# UNIT-I

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Points to be covered in this topic

- ❖ Introduction
- ❖ Principle
- ❖ The NMR phenomenon
- ❖ Chemical shift
- ❖ Factors affecting chemical shift
- ❖ Coupling constant
- ❖ Spin-spin coupling
- ❖ Relaxation
- ❖ Instrumentation
- ❖ Applications

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

❑ INTRODUCTION

- Nuclear magnetic resonance (NMR) is a **spectroscopic** technique that **detects the energy absorbed by changes in the nuclear spin state**.

- A **photon** of light causes a **transition from the ground state to the excited state**.

- For example, in the case of **visible spectroscopy** the absorption of a photon by an electron causes the electron to move from its ground state orbital to an orbital of higher energy, the excited state.

- In the case of NMR, the **absorption of radio-frequency energy, nuclei promotes a nuclear spin** from its ground state to its excited state.

- NMR spectroscopy **differs in a number of important aspects** from other forms of spectroscopy.

- **First,** the generation of the ground and excited NMR states requires the existence of an **external magnetic field.**

- This requirement is a very important distinction of NMR spectroscopy in that it allows one to change the characteristic frequencies of the transitions by **simply changing the applied magnetic field strength**.

- **Second,** the NMR **excited state has a lifetime that is on the order of 10^9 times longer** than the lifetime of the excited electronic states.
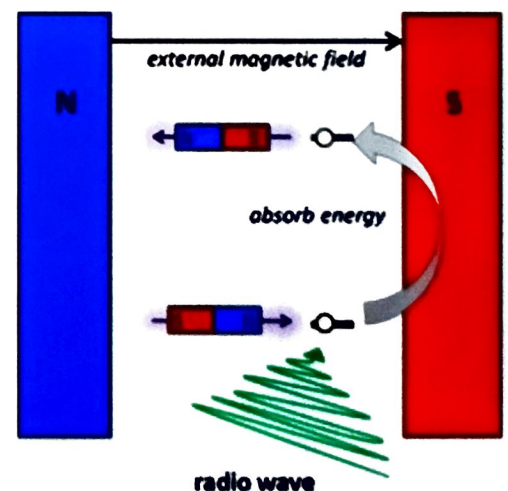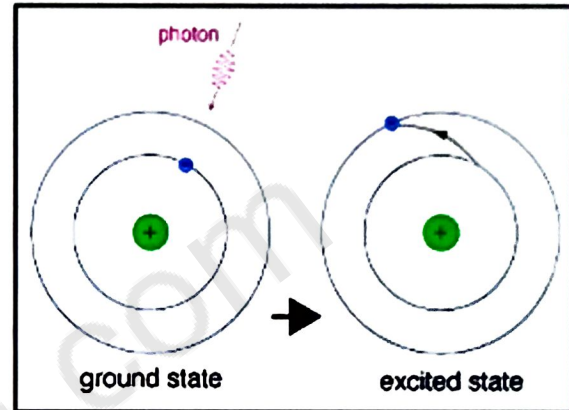
❑ PRINCIPLE

➤ H-NMR

- If **Precessing nuclei irradiate with beam of radiofrequency energy** of a correct frequency, the **low- energy nuclei may absorb this energy and move to a higher energy state.**

- The precessing proton will only **absorb energy from the radiofrequency source**, if the **precessing frequency is the same as the frequency of radiofrequency beam are said to be resonance;** the term as **nuclear magnetic resonance.**

➤ C-NMR

- ^{13}C **nucleus can behave as a little magnet.** ^{12}C nuclei don't have this property. ^{13}C can be aligned with an **external magnetic field or opposed to it.** Again, the **alignment where it is opposed to the field is less stable** (at a higher energy).

- It is possible to **make it flip from the more stable orientation to the less stable one by supplying exactly the right amount of energy.** The energy needed to make this flip depends on the **strength of the external magnetic field used,** but is usually in the range of energies found in radio waves - **at frequencies of about 25-100 MHz.**

- ^{13}C NMR refers to **recording another NMR spectrum but of 'C' atoms rather than hydrogen atoms.**

- In actual practice, these spectra are recorded in such a manner that each **chemically distinct carbon gives to single peak, without any coupling or fine structure.**

- Hence simply a **count of the peak can to see how many carbons are actually present in the molecule.**

❑ THE NMR PHENOMENON

❖ **The spinning nucleus:** The nucleus of the **hydrogen atom** (the proton) **behaves as a spinning bar magnet,** because it possesses both **electric charge and mechanical spin.** Any spinning charged body will generate a magnetic field.


The spinning nucleus
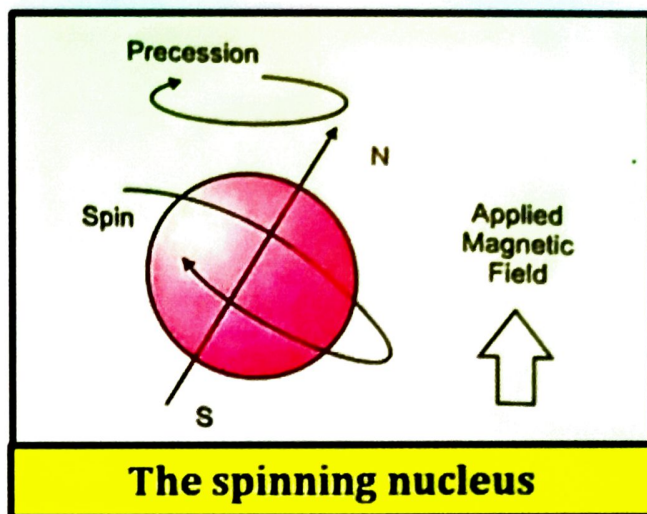
❖ **The effect of an external magnetic field**

- Like all bar magnets, the **proton will respond to the influence of an external magnetic field,** and will tend to **align itself** with that field.

- The proton can adopt **two orientations with respect to an external magnetic field**- either **aligned** with the field (lower energy state) i.e. **parallel** or **opposed to the field** (the higher energy state) i.e. **anti parallel.**

❖ **Precessional motion**

- Because of the **proton is behaving as a spinning magnet,** not only can it align itself with or oppose an external magnetic field, but it will **move in a characteristic way under the influence of an external magnet.**

- Consider the **behavior of a spinning top as well as describing its spinning motion,** the top will (unless absolutely vertical) also perform a slower waltz-like motion, in which the **spinning axis of the top moves slowly around the vertical.**

- This precessional motion, and the **top is said to be precessing around the vertical axis of the earth's gravitational field.**

- The precession arises from **the interaction of spin-that is, gyroscopic motion will precess;** a static will merely fall over.

Precessional motion of nuclei
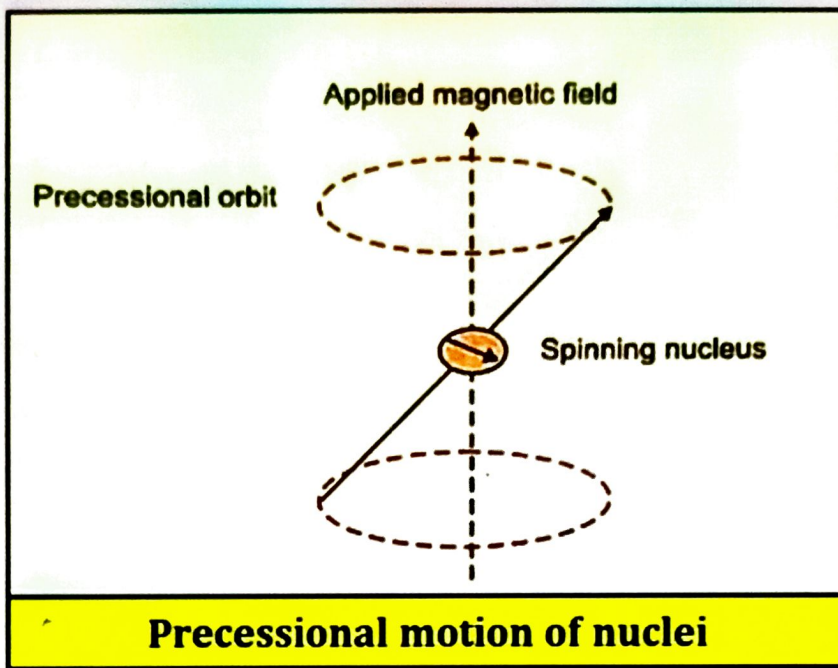
❖ Precessional frequency

- The precessional frequency (U) of the **nucleus is directly proportional to the strength of an external field (B_0)** and depends on the nature of the nuclear magnet. **Magnetic nuclei of different atoms have different characteristic precessional frequency**.

According to Larmor precession theory. $\omega = \gamma B_0$

   Where ω = Larmor precession frequency

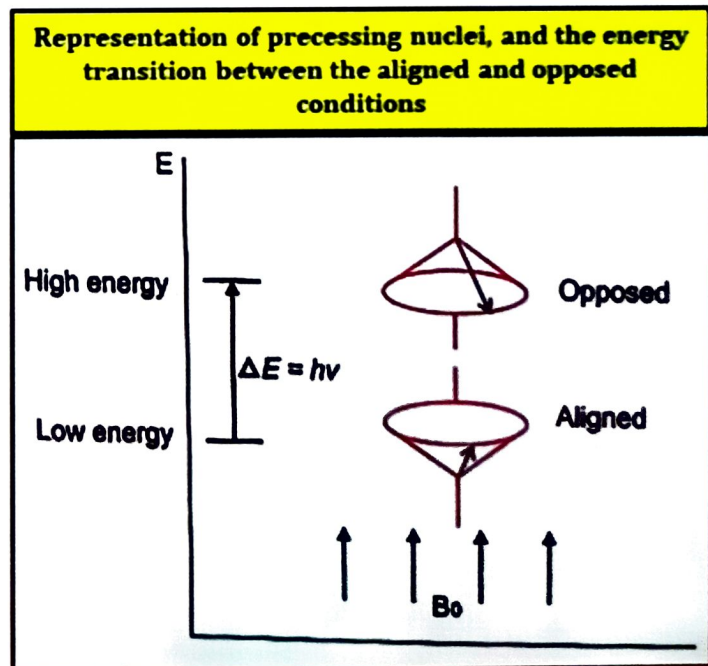   But $\omega = 2\pi v$

   ✓ $2\pi v = \gamma B_0$

   ✓ $v = \dfrac{\gamma}{2\pi} B_0$

   ✓ $v \propto B_0$

❖ Energy Transition

- When **proton is kept in an external magnetic field will precess and can take one of the two orientations** with respect to the axis of the external field aligned or opposed.

Representation of precessing nuclei, and the energy transition between the aligned and opposed conditions

- If a **proton is precessing in the aligned orientation, it can absorb energy and pass into the opposed orientation**; subsequently it can lose this extra energy and relax back into the aligned position.

- **The transition from one energy state to the other is called flipping of the proton**. The transition of two energy states can be brought by the radiofrequency wave radiation.

Excitation process of nucleus
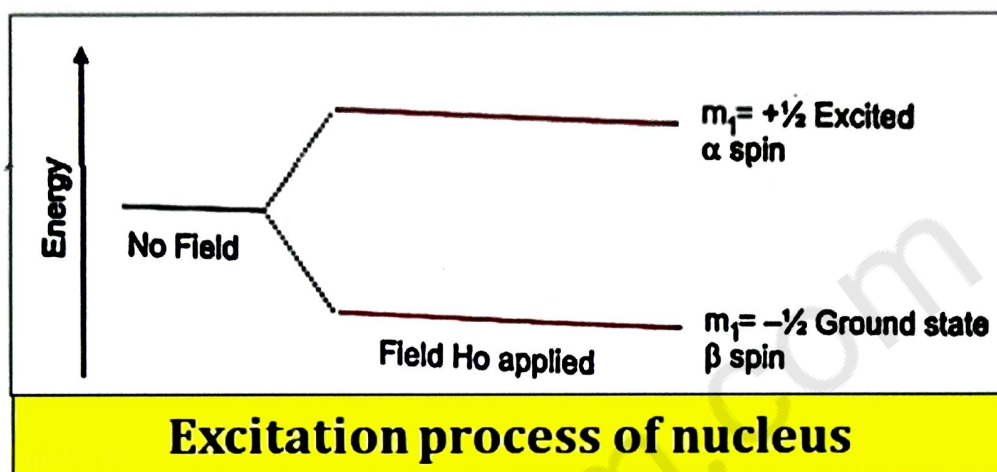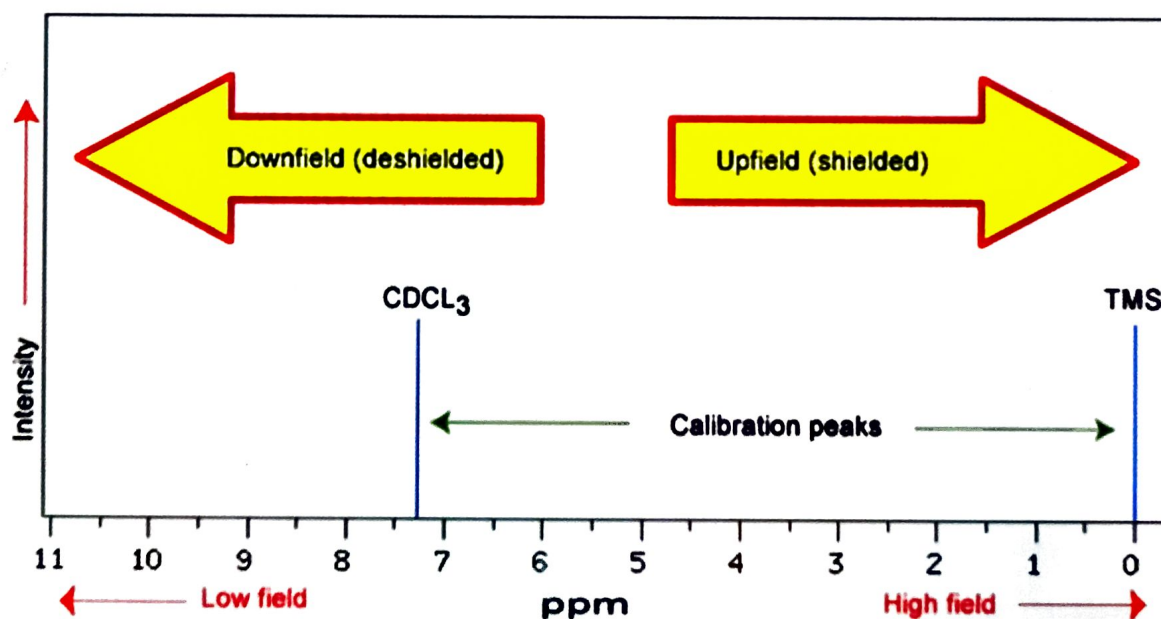
❑ CHEMICAL SHIFT

- When proton is **placed in a magnetic field, its electrons are caused to circulate, so they produce secondary magnetic field** i.e. **induced magnetic field.**

- **Rotation of electrons** (n) electrons about nearby nuclei generates a field that can **either oppose or parallel the applied field at the proton.**

- If **induced field opposes** (diamagnetic) the applied field, then proton is said to be **shielded.** If **induced field parallel** (paramagnetic) to applied field, the proton feels higher field strength and such proton is said to be **deshielded.**

- **Shielding shifts the absorption upfield** and **deshielding shifts the absorption downfield** to get effective field strength necessary for **absorption.**

- The **difference in absorption position in NMR spectrum** is arises due to **shielding and deshielding of protons by the electrons.** The difference in the absorption position of the proton in **parts per million** (ppm) with respect to **standard reference (TMS) signal is called chemical shift.**

- Chemical shift positions are expressed **in δ units.** Since δ unit is proportionality, it is **dimensionless number**; it is **independent of field strength.**

- The relationship between **δ values (in ppm) and frequency (in Hz)** is given by equation:

$$X = \frac{X^- \, TMS}{0}$$

Where,

- **δx=** chemical shift (TMS)
- **Ux and Uᴛᴍꜱ =** frequencies (in Hz) of the signal for X and TMS, respectively
- Do operating frequency of the instrument (in MHz).
- The most chemical shift has δ values **between 0 and 10.**
- τ scale running counter to δ scale. The τ scale **corresponds with low field value on the left and high on the right.**
- The δ and τ scale related by the expression = 10-
- Scale only found in older spectra and are not used at all in current practice.

Chemical shift

❖ Reference standard

- Reference standards commonly used as **internal standard.**

1. TMS (tetramethyl silane), silicon pushes electrons into the methyl groups of TMS by a positive inductive effect, and this powerful shielding effect means TMS protons come to resonance at low frequency, at low δ value (0 ppm).

- TMS is used as internal standard for measuring the position of ^1H, ^{13}C and ^{29}Si in NMR spectroscopy.

- It is referred due to following reasons:

✓ TMS contains twelve protons are all magnetically equivalent.

✓ It is chemically inert and miscible with a large range of solvents.

✓ It is highly volatile and can be easily removed to get back the sample.

✓ It does not take part in intermolecular association with the sample.

✓ Its resonance position is far away from absorption due to protons in most organic molecules.

2. Sodium salt of 3-(trimethyl silyl) propane sulphonate: It is water soluble compound. Generally **used for water soluble samples in** deuterium oxide (D_2O) solvent.

❖ Solvents

- A substance **free of proton should be used as a solvent,** i.e. which does **not give absorption of its own in NMR spectrum.**

- The solvent should be capable of **dissolving at least 10%** of the substance under investigation.

- Following solvents are commonly used in NMR spectroscopy.

1. Carbon tetrachloride (CCl_4)

2. Carbon disulphide (CS_2)

3. Deuterochloride $(CDCl_{l3})$

4. Hexachloroacetone $(CCl_{l3})_2CO$

❏ FACTORS AFFECTING CHEMICAL SHIFT

Following are the factors which affecting the chemical shift:

1. Electronegativity

2. van der Waal's deshielding

3. Anisotropic effects

4. Hydrogen bonding

5. Concentration, solvent and temperature effect

❖ **Electronegativity:** The following table show the chemical shift positions for CH_3 protons when a methyl group is attached to functions of increasing electronegativity. As the electronegativity of the function is increased, the CH_3 protons come to resonance at a higher δ values.

- Chemical shift values for protons attached to groups of varying electronegativity

Compound	Chemical shift
CH_3I	2.16
CH_3Br	2.65
CH_3Cl	3.10
CH_3F	4.26

Consider the following compounds:

    b   a   b   a

(i) CH_3CH_2F (ii) CH_3CH_2Cl

- **Two signals are expected** for each of the two compounds. **Deshielding for protons 'a' in compound (i) is more** than that for similar protons in compound (ii).

- As the **distance from the electronegative atom increases**, the deshielding effect due to it diminishes. **Protons 'b' is comparatively less deshielded and hence will resonate at comparatively lower δ value.**

❖ **Van der Waal's deshielding:** In a rigid molecule, a proton to occupy a sterically hindered position. Electron cloud of a bulky group (hindering group) will tend to repel the electron cloud surrounding the

proton **by electrostatic repulsion. The proton will be deshielded and appear at higher & values than would be predicted** in the absence of the effect.

❖ **Hydrogen bonding:** A hydrogen atom exhibiting property of hydrogen bonding in a compound absorbs at a low field.

- The hydrogen bonded proton being attached to a **highly electronegative atom will have smaller electron density around it.**

- Deshielded in nature, the **field felt by such proton will be more and hence resonance will downfield.** The downfield shift depends upon the strength of hydrogen bonding.

❖ **Concentration, solvent and temperature effect:**

- Chemical shift of a proton attached to carbon of CCl_4 and $CDCl_3$ is independent of concentration and temperature while protons of -OH, -NH_2, -SH groups exhibit a substantial concentration and temperature effect due to hydrogen bonding.

- Interamolecular hydrogen bonding is less affected than intermolecular bonding by concentration change. Both types of hydrogen bonding are affected by temperature variations.

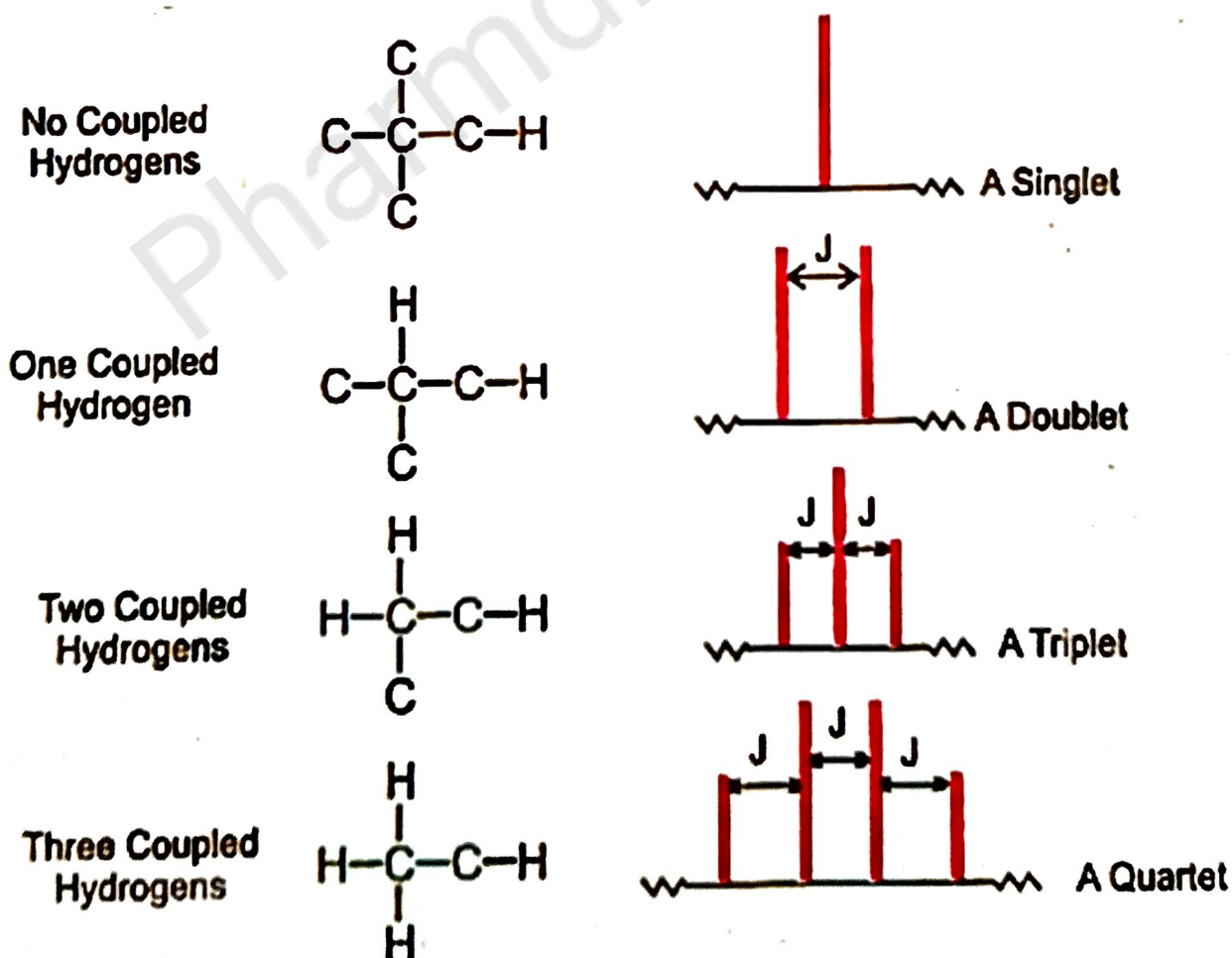❖ **Anisotropic effects:** Anisotropic effect is also called as space effect. The chemical shift positions (δ) for protons when **alone are less than attached to C=C in alkenes is higher. This same thing for aldehydic protons and aromatic protons, whereas alkyne protons appear at relatively low δ.**

- In this case π electrons circulate under the influence of the applied field and can lead to shifts to higher frequency (downfield shift) or to lower frequency (upfield shift).

❑ COUPLING CONSTANT

- The **distance between centers of two adjacent peaks in a multiplet** is constant and is called coupling constant.

- It is denoted by letter **'J'**. It is measured in **Hertz (Hz)** or in **cps (cycles per second)**. The value of coupling constant is **independent of the external field.**

- From the value of coupling constant one can **distinguish between the two singlets and one doublet and also a quarter from two doublets.**

- It can be determined by **recording the spectrum at two different radiofrequencies.**

- If the **separation between the lines does not change, that signal indicates the doublet**, and the **separation of the lines increases with increasing frequency will be two singlets.**

Few examples of 'H' coupling constant (J) are given below.

No Coupled Hydrogens	C–C–C–H (with C above and C below central C)	A Singlet
One Coupled Hydrogen	C–C–C–H (with H above and C below)	A Doublet
Two Coupled Hydrogens	H–C–C–H (with H above and C below)	A Triplet
Three Coupled Hydrogens	H–C–C–H (with H above and H below)	A Quartet

The following general rules summarize important requirements and characteristics for spin 1/2 nuclei:

1) **Nuclei having the same chemical shift (called isochronous)** do not exhibit **spin-splitting**. They may actually be spin-coupled, but the splitting cannot be observed directly.

2) **Nuclei separated by three or fewer bonds** (e.g. **vicinal and geminal nuclei**) will usually be spin-coupled and will show mutual spin-splitting of the resonance signals (same J's), provided they have different chemical-shifts.

3) **The magnitude of the observed spin-splitting** depends on many factors and is given by the coupling constant J (units of Hz). J is the same for both partners in a spin-splitting interaction and is **independent of the external magnetic field strength.**

4) The splitting pattern of a given nucleus (or set of equivalent nuclei) can be predicted by the n+1 rule, where **n is the number of neighboring spin-coupled nuclei with the same (or very similar) Js.**

   - If there are **2 neighboring, spin-coupled,** nuclei the observed signal is **triplet (2+1=3)**; if there are **three spin-coupled** neighbors the signal is a **quartet (3+1-4).**

   - In all cases the **central line(s) of the splitting pattern are stronger than those on the periphery.** The intensity ratio of these lines is given by the numbers in **Pascal's triangle.**

   - Thus a doublet has 1:1 or equal intensities, a triplet has an intensity ratio of 1:2:1, a quartet 1:3:3:1 etc.
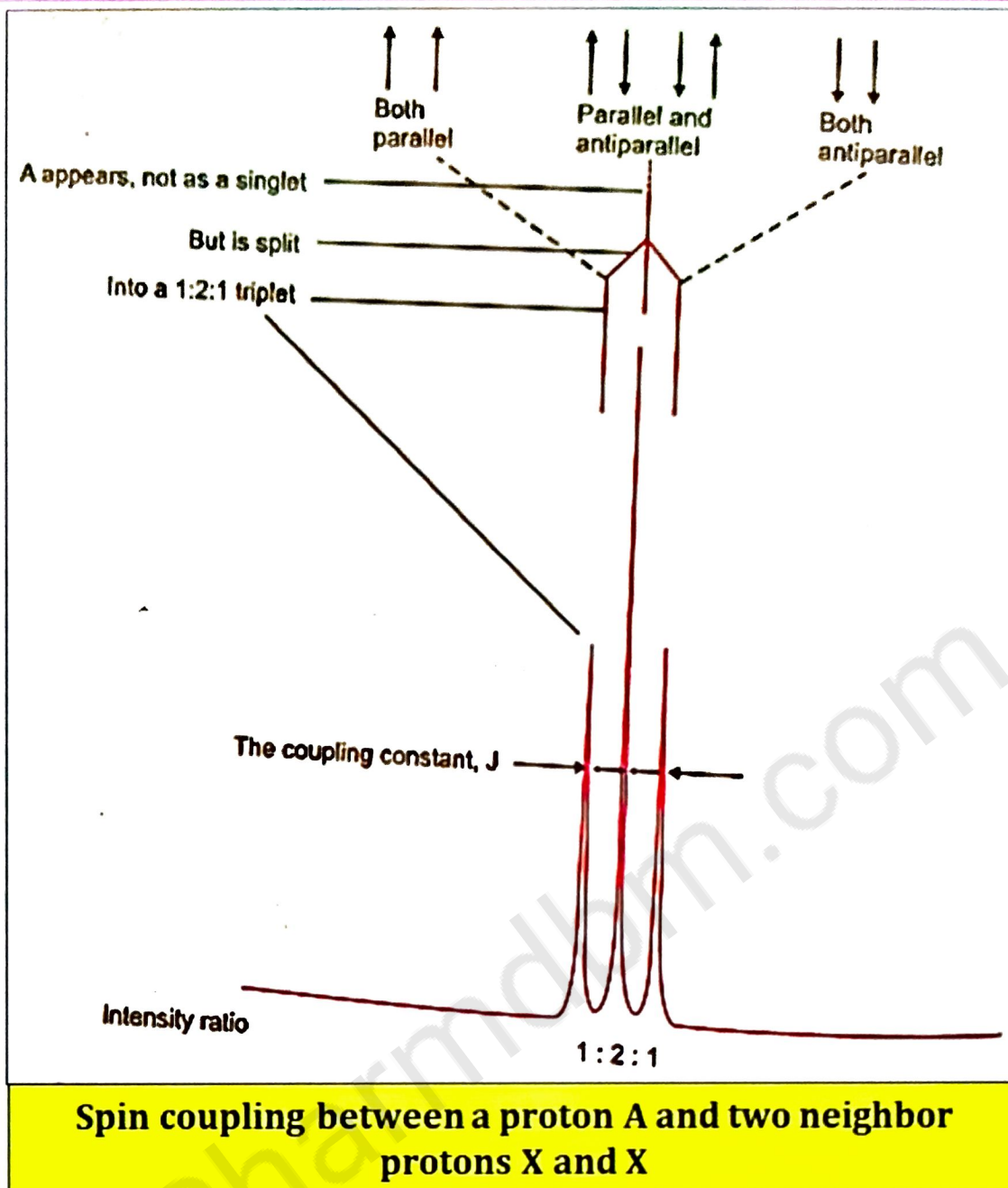

Pascal's Triangle
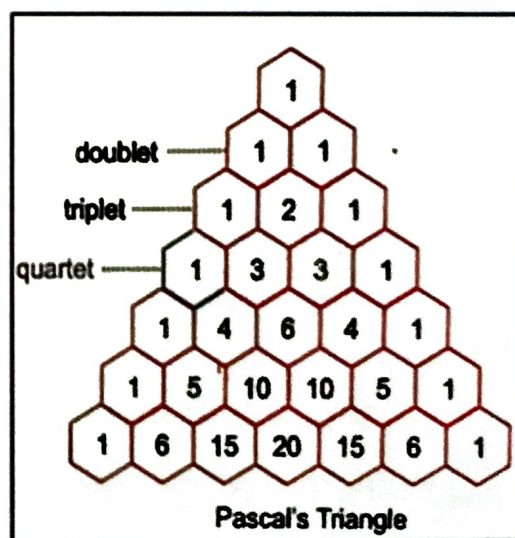
❑ SPIN-SPIN COUPLING

- **Nuclei can interact with each other** to cause mutual splitting of the sharp resonance lines into multiplates, called **spin-spin coupling.**

- The **number of lines (multiplicity) observed in the NMR signal for a group of protons** is not related to the number of protons in that group; the multiplicity of lines is related to the number of **protons in neighboring groups.**

- (n+1) rule: The simple rule **to find the multiplicity of the signal from a group of protons,** count the number of neighbors (n) and add 1.

- Example: **Protons, Hx in have only one neighboring proton, and Hx appears as a two-line signal** (doublet); proton H_A has two neighbors and the signal is split into three lines (triplet).
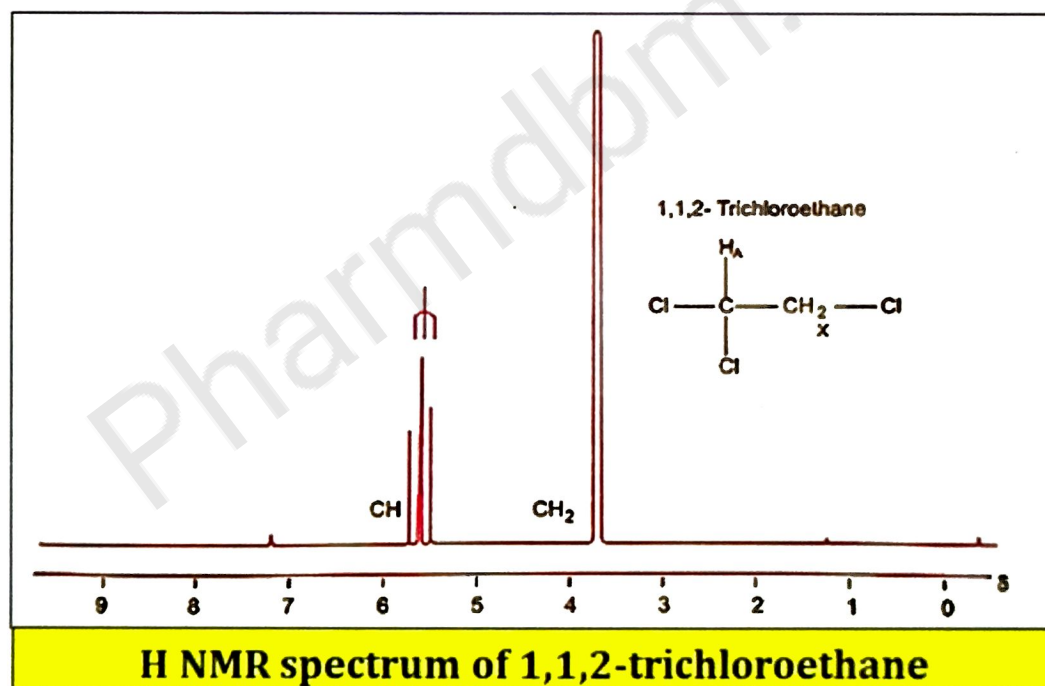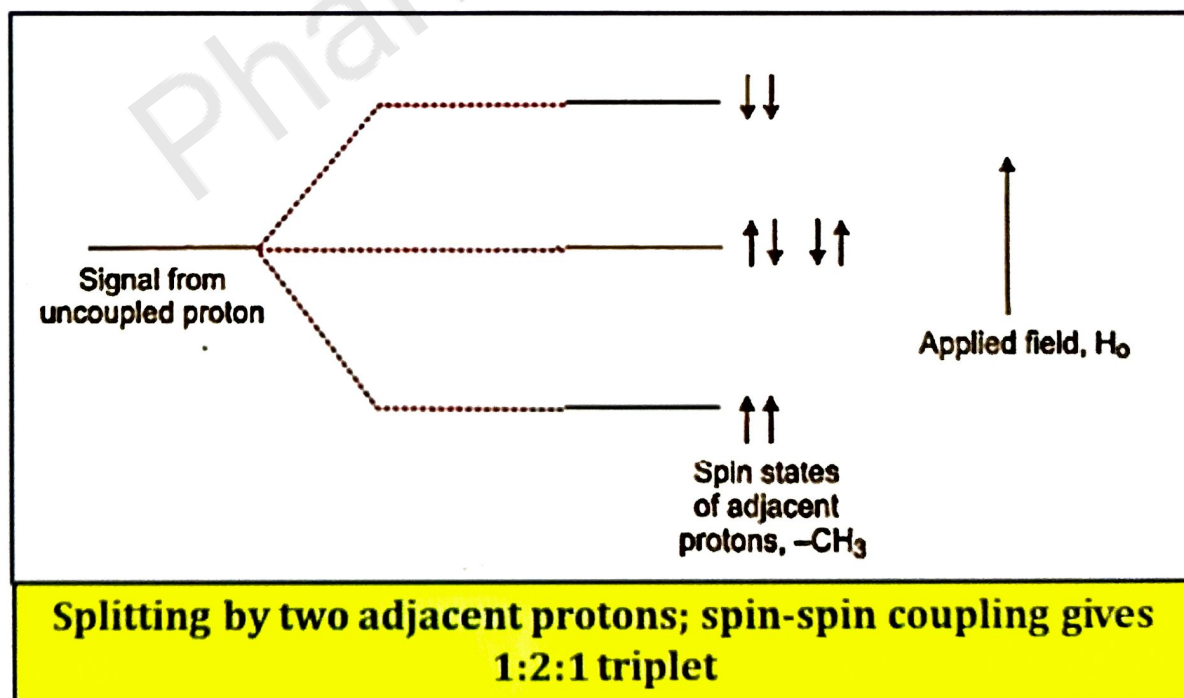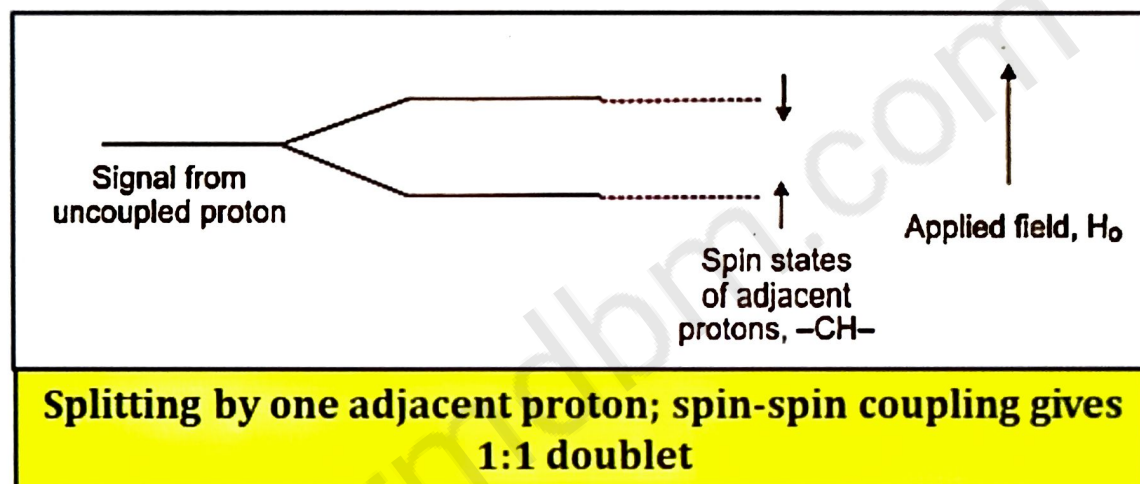

H NMR spectrum of 1,1,2-trichloroethane

- Splitting of the **spectral lines arises because of a coupling interaction between neighbor protons,** and is related to the **number of possible spin orientations that these neighbors can adopt.** The phenomenon is called either spin-spin splitting or spin-spin coupling.

- Example: **NMR spectrum of 1,1,2-trichloroethane.**

Both parallel ↑↑

Parallel and antiparallel ↑↓ ↓↑

Both antiparallel ↓↓

A appears, not as a singlet ────

But is split ────

Into a 1:2:1 triplet ────

The coupling constant, J ───→ ←───

Intensity ratio

1 : 2 : 1

Spin coupling between a proton A and two neighbor protons X and X

- When proton A 'sees' the **two neighboring protons X and X'**, A can see three different possible combinations of spin: the nuclear spins of X and X'

  I. **Both can be parallel to A (↑↑)**

  II. **Both can be antiparallel to A (↓↓)**

  III. **One can parallel and the other antiparallel**, and this can arise in **two ways-X parallel with X' antiparallel** (1) or X antiparallel with X' parallel (1).

- Three distinct energy situations, (1) (2) and (3), are created, and therefore **proton A gives rise to a triplet.**

- The **probability of the first two energy states arising is equal,** but since the **third state can arise in two different ways, it is twice as likely to arise;** the intensity of the signal associated with this state is twice that of the lines associated with the first two states, and we see in spectrum of,1,2-trichloroethane that the relative line intensities in the triplet are 1:2:1.

- The **spectrum of 1,1,2-trichloroethane, consisting of the characteristic doublet and triplet of two protons coupling with one proton.**

Splitting by one adjacent proton; spin-spin coupling gives 1:1 doublet

Splitting by two adjacent protons; spin-spin coupling gives 1:2:1 triplet

❑ RELAXATION

- Relaxation is the process of **transition from exited state to ground state** (or) relaxation processes involve **some non-radiation transitions by which a nucleus in an upper transition state to the lower spin state.**

  1. Spin-lattice or longitudinal relaxation
  2. Spin-spin or transverse relaxation

❖ **<u>Spin - lattice or longitudinal relaxation:</u>** It involves the **transfer of energy from the nucleus in its higher energy state to the molecular lattice.**

- Spin lattice relaxation time T_1 **which T_1 prime is measure of the average life time of the nuclei in the higher energy state.** The energy is transferred to the **components of the lattice as the additional translational, vibrational and rotational energy.** The total energy of the system remains the same.

- T_1 strongly affected by the **mobility of the lattice.** In **crystalline solids and viscous liquids mobilities are low, T_1 is large.** As the mobility of lattice (at higher temperature) the vibrational and rotational frequencies increases.

- By **enhancing the probability of magnetic fluctuations of the proper magnitude,** for a relaxation transition, T_1 becomes shorter. An efficient relaxation process involves a **short time and results in the broadening of absorption peaks.** Smaller, the time of excited stage, greater is the line width. This mechanism is not effective in solids.

❖ **<u>Spin Spin relaxation:</u>** It is due to **mutual exchange of spins by two precessing nuclei** which are **close proximity with each other.** It is also known as **transverse relaxation time T_2.**

- In this **nuclei exchange spins with neighbouring nuclei by interaction of their magnetic moments.** Although no spin energy lost by this mechanism, T_2 values are generally so smaller for crystalline solids or viscous liquids.

- When two neighbouring nuclei of the same kind have identical precession rates, but are in different magnetic quantum states, the magnetic fields of each can interact to cause an interchange of states, a nucleus in the lower spin state is exited while the excited nucleus relaxes to the lower energy state.

- The **total magnetic field felt by any particular nucleus is B_0 plus or minus a factor for small magnetic fields generated by the nuclei of its immediate environment**. Thus, the greater the number of spin-state exchanges the greater are the local field fluctuations, leading to a greater range over which radiofrequency energy may be absorbed, and therefore to line broading.

- **Large values of T_2 results in narrow resonance lines, small values give broad lines.** In liquids and gases local magnetic field variations average to zero, whereas in solids such interactions are finite and lead to very broad absorption bands.

❑ INSTRUMENTATION

Several high resolution NMR spectrometers are available commercially; all may consisting of following major parts.

1. Magnet
2. Magnetic field sweep
3. Radiofrequency source
4. Sample holder
5. Sample probe
6. The receiver
7. Signal detector and recording systems.
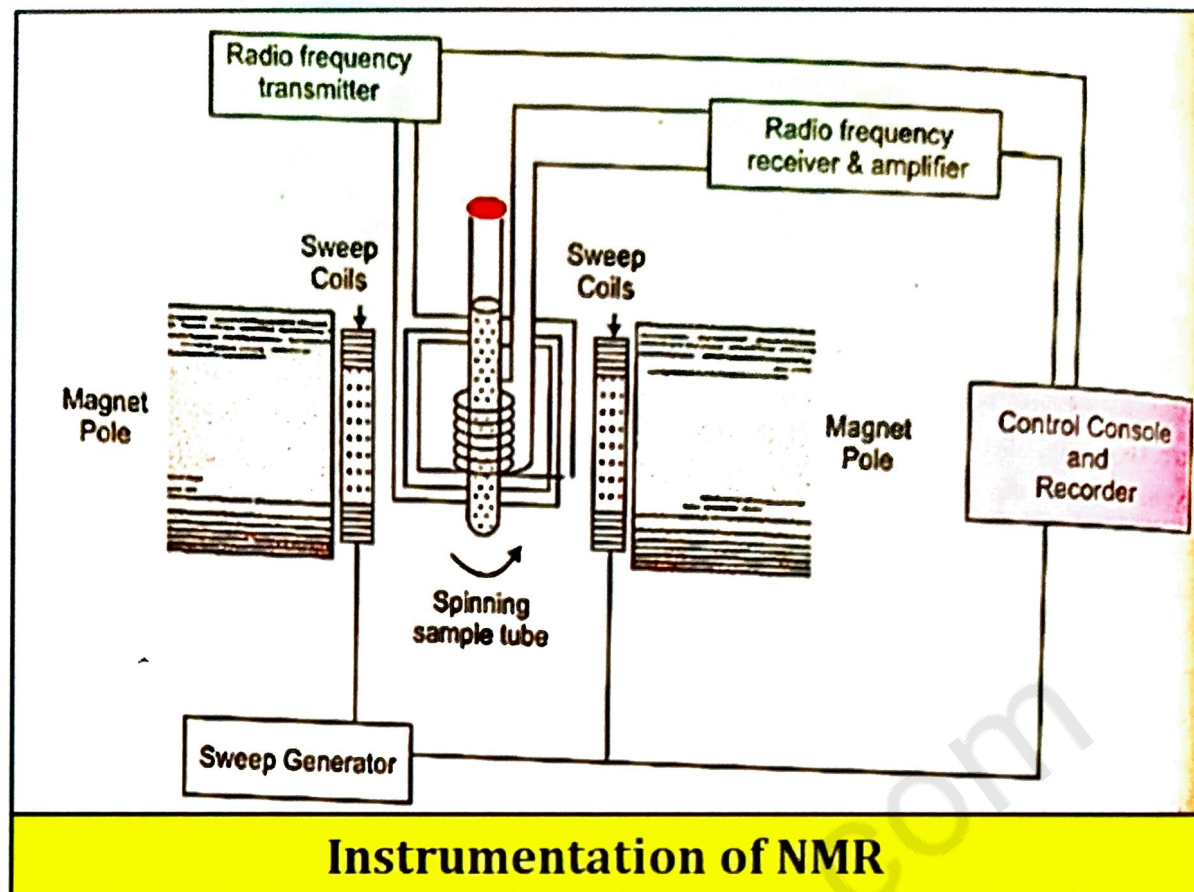
Instrumentation of NMR

❖ Magnet:

- It is used to **supply principle part of field 'B'**. The magnet in most commercial NMR spectrometers may be either an **electromagnet or permanent magnet**, but both must have a **very high degree of field homogeneity between the pole pieces if high resolution work is needed**.

- The strong magnet provides stable and homogeneous field. Magnet size is approximately **15 inches** in diameter.

- The field produced must be homogeneous to 1 part in 10^8 within the sample area and must be stable to similar degree for short period of time.

❖ Magnetic field sweep:

- An alternation over a small range in the applied field may made by making use of a pair of coils (Helmholts coil) located parallel to the magnet face.

- These coils **superimpose on the main field of the magnet**, the additional field required to bring the total resonance condition. **By varying a direct current through these coils, the effective field can be changed by a few hundred miliguass without any loss in field homogeneity**.

- The field strength is changed, however, **automatically and linearly with time and change is synchronized** with the linear drive of a chart recorder.

❖ **Radiofrequency source:** The **signal from a radiofrequency oscillator** or transmitter is fed into a **pair of coils mounted at right angles to the path of the field**. A plane polarized beam of radiation has been obtained. **A fixed oscillator having a capacity of exactly 60 MHz is normally used.** For **high resolution work, the frequency must be constant to about one part in 10^8**.

❖ **Sample holder:** The usual NMR sample cell consists of a **5 mm outer diameter glass tube which has a capacity of about 0.4 ml of liquid**. Microtube for sample volumes is also marketed. The **sample must be in the liquid or solution state for high resolution spectra**. It is contained in a tube which is spun by means of an air turbine.

❖ **Sample probe:** The sample probe is a **device that holds the sample tube in a fixed position in the field** and it is also provided with an **air driven turbine for rotating the sample tube along its longitudinal axis at several hundred RPM**.

- This rotation minimizes the **effects of in homogeneity in the field and as consequences sharper lines** and better resolution are obtained.

- The actual probe design is variable, depending upon the particular instrument. Probe may be either a **single coil or turn coil system**.

- ❖ **The receiver:** The **resonance signal is detected by one of the two methods.** In single coil instruments a radiofrequency bridge like as **Wheatstone bridge**, is employed.

- The **applied signal is balanced against the received signal** and the **absorption or the resonance signal is recorded as an out of balance emf,** which may be amplified and recorded mechanically.

- In the **double coil or nuclear induction method transmitter and receiver coils are set at right angles** to each other about the sample.

- ❖ **Signal detector and recording system:** The receiver coil which is at right angles to the source or transmitter coil and also **surrounds the sample has been used to direct the radiofrequency signal** produced by the resonating nuclei. **The electrical signal generated into the coil must be amplified before it can be recorded.**

- **Resonance line intensity in an NMR spectrum is proportional to the number of nuclei responsible for the signal.** The area under the signal is a direct measure of the intensity and is determined in a cumulative manner by an electronic integrator.

❑ Working:

- The sample under investigation is placed between the **pole faces of the magnet.**

- A **radiofrequency source** (u= 60 megacycles/sec) is made to fall on the sample. It can be **done by feeding energy (radiofrequency source) into a coil wound round the sample tube.**

- A signal is **detected if the nuclei in the sample resonate with the source** i.e. ΔE, energy required to a strip chart recorder after amplification.

- Proton being in electronic environments in a **molecule cannot resonate at exactly 60 megacycles/sec.**

- For practical purpose, **radiofrequency source is held steady at the said frequency and field strength is varied by placing small electromagnet** to the pole faces of the main magnet.

- By increasing the **current flowing** through these electromagnets, the total field strength is increased.

- As the **field strength increases**, the precessional frequency of each proton increases until resonance with the radiofrequency source takes place.

- As a proton (or a set of equivalent protons) comes to resonance, the signal from detector produces a peak on the chart paper.

- The NMR spectrum consists of series of peaks that corresponds to different applied fields strengths. Each peak means a set of protons.

❏ APPLICATIONS

1. **Structure elucidation:** NMR spectroscopy basically used for structure elucidation Organic compounds have hydrogen atoms in their structure and environment of each proton is not same. Hence from NMR spectra following information can be obtained.

   a. **Types of protons:** **No. of peaks** in spectra shows no. of types of protons present in sample.

   b. **Environment of protons:** Whether the proton is shielded or deshielded from the position of the peaks. Shielded protons requires high magnetic field and deshielded protons requires low magnetic field.

   c. **No. of protons of each type:** From the **intensity of peaks**, if the peak is of more height/area, then more protons of that type is present. If peak is of less height/area, then less no. of protons of that type is present.

d. **No. of adjacent protons:** From the **multiplicity of peaks**. If **one adjacent proton is present, then doublet (same peaks of same height) is recorded**. If **two adjacent protons are present triplet (3 peaks in the ratio of 1:2:1) is recorded**. If **three adjacent protons are present, then quartet (4 peaks in the ratio 1:3:3:1) is recorded**. Hence no. of adjacent protons can be known.

2. **Assay of components:** Single component or multicomponent **without separation compounds, can be quantitatively estimated. Specific peak for each component identified** and the **peak area/height ratio given by integral value is found using standard and sample.**

3. **Detection of aromaticity:** Protons attached to benzoyl, polynuclear and heterocyclic compounds whose π electrons follow Huckel's rule [(4n+2)n] electrons. Protons are deshielded due to **circulating ring current of π electrons.** As a result of this the signals for the aromatic protons appear at a very low field than that observed even for benzene. From this, the aromatic character of the compound under investigation can be predicted.

4. **Distinction between Cis-Trans Isomerism:** The **cis-trans isomers of a compound can be easily distinguished** as the concerned protons have different values of the chemical shifts as well as the coupling constant.

5. **Hydrogen bonding:** The NMR spectroscopy can be **used to study the hydrogen bonding in metal chelates, organic compounds**. Proton signal is shifted towards low field in case of hydrogen bonding. This reveals that **hydrogen bond formation results in the decrease in the electron shielding of the proton.**

6. **Materials science:** NMR spectroscopy, in solid state, can be used to investigate new materials of great technological importance such as glasses, ceramics, polymers, synthetic membranes and superconductors. Furthermore, it can be used to investigate reactions taking place in catalytical surfaces.

7. **Food chemistry:** NMR spectroscopy can be used in the verification of the wine aging and authenticity as well as in the identification of the oil's fatty constituents. High resolution NMR techniques have found interesting applications in the analysis of complex mixtures of various extracts of natural products.

8. **Clinical applications:** NMR has found numerous applications in the localization and characterization of metabolites in biological fluids in vivo and in vitro and, thus, it can be utilized in the diagnosis of many kinds of diseases.

9. **NMR as a microscope:** NMR microscopy exhibits a variety of technological applications such as the detection of microscopic defects in plastic tubes, the diamond localization in order to avoid breakings during the procedures that follow their excavation. NMR microscopy can also provide valuable information about the fruits ripening, the best conditions for food handling and even yet about the best cooking temperature conditions.

10. **Magnetic Resonance Imaging (MRI):**

- The blood volume that is conveyed by the heart in one pulse can be measured, allowing the study of the heart in action. Moreover, soft tissues that it is impossible to be detected using X-rays are clearly shown with the MRI technique, allowing the diagnosis of tumors and of other diseases.

- It is well known that during biopsy human tissue is subtracted and the **malignancy of a neoplasm is examined under the microscope**. On the contrary during the NMR tomography no tissue abstraction is taking place.

- Instead, **a chemical compound with specific properties is infused in human's blood**.

- With this methodology, which is completely painless, the insight morphology between, for example, the benign and the malignant breast cancer can be distinguished.

UNIT-I

MASS SPECTROMETRY

Points to be covered in this topic

- ❖ **Principle**
- ❖ **Fragmentation**
- ❖ **Ionization techniques**
  - ➢ Electron impact ionization
  - ➢ Chemical ionization
  - ➢ MALDI
  - ➢ FAB
- ❖ **Analyzers**
  - ➢ Time of flight
 - ➢ Quadrupole
- ❖ **Instrumentation**
- ❖ **Application**

MASS SPECTROMETRY

❑ PRINCIPLE

- The physics behind mass spectrometry is that a **charged particle passing through a magnetic field is deflected along a circular path on a radius that is proportional to the mass to charge ratio, m/z.**

- In an electron impact mass spectrometer, a **high energy beam of electrons** is used to **displace an electron from the organic molecule to form a radical cation** known as the **molecular ion or parent ion**.

- If the molecular ion is too unstable then it can break up in to smaller ion fragment ions or daughter ion.

- The **collection of ions is then focused into** a beam and accelerated into the magnetic field and deflected **along circular paths** according to the masses of the ions. By adjusting the magnetic field, the ions can be focused on the detector and recorded.
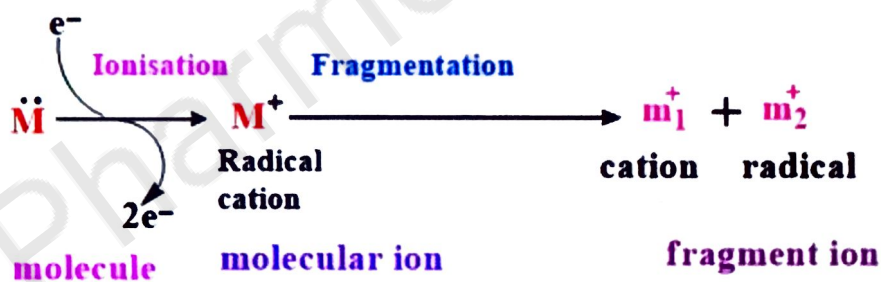
- The molecular ions, the fragment ions and the fragment radical ions are **separated by deflection in variable magnetic field** according to their mass and charge, and generate a current (the ion current) at the collector in proportion to their relative abundances.

- A spectrum is **plot of relative abundance against the ratio mass/charge** (the m/z value).

- For singly charged ions, the **lower the mass the more easily ion deflected in the magnetic field.**

- Doubly charged ions are occasionally formed: **these are deflected twice as much as singly charged ions of the same mass**, and they appear in the mass spectrum at the same value as do singly charged ions of half the mass, since $2m/2z = m/z$.
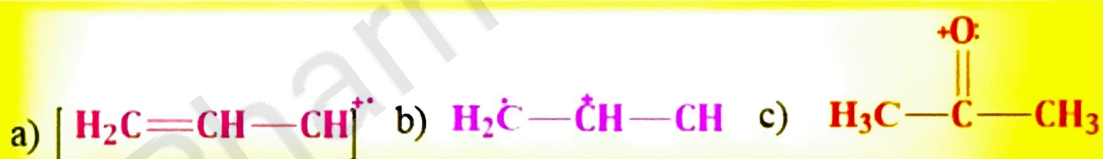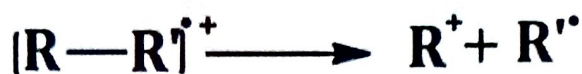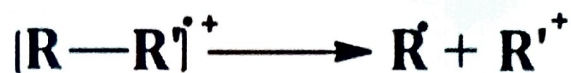
MASS SPECTROMETRY
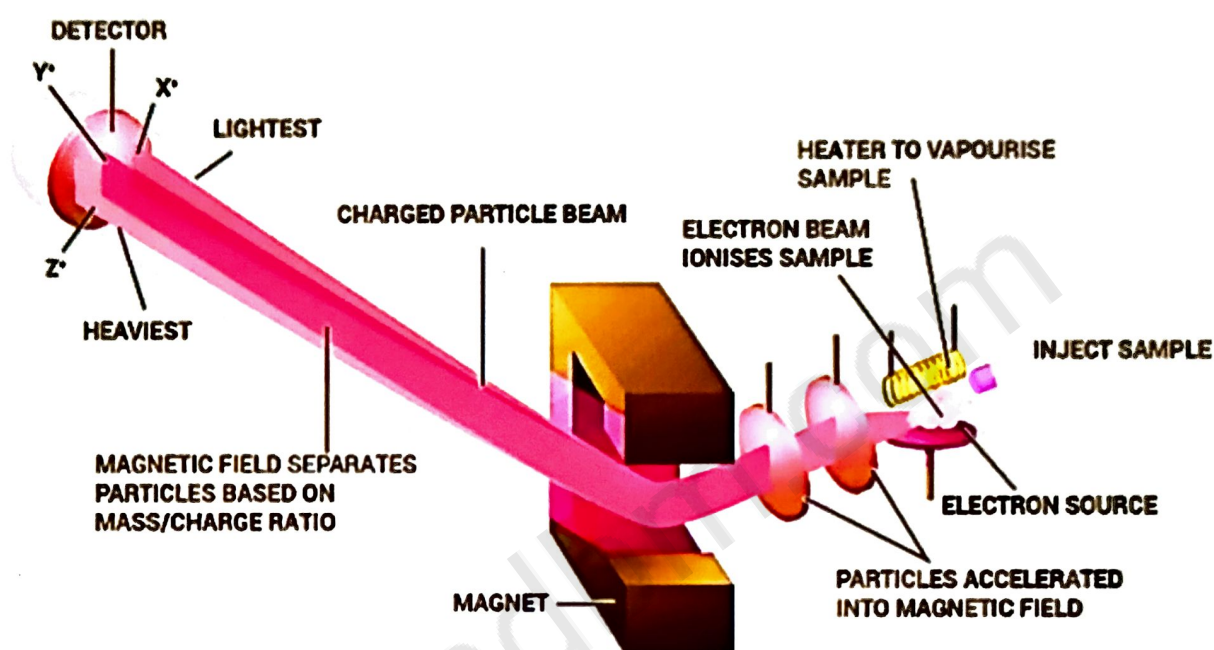
❑ FRAGMENTATION

- In mass spectroscopy, the **information regarding the identity can be very well obtained by the molecular ion.**

- In fragmentation pattern, **molecular ions break up in to smaller ions, called fragment ions or daughter ions.** This ion gives further information regarding the structure of the compound.

- Fragmentation of even simple molecules **produces a large number of ions of different masses.**

- A complex spectrum is obtained which often permits identification of compounds or recognition of the **presence of functional groups** in compounds.

- While the **molecular ion** is one of the most important **peaks in the spectra**, it is also important to **gain information from the peaks** that are a result of fragmentation.

- The goal of **interpreting mass spectra is identifying the structure of the molecular ion** by examining pieces (fragments) of the original molecule.

- The **frequency and size of the fragments is dependent on the structure and bond energy** of the sample molecule.

- This property has resulted in the **creation of unique and reproducible spectrum** for a wide variety of compounds.

- The presence of the **vacuum** in the instrument **prevents collisions with other molecules** allowing these reactive cations to exist.

- Examples represent the charge

  a.   As a delocalized one

  b.   Localized it on either a π bond

  c.   On a heteroatom

a) $\left[H_2C{=}CH{-}CH \right]^{+\bullet}$ b) $H_2\overset{\bullet}{C}{-}\overset{\bullet}{C}H{-}CH$ c) $H_3C{-}\overset{\overset{+O\colon}{\|}}{C}{-}CH_3$

- The process that creates these **observed fragments is the result with their interaction with the energy released from the source.**

- This energy both removes a **single electron, while the excess energy is distributed over various degrees of freedom.** This distribution converts electronic energy into electronic, rotational, and vibration energy.

- The molecular ion is created when the **sample molecule returns to its ground state via a relaxation.** Other times this **energy exceeds the activation energy of fragmentation** and this energy is released via the breaking of bonds.

$$e^- + R{-}R' \longrightarrow [R{-}R']^{\cdot+} + 2e$$

$$[R{-}R']^{\cdot+} \longrightarrow \overset{\cdot}{R} + R'^{+}$$

$$[R{-}R']^{\cdot+} \longrightarrow R^{+} + R''^{\cdot}$$

- The fragmentation of **a single bond** can produce two peaks, one from R + and the other from R'+, since the instrument can only **detect the positive ion**.

- According to **Stevenson's rule,** two fragments are in **competition to produce a cation,** the fragment with the lowest ionization energy will be formed more frequently.

❑ IONIZATION TECHNIQUES

- Ionization of the organic compound is the primary step in obtaining the mass spectrum. Several methods available for inducing the ionization of organic compound.

➢ **Volatile samples**

✓ Electron impact Ionisation

✓ Chemical Ionisation

✓ GC (and LC) inlets

➢ **Non-volatile samples**

✓ Fast Atom Bombardment

✓ Thermospray

✓ Matrix Assisted Laser Desorption Ionisation

✓ Electrospray Ionisation

✓ Atmospheric Pressure Chemical Ionisation - LC (and GC) inlets

❖ Electron impact ionization

- Organic molecules react on **electron impact in two ways:** either **an electron is captured by molecule, giving a radical anion**, or an **electron is removed from the molecule, giving a radical cation:**

$$M^e \longrightarrow M^- \quad \text{or} \quad M^{-e} \longrightarrow M^+ + 2e$$

- The letter is more probable by a **factor of 10^2,** and **positive-ion** mass spectrometry result.

- When **energy of electron impact reaches 10-15 eV molecular ion (M+) produced from organic molecules.**

- The minimum energy required to ionize the organic molecule is called as its ionization potential.

- This ionization potential is of great importance, fragmentation of the molecular ion only reaches substantial proportions at higher impact energies, and **70 eV is used for most organic work.**
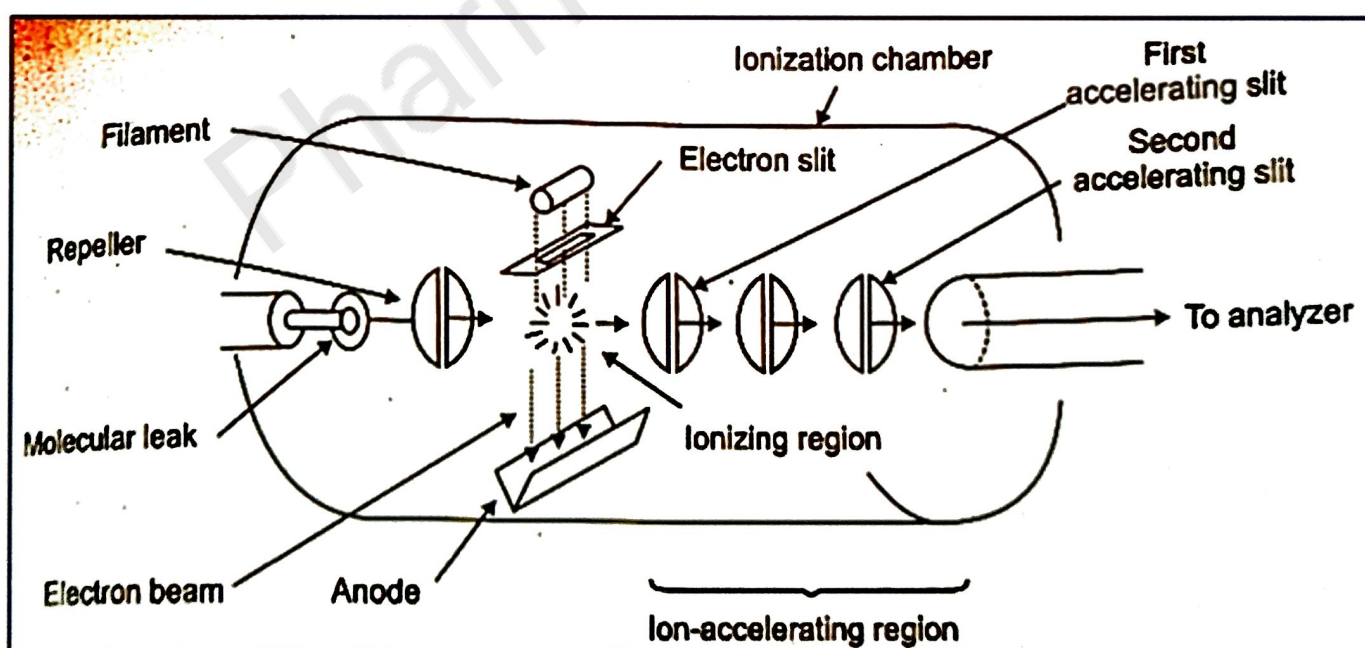
Electron impact ionization

- The electron-impact ion source is the most commonly used and **highly developed ionization method.**

- Once past the molecular leak, the neutral molecules are in a chamber that is maintained at a **pressure of 0.005 torr and a temperature of 200 plus/minus 0.25^{0}C**

- An **electron gun** is located perpendicular to the **incoming gas stream.**

- Electrons emitted from a **glowing filament are drawn off by a pair of positively charged slits** through which the electrons pass into the body of the chamber.

- An **electric field maintained** between these slits accelerates the electrons.

- The **ionizing electrons from the cathode are formed** into a tight helical beam by a **small magnetic field, on the order of 100 G, which is confined in the ionization region.**

- The **number of electrons is controlled by the filament temperature** whereas the energy of the electrons is controlled by the **filament potential.**

- **Ions are formed by the exchange of energy during the collision of the electron beam and sample molecules.**

- This results in a **Franck-Condon transition producing a molecular ion,** which is usually in a high state of electronic and vibrational excitation.

- The filament potential is variable. A range of **6-15 V is used in molecular weight determinations** when it is desirable to avoid fragmentation.

- A **source operating at 70 V, the conventional operating potential, provides sufficient energy to ionize** and cause the characteristic fragmentation of sample molecules.

- Fragmentation can provide **positive identification** of an unknown.

- The **positive ions formed in the ionization chamber are drawn out by a small electrostatic field** between the large repeller plate (charged positive) behind them and the first accelerating slit (charged negative) ahead of them.

- The positive charge on the repeller plate does not affect the un-ionized molecules as they enter the ionization chamber.

- A strong electrostatic field between the **first and second accelerating slits of 400-4000 V accelerates** the ions of masses m1,m2,m3, and so on, to their final velocities.

- The ions emerge from the **final accelerating slit as a collimated ribbon of ions with velocities and kinetic energies given by**

$$zV = \frac{1}{2}m_1 v_1^2 = \frac{1}{2}m_2 v_2^2 = \frac{1}{2}m_3 v_3^2 = \dots$$
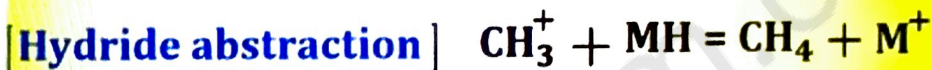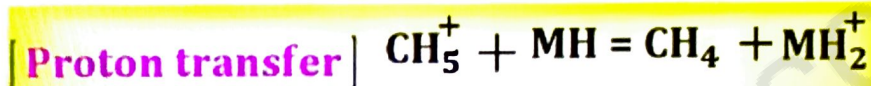
Where z is charge of the ion.

❖ Chemical ionization

- Involves ionization of a gas molecule (CH_4) to protonate the analyte (at pressures of 1 Torr).

- Ionizing gas usually **present at concentrations 10^3-10^4 times the analyte molecules**. Ionizing gas ionized by an **electron beam**. CH_4 produces ions from **analyte molecule by H^+ or H^- transfer**. Other gases used are NH_3, iso-C_4H_{10}.

- It is a **Soft Ionization Technique**. Is an especially useful technique when no molecular ion is observed in EI mass spectrum, Produces molecular ions $[M+H]^+$ called quasimolecular ions (diff from M^+).

- **Positive chemical ionization:** A two part process occurs
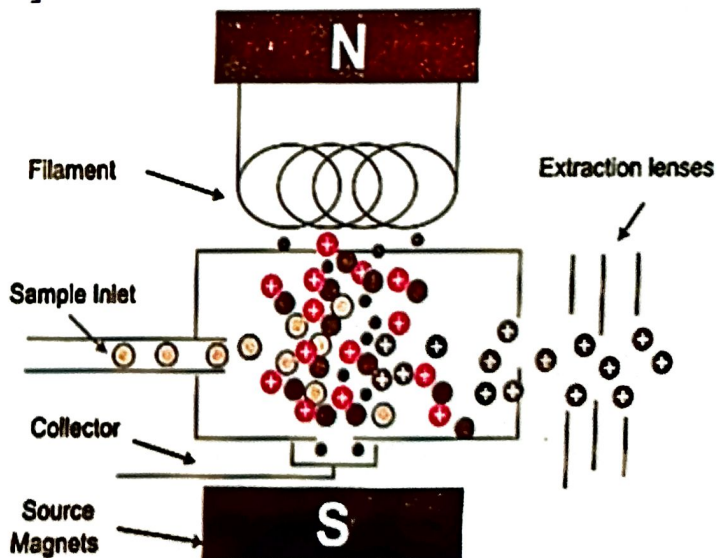 - ✓ First part a **reagent gas is ionized by electron impact ionization** in the source.
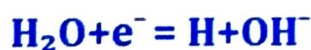
- **Ionizing gas: Methane**

Electron impact	$CH_4 + e^- = CH_3^+ + 2e$
Secondary ions	$CH_3^+ + CH_4 = CH_5^+CH_3$
	$CH_3^+ + CH_4 = C_2H_5^+ + H_2$
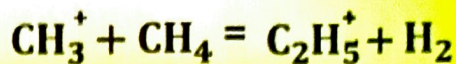
- Second part of the chemical ionization process occurs when a **reagent ion** $(CH_5, C_2H_5^+)$ encounters a sample molecule (MH). The reagent ion and sample molecule react via any of several modes:

[**Proton transfer**]	$CH_5^+ + MH = CH_4 + MH_2^+$
[**Hydride abstraction**]	$CH_3^+ + MH = CH_4 + M^+$
[**Charge transfer**]	$CH_3^+ + MH = CH_4 + MH^+$

- **Negative chemical ionization:** Negative chemical ionization is the **counterpart of positive chemical ionization**. Typical negative ion-forming reactions are:
  - ✓ [Resonance capture of electron] $M + e^- = M^-$
  - ✓ [Dissociative capture of electron] $RCI + e^- = R + Cl^-$

  $H_2O + e^- = H + OH^-$

Chemical ionization

❖ MALDI

- MALDI is the abbreviation for **"Matrix Assisted Laser Desorption/Ionization."**

- The sample for MALDI is **uniformly mixed in a large quantity of matrix.** The matrix **absorbs the ultraviolet light** (nitrogen laser light, wavelength 337 nm) and **converts it to heat energy**.

- A small part of the **matrix** (down to 100 nm from the top outer surface of the Analyte in the diagram) **heats rapidly** (in several nano seconds) and is vaporized, together with the sample.

➤ Working

- A **sample is mixed with an aromatic carboxylic acid matrix;** most common are **4- cyanohydroxycinnamic acid and sinapinic acid.**

- The **sample-matrix mixture is dried on a plate**, then loaded into the mass spectrometer which is **evacuated to a high vacuum.**

- A **laser illuminates the sample and the matrix transfers energy to the sample molecule** which moves into the vapor phase.

- The **energy also ionizes the molecule giving it a charge** (positive ions most commonly used).

- An **electric field accelerates the ion down a tube about 1 meter long.** The kinetic energy of the ion when it reaches the **detector can be calculated using Newtonian physics with the equation:**

$$\text{Energy} = \tfrac{1}{2} \text{ mass} *(\text{velocity})^2.$$

The energy of the ion = charge * voltage,

- Where the **voltage of about 20,000 is known from instrument settings and charge is an integral number, most often 1.**

- From **measurement of the time taken for the ion to move from the plate on which it was loaded to the detector**, the mass can be calculated.

- Although the instrument measures the **time for an ion to move from where it goes into the vapour phase to the detector**, normally results are shown as mass/charge.

- Reflector mode in which an electrostatic lens reflects ion, focusing them, and thus **increasing the resolution between different molecules. Useful up to about 6000.**

**Matrix Assisted Laser Desorption/Ionization**
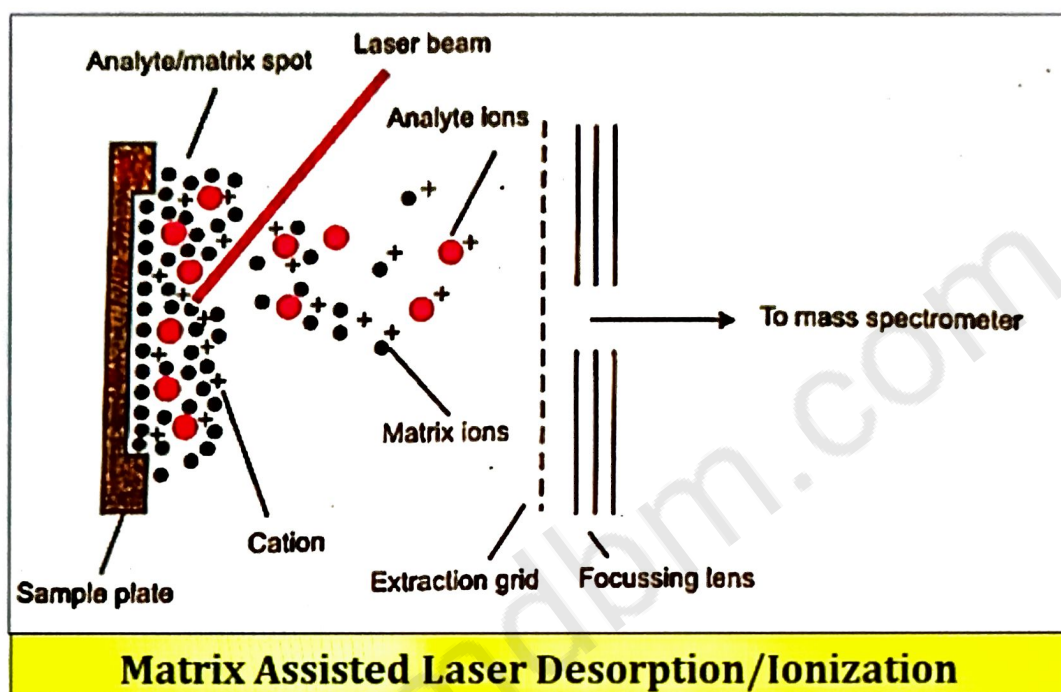
Advantages

- Measure intact mass of proteins, peptides and other molecules.

- Isotopic resolution of molecules up to mass of 3500.

- Mass range of **700 to 70,000.**

- Smaller molecules can be measured in some cases-matrix interferes below 600.

- Non-peptide molecules may not ionize well.

- Positive ions (protonated) used most often; negative ions (deprotonated molecule) use for negatively charged molecules.

- Salt can be removed with Zip Tip or diluted for some samples.

- Some detergents can be tolerated in concentrated samples-need special procedure.

❖ FAB

- **Fast atom bombardment** spectrometry can be considered to be an extension of secondary mass spectrometry.

- **A beam of high energy particles is generated and focused on to the sample**, which is **held a thin film on a clean metal surface**.

- The secondary ions extracted by an appropriate ion optical arrangement into the mass spectrometer analyzer.

- The impact of a **high energy ion striking the surface produces an intense thermal spike**. The energy from this thermal spike dissipated through the outer layer of the sample.

- In this innovative device first development by Barber, neutral atoms of **argon and xenon** were employed, but ultimately these we replaced by charged ions such as Cs^+ and Xe^+.

➢ Working

- Fast moving beam is directed towards the sample.

- **Sample is dissolved in a matrix an placed on target**. Beam **collides producing $+^{ve}$ and $-^{ve}$ ions** from matrix, analyte often added to enhance **$[M+H]^+$ formation**.

- FAB matrix facilitating the **desorption and ionization process**. Constantly replenish the surface with **new sample as it is bombarded by the incident ion beam**.

- By absorbing most the incident energy, the matrix also minimizes sample degradation from the high-energy particle beam. Two of the most common matrices used **m-nitrobenzyl alcohol (NBA) and glycerol**.

- If a dry sample is used, the **surface becomes damaged by the intense energy of incident beam** and the yield of secondary ions is rapidly reduced.
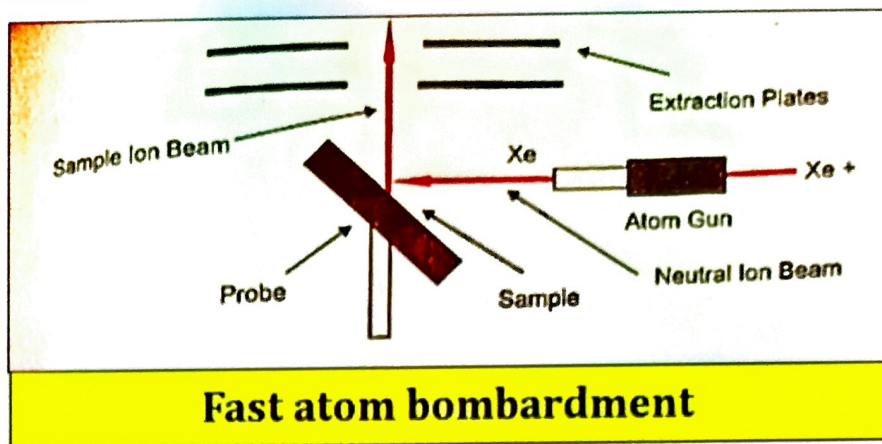
Fast atom bombardment

- However, **if the sample is dissolved (or dispersed) in a liquid matrix** (e.g. glycerol) the liquid surface is continuously renewed and **the high energy primary beam safely used.**

- This **method of ionization is now a standard procedure used to obtained the mass spectra of very polar and labile substances**, The temperature of the glycerol is critical.

- At **-20°C the glycerol is too viscous, and cannot dissipate energy rapidly enough, and at 40 °C the vapour pressure of the glycerol is too high** and it evaporates in the high vacuum of the source. Optimum sensitivity appears to be realized at a temperature of 25°C.

Advantages

- It is **Soft Ionization technique** Used to **analyze polar, ionic, thermally** and energetically labile and high MW compounds that are not amenable to EI/CI.
  - ✓ MW between 300-6000Da
  - ✓ Sample is dissolved in a matrix and bombarded with Ar/Xe atoms (8-15keV) or fast ions
  - ✓ Observed peaks in FAB are those of matrix cluster ions, analyte ions $(M^+$ and $M^-)$, $(Cs^+$ up to 35 keV).

- **Classes that use FAB are:** peptides, proteins, fatty acids, organometallics, surfactants, impurities, and ions of matrix modifiers. Carbohydrates.

❑ ANALYZERS

- In the **mass analyzer ions are separated** based on their **mass to charge (m/z) ratios by applying electric field** (electrostatic analyzer) **or magnetic field** (magnetic analyzer) **or both.**

- The ions are analyzed by **measuring the electric field or both required for getting peak in the spectrum**, or by measuring the time they take to travel a fixed distance.
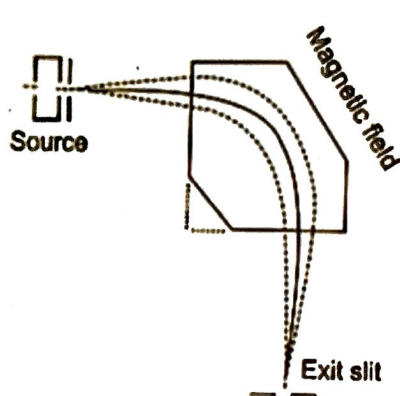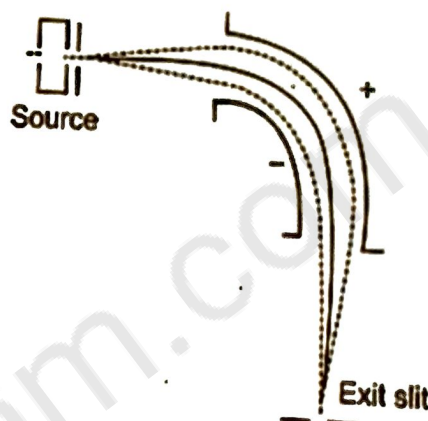
| Magnetic analyzer | Electrostatic analyzer |

- **Separation of ions in analyzers In a magnetic analyzer ions are separated on the basis of m/z values**, and a number of equations can be brought to bear on the behaviour of **ions in the magnetic field**.

- The kinetic energy, E, of an ion of mass m travelling with velocity v is given by the familiar $=\dfrac{1}{2}mv^2$

- The **potential energy of an ion of charge z being repelled by an electrostatic field of voltage V is zV. When the ion is repelled, the** potential energy, zV, is converted into the kinetic energy, $=\dfrac{1}{2}mv^2$ so that

Therefore,
$$zV=\frac{1}{2}mv^2$$

$$v^2=\frac{2zV}{m} \quad \text{......1}$$

❖ Time of flight

- It has been that **all ions leave the acceleration field with different velocities** depending their masses.

- With magnetic focusing, the **ions get separated by changing their directions** However, **if these ions are allowed to travel in a straight line through a magnetic field-free region**, they will take **different times to travel a given distance.**

- The measurement of "this time of flight" forms the **basis for the non-magnetic separator.**
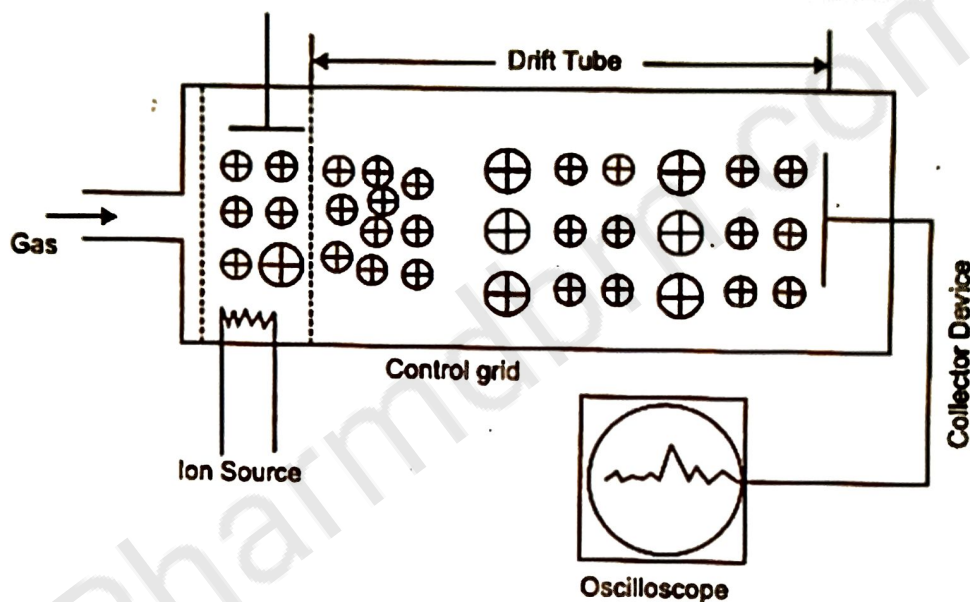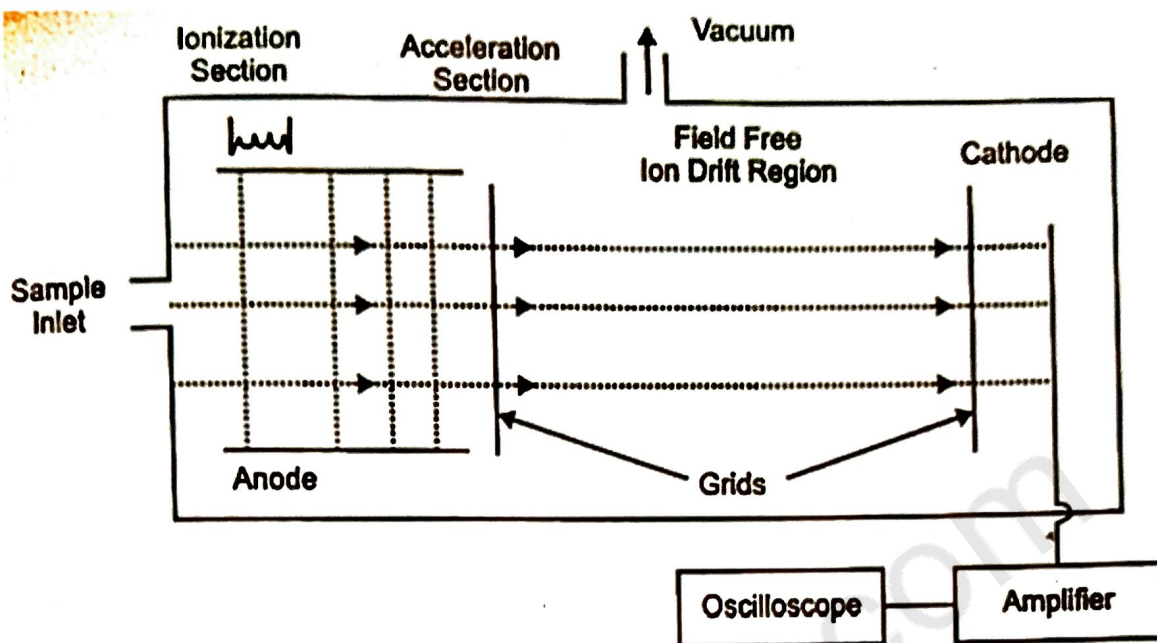

Time of flight

- If the ions are **permitted to enter the drift tube continuously**, the time required for the **ions to travel in non-magnetic separator cannot be measured.**

- The problem has been **solved by inserting a control grid in the electron beam.** By this, the ions will be produced in pulses which will last only for **about 0.25 μ sec at a frequency of 10,000 times per second.**

- The **accelerating grid and collector device** have to be pulsed in the sequence. Time of flight is given by $t = k\sqrt{m/e}$

- ✓ Where k is known as **proportionality constant**
- ✓ Value of k depends on the **length of flight path.**
- ✓ **t is time in microseconds.**

Time of flight mass spectrometer

- An electron stream which is **pulsed every 100 μsec for a short (0.25 μsec) period of time strikes and ionizes** the sample molecule as they enter the chamber.
- These **charged particles pass through the focussing grids** and enter the **field free drift chamber in bursts** which corresponds to the pulsing frequency.
- The **bursts separate into bunches of particles, the faster particles moving ahead of the slower ones.** Each bunch of particles produces a current as they **strike the ion cathode receiver.**
- The produced current is **amplified and recorded on an oscilloscope,** the time base of which is synchronized with the pulse timing.
- The almost **instantaneous display of the complete spectrum, combined with the possibility of observing the spectral changes** in a short period of time, lend itself well to kinetic studies.

❖ Quadrupole

- The quadrapole instruments are the field instruments, which incorporate the **combination of a radiofrequency (RF) and a direct current field**.

- In a quadrapole mass analyzer, focusing of the ions after their acceleration from the ion source is **brought about by the process of filtration.**

- A quadrapole filter **consists of four parallel tungsten rods or poles**. In most instruments rods are **cylindrical, although rods that have a hyperbolic shape are also used.**

- The **accelerated ionic beam from the ionic source passes through a collimating hole** that is aligned with the space between the four rods.

- **Diagonally opposite rods are electrically connected**; i.e. rod 1 is connected to rod 3 and rod 2 is connected to rod 4.

- A **DC potential difference and a radiofrequency AC potential** are simultaneously applied between the two groups of connected rods.

- When **ions produced in the ion source enter the area between two pairs of a rods**, they **travel with a constant velocity in the direction parallel to the poles.**

- Because of the application of the **radio frequency ac potential, the polarity of two tungsten rods constantly changes.**

- Positive ions that **enter the space between the electrodes are repelled by the rods that are momentarily positively charged and attracted to the rods that negatively charged**, because the relatively charge on the sets of rods is continuously changing, the ions follow an irregular oscillating path between the rods.

- Only those ions that can pass through the **space between the rods strike the exit hole** and are measured by the detector. Other ions strike one of the rods and are not detected.

- This continues and results in the complex oscillations in X and Y directions.
- The **force of attraction exerted by the negatively charged tungsten rod** on the positively charged ions is so great for certain ions that the ions collide with the rod and loose their charge.
- Thus, the **ions of certain m/z value do not get recorded** at a **particular value of radiofrequency ac potential.**
- Only a **stable oscillation, for an ion having a definite m/z value can pass through the filter at a particular value of dc potential** difference and ac radio frequency potential.
- All other ions **possess unstable oscillations and strike the poles and are lost.** By **varying dc potential difference and ac radio frequency potential the ions of different m/z values are allowed to pass** through the filter sequentially.
- The lower voltages allow ions of smaller m/z values to pass through the filter.
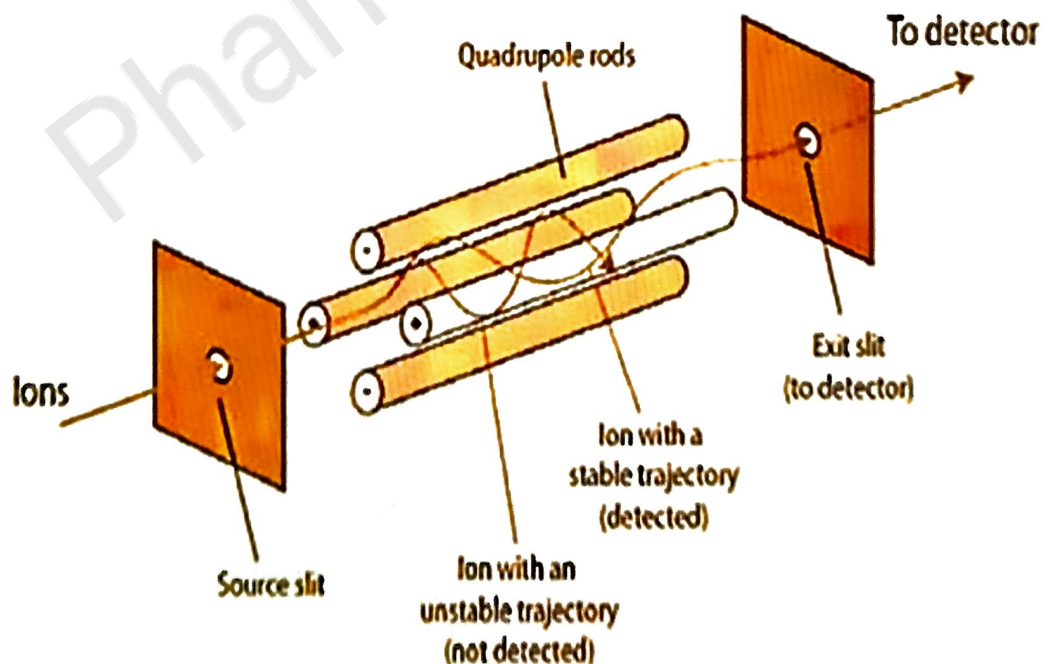

Quadrupole analyzer

❑ INSTRUMENTATION
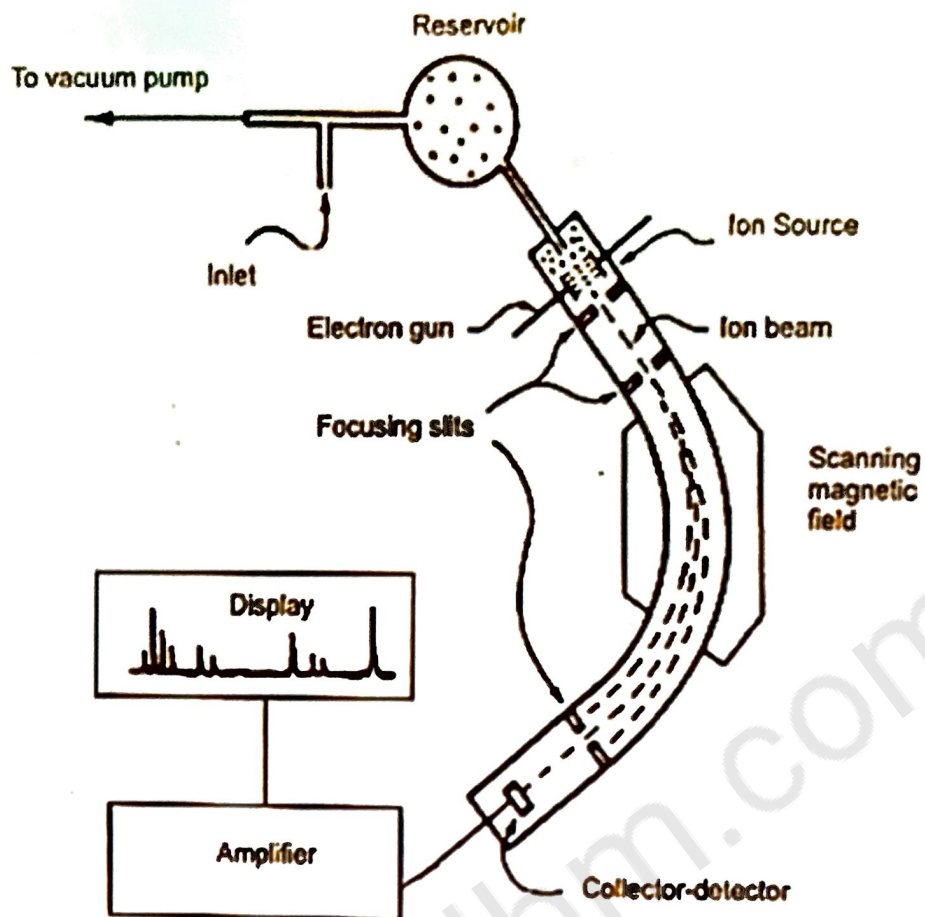
Instrumentation of Mass Spectrophotometer

- Mass spectrometer consists of following basic components and a schematic view of the major components of a mass spectrometer is presented below.

1. **Sample inlet/introduction**

2. **Ionization chamber**

3. **Ion separator or mass analyzer**

4. **Ion collector, detector and read out system**

5. **Vacuum system**
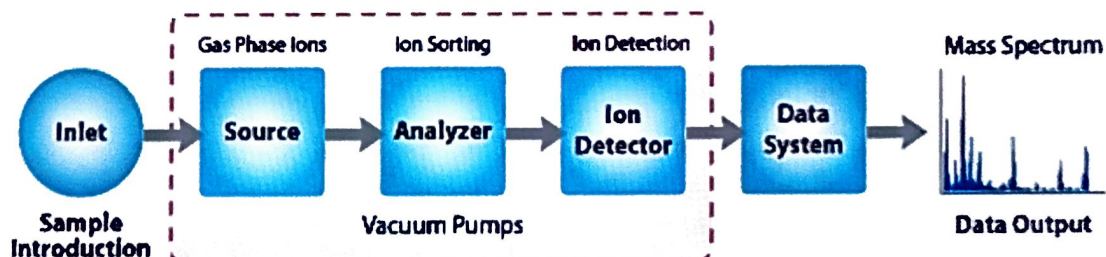
Schematic view of the basic components of the mass spectrometer

- Mass spectrometry is used to **measure molecular masses** of individual compounds or atoms by **converting them to charged ions**.
- Three basic steps are essentially involved in all mass spectrometers:
1. The first step is **formation of gas phase ionic species** (cation or anion).
2. The next step is **separation of ions based on their m/z ratios** in a mass analyzer.
3. The final step is the **detection and amplification of ions** obtained after separation in the mass analyzer.

❖ SAMPLE INLET/INTRODUCTION

- The purpose of the sample handling system is to **introduce the sufficient quantity of the sample** into an **ion source at constant rate without disturbing the vacuum.**
- A micromole sample in **gaseous state is slowly allowed to leak into ionization chamber.**
- The pressure of the sample in the **reservoir is greater than the ionization chamber to maintain the steady flow** of gaseous sample.
- For different types of samples inlet systems are used.

➤ Heated inlet system:

- This type of inlet system generally used for less **volatile liquid sample** having boiling point less than $500°C$ and gaseous samples.
- In this system **sample is vaporised externally and then introducing sample into ion source** without breaking the vacuum.
- The volatile liquids are handled by **'freeze out' technique** in which the sample holder tube is **frozen with liquid nitrogen or dry ice.**
- Examples of low volatile samples- **amino acids, acids, sugars** are converted into **volatile derivatives.**
- **Example: hydroxyl compounds into ethers, acids to esters.**

- ➢ **Direct inlet system:** Solids, non-volatile liquids and thermally unstable compounds are directly introduced into the ion source by means of sample probe which is inserted through a vacuum lock.

- Usually the provision is made for both **cooling and heating of the sample on the probe by which sample can be slowly vaporised** in the electron impact region.

- In this case very low sample is required to **record the spectrum.**

- **Thermal decomposition is eliminated** through this technique.

- **Examples: Non-volatile samples- carbohydrates, polymers, steroids.**

- ➢ **Chromatographic technique:** Sample introduction into the mass spectrometer is achieved either by a direct insertion probe which allows less volatile substances to be introduced through a vacuum lock assembly or from a separating system(GC, LC or CE) where separated analytes are introduced as pure components in gas or liquid stage into the ionization chamber.

❖ IONIZATION CHAMBER/ION SOURCE

- In the ionization chamber, **charged ionic species are generated from neutral molecules prior to their analysis** in the mass analyzer mainly through **electron ejection, electron capture, protonation, deprotonation or adduct formation.**

- The ions formed in the mass spectrometer are then **accelerated by electrostatic field into a mass filter** to be separated and finally detected.

- Several different types of **ion sources have been developed for ionization** of a wide range of compounds. However, the physicochemical properties of the **analytes and the internal energy transferred during ionization are of prime importance** during ionization in the ion source.

❖ MASS ANALYZERS

- After formation of gas phase ions in the ion sources, they are required to be separate according to **their masses or more precisely their m/z ratio.**

- All mass analyzers perform two basic functions **by using static or dynamic electric a magnetic fields:**

    1. **To resolve all masses according to their m/z ratio.**

    2. **To focus all resolved ions to a single point.**

- Different types of mass analyzers are in use and each of them has **trade-offs relating to speed of data acquisition, sensitivity, resolution of separation, mass accuracy** and other operational requirements.

- **Mass accuracy usually expressed in parts per million (ppm) units indicates the accuracy of the m/z provided by the mass analyzer** and is directly linked with the stability and resolution of the mass analyzer.

List of some of the prominent mass analyzers

| Mass analyzer	Resolution
Magnetic Sector	100,000
Time-of-Flight(TOF)	5000
Quadrupole	2000
Ion Trap(IT)	4000

❖ DETECTORS

- A detector **converts the beam of ions into a usable signal through the generation of electric current** which is proportional to the ion abundance.

I. **Faraday cup collector:** Faradays cup are based on the measurement of **direct charge current produced when an ion hits the surface**. It consists of an **insulated conductor directly connected to an electrometer amplifier**. Its cup shape with **suppressor and guard electrode reduces the chances of escape of secondary electrons** liberated by ion impact.
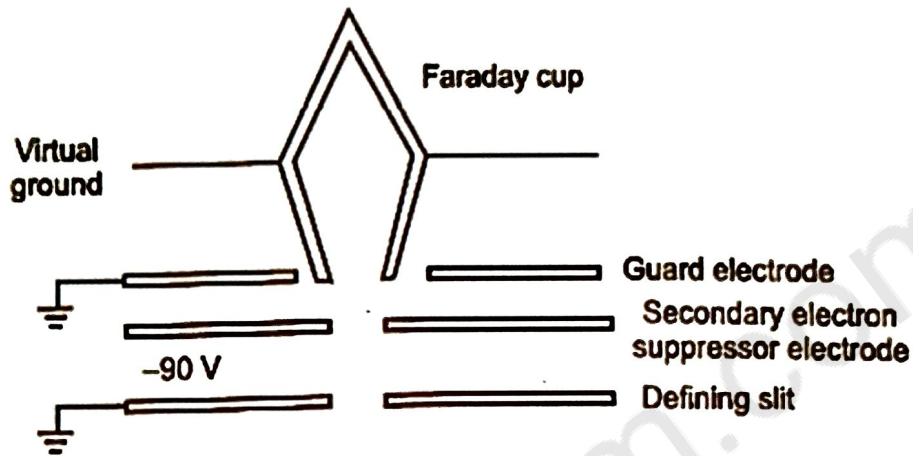
Faraday cup collector

II. **Electron multiplier:** electron multipliers are based on **the kinetic energy transfer of incident ions by collision** with a surface which amplifies the signal by generating secondary electrons.
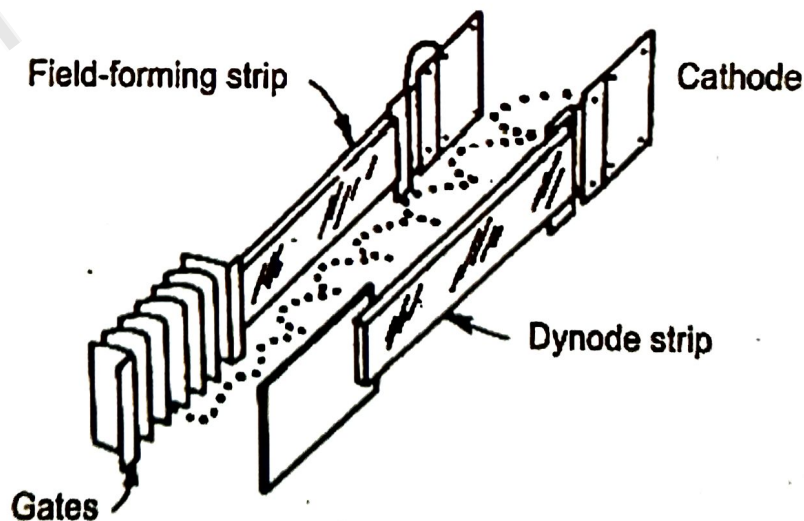
Electron multiplier

III. Photographic plate detection: This detector system is more **sensitive** than any other detector because the photo plate integrates the ion signal over a period of time.

- The photo plates are processed by the usual photographic technique and read with the **aid of densitometer.** /photographic detection is used effectively for high resolution in double focusing spectrometer.

❖ VACUUM SYSTEM

- For the operation of mass spectrometer, **the ion source, the mass analyzer, and the detector** must be kept under high vacuum conditions of 10^{-6} to 10^{-7} torr.

- Both the speed at which the instrument is operational after cleaning or **opening to atmospheric pressure and the efficiency of maintaining a high vacuum** are related to the capacity and speed of vacuum system.

- Most systems use a **combination of oil diffusion pumps to maintain high vacuum and backing rotary pumps** to reduce the initial pressure to approx. 0.001 torr.

- However oil diffusion pumps are being replaced more and **more turbomolecular pumps.**

- Turbomolecular pumps contain **no working fluid**; the pumping effect is purely mechanical.

- Therefore background spectra are practically nonexistent and thus eliminate problems due to accidental venting.

❖ READ OUT SYSTEM

- Electrical detection of **mass spectrum allows operation of the ion-collection system** in one of the several modes.

- Scanning the mass spectrum across the detector is an **extremely inefficient way to collect information** and is seldom done.

- **Peak switching allows the detector to view selected regions of the spectrum,** bypassing those that contain no information or unwanted information.
- In this mode the **magnetic field is varied to scan the spectral regions.**
- On the **modern mass spectrometers the data are digitalized and collected on magnetic tape** or **stored in the memory of a computer** for subsequent processing.

❑ APPLICATION

➢ Determination of molecular weight of compound:

- Mass spectroscopy is best method for **determination of molecular weight of compound.**
- When a substance **bombarded with moving electrons,** and mass spectrum is recorded.
- The **mass spectrum of compound is a line spectrum** obtained by plotting m/z values on X-axis and the intensity of peaks or lines with respect to base line, on Y-axis. A peak 100% intensity is considered as base peak.
- A molecule **losing a single electron after a ionization method produces a molecular ion.** This ion corresponds to the m/z value, which is exactly equal to the molecular weight of that compound.

➢ Determination of molecular formula:

- The correct molecular formula or the elemental composition of a compound can be determined if the exact mass of atoms up to 4 or 6 decimal is known.
- This is possible with **high resolution spectrophotometer or a double focusing spectrophotometer.**

Determination of elemental composition of ions by isotope abundance ratio method:

- The isotope abundance of **easily vaporisable elements** can be determined using a mass spectrophotometer.

- When this information is **coupled with the precise isotopic mass determination, values of the atomic weights** of the elements may be obtained.

Quantitative analysis of mixtures:

- In quantitative analysis of mixture, the **spectra are recorded for component.**

- It should be noted that **samples of each compound must be available in a fairly pure state.**

- From a **careful inspection of the individual mass spectra, known or suspected to be present in the mixture**, it is possible to select analysis peaks on the basis of the intensity and freedom from interferences by the presence of both component.

Thermodynamic studies:

- Mass spectrometry very useful in thermodynamic studies and can be **used over a wide temperature range.**

- A special type of ion source, known as **Knudsen cell ,** has been used for **determining the molecular species volatilized.**

- In Knudsen cell impact of **high energy beam of electrons causes an increase in temperature of the sample upto 2500°C** with resultant emission of ions.

Heat of vapourization:

- Heat of vapourization of high temperature material can be determined by making use of **graph in which ion intensities are plotted against temperature.**

- Change of **partial pressure with temperature** can be calculated from the slope of the line in the graph.

- Heat of vapourization at any temperature can be calculated by **substituting the change of partial pressure in Clapeyron equation.**

- The vapour pressure curves as well as heat of vapourization of **graphite, scandium and lanthanium** have actually been determined by making use of this technique.

Identification of an unknown compound:

- In order to identify the mass spectrum of an unknown compound, **following points are to be kept in mind:**

- **A background spectrum is run before a sample** is introduced. This is done because **weak peaks are often present at m/z 41,43,55 and 57(hydrocarbon background).** Then the sample is introduced into the **ionization chamber and sample spectrum is run.**

- Next step is to **check whether the peak at highest mass is likely to be molecular ion.** Then check whether the peaks immediately **below an assumed molecular ion corresponds to the loss of neutral molecules** or not. Further to find **nature of bonds in molecular ion from molecular ion abundance.** Finally, one has to note molecular weight odd or even.

- From **fragmentation pattern,** one may find out very easily a **preliminary identification about functional groups** and also **partial structural information about the compound.** Also assign any metastable peaks.

- After molecular diagnosis as described above, **the final identification of unknown compound may be done by comparing its mass spectrum with that of an authentic sample**. One important point to remember is that the two mass spectra should be recorded under identical conditions.

➢ Characterization of polymers:

- Mass spectrometry use for the characterization of polymers. First of all, The polymer is **pyrolized (decomposed by heating) and then pyrolized products are fed into the inlet of a mass spectrometer** and identified.

- From the results, one may obtained much information concerning the structure of polymer.

➢ Impurity detection:

- Mass spectrometry used for detection of impurities. The detection impurities in parts per million is only possible if their structures differ considerably from those of the major components.

- If the molecular weight of the impurities much large than major components their detection is easier because their higher mass peaks are from contribution by those of the major components.

- On the other hand if the molecule weights of the impurities are much lower than the major components, their **detection a not an easy job because of the formation of common fragment ions.**

➢ Quantifying Drugs in Plasma:

- The use of tandem quadruple mass spectrometry allow for the separation of analytes in plasma using a high-sensitivity method to achieve low limits of quantitation.

- The method described **alleviates the need for evaporation to dryness and allows for increased sensitivity.**