(UNIT III)

"Mass spectrometry"

(Principle) – Mass spectrometry is the most accurate method for determining the molecular mass of the compound and its element composition.

In this technique molecules are bombarded with a beam of energtic electron. The molecules are ionised and broken up into many fragment, some of which are position ion. Each kind of ions has a particular ration of mass to charge i.e (m/e) value. For most ions, the charge is one and thus (m/e) ~~ration~~ ratio is the simply molecular mass of the ion.

→ Mass Spectra is also called as positive ion spectra. we use electron bombardemont to convert a Neutral molecules to a positive charge one. Theree is no ground or excited state.

→ obtaining mass spectra consist of:

(I) conversion of Neutral molecules into a charged molecules, preferably to a positive charged molecules.

(II) Seperation of the positively charged fragmont formed, based on their masses, by using electrical or magnetic field or both.

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The sample is bombarded with high energy electron beam (70 eV), where an electron is knocked off from every molecules. Hence the molecules become positively charged. when a positive potential (accelerating potential) is applied, as the molecules are positively charged, they get repelled and travel with great speed in a straight line.

potential energy = kinetic energy of molecules

$$eV = \frac{1}{2}mv^2 \quad \text{---}$$

e = charge of ion V = acceleration voltage

m = mass

v = velocity after acceleration

when a magnetic field or electric field is applied, the positive charged fragments which were travelling in straight path, now travels in a curved path. when they travel in a curved path under the influence of magnetic field, the fragments are seperated into different masses because the radius of curvature depends upon their respective masses

Under magnetic field

$$He\underline{v} = \frac{mv^2}{r} \quad \text{---(II)}$$

r = radius of ion path

H = Strength of mag. field

$$v = \frac{reh}{m}$$ — from equation (II)

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Substituting the value of v in first equation

(i.e $= eV = \frac{1}{2} m v^2$)

$$eV = \frac{1}{2} \times m \times \left(\frac{reh}{m}\right)^2$$

$$eV = \frac{1}{2} m \times \frac{r^2 e^2 h^2}{m^2}$$

$$V = \frac{r^2 h^2 e}{2m}$$

$$2mV = r^2 h^2 e$$

$$\left(\frac{m}{e}\right) = \frac{h^2 r^2}{2V} \quad (h \& V \text{ are maintained constant})$$

$$\boxed{\frac{m}{e} \propto r^2}$$

mass $\propto$ (radius of ion path)^2, (since e=1 (unit positive charge)

(Theory)

A parent ion Result when one electron is removed from the parent molecules of the substance.

$$M_{(g)} + e \longrightarrow M^{+}_{(g)} + 2e$$

$M^{+}_{(g)}$ → molecular ion (parent ions)

The (m/e) value of the molecular ions is equal to the molecular mass of the compound. In a few cases the molecular ion peak may be the base peak and can be easily Recognised. In most cases parent ion peak is

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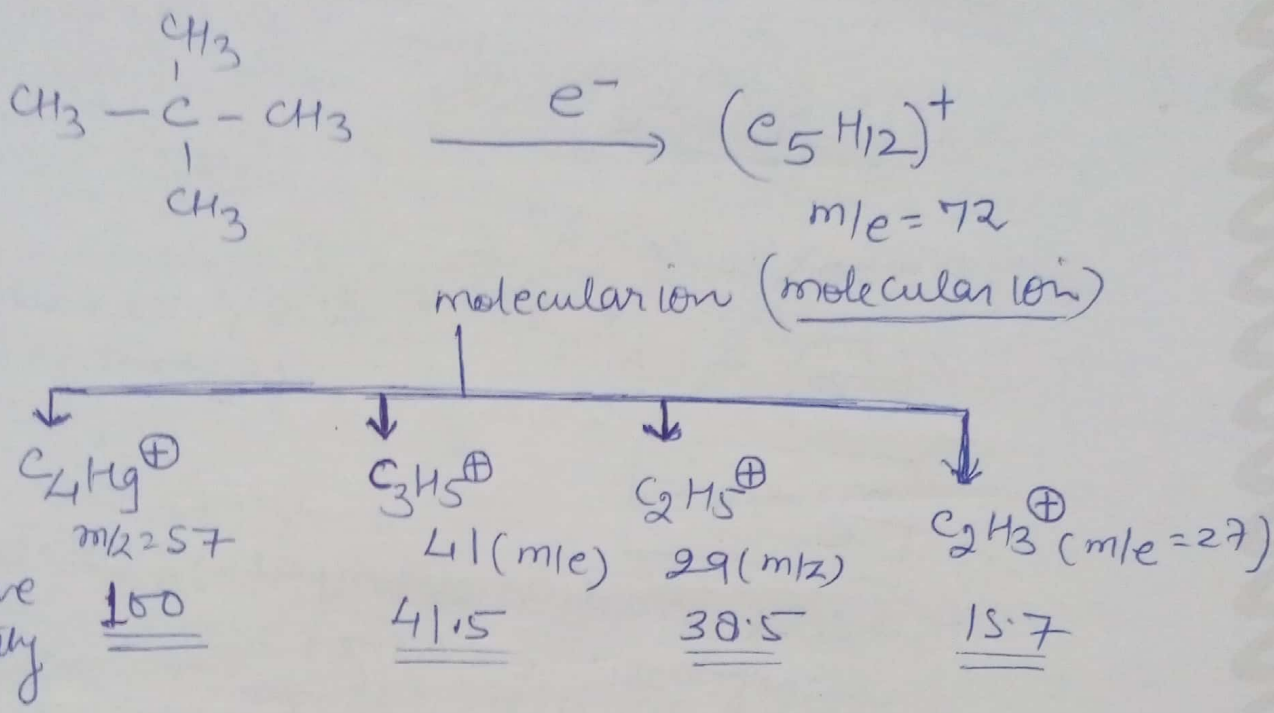
not the base peak and is often of very small Abundance.
The molecular ion peak gives an exact numerical molecular wt.

The mass spectrum of Neopentane



Here molecular ion peak is $(C_5H_{12}^+)$. It is positively charged molecules with unpaired e⁻.

The set of ion are analysed in such a way that a signal is obtained for each value of (m/e). The intensity of each signal represent the "Relative abundance" of the ion producing signal.

→ (Base peak) →

→ most intense peak (max height).
Base peak has value of 100,
The Base peak has 100% abundance.

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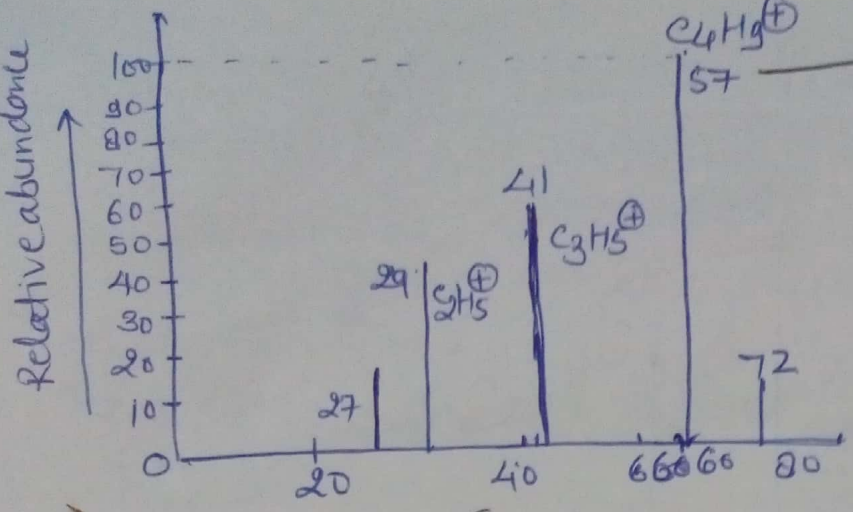

(mass spectrum of Neopantane)

Mass spectrum of ethanol (CH_3-CH_2-OH)

$$CH_3-CH_2-OH \xrightarrow{e^-} CH_3-CH_2-\overset{+\cdot}{O}H$$

$m/z = 46$

(I) $CH_3-CH_2-\overset{+\cdot}{O}-H$ (Heterolytic cleavage)

$$CH_3-\overset{\oplus}{C}H_2 + \cdot\ddot{O}H$$

$(m/z = 29)$

(II) $CH_3-CH_2-\overset{+\cdot}{O}-H \longrightarrow$ (Homolytic cleavage, alpha cleavage, (α))

(α-cleavage) — α — $-\dot{C}H_3$

$$\dot{C}H_3 + CH_2=\overset{\oplus\cdot}{O}H$$

$M/e = (31)$

(Base peak)

The α-cleavage is prefered fragmention mode in ethanol.

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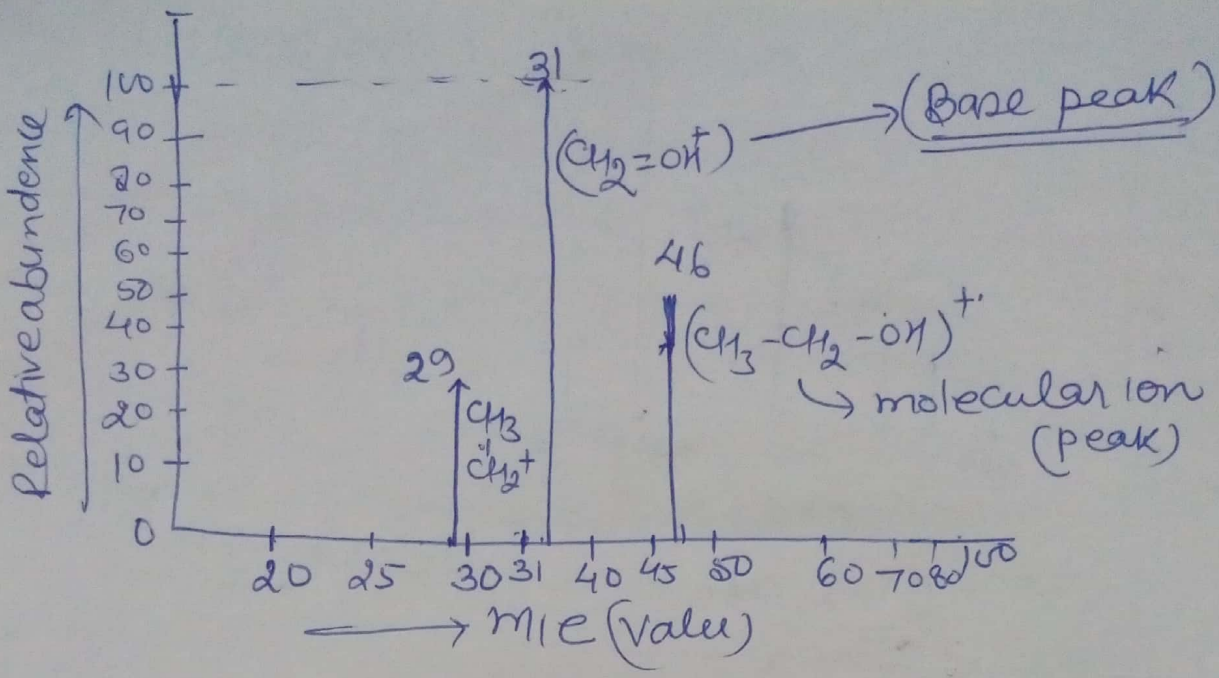


(II) $(C_6H_5-\overset{\overset{O}{\|}}{C}-H)$ - (Benzaldehyde)

$C_6H_5-\overset{\overset{O}{\|}}{C}-H \xrightarrow{-e^-} C_6H_5-\overset{\overset{\overset{+\cdot}{O}:}{\|}}{C}-H$

(M^+) m/e = 106 (1)

(I) $C_6H_5CH=\overset{+\cdot}{O} \xrightarrow{-(C=O)} C_6H_6^{\oplus}$

↓ Heterolytic (cleavage)

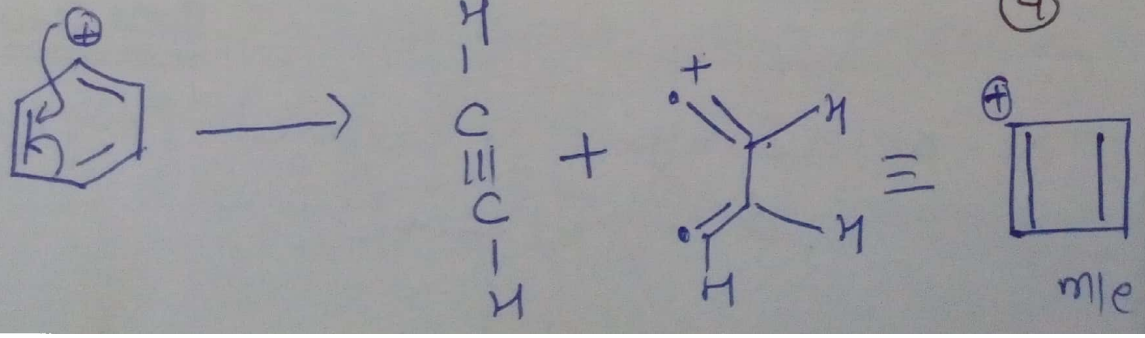
m/e = (78) (2)

(II) $C_6H_6^{\oplus} \xrightarrow{-(H^\bullet)} C_6H_5^{\oplus}$

m/e = 77 (3)

(III) $C_6H_5^{\oplus} \xrightarrow{-(C_2H_2=CH\equiv CH)} C_4H_3^{\oplus}$

m/e = 51 (4)



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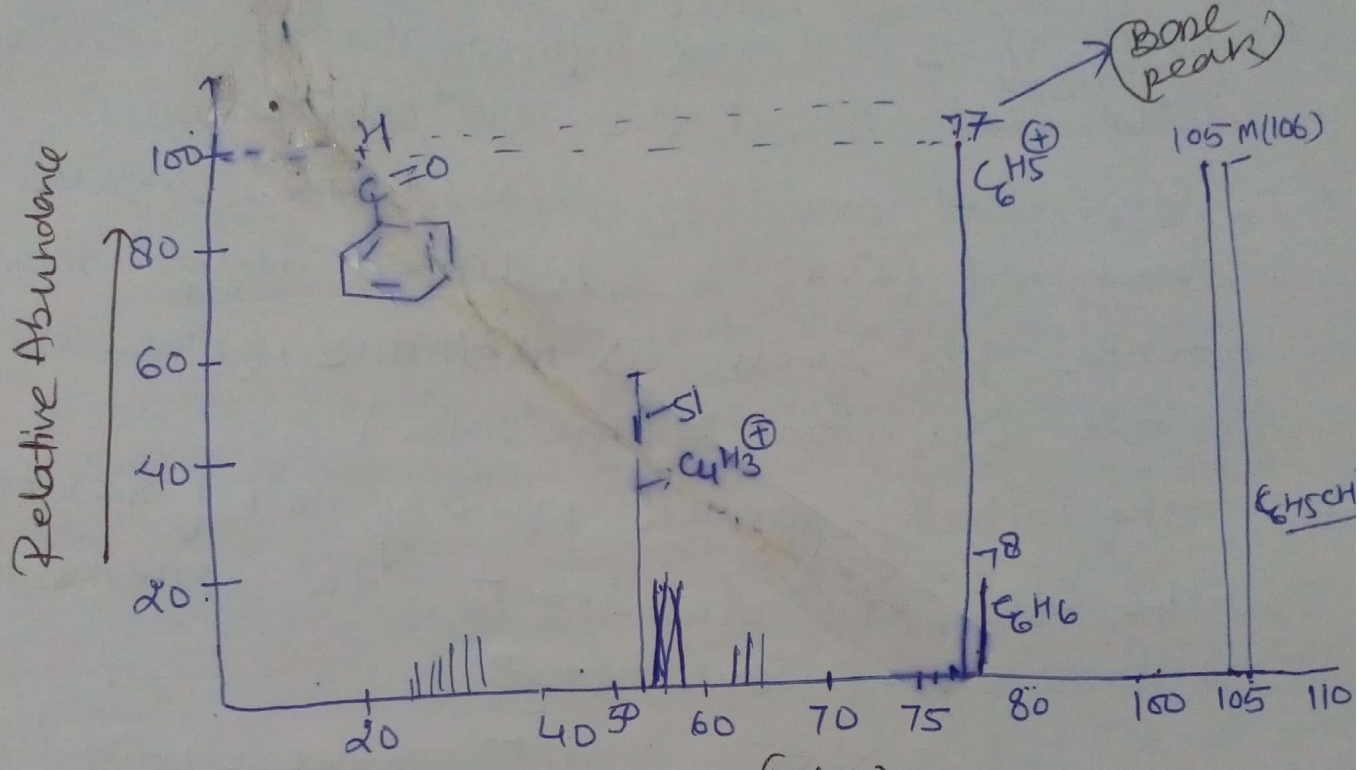
In these compound parent ion peak is intense $(M^{+\cdot}-1)$. $(M^{+\cdot}-28)$ due to the elimination of (C=O) in benzaldehyde are formed.

→ peak at (m/e = 77) due to $C_6H_5^{\oplus}$ followed by the one at (m/e = 51) due to $C_4H_3^{+}$ also.



(Mass spectrum of Benzaldehyde)

(Ionisation technique)

(I) CI (chemical ionisation

(II) FAB (Fast atom bombardment)

(III) ESI (Electrospray ionisation)

(IV) MALDI (Matrix Assisted Lesser Desorption ionisation)

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① Chemical ionisation

⊕ Chemical ionisation is lower energy process than electron ionisation. The lower energy yield lesser or sometimes no fragmention and usually a simple spectrum.

The Lack of fragmention limit the Amount of structural information that can be determined About the ionated species.

⇒ In chemical ionisation method the organic compound (R-H) is Introduced along with carrier gas such as methane. (other carrier gas are Isobutane & Ammonia).

= The Initally formed primary ion from methane Like $CH_4^{+\bullet}$, $CH_3^{\oplus}$ etc. react with methane to yield secondry ion $CH_5^{\oplus}$.

$$CH_4 \xrightarrow{e^-} CH_4^{+\bullet} \text{ (primary ion)}$$

$$CH_4^{+\bullet} + CH_4 \longrightarrow CH_5^{\oplus} + CH_3^{\bullet}$$

(CH_4: gas for ionisation; $CH_5^{\oplus}$: secondry ion)

→ It is an Acid - Base type Reaction.

→ A Secondry ion Like $CH_5^{\oplus}$ is an energetic proton transfer reagent which React with organic compound (R-H) to give $(RH_2)^{+\bullet}$

→ If the Neutral organic compound the Analyte is Represented by

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by 'M' then the ion $((M+H)^+$ or simply MH^+ has an (m/e) value one AMU greater than that of the molecular ion.

- The chemical ionsation produced MH^+ ion are generally prominant and undergo less fragmantion and thus provide a method to Locate the molecular ion.

FAB (Fast Atom Bombardment)

FAB is an ionisation technique used in a mass spectrometry in which a beam of high energy atom strikes a surface to create ion.

In FAB the material to be Analysed is mixed with a non-volatile chemical protection environment called matrix and is bombard under vacuum with a high energy (4000 to 100000 eV) beam of atom. the Atom are typically from an inert gas such as Argon or Xenon.

→ Common matrix included glycerol, thioglycerol, 3-Nitrobenzyl alcohol.

→ FAB is Relatively low fragmantion (soft) ionization technique and produced protonated molecules denoted as $[M+H]^+$ and deprotonated as $(M-H)^-$.

The Nature of its ionisation is simmular to MALDI.

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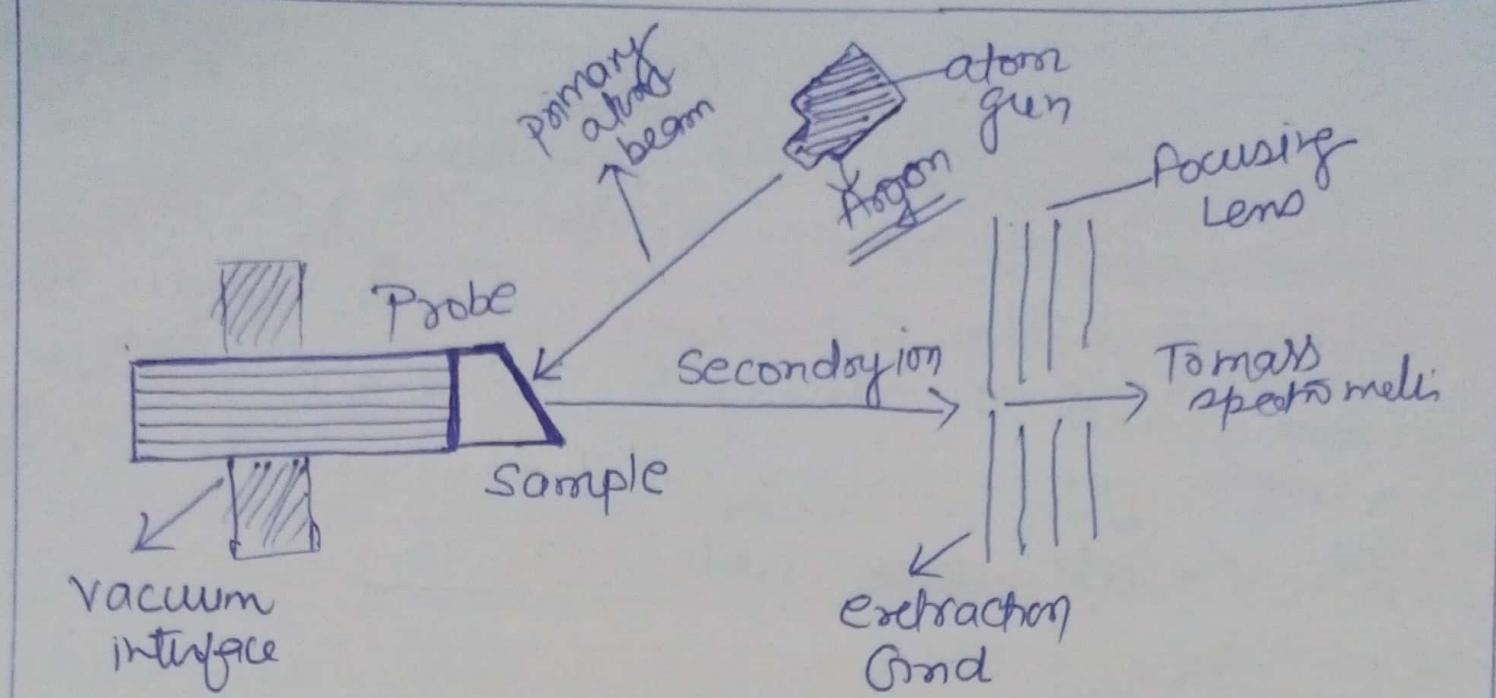
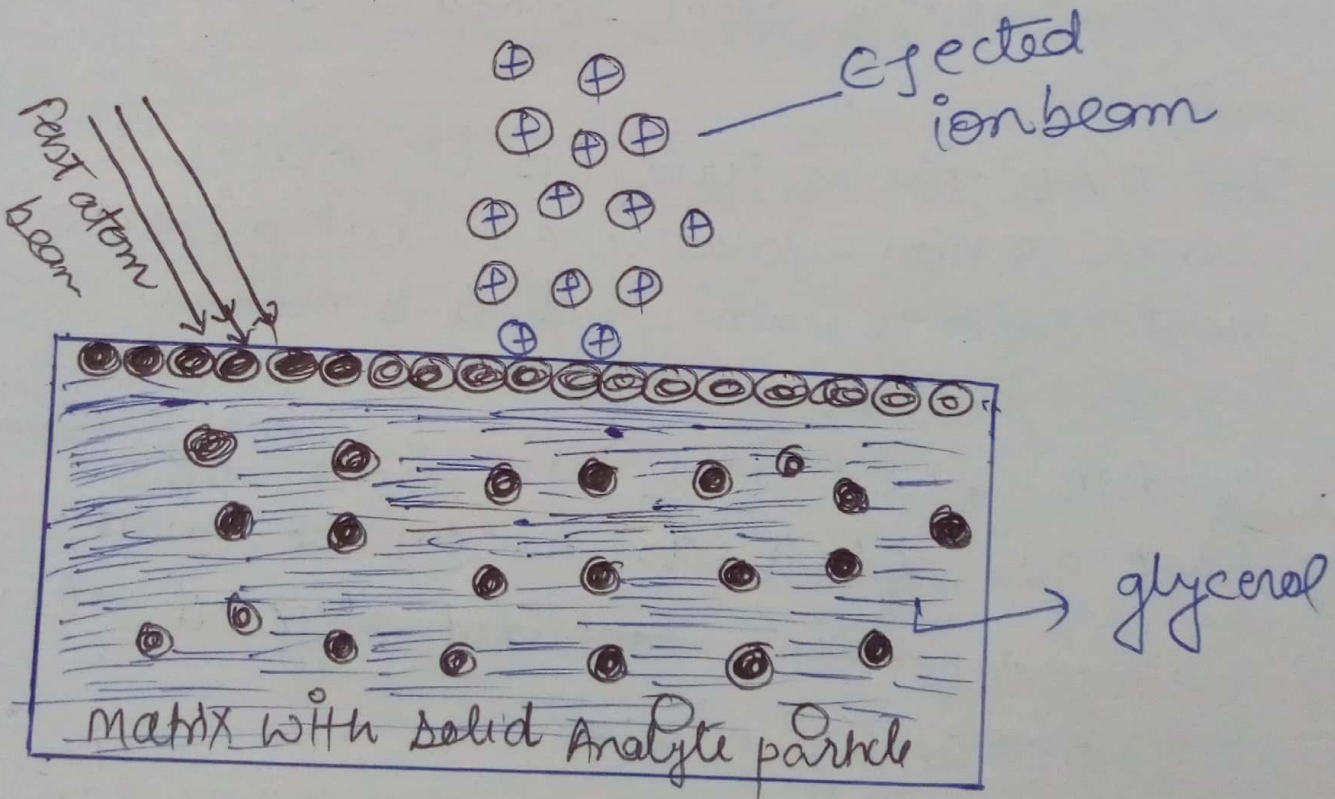


(FAB)

ionization Mechnism



Application - elucidation of Amino Acid sequence.

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MALDI

Matrix - Assisted laser desorption ionization

It is soft ionization technique used in mass spectrometry, allowing the analysis of Biomolecules (Biopolymer such as DNA, protein, peptide & large) and large organic molecules such polymer, dendrimer & other macromolecules) which tend to be fragile and fragment when ionized by more conventional ionization method.

→ MALDI methodology is a three step process first the sample is mixed with a suitable matrix material and applied to metal plate.

→ Second, a pulse laser irradiation the sample triggering Ablation and desorption of the sample and matrix material.

Finally the analyte molecules are ionized by being protonated or deprotonated in the ablated gases.

Matrix & Sample Preparation

Compound	Solvent	Wavelength	Applicat
2,5, Dihydroxy Benzoic Acid (Gentisic Acid)	Acetonitrile water, CH_3OH, CH_3-CH_3, chloroform	337, 355, 260	peptide, Nucleotide, oligonucleotide
3,5, Dimethoxy 4-hydroxy cinnamic Acid (Sinapic Acid)	ACN, water chloroform	337, 266	peptide, protein, Lipid

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4-hydroxy 3-methoxy cinamic Acid (Ferulic Acid)	Acetonitrile, ethanol	337, 355	protein
2-cyno 4-hydroxy cinn-amic Acid	Acetonitrile, water, ethanol, Acetone	337, 265	peptide, Lipid nucleotide
picolinic Acid	ethanol	266	oligonucleotide
3-hydroxy pico-dinic Acid	ethanol	337, 355	oligonucleotide

- The matrix consist of crystallized molecules of which most commonly used are Sinapic Acid (3.5, ~~Dimethoxy~~ 4 hydroxy cinomic Acid). A solution of one of these molecules is made often in a mixture of highly purified water and an organic solvent such as Acetonitrile or ethanol.

→ A counter ion source such as trifluoroAcetic Acid is usually added to generate the (M+H) ion.

→ Sinapic Acid in acetonitrile : water : TFA (50 : 50 : 0.1)

- the matrix solution is mixed with the Analyte (protein sample). A mixture of water and organic solvent allows both Hydrophobic & Hydrophilic molecules to dissolve into solution the solution is spotted onto a mabi ple

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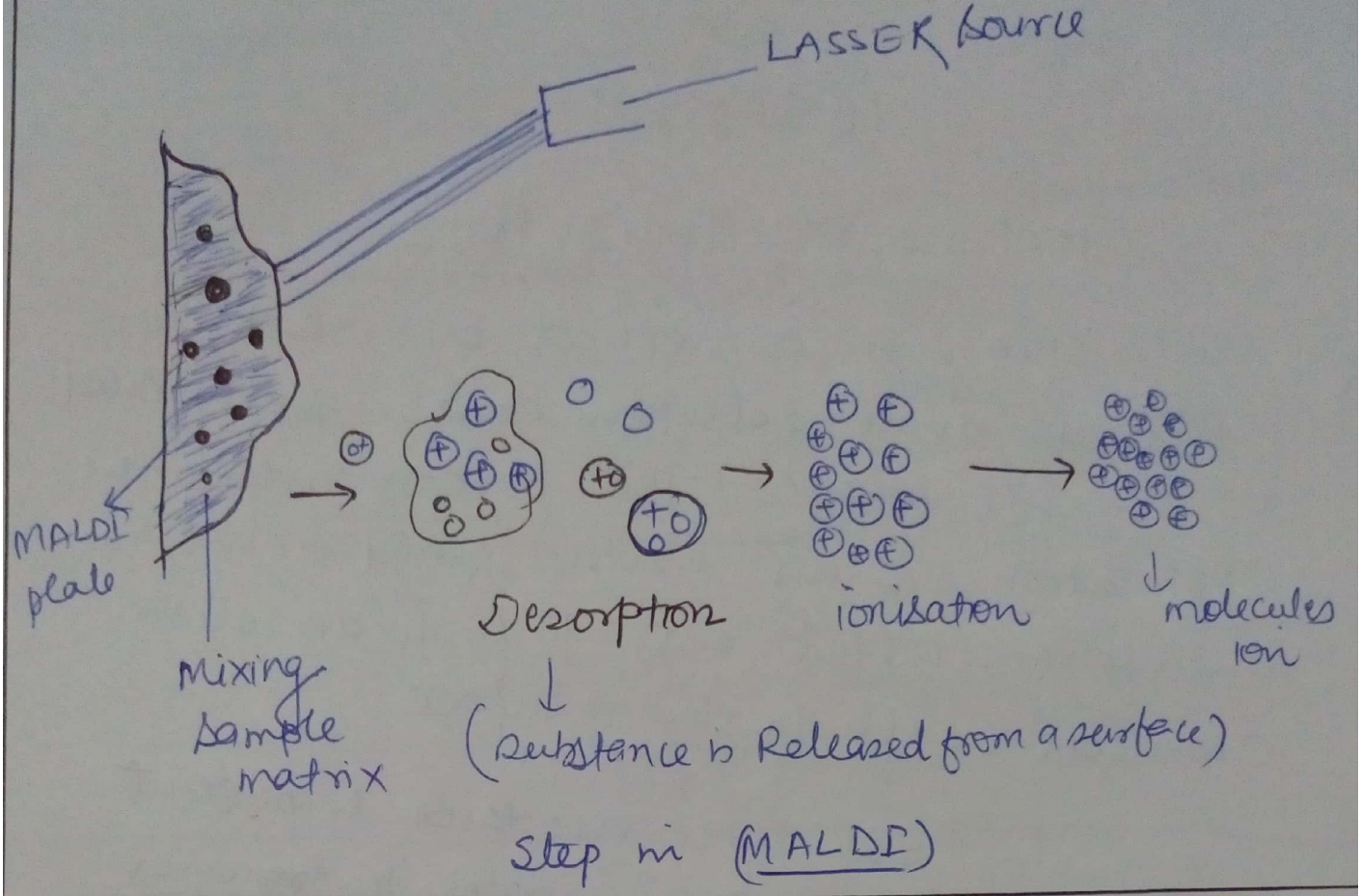

Step in (MALDI)

(Application of MALDI) - Biochemistry

In proteomics, used for the Rapid identification of protein

- organic chemistry - Some Synthetic molecules such as dendrimer, have molecular wt extending into thousand or ten thousand where most ionization technique.

→ polymer chemistry → In polymer chemistry MALDI can be used to determine the molar mass Distribution

→ microbiology: MALDI spectra are used to identification of micro-organism such as bacteria or fungi.

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Medicine used for the Diagnosis of Disease

(ESI)

Electro Spray Ionisation -

electro spray is a method by which ion present in a solution can be transferred to the gas phase. The process involve the application of an electric field across an Interface which acts to form an electro chemical cell in the interface.

In ESI there are three important process that occurs in order to transfer sample molecules.

(I) production of charged droplet at capillary tip

(II) Shrinkage of the charged droplet, leading to fission

(III) production of gas phase ions from small highly charged particle.

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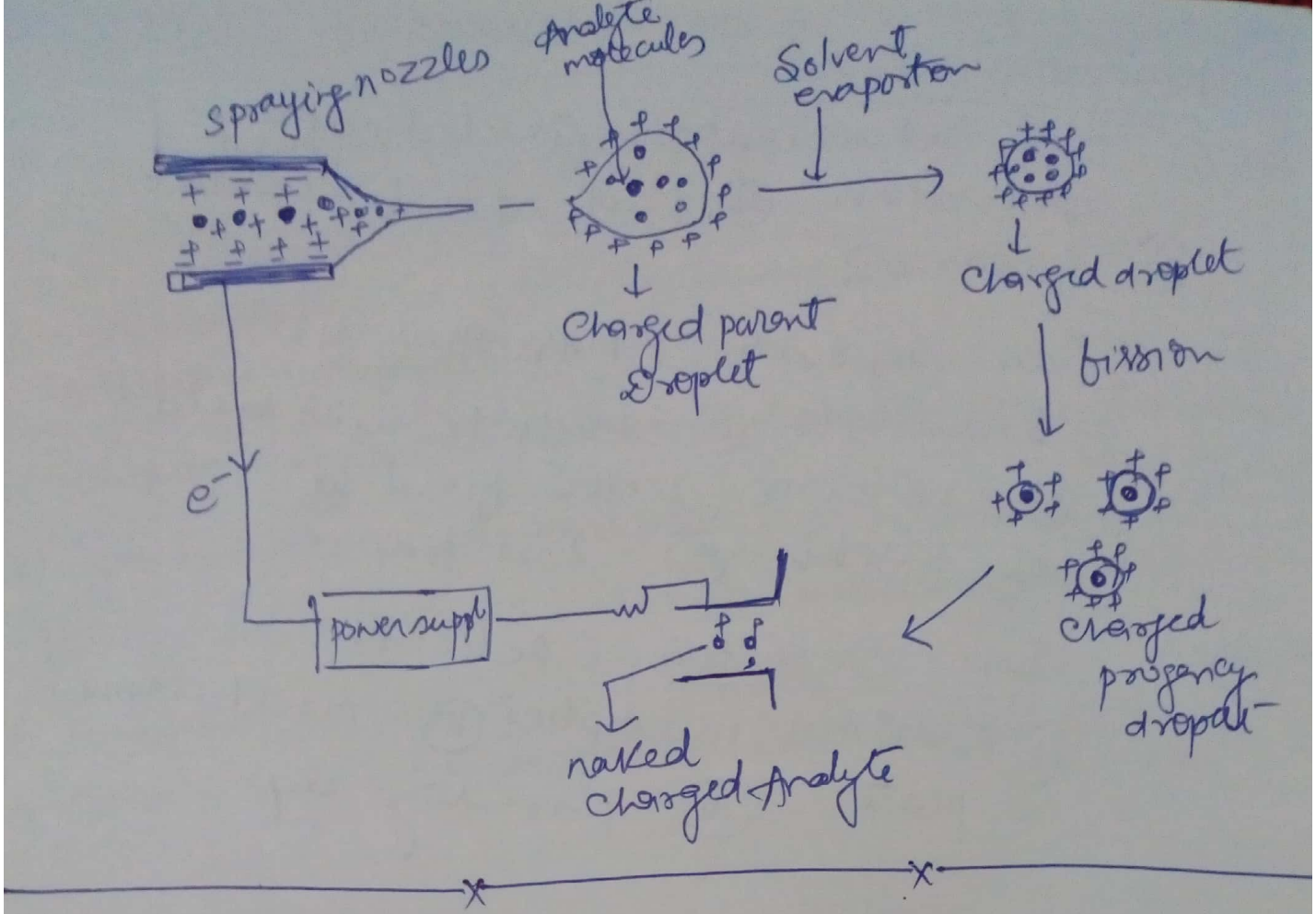
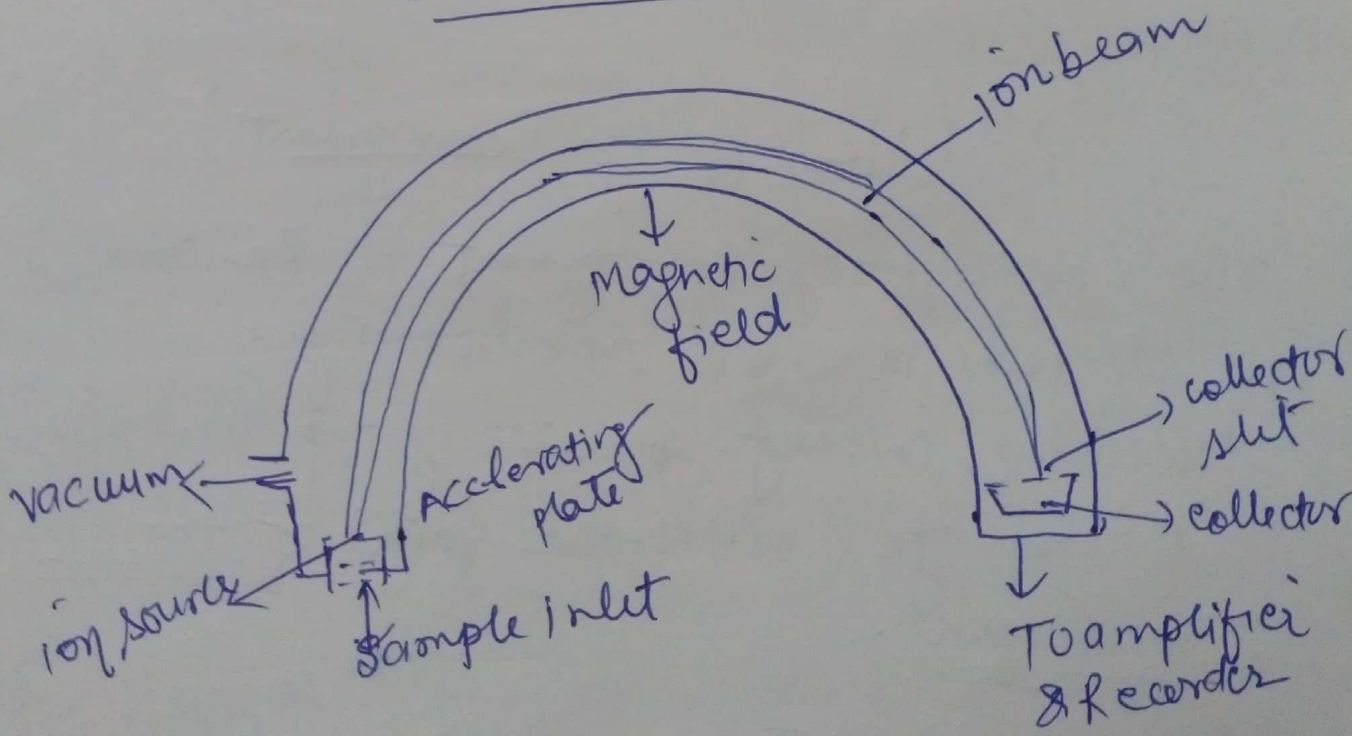


Instrumentation



Sample input → ionisation chamber → mass Analyser → ion detector → mass spectrum → spectrum Analysis

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Ionization chamber:

① chemical ionization ⑪ electrospray ionization ⑪ Fast atm bombardement ⑫ MALDI

② Ion seperation (Mass-Analyser)
① Quadruple ⑧ magnetic sector field ⓒ Electric sector field ⓓ Time of flight ⓔ Ion trap

③ Ion Detector (Detectors)
① electron multiplier ⑩ multichannel plate ⑪ faraday cup.

(Mc Lafferty Rearragment)

The Mc-Lefferty Rearragment is Reaction observed in mass spectrometry. Molecules containg Keto group undergo β-cleavage with the gain of the γ hydrogen.

This Rearragment may take place by Radical or ionic mechnism.

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Application of Mass spectroscopy →

* Elucidation of the structure of the organic and biological molecules.
* Determination of molecular mass of peptides, protein and oligonucleotides.
* monitoring gases in patients · breath during surgery
* Identification of drug abuse and metabolites of drugs of abuse in blood, urine and saliva.
* Analyses of aerosol particles.
* Determination of pesticides residues in food.

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