PES MODERN COLLEGE OF PHARMACY (FOR LADIES), MOSHI

Lecture Synopsis — Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

SR NO.	Topic	Content
1.	Flash Chromatography	Theory
2.	Flash Chromatography	Instrumentation
3.	Flash Chromatography	Applications
4.	Supercritical Fluid Chromatography	Theory
5.	Supercritical Fluid Chromatography	Instrumentation
6.	Supercritical Fluid Chromatography	Instrumentation
7.	Supercritical Fluid Chromatography	Instrumentation
8.	Supercritical Fluid Chromatography	Applications
9.	Automated methods of analysis	Flow injection analysis, Introduction
10.	Automated methods of analysis	Flow injection analysis, Instrumentation
11.	Automated methods of analysis	Flow injection analysis, Applications
12.	Ion exchange Chromatography	Theory
13.	Ion exchange Chromatography	Theory
14.	Ion exchange Chromatography	Instrumentation
15.	Ion exchange Chromatography	Instrumentation
16.	Ion exchange Chromatography	Applications
17.	Mass Spectrometry	Theory
18.	Mass Spectrometry	Theory, RESOLUTION
19.	Mass Spectrometry	Instrumentation
20.	Mass Spectrometry	Instrumentation
21.	Mass Spectrometry	Different methods/techniques of ionization (EI,CI)
22.	Mass Spectrometry	FAB,ESI and MALDI)
23.	Mass Spectrometry	Resolution
24.	applications of mass spectrometry	Applications of mass spectrometry
25.	Mass Spectrometry	Applications
26.	Mass Spectrometry	Introduction to GC-MS
27.	Mass Spectrometry	LC-MS, MS-MS
28.	Nuclear Magnetic resonance	Theory
29.	Nuclear Magnetic resonance	Chemical and Magnetic Equivalence,Solvents
30.	Nuclear Magnetic resonance	Chemical shift, shielding-deshielding
31.	Nuclear Magnetic resonance	Spin-Spin Coupling (Splitting), Coupling Constant,
32.	Nuclear Magnetic resonance	Instrumentation

33.	Nuclear Magnetic resonance	Instrumentation
34.	Nuclear Magnetic resonance	Factors affecting chemical shift
35.	Nuclear Magnetic resonance	Factors affecting chemical shift
36.	Nuclear Magnetic resonance	Factors affecting chemical shift
37.	Nuclear Magnetic resonance	Anisotropy
38.	Nuclear Magnetic resonance	Shift reagents
39.	Nuclear Magnetic resonance	Double resonance
40.	Nuclear Magnetic resonance	Application and simple structure determination.
41.	Nuclear Magnetic resonance	Application and simple structure determination.
42.	Nuclear Magnetic resonance	Introduction to ^{13}C NMR
43.	Electron Spin Resonance	ESR: Introduction, principal
44.	Electron Spin Resonance	ESR: Hyperfine splitting
45.	Electron Spin Resonance	ESR: instrumentation

Lecture No: 1

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Name of topic/lesson – Flash Chromatography (Medium Pressure Liquid Chromatography, MPLC)

Subtopic: Introduction

Topic Outcomes: At the end of topic you should be

1. Able to define flash chromatography
2. Differentiate it from other column chromatographic techniques

---

Flash chromatography is a process in which a vertical glass (**column**) is placed, and that **column** is packed with the solid stationary phase from which the mobile phase (liquid form) is passed through it by gravity or some external pressure.

Flash chromatography is an inexpensive and very useful technique for quickly separating increasing quantities of samples. It is predictable and easy to scale up and down as required.

1. Majorly purification Technique
2. With good performance, improved speed
3. Particle size of stationary phase: 230-400 # (40-60 µm)
4. Medium pressure is used to pump mobile phase (200 psi)
5. Low cost in comparison to HPLC
6. Flow rate of Mobile phase: 1ml-1000ml/min
7. High sample loading
8. Majorly, it is a normal phase chromatography

Used Stationary phases (Silica> Amino> C18> diol, alumina, ion exchange Cyano)

References

1. Principles of Chromatography by KR Mahadik, K G Bothara, 1st edition, Nirali Prakashan.

2. Introduction to Chromatography (Theory and Practice) by VK Srivastav and KK Shrivastav

Lecture No: 2

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Name of topic/lesson – Flash Chromatography (Medium Pressure Liquid Chromatography, MPLC)

Subtopic: Instrumentation

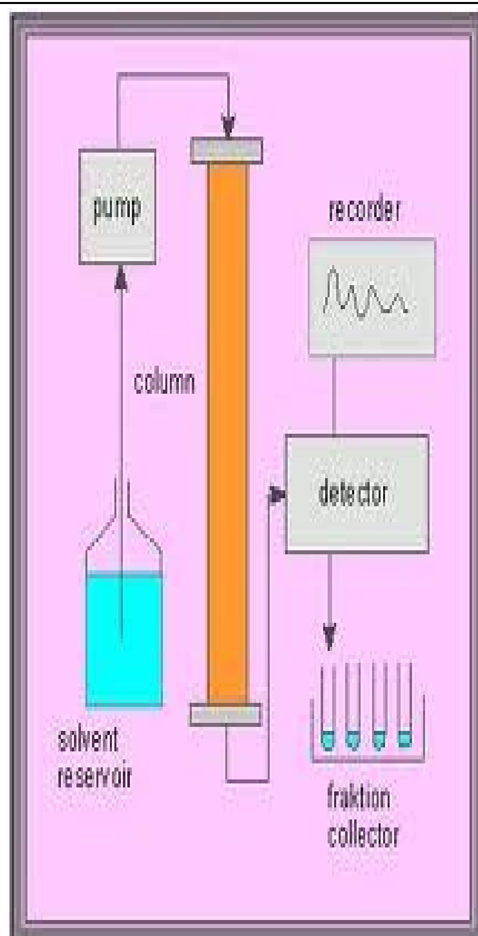
Topic Outcomes: At the end of topic you should be

1. Draw block diagram of Flash chromatography

2. Explain the functions of each part

Important components:

- Solvent Reservoir
- Pump (Gradient formation)
- Sample introduction
- Column
- Detector (UV, ELSD, Refractive index detector)
- Flash fraction collector
- Recorder



References

1. Principles of Chromatography by KR Mahadik, K G Bothara, 1st edition, Nirali Prakashan.

2. Introduction to Chromatography (Theory and Practice) by VK Srivastav and KK Shrivastav

---

Lecture No: 3

Name of topic/lesson – Flash Chromatography (Medium Pressure Liquid Chromatography, MPLC)

Subtopic: Applications

Topic Outcomes: At the end of topic you should be

1. Know the applications of Flash Chromatography

APPLICATIONS OF FLASH CHROMATOGRAPHY

1. Purification Of Peptides
2. Seperation Of Closely Related Organic Compounds
3. For High Speed Fractionation Of Natural Products
4. Purify, Collect and Identify the Aromatic and Heterocyclic Compounds.
5. Isolation of Organic Compounds from Synthetic Mixtures.

References

1. Principles of Chromatography by KR Mahadik, K G Bothara, 1st edition, Nirali Prakashan.

2. Introduction to Chromatography (Theory and Practice) by VK Srivastav and KK Shrivastav

---

Topic: Supercritical Fluid Chromatography

Subtopic: Theory

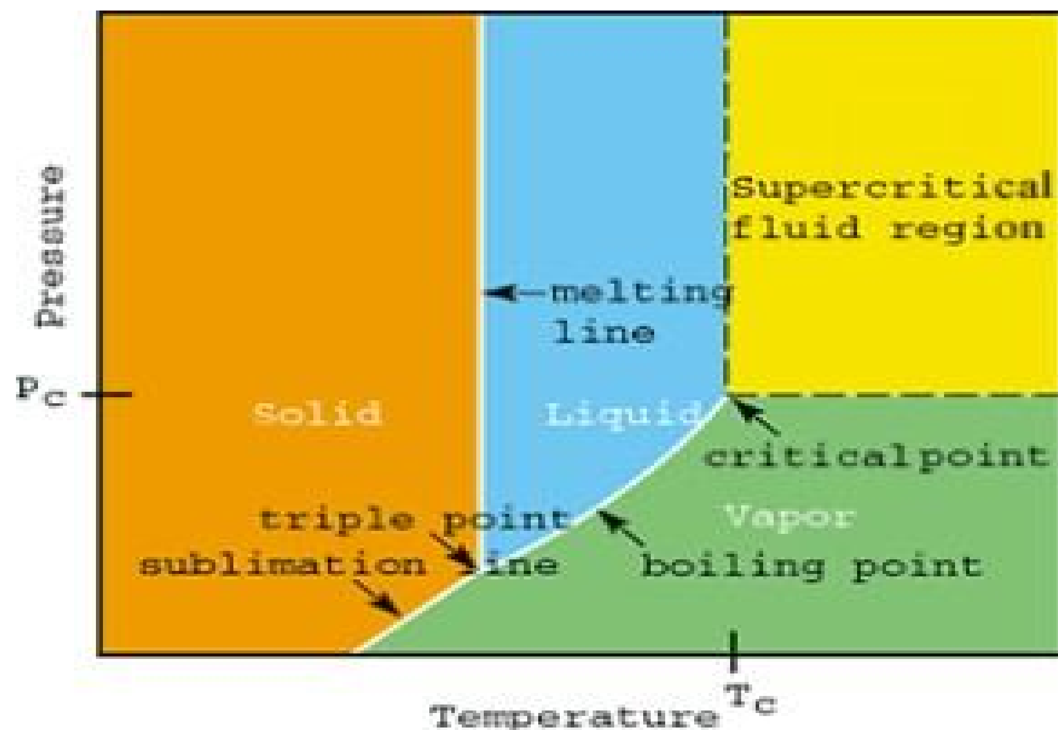
Objectives:

- To study principle of supercritical fluid chromatography.
- To study various supercritical fluids
- To study various stationary phases used in SFC.

Topic outcomes:

- Defines and Know advantages of SFC

A supercritical fluid is the phase of a material at critical temperature and critical pressure of the material. Critical temperature is the temperature at which a gas cannot become liquid as long as there is no extra pressure; and, critical pressure is the minimum amount of pressure to liquefy a gas at its critical temperature. Supercritical fluids combine useful properties of gas and liquid phases, as it can behave like both a gas and a liquid.



Advantages of SFC : Inexpensive, Ecofriendly, Nontoxic

Common SFC: Carbon dioxide, Water, Ethane, Ethylene, Propane, Propylene

Physical Properties of Supercritical Fluids

As mentioned above, SF shares some common features with both gases and liquids. This enables us to take advantage of a correct combination of the properties.

Density

Density characteristic of a supercritical fluid is between that of a gas and a liquid, but closer to that of a liquid. In the supercritical region, density of a supercritical fluid increases with increased pressure (at constant temperature). When pressure is constant, density of the material decreases with increasing temperature. The dissolving effect of a supercritical fluid is dependent on its density value. Supercritical fluids are also better carriers than gases due to their higher density.

Diffusivity

Diffusivity of a supercritical fluid can be 100 x that of a liquid and $^{1}/_{1,000}$ to $^{1}/_{10,000}$ x less than a gas. Because supercritical fluids have more diffusivity than a liquid, it stands to reason a solute can show better diffusivity in a supercritical fluid than in a liquid. Diffusivity is parallel with temperature and contrary with pressure. Increasing pressure affects supercritical fluid molecules to become closer to each other and decreases diffusivity in the material. The greater diffusivity gives supercritical fluids the chance to be faster carriers for analytical applications. Hence, supercritical fluids play an important role for chromatography and extraction methods.

Viscosity

Viscosity for a supercritical fluid is almost the same as a gas, being approximately $^{1}/_{10}$ of that of a liquid. Thus, supercritical fluids are less resistant than liquids towards components flowing through. The viscosity of supercritical fluids is also distinguished from that of liquids in that temperature has a little effect on liquid viscosity, where it can dramatically influence supercritical fluid viscosity.

These properties of viscosity, diffusivity, and density are related to each other. The change in temperature and pressure can affect all of them in different combinations. For instance, increasing pressure causes a rise for viscosity and rising viscosity results in declining diffusivity.

References

1. Instrumental Methods of Analysis by Skoog

Lecture No: 5

Topic: Supercritical Fluid Chromatography

Subtopic: Instrumentation (block Diagram)

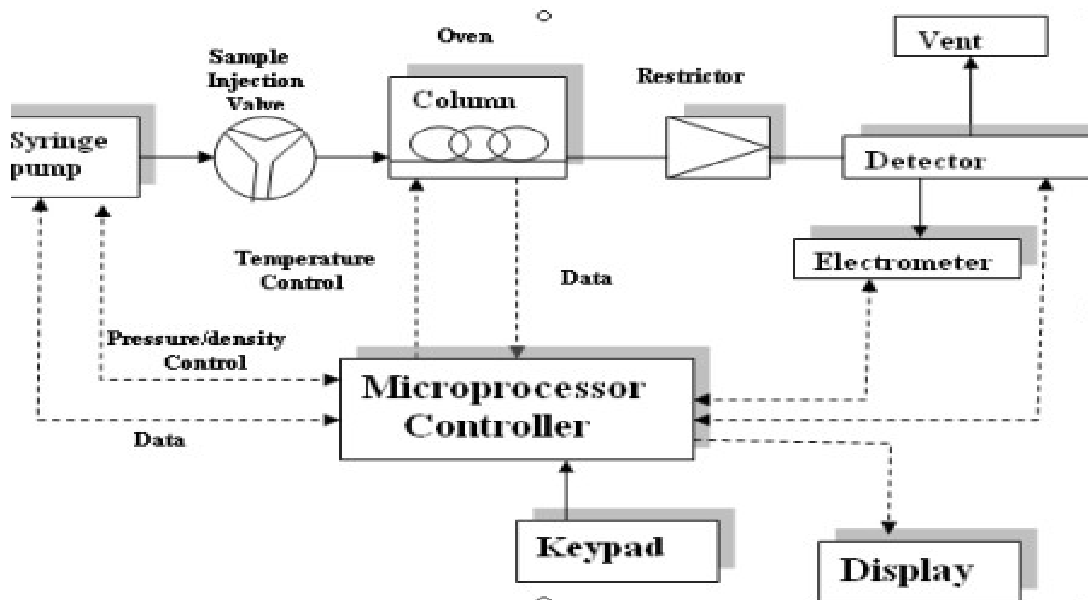
Objectives:

- To study various components of SFC

Topic outcomes:

- You will be able to draw block diagram of SFC
- You will know function of each component

Gas reservoirs, Pumps, Injector, Oven, Columns, Restrictor or Back-Pressure Device, Microprocessor, Detector



References

1. Instrumental Methods of Analysis by Skoog

Lecture No: 6

Topic: Supercritical Fluid Chromatography

Subtopic: Instrumentation (columns and stationary phases)

Objectives:

- To study various types of columns and stationary phases used in SFC

Topic outcomes:

- You will be able to differentiates between various types of stationary phases and columns used in SFC
- you will be able to select correct stationary phase depending upon the analyte nature

Types of Stationary Phases

- Particle based (silica and polymer based)
- Monolithic (silica and polymer based)
- HILIC and mixed beds (Hydrophilic interaction)
- Hybrid Packing
- Packing based on Zirconia or titania

References

1. Instrumental Methods of Analysis by Skoog

Lecture No: 7

Topic: Supercritical Fluid Chromatography

Subtopic: Instrumentation (Mobile Phases, Detector)

Objectives:

- To study various detectors used in SFC

Topic outcomes:

- You will be able to select correct detector depending upon the nature of analyte
- You will be able to compare various types of mobile phases

Mobile Phase:

The most widely used mobile phase for SFC is carbon dioxide. It is an excellent solvent for a variety of organic molecules. In addition, it transmits in the ultraviolet and is odorless, nontoxic, readily available, and remarkably inexpensive when compared with other chromatographic mobile phases.

Detectors

1. High Pressure detector
2. Low pressure detector

We can uses all the detectors used in Gas chromatography as well as HPLC. The supercritical fluid can be converted in to liquid or gaseous phase depending upon the pressure condition and detector has to be selected based on the phase of mobile phase.

References

1. Instrumental Methods of Analysis by Skoog

Lecture No: 8

Topic: Supercritical Fluid Chromatography

Subtopic: Applications

Objectives:

- To study applications of SFC

Topic outcomes:

- You will know various applications of SFC

- **In clinical research laboratories**

Clinical research laboratories have two main goals:

- Identification of molecules in body fluids (blood, urine, cerebrospinal fluid) or tissues that (1) are toxic, (2) indicate a predisposition to disease, or (3) have therapeutic efficacy—any of which may serve as a marker.
- Development of drugs that is safe and effective for specific disease conditions.

In both, the absolute purity of the molecule of interest is of primary importance; undetected impurities may lead to lesser efficacies or toxic side effects. Biomolecules are chiral, and living systems are known to have preferences for specific enantiomers for almost every molecule. The absorption, metabolism, and excretion of enantiomers are also known to be quite different in living systems. For these reasons, the FDA (Food and Drug Administration) in the United States and the CHMP (Committee for Medicinal Products for Human Use) in the European Union have issued guidelines for pharmaceutical drug use—that only therapeutically effective enantiomers of chiral drugs can be released into the market. SFC has met the chromatography needs of the pharmaceutical industry by providing efficient and selective testing capabilities on the analytical, semi-preparative, and preparative scale.
It has been helpful in all stages of pharmaceutical drug preparation:

- Chiral separation of the enantiomers of a molecule
- Purification of each of the enantiomers in sufficient quantities to permit a study of the enantiomer's pharmacokinetic and metabolic properties
- Identification of the enantiomer of choice as a possible therapeutic agent
- Purification on higher (production) scales

References

1. Instrumental Methods of Analysis by Skoog

Lecture No: 9
Name of topic/lesson – Flow injection analysis (FIA)
Subtopic: Theory, Introduction
Topic Outcomes: At the end of topic you will

1.Know principle and theory of Flow injection analysis

FIA is an automated method of chemical analysis in which a sample is injected into a flowing carrier solution that mixes with reagents before reaching a detector.

FIA techniques developed into a wide array of applications using spectrophotometry, fluorescence spectroscopy, atomic absorption spectroscopy, mass spectrometry, and other methods of instrumental analysis for detection.

Automated sample processing, high repeatability, adaptability to micro-miniaturization, containment of chemicals, waste reduction, and reagent economy in a system that operates at microliter levels are all valuable assets that contribute to the application of flow injection to real-world assays.

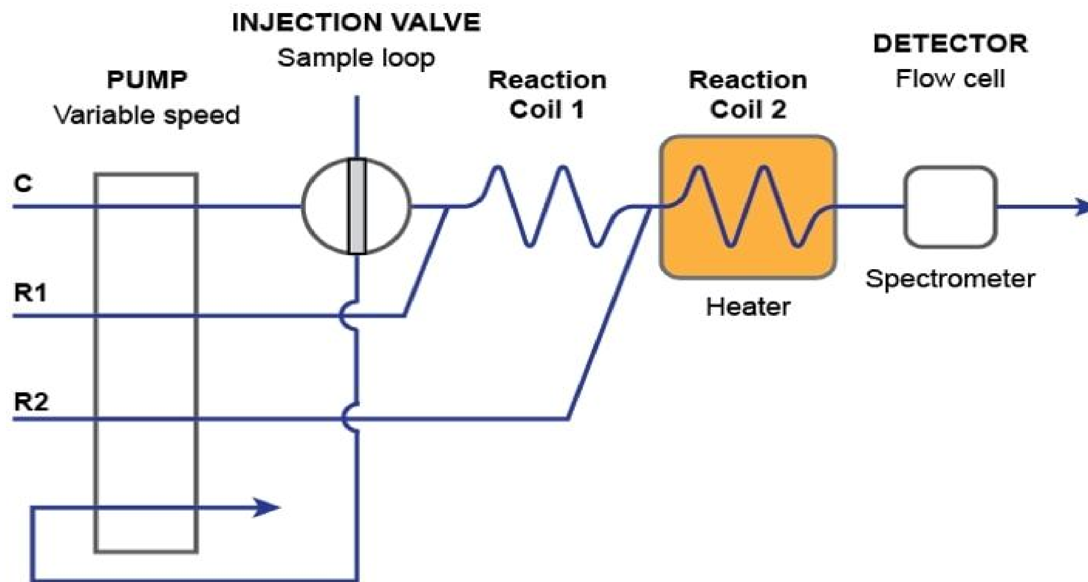
A sample (analyte) is injected into a flowing carrier solution stream that is forced by a peristaltic pump. The injection of the sample is done under controlled dispersion in known volumes. The carrier solution and sample then meet at mixing points with reagents and react. The reaction time is controlled by a pump and reaction coil. The reaction product then flows through a detector. Most often, the detector is a spectrophotometer as the reactions usually produce a colored product. One can then determine the amount of an unknown material in the sample as it is proportional to the absorption spectrum given by the spectrophotometer. After moving through the detector, the sample then flows to waste.

Reference:

https://chem.libretexts.org/Courses/Northeastern_University/13%3A_Kinetic_Methods/13.4%3A_Flow_Injection_Analysis

Lecture No: 10
Name of topic/lesson – Flow injection analysis
Subtopic: Instrumentation
Topic Outcomes: At the end of topic you will

1.Know instrumentation of Flow injection analysis



Instrumentation includes a **pump** for propelling the carrier stream and reagent streams, a means for **injecting the sample** into the carrier stream, and a **detector** for monitoring the composition of the carrier stream. Connecting these units is **a transport system** that brings together separate channels and provides time for the sample to mix with the carrier stream and to react with the reagent streams.

Propelling Unit

The propelling unit moves the carrier stream through the flow injection analyzer. Although several different propelling units have been used, the most common is a **peristaltic pump**. A peristaltic pump consists of a set of rollers attached to the outside of a rotating drum. Tubing from the reagent reservoirs fits between the rollers and a fixed plate. As the drum rotates the rollers squeeze the tubing, forcing the contents of the tubing to move in the direction of the rotation. Peristaltic pumps provide a constant flow rate, which is controlled by the drum's speed of rotation and the inner diameter of the tubing. Flow rates from 0.0005–40 mL/min are possible, which is more than adequate to meet the needs of FIA where flow rates of 0.5–2.5 mL/min are common. One limitation to a peristaltic pump is that it produces a pulsed flow—particularly at higher flow rates—that may lead to oscillations in the signal.

Injector

The sample, typically 5–200 µL, is injected into the carrier stream. Although syringe injections through a rubber septum are possible, the more common method is to use a rotary, or loop injector similar to that used in an HPLC. This type of injector provides for a reproducible sample volume and is easily adaptable to automation, an important feature when high sampling rates are needed.

Detector

The most commonly used detectors for flow injection analysis are the electrochemical and optical detectors used in HPLC. FIA detectors also have been designed around the use of ion selective electrodes and atomic absorption spectroscopy.

A flow-through detector is located downstream from the sample injector and records a chemical physical parameter. Many types of detector can be used such as:

- spectrophotometer
- fluorimeter
- ion-selective electrode
- biosensors
- mass spectrometer

Transport System

The heart of a flow injection analyzer is the transport system that brings together the carrier stream, the sample, and any reagents that react with the sample. Each reagent stream is considered a separate channel, and all channels must merge before the carrier stream reaches the detector. The complete transport system is called a **manifold**.

The simplest manifold includes only a single channel. This type of manifold is commonly used for direct analyses that do not require a chemical reaction. In this case the carrier stream serves only as a means for rapidly and reproducibly transporting the sample to the detector. For example, this manifold design has been used for sample introduction in atomic absorption spectroscopy, achieving sampling rates as high as 700 samples/h. A single-channel manifold also is used for determining a sample's pH or determining the concentration of metal ions using an ion selective electrode.

Reference:

https://chem.libretexts.org/Courses/Northeastern_University/13%3A_Kinetic_Methods/13.4%3A_Flow_Injection_Analysis

Lecture No: 11

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Name of topic/lesson – Flow injection analysis

Subtopic: Applications

Topic Outcomes: At the end of topic you will

1.Know applications of Flow injection analysis

Flow injection analysis has been used to analyze a wide variety of samples, including environmental, clinical, agricultural, industrial, and pharmaceutical samples. The majority of analyses involve environmental and clinical samples.

Quantitative analytical flow injection analytical methods have been developed for cationic, anionic, and molecular pollutants in wastewater, freshwaters, groundwaters, and marine waters.

Flow injection techniques have proven very useful in marine science for both organic and inorganic analytes in marine animal samples/seafood. Flow Injection methods applied to the determination of amino acids (histidine, L-lysine and tyrosine), DNA/RNA, formaldehyde, histamine, hypoxanthine, polycyclic aromatic hydrocarbons, diarrheic shellfish poisoning, paralytic shellfish poisoning, succinate/glutamate, trimethylamine/ total volatile basic nitrogen, total lipid hydroperoxides, total volatile acids, uric acid, vitamin B12, silver, aluminium, arsenic, boron, calcium, cadmium, cobalt, chromium, copper, iron, gallium, mercury, indium, lithium, manganese, molibdenum, nickel, lead, antimony, selenium, tin, strontium, thallium, vanadium, zinc, nitrate/nitrite, phosphorus/phosphate and silicate.

Reference:

https://chem.libretexts.org/Courses/Northeastern_University/13%3A_Kinetic_Methods/13.4%3A_Flow_Injection_Analysis

Lecture No: 12

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Name of topic/lesson – Ion exchange Chromatography

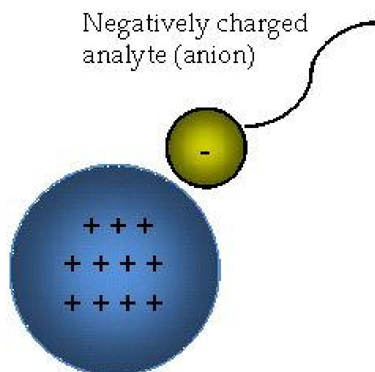
Subtopic: Principle

Topic Outcomes: At the end of topic you will

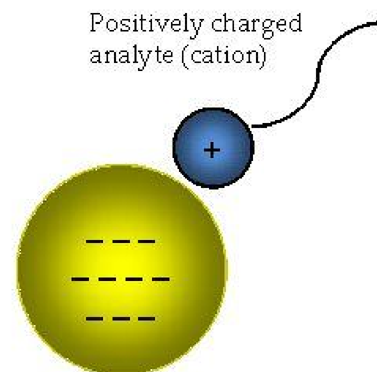
1.Know principle and mechanism involved in IEC

Ion-exchange chromatography (IEC) is part of ion chromatography which is an important analytical technique for the separation and determination of ionic compounds, together with ion-partition/interaction and ion-exclusion chromatography. Ion chromatography separation is based on ionic (or electrostatic) interactions between ionic and polar analytes, ions present in the eluent and ionic functional groups fixed to the chromatographic support. Two distinct mechanisms as follows; ion exchange due to competitive ionic binding (attraction) and ion exclusion due to repulsion between similarly charged analyte ions and the ions fixed on the chromatographic support, play a role in the separation in ion chromatography. Ion exchange has been the predominant form of ion chromatography to date.

The separation is based on the formation of ionic bonds between the charged groups of biomolecules and an ion-exchange gel/support carrying the opposite charge



anion exchanger stationary phase particle



cation exchanger stationary phase particle

References: 1. Instrumental methods of analysis by Willard

2. https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications

Lecture No: 13

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Name of topic/lesson – Ion exchange Chromatography

Subtopic: Theory

Topic Outcomes: At the end of topic you will

1.Know theory of IEC

Ion-exchange chromatography which is designed specifically for the separation of differently charged or ionizable compounds comprises from mobile and stationary phases similar to other forms of column based liquid chromatography techniques. Mobil phases consist an aqueous buffer system into which the mixture to be resolved. The stationary phase usually made from inert organic matrix chemically derivative with ionizable functional groups (fixed ions) which carry displaceable oppositely charged ion. Ions which exist in a state of equilibrium between the mobile phase and stationary phases giving rise to two possible formats, anion and cation exchange are referred to as counter ion. Exchangeable matrix counter ions may include protons (H^+), hydroxide groups (OH^-), single charged mono atomic ions (Na^+, K^+, Cl^-), double charged mono atomic ions (Ca^{2+}, Mg^{2+}), and polyatomic inorganic ions (SO_4^{2-}, PO_4^{3-}) as well as organic bases (NR_2H^+) and acids (COO^-). Cations are separated on cation-exchange resin column and anions on an anion exchange resin column. Separation based on the binding of analytes to positively or negatively charged groups which are fixed on a stationary phase and which are in equilibrium with free counter ions in the mobile phase according to differences in their net surface charge.

Ion exchange chromatography involves separation of ionic and polar analytes using chromatographic supports derivatized with ionic functional groups that have charges opposite that of the analyte ions. The analyte ions and similarly charged ions of the eluent compete to bind to the oppositely charged ionic functional group on the surface of the stationary phase. Assuming that the exchanging ions (analytes and ions in the mobile phase) are cations, the competition can be explained using the following equation;

In this process the cation M^+ of the eluent replaced with the analyte cation C^+bound to the anion X^- which is fixed on the surface of the chromatographic support (S).

In anion exchange chromatography, the exchanging ions are anions and the equation is represented as follow;

S-X+A- + B-↔ S-X+B-+ A-S-X+A- + B-↔ S-X+B-+ A-

The anion B- of the eluent replaced with the analyte cation A- bound to the positively charged ion X+ on the surface of the stationary phase. The adsorption of the analyte to the stationary phase and desorption by the eluent ions is repeated during their journey in the column, resulting in the separation due to ion-exchange.

References: 1. Instrumental methods of analysis by Willard

2.https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications

Lecture No: 14
Name of topic/lesson – Ion exchange Chromatography
Subtopic: Matrices used in IEC
Topic Outcomes: At the end of topic you will

1.Know matrices used in IEC

In order to minimize non-specific interactions with sample components inert matrix should be used. High physical stability provides that the volume of the packed medium remains constant despite extreme changes in salt concentration or pH for improving reproducibility and avoiding the need to repack columns. High physical stability and uniformity of particle size facilitate high flow rates, particularly during cleaning or re-equilibration steps, to improve throughput and productivity. There are pH and pressure limits for each stationary phases. For example pH values higher than 8 should not used in silica based materials which are not coated with organic materials. Matrix stability also should be considered when the chemicals such as organic solvents or oxidizing agents should be required to use or when they are chosen for column cleaning.

Matrices which are obtained by polymerization of polystyrene with varying amounts of divinylbenzene are known as the original matrices for ion exchange chromatography. However these matrices have very hydrophobic surface and proteins are irreversibly damaged due to strong binding. Ion exchangers which are based on cellulose with hydrophilic backbones are more suitable matrices for protein separations. Other ion exchange matrices with hydrophilic properties are based on agarose or dextran.

Several matrix types and their important properties can be listed as follow;

Matrix materials;

Cellulose; Hydrophilic surface, enhanced stability by cross-linking, inexpensive

Dextran; Considerable swelling as a function of ionic milieu, improved materials by cross-linking)

Agarose; Swelling is almost independent of ionic strength and pH, high binding capacity obtained by production of highly porous particles

Polyacrylamide; Swelling behavior similar to dextran

Acrylate-copolymer; High pH stability

Polystyrene-divinilybenzene; Hydrophobic surface, low binding capacity for proteins

Coated polystyrene-divinilybenzene; Hydrophilic surface

Silica; Unstable at pH > 8, rigid particles

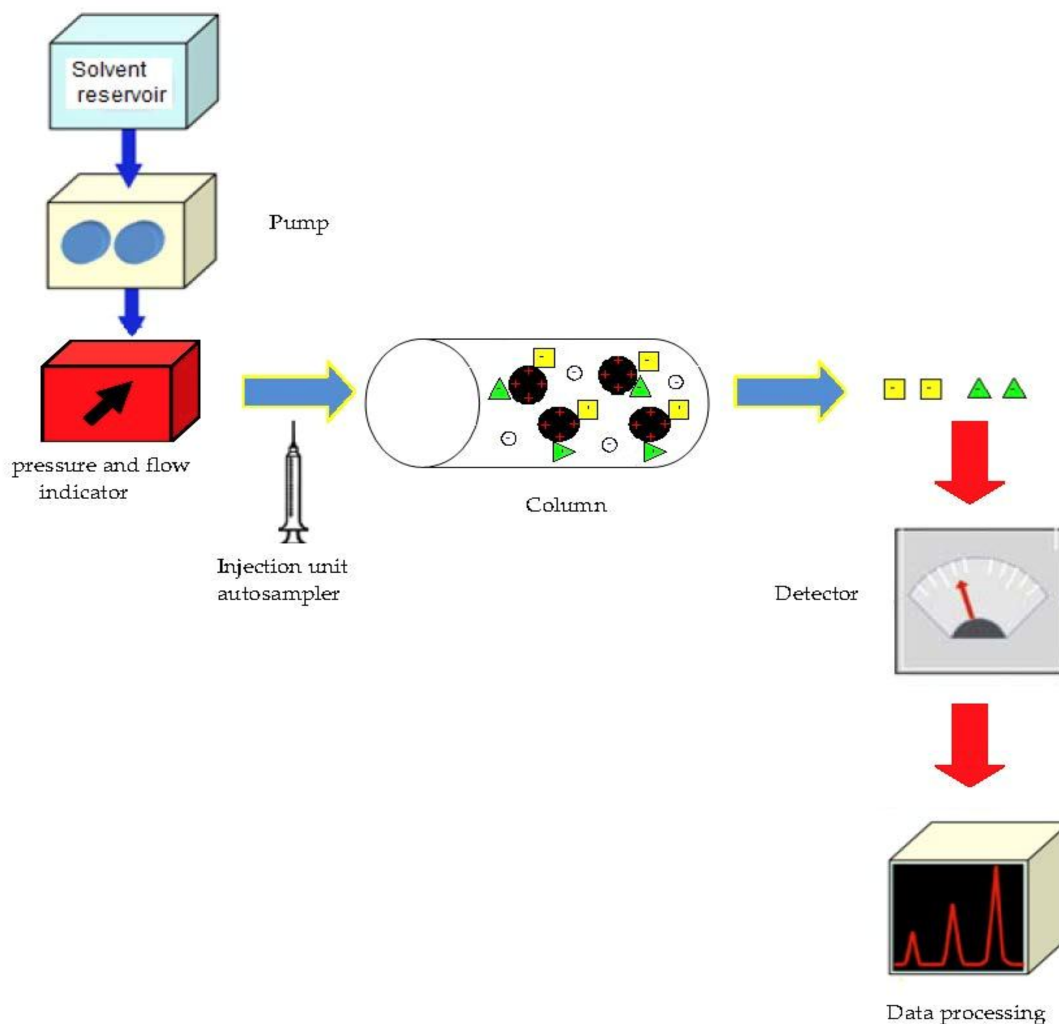
Coated Silica; Hydrophilic surface

Lecture No: 15
Name of topic/lesson – Ion exchange Chromatography
Subtopic: Instrumentation
Topic Outcomes: At the end of topic you will

1.Know instrumentation of IEC

General components of an ion-exchange chromatography are presented as below.

- A high pressure pump with pressure and flow indicator, to deliver the eluent
- An injector for introducing the sample into the eluent stream and onto the column
- A column, to separate the sample mixture into the individual components
- An oven, optional
- A detector, to measure the analyte peaks as eluent from the column
- A data system for collecting and organizing the chromatograms and data



References: 1. Instrumental methods of analysis by Willard

Lecture No: 16
Name of topic/lesson – Ion exchange Chromatography
Subtopic: Applications
Topic Outcomes: At the end of topic you will

1.Know applications of IEC

Ion exchange chromatography can be applied for the separation and purification of many charged or ionizable molecules such as proteins, peptides, enzymes, nucleotides, DNA, antibiotics, vitamins and etc. from natural sources or synthetic origin.

References: 1. Instrumental methods of analysis by Willard

2.https://www.intechopen.com/books/column-chromatography/ion-exchange-chromatography-and-its-applications

Lecture No: 17
Name of topic/lesson – Mass Spectrometry
Subtopic: Principle
Topic Outcomes: At the end of topic you will

1.Know Principle Of Mass Spectrometry

Mass Spectrometry is the generation, separation and characterization of gas phase ions according to their relative mass as a function of charge

Previously, the requirement was that the sample be able to be vaporized (similar limitation to GC), but modern ionization techniques allow the study of such non-volatile molecules as proteins and nucleotides

The technique is a powerful qualitative and quantitative tool, routine analyses are performed down to the femtogram (10^{-15} g) level and as low as the zeptomole (10^{-21} mol) level for proteins

Of all the organic spectroscopic techniques, it is used by more divergent fields – metallurgy, molecular biology, semiconductors, geology, archaeology than any other

Vaccum is needed to-

1. Avoid signal loss

Ions are created in the ion source. However, they may collide with many types of gas, such as carrier gas (He) and residual gas (air, water etc.), on the way to the detector to be unfortunately removed. This collision may happen in a short time with a low vacuum. The average distance of an ion's free flight without collision is called as the "mean free path". If a high vacuum provides a long mean free path exceeding the dimension of the chamber, ions can easily reach the detector. MS is usually operated at around 10-3 to 10-4 Pa pressure, which corresponds to 5m to 50m of a mean free path.

2. Prevent chemical contamination

3. Prevents Arching

Reference: 1. Organic Spectroscopy by P.S. Kalsi

2. Organic Spectroscopy by William Kemp

3. Instrumental methods of Analysis by Skoog

Lecture No: 18
Name of topic/lesson – Mass Spectrometry
Subtopic: THEORY
Topic Outcomes: At the end of topic you will

1.Know theory in Mass Spectrometry

A mass spectrometer needs to perform three functions:

- Creation of ions – the sample molecules are subjected to a high energy beam of electrons, converting some of them to ions
- Separation of ions – as they are accelerated in an electric field, the ions are separated according to mass-to-charge ratio (m/z)
- Detection of ions – as each separated population of ions is generated, the spectrometer needs to qualify and quantify them

The differences in mass spectrometer types are in the different means to carry out these three functions

Common to all is the need for very high vacuum (~ 10^{-6} torr), while still allowing the introduction of the sample

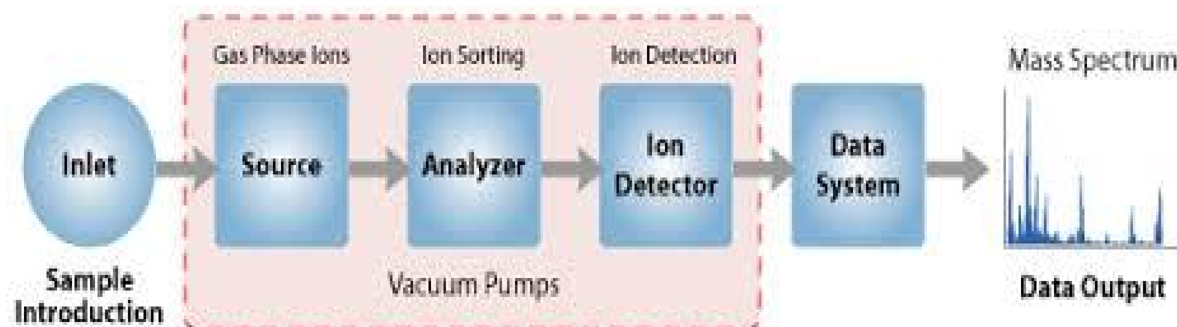
Reference: 1. Organic Spectroscopy by P.S. Kalsi

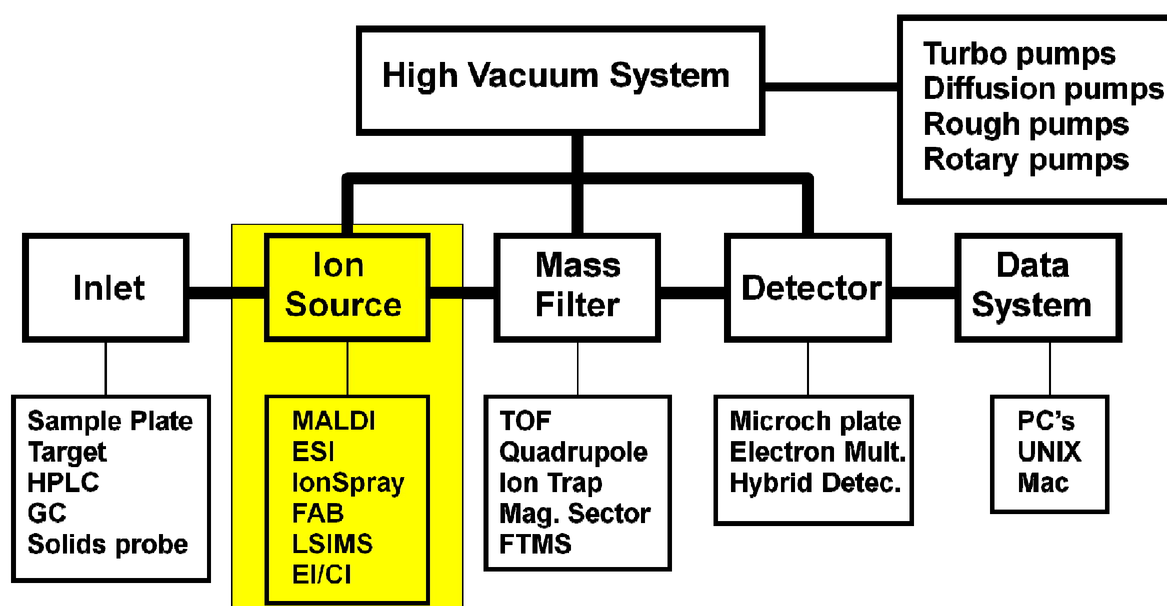
2. Organic Spectroscopy by William Kemp

3. Instrumental methods of Analysis by Skoog

Lecture No: 19
Name of topic/lesson – Mass Spectrometry
Subtopic: Instrumentation
Topic Outcomes: At the end of topic you will

1.Know block diagram of Mass Spectrometry with function of each part





The Mass Spectrometer

A. **Single Focusing Mass Spectrometer**
B. **Double Focusing Mass Spectrometer**
C. **Quadrupole Mass Spectrometer**
D. **Time of Flight Mass Spectrometer**

Reference:
1. Organic Spectroscopy by P.S. Kalsi
2. Organic Spectroscopy by William Kemp
3. Instrumental methods of Analysis by Skoog

Lecture No: 20
Name of topic/lesson – Mass Spectrometry
Subtopic: Instrumentation

Topic Outcomes: At the end of topic you will

1.Know difference between different ionization techniques

Sr. No.	Hard ionisation techniques	Soft ionisation techniques
1.	Causes extensive fragmentation of molecule	Causes little or no fragmentation of molecule
2.	Identity of molecular ion is lost most of the times	Identity of molecular ion canbe done so useful for determination of molecular weight
3.	Compound is vapourised first and then converted to gaseous ions	Compound is directly converted into gaseous ions.
4.	Used for compounds with low boiling point or molecular weight upto 10^3 daltons	Used for compounds with high boiling point or molecular weight upto 10^5 daltons
5.	Eg. Electron Impact, Chemical Impact	Eg. Fast Atomic Bombardment, Matrix assisted laser desorption ionization

Reference: 1. Organic Spectroscopy by P.S. Kalsi

2. Organic Spectroscopy by William Kemp

3. Instrumental methods of Analysis by Skoog

Lecture No: 21

Name of topic/lesson – Mass Spectrometry

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Subtopic: Instrumentation

Topic Outcomes: At the end of topic you will

1.Know different methods/techniques of ionization (EI,CI)

Hard ionization technique	Soft Ionization technique
Electron Impact, EI	Matrix Assisted Laser Desorption Ionization (MALDI)
Chemical Ionization, CI	Atmospheric Pressure Chemical Ionization, APCI
Field Ionization	Fast Atom Bombardment, FAB
	Electro Spray Ionization

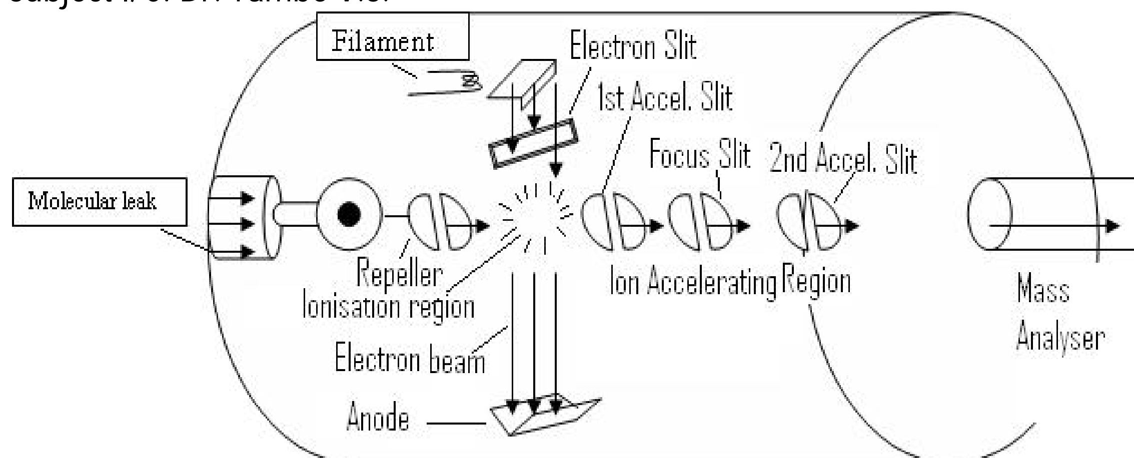
Electron Impact, as an Ionization source

- Ionization involves converting molecule into molecular ion
- It is a hard ionization technique
- Electron Impact, EI, most common
- Molecule is bombarded with electron with 70 eV standard ionization energy
- electron is deflected but transfers much of its energy to the molecule
- This energy-rich species ejects an electron.
- $M + e^- \Rightarrow M^+ + 2e^-$
- But sometime 70 eV too powerful

Molecular ion passes between poles of a magnet and is deflected by magnetic field. amount of deflection depends on mass-to-charge ratio highest m/z deflected least
lowest m/z deflected most.

If the only ion that is present is the molecular ion, mass spectrometry provides a way to measure the molecular weight of a compound and is often used for this purpose.

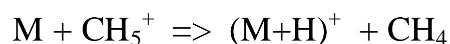
However, the molecular ion often fragments to a mixture of species of lower m/z.



Chemical Ionization

A softer ionization technique

Use ionized molecules to transfer protons (+)



$M + CH_5^+ \Rightarrow (M+H)^+ + CH_4$

Chemical Ionization gases: methane, isobutane

Reference: 1. Organic Spectroscopy by P.S. Kalsi

2. Organic Spectroscopy by William Kemp

3. Instrumental methods of Analysis by Skoog

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Lecture No: 22

Name of topic/lesson – Mass Spectrometry

Subtopic: Instrumentation

Topic Outcomes: At the end of topic you will

1.Know different methods/techniques of ionization (FAB,ESI and MALDI)

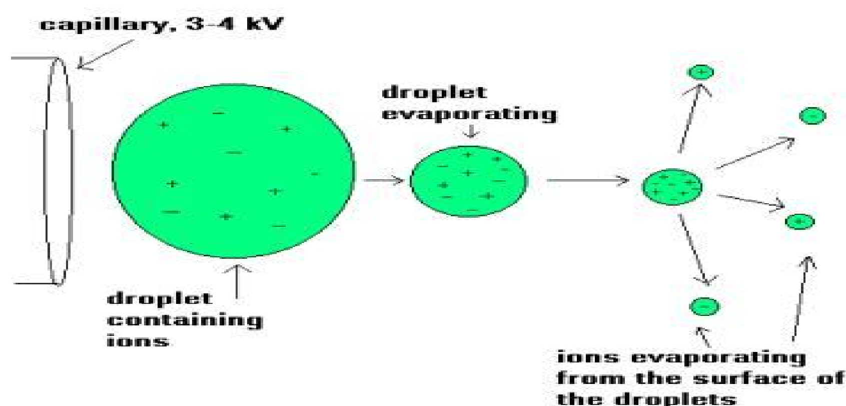
Electrospray mass spectrometry (ESI-MS)

Liquid containing analyte is forced through a steel capillary at high voltage to electrostatically disperse analyte. Charge imparted from rapidly evaporating liquid.

Matrix-assisted laser desorption ionization (MALDI)

Analyte (protein) is mixed with large excess of matrix (small organic molecule)

Irradiated with short pulse of laser light. Wavelength of laser is the same as absorbance max of matrix.



MALDI

- Sample is ionized by bombarding sample with laser light
- Sample is mixed with a UV absorbant matrix (sinapinic acid for proteins, 4-hydroxycinnaminic acid for peptides)
- Light wavelength matches that of absorbance maximum of matrix so that the matrix transfers some of its energy to the analyte (leads to ion sputtering)

Reference: 1. Organic Spectroscopy by P.S. Kalsi

2. Organic Spectroscopy by William Kemp

3. Instrumental methods of Analysis by Skoog

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Lecture No: 23

Name of topic/lesson – Mass Spectrometry

Subtopic: Resolution

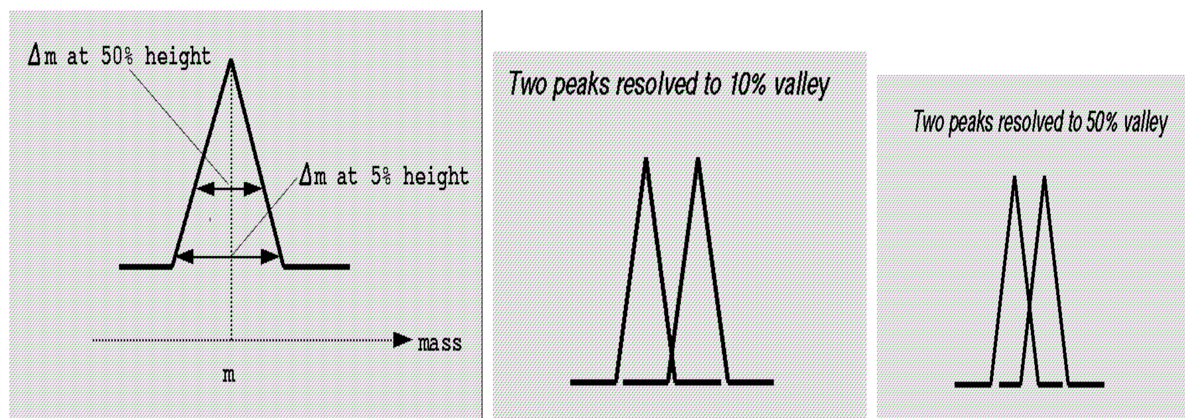
Topic Outcomes: At the end of topic you will

1.Know resolution of Mass Spectrometry

- Width of peak indicates the resolution of the MS instrument
- The better the resolution or resolving power, the better the instrument and the better the mass accuracy

Resolving power is defined as: ΔM/M

M is the mass number of the observed mass (DM) is the difference between two masses that can be separated



Reference: 1. Organic Spectroscopy by P.S. Kalsi

2. Organic Spectroscopy by William Kemp

3. Instrumental methods of Analysis by Skoog

Subject I/C: Dr. Tambe V.S.

Lecture No: 24

Name of topic/lesson – Mass Spectrometry

Subtopic: Applications of mass spectrometry

Topic Outcomes: At the end of topic you will

1. Know applications of Mass Spectrometry

- Detect and identify the use of steroids in athletes
- Monitor the breath of patients by anesthesiologists during surgery
- Determine the composition of molecular species found in space
- Determine whether honey is adulterated with corn syrup
- Monitor fermentation processes for the biotechnology industry
- Detect dioxins in contaminated fish
- Establish the elemental composition of semiconductor materials
- Perform forensic analysis – arson identification
- Determine exact atomic mass and isotope abundance

Reference:
1. Organic Spectroscopy by P.S. Kalsi
2. Organic Spectroscopy by William Kemp
3. Instrumental methods of Analysis by Skoog

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Lecture No: 25

Name of topic/lesson – Mass Spectrometry

Subtopic: Applications of mass spectrometry

Topic Outcomes: At the end of topic you will

1.Know applications of Mass Spectrometry

- Pharmaceutical analysis
- Bioavailability studies
- Drug metabolism studies, pharmacokinetics
- Characterization of potential drugs
- Drug degradation product analysis
- Screening of drug candidates
- Identifying drug targets
- Biomolecule characterization
- Proteins and peptides
- Oligonucleotides
- Environmental analysis
- Pesticides on foods
- Soil and groundwater contamination
- Forensic analysis/clinical

Reference: 1. Organic Spectroscopy by P.S. Kalsi

2. Organic Spectroscopy by William Kemp

3. Instrumental methods of Analysis by Skoog

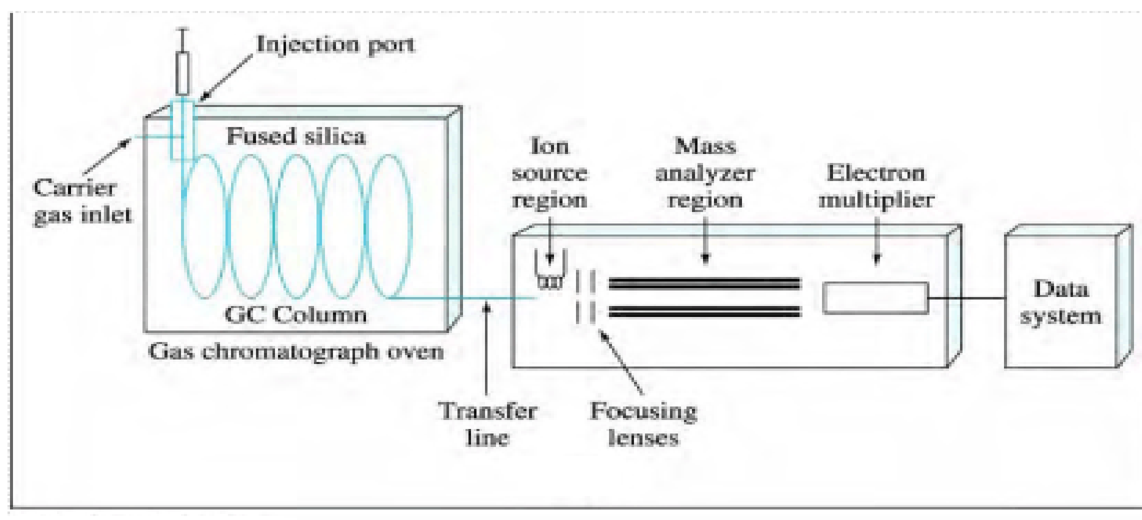
Lecture No: 26

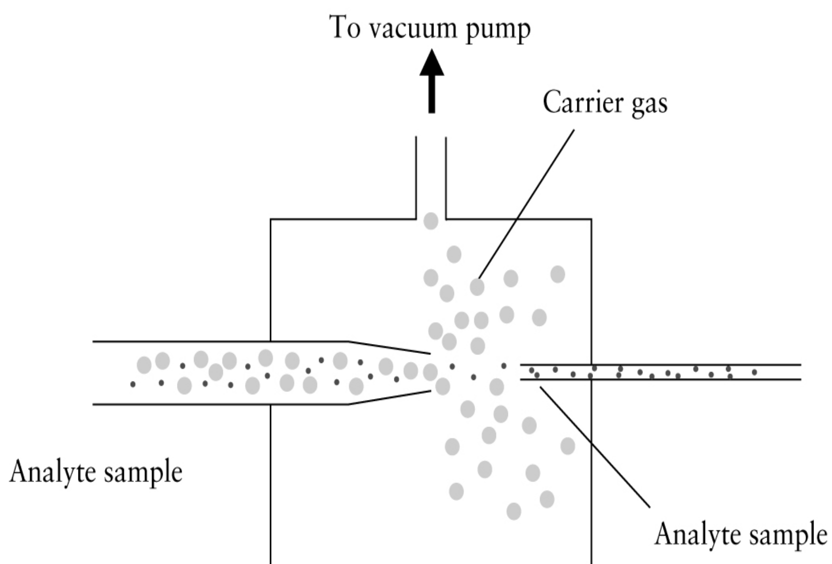
Name of topic/lesson – Mass Spectrometry

Subtopic: Introduction to GC-MS

Topic Outcomes: At the end of topic you will

1. Know principle, interphases and applications of GC-MS





Reference: 1. Organic Spectroscopy by P.S. Kalsi

2. Organic Spectroscopy by William Kemp

3. Instrumental methods of Analysis by Skoog

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

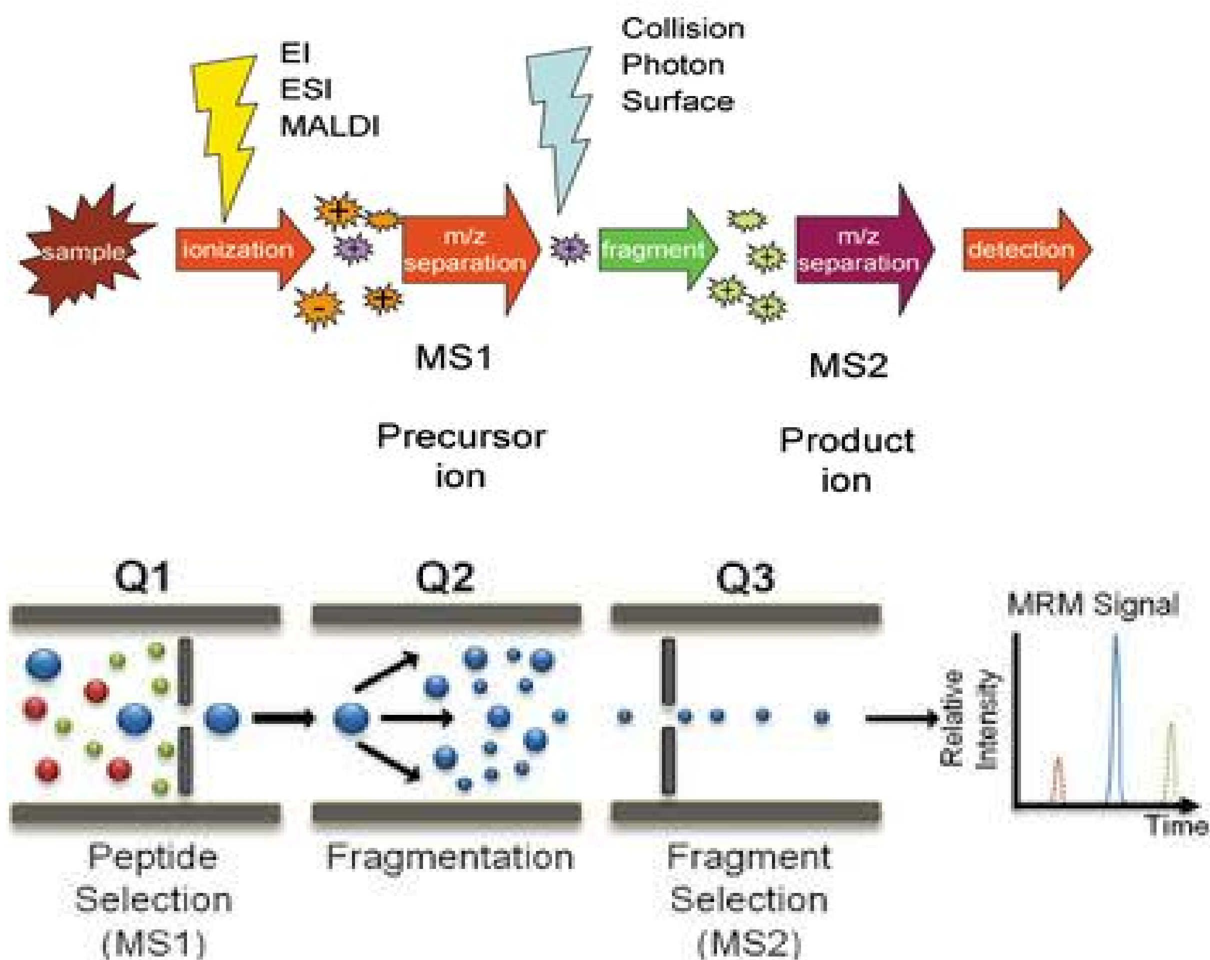
Lecture No: 27

Name of topic/lesson – Mass Spectrometry

Subtopic: Introduction to LC-MS, MS-MS

Topic Outcomes: At the end of topic you will

1. Know principle, interphases and applications of LC-MS, MS-MS



Reference: 1. Organic Spectroscopy by P.S. Kalsi

2. Organic Spectroscopy by William Kemp

3. Instrumental methods of Analysis by Skoog

PES MODERN COLLEGE OF PHARMACY (FOR LADIES), MOSHI

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Lecture No: 28

Name of topic/lesson – NUCLEAR MAGNETIC RESONANCE (NMR)

Subtopic: Theory

Topic Outcomes: At the end of topic you should be

1.Able to describe principle and theory of NMR

Bloch and Purcell demonstration - nuclei absorb Electromagnetic radiation in strong magnetic field due to the energy level splitting induced by magnetic field, (Nobel Prize1952)

uclear: manipulation of nuclear spin

lagnetic: magnetic field strength influences ΔE

esonance: tendency of a system to oscillate at maximum amplitude at a certain frequency

NMR

^{1}H nucleus = a proton → ^{1}H-NMR = proton NMR

Spin Quantum Numbers of Some Common Nuclei

The most abundant isotopes of C and O do not have spin.

Element	^{1}H	^{2}H	^{12}C	^{13}C	^{14}N	^{16}O	^{17}O	^{19}F
Nuclear Spin Quantum No (I)	1/2	1	0	1/2	1	0	5/2	1/2
No. of Spin States	2	3	0	2	3	0	6	2

Elements with either odd mass or odd atomic number have the property of nuclear "spin".

The number of spin states is 2I + 1, where I is the spin quantum number. Nuclei with odd mass no B 11, N 15, O17

Properties of the Nucleus

Nuclear spin

Nuclear magnetic moments

The Nucleus in a Magnetic Field

Precession and the Larmor frequency

Nuclear Zeeman effect & Boltzmann distribution

When the Nucleus Meet the right Magnet and radio wave

Nuclear Magnetic Resonance

Energy difference is proportional to the magnetic field strength.

$$\Delta E = h\nu = \gamma \frac{h}{2\pi} B_0$$

Gyromagnetic ratio, γ, is a constant for each nucleus (26,753 $s^{-1}gauss^{-1}$ for H).

In a 14,092 gauss field, a 60 MHz photon is required to flip a proton.

Low energy, radio frequency.

References: 1. Organic Spectroscopy by P.S. Kalsi

2. Organic Spectroscopy by William Kemp

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Lecture No: 29

Name of topic/lesson – NUCLEAR MAGNETIC RESONANCE (NMR)

Subtopic: Chemical and Magnetic Equivalence, Solvents

OBJECTIVE: To Understand Chemical and Magnetic Equivalence

To study the requirements of a solvent from NMR along with their examples.

Topic Outcomes: At the end of topic you should be

1.Able to identify the number of signals shown by compound

Number of Signals (Proton Equivalency)

- **NMR signal due to photon absorption**
- **Photon energy controlled by magnetic environment of nucleus**
- **Nuclei in same magnetic environment = equivalent**
- **Multiple magnetic environments → multiple signals**

Number of signals = number of equivalent proton sets

Magnetic equivalence:

The protons which are coupling equivalent are called as magnetically equivalent.

Characteristics of solvent used:

1. It should be **chemically inert** and **magnetically isotropic.**
2. It should be **devoid of hydrogen atom**.
3. It should **dissolve the sample** to a reasonable extent.
4. **Non-toxic** liquid - safe to use
5. Even **small quantity** gives a **strong peak**.

6.Protons are **highly shielded** and are **chemically inert**.

7. Volatile-can be distilled off & **used again**

8. Soluble in most organic solvents

9. 12 chemically equivalent hydrogen atoms - **single intense peak**

CCl_4	**Carbon tetrachloride**
CS_2	Carbon disulfide
$CDCl_3$	Deuteriochloroform (chloroform-d)
C_6D_6	Hexadeuteriobenzene (benzene- d_6)
D_2O	Deuterium oxide (heavy water)
$(CD_3)_2SO$	Hexadeuteriodimethylsulfoxide (DMSO-d_6)
$(CD_3)_2CO$	Hexadeuterioacetone (acetone-d_6)
$(CCl_3)_2CO$	Hexachloroacetone

References: 1. Organic Spectroscopy by P.S. Kalsi

2. Organic Spectroscopy by William Kemp

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Lecture No: 30

Name of topic/lesson – NUCLEAR MAGNETIC RESONANCE (NMR)

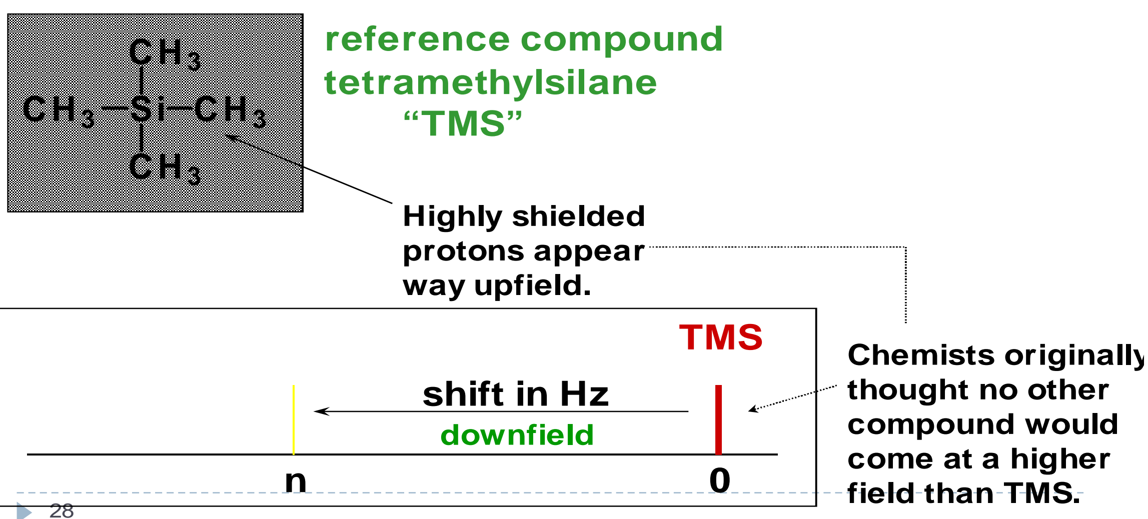
Subtopic: CHEMICAL SHIFT, SHIELDING AND DESHIELDING

Objective: To Understand Chemical Shift, Shielding and Deshielding

Topic Outcomes: At the end of topic you should be

1. Able to identify the position of proton signals shown by compound

---



Reasons for using TMS as an internal standard

- **Signal** produced **outside range of most** protons
- For Proton NMR spectroscopy usually **non**
- **viscous** solvents are used. Many of which are
- normal organic solvents in which **hydrogen has**
- **been replaced by deuterium.** A substance free of
- proton should be used as a solvent

References: 1. Organic Spectroscopy by P.S. Kalsi

2. Organic Spectroscopy by William Kemp

PES MODERN COLLEGE OF PHARMACY (FOR LADIES), MOSHI

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Lecture No: 31

Name of topic/lesson – NUCLEAR MAGNETIC RESONANCE (NMR)

Subtopic: Spin-Spin Coupling (Splitting), Coupling Constant

Objective: To Understand Spin-Spin Coupling (Splitting) and significance Coupling Constant

Topic Outcomes: At the end of topic you should be

1. Able to predict splitting pattern and line intensities of NMR signals

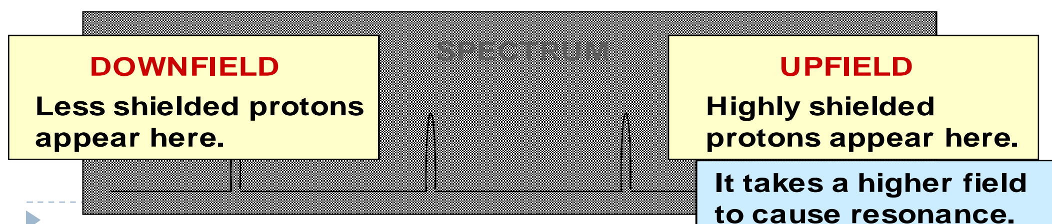
---

PROTONS DIFFER IN THEIR SHIELDING

All different types of protons in a molecule have a different amounts of shielding.

They all respond differently to the applied magnetic field and appear at different places in the spectrum.

This is why an NMR spectrum contains useful information (different types of protons appear in predictable places).



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).

We can adjust the shift to a field-independent value, the "chemical shift" in the following way:

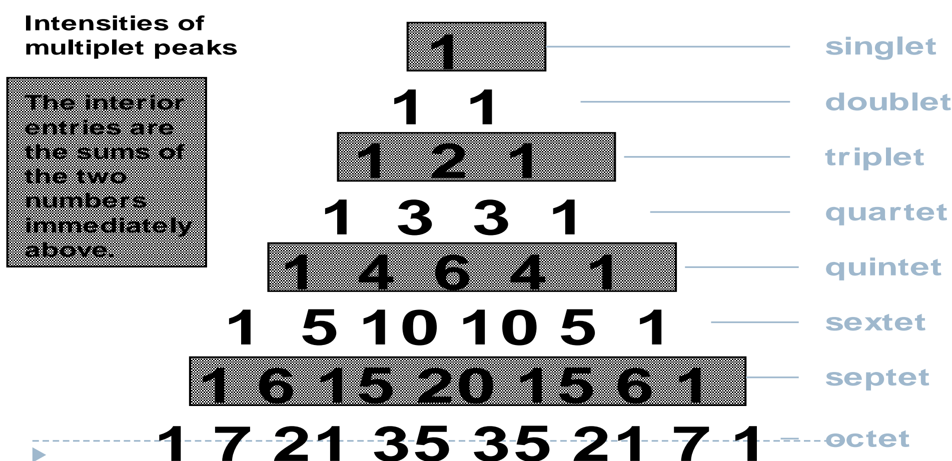
SPIN-SPIN SPLITTING IN NMR

Often a group of hydrogens will appear as a multiplet rather than as a single peak. If a signal is split by N equivalent protons, it is split into $N + 1$ peaks.

Multiplets are named as follows:

Singlet **Quintet**
Doublet **Septet**
Triplet **Octet**
Quartet **Nonet**

This happens because of interaction with neighboring hydrogens and is called SPIN-SPIN SPLITTING



- **Equivalent protons do not split each other.**
- **Protons bonded to the same carbon will split each other only if they are not equivalent.**
- **Protons on adjacent carbons normally will couple.**
- **Protons separated by four or more bonds will not couple.**

COUPLING CONSTANT

- **Distance between the peaks of multiplet**
- **Measured in Hz**
- **Not dependent on strength of the external field**
- **Multiplets with the same coupling constants may come from adjacent groups of protons that split each other.**

References: 1. Organic Spectroscopy by P.S. Kalsi

2. Organic Spectroscopy by William Kemp

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Lecture No: 32

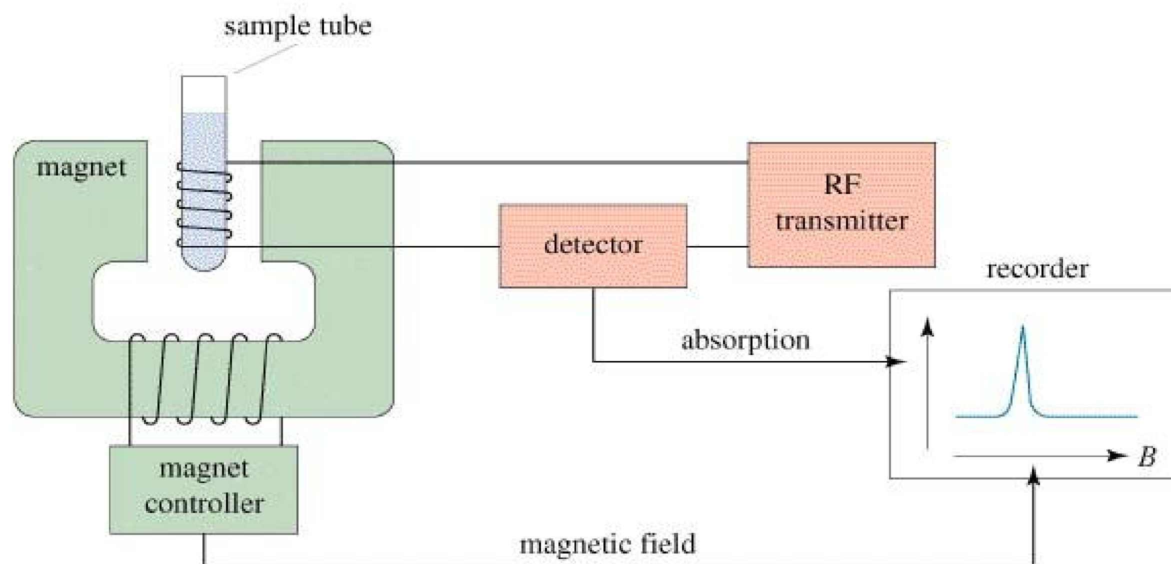
Name of topic/lesson – NUCLEAR MAGNETIC RESONANCE (NMR)

Subtopic: Instrumentation

Objective: To study the instrumentation of NMR

Topic Outcomes: At the end of topic you will

1. Know block diagram of NMR with function of each part



- **Sample Preparation,**
- **Standards,**
- **The Probe**
- **Tuning and Matching,**
- **Locking, and Shimming.**

MR samples are prepared and run in 5 mm glass NMR tubes. Always fill your NMR tubes to the same height with lock solvent. Deuteron resonance serves as lock- signal for the stabilization of the spectrometer magnetic field.

Reference: Instrumental methods of Analysis by Skoog

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Lecture No: 33

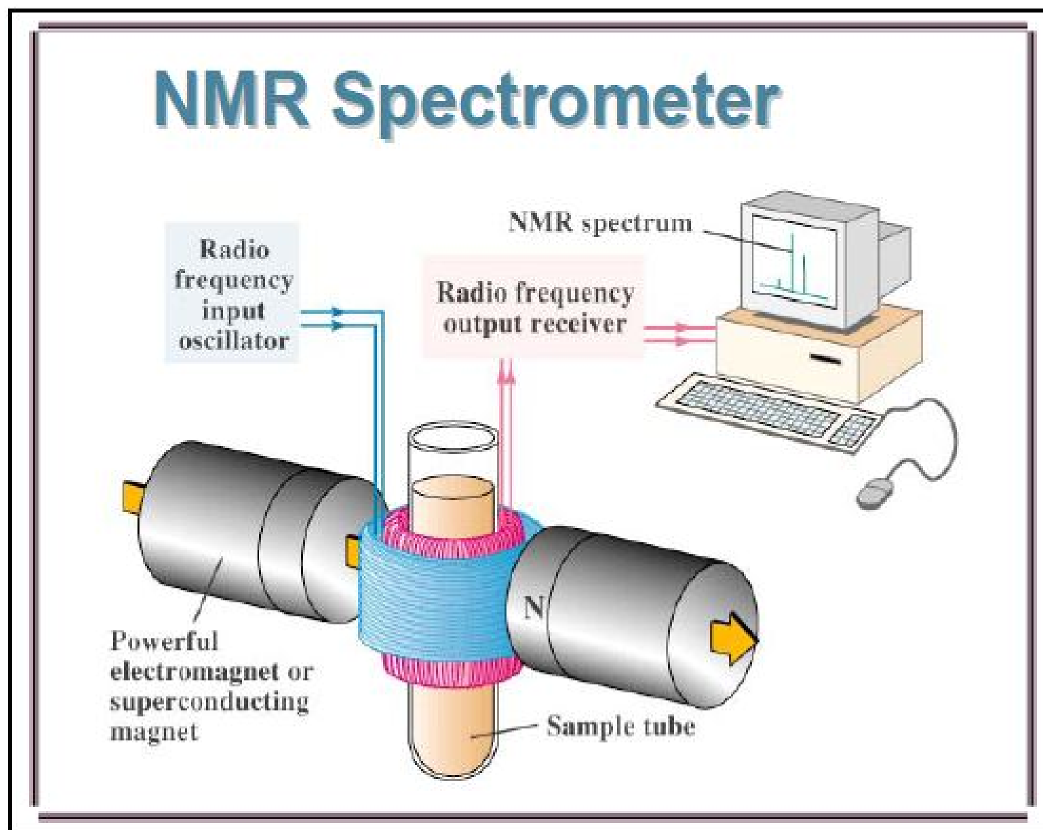
Name of topic/lesson – NUCLEAR MAGNETIC RESONANCE (NMR)

Subtopic: Instrumentation

Objective: To study the instrumentation of NMR

Topic Outcomes: At the end of topic you will

1. Know each part of NMR in detail



References: 1. Organic Spectroscopy by P.S. Kalsi

2. Organic Spectroscopy by William Kemp

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Lecture No: 34

Name of topic/lesson – NUCLEAR MAGNETIC RESONANCE (NMR)

Subtopic: Chemical Shift

Objective: To study Factors affecting chemical shift

Topic Outcomes: At the end of topic you will

1. Know Factors affecting chemical shift
2. Perform structural elucidation of organic compound

FACTORS AFFECTING CHEMICAL SHIFT

A. **Electronegativity – Shielding and Deshielding**

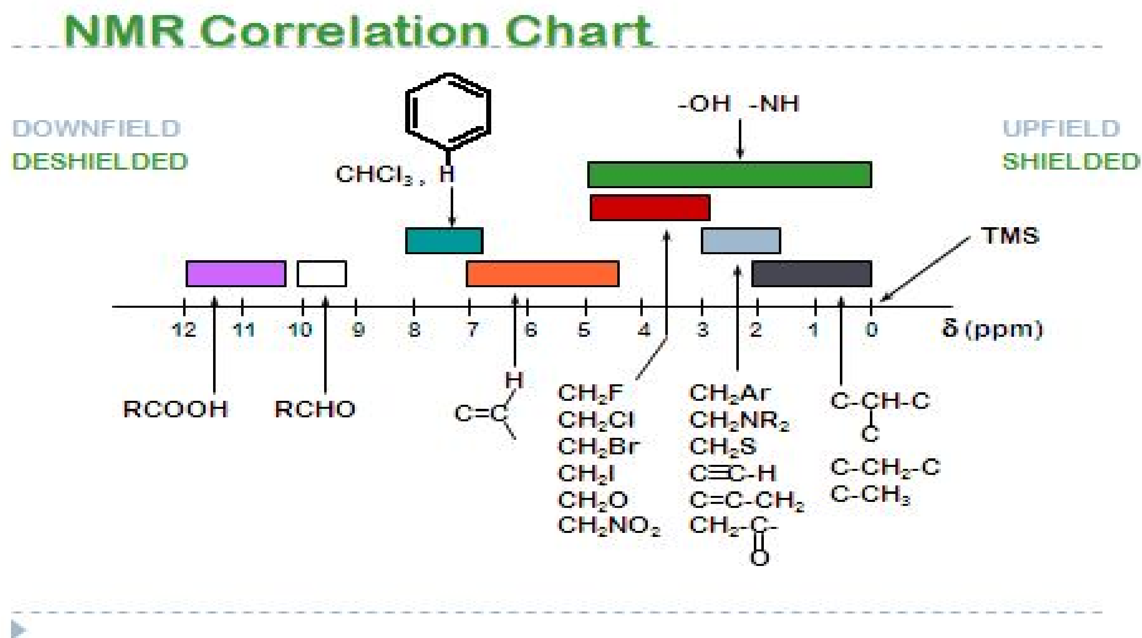
B. **Van Der Waals Deshielding**

C. **Hydrogen Bonding**

D. **Anisotropic Effects**

Chlorine "**deshields**" proton, i.e. takes valence electron density away from carbon, which in turn takes more density from hydrogen deshielding the proton.

NMR CHART



References: 1. Organic Spectroscopy by P.S. Kalsi

2. Organic Spectroscopy by William Kemp

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Lecture No: 35

Name of topic/lesson – NUCLEAR MAGNETIC RESONANCE (NMR)

Subtopic: Chemical Shift

Objective: To study Factors affecting chemical shift

Topic Outcomes: At the end of topic you will

1. Know Factors affecting chemical shift

2.Perform structural elucidation of organic

Van Der Waals Deshielding:-

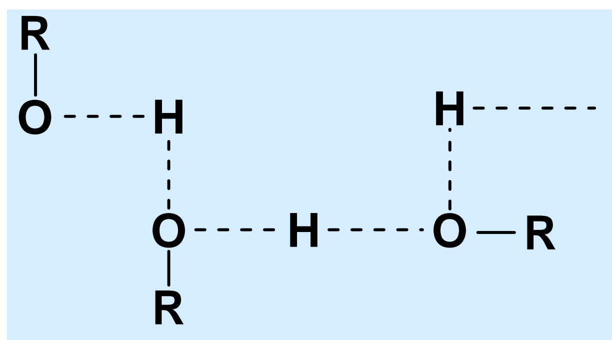
- In rigid molecule, **proton occupy stearically hindered position**
- Electron cloud of hindering group tend to repel the electron cloud surrounding the proton **(ELECTROSTATIC REPULSION)**
- **Proton** will be **deshielded** and appear at **higher δ value**

Hydrogen Bonding:-

Hydrogen bonding shifts resonance signal proton to lower field (higher frequency) – deshields protons & lengthens –OH bond

- ❑ NH, SH & OH protons NMR signals moved on changing to solvents of different polarity
- ❑ **Higher temperatures** reduces intermolecular hydrogen bonding means **lower δ** values
- ❑ **Intramolecular hydrogen bonding** is **unchanged by dilution**

HYDROGEN BONDING DESHIELDS PROTONS



References: 1. Organic Spectroscopy by P.S. Kalsi

2. Organic Spectroscopy by William Kemp

PES MODERN COLLEGE OF PHARMACY (FOR LADIES), MOSHI

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Lecture No: 36

Name of topic/lesson – NUCLEAR MAGNETIC RESONANCE (NMR)

Subtopic: Chemical Shift

Objective: To study Factors affecting chemical shift

Topic Outcomes: At the end of topic you will

1. Know Factors affecting chemical shift

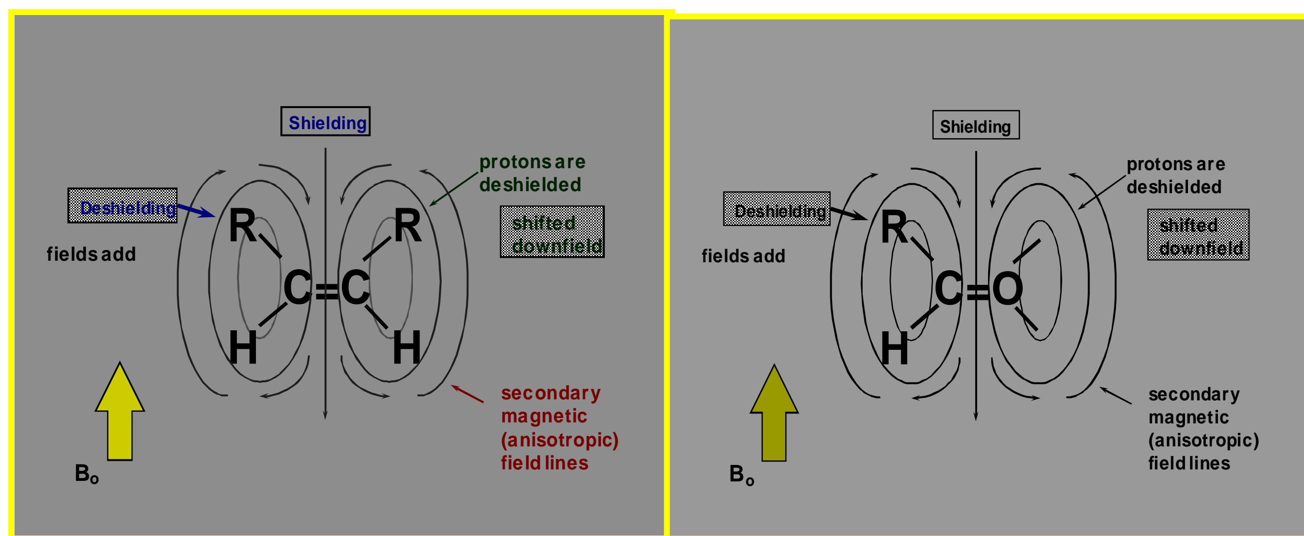
2.Perform structural elucidation of organic

Phenomenon **(spatial variation) of shielding and deshielding** depending on orientation of molecule w.r.t applied magnetic field is called **Anisotropy**

Anisotropy is property of molecule in different orientations which show **variations in physical properties along different axes of molecule**

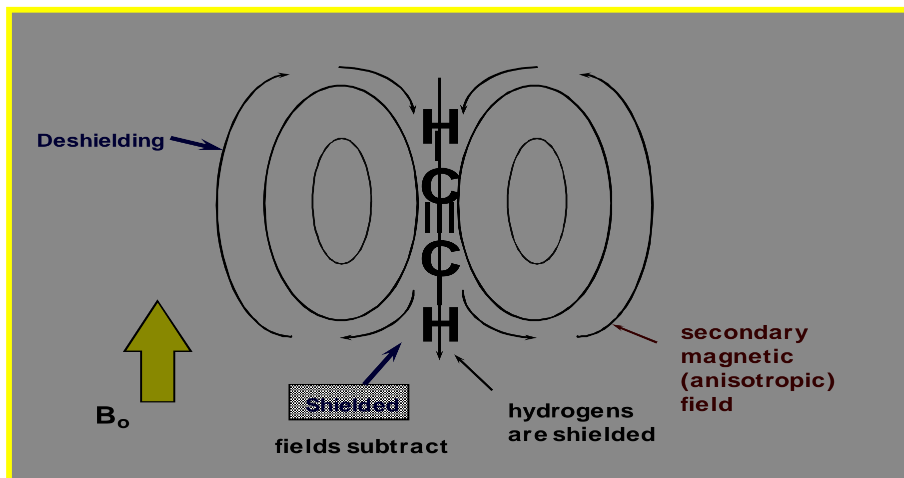
1. Alkenes

Deshielding occurs in cone-shaped zones, here δ values tend to be higher Shielding is found outside cones, here δ values tend to be lower



2. Carbonyl Compounds
   - Protons within 2 cones, lie parallel to axis of C=O bond, experience deshielding
   - So aldehydic , formyl protons of formate esters, appear at high δ values

3. Alkynes
   - Axis of alkyl group parallel to that of B_0
   - Cylindrical sheath of π electrons induced to circulate around axis
   - Resultant annulus-shaped magnetic field - in opposite direction to B_0
   - Therefore, acetylenic protons appear at low δ values



References: 1. Organic Spectroscopy by P.S. Kalsi

2. Organic Spectroscopy by William Kemp

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Lecture No: 37

Name of topic/lesson – NUCLEAR MAGNETIC RESONANCE (NMR)

Subtopic: Anisotropy

Objective: To study Anisotropy

Topic Outcomes: At the end of topic you will

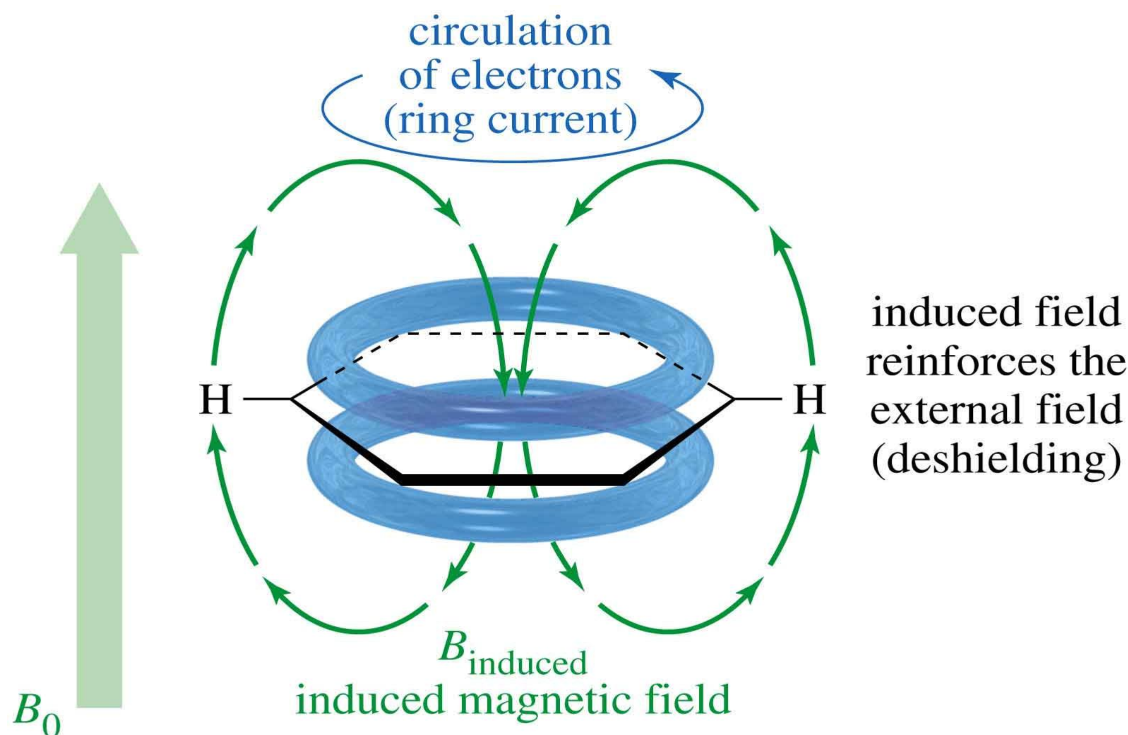
1. Know meaning of anisotropy and how it affects chemical shift

---

Anisotropy is the property of being directionally dependent, which implies different properties in different directions, as opposed to isotropy.

Aromatic Compounds

- Delocalized electrons, in presence of applied field $B_{0,}$ produce **ring current**
- Induced field is **diamagnetic,** in **centre of the ring** (therefore protons have lower δ value)
- Returning flux **outside ring** is **paramagnetic** (so protons have higher δ value)
- **Aromatic Protons, δ7-δ8**



References: 1. Organic Spectroscopy by P.S. Kalsi

2. Organic Spectroscopy by William Kemp

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Lecture No: 38

Name of topic/lesson – NUCLEAR MAGNETIC RESONANCE (NMR)

Subtopic: Shift reagent

Objective: To study Shift reagent

Topic Outcomes: At the end of topic you will

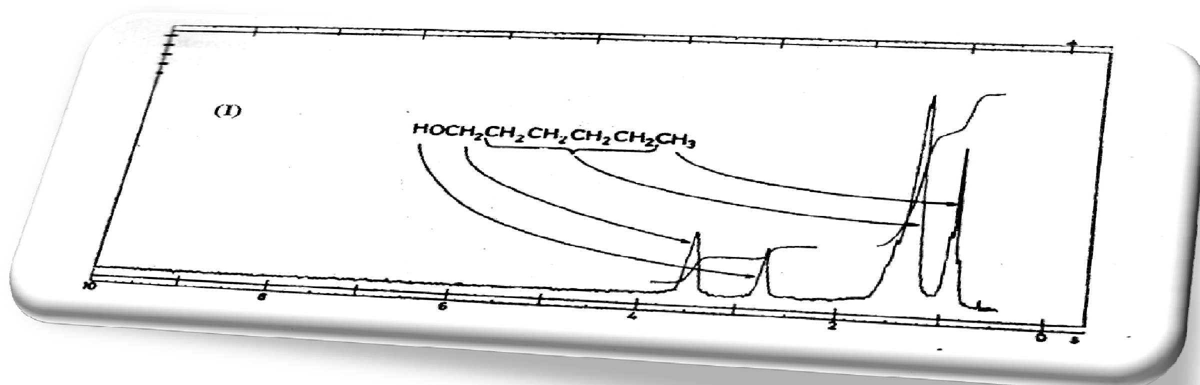
1. Know ways to simplify complex NMR Spectra along with role of shift reagent

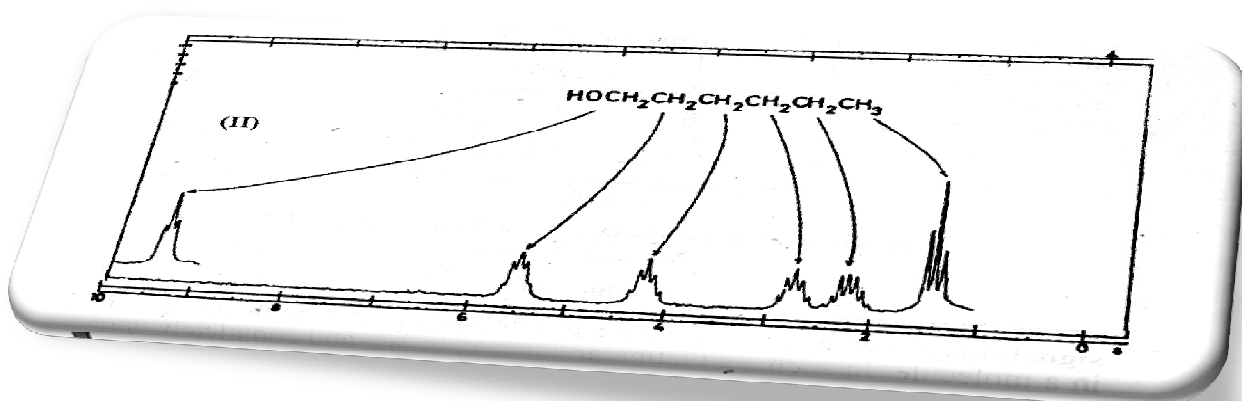
---

USE OF SHIFT'S REAGENT / LANTHENIDE SHIFT REAGENT:-

Europium complexes produce shifts to higher δ, while praseodymium complexes produce shifts to lower δ

- Mechanism:- Unpaired electron spin in the paramagnetic ion (for eg. Eu(III) is partially transferred through the intervening bonds to the protons of the organic substrate
- Spinning paramagnetic ion generates magnetic vectors operating through space creating secondary fields around the proton (predominates in the case of the lanthanide ions).





References: 1. Organic Spectroscopy by P.S. Kalsi

Lecture No: 39

Name of topic/lesson – NUCLEAR MAGNETIC RESONANCE (NMR)

Subtopic: Double resonance

Objective: To study technique of Double resonance

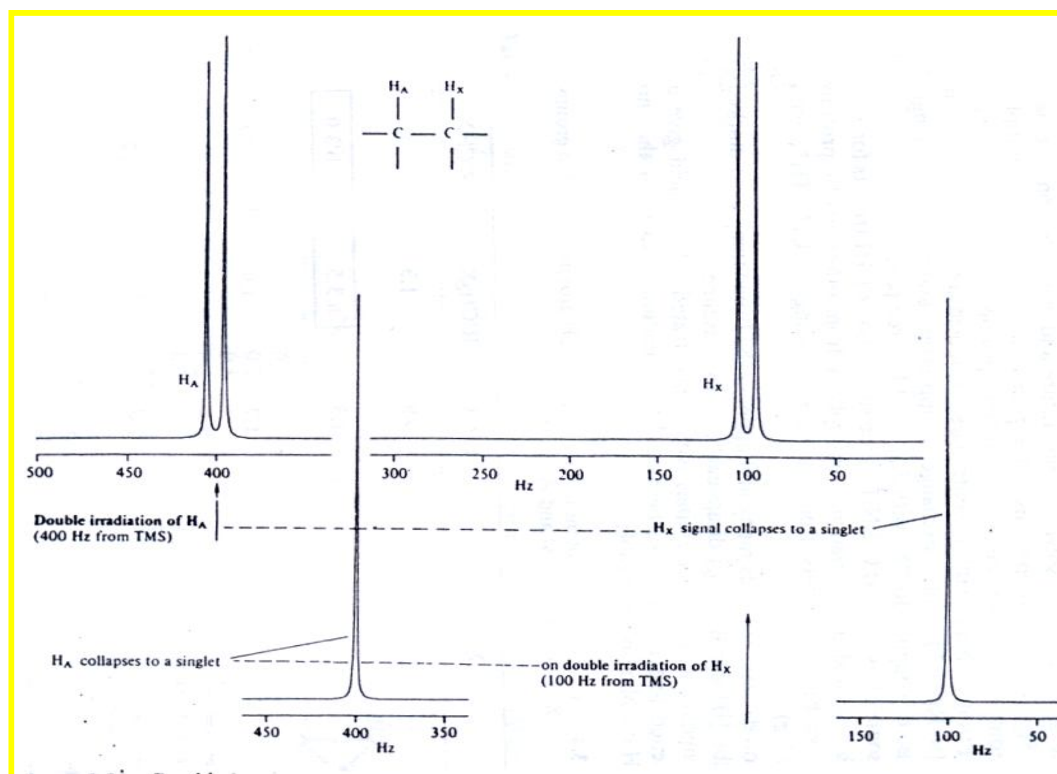
Topic Outcomes: At the end of topic you will

1. Know use of double resonance in simplification of NMR Spectra along with its principle

---

DOUBLE RESONANCE

- Irradiation of a nucleus at its resonance frequency using a second radio frequency oscillator
- Extra energy imparted to particular nucleus leads to rapid transitions between its nuclear spin states
- Adjacent proton (i.e nucleus) will only see, one time averaged view of nuclear spin states of nucleus being irradiated
- As a result splitting pattern disappear



References: 1. Organic Spectroscopy by P.S. Kalsi

2. Organic Spectroscopy by William Kemp

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Lecture No: 40, 41

Name of topic/lesson – NUCLEAR MAGNETIC RESONANCE (NMR)

Subtopic: Application and simple structure determination

Objective: To study applications of NMR

Topic Outcomes: At the end of topic you will

1. Know application of NMR

There are many useful NMR applications in biotechnology and pharmaceutical industries. ^{1}H and multinuclear NMR Spectroscopy (*e.g.*, ^{13}C, ^{19}F, ^{31}P) are essential tools for structural elucidation and drug discovery. Additionally, they can be used for structural confirmation of both known synthetic drugs and natural products with pharmaceutical effects. See for example, the series of non-steroidal anti-inflammatory drugs (NSAIDs). In addition to qualitative analysis, the quantitative nature of NMR can address a number of inquiries including:

1. Impurity Profiles

2. Composition

3. Metabolic products in bodily fluids

4. Reaction monitoring/ Kinetic profiling

References: 1. Organic Spectroscopy by P.S. Kalsi

2. Organic Spectroscopy by William Kemp

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Lecture No: 42

Name of topic/lesson – NUCLEAR MAGNETIC RESONANCE (NMR)

Subtopic: Introduction to ^{13}C NMR

Objective: To study theory of ^{13}C NMR

Topic Outcomes: At the end of topic you will

1. Know theory of ^{13}C NMR

2. Understand the differences between H1-NMR and C13 NMR

Sr. No.	PMR	CMR
1.	It is study of spin changes of proton nuclei	It is study of spin changes of carbon nuclei
2.	Chemical shift range is 0-14 ppm	Chemical shift range is 0-240 ppm
3.	Continuous wave method is used	Fourier transform technique is used
4.	Slow process	Very fast process
5.	Precessional frequency is 4 times higher than Carbon at given magnetic field	
6.	Natural abundance of 1H is 99.9844	Natural abundance of 13C is 1.108
7.	Relative Sensitivity At Constant Field Is 1.000	Relative Sensitivity At Constant Field Is 1.59* 10-5
8.	Magnetic moment 2.792	Magnetic moment 0.70220
9.	Magnetogyric Ratio 26,753	Magnetogyric Ratio 6,728

References: 1. Organic Spectroscopy by P.S. Kalsi

2. Organic Spectroscopy by William Kemp

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Lecture No: 43

Name of topic/lesson – Electron Spin Resonance (ESR)

Subtopic: Introduction, principal

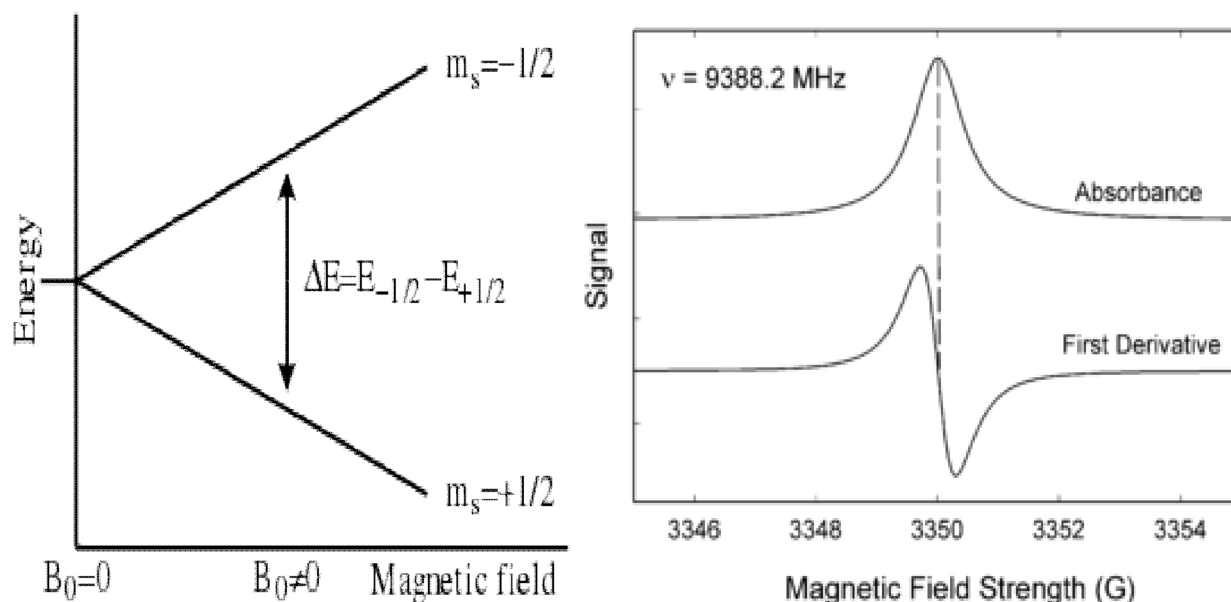
Objective: To study Principle of ESR

Topic Outcomes: At the end of topic you will

1. Know Principle of ESR

Electron paramagnetic resonance (**EPR**) or **electron spin resonance** (**ESR**) spectroscopy is a method for studying materials with unpaired electrons. The basic concepts of EPR are analogous to those of nuclear magnetic resonance (NMR), but it is electron spins that are excited instead of the spins of atomic nuclei. EPR spectroscopy is particularly useful for studying metal complexes or organic radicals.

Appearance of ESR spectra



Every electron has a magnetic moment and spin quantum number s, of 1/2, with magnetic components +1/2 and -1/2. In the presence of an external magnetic field with strength Bo, the electron's magnetic moment aligns itself either parallel or antiparallel to the field, each alignment having a specific energy due to the Zeeman effect.

References: 1. Organic Spectroscopy by William Kemp

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Lecture No: 44

Name of topic/lesson – ESR

Subtopic: Hyperfine splitting

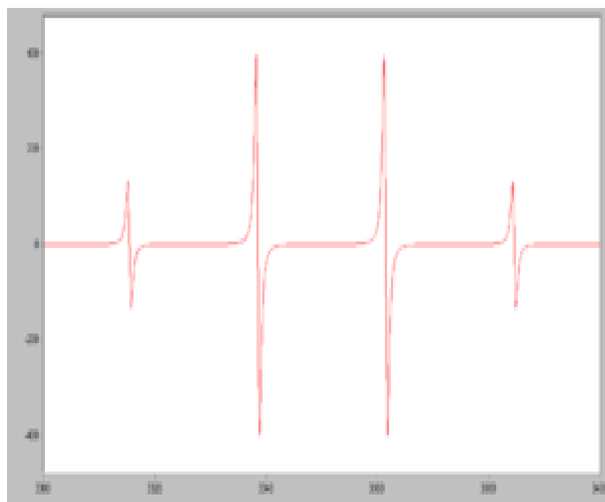
Objective: To study Hyperfine splitting pattern seen in ESR

Topic Outcomes: At the end of topic you will

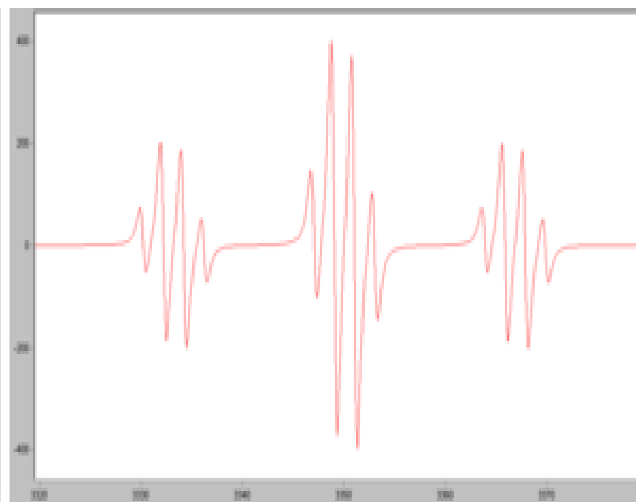
1. Know the appearance of ESR spectra

2. Draw the ESR spectra of given free radical

- For a radical having M equivalent nuclei, each with a spin of I, the number of EPR lines expected is $2MI + 1$. As an example, the methyl radical, CH_3, has three ^1H nuclei, each with $I = 1/2$, and so the number of lines expected is $2MI + 1 = 2(3)(1/2) + 1 = 4$, which is as observed.
- For a radical having M_1 equivalent nuclei, each with a spin of I_1, and a group of M_2 equivalent nuclei, each with a spin of I_2, the number of lines expected is $(2M_1I_1 + 1)(2M_2I_2 + 1)$. As an example, the methoxymethyl radical, $H_2C(OCH_3)$, has two equivalent ^1H nuclei, each with $I = 1/2$ and three equivalent ^1H nuclei each with $I = 1/2$, and so the number of lines expected is $(2M_1I_1 + 1)(2M_2I_2 + 1) = [2(2)(1/2) + 1][2(3)(1/2) + 1] = 3\times4 = 12$, again as observed.



Simulated EPR spectrum of the CH_3 radical



Simulated EPR spectrum of the $H_2C(OCH_3)$ radical

References: 1. Organic Spectroscopy by William Kemp

Lecture Synopsis Sub: Pharmaceutical Analysis-VI

Subject I/C: Dr. Tambe V.S.

Lecture No: 45

Name of topic/lesson – ESR

Subtopic: Instrumentation of ESR

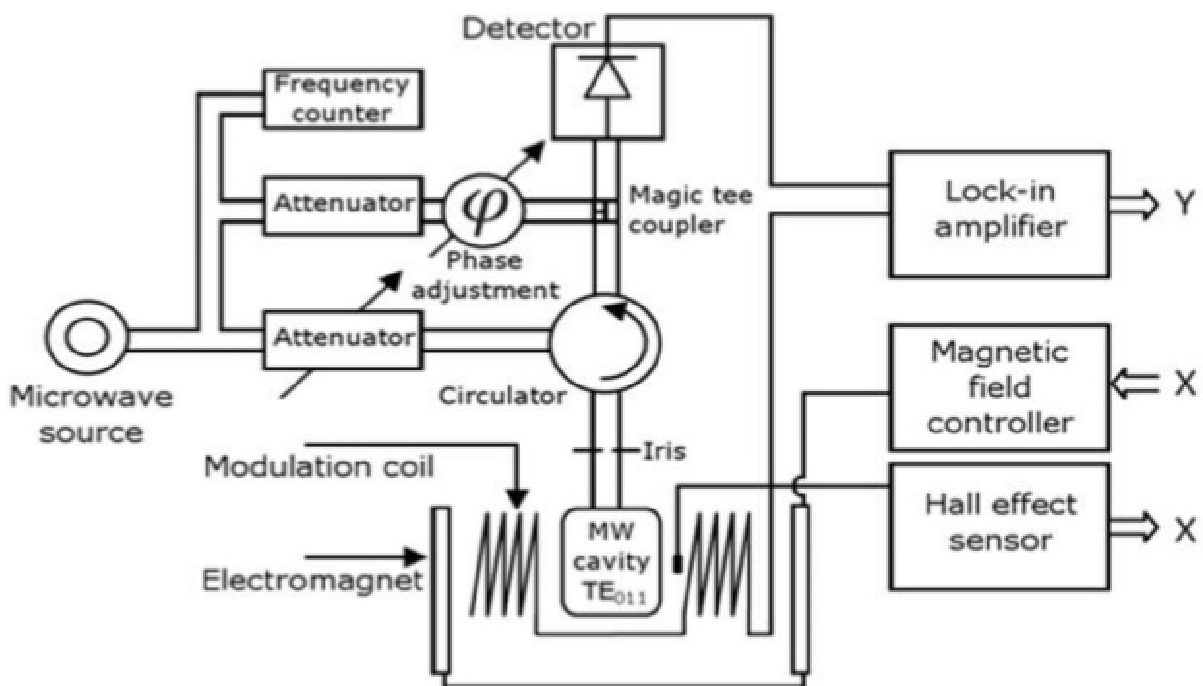
Objective: To study instrumentation of ESR

Topic Outcomes: At the end of topic you will

1. Know components of ESR along with function of each part

EPR has been used to investigate kinetics, mechanisms, and structures of paramagnetic species and along with general chemistry and physics, has applications in biochemistry, polymer science, and geosciences.

SCHEMATIC DIAGRAM OF AN ESR SPECTROMETER



(1) KLYSTRONS

Klystron tube acts as the source of radiation. It is stabilized against temperature fluctuation by immersion in an oil bath or by forced air cooling. The frequency of the monochromatic radiation is determined by the voltage applied to klystron. It is kept a fixed frequency by an automatic control circuit and provides a power output of about 300 milli watts.

(2) WAVE GUIDE OR WAVEMETER

The wave meter is put in between the oscillator and attenuator to know the frequency of microwaves produced by klystron oscillator. The wave meter is usually calibrated in frequency unit (megahertz)

instead of wavelength. Wave guide is a hollow, rectangular brass tube. It is used to convey the wave radiation to the sample and crystal.

(3) ATTENUATORS

The power propagated down the wave guide may be continuously decreased by inserting a piece of resistive material into the wave guide. The piece is called variable attenuator and used in varying the power of the sample from the full power of klystron to one attenuated by a force 100 or more.

(4) ISOLATORS

It is a non-reciprocal device which minimizes vibrations in the frequency of microwaves produced by klystron oscillator. Isolators are used to prevent the reflection of microwave power back into the radiation source. It is a strip of ferrite material which allows micro waves in one direction only. It also is being stabilizing the frequency of the klystron.

(5) SAMPLE CAVITIES

The heart of the ESR spectrometer is the resonant cavity containing the sample. The sample is contained in a resonance cavity. Rectangular TE120 cavity and cylindrical TE011 cavity have widely been used. In most of the ESR spectrometers, dual sample cavities are generally used. This is done for simultaneous observation of a sample and a reference material. Since magnetic field interacts with the sample to cause spin resonance the sample is placed where the intensity of magnetic field is greatest. A measure of quality of the cavity is 'Q factor' which is defined as

The sensitivity of the spectrometer is directly proportional to this value of Q.

Rotable cavities and dual cavities have also been used respectively for study in anisotropic effect in single crystal land simultaneous spectroscopic observation of a sample and standard.

(6) COUPLERS AND MATCHING SCREWS

The various components of the micro wave assembly to be coupled together by making use of irises or slots of various sizes.

(7) CRYSTAL DETECTORS AND HOLDERS

A Silicon crystal detectors, which converts the radiation in D.C., has widely been used as a detector of microwave radiation. Microwave Bridge such as magic T and hybrid ring variety are most common.

(8) MAGNET SYSTEM

The resonant cavity is placed between the poles pieces of an electromagnet. An electro magnet capable of producing magnetic field of at least 5000 gauss is required for ESR. The field should be stable and uniform over the sample volume. The stabilityof field is achieved by energizing the magnet with a highly regulated power supply.

The ESR spectrum is recorded by slowly varying the magnetic field through the resonance condense by sweeping the current supplied to the magnet by the power supply. This sweep is usually accomplished by with a variable speed motor drive. Both the magnet as well as the power supply may require water cooling.

(9) MODULATION COIL

The modulation of the signal at a frequency consistent with good signal noise ratio in the crystal detector is accomplished by a small alternating variation of the magnetic field. The variation is produced by supplying an A.C. signal to modulation coil oriented with respect the sample in the same direction as the magnetic field. If the modulation is of low frequency (400 cycles/sec or less), the coils can be mounted outside the cavity and even on the magnet pole pieces. For higher modulation frequencies, modulation coils must be mounted inside the resonant cavity or cavities constructed of a non-metallic material e.g., Quartz with a tin silvered plating, because metallic penetration is not very effective in case of higher modulation frequencies.

(10) DISPLAY DEVICES

In order to adjust the spectrometer and to observe the signal, a cathode ray oscilloscope has been employed. A strip chart or X-Y recorder is used for recording the signal.

EPR spectra are usually displayed in derivative form to improve the signal-to-noise ratio.

Reference: PHARMATUTOR-ART-1579