstain from any meditation for at least one week and to fast over night prior to and for a minimum of 4hrs after dosing.
- A minimum of 10 half-lives should be allowed for any two studies on the same subject.

ESTIMATION / MEASUREMENT OF BIOAVAILABILITY



There are three methods to measure the bioavailability.

They are :

1) Pharmacokinetic methods
2) Pharmacodynamic methods
3) In vitro dissolution testing studies

- Pharmacokinetic methods and Pharmacodynamic methods are together called as in vivo methods.

- In vitro dissolution testing is the preliminary test carried on any dosage form for assessing rate and extent of drug release.

Pharmacokinetic Method

(Indirect method of estimation)

1. Plasma concentration Vs time profile studies which includes

   a) single dose studies

   b) multiple dose studies

2. Urinary Excretion studies which includes

   a) single dose studies

   b) multiple dose studies

Pharmacodynamic Method

(Direct method of estimation)



It includes two methods :

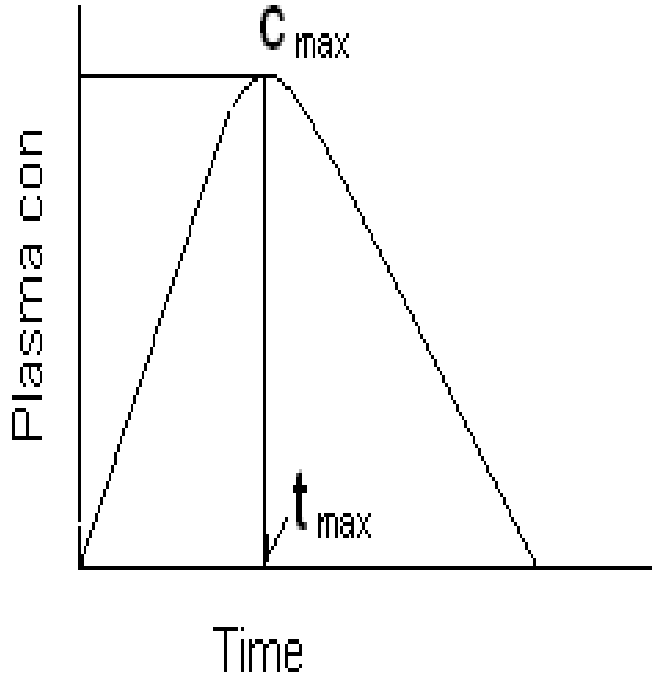
1. Acute pharmacological studies
2. Pharmacotherapeutic / Clinical response studies

PHARMACOKINETIC STUDIES

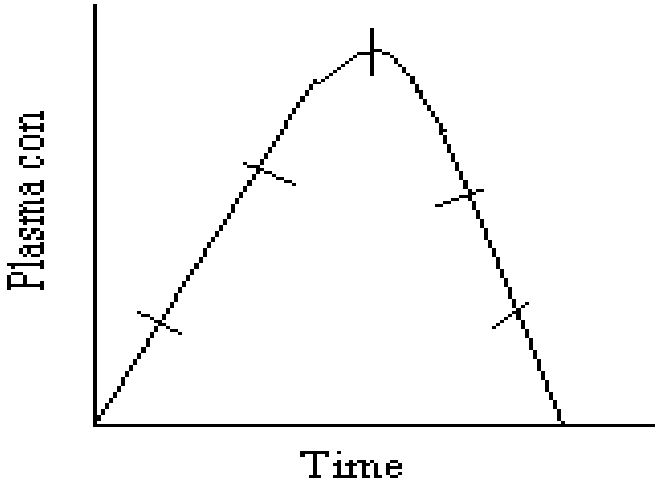
Plasma concentration Vs Time profile:

Single dose

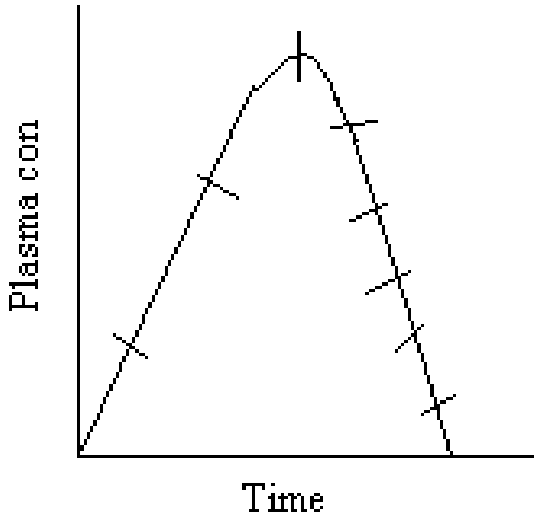
- In general single dose bioavailability studies are conducted.
- From the point administration of dosage form the samples are withdrawn for about 2-3 biological half lives.
- Number of samples to be withdrawn depends on the nature of the dosage form i.e., route of administration.
- From the graph AUC, Tmax, and Cmax were determined.



- In one compartment model 3 samples are taken on descending curve and 3 samples on ascending curve.
- In multi compartment model 3 samples are taken on ascending curve and 5-6 samples on descending curve.



One compartment



Multi compartment

- **Multiple dose is administered to attain steady state concentration.**

- **It is generally done to lessen the intersubject variability.**

- **Number of doses to be administered is to the extent of 5 to 6 biological half lives.**

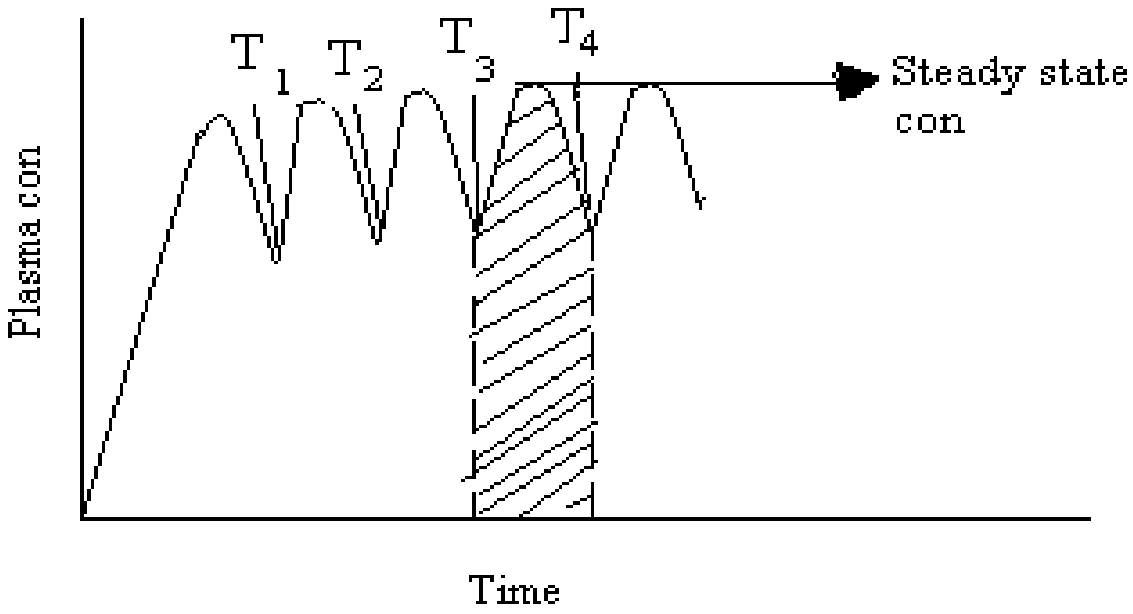
- **Sample is withdrawn prior to the administration of next dose.**
- **Cmax Tmax and Auc**

- **Samples are withdrawn at stipulated time intervals after attaining the steady state concentration.**

$$Fr = \frac{[AUC]\ test\ D_{std}\ Ttest}{[AUC]std\ Dtest\ Tstd}$$

- **Bioavailability can be assessed by Cmax at steady state concentration.**

$$Fr = \frac{(Cmax\ ss)test\ Dstd\ Ttest}{(Cmax\ ss)std\ Dtest\ Tstandard}$$



URINARY EXCRETION DATA



- Most of the drugs are excreted in unchanged form in urine.
- As a rule determination of bioavailability of urinary excretion data should be conducted only if at least 20% of administered dose is excreted unchanged in urine.

  Ex : Thiazide diuretics and sulphonamides

The method involves

1. Collection of urine at regular intervals for a time span equal to 7 biological half lives.
2. Analysis of unchanged drug in the collected sample.
3. Determination of the amount of drug excreted in each interval and cumulative amount excreted.

CRITERIA TO OBTAIN VALID RESULTS:



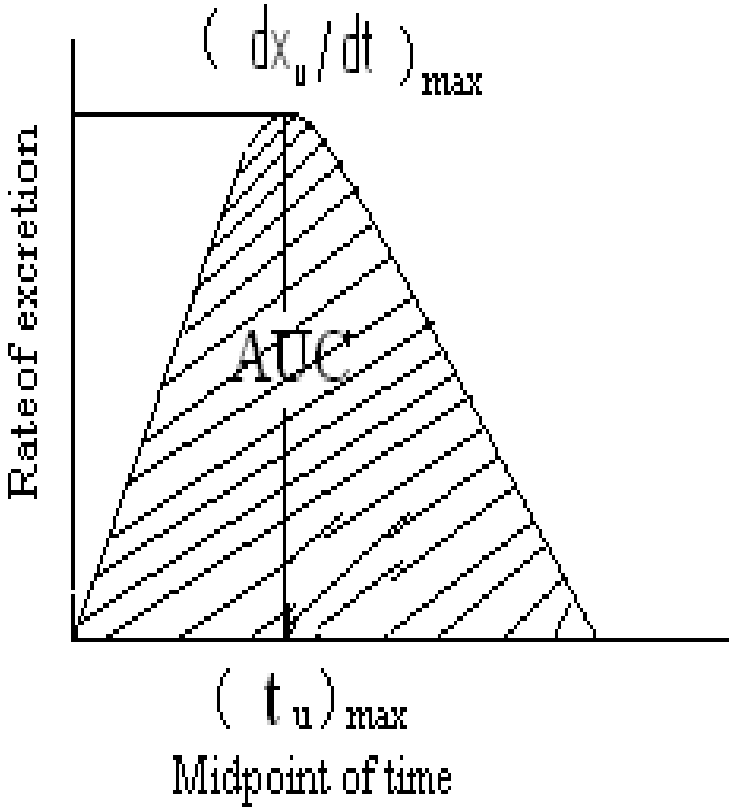
- At each sample collection total emptying of bladder is necessary to avoid errors resulting from addition of residual amount of next urine sample.

- Frequent sampling of urine is also essential in the beginning in order to compute correctly the rate of absorption.

- The fraction excreted unchanged in urine must remain constant.

Three Parameters Examined In Single Dose Study :

1. **The maximum urinary excretion rate (dXu/dt)max :** it is obtained from the peak of plot between rate of excretion Vs midpoint time of urine collection period. It is analogous to the Cmax .
2. **The time For maximum excretion rate (tu)max :** It is analogous to Tmax of plasma level data.
3. **The cumulative amount of drug excreted in the urine Xu~:** It is related to AUC of plasma level data.



- The extent of bioavailability can be calculated by using

$$F = \frac{(Xu)oral\ Div}{(Xu)iv\ Doral} \qquad Fr = \frac{(Xu)test\ Dstd}{(Xu)std\ \ Dtest}$$

- With multiple dose study to steady state the equation for computing bioavailability is :

$$Fr = \frac{(Xu, ss)test\ \ Dstd\ \ Ttest}{(Xu, ss)std\ \ Dtest\ \ Tstd}$$

PHARMACODYNAMIC STUDIES



- When bioavailability measurement by pharmacokinetic method is difficult , inaccurate or non reproducible, an acute pharmacological effect such as a change in ECG or EEG readings, pupil diameter etc is related to time course of related drug.
- Bioavailability can then be determined by construction of pharmacological effect-time curve as well as dose-response graphs.
- This method requires measurement of responses for atlease 3 biological half lives of the drug in order to obtain a good estimate of AUC.

THERAPEUTIC RESPONSE METHOD

- This method is based on observing the clinical response to a drug formulation given to patients suffering from disease for which it is intended to be used.

Drawbacks :

1. Quantitation of observed response is too improper to allow for reasonable assessment of bioavailability.
2. Bioequivalence studies are conducted using a crossover design and the physiological status of the subject should not change over duration of study.
3. Unless multiple dose protocols are employed a patient who required the drug for a disease would be able to receive only a single dose of drug.
4. Many patients receive more than one drug and the results obtained from a bioavailability study could be compromised because of drug-drug interaction.

INVITRO DISSOLUTION TESTING

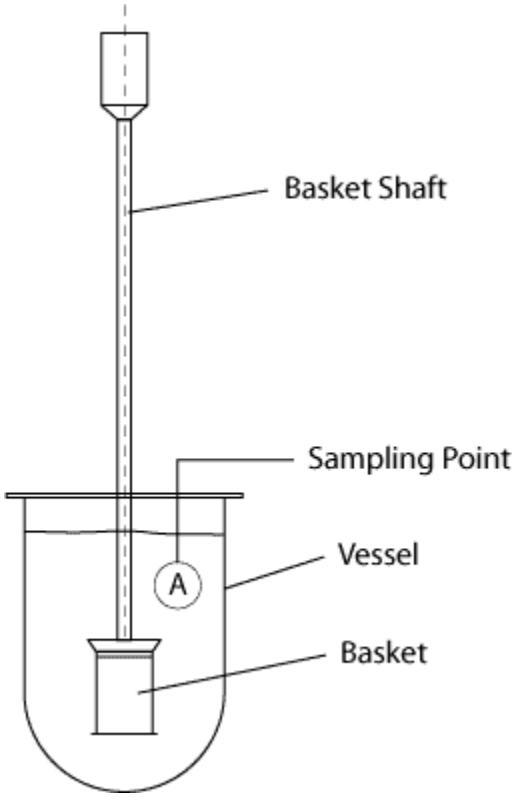


- The best available tool today which can at least quantitatively assure about the biological availability of a drug from its formulation is its in vitro dissolution test.

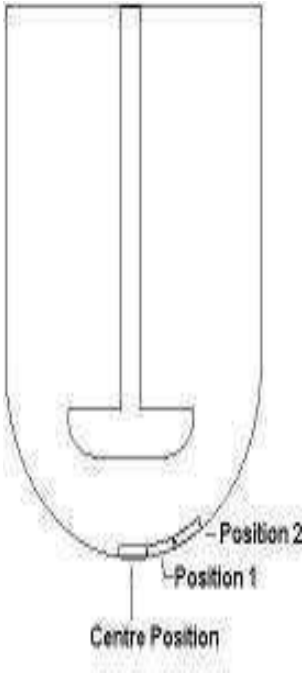
Factors Considered In Design Of Dissolution Test :

1. Factors relating to dissolution apparatus.
2. Factors relating to dissolution fluid.
3. Process parameters.

USP dissolution apparatus 1



USP dissolution apparatus 2



DISSOLUTION ACCEPTANCE CRITERIA

S1	6	No dosage unit is less than Q+5%
S2	6	Average of 12 dosage units (S1+S2) >Q% and no dosage unit is less than Q-15%
S3	12	Average of 24 dosage units (S1+S2+S3) > Q% and not more thatn 2 dosage units are less than Q-15% and no dosage unit is less than Q-25%

INVITRO - INVIVO CORRELATION



- It is defined as the predictive mathematical model that describes the relationship between an in-vitro property of a dosage form and an in-vivo response.

- The main objective of developing and evaluating an IVIVC is to enable the dissolution test to serve as a surrogate for in vivo bio availability studies in human beings.

Objectives:

1. To ensure batch to batch consistency in the physiological performance of a drug product by use of such in vitro values.

2. To serve as a tool in the development of a new dosage form with desired in vivo performance.

3. To assist in validating or setting dissolution specifications.

Approaches for invivo-invitro correlation:



1. By establishing a relationship usually linear between the in vitro dissolution and the in vivo bioavailability parameters.

- Correlations based on the plasma level data
- Correlations based on the urinary excreation data.
- Correlations based on the pharmacologic response.

2. By using data from previous bioavailability studies to modify the dissolution methodology in order to arrive at meaningful in vitro- in vivo correlation.

Correlations based on plasma level data :

Here linear relationships between dissolution parameters and plasma level data are established.



In vitro dissolution parameters

- Time for specific amount of drug to dissolve
- Amount dissolved at a specific time point
- Mean dissolution time
- Parameter estimated after modeling the dissolution process.

In vivo plasma data parameters

- AUC , Cmax
- Fraction absorbed , absorption rate constant Ka
- Mean residence time ,mean dissolution time, mean absorption time
- Concentration at time t, amount absorbed at time t.

Correlations based on urinary excretion data

- Here dissolution parameters are correlated to the amount of drug excreted unchanged in urine, cumulative amount of drug excreted as a function of time etc.,

Correlations based on pharmacological response :

- An acute pharmacological effect such as LD 50 in animals is related to any of the dissolution parameters.

- **Equivalence** is more a general,relative term that indicates a comparision of one drug product with another or with a set of established standards.Equivalence may be defined in several ways:

- **Chemical equivalence** indicates that two or more dosage forms contain the same labeled quantities (plus or minus specified range limits) of the drug.

- **Clinical equivalence** occurs when the same drug from two or more dosage forms gives identical *in vivo* effects as measured by a pharmacological response or by control of a symptom or disease.

- **Therapeutic equivalence** implies that two brands of a drug product are expected to yield the same clinical result. The FDA specifically uses the term therapeutic equivalence in the evaluation of multisource prescription drug products.

- **Bioequivalence** indicates that a drug in two or more similar dosage forms reaches the general circulation at the same relative rate and the same relativ extent(i.e, that the plasma level profiles of the drug obtained using the two dosage forms are the same).

- **Pharmaceutical equivalence** refers to two drug products with the same dosage form and same strength.

EXPERIMENTAL DESIGN:

Parallel Design:

- Two formulations are administered to two groups of volunteers.
- To avoid bias two formulations are administered randomly to the volunteers.

Disadvantages:

- inter-subject variation is not corrected.
- Inter-subject variation is greater than the variation between any formulations

EXPERIMENTAL DESIGN:



Cross- Over Design:

- Each subject receives the test drug product and the reference drug product
- Minimizes the effect of inter-subject variability in the study

3 Types:-

- Latin Square crossover Design
- Balanced Incomplete Block Design (BIBD)
- Replicated crossover design

Latin Square crossover Design:

- ✓ Each subject receives each formulation only once.
- ✓ Each formulation is administered only once in each study period.
- ✓ Each subject acts as his own control.

Table 11.2 Latin Square Designs

Two-Way Crossover		
Group No.	*Subjects in Group*	*Treatment for Period No.*
		I II
1.	1,2,3,4,5,6	A B
2.	7,8,9,10,11,12	B A
Three-Way Crossover		
Group No.	*Subjects in Group*	*Treatment for Period No.*
		I II III
1.	1,2,3,4,5,6	A C B
2.	7,8,9,10,11,12	B A C
3.	13,14,15,16,17,18	C B A
Four-Way Crossover		
Group No.	*Subjects in Group*	*Treatment for Period No.*
		I II III IV
1.	1,2,3,4,5,6	A B C D
2.	7,8,9,10,11,12	B D A C
3.	13,14,15,16,17,18	C A D B
4.	19,20,21,22,23,24	D C B A

Latin Square crossover Design:



Advantages

- ✓ Minimizes the carry-over effects.
- ✓ Minimizes the time effect on bioavailability.
- ✓ Requires less number of subjects to get good results.

Disadvantages

- ✓ Requires longer time to complete the study.
- ✓ Time to complete the study also depends on the number of formulations to be evaluated.
- ✓ Increased number of study periods leads to high subject drop outs.
- ✓ Medical ethics does not allow too many trials on a subject for a longer time.

Balanced incomplete Block Design (BIBD) :

- Each subject receives not more than 2 formulations
- Each formulation is administered same number of times
- Each pair of formulations occurs together in the same number of subjects

Table 11.3 Balanced incomplete block design (BIBD) for four formulations

Subject	Treatment	for Period No.
	I	II
1	A	B
2	B	A
3	A	C
4	C	A
5	A	D
6	D	A
7	B	C
8	C	B
9	B	D
10	D	B
11	C	D
12	D	C

Replicated crossover design :

- To determine individual bio-equivalence.
- To estimate within-subject variance for both the Test and Reference drug products.
- To estimate subject-by-formulation interaction variance.
- Reference-Reference and Test-Test comparisons may be made.

	Period 1	**Period 2**	**Period 3**	**Period 4**
Sequence 1	T	R	T	R
Sequence 2	R	T	R	T

Wash out period:

The time interval between two treatments

- 10 Half-lives
- Elimination of 99.9% of administered dose
- Elimination of metabolites

No: of washout periods:

- ✓ Study design used
- ✓ No: of formulations to be evaluated

Example:

- ✓ Digitoxin – Half-life = 6-9 days
- ✓ Most drugs – Half-life = 1-10 hrs

DRUG PRODUCTS:

Test Products

- Newdrug formulation
- New dosage form

Reference standard

- Any innovator's drug product
- RLD (reference listed drug)
- Approved drug products with therapeutic equivalence evaluations (Orange book)

BIOAVAILABILITY AND BIOEQUIVALENCE STUDIES

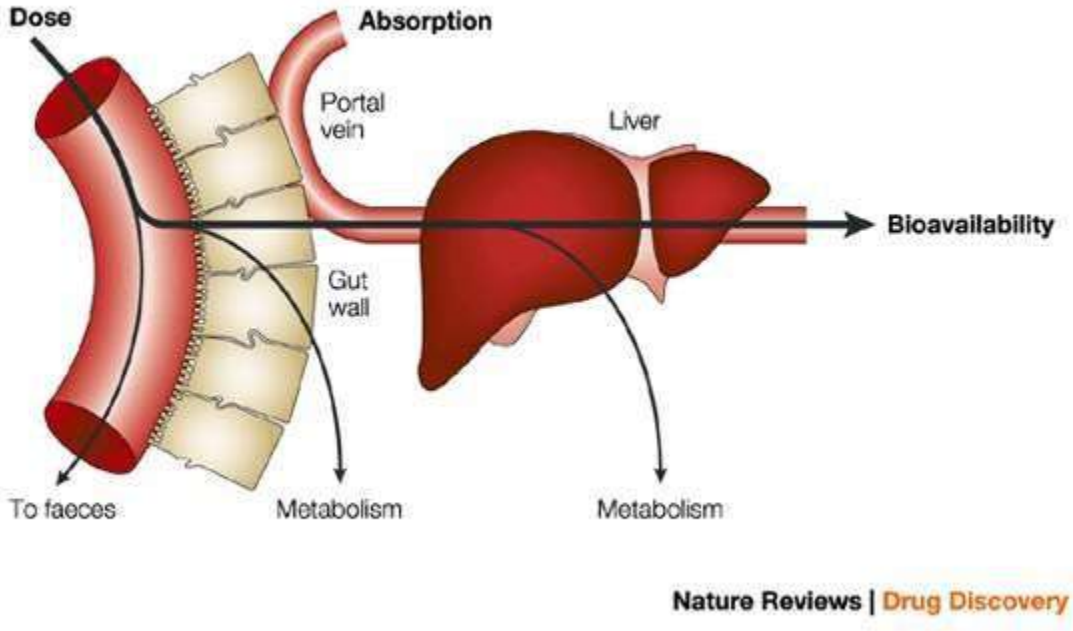
G.NATESH

M. Pharm,

Vikas College Of Pharmaceutical Sciences

BIOAVAILABILITY :

Bioavailability is the rate and extent of drug that reaches systemic circulation in an unchanged form so as to provide desired therapeutic activity.



OBJECTIVES

- *Development of a **new dosage form** for a **new drug entity.***

- *Determination of influence of **excipients**, **patient related factors** and possible **interaction** with other drugs on the **efficiency of absorption.***

- ***Control of quality** of a drug product during the early stages of marketing in order to determine the influence of **processing factors, storage and stability on drug absorption.***

➢*Development of **new formulations** for the existing drug.*

Ex: Aspirin



Normal Tablets



Soluble tablets



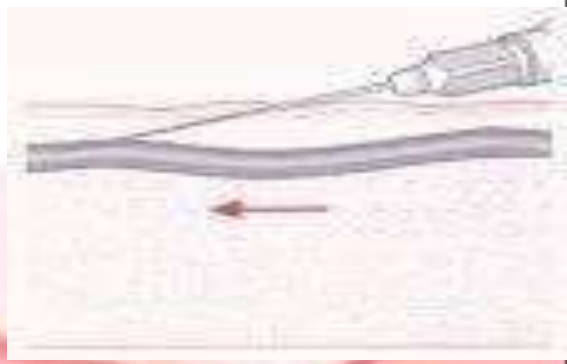
Buffered Aspirin Tablets

BIOAVAILABILITY –ABSOLUTE (VS) RELATIVE

Absolute bioavailability* (F) :-** *it is the measure of* ***systemic availability *of drug moiety in comparison with the* ***intravenously*** *administered dosage form of the* ***same drug.***

$$F = \frac{[AUC]_{oral} . DOSE_{iv}}{[AUC]_{iv} . DOSE_{oral}}$$





Significance: *To characterize a drugs* ***inherent absorption property*** *from the extra vascular site.*

***Relative bioavailability* (Fr) :-** *it is the measure of systemic availability of a particular drug from a test dosage form is in comparison with the standard dosage form of similar type.*

$$Fr = \frac{[AUC]test.\ DOSE\ std}{[AUC]std\ .DOSE\ test}$$

Significance:

To characterize absorption of a drug from its formulation.

F and Fr are generally expressed in percentages.

Standard



Test



HUMANVOLUNTEERS- healthy subjects (vs.) patients (as per FDA)





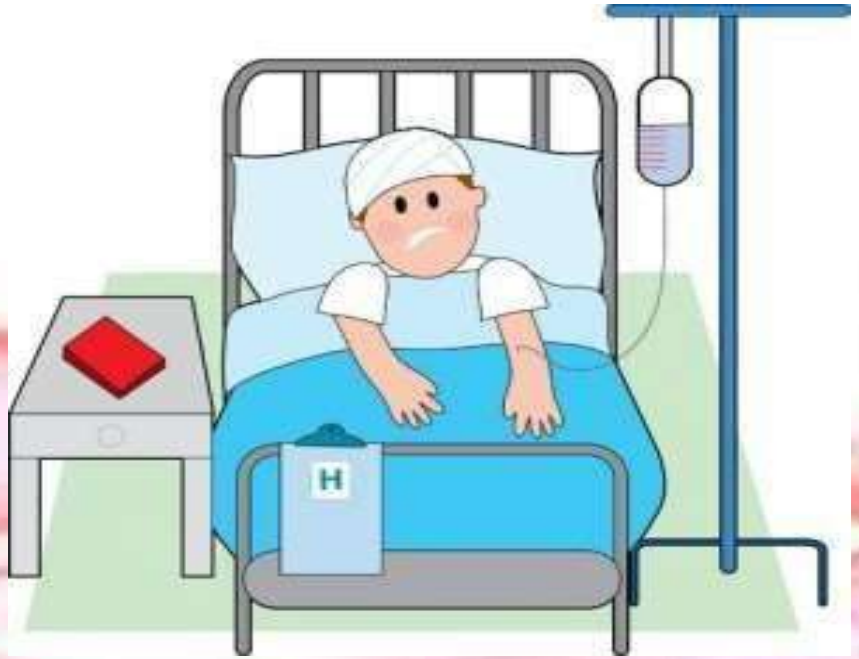


Disadvantages

- *Disease, other drugs, physiological changes may modify the drug absorption pattern.*

Advantages

- *The patient will be benefited.*
- *Reflects the therapeutic efficacy of the drug.*
- *Drug absorption pattern in disease state can be evaluated.*
- *Avoids the ethical quandary of administering of drugs to a healthy subjects.*



SELECTION OF HUMAN VOLUNTEERS

(HEALTHY SUBJECTS)

As per FDA Regulations

- *Adult healthy human of age 20-40, with uniform weight should be considered.*
- *For certain drugs female volunteers of age 20-40 can be selected.*
- *The volunteers selected should be clinically examined for any diseases.*

- *Volunteers **consent** must be obtained before involvement in testing.*



- *The volunteers should be **insured**.*
- *Volunteers must be advised to **abstain** from **any medication** for at least **one week**, and to **fast over night** prior to and for a minimum of **4hrs** after dosing.*
- ***A minimum of 10 half-lives** should be allowed for any two studies on the same subject.*



- *The human volunteers selected should be kept under **restricted dietary conditions** prior to **24hrs** of drug administration.*

- *The volunteers are **advised not** to undergo **vigorous physical exercise** during the testing*

ESTIMATION / MEASUREMENT OF BIOAVAILABILITY



There are three methods to measure the bioavailability.

They are :

1) *Pharmacokinetic methods*
2) *Pharmacodynamic methods*
3) *In vitro dissolution testing studies*

- *Pharmacokinetic methods and Pharmacodynamic methods are together called as in vivo methods.*

- ***In vitro dissolution testing is the preliminary test carried on any dosage form for assessing rate and extent of drug release.***

Pharmacokinetic Method

(Indirect method of estimation)



1.Plasma concentration Vs time profile studies which includes

a) single dose studies

b) multiple dose studies

2. Urinary Excretion studies which includes

a) single dose studies

b) multiple dose studies

Pharmacodynamic Method

(Direct method of estimation)

It includes two methods :

1. *Acute pharmacological studies*
2. *Pharmacotherapeutic / Clinical response studies*

Typical Plasma Concentration time Profile

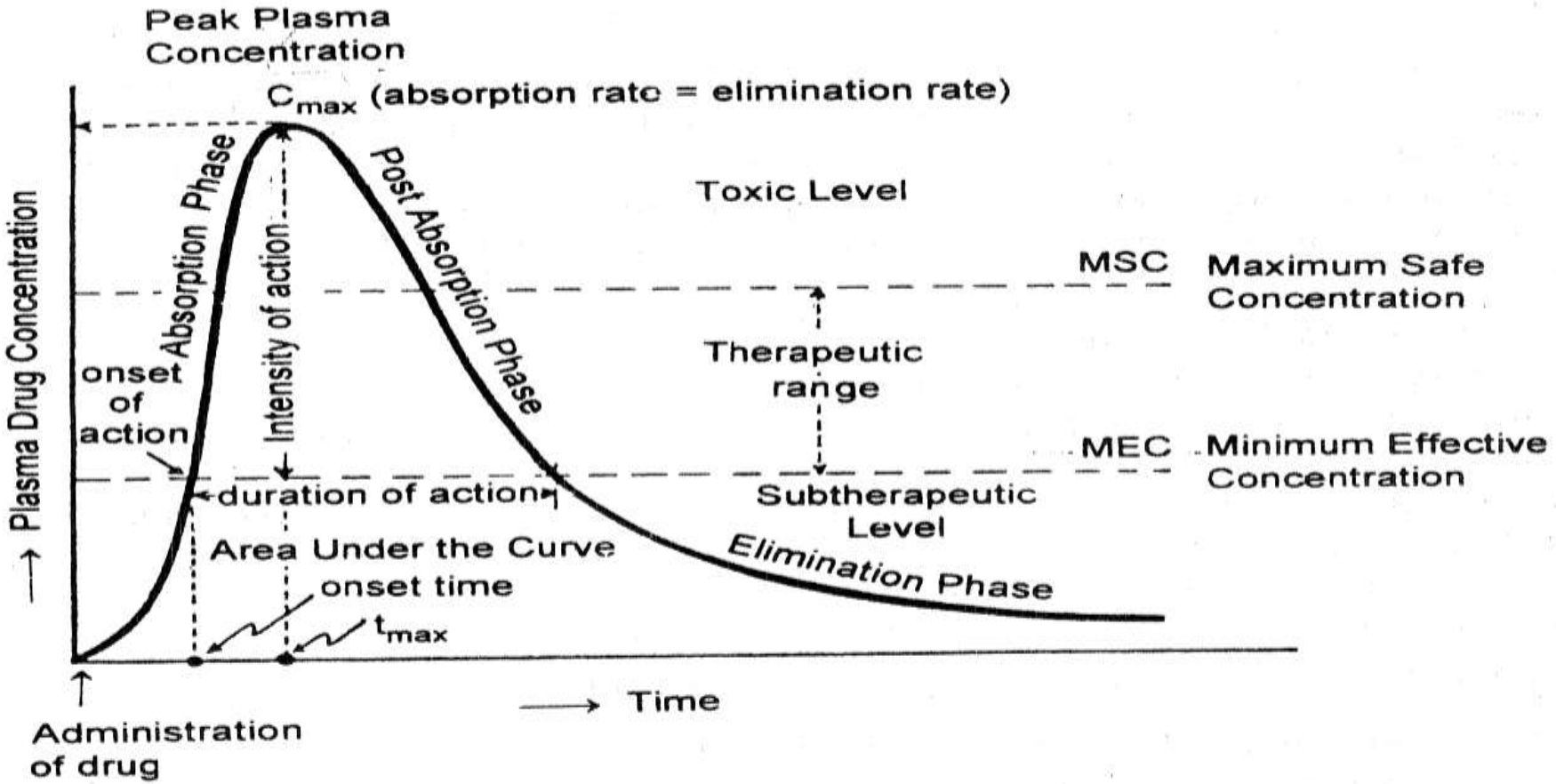


Fig. 9.1 A typical plasma concentration-time profile showing pharmacokinetic and pharmacodynamic parameters, obtained after oral administration of single dose of a drug.

SINGLE DOSE Vs MULTIPLE DOSE STUDIES



Single dose:-

Samples are withdrawn to the extent of 2-3 biological half lives.

- *In one compartment model 3 samples are taken on descending curve and 3 samples on ascending curve.*
- *In multi compartment model 3 samples are taken on ascending curve and 5-6 samples on descending curve.*

ADVANTAGES:



- They are easy.
- Offer less exposure to drugs.
- Less tedious.

DISADVANTAGES:



- Difficult to predict-steady state concentration.
- Lot of errors.
- Inter subject variability.
- More samples are required.

MULTIPLE DOSE STUDIES

- ***Samples are withdrawn to the extent of 5-6 biological half lives.***

- ***Sample is withdrawn prior to the administration of next dose.***



ADVANTAGES:



- More accurate.
- Steady state concentration is achieved.
- No. of subjects required is less.
- Inter subject variability is less.

DISADVANTAGES:



- Need expertise.
- Costly.
- Greater exposure to test drug. (adverse reactions)

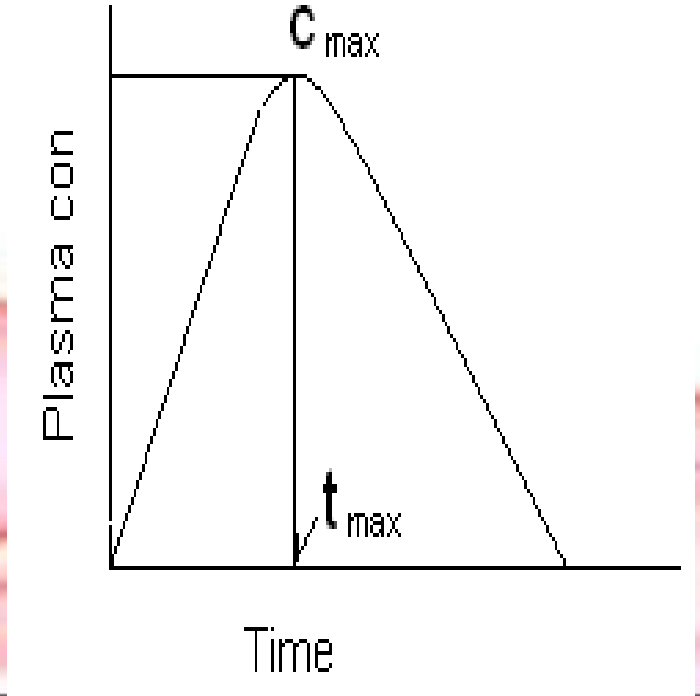
PHARMACOKINETIC STUDIES

Plasma concentration Vs Time profile

Single dose

- *From the graph **AUC, Tmax, and Cmax** were determined.*

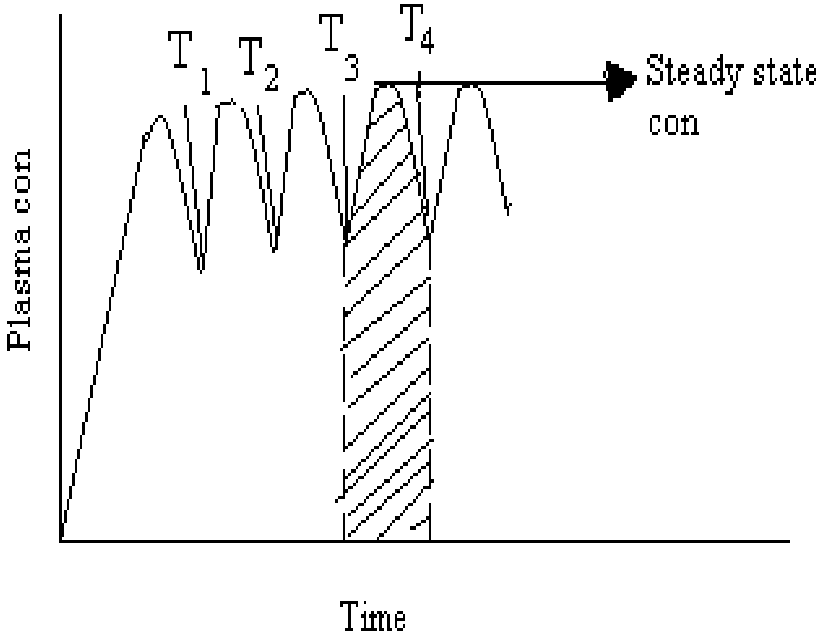
.



Multiple dose

- *Samples are withdrawn at stipulated time intervals after attaining the steady state concentration.*

$$Fr = \frac{[AUC]\ test\ D_{std}\ T_{test}}{[AUC]std\ \ Dtest\ Tstd}$$



- *Bioavailability can be assessed by Cmax at steady state concentration.*

$$\mathbf{Fr = \frac{(Cmax\ ss)test\ Dstd\ Ttest}{(Cmax\ ss)std\ DtestTstandard}}$$

URINARY EXCRETION DATA

- *Some of the drugs are excreted in unchanged form in urine.*
- *As a rule determination of bioavailability of urinary excretion data should be conducted only if at least 20% of administered dose is excreted unchanged in urine.*

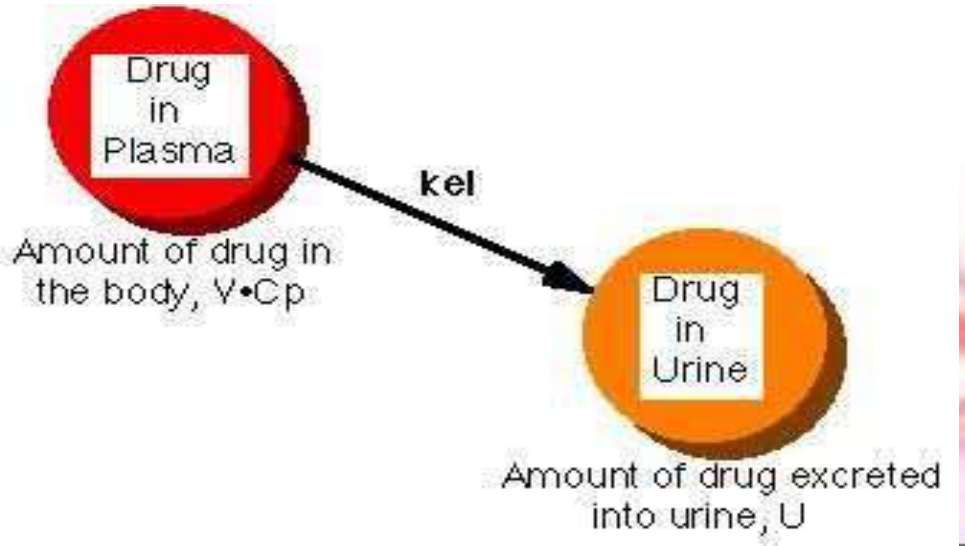
Ex : Thiazide diuretics and sulphonamides



- *The method involves*
- *Collection of urine at regular intervals for a time span equal to 7 biological half lives.*
- *Analysis of unchanged drug in the collected sample.*
- *Determination of the amount of drug excreted in each interval and cumulative amount excreted.*

CRITERIA TO OBTAIN VALID RESULTS

- *At each sample collection total emptying of bladder is necessary to avoid errors resulting from addition of residual amount IN next urine sample.*
- *Frequent sampling of urine is also essential in the beginning in order to compute correctly the rate of absorption.*
- *The fraction excreted unchanged in urine must remain constant.*

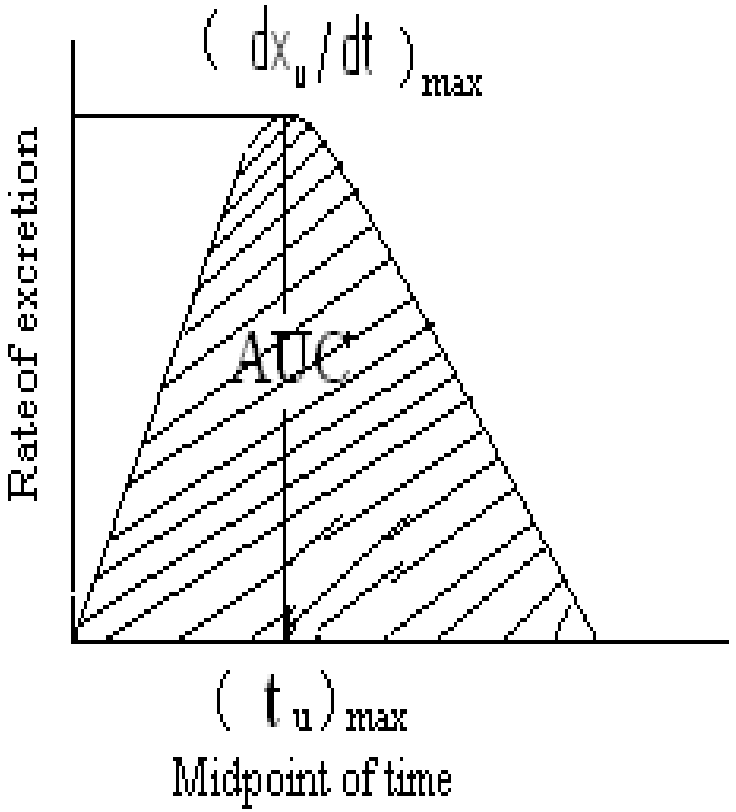


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2. ***The time For maximum excretion rate (tu)max :*** *It is analogous to Tmax of plasma level data.*

3. ***The cumulative amount of drug excreted in the urine Xu~:*** *It is related to AUC of plasma level data.*



- *The extent of bioavailability can be calculated by using*

$$F = \frac{(Xu)\text{oral Div}}{(Xu)\text{iv Doral}} \qquad Fr = \frac{(Xu)\text{test Dstd}}{(Xu)\text{std Dtest}}$$

- *With multiple dose study to steady state the equation for computing bioavailability is :*

$$Fr = \frac{(Xu, ss)\text{test Dstd Ttest}}{(Xu, ss)\text{std Dtest Tstd}}$$

PHARMACODYNAMIC STUDIES



- *When bioavailability measurement by pharmacokinetic method is difficult , inaccurate or non reproducible, an acute pharmacological effect such as a change in ECG readings, pupil diameter etc is related to time course of related drug.*
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- *This method requires measurement of responses for at least 3 biological half lives of the drug in order to obtain a good estimate of AUC.*

Dilation Of Pupil



THERAPEUTIC RESPONSE METHOD

- *This method is based on observing the clinical response to a drug formulation given to patients suffering from disease for which it is intended to be used.*

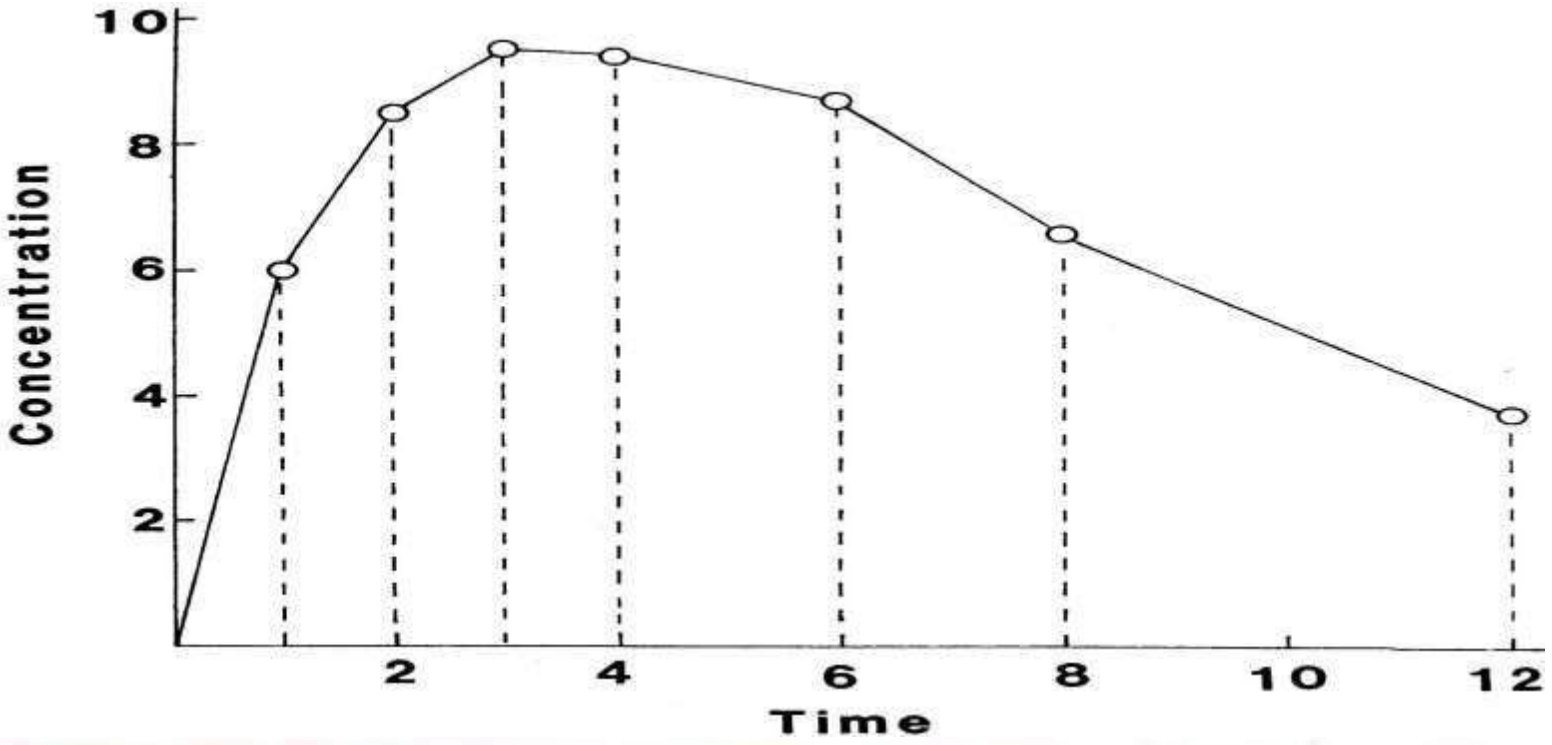






How Far It Is Cured ?????

ESTIMATION OF AREA UNDER THE CURVE



The area of trapezoids can be calculated by :

Area=1/2(C1+C2)(t2-t1)+

1/2(C2+C3)(t3-t2)…+

1/2(Cn-1+Cn)(t n – tn-1)

Example :

The area of 1st and 5th trapezoids can be calculated as:

Area (1)= ½(0+6.6)(1-0)=3.3μgm-hr/ml

Area (5)= ½ (9.4+8.7)(6-4)=18.10 μgm-hr/ml



Drug Concentration as a Function Of Time After Oral Administration

sample	Time	Concentration	Area (μgm-hr/ml)
1	0	0.0	3.30
2	1	6.6	7.55
3	2	8.5	9.00
4	3	9.5	9.45
5	4	9.4	18.10
6	6	8.7	15.30
7	8	6.6	20.60
8	12	3.7	_
		total	83.3

BIOEQUIVALENCE



EQUIVALENCE

It is a relative term that compares drug products with respect to a specific characteristic or function or to a defined set of standards.

CHEMICAL EQUIVALENCE

It indicates that two or more drug products contain the same labeled chemical substance as an active ingredient in the same amount.

PHARMACEUTICAL EQUIVALENCE

This term implies that two or more drug products are identical in strength, quality, purity, content uniformity and disintegration and dissolution characteristics; they may however differ in containing different excipients.

BIOEQUIVALENCE

It is a relative term which denotes that the drug substance in two or more identical dosage forms reaches the systematic circulation at the same relative rate and to the same relative extent i.e., their plasma concentration –time profiles will be identical without significant statistical differences.

THERAPEUTIC EQUIVALENCE

This term indicates that two or more drug products that contain the same therapeutically active ingredient, elicit identical pharmacologic effects and can control the disease to the same extent.

TESTING PROTOCOL FOR BIOAVAILABILITY AND BIOEQUIVALENCE STUDIES

Parallel Design:

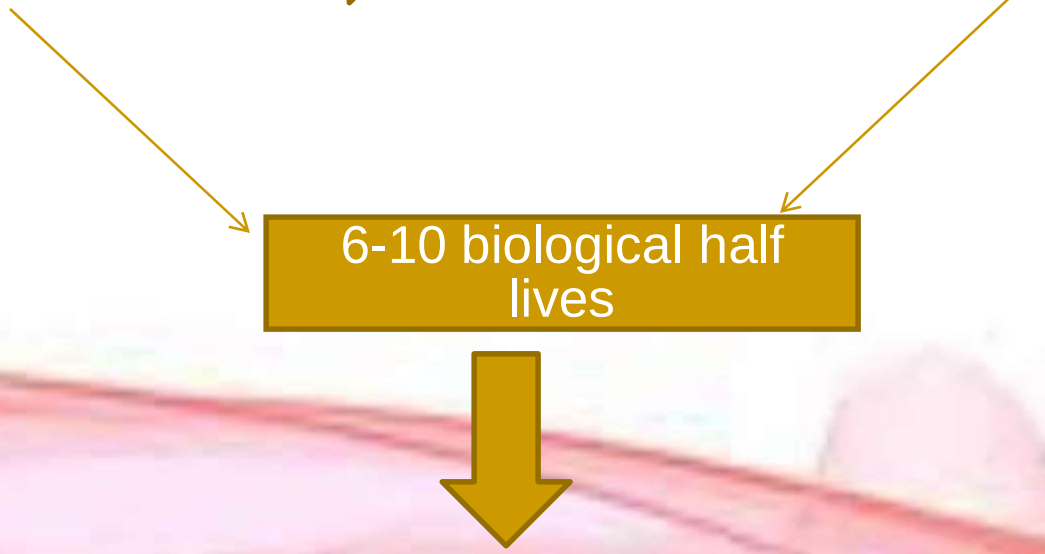
- Two formulations are administered to two groups of volunteers.
- To avoid bias two formulations are administered randomly to the volunteers.

Disadvantages:

- inter-subject variation is not corrected.
- Inter-subject variation is greater than the variation between any formulations

PARALLEL DESIGN

- GROUP A
- **Standard** → **(SAME DOSE)** ← **Test** ← GROUP B



Cross Over Design:

- Each subject receives the test drug product and the reference drug product
- Minimizes the effect of inter-subject variability in the study

3 Types:-

- Latin Square crossover Design
- Balanced Incomplete Block Design (BIBD)
- Replicated crossover design

LATIN SQUARE CROSS OVER DESIGN



- Two square cross over (T+S)
- Three square cross over (S+2T)
- Four square cross square (S+3T)

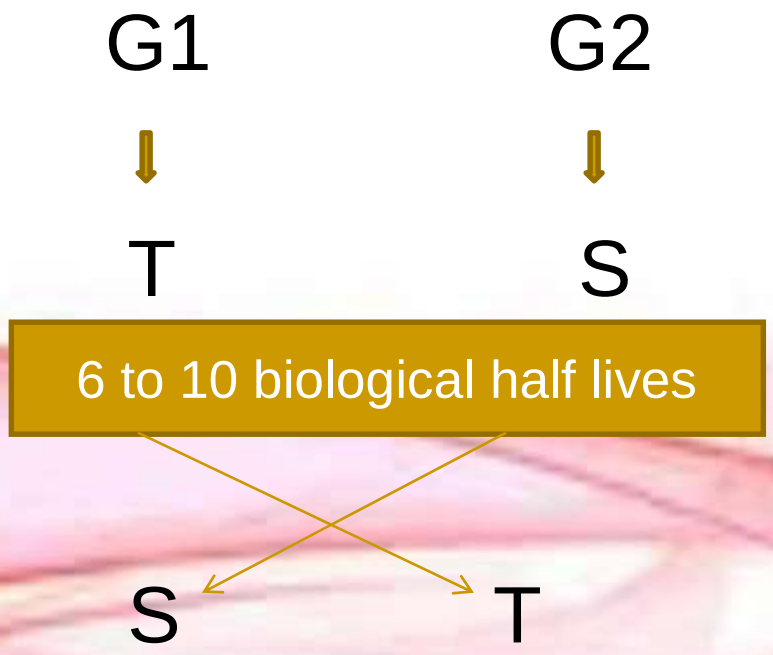
✓ Each subject receives each formulation only once.

✓ Each formulation is administered only once in each study period.

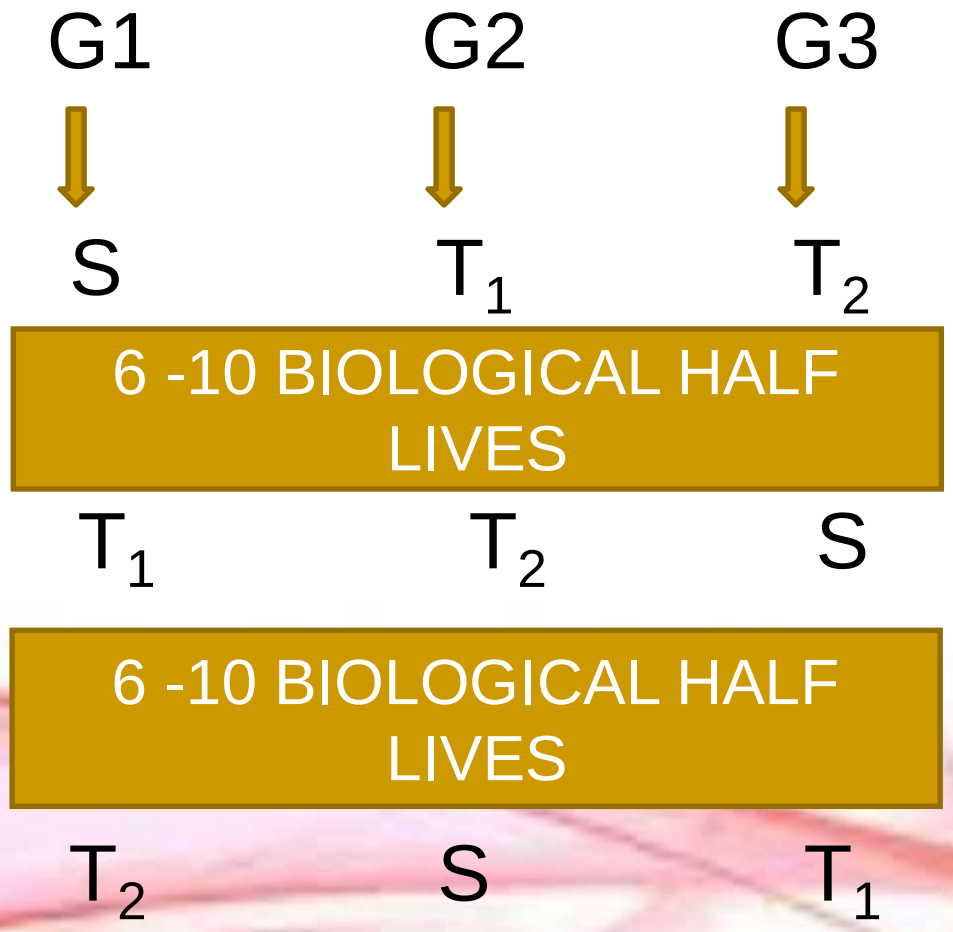
✓ Each subject acts as his own control.

LATIN SQUARE CROSS OVER DESIGN

- Two square cross over (T+S)

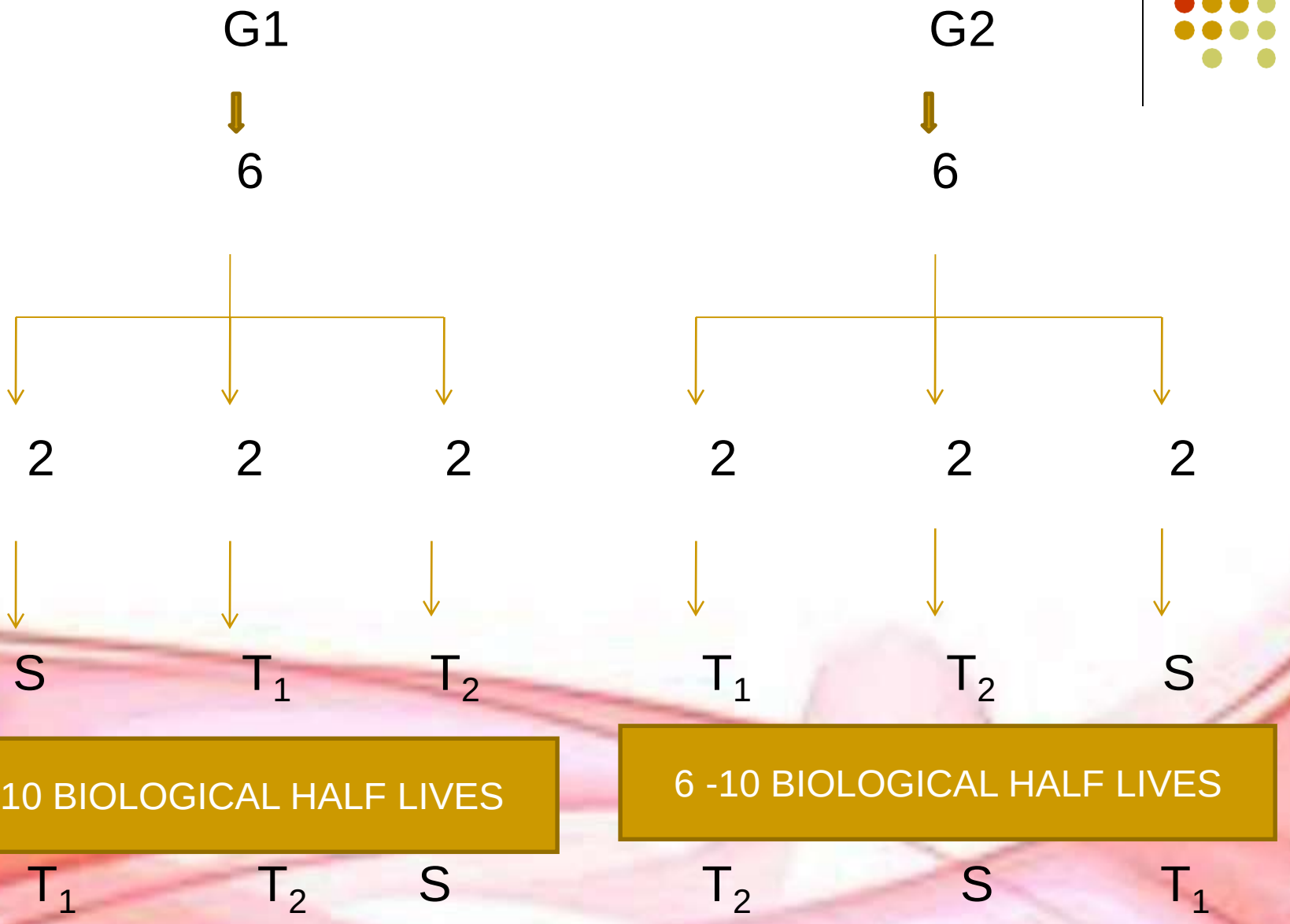


THREE SQUARE CROSS OVER DESIGN



Similarly four square cross over design is performed

Balanced Incomplete block Design



Balanced incomplete Block Design (BIBD) :

- Each subject receives not more than 2 formulations
- Each formulation is administered same number of times
- Each pair of formulations occurs together in the same number of subjects

Replicated crossover design :

- To determine individual bio-equivalence.
- To estimate within-subject variance for both the Test and Reference drug products.
- To estimate subject-by-formulation interaction variance.
- Reference-Reference and Test-Test comparisons may be made.

	Period 1	Period 2	Period 3	Period 4
Sequence 1	T	R	T	R
Sequence 2	R	T	R	T


Thank U

CLINICAL TRAILS

Dr.Y.Kiran Kumar
Associate Professor, VCPS

Clinical Trails

- Clinical trials are studies performed with human subjects to test new drugs or combinations of drugs, new approaches to surgery or radiotherapy or procedures to improve the diagnosis of disease and the quality of life of the patient.
- clinical trials are generally considered to be biomedical or health-related research studies in human beings that follow a pre-defined protocol.

- **Meinert**: “ a planned experiment designed to assess the efficacy of a treatment in man by comparing the outcomes in a group of patients treated with the test treatment with those observed in a comparable group of patients receiving a control treatment, where patients in both groups are enrolled, treated, and followed over the same time period."

Clinical trials help physicians discover the answers to the following questions

- Is the treatment safe and effective
- Is the treatment potentially better than the treatments currently available
- What are the side effects of the treatment?
- Does the treatment have any possible risks?
- How well does the treatment work

- The first clinical trial of a novel therapy was conducted accidentally by the famous surgeon Ambroise Pare in 1537. In 1537 while serving with the Mareschal de Motegni he was responsible for the treatment of the battlefield wounded soldiers. As the number of wounded was high and the supply of conventional treatment – oil was not adequate to treat all the wounded, he had to resort to unconventional treatment. He describes,' at length my oil lacked and I was constrained to apply in its place a digestive made of yolks of eggs, oil of roses and turpentine
- One of the most famous clinical trials was James Lind's demonstration in 1747 that citrus friuts cure scurvy. He compared the effects of various different acidic substances, ranging from vinegar to cider, on groups of afflicted sailors, and found that the group who were given oranges and lemons had largely recovered from scurvy after 6 days.

Historical Events in the Development of Clinical Trials.

Date	Author	Event
1747	Lind	Experiment with untreated control group (Lind, 1753)
1799	Haygarth	Use of sham procedure (A Placebo Surgery)
1800	Waterhouse	U.S. based smallpox trial
1863	Gull	Use of placebo treatment

Classification of clinical trials

The U.S. National Institute of Health(NIH) organizes trials into five different types:

- **Prevention trials**:

  Look for better ways to prevent disease in people who have never had the disease or to prevent a disease from returning. These approaches may include medicines, vitamins, vaccines or minerals.

- **Screening trials**:

  Test the best way to detect certain diseases or health conditions.

- **Diagnostic trials**:

  Conducted to find better tests or procedures for diagnosing a particular disease or condition.

- **Treatment trials**:

  Test experimental treatments, new combinations of drugs, or new approaches to surgery or radiation therapy.

- **Quality of life trials**:

  Explore ways to improve comfort and the quality of life for individuals with a chronic illness.

- **Compassionate use trials** :

  Usually, this involves a disease for which no effective therapy exists, or a patient that has already attempted and failed all other standard treatments and whose health is so poor that he does not qualify for participation in randomized clinical trials.

Primary responsibility for regulation of clinical trials rests with agencies of the U.S. Department of Health and Human Services (HHS).

- Office for Human Research Protections (OHRP)
- Office of Civil Rights (OCR)
- Food and Drug Administration (FDA)
- Office of Research Integrity (ORI)
- National Institutes of Health (NIH)
- Centers for Medicare and Medicaid Services (CMS)

Regulatory Agencies in INDIA

- Ministry of Health and Family Welfare
- Central Drug Standards Control Organisation
- Indian Council of Medical research
- Ministry of chemical and fertilizers

Ethics of Clinical Trials

- **Respect for Persons:** Treatment of person as autonomous

- **Beneficence:** conflict between good of society vs. individual

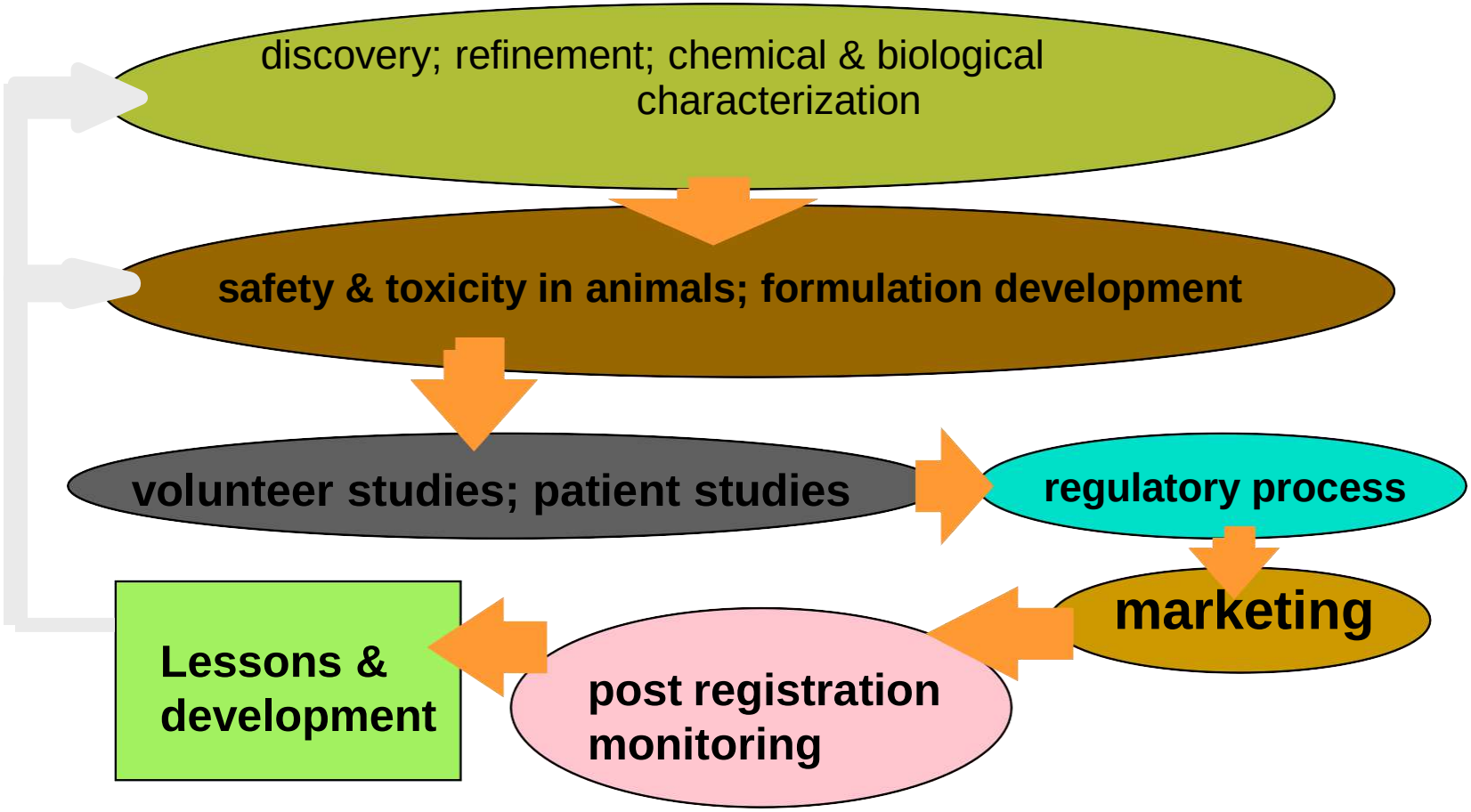
- **Justice:** Treatment of all fairly & all equally share benefits & risks

How are patients benefited and protected?

Rationale of Clinical Trials

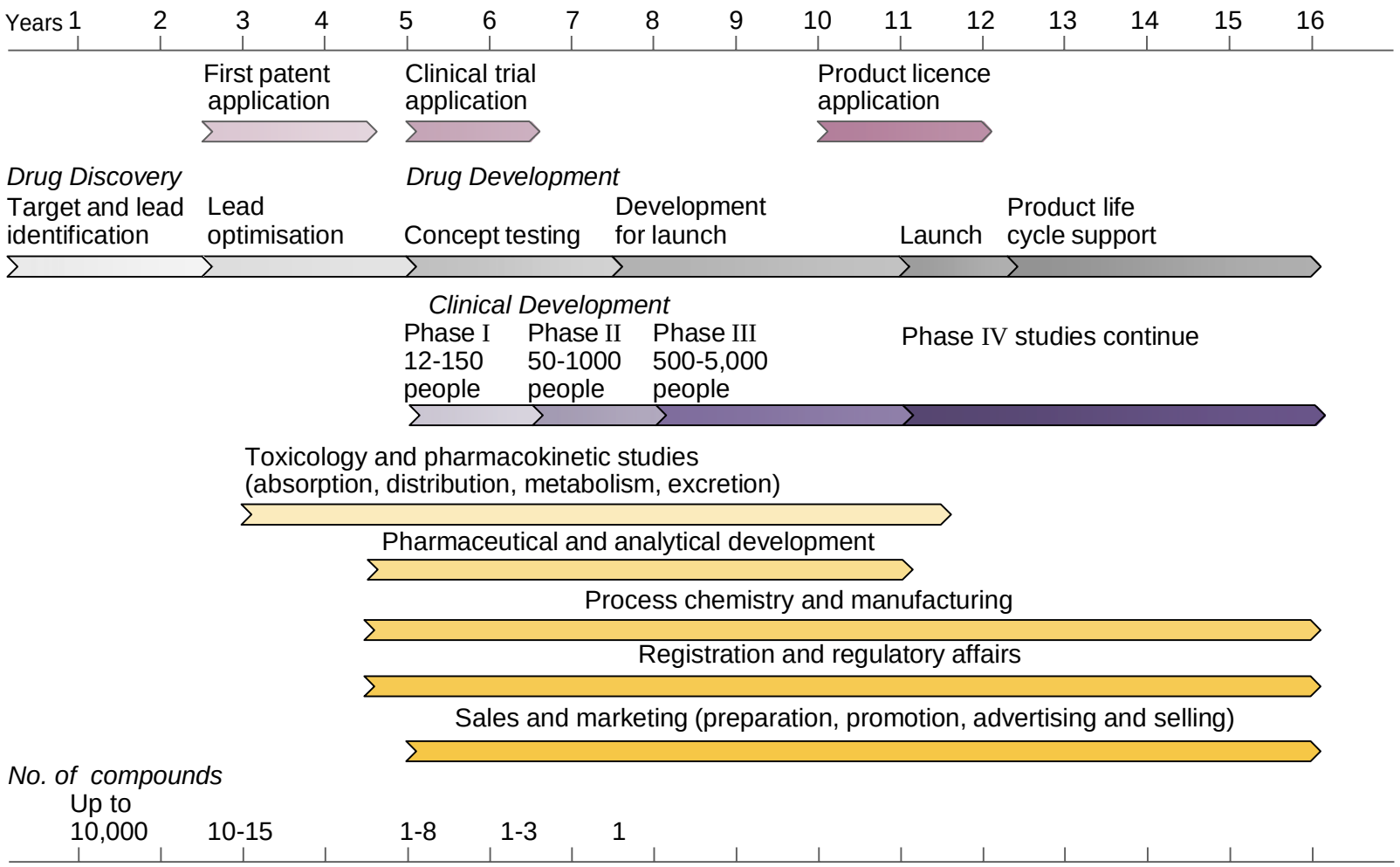
- Clinical trials are the method for determining whether an intervention has a postulated effect.
- Clinical trials develop scientific evidence with acceptable levels of risk and sufficient scientific defensibility.
- Variability in measurement and subjects is inherent but accounted for in such studies.
- Bias, which may exist in observational studies, is avoided through randomization and other techniques.

Drug discovery/development process



Discovery=find new active structure : Development=convert it to a useful drug

The path to a new medicine



PRE-CLINICAL TRIALS

As described earlier, a preclinical trial involves in vitro (test tube or cell culture) and in vivo (animal) experiments using wide-ranging doses of the study drug to obtain preliminary efficacy, toxicity and pharmacokinetic information.

Steps involved in designing a Pre-Clinical Trial/Study:

• **Identifying a Drug Target:** All drugs target specific points in biochemical pathways. Almost all illnesses except infectious diseases are caused by problems associated with specific biochemical pathways. Identifying the appropriate target step in the biochemical pathway is critical and can determine the chances of success of the prospective drug molecule.

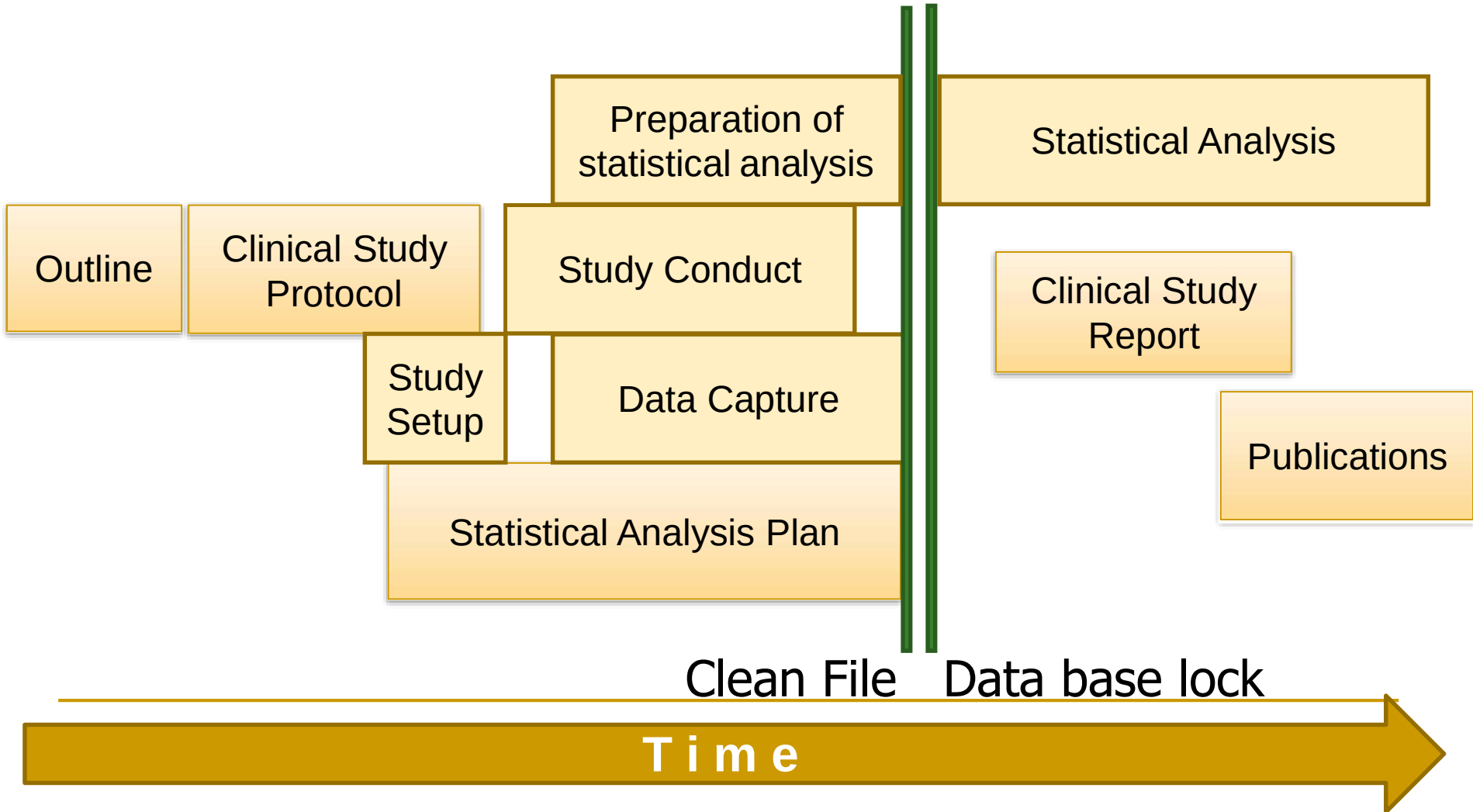
• **Developing a Bioassay:** A bioassay is a "live" system that is devised to measure the effects of a drug. It varies from a cell or tissue culture system to organs or even a whole living being. For example, a zebra fish embryo can be used to observe the effects of drugs on bone density, blood vessel growth, among other systems.

•**Screening the drug in the Bioassay:** This is a screening test done with the bioassay to determine the safety and effectiveness of the molecule. The drug must clear this step.

•**Establishing effective and toxic doses**: This step involves establishing the safe and toxic dose ranges. Future studies take cues from here about the dose ranges to be tested in humans.

•**Filing for an approval as an IND (Investigational New Drug):** After all these steps are cleared the drug is fit for an application to the FDA as an IND.

The Clinical Study Process



Essential elements

- Human subjects
- Intervention
- Prospective in nature with follow-up observations
- Inclusion and exclusion criteria
- Risks and benefits for patients
- Informed consent required
- High cost
- Limited number of subjects
- Cannot completely control a subject's environment

Clinical Trial Protocol

- A **clinical trial protocol** is a document that describes the objective, design, methodology, statistical considerations, and organization of a clinical trial.

1 General Information

Protocol title, protocol identifying number, and date.

Name and address of the sponsor and monitor

Name and title of the person(s) authorized to sign the protocol and the protocol amendment(s) for the sponsor.

Name, title, address, and telephone number(s) of the sponsor's medical expert for the trial.

Name and title of the investigator(s) who is (are) responsible for conducting the trial, and the address and telephone number(s) of the trial site(s).

Name, title, address, and telephone number(s) of the qualified physician, who is responsible for all trial-site related medical decisions (if other than investigator).

Name(s) and address(es) of the clinical laboratory(ies) and other medical and/or technical department(s) and/or institutions involved in the trial.

2. **Background Information**

Name and description of the investigational product(s).

A summary of findings from non clinical studies that potentially have clinical significance and from clinical trials thatare relevant to the trial.

Summary of the known and potential risks and benefits, if any, to human subjects.

Description of and justification for the route of administration, dosage, dosage regimen, and treatment period(s).

A statement that the trial will be conducted in compliance with the protocol, GCP and the applicable regulatory requirement(s).

Description of the population to be studied.

References to literature and data that are relevant to the trial, and that provide background for the trial.

3 **Trial Objectives and Purpose**.

4 **Trial Design**. The scientific integrity of the trial and the credibility of the data from the trial

A description of the trial design, should include:

A description of the type/design of trial to be conducted (e.g. double-blind, placebo-controlled, parallel design) and a schematic diagram of trial design, procedures and stages.

A description of the measures taken to minimize/avoid bias.

Accountability procedures for the investigational product(s), including the placebo(s) and comparator(s), if any.

5 **Selection and Withdrawal of Subjects**

Subject inclusion criteria.

Subject exclusion criteria

Subject withdrawal criteria

6 **Treatment of Subjects**

The treatment(s) to be administered, including the name(s) of all the product(s), the dose(s), the dosing schedule(s),
Medication(s)/treatment(s) permitted (including rescue medication) and not permitted before and/or during the trial.

Assessment of Efficacy

Assessment of Safety

Statistics

Procedure for accounting for missing, unused, and spurious data.

7 Data Handling and Record Keeping

Study Participant Recruitment

Identify eligible participants

Explain study

Provide informed consent

Reassess eligibility

Assign to one group

Participants should be told

May have side effects (adverse effects)

Time commitment

Benefits & risks

May withdraw at any time

Enrollment 100% voluntary

Institutional Review board

- Participants' rights are protected by Institutional Review Boards [IRBs]
- An IRB is defined as any board, committee or other group formally designated by an institution to review, to approve the initiation of, and to conduct periodic review of biomedical research involving human subjects"

Informed Consent

Objectives of Informed Consent

To Ensure

- Voluntaries
- Comprehension
- Information

To Demonstrate That

- Person freely gave consent to participate
- Consent given by a competent person
- Person has been given all information
- Person knows this is research – not treatment

The Possible World of Clinical Trial Designs

- Randomized/blinded trial
- Randomized/double blinded trial
- Non-randomized concurrent controlled trial
- Placebo trial
- Parallel group design
- Crossover design
- Withdrawal trial

- **Randomized:** Schemes used to assign participant to one group. o Ex: Every 3 gets higher dose

- **Blinded:** Participants do not know if in experimental or control group

- **Double Blinded:** Participants and staff do not know group assignment

- **Placebo:** Inactive pill w/ no therapeutic value

Parallel group design

- The most common trial design in which subjects are randomized to one or two or more arms, each arm being allocated a different treatment.
- These treatments include the investigations at one or more doses and one or more control treatments such as placebo and or active comparator.

Cross over design

- Each subject is randomized to a sequence of two or more treatments and hence acts as his own control for treatment comparisons.
- In the simplest 2 X 2 cross over design each subject receives each of two treatments in randomized manner in two successive treatment periods.

PHASES OF CLINICAL TRAILS

Phase 0

- It is a recent designation for exploratory, first-in-human trials conducted in accordance with the U.S. Food and Drug Administration's (FDA) 2006 Guidance on Exploratory Investigational New Drug (IND) Studies.
- Phase 0 trials are also known as human micro dosing studies and are designed to speed up the development of promising drugs or imaging agents by establishing very early on whether the drug or agent behaves in human subjects as was expected from preclinical studies and pharmacodynamics (how the drug works in the body).

- Distinctive features of Phase 0 trials include the administration of single sub therapeutic doses of the study drug to a small number of subjects (10 to 15) to gather preliminary data on the agent's pharmacokinetics (how the body processes the drug)

- A Phase 0 study gives no data on safety or efficacy, being by definition a dose too low to cause any therapeutic effect.

- **Phase I** - The first studies in humans are Phase I trials. They are performed with small numbers of patients or healthy volunteers and are used to answer questions such as what dose of the drug is likely to be effective and what side effects might occur.

- **Phase II** - The trials with larger numbers of patients and focus on how well the treatment or procedure works, perhaps in particular situations or groups of patients.

Phase III

- The trials enroll large numbers of patients and are used to compare the effectiveness and safety of the new treatment with that of the standard existing treatment.

- Information obtained from Phase III trials that demonstrates the benefits a new drug over the existing treatments are presented to regulatory authorities in order to obtain a licensee to market and sell the drug.

Phase IV trial

- Also known as **Post Marketing Surveillance Trial**.
- Phase IV trials involve the safety surveillance (pharmacovigilance) and ongoing technical support of a drug after it receives permission to be sold.
- Phase IV studies may be required by regulatory authorities or may be undertaken by the sponsoring company for competitive (finding a new market for the drug) or other reasons

- The safety surveillance is designed to detect any rare or long term adverse effects over a much larger patient population and longer time period than was possible during the Phase I-III clinical trials.
- Recent examples involve cerivastatin (brand names Baycol and Lipobay), troglitazone (Rezulin) and rofecoxib (Vioxx).

Documents for the conduct of clinical trial

- The list of documents to be maintained and submitted in the clinical research study are grouped in to three sections according to the stage of the clinical trial.

1. Before the clinical phase of the trial commences.
2. During the conduct of the trial.
3. After completion of the trial.

Before the clinical phase of the trial commences

- Investigators brochure.
- Signed protocol and amendments and sample case report form.
- Information given to the trial subject.
  1. Informed consent form
  2. Advertisement for subject recruitment.
  3. Any other information.
- Financial aspects of the trial.
- Insurance statement.
- Signed agreement between involved parties.
- IRB/IEC composition.
- Regulatory authority approval
- Meical/ laboratory procedures certification.
- Sample of label attached to investigators drug containers.
- Trial initiation monitoring report.

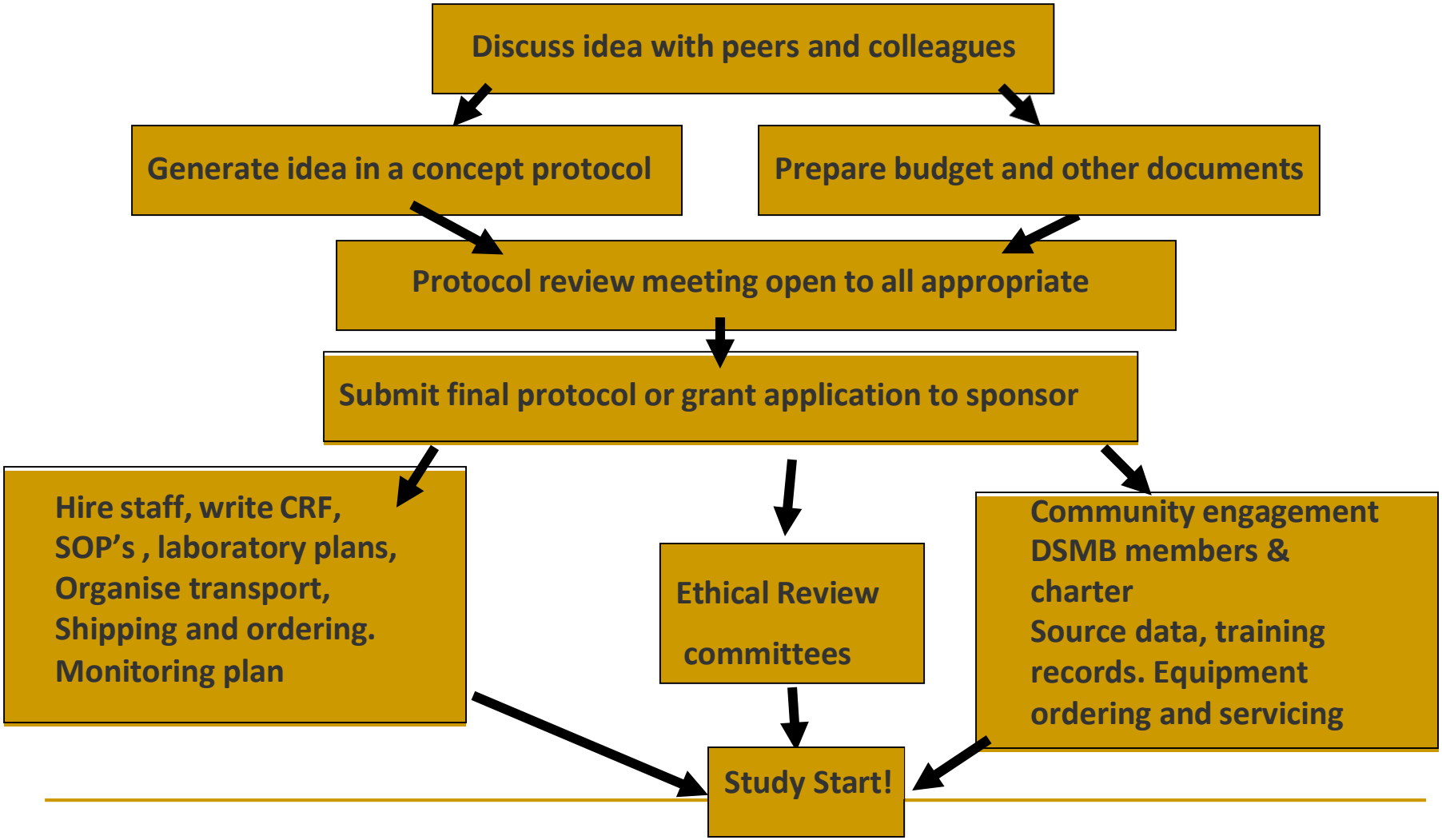
During the conduct of the trial

- Investigator brochure updates
- Any revision of protocol, informed consent form.
- Regulatory authority approvals.
- Cirriculam vitae for new investigators or sub investigators.
- Updates of Meical/ laboratory procedures certification.
- Monitoring visit reports.
- Signed informed consent forms.
- Notification of investigator or sponsor of serious adverse events & reports.
- Notification by sponsor or investigator to authority & IRB /IEC of unexpected serious ADR.
- Subject screning and enrollment log.

After completion of the trial

- Investigational product accountability at site.
- Documentation of investigational product destruction.
- Completed subject identification list.
- Audit certificate.
- Final trial close out monitoring report.
- Final report by investigator to IRB/ IEC where required and applicable to authority.

How a trial is started...?



TARGATED DRUD DELIVERY SYSTEM

G.Natesh
VCPS

DEFINITION

Colon: The long, coiled, tube like organ that removes water from digested food. The remaining material, solid waste called stool, moves through the colon to the rectum and leaves the body through the anus. Also known as large bowel and large intestine.

Colon drug delivery system: It refers to targeted delivery of drug in to the lower parts of GI tract , mainly large intestine.

Objectives

- Oral route is considered to be most convenient for administration of drug to patient.
- Colon is used as site of Targeted drug delivery.
- Colon was considered as a BLACK-BOX , as most of the drug are absorbed from the upper part of the GI tract.
- Prime objective-Beneficial in the treatment of colon diseases.
  - Increase the pharmacological activity.
  - Reduce dosing & side effects.
  - Prevent drug from degradation.

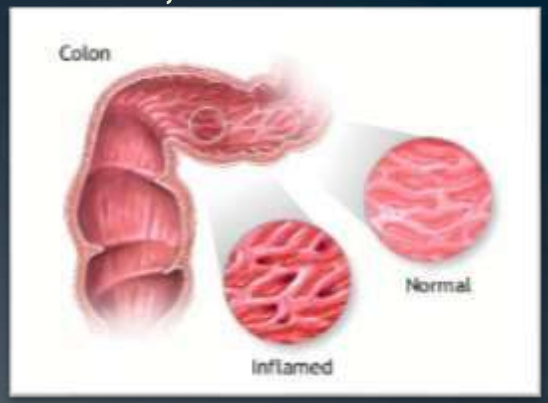
- As most of the conventional drug delivery systems for treating colon disorders such as inflammatory bowel diseases, infectious diseases and colon cancer are failing as the drugs don't reach the site of action in appropriate concentration.
- In recent times the colon-specific delivery systems(CSDDS) are also gaining importance for the systemic delivery of protein and peptide drugs . This is because,

i)As the peptide and protein drugs are destroyed and inactivated in acidic environment of stomach or by pancreatic enzymes (or) by parenteral route which is inconvenient and expensive.

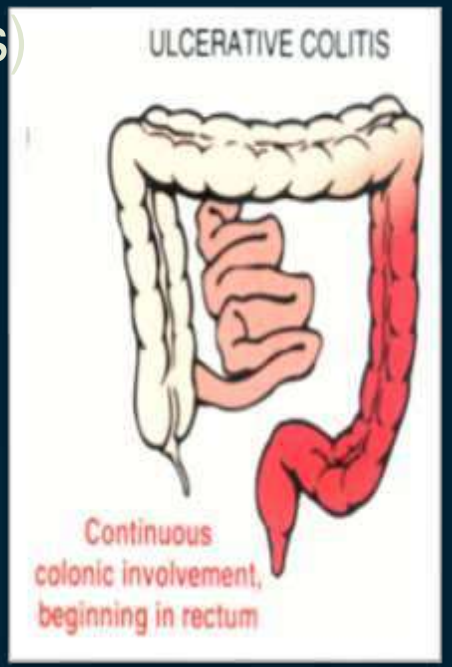
ii) Due to the negligible activity of brush border membrane peptidase activity and less activity of pancreatic enzymes the colon is considered as the most suitable site.

ADVANTAGES

- The site specific delivery of drug to lower part of GIT, for localized treatment of several colonic diseases. (ulcerative colitis, Crohn's disease, carcinomas and infections)







- Prevent drug from degradation
- Ensure direct treatment at disease site.
- Suitable absorption site for Protein & Peptide drug.
- Used to prolong the drug therapy.
- Improved drug utilization.

LIMITATIONS

- Bioavailability of drug may be low due to potentially binding of drug in a nonspecific way to dietary residues, intestinal secretions, mucus or fecal matter.
- Non availability of an appropriate dissolution testing method to evaluate the dosage form in-vitro.
- Drug should be in solution form before absorption and there for rate limiting step for poor soluble drugs.
- Substantial variation in gastric retention time may affect drug delivery.
- Diseased condition may affect the colonic transit time and drug release profile.
- pH level of colon may vary between individuals due to disease, state and temperature of food consumed.

TARGET SITES	DISEASE CONDITIONS	DRUG AND ACTIVE AGENTS
Topical action	Inflammatory Bowel Diseases, Irritable bowel disease and Crohn's disease. Chronic pancreatitis	Hydrocortisone, Budenoside, Prednisolone, Sulfaselazine, Olsalazine, Mesalazine, Balsalazide
Local action	Pancreatactomy and cystic fibrosis, Colorectal cancer	Digestive enzyme supplements 5-Flourouracil
Systemic action	To prevent gastric irritation To prevent first pass metabolism of orally ingested drugs Oral delivery of peptides Oral delivery of vaccines	NSAIDS Steroids Insulin

CRITERIA OF DRUG SELECTION

- Drugs used for local effects in colon against GIT diseases.
- Drugs poorly absorbed from upper GIT.
- Drugs for colon cancer.
- Drugs that degrade in stomach and small intestine.
- Drugs that undergo extensive first pass metabolism.
- Drugs for targeting.

Factors affecting Colon drug delivery

- Physicochemical parameters
- Intestinal transit time
- Colonic Microflora
- Role of absorption enhancers

PRIMARY APPROACHES FOR CDDS

- pH sensitive systems
- Microbially triggered system
  - Prodrugs
  - Polysaccharide based systems
- Delayed release systems

NEWLY DEVELOPED APPROACHES FOR CDDDS

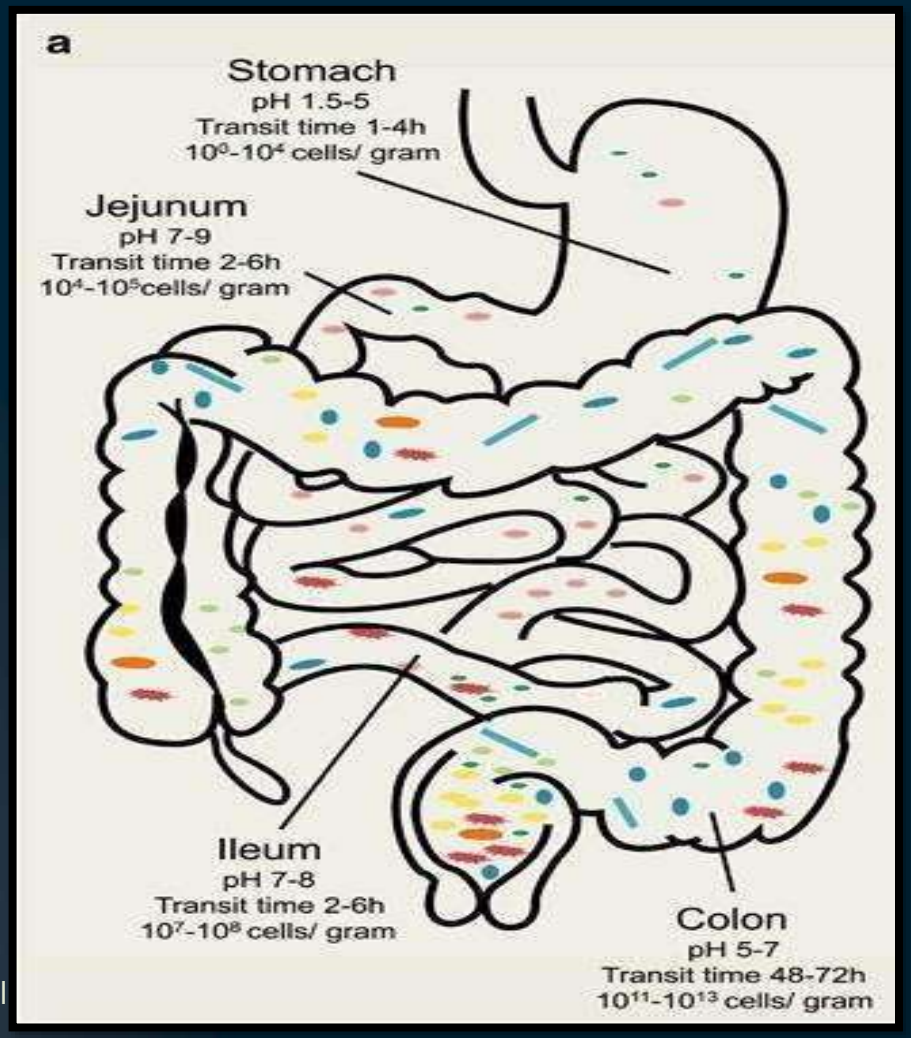
- Pressure controlled drug delivery systems
- Novel colon targeted delivery systems
- Osmotic controlled drug delivery systems

1. PRIMARY APPROACHES

a) pH

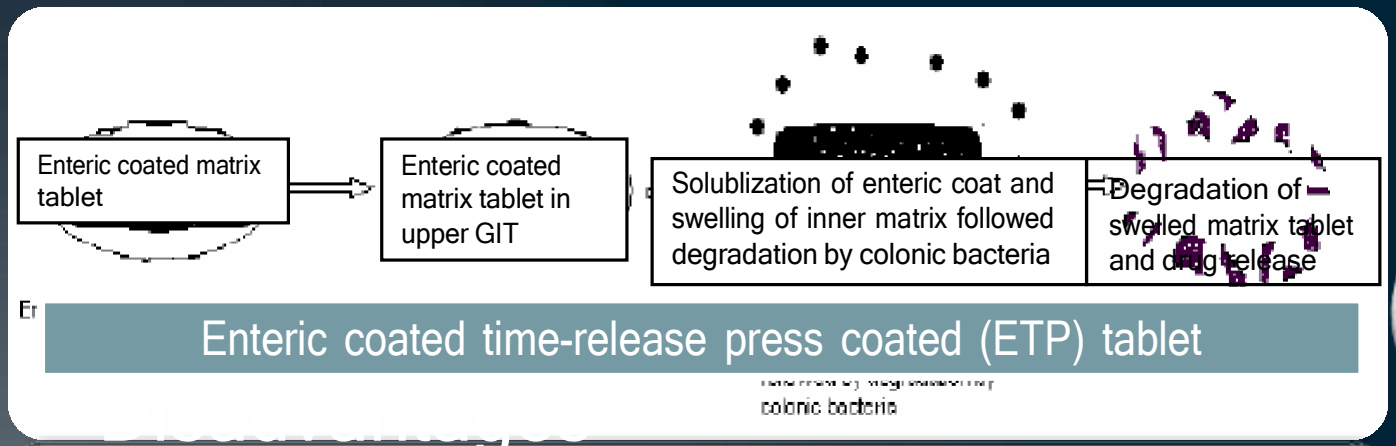
to colon

LOCATION	pH
1. STOMACH:	1.5 - 2.0
Fasted	3.0 - 5.0
Fed	5.0 - 6.5
2. SMALL INTESTINE:	6.0 - 7.5
Jejunum	6.4
Ileum	6.7 - 7.3
3. LARGE INTESTINE:	6.5 - 7.0
Right colon	5.7
Mid colon	6.6
Left colon	7.0



CHI

b) Delayed release drug delivery to colon





- Gastrointestinal Transit
- Gastric emptying of various dosage form is highly inconsistent & depends primary on whether the subject is fed or fasting & properties of dosage form.
- Accelerated transit was observed in IBD and ulcerative colitis patients

- The microflora of the colon is in the range of 1011 -1012 CFU/ mL, consisting mainly of anaerobic bacteria, e.g. bacteroides, bifidobacteria, eubacteria, clostridia, enterococci, enterobacteria and ruminococcus etc.
- Microflora produces a vast number of enzymes like glucoronidase, xylosidase, arabinosidase, galactosidase, nitroreductase, azareducatase, deaminase, and urea dehydroxylase.
- Presence of the biodegradable enzymes only in the colon, the use of biodegradable polymers for colon-specific drug delivery.
- These polymers shield the drug from the environments of stomach and small intestine, and are able to deliver the drug to the colon.

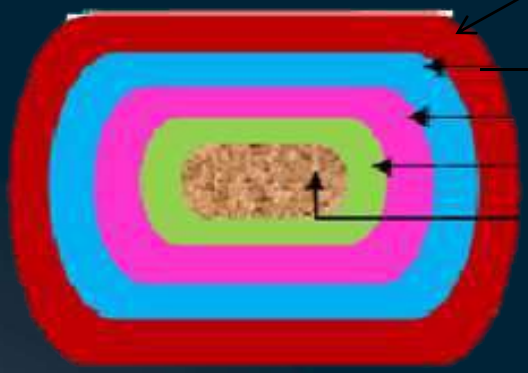
1. Prodrug Approach – Not a versatile approach.
2. Azo-polymeric prodrugs
3. Polysaccharide based delivery - GRAS

2. NEW DRUG APPROACHES

a) Pressure controlled delivery

- As a result of peristalsis, higher pressures are encountered in colon than in small intestine.
- Takaya et al. developed pressure controlled drug delivery capsules are prepared using ethyl cellulose, which is water insoluble.
- Thickness of polymer membrane and capsule size and its density are important factors for the disintegration of formulation.
- Due to reabsorption of water from colon, viscosity of luminal content seems to be higher in colon than in small intestine.
- Lag time of 3 to 5 hrs. in relation to drug absorption.

b) Novel colon targeted delivery



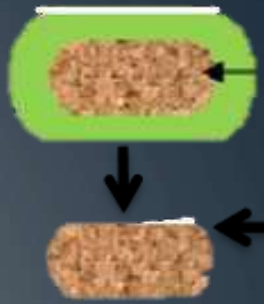
Enteric coating
Barrier layer coating
Acid soluble coating
Barrier layer coating
Citric acid consisting Minitab

In Stomach



Acid soluble coating
Barrier layer coating
Citric acid consisting Minitab

In Small intestine



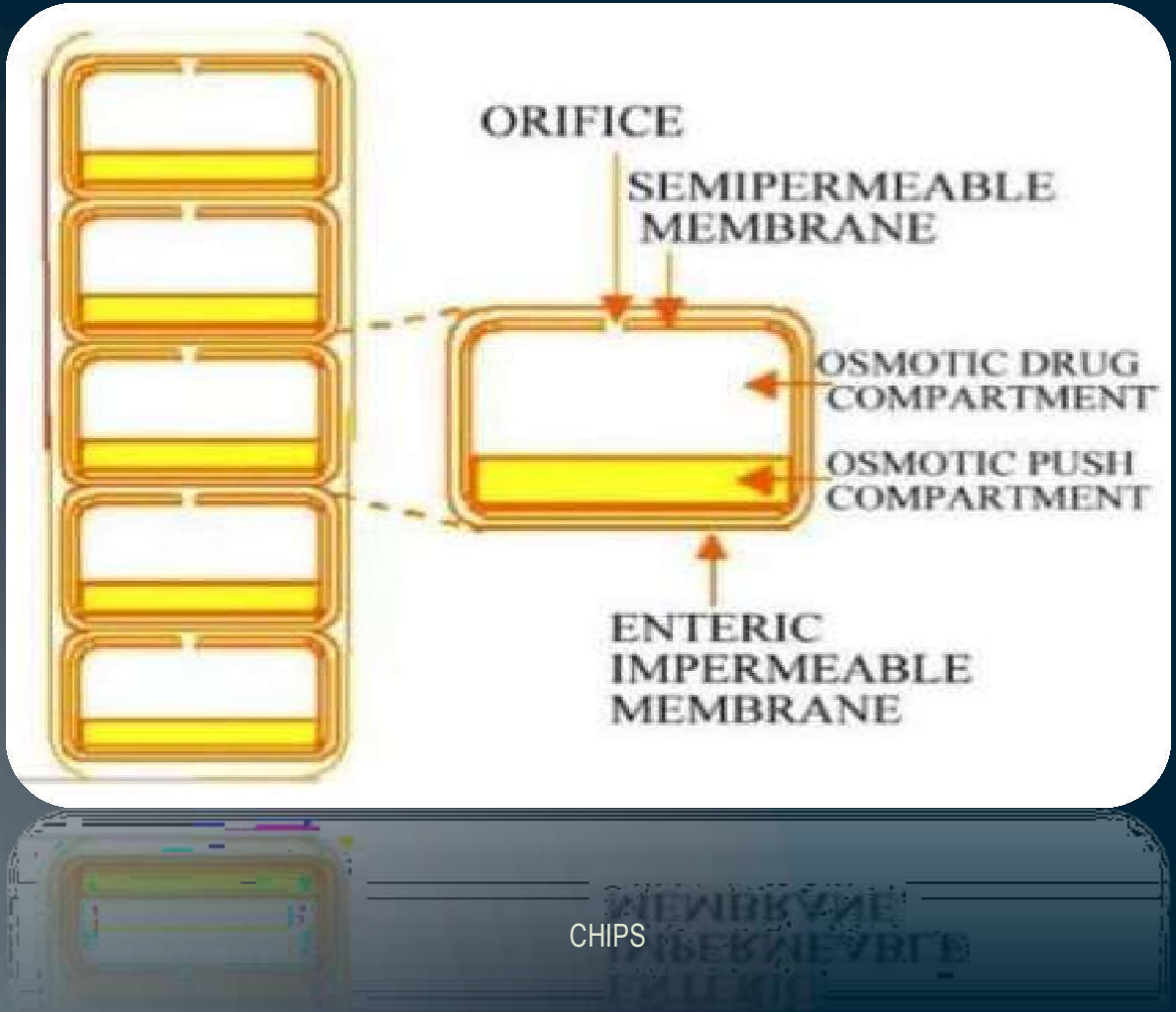
Citric acid

Dissolve acid soluble coat by Microflora

Drug release from Minitab

In Colon

c) Osmotic controlled drug delivery



APPLICATIONS

- **LOCAL ACTIONS**

1. Ulcerative colitis.
2. CROHN'S disease.
3. Irritable bowel syndrome.
4. Metastatic human colon cancer.

- **SYSTEMIC ACTIONS**

1. Molecules degraded/poorly absorbed from upper GIT such as peptides and proteins are better absorbed from colon.
2. For achieving chemotherapy for diseases that are sensitive to circadian rhythm such as Asthma, angina, arthritis.

MARKETED PRODUCTS

Sr. no.	Marketed name	Company name	Disease	Drug content
1)	Mesacol tablet	Sun pharma, India	Ulcerative colitis	Mesalamine
2)	SAZO	Wallace , India	Ulcerative colitis, crohn's disease	Sulphasalazine
3)	BUSCOPAN	German remedies	Colonic motility	Hyoscine butyl bromide
4)	Entofoam	Cipla, India	Ulcerative colitis	Hydrocortisone acetate

EVALUATION TESTS

- For evaluation, not any standardized evaluation technique is available for evaluation of CDDS because an ideal in vitro model should posses the in-vivo conditions of GIT such as pH, volume, stirring, bacteria, enzymes, enzyme activity, and other components of food.
- These conditions are influenced by the diet, physical stress, and these factors make it difficult to design a standard in-vitro model.

1. In vitro dissolution study - Conventional basket method, BIO-DIS III
2. In vitro enzymatic degradation test
3. In vivo evaluation
4. Drug delivery index (DDI)
5. Clinical evaluation



BIO-DIS III

2. In vitro Enzymatic tests

Method 1:

- Drug release in buffer medium containing enzymes (ex. pectinase, dextranase) or rat or guinea pig or rabbit decal contents

↓

- Amount of drug release in particular time directly proportional to the rate of degradation of polymer carrier.

Method 2:

- Incubating carrier drug system in fermenter

↓

- Suitable medium containing colonic bacteria (streptococcus faecium or B.ovatus)

↓

- Amount of drug released at different time intervals determined.

3. In vivo Evaluation

- Rats, mice, pigs and dogs animal models were reported for colon targeted drug delivery systems.
- For simulating the human physiological environment of the colon, appropriate animal model selection is depends on its approach and design of system.
- For example, guinea pigs have glycosidase and glucuronidase activities in the colon and digestive anatomy and physiology is similar to that of human, so they are appropriate in evaluating prodrugs containing glucoside and glucuronate conjugated for colonic delivery.

4. Drug Delivery Index (DDI)

$$DDI = \frac{Relative\ colonic\ exposure\ to\ the\ drug(RCE)}{Relative\ systemic\ exposure\ to\ the\ drug(RSC)}$$

- Absorption of drug from colon is monitored by
  - String technique
  - Endoscopy
  - Radiotelemetry
  - Roentegenography
  - Gamma scintigraphy

CONCLUSION

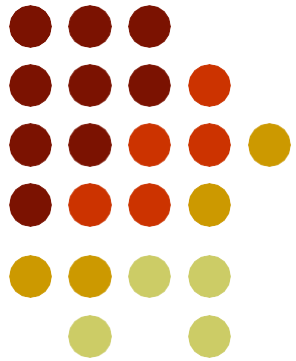
- The colonic region of the GIT has become an important site for drug delivery that considerably offers therapeutic benefits to patients in terms of both local and systemic.
- Colon specificity is more likely to be achieved with natural materials that are degraded by colonic bacterial enzymes.
- Uncertainty of current dissolution methods in establishing possible in-vitro/in-vivo correlation, and remains as a challenge for the pharmaceutical scientists to develop and validate a dissolution method that incorporates the physiological features of the colon, and yet can be used routinely in an industry setting for the evaluation of CDDS.

THANK YOU
PHARMACIST

CRYSTALLIZATION

G.NATESH

M. Pharm,
VCPS



DEFINITION

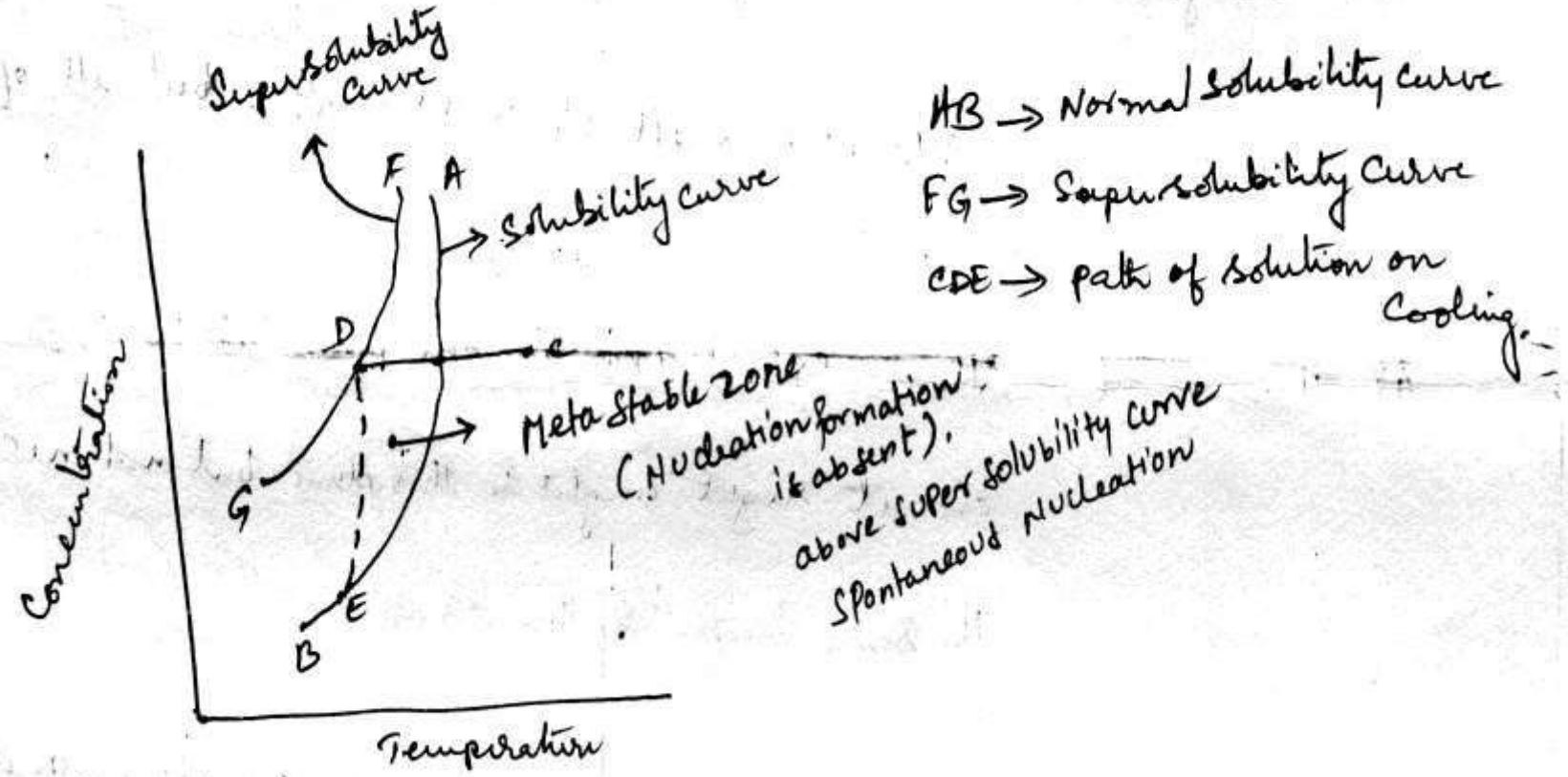
- Crystallization is the formation of solid particles within a homogeneous phase.

Various forms of Crystals

- Cubic
- Tetragonal
- Orthorhombic
- Hexagonal
- Monoclinic
- Triclinic

Theory of Crystallization

Miers Supersaturation theory:



HB → Normal solubility curve

FG → Supersolubility Curve

CDE → path of solution on Cooling.

above super solubility curve Spontaneous Nucleation

Limitations of Miers theory:

1) It is known that sufficiently great lengths of time nuclei can form even well below the supersolubility curve. The formation of such nuclei depends on such accidental collisions of molecules of solute into aggregates large enough to persist. It would seem that the larger the volume of the solution the more chance of such cases. (This phenomenon makes it doubtful that any exact line such as FG. can be drawn)

2) Miers theory hold good only for the pure saturated solution. But it has been found that any foreign body can act as the nucleus. It is unavoidable that in commercial practice, when solutions are exposed to air, millions of dust particles may fall into the solution. Any foreign body may fall into the solution may act as nucleus so in actual practice the curve FG is not possible.

Significance of Miers theory:

1) It points out that greater the degree of super saturation there is more chance of nuclei formation.

2) If the supersaturation passes a certain range of values, nucleus formation is apt to be extremely rapid.

Nucleation

- Nucleation is process of giving birth to a very small solid body in a super saturated homogenous medium.

Rate of Nucleation

- Is the formation of number of new solid particles per unit time per unit volume of mother liquor.

Types of Nucleation

- Homogenous
- Heterogenous
- Spurious
  a) Needle Breeding
  b) Vieled Growth

Stages of Crystal Growth

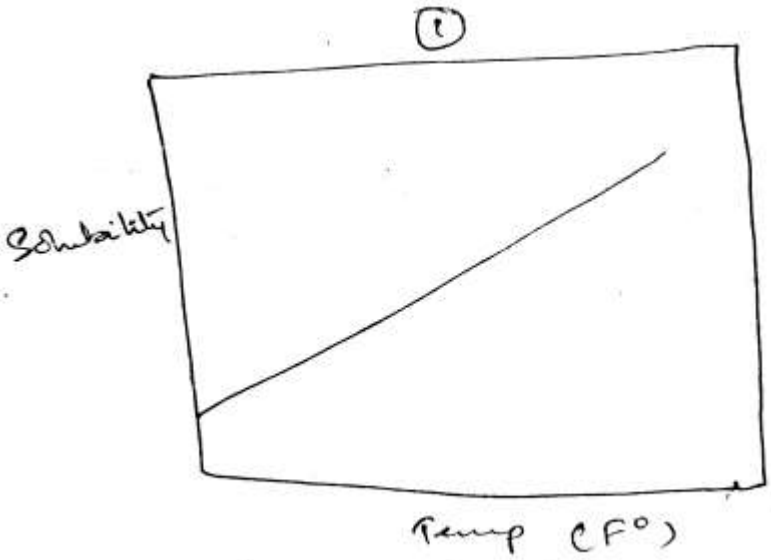
- Cluster $\longleftrightarrow$ Embryo $\longleftrightarrow$ Nucleus $\longrightarrow$ Crystal

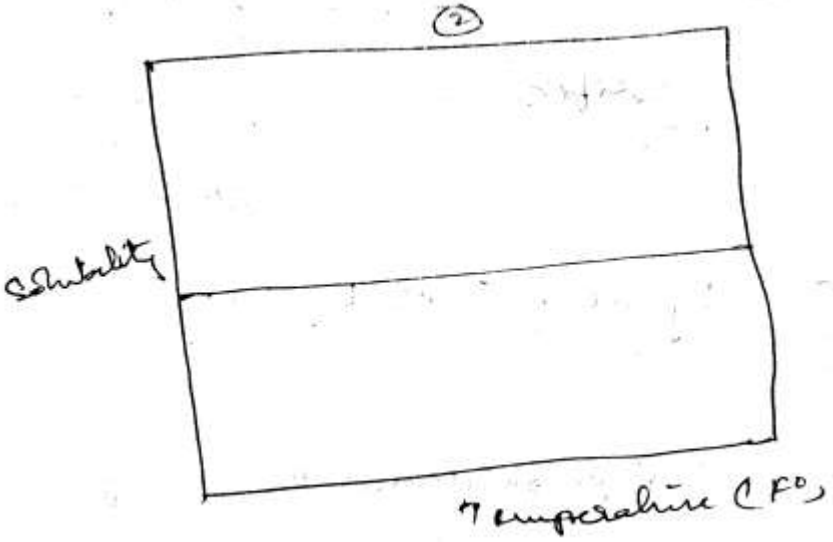
Rate of Crystal Growth

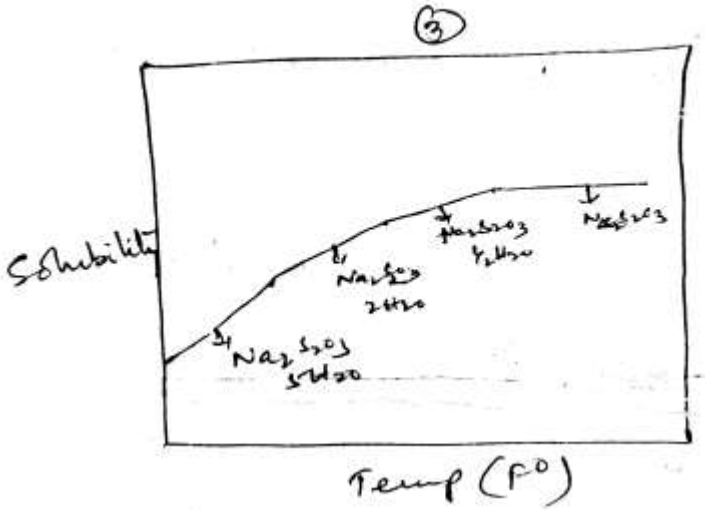
Factors :

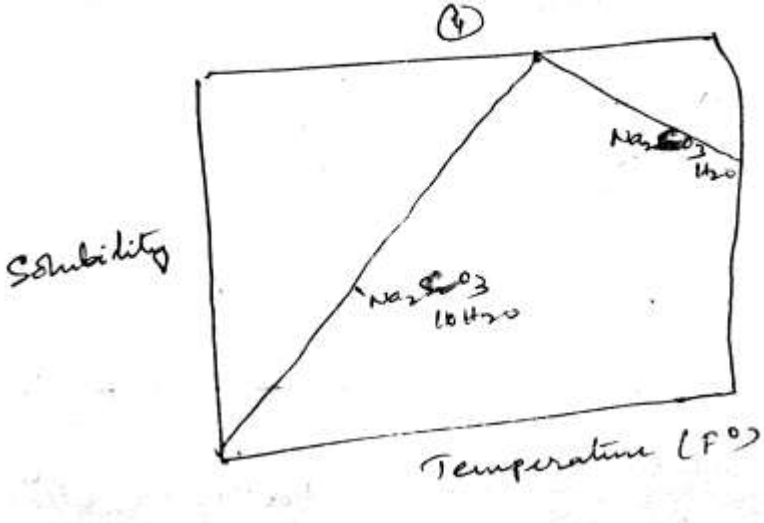
a) Rate of Diffusion
b) Effect of Viscosity

Solubility Curves









Classification of Crystallizers

1) Supersaturation by Cooling
   - A) Batch process
     - I) Tank crystallization
     - II) Agitated batch crystallizer
   - B) Continuous process
     - I) Swenson walker crystallizer
     - II) others
2) Supersaturation by adiabatic cooling
   - A) Vacuum crystallizers
     - I) without external classifying seed bed
     - II) with external classifying seed bed
3) Supersaturation by Evaporation
   - A) Salting Evaporators
   - B) Krystal Evaporators.

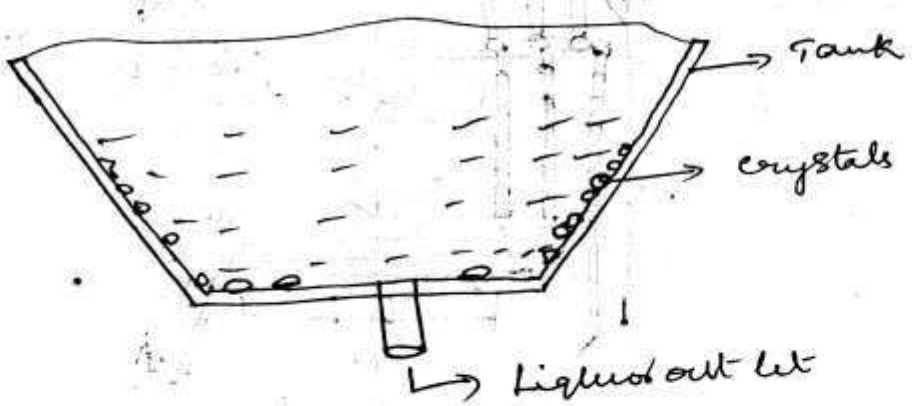
Disadvantages:

1) crystal formation is slow

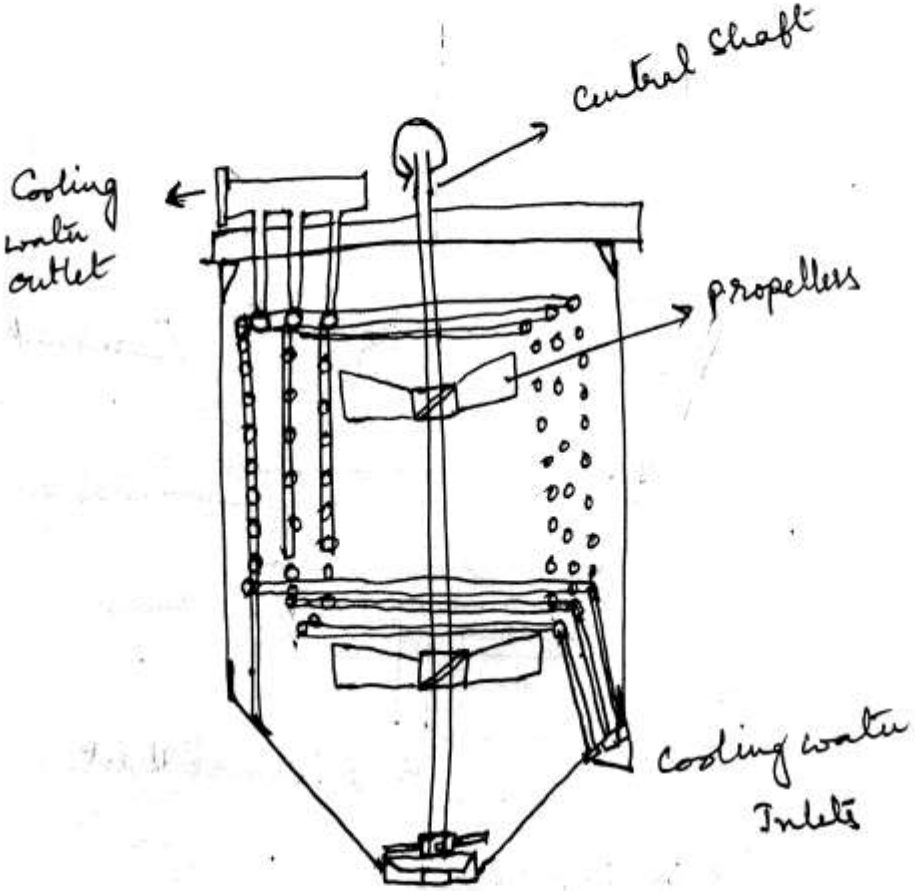
2) crystals formed are large and interlocked which results in occulsion of mother liquor, thus introducing impurities.

3) Requires much labour

4) Requires more floor space.



Tank crystallizer



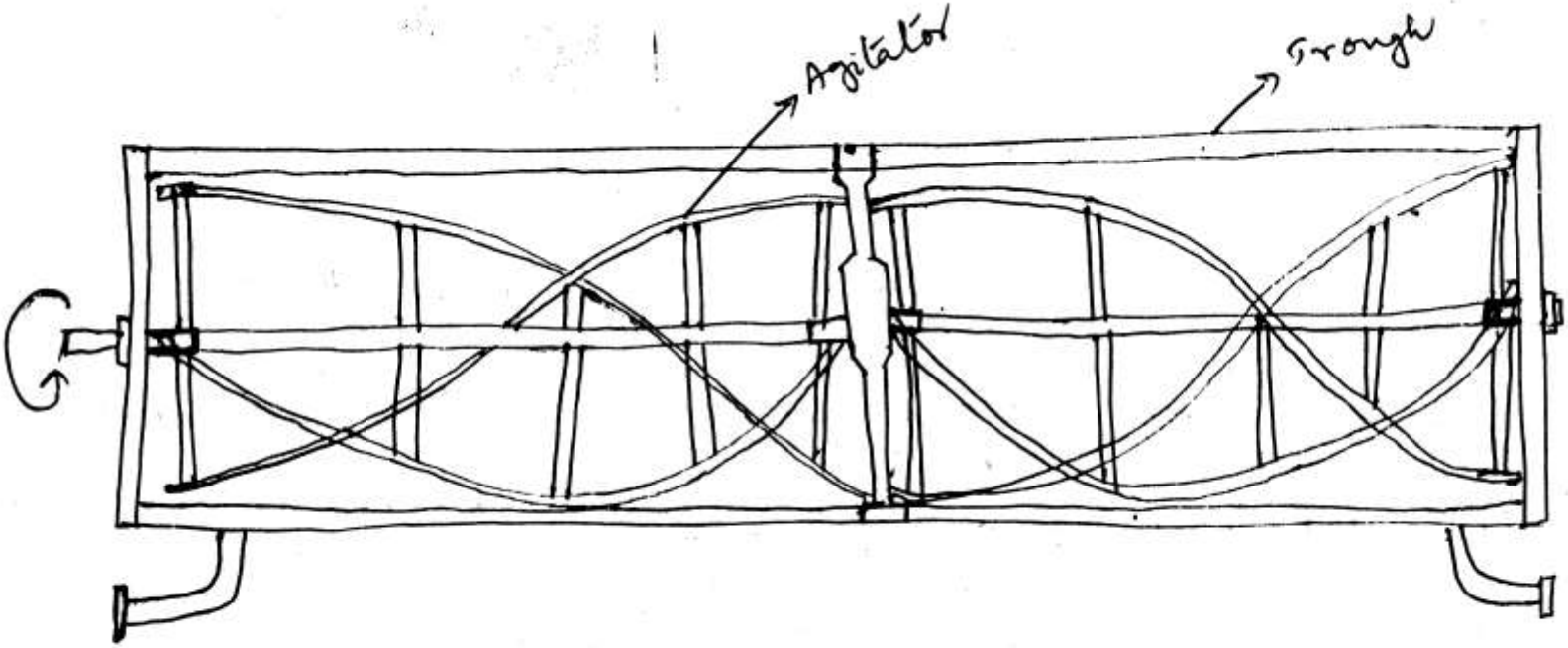
Agitated Batch Crystallizer

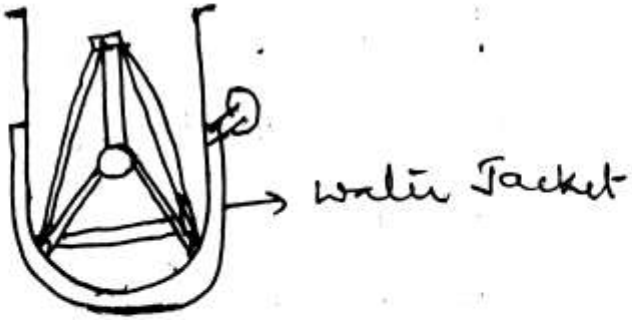
Advantages:

1) The product obtained is more uniform and finer than the tank crystallizer.

Disadvantages: 1) It is a batch or discontinuous process.

2) Solubility is least at the surface of the cooling coils. Crystal growth is most rapid at this period and coils rapidly build up with a mass of crystals that lowers the rate of heat transfer.





Swenson - walker crystallizer

Swenson Walker Crystallizer

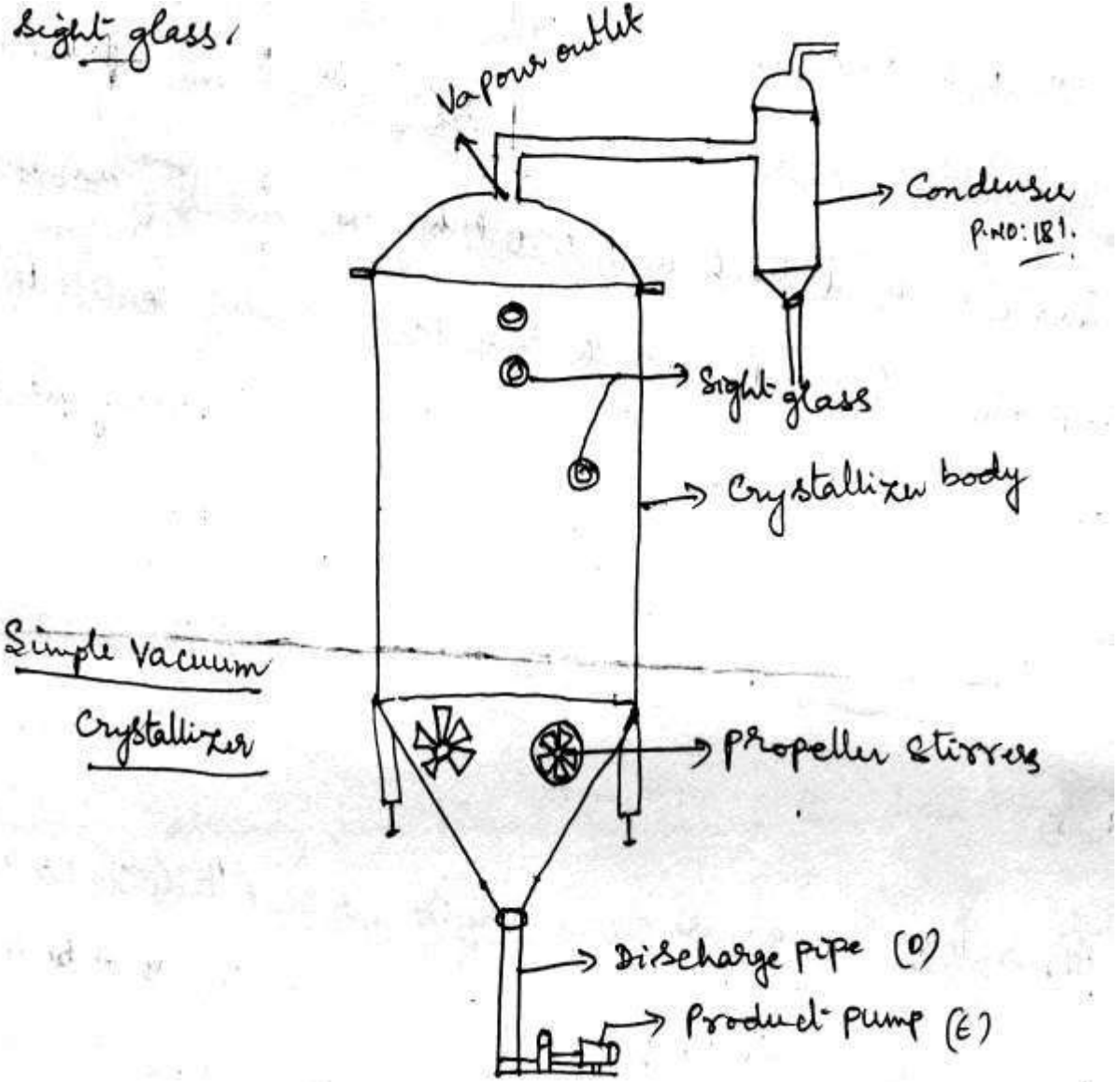
Advantages

- It can be operated continuously
- It produces uniform size and shaped crystals.
- Spiral agitator helps in proper crystal growth and prevents the crystals to accumulate on the surface of the crystallizer.

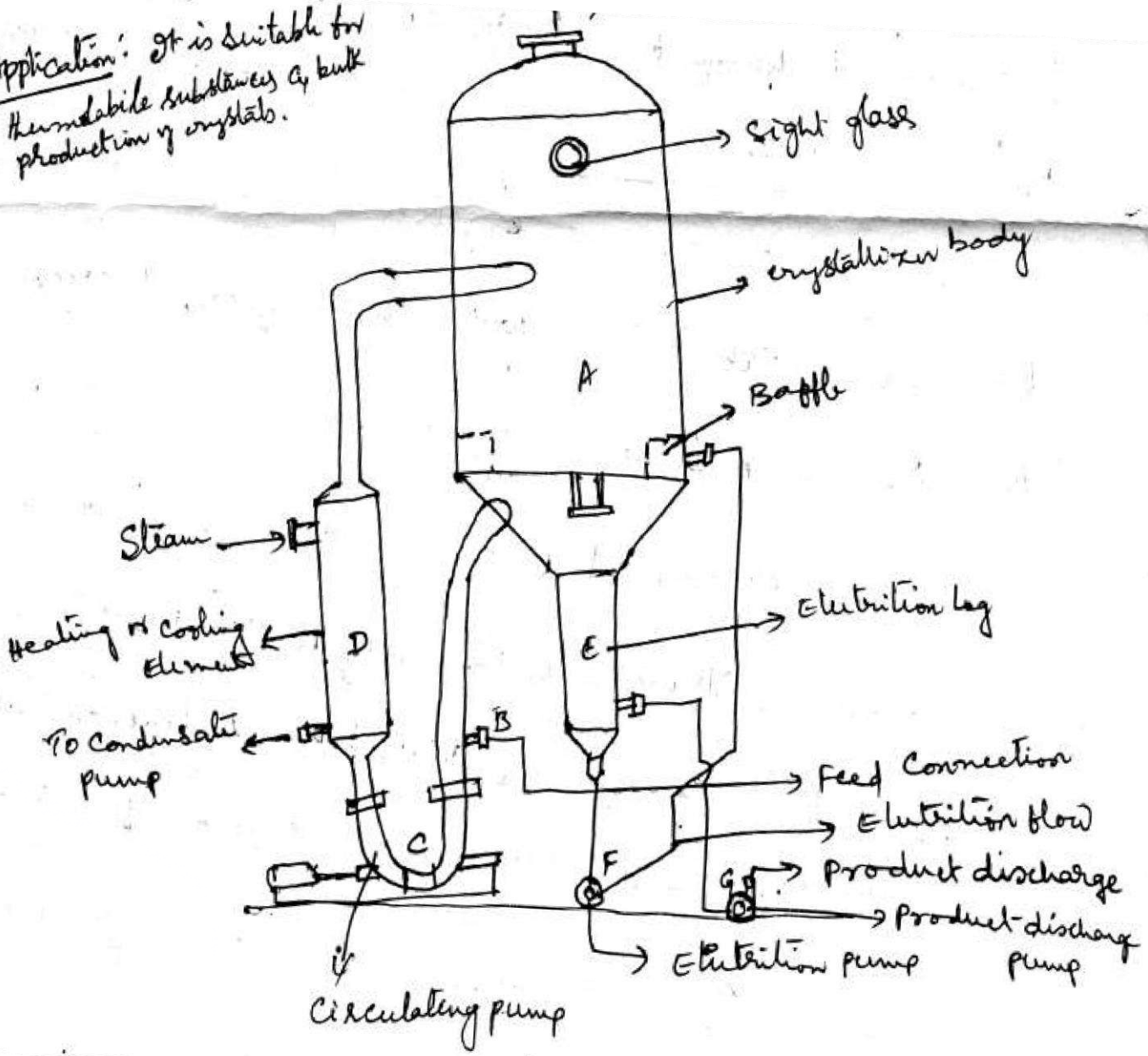
Disadvantages

- The apparatus is costly and its maintenance is difficult.

Sight glass:


Vapour outlet
Condenser
P.NO: 181.
Sight glass
Crystallizer body
Simple Vacuum Crystallizer
Propeller stirrer
Discharge pipe (D)
Product pump (E)

Application: It is suitable for thermolabile substances & bulk production of crystals.

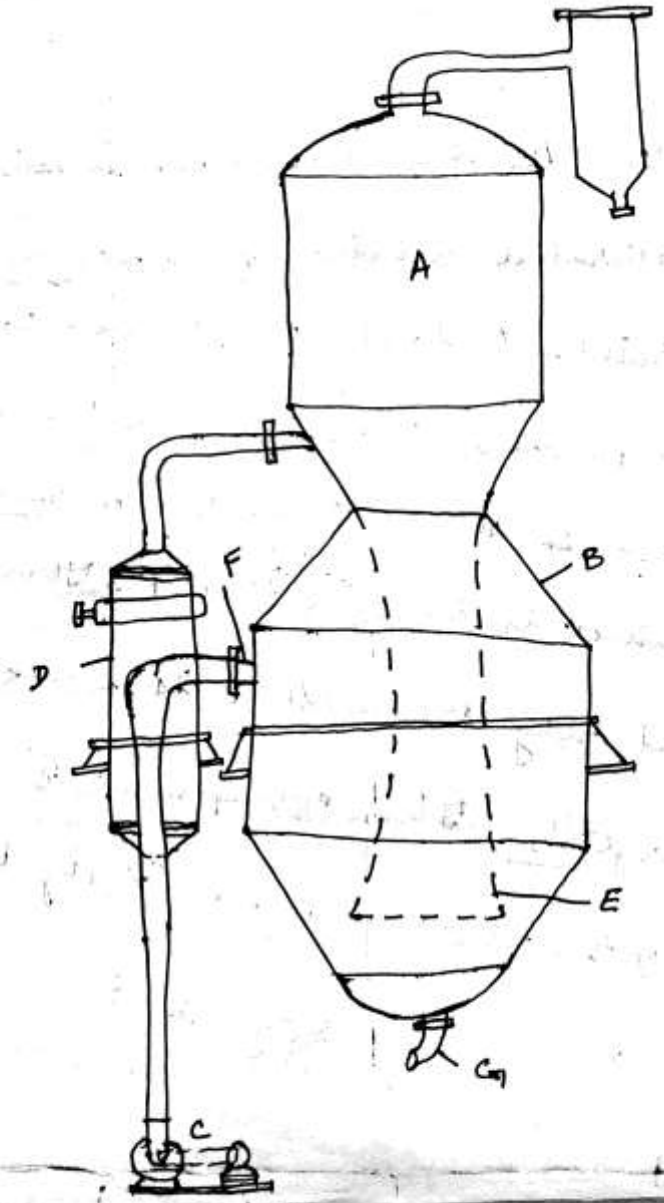


Advantages

- It is very simple without any moving parts.
- Corrosive materials can be used as inner surface can be made acid resistant.
- It can be constructed as large as desired.
- It can be operated continuously or batch wise.

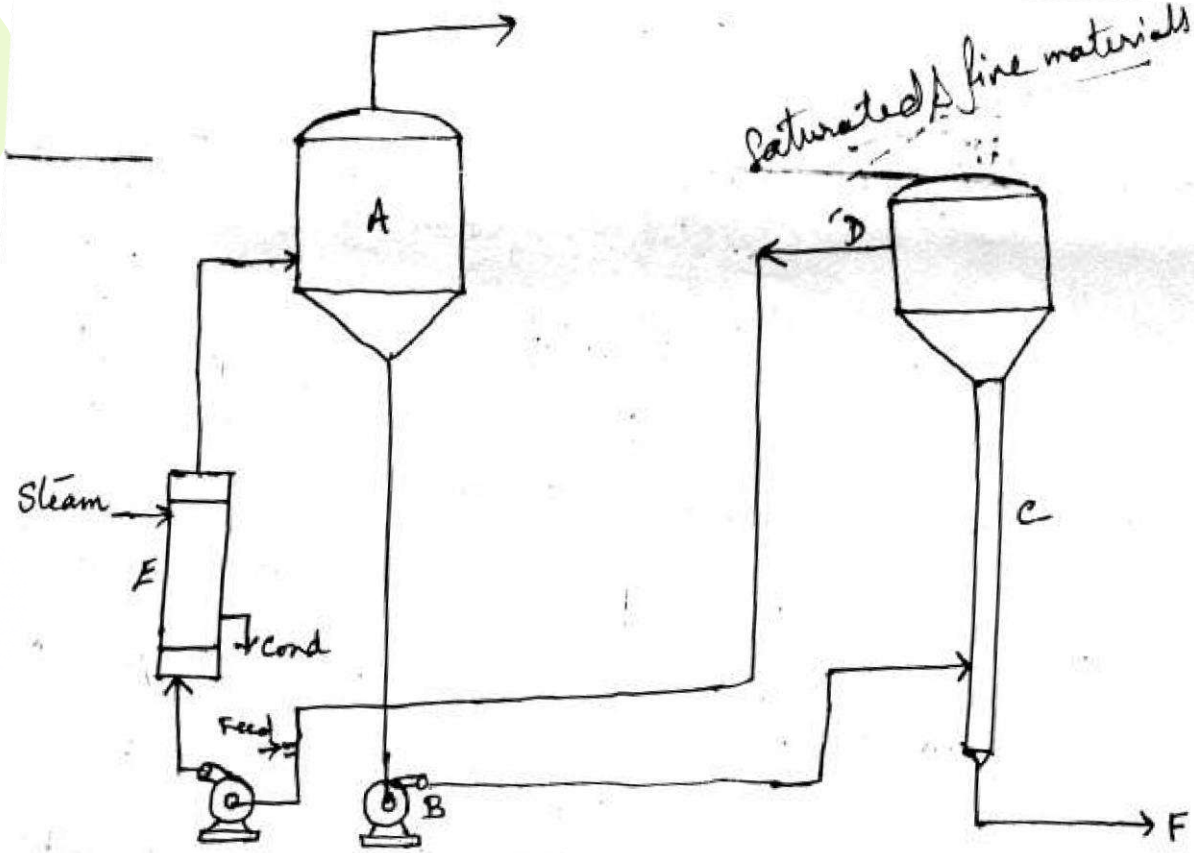
Disadvantages

- Requires many number of pumps.
- Complex piece of equipment.



A = Vapour Head
B = Crystal Growth Chamber
C = Circulating pump
D = Heater or Cooler
E = Discharge tube to bottom of crystal bed
F = overflowing to circulating pump
G = product discharge

Krystal Crystallizer



Growth-type crystallizer

A = Vapour head

B = Transfer pump

C = Growth column

D = overflow from growth column

E = Heater

Uses

- It is used for crystallization of Sodium Chloride & Magnesium Sulphate.

Advantages

- It is preferred when large quantity of crystals of controlled sizes are required.
- It is Available in very large sizes with a body upto 4.5 mts diameter and 6.0 mts height.

Caking of Crystals

- *Caking* can be defined as the process of formation of clumps or cakes when crystals are improperly stored.
- *Crystal Humidity* is the humidity above which crystals absorb moisture and below which they donot absorb moisture.

Factors effecting Caking

- Size of the crystals
- Shape of the crystals
- Humidity
- Time of exposure
- Impurities in crystals
- Melting point
- Temperature Fluctuations

Prevention of Caking

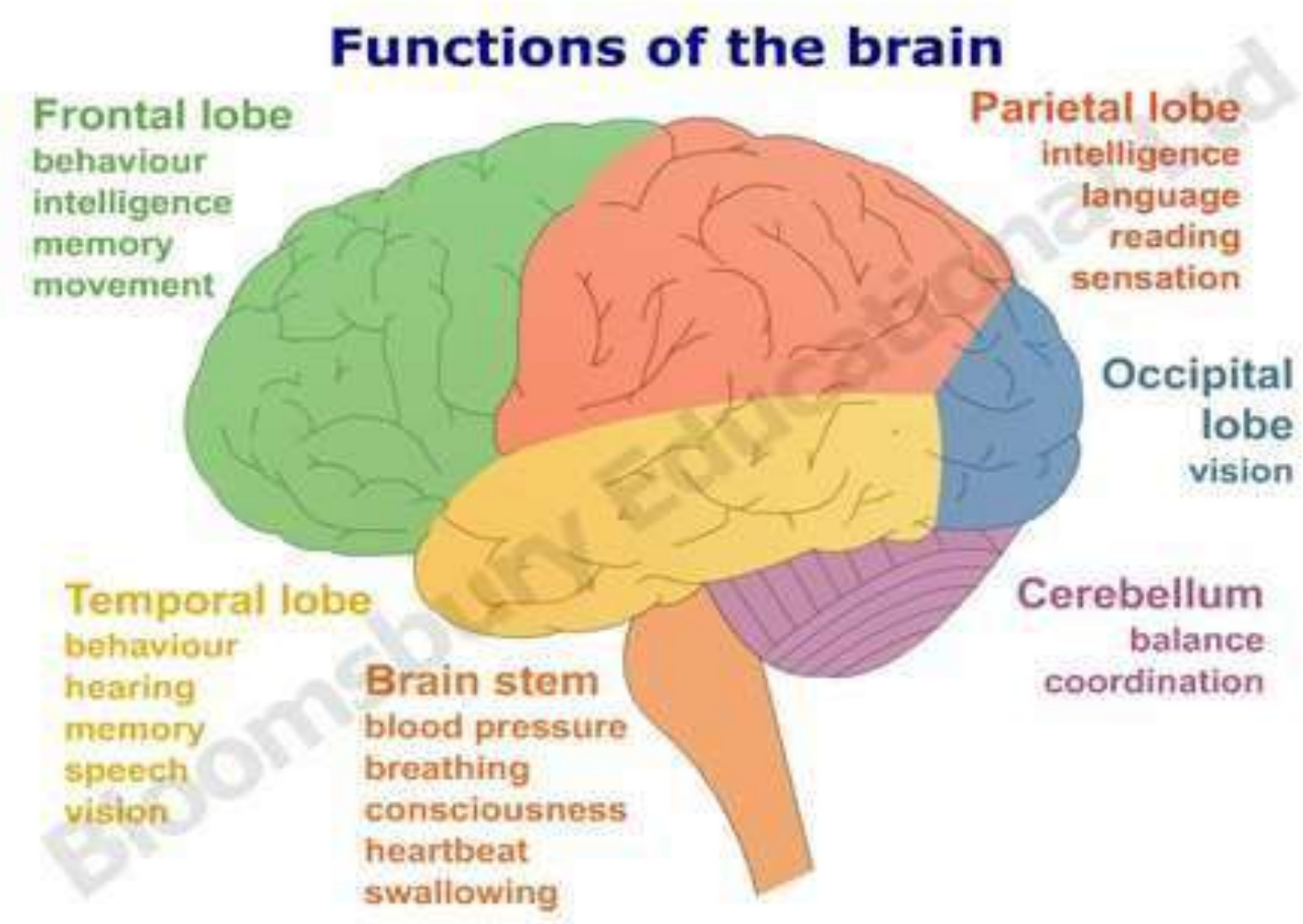
- Crystals must be more spherical in shape with least points of contact.
- Crystals must be larger in size with more void spaces & must have narrow size distribution.
- Crystals must have highest possible critical humidity.
- Crystals must be coated with powdery inert material to prevent absorption of moisture. Ex: Table salt is coated with Magnesia or TCP.

  Flake Calcium Chloride is coated with anhydrous Calcium Chloride.

Thank you

Drug Delivery to Brain and Targeting of Drugs to Brain

N.Shanthi


Functions of the brain
Frontal lobe
behaviour
intelligence
memory
movement
Parietal lobe
intelligence
language
reading
sensation
Occipital lobe
vision
Temporal lobe
behaviour
hearing
memory
speech
vision
Brain stem
blood pressure
breathing
consciousness
heartbeat
swallowing
Cerebellum
balance
coordination
Pascalis Spyrou

Introduction

- The CNS is the most dynamic expression of the harmonious co-ordination found in the human beings.
- Brain is the important part of CNS.
- Certain diseases that attack brain mainly arise from local or peripheral physiological disorders.
- Ex: Epilepsy, Parkinson's disease etc.
- Some are caused by brain infections.

Ex: **Encephalitis**, is an acute inflammation of the brain.

Meningitis. is an inflammation of the membranes that surround the brain and spinal cord . Meningitis is most commonly caused by infections with various pathogens, examples of which are *Streptococcus pneumoniae* and *Haemophilus influenzae*.

Factors that Influence Uptake and Distribution of Drug into Brain

- Blood flow.
- Binding of proteins in blood plasma.
- Clearance from blood.
- Metabolism.

- Drugs that are effective against diseases in CNS should reach the brain by passing through blood brain barrier.
- The major challenge to CNS drug delivery is the blood-brain barrier (BBB), which limits the access of drugs to the brain substance.
- BBB is formed by complex system of endothelial cells, astroglia, pericytes and basal lamina.
- The BBB is the rate limiting factor in the permeation of drug into brain and also influences the steady state distribution.

Barriers in Brain drug delivery

1) Blood-Brain Barrier (BBB)

- Build up by endothelial cells of the brain capillaries
- Protects brain from foreign and toxic substances as well as neurotransmitters and hormone

2) Blood-CSF Barrier (BCSFB)

- Formed by the epithelia of the choroid plexus and the circumventricular organs
- More permeable than BBB

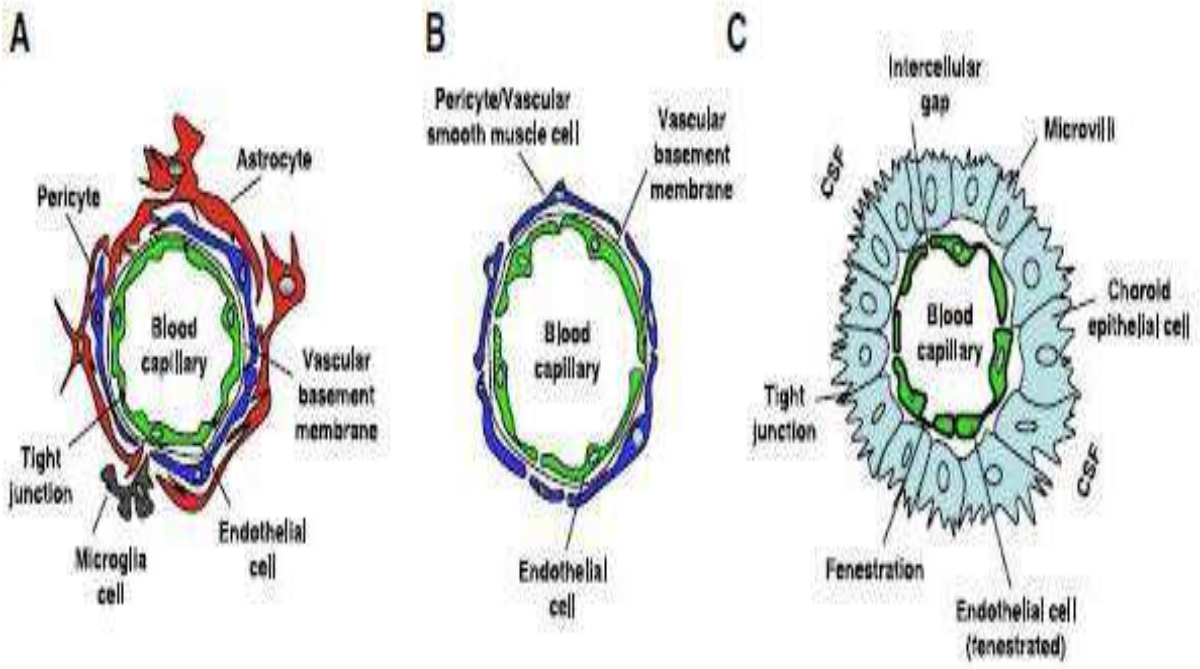
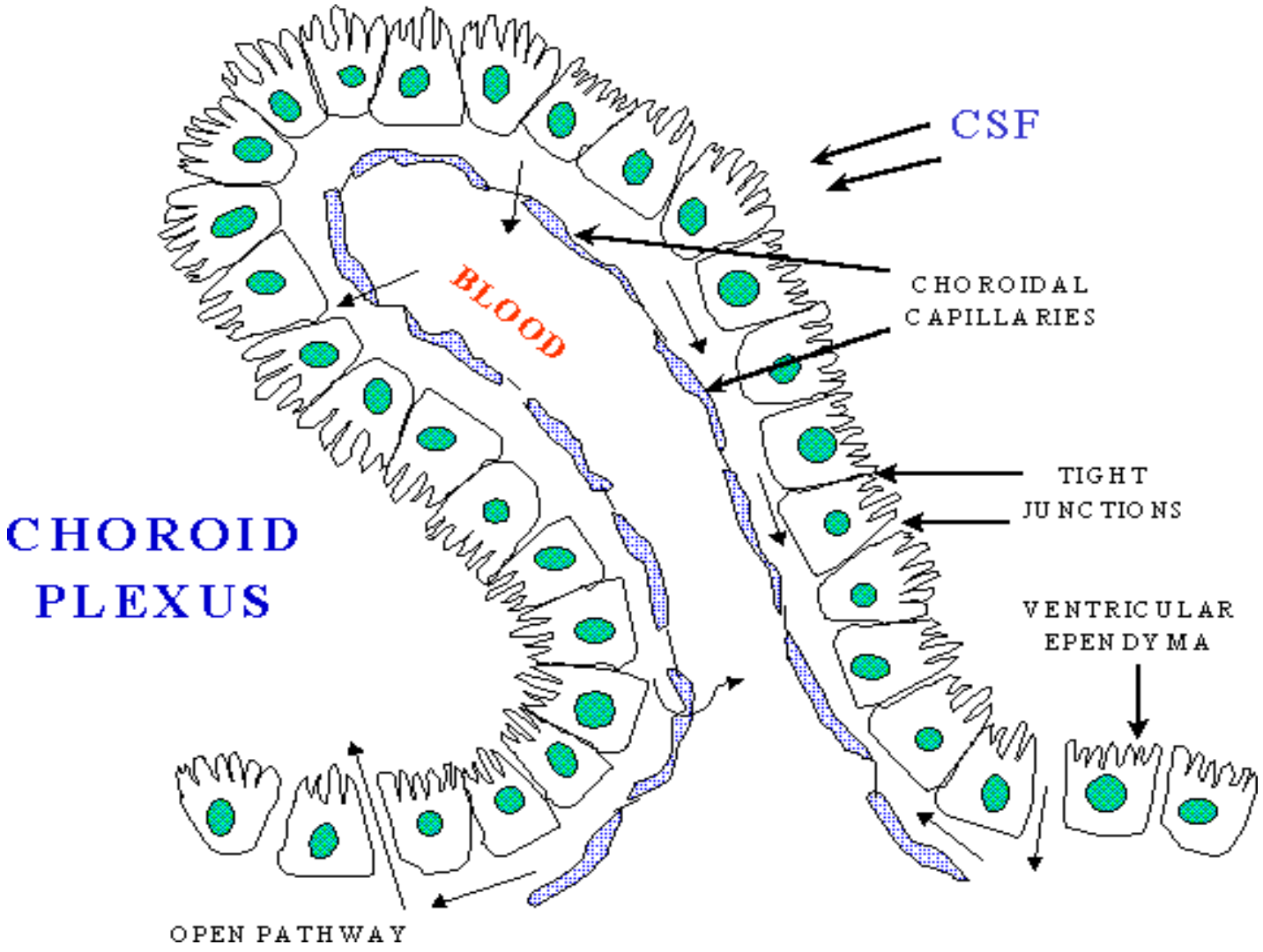


Fig . NO. 1 Structure of

A. Blood brain barrier
B. Peripheral capillaries
C. B-CSF barrier

- The brain micro vessel endothelial cell (BMEC) that form the BBB, display important morphological characteristics such as the presence of tight junctions between the cells, the absence of fenestrations that together help to restrict the passage of compounds from the blood into the extra cellular environment of the brain.
- This barrier permits the exchange of essential gases and nutrients between the bloodstream and the brain, while blocking larger entities such as microbes, immune cells and most drugs from entering.


CSF
BLOOD
CHOROIDAL
CAPILLARIES
TIGHT
JUNCTIONS
CHOROID
PLEXUS
VENTRICULAR
EPENDYMA
OPEN PATHWAY

Mechanism of transporting drugs into brain

- Permeation of compounds across BBB depends on their lipophilicity.
- Some drugs like VINCRISTINE, VINBLASTINE although highly lipophilic, the permeation of these drugs across BBB is very slow.
- This indicates the existence of multiple mechanisms of drug transport through BBB.

VARIOUS BBB TRANSPORT MECHANISMS

1. **Active efflux transport by p-glycoprotein at BBB.**

- PGP at BBB is important for limiting access of endogenous and exogenous toxic agents.

- Pharmacological opening of BBB by inhibition of PGP function leads to increased uptake of some drugs like Anti-Cancer drugs.

2. Carrier mediated BBB transport of drugs

- Mono carboxylic acid transport systems

  Ex: lactate, pyruvate.
- Amine transporters

  Ex: Endogenous hydrophilic amine choline.
- Neutral amino acid transport system for drugs like leucine, l-dopa, gabapentin.
- Basic amino acid transport system for lysine.
- Beta amino acid transport system for beta-Alanine.

3. Transport of peptide across BBB

- Peptides are generally relatively large hydrophilic and unstable. So specific delivery strategy is employed.

Carrier Mediated Transport of Peptides

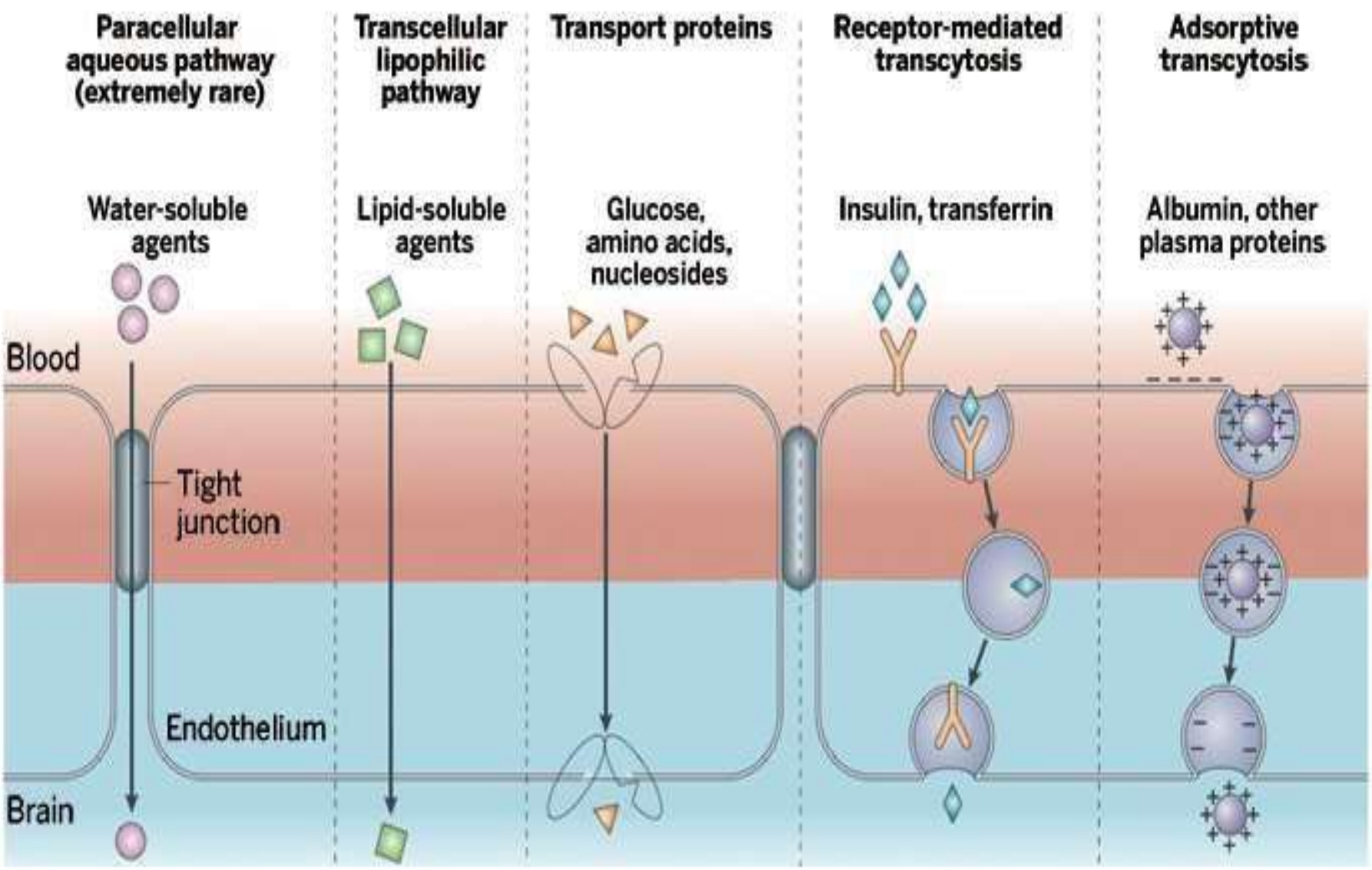
- It is suitable for small Di and Tri peptides.

Adsorptive Mediated Endocytosis

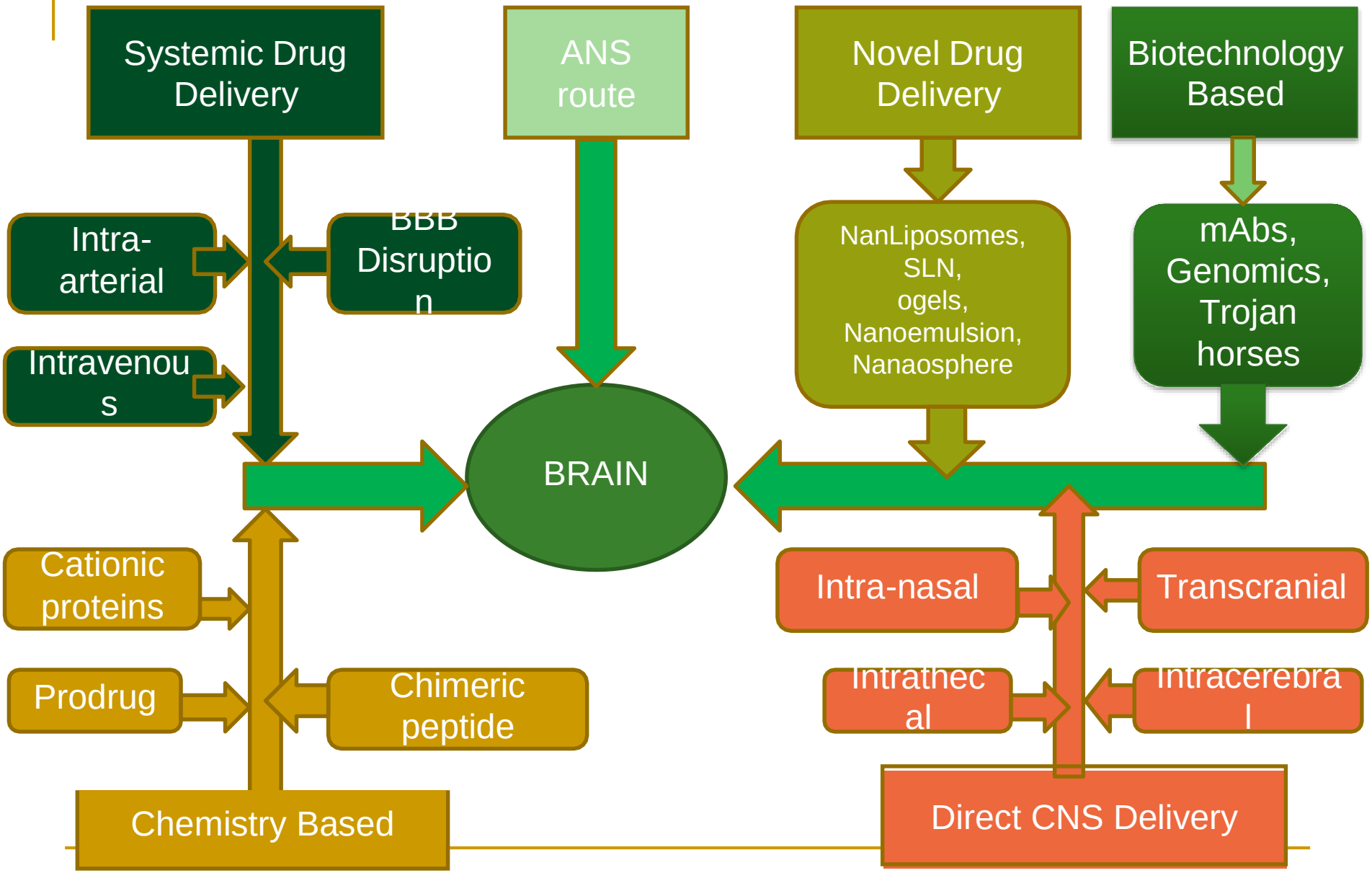
- It is triggered by electrostatic interaction between a positively charged peptide and negatively charged plasma membrane surface region.

4. Efflux transport system for drugs

- It is expressed at luminal and for albuminal membranes of basal capillary endothelial cells which restricts accumulation of some drugs.
- PGP is one such Efflux transporter.


Paracellular aqueous pathway (extremely rare)
Transcellular lipophilic pathway
Transport proteins
Receptor-mediated transcytosis
Adsorptive transcytosis
Water-soluble agents
Lipid-soluble agents
Glucose, amino acids, nucleosides
Insulin, transferrin
Albumin, other plasma proteins
Blood
Tight junction
Endothelium
Brain

Approaches for Brain Drug Delivery



OVERVIEW OF DIFFERENT APPROACHES OF BRAIN TARGETING

Pathways for drug administration into Brain

- BBB
- Nasal route

  Ex: Vitamin B12, Apomorphine, Analgesics Anti migraine drugs etc…
- Intra cerebroventricular administration: surgical implantation.

  Ex: water soluble Anti Cancer drugs, Morphine.

Strategies for brain delivery of drug

INVASIVE	PHYSIOLOGICAL	PHARMACOLOGICAL
BBB disruption	Pseudonutrients	Liposome
Intracerebral Implants	ligand binding proteins	Nanoparticles
	Chimeric Peptides	Chemical Drug Delivery

Intra nasal drug delivery

- After nasal delivery drugs first reach the respiratory epithelium, where compounds can be absorbed into the systemic circulation by Tran cellular and Para cellular passive absorption, carrier-mediated transport, and absorption through trancytosis.
- When a nasal drug formulation is delivered deep and high enough into the nasal cavity, the olfactory mucosa may be reached and drug transport into the brain and/or CSF via the olfactory receptor neurons may occur.

Invasive Delivery Strategies

- Invasive approach to drug delivery require surgical intervention. This includes the methods that physically bypass the BBB by direct delivery into CSF.

1. Drug delivery based on BBB disruption.

   This method involves temporary physiochemical disruption of endothelial cells.

Intraventricular / Intrathecaldelivery

- Here using a plastic reservoir which implanted subcutaneously in the scalp and connected to the ventricles within the brain by an outlet catheter. Drug injection into the CSF is a suitable strategy for sites close to the ventricles only.

- Hyper Osmolar Barrier Opening

  It is used for delivery of small molecular weight cytostatic agents to brain tumors.

Mechanisms:

- Endothelial cell shrinkage.
- Disruption of tight junctions.
- Vasodilation by osmotic shift.

Biochemical barrier opening

- BBB opening may also be achieved by receptor mediated mechanisms.
- Vasoactive compounds such as prostaglandins, serotonin, histamine, bradykinin induce BBB leakage.
- Effects of bradykinin are more pronounced on blood tumor barrier than normal BBB.

2.Physiological Delivery Strategies

- Drug treatments of chronic degenerative disorders will require long term therapy.
- This implies a need to develop a non invasive approach for brain delivery via systemic route.

1. Receptor mediated uptake:

   This involves transport of peptides and proteins at BBB.

   The overall process is designated as transcytosis and is composed of binding to a luminal plasma membrane receptor, Endocytosis, transfer through endothelial cytoplasm.

2. Absorptive mediated uptake of lecithins and cationic peptides/proteins

- A mechanism of brain uptake related to receptor-mediated transcytosis operates for cationic peptides/proteins and some lecithins.

- Initial binding to luminal plasma membrane is mediated by electrostatic interactions with anionic sites or by specific interaction with sugar residues [absorptive mediated transcytosis]

3. Drug delivery trough chimeric peptides

these are generated by linking a drug that lacks transport at BBB to a vector.

binding of vector at luminal membrane of brain capillary endothelial cells initiates receptor mediated or adsorptive mediated transcytosis.

Chemical Delivery Systems

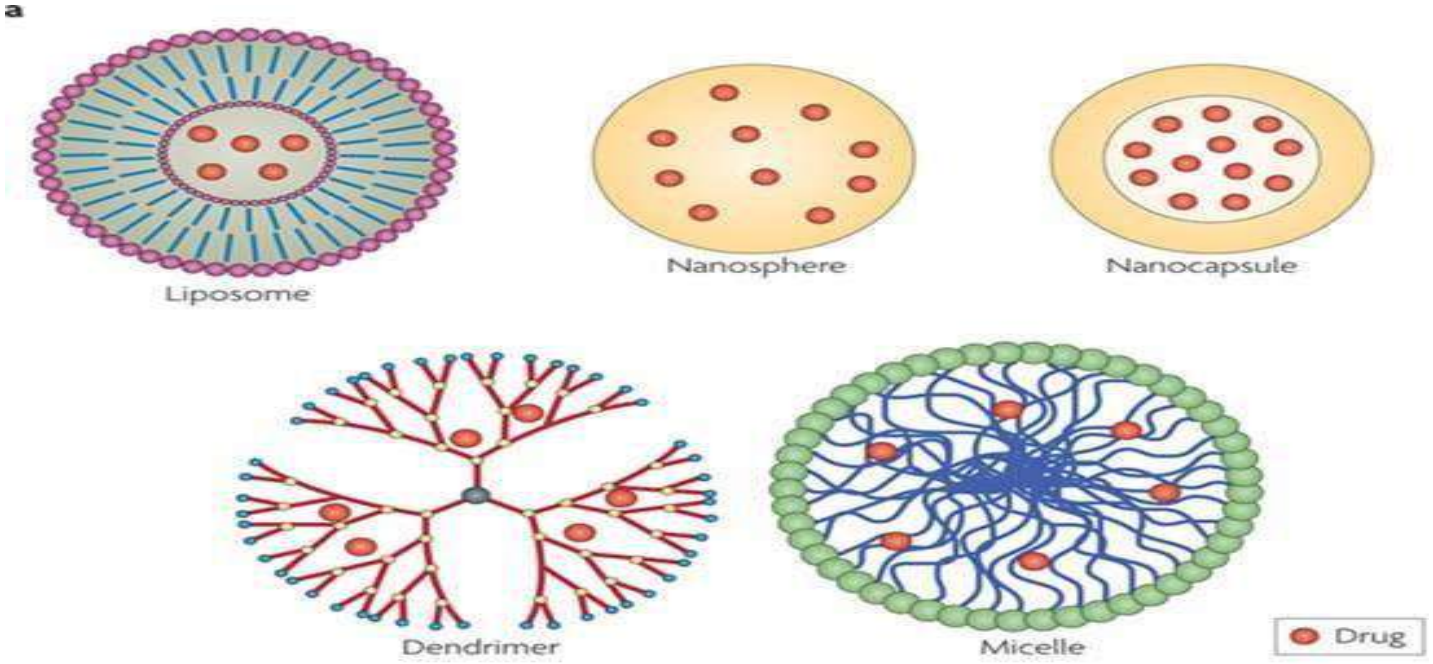
- Brain targeted chemical delivery systems represent a rational drug design approach that exploits sequential metabolism not only to deliver but also to target drugs to their site of action.

- By localizing drugs at desired site of action one can reduce toxicity and increase treatment efficacy.

- The use of redox chemical system for drug targeting to brain is of great significance for two categories of drugs.

    1. Which do not cross BBB at all.
    2. The drugs that readily penetrate BBB.

PHARMACOLOGICAL STRATEGIES



Nature Reviews | Neuroscience

NANOPARTICLES

Nanoparticles are solid colloidal particles ranging in size 1-1000nm.

- They consist of macromolecular materials in which the active principle is dissolved, entrapped or encapsulated.
- Drugs may be bound inform of a solid solution or dispersion or be adsorbed to the surface or chemically attached.
- Poly (butylcyanoacrylate) nanoparticles represent the only nanoparticles that were so far successfully used for the in vivo delivery of drugs to the brain.
- The first drug that was de-livered to the brain using nanoparticles was the hexapeptidedalargin

Mechanisms of Nanoparticles mediated transport.

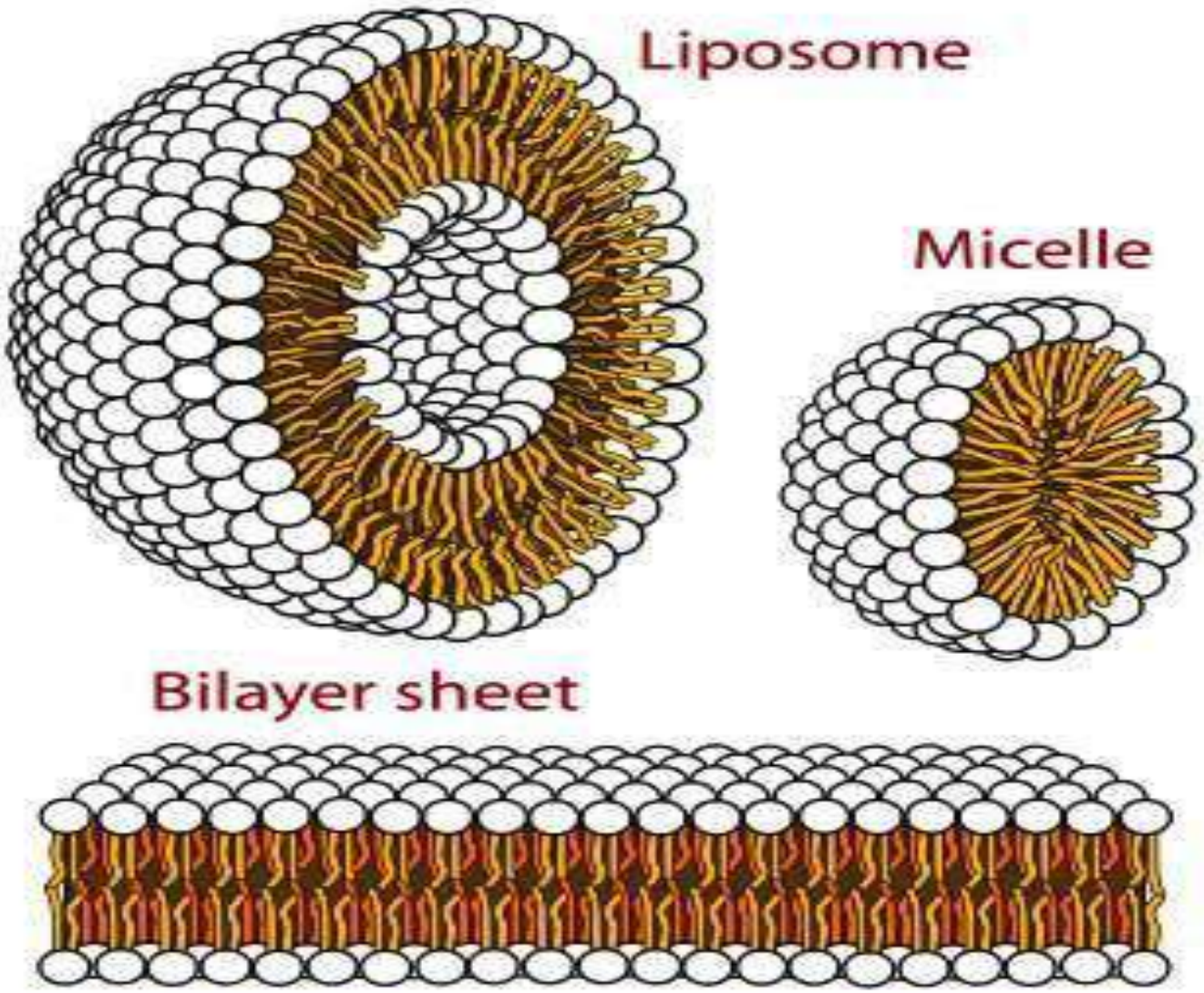
1. Adhesion of Nanoparticles to brain blood vessel walls.
2. Fluidization of endothelium by surfactants
3. Opening of tight junctions of endothelium
4. Endocytosis by the brain blood vessel endothelial cells.
5. Blockage of p-glycoprotein in the brain cells.

- Nanoparticles provide massive advantages regarding drug targeting, delivery and release and, with their additional potential to combine diagnosis and therapy, emerge as one of the major tools in nanomedicine.
- The main goals are to improve their stability in the biological environment, to mediate the bio-distribution of active compounds, improve drug loading, targeting, transport, release, and interaction with biological barriers.
- The cytotoxicity of nanoparticles or their degradation products remains a major problem, and improvements in biocompatibility obviously are a main concern of future research.

Liposomes

- Liposomes are lipid vesicles that offer an advantage of targeting the drug to the tissues.
- Liposomes are biocompatible, non-toxic and biodegradable which offer possibility of carrying hydrophilic, hydrophobic and amphoteric molecules.
- They can act as carriers for drugs, enzymes, proteins, anticancer substances etc…
- These modify therapeutic profile of selected anitumor drugs in favorable manner by reducing toxicity.

- In some cases liposomes attach to cellular membranes and appear to fuse with them, releasing their or drugs into the cell.
- In the case of phagocytic cells, the liposomes are taken up, the phospholipid walls are acted upon by organelles called lysosomes, and the medication is released.
- Liposomal delivery systems are still largely experimental; the precise mechanisms of their action in the body are under study, as are ways in which to target them to specific diseased tissues.


Liposome
Micelle
Bilayer sheet

Monocytes:

- The unusual ability of Monocytes to cross BBB makes it possible to consider these cells to transport agents into CNS.

- Monocytes express certain receptors on their membrane which are involved in receptor mediated Endocytosis upon interaction with suitable ligands.

Recent advances

- Cationic Liposomes were developed and these were found to undergo absorptive mediated Endocytosis into cells.

- The addition of sulfatide to liposome composition improves their ability to penetrate BBB.

- Prolonged circulation of Liposomes is achieved by insertion of gangliosides or PEG derivatised lipids within the bi layer of Liposomes.

In vivo techniques for assessing pharmacokinetics of drugs targeted to brain

- The various in vivo techniques include

1. Brain uptake index.
2. Brain efflux index.
3. Brain perfusion.
4. Unit impulse method.
5. Micro dialysis.

- The brain uptake index (BUI), as originally described and refined by Oldendorf. The BUI employs a rapid bolus injection of radiolabeled test and reference substances into the common carotid artery of anesthetized animals (e.g., in the rat 0.2 ml in less than 0.5 s).
- After the 1- to 2-s passage time through the brain capillaries, brain uptake is measured as the single pass extraction, E, by brain tissue sampling after decapitation, which is performed within 5-15 s.
- A second reference substance is co injected, which does not penetrate the BBB, to correct for the fraction of the bolus remaining in the vascular lumen at the time of brain sampling (about 2%).
- Suitable combinations of radioisotopes must be available to allow for double or triple isotope counting of the tracers in injectate and brain tissue, respectively. The calculation of BUI (as percentage) is defined by the equation:

$$BUI = 100[(E_{test} - E_{refv})] E_{refp}$$

- where E_{test} is the brain extraction of the unknown test substance, E_{refV} is the apparent extraction of a vascular marker (non permeant reference) and E_{refP} is the extraction of the permeant reference.

IN SITU BRAIN PERFUSION.

- A major approach to measure brain penetration in whole animals has been brain perfusion method of Takasatu and colleagues, which measures the rate of entry across brain endothelium *in situ*.
- Unlike earlier methods, such as the brain uptake index, which is mostly suitable for fast BBB-penetrating compounds, the brain perfusion method can be used for both slow and fast brain-penetrating compounds.
- The method utilizes catheterization of the common carotid artery in the anesthetized rat, together with ligation of the external carotid artery.
- The brain is then perfused via the internal carotid using an oxygenated physiological saline buffer containing the test substance.
- Once perfusion is complete, the brain is removed for analysis and uptake (volume of distribution, Vd) determined according to the following equation:

$$V_d = \text{tissue concentration} / \text{perfusate concentration}$$

- With a constant concentration of the test compound during the perfusion over time, t, the rate of compound transfer (K_{in}) can be determined according to the following equation:

$$K_{in} = V_d / t$$

- *In situ* perfusion therefore provides a kinetic measure of the uptake of a compound into brain.

Micro Dialysis

- Intracerebral micro dialysis is particularly suitable for estimating extra cellular unbound drug concentration.
- The kinetics of unbound drug in blood is known Intracerebral micro dialysis can be used to characterize drug transport across BBB under various conditions.
- Intracerebral dialysis involves the implantation of a micro dialysis probe into brain.

Advantages:

It provides samples obtained from multiple time points from an individual animal.

Disadvantage:

It involves an implantation of probe which may cause tissue trauma.

DRUG	MECHANISM OF TRANSPORT	USE OF DRUG
GLUCOSE MANNOSE	HEXOSE TRANSPORT SYSTEM	SOURSE OF CARBOHYDRATE IN PARENTERAL NUTRITION
FLUOROURACIL RANIMUSTIN	PHARMACOLOGICAL OPENING OF BBB BY INHIBITION OF PGP	ANTICANCER
ROVASTATIN	MONO CARBOXYLIC ACID TRANSPORT SYSTEM	TREATMENT OF HYPER LIPIDAEMIAS
EBIRATIDE	ADSORPTIVE MEDIATED ENDOCYTOSIS	ALZHEIMERS DISEASE
ß-LACTAM ANTIBIOTICS	OLIGOPEPTIDE TRANSPORTERS[PEP T1, PEP T2]	ANTIBIOTIC
PHENYL ALANINE	NEUTRAL AMINO ACID TRANSPORT SYSTEM	DIETARY SUPPLEMENT
ASPARATE	ACIDIC AMINO ACID TRANSPORT SYSTEM	DIETARY SUPPLEMENT

Thank you

DRUG DELIVERY TO RESPIRATORY SYSTEM

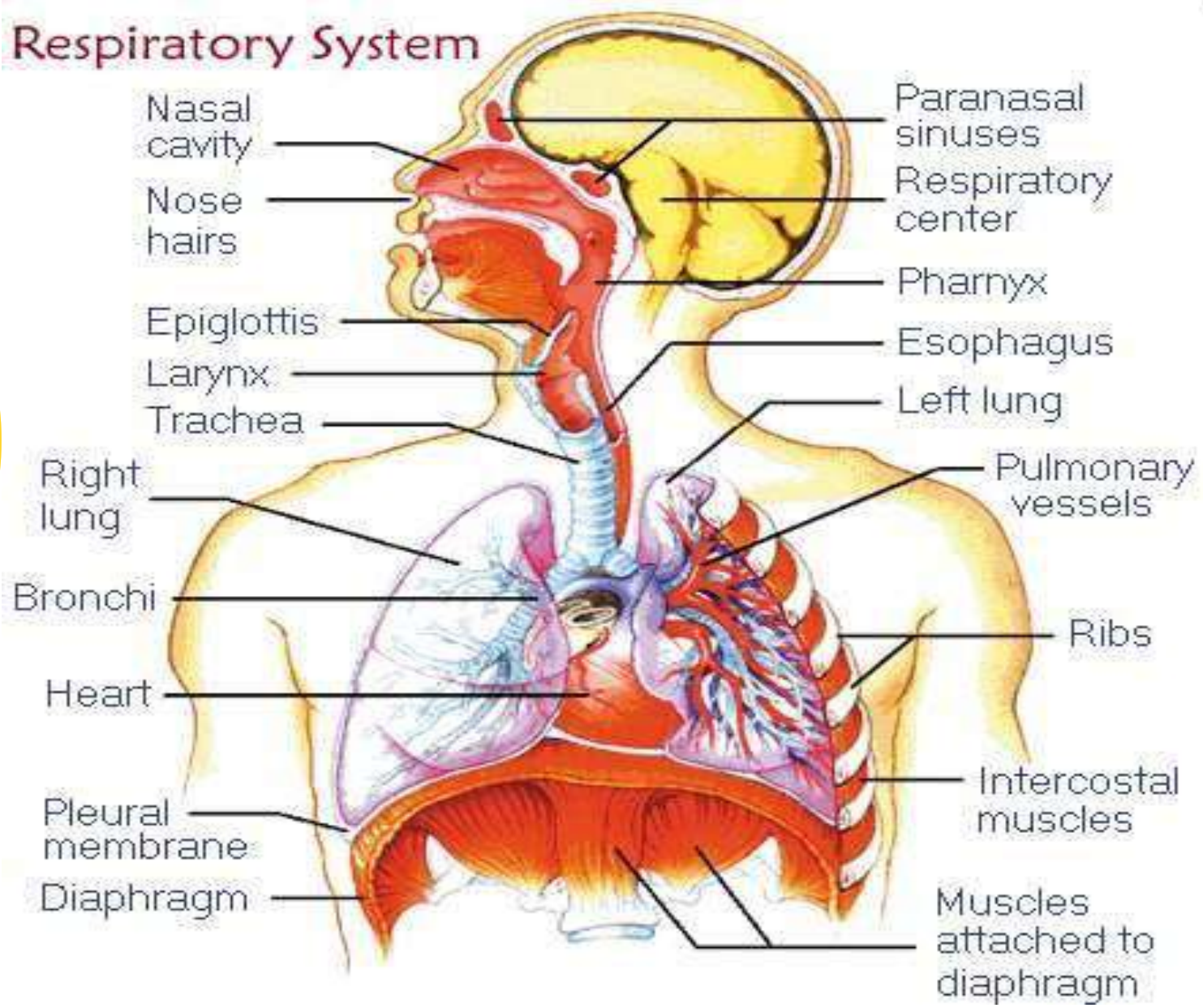
B.Rajewsar
Associate Professor, VCPS

General Respiratory Anatomy and Physiology

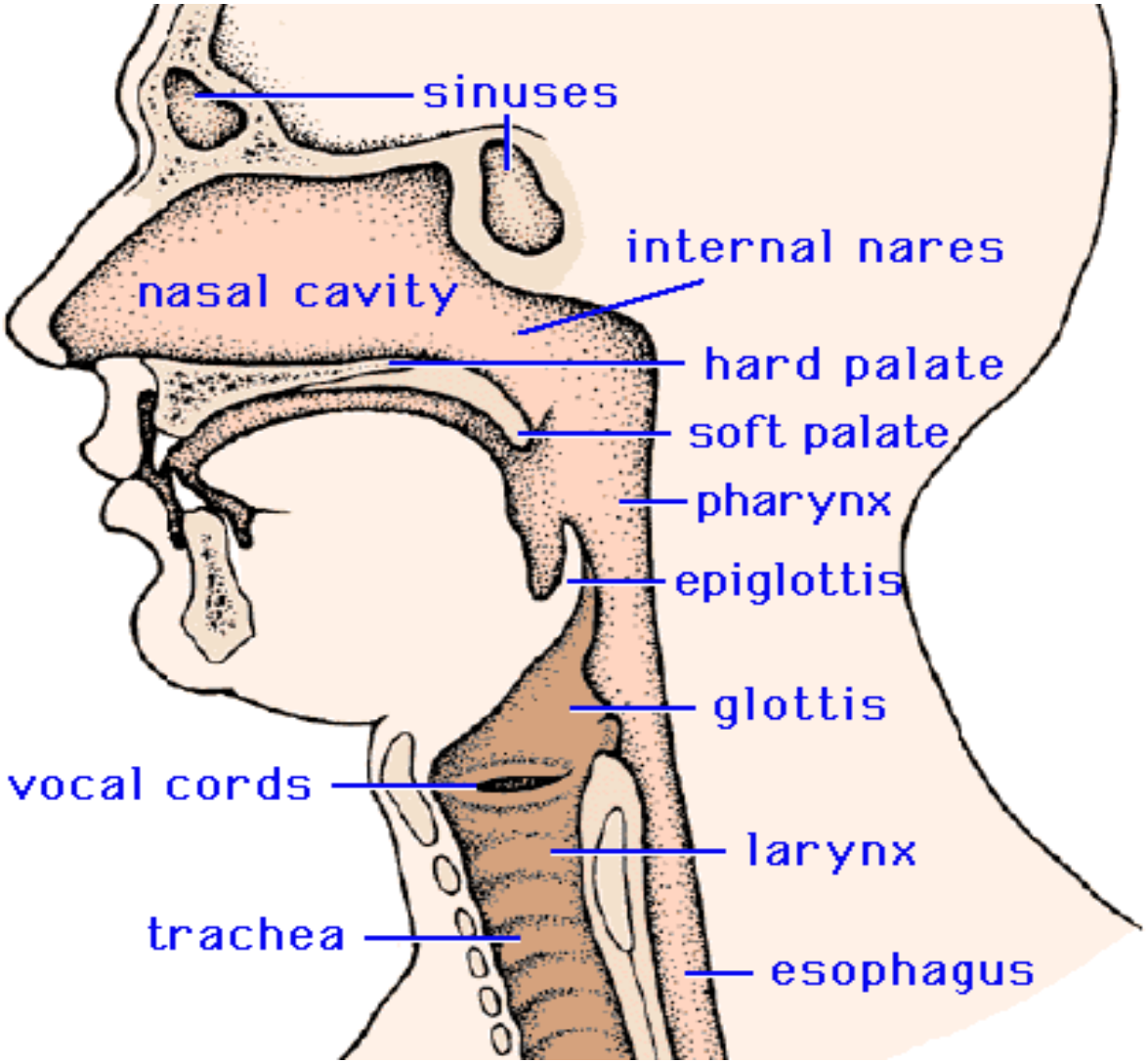
A. The respiratory system is comprised of the upper airway and lower airway structures.

B. The upper respiratory system filters, moistens and warms air during inspiration

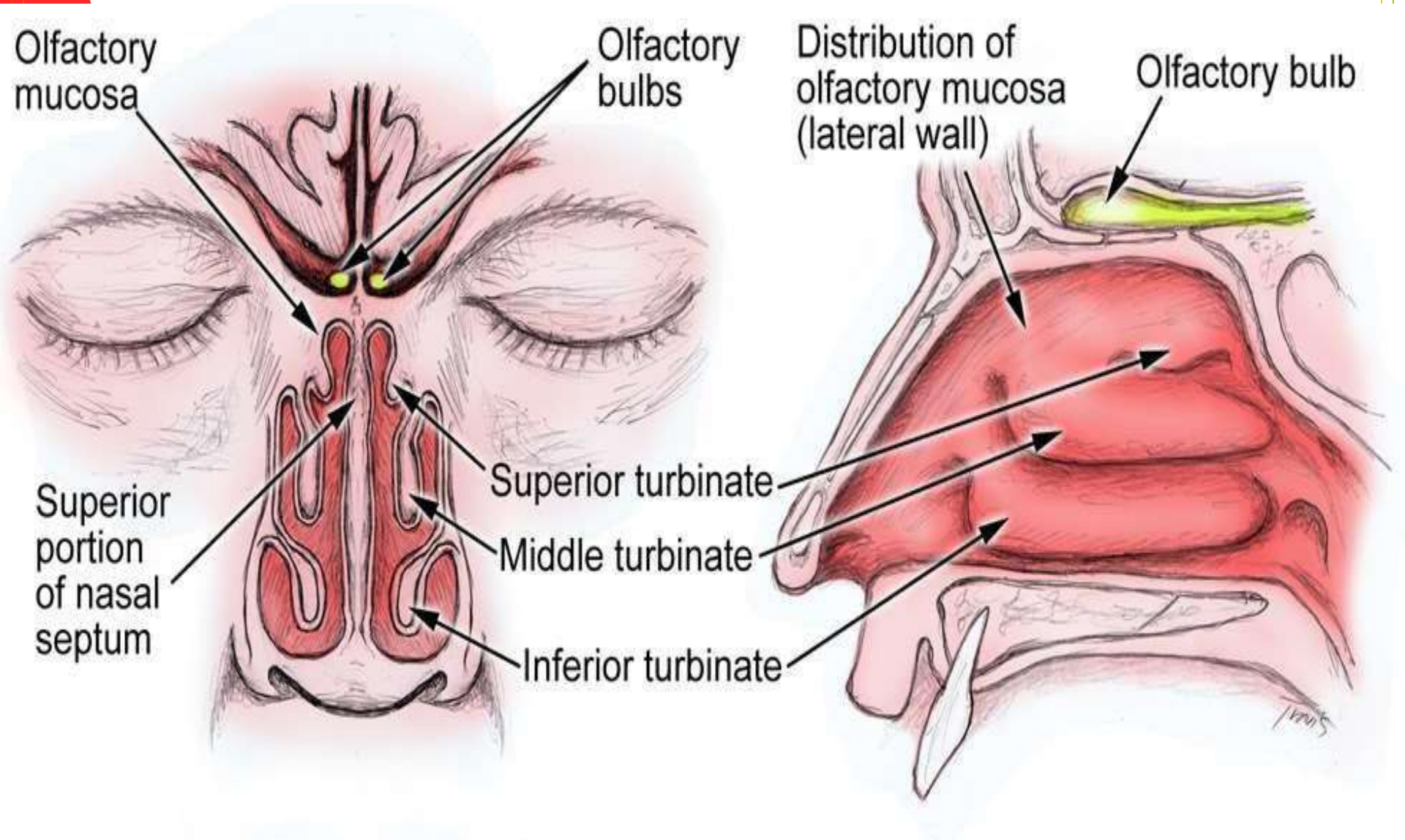
C. The lower respiratory system enables the exchange of gases to regulate serum PaO_2 (partial pressure of oxygen in arterial blood) , $PaCO_2$ (partial pressure of carbon dioxide in arterial blood) and Ph (partial pressure of hydrogen in blood) .

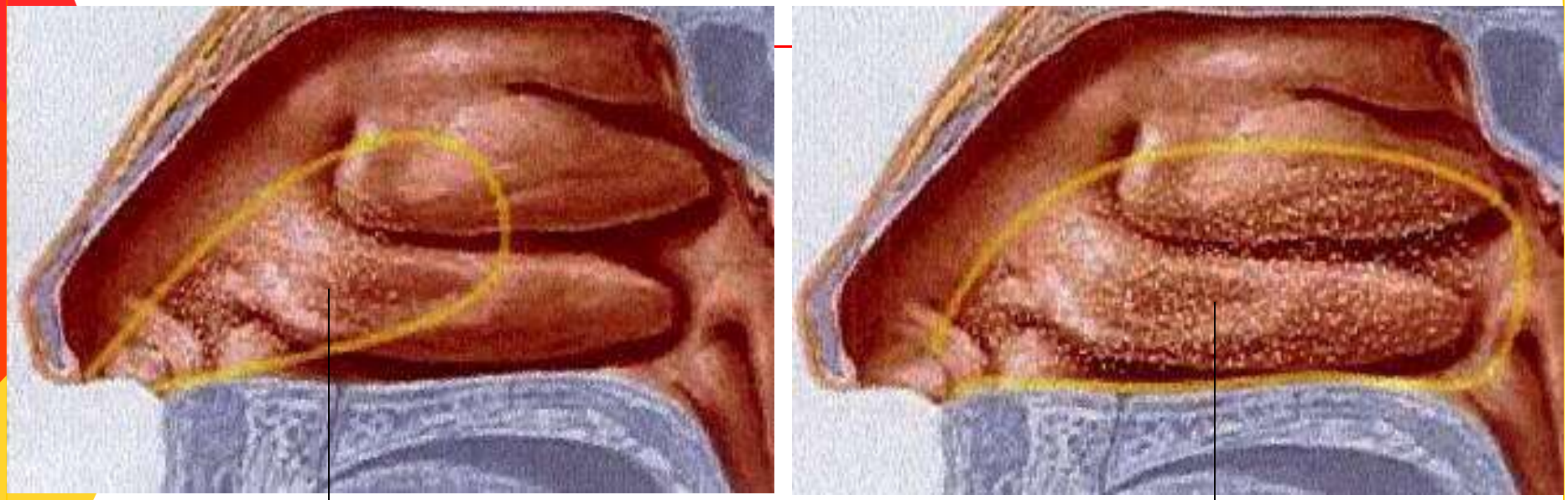

Respiratory System
Nasal cavity
Nose hairs
Epiglottis
Larynx
Trachea
Right lung
Bronchi
Heart
Pleural membrane
Diaphragm
Paranasal sinuses
Respiratory center
Pharnyx
Esophagus
Left lung
Pulmonary vessels
Ribs
Intercostal muscles
Muscles attached to diaphragm

UPPER RESPIRATORY TRACT



ANOTOMY OF HUMAN NASAL CAVITY





Site of drug spray & absorption

Advantages of Nasal Drug Delivery System

1) Drug degradation that is observed in the gastrointestinal tract is absent.
2) Avoidance of Hepatic first – pass metabolism..
3) Rapid drug absorption and quick onset of action can be achieved.
4) The bioavailability of larger drug molecules can be improved by means of absorption enhancer or other approach.
5) Direct transport into systemic circulation and CNS is possible

5) The nasal bioavailability for smaller drug molecules is good.

6) Drugs that are orally not absorbed can be delivered to the systemic circulation by nasal drug delivery.

7) Convenient for the patients, especially for those on long term therapy, when compared with parenteral medication.

8) Good penetration, especially lipophilic, low molecular weight drugs through the nasal mucosa. For instance the absolute nasal availability of fentanyl is about 80%.

LIMITATIONS

1) The histological toxicity of absorption enhancers used in nasal drug delivery system is not yet clearly established.
2) Relatively inconvenient to patients when compared to oral delivery systems since there is a possibility of nasal irritation.
3) Nasal cavity provides smaller absorption surface area when compared to GIT.
4) Volume that can be delivered into nasal cavity is restricted to 25–200 µl.
5) Normal defense mechanisms like mucocillary clearance and ciliary's beating affects the permeability of drugs

Therapeutic class of drugs

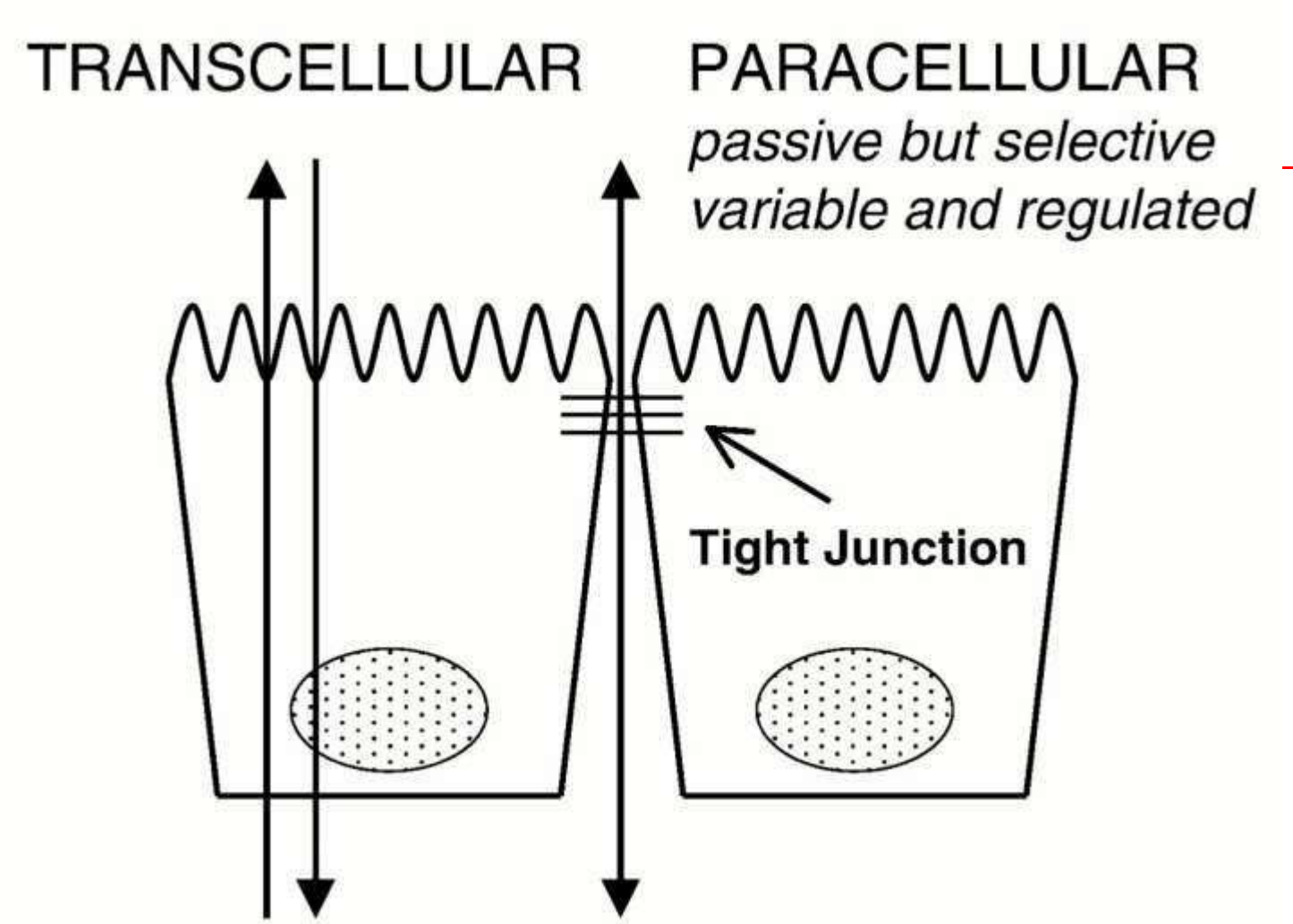
1. β_2 adrenergic agonists
2. Corticosteroids
3. Antiviral
4. Antibiotics
5. Antifungal
6. More recently, vaccines

MECHANISM OF DRUG ABSORPTION

- The first step in the absorption of drug from the nasal cavity is passage through the mucus. Small, uncharged particles easily pass through this layer. However, large or charged particles may find it more difficult to cross.
- The following two mechanisms have been considered predominantly to explain nasal absorption

1. Paracellular route
2. Transcellular process

1. The first mechanism involves an aqueous route of transport, which is also known as the paracellular route. This route is slow and passive. Poor bioavailability was observed for drugs with a molecular weight greater than 1000 Daltons.
2. The second mechanism involves transport through a lipoidal route that is also known as the transcellular process and is responsible for the transport of lipophilic drugs that show a rate dependency on their lipophilicity.


TRANSCELLULAR
PARACELLULAR
passive but selective
variable and regulated
Tight Junction

Factors effecting the bioavailability of drugs following intranasal administration

- **Physiological factors**
  - Speed of mucous flow
  - Blood flow
  - Pathological conditions of Nasal Cavity
  - Change in Physiological State
- **Dosage form factors**
  - Concentration of active drug
  - Physicochemical properties of drug
  - pH, Viscosity & Lipophilicity of drug
- **Administration factors**
  - Volume administered
  - Site of deposition
  - Mechanical loss into esophagus & other regions

NASAL PHYSIOLOGICAL FACTORS

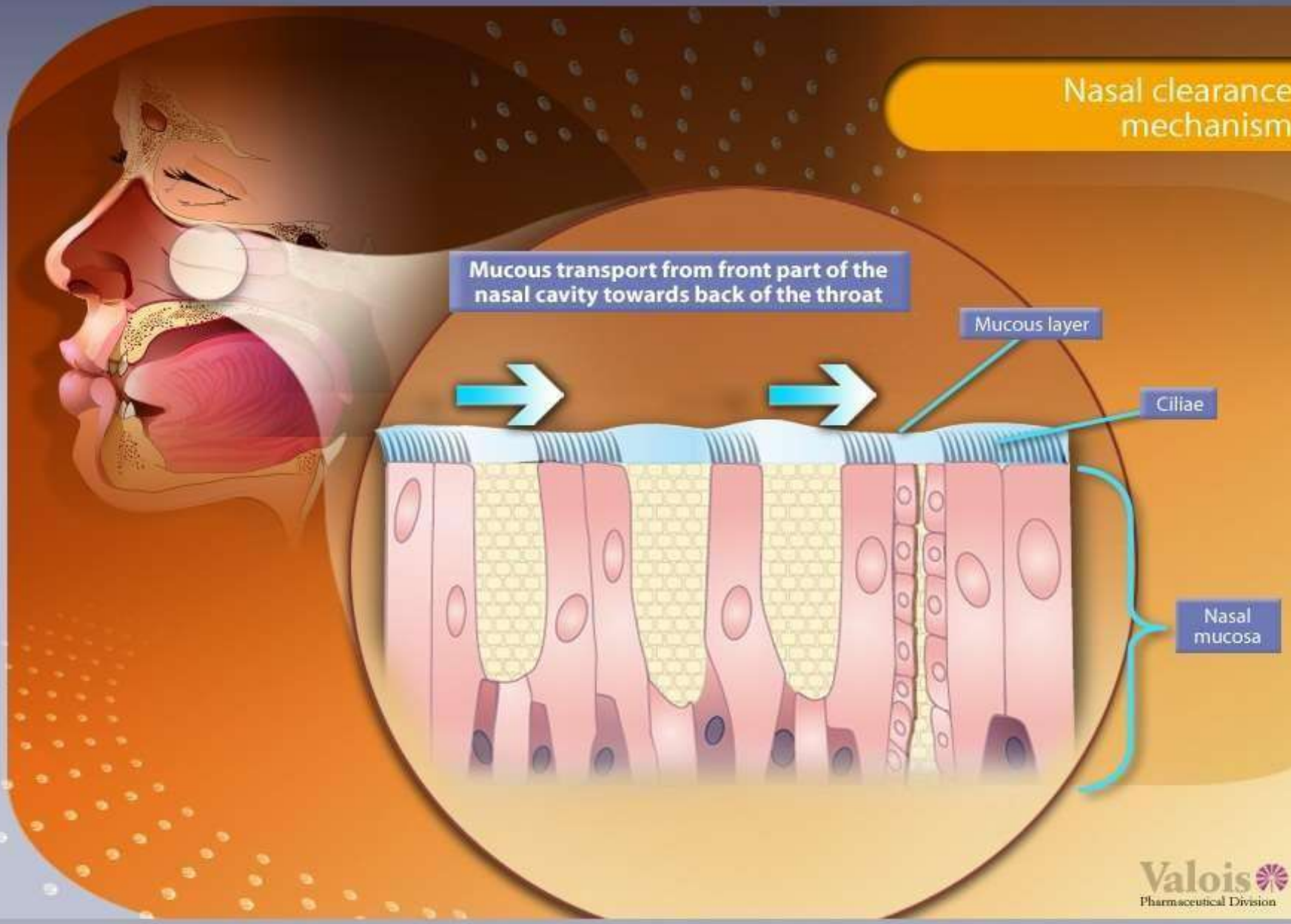
- ***Blood flow***

As the blood flow rate increases the amount of drug that reaches the general circulation also increases.

Mucociliary clearance (MCC)

One of the functions of the upper respiratory tract is to prevent noxious substances (allergens , bacteria , viruses , toxins etc) from reaching the lungs . When such materials adhere to , or dissolve in the mucus lining of the nasal cavity , they are transported towards the nasopharynx for eventual discharge into the GIT. Clearance of this mucus and the absorbed / dissolved substances is called the MCC.

Nasal clearance mechanism



Valois
Pharmaceutical Division

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FORMULATION FACTORS

pH, Concentration,

The pH of the nasal formulation should be adjusted to 4.5-6.5.
Concentration gradient plays very important role in the absorption / permeation process of drug through the nasal membrane due to nasal mucosal damage.

Viscosity

A higher viscosity increases contact time between the drug and the nasal mucosa thereby increasing the time for permeation.
At the same time, highly viscous formulations interfere with the normal functions like ciliary beating or mucociliary clearance and thus alter the permeability of drugs.

Osmolarity

Drug absorption can be affected by tonicity of the formulation. Shrinkage of epithelial cells has been observed in the presence of hypertonic solutions. Hypertonic saline solutions also inhibit or cease ciliary activity.

FACTORS RELATED TO DRUG

Molecular weight, lipophilicity and pKa

- On increasing lipophilicity, the permeation of the compound normally increases through nasal mucosa.
- In general, the passage across biomembranes is affected not only by lipophilicity/hydrophilicity, but also by the amount of drug existing as uncharged species. This depends on the drug pKa and the pH of the absorption site (5.0-6.5 in human nasal mucosa)
- Large particles (> 7 microns) will be lost in the gastrointestinal tract
- Small particles (< 3 microns) will be lost in exhaled breathe
- Intermediate particles (3 to 7 microns) reach the actual site of action

- Since the rate of absorption for most compounds is rapid, the extent of absorption is dependent on physiological factors such as rate of nasal secretion, ciliary movement and metabolism.

- The greater the rate of nasal secretion and the faster the ciliary movement, the smaller the bioavailability will be

Pharmacokinetics of Nasal Absorption

- The bioavailability of drug following intranasal route is given as

$$Ae = \frac{(AUC)_{i.n}\ (Dose)_{i.v}}{(AUC)_{i.v}\ (Dose)_{i.n}}$$

Zero order Kinetics

$$dX_B/dt = Ko - Ke\ X_B$$

where Ko is Zero order absorption rate constant
Ke is overall elimination constant

$$Cp = Ko/Cl\ (1 - e^{-ket})$$

where Cl is total body clearance
t time interval following drug administration

- First order kinetics

$$dX_B/dt = F\ X_{IN}\ Ka - Ke\ K_B$$

where Ka is first order absorption rate constant

F is fraction of applied dose absorbed

X_{IN} is amount of drug administered to absorption site

X_B is the amount of drug in central compartment

Ke is overall elimination constant

$$Cp = \frac{F\ XO_{IN}\ Ka}{V(\ Ka-Ke)}\ e - {}^{Ke t}\ - e^{-k\ a^t}$$

where XO_{IN} is initial dose applied to site of absorption at time zero

Nasal Administration

- The drugs employed for nasal delivery can be classified as
  1. Drugs administered for local action
  2. Drugs administered for systemic effects

Profile of an ‘ideal’ drug candidate for nasal delivery

- Appropriate aqueous solubility to provide the desired dose are about 0.25 to 0.3 ml per nostril.
- Appropriate nasal absorption properties
- No nasal irritation from the drug
- Low dose. Generally, below 25 mg per dose
- No toxic nasal metabolites
- Suitable stability characteristics

Formulation Excipients

SOLUBILIZERS

- Sometimes, it is necessary to use solubilizers in nasal formulations.
- One can use the conventional approach which includes the use of co-solvents. Some commonly used co-solvents include glycols, small quantities of alcohol.

BUFFER COMPONENTS

- Various conventional buffer systems can be used to buffer nasal formulations.
- It should be kept in mind that secretions can alter the formulation pH. A high buffer capacity is important to maintain in situ formulation pH.

ANTIOXIDANTS

- Depending upon the stability profile of a given drug in the formulation chosen, it may be necessary to use antioxidants to prevent drug degradation.
- Typically, sodium metabisulfite, sodium bisulfite, are used. Usually, antioxidants are used in small quantities and they may not affect drug absorption or cause any nasal irritation.

PRESERVATIVES

- Nasal formulations usually contain preservatives to protect them from microbial contamination.
- Parabens, benzalkonium chloride and benzoyl alcohol are some typically used preservatives.

HUMECTANTS

- To avoid any nasal irritation by formulation humectants are usually added to formulations.
- Some common humectants used include glycerin, sorbitol, and mannitol.

GELLING /VISCOFYING AGENTS

- Some formulations need to be gelled or made more viscous as formulation viscosity can affect drug absorption.

ENHANCEMENT IN NASAL ABSORPTION

Generally, the absorption enhancers act via one of the following mechanisms

- Inhibit enzyme activity.
- Reduce mucus viscosity or elasticity.
- Decrease mucociliary clearance.
- Open tight junctions.
- Solubilize or stabilize the drug.

General Approaches

1. Viscosity Modifiers
2. Absorption Enhancers
3. Bio adhesive Polymers
4. Colloidal Drug Delivery Systems

VISCOSITY MODIFIERS

- Increase nasal residence time of formulation and thus drug.
- Spray solutions with 0.25% methyl cellulose for administration of Desmopression and HPMC in other formulations.

ABSORPTION ENHANCERS

- Penetration enhancers act by increase in membrane fluidity by extracting proteins from nasal membrane & creating hydrophilic pores, altering the properties of mucous layer, facilitating the leaking of lipids and by opening the tight junctions between epithelial cells

Mucoadhesive drug delivery systems

- MCC is one of the most important limiting factors for nasal drug delivery, because it reduces the time allowed for drug absorption.
- Thus, improving nasal drug absorption can also be achieved prolonging the contact time between drug and nasal mucosa.
- Mucoadhesion implies the attachment of the drug delivery system to the mucus, involving an interaction between mucin and a synthetic or natural polymer called mucoadhesive.
- Mucoadhesives mostly used in intranasal drug delivery are chitosan, alginate and cellulose or its derivatives.
- Some of them may present other important characteristics which also enhance drug absorption.
- For example, chitosan is mucoadhesive and also causes a transient widening of epithelial tight junctions.

PERMEATION ENHANCER	EXAMPLE
Surfactants	Laureth -9
Bile salts	Deoxycholate
Chelators	EDTA, Citric acid
Fatty acid salts	Oleic, Lauric etc
Cyclodextrins	α- β- Cyclodextrins
Particulate carriers	Microspheres

COLLOIDAL CARRIERS

Ex: Starch, Dextran , Albumin etc

BIOADHESIVE POLYMERS

- They increase the time of contact between the delivery system and mucosa via bioadhesion
- Release the drug in controlled and sustained manner
- Protects the drug from enzymatic degradation in nasal cavity

Types of dosage forms and delivery systems

NASAL DROPS

- Advantage of this type of delivery is that it is perhaps the simplest and most convenient form of administering drug formulations into the nose.
- Disadvantage of this is that an exact amount of the formulation cannot be delivered.
- This dosage form was most popular

in the past because no

metered dose nasal devices

were available



SOLUTION SPRAYS

- As more sophisticated drug delivery devices became available, especially, metered dose nasal actuators, solution formulations were packaged in such delivery systems.
- Today, spray solutions are most commonly delivered through metered dose nasal actuator systems. These systems can deliver actuation volumes as low as 25 µl.

.



SUSPENSION SPRAYS

- Suspension dosage forms are also administered by using the metered dose nasal actuator systems .
- The actuator may have to be designed according to the specific needs taking into consideration the particle size and morphology of the drug particles

GELS

- Gels are either thickened / gelled solutions or suspensions of drugs delivered by metered dose gel devices.

Gels can offer the following advantages over other dosage forms

- Gels reduce anterior leakage of the drug out of the nasal cavity
- Gels also help localize formulation on the thereby providing a better chance for the drug to be absorbed.

NASAL DELIVERY DEVICES

Common devices are

- Droppers
- Squeeze bottles
- Spray pumps / atomizers (Accuspray Nasal Atomizer, MAD (Mucosal Atomization Device, nasal)
- Gel applicators
- Nasal Nebulisers (Sinus Nebuliser Rhino Clear)
- Pressurized Metered Dose Inhalers (pMDIs) Nasal (Ex: Landmark®)
- Disposable Unit/Bi-dose dispensing devices
- Powder Dispensing Systems





MA

Pulmonary Drug Delivery

- Drug delivery to or via the respiratory tree for the treatment of diseases has been long standing objective.

ADVANTAGES OF PULMONARY DRUG DELIVERY

1) It is needle free pulmonary delivery.
2) It requires small and fraction of oral dose.
3) Low concentration in the systemic circulation are associated with reduced systemic side effects.
4) Rapid Onset of action
5) Avoidance of gastrointestinal upset
6) Degradation of drug by liver is avoided in pulmonary drug delivery

DISADVANTAGES OF PULMONARY DRUG DELIVERY

1) Oropharyngeal deposition gives local side effect.
2) Patient may have difficulty using the pulmonary drug devices correctly
3) Drug absorption may be limited by the physical barrier of the mucus layer.
4) Various factors affect the reproducibility on drug delivery on the lungs, including physiological and pharmaceutical barrier.

- Generally, lung physiological investigations show that the airway and alveolar epithelia, not the interstitium and the endothelium, constitute the main barrier that restricts the movement of drugs and solutes from the airway lumen into the blood circulation.

1. Transcellular diffusion
2. Paracellular diffusion
3. Carrier-mediated transport
4. Vesicle-mediated transcytosis
5. Efflux transport

- Drug delivery to or via respiratory tree for the treatment of diseases like asthma, bronchitis has been a long standing objective.

- Drug delivery to the lungs can be achieved by two mechanisms, one is by aerosol drug delivery and second one is by direct instillation.

1. **AEROSOLS**
2. Nebulizers
3. Metered Dose Inhalers

They are the dosage forms containing therapeutically active ingredients that are packaged under pressure in a sealed container and are released as a fine mist of spray upon activation of a suitable valve system4.

Basic components

- 1. The container
- 2. Propellants
- 3. Product concentrate (containing API)
- 4. Valve and Actuators.

AEROSOLS

- Aerosols delivery systems consists of finely divided liquid droplets or solid particles in a gaseous suspension.
- Pharmaceutical aerosols may be classified as **space spray** and **surface coating** aerosols.
- **Space spray** disperse drug as a finely divided spray with particles less than 50μ in size and are suitable pulmonary drug delivery.
- Surface coating aerosols generate coarse and wet particles and are not suitable for pulmonary delivery.

- Limitations of aerosols include low patient compliance, drug metabolism, cost.
- The inhalation drug delivery systems are classified into

1. Nebulizers.
2. Metered dose inhalers.
3. Dry powder inhalers.

- The design of these systems is influenced by physicochemical properties of drug, target population and clinical objective to be met.

Nebulizers

- These are designed with aqueous solutions or suspensions .
- Normally water is used to prepare nebulizer solution and cosolvents like glycerine, ethanol andpropylene glycol may also be used.
- Nebulizers are classified as pneumatic type and electric type.
- For medical applications the pneumatic nebulizers can be jet or hydrodynamic while electric are ultrasonic type.

- The type of nebulizer and its operation determine the output and particle size of the aerosol generated.
- The droplets generated from nebulizers vary in size from1-10 μ.
- The advantages of nebulizers are the adjustment of flow rate with the inhalation rate of patient, large quantity of drug can be delivered through continuous nebulization.

Nebulizers

- In jet nebulizers, an aerosol is prepared by a high velocity air stream from a pressurized source directed against a thin layer of liquid solution.
- Ultrasonic nebulizers include the vibration of a piezoelectric crystal aerosolizing the solution.
- The nebulizer can transport more drugs to the lungs than MDI or DPI, the most common disadvantage of nebulizer are lack of possibility, higher costs of drug delivery as a result of the larger need for assistance from healthcare professionals, and the need for higher drug doses to achieve a therapeutic result.

NEBULIZERS



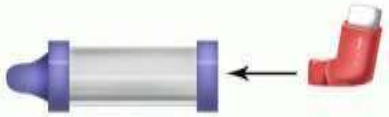


METERED DOSE INHALERS

How to Use a Metered-Dose Inhaler with a Spacer



1. Shake the medicine.



2. Insert the mouthpiece of the inhaler into the rubber-sealed end of the spacer.



3. Breathe all of the air out of your lungs. Then put the spacer into your mouth between your teeth. Make a tight seal around the mouthpiece with your lips.



4. Press the metered-dose inhaler down once to release a spray of medicine. The medicine will be trapped in the spacer. Breathe in slowly and deeply.



5. Hold your breath for at least 5 to 10 seconds. Breathe out slowly.

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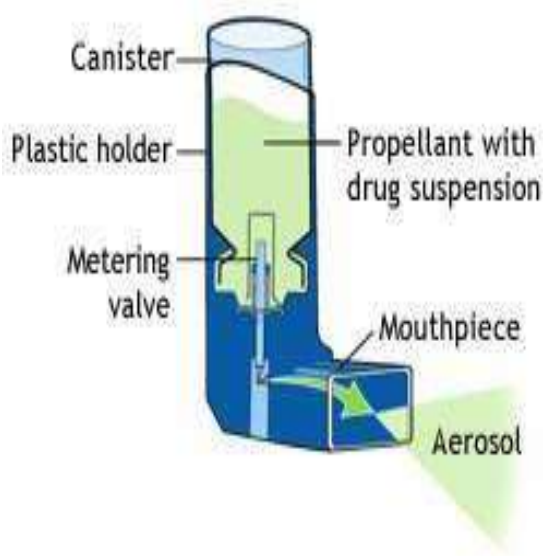

Spacer
Inhaler
Inhaler
AeroChamber
Spacer

Meterd Dose Inhalers

- MDI are sophisticated and safe dosage forms that can deliver accurate doses.

A typical MDI is composed of 4 components.

- The base formulation
- The container
- The metering valve
- The actuator or Mouth peice



Drug Targeting to Respiratory Tract

Physical targeting Or Site Specific targeting

- Non Spherical elongated Particles
- Salts And Precipitates
- Colloidal carriers

Chemical targeting

DRYING

G.SAMATHA
VCPS

Drying is defined as the process in which the removal of liquid from the material is done by the application of heat &is accomplished by transfer of liquid from the surface in to an unsaturated vapour phase.

OBJECTIVES:

- Drying is required during bulk manufacturing of granules, final stage compression as tablets or filling them in to capsule shells.
- Some of the products are obtained only by final stage drying process during their preparation. eg:Aluminum hydroxide, spray dried lactose, spray dried starch etc.,
- The bulkiness & weight of material can be reduced by drying.
- Certain drugs are to be dried for size reduction & also for extraction of active principles from them.
- Drying improves the stability of products.

FACTORS TO BE CONSIDERED IN SELECTING A DRYER FOR SUITABLE PURPOSE

- Whether the product is heat sensitive or not.
- The physical characters of the material to be dried.
- Nature of the solvent to be evaporated.
- Whether the process is to be carried out under aseptic conditions or not.
- The type & source of heat used.
- The quantity of material to be dried.
- The cost factor involved.

THEORY OF DRYING

- Drying involves both heat & mass transfer operations.

- Heat transfer involves application of heat to the material to be dried to undergo latent heat of vaporization of liquid present in it.

Heat transfer:- $dw / d\theta = q / \lambda$

Mass transfer:- Mass transfer depends upon rate of diffusion.

$$dw / d\theta = k^1 A (Hs - Hg)$$

$$k^1 = CGn$$

- The rate of heat transfer should be equal to rate of mass transfer.

$$dw / d\theta = q / \lambda = k^1 A (Hs - Hg)$$

- .

FACTORS INFLUENCING RATE OF DRYING

- Nature of material to be dried.
- Air flow
- Thickness of the solid bed & surface area
- Mode of heat transfer.

EQUILIBRIUM MOISTURE CONTENT

" EMC means vapour pressure exerted by the moisture present in the material is in equilibrium with that of vapour pressure exerted by moisture in the atmosphere".

- At EMC , the material is more stable & it does not absorb or loose moisture.

Significance of EMC :-

- It is advantageous to stop the drying process at EMC.
- Thermolabile materials can be saved from over drying.
- Length & time of drying can be saved.

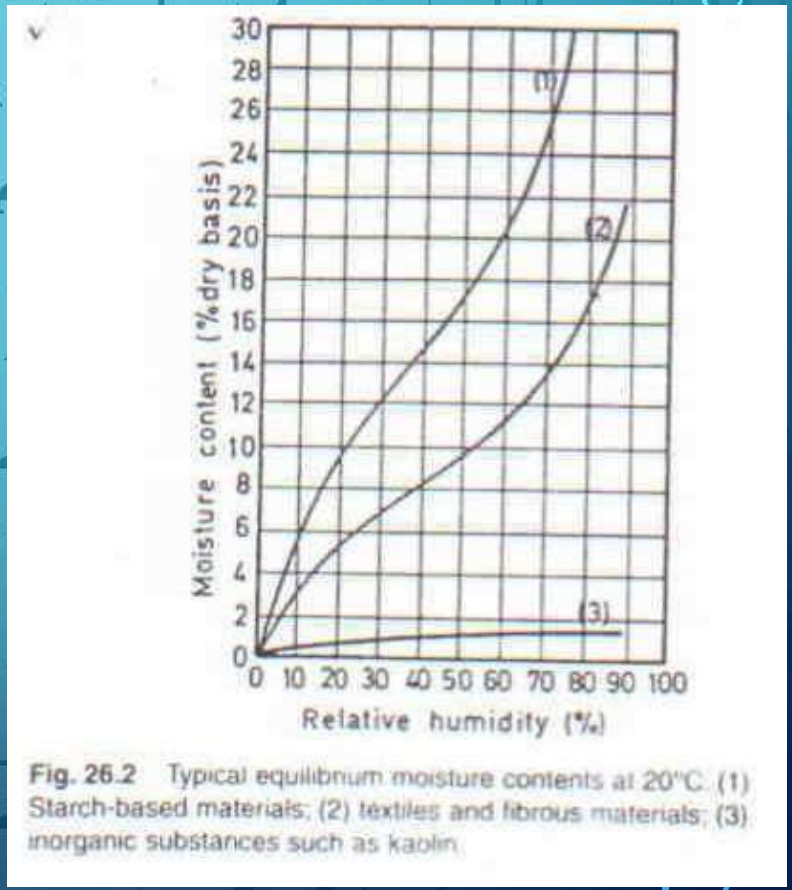


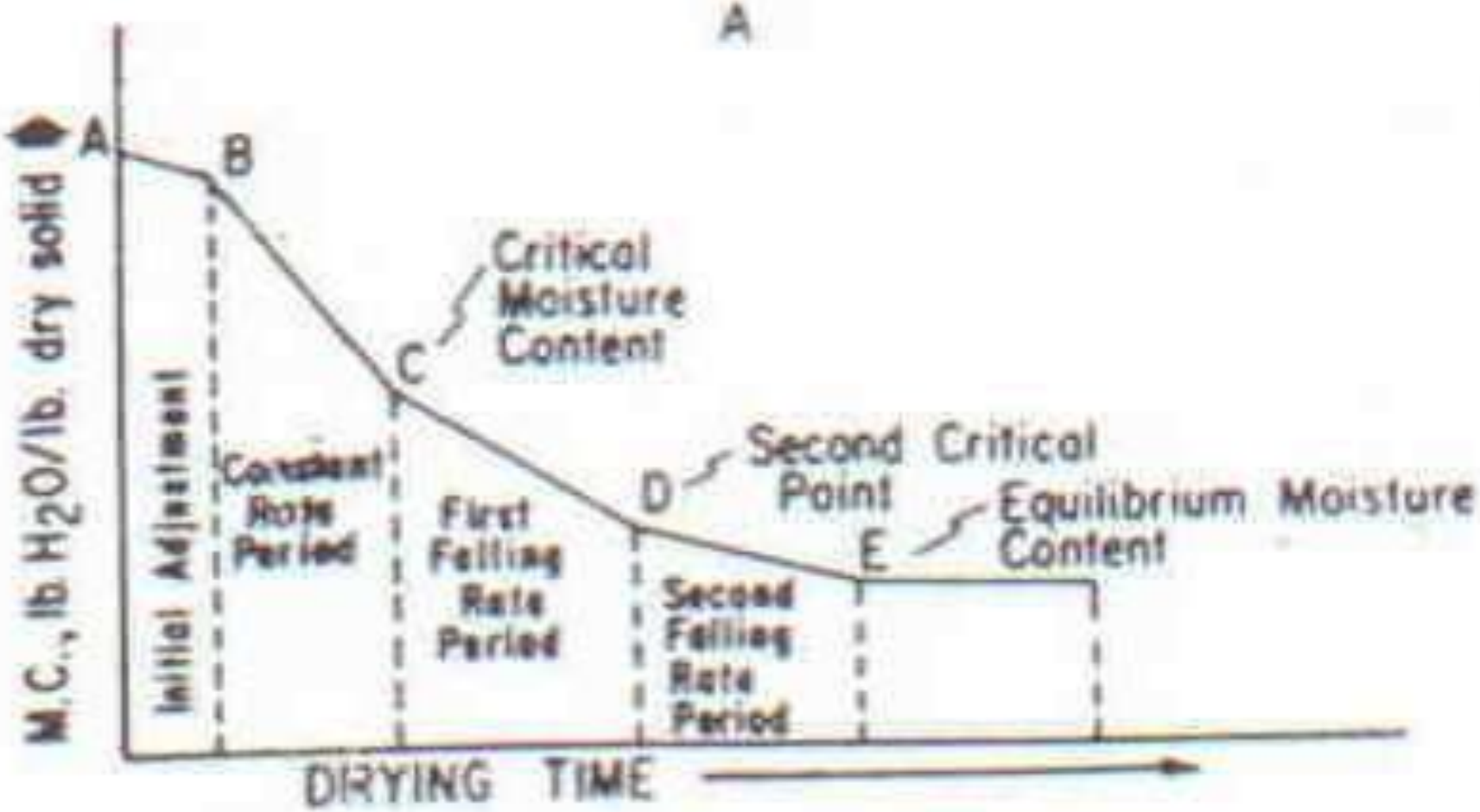
Fig. 26.2 Typical equilibrium moisture contents at 20°C. (1) Starch-based materials; (2) textiles and fibrous materials; (3) inorganic substances such as kaolin

EFFECT OF SHRINKAGE ON DRYING PROCESS

There are 3 different types of shrinkage.

1. Change in the surface of material per unit weight
2. Formation of hardened layer upon the surface
3. Warping or checking of material

DRYING RATE CURVES



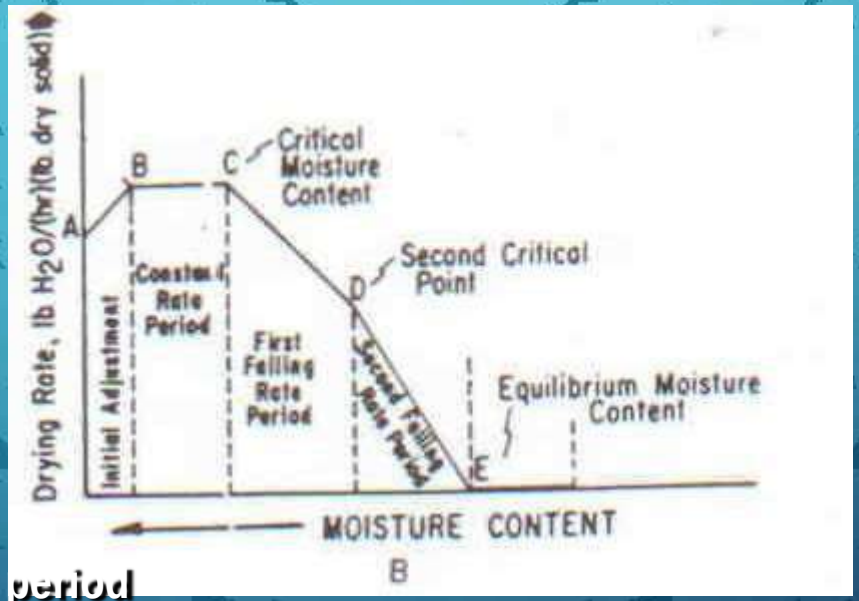
A-B : Initial adjustment period

B-C : Constant rate period

C-E : Falling rate period

C-D : First falling rate period

: Second falling rate period



A-B : Initial adjustment period
B-C : Constant rate period
C-E : Falling rate period
C-D : First falling rate period
D-E : Second falling rate period

CLASSIFICATION OF DRYERS

Based upon nature of material subjected to drying :

1. Dryers for drying of solutions, suspensions and emulsions. Eg: spray dryer, drum dryer
2. Dryers for drying of semi solid or sticky masses. Eg: pan dryer, agitated batch dryer.
3. Dryers for drying of damp solid masses. Eg: tray dryer, tray and truck dryer, tunnel and conveyer dryer, turbo tray dryer, rotary dryer, vacuum oven, freeze dryer, fluid bed dryer.

Based upon the mode of heat transfer:

1. Conductive dryers: Eg: Pan dryer, Batch dryer, vacuum oven, freeze dryer.

2. Convective dryers. Eg: tray dryer, tray and truck dryer, turbo and tray dryer, rotary dryer, FBD, spray dryer.

3. Radiant dryers. Eg: vacuum oven, tunnel and conveyor dryer.

Based upon the movement of material to be dried:

1. **Static bed dryers :**

Eg: drum dryer, tray dryer, tray and truck dryer, tunnel and conveyor dryer, freeze dryer, vacuum oven.

2. **Moving bed dryers :**

Eg: spray dryer, pan dryer agitated bed dryer, turbo and tray dryer, rotary dryer, FBD.

TRAY & TRUCK DRYER

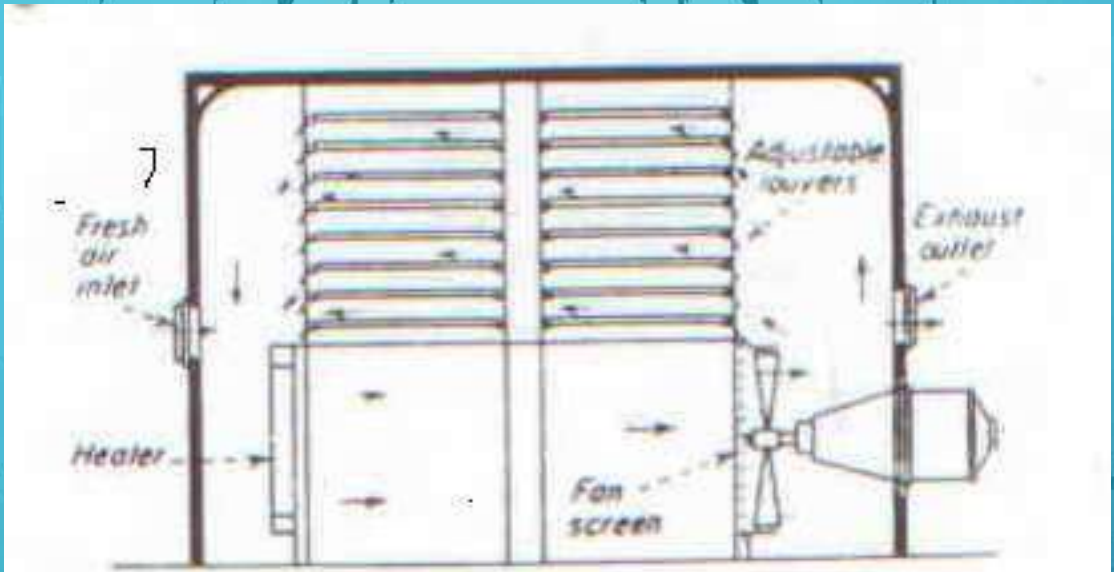


FIG. 3-6. *Tray dryer. (Courtesy of the Proctor and Schwartz Company.)*

ADVANTAGES:

- These are batch operated dryers.
- In pharmaceutical processes the maximum lot of material subjected may be up to 100-200 pounds which does not require continuous operations.
- Different kinds of materials can be dried in a batch wise process.

TUNNEL & CONVEYOR DRYER

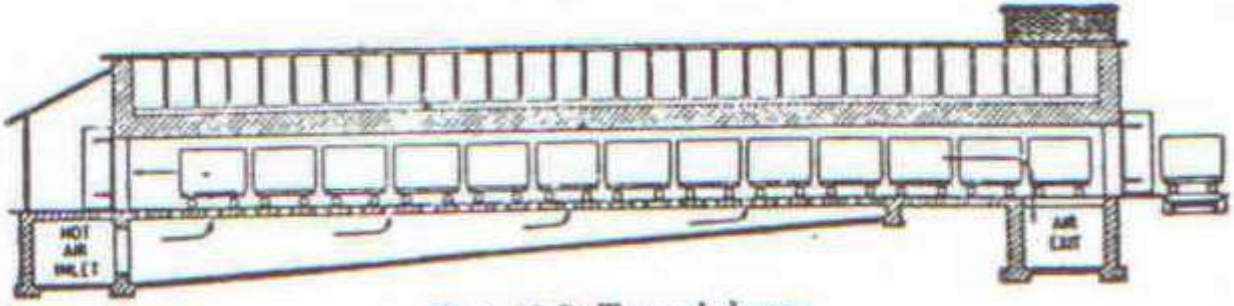
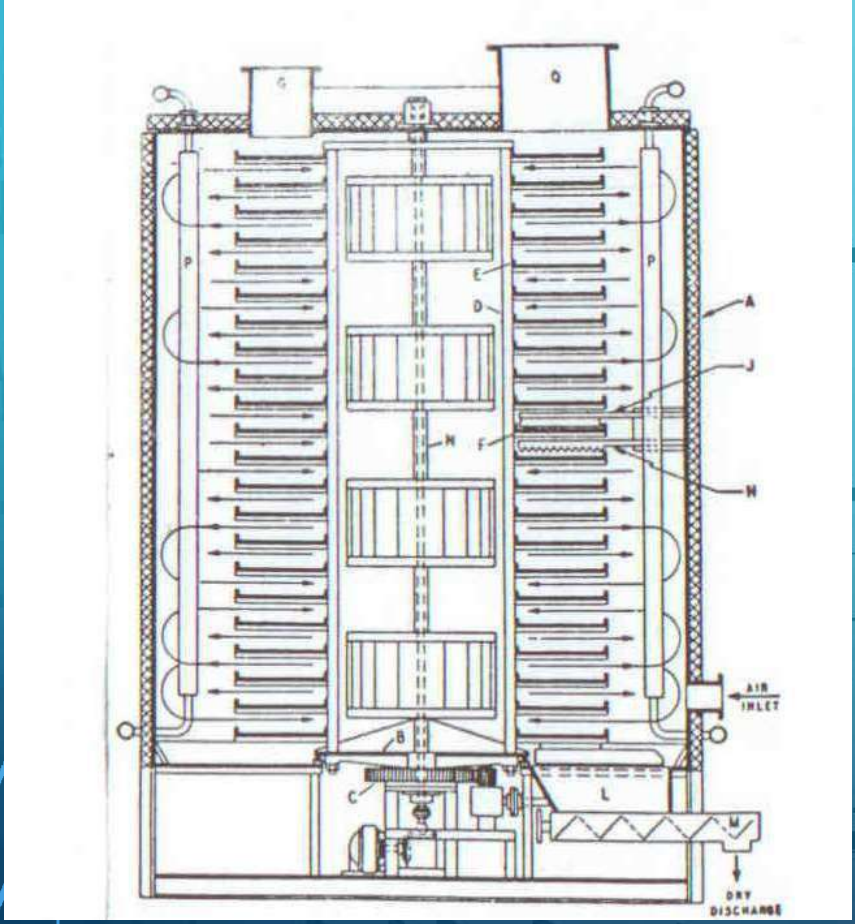
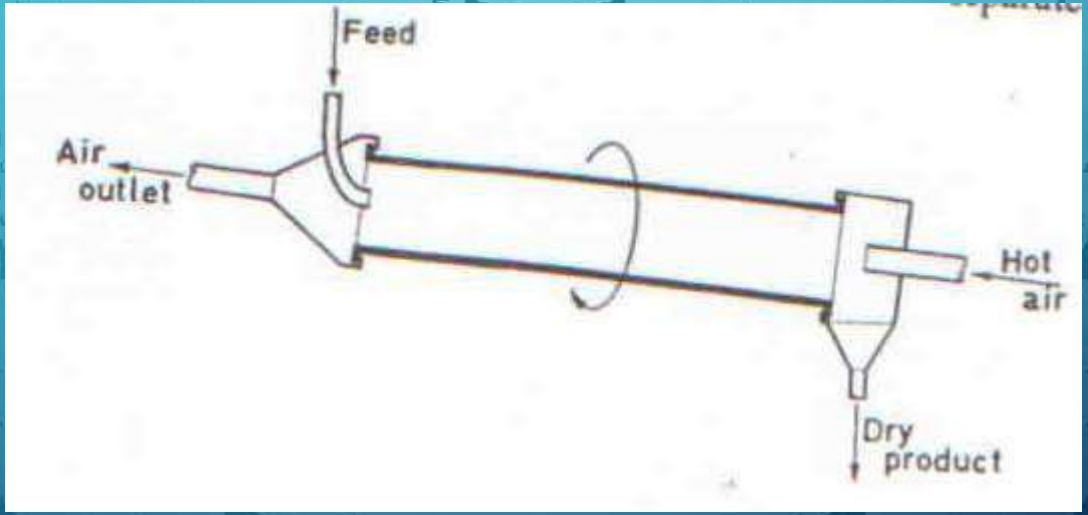


FIG. 10-3. Tunnel dryer.

TURBO & TRAY DRYER



ROTARY DRYER



AGITATED BATCH DRYER

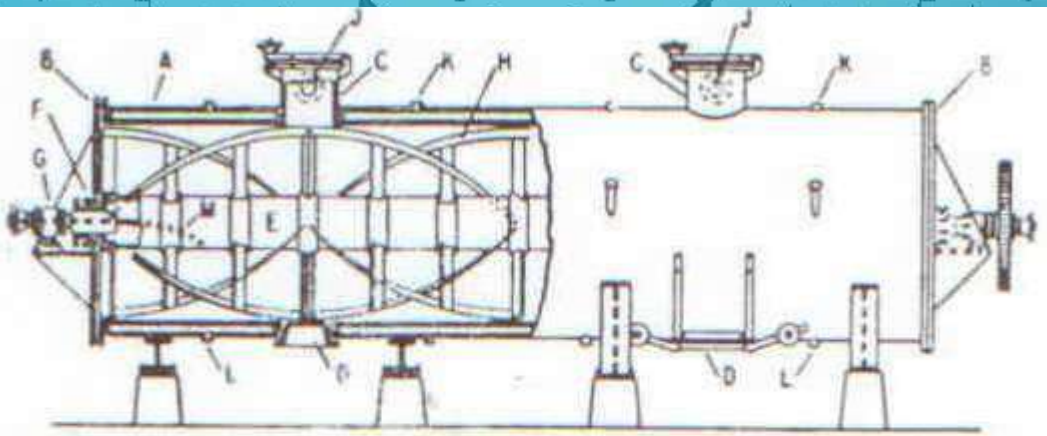
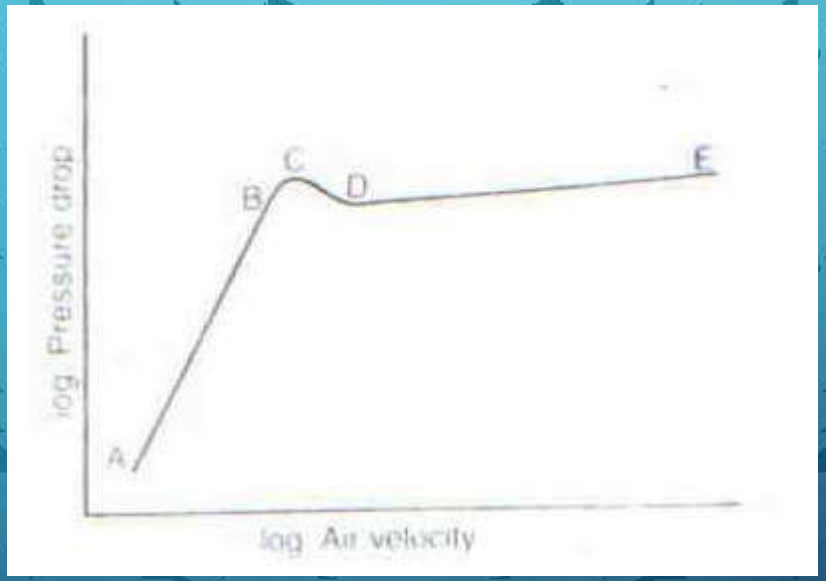
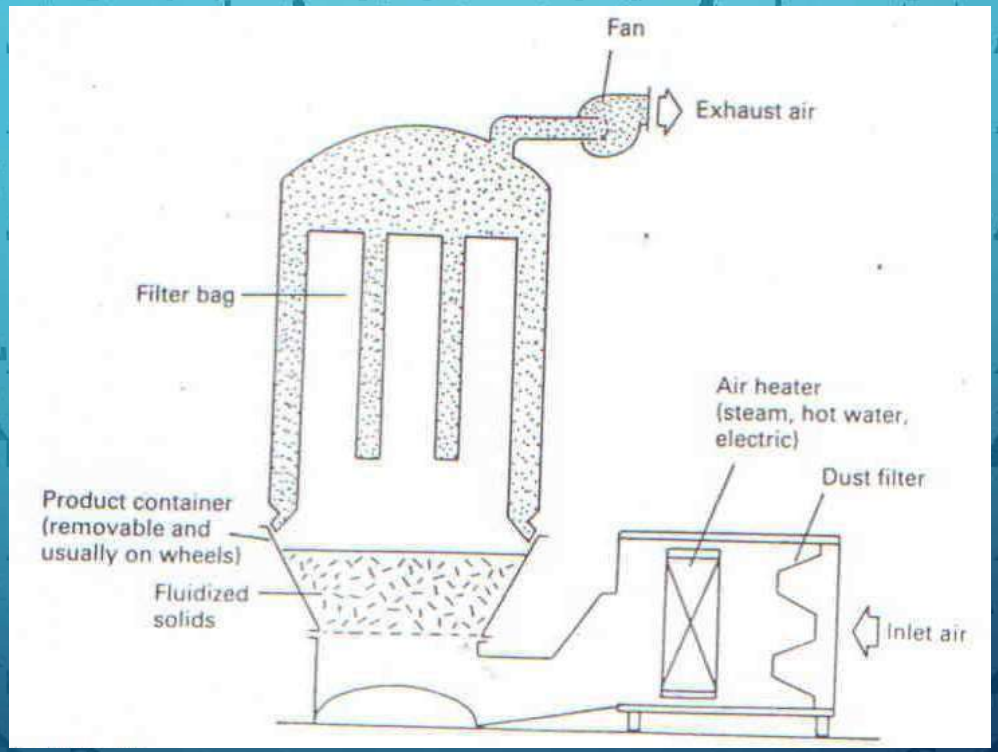


FIG. 10-10. Agitated-batch dryer: *A*, jacketed shell; *B*, heads; *C*, charging connections; *D*, discharge doors; *E*, agitator shaft; *F*, stuffing box; *G*, shaft bearings; *H*, agitator blades; *J*, vapor outlets; *K*, steam inlets; *L*, condensate outlets; *M*, discharge siphon for shaft condensate. (*Buflovac.*)

FLUID BED DRYER




Fan
Exhaust air
Filter bag
Air heater
(steam, hot water,
electric)
Dust filter
Product container
(removable and
usually on wheels)
Fluidized
solids
Inlet air

ADVANTAGES :

- The temperature of FBD is constant and drying time is very short.
- The fluidized state produces a free flowing product.
- The free flowing of individual particles eliminates the risk of soluble material migration.
- The containers are mobile, making handling simple & reducing the labor costs.

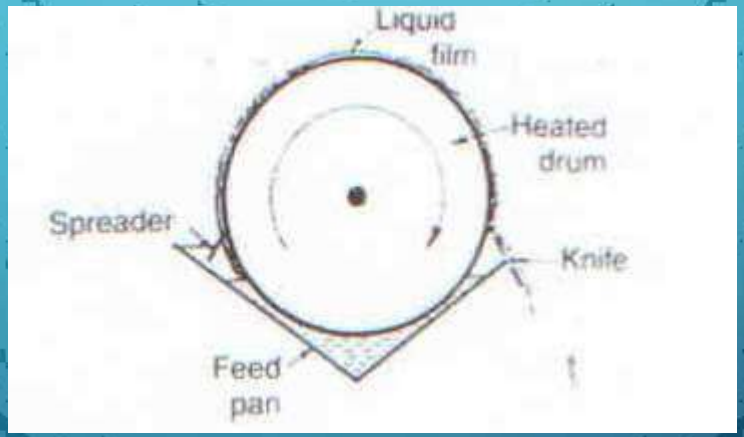
DISADVANTAGES :

- The turbulence of fluid state may lead to production of fines.
- Fine particles may become entrained and must be collected by bag filters.
- The vigorous movement of particles can lead to generation of static charges.

APPLICATIONS :

- It is mainly used for drying of solid powder material & tablet granules.
- It is used for production of spansules or beads for micro encapsulation of products. Egg: ferrous sulphate beads.
- It is used for production of pelletization of drugs which undergo degradation. Eg: omeprazole pellets.
- It is used for the coating of granules or small tablets for their enteric or film coating.

DRUM DRYER



ADVANTAGES:

- It is versatile piece of equipment.
- It offers rapid drying in few seconds.
- It produces a flaky mass of solid material.
- It can also be used for thermolabile materials under vacuum.
- It can be continuously operated for one type of material.

DISADVANTAGES:

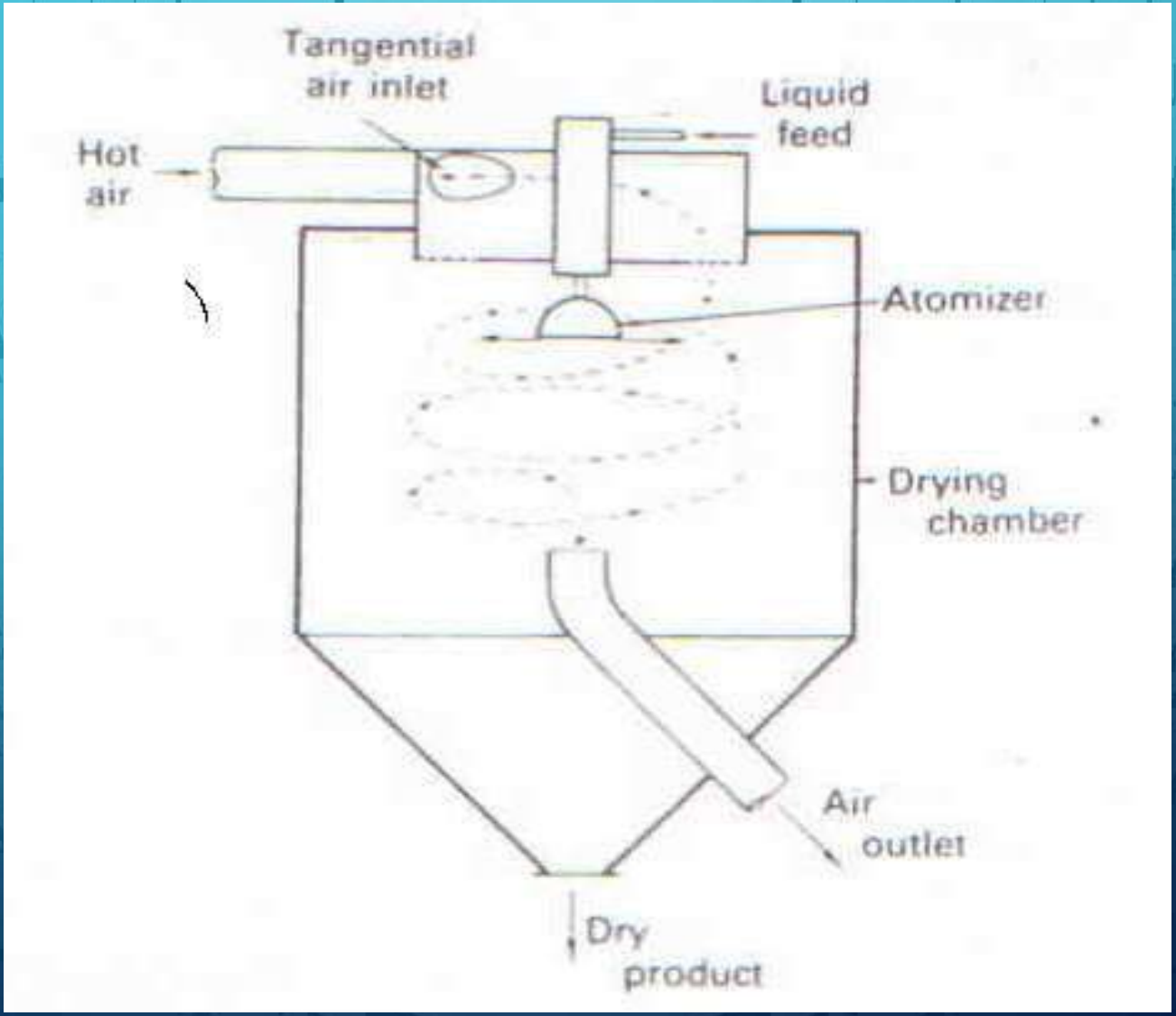
- The drying rate is highly critical.
- Not suitable for salt solutions containing insoluble or poorly soluble substances.

APPLICATIONS:

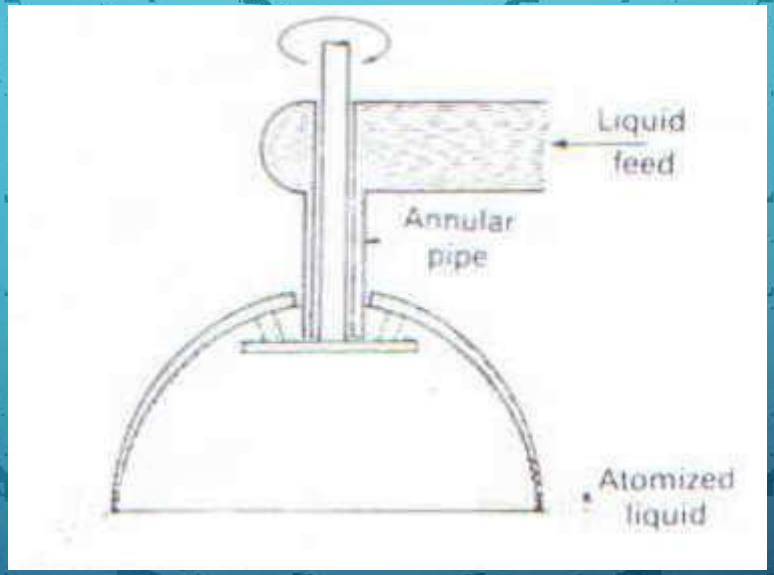
- It is mainly used for drying of solutions or suspensions.

- Used for drying of materials such as milk products, starch products, ferrous containing solutions, suspension of kaolin, ZnO, yeast suspension, pigment solution, malt extract, antibiotics, calcium carbonate, barium carbonate etc.

SPRAY DRYER


Tangential
air inlet
Liquid
feed
Hot
air
Atomizer
Drying
chamber
Air
outlet
Dry
product

ROTARY AUTOMIZER



ADVANTAGES:

- The products obtained has low density , improved solubility & bulky in nature.
- Rate of drying is very rapid.
- Spherical shaped granular products are produced which are directly compressible.

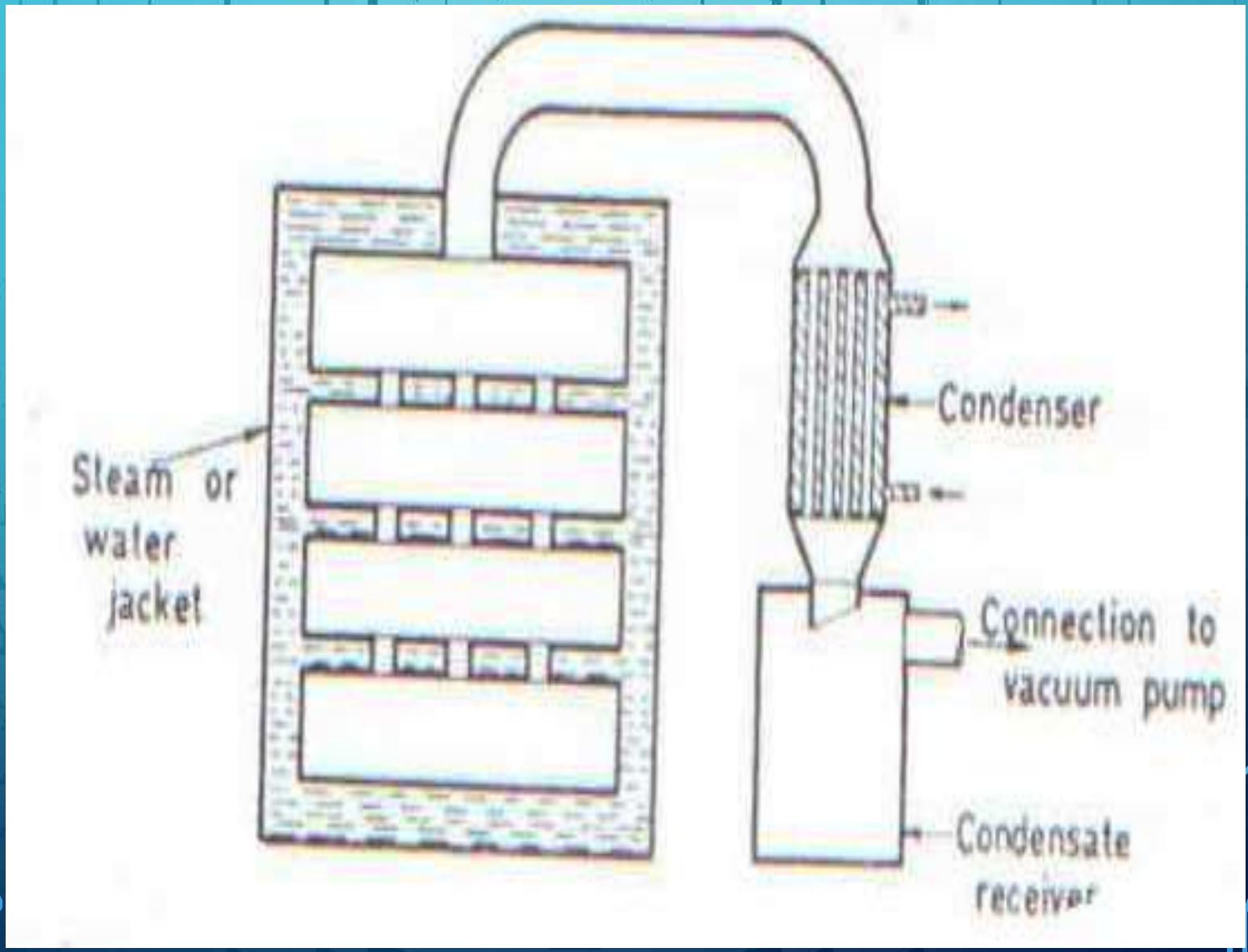
DISADVANTAGES:

- A typical spray dryer occupies 15m height& 6m diameter.
- The thermal efficiency is rather low.

APPLICATIONS :

- It is highly suitable for drying of thermolabile materials, suspensions and emulsions.
- For micro encapsulation of oily substances like vitamin-A, D by using polymer.
- For producing reconstitutable products.
- Masking bitter taste by incorporation of organic polymer to coat the dry products.
- Drying of materials aseptically .Eg:serum hydrolysate.
- Widely used for drying of milk products, milk etc .
- The spray dried products are borax, barium sulphate, calcium phosphate, soaps & detergents.
- It is also used for drying of serum hydrolysate, coffee & tea extracts, vitamins, hormones, plasma products, yeast products ,bacitracin, methyl cellulose, vaccines, chloramphenicol, pancreatin, penicillin etc.

VACUUM OVEN


Steam or
water
jacket
Condenser
Connection to
vacuum pump
Condensate
receiver

ADVANTAGES :

- Thermolabile substances can be dried at low temperatures.
- A porous & friable products can be obtained during drying, suitable for tablet manufacturing.
- Costlier solvents can be recovered by using condenser.

DISADVANTAGES :

- Heat transfer may be low & non uniform.
- The capacity is limited.
- The labour & running costs are very high
- There is a danger of entrainment of fines.

APPLICATIONS :

- It is used for heat sensitive materials which undergo decomposition under high temperatures.eg: Alkaloids,glycosidal extracts.

- It is used for drying of hygroscopic & dusty materials.

- Used for the purpose of producing drugs in porous form.

FREEZE DRYER

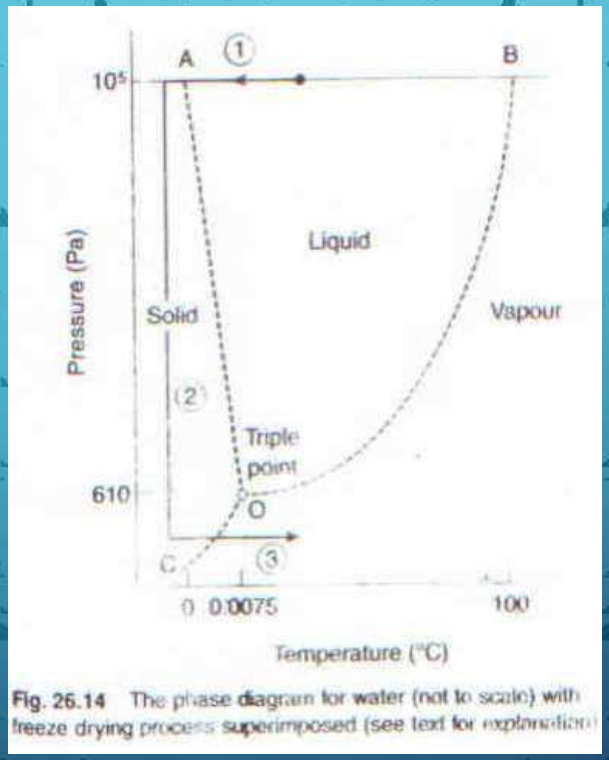


Fig. 26.14 The phase diagram for water (not to scale) with freeze drying process superimposed (see text for explanation)

PRECAUTIONS :

- The material to be dried should be maintained below the triple point conditions ie.-10 to -30c
- The surface area provided should be increased.
- The vapours raised should be removed as quickly as possible.
- The packing of the product should be done at the similar vacuum conditions.

STEPS INVOLVED IN FREEZE DRYING:

- Freezing :

  1.shell freezing.

  2.Vertical spin freezing.

  3.Centrifugal evaporative freezing
- Vacuum application or primary drying
- Heat supply
- Vapor removal
- Secondary drying
- Packing

ADVANTAGES:

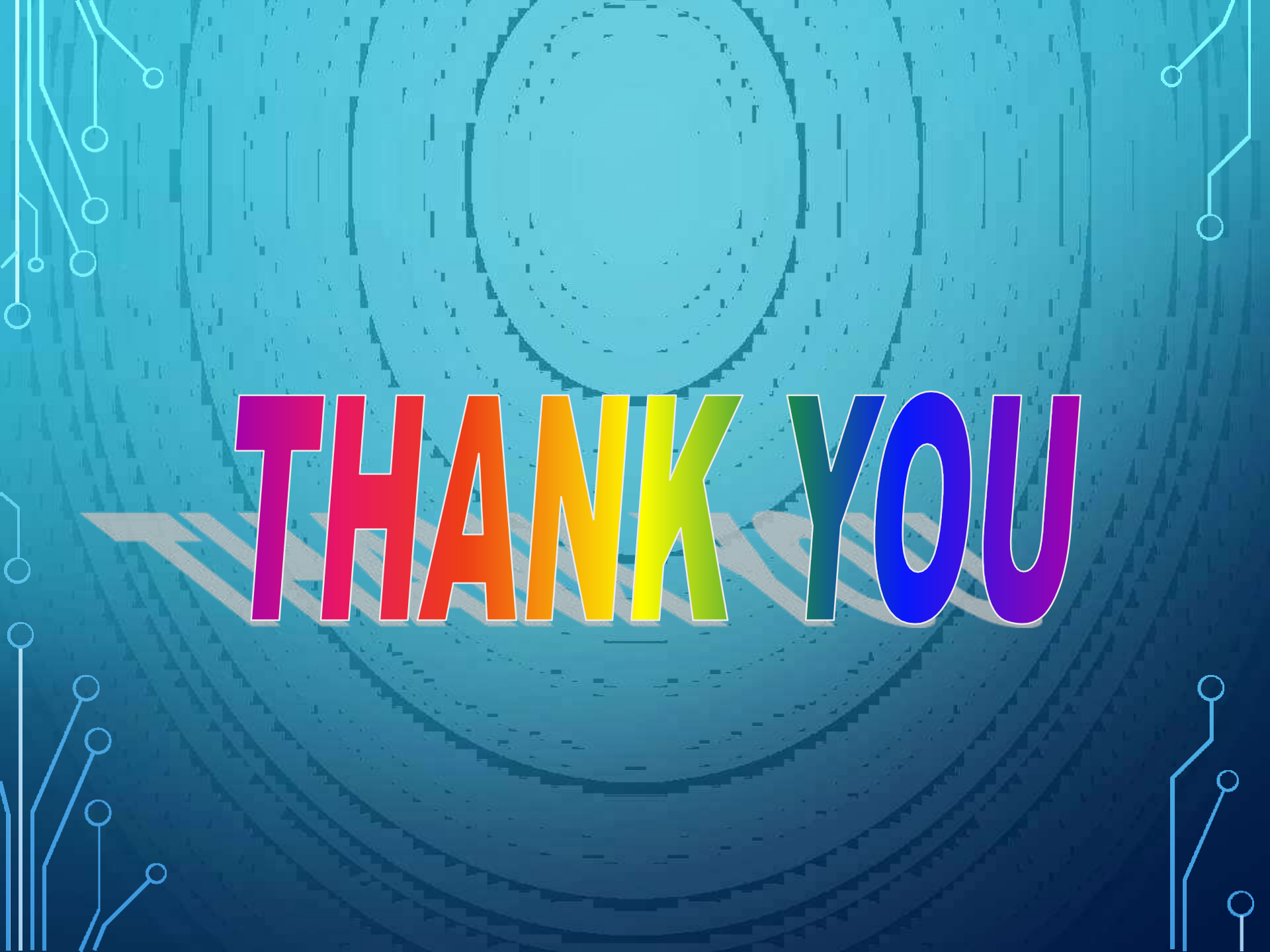
- Thermo labile products can be freeze dried without undergoing oxidation or hydrolysis.
- The product obtained is light, porous & improves the solubility.
- There is no concentration of solutions prior to drying.
- Under high vacuum the drying process can be carried out in a highly aseptic conditions.

DISADVANTAGES:

- The product obtained by freeze drying is highly hygroscopic in nature.
- The process is very slow and requires a very complicated plant which is highly expensive.
- It is difficult to dry solutions containing hot aqueous solvents.

APLLICATIONS:

1. Freeze drying is mainly used for drying of biological products such as vaccines, seras, enzymes, blood products, antibiotics, etc.
2. It is used in maintenance and preservation of microbial cultures.
3. Fibrinogen dissolved in sodium chloride injection and then clotted by addition of human thrombin is freeze dried.
4. A solution of gelatin containing formaldehyde is freeze dried and is used for surgical dressing.
5. Freeze dried hemoglobin formulated with sucrose is widely used to prevent dehydration of hemoglobin.
6. Vaccines produced by freeze drying are BCG, yellow fever, small pox, chicken pox, tetanus, hepatitis B vaccines etc.


THANK YOU

Ethical Guidelines in Clinical Research

Dr.Y.Kiran
Associate Professor, VCPS

Physician/Patient Relationship

- Dilemmas of physicians and health care providers with conflicting roles of helping the patient and gaining scientific knowledge.

- A properly designed and conducted clinical trial is an ethically appropriate way to acquire new knowledge.

- Clinical decisions for patient treatment without strong evidence of rigorous scientific support has ethical questions.

Clinical Research or Clinical Practice???

- How often is a physician certain of the outcome from a specific therapy for a particular patient?
  - If patient's reaction is predictable, applying treatment is practice.
  - Physician is unsure of the outcome, applying the treatment could be considered research.
- Actions by the physician for the benefit of individual patients have the potential of increasing scientific knowledge.
- Scientific knowledge gained from research can be of benefit to individual patients.

When do ethical questions arise?

- Unproven therapies are proposed to replace proven ones and are particularly acute for chronic or fatal illness.

- Clinical trials are one of several settings in which the physician's duty extends beyond his/her responsibility to the individual patient.

- Example: vaccinations against communicable disease are promoted by physicians, yet the individual vaccinated incurs a small risk to benefit the population as whole.

Randomization (1)

- Physicians and patients may feel it is inappropriate to base a patient's treatment on chance.
- Physicians may feel an obligation to have a preference even when the evidence does not favor any particular treatment.
- Randomization is justified when there is relative ignorance about the best treatment.

Randomization (2)

- Physicians and patients with firm preferences for treating a particular indication should not participate in a clinical trial.
- Patients with strong preference about a treatment are likely to become easily dissatisfied with randomization to a treatment in the clinical trial.
- Physicians with strong convictions could bias the clinical trial in a different direction.

Informed Consent

- Patients (or parents/guardians/family members) must sign an informed consent prior to participation in a research study.
- Sick and dying patients and their families maybe vulnerable and technical information may be presented in complicated ways!!!
- Informed consent procedures were developed to protect patients from exploitation.
- Currently informed consent is viewed as protection from litigation.
- IRB must evaluate the informed consent form prior to the start of the study.

Confidentiality

- The U.S. Department of Health and Human Service (HHS) sets the Standards for Privacy of Individually Identifiable Health Information under the Health Insurance Portability and Accountability Act of 1996 (HIPAA).

- This provided the first comprehensive Federal protection for the privacy of personal health information.

- Took effect April 14, 2003.

- The DHHS (web site http://privacyruleandresearch.nih.gov) for further information about HIPAA.

Oversight Groups

- Institutional Review Boards
  - Checklist for informed consent
  - Informed consent sample information
- Scientific Review Committees
- Data and Safety Monitoring Boards
  - NCI policy on DSMBs
- Office for Protection from Research Risks (OPRR)

Other Committees

Steering committee

Executive committee

Treatment effects monitoring committee (DSMB)

Advisory review committee (Conduct)

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Active-control Trials

- Placebo trials are not always ethical.
- Placebo control is untenable when the disease is life-threatening and an effective therapy is available.
- Comparison is made between active control and the experimental treatment or therapy.

Historical Perspective

- Nuremberg trials (1946-47): atrocities in WWII concentration camps committed by Nazi physicians.
- 20 of the 23 tried for the crimes were physicians.
- No international standards for ethical conduct in human experimentation during this time.
- Results: Nuremberg Code adopted in 1947.

Nuremberg Code

- Voluntary consent is essential
- Must be no reasonable alternative to conducting the experiment
- Anticipated results must have a basis in biological knowledge and animal experimentation must have potential for meaningful results for the good of society.
- Avoid unnecessary physical and mental suffering and injury.
- No expectation of death or disability.
- Risk should not exceed humanitarian importance.
- Protection against even a remote possibility of death or injury.
- Qualified scientists.
- Subject can stop at will.
- Investigator has an obligation to terminate the experiment if injury or death seems likely.

Helsinki Declaration

- The World Medical Association (WMA) adopted a formal code of ethics for physicians engaged in clinical research in 1964 in Helsinki, Finland.

- Latest complete revision in 2000 (Helsinki Declaration).

- It reiterates the principles of Nuremberg Code with particular attention to the duty of physician to protect the life and health of human subjects.

- Written protocol must be reviewed by an ethical review committee independent from the investigator.

Other International Guidelines

1. International Covenant of Civil and Political Rights (1976) adopted by the United Nations General Assembly.

2. The World Health Organization (WHO)

3. The Council for International Organizations of Medical Sciences (CIOMS) issued the International Ethical Guidelines for Biomedical Research Involving Human Subjects.

Tuskegee Syphilis Experiment

- The U.S. government's 40-year experiment on black men with syphilis in Tuskegee, Alabama (1936).
- Long after the availability of penicillin, a proven cure in the 50's, the US Public Health Service studied untreated black men.
- The study was stopped in the early 1970's after it was publicized.
- President Clinton's apology for the Tuskegee Syphilis Experiment to the eight remaining survivors, May 16, 1997.

Modern Perspective

- Congress established the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research through the 1974 National Research Act.

- The Commission produced the Belmont Report in 1974 which distilled basic ethical guidelines in research with human subjects.

- Three principles: respect of persons or individual autonomy, beneficence and justice.

Individual Autonomy – patients have the right to decide what should be done for them with respect to their illness unless the result would be clearly detrimental to others.

Beneficence – patient's right to receive advantageous or favorable treatment.

Justice – fairly distributing the benefits and burdens of research.

IRB

- The U.S. National Institute of Health Policies for the Protection of Human Subjects (1966) established the IRB to protect human participants in research.

- In 1981, U.S. regulations required IRB approval for all drugs or products regulated by the FDA.

- Prerequisites set forth by the FDA:
  - Risks to participants are minimized
  - Risks are reasonable in relation to the anticipated benefits
  - Selection of study participants is equitable
  - Informed consent obtained appropriately
  - Adequate provisions for monitoring data collection
  - Privacy of the participants and the confidentiality of the data protected

Components of Informed Consent

- Research nature of the study
- Reasonable foreseeable risks and discomfort
- Potential benefits and alternatives
- Procedures for maintaining privacy
- Treatment for injuries incurred
- Individuals to contact for questions
- Voluntary nature of the study and the possibility of withdrawal at any time
- Not entering the study does not lead to loss of benefits

EVAPORATION

By

G.Natesh

VCPS

Evaporation

- It may be defined as the vaporization of a portion of solvent from a solution of a solid, leaving a concentrated liquid residue.
- **Objectives:**

1. Liquid, soft and dry extracts are prepared employing evaporation process.
2. It is also used in the concentration of blood plasma and serum.
3. Used in the preparation of medicaments containing enzymes, hormones, antibiotics and many other agents.

Differences between Evaporation, Distillation & Drying

EVAPORATION	DISTILLATION	DRYING
Components are heated below their boiling points	Components are heated at their respective boiling points	Components are heated above their boiling points
Vaporization takes place from surface of liquid	Vaporization takes place from entire body of liquid	Vaporization takes place from entire solid bed
Condensation of vapors are not required	Condensation of vapors are required to get purified product	Condensation of vapors are not necessary

General Principles of Evaporation

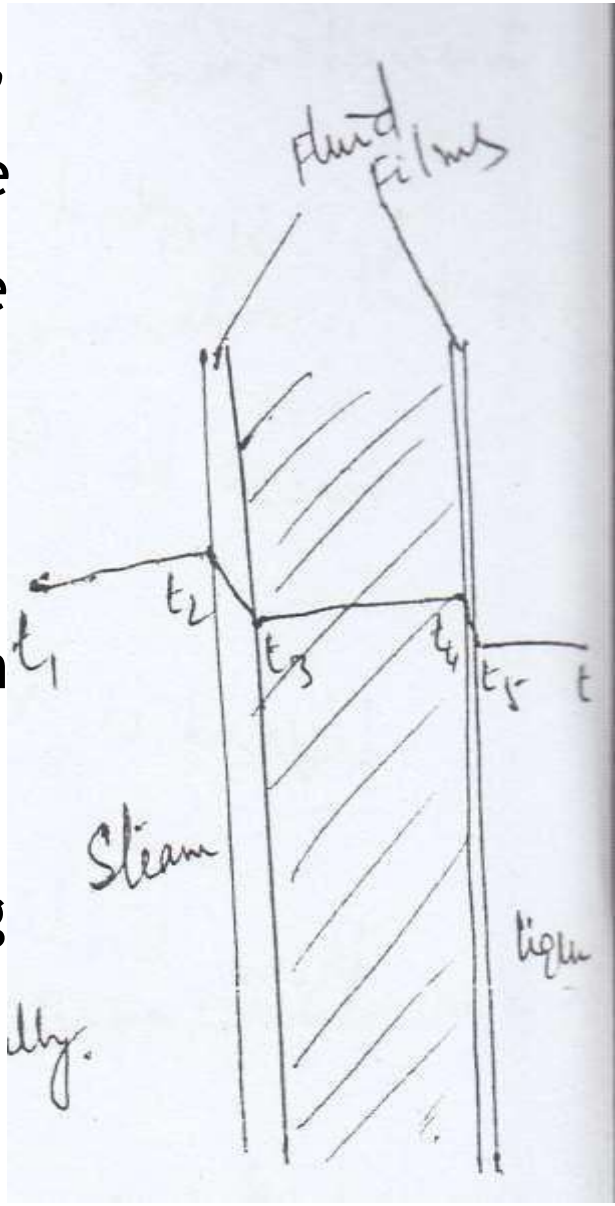
- Various factors influencing the heat transfer co-efficient of an evaporator are;

1. Vapor pressure
2. Boiling point
3. Effect of dissolved substances
4. Heat supply and vapor removal
5. Heat transfer

Heat Transfer

- When steam is used as a source of heat, there are three obstructions to the transfer of heat from the steam to the substance being heated. They are;

1. The Wall of the evaporator.
2. The film of condensed steam on steam side of wall.
3. The liquid film of substance being heated on the other side of the wall.



Film Co-efficients

- The general equation for heat transfer can be expressed as follows;

$$q = UA\Delta t$$

- Flow of heat through a solid can be represented by equation;

$$\frac{q}{A} = \frac{\Delta t K}{L}$$

- Flow of heat through a fluid film is expressed by equation;

$$\frac{q}{A} = \Delta th$$

- Flow of heat through fluid films is expressed by equations;

$$\frac{q}{A} = \Delta th1 \qquad \frac{q}{A} = \Delta th2$$

- The total resistance per unit of cross sectional area is the summation of fluid film resistances, the wall resistances and equals;

$$\frac{1}{h1}+\frac{1}{K}+\frac{1}{h2}$$

Where h1 and h2 are the film coefficients of the steam film and the liquid film respectively.

- The reciprocal of this resistance can be treated as a conductance U, so that

$$\frac{1}{U}=\frac{1}{h1}+\frac{1}{K}+\frac{1}{h2}$$

- The rate of heat flow from the steam side to liquid side is

$$\frac{q}{A}=\frac{t6-t1}{1/U}=(\mathrm{t6}-\mathrm{t1})\mathrm{U}$$

U is over all heat transfer coefficient

Factors influencing Rate of Evaporation

- Various factors influencing the rate of evaporation are;

1. Temperature
2. Temperature and time of evaporation
3. Temperature and moisture content
4. Type of product required
5. Effect of concentration

Classification of Evaporators

- They are classified based upon the movement of heat transfer.They are;

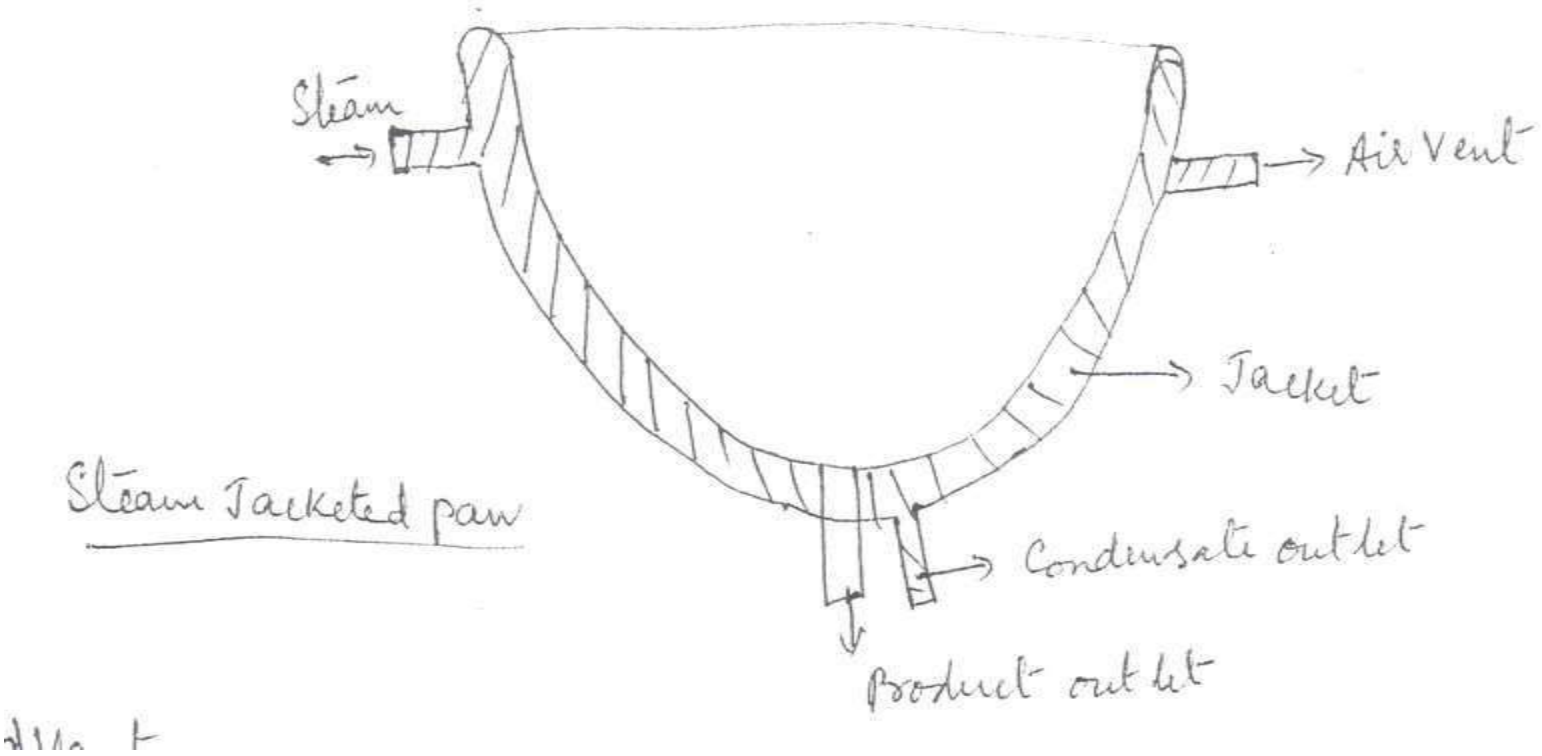
1. Natural circulation evaporators
2. Forced circulation evaporators
3. Film evaporators

Natural Circulation Evaporators

- They are of three types;

1. Evaporating pans or Steam jacketed pans
2. Evaporating stills
3. Short tube evaporator

Evaporating pans or Steam jacketed pans



- **Advantages:**

1. It is simple and cheap to construct.
2. It is easy to use, clean and maintain.

- **Disadvantages:**

1. Heat transfer in these pans is very poor and solids are likely to deposit on the surface, leading to decomposition of the product. Also many products give rise to foaming when boiled under conditions of natural convection.
2. The entire liquid is heated all the time, which is not suitable for thermolabile materials.
3. The heating surface is limited and decreases proportionally as the size of the pan increases. Further more the heating area will decrease as concentration of the product occurs.

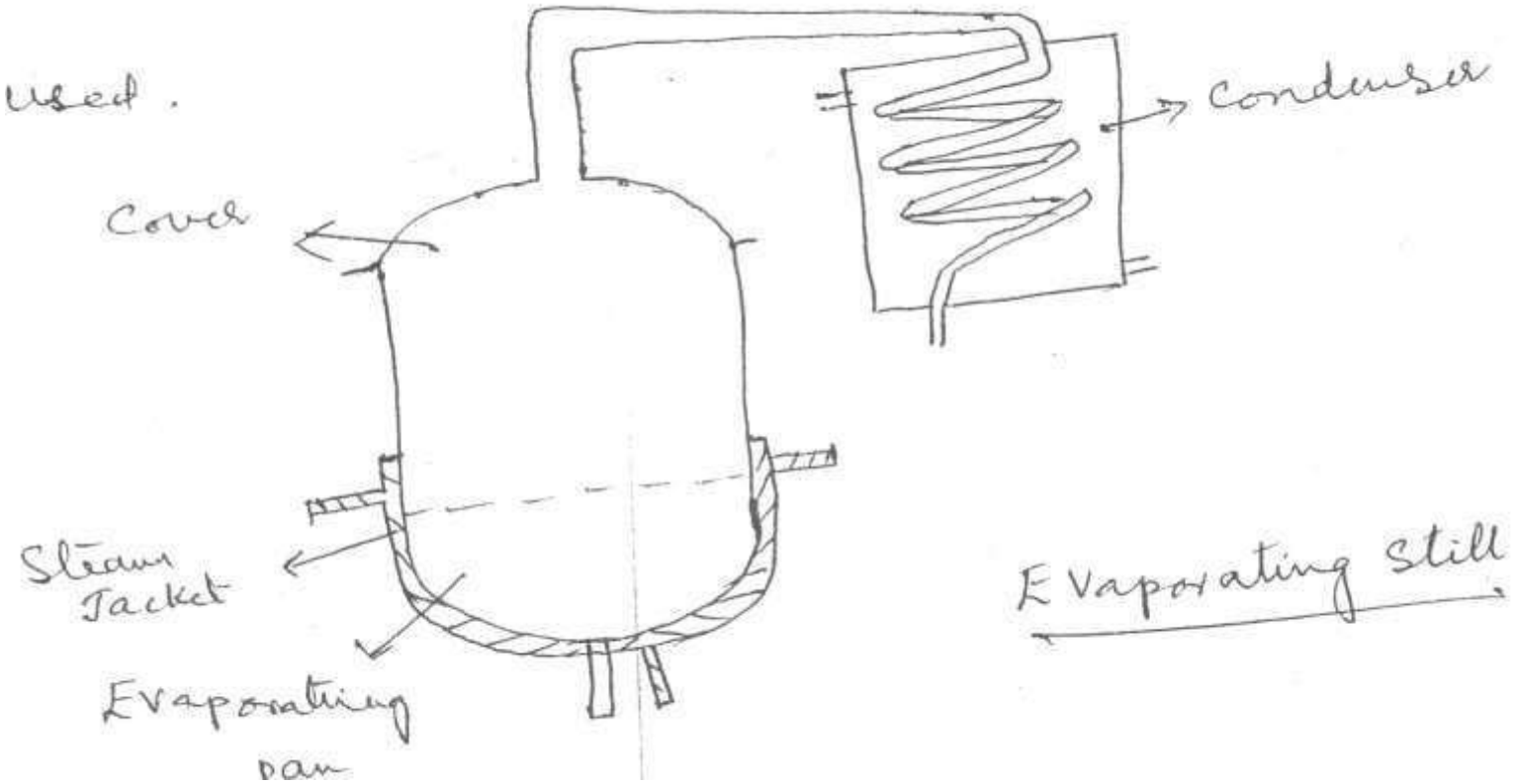
- **Disadvantages:**

4. The pan is open, so that the vapor passes to the atmosphere which can lead to the saturation of the atmosphere and pollution cannot be controlled.
5. These pans are limited only to aqueous liquids evaporation.
6. Vacuum cannot be used with these pans, so that the temperature of boiling cannot be lowered.

- **Applications:**

1. It is used for the concentration of aqueous and thermostable liquors. Eg: Extract of Liquorice
2. It is used for the preparation of final stage process of soft extracts.

Evaporating Stills



- **Advantages:**

1. It is quite simple to construct and easy to clean and maintain.
2. The vapor is condensed, which speeds evaporation and allows equipment to be used for any type of solvents. Eg: Ethanol
3. A receiver and vacuum pump can be fitted to the condenser, permitting operation under reduced pressure and hence at lower temperatures.

- **Disadvantages:**

1. Natural convection only.

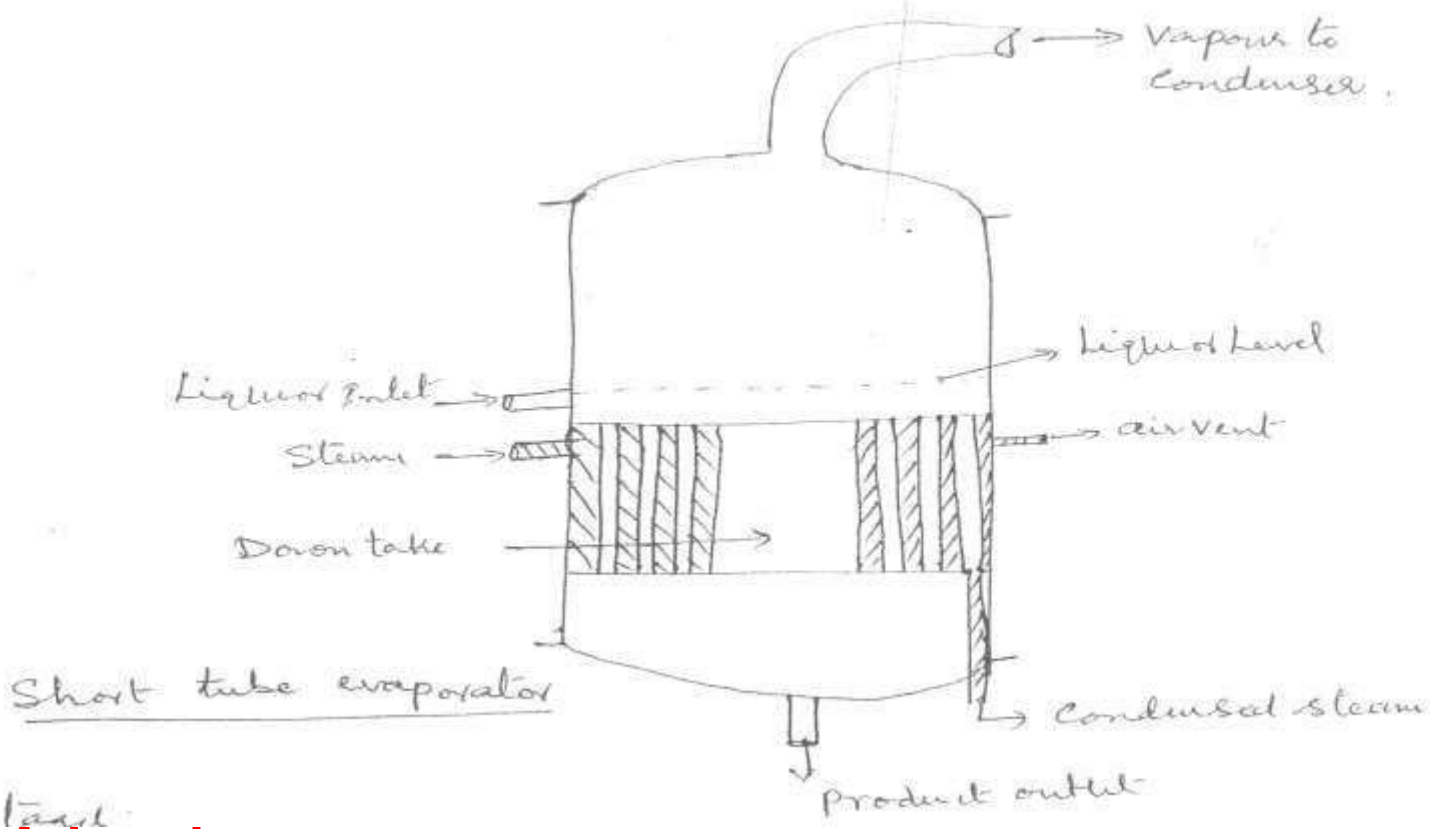
2. All the liquid is heated all the time.

3. The heating surface is limited.

- **Applications:**

1. The still can be used for evaporating liquors in general, with aqueous or other solvents.
2. Thermolabile materials can be evaporated under reduced pressure.
3. Easy removability of the still head makes it convenient for evaporating extracts to dryness.
4. This plant can be useful to deal with small batches of variety of materials.

Short Tube Evaporator



- **Advantages:**

1. It increases the heating area, possibly by 10 to 15 times when compared with that of an external jacket.
2. Vigorous circulation reduces boundary layers and keeps solids in suspension, so increases the arte of heat transfer. As a result, over all coefficients of heat transfer are from 3 to 5 times greater than those founding in evaporating pans.

- 3. Like the still, a condensor and receiver can be attached, to collect the condensed liquid.
- **Disadvantages:**

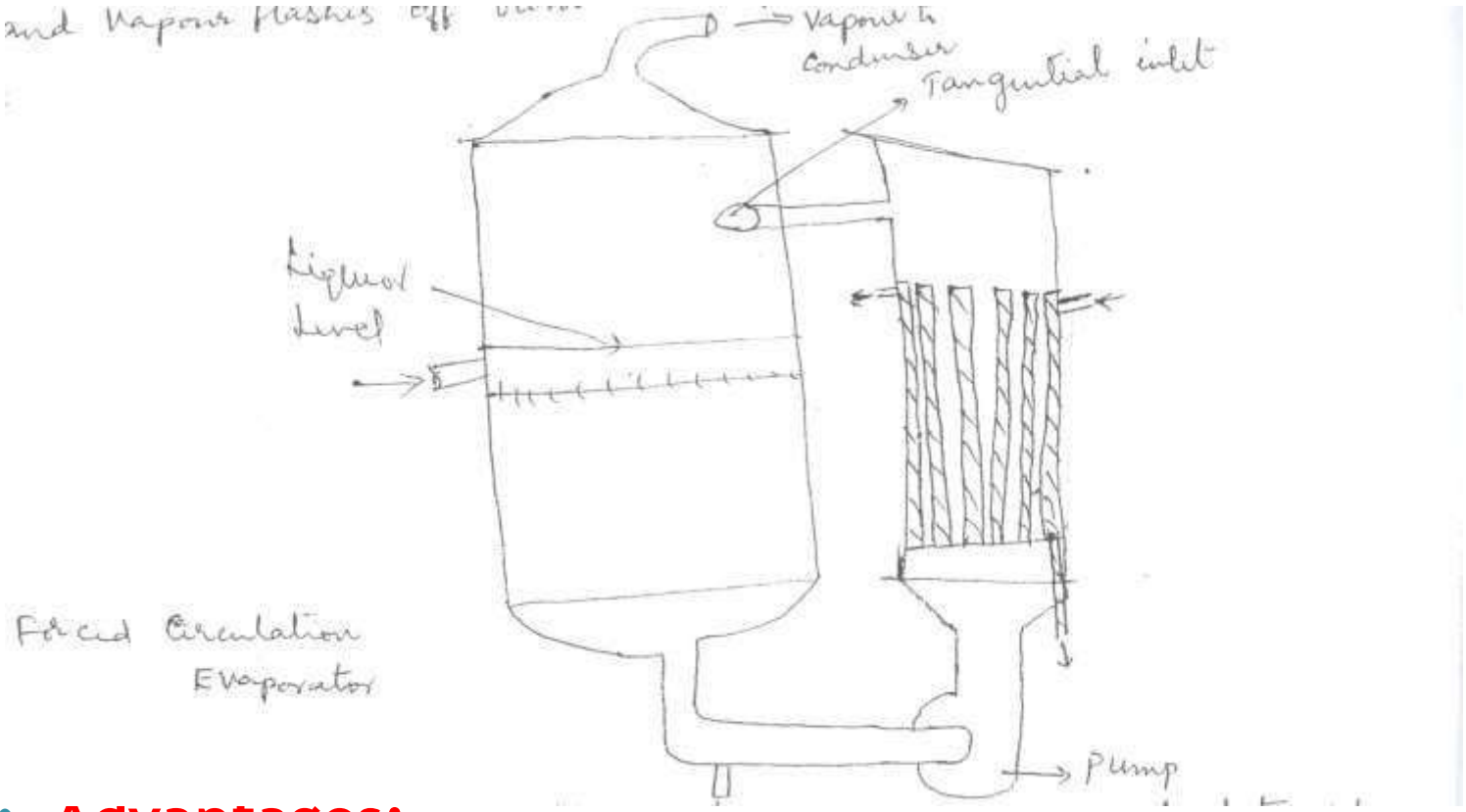
1. Since the evaporator is fitted to a point above the level of the calandria, a considerable amount of the liquid is heated for a long time.
2. The plant is much more complicated, making it expensive to construct and increasing the difficulty of cleaning and maintenance compared with the simple still.
3. The head of liquor increases the pressure at bottom of vesel especially in large evaporators where the liquor depth may be 2m, which may give pressure of about 0.25 bars, leading

to elevation of boiling point by 5 or 6 degrees.

- **Applications:**

1. This equipment is most useful for products manufactured on large scale, where the process can be operated continuously on one type of material.
2. This method is used for extracts manufactured in large quantities such as cascara, sugar, salt or caustic soda.

Forced Circulation Evaporator



- **Advantages:**

1. The rapid liquid movement improves heat transfer, especially with viscous liquids or materials that deposit solids of foam readily.
2. This equipment is particularly suitable for operation under reduced pressure.
3. The evaporation rate is rapid.

- **Applications:**

1. This is more suitable for thermolabile materials.

   Eg: It is used in practice for the concentration of insulin and liver extracts.

FILM EVAPORATORS

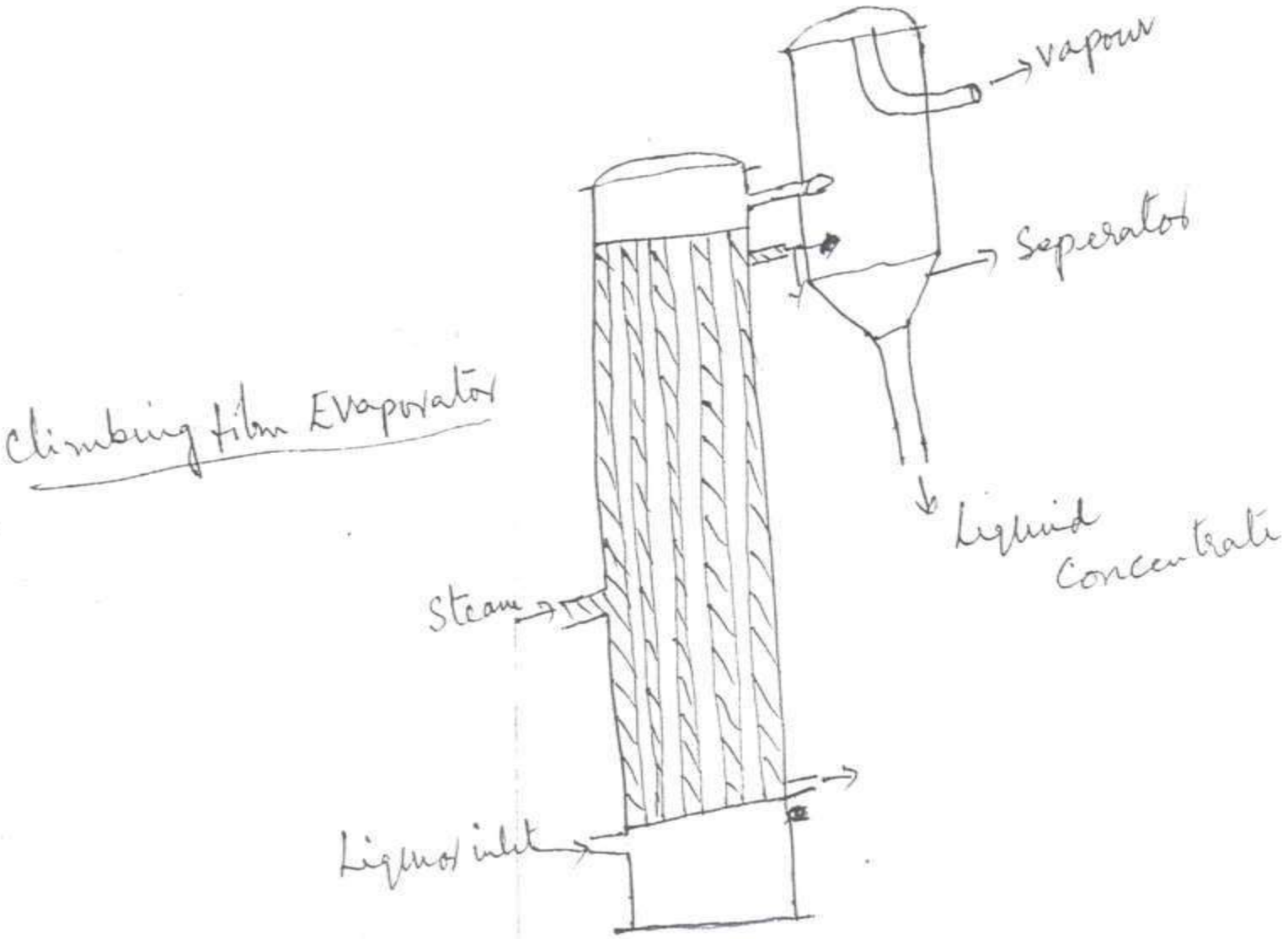
They are of several types.

(a) Long tube evaporators – Climbing film evaporators
– Falling film evaporators
– Horizontal film evaporators

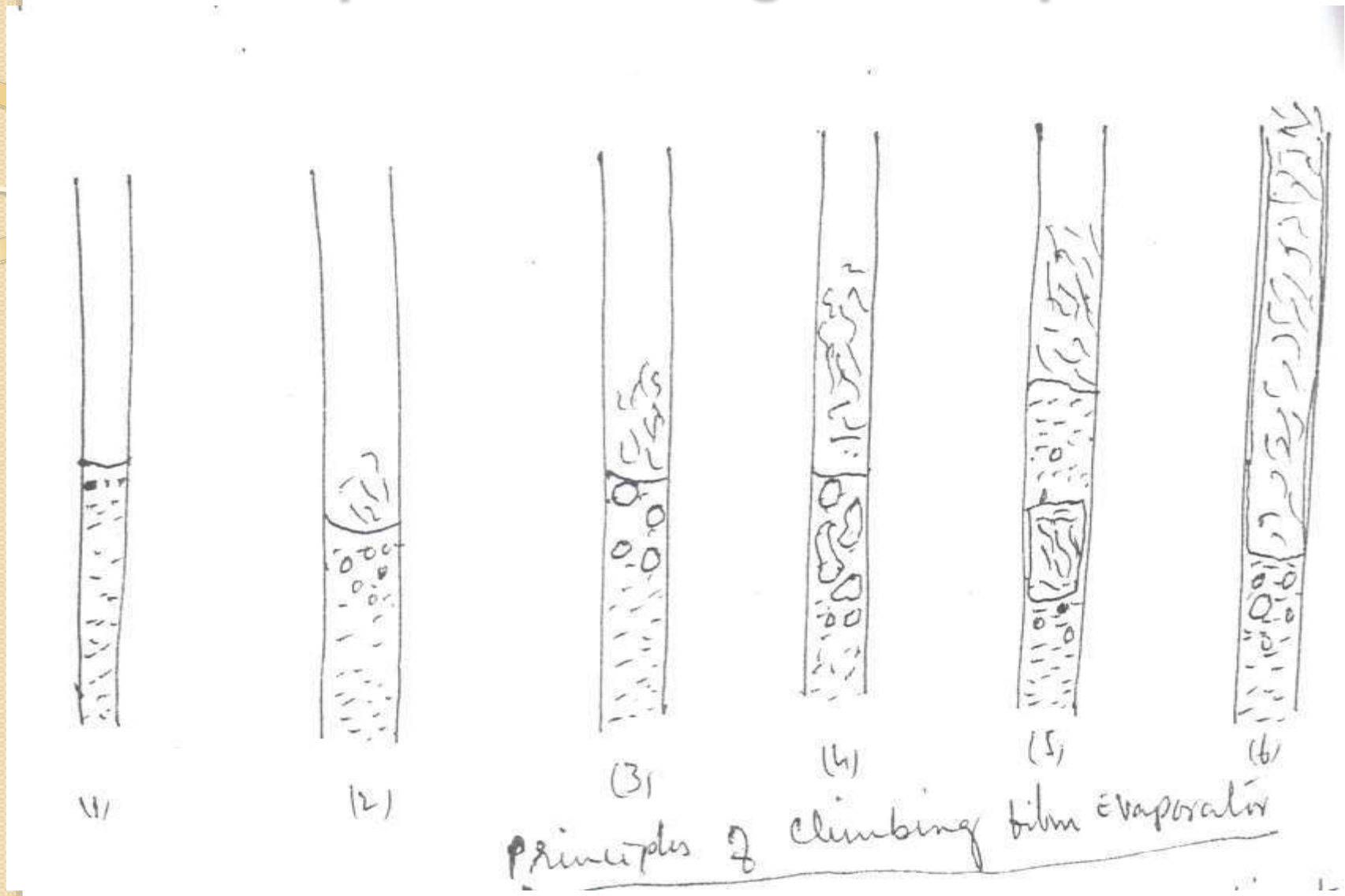
(b) Others – Wiped film evaporator
– Agitated film evaporator

(c) Miscellaneous – Multiple effect evaporator

Climbing Film Evaporator



Principles of Climbing Film Evaporator



- **Advantages:**

1. The very high film velocity reduces boundary layers to a minimum giving improved heat transfer.
2. Use of long narrow tubes provide large areas for heat transfer.
3. Because of increased efficiency of heat transfer, a small temperature difference is sufficient, with loss of damage to thermolabile materials.
4. The time of contact between liqour and heating surface is very short when compared to conventional type (1-20 sec) which is more advantageous for heat sensitive materials.

5. Evaporation rate is very high, since the film formation gives an extremely large surface area in relation to the volume of liquid.

6. The mixture of liquid and vapor enters the separator at high velocity, which improves the separation efficiency and makes the method especially suitable for materials that foam.

7. Although the tubes are very long, they are not submerged as in the short-tube evaporator, so that there is no elevation of boiling point due to hydrostatic head.

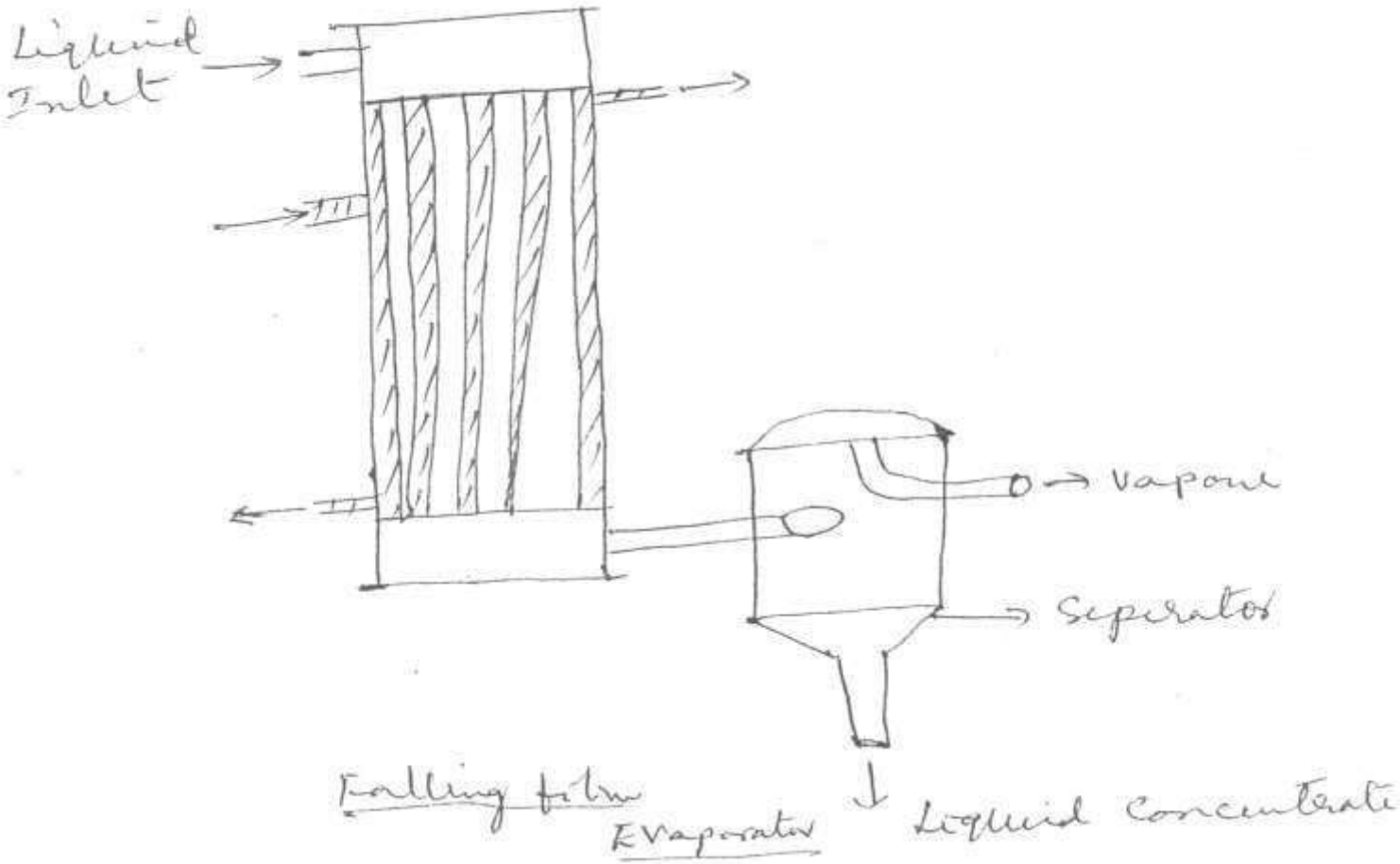
- **Disadvantages:**

1. It is expensive to manufacture and it is difficult to clean and maintain.
2. The feeding rate is critical.
   (a) If the feed rate is too high, the liquid may be concentrated insufficiently.
   (b) If the feed rate is too low, the film cannot be maintained and dry patches may form on tube wall.

- **Applications:**

1. Due to rapid evaporation, minimum temperature gradient and short heating time, this method approaches the perfect method for pharmaceutical products.
2. Thermolabile materials can be suitably evaporated.
   Eg: Concentration of solutions such as insulin, liver extracts and vitamins.

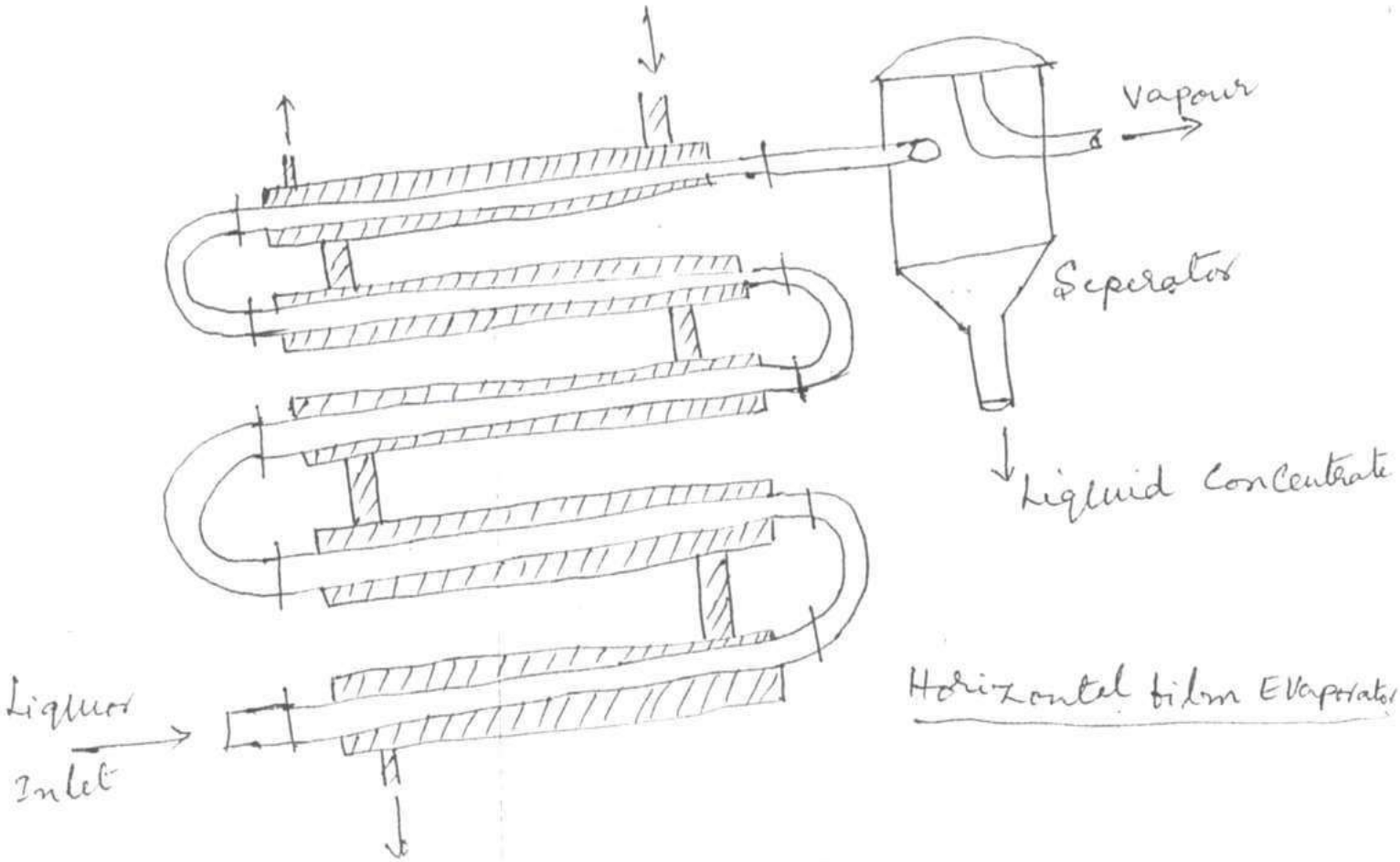
Falling Film Evaporator



- **Advantage:**

1. The movement of the liquid film is assisted by gravity, enabling more viscous liquids to be handled.

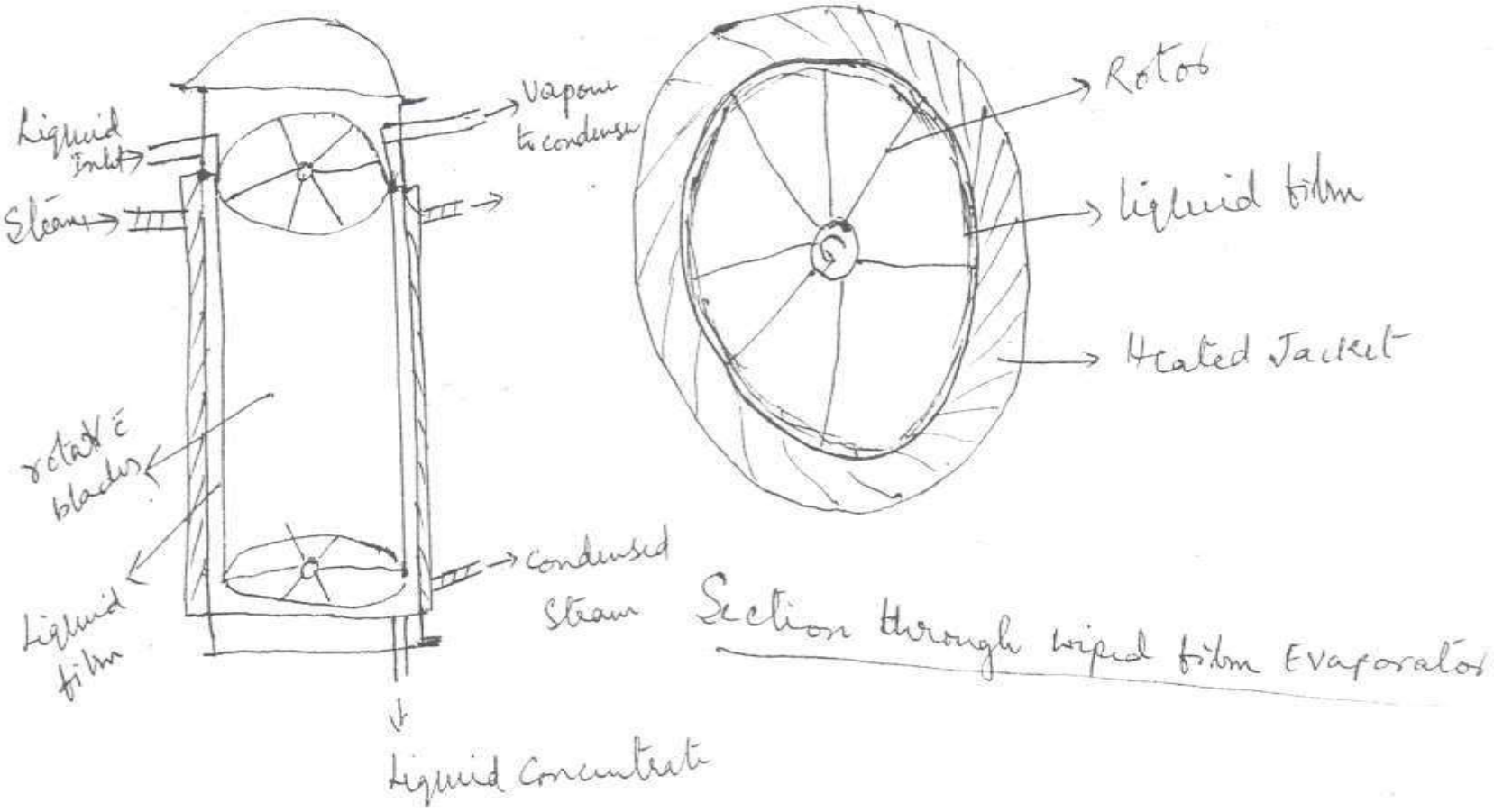
Horizontal Film Evaporator



- **Advantages:**

1. Occupies very small space and U bends can be easily removed.
2. Used safely with small batches of different materials.

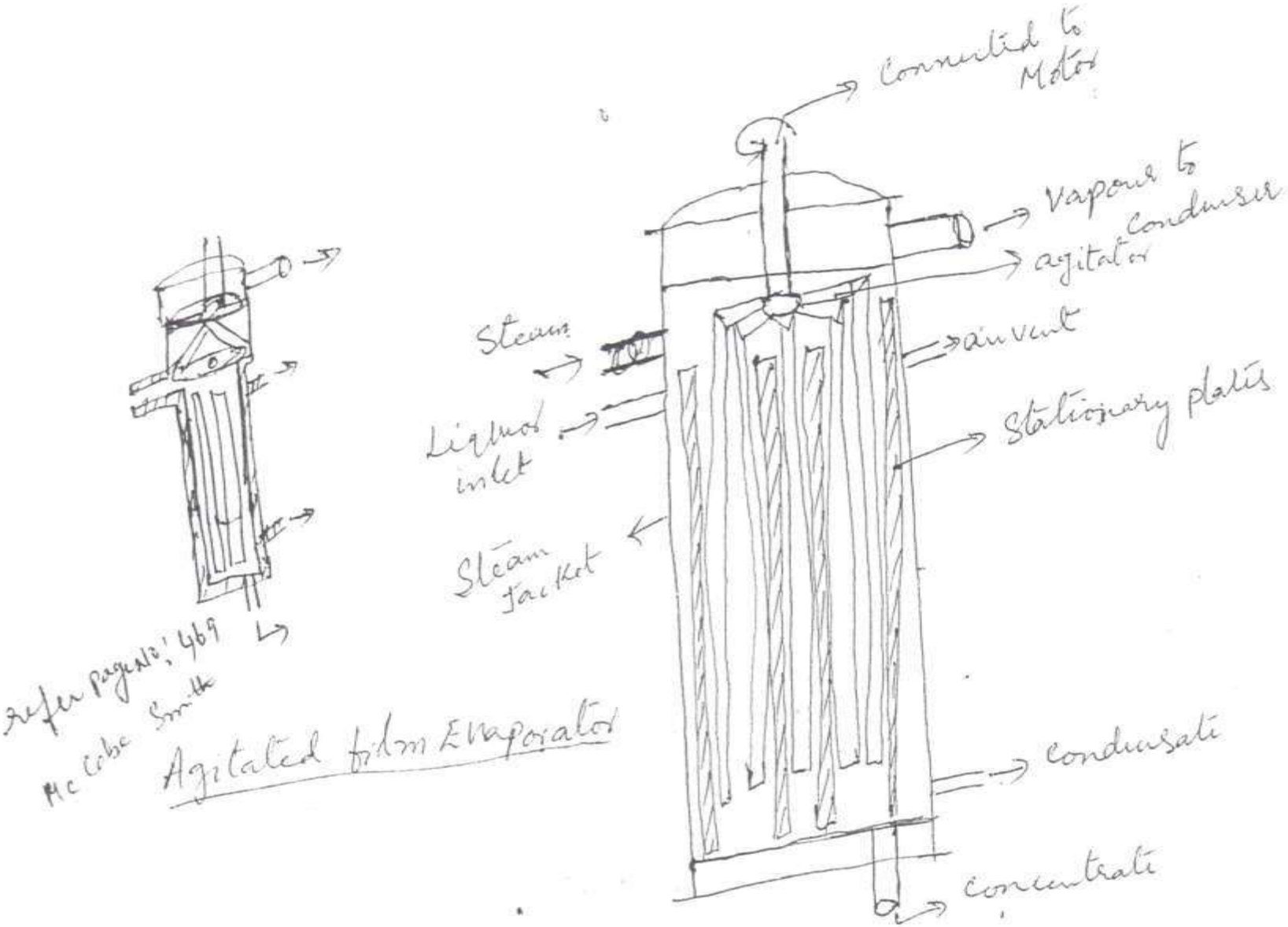
Wiped Film or Rotary Film Evaporator



- **Advantage:**

1. Because of good heat transfer, this method is especially useful with liquids that are too viscous to be processed in units in which the film is formed naturally.

Agitated Film Evaporator



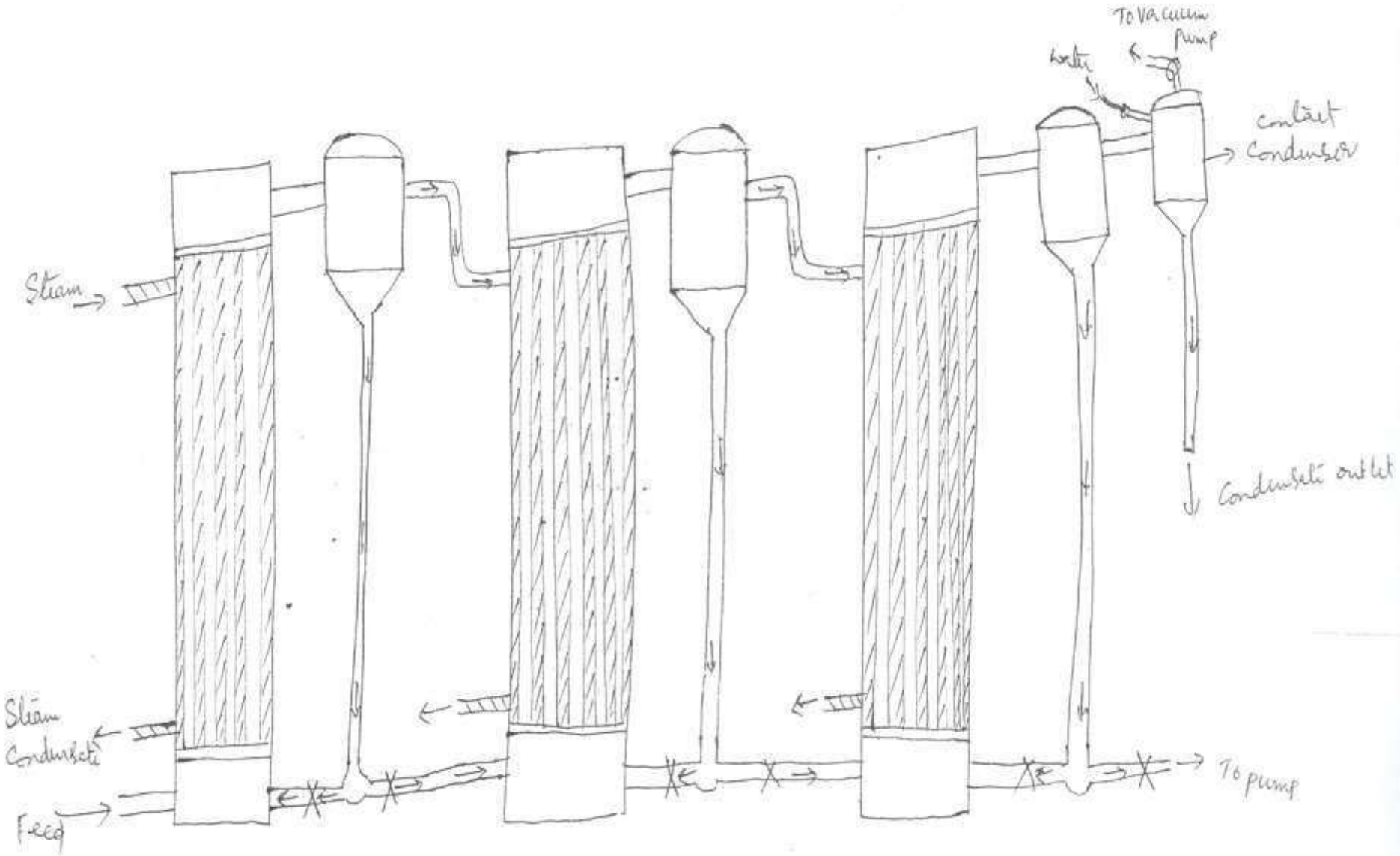
- **Advantages:**

1. This equipment is more suitable to provide high rates of heat transfer with viscous liquids.
2. This is particularly effective with such viscous heat-sensitive products as gelatin, rubber, latex, antibiotics and fruit juices.

Disadvantages:

1. Its cost is very high.
2. It consists of various internal moving parts, which may need considerable maintenance.

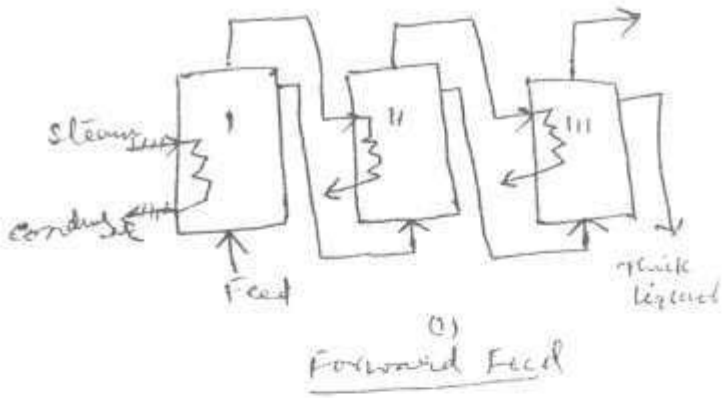
Multiple Effect Evaporator



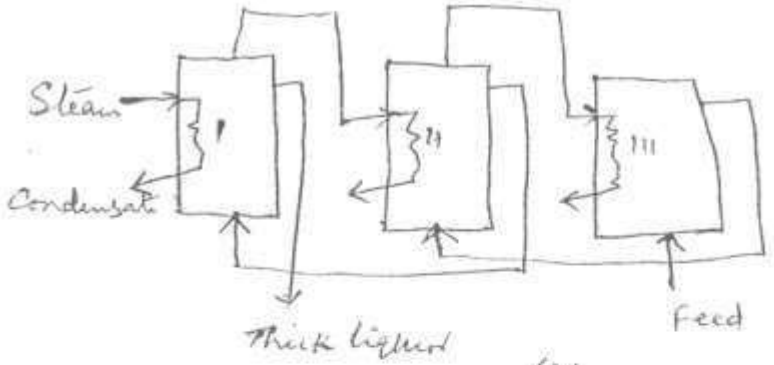
Various Feeding Techniques

- Various liquor feeding techniques are;

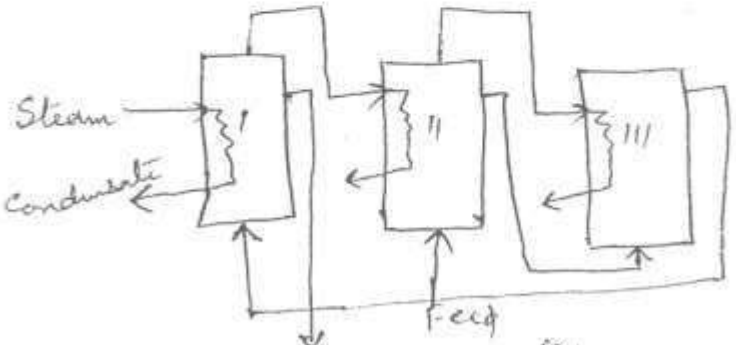
  1. Forward feed 2. Backward feed 3. Mixed feed 4. Parallel feed



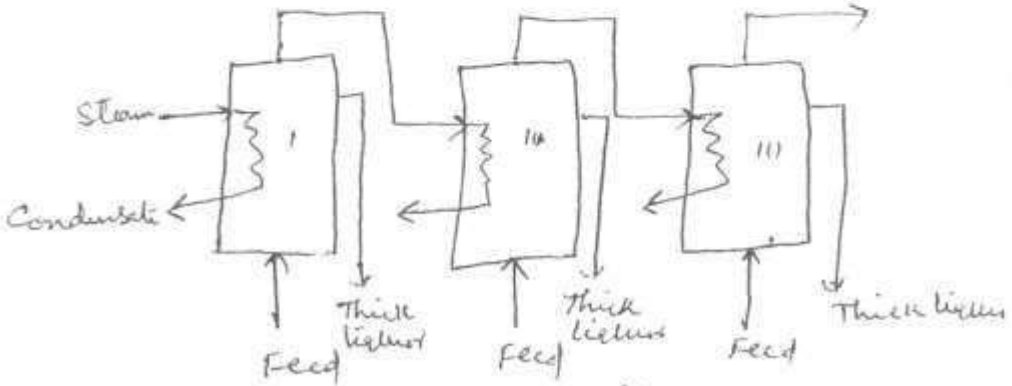
(1) Forward Feed



(2) Backward Feed



(3) Mixed Feed



(4) Parallel Feed

Capacity and Economy of Multiple Effect Evaporator

1. Multiple effect evaporator is more economical than any other conventional evaporator.
2. Capacity of multiple effect evaporator is no way greater than single conventional evaporator. This is due to elevation of boiling point. It remains same in conventional and multiple effect evaporators.

- If heating load and heat of dilution are neglected, the capacity of an evaporator is directly proportional to rate of heat transfer.The heat transferred in 3 effects is given by;

$q1 = U_1 A_1 \Delta T_1$ $q2 = U_2 A_2 \Delta T_2$ $q3 = U_3 A_3 \Delta T_3$

- Assume that the surface area is A sq.mts in each effect and that the overall coefficient U is also same in each effect.

$$qT = U_A (\Delta T_1 + \Delta T_2 + \Delta T_3) = UA\Delta T$$

Where ΔT is the total temperature drop between the steam in the first effect and the vapor in the last effect.

- If the overall coefficient is the same as in each effect of the triple effect evaporator, the rate of heat transfer in the single effect is

$$qT = UA\Delta T$$

THANK YOU

FILTRATION

P.GEETHA

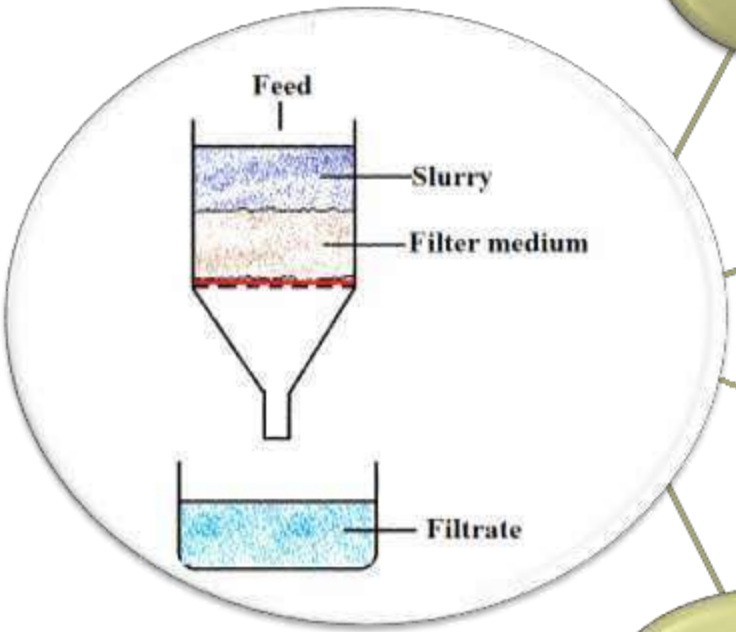
VCPS

DEFINITION

- Filtration: It may be define as a process of separation of solids from a fluid by passing the same through a porous medium that retains the solids but allows the fluid to pass through.

- Clarification: When solid are present in very low concentration, i.e., not exceeding 1.0% w/v, the process of its separation from liquid is called clarification.

TERMS USED IN FILTRATION



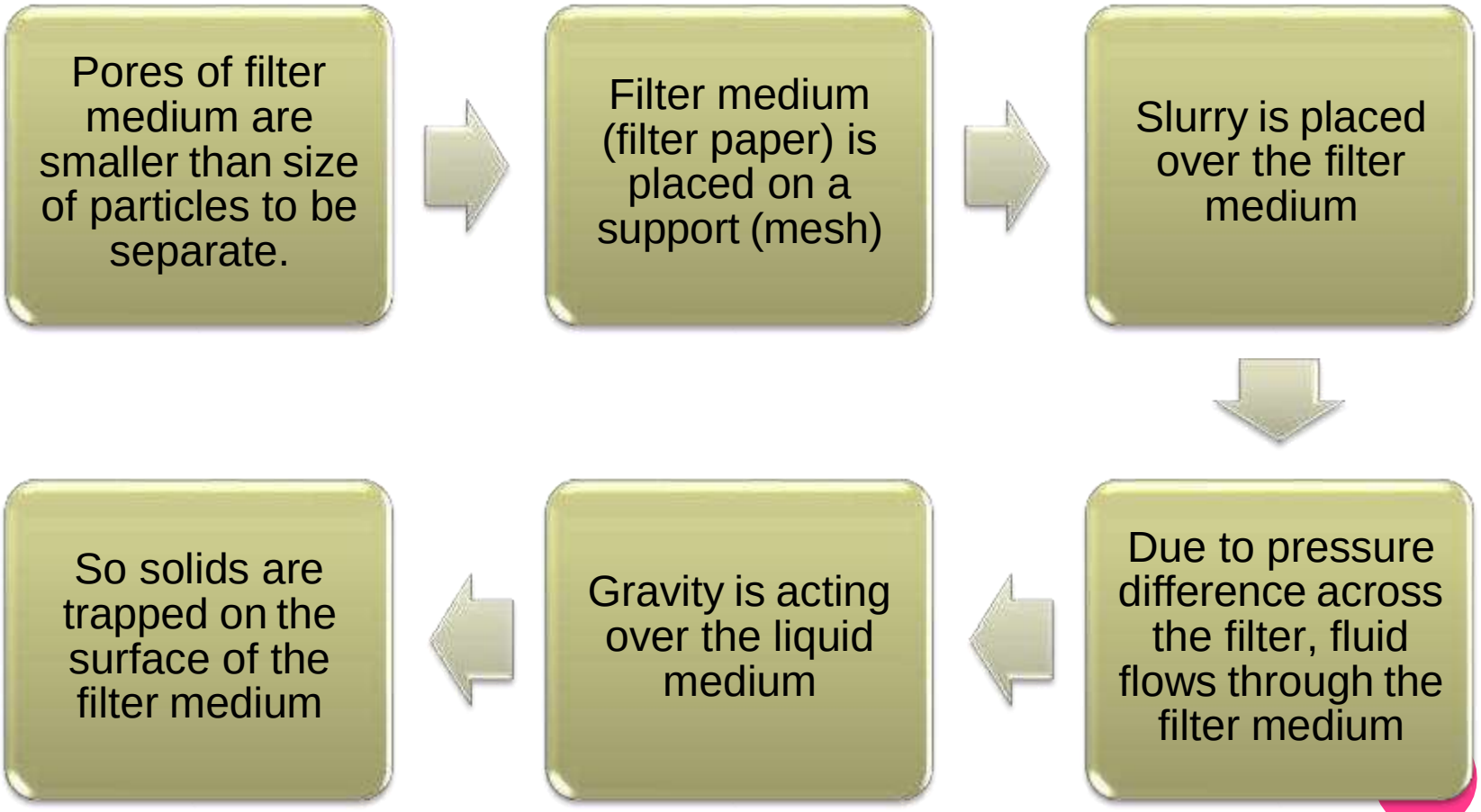
Slurry
- Suspension to be filtered

Filter medium
- Porous medium used to retain solid

Filter cake
- Accumulated solids on the filter

Filtrate
- Clear liquid passing through the filter

PROCESS OF FILTRATION



APPLICATIONS OF FILTRATION

- Production of sterile products:
  - ✓ HEPA filters or laminar air bench
  - ✓ Membrane filters.
- Production of bulk drugs
- Production of liquid dosage
- Effluents and waste water treatment

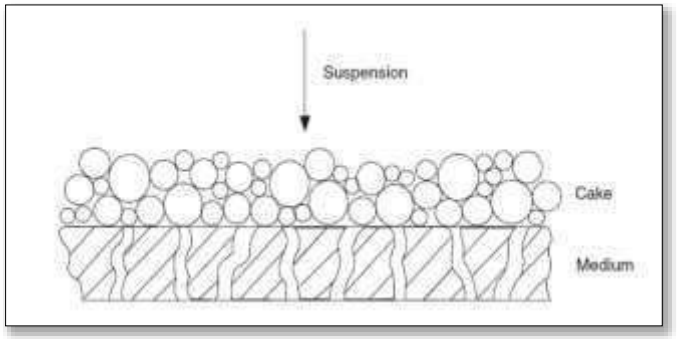
MECHANISM OF FILTRATION

- The mechanism whereby particles are retained by a filter is significant only in initial stages of filtration.

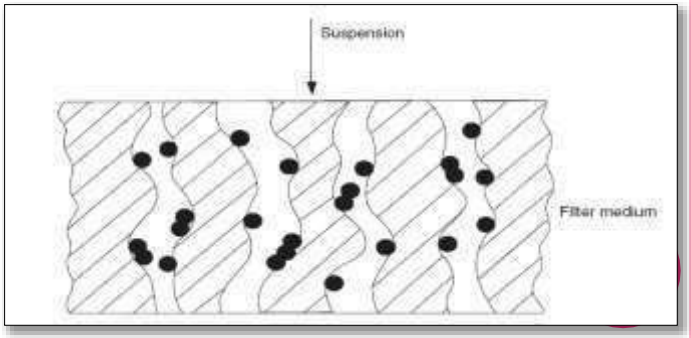
Straining	• Similar to sieving, i.e., particles of larger size can't pass through smaller pore size of filter medium.
Impingement	• Solids having the momentum move along the path of streaming flow and strike (impinge) the filter medium. Thus the solids are retained on the filter medium.
Entanglement	• Particles become entwined (entangled) in the masses of fibres (of cloths with fine hairy surface or porous felt) due to smaller size of particles than the pore size. Thus solids are retained within filter medium.
Attractive forces	• Solids are retained on the filter medium as a result of attractive force between particles and filter medium, as in case of electrostatic filtration.

TYPES OF FILTRATION

- **Surface/ screen filtration**
  - It is a screening action by which pores or holes of medium prevent the passage of solids.
  - Mechanism involved : straining and impingement
  - For this, plates with holes or woven sieves are used.
  - Efficacy is defined in terms of mean or maximum pore size.



- **Depth filtration**
  - In this slurry penetrates to a point where the diameter of solid particles is greater than that of the tortuous void or channel.
  - Mechanism : Entanglement
  - The solids are retained with a gradient density structure by physical restriction or by adsorption properties of medium.



Difference between surface and depth Filtration

Surface filtration

- The size of particles retained is slightly higher than the mean pore size of medium.
- Mechanical strength of filter medium is less, unless it is made of stainless steel.
- It has low capacity.
- The size of particles retained is more predictable.
- Equipment is expensive because ancillary equipment such as edge clamps is required.
- Ex. Cellulose membrane filter.

Depth filtration

- The size of particles retained is much smaller than the mean pore size of medium.
- Mechanical strength of filter medium is high.
- It has high capacity.
- The size of particles retained is less predictable.
- Equipment is cheaper because ancillary equipment is not required.
- Ex. Ceramic filters and sintered filters.

THEORIES OF FILTRATION

- The flow of liquid through a filter follows the basic rules that govern the flow of any liquid through the medium offering resistance.
- The rate of flow may be expressed as-

Rate = driving force / resistance

- The rate of filtration may be expressed as volume (litres) per unit time (dv/dt).
- Driving force = pressure upstream – pressure downstream
- Resistance is not constant.
- It increases with an increase in the deposition of solids on the filter medium.
- Therefore filtration is not a steady state.

CONTINUE……..

- The rate of flow will be greatest at the beginning of filtration process, since the resistance is minimum.
- After forming of filter cake, its surface acts as filter medium and solids continuously deposit adding to thickness of the cake.

Powder or granule bed visualized as a bundle of capillaries

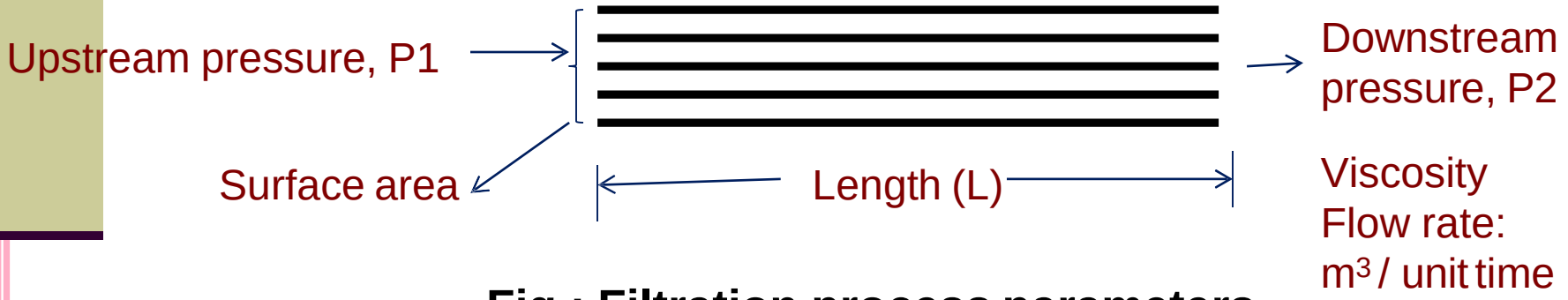


Fig.: Filtration process parameters.

- Resistance to flow is related to several factors given in fig.

Resistance to movement={pressure upstream- pressure downstream}/ length of capillaries

POISEULLIE'S EQUATION

- Poiseullie considered that filtration is similar to the streamline
  - flow of liquid under pressure through capillaries.
- Poiseullie's Equation is-

$$V = \frac{\pi \Delta P r^4}{8 L \eta}$$

- Where, V = rate of flow, m^3/s (l/s)
  - ΔP= Pressure difference across the filter, Pa r = radius of capillary in the filter bed, m
  - L = thickness of filter cake (capillary length), m

DARCY'S EQUATION

- Poiseullie's law assumes that the capillaries found in the filter are highly irregular and non-uniform.
- Therefore, if the length of capillary is taken as the thickness of bed, a correction factor for radius is applied so that the rate is closely approximated and simplified.
- The factors influencing the rate of filtration has been incorporated into an equation by Darcy, which is:

$$V = \frac{KA\Delta P}{\eta L}$$

- Where, K = permeability coefficient of cake, m^2

  A = surface area of porous bed (filter medium), m^2

Other terms are same as previous equation

- K depends on characteristics of cake, such as porosity, specific surface area and compressibility.

CONTINUE ....

- Permeability may be defined quantitatively as the flow rate of a liquid of unit viscosity across a unit area of cake having unit thickness under a pressure gradient of unity.
- This equation is valid for liquids flowing through sand, glass beds and various porous media.
- This model is applied to filter beds or cakes and other types of depth filter.
- This equation is further modified by including characteristics of K by Kozeny-Carman.

KOZENY-CARMAN (K-C) EQUATION

- Kozeny-Carman equation is widely used for filtration.

$$V = \frac{A}{\eta S^2} \times \frac{\Delta P}{KL} \times \frac{\varepsilon^3}{(1-\varepsilon)^2}$$

- Where,

s=porosity of cake (bed)

S = specific surface area of particles comprising the cake m^2 / m^3

K = Kozeny constant (usually taken as 5)

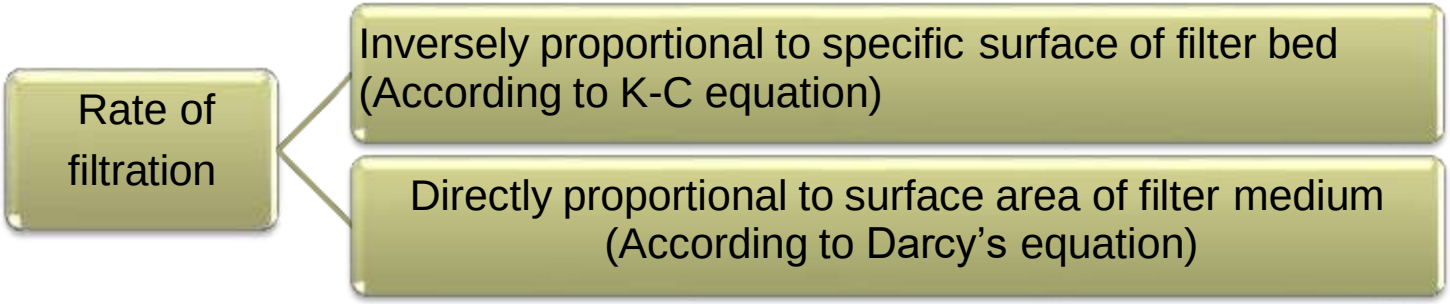
Other terms are same as previousequations

- Limitations:
  - It does not consider the fact that depth of granular bed is lesser than the actual path traversed by the fluid.
  - The actual path is not same through out the bed, but it is sinuous or tortuous.

FACTORS INFLUENCING FILTRATION

Properties of solid	Properties of liquids	Properties of solid in slurry	Objectives	Temperature
• Particle shape • Particle size • Particle charge • Density • Particle size distribution • Rigidity or compressibility of solid under pressure • Tendency of particle to flocculate or adhere together	• Density • Viscosity • corrosiveness	• Rate of formation of filter cake especially in early stage of filtration	• Whether the solid or liquid or both are to be collected	• Temperature of suspension

SURFACE AREA OF FILTER MEDIUM



- Rate can be increased either using large filter or connecting a number of small units in parallel.
- Filter press works on principle of connecting units in parallel.

PRESSURE DROP ACROSS THE FILTER MEDIUM

- According to K-C equation the rate of filtration is proportional to the overall pressure drop across both the filter medium and filter cake.
- The pressure drop can be achieved in a number of ways:

Gravity
- A pressure difference could be obtained by maintaining a head of slurry above the filter medium.
- The pressure developed will depend on the density of the slurry

Vacuum (Reducing pressure)
- The pressure below the filter medium may be reduced below atmospheric pressure by connecting the filtrate receiver to a vacuum pump and creating a pressure difference across the filter.

Pressure
- The simplest method being to pump the slurry into the filter under pressure.

Centrifugal force
- The gravitational force could be replaced by centrifugal force in particle separation

VISCOSITY OF FILTRATE

- According to K-C equation rate of filtration is inversely proportional to the viscosity of the fluid.
- Reason behind this is an increase in the viscosity of the filtrate will increase the resistance of flow.
- This problem can be overcome by two methods:
  - The rate of filtration may be increased by raising the temperature of the liquid, which lowers its viscosity. However, it is not practicable if thermolabile materials are involved or if the filtrate is volatile.
  - Dilution is another alternative but the rate must be doubled.

Filter Media

- The surface upon which solids are deposited in a filter is called the “Filter medium”
- Properties of ideal filter medium:
- It should-

1) be capable of delivering a clear filtrate at a suitable production rate.
2) have sufficient mechanical strength.
3) be inert.
4) retain the solids without plugging at the start of filtration.
5) Not absorb dissolve material.
6) Sterile filtration imposes a special requirement since the pore size must not exceed the dimension of bacteria or spores.

MATERIAL USED AS FILTER MEDIA

Woven material

- Made up of wool, silk, metal or synthetic fibres (rayon, nylon etc.).
- These include a- wire screening and b- fabrics of cotton, wool, nylon.
- Wire screening e.g. stainless steel is durable, resistance to plugging and easily cleaned.
- Cotton is a common filter ,however, Nylon is superior for pharmaceutical use, since it is unaffected by mold, fungus or bacteria and has negligible absorption properties .
- The choice of fibre depends on chemical

Perforated sheet metal

- Stainless steel plates have pores which act as channels as in case of meta filters.

Bed of granular solid built up on supporting medium

- In some processes, a bed of graded solids may be formed to reduce resistance of flow.
- Ex. Of granular solids are gravel, sand, asbestos, paper pulp and keiselgur.
- Choice of solids depends on size of solids in process.

PREFABRICATED POROUS SOLID UNITS

- Used for its convenience and effectiveness.
- Sintered glass, sintered metal, earthenware and porous plastics are used for fabrication.

Membrane filter media

- These are cartridge units and are economical and available in pore size of 100 µm to even less than 0.2 µm.
- Can be either surface cartridges or depth type cartridges.
- **Surface cartridges**
- These are corrugated and resin treated papers and used in hydraulic lines.
- Ceramic cartridges and porcelain filter candles are examples.
- Can be reuse after cleaning.
- **Depth type cartridges:**
- Made up of cotton, asbestos or cellulose.
- These are disposable items, since cleaning is not feasible.

FILTER AIDS

- The objective of filter aid is to prevent the medium from becoming blocked and to form an open, porous cake, hence, reducing the resistance to flow of the filtrate.
- Filter aid forms a surface deposit which screens out the solids and also prevents the plugging of supporting filter medium.

Characteristics of filter aids:

- Chemically inert and free from impurities.
- Low specific gravity, so remain suspended in liquids.
- Porous rather than dense, so that pervious cake can be formed.
- Recoverable.

Disadvantages:

- Remove the coloured substances by absorbing them.
- Sometimes active principles such as alkaloids are absorbed on filter aid.
- Rarely, filters are source of contamination such as soluble iron salts, which can provoke degradation of sensitive ingredient.

HANDLING OF FILTER AIDS

- Filter aids may be used in either or both two ways:

1) Pre- coating technique: by forming a pre-coat over the filter medium by filtering a suspension of the filter aid .
2) Body- mix technique: A small proportion of the filter aid (0.1-0.5 %) is added to the slurry to be filtered. This slurry is recirculated through the filter until a clear filtrate is obtained, filtration then proceeds to completion.

- Different flow rates can be achieved depending on grade of aid-

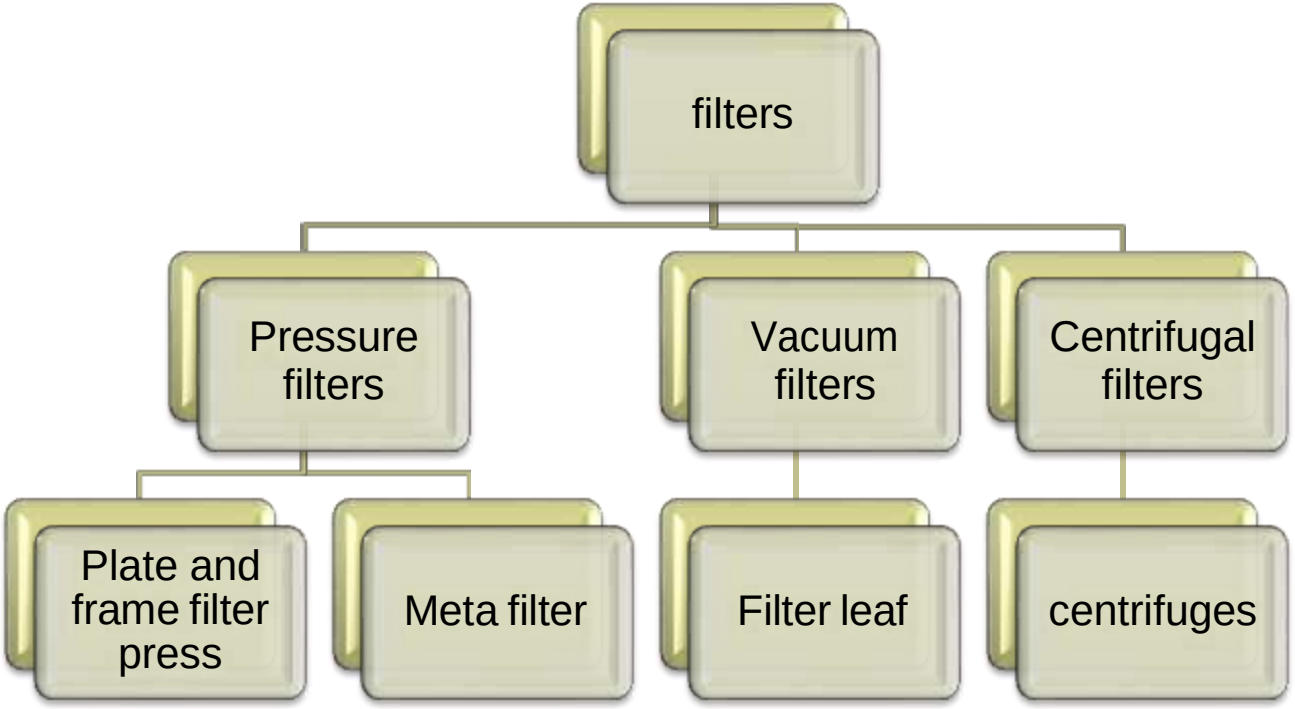
1. Low flow rate: fine grade filter aids- mainly used for clarity
2. Fast flow rate: coarse grade filter aids- acceptable filtrate.

Examples of filter aids

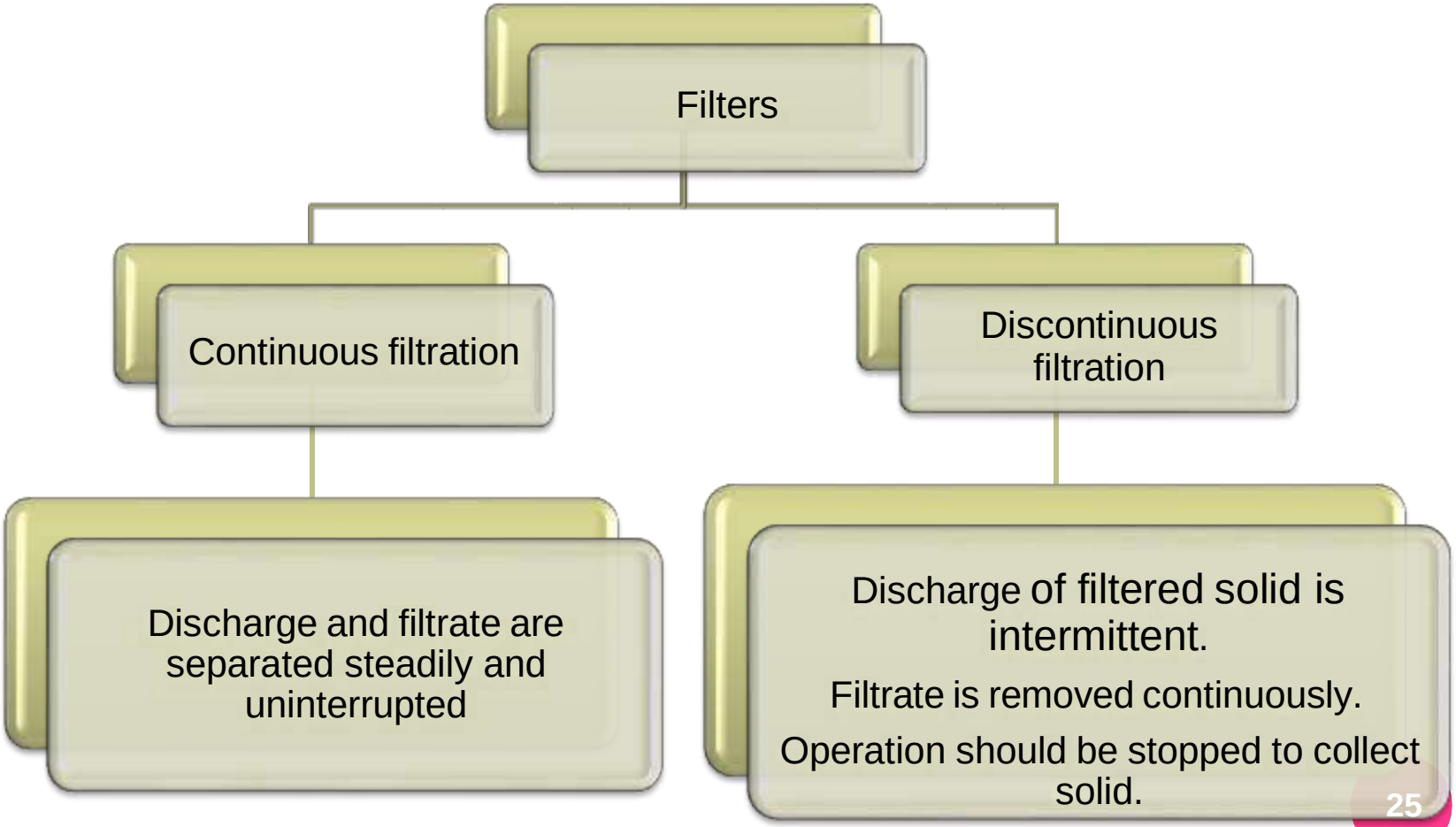
- Diatomite (Keiselgur) , obtained from natural siliceous deposits.
- Perlite , it is an aluminium silicate. Cellulose, Asbestos, charcoal, talc, bentonite , fullers earth etc.

CLASSIFICATION OF FILTRATION EQUIPMENTS

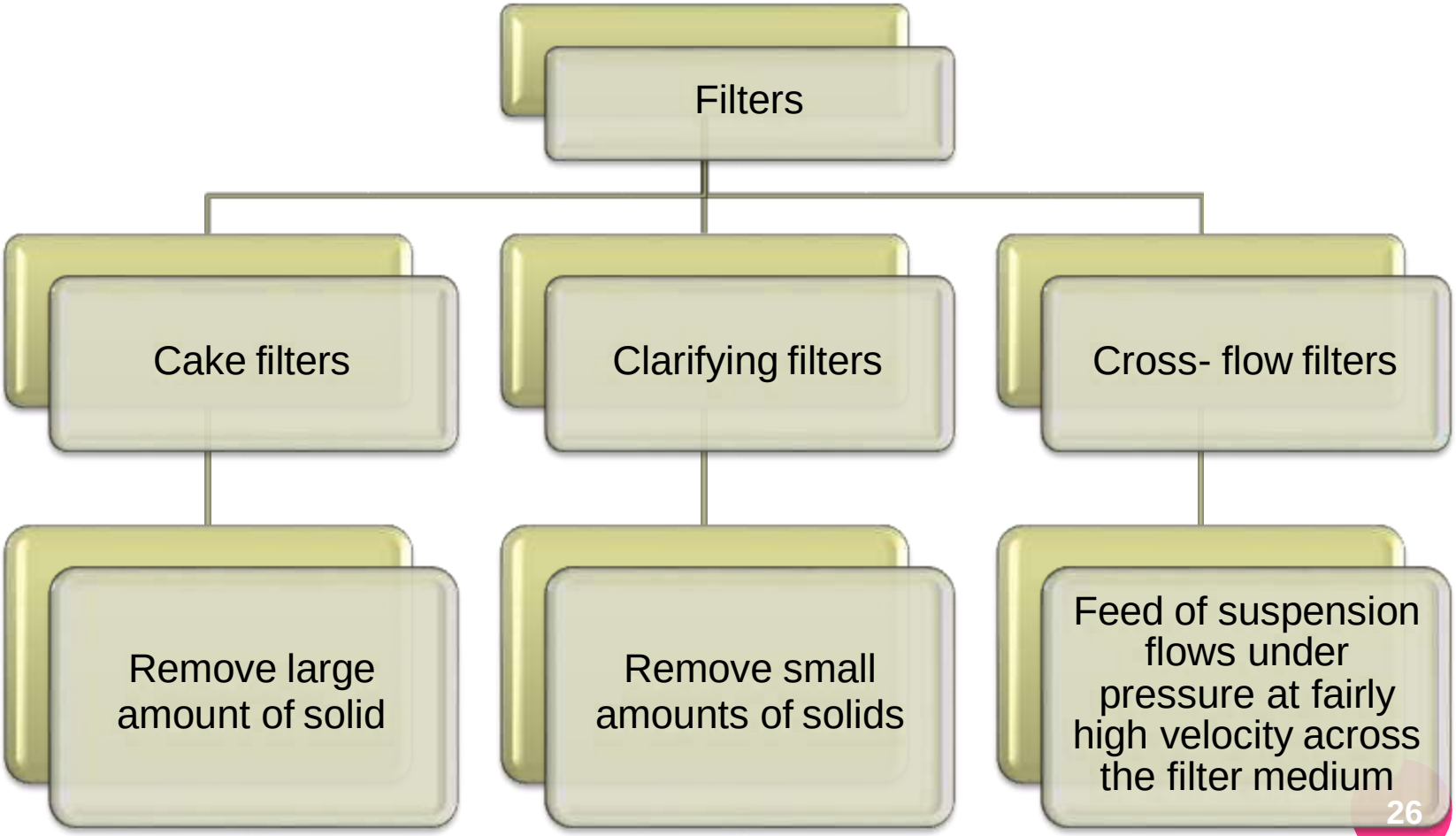
- Based on application of external force:



BASED ON OPERATION OF FILTRATION



BASED ON NATURE OF FILTRATION



LABORATORY SCALE FILTRATION

- Filter paper and funnel
- Buchner funnel and filter paper
- Sintered glass filter
- Seitz filter
- Membrane filter

SELECTION OF FILTERSDEPENDS UPON below listed factors

Material related

- Properties of fluid- viscosity
- Nature of solids- size, shape, distribution and packing characteristics of particles
- Conc. Of solid in suspension
- Quantity of material to be handle
- Whether it is necessary to wash the solid
- Whether any form of pretreatment will be helpful.

Equipment and process related

- Flow rate
- Should be absolute in sense, limit to size of particles passing through the filter should be known.
- Should be sterilisable by heat, radiation or gas
- Should be economical.

PLATE AND FRAME FILTER PRESS

- Principle:
  - Mechanism is surface filtration.
  - The slurry enters the frame by pressure and flows through filter medium.
  - The filtrate is collected on the plates and send to outlet.
  - A number of frames and plates are used so that surface area increases and consequently large volumes of slurry can be processed simultaneously with or without washing.

DIAGRAM

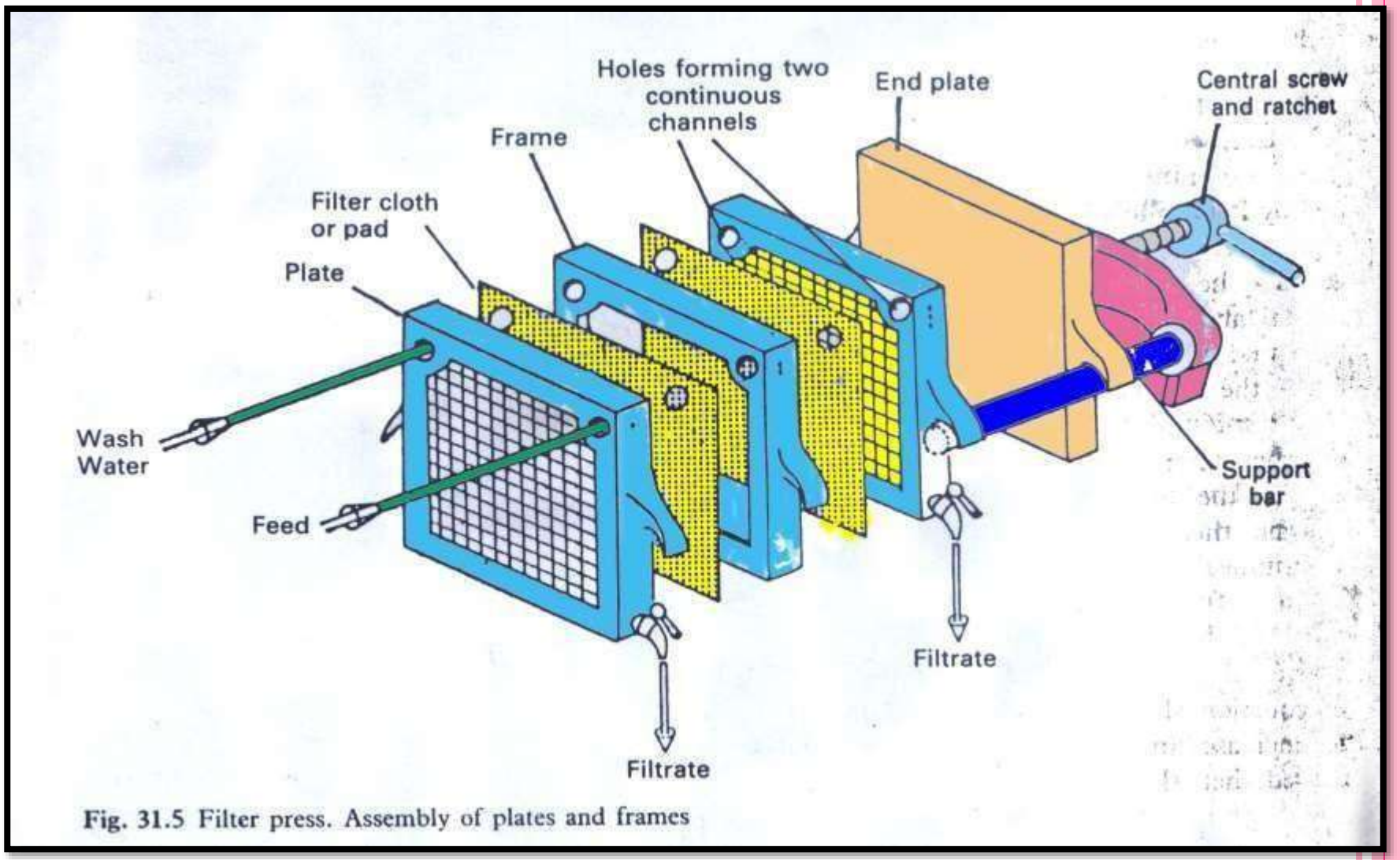
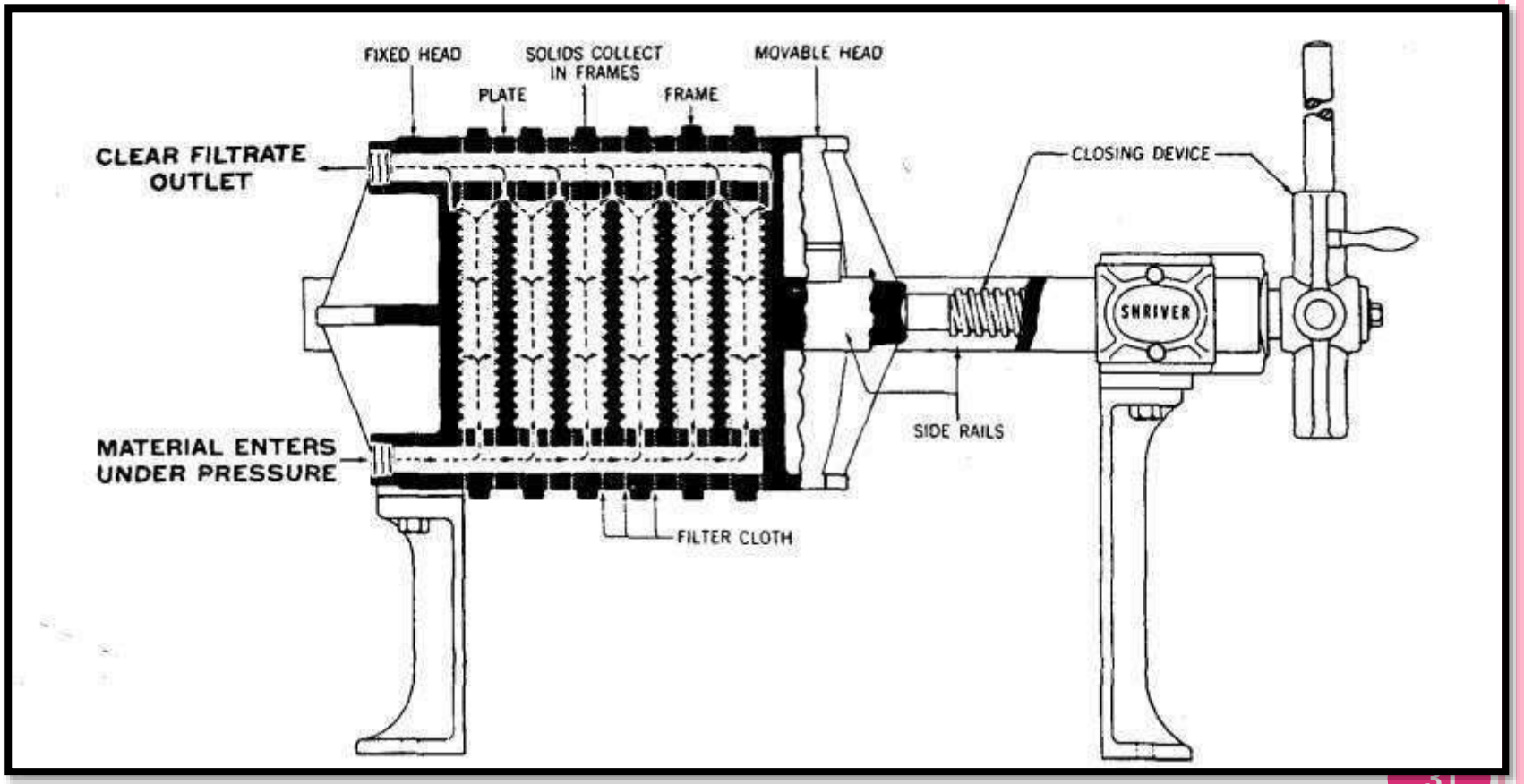


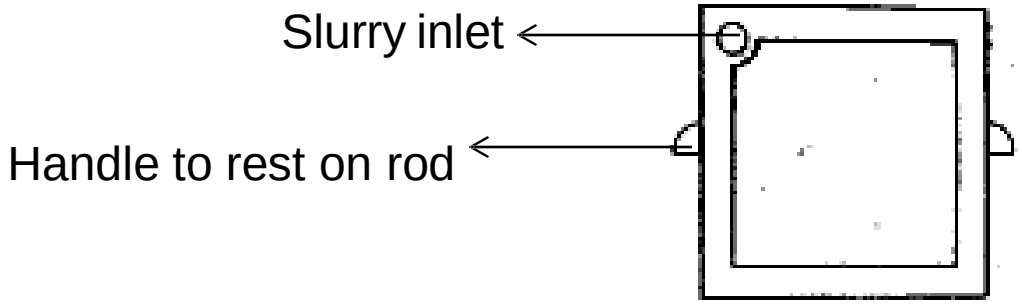
Fig. 31.5 Filter press. Assembly of plates and frames

ASSEMBLY OF PLATE AND FRAME FILTER



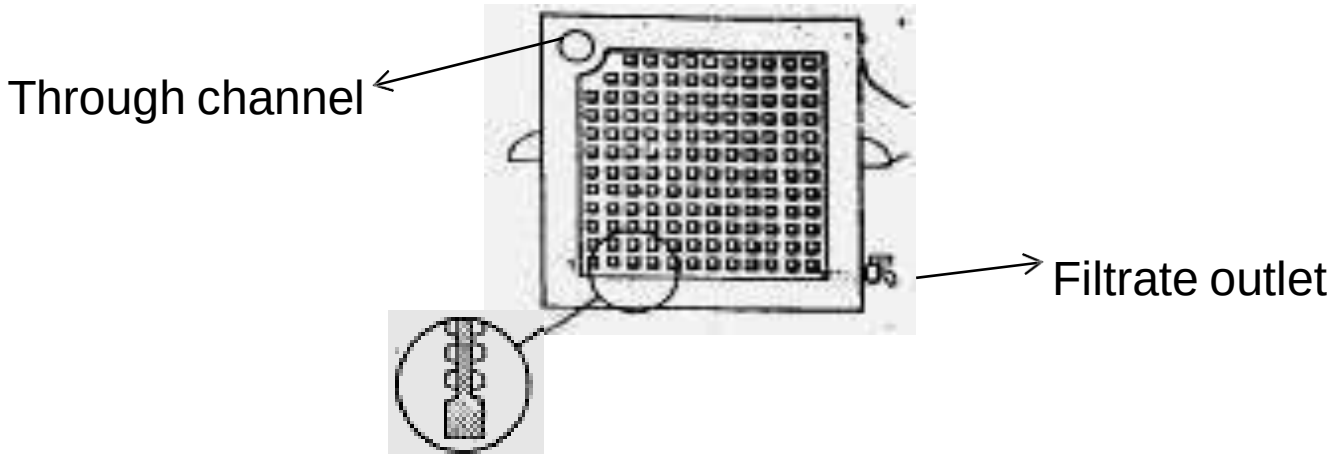
CONSTRUCTION

- The Filter press is made of two types of units, plate and frames.
- Usually made of aluminium alloy.
- Sometimes, these are also lacquered for protection against corrosive chemicals and made suitable for steam sterilization.
- **Frame**
  - It contains a open space inside wherein the slurry reservoir is maintained for filtration and an inlet to receive the slurry.
  - It is indicated by two dots in description.



CONTINUE ....

- Frames of different thickness are available.
- It is selected based on the thickness of cake formed during filtration.
- Optimum thickness of frame should be chosen.
- **Plate**
  - The plate has a studded or grooved surface to support the filter cloth and an outlet.
  - It is indicated by one dot in description.



CONTINUE ....

- Plate supports the filter medium, receiving the filtrate and outlet.
- The filter medium usually cloth is interposed between plate and frame.
- Plate, filter medium, frame, filter medium and plate are arranged in sequence and clamed to a supporting structure.
- It is normally described by dots as 1.2.1.2.1 so on.
- A number of plates and frames are employed so that the filtration area is as large as necessary.
- Number of filtration units are operated in parallel.
- Channels for slurry inlet and filtrate outlet can be arranged by fitting eyes to the plates and frames, these join together to form a channel.
- In some types only one inlet channel is formed, while each plate is having individual outlets controlled by valves.

WORKING

- Working can be divided into two steps-

1. Filtration operation
2. Washing of cake (if desirable)

- Filtration operation

Frame- marked by 2 dots
Plate – marked by 1 dot

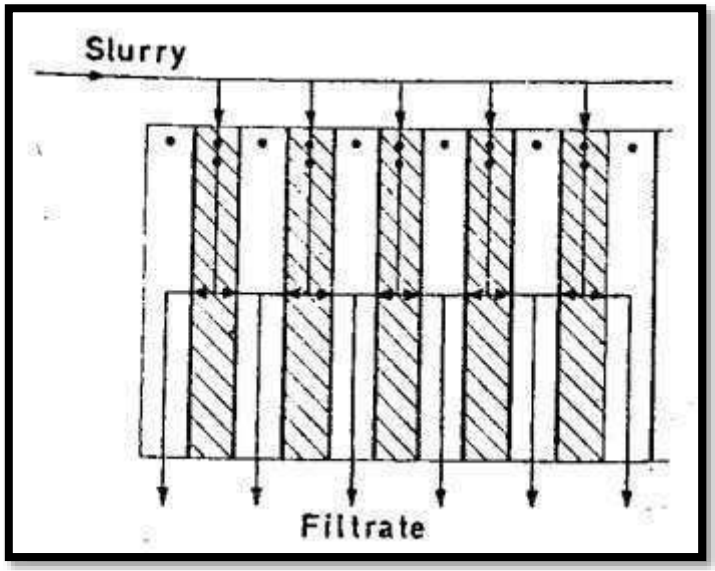
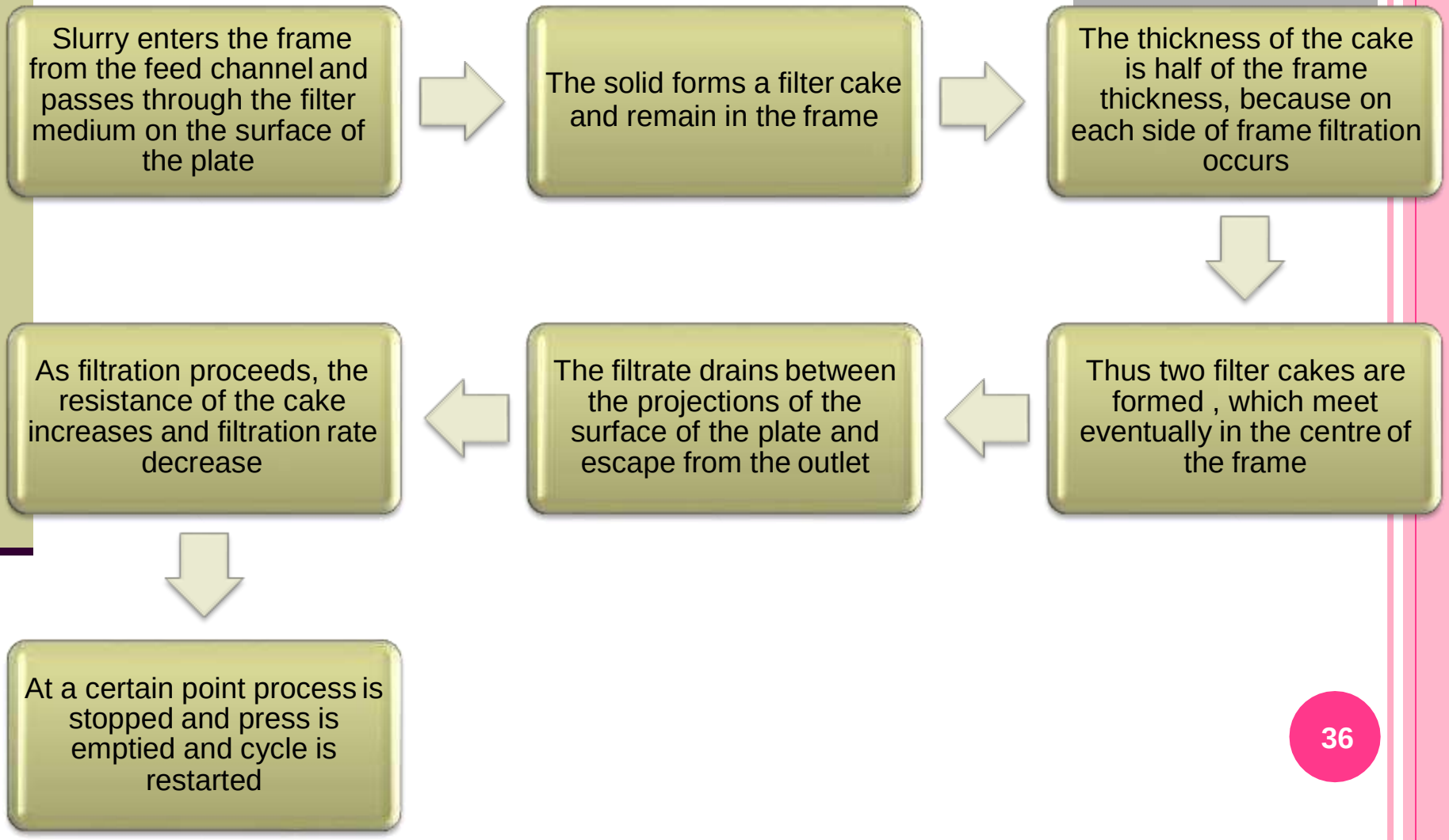


Plate and frame filter press, principle of filtration operation

CONTINUE……


Slurry enters the frame from the feed channel and passes through the filter medium on the surface of the plate
The solid forms a filter cake and remain in the frame
The thickness of the cake is half of the frame thickness, because on each side of frame filtration occurs
Thus two filter cakes are formed , which meet eventually in the centre of the frame
The filtrate drains between the projections of the surface of the plate and escape from the outlet
As filtration proceeds, the resistance of the cake increases and filtration rate decrease
At a certain point process is stopped and press is emptied and cycle is restarted

WASHING OPERATION

- When washing of cake is also required modified plate and frame filter is used.
- For this purpose an additional channel is included called as washing plate and are identified by 3 dots.
- In the half of the washing plate, there is a connection from wash water cannel to the surface of plate.
- The sequence of arrangement of plates and frames can be represented by dots as 1.2.3.2.1.2.3.2.1 so on (between 1 and 1, 2.3.2 must be arranged.

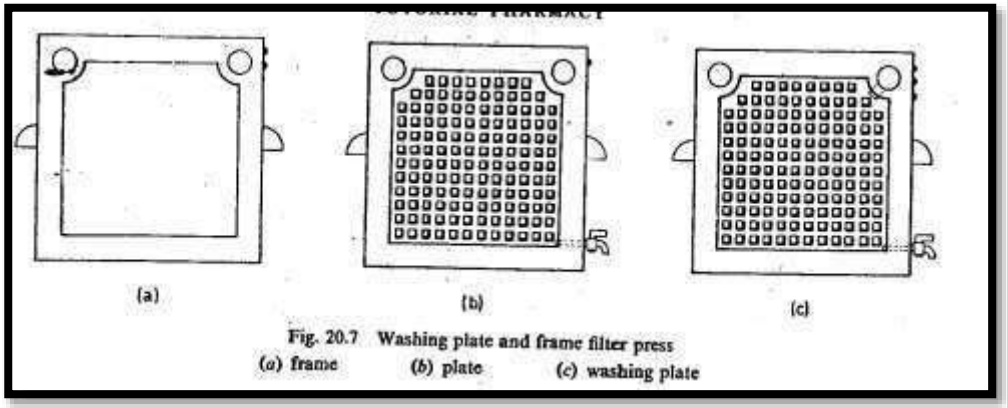


Fig. 20.7 Washing plate and frame filter press
(*a*) frame (*b*) plate (*c*) washing plate

PROCEDURE FOR WASHING THE PRESS

Step 1
- Filtration proceeds in the ordinary way until the frames are filled with cake.

Step 2
- To wash the filter cake, the outlets of washing plates are closed.

Step 3
- Wash water is pumped in the washing channel.
- The water enters through the inlets on the surface of washing plate.

Step 4
- Water passes through the filter cloth and enters frame which contains the cake.
- Then water washes the cake, passes through the filter cloth and enters the plate down the surface.

Step 5
- Finally washed water escapes through the outlet of that plate.

DIAGRAMMATIC PROCEDURE

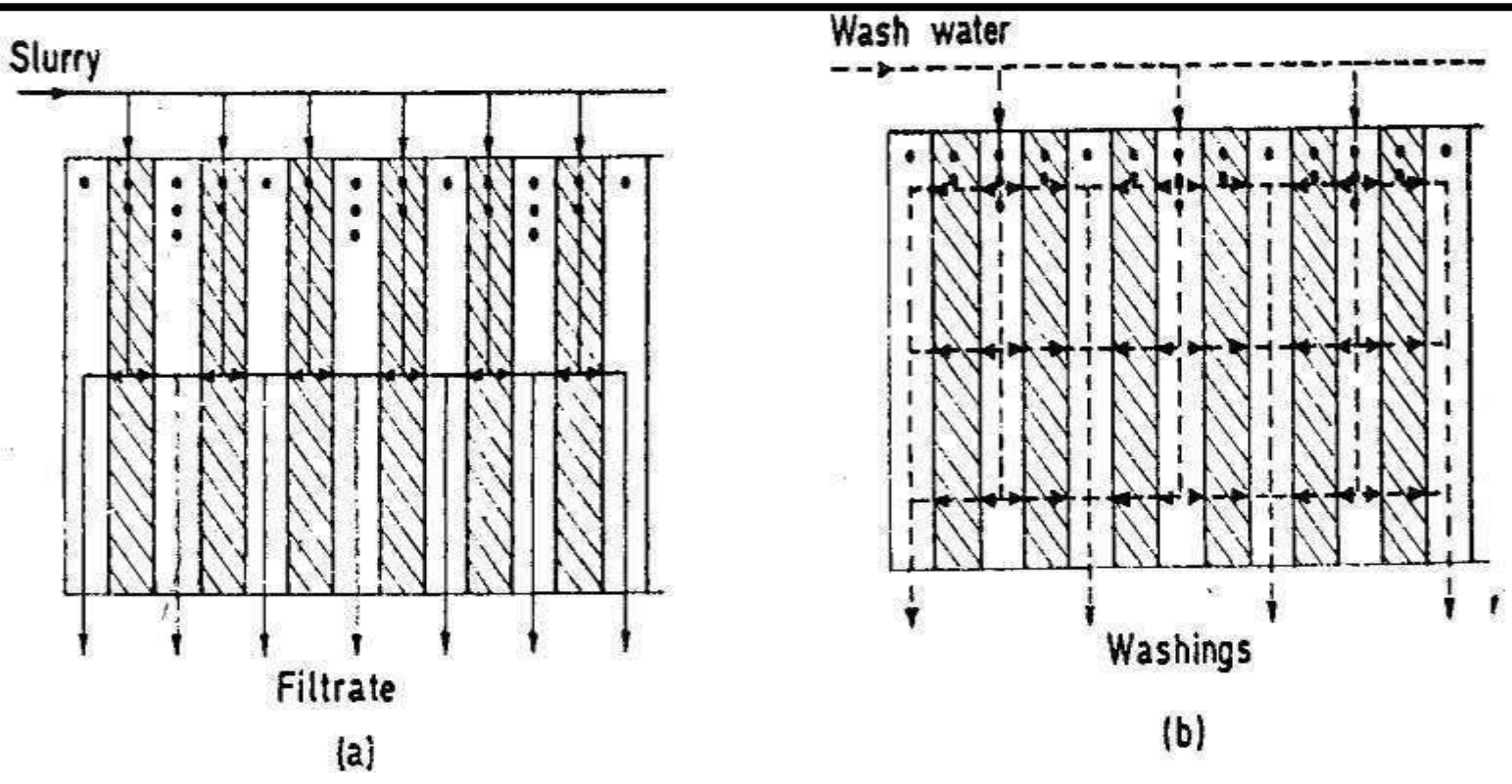


Fig. 20.6 Washing plate and frame filter press: principles of operation
(*a*) filtering (*b*) washing

THINGS TO BE NOTED

- Water – wash is efficient only if the frames are full with filter cake.
- If the solid do not fill the frame completely, the wash water causes the cake to break (on the washing plate side of the frame), then washing will be less effective.
- Hence it is essential to allow the frames become completely filled with washing cake.
- This helps not only in emptying the frames but also helps in washing the cake correctly.

Special provisions and Uses

- Special provisions:
- 1. Any possible contamination can be observed by passing the filtrate through a glass tube or sight glass from the outlet on each plate.
- This permits the inspection of quality of filtrate. The filtrate goes
- through the control valves to an outlet channel.
- 2. The filtration process from each plate can be seen.
- In the event of broken cloth, the faulty plate can be isolated and filtration can be continued with one plate less.
- Uses:

heating/cooling coils in the press.

ADVANTAGES

- Construction of filter press is very simple and a variety of materials can be used.

I. Cast iron – for handling common substances.
II. Bronze - for smaller units.
III. Stainless steel – contamination can be avoided.
IV. Hard rubber and plastic- used where metals must be avoided.
V. Wood- for lightness though it must be kept wet.

- Provide large filtration area in relatively small floor space. The capacity being variable according to thickness of frames and number used.
- Sturdy construction permits the use of considerable pressure difference. (2000 Kilopascals normally used)
- Efficient washing of cake is possible.
- Operation and maintenance is easy.
- It produce dry cake in form of slab.

DISADVANTAGES

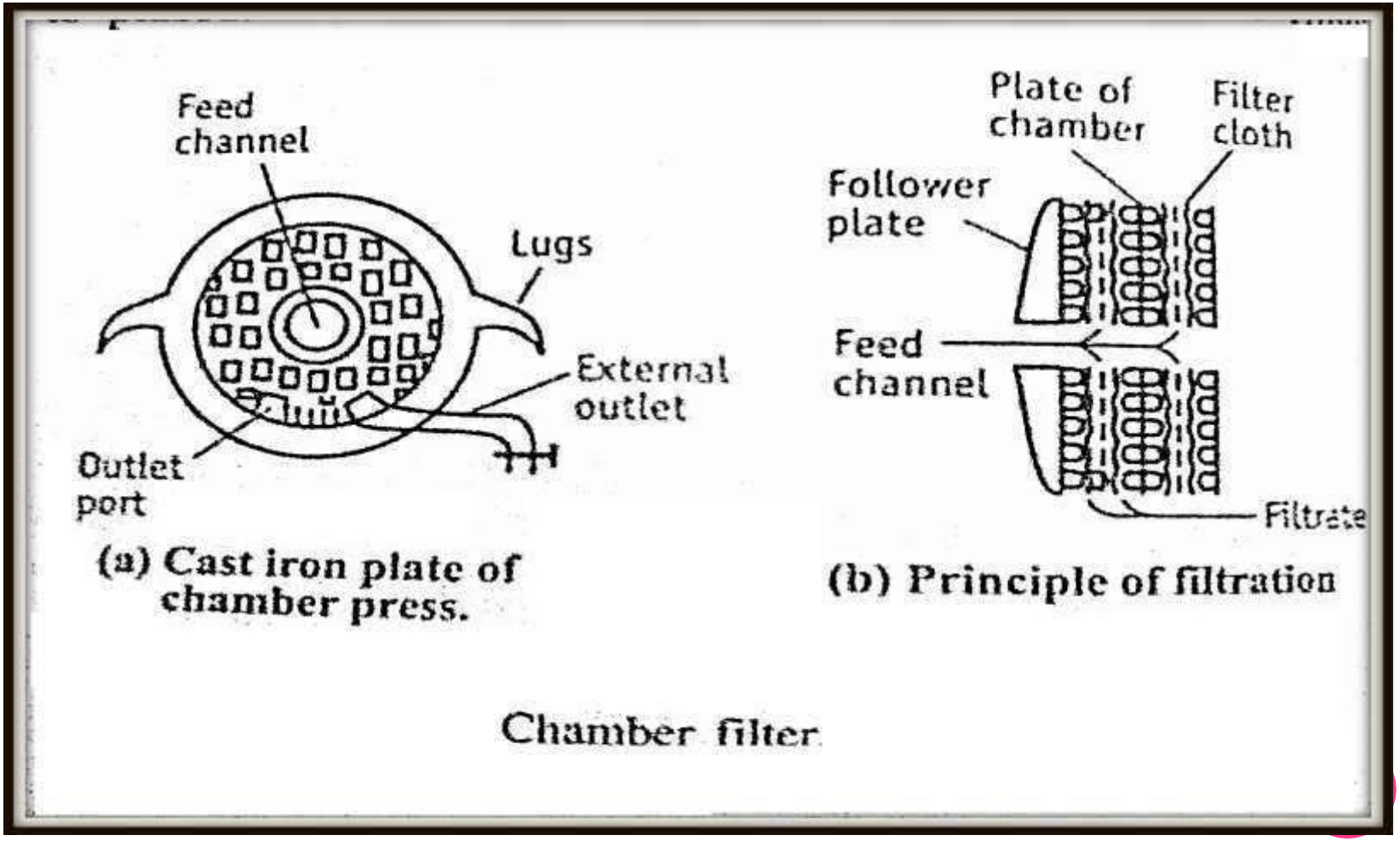
- It is a batch filter, so it is a time consuming.
- The filter press is an expensive filter, the emptying time, the labour involved, and the wear and tear on the cloths resulting in high costs.
- Operation is critical, as the frames should be full, otherwise washing is inefficient and the cake is difficult to remove.
- The filter press is used for slurries containing less about 5 % solids
- In view of the high labour costs , it is most suitable for expensive materials e.g. the removal of precipitated proteins from insulin liquors.

CHAMBER PRESS

Principle

- Mechanism is surface filtration.
- The slurry enters the frame by pressure and flows through the filter cloth.
- The filtrate is collected on the plates and send to the outlet.
- A number of head plates are used, so that the surface area can be increases and consequently large volumes of slurry can be processed simultaneously.

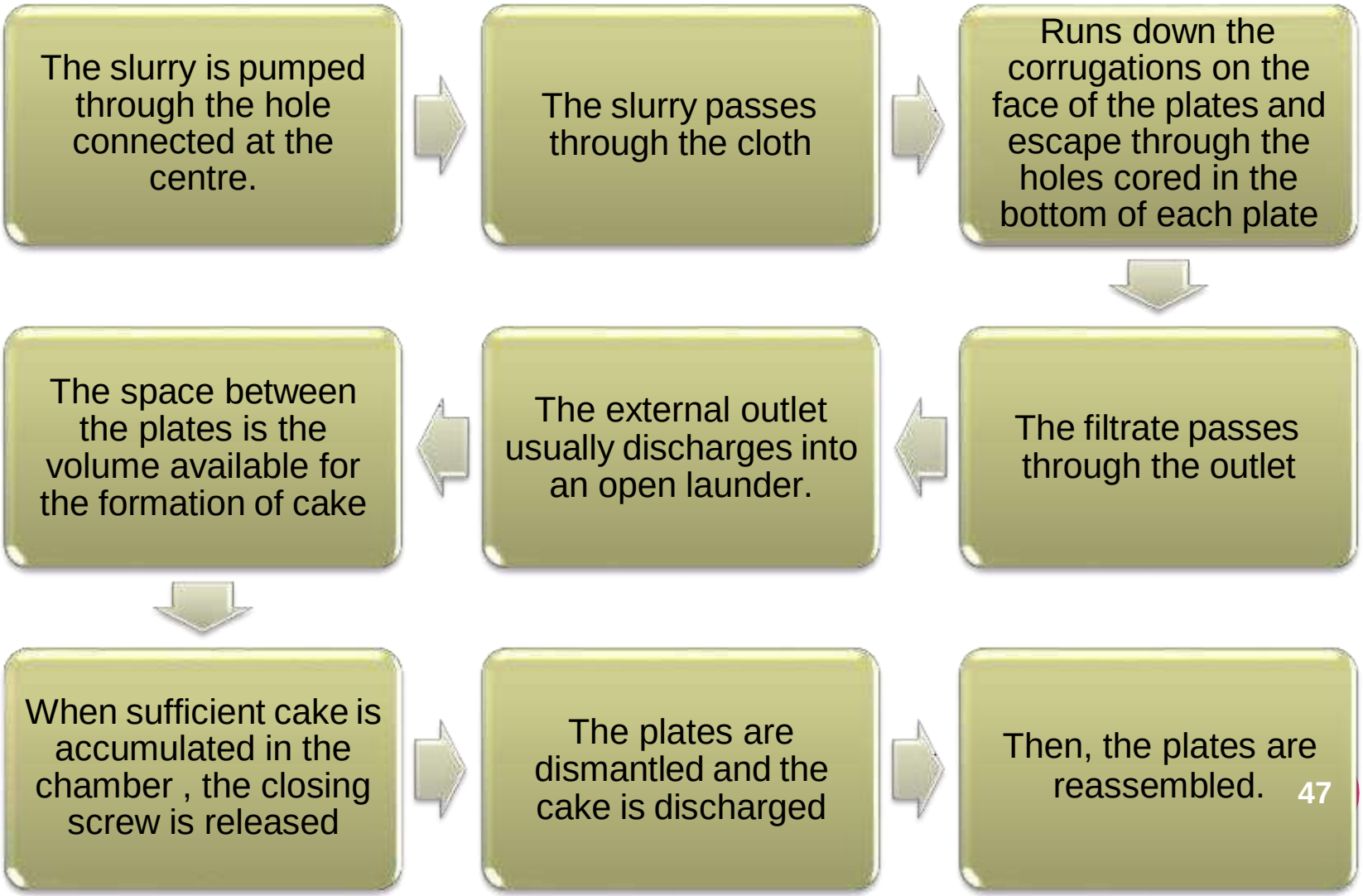
DIAGRAM


Feed channel
Lugs
External outlet
Outlet port
(a) Cast iron plate of chamber press.
Plate of chamber
Filter cloth
Follower plate
Feed channel
Filtrate
(b) Principle of filtration
Chamber filter

CONSTRUCTION

- It consist of heavy fixed head plate made of cast iron and mounted on
- suitable frame.
- Extending from the head plate, two horizontal bars support on both ends.
- The bar supports the plate by lugs.
- At the center of the each plate, a hole is provided for introducing the
- feed.
- The holes of the all plate are connected on the head of the press.
- Over each press plate, a sheet of filter cloth with a hole cut in the centre and is placed.
- The filter cloth is fastened by means of rings called grommets and
- either screwed together or locked.
- These draw down the cloth, so that it is shaped round the plate.
- The cloth serves as a gasket between the adjacent plates.
- All such plates are arranged and a heavy follower plate is placed behind and tighten.

WORKING


The slurry is pumped through the hole connected at the centre.
The slurry passes through the cloth
Runs down the corrugations on the face of the plates and escape through the holes cored in the bottom of each plate
The filtrate passes through the outlet
The external outlet usually discharges into an open launder.
The space between the plates is the volume available for the formation of cake
When sufficient cake is accumulated in the chamber , the closing screw is released
The plates are dismantled and the cake is discharged
Then, the plates are reassembled.

USES, ADVANTAGES AND DISADVANTAGES

Uses:

- used for clarification of syrups and filtrating of injection solutions.

Advantages:

- Provide large filtering area in relatively small floor space.
- A number of head plates can be increased or decreased as per need.
- Operation and maintenance are straight forward (no moving part).
- Filter cloths are easily renewable.

Disadvantages:

- Not adaptable for washing of cake.
- The wear of cloth is severe.
- The filtrate is usually not clear.

FILTER LEAF

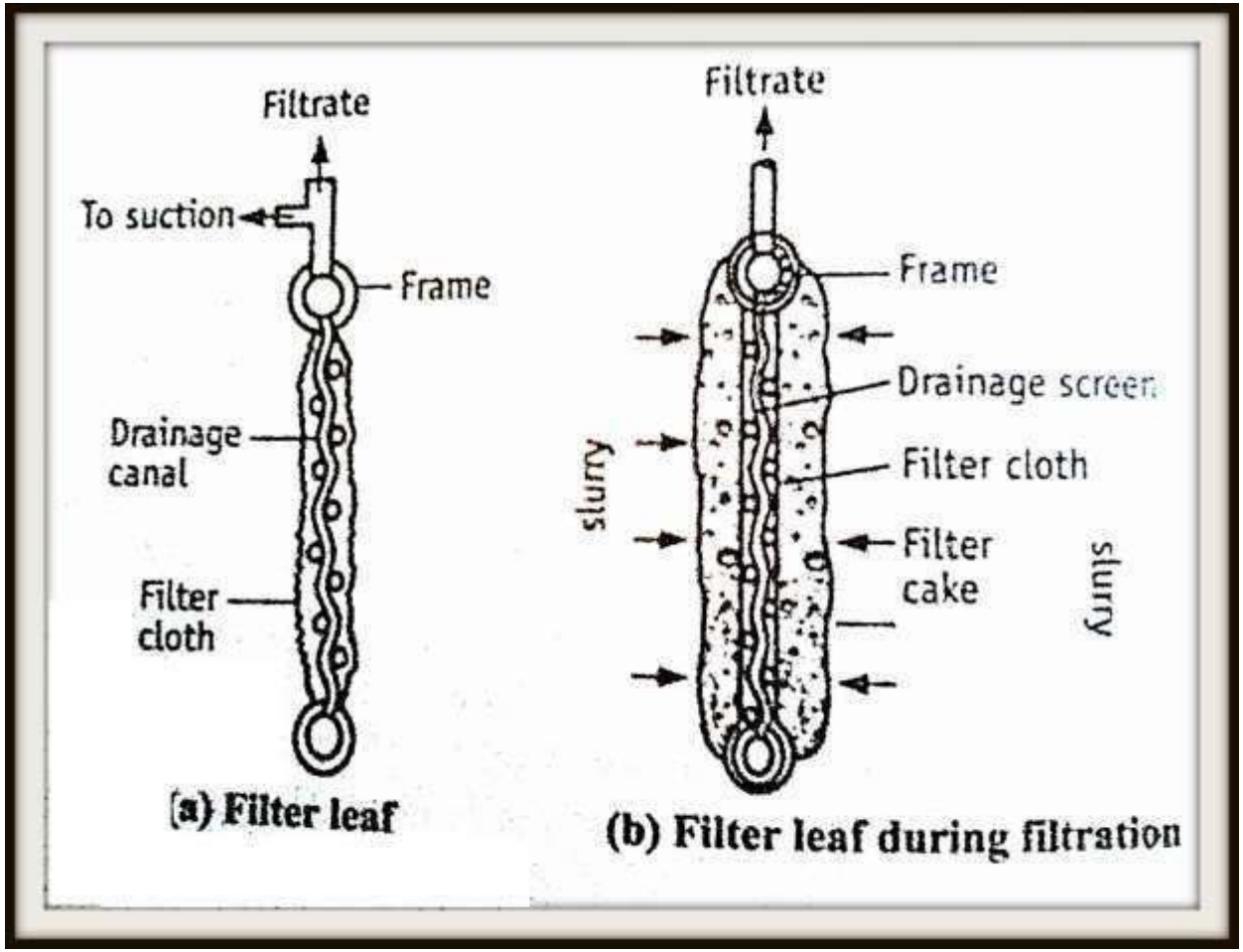
Principle:

- It is an apparatus consisting of a longitudinal drainage screen covered with a filter cloth.
- The mechanism is surface filtration and acts as sieve or strainer.
- Vacuum or pressure can be applied to increase the rate of filtration.

Construction:

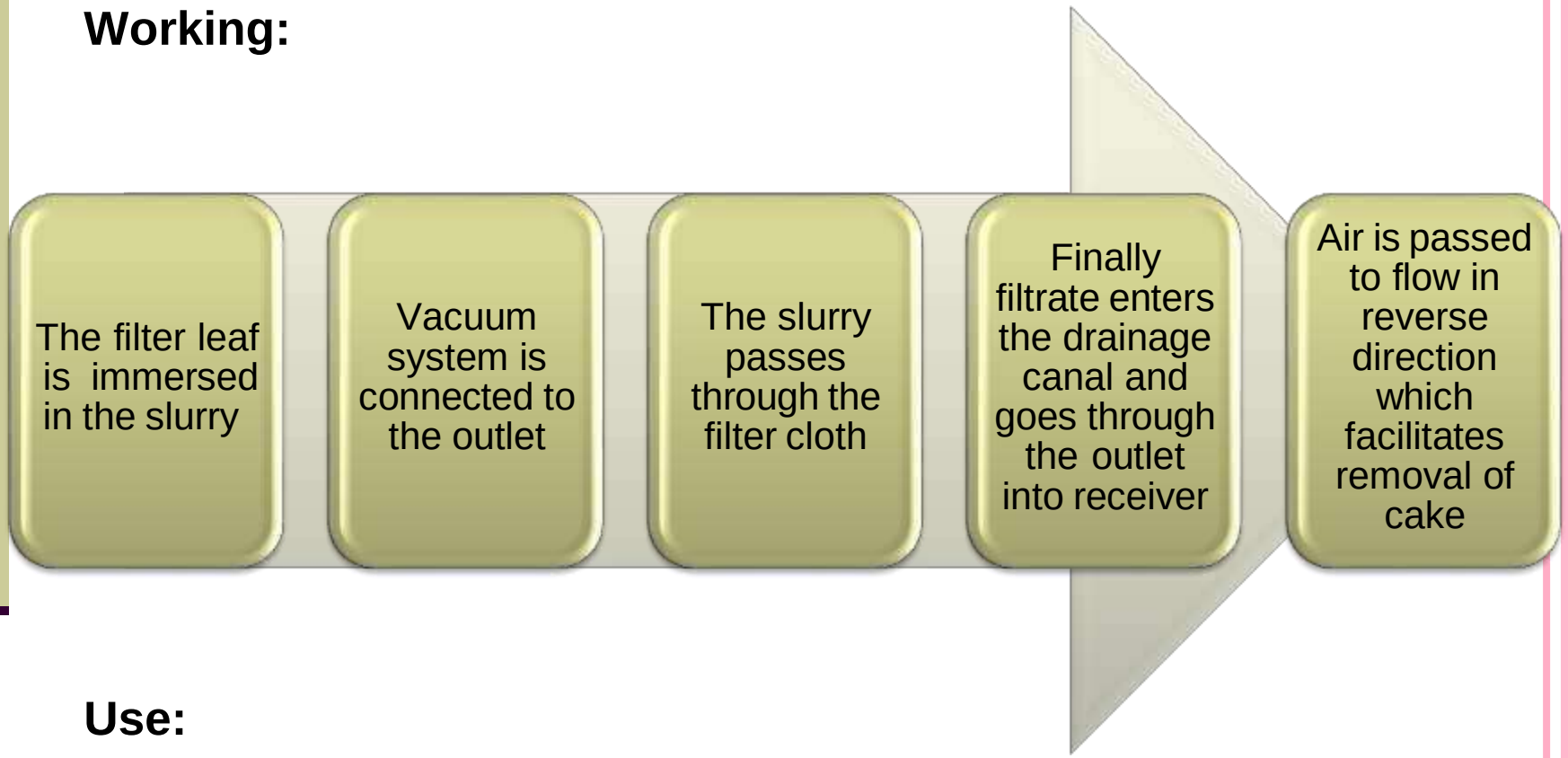
- The leaf filter is consisting of a frame enclosing a drainage screen or grooved plate.
- The frame may be any shape circular, square or rectangular.
- The whole unite being covered with filter cloth.
- The outlet for the filtrate connects to the inside of the frame through suction.

DIAGRAM


Filtrate
To suction
Frame
Drainage canal
Filter cloth
(a) Filter leaf
Filtrate
Frame
Drainage screen
Filter cloth
Filter cake
slurry
slurry
(b) Filter leaf during filtration

WORKING AND USES

Working:



Use:

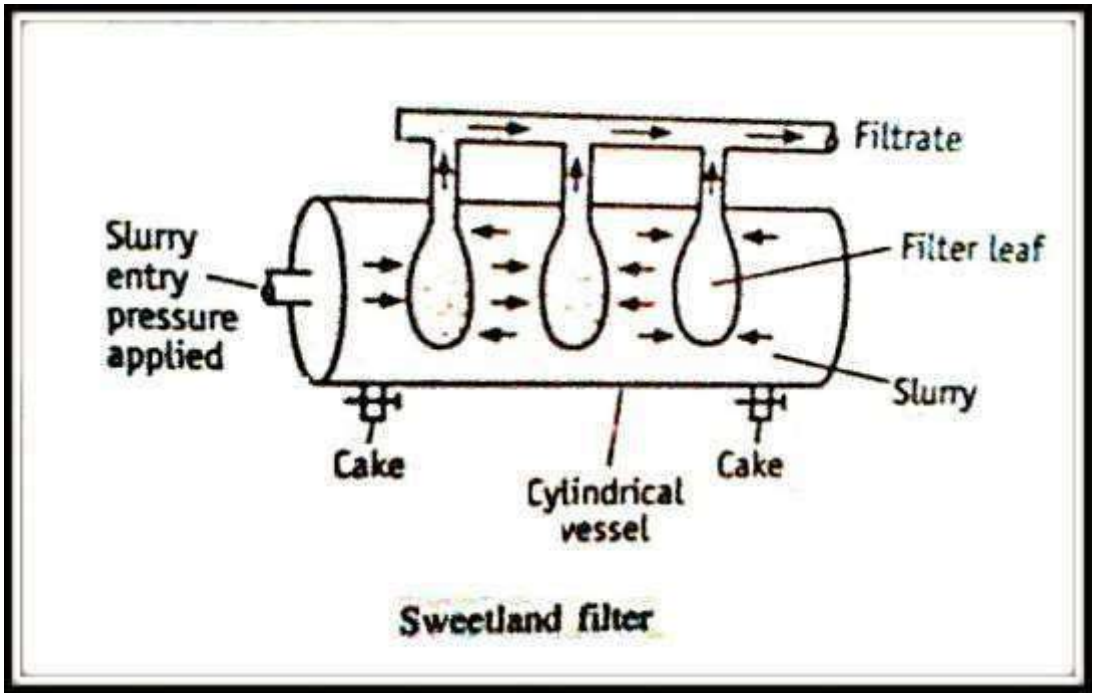
- Use for the filtration of slurry which do not contain high solid content, about 5%, i.e. dilute suspensions.

ADVANTAGES

- Simplest form of filter used for batch process.
- A number of units can be connected in parallel to increase the surface area of filtration.
- Pressure difference can be obtained either with vacuum or using pressure up to the order of 800 kilopascals.
- Labour costs for operating the filter leaf are fairly moderate.
- The efficiency of washing is high.
- The slurry can be filtered from any vessel.
- The cake can be washed simply by immersing the filter in a vessel of Water.

SWEETLAND FILTER (VARIANT OF FILTER LEAF)

- An alternative method is to enclose the filter leaf in a special vessel into which the slurry is pumped under pressure.
- A number of leaves are connected to a common outlet, to provide a large area for filtration.



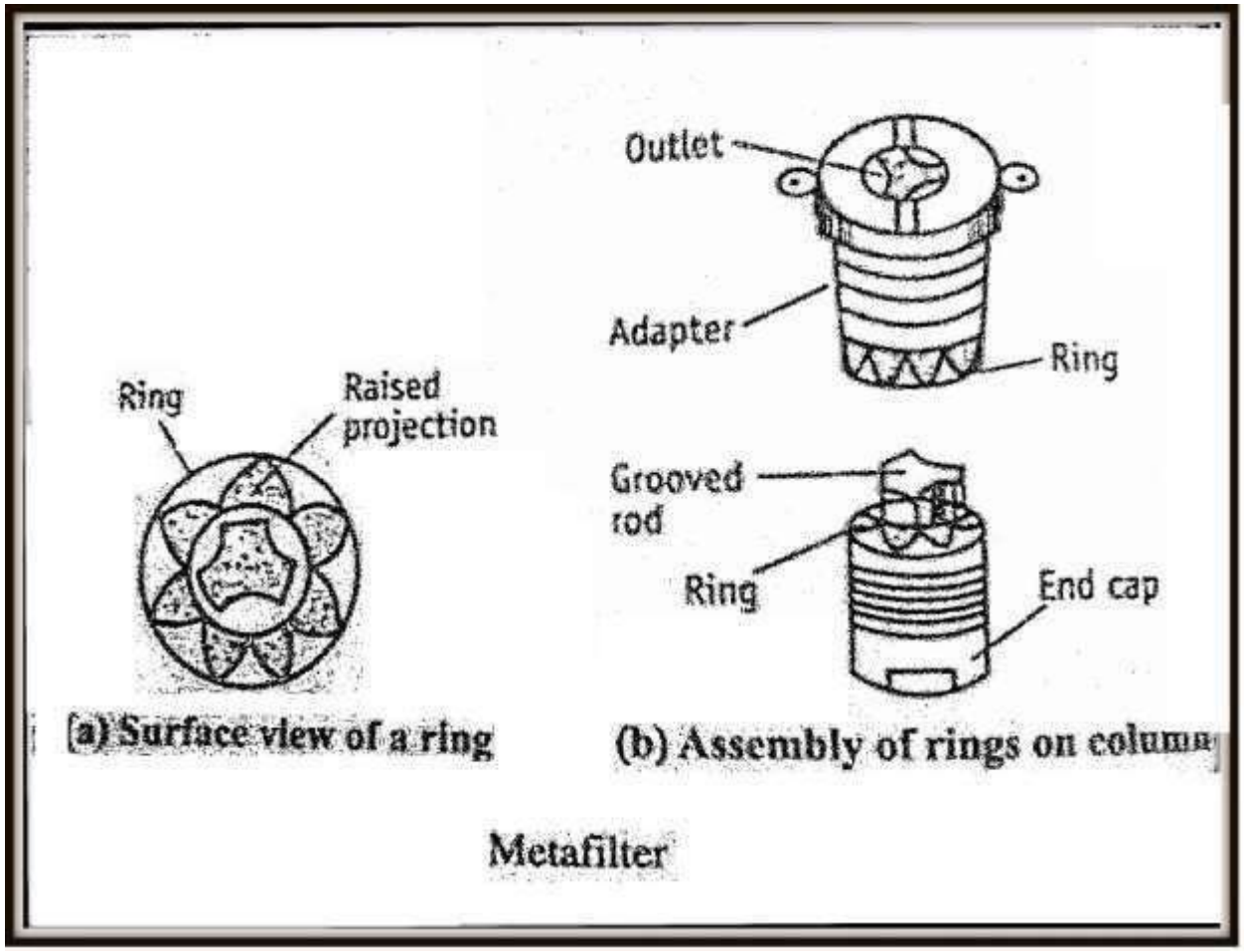
METAFILTER

- **Principle:**
- Mechanism is surface filtration.
- In this, metal rings contain semicircular projections, which are arranged as a nest to form channels on the edges.
- This channel offers resistance (strainer) to the flow of solids (coarse particles).
- The clear liquid is collected into receiver from the top.

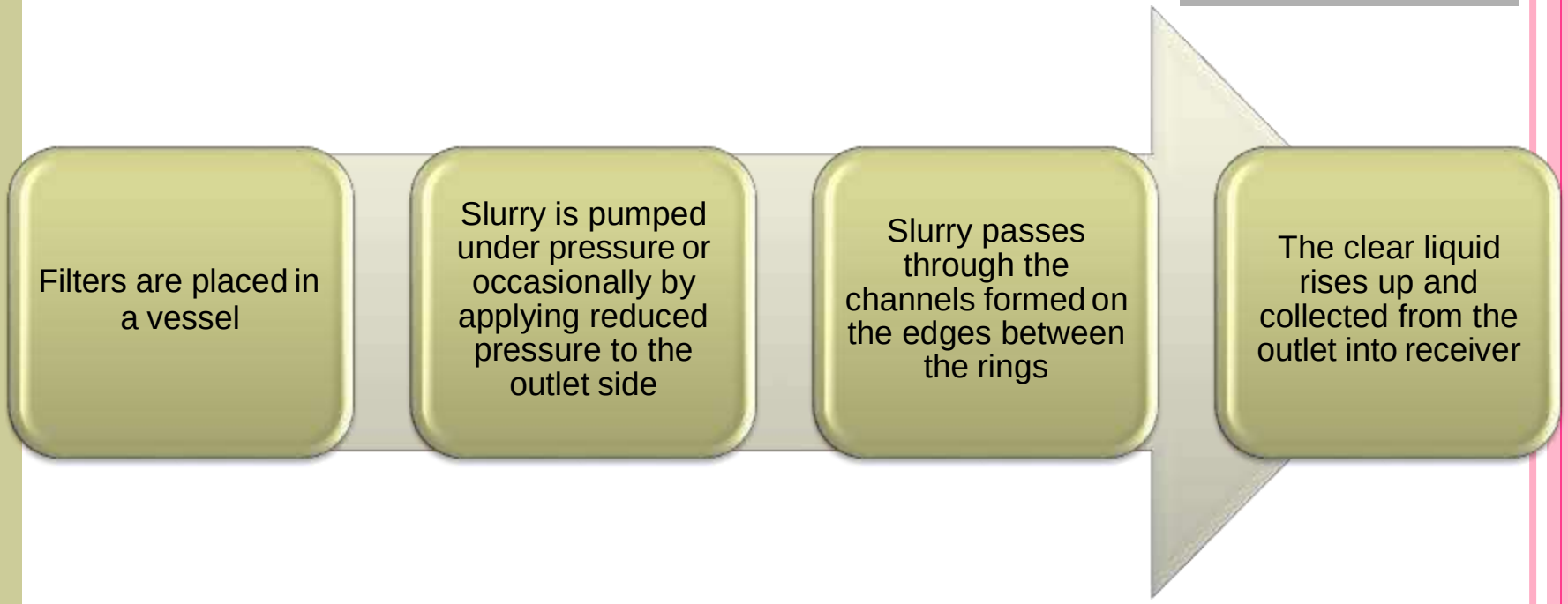
CONSTRUCTION

- Metafilter consists of a series of metal rings.
- These are threaded so that a channel is formed on the edges.
- It contains a grooved drainage column on which a series of metal rings are packed.
- These rings are usually made up of stainless steel and have dimensions of about 15.0 mm internal diameter and 22.0 mm external diameter.
- Each metal ring has a number of semicircular projections (0.8 mm in thickness) on one side of surface.
- The projections are arranged as a nest to form channels on the edges.
- These rings are tightened on the drainage column with a nut.
- Metafilters are also known as edge filters.

DIAGRAM


Outlet
Adapter
Ring
Ring
Raised projection
Grooved rod
Ring
End cap
(a) Surface view of a ring
(b) Assembly of rings on columa
Metafilter

WORKING



- For separation of fine particles, a bed of suitable materials such kieselguhr is first built up.
- The pack of rings serves essentially as a base on which the true filter medium is supported.

USES

- Metafilters can be used for-
  - Clarification of syrups
  - Filtration of injection solutions
  - Clarification of insulin liquors
  - Filtration of viscous liquids can be achieved by applying pressure.

ADVANTAGES

- Can be used under high pressures, without any danger of bursting the filter medium.
- Running cost are low, as separate filter medium is not used.
- Can be constructed from a material that can provide excellent resistance to corrosion and avoid contamination of sensitive products.
- It is extremely versatile filter because fine as well as large both type of particles can be separated.
- Removal of cake can be carried out by simply back- flushing with water.
- Change over from one batch to another or one product to another is easy.
- Sterile products can be handled.

CARTRIDGE FILTER

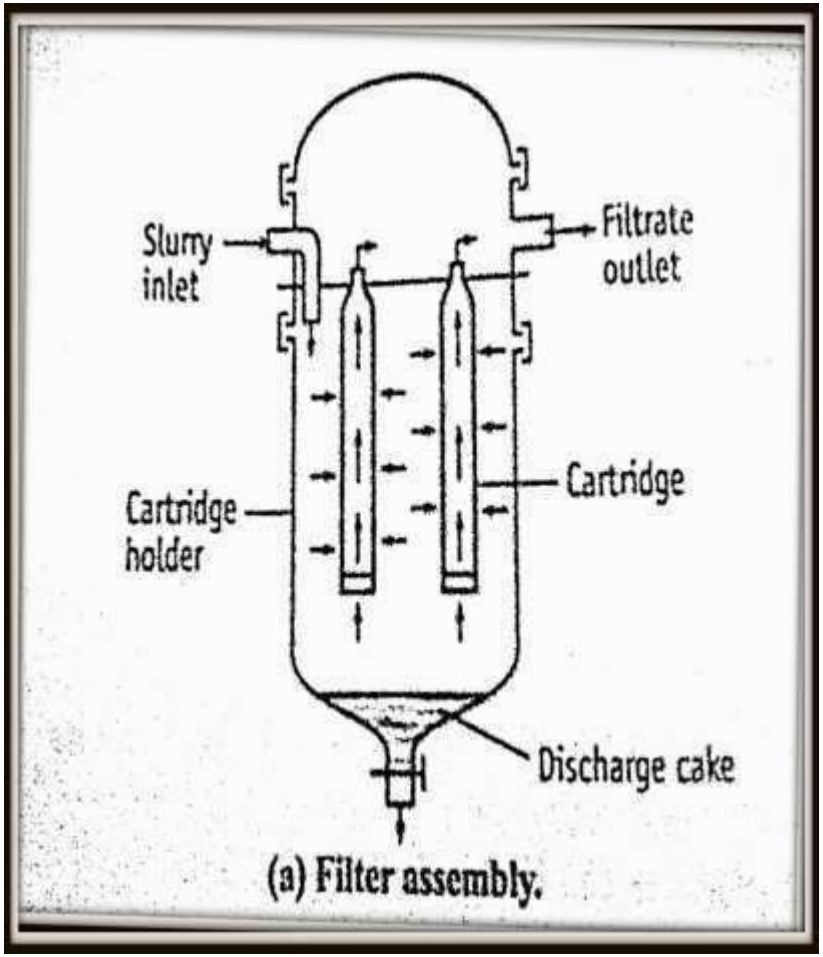
Principle:

- It is a thin porous membrane in which pre filter and membrane filter are combined in a single unit.
- The filtration action is mainly sieve like and particles are retained on the surface.

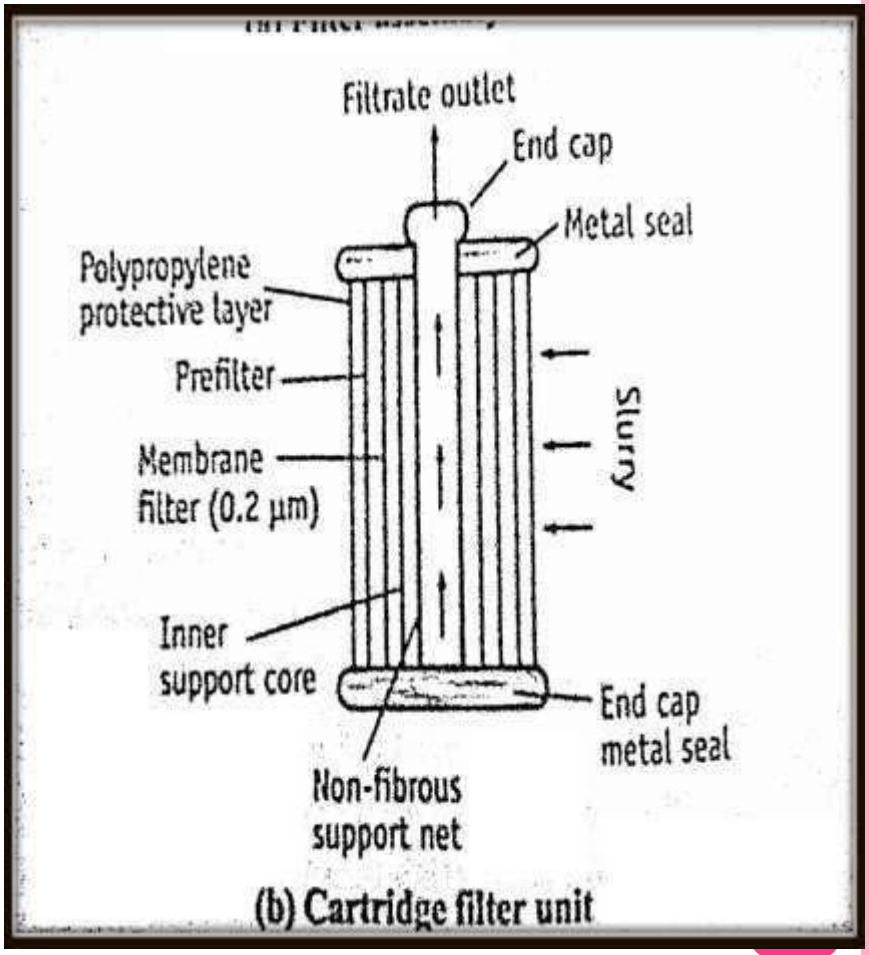
Construction:

- It has cylindrical configuration made with disposable or changeable filter media.
- Made up of either plastic or metal.
- Consist of two membrane filters (sieve like) made of polypropylene: pre filter and actual filter for filtration.
- A protective layer surrounds them.
- The cartridge are housed in a holder and a number of cartridges can be placed in a same housing.
- The housing is closed with the lid.
- Housing has provisions for slurry inlet and outlets.

DIAGRAM



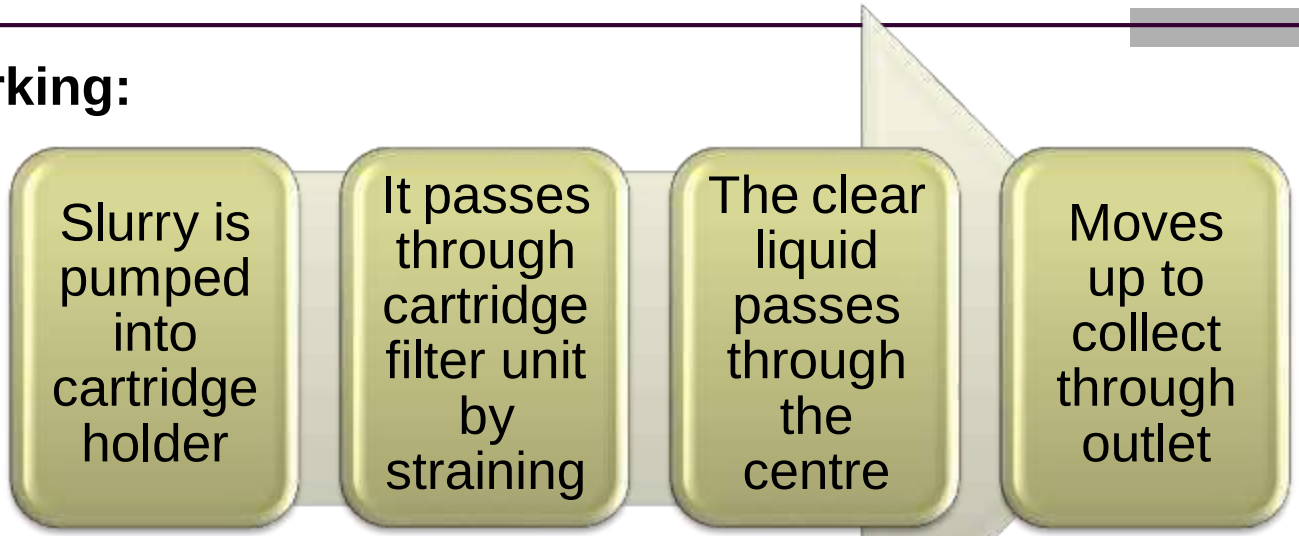
(a) Filter assembly.



(b) Cartridge filter unit

WORKING AND USES

Working:



Uses:

- Particularly useful for preparation of particulate free solutions for parenterals and ophthalmic uses.
- This filter holder will process 1000 – 15000 litres of sterile solution per hour.

ADVANTAGES AND DISADVANTAGES

Advantages:

- Autoclaving can be done for sterile operations due to stainless steel construction.
- Cartridge with self cleaning devices are advantageous.
- Rapid disassembling as well as reusing of filter medium is possible.
- Cartridge are not brittle, when they are dry.
- Used as in-line continuous filtration, which reduces handling of solutions. It minimize chances of contaminations.

Disadvantages:

- A number of manufactures provide the components, which are generally not interchangeable between suppliers.
- Cost of disposable elements offsets the labour saving in terms of assembly and cleaning of cartridge clarifiers.

ROTARY DRUM FILTER

- **Principle:**
  - Slurry filtered through sieve like mechanism on the rotation drum surface, under the condition of vacuum.
  - In addition compression, drying (using hot air), and removing the filter cake (using knife) are possible.
- **Construction:**
  - It consist of a metal cylinder mounted horizontally.
  - The drum may be up to 3 meters in diameter and meters in length and gives surface area of 20meter square.

DIAGRAM

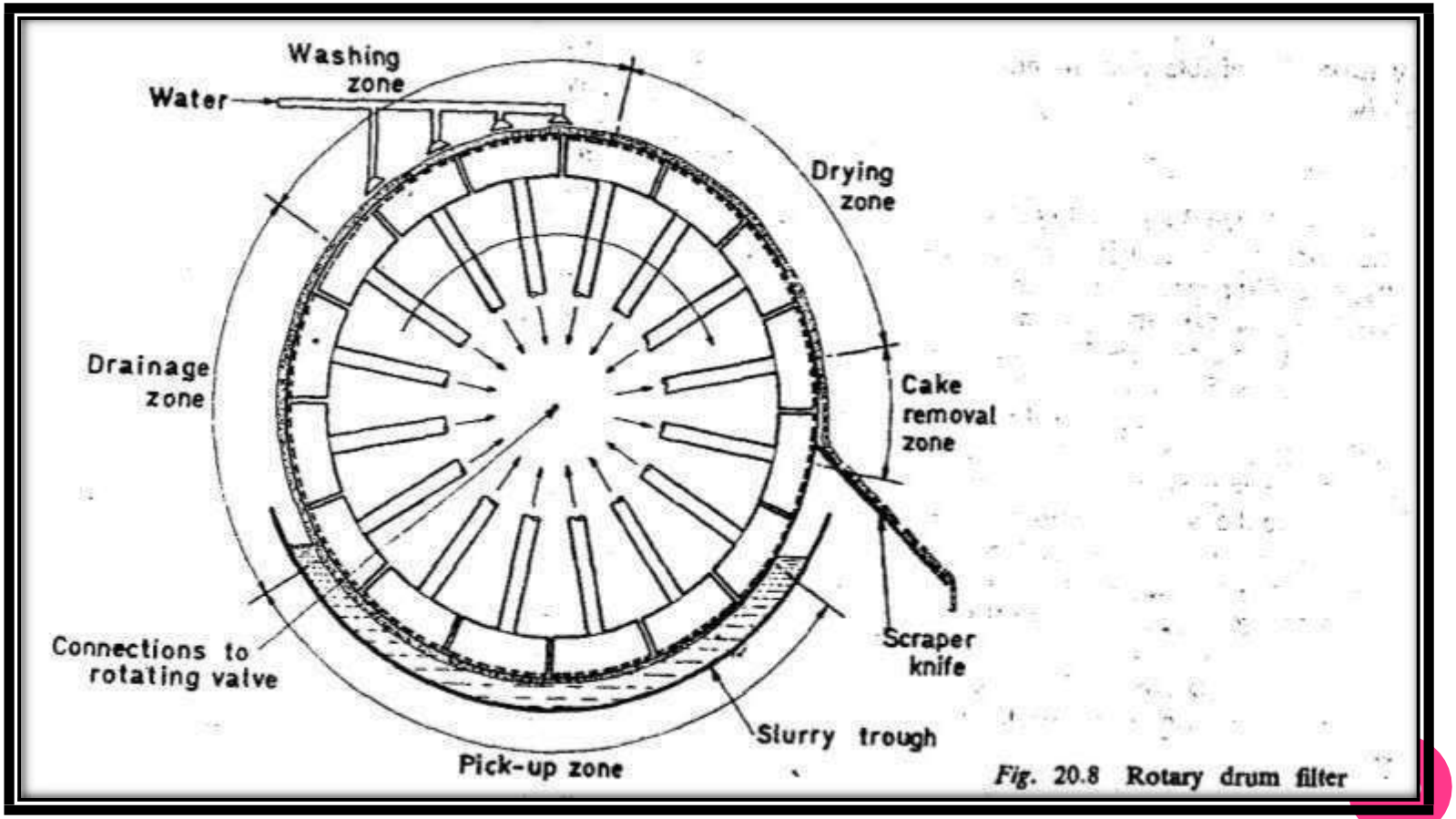


Fig. 20.8 Rotary drum filter

WORKING

- The drum is dipped into the slurry and vacuum applied to the outlet, which is connected to the filtrate receiver.
- When the cake has formed, the cake drained or partially dried by vacuum.
- The drum is sprayed with water to wash the cake.
- Retaining the vacuum connection drains the cake and produces partial dryness then, removed by a doctor knife.
- When the solids of the slurry are too much that the filter cloth becomes blocked with the particles, a pre-coat filter may be used.
- A pre-coat of filter aid is deposited on the drum prior to the filtration process.

Uses

- The rotary filter for continuous operation on large quantities of
  - slurry.
- Suitable for slurry contains considerable amounts of solids in the range 15-30%.

- Examples of pharmaceutical application include
  - the collection of calcium carbonate, magnesium carbonate, and starch.
  - The separation of the mycelium from the fermentation liquor in
  - the manufacture of antibiotics.

ADVANTAGES

- The rotary filter is automatic and is continuous in operation, so that the labour costs are very low.
- The filter has a large capacity , so it is suitable for the filtration of highly concentrated solutions.
- Variation of the speed of rotation enables the cake thickness to be controlled.
- Pre-coat of filter aid could used to accelerate the filtration rate.
- Filter has large surface area.

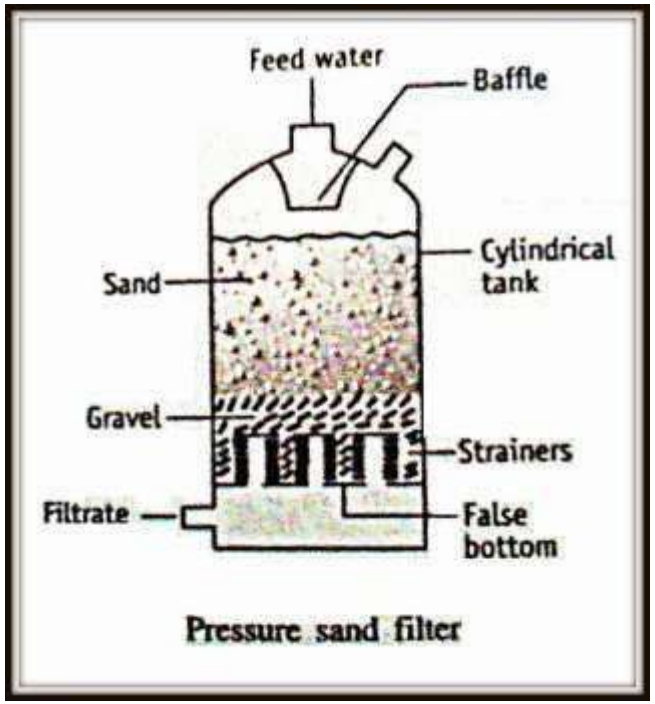
DISADVANTAGES

- The rotary filter is a complex piece of equipment , with many
- moving parts and is very expensive,.
- In addition to the filter itself, some accessories are connected
- ,e.g., a vacuum pump, vacuum receivers , slurry pumpsand agitators are required .
- The cake tends to crack due to the air drawn through by the
- vacuum system, so that washing and drying are not efficient.
- Being a vacuum filter, the pressure difference is

PRESSURE AND SAND FILTER

Principle:

- Depth filtration
- Mechanism is impingement and entanglement of solids on account of low pressure differential.
- The slurry enters the sand bed and the clear liquid is collected from the bottom outlet.

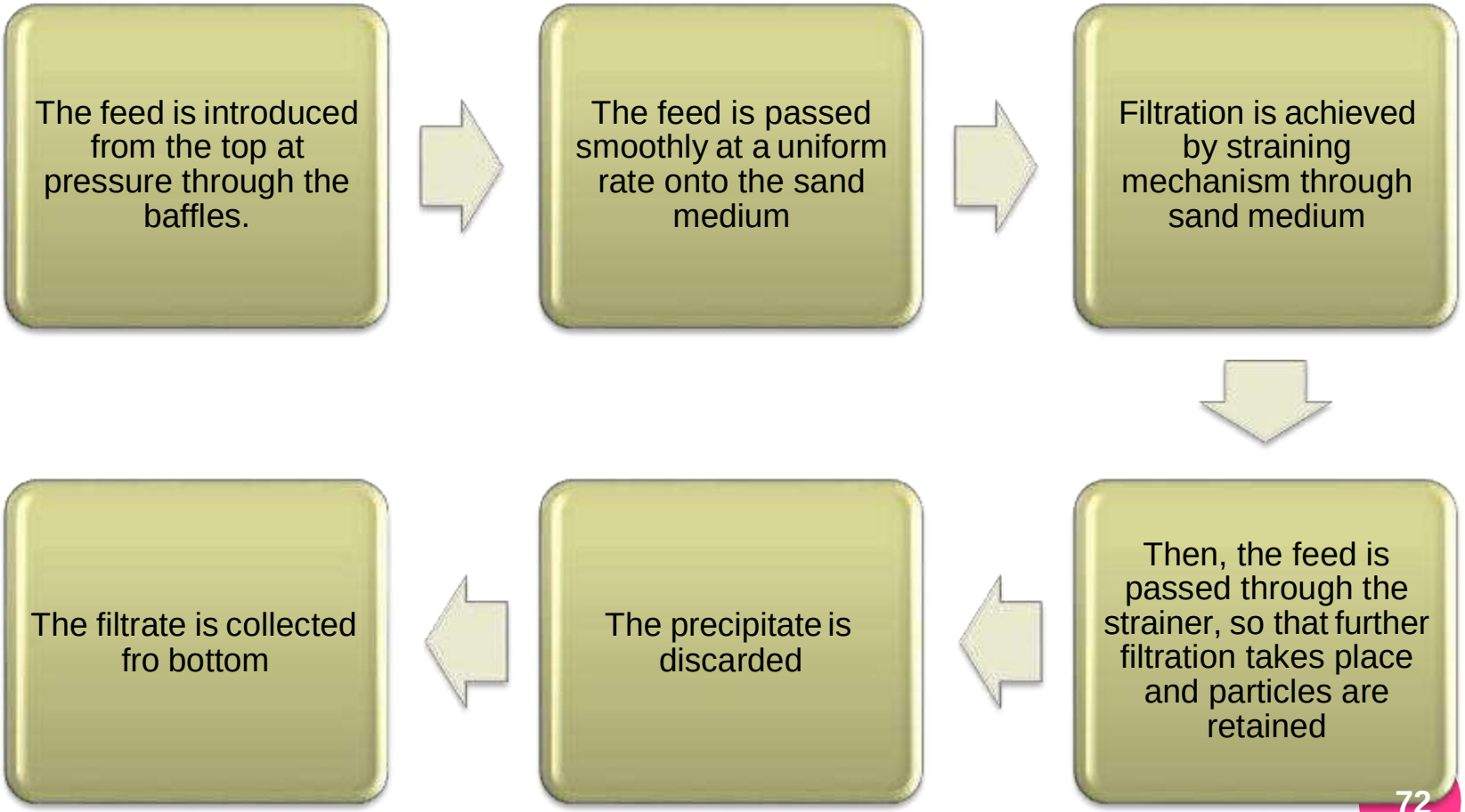


Pressure sand filter

CONSTRUCTION

- It is a closed system and consists of cylindrical tank with a bottom containing a number of brass strainers.
- These are mounted on false bottom or connected to a modified embedded in concrete.
- The strainers have narrow slots sawed in them.
- Above this 6-12 m depth sand layer is placed, which acts as a filter medium.
- Baffles are arranged at the point of feed inlet, in order to prevent the disturbance of sand by direct stream.
- Provisions is made at the bottom for collecting the filtrate.

WORKING


The feed is introduced from the top at pressure through the baffles.
The feed is passed smoothly at a uniform rate onto the sand medium
Filtration is achieved by straining mechanism through sand medium
Then, the feed is passed through the strainer, so that further filtration takes place and particles are retained
The precipitate is discarded
The filtrate is collected fro bottom

CONTINUE…..

- When the precipitate clogs the sand (indicated by reduced filtration rate), back washing permits the regeneration of the filter bed.
- During this operation, water moves up through the sand bed and moves out from the inlet.

Uses:

- Used for filtration of boiler feed or water for similar purposes.
- Mainly used when solids are little and large volume of feed is to be handled.
- They are applicable only to the separation of precipitates that can be removed from the sand.

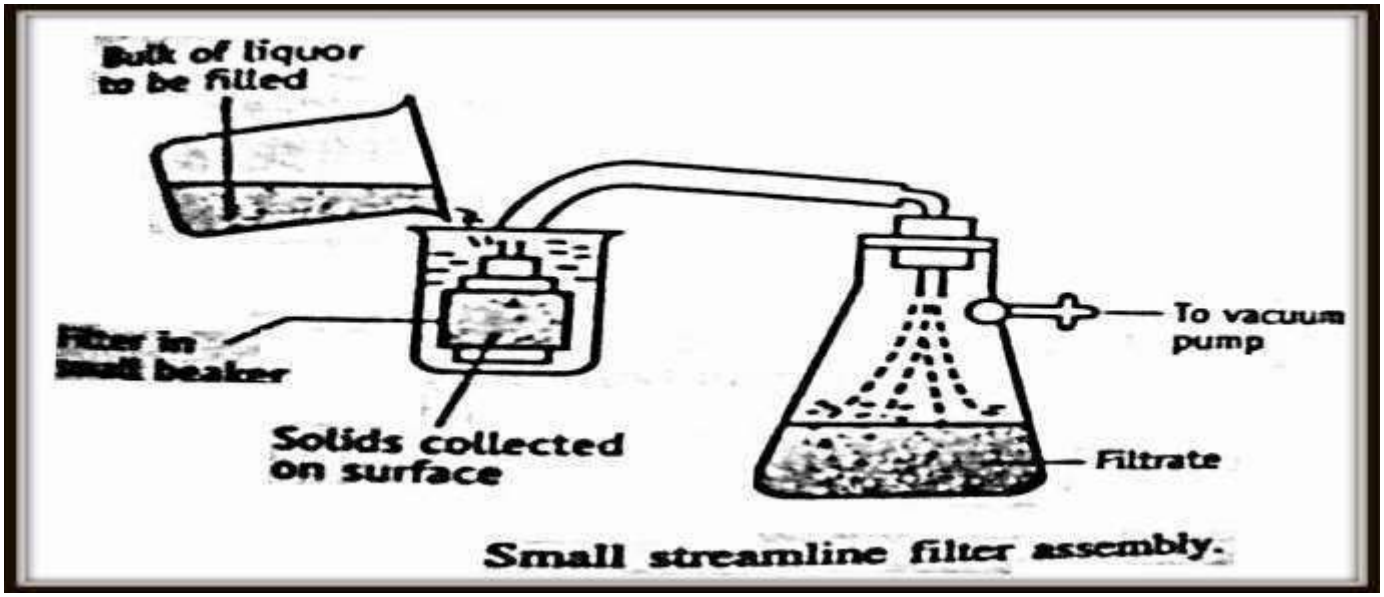
DISADVANTAGES

- Feed containing precipitates that are gelatinous or form a coat on sand can't be filtered through the sand, because back washing does not permit regeneration.
- Feed containing finely divided solids can't be separate by this.
- Can't remove bacteria. In such cases, coagulants such as ferrous sulphate, are added to feed before filtration.

STREAMLINE FILTER

Principle:

- It consists of column of filter elements held tightly together, so that the slurry enters from the side of the column and passes through vary narrow channels between the elements.
- The filters employ paper discs.
- The filter act as a strainer to retain solids.

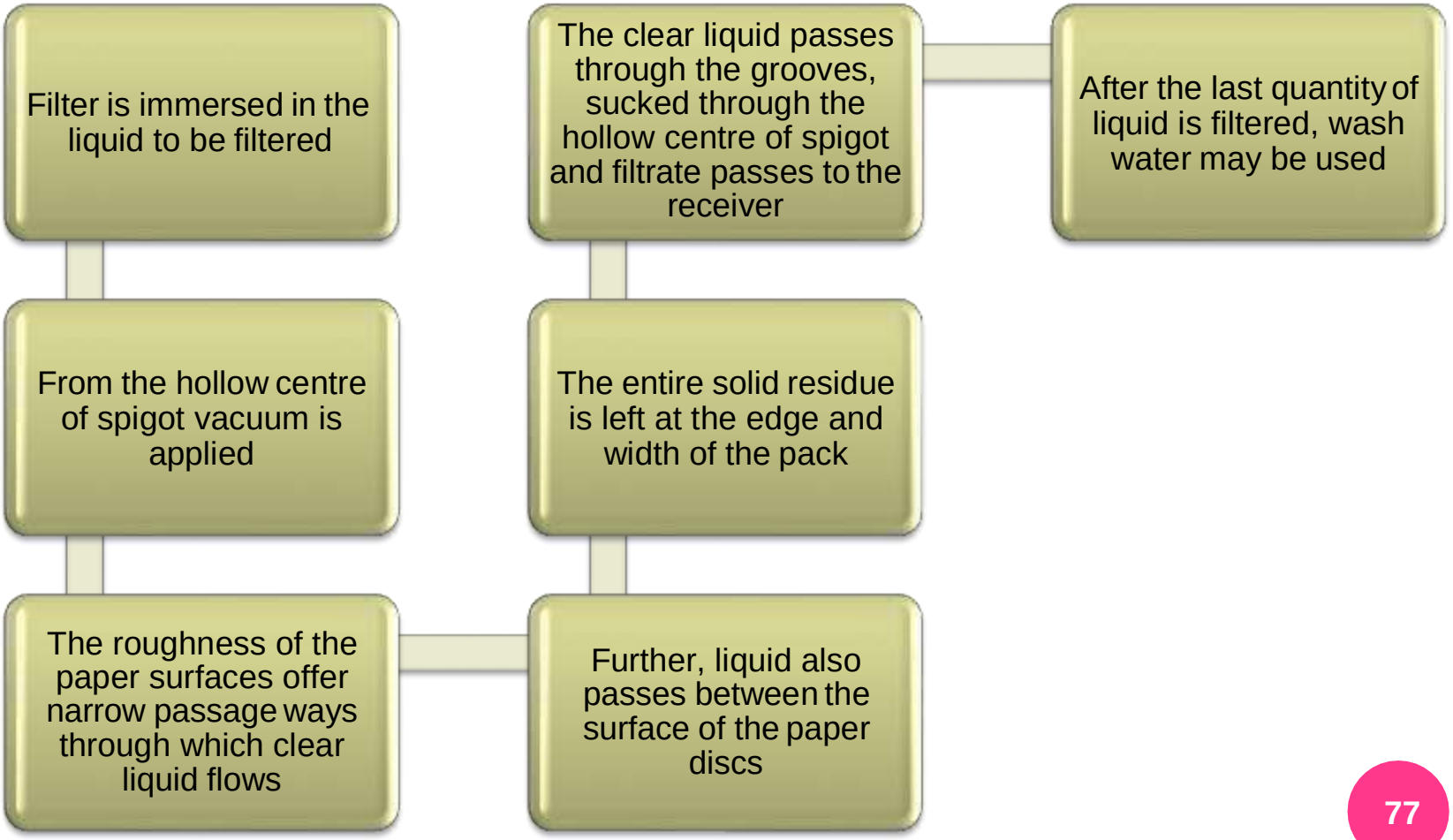


Small streamline filter assembly.

ASSEMBLY

- It consists of specially treated filter paper discs arranged in the form of a compressed pack on an upright rod or spigot.
- In assembling the filter, the two parts of the filter can be unscrewed.
- The required quantity of rings are placed on the spigot portion and two parts are then screwed together to give a compact stack of filter elements.
- The extent of compression on the disc may be varied depending on the size of particles to be removed.
- For small scale filtration, ebonite material made spigots are used.
- The spigot is hollow and grooves run down the length.
- From hollow centre of the spigot, vacuum is applied.

WORKING


Filter is immersed in the liquid to be filtered
From the hollow centre of spigot vacuum is applied
The roughness of the paper surfaces offer narrow passage ways through which clear liquid flows
Further, liquid also passes between the surface of the paper discs
The entire solid residue is left at the edge and width of the pack
The clear liquid passes through the grooves, sucked through the hollow centre of spigot and filtrate passes to the receiver
After the last quantity of liquid is filtered, wash water may be used

CONTINUE ....

- Cleaning of streamline filter is attempted by connecting the spigot to the water tap.
- The water is passed in opposite direction.
- The residue is washed away from the edges of the stack.
- If solid residue is required, air may be blown, so that solid is peeled of, which may be collected.

USES AND ADVANTAGES

Uses:

- It removes suspended matter, trace of fines and even submicron size colloidal matter from large volumes of the slurry.
- The two parts of spigots are screwed tightly and fine precipitates can be separated for achieving high grade polishing.
- Oily, aqueous and alcoholic solutions which do not soften the fibre of the pack can also be filtered.
- Heat resistant porcelain filters are used for strongly acidic or hot strong alkaline solutions.

Advantages:

- Instead of vacuum, pressure may be applied.
- In this case, the container (in which assembly is immersed) must be sealed and should be able to withstand applied pressure.


Thank You!
Http://maomao520.yeah.net

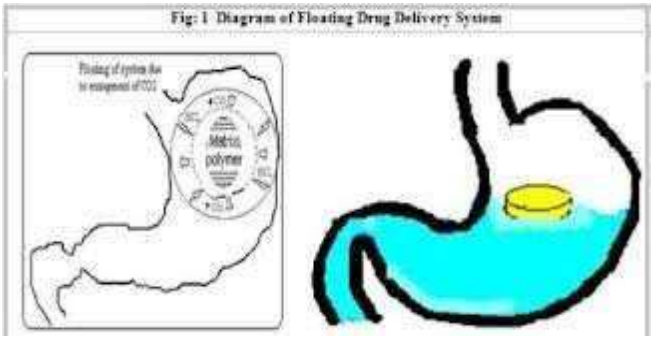
FLOATING DRUG DELIVERY SYSTEM

G.Shankar

VCPS

INTRODUCTION

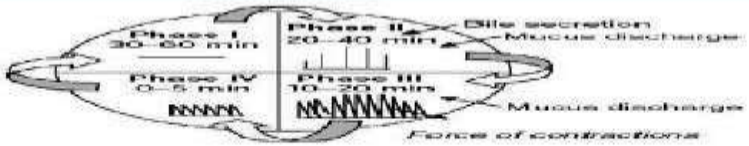
- Floating drugs are gastro retentive systems which remain in gastro region for several hrs i.e it prolong gastro residual time of drugs
- FDDS enhance GRT&Control fluctuation in plasma drug concentration
- **NEED OF FDDS**
- Gastric emptying time in humans avg(2-3 hrs) through major absorption zone can result incomplete drug release from drug delivery leads to reduce in administered dose efficacy
- Beneficial in gastro intestinal diseases
- Lower dosing&lesser side effects

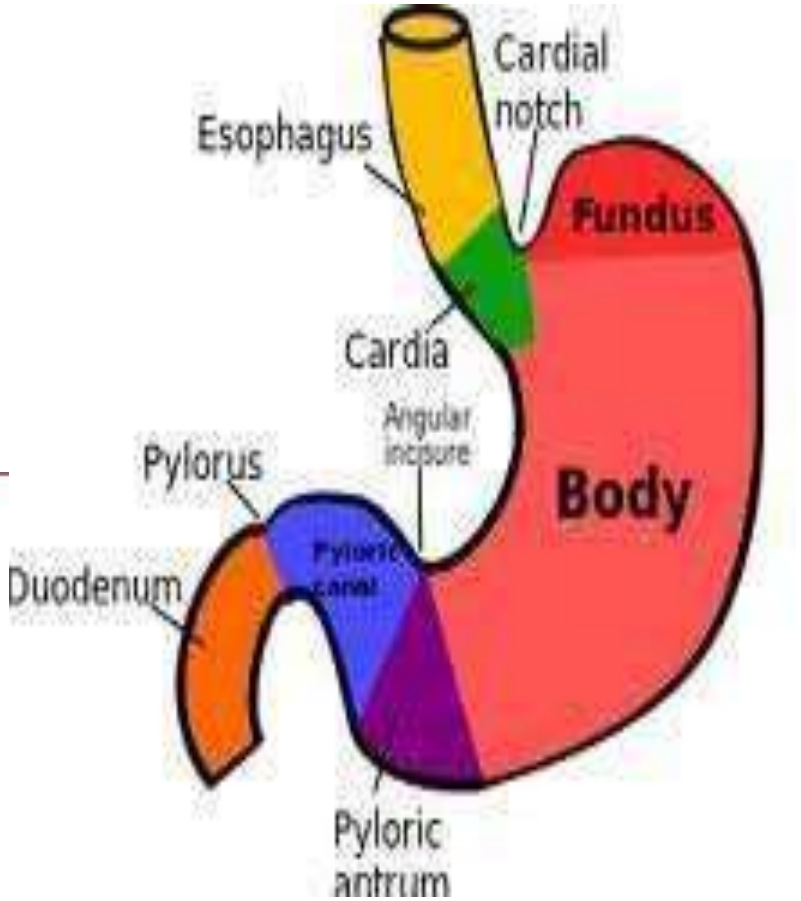


BASIC GASTRO INTESTINAL TRACT

Stomach:

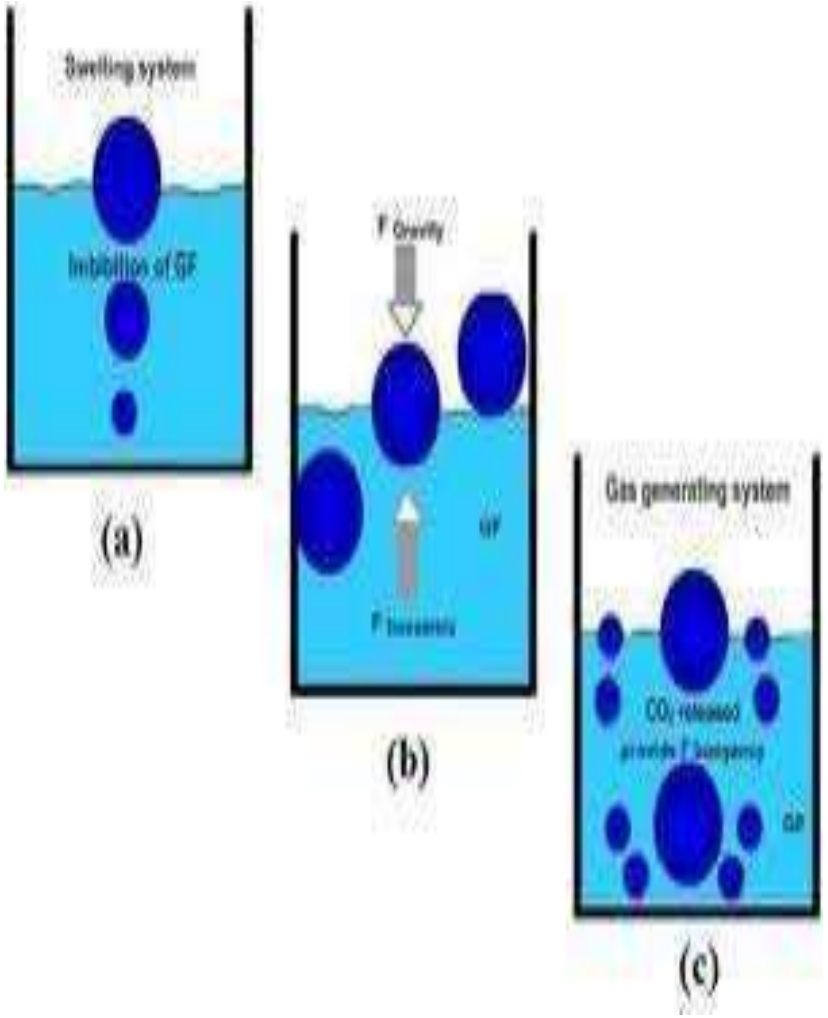
1. Fundus
2. Body
3. Antrum





MECHANISM OF FLOATING SYSTEMS

- FDDS have less bulk density than GI fluids&so remain buoyant in the stomach without affecting GER for prolonged period of time
- F=F Buoyancy-F gravity
- =(Df-Ds)gv ---(1)
- Where,F =total vertical force,Df=fluid density
- Ds=object density,v=volume,g=acceleration due to gravity



Factors affecting floating time

- Density, size & Shape of Dosage form
- Single or Multiple unit operation
- FED or UNFED state
- Nature of Meal
- Frequency of Feed
- Age & Gender
- BIOLOGICAL factors

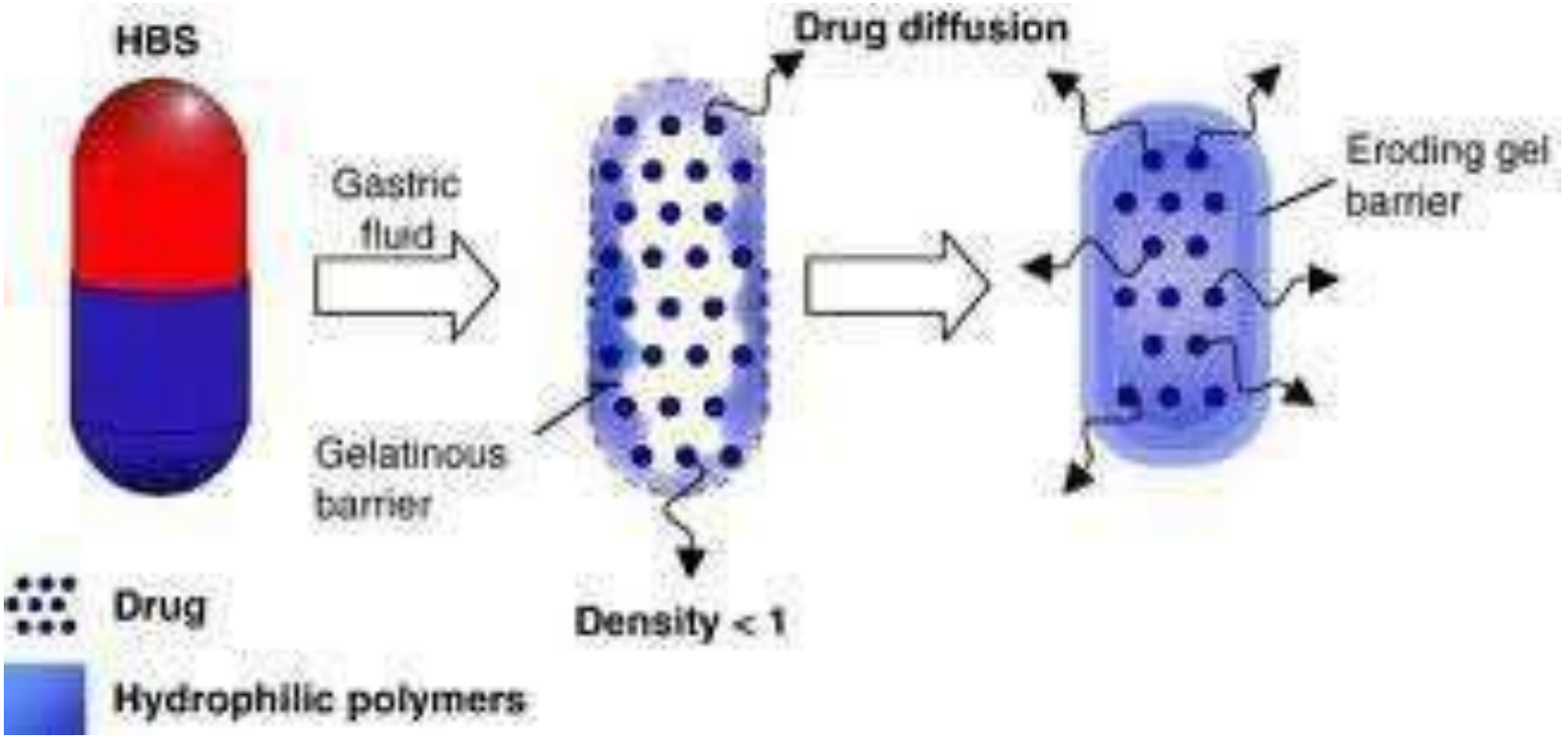
CLASSIFICATION OF FDDS

A Single Unit Floating Dosage Systems

1. Non-effervescent Systems
2. Effervescent Systems(gas generating system)

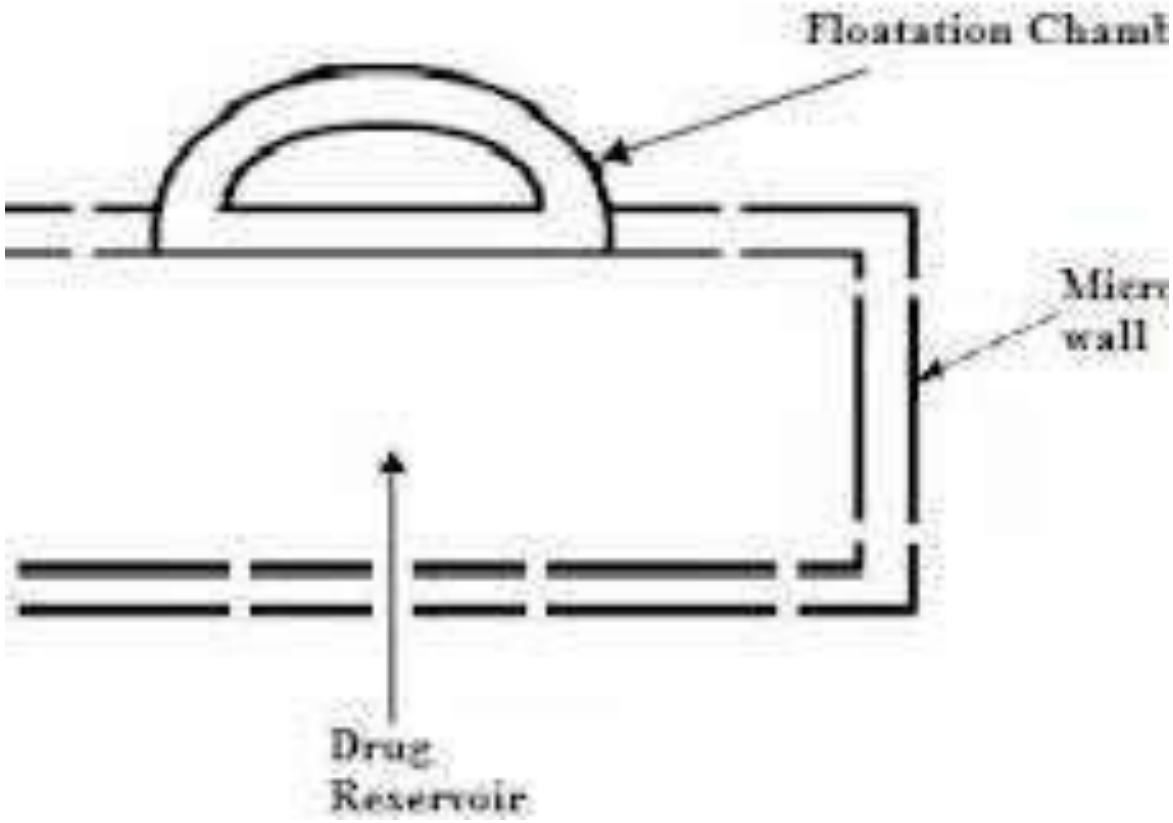
B. Multiple Unit Floating Dosage Systems

1. non-effervescent Systems
2. Effervescent systems
3. Raft Forming Systems
4. Hollow Microspheres



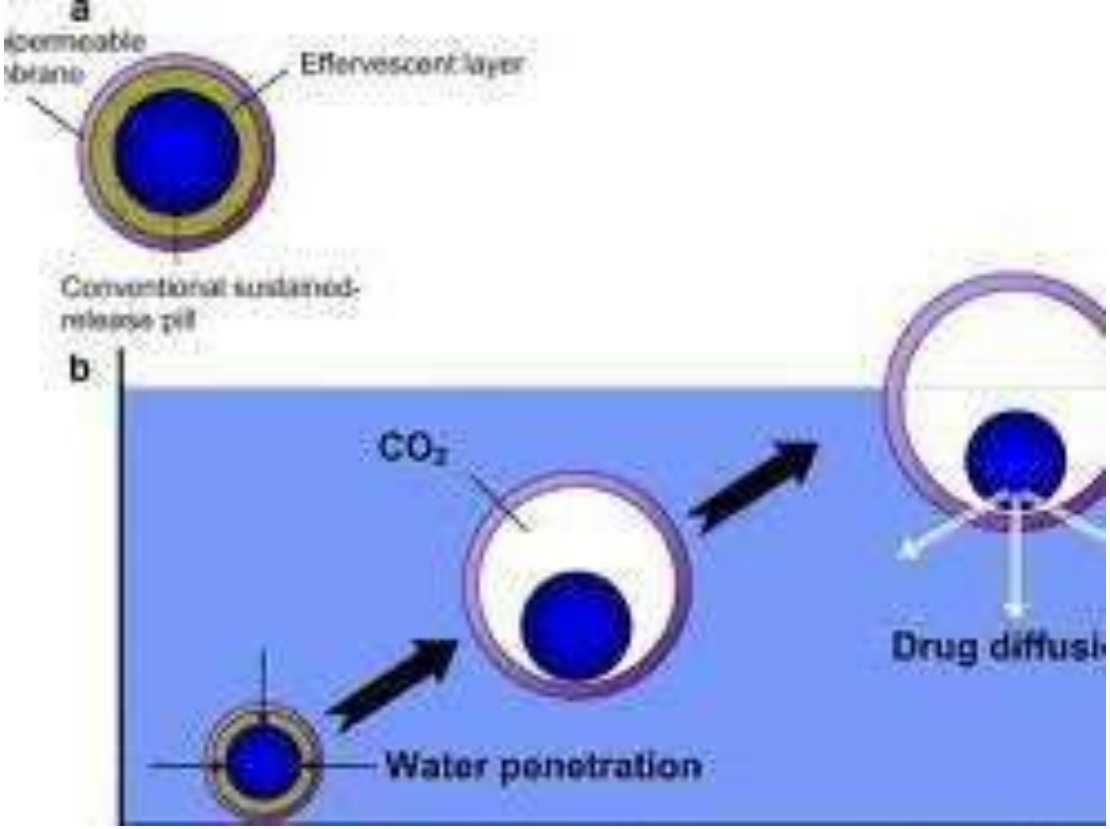
HYDRODYNAMIC BALANCED SYSTEMS

This system contains drug with gel forming hydrocolloids meant to remain buoyant on the stomach content



FLOATATION CHAMBER

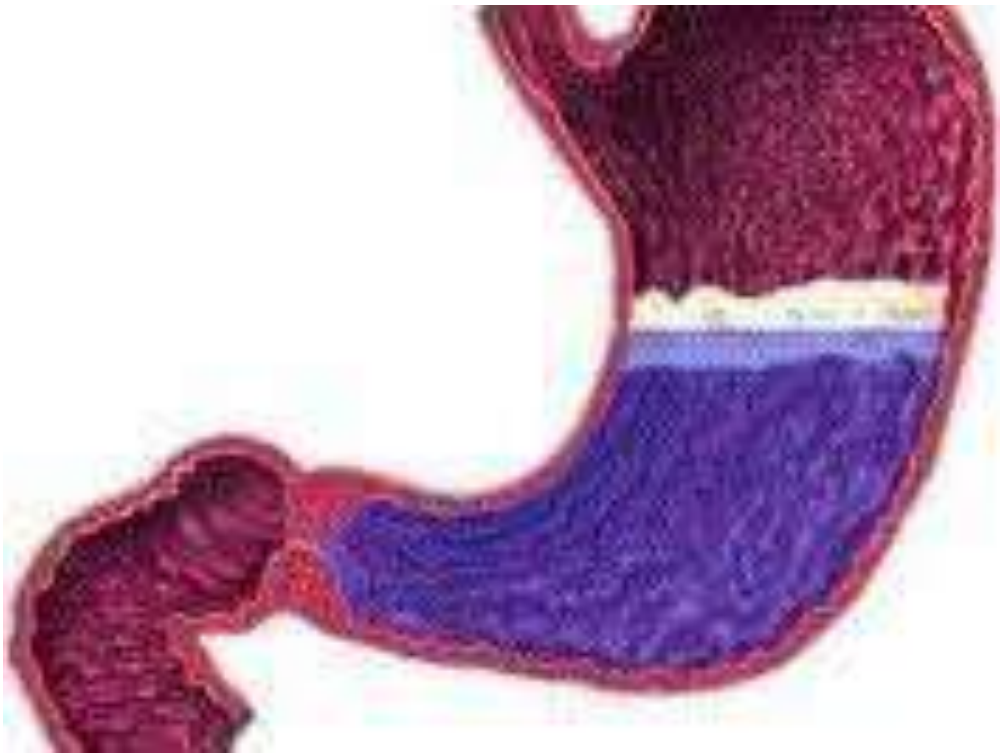
Fluid filled floating chamber which includes incorporation of a gas filled floatation chamber into a micro porous component that houses a drug reservoir



EFFERVESCENT FLOATING DOSAGE FORMS

These are matrix type of systems prepared with the help of swellable polymers such as methyl cellulose and chitosan and various effervescent compounds e.g, sodium bicarbonate,citric acid

They are formulated in such a way that when in contact with the acidic gastric contents co2 is liberated and gets entrapped in swollen hydro colliods



RAFT FORMING SYSTEMS

1. On contact with fluid a gel forming solution is formed this contains co2 bubbles
2. This forms a raft layer on the Gastro intestinal liquid

Advantages & disadvantages of FDDS

ADVANTAGES

✓ These type of drugs can benefit FDDS,they are Drugs acting in Stomach,Poorly soluble in alkaline pH,Rapidly absorbed in GI tract,Degrade in stomach.

DISADVANTAGES

- It requires sufficient high level of fluids in the stomach for the drug delivery to float
- There are certain situations where gastric retention is not desirable

EVALUATION OF FDDS

A.Invitro Methods

1.Floating lag time & floating time

2.Dissolution study

3.Resultant weight test

B.Invivo methods

4.X ray method

5.Gamma-scintigraphy

6.Gastrophy

7.Ultra sonography

CONCLUSION

- FDDS promises to be a potential approach for Gastric retention
- Dosage forms with a prolonged GRT will bring about new and important therapeutic options
- A large no of companies are focusing toward commercializing this technique.
- REFERENCE
- Wikipedia,

FLOW OF HEAT

G.Natesh

VCPS

Classification of Heat–Flow Process

1. Conduction

Ex: Flow of heat through the brick wall of a furnace or the metal shell of a boiler takes place by conduction.

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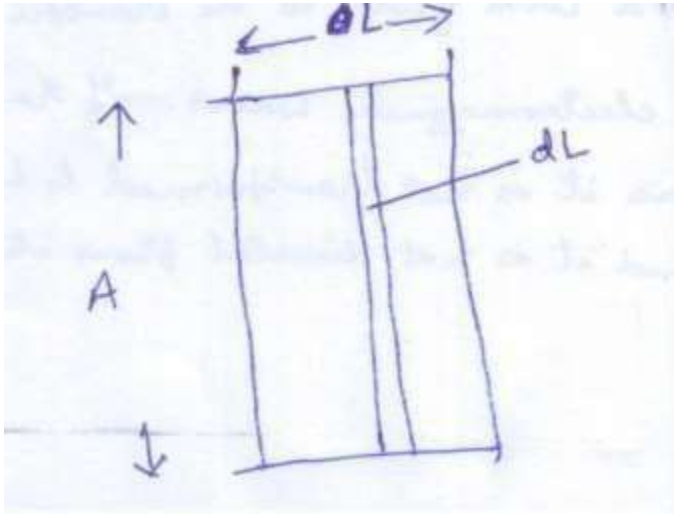
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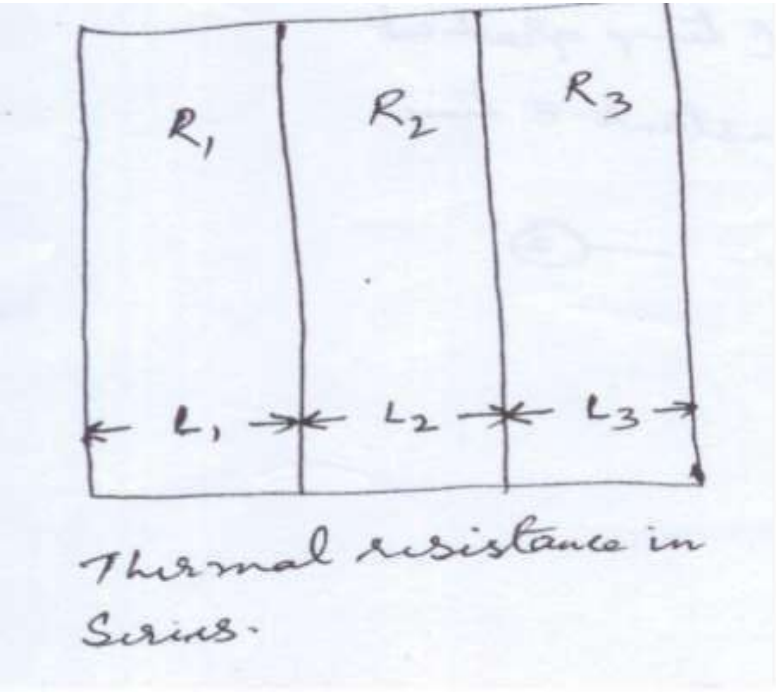
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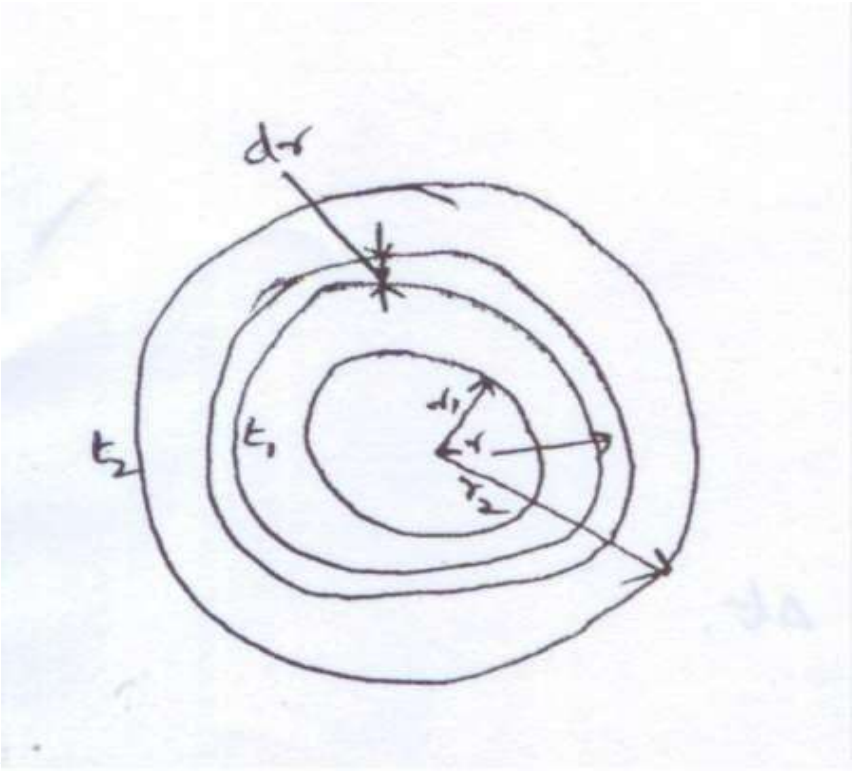
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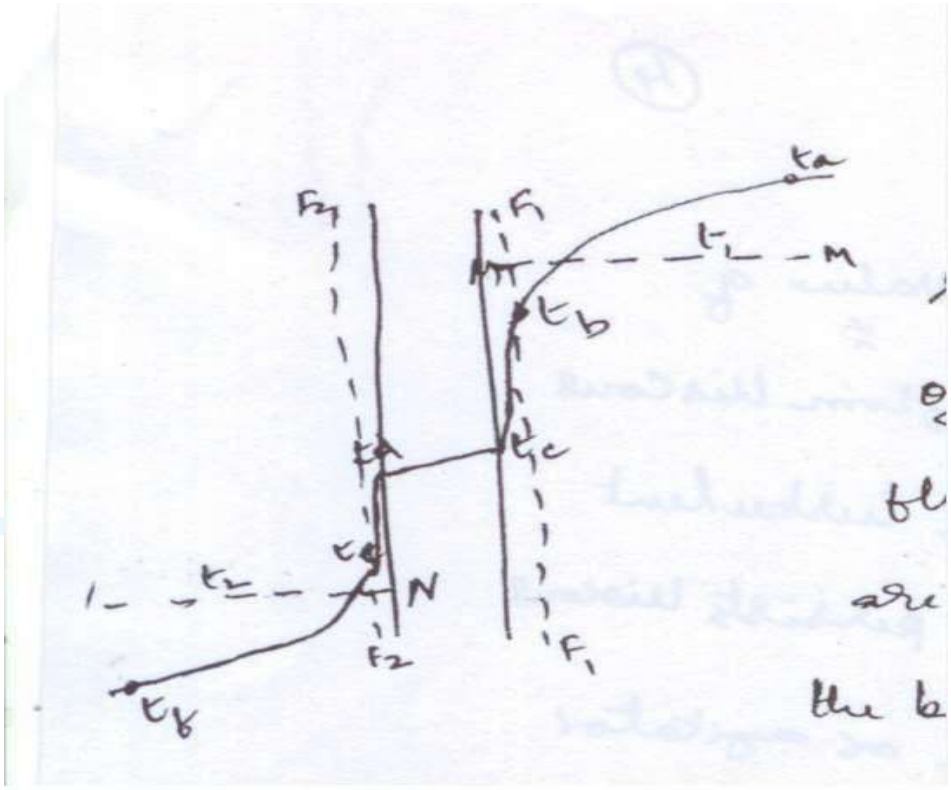
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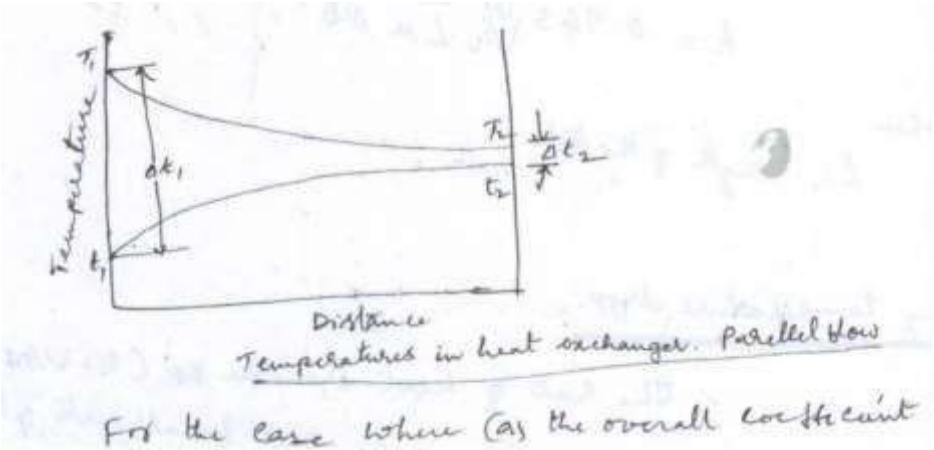
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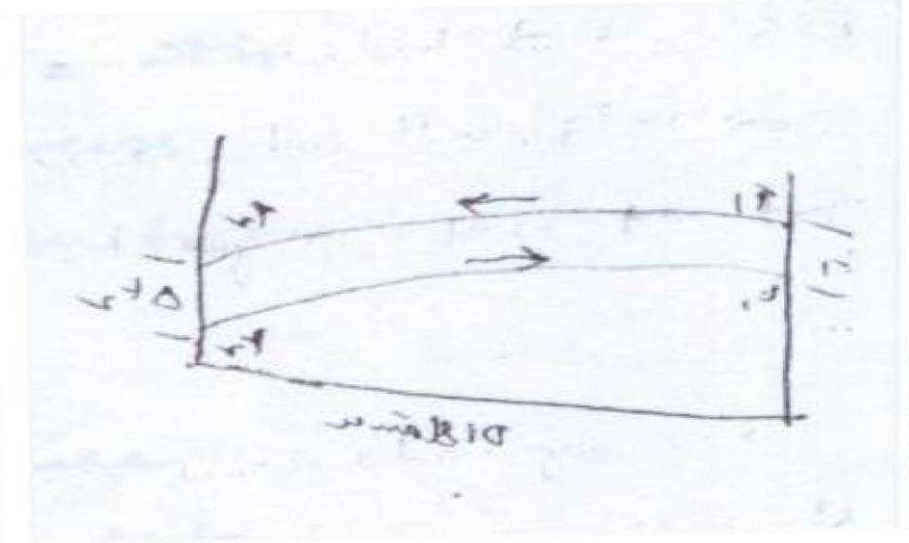
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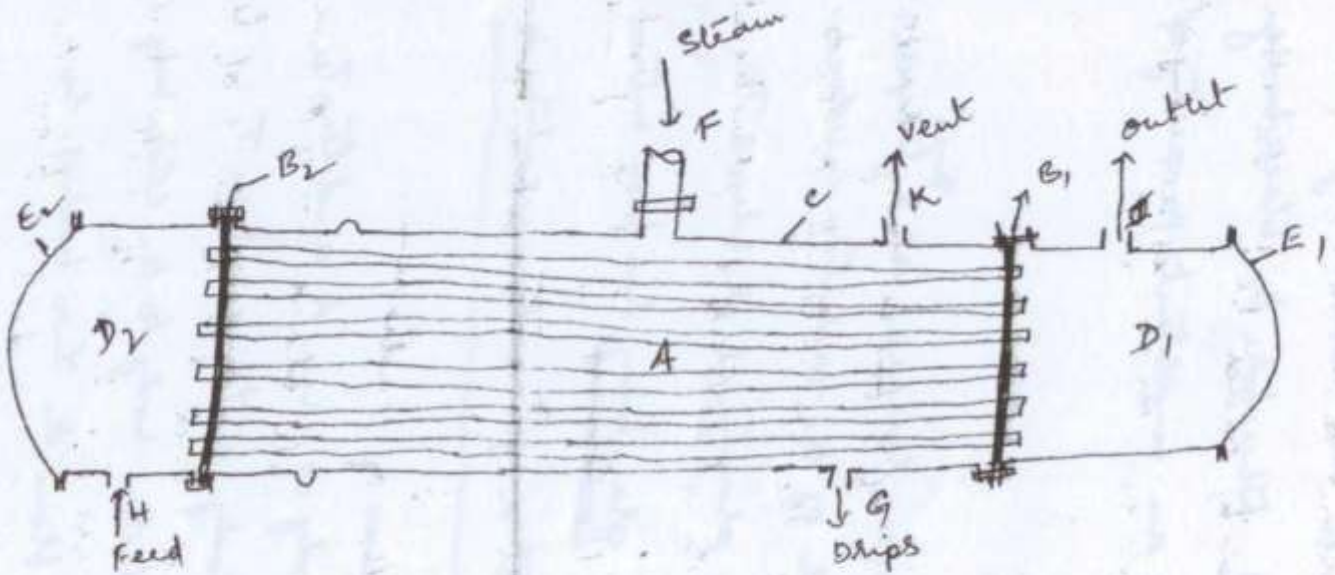
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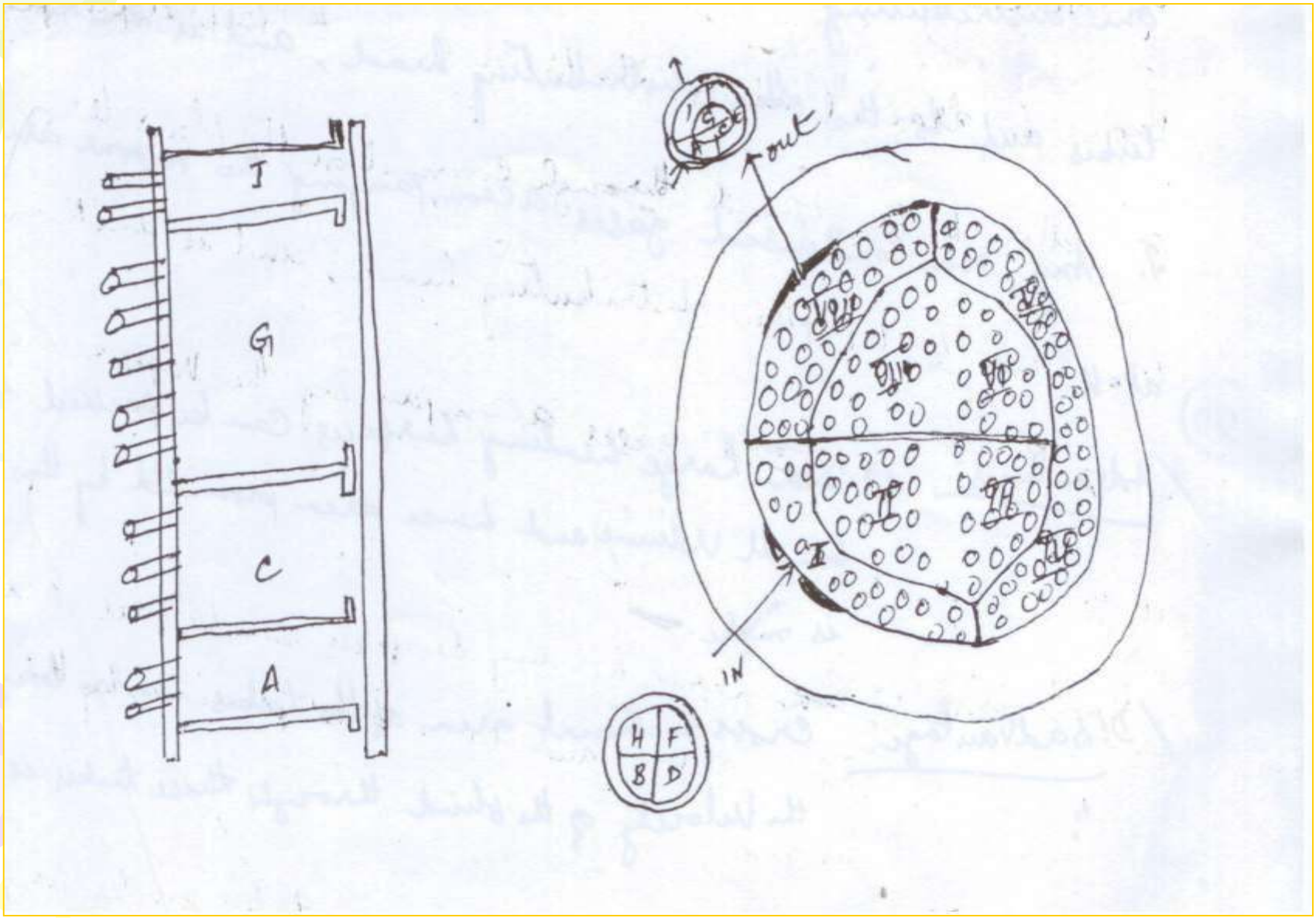
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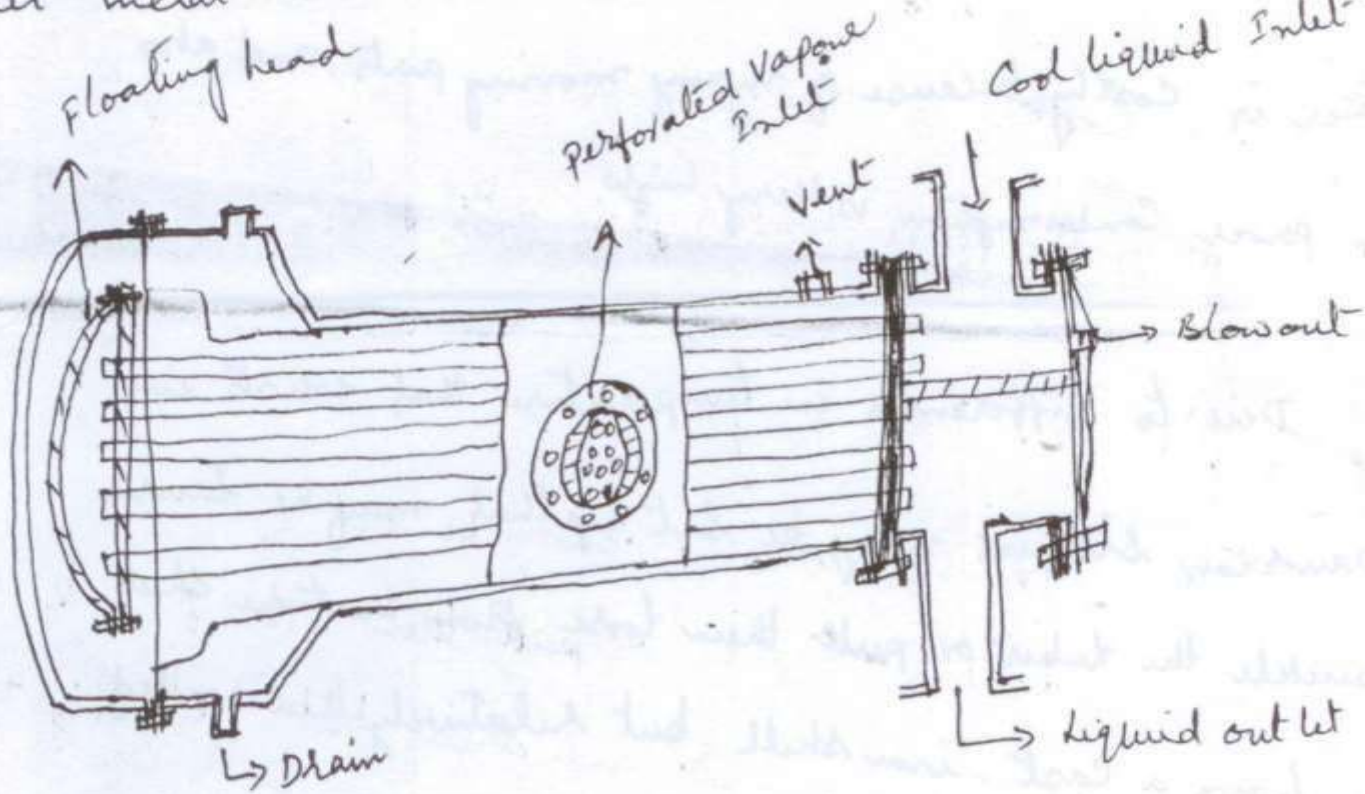
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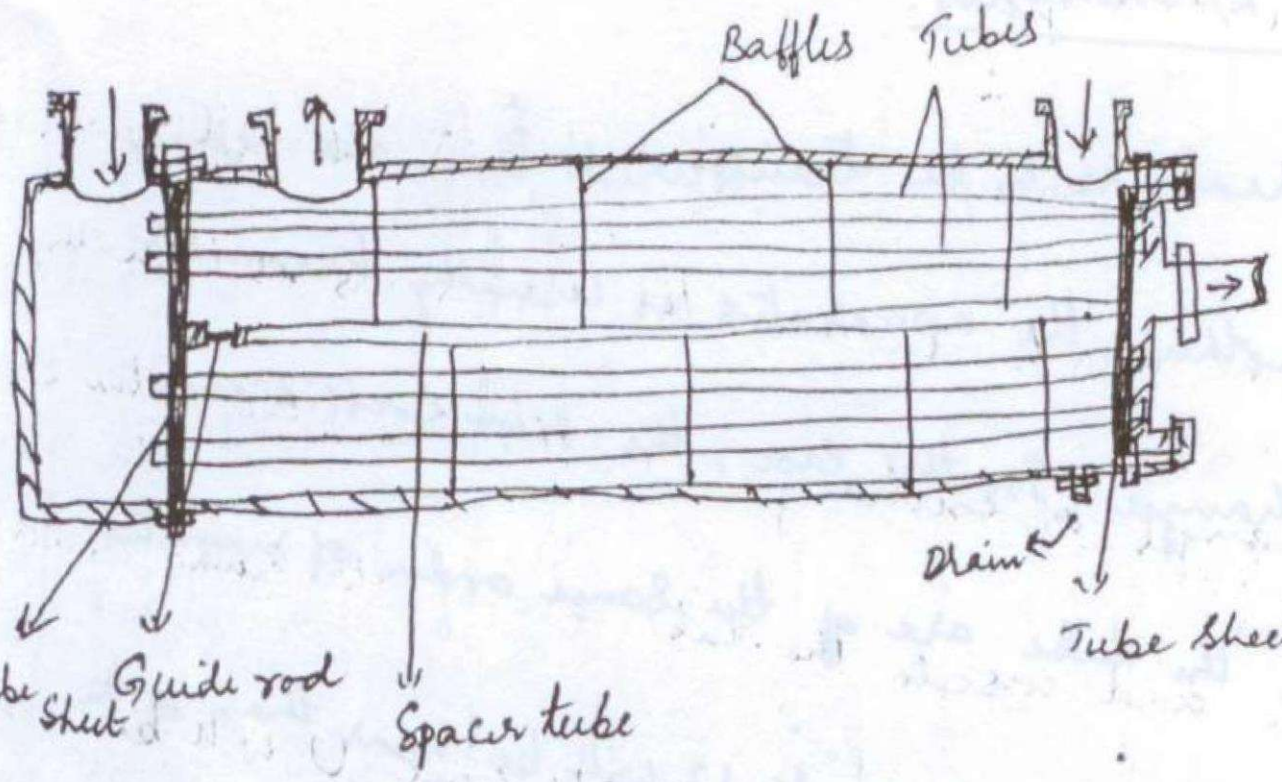
1) The heater is slightly more complicated.
2) Friction drop through the apparatus is increased because of the effect of velocity on friction drop and the miltiplication of exit and entrance losses.
3) The heater is costly because of many moving parts and also cost of the power consumption is very high.

Sheet metal is

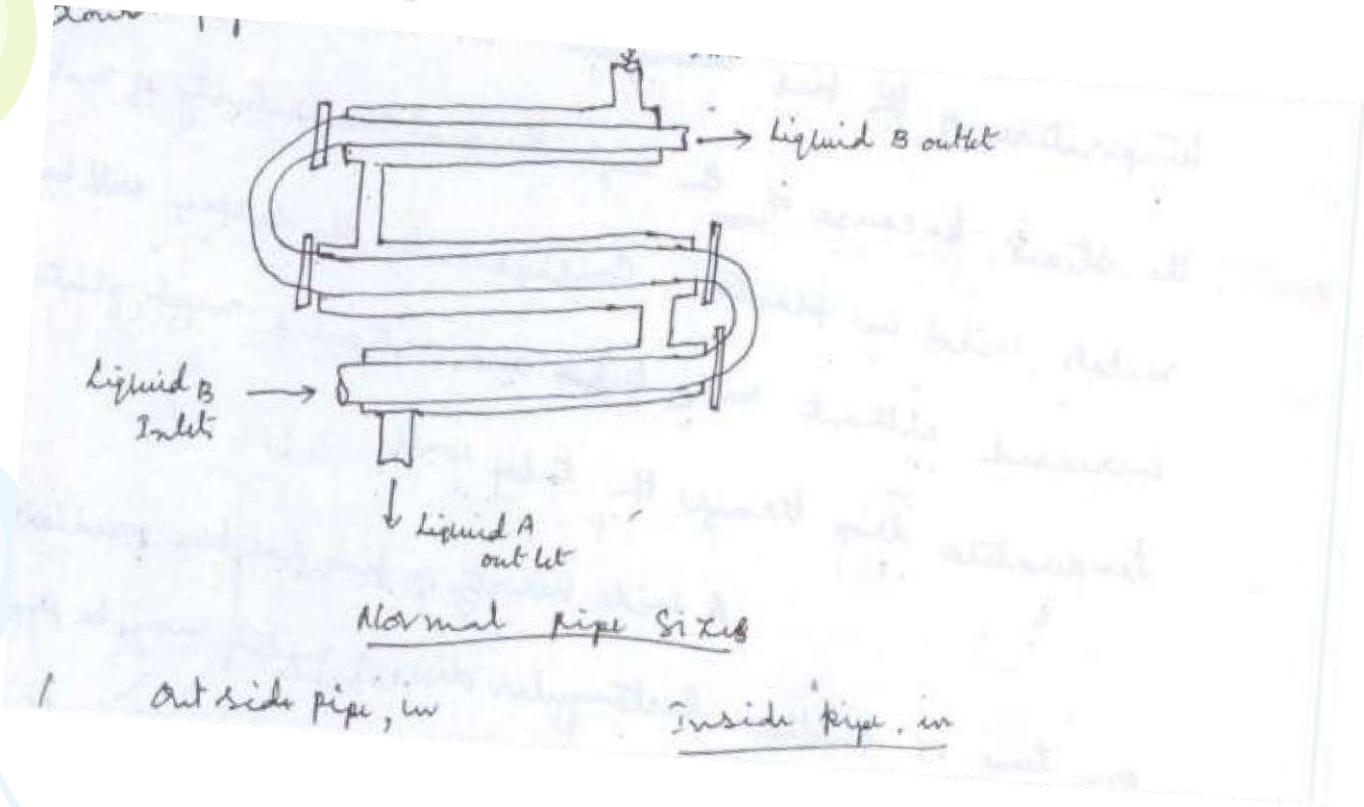


Two-pass Floating Head Heater

Liquid to Liquid Heat Interchanger:



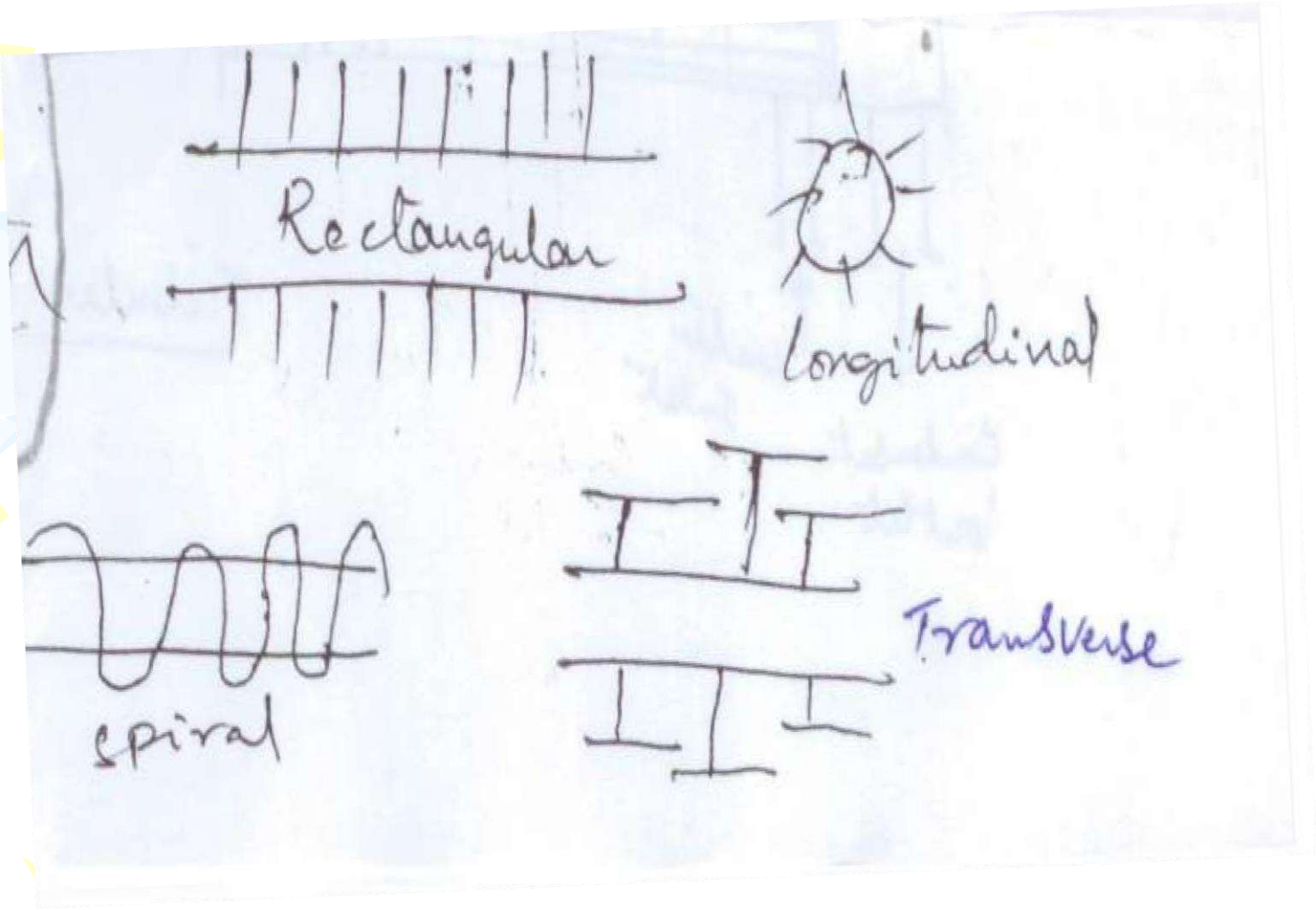
Double Pipe Heat Interchanger



Normal Pipe sizes

Out side pipe(inch)	Inside pipe(inch)
2	1 ¼
2 ½	1 ¼
3	2
4	3

Finned Tubes




Thank You

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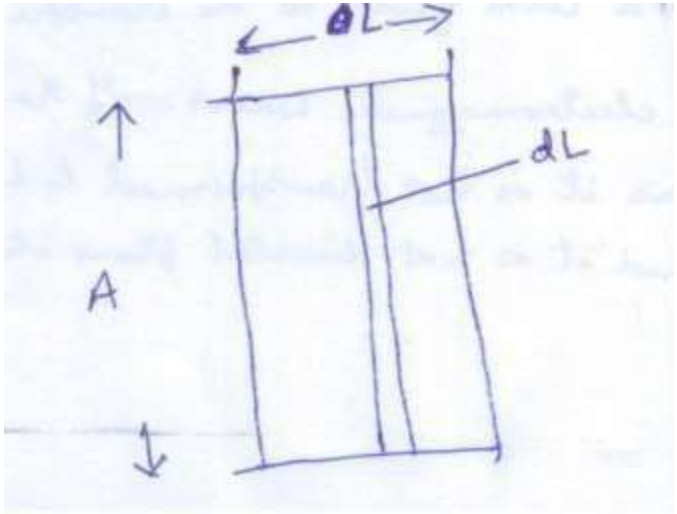
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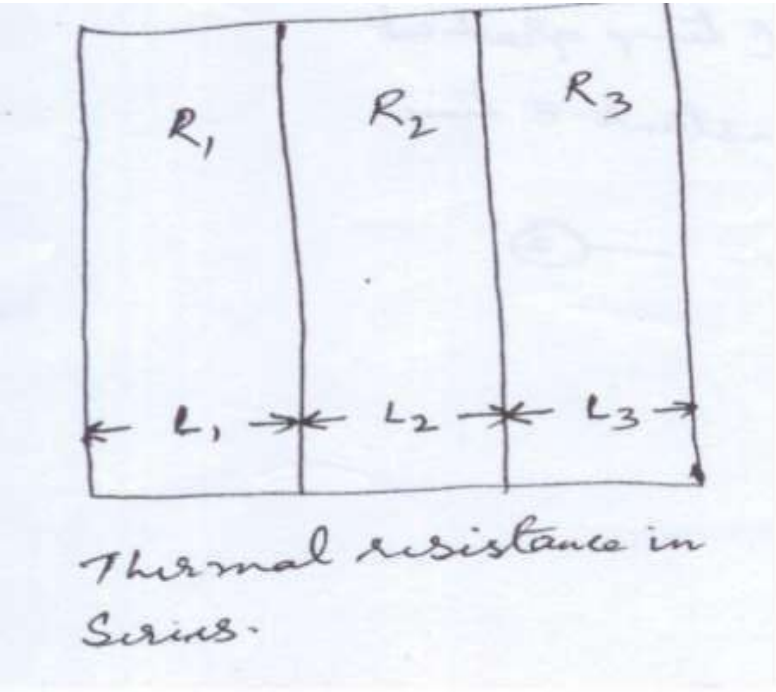
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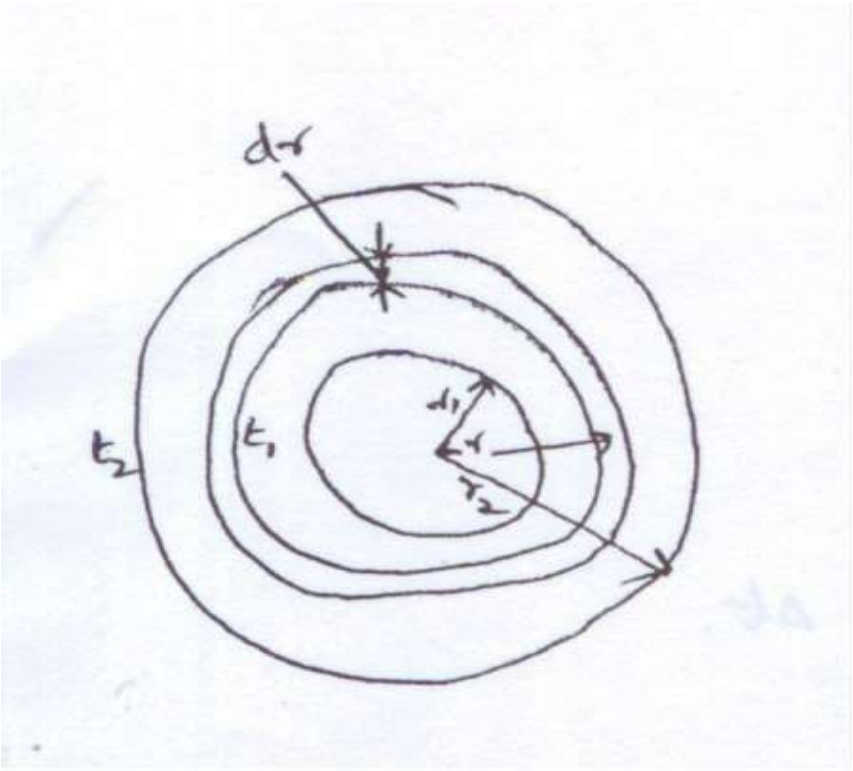
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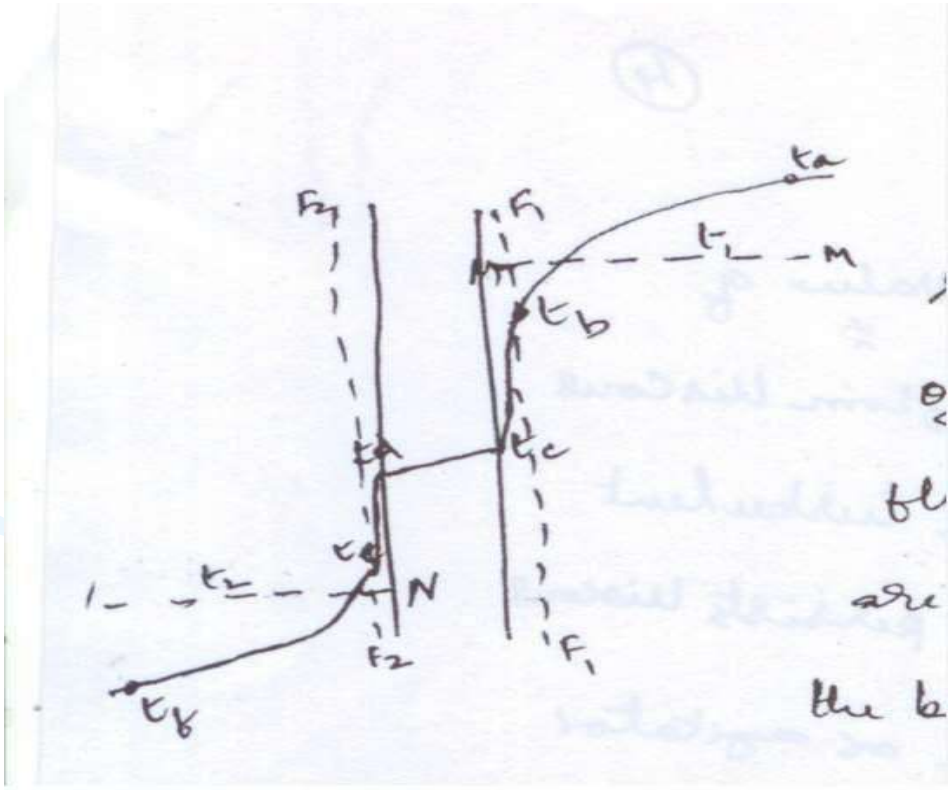
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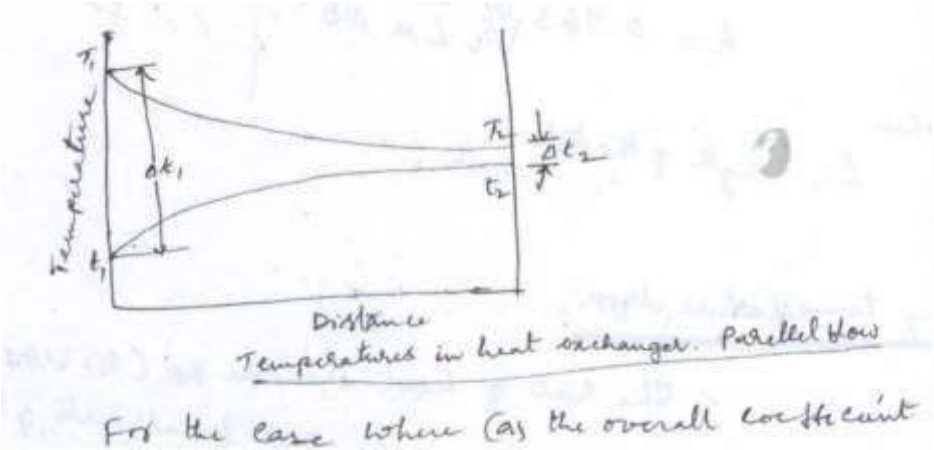
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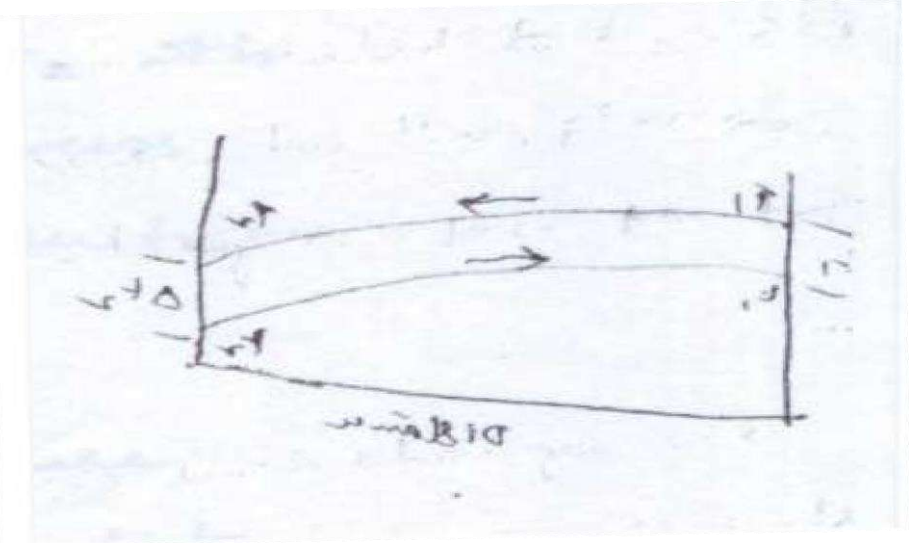
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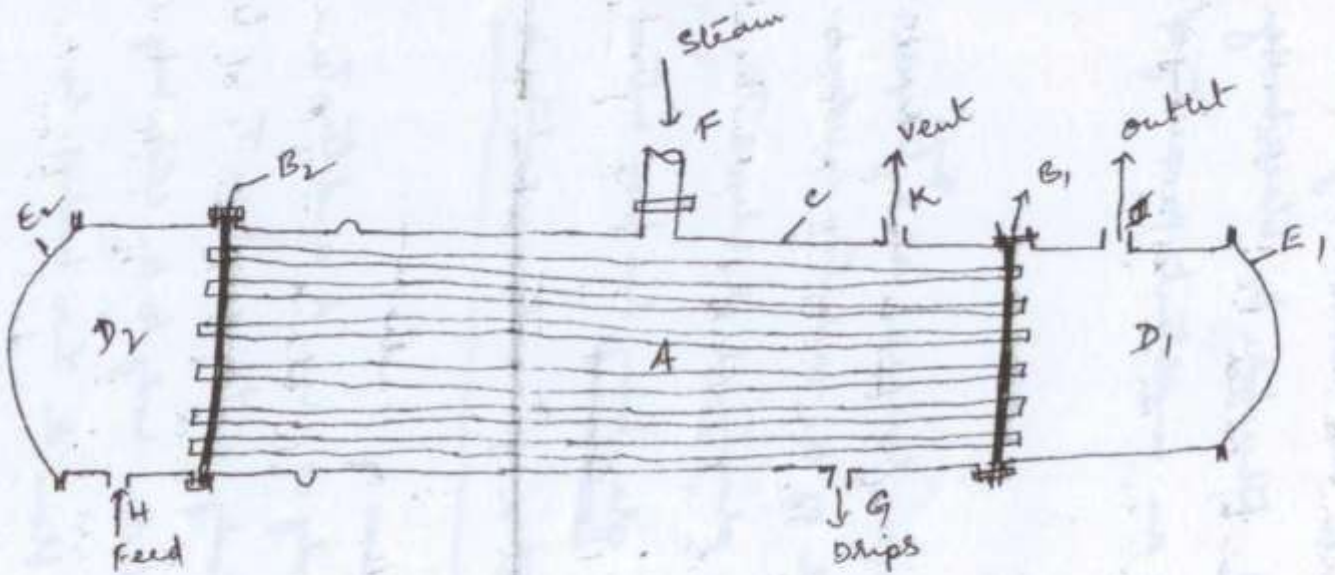
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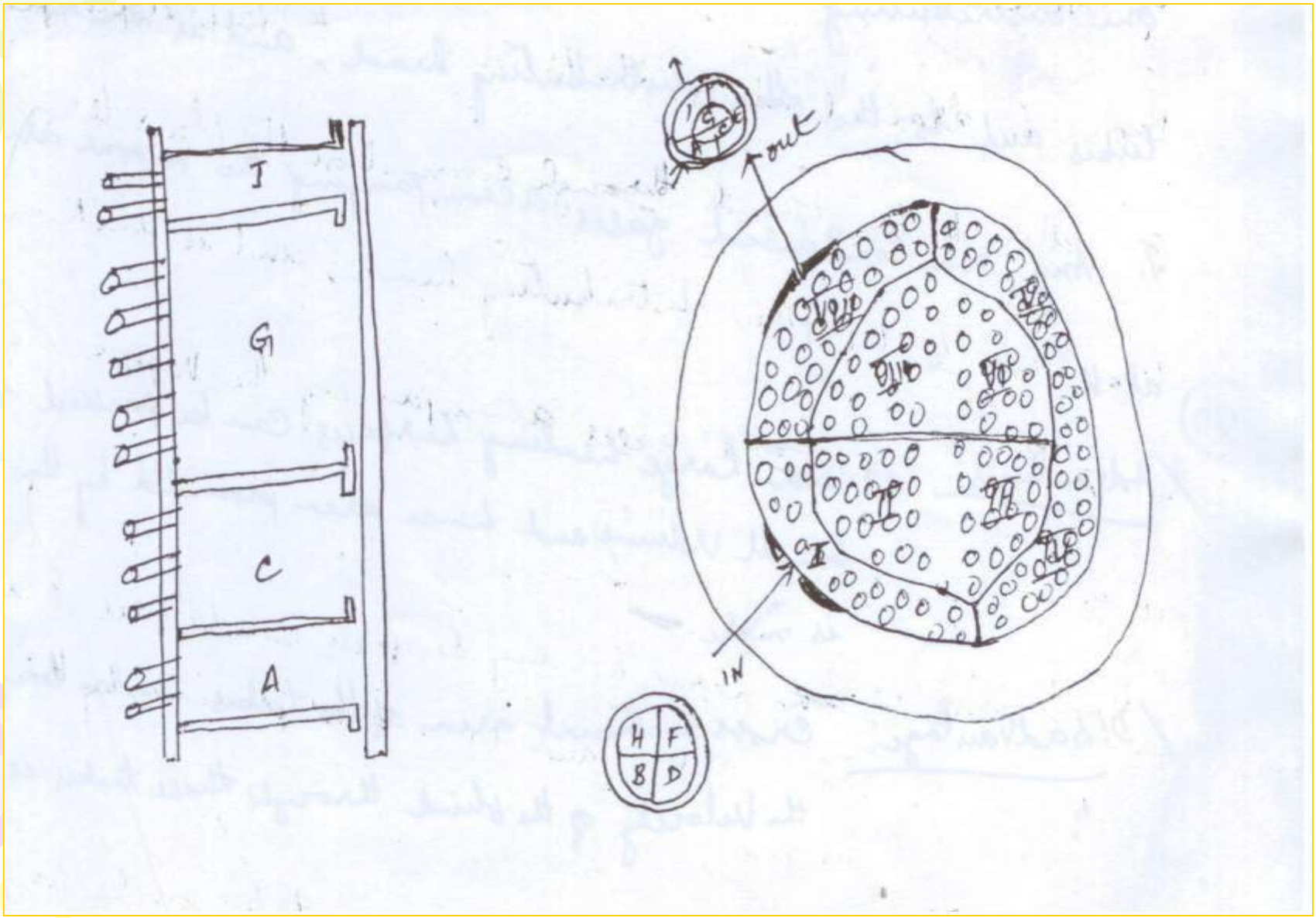
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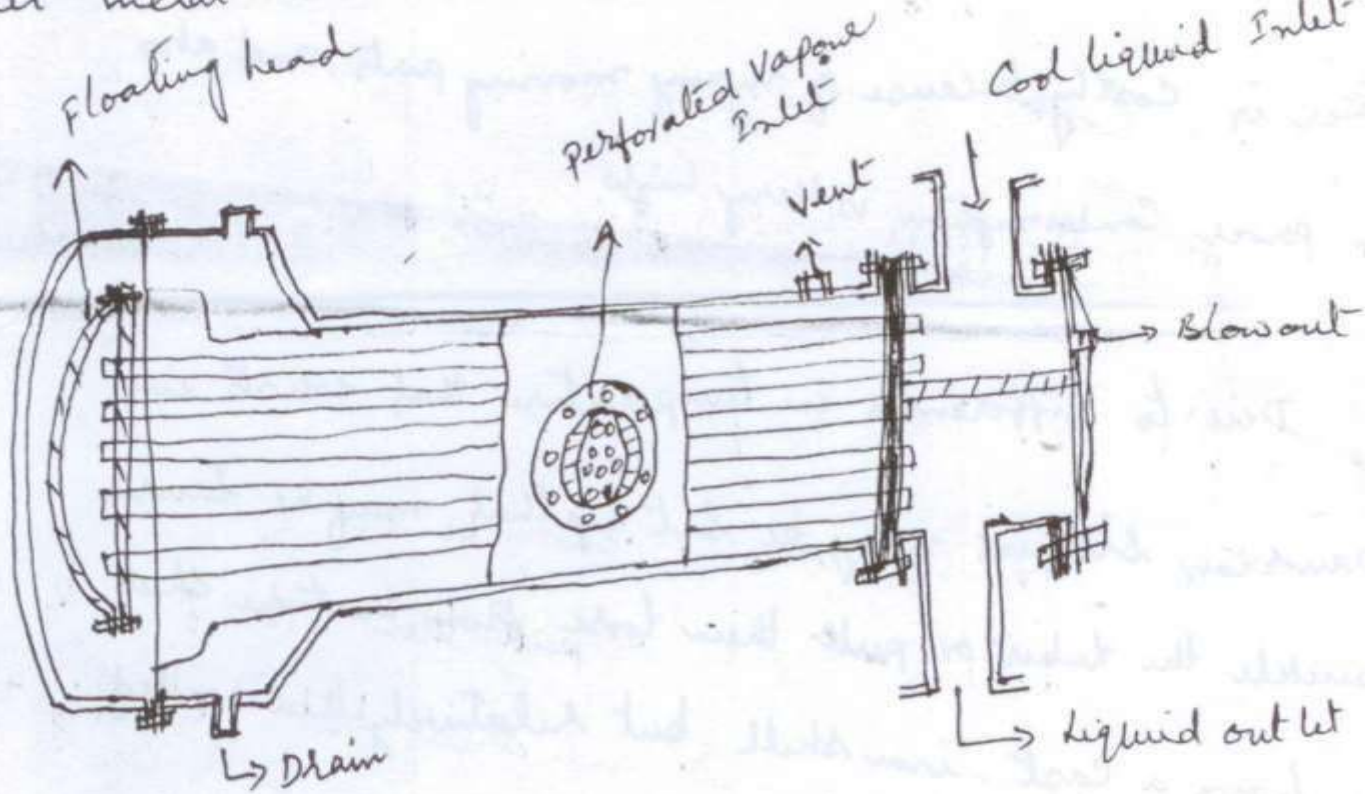
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- Multi pass construction ↓ses the cross section of the fluid path and ↑ ses the fluid velocity with a corresponding increase in the heat transfer co-effecient.

Disadvantages:

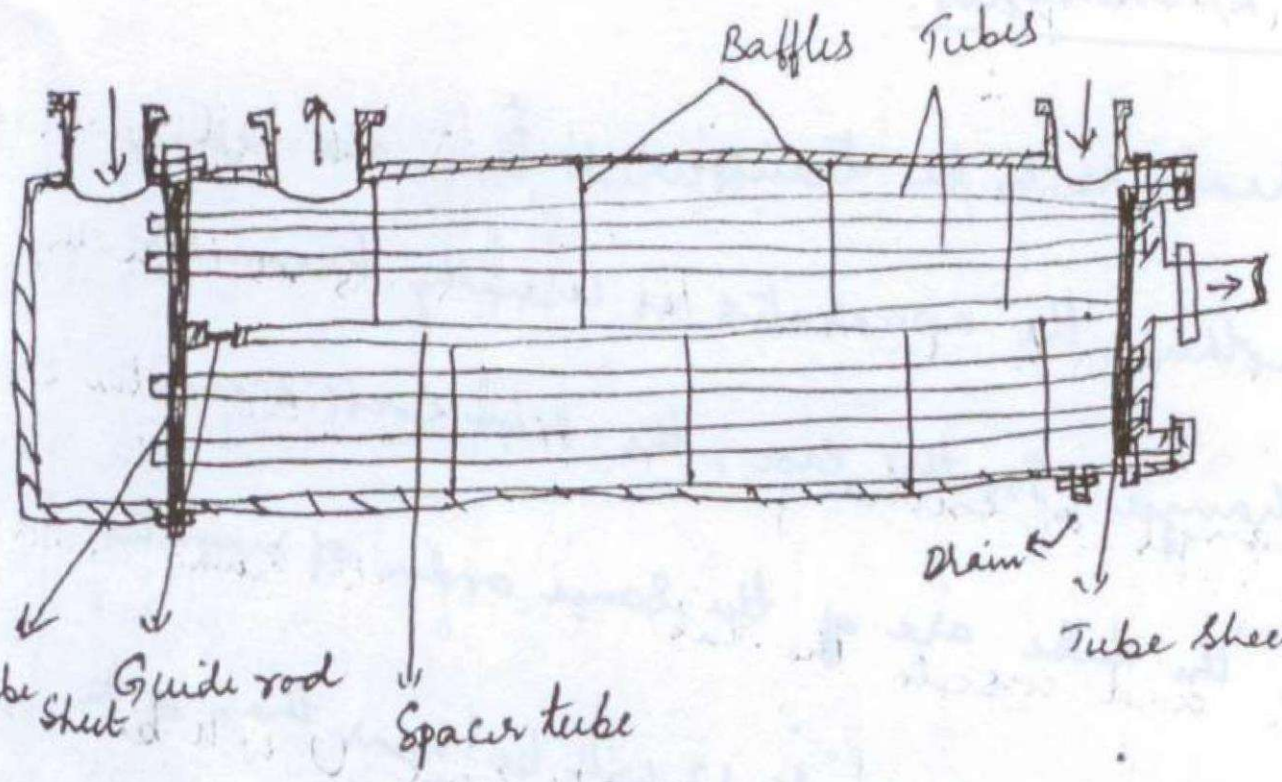
1) The heater is slightly more complicated.
2) Friction drop through the apparatus is increased because of the effect of velocity on friction drop and the miltiplication of exit and entrance losses.
3) The heater is costly because of many moving parts and also cost of the power consumption is very high.

Sheet metal is

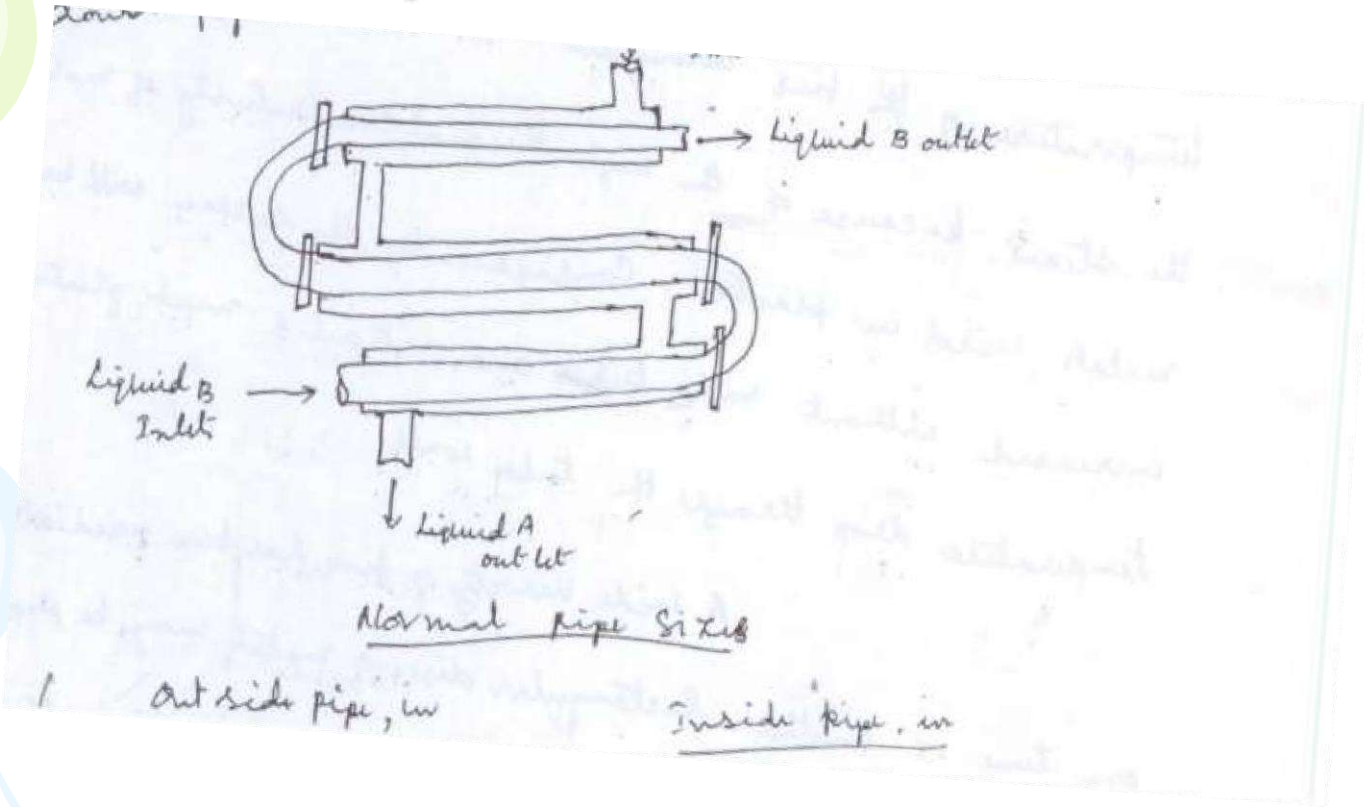


Two-pass Floating Head Heater

Liquid to Liquid Heat Interchanger:



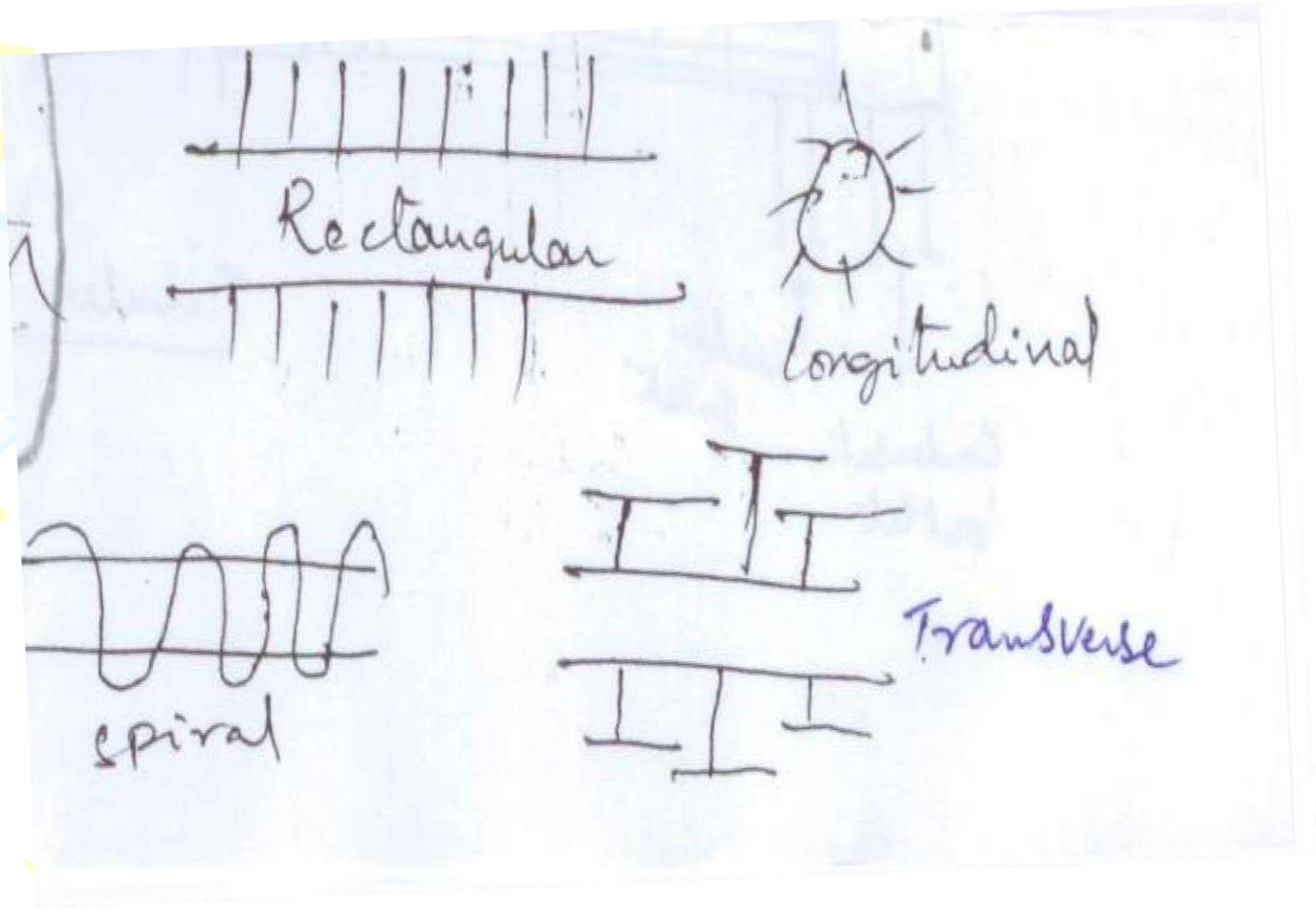
Double Pipe Heat Interchanger



Normal Pipe sizes

Out side pipe(inch)	Inside pipe(inch)
2	1 ¼
2 ½	1 ¼
3	2
4	3

Finned Tubes




Thank You

s

GOOD CLINICAL PRACTICES

Dr.Y.KIRAN

Definition

- Good Clinical Practice (GCP) is defined as a ‘standard for the design, conduct, performance, monitoring, auditing, recording, analyses and reporting of clinical trials that provides assurance that the data and reported results are credible and accurate, and that the rights, integrity and confidentiality of trial subjects are protected’

GCP

- Are mainly focused on the protection of human rights in clinical trial.
- Provide assurance of the safety of the newly developed compounds.
- Provide standards on how clinical trials should be conducted.
- Define the roles and responsibilities of -
  - Clinical Sponsors,
  - Clinical Research Investigators,
  - Clinical Research Associates, And
  - Monitors.

- GCPs are generally accepted, international best practices for conducting clinical trials and device studies
- They are defined as an international ethical and scientific standard for designing, conducting, recording and reporting trials that involve the participation of human subjects
- Compliance with GCPs provide public assurance that the rights and safety of participants in human subject research are protected and that the data that arises from the study is credible

Good Clinical Practice Guidelines

- Clinical trials should be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with GCP and the applicable regulatory requirements
- Before a trial is initiated, foreseeable risks and inconveniences should be weighed against the anticipated benefit for the individual trial subject and society. A trial should be initiated and continued only if the anticipated benefits justify the risks
- The rights, safety, and well-being of the trial subjects are the most important considerations and should prevail over interests of science and society
- The available non clinical and clinical information on an investigational product should be adequate to support the proposed clinical trial

- Clinical trials should be scientifically sound, and described in a clear, detailed protocol
- A trial should be conducted in compliance with the protocol that has received prior institutional review board (IRB)/independent ethics committee (IEC) approval/favorable opinion
- The medical care given to, and medical decisions made on behalf of, subjects should always be the responsibility of a qualified physician or, when appropriate, of a qualified dentist

- Each individual involved in conducting a trial should be qualified by education, training, and experience to perform his or her respective tasks
- Freely given informed consent should be obtained from every subject prior to clinical trial participation
- All clinical trial information should be recorded, handled, and stored in a way that allows its accurate reporting, interpretation, and verification

- The confidentiality of records that could identify subjects should be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirements
- Investigational products should be manufactured, handled, and stored in accordance with applicable good manufacturing practice (GMP). They should be used in accordance with the approved protocol
- Systems with procedures that assure the quality of every aspect of the trial should be implemented

Good Clinical Practices (GCP)

1. PROVISIONS AND PREREQUISITES FOR A CLINICAL TRIAL

    Justification for the trial

    Ethical principles

    Supporting data for the investigational product 1.4

    Investigator and site(s) of investigation

    1.5 Regulatory requirements

2. THE PROTOCOL

3. PROTECTION OF TRIAL SUBJECTS

    Declaration of Helsinki

    Ethics committee

    Informed consent

    Confidentiality

4. RESPONSIBILITIES OF THE INVESTIGATOR

- Medical care of trial subjects
- Qualifications
- Selection of trial subjects
- Compliance with the protocol
- Information for subjects and informed consent
- The investigational product
- The trial site
- Notification of the trial or submission to the DRA
- Review by an ethics committee
- Serious adverse events or reactions
- Financing
- Monitoring, auditing and inspection
- Record-keeping and handling of data
- Handling of and accountability for pharmaceutical products for trial
- Termination of trial
- Final report
- Trials in which the investigator is the sponsor

5. RESPONSIBILITIES OF THE SPONSOR

- Selection of the Investigator(s)
- Delegation of responsibilities
- Compliance with the protocol and procedures 5.4
- Product information
  - Safety information
  - Investigational product
  - Trial management and handling of data
  - Standard operating procedures
- Compensation for subjects and investigators 5.10
- Monitoring
  - Quality assurance
  - Study reports
  - Handling of adverse events
  - Termination of trial

6. RESPONSIBILITIES OF THE MONITOR
   - Qualifications
   - Assessment of the trial site
   - Staff education and compliance
   - Data management
   - Case-report forms
   - Investigational product
   - Communication
   - Notification of the trial or submission to the regulatory authority
   - Reports

7. MONITORING OF SAFETY
   - Handling and recording adverse events
   - Reporting adverse events

8. RECORD-KEEPING AND HANDLING OF DATA
   - Responsibilities of the investigator
   - Responsibilities of the sponsor and the monitor
   - Archiving of data

9. STATISTICS AND CALCULATIONS
    - Experimental design
    - Randomization and blinding
    - Statistical analysis

10. HANDLING OF AND ACCOUNTABILITY FOR PHARMACEUTICAL PRODUCTS
    - Supply and storage
    - Investigational labelling and packaging
    - Responsibilities of the investigator
    - Responsibilities of the sponsor and the monitor
11. ROLE OF THE DRUG REGULATORY AUTHORITY
    - General responsibilities
    - On-site inspections

12. QUALITY ASSURANCE FOR THE CONDUCT OF A CLINICAL TRIAL

IMPLANTABLE THERAPEUTICS

By

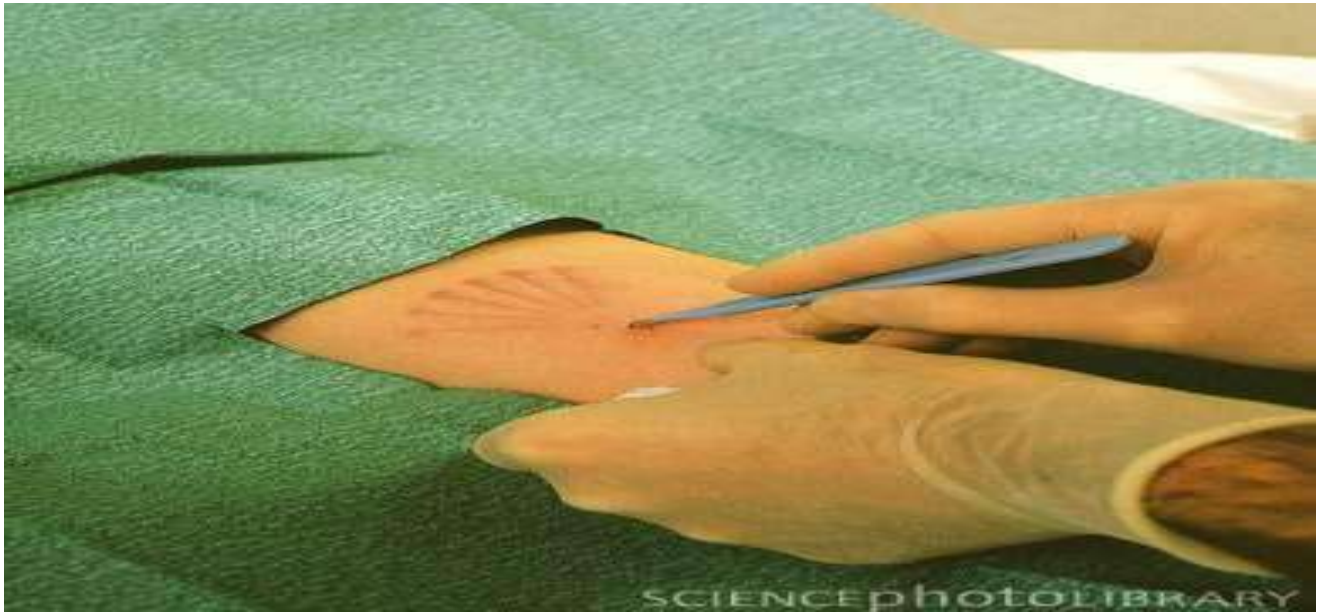
Dr. Y. Kiran

VCPS

WHAT ARE IMPLANTS.....???



- ✓ These are drug loaded devices administred into the subcutaneous region of the skin,by making a small incision on the skin surface, generally ment for delivering the drug at constant rate over a prolonged durationof time.



Ideal Requirements Of Implants

1. Environmentally stable.
2. Biocompatible.
3. Sterile.
4. Biostable.
5. Improve patient compliance.
6. Release the drug in a rate controlled manner which lead to enhanced effectiveness and reduced side effects.
7. Readily retrievable by medical personnel to terminate medication.
8. Easy to manufacture and relatively inexpensive.

Advantages



- Mainly meant for extended drug release over a prolonged duration at a steady state.
- Quiet suitable for chronic diseases & also for contraception purpose.

Disadvantages



- *Invasive*
- *Termination*
- *Danger of device failure*
- *Adverse reactions*
- *Biocompatible issues*

Approaches to design implantable therapeutics..

✓ **1) Controlled drug release by diffusion.....**

→ **MEMBRANE PERMEATION- CONTROLLED DRUG DELIVERY**

A) NON POROUS MEMBRANES

B) MICROPOROUS MEMBRANES

C) SEMIPERMEABLE MEMBRANES

→ **MATRIX DIFFUSION CONTROLLED DRUG DELIVERY USING;**

A) LIPOPHILIC POLYMERS

B) HYDROPHILIC POLYMERS

C) POROUS POLYMERS

→ **MICRO RESERVOIR DISSOLUTION CONTROL DRUG DELIVERY**

A. HYDROPHILIC RESERVOIR/ LIPOPHILIC MATRIX

B. LIPOPHILIC RESERVOIR/ HYDROPHILIC MATRIX

Controlled drug delivery by activation process

- ✓ **Osmotic pressure – activated drug delivery**
- ➢ **Vapor pressure - activated drug delivery**
- ❖ **Magnetically - activated drug delivery**
- ○ **ultrasonic - activated drug delivery**
- ❑ **Hydrolysis - activated drug delivery**

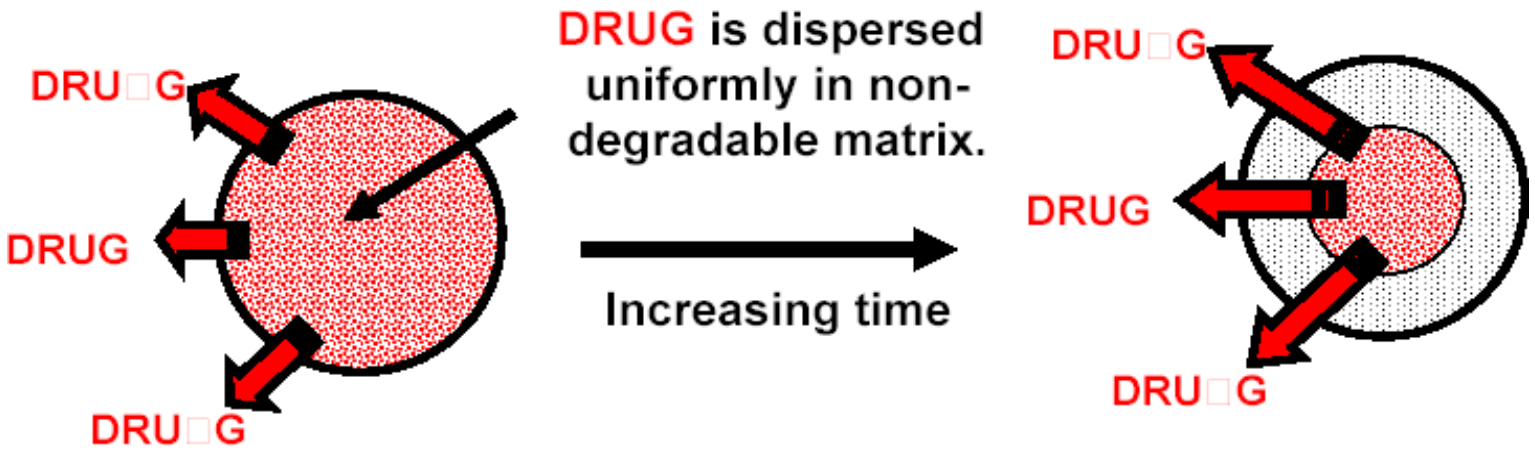
Controlled drug delivery by feed back regulated mechanism

- ❖ **Bioerosion regulated drug delivery**
- ❖ **Bioresponsive drug delivery**

Polymer membrane permeation- controlled drug delivery

In this type, the drug reservoir is encapsulated with in a compartment totally enclosed by a rate controlling polymeric membrane.

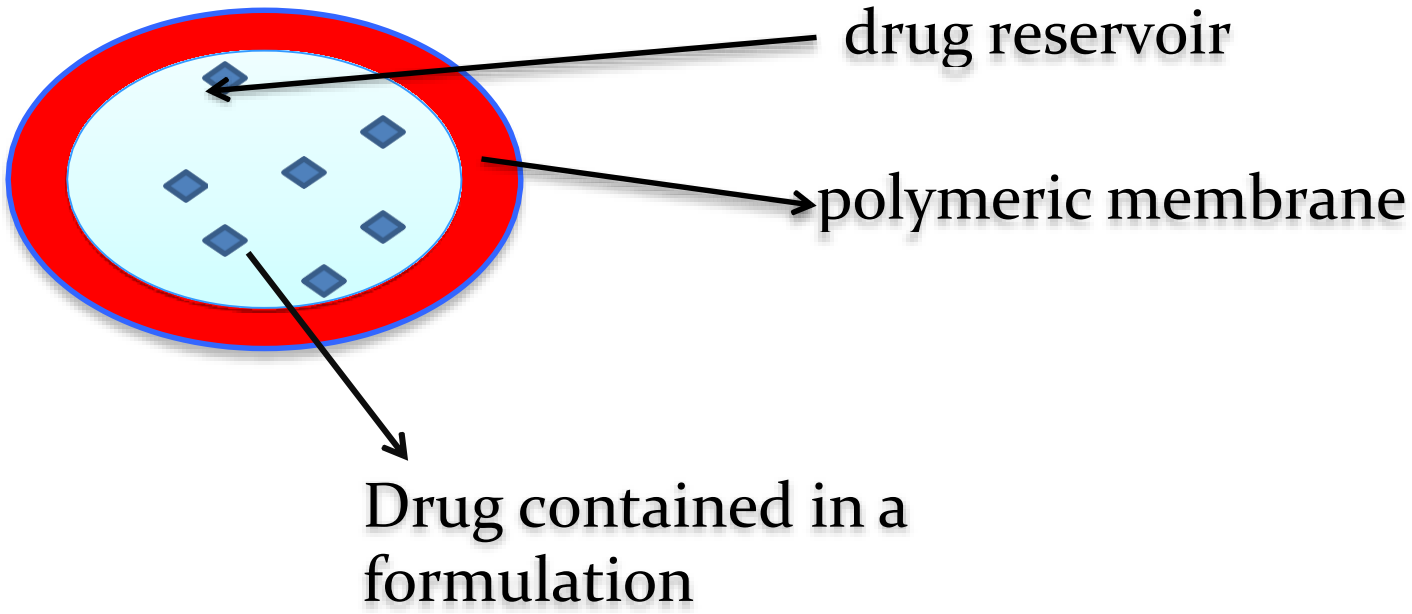
The drug reservoir can be either drug solid particles or a dispersion of drug solid particles in a liquid or a solid type dispersing medium

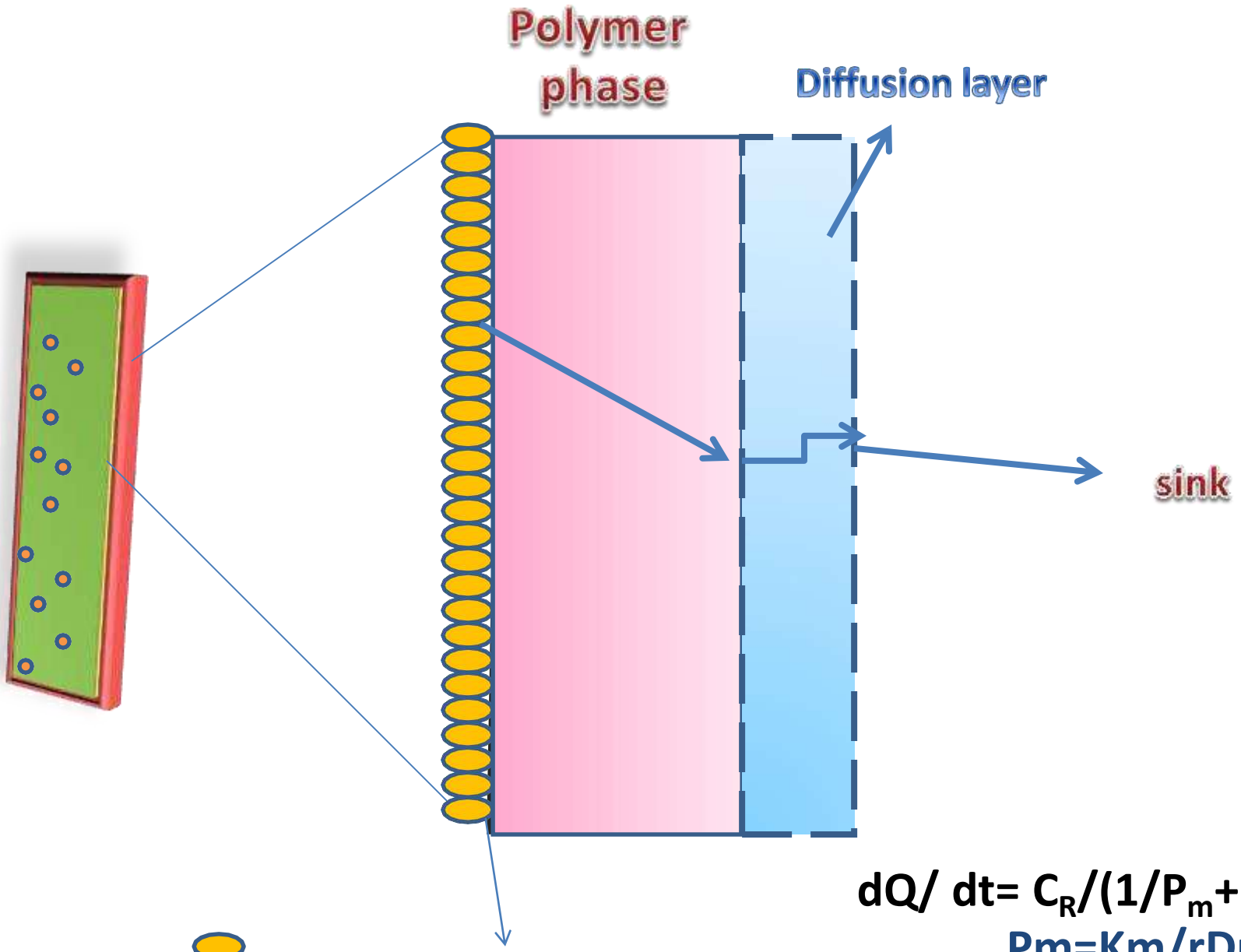


- Polymeric membrane can be fabricated from a homogeneous or a heterogeneous non porous polymeric material or a microporous membrane.

- Encapsulation of drug formulation in to the reservoir compartment can be done by

  Moulding
  Spray coating
  Microencapsulation





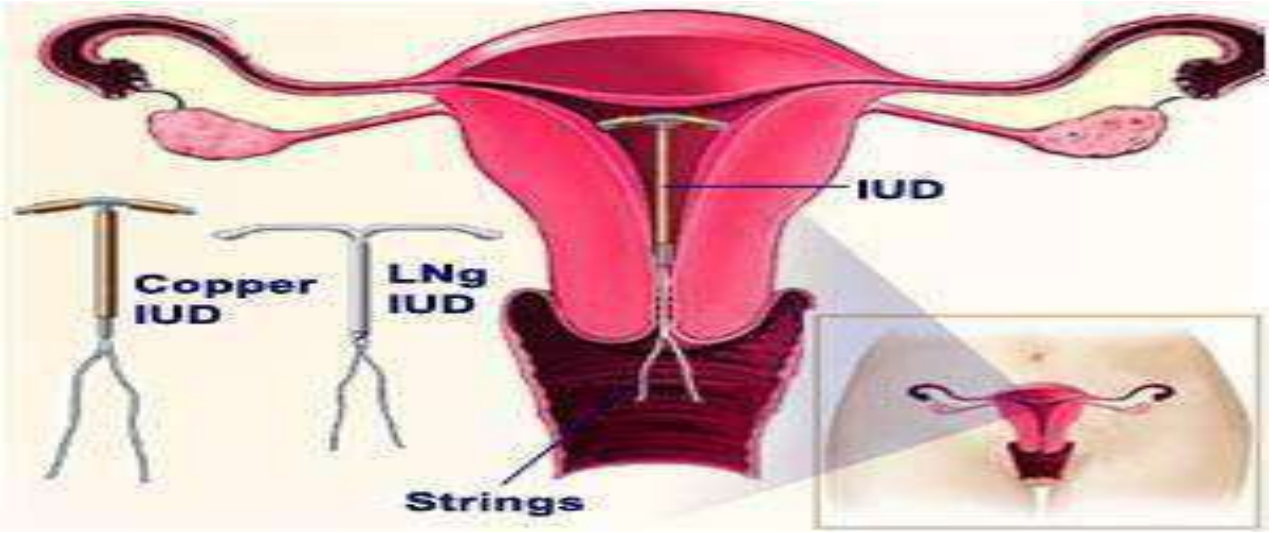
$$dQ/dt = C_R/(1/P_m + 1/P_d)$$

$$Pm = Km/rDm/\delta m$$

$$Pd = Ka/mDa/\delta d$$

Examples

1) Progestasert IUD



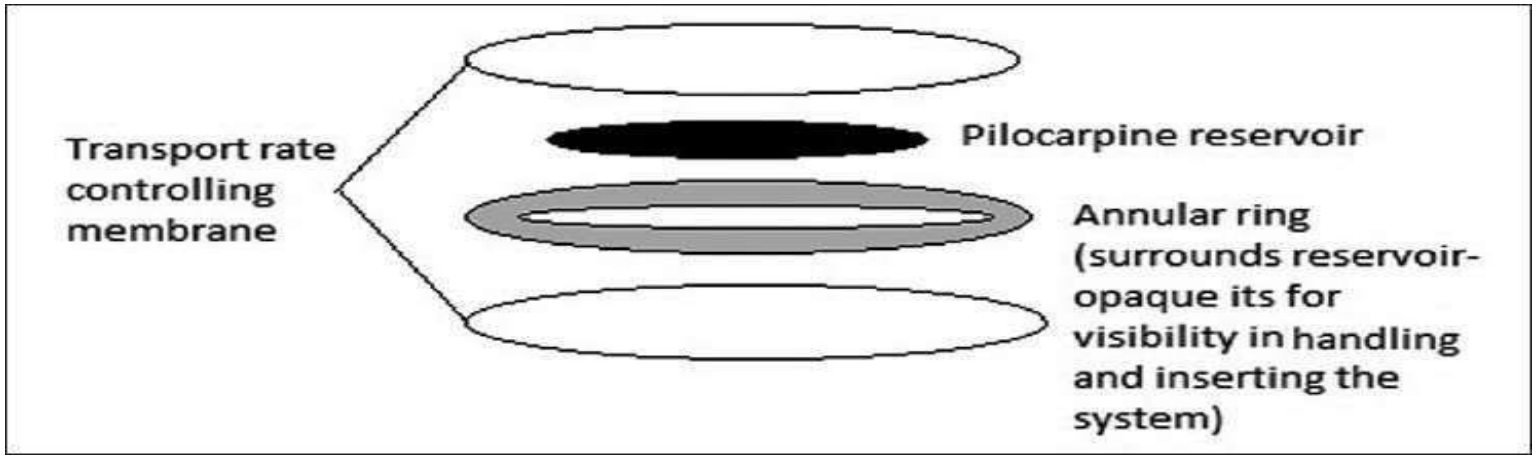


The drug reservoir is a suspension of progesterone crystals in liquid silicon polymer is encapsulated in a T shaped Intra Uterine device(IUD) enclosed by a non porous membrane of Ethylene Vinyl acetate co-polymer.

Ocusert system..



Encapsulated cell technology:
Schematic diagram of implant placed in vitreous cavity of human eye



- In this a thin disc of Pilocarpine alginate is sandwiched between two transparent sheets of micro porous membrane fabricated from ethylene vinyl acetate co-polymer.

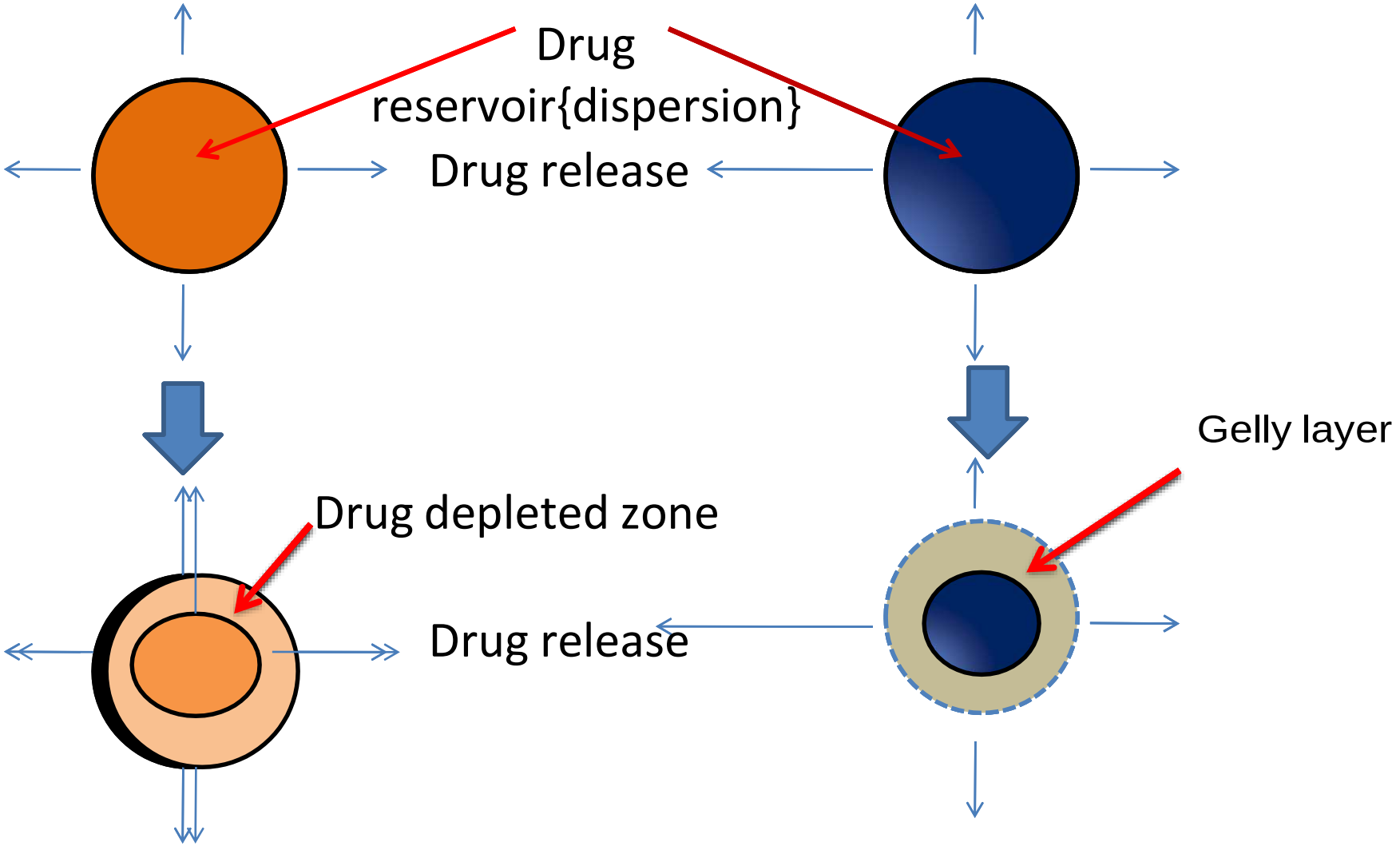
Matrix diffusion-controlled drug delivery

- In this type of controlled release drug delivery system the drug reservoir is formed by homogenous dispersion of drug particles in a rate controlling polymer matrix fabricated from either a lipophillic or a hydrophilic polymer

The drug dispersion in a polymer matrix is done by

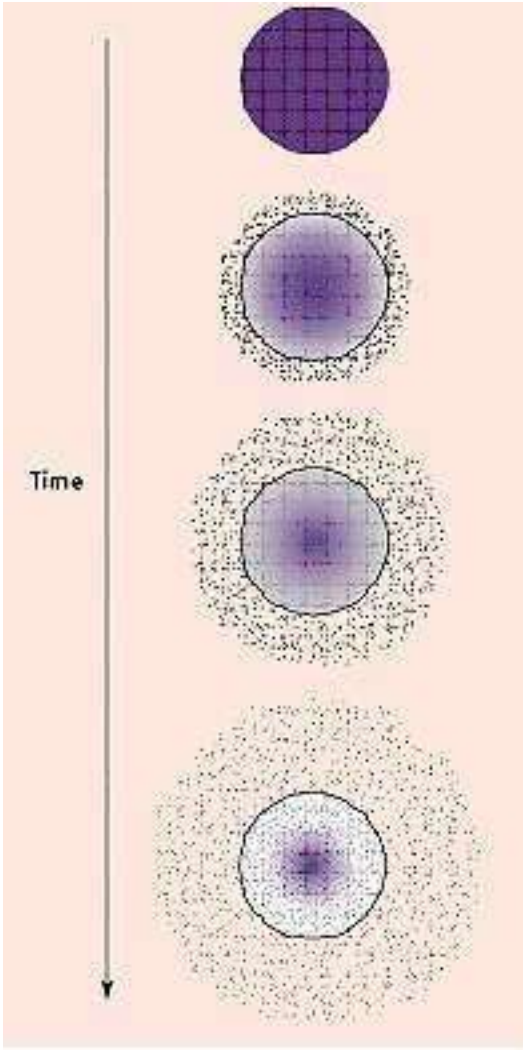
1. Blending finely divided drug particles with a liquid polymer or a viscous base followed by cross linking of the polymer chain
2. Mixing the drug with a polymer at an elevated temperature
3. Dissolving drug and polymer in a common solvent followed by solvent evaporation at elevated temperature or under vacuum

- The resultant drug polymer dispersion is then molded or extruded to form a drug delivery devices of various shapes
- Ex: Contraceptive vaginal ring, Syncro mate B implant, Compudose implant



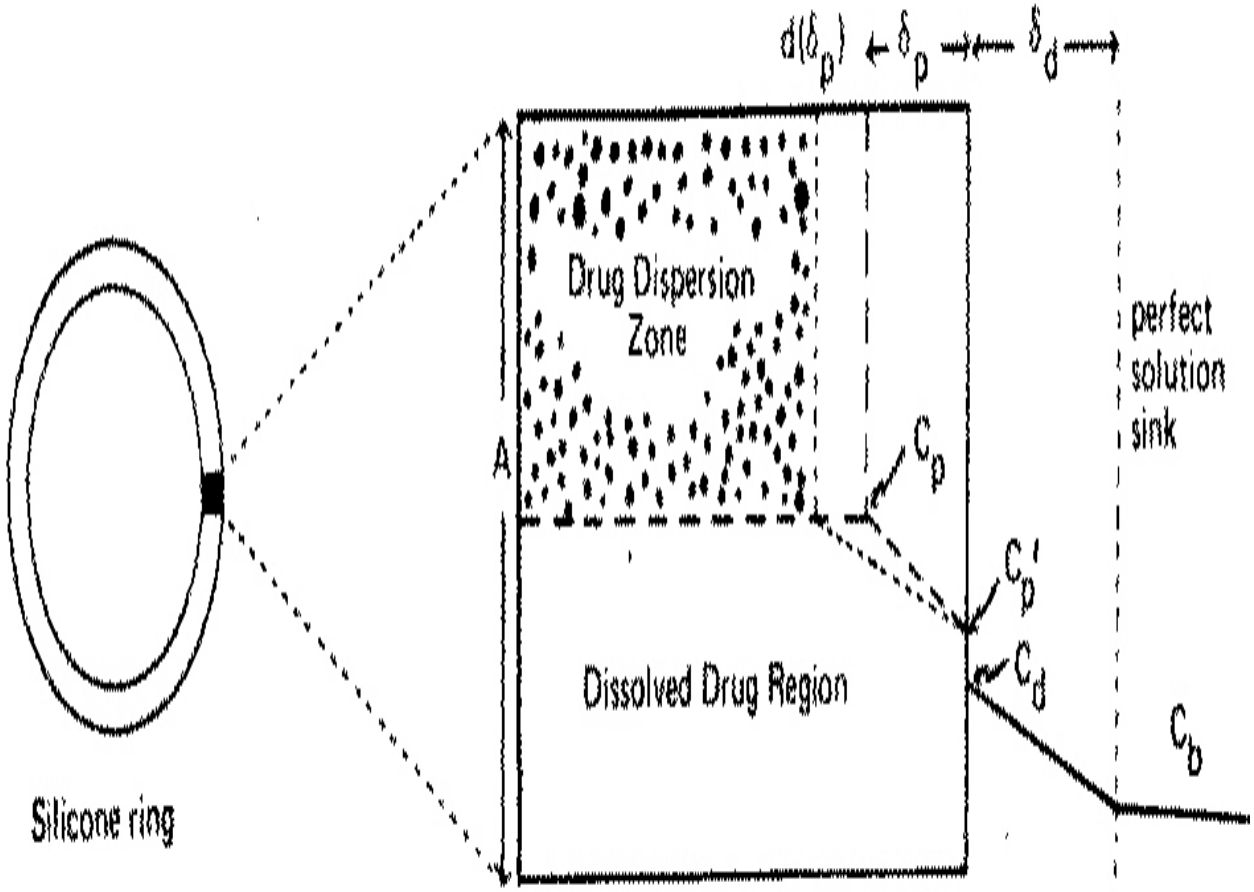
Lipophillic polymer
Non swellable matrix

Hydrophilic polymer
Swellable **matrix**

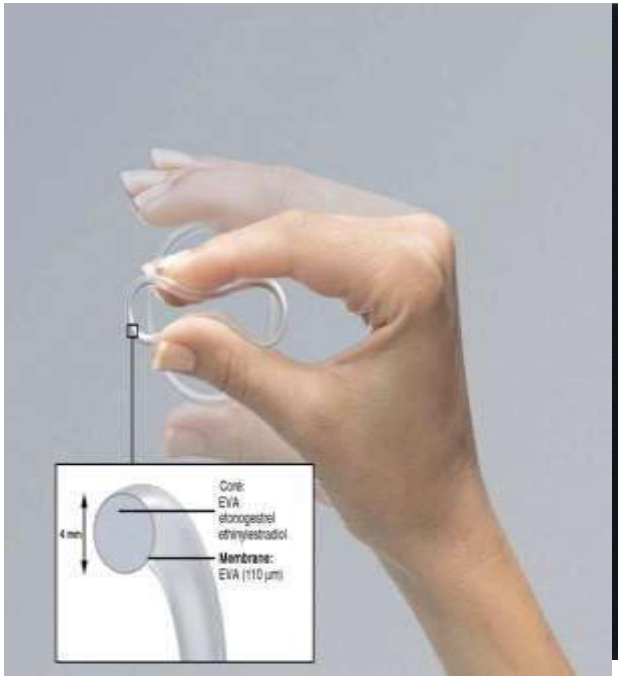


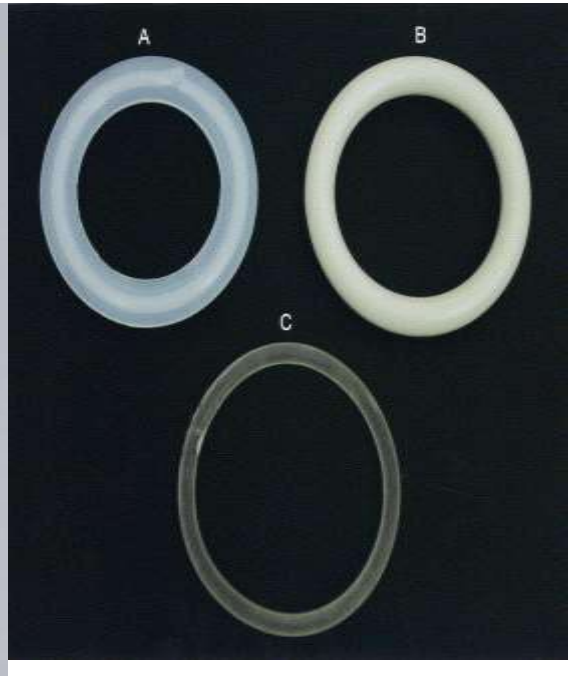
$$dQ/dt = (ACpDp/2t)^{1/2}$$

$$Q/t^{1/2} = (2ACpDp)^{1/2}$$



Contraceptive Vaginal Ring.....





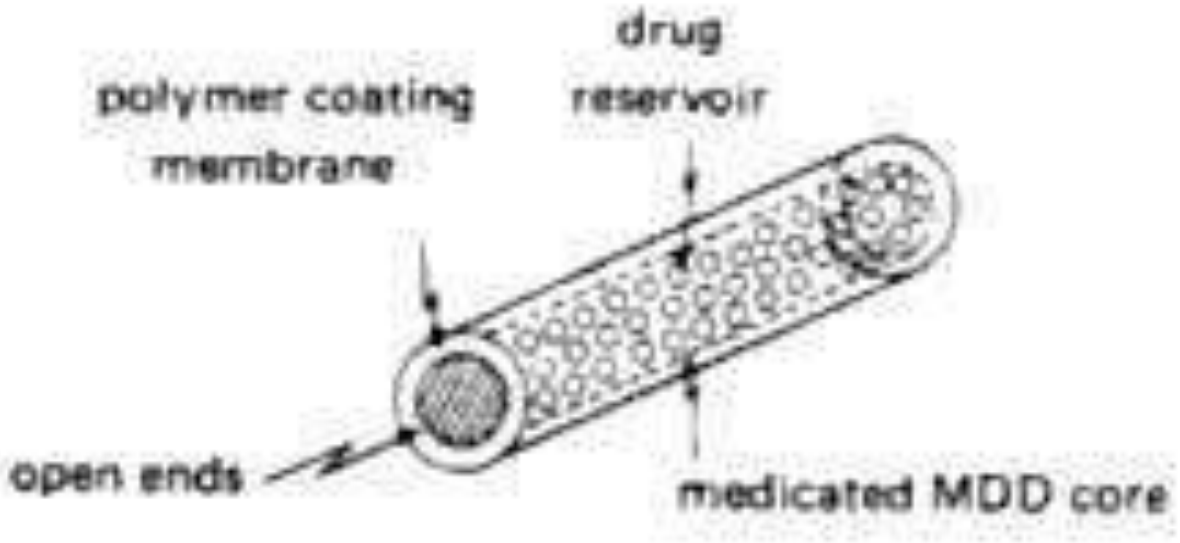


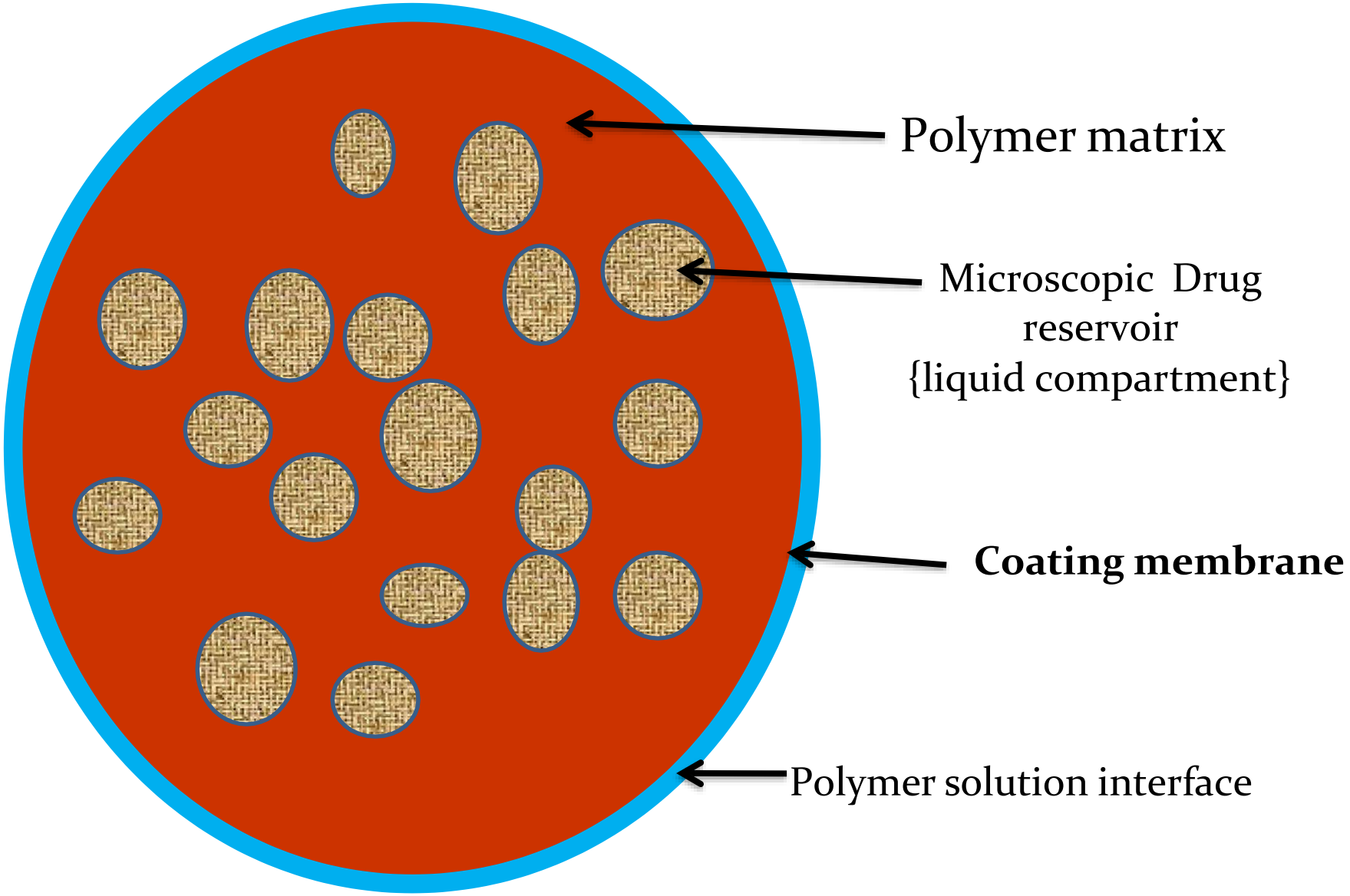
- It is fabricated by dispersing Contraceptive steroid ,e.g. Medroxy Progesterone acetate in viscous mixture of silicon elastomer then extruding the steroid- polymer dispersion into a mould to form a donut shape Vaginal ring.
- Others :Synchromate B implant,Compudo Implant

Microreservior partition-controlled drug delivery system

- In this type, drug reservoir is fabricated by micro dispersion of an aqueous suspension of drug in biocompatible polymer to form homogeneous dispersion.

  Ex. Syncromate-C.



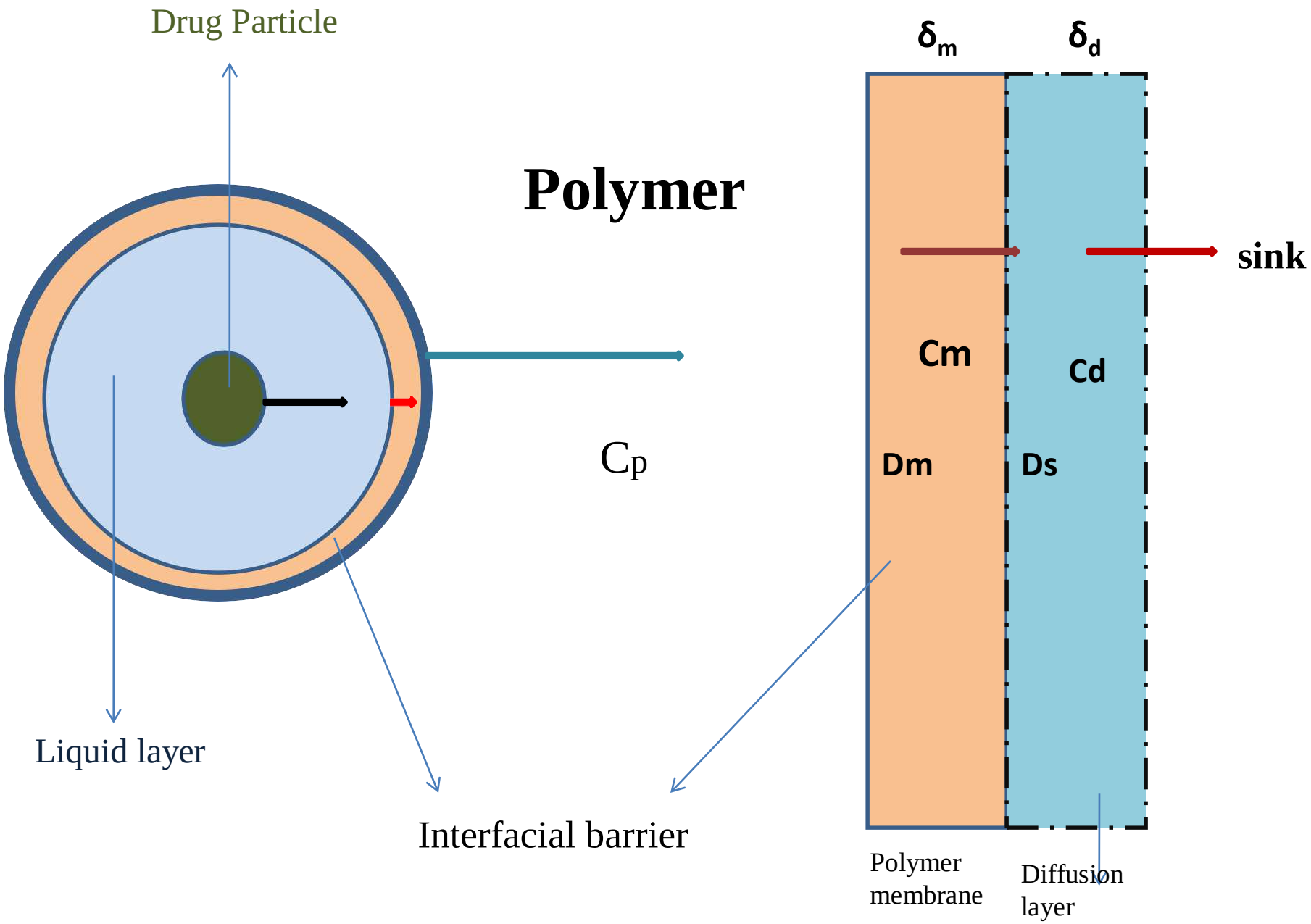

Polymer matrix
Microscopic Drug reservoir {liquid compartment}
Coating membrane
Polymer solution interface

Syncro-Mate-c Implant

- It is fabricated by dispersing the drug reservoir which is a suspension of Norgestromet in an aqueous solution of PEG 400 to form millions of microscopic drug reservoirs in a viscous mixture of Silicon elastomers.

Dual Release Vaginal Contraceptive Ring

- It is a suspension of progestin and an Estrogen in an aqueous solution of PEG 400 to form many microscopic drug reservoirs in a viscous mixture of silicon elastomers.


Drug Particle
Polymer
Cp
Liquid layer
Interfacial barrier
δm
δd
sink
Cm
Cd
Dm
Ds
Polymer membrane
Diffusion layer

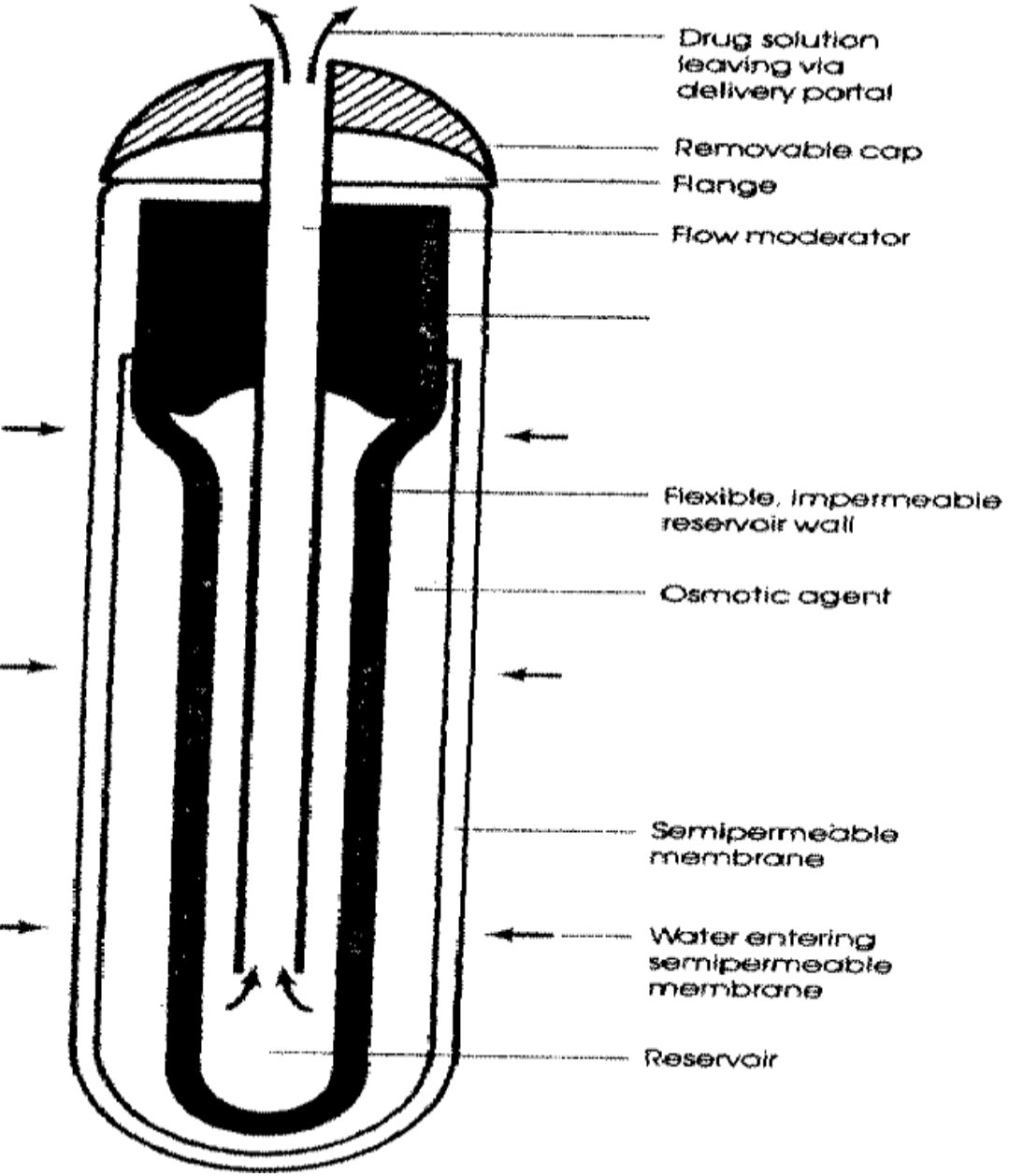
Controlled drug delivery by activation process

- ✓ In this type release of the drug is activated by some physical, chemical, or biological process and/or by the energy supplied externally and the rate of release is then regulated by the processes applied or input of energy

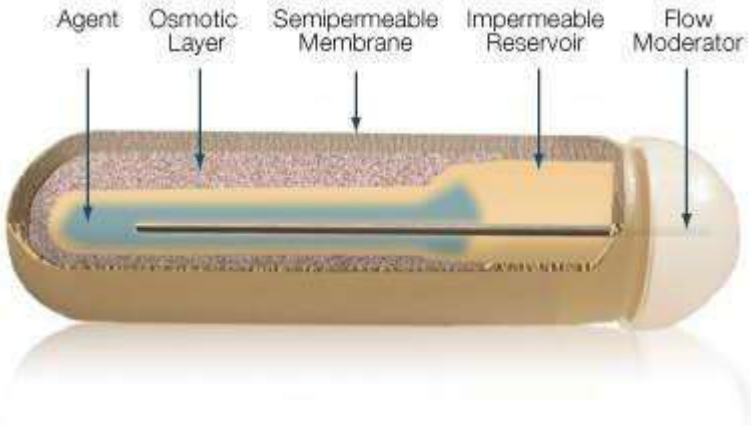
- ✓ Based on the processed applied these activation modulated drug delivery system can be classified in to

1. Osmotic pressure activated
2. Vapor pressure activated
3. Magnetically activated
4. Hydrolytic-activated
5. Hydration activated

Osmotic pressure activated drug delivery system

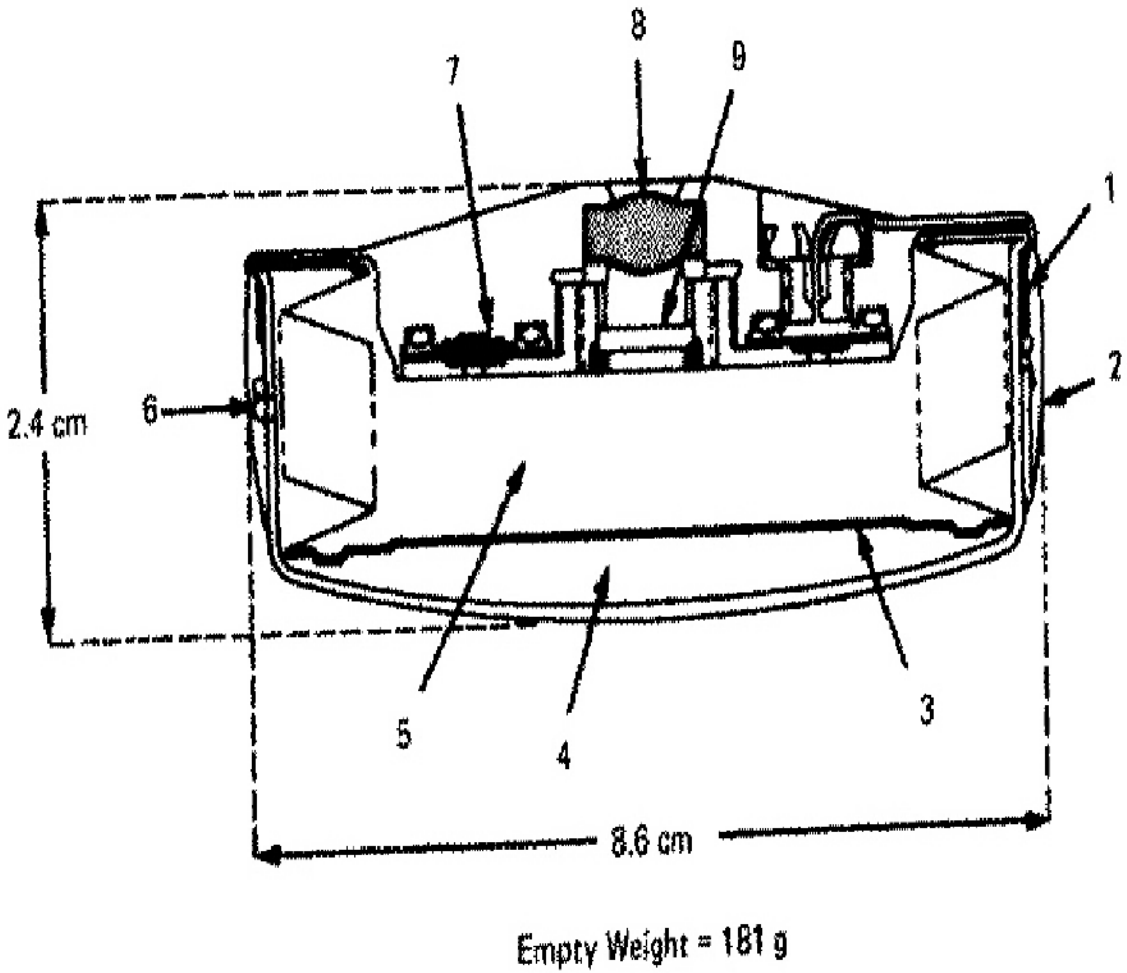


$$dQ/dt = P_w A_m / h_m(\pi_s - \pi_e)$$



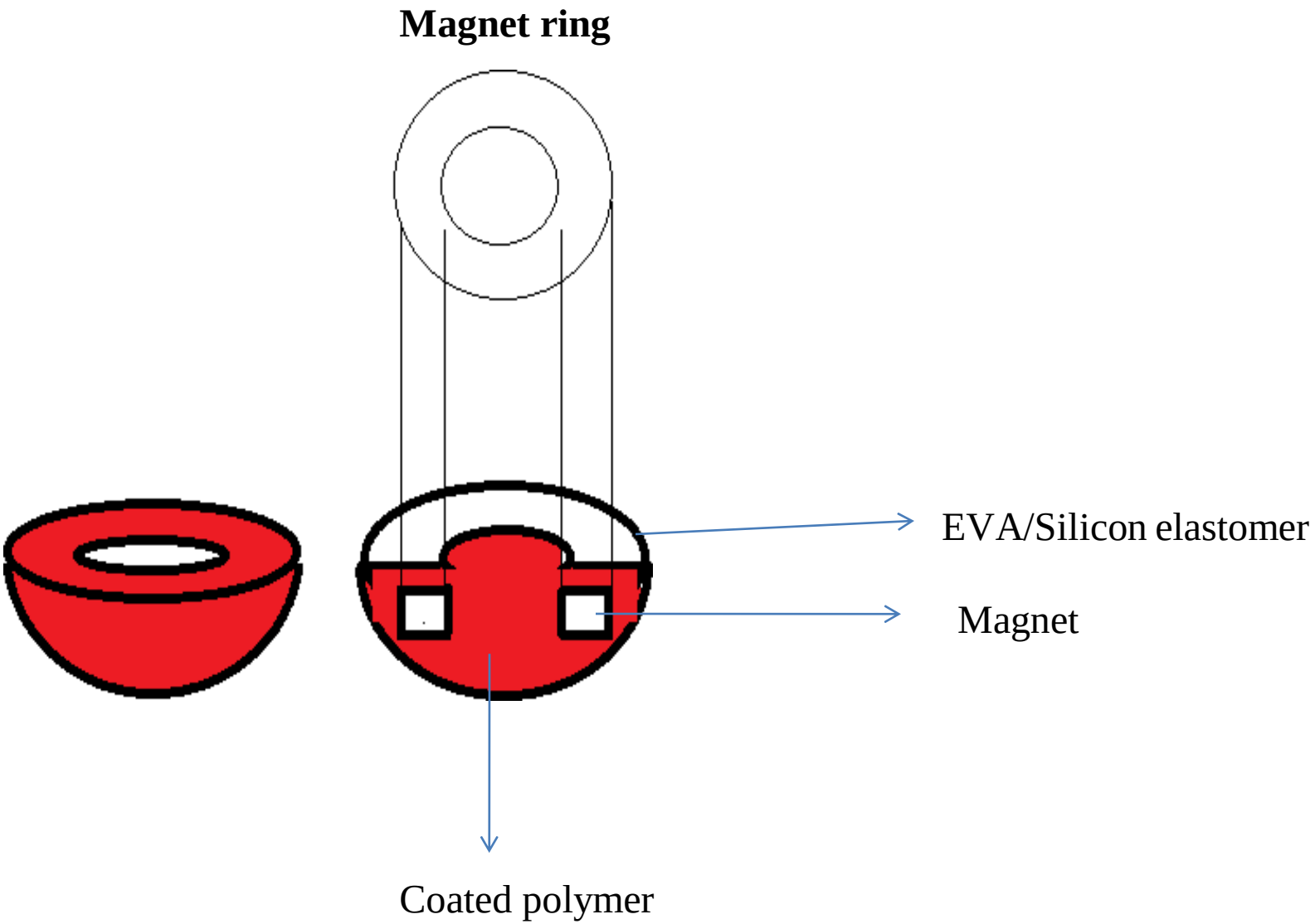
alzet® OSMOTIC PUMPS

. Vapour pressure- Activated drug delivery



1. Flow regulator
2. Silicon polymer coating
3. Bellows
4. Flouro-carbon chamber
5. Infusate chamber
6. Flourocarbon fluid filling tube
7. Filter assembly

Magnetic- activated drug delivery……



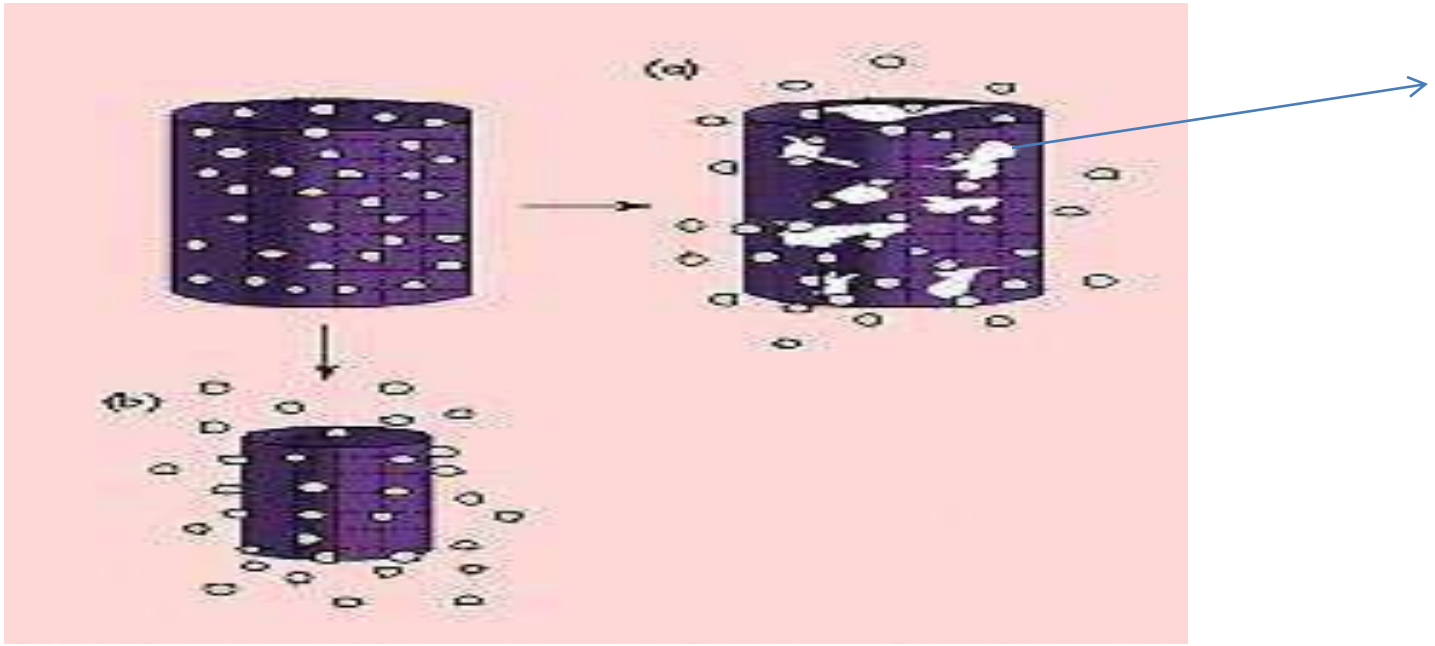
. *Ultrasound- activated drug delivery*

- This implant utilizes ultrasonic energy to activate the delivery of drug from polymer, this system can be fabricated from either a nondegradable polymer such as ethylen vinyl acetate copolymer or bioerodible polymer such as poly[bis(p- carboxyphenoxy)alkane anhydride].
- Its modulation is still undergoing evaluation.

Hydrolysis- activated drug delivery

Release of drug is activated by hydrolysis of a bioerodible polymer by the cell fluid at the implantation site.
Biodegradable polymers like
1.Poly(lactide/glycolide) copolymer
2.Poly(orthoester)
3.Poly(anhydride) are used in fabrication of this type of implantable drug delivery system.

✓ This system is made by dispersing loading dose of a drug with in a bioerodible or biodegradable polymer , which is then molded in to pellet or a bead shaped implant.

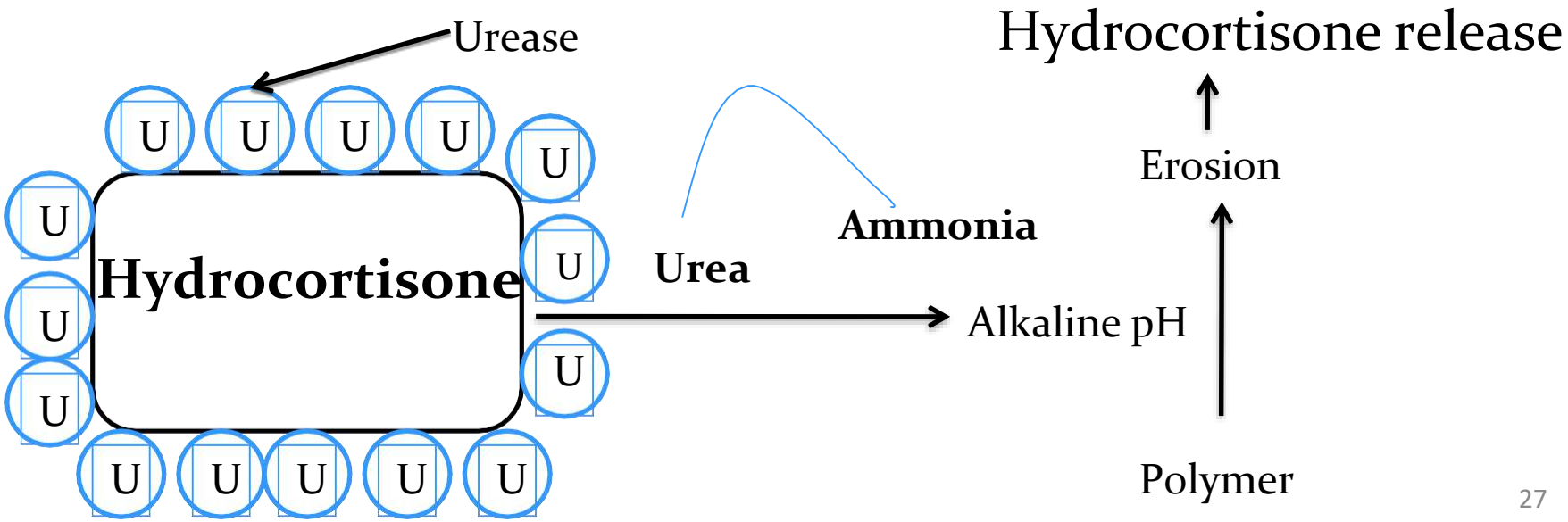


Controlled drug delivery by feed back regulated mechanism

- Using this group of controlled drug delivery system the release of a drug is activated by some biochemical molecule in the body and its concentration at the implantable site via feedback mechanism
- And the rate of controlled release of drug is regulated by the concentration of biochemical substance detected by a sensor in the feedback mechanism

Bioerosion regulated drug delivery system

- This system consist of a drug dispersed in to a biodegradable polymer matrix like poly vinyl methyl ether and is coated with immobilized urease in a neutral pH..
- Urease at the surface of drug delivery system metabolize urea to form ammonia causing increase in pH at which polymer degrades leading to drug release.



Medical aspects of implantation….

A) The Environment of Living Tissues

B) Reaction of Host to Implant

c) **Reaction of implant to Host.**


Thank You

RESINS AND RESIN COMBINATIONS

K.Umamaheswari
VCPS

Resins and resin combinations

- The term ‘resin’ is applied to more or less solid, amorphous products of complex chemical nature. These are amorphous mixtures of essential oils, oxygenated products of terpenes and carboxylic acids

- Resins and related resinous products are produced in plants during normal growth or secreted as a result of injury to the plants
- They are usually occur in schizogenous or schizolysigenous cavities or ducts

General properties

- Physical characters –
- All resins are heavier than water , they are usually amorphous ,hard, and brittle solids.
- They are insoluble in water and usually insoluble in petroleum ether but dissolve more or less completely in alcohol, chloroform and ether.

- Chemically, resins are complex mixtures of resin acids, resin alcohols (resinols), resin phenols (resinotannols), esters and chemically inert compounds known as resenes.
- Many resins ,when boiled with alkalies yield soaps

- By the action of heat they soften yielding clear, adhesive fluids , Resins burn with a characteristic, smoky flame.
- Resins are often associated with volatile oils (*oleoresins*), with gums (*gum-resins*) or with oil and gum (*oleo-gum-resins*).
- Resins may also be combined in a glycosidal manner with sugars.

Chemical nature :

- Chemically resins are not pure substances but complex mixtures of several resinous substances as resin acids, resin alcohols, resin esters, and neutral resins.
- Resins do not contain nitrogen elements
- (Non nitrogenous compounds)

Classification of resins

- Resins are classified in different three ways:

1. Taxonomical classification, i.e. according to botanical origin, e.g. *Berberidaceae* resins.
2. Classification according to predominating chemical constituent; e.g. acid resins, resene resins, glycosidal resins; etc.

3.*Resins may be classified according to the portion of the main constituents of the resin or resin combination*; e.g. resins, oleoresins, oleo-gum-resins, balsams.

Classification of resins

- 1. Acid resins –
- Here the resins occur their along with acids. Examples –
- Colophony - Abietic acid
- Sandrac - Sandracolic acid
- Myrrh - Commiphoric acid
- Copaiba - Copaivic acid

- 2. Ester resins –
- This group contains esters as the chief constituents of the resins- Examples
- Benzoin and Storax ,
- Benzoin contains benzyl benzoate,
- Storax contains cinnamyl cinnama te

- 3.Resin alcohols –
- They occurs as in free state or as esters , examples –
- Balsam of peru with perru resino tannol
- Guaiaccum resin with guaic resinol

- They are also further classified into –
  Resins: colophony, cannabis.

- Oleoresins: copaiba, ginger.
- Oleo-gum-resins: asafoetida, myrrh.
- Balsams: balsam of Tolu, balsam of Peru.
- Glycoresins : jalap
- Resenes : Asafoetida, colophony

Balsams

- Balsams are resinous mixtures that contain large proportions of cinnamic acid, benzoic acid or both or esters of these acids.
- The term “balsam” is often wrongly applied to oleoresins and should be reserved for such substances as *balsam of Peru*, *balsam of Tolu* and *storax*, which contain a high proportion of aromatic balsamic acids.

Preparation of resins

- Two general classes of resinous substances are recognized and this classification is based on the method used in preparing them:

1. Natural resins, occur as exudates from plants, produced normally or as result of pathogenic conditions

- Example by artificial punctures e.g. mastic; or deep cuts in the wood of the plant e.g. turpentine, or by hammering and scorching, e.g. balsam of Peru.

2. Prepared resins; are obtained by different methods. The drug containing *resins* is powdered and extracted with alcohol till exhaustion. The Concentrated alcoholic extract is either evaporated, or poured into water and the precipitated resin is collected, washed and carefully dried.

In the preparation of *oleoresins*; ether or acetone having lower boiling point are used. The volatile oil portion is removed through distillation.

When the resin occurs associated with gum (*gum-resins*), the resin is extracted with alcohol leaving the gum insoluble.

Crude drugs containing resins And resin combinations

1. CANNABIS

Synonym –

Ganja

- It consists of dried flowering tops of the

Biological source –

cultivated female plant s of *Cannabis sativa*

- Family - Cannabinaceae

- Diagnostic characters –
- 1.Trichomes – Numerous, both covering and glandular trichomes are present.
- Covering trichomes-
- Unicellur, slightly curved, pointed at one end and enlarged and contains cystolith (Dragger shaped)
- Glandular trichomes – Two types of glandular trichomes are present .

- 1.Multiseriate and multicellur tongue shaped stalk with a globular head.
- Short one celled stalk and 8 celled head
- Chemical constituents -
- It contains 15-20% of resin, which contain major active principle 1,3,4 Tetra hydro cannabinol
- (Commonly known as Δ THC)

- The resin also contains cannabinol, cannabidiol, cannabidiolic acid etc
- Indian hemp seeds contains about 20% fixed oil.
- Uses –
- 1.Sedative
- 2. Narcotic analgesic
- 3.Hypnotic (Induce sleep)

- 4. It has psychotropic properties due to 1,3,4 Tetra hydro cannabinol
- 5.Used as an antibacterial agent

2. Podophyllum

- Synonym – Indian podophyllum
- Biological source –
- It consists of the dried rhizome and root of *Podophyllum hexandrum* (*Podophyllum emodi*)
- Family - Berberidaceae

- Diagnostic characters –
- 1. Sclereids –
- In groups, uniformly thickened and rectangular in shape
- 2. Wood elements –
- Large number of vessels, either entire or fragments of the same showing reticulate thickening

- 3.Starch grains –
- Abundant, simple (Spherical to ovoid) and compound (3-8)
- 4. Parenchyma –
- Parenchyma fully loaded with starch grains

- Chemical constituents –
- It contains 7-15% of resin known as podophyllin . Roots contain more resin than the rhizomes.
- The active principle in podophyllin resin is known as podophyllotoxin(40%) in Indian variety , alpha and β peltatins in the American podophyllum.
- It also contains Quercetin , Kaempferol, astragalin, essential oil

- Uses –
- Used in the treatment of cancer
- Used as purgative
- Used as bitter tonic

3.Ginger

- Biological source –
- It consists of rhizomes of *Zingiber officinalae* the
- Family – Zingiberaceae
- Diagnostic characters –
- 1. Odour – Pleasant aromatic
- 2.Taste - Pungent

- 3. Parenchyma –
- Some of the cells contain yellowish brown oleo-resinous cells which occur either in fragments OR droplets
- 4. Starch grains –
- Characteristic , abundant, simple, ovoid or sack shaped

- 5. Fibres and Vessels –
- Fibres (Non – lignified) in groups associated with vessels
- Chemical constituents –
- It contains 1-2% ofvolatile oil, 5-8% pungent principle, resinous mass and
- starch

The chief constituent of volatile oil is Zingiberine .

It also contains gingerol and traces of shogaol

- Uses –
- 1. Used as stomachic
- 2. Used as an aromatic
- 3. Used as a carminative
- 4. Used as stimulant
- 5.Used as flavouring agent
- 6.Ginger oil is used in mouth washes, ginger bevarages and liquors

4.Capsicum

- Synonym – Chillies
- Biological source –
- It consists of the dried ripe fruits of Capsicum frutescens or Capsicum annum or capsicum minimum
- Family – Solanaceae

- Diagnostic characters –
- 1. Oil globules –
- Abundant and red colored
- 2.Sclerides –
- From the endocarp evenly thickened and pitted (in surface view)
- 3.Epidermis of the testa –
- Unevenly thickened and yellowish green in colour
- (in surface view)

- Chemical constituents –
- It contains an extremely pungent principle Capsaicin, red colouring matter Capsanthin.
- It also contains Ascorbic acid, carotin, red pigments etc

- Uses –
- 1. Used as a spice
- 2. Used as carminative
- 3. Used as nervine stimulant
- 4. Used as a source of vitamin- C
- 5. Used as an appetizer
- 6 . Used as a stomachic
- 7.Externally used as counter- irritant in rheumatism

5.Benzoin

- Synonyms –
- Gum benzoin, Luban, Loban, Sambrani, Lobana (Sumatra benzoin)
- Biological source- Benzoin is the balsamic resin obtained from the incised stem of *Styrax benzoin, Styrax parallelloneurus*
- Family – Styraceae
- Siam benzoin – It consists of balsamic resin of *Styrax tonkinensis,* Family - Styraceae

- Chemical constituents –
- It contains 23% of balsamic acids – Cinnamic acid and benzoic acid
- It contains 70-80% resin consisting of triterpenoids, siaresinolic acid and suma resinolic acid
- It also contains vanillin, sterol, phenyl propyl cinnamate responsible for the aromatic smell

Chemical tests -

- 1. Heat small amount of benzoin slowly in a dry test tube --- melts and evolves white fumes is produced. Which on condensation form crystalline sublimate
- 2.Benzoin heated with potassium permanganate solution --- odour of benzaldehyde (Sumatra benzoin)

- 3. Benzoin is extracted with alcohol and to the extract add water --- milky white solution is formed
- *4. Digest benzoin with few drops of petroleum ether for 5 minutes . Pour 1ml of the etherial solution in a porcelain dish containing 2-3 drops of con. Sulphuric acid and rotate the dish --- reddish brown colour is produced (Benzoin confirmed)

Uses -

- Used as an antiseptic
- Used as an expectorant
- Used as a stimulant
- It is used in the preparationof Compound benzoin tincture

Siam benzoin

- Chemical constituents – It contains about 70% crystalline and 10% amorphous coniferyl benzoate, 10% free benzoic acid, 6% siaresinolic acid, vanillin, cinnamyl benzoate (Absence of cinnamic acid)
- Uses – Antiseptic, expectorant, used in perfumery, cosmotics etc

6. **Asafoetida**

- Synonyms –
- Heeng, hing, hingu
- Biological source – It is the oleo-gum-resin obtained by incising the living rhizomes and roots of Ferula foetida, Ferula asafoetida
- Family - Umbelliferae

Chemical constituents -

- It contains 4-20% of volatile oil, 45-60% of resin and 20% of gum.
- Volatile oil contains Pinene, organic disulphide (isobutylpropenyl disulphide responsible for alliaceous odour)
- Resin contains free asaresinotannaol and in combination with ferulic acid

Chemical tests -

- 1.Powder triturated with water – yellowish orange emulsion is produced.
- *2. Combined umbelliferone test –
- Drug is boiled with hydrochloric acid for 5minutes .It is filtered and ammonia is added to the filtrate – A blue fluorescence is produced

.

- 3. Drug is treated with few drops of 50% nitric acid – green colour is produced
- 4. Drug is treated with few drops of sulphuric acid – red colour is produced which changes to violet on washing with water.
- **Uses –**
- As a carminative, an expectorant, an antispasmodic , as a laxative, nervine tonic

7.Colophony

- Synonyms – Chir, long needle pine
- Biological source –
- Colophony is the solid residue obtained after distilling the oleo-resin from various species of pinus- pinus longifolia, pinus palustris, pinus maritima
- Family - Pinaceae

Chemical constituents-

- It contains resin acids – abietic acid , neutral inert substance – resenes, esters of fatty acid.
- Chemical tests –
- 1.Dissolve the powdered drug in 2-3ml of acetic anhydride in a test tube and a drop of con. Sulphuric acid – violet colour is produced

- 2. An alcoholic solution of colophony is acidic to litmus
- 3.Colophony is dissolved in light petroleum and filtered. To the filtrate 2-3 times its volume , dilute copper acetate solution is added – emerald green colour is seen in the petroleum layer (upper layer)

- **Uses –**
- Used in the preparation of plasters and ointments.
- Used in the manufacture of varnishes and disinfecting liquids.

“Talk doesn’t cook rice”. - **Chinese proverb**

THANK YOUFOR YOUR ATTENTION

Drugs Containing Saponin glycosides (LIQUORICE, BRAHMIGINSENG & DIOSCOREA)

P.Mamatha
VCPS

General Characters of Saponins

1. Widely distributed in higher plants.
2. Bitter, acrid taste & sternutatory(irritant to mucousmembranes).
3. Form colloidal solutions in H_2O → foam on shaking → lowers surfacetension in aqueous solution.
4. Destroy RBCs → blood haemolysis.
6. Toxic by i.v.injection & harmless by oralroute.

Chemical Characters

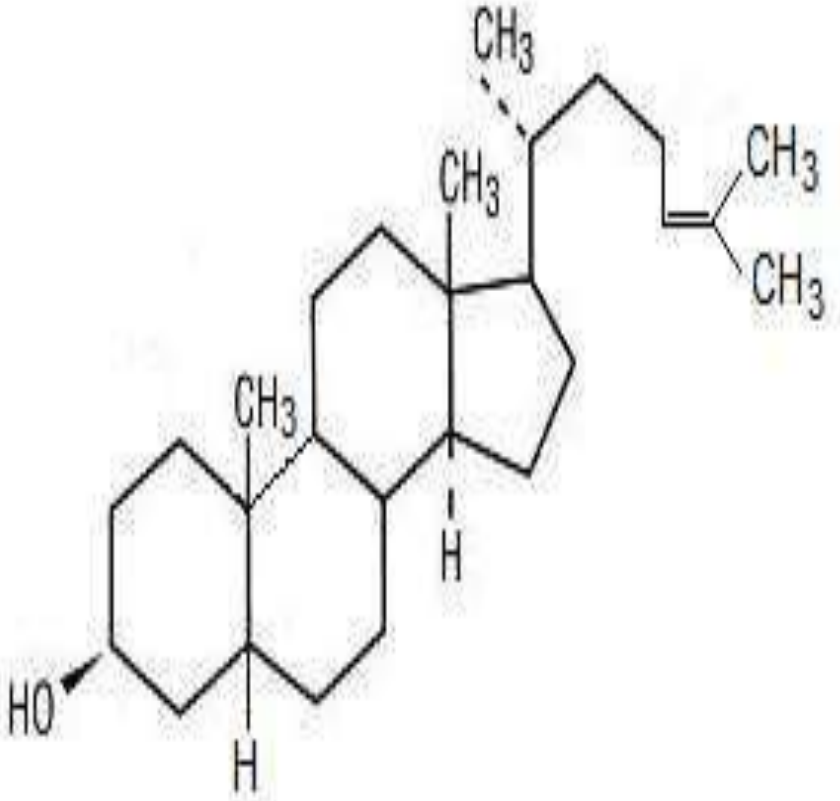
- 1.O-glycosides on hydrolysis aglycone(sapogenin)+sugarmoiety.
- 2.Aglycone:
- –triterpenoidal(C-30)[mainly in Dicotyledons]
- –steroidal(C-27)[mainly in Monocotyledons]
- 3.Sugarmoiety:
- –Often contain uronicacids or acylresidues.
- –Usually glycosylation is at C-3.

Physical Characters

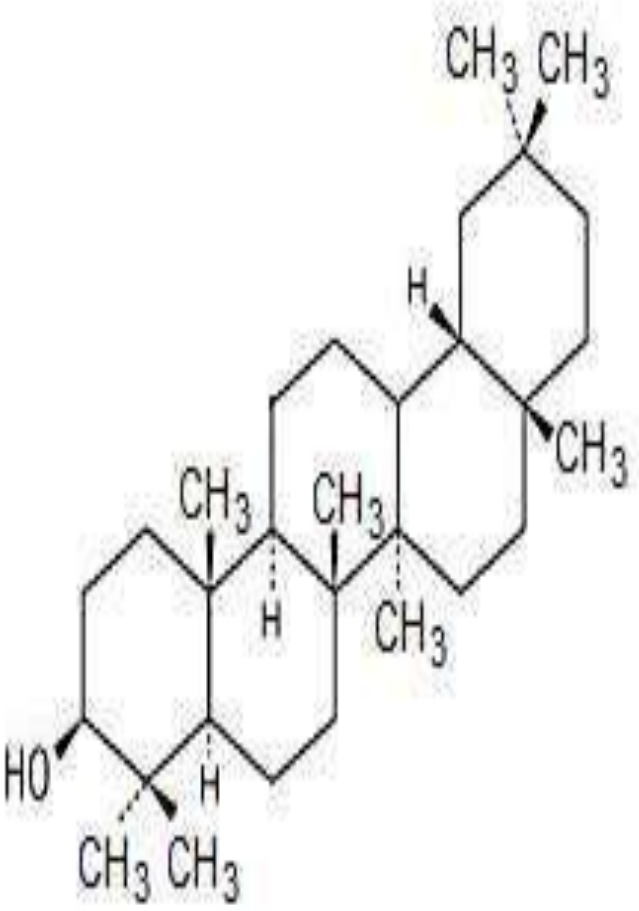
1. Rarely crystalline & generally amorphous powder with high MPs
2. Soluble in water and form colloidal solutions
3. Soluble in ethyl & methyl alcohol
4. Insoluble in organic solvents like petroleum ether, chloroform and acetone etc.
5. Bitter in taste
6. Non-alkaline in nature
7. Produce sneezing and have property of lowering surface tension
8. Hydrolysed by acids, alkalies to yield aglycone called sapogenin

Physiological Properties:

1. Extremely toxic to fishes but not poisonous to man when taken orally 2. Very dilute solution of saponins hemolyses RBC (hemolysis takes place due to the formation of complex with the cholesterol or erythrocyte membrane causing its destruction (chief property of saponin, very rarely shown by any other plant product. 3. Accelerate the germination and growth of the seeds. 4. Shows fungicidal, antifertility, molluscidal, blood purifying, abortifacient, anthelmintic, sedative & antispasmodic effects. Occurrence: 1. In whole, 75% of the families showed the presence of saponins 2. Function in the plant is as storage in form of carbohydrate and acts as immune system.



Steroidal skeleton



Tri-terpenoidal skeleton

Economical & Medicinal Importance

- **Economical uses**
- **1.Cleaning industrial equipment & finefabrics.**
- **2.Powerful emulsifier.**
- **3.Steroidal sapogenins used in semisynthesis of cortisone & sexhormones.**
- **Medicinal uses**
- **1.Expectorant**
- **2.Immunostimulant**
- **3.Controlofschistosomiasissnails(molluscicides)**
- **4.Hypoglycemic.**

Tests for Identification

- **1.Froth test:**

1 ml of aqueous solution of saponin or plant extract + shake →persistent & voluminous froth.

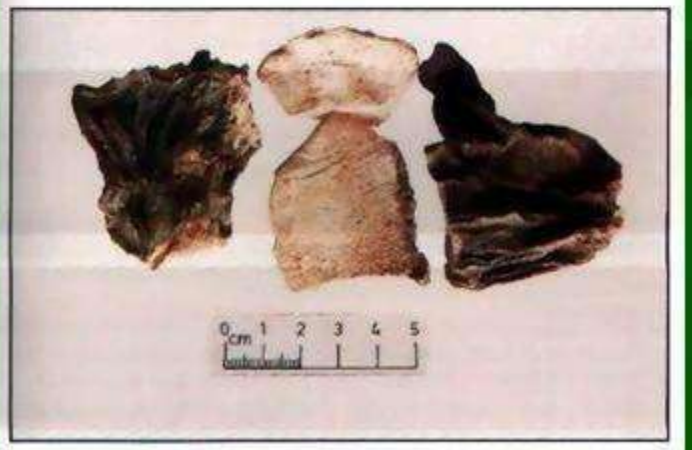
- **2.Haemolysis test:**

Suspension of RBCs in normal saline + equal volume of plant extract in normalsaline+ Shake gently →clear red solution indicating heamolysis of RBCs(compared with blank).

DIOSCOREA



DIOSCOREA COMPOSITA



DIOSCOREA COMPOSITA : TUBERS

Synonym: Yam, Rheumatism root

Biological Source:- Dried tubers of plant *Dioscorea deltoidea, D. composita* and other species of Dioscorea

Family: Discoreaceae

Organoleptic Characters:

Colour: Slightly brown.

Odour: - Odourless.

Taste: - Bitter.

Size: varies depending upon age of rhizomes. Rhizomes are soft.

Chemical Constituents

1. It contains 75% of starch which is non edible because it is bitter in taste.
2. Chief active constituent is a steroidal sapogenin known as diosgenin.

Uses

1. It is used in treatment of rheumatic arthritis.
2. Used for extraction of diosgenin.
3. Diosgenin is steroidal in nature and used as precursor for synthesis of several corticosteroids, sex-hormones and oral contraceptives.

A.R.CHAUDHARY

- **DIOSCOREA (STEROIDAL SAPONIN) Dried tubers of Dioscorea deltoidea, D. composita & other species of Source Dioscorea Family Dioscoreaceae Syn. Yam, Rheumatism root**

- **G.S. North western Himalaya, USA, Mexico Non-edible as very bitter.**
- **ChemicalConstituents : Rhizome: 75% starch & phenol; Roots: Diosgenin (4-6%) steroidal sapogenin, glycoside: smilagenin, epismilagenin, B-isomer of yammogenin, Enzyme: sapogenase;**
- **The group contains the sapogenins with pentacyclic triterpenoids nucleus which is linked with sugars and uronic acids. The sapogenins are further divided in to α-amyrin, β-amyrin & Lupeol.**
-

- Important derivative of this group is triterpenoid acid formed via substitution of carboxylic acid group at C4, C17 & C20 positions
- USES:

Diosgenin is hydrolytic product of saponin dioscin. Source of STEROIDS (in manufacturing progesterone, steroidal drugs, contraceptive) & in treatment of arthritis

Licorice Root(RADIX GLYCYRRHIZAE)

- **Botanical origin : The dried peeled or unpeeled roots and stolons of Glycyrrhizaglabra L.and its varieties (Family Leguminosae).**
- **Geographical Source:Licoriceisnative to the Mideterranean region, as Spain,Italy, England, France Germany,U.S.A.,Russia and Egypt.**



- **Deep well cultivated fertile moistened retentive soil for good root production -prefers a sandy soil with abundant moisture and does not flourish in clay. -slightly alkaline condition gives best production**
- **Cultivation -thrives in maritime climate -propagated using seeds and roots -seeds are presoaked for 24 h in warm water and then sown in spring or autumn in a green house -The roots are usually harvested after 3 to 4 years from its plantation when they mostly display enough growth.**
- **The rhizomes and roots are normally harvested in the month of October, particularly from all such plants that have not yet borne the fruits, thereby ascertaining maximum sweetness of the sap.**
- **The rootlets and buds are removed manually and**

- **Preparation -The drug is first dried under the sun and subsequently under the shade till it loses almost 50% of its initial weight. -The large thick roots of the Russian Liquorice are usually peeled before drying. - It is an usual practice in Turkey, Spain and Israel to extract a substantial quantity of the drug with water, the resulting liquid is filtered and evaporated under vacuo and the concentrated extract is molded either into sticks or other suitable forms.**
- **MORPHOLOGY: Color Unpeeled Liquorice-Externally, yellowish brown or dark brown; and internally, yellowish colour Odour Faint and characteristic**
- **Shape Unpeeled drug—Straight and nearly cylindrical Peeled drug— Mostly angular Size Length 20 to 50 cm; Diameter 2 cm, Taste Sweet**
- **Fracture Fibrous in bark; and splintery in the wood**

CHEMICAL CONSTITUENTS

Glycyrrhizin (6-8%) is found to be 50 times as sweet as sugar. - Glycyrrhizin upon hydrolysis loses its sweet taste and gives rise to the aglycone glycyrrhetinic acid (glycyrrhetic acid) together with two moles of glucuronic acid.

-Color of drug is due to Chalcone glycoside-isoliquiritin -A host of other chemical constituents essentially include are namely: coumarin derivatives e.g., umbelliferone and herniarin;

flavonoid glycoside e.g., liquiritoside; isoliquiritoside, liquiritin; isoliquiritin, rhanoliquiritin, and rhamnoisoliquiritin;

Usparagines; 22-33-dihyrostigmasterol; glucose; mannitol and about 20% of starch.

Interestingly, carbenoxolone, which is an oleandane Chemicalderivative

Section/Powder + 80% H2SO4 orange yellow color Test (transformation of flavones glycoside liquiritin to chalcone glycoside isoliquiritin

USES -demulscent and expectorant; -as a masking agent for bitter drugs in pharmaceutical formulations, such as: Uses quinine, aloe, ammonium chloride etc. -Ammoniated glycyrrhiza: as a flavouring agent in beverages, pharmaceuticalsand confectionary.

- **The presence of glycyrrhetinic acid exert mineralocorticoid activity and hence it is used in the treatment of inflamations, rhematoid arthritis and Addison's disease.**
- **- liquid extract: as a foam stabilizer in the foam type-fire-extinguisher**
- **in the treatment of peptic ulcer.**
- **- In Europe the glycyrrhetic acid: in dermatological formulations;**
- **anti- iinflammatory properties.**
- **Substitutes/Adulterants**

1. **Glycyrrhiza uralansis, also known as Manchurian Liquorice, which is pale chocholate brown in appearance having wavy medullary rays and & exfoliated cork**
2. **Russian Liquorice is also used as an adulterant, because the drug is purplish in appreance, has long roots but having no stolons.**

GINSENG



Ginseng, *Panax ginseng* (Araliaceae). Left : Plants with green stems and leaves supported on bamboos showing clusters of bright red berries. Right : Fresh white ginseng roots. (See page 254)

Synonym: Five fingers, Ninjin, Schinset

Biological Source:- Dried roots of *Panax ginseng* (Asian ginseng) or *P.quinquefolium*(American ginseng)

Family: Araliaceae

Organoleptic Characters: White, yellowish brown or red, fleshy

Chemical Constituents

It contains mixture of saponine glycosides, they can be grouped as

1. Ginsenosides.
2. Panaxosides.
3. Chikusetsusaponin.

Gynsenosides are -Ro,-Rb,-Rb2,-Rc,-Rd,-Re,-Rf, -Rg2,-Rg3 and -Rh.

Uses

1. Used as tonic, stimulant and aphrodisiac,
2. It regulates catabolic and anabolic processes of cells.
3. It stimulates immunological function
4. It lowers blood pressure, blood sugar
5. It stimulates pituitary and adrenal glands
6. Inhibits tumor growth

Ginseng Root

- RootsofPanaxquinquefolius(Americanginseng)&P.ginseng(Asianginseng),Araliaceae.
- Containsacomplexmixtureoftriterpenoidalsaponinswithatetracyclic(steroids)orpentacyclicstructure(in its cork).

- Classification
- Classified into 2 types:
- 1.Ginsenosides,
- 2.Panaxosides (differ from ginsenosides in the sugar moiety).
- Aerial parts
- Roots

- **GINSENG (TRI TERPENOID SAPONIN) Dried roots of Panax ginseng (Korea) and other species of Panax Source P. japonicas (Japan), P. pseudoginseng (Himalaya), P. quinquefolius (American), P. trifolius (Dwarf), P. vietnamensis (Vietnamese), P. notoginseng (Chinese) Family Araliaceae Syn. Ninjin, Pannag, Energofit Image G.S. Korea, China, Russia now cultivated in Japan, Canada & US The term panax (derived from greek) panaceae = cure all History The term ginseng (derived from chinese) shen sang = man root Shape of human body - Ginsenosides, Panaxosides and Chikusetsu Saponins. Chemical - Ginsenoside consists of aglycone dammarolConstituents - Panaxoside have aglycone as oleanolic acid**
- **- Starch, Gum, Resin, volatile oil, flavonoids, sesqui terpenoids**
- **USES-important immunomodulatory drug, increase natural resistance and overcome illness -both stimulant & sedative property Uses -aphordiasic & adrenal & thyroid disfuntioning -blood sugar, anaemia, gastritis etc [toxic on prolonged usage**

Ginseng -Therapeutic uses

- **Adaptogenic(antistress):enhancesbodynonspecificresistancetoexternalstress(physical,chemicalorbiological).**

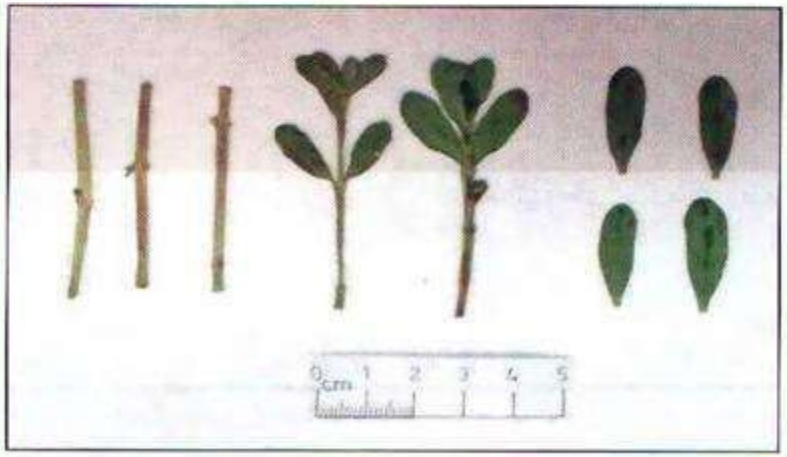
- **Improvesphysical&mentalperformancee.g.learning,memory&physicalcapabilities.**
- **Tonic,stimulant,diuretic & carminative.**
- **Improves immunefunction &metabolism.**
- **Used in anemia, Hepatoprotective(ginsenosides), diabetes(saponinsandpolysaccharides), insomnia, gastritis, Antitumor(polyacetylenes and polysaccharides) & sexual impotence.**

- **Contraindicatedincaseofhypertension&duringpregnancy.**

BRAHMI



BACOPA MONNIERI



BACOPA MONNIERI: LEAVES AND STEMS

- Synonym: Jalbrahmi
- Biological Source:- Fresh & dried leaves and stems of plant *Bacopa moniera (Herpestis moniera)*
- Family: Scrophulariaceae
- Organoleptic Characters:
- Colour: Green.
- Odour: - None.
- Taste: - Bitter.
- Shape: Leaves are obovate, entire, sessile with dotted lower surface, flowers are small with bluish white colour, five sepals.
- Chemical Constituents
- 1. It contains alkaloids important are brahmine, herpestine, bacosides A and B.
- Uses
- It is used as nervine tonic.
- In treatment of asthma, epilepsy and insanity.
- Alcoholic extract of plant is anti-cancer.

A.R.CHAUDHARY

BACOPA

- Source: Fresh leaves and stem of Bacopa monnieri (Herpestis monnieri) Family Scrophulariaceae Syn. Jalbrahmi, Neerbrahmi
- G.S. Throughout India in wet, damp & marshy places up to 1200 m elevation –
- ChemicalConstituents: saponin glycosides known as bacoside A and bacoside B - on acid hydrolysis triterpenoid aglycone bacogenin A and bacogenin B - asiatic acid and brahmic acid ChemicalConstituents

- treatment of insanity and epilepsy, asthma Uses - potent nervetonic, cardiotonic and diuretic - mild laxative.

HYDROCOTYL

Source: dried aerial parts of Centella asiatica (Hydrocotyl asiatica) Family Umbelliferae Syn. Indian pennywort, Mangosteen,Mandukparni

G.S.: India, Pakistan, Srilanka, Madagaskar

ChemicalConstituents: Tri terpenoid saponin glycoside in form of α-amyrin derivative: asiaticoside,madecassoside - Asiaticoside hydrolysis asiatic acid + 2 glucose + rhamnose -Madecassoside hydrolysis madecassic acid + 2 glucose + rhamnose Chennai & Lucknow variety Brahmoside, Brahminoside hydrolysis brahmic acid & iso brahmic acid , arabinose, glucose, rhamnose

USES -nervine tonic, spasmolytic, anti anxiety, anti-stress, sedative Uses -skin diseases, leprosy, syphilis

SQUILL (CARDIAC GLYCOSIDE)

- **Dried slices of the bulb of white variety Urginea maritima Source Red variety: Urginea maritima Family Liliaceae Syn. Scillae bulbus, Urginea scilla, Drimia maritime, European Scilla**
- **G.S. Spain, Portugal, Morocco, Algeria, Southern France, Italy, Dalmatia, Greece, Syria White variety**
- **Cardiac glycoside: Bufadienolide: Scillaren A (2/3rd of total glycoside content, responsible for activity) & Scillaren B.**
- **Scillaren A on acid hydrolysis Scillarenin + Scillabiose (Glucose + Rhamnose) Scillaren A enzyme hydrolysis Proscillaridin A + Glucose Glucoscillaren A: Scillarenin + Rhamnose + Glucose + Glucose**
- **Proscillaridin A acid hydrolysis Scillarenin A + Rhamnose ; Xanthoscillide, flavonoids, mucilage, Calcium oxalate, sinistrin (carbohydrate similar to inulin), volatile substances (causing irritation) Red variety Anthocyanin (red color), Scilliroside (glycoside which is toxic to rat) White & Red variety is chemical races.**

Chemical Test -NOT POSITIVE Baljet Test & Legal Test: Liberman's sterol Test: Squill glycoside

Squill mesophyll region: mucilage, calcium oxalate and yellow coloring matter xanthoscillide is present. Mucilage not pink color with ruthenium red but stains red with corallin soda & pale yellow with iodine

Due to mucilage, it is very much susceptible for moisture & with moisture it forms clumpy mass. Moisture hydrolyses glycoside content aglycone become less active Calcium oxalate, as a bundle of long acicular crystal, which easily penetrate skin when bulbs are handled, cause intense irritation, sometimes eruptions Stimulating, expectorant, diuretic property, cardiac tonic, same like Uses digitalis (but more irritating to GI mucus membrane), chronic bronchitis, catarrhal affection, asthma

INDIAN SQUILL

Source: Dried slices of the bulb of Urginea indica Family Liliaceae Syn. Sea onion, jungali pyaj Image G.S. Westernal Himalaya, Konkan, Coramandal coast, Bihar Chemical Cardiac glycoside similar to European squill, Mucilage in mesophyll cellConstituents Mesophyll Colarin solution red.

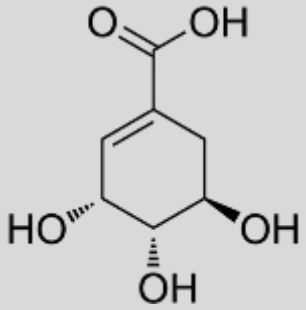
Chemical Test Mucilage reddish purple with iodine water while European squill does not

- **Cardiotonic, Expectorant, Stimulant, Diuretic, Cathartic, Bronchodilator & Uses anti cancer**

Shikimic Acid Pathway

G.Nellamma
VCPS

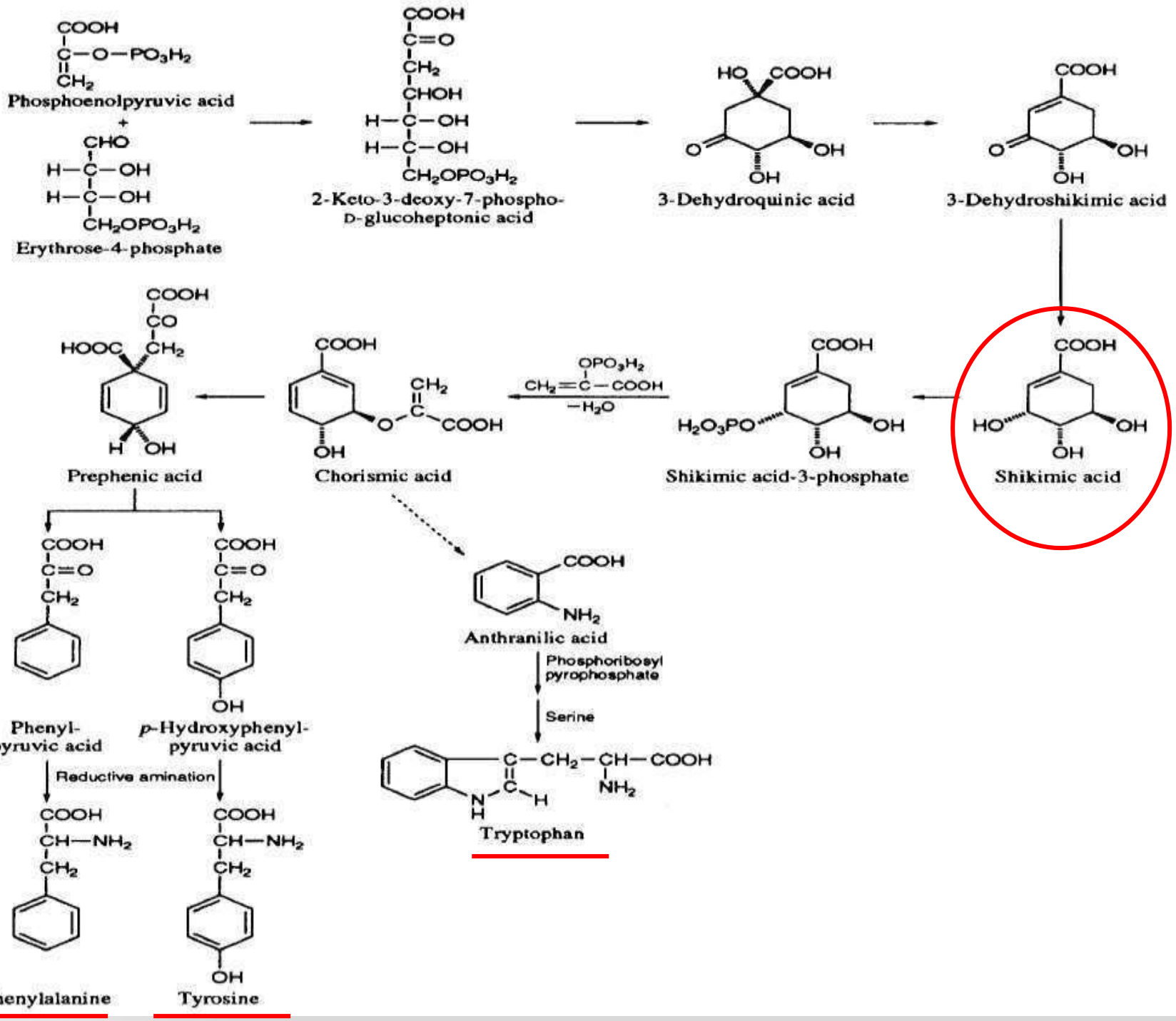
Shikimic acid



- Commonly known as its anionic form **shikimate**, is a cyclohexene, a cyclitol and a cyclohexanecarboxylic acid.
- It is an important biochemical metabolite in plants and microorganisms.
- Its name comes from the Japanese flower *shikimi* the Japanese star anise, *Illicium anisatum*), from which it was first isolated in 1885 by Johan Fredrik Eykman.
- The elucidation of its structure was made nearly 50 years later.
- Shikimic acid is also the glycoside part of some hydrolysable tannins.

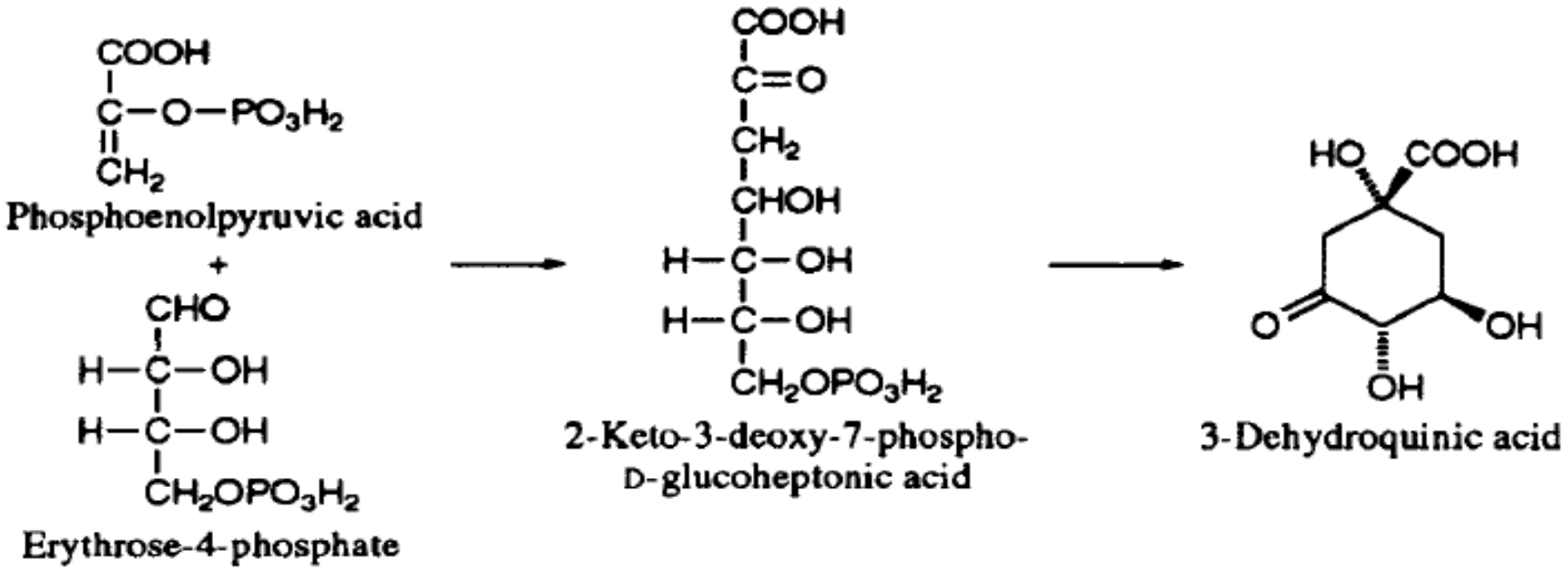
- The shikimate pathway is a seven step metabolic route used by *bacteria*, *fungi*, *algae*, *parasites, and plants* for the biosynthesis of aromatic amino acids (*phenylalanine, tyrosine, and tryptophan*).
  - This pathway is not found in animals; therefore, phenylalanine and tryptophan represent *essential amino acids* that must be obtained from the animal's diet

  - Animals can synthesize tyrosine from phenylalanine, and therefor is not an essential amino acid except for *individuals unable to hydroxylate phenylalanine to tyrosine).*

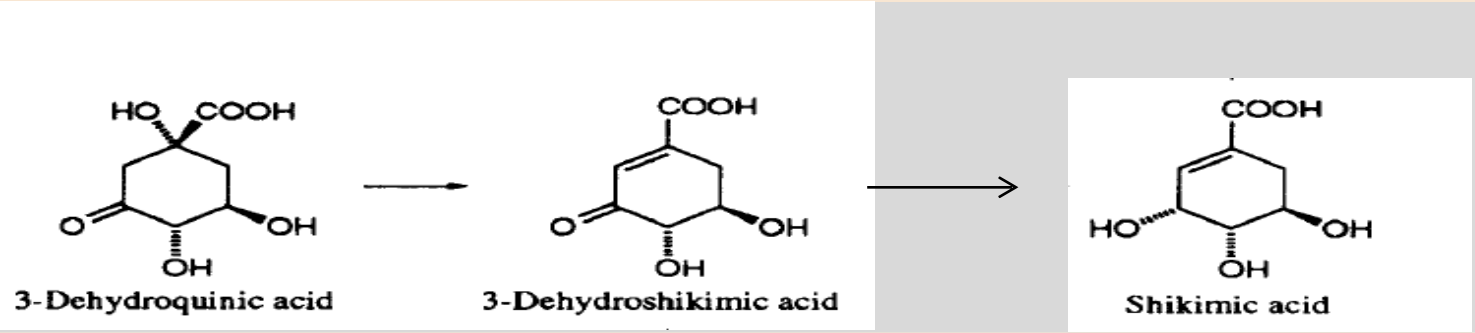

Phosphoenolpyruvic acid
Erythrose-4-phosphate
2-Keto-3-deoxy-7-phospho-D-glucoheptonic acid
3-Dehydroquinic acid
3-Dehydroshikimic acid
Shikimic acid
Shikimic acid-3-phosphate
Chorismic acid
Prephenic acid
Anthranilic acid
Phosphoribosyl pyrophosphate
Serine
Tryptophan
Phenyl-pyruvic acid
p-Hydroxyphenyl-pyruvic acid
Reductive amination
Phenylalanine
Tyrosine

- *Phosphoenolpyruvate* and *erythrose-4-phosphate* react to form *2-keto3-deoxy7phosphoglucoheptonic acid*, in a reaction catalyzed by the enzyme **DAHP synthase.**
- *2-keto3-deoxy7phosphoglucoheptonic acid* is then transformed to *3-dehydroquinate (DHQ)*, in a reaction catalyzed by **DHQ synthase.**
- Although this reaction requires nicotinamide adenine dinucleotide (NAD) as a cofactor,

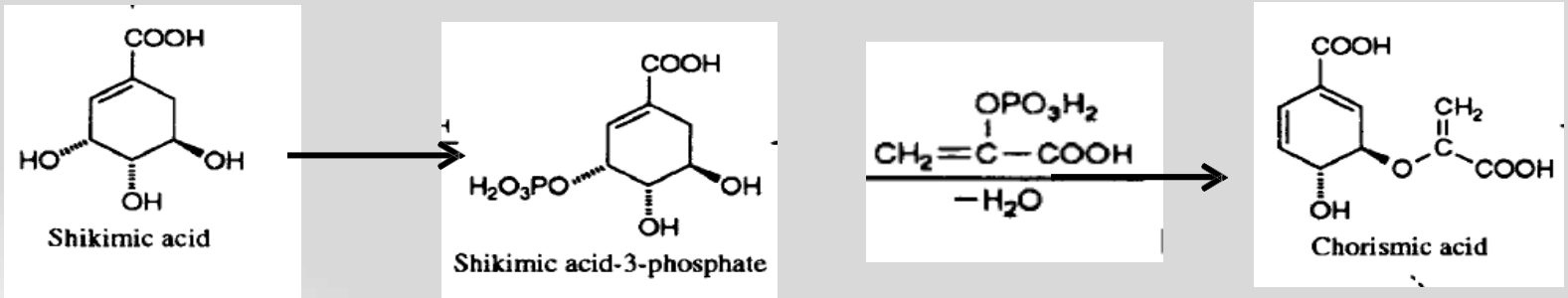
the enzymic mechanism regenerates it, resulting in the net use of no NAD.



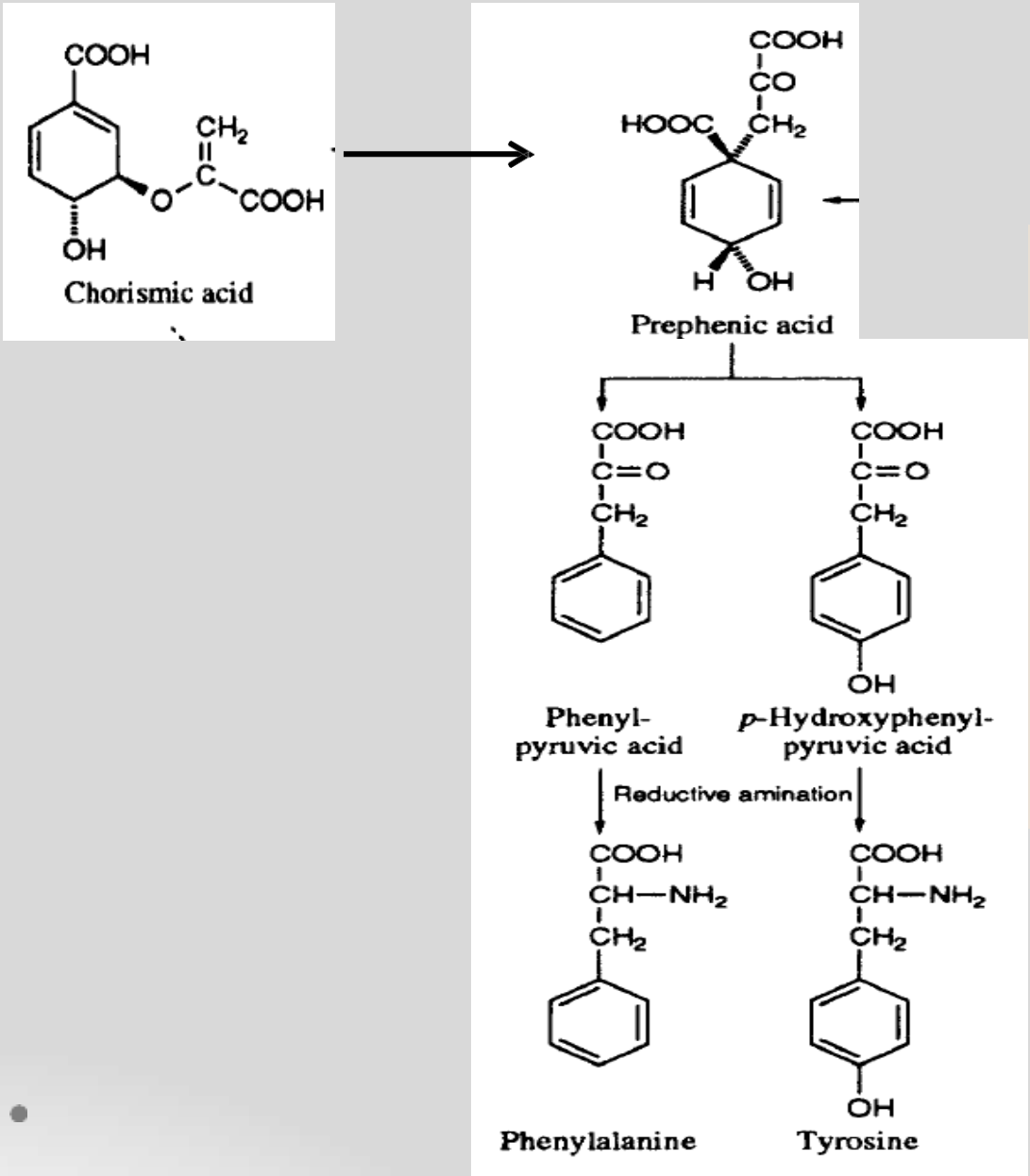
- DHQ is dehydrated to *3-dehydroshikimic acid* by the enzyme *3-dehydroquinate dehydratase*, which is reduced to *shikimic acid* by the enzyme *shikimate dehydrogenase*, which uses nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor.



- The next enzyme involved is *shikimate kinase*, an enzyme that catalyzes the ATP-dependent phosphorylation of shikimate to form *shikimate 3-phosphate*. Shikimate 3-phosphate is then coupled with *phosphoenol pyruvate* to give *5-enolpyruvylshikimate-3-phosphate* via the enzyme *5-enolpyruvylshikimate-3-phosphate* ***(EPSP) synthase***.
- Then 5-enolpyruvylshikimate-3-phosphate is transformed into chorismate by a ***chorismate synthase.***



- *Prephenic acid* is then synthesized by a *Claisen rearrangement* of chorismate by ***Chorismate mutase***.



Prephenate is *oxidatively decarboxylated* with retention of the hydroxyl group by **Prephenate dehydrogenase** to give *p-hydroxyphenylpyruvate*, which is transaminated using glutamate as the nitrogen source to give *tyrosine and α-ketoglutarate*.

Role of Shikimic Acid Pathway:

•Starting Point in The Biosynthesis of Some Phenolics

Phenyl alanine and tyrosine are the precursors used in the biosynthesis of phenylpropanoids. The phenylpropanoids are then used to produce the *flavonoids, coumarins, tannins and lignin.*

•Gallic acid biosynthesis

Gallic acid is formed from *3-dehydroshikimate* by the action of the *enzyme shikimate dehydrogenase* to produce *3,5-didehydroshikimate*. The latter compound spontaneously rearranges to gallic acid.

- **Other compounds**
  - •Shikimic acid is a precursor for:
    - ➢ indole, indole derivatives and aromatic amino acid tryptophan and tryptophan derivatives such as the psychedelic compounddimethyltryptamine.
    - ➢ many alkaloids and other aromaticmetabolites.

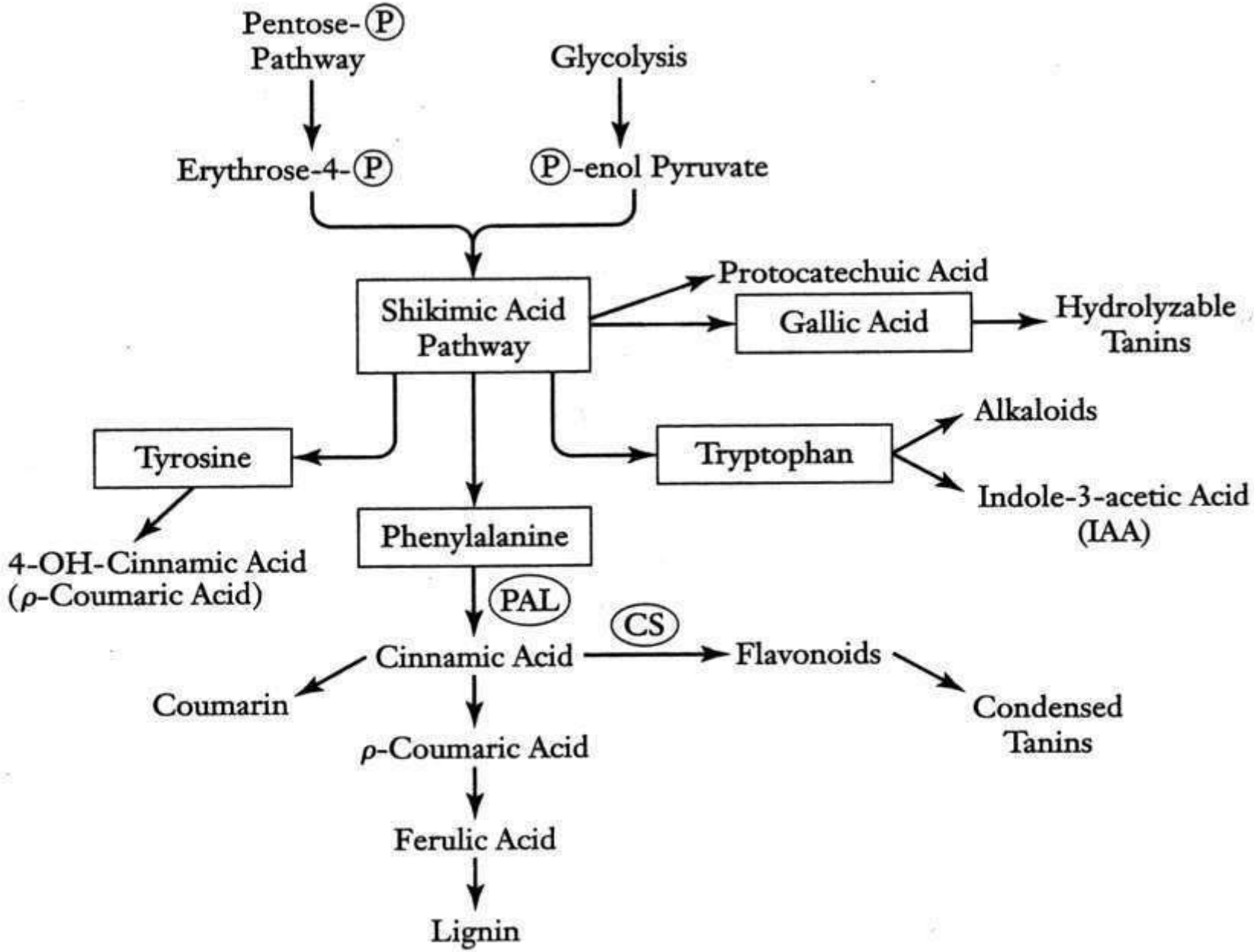


FIGURE 14.14 The central role of the shikimic acid pathway in the synthesis of various primary and secondary metabolites. PAL = phenylalanine ammonia lyase. CS = chalcone synthase.

Uses:

- In the pharmaceutical industry, shikimic acid from the Chinese star anise (*Illicium verum*) is used as a base material for production of *oseltamivir (Tamiflu)*.

Target for drugs

- Shikimate can be used to synthesize *(6S)-6-Fluoroshikimic acid*, an antibiotic which inhibits the aromatic biosynthetic pathway.
- **Glyphosate**, the active ingredient in the **herbicide Roundup**, kills plants by interfering with the shikimate pathway in plants. More specifically, glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). "Roundup Ready" genetically modified crops overcome that inhibition.

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STANDARDISATION OF HERBAL DRUGS

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HERBS

- ARE - crude plant material such as leaves, flowers, fruit, seed, stems, wood, bark, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered.

HERBAL DRUGS/ HERBAL FORMULATION

- **ARE – Finished labelled products that contain active ingredients such as aerial or underground parts of plant or other plant material or combinations thereof, whether in the crude state or as plant preparations.**

HERBAL DRUGS

¬ Phytomedicines or Phytopharmaceuticals sold as Over The Counter (OTC) products in modern dosage forms such as Tablets, Capsules & Liquids for oral use.

¬Dietary Suppliments containing Herbal Products, also called Neutraceuticals available in modern dosage forms.

¬Herbal Medicines consisting of either Crude, Semi Processed or Processed Medicinal Plants.

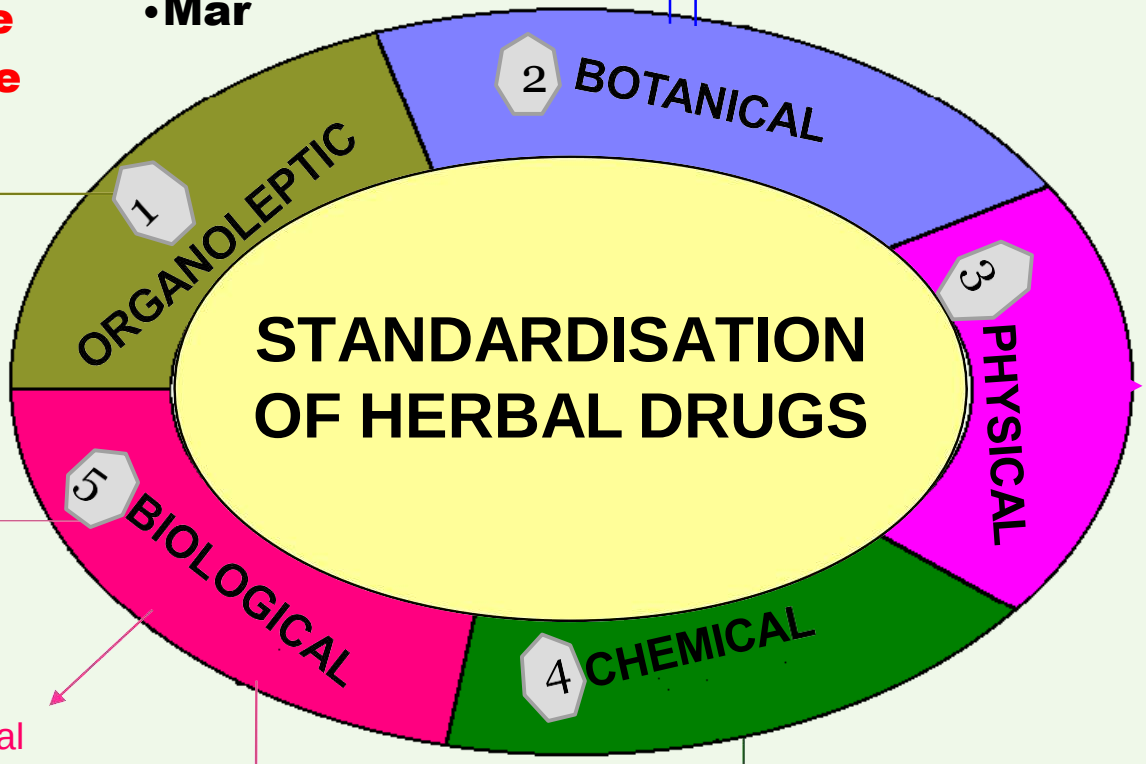
STANDARDISATION

- Standardization of drug means

  "confirmation of its identity and determination of its quality and purity and detection of nature of adulterant by various parameters like morphological, microscopical, physical, chemical and biological observations."



Standardization & Quality Evaluation of Herbal drugs



- Colour
- Odour
- Taste
- Texture
- Fracture

- Shape
- External
- Marking

Macroscopic

Microscopic

- Qualitative
- Quantitative
- SEM Studies
- Powder Studies

- Moist. Cont.
- Extrac. Values
- Ash Values
- Fluores. Analy.

Microbial Contamination

A) Total viable aerobic count

B) Determination of pathogens

C) Aflatoxins content

- Pharmacological

Bitterness value
Haemolytic activity
Foaming index
Swelling index

- Toxicological

Determination of pesticide residues
Determination of heavy metals

Antagonistic

- Bacterial
- Fungal

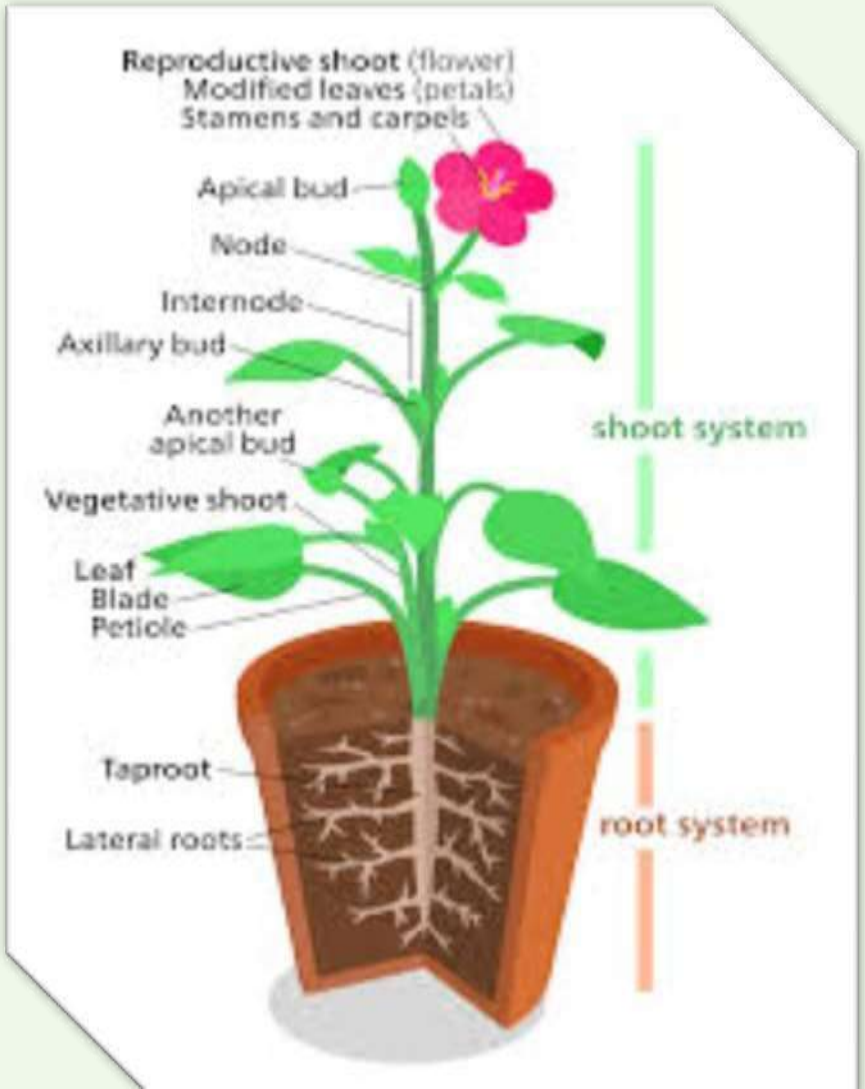
HPTLC
GLC
HPLC

- Qualitative
- Quantitative
- Chromatography
- Radioactive contamination

HPTLC Finger printing
Sec. Metabolites
DNA Finger printing

Macroscopic study

- Visual inspection provides the simplest and quickest means by which to establish identity, purity and quality.
- Macroscopic identity of medicinal plant materials is based on shape, size, colour, surface characteristics, texture, fracture characteristics and appearance of the cut surface.



Microscopic study



- Detail of cell structure and arrangement of the cells useful for differentiating similar species.
- Select a representative sample of the material & If it is dried parts of a plant than it may require softening before preparation for microscopy, preferably by being placed in a moist atmosphere, or by soaking in water.
- Any water-soluble contents can be removed from the cells by soaking in water. Starch grains can be gelatinized by heating in water.

- Histochemical detection

- Starch grains
- Aleurone grains
- Fats, fatty oils, volatile oils and resins
- Calcium oxalate/carbonate crystals
- Lignified cell wall
- Cellulose cell wall
- Mucilage
- Tannin

- Measurement of specimen

- Stomatal number
- Stomatal index
- Palisade ratio
- Vein-islet number
- Vein termination number
- Lycopodium spore method

Foreign organic matter

- Parts of the medicinal plant material or materials other than those named with the limits specified for the plant material concerned;
- Any organism, part or product of an organism, other than that named in the specification and description of the plant material concerned;
- Mineral admixtures that is adhering to the medicinal plant materials, such as soil, stones, sand, and dust.



Foreign matter: NMT 2%w/w

Ash value

- It involves non-volatile inorganic components.
- High ash value is the indicative of contamination, substitution, adulteration or carelessness in preparing the crude drugs.



Total ash

- Total ash is designed to measure the total amount of material produced after complete incineration of the drug material at as low temperature as possible (about 450°C) to remove all the carbons.

- Total ash usually consists of carbonates, phosphates, silicates and silica.
- IP and USP: 675±25°C
- BP : 600±25°C
- WHO: 500-600°C

Acid insoluble ash

- Ash insoluble in HCl is the residue obtained after extracting the total ash with HCl. It gives idea about the earthy matter

- IP method: 25mL 2M HCL solution
- USP method: 25mL 3N HCL solution
- BP method: 15mLwater and 10mL HCL
- WHO method: 25 ml of hydrochloric acid (~70g/l)

Water soluble ash

- Total ash content which is soluble in water. It’s good indicator of presence of previous extraction of water soluble salts in the drug or incorrect preparation or amount of inorg. matter
- Carbonated ash: Ash is treated with ammonium carbonate.
- Nitrated ash: Ash is treated with dilute nitric acid.

Extractive value

- Amount of the active constituents present in crude drug material when extracted with specific solvent.
- There are following Methods for determin--nation of Extractive value.

a) Cold method
b) Hot method
c) Soxhlet method



COLD EXTRACTION METHOD :



Cold extraction



Cold extracts on water bath

- Water soluble extractive value
- Alcohol soluble extractive value: Solvent strength: 20-95% v/v
- Solvent Hexane soluble extractive value: Continuous extraction for 20 hours *eg.Phyllanthus amarus*: NLT 3%
- Volatile ether soluble extractive value: Anhydrous ether-continuous extraction for 20hours
- Nonvolatile ether soluble extractive value:

  Drugs having lipid content, fixed oils eg.Colocynth fruits : NMT 3% (Pulp-medicinal value)

Insoluble matter:

- Presence of woody matter or vegetable debris or pieces of bark materials.

- Eg. In catechu
  Water insoluble matter: NMT 33%
  Alcohol insoluble matter: NMT 30%

Total solid content

- The residue obtained when prescribed amount of preparation is dried to constant weight under the specified condition (Residue on evaporation)
- Powdered extract: NLT 95%
- Semisolid extract: NLT 70%



Water Content

- Loss on drying (Gravimetric determination)
- Volumetric Azeotropic distillation (toluene distillation) method
- Titrimetric Karl fisher method
- Gas chromatographic method

Volatile oil content

- Volatile oils are the liquid components of the plant cells, immiscible with water, volatile at ordinary temperature and can be steam distilled at ordinary pressure
- Many herbal drugs contain volatile oil which is used as flavourig agent.
- E.g. Clove: NLT 15%v/w



Bitterness value



- Medicinal plant materials that have a strong bitter taste ("bitters") are employed therapeutically, mostly as appetizing agents. Their bitterness stimulates secretions in the gastrointestinal tract, especially of gastric juice.

- The bitter properties of plant material are determined by comparing the threshold bitter concentration of an extract of the materials with that of a dilute solution of quinine hydrochloride.

- The bitterness value is expressed in units equivalent to the bitterness of a solution containing 1g of quinine hydrochloride R in 2000 ml.

- Bitterness value calculated in units per g using the following formula:

$$\frac{2000 \times c}{a \times b}$$

Where,

a= the concentration of the stock test solution (S_T) (mg/ml),

b = the volume of test solution S_T(in ml) in the tube with the threshold bitter concentration,

c = the volume of quinine hydrochloride R (in mg) in the tube with the threshold bitter concentration.

Haemolytic activity



. Hemolytic activity of the crude extract on blood agar plate. (PBS buffer, pH 7.4), T: crude extract.

- Many medicinal plant materials, of the families Caryophyllaceae, Araliaceae, Sapindaceae, Primulaceae, and Dioscoreaceae contain saponins.
- The most characteristic property of saponins is their ability to cause haemolysis; when added to a suspension of blood, saponins produce changes in erythrocyte membranes, causing haemoglobin to diffuse into the surrounding medium.
- The haemolytic activity of plant materials, or a preparation containing saponins, is determined by comparison with that of a reference material, saponin R, which has a haemolytic activity of 1000 units per g.

Serial dilution for the preliminary test

	Tube no.			
	1	2	3	4
Plant material extract (ml)	0.10	0.20	0.50	1.00
Phosphate buffer pH 7.4 TS (ml)	0.90	0.80	0.50	-
Blood suspension (2%) (ml)	1.00	1.00	1.00	1.00

- Calculate the haemolytic activity of the medicinal plant material using the following formula:

$$1000 \times a/b$$

Where,

1000 = the defined haemolytic activity of saponin R in relation to ox blood,

a **= quantity of saponin R that produces total haemolysis (g)**

b **= quantity of plant material that produces total haemolysis (g)**

Determination of tannins

- Tannins (or tanning substances) are substances capable of turning animal hides into leather by binding proteins to form water-insoluble substances that are resistant to proteolytic enzymes.



- This process, when applied to living tissue, is known as an "astringent" action and is the reason for the therapeutic application of tannins.

- Chemically, tannins are complex substances; usually occur as mixtures of polyphenols that are difficult to separate and

- Calculate the quantity of tannins as a percentage using the following formula:

$$\frac{[T_1 - (T_2 - T_0)]x500}{w}$$

where w = the weight of the plant material in grams
T1= Weight of material extracted in water
T2= Weight of material not bound to hide powder
T0= Weight of hide powder material soluble in water

Determination of swelling index

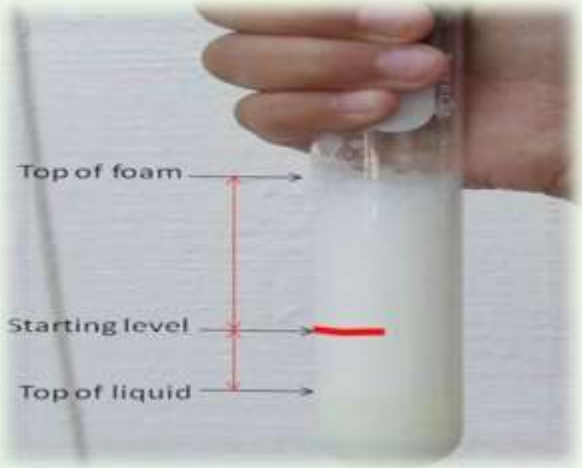
- The swelling index is the volume in ml taken up by the swelling of 1 g of plant material under specified conditions.
- Its determination is based on the addition of water or a swelling agent as specified in the test procedure for each individual plant material (either whole, cut or pulverized).



Determination of foaming index

- Many medicinal plant materials contain saponins that can cause a persistent foam when an aqueous decoction is shaken.

- The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of a foaming index. Calculate the foaming index using the following formula:



$$\text{foaming index} = \frac{1000}{a}$$

where a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm is observed.

Determination of pesticide residues

- Not more than 1%
- An ARL (in mg of pesticide per kg of plant material) can be calculated on the basis of the maximum acceptable daily intake of the pesticide for humans (ADI), as, recommended WHO, and the mean daily intake (MDI) of the medicinal plant material.

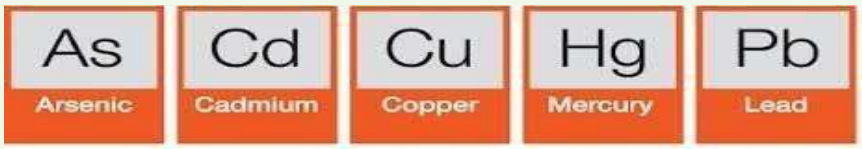
$$ARL = \frac{ADI \times E \times 60}{MDI \times 100}$$

ADI = **maximum acceptable daily intake** of pesticide (mg/kg of body weight);
E = extraction factor, which determines the transition rate of the pesticide from the plant material into the dosage form;
MDI = **mean daily intake** of medicinal plant product.

Determination of arsenic and heavy metals

- Contamination of medicinal plant materials with arsenic and heavy metals can be attributed to many causes including environmental pollution and traces of pesticides.
- Limit test for arsenic
- Limit test for cadmium and lead
- The contents of lead and cadmium may be determined by inverse voltametry or by atomic emission spectrophotometry.
- The following maximum amounts in dried plant materials, which are based on the ADI values, are proposed:

  - lead, 10 mg/kg;
  - cadmium, 0.3 mg/kg.



Determination of microorganisms

Test strains and culture media for use in validating the tests for specific microorganisms

Microorganism	Strain number[a]	Medium
Escherichia coli	e.g. NCIMB 8545 (ATCC 8739, CIP 53.126)	lactose broth
Pseudomonas aeruginosa	e.g. NCIMB 8626 (ATCC 9027, CIP 82.118)	soybean-casein digest medium
Salmonella typhimurium	No strain number is recommended. Species not pathogenic for humans, such as Salmonella abony (NCTC 6017, CIP 80.39), may be used	lactose broth
Staphylococcus aureus	e.g. NCIMB 8625 (ATCC 6538 P, CIP 53.156) or NCIMB 9518 (ATCC 6538, CIP 4.83)	soybean-casein digest medium

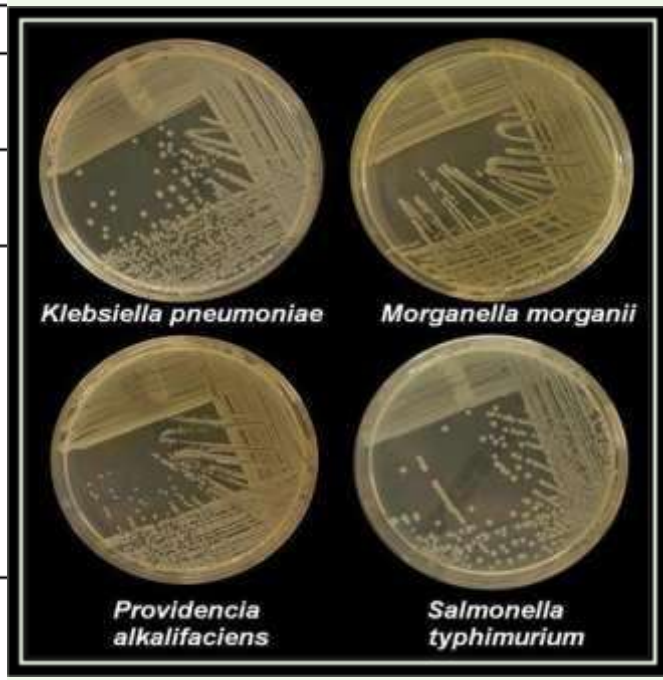


Table 1. Limits for microbial contaminants in finished products & Raw materials

Microorganism	Finished product	Raw materials
E. coli	10^1	10^4
Salmonella	-	-
Total aerobic bacteria	10^5	-
Enterobacteria	10^3	-

Aflatoxins Content



- Aflatoxins are naturally occuring mycotoxins produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*.
- The presence of aflatoxins can be determined by chromatographic methods using standard aflatoxins B1, B2, G1, G2 mixtures.
- IP method: NMT 2 µg/kg of aflatoxins B1& Total aflatoxins 4 µg/kg
- USP method: NMT 5ppb of aflatoxins B1& Total aflatoxins 20ppb



Radioactive contamination

- The range of radionuclides that may be released into the environment as the result of a nuclear accident might include long-lived and short-lived fission products, actinides, and activation products.

- Microbial growth in herbals is usually avoided by irradiation. This process may sterilize the plant material but the radioactivity hazard should be taken into account.

- The nature and the intensity of radionuclides released may differ markedly and depend on the source (reactor, reprocessing plant, fuel fabrication plant, isotope production unit, etc.).
- The radioactivity of the plant samples should be checked accordingly to the guidelines of International Atomic research centre

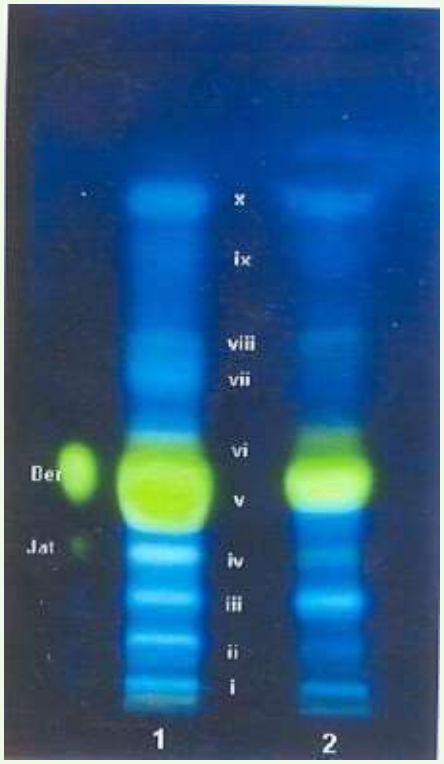


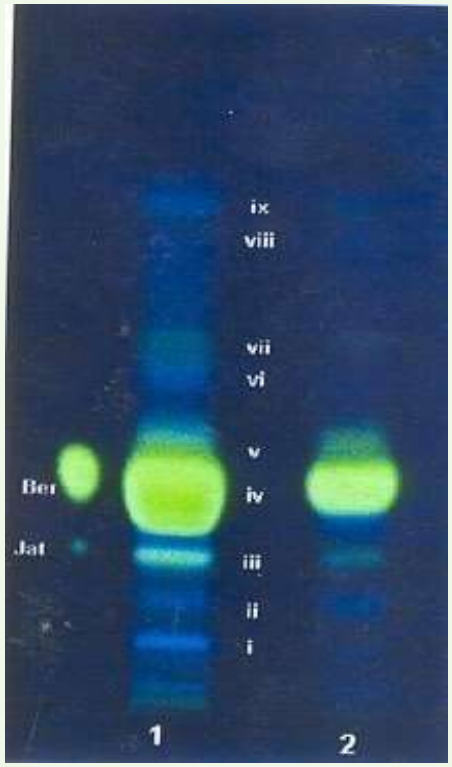
CHROMATOGRAPHY OF HERBAL DRUG

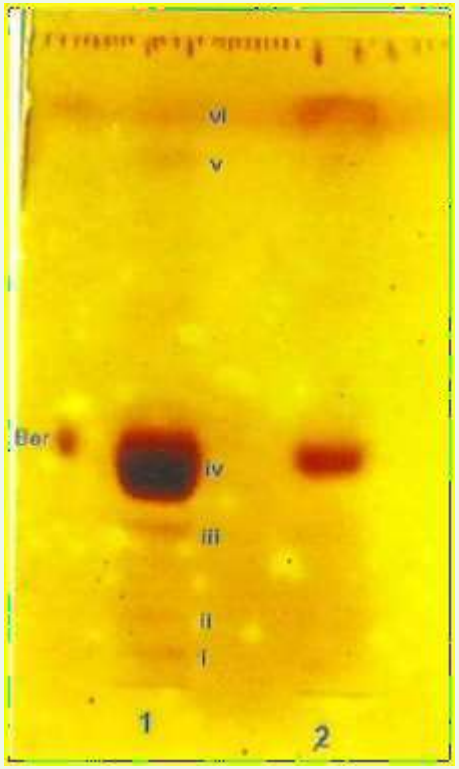
- Seperation, identification, impurity detection and assay of herbal drug in the formulation or in the extract are carried out by following methods :-

a) TLC
b) HPTLC
c) HPLC/Densitometric chromatography
d) GLC

a) **T.L.C. Finger-print profile of Methanolic extract of *Coscinium fenestratum***



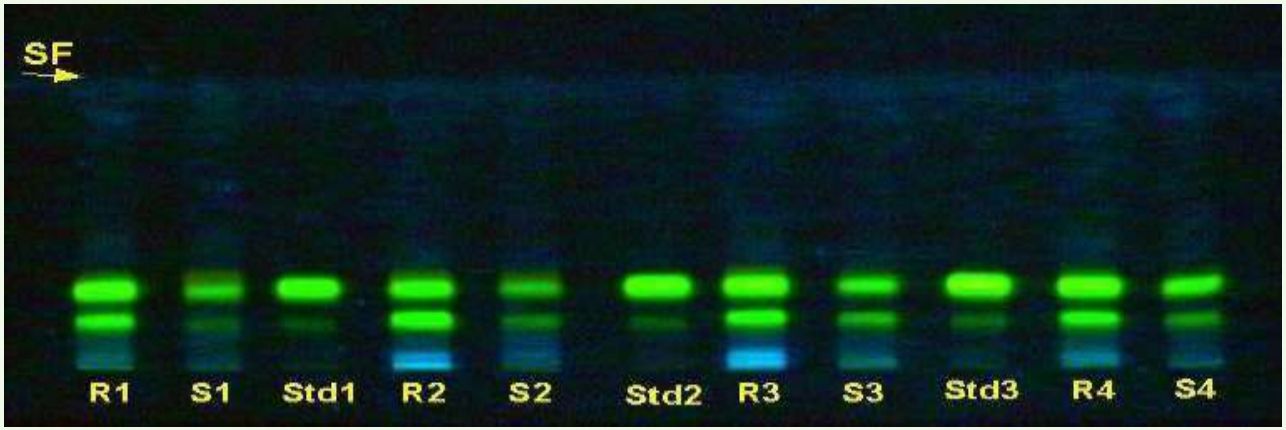


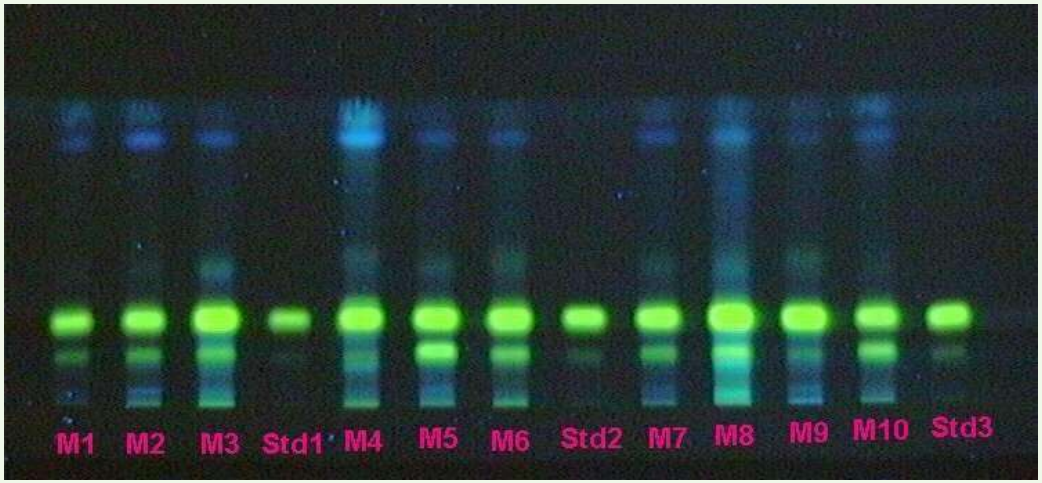


Dectetion :

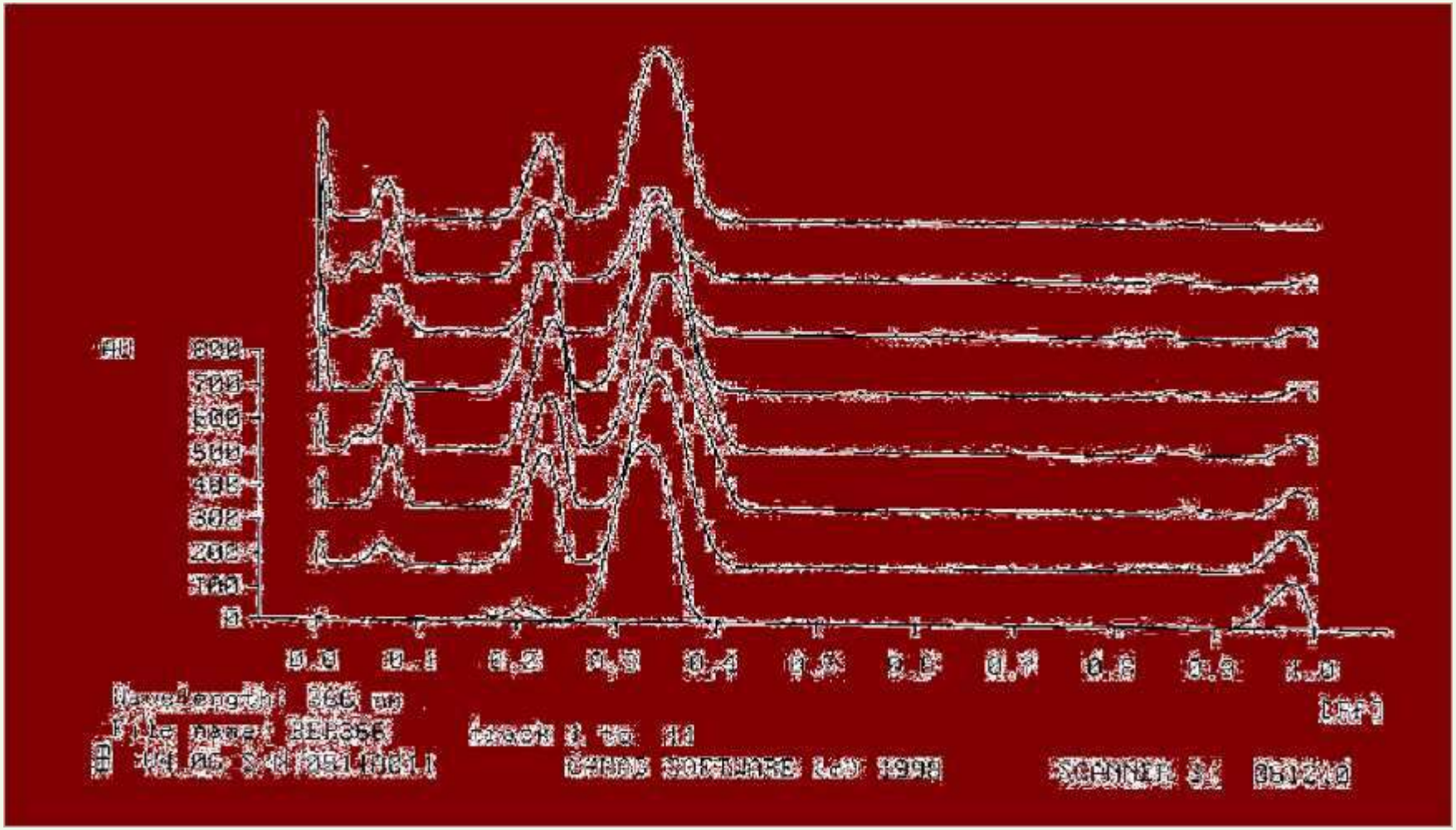
(a) Under UV 366nm **(b) Under UV 254nm. (c) Under visible light after**

b) Comparative HPTLC profile of Berberine in different market samples





c) Densitometric scan of different samples of Berberis spp. at UV 266 nm



ANALYTICAL SPECIFICATIONS OF VATI/GUTIKA (TABLET/PILLS)



1. Description
   Colour
   Odour
2. Weight variation
3. Disintegration time-Not more than 15 min
4. Identification TLC/HPTLC/GLC
5. Assay
6. Test for heavy/Toxic metals
   Lead
   Cadmium
   Mercury
   Arsenic
7. Microbial contamination
   Total bacterial count
   Total fungal count
8. Test for specific Pathogen
   E. coli
   Salmonela spp.
   S.aureus
   Pseudomonas aeruginosa
9. Pesticide residue
   Organochlorine pesticides
   Organophosphorus pesticides
   Pyrethroids
11 Test for Aflatoxins (B1, B2, G1, G2)

ANALYTICAL SPECIFICATIONS OF SYRUP (LIQUID ORAL)



1. Description, Colour
2. Odour
3. Total–ash
4. Acid– insoluble ash
5. Water-soluble extractive
6. Alcohol– soluble extractive
7. PH
8. Total sugar content
9. Viscosity
10. Identification TLC/HPTLC/HPLC
11. Test for heavy metals
    - Lead
    - Cadmiu
    - m
    - Mercury
12. Microbial contamination
    - Total bacterial count
    - Total fungal count
13. Test for specific Pathogen
    - E. coli
    - Salmonela spp.
    - S.aureus
    - Pseudomonas aeruginosa
14. Pesticide residue
    - Organochlorine pesticides
    - Organophosphorus pesticides
    - Pyrethroids

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Tannins

G.Nellamma
VCPS

Introduction

- **Tannins** are polyphenolic substances found in many plants product of secondary metabolism. Its water-soluble nature allows easy extraction and is useful in various applications in the chemical and pharmaceutical industry.

- **Definition**-the complex,organic,non-nitrogenous,polyphenolic substances of higher molecular weight. They are used as antiseptics and in git diseases like diarrhea and also used in leather industries.

Properties

Pale yellow to light brown-red amorphous substances widely distributed in plants and used chiefly in tanning leather, dyeing fabric, and making ink.

- Their solutions are acid and have an astringent taste.
- They are isolated from oak bark, sumac, and galls.
- Tannins give tea astringency, color, and flavor. Tannins are phenol glycosides.

Physical properties

- Tannins are **non-crystallizable** compounds.
- They are **soluble in water** forming colloidal solutions with acidic reaction and sharp astringent taste.
- They are soluble in dilute alkalis, alcohol, glycerol and acetone, but only sparingly soluble in other organic solvents.
- Their solutions precipitate heavy metals, alkaloids, glycosides and protein (e.g. gelatin).

Medicinal and biological properties

- Tannin-containing drugs **precipitate proteins** and have been traditionally used as **styptics** (stop hemorrhage) and internally for the **protection of inflamed surfaces** of mouth and throat.
- They play an important role in the **treatment of burns**. They form a mild antiseptic protective layer on the surface of the injured skin below which regeneration of new tissue takes place.

- They act as **anti-diarrheals**, although not recommended in this respect as they usually delay elimination of bacterial toxins from the body.

- Tannins have been employed as **antidote** in **poisoning by heavy metals**, **alkaloids** and **certain glycosides** due to their **precipitation as tannates**.
- Recently **tannins as most polyphenols** were proved to have a potent **antioxidant effect**.

Uses

- Dyes
- Fibers
- Glues
- Oils
- Waxes
- Flavoring agents
- Drugs
- Perfumes
- Potential sources of new natural drugs, antibiotics, insecticides and herbicides

Importance of tannins

Medicinal Uses:

Antidote

Antiseptic

Algicidals

Astringents

Anti-carcinogenic

Industrial Uses:

Ink manufacture

Vegetable tanning

Preservatives

Biological Activities:

Inhibition of lipid per oxidation

Decrease in blood urea nitrogen content

Inhibition of plasmin

Lipolysis in fat cells

CLASSIFICATION OF TANNINS

True Tannins

(High molecular weight compounds)

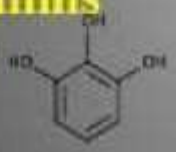
Pseudotannins

Low molecular weight compounds

e.g. Gallic acid, Flavan-3,4-diol

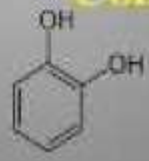
Hydrolysable Tannins

(Pyrogallol)

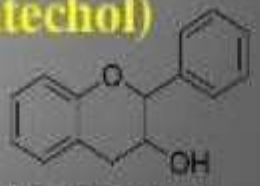


Condensed Tannins

(Catechol)

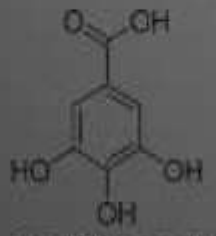


Complex tannins



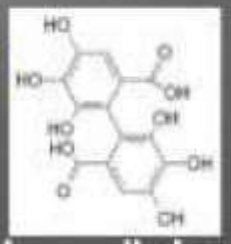
Flavan-3-ol

Gallitannins



Gallic acid

Ellagitannins

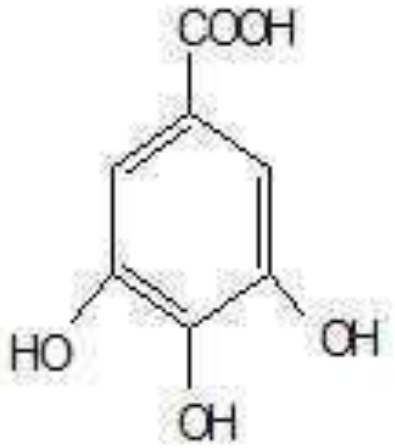


Hexahydroxydiphenic acid

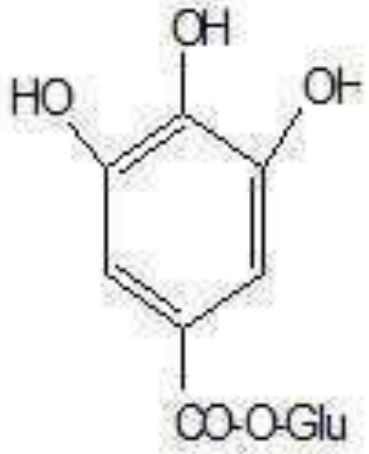
Classification

- The tannins are broadly classified into two groups based on complexity of their chemical nature and according to their behaviour on dry distillation

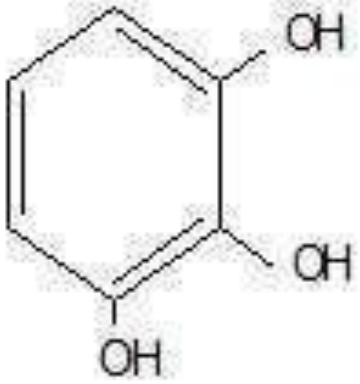
- **Hydrolysable tannins** :
- As the name indicates, these tannins are **hydrolyzed** by **acids or enzymes** quickly and the products of hydrolysis are Gallic acid or ellagic acid. On dry distillation, Gallic acid and other components get converted to pyrogallol. They respond to ferric chloride solution, producing blue colour. The examples of hydrolysable tannins are- Gallo tannin in nutgall, rhubarb, amla, clove and chestnut; ellagi tannin from oak, myrobalans and pomegranate bark.



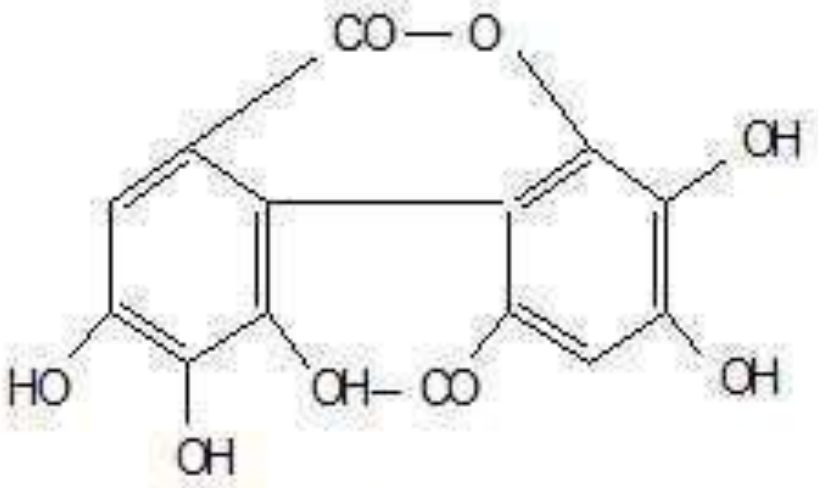
Gallic Acid



Glucogallin



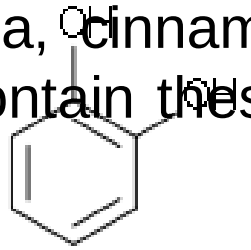
Pyrogallol



Ellagic Acid

Continued…

- **Condensed tannins :** They are also called as **non-hydrolysable** tannins, phiobatannins or proanthocyanidins. They are much **resistant to hydrolysis**. They are related to flavonoid pigments, because they are formed via derivatives of flavones, like catechin or flavan-3-ol or flavan-3,4-diols. Unlike the hydrolysable tannins, on treatment with enzymes or mineral acids, they are polymerized or decomposed into red colored substances called phlobaphenes. which are insoluble in water and indicate the typical brownish-red colour of many plants and drugs colour. On dry distillation they yield **catechol**. Tannins with ferric chloride produce brownish-green. They are distributed in different parts of plants. The green tea and hamamelis leaves, cinchona, cinnamon and wild cherry bark, pale and black catechu contain these types of tannins.



Catechol

Continued..

- **Pseudo tannins-** This is not as such a separate group of tannins, but may be treated as **sub group** because they do not obey to goldbeaters skin test and are low molecular weight compounds.

Chlorogenic acid in coffee and nuxvomica, ipecacuanhic acid in ipecacuanha and catechins in cocoa are examples of pseudotannins. The detection test for chlorogenic acid is carried out by extracting the drug with

water.

Tannin containing drug

❖ Hydrolysable tannin

1. Myrobalan
2. Bahera
3. Amla
4. Arjuna

❖ *Non- hydrolysable tannine*

1. Ashoka
2. Black catechu
3. Pale catechu
4. Pterocarpus

Extraction and Isolation of tannins

- The various types of the methods of extraction depending upon the source of tannins are employed. As the tannins are high molecular weight compounds so it becomes difficult to isolate the tannins in pure form. Thus the solvents used are the mixture of polar, non-polar and semi-polar solvent like alcohol, ether,water,acetone etc.

Identification test

- **1. Gelatin test:**
- To a solution of tannin, aqueous solution of 1% gelatin and 10% sodium chloride are added. **A white buff colored** precipitate is formed. Conforms the presence of tannins and pseudo tannins
- **2. Goldbeater's skin test:**
- A small piece of goldbeater skin (membrane prepared from the intestine of an ox) is soaked in 20% hydrochloric acid, rinsed with distilled water and placed in a solution of tannin for 5 minutes. The skin piece is washed with distilled water and kept in a 1% solution of ferrous sulphate. A **brown or black colour** is produced on the skin due tob presence of tannins.
- **3. Phenazone test:**
- A 10ml of aqueous extract of a tannin and sodium acid phosphate is heated and cooled and filtered. A 2% solution of phenazone is added to the filtrate. A bulky colored precipitate is formed.

Continue..

- **Match stick test (Catechin test):**
- A match stick is dipped in aqueous plant extract, dried near burner and moistened with concentrated hydrochloric acid. On warming near flame, the matchstick wood turns pink or red due to formation of phloroglucinol.

Test with ferric chloride-To the solution of tannins add ferric chloride solution. A blue,black,violet or green precipitate or colour confirms the presence of tannins.

DRUGS CONTAINING TANNINS

CATECHU

- Synonyms – Pale catechu, gambier, kattha
- Biological source – It consists of the dried aqueous extract prepared from the leaves of *Uncaria gambier*
- Family - Rubiaceae

- Chemical constituents –
- It contains tannins like catechins and catechu tannic acid
- It contains flavonoids like quercetin and fluorescent substances Gambier fluorescein.
- It also contains catechu- red, pyrogallol, fixed oil and waxes

- Uses –
- Used as an astringent
- Used in the treatment of diarrhoea
- Used in the preparation of lozenges





BLACK CATECHU

Synonyms- Catechu nigrum, Catechu

- Biological source –
- It consists of the dried aqueous extract prepared from heart wood of *Acacia catechu* and *Acacia chundra*
- Family - Leguminosae

- Chemical constituents –
- It contains tannins like catechins and catechu tannic acid
- It contains flavonoids like quercetin.
- Others like Catechu- red and gum
- Uses –
- Used as an astringent, used in diarrhoea
- Used as a cooling and digestive agent


1
4
5
2
3
ACACIA CATECHU, Willd.
M&N Hazhart imp.



TANNIC ACID

Synonyms- Galla, Blue galls, Aleppo galls

- Biological source –

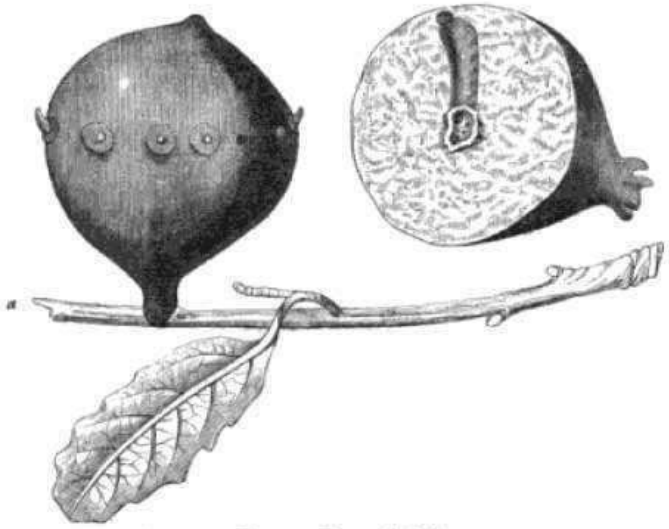
  It is obtained from the nutgalls. Galls are vegetable outgrowths found on the young twigs of *Quercus infectoria*

- Family - Fagaceae

- Tannic acid is a hydrolysable tannin and yields Gallic acid and glucose on hydrolysis

- Uses –
- Used as an astringent
- Used externally in the treatment of burns
- Used as an antidote for alkaloid poisoning

FIG. 157. *b*



Mecca or Bussorah Galls.

a, Branch bearing a gall. *b*, Section of a gall.



Amla

- **Synonyms:** Emblic Myrobalan Tree,Indian Gooseberry, Emblica, Amalk,Amla,Aonla,Auna.
- **Biological Source:** Amla consists of the fresh or dried fruits of *Emblica officinalis* Gaertn. (syn. *Phyllanthus emblica* Linn). **Family** : Euphorbiaceae.
- **Habitat** : The plant is a middle-sized tree commonly found in the mixed deciduous forests of India,Sri Lanka. China and Malaya.
- **Chemical Constituents:** The principal chemical constituent of Amla is vitamin C (650 - 900 mg/lOO g). It also contains tannins (5%), glucose, pectin. and minerals like iron,phosphorus and calcium. Tannins are mixture of gallic acid, ellagic acid and phyllembin. The presence of the tannins prevents the oxidation of vitamin.

Uses of Amla : .

- Treats frequent urination.
- Hair Loss.
- Helps to improve immunity.
- It is very helpful in skin diseases.
- Helps to accelerate the wound healing process.
- Helps to reduce the risk of cancers.
- It is helpful in improving liver function.
- It acts as an effective natural cure for indigestion, acidity, constipation, gastric troubles, ulcers and flatulence.
- It also acts as anti-inflammatory agent thereby suppresses pain and swelling.
- It provides nourishment to the nerves and is helpful in paralytic conditions.
- It also works as brain tonic and helps to alertness and memory.
- It helps in lowering cholesterol and blood sugar level.
- Lowers the risk of cold, cough and other infections of respiratory tract.





ARJUNA

- **Synonyms** : Arjuna (Sans.); Arjun (H).
- **Biological Source**: Drug is collected from dried bark of Terminalia Arjuna W. & A. **Family** : Cornbretaceae.
- **Habitat** : The plant is found throughout India.
- **Chemical Constituents:**

  Terminalia Arjuna contains Triterpene glycosides, arjunetin, arjunetoside, arjunaphthanoloside, together with oleanolic and arjunic acids, terminic acid, Antioxidants (flavones, tannins, proanthocyanidins) have been isolated from the root bark of Terminalia Arjuna. The bark contains phytosterols (ß-sitosterol), ellagic and oleanolinic acids, magnesium, zinc, copper and tannins. The whole tree is so rich on calcium that even the soil it grows on becomes rich on this element too. Arjuna also contains coenzyme Q10.

ARJUNA

- **Uses :**
- Arjuna bark is used as astringent (causing the contraction of skin cells and other body tissues).
- It is mainly used for both prevention and treatment of heart diseases including angina, heart failure and hypercholesterolemia, hypertension.
- It is also used in treating asthma, impotence and to help bones regain their strength.
- As a diuretic.
- As an antidote.





Ashoka

- **Synonyms** : Ashoka (Hindi); Asoka (Bengali).
- **Biological Source** : Ashoka is the dried bark of stem of *Saraca indica* Linn.
- **Family** : Leguminosae.
- **Habitat** : Ashoka tree is found in central and eastern Himalayas, eastern Bengal. western Peninsula, Burma, Sri Lanka and Malaya.
- **Chemical Constituents** : Ashoka bark contains tannins (6%), catechol, sterol, haemotoxyline, phlobaphenes. organic calcium compound and a keto sterol.
- **Uses:**
- Ashoka bark is used as astringent,
- It is used in uterine infections, biliousness, dyspepsia, dysentery, colic, piles, ulcers, pimples and
- In menorrhagia (excessive mensturation), Dysmenorrhoea.





Bahera

- Synonyms : Beleric Myrobalan; Bahira (Sanskrit).
- Biological Source : Bahera is the dried ripe fruit of *Terminalia bellerica* Roxb. Family : Combretaceae.
- Habitat : The plant is found throughout the forests of India,Burma and Sri Lanka
- Chemical Constituents: Bahera contains tannins (20-25%), phyllemblin, p-sitosterol, mannitol, glucose, fructose, rhamnose, fixed oil (30-40%) and hydrocarbons such as tetratriacontane, ditriacontane and tritriacontane. The fixed oil contains the esters of palmitic, stearic, oleic and linoleic acids.

Bahera

- Use:
- Bahera is analgesic, anti-inflammatory, haemostatic and gives black colour to skin and hair.
- Bahera oil is hair tonic.
- As laxative and anthelmintic.
- Bahera half ripe fruit is purgative and ripe fruit is astringent.
- Bahera is antiemtic and reduces excessive thirst.
- Bahera is used as a blood coagulant due to astringent property.
- Bahera helps in asthma and cough by reducing the inflammation of the bronchi.
- Bahera pulp is an aphrodisiac.
- Bahera pulp is intoxicant.






thank
you

VOLATILE OILS

K.Umamaheswari
VCPS

LEMON PEEL

Definition: Dried lemon (*Limonis Cortex*) peel is obtained from the fruit of *Citrus limon* (Rutaceae).

Botanical Description:

Small tree, growing to 3-5 m in height.

Geographical Sources

Mediterranean Regions



Limonis Cortex – COLLECTION & PREPARATION

Lemons are collected in January, August & November, before the green colour changes to yellow.

The smaller fruit, which would not be sold on the produce market, are used in the preparation of oil of lemon - the peel is removed with a sharp knife in the form of a spiral band.



Limonis Cortex – CHARACTERISTICS

Dried lemon peel occurs in spiral bands (2 cm wide; 2-3 mm thick).

The **outer** surface is rough & yellow; the **inner** surface is pulpy & white (anatomically similar to that of an orange peel).

Odour: Strong & characteristic

Taste: aromatic & bitter



Limonis Cortex – CONSTITUENTS & USES

CONSTITUENTS

Should contain at least 2.5% volatile oil

Vitamin C

Hesperidin (Flavonoid)

Mucilage

Calcium Oxalate



USES

Flavouring purposes

LEMON OILS - *Oleum limonis*

Definition: Lemon oil is the oil expressed from the outer part of the fresh pericarp of the ripe or nearly ripe fruit of *Citrus limon* (Rutaceae).

BP: oil should be obtained by suitable mechanical means, without the use of heat, from the fresh peel.

Much oil is derived via **steam distillation**, but this process yields oil of **inferior quality**.

Distilled oil of lemon is much cheaper than that prepared by expression. Large amounts are used for non-pharmaceutical purposes.

Geographical Sources:

Mediterranean, North & South America, Australia & parts of Africa.

Preparation of Lemon Oils

i. **Hand Methods**

No longer applicable to pharmaceutical oils



Production of Lemon Oils

ii. Machine Processes

Quality is inferior to the best hand-pressed oils.

Machines are designed to release oils from the peel via puncture, rasting or cutting and by imitating the gentle squeezing action of the sponge method. (Superiority of the sponge method is due to the fact that there is no contact between the oil & the inner white part of the skin.

The newer machines extract oil more completely than the older ones and give a higher yeild

iii. Distilled Oils

Although not official, some lemon oils are produced by distillation, mainly from the residue of the expression processes. It is much cheaper than hand-pressed or machine-made oil.

Oleum limonis - CONSTITUENTS

Terpenes – mainly limonene

Sesquiterpenes

Aldehydes (Citral & Citronella)

Esters

Lemon oil has a tendency to **resinify** and should be protected from the action of air & light as much as possible.

Oleum limonis – ADULTERATION & USES

ADULTERATION

Oil of turpentine

Terpenes from ‘terpeneless oil lemon’

Distilled oil of lemon

Oil of lemon-grass



USES

Perfumery

Flavouring

TERPENELESS LEMON OIL

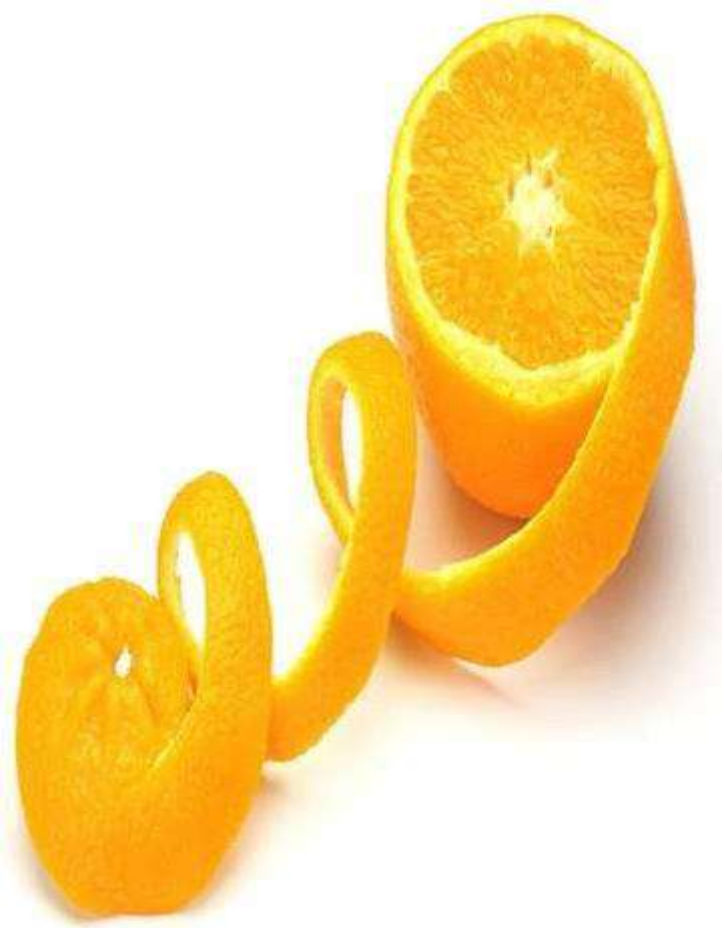
Definition: Oil prepared by concentrating lemon oil in vacuum until most of the terpenes have been removed, or by solvent partition. The concentrate is a terpeneless oil, which has a citral content of 40-50 %.

It is equal in flavouring to 10-15 times its volume of lemon oil



BITTER ORANGE PEEL

Definition: Dried bitter orange peel is obtained from the fruit of *Citrus aurantii* (Rutaceae).



Botanical Description:

Small tree, growing to 3-5 m in height.

Geographical Sources

Mediterranean Regions

Definition: Bitter orange peel oil is the oil expressed from the outer part of the fresh pericarp of the ripe or nearly ripe fruit of *Citrus aurantiii* (Rutaceae).

BP: oil should be obtained by suitable mechanical means, without the use of heat, from the fresh peel.

Much oil is derived via **steam distillation**, but this process yields oil of **inferior quality**.

Distilled oil of lemon is much cheaper than that prepared by expression. Large amounts are used for non-pharmaceutical purposes.

Geographical Sources:

Mediterranean, North & South America, Australia & parts of Africa.

Bitter orange peel
CONSTITUENTS & USES

CONSTITUENTS

Should contain at least 2.5% volatile oil

Vitamin C

Hesperidin (Flavonoid)

Mucilage

Calcium Oxalate

Volatile oil consits of Limonene and traces of citral.

USES

Flavouring purposes

EUCALYPTUS LEAF

DEFINTION: Eucalyptus leaf consists of the whole or cut dried leaves of the older branches of *Eucalyptus globulus*, (Myrtaceae).

GEOGRAPHICAL SOURCES

Portugal, SA, Spain, China, Brazil, Australia, India & Paraguay.



Eucalyptus - DESCRIPTION

MACROSCOPICAL

- Older dried leaves are grey-brown & have lateral veins. Secretory oil cells are visible in leaves held to the light.

MICROSCOPIC

- Epidermal cells have a thick cuticle.
- Anisocytic stomata
- Mesophyll has schizogenous oil glands
- Calcium oxalate crystals: Prisms & Cluster crystals



Eucalyptus - CONSTITUENTS

Volatile Oil (at least 2 %)

sesquiterpene - Anti-bacterial action against oral pathogens.



EUCALYPTUS OIL

Oil of eucalyptus is distilled from the fresh leaves of various species of *Eucalyptus* and rectified. They are produced in the same countries which produce the dry herb.

Only a certain amount of species produce oil suitable for medicinal use – the main criteria is a **high cineole** content and **low** amounts of **phellandrene and aldehydes.**

Suitable oils are obtained from *E. polybractea, E. smithii,* ***E. globulus*** and ***E. australiana*****.**

CHARACTERISTICS & CONSTITUENTS

CHARACTERISTICS

Colourless or pale yellow liquid

Aromatic & camphoraceous in odour.

Pungent & camphoraceous in taste, which is followed by a sensation of cold.

CONSTITUENTS

At least 70 volatile oils (mainly cineole).



EUCALYPTUS OIL - USES

Alleviating the symptoms of nasopharyngeal infections

Treating coughs

Decongestant.

Official preparations

Mixtures, inhalations, lozenges and pastilles; also applied externally as ointments and liniments.



PEPPERMINT
& PEPPERMINT OIL

DEFINITION: Peppermint is the dried leaves of *Mentha piperita* (Labiate). It should contain at least 1.2 % volatile oil.

GEOGRAPHICAL SOURCES:

Europe & America



MACROSCOPICAL FEATURES

All mints have a square stem & creeping rhizome.

Black mint, which is the most commonly cultivated variety in England, has purple stems and dark green petiolate leaves tinged with purple. Leaf blades are 3-9 cm long with a grooved petiolate up to 1 cm long.

Pinnate venation.

Glandular trichomes: bright yellow points (hand lens)

Small purple flowers appear in late summer.







MICROSCOPIC FEATURES

Diacytic stomata

Multicellular clothing trichomes

2 types of glandular trichomes (one with a unicellar head; with a multicellular head).

Calcium oxalate is absent.



Oleum Menthae

Oil of peppermint is obtained from *Mentha piperita* via steam distillation using the flowering tops.

Oil should contain at least 44 % menthol, 15-32 & menthone and 4.5-10 % menthyl acetate.



Oleum Menthae - CONSTITUENTS

Menthol
Menthone & isomenthone
Menthyl acetate
Limonene
Cineole
Menthofuran
Pulegone
Cineole

OIL COMPOSITION: greatly influenced by genetic factors & seasonal variation.



PEPPERMINT & PEPPERMINT OIL USES

OIL: anti-bacterial, cooling, carminative

HERB: Carminative



SANDALWOOD OIL

SYNONYM-

Yellow sandalwood , Chandan oil

BIOLOGICAL SOURCE-

It is dried heartwood of *Santalum album* (SANTALACEAE).

PREPARATION-

30 year old plant selected for collection of volatile oil. Plant is uprooted , bark is removed from stem and root. Wood is cut into small pieces for steam distillation.

Oil is yellowish or pale reddish viscous liquid, strong fragrance, bitter taste.

CHEMICAL CONSTITUENTS-

Alfa & beta santalol(90)
Alfa & beta santalene, Alfa & beta santalic acid
Santalone
Santanone, santene

Chemical and Therapeutic properties

- The main chemical components are santalol, santyl acetate and santalene. Our essential oil contains 90% santalol.

- The therapeutic properties of sandalwood oil are antiphlogistic, antiseptic, antispasmodic, astringent, carminative, diuretic, emollient, expectorant, sedative and tonic.

OIL OF CITRONELLA



CITRONELLA

- Citronella oil is an essential oil containing citronellal, geraniol and hydroxy citronellol and other high value perfumery bases obtained on steam distillation of citronella grass
- 5-6 harvests can be taken per year at 2 months intervals
- Oil yield is around 150 kg/acre/year

USES

- Insect/ Mosquito repellent
- Used in hair oils
- Used in preparation of soaps and brilliantines

ALKALOIDS

Dr.G.Neelamma
VCPS

CINCHONA:

- Cinchona is a dried bark obtained from the cultivated trees of ***Cinchona calisaya, Cinchona ledgeriana, Cinchona officinalis, Cinchona succirubra*** of the family Rubiaceae.
- Cinchona is called as *Jesuit's bark* as this bark was identified and used by the Jesuits for its anti-pyretic property. As it was first discovered in Peru it is also known as *Peruvian bark*.

Cultivation

- Propagation: either seeds or budding or layering, in WB only budding is used and in TN budding or layering methods are applied.
- sub-tropical/tropical climates, height of about 1000-3000 m. If growing below this height, found to have less % of quinine.
- Rainfall: Uniform, 250-380 cm in a year. Atmospheric temp.: 16-24 degree C
- Soil: drained forest soil and rich in organic matter, acidic soil having pH of 4.5. to 6, small amount of N favorable for growth.
- Specification: Needs slopping situation, high humidity and protection from wind.
- Seeds are very small and light in weight, about 1 gm seeds contain 3500 seeds so that seeds are admixed with soil during sowing Seeds immediately used for propagation , on storage they lose their viability Germination take place in 3-6 weeks.

- Seedlings with 2 pairs of leaves are transplanted and space of 6-10 cm is maintained in between 2 seedlings and 2 rows.Young seedlings are protected from direct sunlight. In forest soil, transplanted after 15 months of growth and before heavy rainfall. Distance of 2X2m is maintained between 2 plants.
- Collection: 4-20 yrs of age, plants, selected for harvesting, but the maximum alkaloid content is found in 6-10 yrs old plants. Bark is collected by coppicing method, vertical incisions are made on branches , trunk of tree and incisions are connected by horizontal circles, the bark is then stripped off. Preparation: Dried in sun light and further by artificial heat, drying is done below 80 degree C. During drying loses 70% of its wt. Avoid molding and fermentation during drying.



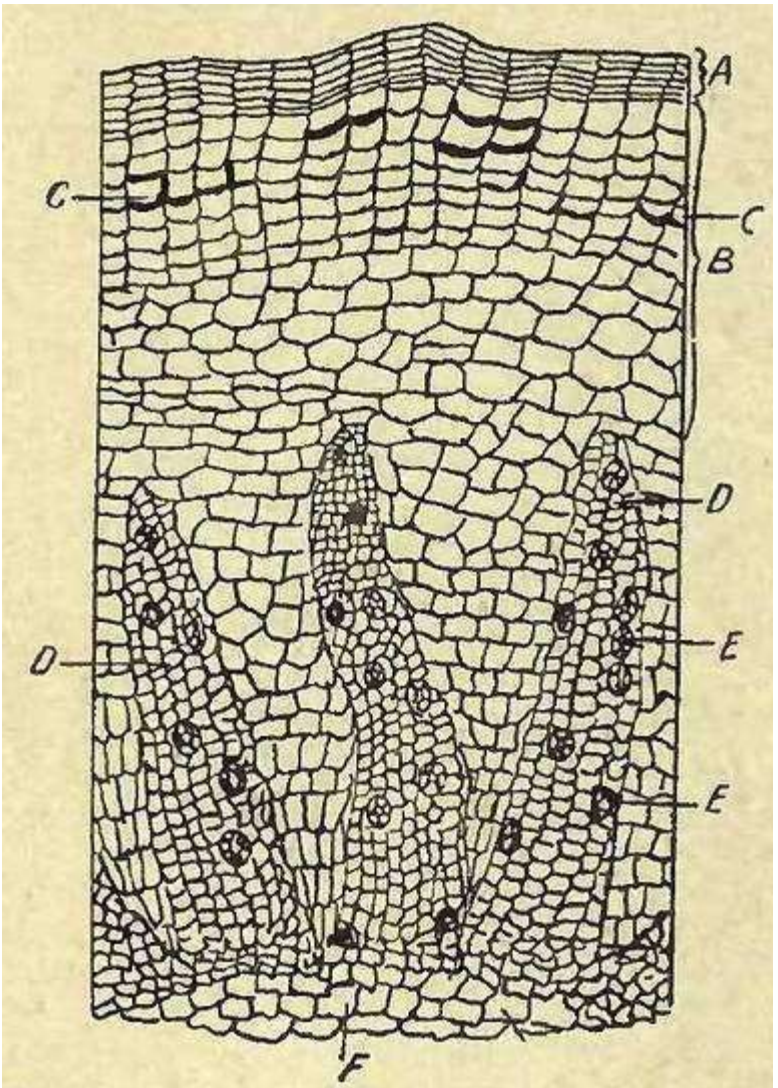


FIG. 232.—*Cinchona rubra*—Cross-section of bark. *A*. Cork cells. *B*. Cortical parenchyma. *C*. Coloring matter, deposits between and within the cells. *D*. Medullary ray. (Black line from *D* should not extend beyond the first two rows of cells of the figure.) *E*. Bast fibers.

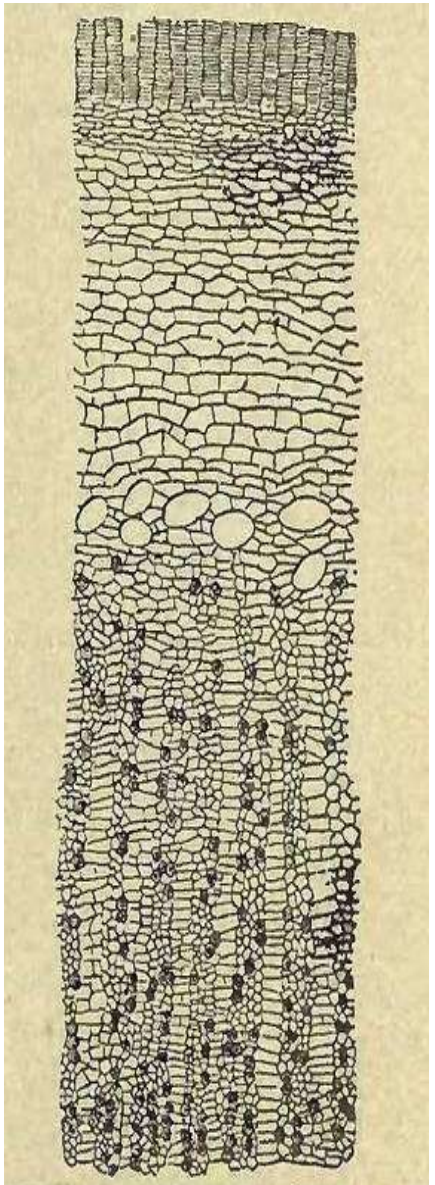


FIG. 230.—Cross-section of *Cinchona calisaya* bark (var. Micrantha).

Morphology

- Odor: slight and characteristic odor
- Taste: astringent and bitter Form : quills and curved pieces. Stem Bark 1. Length about 30 cm
- 2.Thickness about 2-6 cm
- Root bark Length about 2-7 cm
- 3. Outer surface is dull brown grey or Inner and outer surfaces are similar in grey color, color inner surface is pale yellowish-brown to deep reddish in color.
- 4. Presence of mosses and lichens no mosses and lichens Bark is rough and has transverse fissures Bark is scaly and shows depression.
- Microscopy: 1.Cork 2.Phellogen 3.Phelloderm 4.Cortex 5.Starch grains 6.Secretory cels 7.Medullary rays 8.Lignified Phloem fibres 9.Secondary Phloem

- **Extraction of Quinine**
- The bark is powdered and extracted with benzene or toulene in the presence of alkali and are extracted with dilute sulphuric acid. When it is made neutral, quinine sulphate separates as it is sparingly soluble. Cinchona bark has slight, characteristic odour and astringent and intensely bitter taste. It is available in the form of quills and curved pieces.

Chemical constituents of Cinchona:

- Cinchona bark consists of alkaloids which belong to quinoline group.
- quinine, quinidine, cinchonine and cinchonidine, quinicine, cinchonicine hydroquinine, hydrocinchonidine and homocinchonidine.
- Quinidine is also obtained commercially from cuprea bark, Ramijia pendunculata belonging to the family Rubiaceae.
- Quinine and quinidine form many salts, among their salts, sulphates are more significant.
- Quinine and quinidine are stereoisomers of each other.
- Cinchonine and cinchonidine are also isomers of each other.

Chemical Tests:

- 1.Powdered cinchona is slightly moistened with glacial acetic acid & heated in an ignition tube, purple vapours are produced at the upper part of tube.
- 2.Thalleoquin test: Powdered drug gives emrald green color with bromine water and dil. Ammonia solution.
- 3.Quinidine solution gives a white ppt. with silver nitrate solution, which is soluble in nitric acid ,

- Cinchona also contains
  - quinic acid
  - cinchotannic acid
  - a glycoside called quinovin,
  - tannins and
  - bitter essential oil.
- Quinine occurs as white crystals and in dark it shows fluorescent properties.
- It gives blue fluorescence in ultra-violet light.

- **Uses of Cinchona :**

✓ Cinchona bark is ***antimalaria***l in nature

✓ Cinchona preparations like cinchona extract, compound cinchona tincture are used as bitter ***stomachics*** and ***antipyretics***

✓ Quinine and its salts are used in the treatment of ***malaria***.

✓ Quinine is a protoplasmic poison for protozoa like *Plasmodium vivax*, *P.falciparum*, *P.malarie*, *P.fatal*. Hence, used as a powerful antimalarial drug

✓ Quinine is active against *Trypanosoma cruzi* epimastigotes.

✓ It has cardiac ***depressant*** activities.

✓ Used for the prevention of arrhythmias and tachycardia.

✓ Used for preventing atrial fibrillation.

Substituents:

- Cuprea Bark (Remijia pedunculata)
- Cuprea Bark (Remijia purdiena)
- C. Colombia

Isoquinoline alkaloids-Ipecac

- B.S: Dried roots and rhizomes of Cephalis ipecacuanha, family Rubiaceae.

- G.S: C.ipecacuanha is called Rio or Brazil ipecac, obtained from Brazil, Myanmar, Malaysia. C.acuminata called Panama ipecac, obtained from Columbia, Panama, India.

HISTORY

- 1817- Pelletier and Magnedi first isolated emetine in crude form.
- 1894- Paul and Cownley separated emetine in pure form.
- 1917- Pyman isolated emetamine, o-methyl psychotrine.

CULTIVATION & COLLECTION

- Propagation done by sowing seeds in mid January to February.
- Germination improved by treatment of seeds with lime water.
- 2months old seedlings are transplanted at spacing of 10x10cm.
- Temperature is 23-38C.
- Rainfall 300cm needed.
- Harvesting of roots done after three years.

DESCRIPTION

- **Brazilian characters:**
- **(a) Root:**
- Colour - Dark brick red to dark brown
- Odour - Faint
- Taste - Bitter
- Size - Up to 150 mm in length and 6 mm in thickness
- Shape - Roots are found in tortuous pieces.

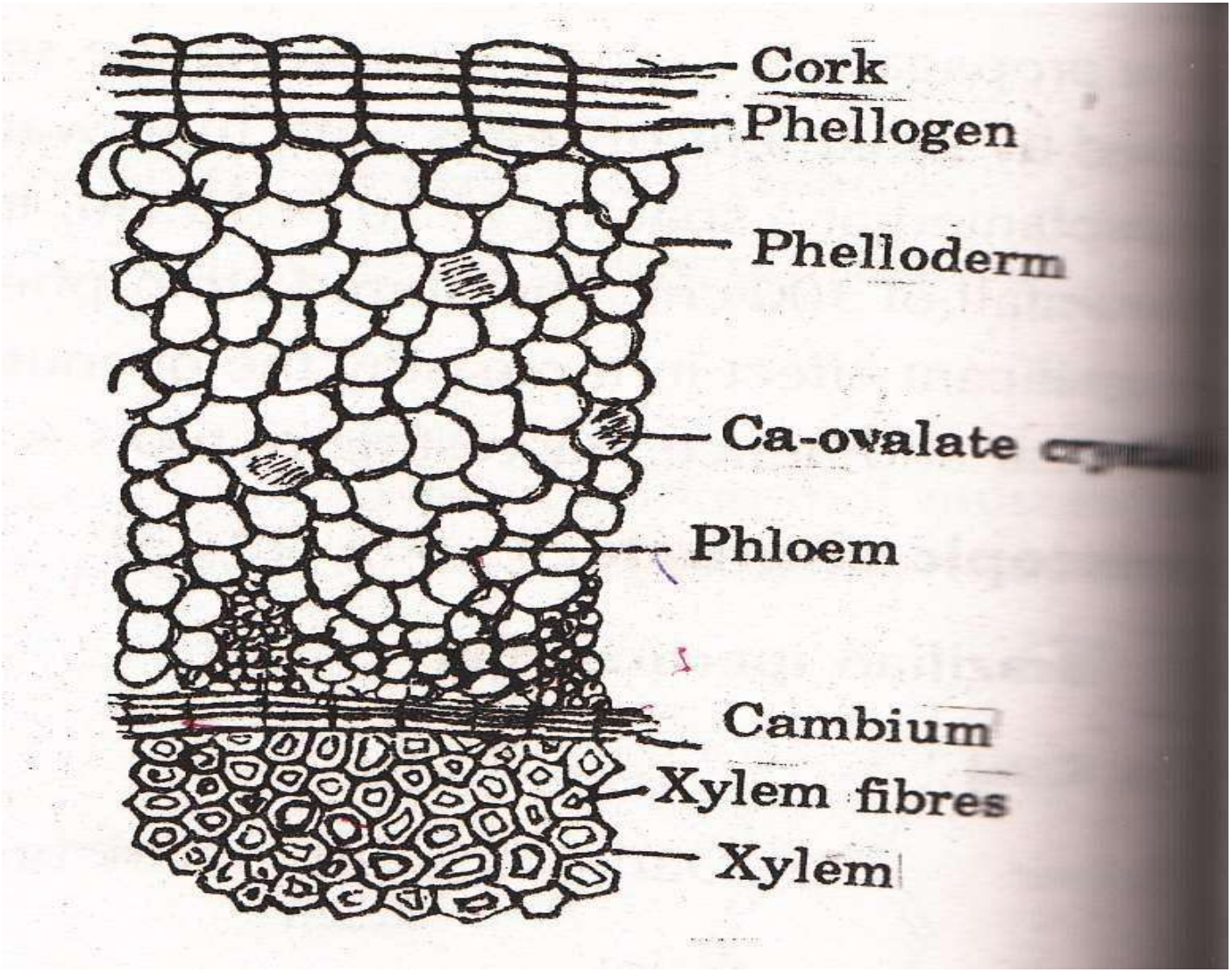
- **Rhizomes:**
- Color - Brick red to dark brown.
- Size - About 2 mm in diameter and short, attached to the roots
- Shape - Cylindrical.

Panama ipecacuanha

- Colour - Greyish-brown to reddish brown
- Odour and taste - Same as in Brazilian variety
- Size - upto 9 mm in thickness.
- Shape - Cylindrical

MICROSCOPY

- **Roots:**
- presence of cork layer with brown contents, which is followed by phelloderm composed of thin walled parenchyma .
- It is full of starch grains and acicular crystals of calcium oxalate.
- Xylem consists of tracheids and small vessel. Secondary medullary ray with starch gains and Size is upto 15 microns.

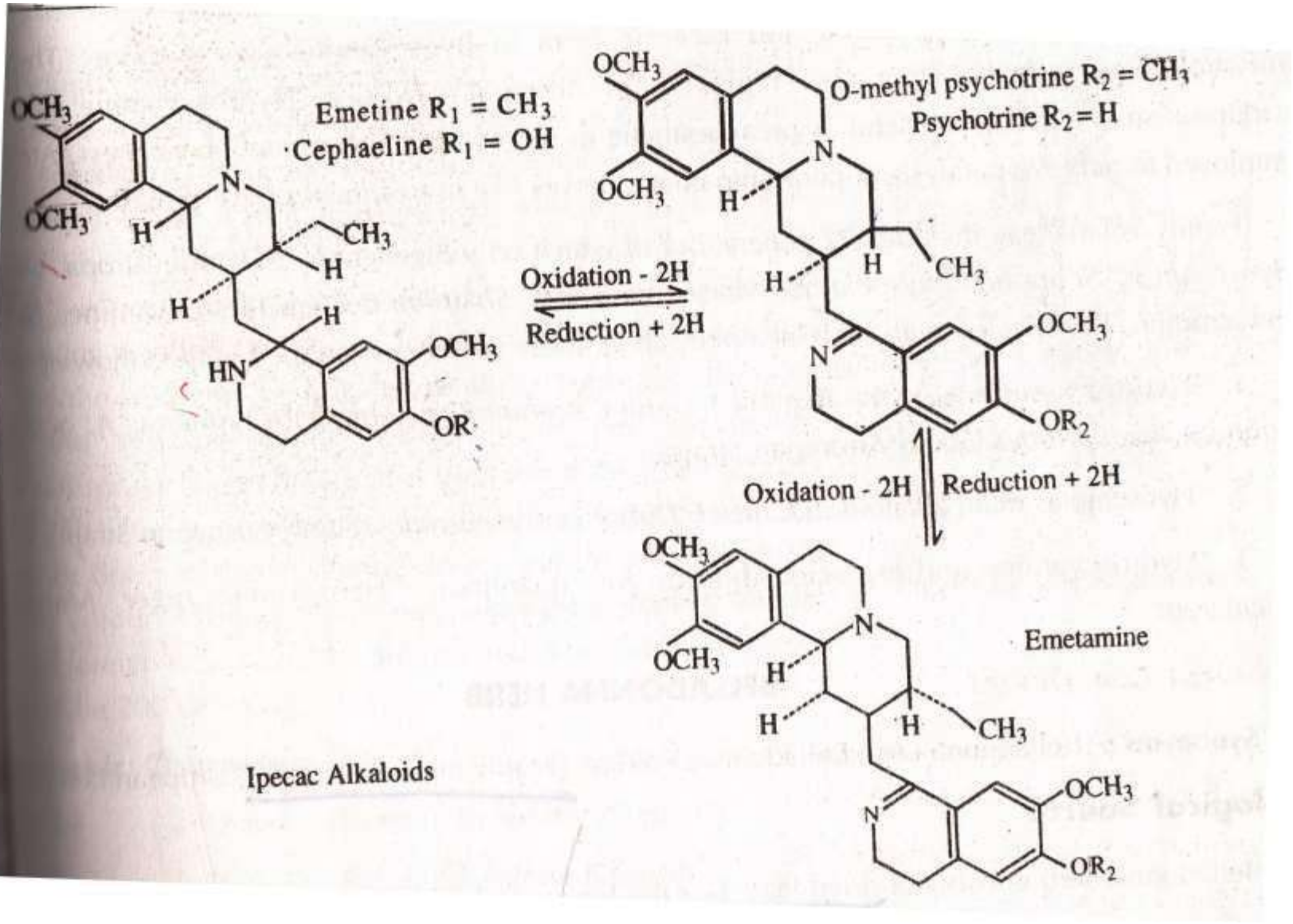

Cork
Phellogen
Phellodern
Ca-ovalate
Phloem
Cambium
Xylem fibres
Xylem

Contd..

- **Rhizomes:** Pericycle is with thick walled scleried, protoxlyem and spiral vessels. Columbinan variety differs only in one aspect i.e.The starch gains are large and upto 22 microns.

CHEMICAL CONSTITUENT

- Isoquinoline alkaloids which belong to phenolic and non phenolic groups.
- The total alkaloids in Rio-ipecac are upto 2%, and in Panama ipecac, and emetamine.
- The portion of emetine and cephaeline in different varieties is 4:1 (Rio-ipecas), minor bases like phychotrine, emetamine and o-methyl psychotrine


Emetine $R_1 = CH_3$
Cephaeline $R_1 = OH$
O-methyl psychotrine $R_2 = CH_3$
Psychotrine $R_2 = H$
Oxidation - 2H
Reduction + 2H
Oxidation - 2H
Reduction + 2H
Emetamine
Ipecac Alkaloids

- Cephaline is conveted into emetine by methylation of phenolic C (6) hydroxyl group.
- Non phenolic alkaloidal group includes emetine and o-methyl psychotrine, while phenolic alkaloidal group includes cephaeline and psychotrine. Ipecac also cotaines ipecauanhic acid, glycoside ipecacuanhin.

CHEMICAL TESTS

- 2.5 g powdered dug + 20 ml hydrochloric acid + 5 ml ether. Shake well and filter.To the filtrate, add 0.5 g Potassium Chlorate.The presence of yellow colur gradually changing to red, after standing, due to emetine.
- The addition of Sulphuric acid and Sodium Molybdate (Frohde's reagent) to small quantity of emeting gives bright green colour.

USES

- Ipecacuanha is expectorant in small doses and and emetic in higer doses. Cephaeline has more emetic and less expectorant action as compared to emetine.
- Emetine hydrochloride is used as antiprotozoal, as it is highly toxic to amoeba i.e. Entamoeba histolytica, Hence, it is used by administering parentally, in treatement of amoebic dysentery.
- Ipecacuanha is used for isolated of emetine and cephaline.
- emeting has anti-tumour properties.

Adulterants and Substitutes

Opium

- **Raw opium**: is the air dried latex obtained by the incisions from the unripe capsules of ***Papave r s o m nife rum*** Papaveraceae.

- India, Pakisthan, Afganisthan,Turkey, Russia, China, Iran.

Overview

- **Opiates** stneutitsnoc fo sevitavired ro stneutitsnoc era .muipo ni dnuof
- Opiates are narcotic analgesic.
- Narcotic refers to the pain-relieving and sleep-inducing properties of these highly-addictive alkaloids, including morphine, codeine and thebaine .
- Morphine is named after Morpheus the god of sleep, while (**somniferum** .nitaL ni "peels ot" snaem)

History:

- First cultivated in Mediterranean regions and brought by Alexander in 327 B.C to India.
- 1803 Narcotine was first isolated by Derosne.
- 1804 morphine isolated by Segnin.
- 1818 first introduced in medical practice by Magendi and Bally.
- 1833 Codiene isolated by Robiquet.
- 1848 Merck company isolated Papverine.
- 1923 Morphine structure elucidation by Gulland and Robinson.

Cultivation, collection and Prepation:

- Poppy is an erect plant attaining 60-120 cm height. It is rarely branched. The leaves are linear, oblong or ovate oblong and have a dentate or serrate margin. It bars bluish white, purple or violet coloured large flowers. Accrodingly, the varienties P. sommniferum var. glabrum, P. someniferum var. album, P. somniferum as only legal source of opium to many countries including United States of America and Britain



- Opium poppy is grown from November to march. Propagation is done by sowing the seed, for wish 3-4 kg of seed per hectare are necessary.The seeds admixed with about 3-4 parts of sand are sown.
- Opium poppy requires, highly fertile, well drained loamy soil with fine sand.The soil should contain organic matter, nitrogen and should have a pH around 7.The distance between two plants maintained is usually 25 cm and the plant reaches maximum height of one meter.



- After sowing within 3-4months the plant bears flowers, which are converted to capsules within few days attain maturity after 15-20days. At maturity the capsule exudes maximum latex.Vertical incision given to capsule upto 2mm.The incision repeats for about 4times on same capsule with 2days interval.

- The average yield of opium is about dried in open areas and further the seeds , it is from 4-5 quintals per hectare .Opium is exported traditionally from India.

Description:

- **Indian Opium**: Dark brown in color . It is found in the form of cubical pieces weighing about 900 g for marketing purposes . It is enclosed in tissue paper and is brittle and plastic in nature. Internally it is homogenous . Depending upon the requirement/the powdered from is available in the pack of 5 to 10 kg

- **Persian Opium** : Dark brown in colour ,found in the form of brick shaped masses , weighing 50 g . It is hygroscopic in nature , granular or nearly smooth with brittle fracture

- **Natural Turkish or European opium:** Brown or dark brown in colour . it is found in conical or rounded and somewhat flattened masses, weighing 250 to 1000g. On keeping, it becomes hard and brittle. It is covered with poppy leaves.

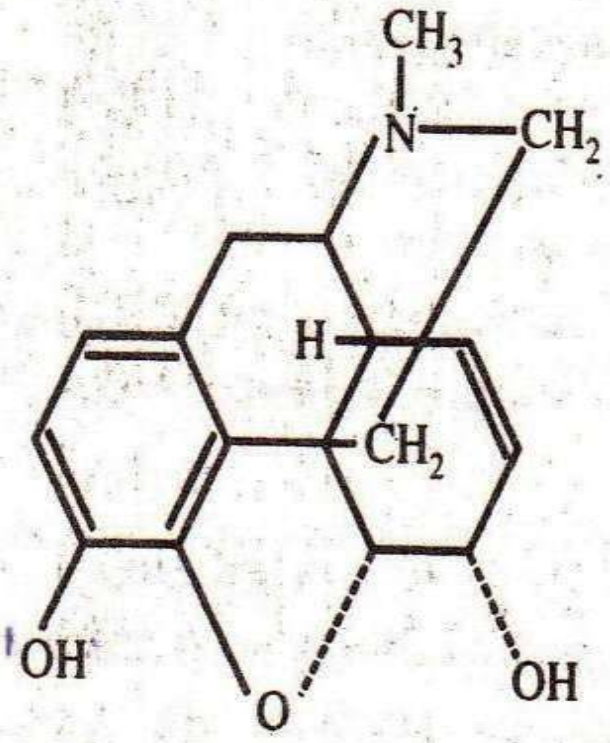
- **Manipulated Turkish opium :** It is chocolate brown or dark brown internally and covered with broken poppy leaves externally.The masses of this type are oval and flattend on upper and lower surface weighing about 200g .It is somewhat plastic or even brittle.

- **Manipulated European opium:** It is dark brown in colour internally and covered with broken leaves. It is found in the form of elongated masses with rounded ends weighing 150 to 500 g. It is firm, plastic and with brittle fracture.

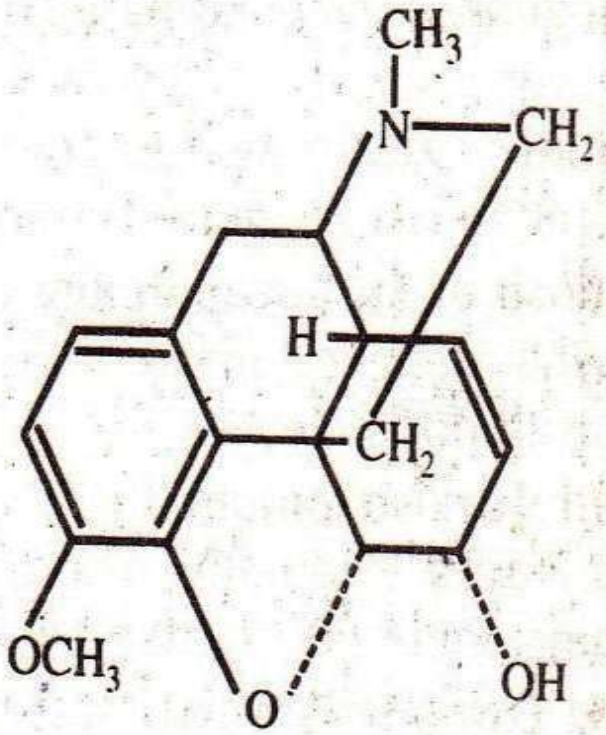
Chemical Constituents:

- The latex conations mainly the alkaloids derived from amino acids Phenylalanine and tyrosine. Chemically ,they are placed under bezylisoquinoline and phenantherene types.

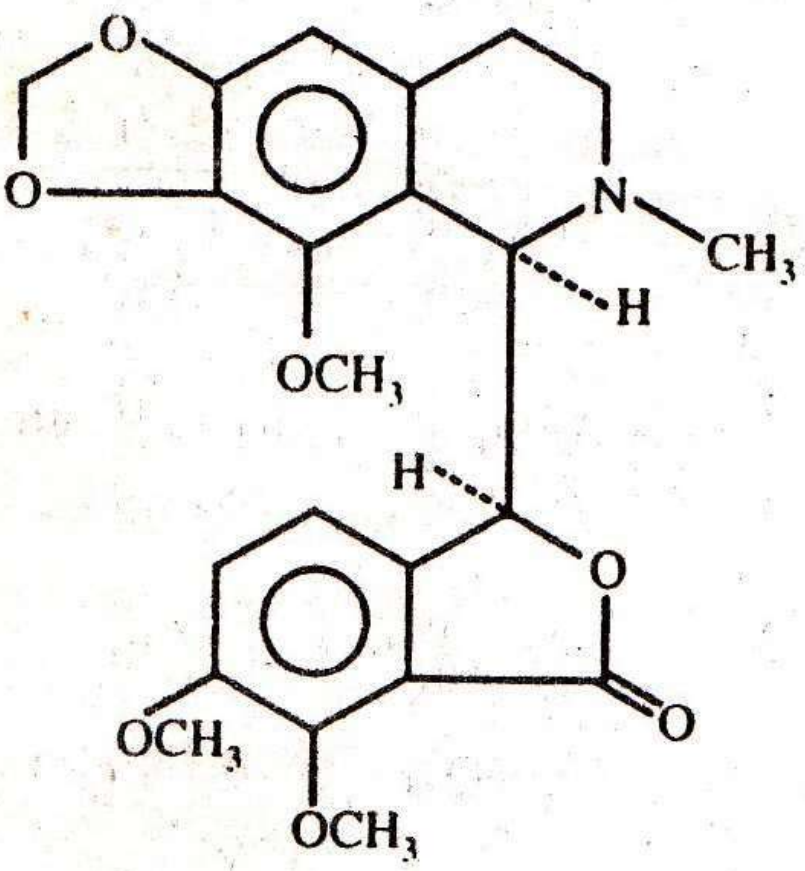
- Morphine is monoacidic ,laevorotatory phenolic alkaloid and also contains an alcoholic hydroxyl group at c(6) position.
- Codeine (methyl morphine) is a strong monoacidic base and inactive optically. It is it is soluble in water and organic solvent.
- Papaverine is a weak monoacidic base and inactive optically. it is slightly soluble in organic solvents, but insoluble in water
- The other important benzylisoquinoline alkaloid narcotine is a weak monoacidic base and is laeavorotatory, while its salt are dextrorotatory.



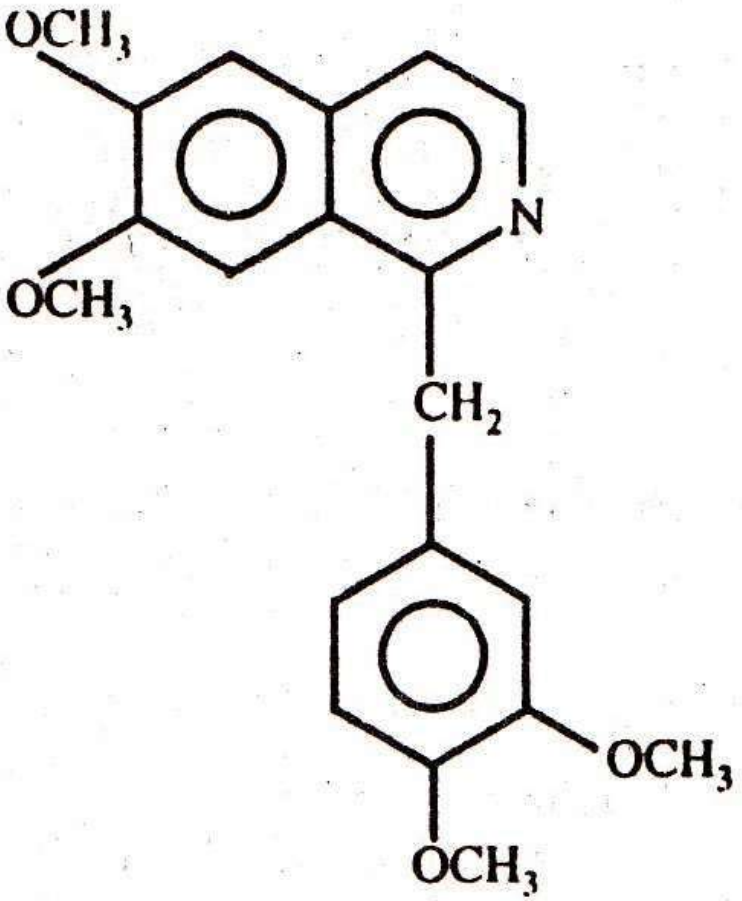
Morphine



Codeine



Narcotine



Papaverine

- The major biologically active alkaloids found in opium are morphine, codeine, thebaine, papaverine and noscapine.
- Papaverine and noscapine, have little to no effect on the human central nervous system, and are not usually considered to be narcotic or opiates, they have have a different mechanism of action.
- papaverine is smooth-muscle relaxant and noscapine is antitussive.

Classification

- **Natural opiates** muipo fo xetal eht ni deniatnoc sdiolakla era: .eniabeht dna ,eniedoc ,enihprom sa
- **Semi-synthetic opioids** ,setaipo larutan eht morf detaerc : morf devired era enodocordyh dna ,enodocyxo ,nioreh sa hcus .eniabeht dna ,eniedoc ,enihprom
- Heroin is diacetylmorphine.

Chemical tests :

- The general test to detect opium is testing presence of meconic acid .The alkaloids are present as the salts of meconic acid.

- (1) Opium is dissolved in water and to the filtrate, ferric chloride solution is added by which deep reddish purple colour is obtained, which persists even on addition of hydrochloric acid.

- **(2)** Morphine when sprinkled on nitric acid give orange red colour codeine does not respond to this test
- **(3)** The treatment of morphine solution with potassium ferricyanide and ferric chloride solutions gives bluish green colour . Codeine does not respond to this test.
- **(4)** Papavering solution in hydrochloric acid gives a lemon yellow colour with potassium ferricyanide solution.

Uses:

- Opium belongs to the category of hypnotic sedative and analgesic in which the action in mainly due to morphine.
- Morphine is a potent analgesic.
- In the medulla ,it sedates the respiratory, centre, emetic centre and the cough reflex.
- Codeine relieves local irritation in the bronchial tact and an antitussive used in various cough medicines.

- Papaverine has relaxant effects on smooth muscles of intestinal and bronchial tract and the blood vessels.
- Diacetyl morphine (heroin) has more narcotic analgesic property than morphine.

- **Fully synthetic opioids**.lodamart ,enodahtem ,enidihtep sa hcus :
- **Endogenous opioid** ,ydob eht ni yllarutan decudorp ,seditpep .snihpromodne dna ,snihpronyd ,snilahpekne ,snihprodne sa hcus

Pharmacological uses

- Opiates and Opioids bind to specific opioid receptors in the central nervous system and other tissues.

.

- They have long been used to treat acute pain such as post-operative pain .

- They have also been found to be valuable in palliative care (cancer, and degenerative conditions such as rheumatoid arthritis)

Commercial Varieties of Opium

- Indian Opium: It is bark brown in colour and found in from of cubical pieces weighing 900g. It is brittle and plastic in nature. The powdered form is available as 5 to 10 kg packs. It contains 10 anhydrous morphine.
- **Persian Opium:** It is dark brown in colour and available as brick shaped masses of 450g .it is hygroscopic, granular or smooth.
- **Turkish Opium:** it is commonly called as druggist's opium or opium. It brown or dark brown in color and available as conical rounded or flattened masses.
- **Chinese Opium:** it comes in market in the form of globular cakes and contains 4-11

Adulteration:

- The production of opium is under government control and hence, normally it is not found to be adulterated. The adulterated forms show presence of opium capsules in powdered from, gum and sugary fruits.

Allied Plants:

- The various other species of poppy ,which do not contain morphine are Papaver aremone, P. dubium, P.orientate, P.bracteatum, P.strigosum, P.intermedia, P.paeoniflorum, hybrid of P. somniferum and P.orientate, P. psendo orientale.

Opiate poisoning

- Opiate poisoning can range from discomfort like constipation to death from respiratory depression.

- Alcohol and other sedatives enhance the effect of opiates, especially respiratory depression.

Vinca

B. Source:

Catharanthus or Vinca is the dried whole plant of *Catharanthus roseus* G. Don (or *Vinca rosea* L), Fam. Apocynaceae.

G. Source: South Africa, India, U.S.A, Europe, Australia.

HISTORY : Known since B.C 50 in European countries as Antidysentric, Antihaemorrhagic, Diuretic, Woundhealing.

Cultivation collection preparation:

- Vinca grows all over india upto 500meters. Rainfall 100cm. Fresh seed are sown in nursery beds or sometimes direct sowing also done.
- 2.5 lag of seeds per hectare are required. When the plants are sufficiently grown up, they are thinned out.
- In february or march they are sown in nursery and transplanted to open fields at 45cm x 30cm distance and about 74,000 plants per hectare is necessary.
- Leaf stripping is done 6 and 9 months after sowing.


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- Stems are cut about 7-8cm above the ground level after one year of growth.
- Leaves, stems and seeds are separated and air dried.
- Seeds are collected from matured fruits for next propagation.
- The yield of dried roots, stems and leaves per hectare in irrigated land is 1 and 3 tonnes respectively.

Description:

C- leaves -> green,

roots-> pale grey

Flowers-> violet pink- white or carmine red

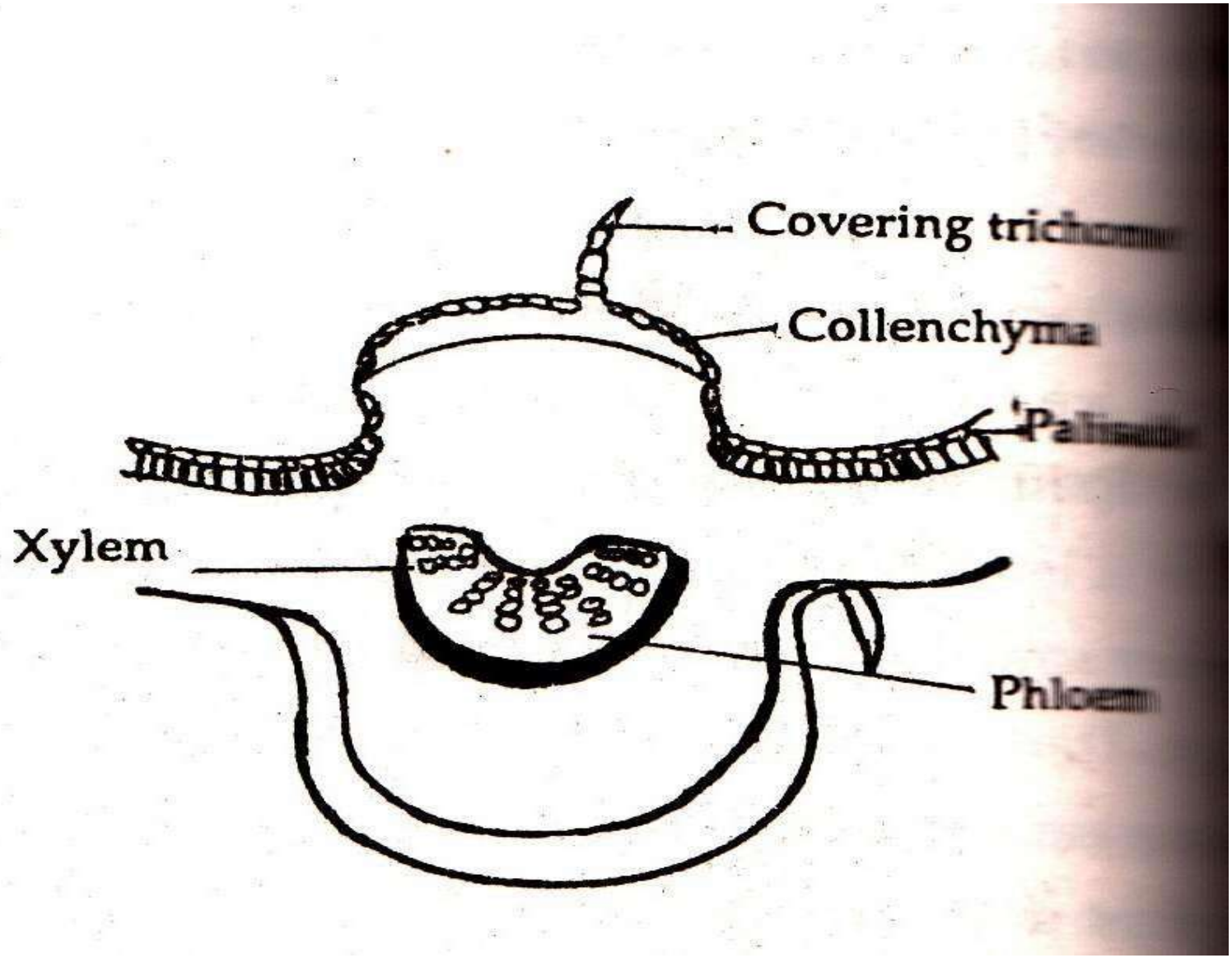
O- characteristic

T- bitter

Sh- vinca is erect, pubescent herb

Microscopic characters:

- Cruciferous stomata
- Upper surface shows presence of single layer of rectangular celled epidermis with unicellular covering trichomes.
- Spongy parenchyma is 5 to 8 layered with intercellular spaces.
- Midrib shows presence of collenchyma.
- Xylem and phloem are present.
- Calcium oxalate crystals absent.


Covering tric
Collenchyma
Xylem
Phlo

- **Chemical constituents & Classification:**

1- Monomeric Alkaloids:

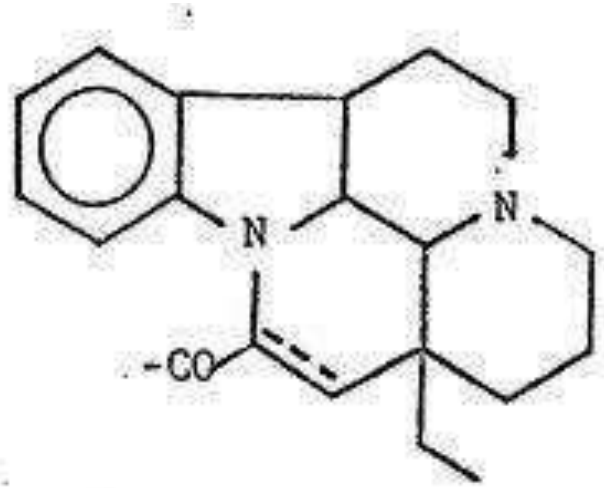
These are alkaloids that contain either indole or indoline:

- **Indole monomers e.g. Catharanthine**
- **Indoline monomers e.g.Vindoline and Vincamine.**

2- Dimeric Alkaloids:

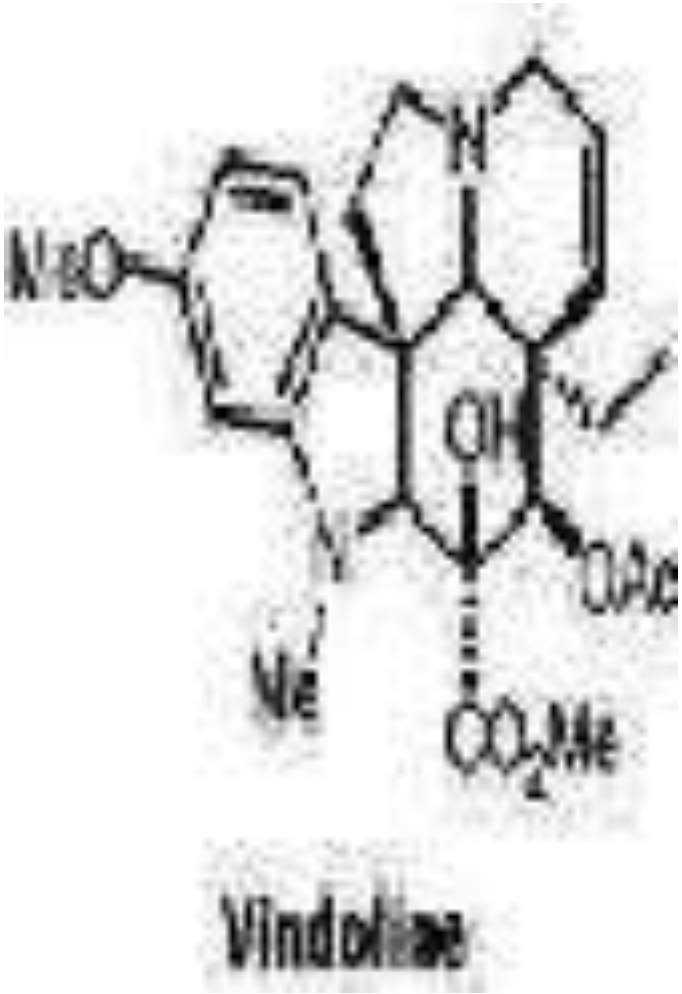
- **Homogenic dimmers: Composed of two indole or indoline monomers.**
- **Mixed dimmers: One indole and one indoline monomers e.g.Vincristine and Vinblastine.**

1- Monomeric Alkaloids:

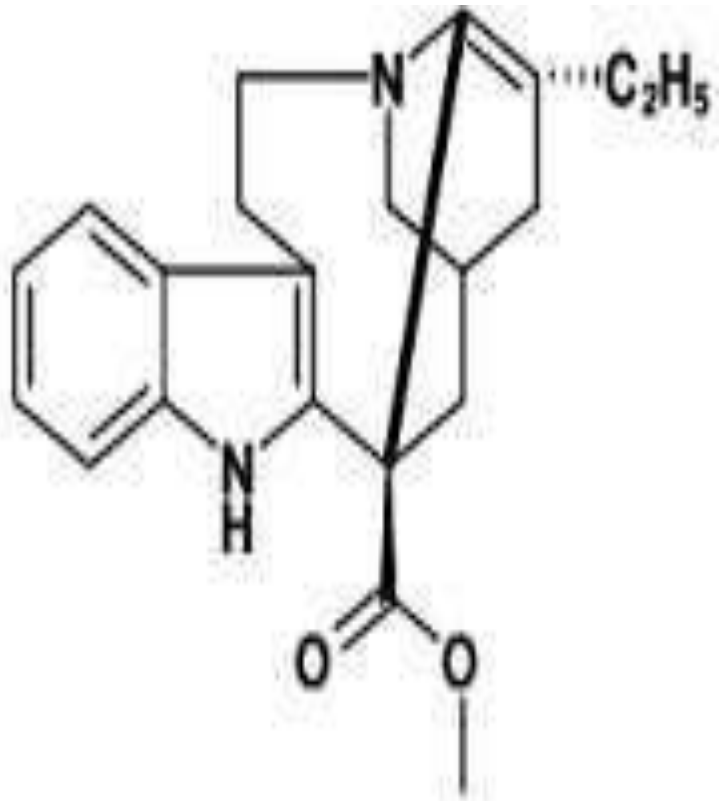


- **Vincamine**

  Enhances the cerebral blood flow, facilitate cerebral circulation metabolism and increase general activity. Vincamine is used in cerebral vascular deficiency and atherosclerosis in elderly patients.



Vindoline



Catharanthine

2- Dimeric Alkaloids:

Mixed dimmers

- ***These are dimeric alkaloids having indole and indoline (dihydro-indole) nuclei e.g.*** **Vinblastine and Vincristine**

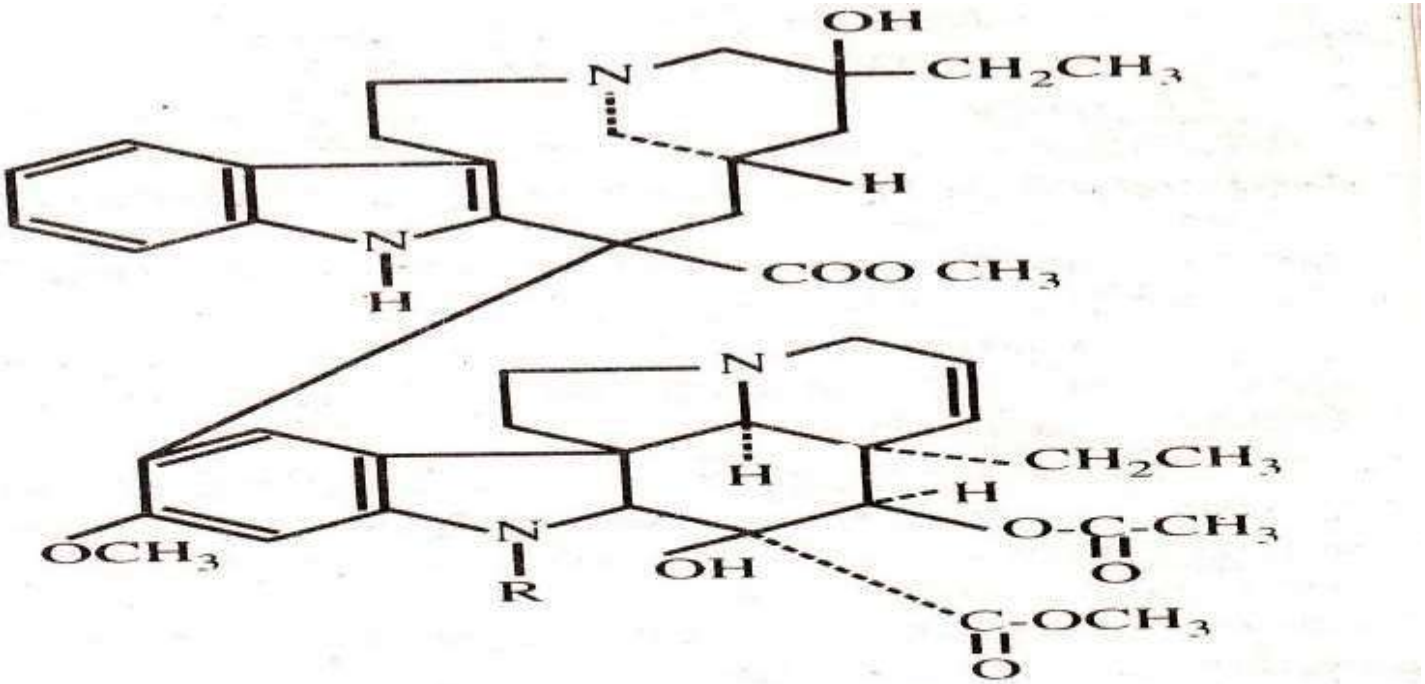
- **Vinblastine and Vincristine**
- **They occur in very minute amounts in *Vinca* (0.003- 0.005); 500 Kg of the plant yield only 1 gm of vincristine.**

- **They are very important for cancer treatment.**

- **Vincristine is more active but isolated in smaller amounts than Vinblastine.Vinblastine can be converted to vincristine chemically or by microbial transformation using *Streptomyces albogriseolu* .**

Vinblastine (Vincaleukoblastine) is produced by coupling of Catharanthine and Vindoline.

Vincristine (leurocristine) has CHO instead of CH_3 in the vindoline part of Vinblastine.



Vinblastine R = CH_3

Vincristine R = CHO

- **Tests for identification:**

- **1-Vanillin /HCl reagent** gives with:

  Vinblastine a **pink** color.

  Vincristine an **orange-yellow** color**.**

- **2-Van-Urk's reagent:**

  → **Reddish-brown color**.

- **Uses :**
- **Vinblastine is used for treatment of Hodgkin's disease (Pseudoleukemia or Lymphatic anaemia) and carcinoma resistant to other therapy.**

- **Vincristine has a cytotoxic effect .It is useful in the treatment of leukemia in children, small cell lung cancer, cervical and vaginal cancers.**

- **Mechanism:**
- Both alkaloids are Antimetabolites interfere with the syntheses of Desoxyribonucleic acids.

Indole Alkaloids

Ergot Alkaloids

Ergot is the dried **sclerotium** of a **fungus**, *Cl aviceps purpurea* (Fam. Claviceptiaceae) that arise on the ovaries of the **rye plant** (*Se ca le ce re a le* , Fam. Gramineae).

- Switzerland, hungary, czech republic.

History:

- “Argot” french word.
- Middle ages toxic effects called st. Anthony’s fire.

- Ergotism is due to consuming cereals contaminated with the fungus.
- **Ergotism**.gninosiop togre mret-gnol fo tceffe eht si
- More recently by the action of a number of ergoline-based drugs.
- 1836 Ergot introduced in London Pharmacopoeia.
- History of *Cla vic e ps purpu re a* first coined by Tulsane in 1853.

Ergotism, historical overview

- In middle ages, ergotism is known to have occurred on a large scale in some regions of Europe.
- In 857, for example, there was an epidemic in Germany characterized by necrosis of limbs and culminating in death.
- In 944, some 40,000 people in the south of France died from ergot poisoning.
- Between 837 and 1347, some fifty epidemics were recorded in Central Europe.

Ergotism, historical overview (cont.)

- In 1670, a French physician, Dr Thuillier, suggested that food could play a role in this disorder.
- Later, others showed that when ergot was fed to animals, they died.
- All the victims had eaten rye bread.
- In August 1951, in France more than a hundred people were poisoned and several died as a result.
- During the 1st half of the twenty century an intoxication have been reported in Egypt and was owed to ergot-contaminated wheat flour.
-

Collection and preparation:

- Rye plant is the host and ergot is a parasite. 600 plants from different families of wild and cultivated plants act as host for ergot fungus. Other species of ergot are C. microcephala, C. nigricans, C. paspali
- Scleroital stage or a dormant stage contains the maximum amount of drug.

Life cycle

- An ergot fo terolf fo yravo eht nehw spoleved *muitoreles* eht fo seiceps lagnuf a fo serops yb detcefni si laerec ro ssarg suneg*Claviceps* .

- The proliferating fungus destroys the plant ovary and connects with the vascular tissue of the plant .

- The first stage of ergot infection is a white soft tissue (known as *s pha ce lia s ta ge* wedyenoh yragus gnicudorp) eht fo tuo spord netfo hcihw ,)ecnatsbus ykcits hcir-ragus(.seiravo detcefni
- This honeydew contains millions of spores which are dispersed to other florets by insects .
- Later, the sphacelia convert into a hard dry **sclerotium** .seiravo eht edisni
- At this stage, alkaloids (mycotoxins) accumulate in the sclerotium .

- When a mature sclerotium drops to the ground, the fungus remains dormant until proper conditions (onset of spring, rain period, climate etc).
- It germinates, forming one or several fruiting bodies with head and stalk.
- In the head, spores are formed, which are ejected simultaneously (at the same time).

Ergot fungus on wheat





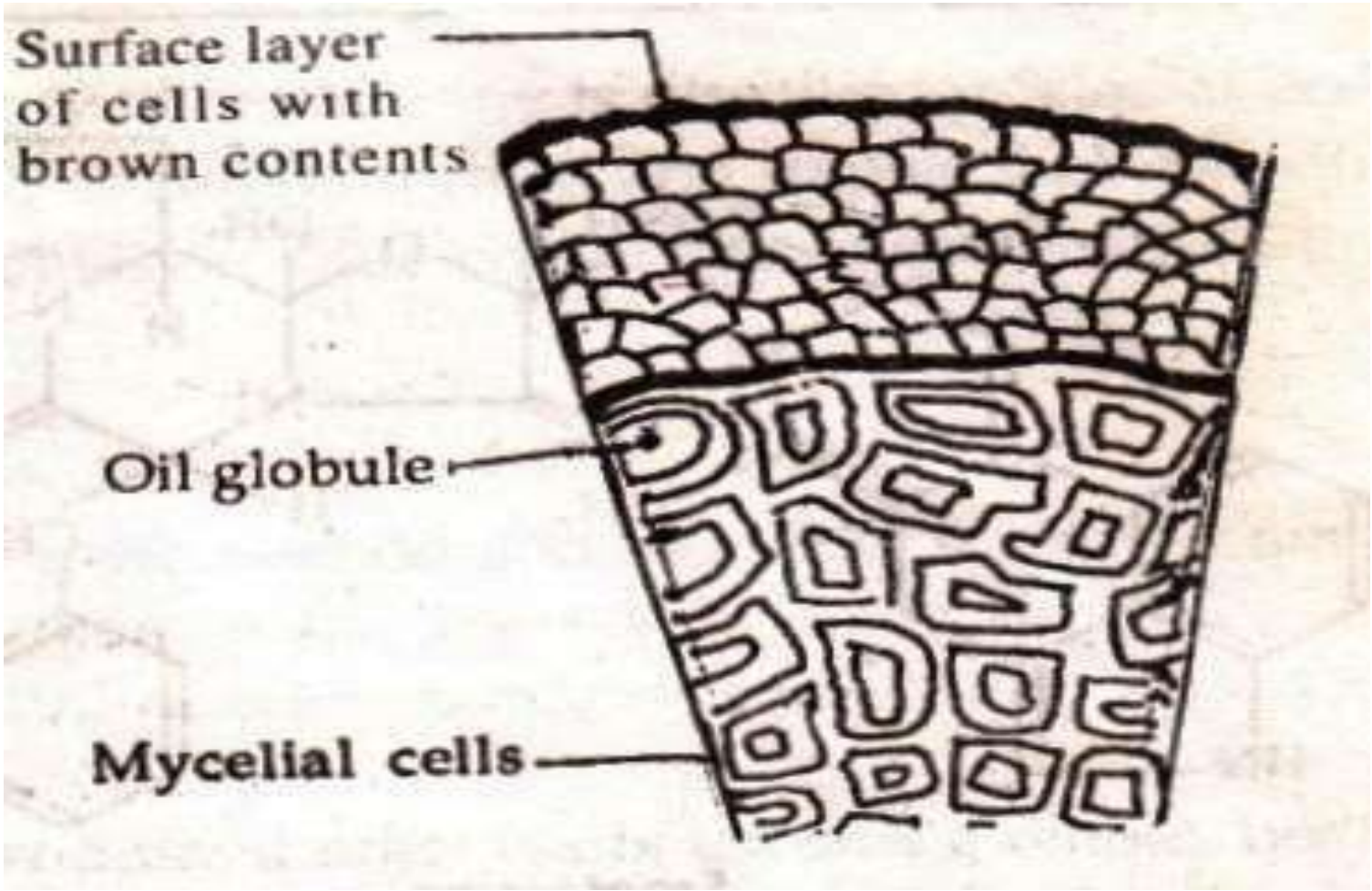
Macroscopy:

- Color- externally dark violet to black, internally pinkish white.
- Odour- disagreeable and faint.
- Taste- unpleasant.
- Size- sclerotia are 1-3cm in length 1-5mm in width.
- Shape- sclerotia are fusiform, triangular.

Microscopy:

- Outermost layer of the sclerotium is made up of few thin, flattened, polygonal cells of puple to dark brown color.
- Inner part is made up of dense pseudo parenchymatous cells composed of chitin.
- Mycelial cells are round or oval, thick with high refractive walls.
- Fixed oils.
- Sclerotium does not contain starch, calcium oxalate or any of the lignified tissue.

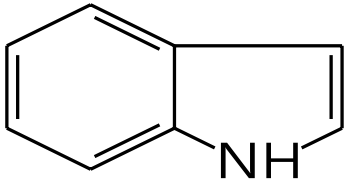
T.S. of Sclerotium of Ergot:



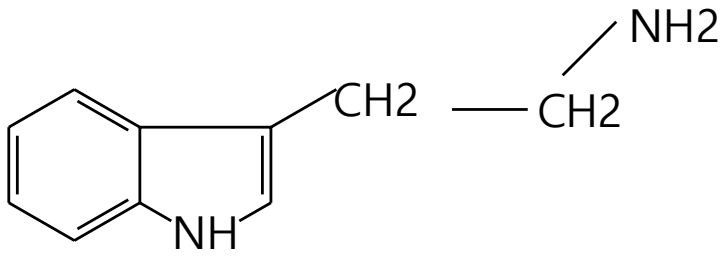
Chemical constituents:

- They are medicinally important indole alkaloids (0.1-0.25%) which are derivatives of lysergic acid.
- Biogenetically they are derived from the amino acid tryptophan.

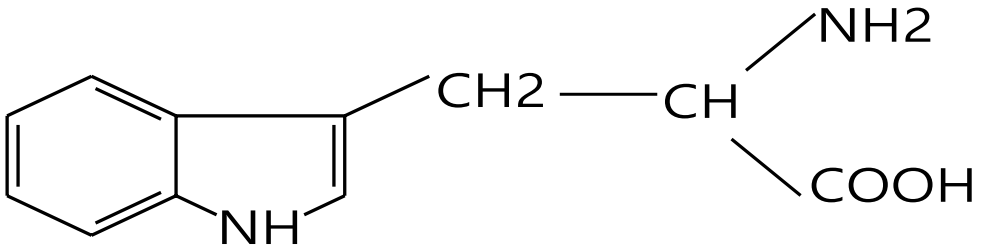
- Indole



- Tryptamine

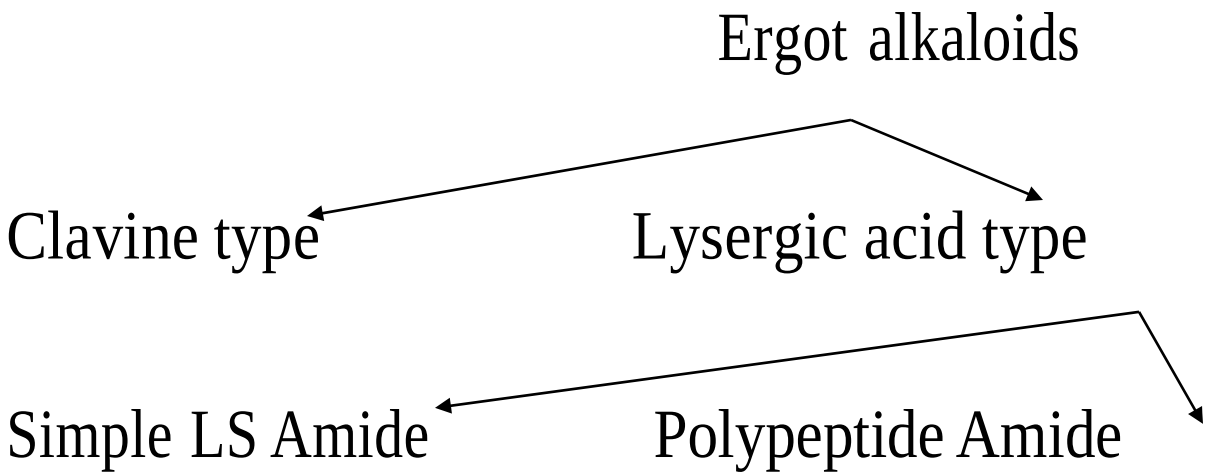


- Tryptophan



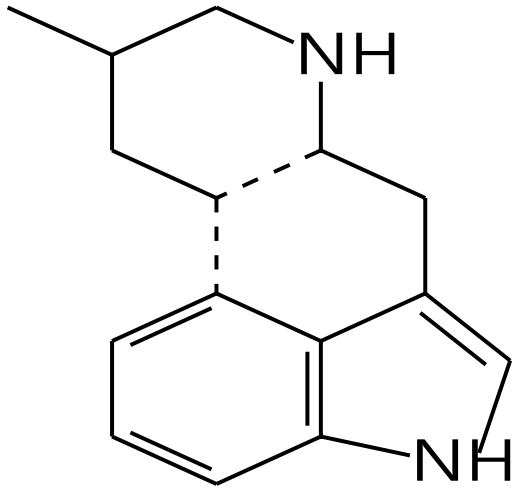
Classification

- There are two main groups of ergot alkaloids :
- A-The clavine type .
- B-The lysergic acid derivatives type.


Ergot alkaloids
Clavine type
Lysergic acid type
Simple LS Amide
Polypeptide Amide

Ergot alkaloids

- The clavine type alkaloids as well as the lysergic acid derivative alkaloids are product of condensation of tryptophan (or tryptamine) with isoprenyl moiety (5 Carbons)
- Ergot alkaloids

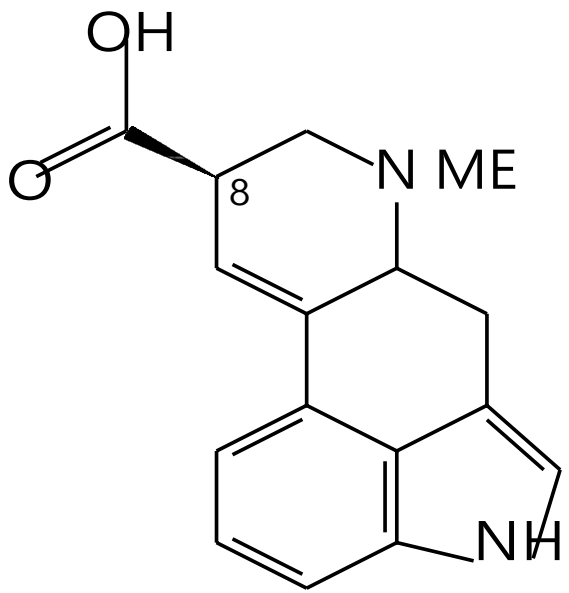


A-The clavine type alkaloids

- Alkaloids of this class all end with the suffix (-clavine) e.g. agroclavine, elemoclavine…..
- They are all simple water soluble bases.
- Not physiologically active as the lysergic acid derivatives.
- Recently few examples show significant oxytocic activity.
- Agroclavine is a powerful uterine stimulant.

B-The lysergic acid derivatives
i-The simple lysergic acid amides

- All are water soluble alkaloids.
- Low molecular weight.
- All are amides of lysergic acid with simple amines.



General Characters:

- Ergot alkaloids are N-monosubstituted amide derivatives of both lysergic acid and its isomer isolysergic acid that differ only in configuration at C-8.
- On treatment with **ammonia lysergic** and **isolysergic acids** give the corresponding amides **ergine** and **isoergine** respectively.

Lysergic acid

Isolysergic acid

Ergine

- Members related to **lysergic acid** (e.g. **ergotamine** and **ergometrine**) are **levorotatory**, **more active** and designated by suffix **"ine"**.
- Members related to **isolysergic acid** (e.g. **ergotaminine** and **ergometrinine)**, are **dextrorotatory, less active** and designated by suffix **"inine"**.

- The simplest naturally existing is ergine (lysergic acid amide, LSA) and erginine (isolysergic acid amide).
- Ergine is a psychoactive component, it is known as natural LSD.
- The semithynthetic LSD is more potent than ergine as a psychedelics drug.
- Psychedelics are part of a wider class of psychoactive drugs known as hallucinogens .

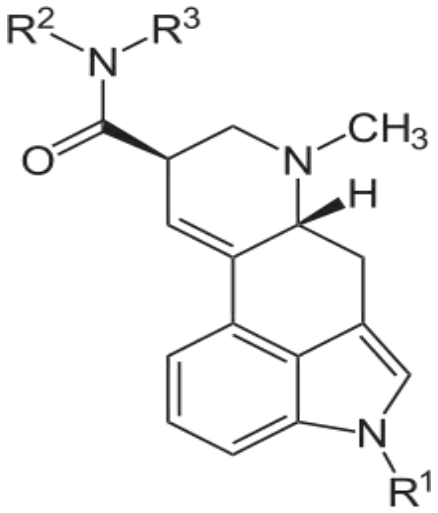
i-The simple lysergic acid amides (cont.)

- The most important members of this group is the potent oxytocic alkaloid ergometrine.
- Ergometrine is the amide of lysergic acid with 2-amino propanol.
- And the famous hallucinogen (LSD) lysergic acid diethylamide.
- LSD is a semisynthetic drug with a very potent action on the CNS in extremely small doses.

LSD

- LSD was first semi-synthesized by Albert Hofmann in 1938 from ergot .
- The short form LSD comes from its early code name *LSD*--eruäS gresyL" namreG eht rof noitaiverbba na si hcihw ,*25* .rebmun laitneuqes a yb dewollof "dimalyhteid
- It is commonly semi-synthesised by:
- reacting diethylamine with lysergic acid .
- or from ergine (lysergic acid amide).

General structure of lysergic acid amides



Name	R1	R2	R3
Ergine	H	H	H
Ergometrine	H	CH(CH3)CH2OH	H
Methergine	H	CH(CH2CH3)CH2OH	H
Methysergide	CH3	CH(CH2CH3)CH2OH	H
LSD	H	CH2-CH3	CH2-CH3

(–) Laevorotatory alkaloids		(+) Dextrorotatory alkaloids
Ergometrine	Water-Soluble	Ergometrinine
Ergotamine	Water insoluble	Ergotaminine
Ergosine		Ergosinine
Ergocristine		Ergocristinine
Ergocryptine		Ergocryptinine
Ergocornine		Ergocorninine

Ergotamine

- **Characters:**
  - Its (d) isomer is called Ergotaminine.
  - The peptide moiety is composed of 3 amino acids:

    α-Hydroxyalanine

    Proline

    Phenylalanine

- **Uses:**

  Treatment of migraine as it constricts the peripheral blood vessels.

  Has some oxytocic (ecobolic) activity.

Ergometrine

- Is a potent oxytocic alkaloid.
- Contraction of uterus.
- Facilitate labour.
- Prevent postpartum hemorrhage.

Chemical tests:

- 1) Ergot powder + p-dimethylaminobenzaldehyde ---- → blue.
- 2) Ergot + solvent ether + sulphuric acid→ Filtrate obtained shows red violet color in its aqueous layer, when treated with saturated sol of sodium bicarbonate.
- 3) Ergometrine gives blue fluorescence in water.

Uses:

- Ergot alkaloids have a wide range of biological activities.
- 1- Ergotamine is used in the treatment of migraine.
- It has the ability to constrict dilated blood vessels in the cerebral membranes.
- Hypertension is a side effect.
- 2- Ergometrine is used during childbirth to enhance labour pains in delivery cases. –after expulsion of the placenta– to stimulate uterine contractions and to prevent postpartum hemorrhage.

Rauwolfia

- Synonyms: Rauwolfia root, Serpentina root, Chhotachand. Source: Dried roots of Rauwolfia serpentina
- Family: Apocynaceae. It contains NLT 0.15% of reserpine and ajmalcine.
- GS: Tropical regions of Asia, America and Africa. Commercially, In India, Sri lanka, Myanmar,Thailand and America. In India UP, Bihar, Orissa, Tamil Nadu,West Bengal, Karnataka, Maharashtra and Gujarat.

Cultivation

- Climate: wide range of, best suited humid, shade
- Soil: wide range of with large amount of sand makes plants more susceptible for disease Clay: loamy soil,
- pH: acidic up to 4 Temperature: 10-38 degree C,
- Rainfall: 250-500 cm
- Propagation: seeds, roots, cutting, root stumps Initially seeds are sown into the nursery beds.
- Sowing period: May or breakdown of monsoon Implanting seedling: August, at a distance of 16-30 cm Various fertilizer, manures: ammonium sulphate, urea, bone meal. Collection: when plants are 3-4 year old. Uprooting, roots are cut properly, washed as to remove earthy matter & dried in air.

Morphology

- Color: bark: greyish yellow,Wood: pale yellow Odor: odorless Taste: bitter Size: 10-18 cm long; 1-3 cm in diameter Shape: sub-cylindrical, slightly tapering & tortuous
- Microscopy:
- Cork: striated Phelloderm (few layers of parenchyma) Narrow phloem: small scattered sieve tubes Secondary phloem: Calcium oxalate crystals Xylem:Vessels, tracheids, wood parenchyma, wood parenchyma & wood fibre

Constituents:

- About 30 indole alkaloids; range from 0.7-3% on GS
- Mostly in bark, root Indole, Indoline, Indolenine ,Oxyindole, Pseudo indoxyl alkaloids Reserpine Oleo-resin, phytosterol, fatty acid, alcohol, sugar .
- Ajmalicine, ajmaline, rauwolfinine, rescinnamine, reserpinine, yohimbine, serpentine, serpentinine Reserpine: ester of trimethoxy benzoic acid Rescinnamine: ester of trimethoxycinnamic acid
- Chemical test :With conc. HNO_3 →red coloration of medullary rays
- Reserpine: with vanillin in acetic acid → violet red color alkaloids: powdered rauwolfia + H_2SO_4 + p-dimethyl amino benzaldehyde →violet-red color

- USES: Reserpine: lowers BP by depleting store of catecholamine at nerve ending, prevent re-uptake of nor-epinephrine at storage sites, allowing enzymatic destruction of neuronal transmitter. Neuropsychiatric disorder Rescinnamine: anti hypertensive but Higher dose □ depression Dereserpine: anti hypertensive & tranquiliser Ajmalicine: treatment of circulatory diseases, relief of obstruction of normal cerebral blood flow. Syrosingopine: same as above but milder
- Allied Species: Rauwolfia vomitoria R. obscura R. Rosea

Nux-Vomica:

- Synonyms: Crow fig, Semen Strychni
- Source: Dried ripe seeds of Strychnous nuxvomica Linn.
- Family: Loganiaceae.
- Contain NLT 1.2 % of total alkaloids calculated as strychinine.
- GS: East India, Sri lanka, Northern Australia, and India. Abundantly in South India i.e.Tamil nadu, Kerala and on Malabar coast. Also available in Bihar, Orrisa, Konkan, Mysore and Gorakhpur.

Collection & Preparation

- Found throughout the tropical area, 1300 m above sea level. -Plant height: 10-12 m, crooked trunk, several branches. -Leaves are orange, oppositely arrange, oval shape, entire margin and acute apex. -Flowers are greenish white and the bark is greyish to yellow. -Fruit are orange yellow, consists a berry about the size of a small orange. -When ripe it has a hard orange-yellow pericarp and a white, pulpy interior – containing 4-5 seeds & Seeds are washed to remove pulp and dried. -Unripen seeds are separated by the floating test in water. -Seeds are dried on mat and packed in gunny bags for marketing. -Collection of fruit and seeds is carried out from NovemberFebruary. -Exported in small sacks known as pockets.

- Morphology: Hilum, ridge on testa and micropyle Color: greenish brown Taste: bitter
- Microscopic Characters: Lignified Trichomes, layer of collapsed cells and endosperm
- Chemical Constituents: 1.5-5% bitter Indole alkaloids, Strychnine and Brucine -Vomicine, α-colubrine, pseudostrychnine, Isostrychnine, N-oxystrychnine, protostrychnine, β-colubrine and novacine. Glycosides: loganin, chlorogenic acid and fixed oil
- Chemical test s:Thin sections of nux vomica seeds are defatted and following test are performed. 1. Stain the TS of nux vomica with conc. HNO3 → endospermic cells take yellow color due to presence of brucine.
- 2. Stain the TS of nux vomica with ammoium vandate & H2SO4 → Manddin's reagent →endospermic cells become purple due to strychine
- 3. Strychnine + H2SO4 + Potassium dichromate → violet color red → finally yellow

- Properties: Brucine is the dimethoxy derivative of Strychnine. Both alkaloids contains 2 Nitrogen atoms. Hemitoxiferine is a degradation product of strychnine. Dimerization of hemitoxiferine produces a valuable skeletal muscle relaxant Toxiferine
- USE -Bitter stomachic, tonic, CNS stimulant, increases BP, certain forms of cardiac failure, stimulant for respiratory & CVS -Brucine: very less physiological action, 1/6 th potency as compared to strychine, 4 times bitter than strychine. Brucine: denaturinng alcohol, inedible fats, dog poison.
- Allied specids: Strychnos nuxblanda, S. potatorum

Introduction to Pharmacognosy

P
K.Umamaheswari

Pharmacognosy

- Pharmacognosy is derived from two Greek words, *Pharmakon* & *Gnosis*
- **Pharmakon** – means **'drug'.**
- **Gnosis** – means **'knowledge'.**
- In brief, Pharmacognosy means **'knowledge of drugs'.**

➢ It is a study of drugs that originate in the **plant & animal kingdoms.**

Pharmacognosy is a branch of pharmacy which deals with the basic resources of medicines from nature (plant & animal) and their uses as medicaments from ancient time to present day.

Pharmacognosy

Pharmacognosy may be defined as an important branch of Pharmacy which deals with the study of structural, physical, chemical, biochemical and sensory characters of natural drugs of plant and animal origin. It also includes a study of their history, distribution, cultivation, collection, identification, preparation, evaluation, preservation, use and commerce.

- **At present pharmacognosy involves not only the crude drugs but also their natural derivatives (pure compounds or constituents).**
- **Digitalis leaf and its isolated glycoside, digitoxin; Rauwolfia root and its purified alkaloid, reserpine; and thyroid gland with its extracted hormone, thyroxine, are all part of the subject matter of pharmacognosy.**

Pharmacognosy - History

History of pharmacognosy is actually the history of medicine that is medicinal plants.

Pre-history:

- The first or beginning of pre-history on use of medicinal plants or herbs or animals, and the place where and how used were not well known, and those information were unwritten for a long time. As a result, the pre-history on herbs was almost lost.
- However, some information was recorded by oral transmission from generation to generation.

Pharmacognosy - History

Written History:

- The written history has originated which was based on region, religion and culture etc.

The written history was divided into the following:

1. The western medicine
2. The Unani (Islam)
3. The Ayurveda (Indian)
4. The orient
5. The Greek History
6. The African System

1. The western medicine:

- ✓ This is originated in Mesopotamia and Egypt. Mesopotamia is considered as the first origin of human civilization. The Sumerians (peoples of ancient Mesopotamia) developed cuneiform tablet of herbal medicines. Those tablets is preserved in British museum.
- ✓ In Egypt, information had written on paper – *Papyrus ebers* (1600BC). It consisted of 800 prescriptions, mentioning 700 drugs.
- ✓ The first pharmacopoeia named London Pharmacopoeia was published in 1618 and then British Pharmacopoeia was published in 1864.

2. The Unani (Islam)

- This herbal system was developed by Arabian Muslim Ibn Sina (980 – 1037 AD). He was a prince and ruler. He was a very brilliant pharmacist and physician who wrote a book – "Kitab-Al-Shifa", means 'Book of Healing'.
- The book was written on Arabic language. This is a great contribution of Ibn Sina on medical and pharmaceutical sciences.

3. The Ayurveda (Indian, 2500-600 BC):

Ayurveda is the term for traditional medicine of ancient India.

The word "Ayur" means 'Life' and "veda" means 'The study of' that is "Study of Life".

The Ayurvedic writings were divided into three systems:

1) Charaka Samhita, 2) Sushruta Samhita and 3) Astanga samhita.

The oldest writing was Charaka Samhita (six to seven century before Christ).

The book describes uses of many metallic drugs eg., iron, mercury, sulphur, cupper etc with

4. The orient (2700 BC):

- This is originated from Chinese, Japanese and Tibet etc. The orient herbalism was very old (142 – 220 BC) and called “Kampo”. The written documents were made by the King ‘Shen Nung’ (2700 BC) and Shang (1766 – 1122 BC) etc.
- Shen Nung investigated medicinal value of several herbs and written a book – “Pen T-Sao” or native herbal.

5. The Greek History:

Some of the early naturalists, scientists and physicians who contributed enormously to the development of human knowledge about medicinal plants include following:

- **Hippocrates (Father of Medicine, 460-370 BC):**

He was the first natural doctor who utilized simple remedies such as vinegar, honey, herbs etc in healing. He is also known to have collected and identified a number of medicinal plants.

- **Aristotle (384-322 BC):**

He gave the philosophy of medicine. He listed more than 500 plants of medicinal importance.

- **Theophrastus (340 BC):**

gave scientific basis of use of plants as medicine.

- **Galen (131-200 AD):**

a Greek pharmacist-physician. He developed the methods of preparing and compounding medicines by mechanical means. He was the originator of the formulae for a cold cream.

6. The African System (Tropical Africa, North and South America):

- They keep information in their groups or tribes. The information transmitted from one generation to another.
- These regions are richest sources of medicinal plants and needs to explore for new drug discovery.

Scope of Pharmacognosy

Pharmacognosy deals primarily with information on the sources and constituents of natural drugs.

1. Primary source of medicines (from ancient time to present day), for example – hyoscine, morphine, ergotamine, ouabain etc.

2. Providing Template/guide for the discovery of new drugs, for example – Pathidine (analgesic drug) designed from morphine.

Terms & Phrase used in Pharmacognosy

Drug

Standard dictionaries define drug as "an original, simple medicinal substance, organic or inorganic, used by itself or as an ingredient in medicine".

According to WHO's definition – "any substance used in a pharmaceutical product that is intended to modify or explore physiological systems or pathological states for the benefit of the recipient".

Pharmaceutical product/Medicine means "a dosage form (tablet/capsule/syrup/IV/IM) containing one or more drugs along with other substances included during the manufacturing process".

Application of Drug

- **Prevention of a disease : for example, vaccine.**
- **Fight against an infection: for example, antibiotics.**
- **Temporary blocking of a normal function: for example, general & local anesthetics**
- **Detoxification of the body: for example, antidotes.**
- **Diagnostic agents: for example, radioisotopes.**
- **Correction of dysfunction: for example, cardiotonics (eg, digoxin) for the treatment of congestive heart failure.**
- **Correction of hyperfuntion: for example, Rauwolfia root (eg., reserpine) for the treatment of hypertension.**

Crude Drug:

The term "Crude", as used in relation to natural products. A crude drug is a natural drug of plant or animal origin which has undergone no treatment other than collection and drying, that is, the quality or appearance of the drug has not been advanced in value or improved in condition by any physical or chemical treatment.

For example: Digitalis leaf, Rauwolfia root etc.

Indigenous vs Naturalized

Indigenous :

Plants growing in their native countries are said to be indigenous to those regions.

For example: Aconite (*Aconitum napellus)* in the mountainous region of Europe. It is highly poisonous used as diuretic in Homeopathy preparation

Naturalized:

Plants are said to be naturalized when they grow in a foreign land or in a locality other than their native homes.

For example: Datura (*Datura stramonium)* which was introduced into the USA from Europe.

Official Books

It provides guideline for the manufacturing, quality control, packaging, storage, dose regimen, indication, contraindication etc of different pharmaceutical products/medicine for the treatment of diseases.

- **USP – United States Pharmacopoeia**
- **BP – British Pharmacopoeia**
- **NF – National Formulary**
- **BNF – British National Formulary**
- **BDNF – Bangladesh National Formulary**
- **BPC – British Pharmaceutical Codex**
- **Martin Dale Extra Pharmacopoeia**
- **Materia Medica**
- **Physician's Index**
- **Goodman's & Gilmann's: The Pharmacological Basis of Therapeutics.**

Official vs Unofficial vs Nonofficial drug

Official drug:

Any drug (crude or prepared) which is included in pharmacopoea or in national formulary or in recognized books is called an 'official drug'.

For example: quinine, morphine, codeine, paracetamol are included in BP, USP, NF and so on.

Unofficial drug:

A drug which has been recognized earlier in the pharmacopoeia or in national formulary or in recognized books but not found in the current issue is designated as an 'unofficial drug'. Those substances were excluded from the recognized books due to their severe toxic effects on humans. **For example:** Sucralfate (hyperacidity), mercurial compounds (diuretics), benzoic acid (preservative) etc.

Official vs Unofficial vs Nonofficial drug

Nonofficial drug

Substance that has never been appeared in either of the official books may be called nonoffical. Such types of compounds may be published in current journals having proven clinical value, but we do not know about their side effects.

For example - Curcumine (sinusitis), etc.

Extractive

The crude mixtures of chemical constituents that are removed from plants or animals by various extraction processes are called extractives or derivatives.

Secondery metabolites

These are substances synthesized or produced as by-products by plants during their metabolic activities. Apparently they are of no primary use of plants.

They are therefore also regarded as ‘waste products of metabolism, which are usually accumulated in some parts of the plant and are physiologically active on living organisms.

Monograph

The descriptive material pertaining to any drug, therapeutic agent included in the pharmacopoeia is known as the monogram.

The monogram of a drug includes the following information on the drug:

official title, synonyms, definition, description, collection or preparation, identity tests, tests for adulterants, method of assay, storage, uses and doses.

Monogram of
Trigonella foenum-graecum (Family: Papilionacacae)

Common name: Fenugreek (Eng.), Methi (Bengali)

Cultivation: It is cultivated in different area of Bangladesh, India, Pakistan, Middle East, Africa.

Description: The Latin species name *foenum-graecum* means 'Greek hay', which is a dried plant's (leaves or seeds) and has a strong hay-like scent. It has a strong, pleasant and a peculiar odor and grows best in well-drained soils with a low rainfall into brownish- yellow rhombic shape seeds.

Parts used: Ripe, dried seeds

Chemical constituents: Seeds are rich in mucilage (maily galactomannans), fixed oil, fatty acids and protein. They also contain alkaloids, saponine, glycosides etc.

Uses: Seeds are diuretics, astringent. They are popularly used in the treatment of loss of appetite, weight loss, menstrual disorder. Also used in hypertension, diabetes, sexual problem etc. It lowers TG, cholesterol, LDL. **Dose:** Daily dose, 18 g.

Pharmacognosy & Modern Medicine

- Simultaneous advancement in the field of chemistry, biochemistry, biosynthesis and pharmacology has developed pharmacognosy.
  - Various active compounds have been isolated from plants which are used in modern medicine.
- With the advancement of synthetic organic chemistry most of the active constituents of plants have been synthesized.
  - More than 100 plants are used in modern medicine in various parts of the word.

Important active constituents of plants used in medicine

Active constituents	Plants	Pharmacological activity
Morphine, Codeine, Papaverine	*Papaver somniferum* L.	Sedative, smooth muscle relaxant
Quinine, Quinidine	*Cinchona* sp.	Antimalarial, antiarrthythmic
Hyoscine, Atropine	*Datura* sp.,	Parasympatholytic
Digitoxin, Digoxin	*Digitalis lanata*	Cardiotonic
Reserpine, Rescinamine	*Rauwolfia* sp.	Hypotensive, vasodialator
Vincristine, Vinblastin	*Catharanthus roseus*	Anticancer

Important active constituents of plants used in medicine

Active constituents	Plants	Pharmacological activity
Caffeine	*Camellia sinenssis*	CNS stimulant
Cocaine	*Erythroxylum coca*	Anaesthetic
Ephedrine	*Ephedra* sp.	Sympathomimetic
Pilocarpine	*Pilocarpus jaborandi*	Parasympathomimetic
Ergometrine Ergotamine Ergotoxine	*Claviceps purpurea*	Oxytocic Vasoconstrictor Vasodialator

Important active constituents of plants used in medicine

Active constituents	Plants	Pharmacological activity
Psyllium mucilage	*Plantago ovata*	Laxative
Sennosides	*Cassia angustifolia*	Laxative
Theophylline	*Caffea arabica*	CNS stimulant Diuretic
Steroid hormones	*Solanum* sp.	Anti-inflammatory, antiarthriytic

Application of Pharmacognosy

- Plants always provide novel molecular structure, which played important role for the molecular design and development of novel potent, less toxic or nontoxic drugs natural morphine has got analgesic & narcotic effect but meperidine a synthetic drug originates from morphine basic skeleton is non-narcotic.

- Plant & animal products give potential biological effect without any undesirable effects.

- Plant & animal products can be taken with very

Limitation of Pharmacognosy

Drugs obtained from natural sources have a variety of limitations. The majors are;

- **A natural compound may be highly active but usually this is associated with high toxicity problem, ex- the toxic and therapeutic dose of digitalis are very close which create serious problem to use this medicine by the patient alone.**
- **Some compounds are found in natures that give beneficial pharmacological actions but their potencies are too low to be employed therapeutically.**
- **The yield of active natural products may be very low and thus the production cost of those drugs will be very high**
- **The source of a natural drug may be very limited with respect to geography, season and climate etc.**

Sources of Medicaments

- At present the prime sources of modern or allopathic medicines are **synthesized** in the laboratory by organic chemist.
- Only a few of compounds are isolated from plants, which are cannot be replaced by synthetic method.
- **For example: morphine** isolated from *Papaver somniferum;*

vincristin & vinblastin (anticancer drug) from *Vinca rosea*,

the most potent anticancer drug **taxol** from *Texus brevifolia;*

the constituents of **digitalis** cannot be properly replaced by any synthetic drug like **quinidine** which is used for the treatment of arrhythmia.

Classification of Drugs

In pharmacognosy, drugs may be classified according to -

1. Their morphology
2. Thetaxonomy of the plants and animals from which they are obtained
3. Their therapeutic application
4. Their chemical constituents or active principles.

Each of these methods of classification has advantages and disadvantages.

1. Morphological classification

- In this system, the drugs are grouped according to the part of the plants, such as roots, leaves, stems, barks, flowers, seeds etc.
- The drugs obtained from the direct parts of the plants are called as organized drugs. They are made up of whole plants or any parts derived from them.

eg., Root – Rauwolfia, aconite, ginger; Bark – Cinnamon, Cinchona Fruit – Amla, Bahera, Capsicum, Leaf – Digitalis, Senna, Tulsi, coca; Wood – Sandal wood.

- The drugs which are prepared from plants by some intermediate physical process such as incision, drying or extraction with a solvent are called unorganized drugs, eg., Dried juice (Aloe juice), Dried extract (agar), Dried latex (Opium latex), Honey, Beewax etc.

The main drawback of morphological classification is that there is no co-relation of chemical constituents with the therapeutic actions.

2. Taxonomical classification

- In this system crude drugs are arranged according to the natural groups (e.g. Families) of their source.
- For example: all the drugs obtained from Solanaceae are grouped together as Solanaceous drugs.
- Tropane alkaloids are the main constituents of Solanaceous drugs.

Family	Drugs
Solanaceae	Solanaceous drugs. eg. Tropane alkaloids
Umbelliferae	Umbelliferous drugs. eg., volatile oils

3. Pharmacological classification

- In pharmacological classification the drugs are grouped according to their therapeutic use.
- For example cardiotonic drug include digitalis, purgative drugs include castrol oil.

Pharmacological action	Drugs
Anticancer	Vinca
Analgesic	Opium, cannabis
Purgatives	Senna, Aloe, Castrol oil, Plantago husk
Cardiotonic	Digitalis, strophanthus
Tranquillizer	Rauwolfia root
Anti-inflammatory	Tumeric, colchicum

The main drawback of this classification is that a drug can be placed in various classes according to its therapeutic use. For example: Cinchona (quinine) can be grouped in antimalarial and antiarrhythmic catagories.

4. Chemical classification

- The biological activity of a drug is due to the presence of certain chemical constituents in the drug.
- Plants and animals synthesize chemical compounds such as carbohydrates, protein, fat, volatile oils, alkaloids, resin etc.

•The chemical classification of drugs is dependent upon the grouping of drugs with identical chemical constituents.

Chemical classification of drugs

Chemical constituents	Drugs
1. Carbohydrates	
a) Monosaccharidase	- Dextrose, fructose, galactose
b) Disaccharide	-Sucrose, Lactose, Maltose
c) Polysaccharide Gum	-Starch
Mucilages Cellulose	-Acacia, Tragacanth
	-Plantago seed
	-Cotton
2. Glycosides	
a) Cardiac	-Digitalis, strophanthus
b) Anthraquinone	-Aloe, Cascara, senna
c) Saponins	-Arjuna
d) Cyanophore	-Wild cherry bark
3. Tanins	Amla, Bohera, Ashoka bark
4. Volatile oil	Clove oil, rose oil, peppermint oil, tulsi etc
5. Lipids	
a) Fixed oils & fats	-Olive oil, castor oil, coconut oil etc
b) Waxes	-Bees wax

Chemical classification of drugs

Chemical constituents	Drugs
6. Resins	ginger, capsicum etc.
7. Alkaloids a) Pyridine & piperidien b) Tropane c) Quinoline d) Isoquinoline e) Indole f) Steroidal g) Purine	 a) Nicotiana, areca nut b) Coca, Belladonna, Datura c) Cinchona d) Opium, Ipecac e) Ergot, Nuxvomica, Rauwolfia, catharanthus, Physostigma f) Kurchi g) Tea, Coffee
8. Protein	Gelatin, gluten etc
9. Vitamins	Thiamine (B1), Riboflavin (B2), Ascorbic acid etc
10. Antibiotics	Penicillin, streptomycin, tetracycline etc
11. Hormones	Adrenaline, thyroxine etc

Evaluation of Drugs

- A natural substance is considered as food if it fills stomach in every day life without any harmful effect.
- A substance become drug if it change a pathological or disease state of human/animal to normal physiological condition having no undesirable effect in specific dose.
- A long-term studies (chemical, biological and physical etc) are required to establish whether a substance will be considered as drug or food or eliminate for consumption. Those studies are referred as evaluation.

Evaluation of drug means –

- Identification
- Determination of quality
- Determination of purity

Identification

- ✓ **The identification can be established by careful observational study of the collected drug, and then compared with authentic specimen by the collector.**
- ✓ **Therefore, for proper identification of a drug from plant or animal sources, a collector must be educated about plant taxonomy and very much experienced with his/her job.**
- ✓ **Therefore, drugs from plants/animals are identified by –**
  - o **A qualified, specialized & experienced personnel**
  - o **Comparison with the authentic sample specimen.**
- ✓ **In every country, there is a national herbarium where most of plants specimen are preserved. A number of specialists are working on plant identification there.**

Quality

- The word "quality" refers to the intrinsic value of the drug, i.e., the amount of medicinal principles or active constituents present. These principles are classified as carbohydrate, alkaloid, glycoside, volatile oil, lipid, antibiotics and steroids etc.
- A high grade of quality in a drug is of primary importance. An effort should be made to obtain and maintain high quality.
- To maintain high quality products one should do the following:

1. Select proper source (wild or cultivated)
2. Appropriate time of collection
3. Collection of required parts of plants (bark, leaf, stem, rhizome, root)
4. Preparation of the collected drug by proper cleaning, drying.
5. Proper preservation to avoid contamination by microorganisms and moisture, heat, air and light.

Purity

- **The purity of drug can be achieved by –**

1. **Proper identification**
2. **Quality assurance.**

Evaluation Method

- **The evaluation of a drug involves a number of methods, which may be classified as follows:**

1. **Organoleptic**
2. **Microscopic**
3. **Biological**
4. **Chemical**
5. **Physical**

1. Organoleptic evaluation of drug

- **Organoleptic evaluation means the study of a drug with the help of organs of sense.**
- **It includes any drug's macroscopic or external appearance, color, odor, taste & sounds of its fracture etc.**
- **The macroscopic or external characteristic of a drug may be divided into 7 headings -**

1. **Shape**
2. **Size**
3. **Color**
4. **Fracture & internal color**
5. **Odor**
6. **Taste**

2. Microscopic evaluation of drug

Microscopic evaluation of drug can be done in the laboratory by the use of microscopes and utilizes various microscopic characters of the drugs, such as types and arrangement of various cells and tissues.

3. Chemical evaluation of drug

- **Chemical evaluation of drugs involves both qualitative and quantitative determination of their active principles.**

•In this method characteristic qualitative chemical tests are employed to identify crude drugs and their constituents.

4. Biological evaluation of drug

- **The biological evaluation of crude drugs is very useful in determining the pharmacological activity of the drug.**
- **Since living organism or their isolated living tissues are used, this method is also called the biological method or bioassay.**
- **Many drugs, particularly the antibiotics, toxins and toxoids and also vitamines are assayed by this method.**

5. Physical evaluation of drug

The physical evaluation of crude drugs is accomplished by the determination of various physical characteristics using various physico-chemical techniques, for example, specific gravity (of fats and volatile oils), melting points (of alkaloids), optical rotation (of alkaloid and volatile oils), etc.



Plant growth hormones

Prepared by:
P.Nagamani

-:CONTENTS:-

- INTRODUCTION
- CHARACTERISTICS
- CLASSES OF PLANT HORMONES

  a)Auxin b)Gibberellin

  c)Cytokinin d)Ethylene e)Absisic acid
- OTHER KNOWN HORMONES
- POTENTIAL MEDICAL APPLICATION
- CONCLUSION
- REFERENCES

INTRODUCTION:-

- Plant hormones are also known as phytohormones.
- Plant hormones are chemicals that regulate plant growth.
- Hormones also determine the formation of flowers,stems,leaves,the shedding of leaves, and the development and ripening of fruit.
- Hormones are vital to plant growth, and lacking them, palnts would be mostly a mass of undifferentiated cells.so they are also known as growth factors or growth hormones.
- The term “PHYTOHORMONES” was coined by ‘THIMANN’ in

1948.

CHARACTERISTICS:-

- The word hormone is derived from Greek , meaning set in motion. plant hormones affect gene expression and transcription levels , cellular division and growth.
- They are naturally produced within plants , though very similar chemicals are produced by fungi and bacteria , that can also affect plant growth.
- Plant hormones are not nutrients , but chemicals that in small amount promote and influence the growth, development , and differentiation of cells and tissues.

CLASSES OF PLANT HORMONES:-

- In general , it is accepted there are five major classes of plants hormones , some of which are made up of many different chemicals that can vary in structure from one plant to the next.
- The five major classes are :-

a)AUXIN

b)GIBBERELLIN c)CYTOKININ

d)ETHYLINE e)ABSISIC ACID

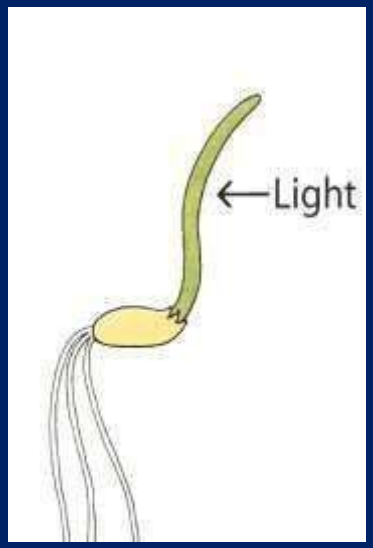
Auxin :-

- Discovered in 1881 by CHARLES AND FRANCIS DARWIN.
- They reported experiments on the response of growing plants to light.
- ➢ Grass seedlings do not bend if the tip is covered with a light proof cap.
- ➢ They do not bend when a collar is placed below the tip.
- ➢ 30 year later, PETER BOYSEN JENSEN AND ARPAD PAAL demonstrated that the influence was actually a chemical.

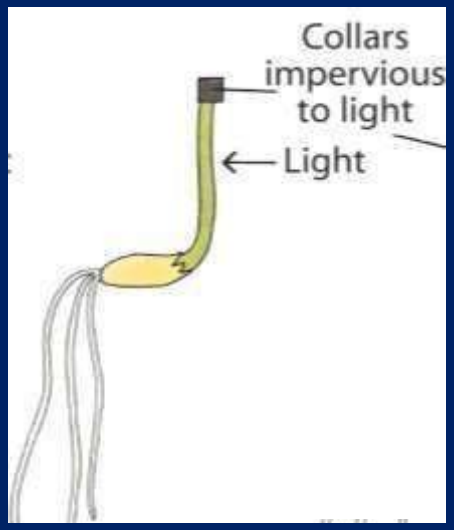
AUXIN:-

- **PRODUCTION**
  - Shoot tips
  - Developing seeds
- **SOME KNOWN ACTION**
  - Establish of polarity of root –shoot axis during embryogenesis.
  - Cel elongation.
  - Cel differentiation.
  - Apical dominance.
  - Lateral root formation & adventitious root formation.
  - Fruit formation

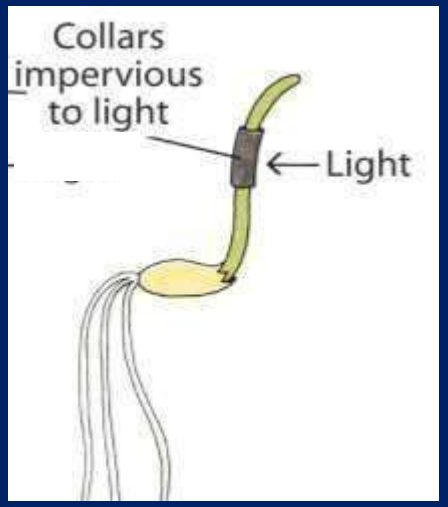
Darwins' (Charles and son) experiment



Under normal conditions, shoot tips bend towards the light



Without light on the tip, no bending



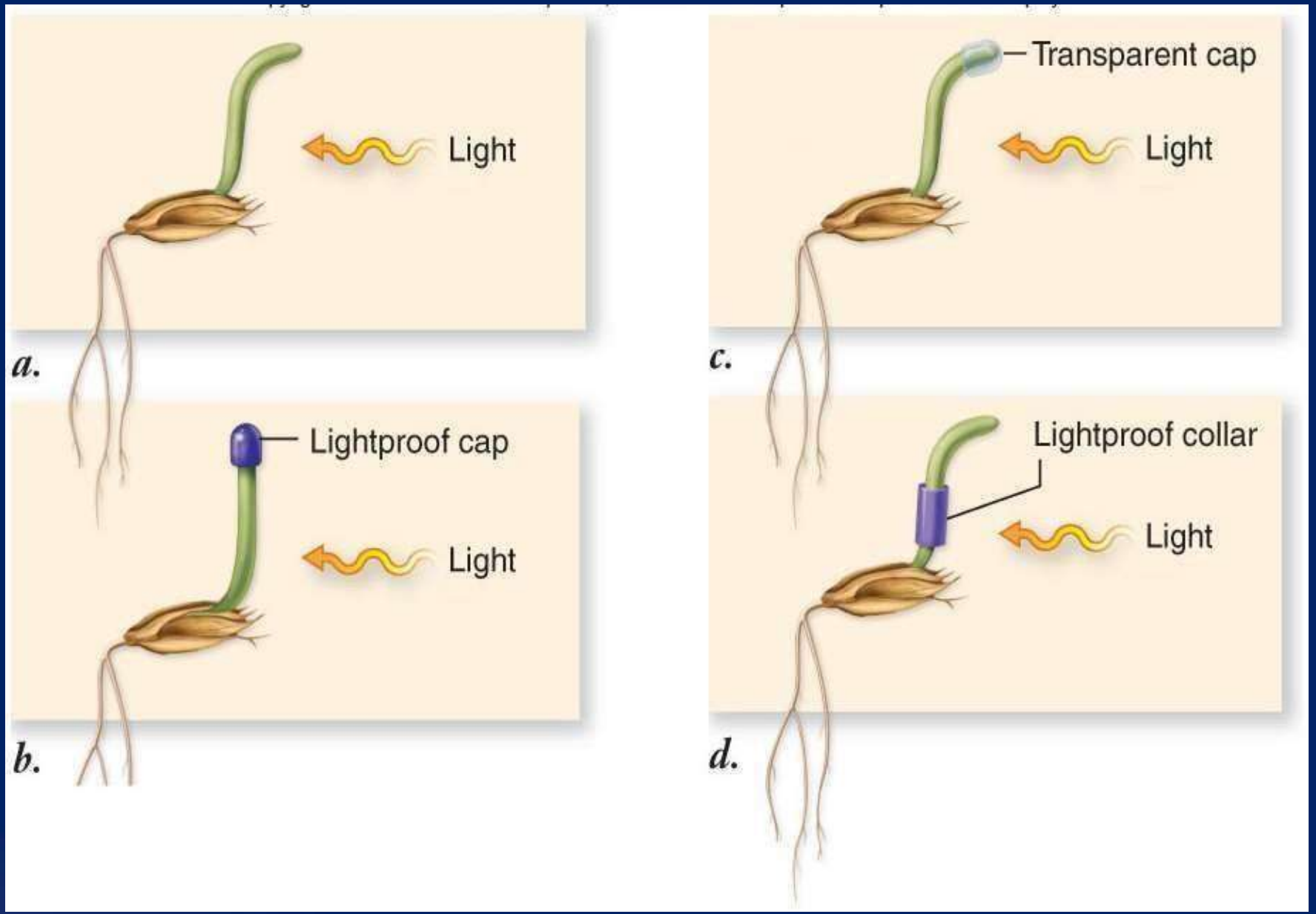
When not at tip, collar doesn't prevent bending

Conclusion: Light is sensed at the tip, but response not at tip

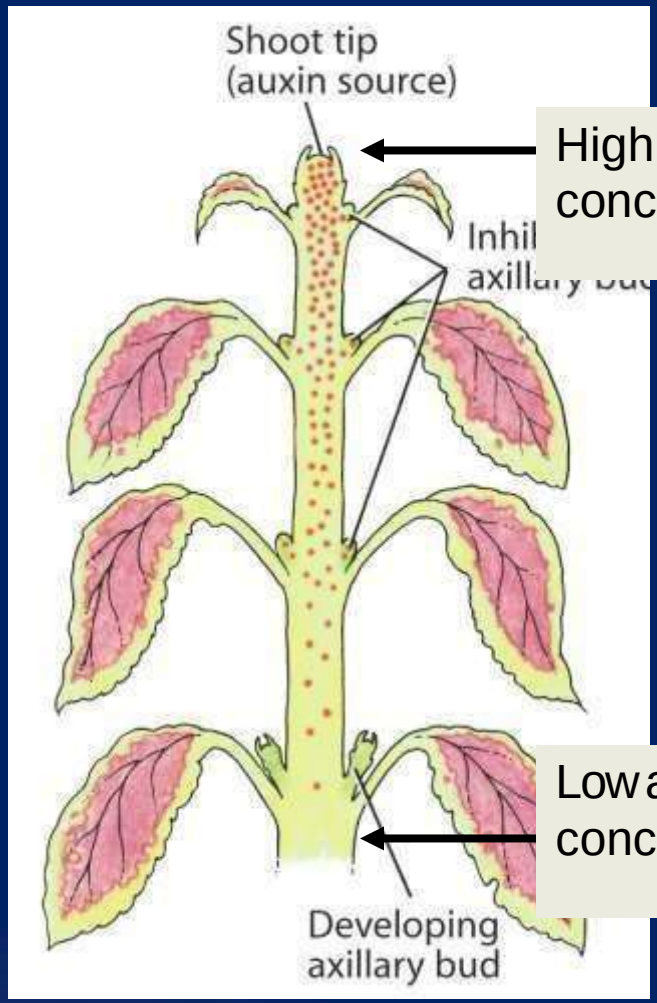
New hypothesis: A substance or chemical is transported

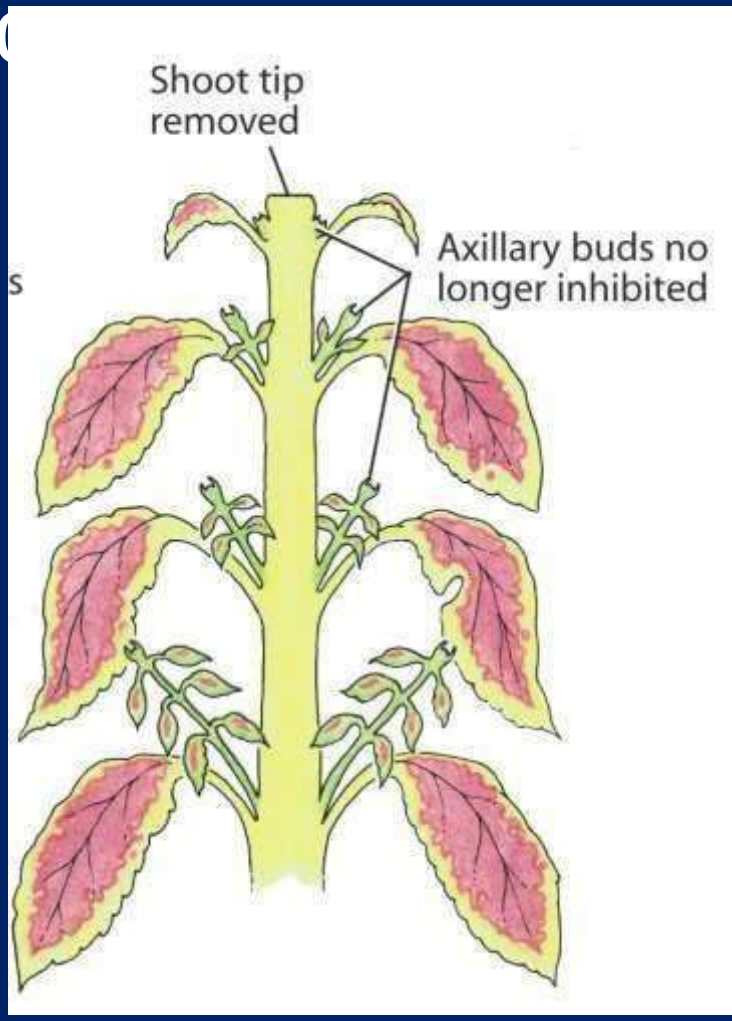
Auxin later isolated from shoot tips and established to be involved in cell elongation

Drawings depicting seedlings of *Zea* (Gramineae family)


Light
a.
Transparent cap
Light
c.
Lightproof cap
Light
b.
Lightproof collar
Light
d.

Evidence for the role of auxin in apical dominanc

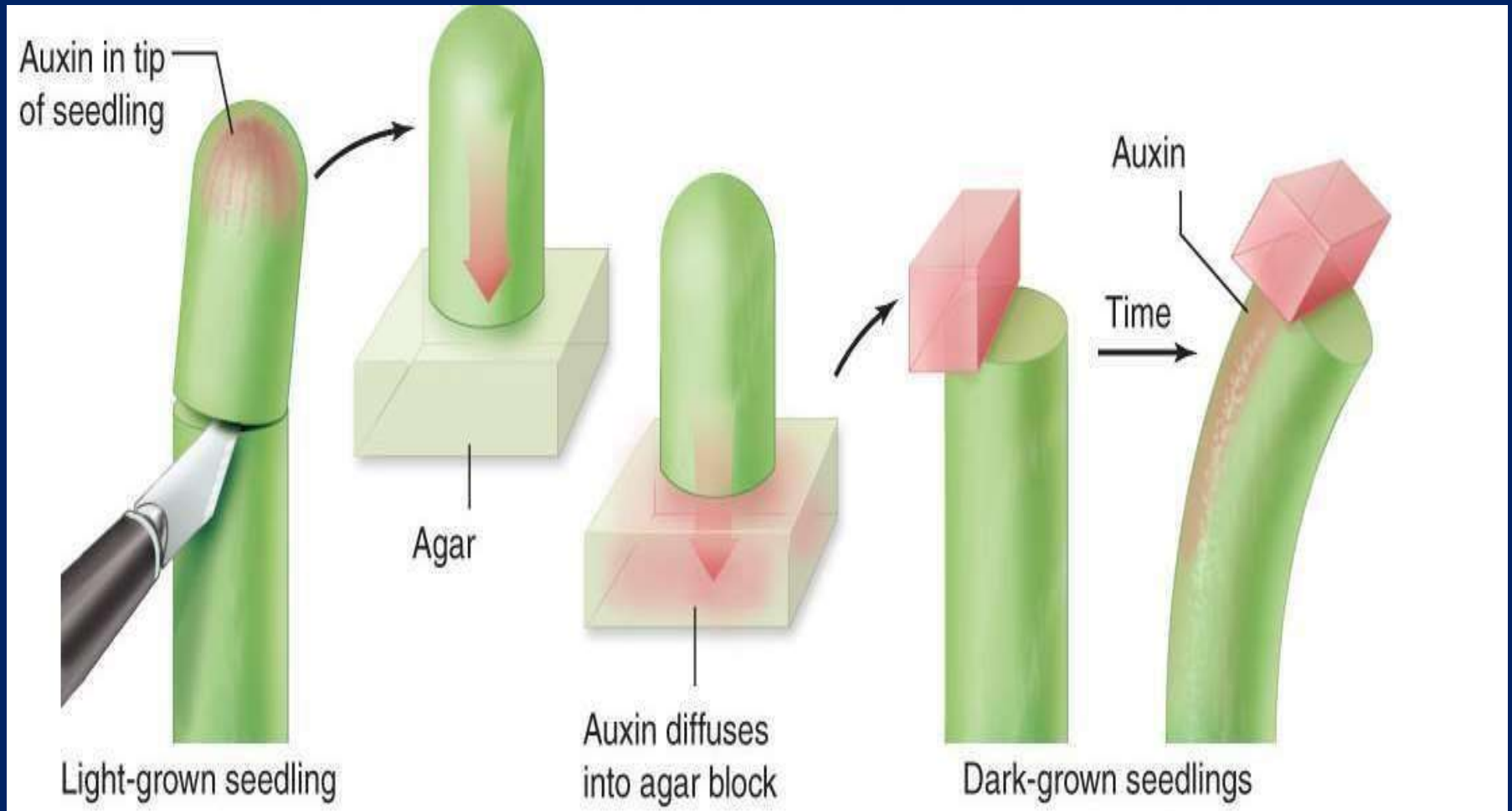




Drawings depicting *Coleus* (Lamiaceae family)

Auxin

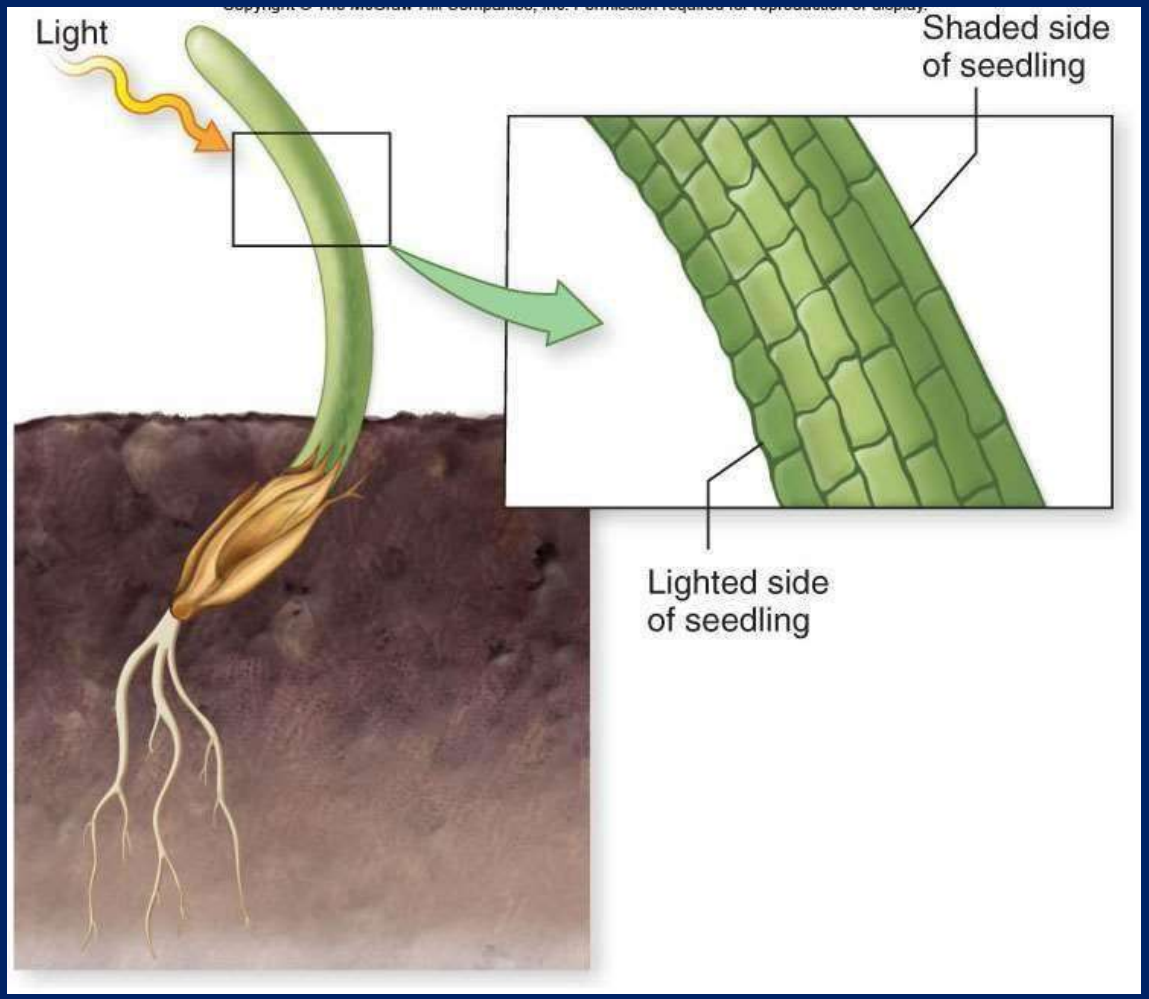
- In 1926, Frits Went performed an experiment that explained all of the previous results
- He named the chemical messenger auxin
- It accumulated on the side of an oat seedling away from light
- Promoted these cells to grow faster than those on the lighted side
- Cell elongation causes the plant to bend towards light



1. Went removed the tips of oat seedlings and put them on agar, an inert, gelatinous substance.

2. Blocks of agar were then placed off-center on the ends of other oat seedlings from which the tips had been removed.

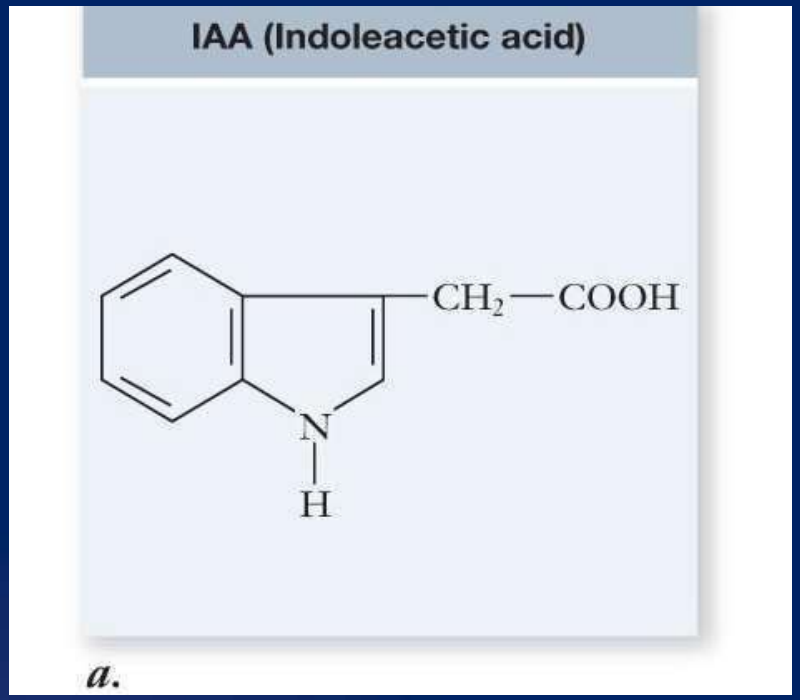
3. The seedlings bent away from the side on which the agar block was placed.

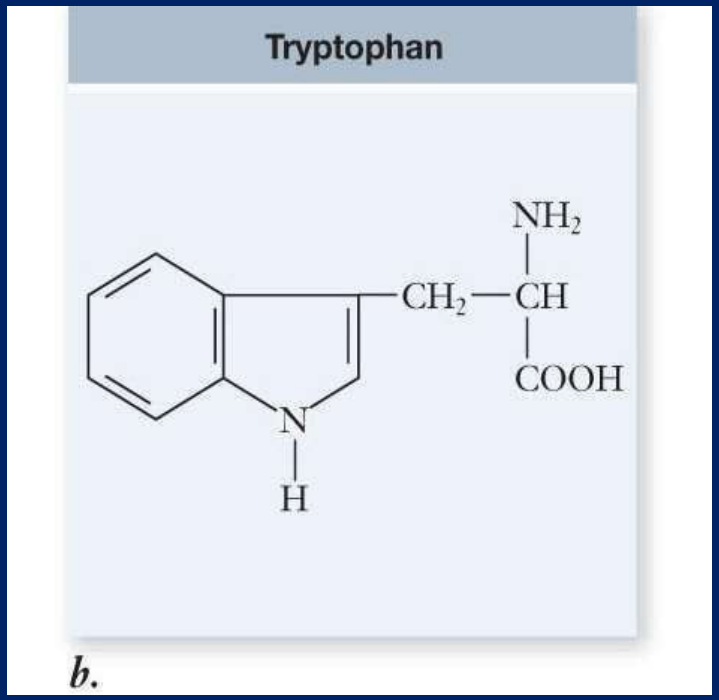


- Chemical enhanced rather than retarded cell elongation
- Frits Went named the substance that he had discovered auxin

How Auxin Works

- Indoleacetic acid (IAA) is the most common natural auxin
- Probably synthesized from tryptophan





Synthetic Auxins

- Naphthalene acetic acid (NAA) and indolebutyric acid (IBA) have many uses in agriculture and horticulture
- Prevent abscission in apples and berries
- Promote flowering and fruiting in pineapples
- 2,4-dichlorophenoxyacetic acid (2,4-D) is a herbicide commonly used to kill weeds

Gibberellins

- Named after the fungus *Gibberella fujikuroi*

which causes rice plants to grow very tall

- Gibberellins belong to a large class of over 100 naturally occurring plant hormones
  - All are acidic and abbreviated GA
  - Have important effects on stem elongation
    - Enhanced if auxin present

- Adding gibberellins to certain dwarf mutants restores normal growth and development



- GA is used as a signal from the embryo that turns on transcription of genes encoding hydrolytic enzymes in the aleurone layer
- When GA binds to its receptor, it frees GA- dependent transcription factors from a repressor
- These transcription factors can now directly affect gene expression

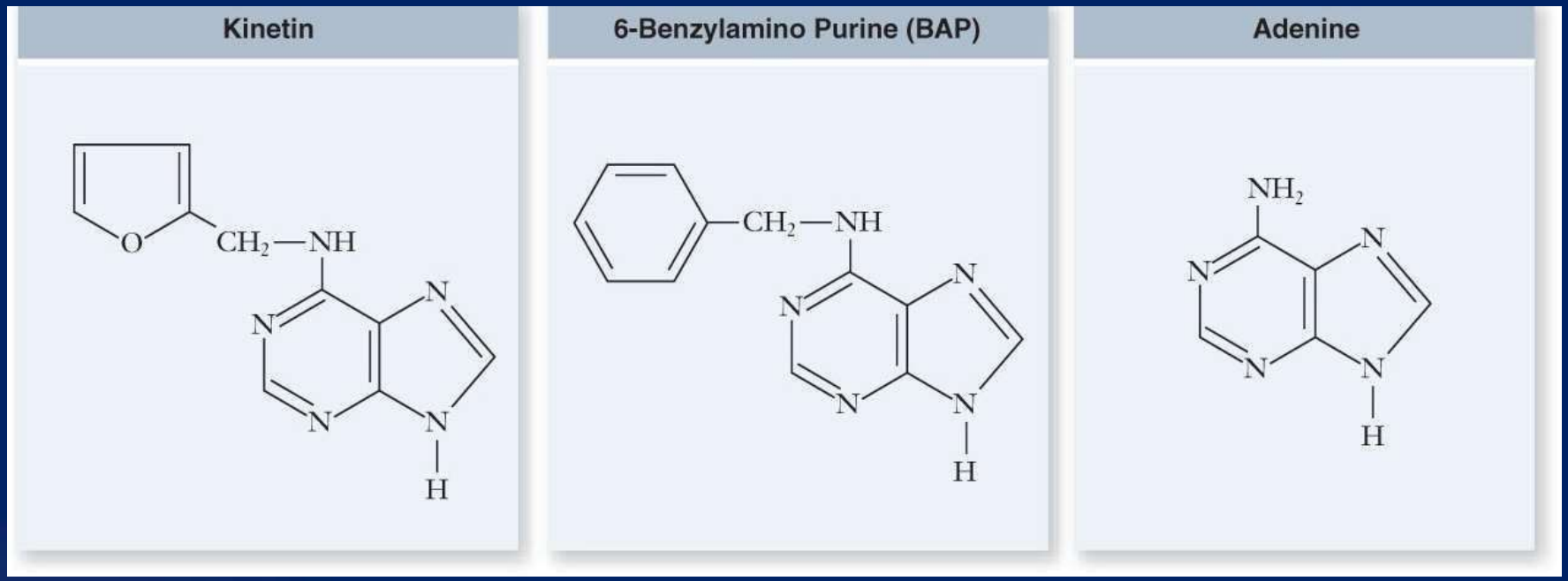
Gibberellins

- Hasten seed germination
- Used commercially to extend internode length in grapes
  - Result is larger grapes



Cytokinins

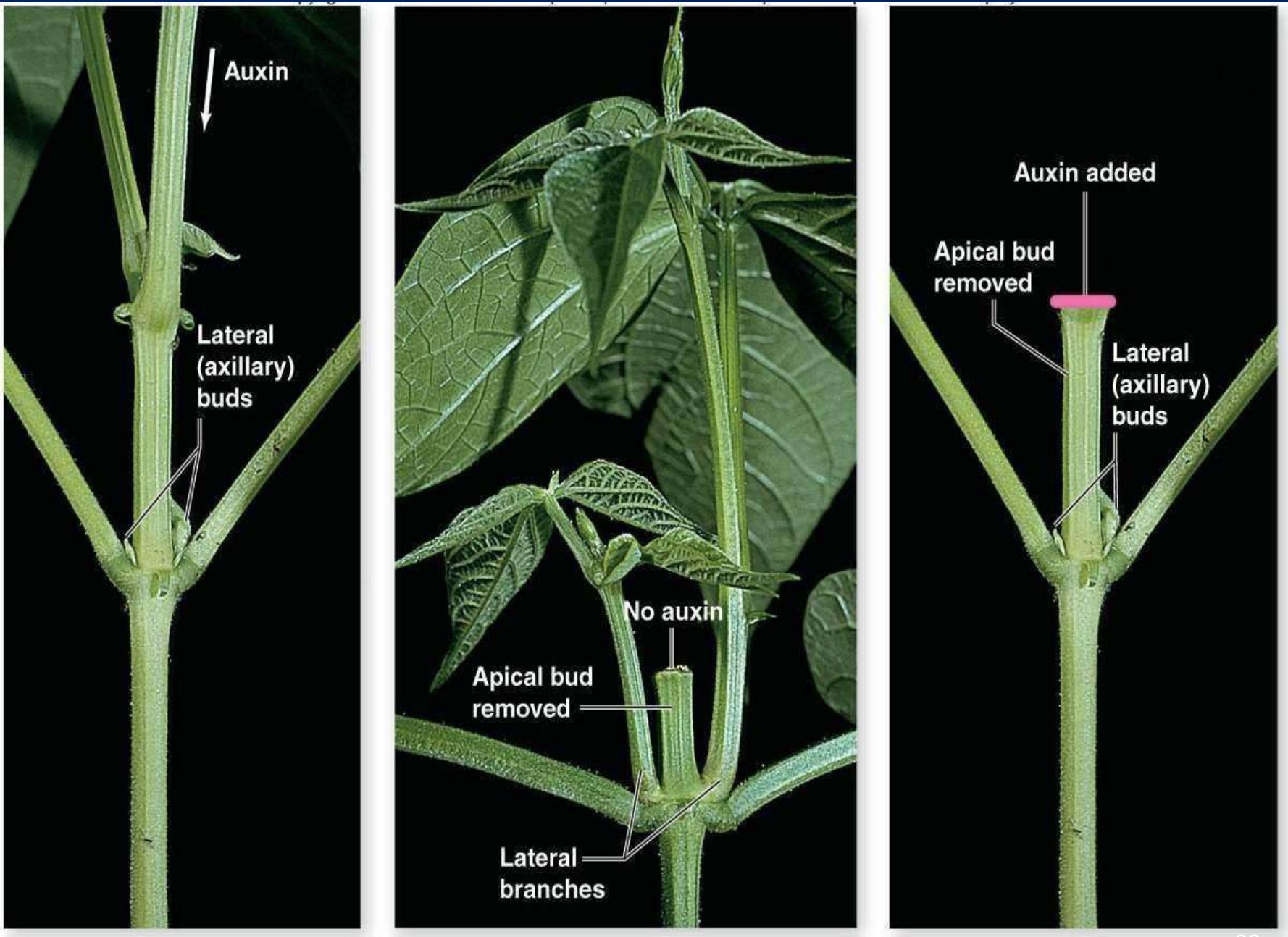
- Plant hormone that, in combination with auxin, stimulates cell division and differentiation



Synthetic cytokinins

- Produced in the root apical meristems and developing fruits
- In all plants, cytokinins, working with other hormones, seem to regulate growth patterns
- Promote the growth of lateral buds into branches
- Inhibit the formation of lateral roots

– Auxin promotes their formation

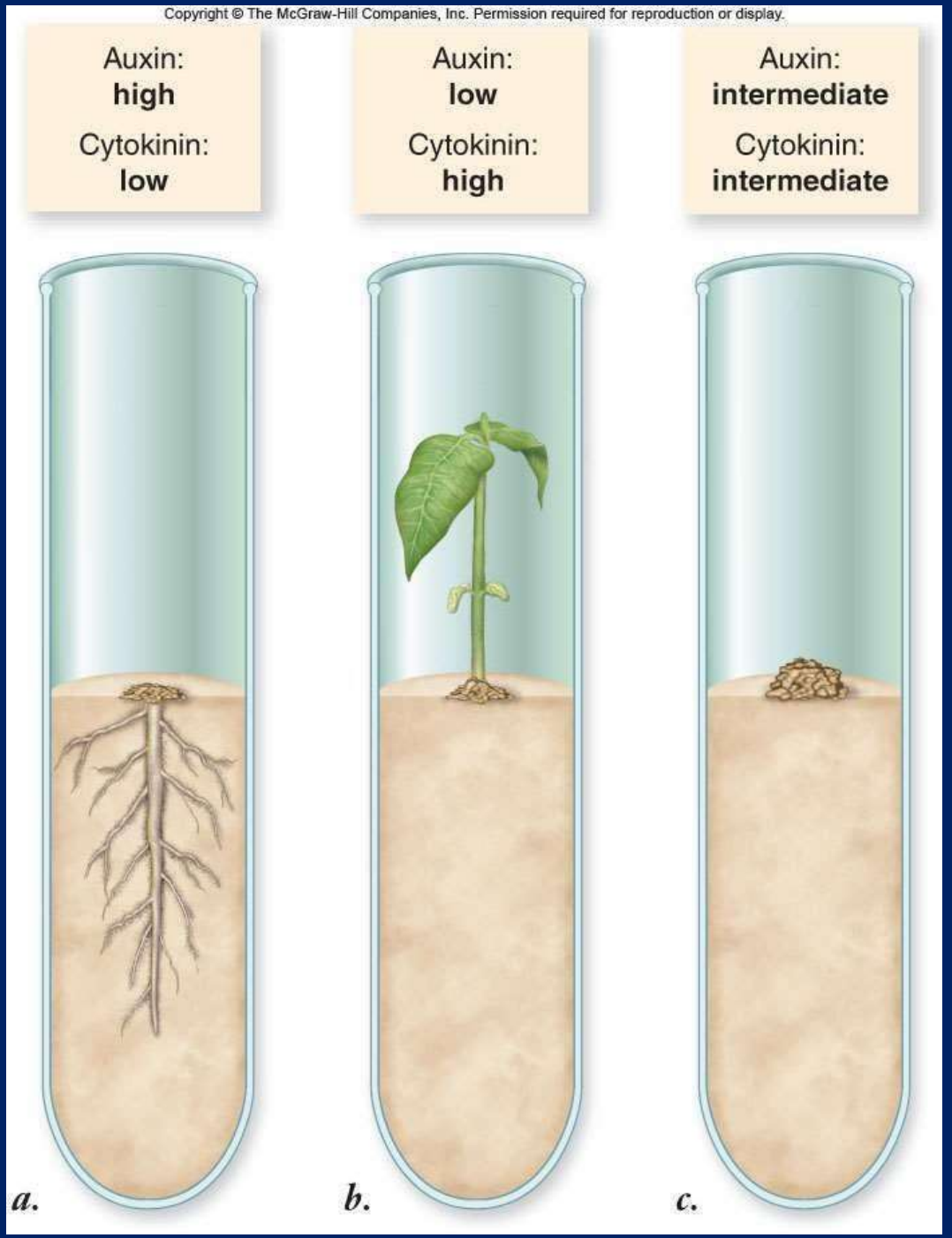

Auxin
Lateral (axillary) buds
No auxin
Apical bud removed
Lateral branches
Auxin added
Apical bud removed
Lateral (axillary) buds

Cytokinins

- Promote the synthesis or activation of cytokinesis proteins
- Also function as antiaging hormones
- *Agrobacterium* inserts genes that increase rate of cytokinin and auxin production
  - Causes massive cell division
  - Formation of crown gall tumor



- Plant tissue can form shoots, roots, or an undifferentiated mass depending on the relative amounts of auxin and cytokinin



Ethylene

- Gaseous hydrocarbon ($H_2C—CH_2$)
- Auxin stimulates ethylene production in the tissues around the lateral bud and thus retards their growth
- Ethylene also suppresses stem and root elongation
- Major role in fruit development – hastens ripening
  - Transgenic tomato plant can't make ethylene
  - Shipped without ripening and rotting

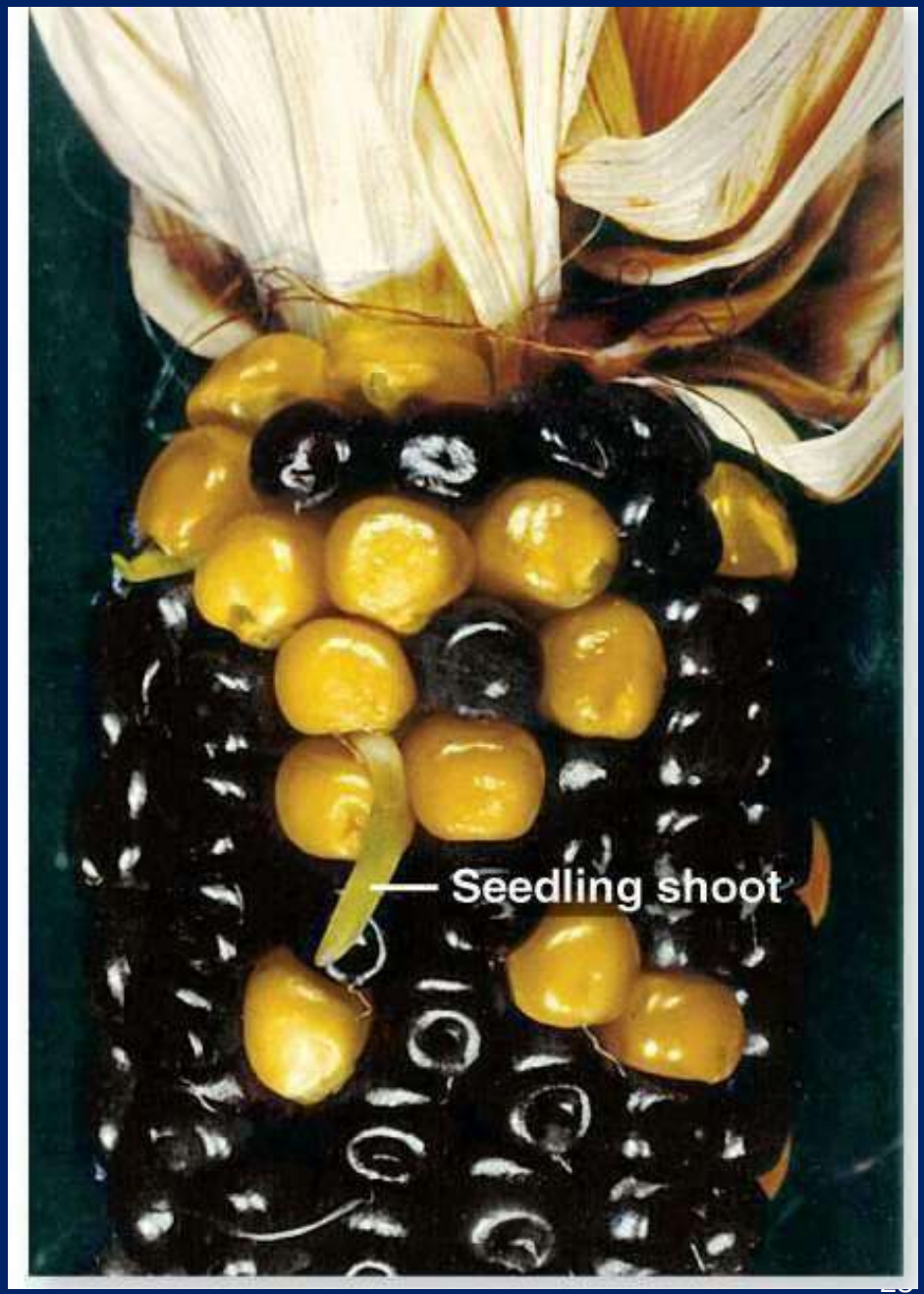
Abscisic Acid

- Synthesized mainly in mature green leaves, fruits, and root caps
- Little evidence that this hormone plays a role in abscission
- Induces formation of dormant winter buds
- Counteracts gibberellins by suppressing bud growth and elongation
- Counteracts auxin by promoting senescence

Abscisic Acid

- Necessary for dormancy in seeds
  - Prevents precocious germination called vivipary
- Important in the opening and closing of stomata


Dormant bud

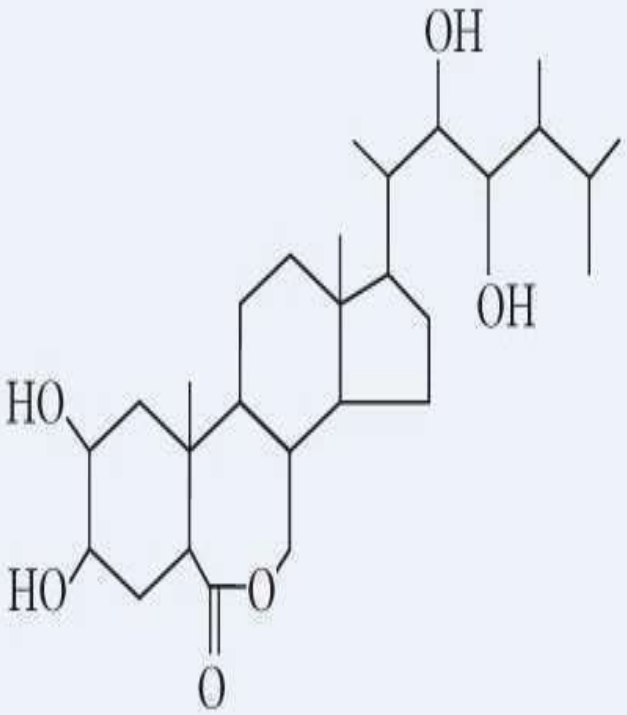
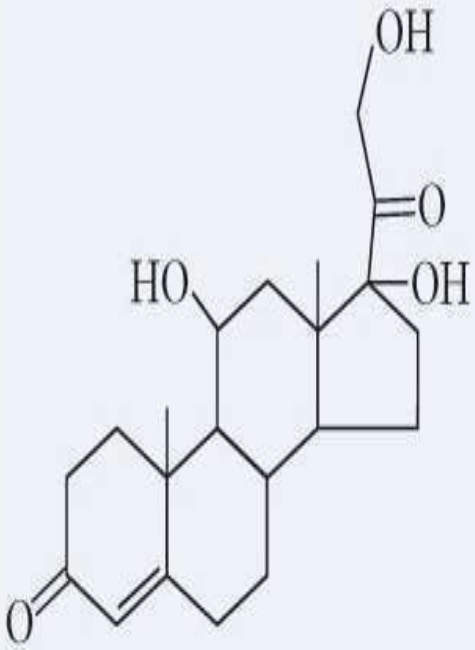
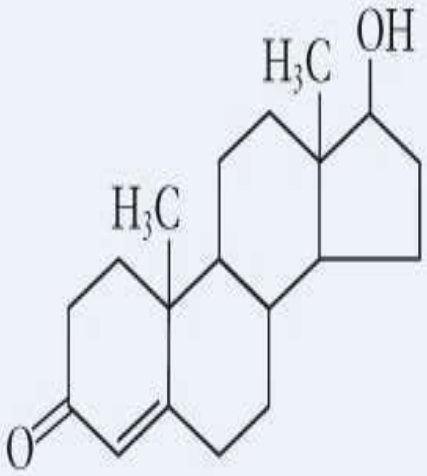

Seedling shoot

Other known hormones:-

- Other identified plant growth regulators include-

a)BRASSINOSTEROID:-

- First discovered in the pollen of brassica spp.
- Are structurally simillar to steroid hormones.

Plant	Animal	
Brassinolide	Cortisol	Testosterone
		

Brassinosteroids

- Functional overlap with other plant hormones, especially auxins and gibberellins
- Broad spectrum of physiological effects

– Elongation, cell division, stem bending, vascular tissue development, delayed senescence, membrane polarization and reproductive development

Salicylic acid:-

- Activate genes in some plants that produce chemicals that aid in the defense against pathogenic invader.

Jasmonates :-

- Are produced from fatty acids & seems to promote the production of defense proteins that are used to fend off invading organisms.
- Also have a role in seed germination.

plant peptide hormones:-

- Involved in cell to cell signaling.
- Roles in plant growth &development , including defense mechanism.

Polyamines:-

- Are strongly basic molecule with low molecular weight that

have been found in all organism studied thus far.

Nitric oxide:-

- Serves as signals in hormonal &defense response.
- E.G-nitrogen fixation , stomata closure , germination , cell

death.

Karrikins:-

- Not plant hormones because they are not made by plants, but are a group of plant growth regulator found in the smoke of burning plant materials that have the ability to stimulates the germination of seeds.

Strigolactones :-

- Implicated in the inhibition of shoot branching.

Triacontanol :-

- A fatty alcohol that acts as a growth stimulant, especially initiating new basal breaks in the rose family.
- It is found in ALFALFA (Lucerne),BEE'S WAX.

POTENTIALMEDICALAPPLICATION:-

- Plant stress hormones activate cellular response, including cell death to diverse stress situation in plants.
- Researchers have found that some plant stress hormone share the ability to adversely affect human cancer cells.
- E.G:-sodium salicylate has been found to suppress proliferation of LYMPHOBLASTIC LEUKEMIA,PROSTATE &MELANOMA human cancer cells.
- Methyl jasmonate has been found to induce cell death in a number of cancer cell lines.

conclusion

- There exists a certain elegance by which the hormones counteract each other, regulate each other, and create completely unique and unexpected effects in tandem.
- The balance of auxin and cytokinin, not either hormone in isolation, allow for calluses to differentiate, while abscisic acid and gibberelins struggle against each other in regulation of seed dormancy.


thank you so much

PLANT TISSUE CULTURE

P.Mamatha

PLANT TISSUE CULTURE

Definition

Tissue culture is in vitro cultivation of plant cell or tissue under aseptic and controlled environmental conditions, in liquid or on semisolid well defined nutrient medium for the production of primary and secondary metabolites or to regenerate plant.

Tissue culture relies on three fundamental abilities of plant there are:

Totipotency

Dedifferentiation

competency



- **1902: Haberlandt attempted to the culture mesophyll tissue and root hair cells.**

  This was the first attempt of *in vitro* culture.
- **1904: Haning attempted to culture excised embryos from mature seeds.**
- **1922: Kotte was successful in obtaining growth from isolated root tips on inorganic media.**
- **Robbins reported similar success from root tip and stem tip.**
- **1934: Used yeast extract (vit B) with inorganic salts to repeatedly culture root tips of tomato by White.**

Contd..

- **1939: Tobacco crown gall culture, callus obtained: called as Plant Cancer.**
- **1940: Coconut milk used in plant cultures to obtain heart-shaped embyos.**
- **1950:Skoog used adenine sulfate to obtain buds on tobacco segments:**
- **identified: kinetin**
- **1958: Stewart and Reinert obtained somatic embryos from carrot cells.**
- **1950-60s: Botanists turned to plant tissue culture to study plant development.**

Contd..

- **1960: Jone, Hildebrandt, Ricker and Wu examined growth in hanging drop cultured of seperated cells from a callus of hybrid tobaccos.**
- **1962: Murashige and Skoog developed MS media for tobacco.**
- **1966: Guha and Maheshwari obtained first haploid plants (Delhi Univ., India)**
- **1970: Discovery of restriction endonuclease (Daniell Nathan). Plasmids were already known.**

Contd..

- **1972-73: First recombinant molecule created by Stanley Cohen, Stanford Univ.**
- **1974: Discovery of Ti plasmid in *Agrobacterium tumefaciens* (by Zaenen in Ghent Univ., Belgium)**
- **1970-80s:Ti plasmid analysis (Nester, Seattle; Van Montagu, Ghent)**
- **1983: First transgenic plant. (Monsanto, Ghent, Washington Univ).**
- **1985: Leaf disk transformation method (Monsanto)**

Tissue Culture:
De-differentiation to Regeneration



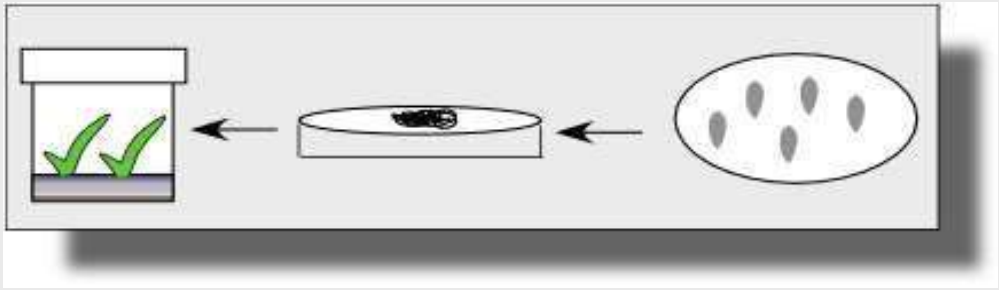
Wheat inflorescence



seeds



embryos



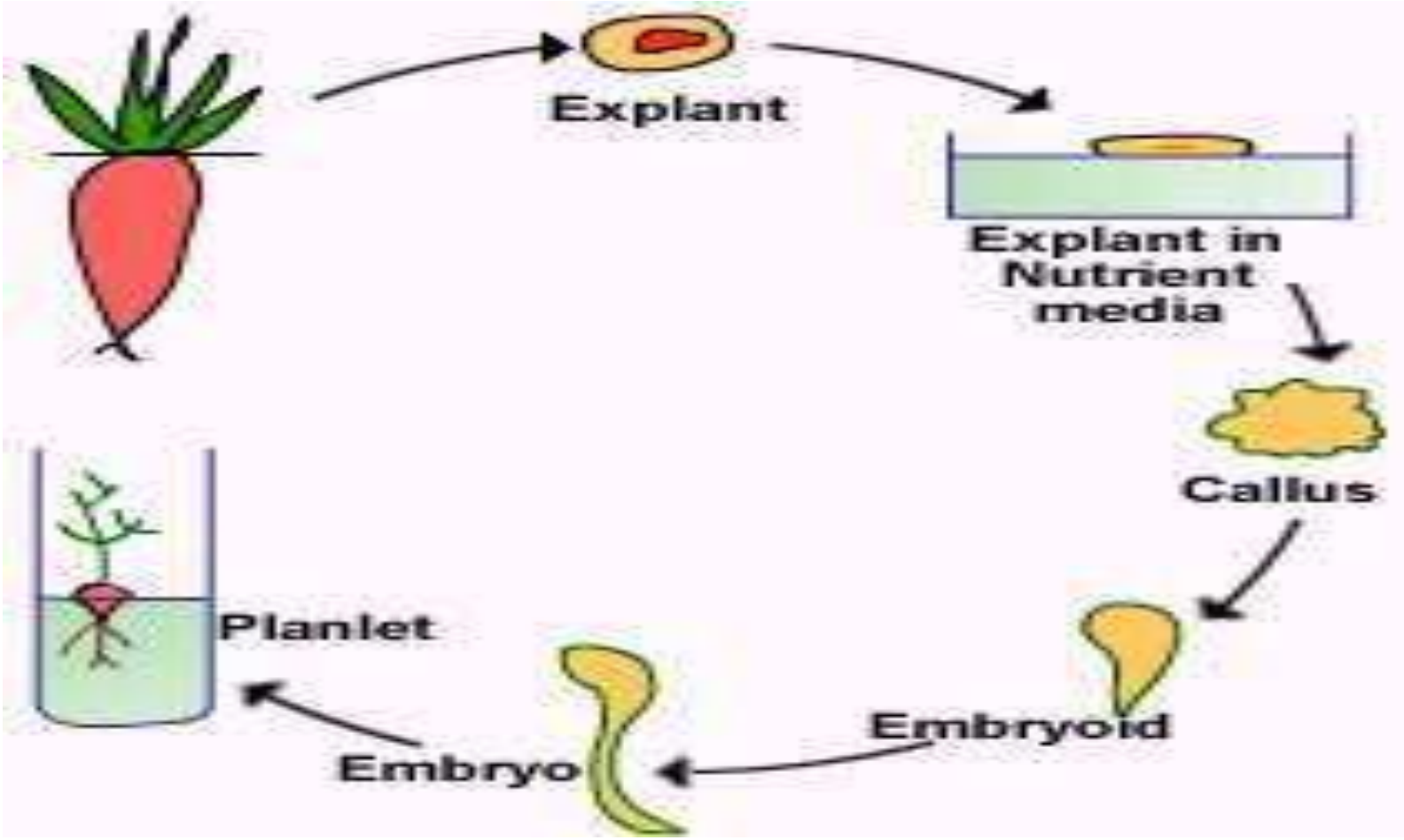
- auxin/ + cytokinin

Callus

+ auxin

Plant cells are totipotent

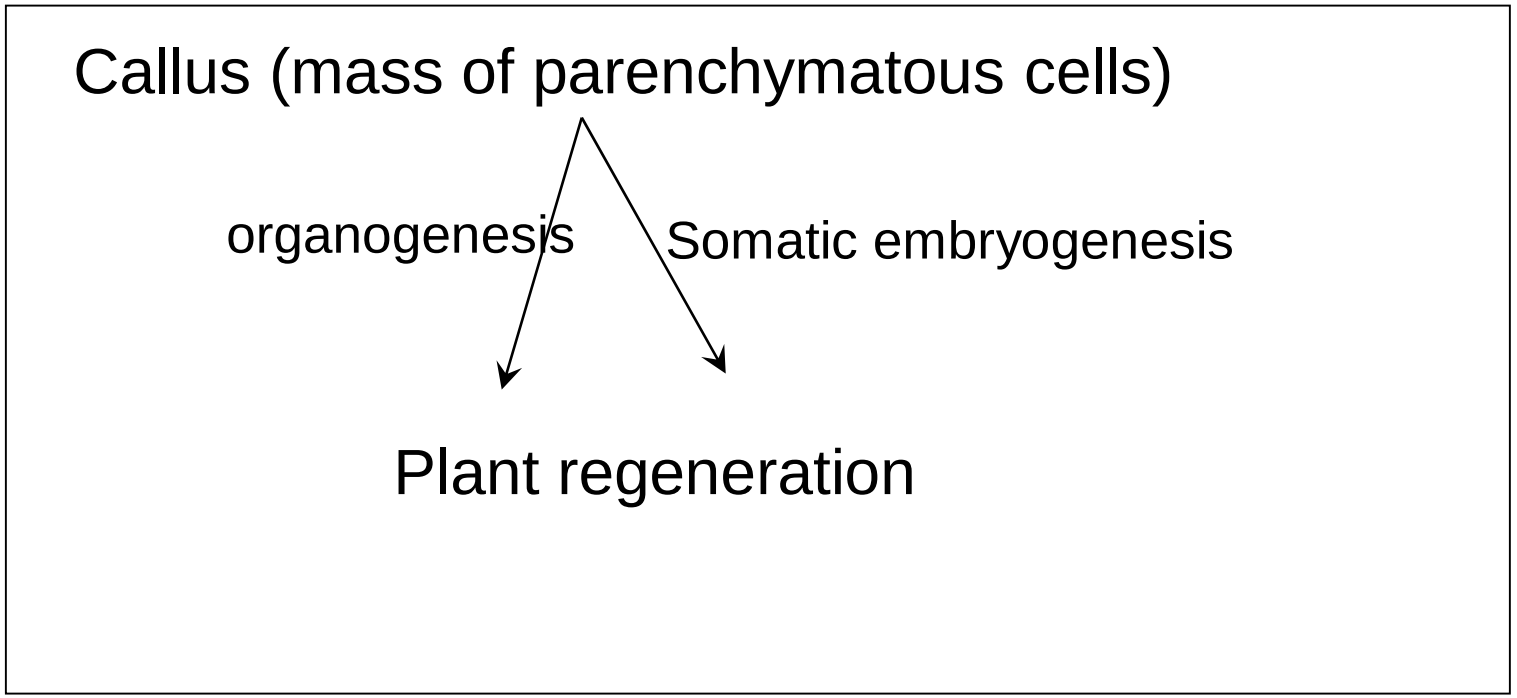
Totipotency: Ability of a cell or tissue or organ to grow and develop into a fully differentiated organism.



Explant: any living tissue: leaf, root, zygotic embryos

Culture Media or Nutrients:

1. Inorganic salts
2. vitamins
3. Carbon source
4. Plant Growth Regulators
5. Organic supplements



Organogenesis

Unique to plants. Plant tissue *in vitro* may produce many types of primordia such as shoot and root

Explant → Callus → meristemoid → organ primordia

Explant → meristemoid → organ primordia

Explant → de-differentiation → induction → differentiation → organ

Applications of Plant Tissue Culture

1. **Haploid production (rice, wheat and barley)**
2. **Triploid production (fruits)**
3. **Embryo Rescue/ Wide hybridization (numerous examples)**
4. **Somatic hybridization (scientific examples, few commercial products)**
5. **Somaclonal Variations (Tomato with altered color, taste and texture by Fresh World Farms; Imidazolinone resistant maize.**

Contd..

6. Production of disease resistant plants.

7. Clonal propagation

8. Secondary metabolite production (eg. Taxol production from cell cultures derived from the bark cuttings of pacific yew tree)

9. Germplasm conservation (cryopreservation)

Applications in detail

- To conserve rare or endangered plant species.
- A plant breeder may use tissue culture to screen cells rather than plants for advantageous characters, e.g. herbicide resistance/tolerance.
- Large-scale growth of plant cells in liquid culture in bioreactors for production of valuable compounds, like plant-derived secondary metabolites and recombinant proteins used as biopharmaceuticals.

Contd..

- To cross distantly related species by protoplast fusion and regeneration of the novel hybrid.
- To rapidly study the molecular basis for physiological, biochemical, and reproductive mechanisms in plants, for example in vitro selection for stress tolerant plants, and in vitro flowering studies.
- To cross-pollinate distantly related species and then tissue culture the resulting embryo which would otherwise normally die (Embryo Rescue).

Contd..

- For chromosome doubling and induction of polyploidy, for example doubled haploids, tetraploids, and other forms of polyploids.
- This is usually achieved by application of antimitotic agents such as colchicine or oryzalin.
- As a tissue for transformation, followed by either short-term testing of genetic constructs or regeneration of transgenic plants.

Contd..

- Certain techniques such as meristem tip culture can be used to produce clean plant material from virused stock, such as potatoes and many species of soft fruit.
- Production of identical sterile hybrid species can be obtained.

QUALITY CONTROL OF HERBS

G.Nellamma
VCPS

III- Safety measures:

Drug adulteration

The adulteration and substitution of herbal drugs is the burning problem in herbal industry and it has caused a major effect in the commercial use of natural products.

Adulteration in market samples is one of the greatest drawbacks in promotion of herbal products. Adulteration it is a practice of substituting the original crude drug partially or fully with other substances which is either free from or inferior in therapeutic and chemical properties or addition of low grade or spoiled drugs or entirely different drug similar to that of original drug substituted with an intention of enhancement of profits. Or adulteration may be defined as mixing or substituting the original drug material with other spurious, inferior, defective, spoiled, useless other parts of same or different plant or harmful substances or drug which do not confirm with the official standards

Adulteration may takes place by two ways:

- Direct or intentional adulteration
- Indirect or unintentional adulteration

1- Direct or intentional adulteration

Direct or intentional adulteration is done intentionally which usually includes practices in which an herbal drug is substituted partially or fully with other inferior products. Due to morphological resemblance to the authentic herb, many different inferior commercial varieties are used as adulterants. These may or may not have any chemical or therapeutic potential. Substitution by “exhausted” drugs entails adulteration of the plant material with the same plant material devoid of the active constituents. This practice is most common in the case of volatile oil-containing materials, where the dried exhausted material resembles the original drug but is free of the essential oils. Foreign matter such as other parts of the same plant with no active ingredients, sand and stones, manufactured artifacts, and synthetic inferior principles are used as substitutes.

*** With artificially manufactured materials**

Substances artificially manufactured being resemble with original drug are used as substitutes. This practice is generally followed for much costlier drug e.g. nutmeg is adulterated with basswood prepared to the required shape and size, the colored paraffin wax is used in place of beeswax.

*** With inferior quality materials**

Inferior quality material may or may not have same chemical or therapeutic value as that of original natural drug due to their morphological resemblance to authentic drug, they are marketed as adulterants e.g. *Belladonna* leaves are substituted with *Ailanthus* leaves, *papaya* seeds to adulterate *Piper nigrum*, mother cloves and clove stalks are mixed with clove, beeswax is substituted by Japan wax.

*** With exhausted material**

Many drugs extracted on large scale for isolation of active principle, volatile oils etc. the exhausted material may be used entirely or in part as a substituent for the genuine drug e.g. umbelliferous fruits and cloves (without volatile oils) are adulterated with exhausted (without volatile oils) original drugs, exhausted jalap and Indian hemp (without resins) are used as adulterant.

*** With foreign matter**

Sometimes synthetic chemicals are used to enhance the natural character e.g. addition of benzyl benzoate to balsam of Peru, citral to citrus oils like oil of lemon and orange oil etc.

*** With harmful / Fictitious substances**

Sometimes the wastes from market are collected and admixed with authentic drugs particularly for liquids or unorganized drugs e.g. pieces of amber colored glass in colophony, limestone in asafetida, lead shot in opium, white oil in coconut oil, cocoa butter with stearin or paraffin.

*** Adulteration of powders**

Besides entire drug powder form frequently found to be adulterated e.g. powder liquorice or gentian admixed with powder olive stones, under the name of cinchona, *C. calisaya* wedd., *C. officinalis* Linn.f., *C. ledgeriana* and *C. succirubra* are available as mixtures.

2- Indirect or unintentional adulteration

Unintentional or undeliberately adulteration which sometimes occurs without bad intention of the manufacturer or supplier. Sometimes in the absence of proper means of evaluation, an authentic drug partially or fully devoid of the active ingredients may enter the market. Factors such as geographical sources, growing conditions, processing, and storage are all factors that influence the quality of the drug.

*** Faulty collection**

Some of the herbal adulteration is due to the carelessness of herbal collectors and suppliers. The correct part of genuine plant should be collected. Other less valuable part of the genuine plant should not be collected. Moreover collection should be carried out at a proper season and time when the active constituents reach maximum. *Datura strumarium* leaves should be collected during flowering stage and wild cherry bark in autumn etc. collection from other plant by ignorance, due to similarity in the appearance, color, lack of knowledge may lead to adulteration.

*** Imperfect preparation**

Non removal of associated structures eg. stems are collected with leaves, flowers, fruits. Non-removal of undesirable parts or structures e.g. cork should be removed from ginger rhizome. Proper drying conditions should be adhered. Improper drying may lead to unintentional adulteration e.g. if digitalis leaves are dried above 65^{o}c decomposition of glycosides by enzymatic hydrolysis.

* Incorrect storage

Deterioration especially during storage, leading to the loss of the active ingredients, production of metabolites with no activity and, in extreme cases, the production of toxic metabolites. Physical factors such as air (oxygen), humidity, light, and temperature can bring about deterioration directly or indirectly. These factors, alone or in combination, can lead to the development of organisms such as molds, mites, and bacteria. Oxidation of the constituents of a drug can be brought about by oxygen in the air, causing some products, such as essential oils, to resinify or to become rancid.

Moisture or humidity and elevated temperatures can accelerate enzymatic activities, leading to changes in the physical appearance and decomposition of the herb. For example volatile oils should be protected from light and stored in well closed containers in cool place. Belladonna leaf should be stored in moisture free containers, which may cause enzymatic action lead to decomposition of medicinally active constituents. Mites, nematode worms, insects/moths, and beetles can also destroy herbal drugs during storage.

*** Gross substitution with plant material**

Due to morphological resemblance i.e similarity in appearance, colors etc the genuine crude drugs are substituted with others are very often sold in the market e.g. *Podophyllum peltatum* L. is used as a substitute for *P. hexandrum*, *Belladona* leaves are substituted with Ailanthus leaves, saffron is admixed with dried flowers of *Carthamus tinctorius*, mother cloves and clove stalks are mixed with clove.

*** Substitution with exhausted drugs**

In this type, the same drug is admixed but devoid of any medicinally active constituents as they are already extracted out. This practice is more common in case of volatile oil containing drugs like fennel, clove, coriander, caraway etc. sometime, natural characters of exhausted drugs like color and taste are manipulated by adding other additives and then it is substituted eg. exhausted gentian made bitter with aloes.

Adulteration involves a number of different conditions:

inferiority, spoilage, deterioration, admixture, sophistication and substitution. From the standpoint of present day commerce, inferior, spoiled or deteriorated drug represent the greatest percentage of cases of drug adulteration.

1- Inferiority:

Inferiority refers to any substandard drug or substance. The more restricted definition as applied to **foods, drugs** and **materials produced** by nature indicates a **naturals substandard condition**.

The dried, ripe seeds of ***Strychnos nuxvomica*** containing **less than 1.15%** of **strychnine** would be an inferior or substandard drug. If, on the other hand, this drug had **originally** contained the required **1.15%** of strychnine had been partially abstracted with a solvent, the drug would be considered as adulterated or deteriorated.

Milk, offered for sale at retail, is usually required to contain **not less than 3%** of **butterfat**. If milk containing **4%** of butterfat has **water** added to it to reduce the butterfat to **3%,** it is considered **adulterated**, but if the same milk has all the butterfat separated from it in the form of cream and then just enough of this cream is restored to the milk to give the required 3% of butterfat, it is **not considered adulterated**.

The standard for **powdered opium** is not **less than 10%** and not **more than 10.5%** of anhydrous **morphine**. Powdered opium containing a higher percentage of alkaloids may be admixed with inferior or exhausted powdered opium to bring the amount of alkaloids in the mixture to the stated percentage. Such an admixture is **not constructed as adulteration**.

The official compendia permit this practice to be followed for powdered extracts and they list the permissible diluents that may be used.

2- Spoilage:

Spoilage refers to a form of substandard drug in which the **quality** or **value** or **usefulness** of the article has been **impaired or destroyed** by the action of **fungi** and **bacteria** as to render the article unfit for human consumption.

Many examples of spoilage are found in the food industry, particularly in fresh fruits, vegetables, meats, fish and seafood.

All drugs which are unfit for human or animal consumption are legally considered as adulterated.

3- Deterioration: فساد

It is applied to any **impairment** of the **quality** or **value** of an article by the **abstraction** or d**estruction** of valuable constituents by **distillation, extraction, aging, moisture, heat, fungi, insects.**

1- Whole **cloves** from which part of the volatile oil has been removed by distillation "spent cloves".

2- Ground **linseed** from which part of the fixed oil has been expressed "linseed cake".

3- **Lard** in which the fats have to some extend decomposed to form fatty acids" rancid lard".

4- Powdered **squill** that has hardened through absorption of moisture.

5- **Coffee** that has largely lost its caffeine through over roasting.

6- **Ergot** that is moldy and **rhubarb** that has become "wormy" are examples of deterioration.

4- Admixture: مزيج

It is the addition of one article to another through **accident**, **ignorance** or **carelessness.** If the admixture is done **intentionally** to **defraud**, it is sophistication. **Buchu** containing a few stem or root with some adhering soil would be classed as admixtures. If, however, an admixture exceeds the established standard, it legally becomes an adulteration.

5- Sophistication:

It is addition of a spurious or inferior material to any article with **intent to defraud**. The addition of **wheat flour** to powdered **ginger**, with enough **capsicum** to restore or enhance the **pungency** and enough **curcuma** to maintain the **color**, would represent a typical example of **sophistication (which is sometimes referred to as true adulteration).**

6- Substitution:

It is occur when an **entirely different article** is used or sold in place of the one required or requested. A **complete substitution**, even through **intentional** and **fraudulent**, is none of the true article is present. All types of substitution are considered legally as **adulteration**. **Cottonseed oil** sold as **olive oil** and **American saffron** sold as **Spanish saffron** are examples of substitution.

Too often we hear the expression: **“Since herbal products are natural, they are safe”.** Unfortunately, this is **not always true**, due to a many factors including the following:

1- Factual information about many herbs is often **lacking**.

2- **A portion** of the information available about many herbs currently on the market may be **exaggerated** or **misleading.**

This is especially true of some individuals and organizations that are mainly interested in marketing their products **only for profit**, with little regard for the consumer.

3-**The lack** of **adequate quality control** present in some herbal product companies around the world leads to **variability** in the **quantity and quality** of the products' content. Added to this is the **practice of mislabeling** some herbal supplements, making the use of these products confusing, risky, or simply ineffective.

4- Fortunately, situation is now changing, as some herbal organizations that include competent technical staff are now establishing good manufacturing practices (GMP).

5- Some of the multilevel marketing schemes involving herbal products and other supplements tend to foster self-diagnosis, as well as self-medication, both of which can be potentially dangerous to the consumer.

6- Some herbal products which are new to the Western market, originating in Asia, Africa or South America, for example, have not been adequately tested for purity, safety or efficacy, prior to their introduction into the United States.

7- Some plants have not been adequately studied with respect to their use by special populations, including pregnant or lactating women, small children and the elderly, for example.

8- Interactions between herbal products and conventional medications can sometimes be serious. Much still needs to be learned about this important topic.



What are the risks of using natural herbs during pregnancy?

Although herbs are natural, not all herbs are safe to take during pregnancy. **The FDA urges pregnant women** not to take any herbal products without talking to doctors first and to consult a trained and experienced herbalist if they want to take herbs during their pregnancies.

Herbs may contain substances that can cause: miscarriage, premature birth, uterine contractions, or injury to the fetus.

Herbs to avoid during pregnancy

Rosemary is considered **Likely Safe** when used orally in amounts typically found in foods. (Rosemary has a Generally Recognized as Safe (GRAS) status in the US.) **But** in pregnancy, Rosemary is considered Possibly UnSafe when used orally in medicinal amounts. Rosemary might have **uterine and menstrual flow stimulant effects**. avoid using.

Garlic, **Sage, Ginger** and **Tumeric**, All of these herbs could be contraindicated in pregnancy when used in large or concentrated doses, but are considered safe when used in amounts found in food.

Saw Palmetto - when used orally, has hormonal activity

Goldenseal - when used orally, may cross the placenta

Dong Quai - when used orally, due to uterine stimulant and relaxant effects

Blue Cohosh - when used orally; uterine stimulant and can induce labor

Roman Chamomile - when used orally in medicinal amounts

Are there any herbs that are recommended in pregnancy?

Choosing to use herbs during pregnancy is a personal choice, but to ensure the best outcome for you and your baby, you should be **well educated on**

1- the types of herbs,

2- parts of the herb (root, leaf, etc…) and

3- the way that they could be used (caplet, tonic, tea).

The herbs that are considered safe to use during pregnancy are often **food** or **tonic herbs**. These often will be found in either tablet form, tea, or infusion form.

Herbs used in Pregnancy

The following herbs have been rated **Likely Safe** or **Possibly Safe** for use during pregnancy:

1- Red Raspberry Leaf - Rich in iron, this herb has

- helped tone the uterus,
- increase milk production,
- decrease nausea,
- ease labor pains.

Some studies have even reported that using **red raspberry leaf** during pregnancy can **reduce complications** during birth.

Pregnancy teas that are made from **red raspberry leaf** to help **promote uterine health during pregnancy**.

There is some controversy about whether this should be used throughout pregnancy or just in the second and third trimester, so many health care providers remain cautious and **only** recommend using it **after the first trimester**.

2- Peppermint Leaf - Helpful in relieving nausea/morning sickness and flatulence.

3- Ginger root - Helps relieve nausea and vomiting

4- Slippery Elm Bark - (when the inner bark is used orally in amounts used in foods) Used to help relieve nausea, heartburn, and vaginal irritations

5- Oats & Oat Straw - Rich in calcium and magnesium; helps relieve anxiety, restlessness, and irritated skin

6- Garlic - when used orally in amounts commonly found in foods

7- Capsicum (hot pepper) - when used topically and appropriately.

The following herbs have been rated as having Insufficient Reliable Information Available by the Natural Medicines Database, although many are recommended by homeopathic physicians, herbalists, and midwives who treat pregnant women.

1- Dandelion - Rich in Vitamin A, calcium, and iron; dandelion root and leaf can also help relieve mild edema and nourish the liver.

2- Chamomile (German) - High in calcium and magnesium; also helps with sleeplessness and inflammation of joints

3- Nettles (Stinging Nettles) - High in vitamins A, C, k , calcium, potassium, and iron. Used in many pregnancy teas because it is a great all around pregnancy tonic.

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