

MASS SPECTROMETRY

- Mass spectrometry is a correct name of mass spectroscopy because of its speed reliability and wealth of information obtained by using only microgram quantity of analyte sample.

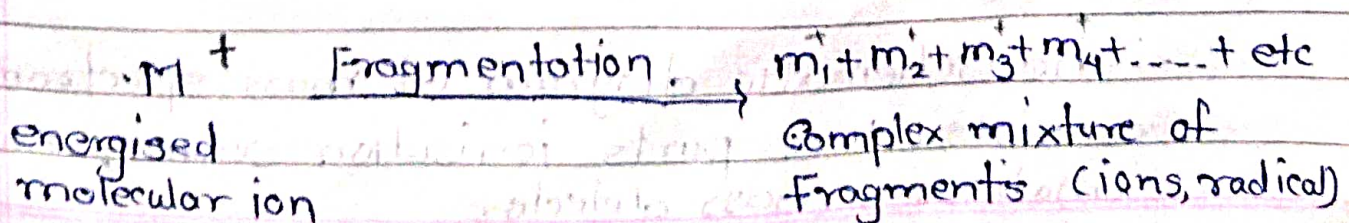
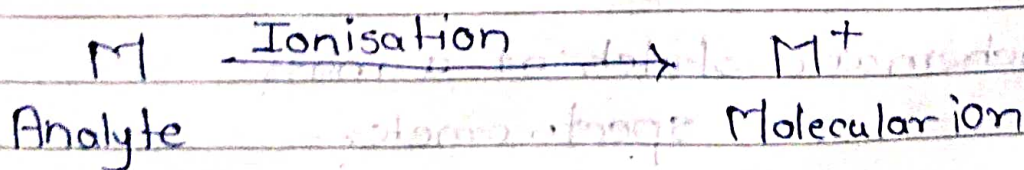
Principle -

- Charge particles are affected by electrical & magnetic field this is some simple theory of mass spectrometry it involve ionisation & fragmentation of neutral analyte into gas phase ionic mixture by suitable method.

- The accelerated ion when pass through scanning magnetic field cations deffering in mass to charge ratio (m/e) defect deflect varying degrees and get separated into discrete cationic beams.

- The intensity of each cationic beam corresponds to relative abundance of ionic type it carries.

- The ionic beam intensity ^{measure} ~~major~~ in terms of ionic current signal and recorded as a peaks to give readymade mass spectrum.



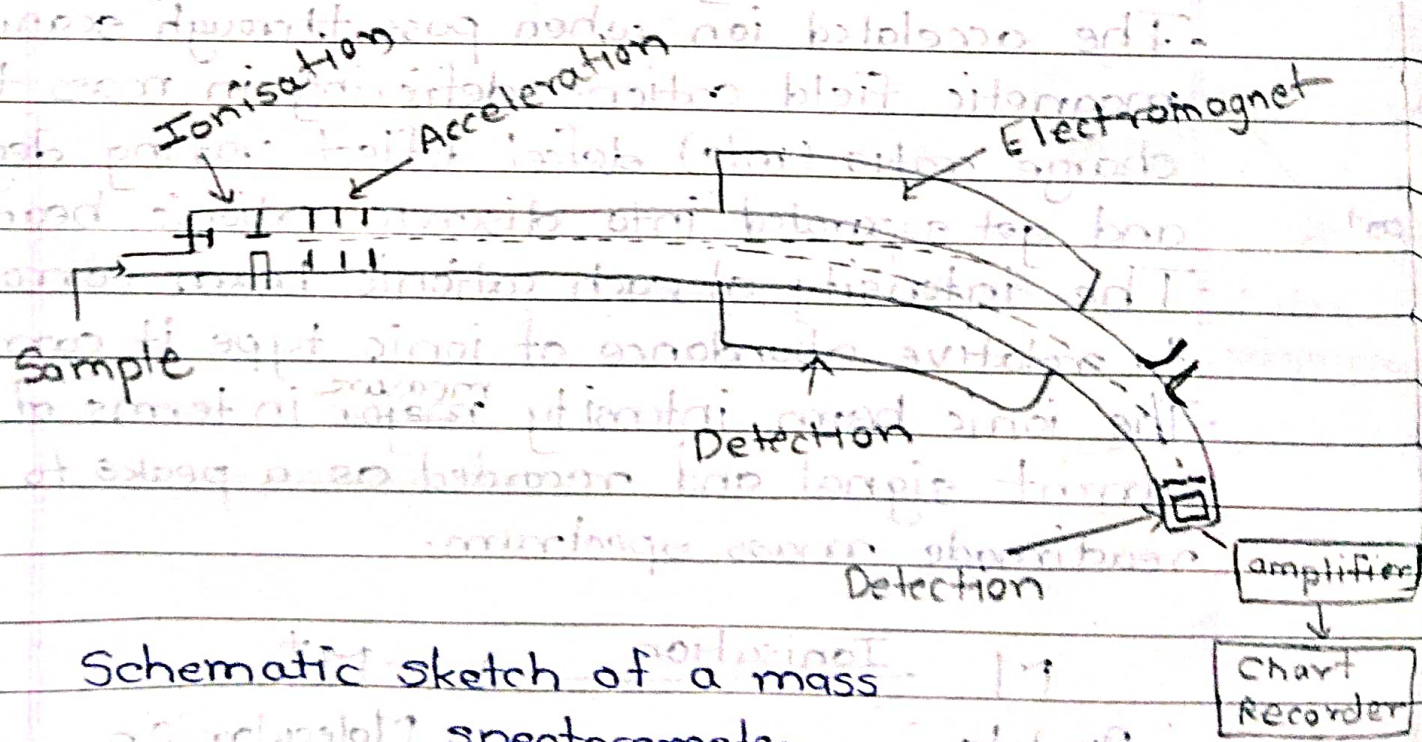
mix. of ions $\xrightarrow{\text{Variable magnetic field}}$ discrete beam of cation having specific m/e ratio.

Ionic current \leftarrow Detector
(detected and recorded as a peaks in spectra)

A mass spectrometer records the cations only. Neutral particles (molecules & free radical) remain undetected and then simply get lost by.

Instrumentation

• Diagram —



Schematic sketch of a mass spectrometer.

- The schematic diagram of mass spectrometry consists three parts ionisation source, mass analyzer, mass detector.

① Ionization source (chamber) -

- This is a chamber in which ionisation of molecule occurs.
- The sample is introduced from a reservoir into ionisation chamber where sample is injected.
- The ionisation chamber is maintained at pressure lower than that of reservoir so that sample molecules are drawn into it easily.
- Further this vacuum gives ion a reasonable chance to travel from one into another without encountering any hindrance from air molecules. Thus a stream of molecule is led into the source without any problem when sample is gas or liquid.
- For non-volatile solid sample, a direct probe tech is used for this purpose.
- The sample is placed on tip of the probe and heated. It is then with help of vacuum lock allowed to enter the chamber.

② Mass Analyzer -

- This is curved tube that is maintained under high vacuum through which ion beam passes from ion source to collector. The major function of mass analyzer is two fold, one to resolve ion having identical M/E ratio from all other ions and second to focus the individual ion beams of discrete mass into a detector or into a second ionization chamber or into collision cell.

The energetics of electron removal

For most organic compounds the ionization potential required lies between 8.5 to 15 eV but electron beam has to be powered with 50-70 eV to generate the ions.

The kinetic energy of an accelerated ions is given by,

$$\frac{1}{2} mV^2 = eV$$

- Where, m = mass of ions
- V = Velocity of ions
- e = Charge of ions
- V = Potential difference of ion acceleration plates

A charged particles follows curved flight path in the presence of magnetic field whose radius of curvature is given by;

$$r = \frac{mV}{eH}$$

From these two equation we get

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

Obviously, higher radius of curvature is associated with higher m/e value. Hence it is this eqⁿ that describes this behaviour of an ion in terms of its m/e for detection in mass analyser.

It is possible to vary V with H as constant so as to bring each m/e species to the same focus.

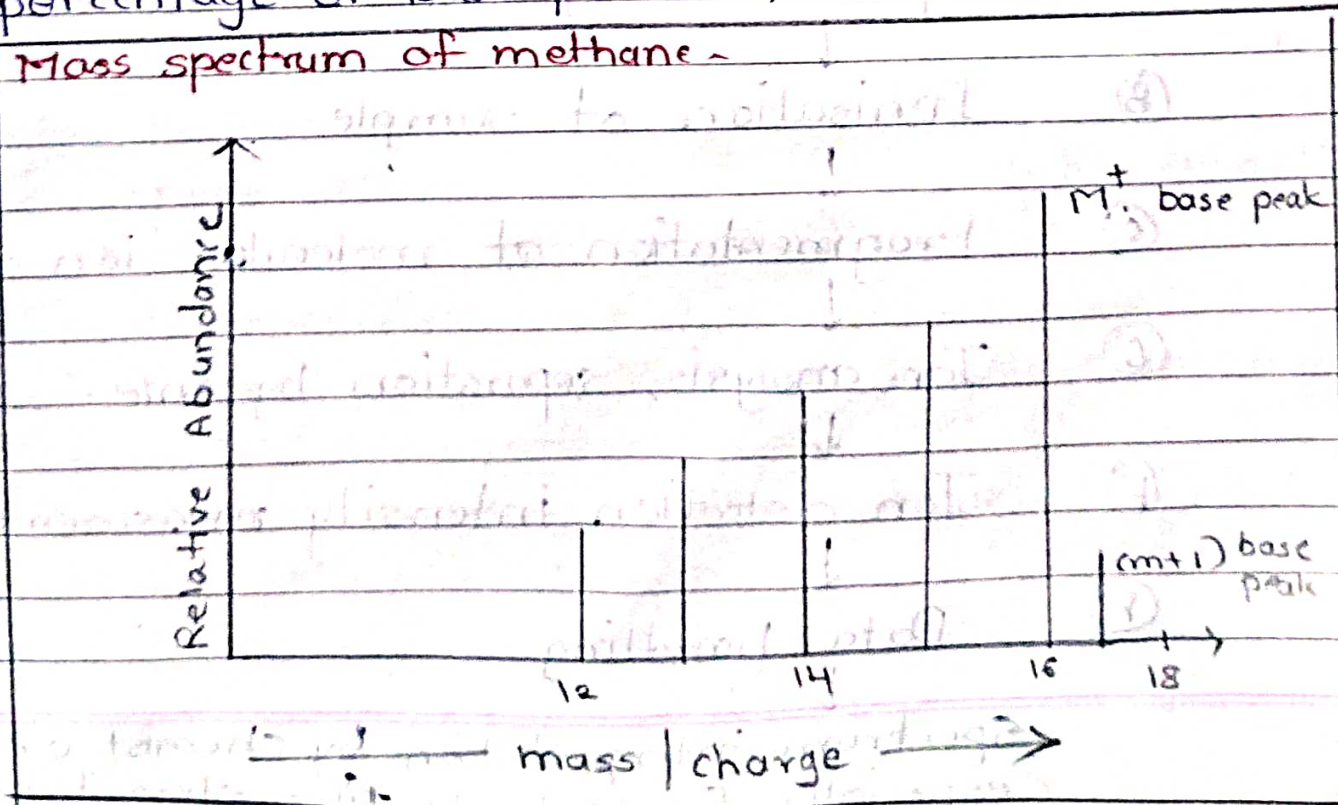
③ Detector - possibility for secondary of still
 If the molecular ion or any of its fragment have lifetimes of at least 10^{-6} s they are able to reach the detector. Detector is a counter.

- It generates an ion electrical signal when struck by an ion as the current is directly related to the no. of particles striking it the detector records the abundance of each mole particle.

* Mass Spectrum! —

- A mass spectrum is plot of positively charged ions (including molecular cations) versus their relative abundance.
- The most intense peak is the spectrum is called base peak and given as arbitrary abundance 100. All other intensities are expressed as percentage of base peak (i.e., relative abundance).

Mass spectrum of methane -



- Due to presence of different isotopes C, H, N, O and halides additional peaks are observed.

- Thus because of small but observable natural abundance of ^{13}C & ^2H there is generally a small peak appearing one mass unit higher than the parent peak, so it is called $m+1$ peak.

- If the heavy isotopes of hydrogen happen to be in the same mole, there is even small peak at two mass unit higher than parent peak & it is called $(m+2)$ chlorine & bromine yield quite higher $(m+2)$ peak because of their higher isotopic abundance, such peaks are called isotopic or satellite peak.

They are valuable to draw imp structural

* Steps of mass spectral analysis -

- (A) Sample inlet system.
- ↓
- (B) Ionisation of sample.
- ↓
- (C) Fragmentation of molecular ion.
- ↓
- (D) Ion analysis, separation by m/e.
- ↓
- (E) Ion detection intensity measurement.
- ↓
- (F) Data handling.

↓
Spectrum interpretation by chemist or computer (especially for molecule like steroids)

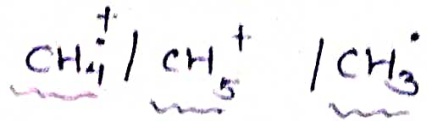
* Ion production: -

1) Electron impact (EI) Ionisation: -

- The first method EI ionisation is hard ionisation. This is because it introduces a large amount of energy into molecules.
- Consequently the molecular ion does not appear or contributes weaker smaller peaks in m/z spectrum. So, in EI technique sample is volatilised into gas phase by heating in vacuum followed by bombarding by a stream of electrons. These electrons are generated by metallic filament and accelerated through a potential difference so that they attend a typical energy of 70 eV; and their impact on gaseous sample molecules result in the ionisation of this molecule.

2) Characteristics of EI technique -

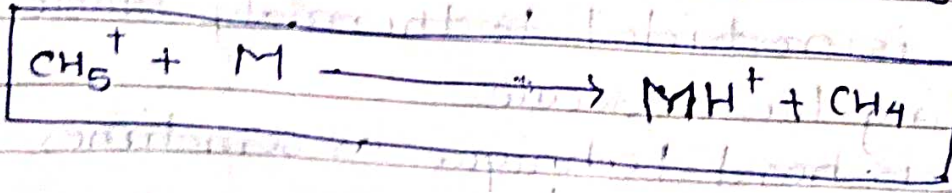
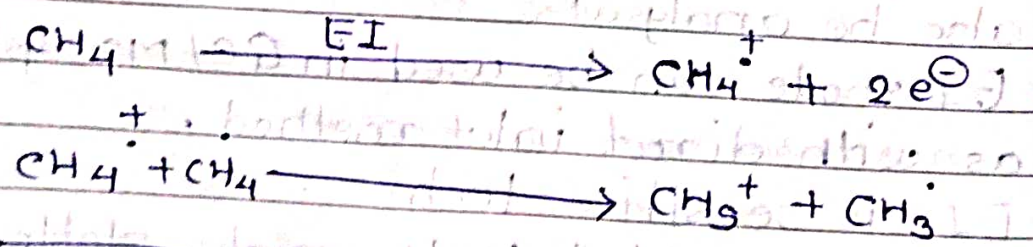
- Relatively easy to obtain EI spectra.
- Compound that are air & moisture sensitive can also be analysed.
- EI mode can be used in GC/MS system as well as with direct inlet method.
- EI is sensitive tech.
- EI is restricted to thermally stable compound having low molecule.
- EI is hard technique as sometimes M^+ peak are not observed.



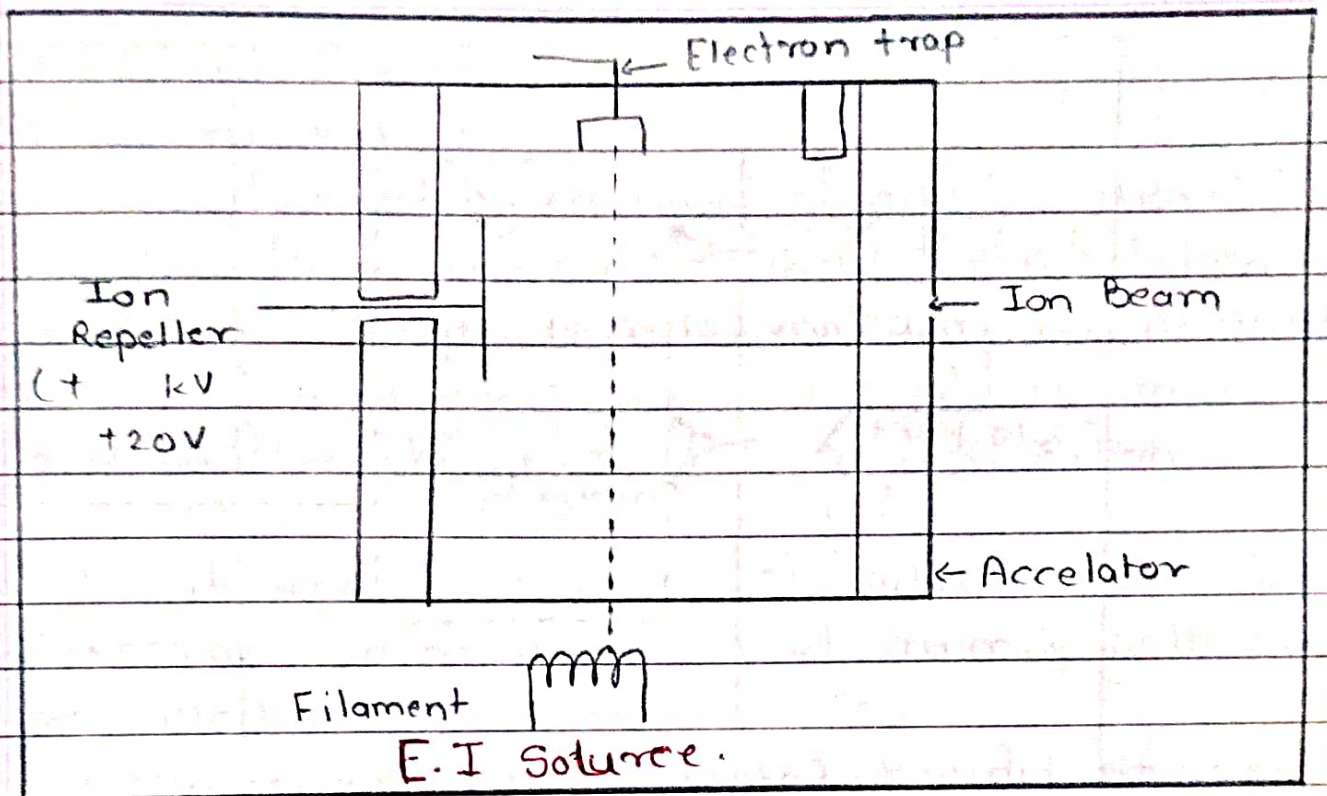
② Chemical ionisation (CI)

- It is closely related to electron impact
- It also uses a stream of electron in the ionisation process. In this case however it is not the sample molecule which are ionised but a reagent gas usually ammonia or methane which is present at a much higher concentration. The sample in this technique is must also be volatilised by heating in a vacume but the main difference between EI & CI tech is that in CI the sample is ionised by a strong acid produce by ionisation of reagent gas.

For eg., Consider the electron impact on the reagent gas methane which leads to a molecular ion CH_4^+ which reacts with CH_4 reagent gas to give strong acid CH_5^+ and a radical CH_3^{\cdot} . It is this strong acid CH_5^+ which ionises the sample by protonation.

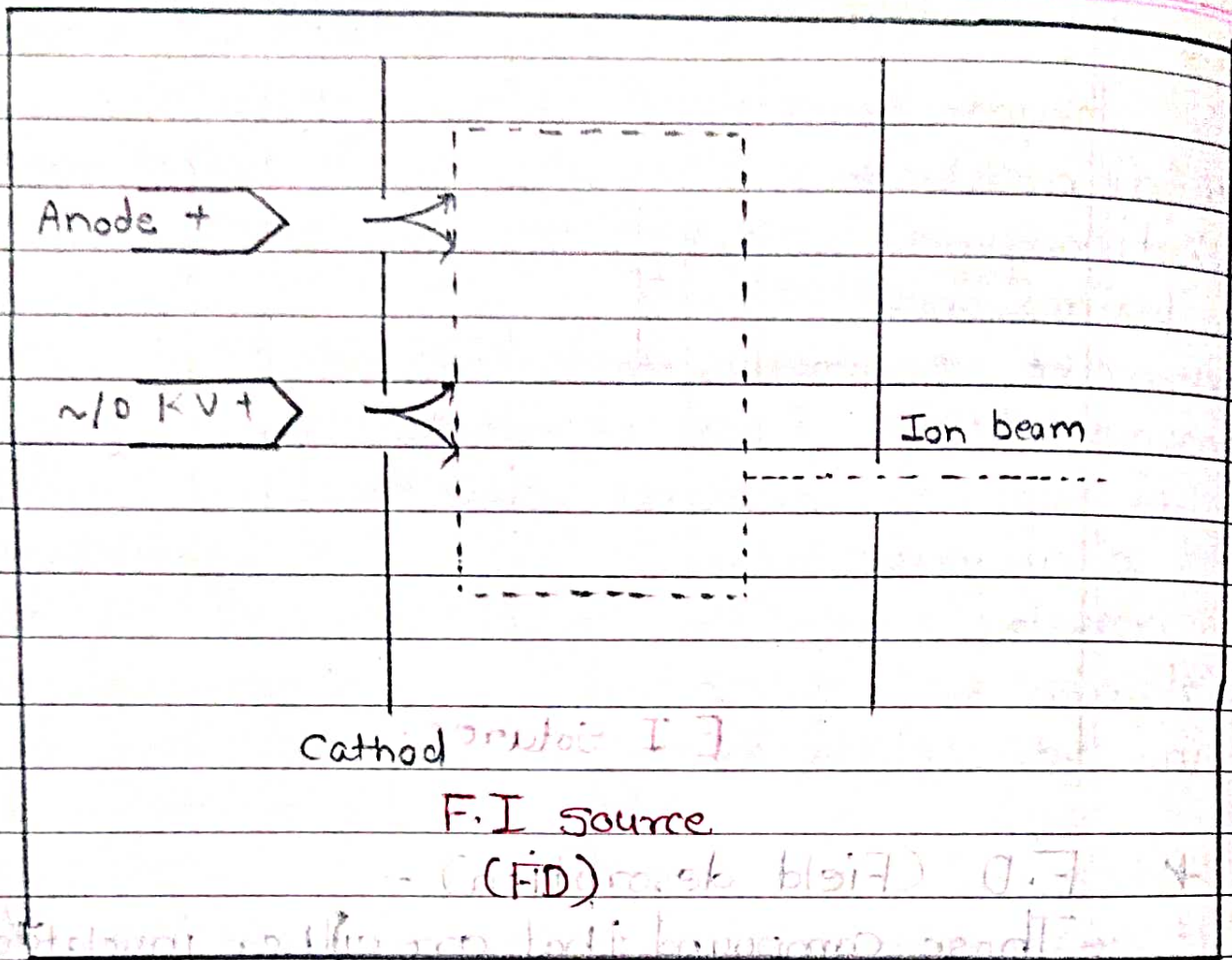


Location of M^+ ion via $(\text{M}+1)$ peaks.



* F.D (Field description) -

- Those compound that are either involatile or thermally unstable can not be analysed by electron impact or the chemical ionisation technique.
- For such compound another technique called field description (F.D) or description ionisation (D.I) is used.
- In this technique the sample is placed on anode of pair of electrode. An intense electric field is passed betⁿ pair of electrodes.
- The sample that was observed not gets desorbed and both molecular ions are generated $[M+1]^+$ i.e., $M+1$. The FI source is shown below.



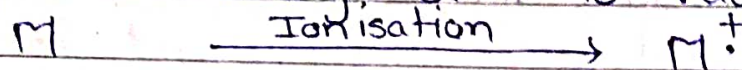
- Thus while EI method fails to give peak at m/e 284, for molecular ion of xanthosine, the FI (FID) ionisation technique does give the M^+ peak at m/e 284.

- This technique works well for polymer upto 1000 Da but is unsuccessful with polymers having molecular mass less than 10,000 Da. For such molecules the FAB technique is popular one.

★ Types of ions -

1] Molecular ion -

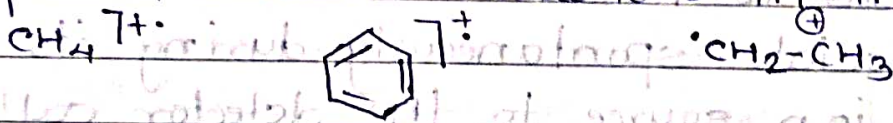
An ion formed by removal of one (or more) electron from molecule without fragmentation of molecular strength is called molecular ion or parent ion. A formed mole ion carries odd electron and positive charge so it is radical cation.



analyte molecule - Molecular or parent ion.

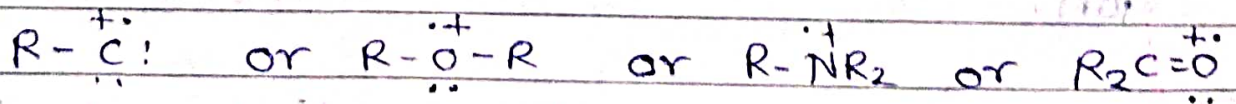
- Molecular ion represent intact molecule with only one electron less than molecule.

- Molecular ion denoted as $M^{\cdot+}$ the dot represent unpaired electron somewhere in the ion.



- The non-bonding electron of O, N & π electron of aliphatic and aromatic molecule are loosely held than C-C & C-H σ bond electron.

So, they are represented by locating odd electron and positive charge on it.



The stability of molecular ion increases if molecule contain π electron system. Because it adjusts more readily to loss electron than σ bond.

① Stability of molecular ion -

Aromatic comp. > conjugated olefins > aldehyde >

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cycloalkane > unbranched ketone > amine > ester > ether > carboxylic acid > branched hydrocarbon > alcohol > etc.

(b) Multiply-charged ion -

The multiply charged ions are ions that have gained or lost more than one electron.

(c) Isotopic ion: - Any ion containing one or more of naturally occurring isotopes.

(d) Metastable ion: - An ion of short life span formed with sufficient excitation energy to dissociate spontaneously during its flight from ion source to the detector called metastable ion.

(e) Rearrangement ion - An ionic species formed in fragmentation but which are not part of the original molecule is called rearrangement ion.

(f) Negative ion - Atom, radical, molecule in vapour phase which has gained one or more electron, acquiring negative charge called negative ion.

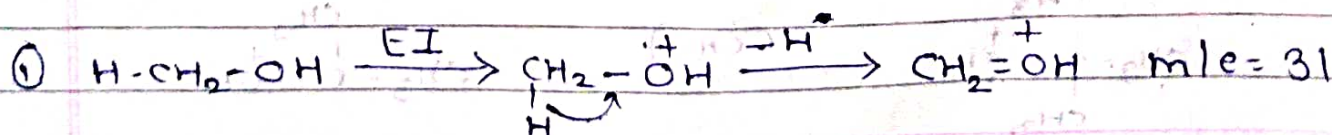
(g) Fragment ion - They are electrically charged dissociation or decomposition fragment derived

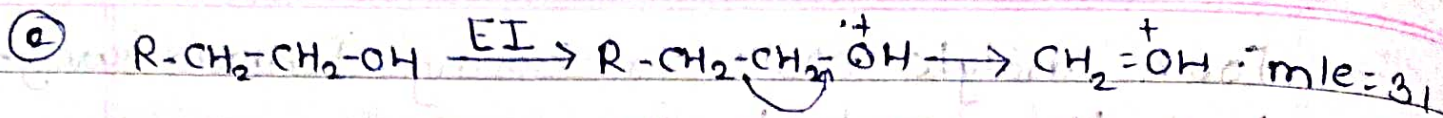
form analyte mole and having formula weight less than molecular ion.

(b) Radical ion -
Anion & cation having unpaired electron.

(i) Adduct ion -
Formed by interaction of two species usually ion & molecule.

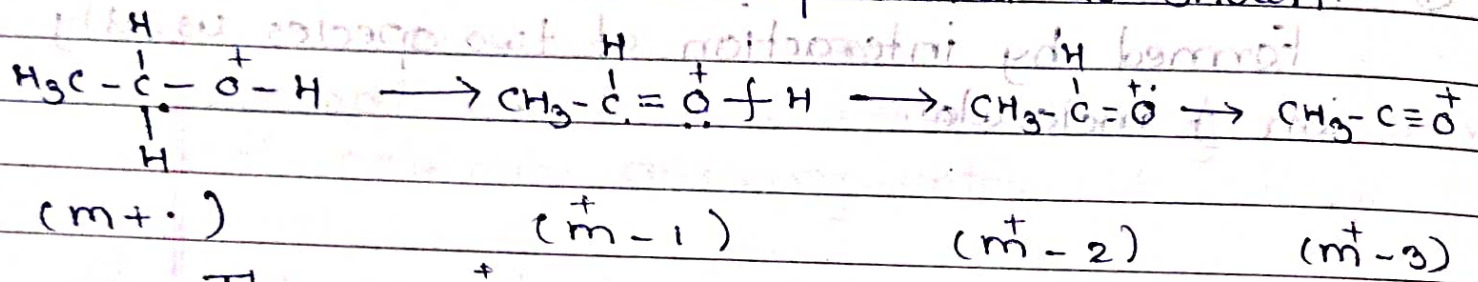
- (1) Alcohol -
- The molecule ion peak of primary and secondary alcohol are of low abundance.
 - For 3° alcohol it is not observed.
 - Parent ion peak is formed by removal of electron from lone pair of oxygen atom of primary and secondary alcohol.
 - The no. of fragmentation made depends on nature of alcohol.
 - The fragmentation of C-C bond adjacent to -OH group is max preferred.
 - For methanol and 1° alcohol the peak at m/e ratio 31 appear in large abundance due to formation of oxonium ion (CH₂=OH)⁺ & is formed by the cleavage of carbon hydrogen bond in methanol.





Primary alcohol show (M-1) peak due to loss of water.

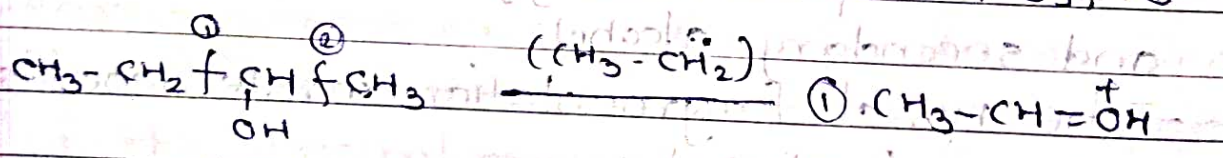
- Long chain members may show peaks corresponding to successive loss of H-radical at (m-1), (m-2), (m-3) it can be represented as shown.



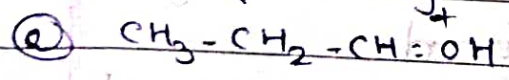
- The $CH_2=O^+H$ is most significant peak in spectra of primary alcohol.

• - 2° alcohol -

- In 2° alcohol cleavage gives prominent peak due to $R-CH=O^+H$ at m/e value 45, 59, 73.

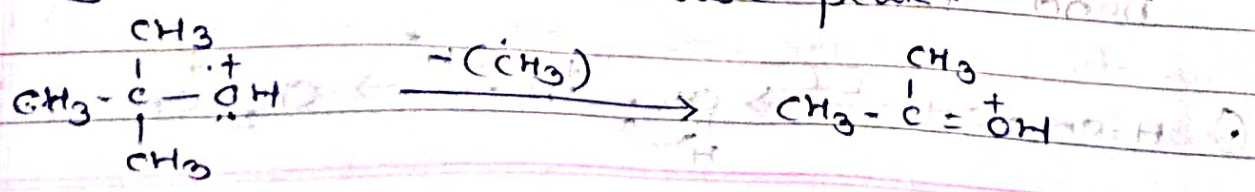


↓ α-cleavage

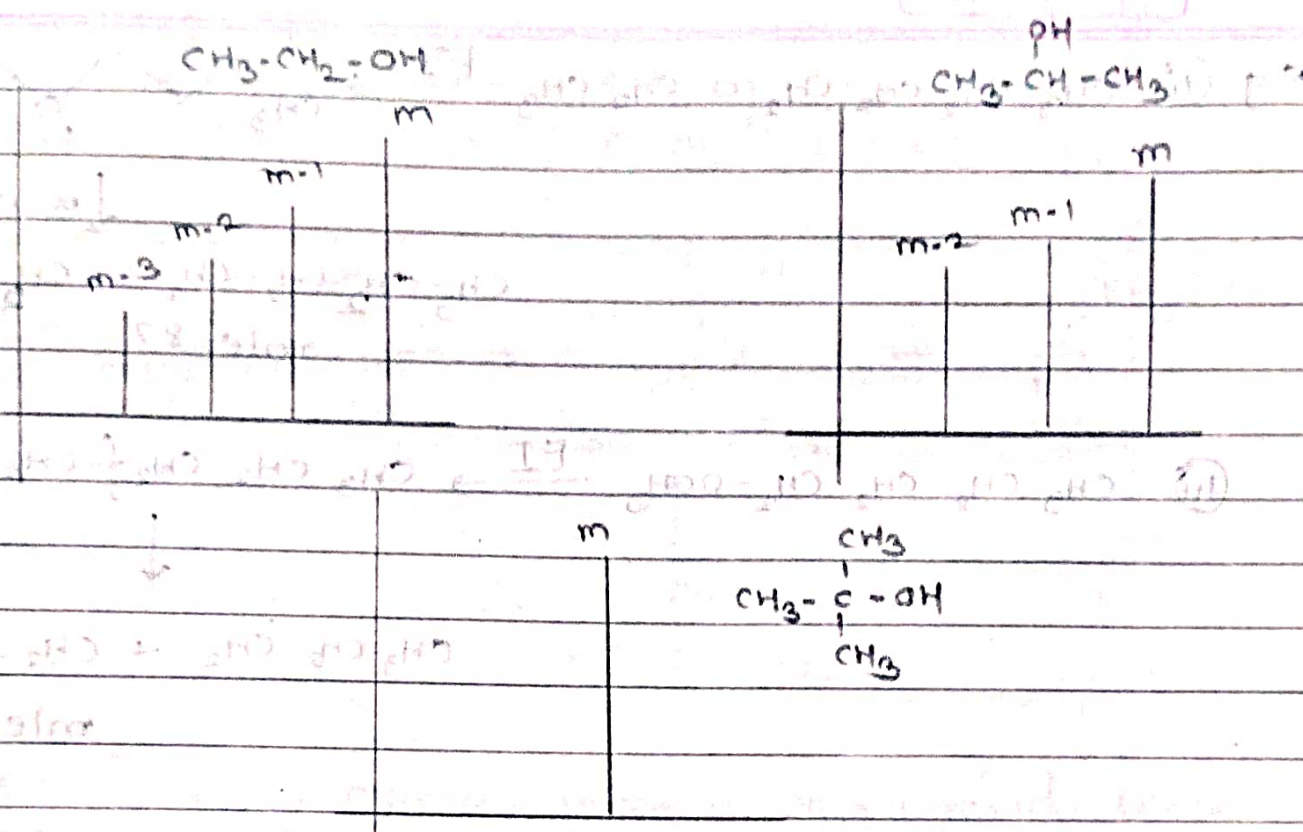


• - 3° alcohol -

- In case of 3° alcohol the molecular ion peak is not for the signal due to α-cleavage is more significant and it is base peak.

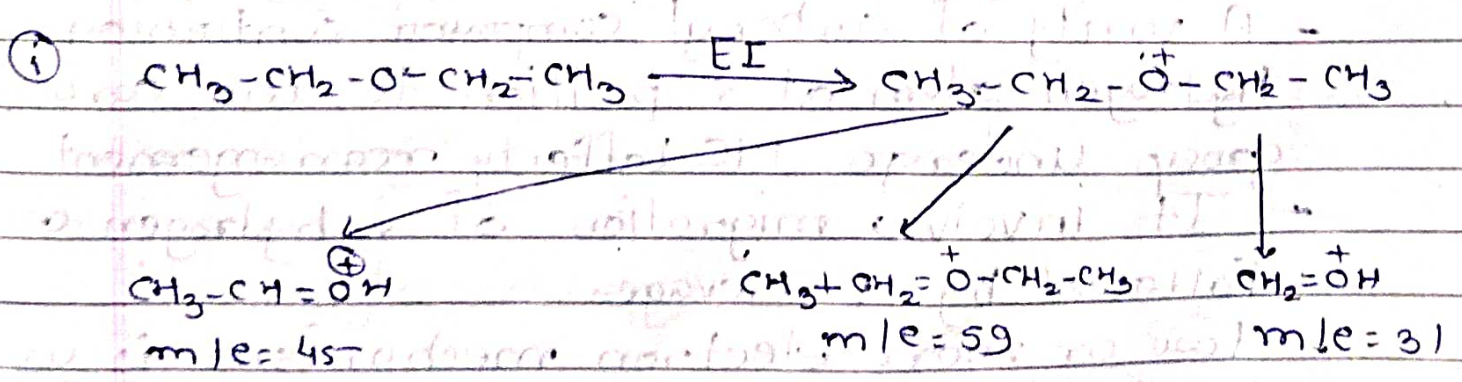


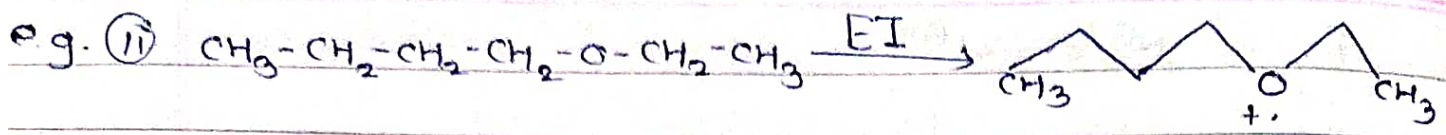
m/e = 74



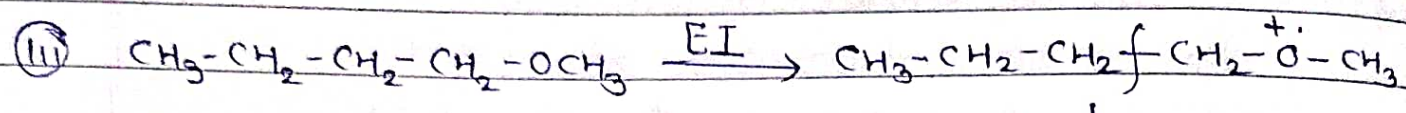
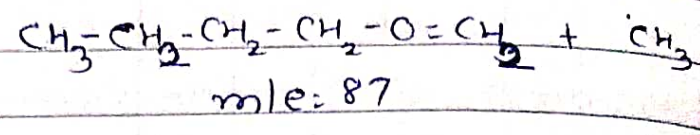
*** Ether:**

- Parent ion peak is due to removal of one electron from lone pair of oxygen atom of ether.
- The ether fragmentation takes place to cleavage at α -position by homolytically or heterolytically.
- The fragmentation of ether gives strong peak at m/e value 31, 45, 59.

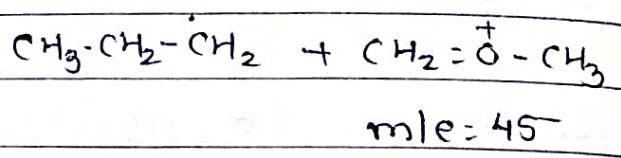




↓ α -cleavage.



↓



★ MC Lafferty rearrangement —

3 Marks

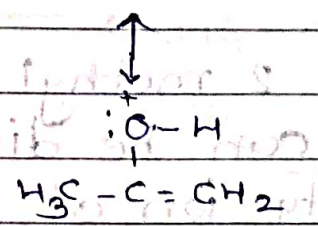
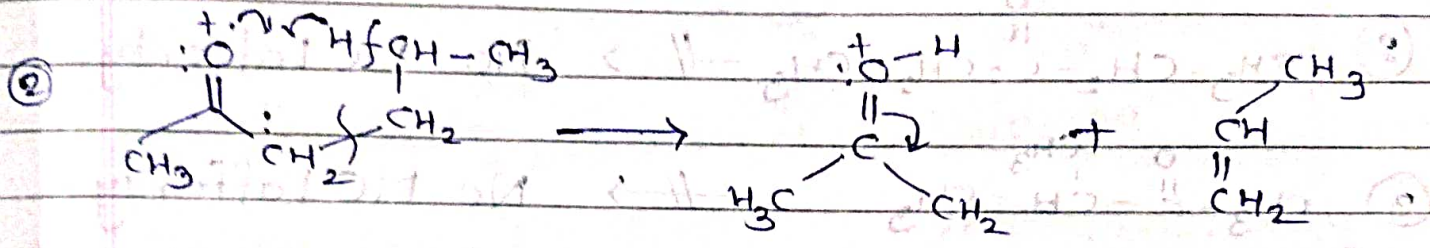
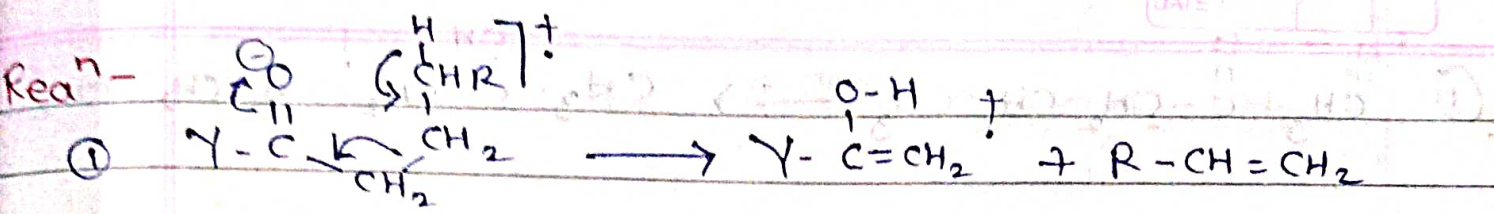
MC Lafferty rearrangement is in migration of γ -hydrogen to carbonyl oxygen atom leading to rearrangement and fragmentation of molecule to form MC Lafferty ion.

- A fragment radical cation having even m/e value form by loss of neutral molecule (alkene) from a molecular ion is called MC Lafferty ion.

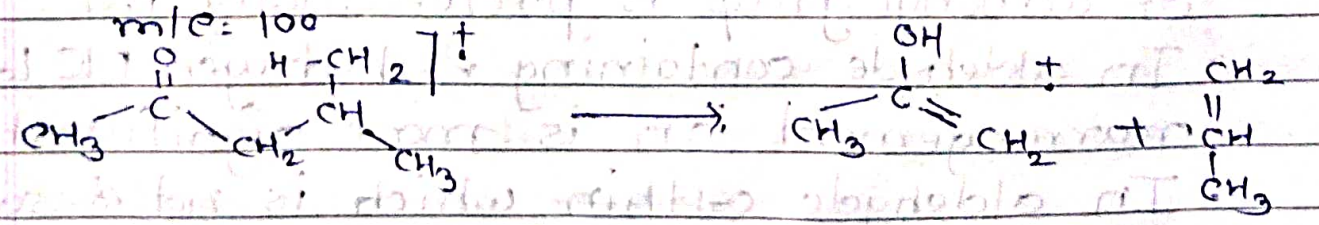
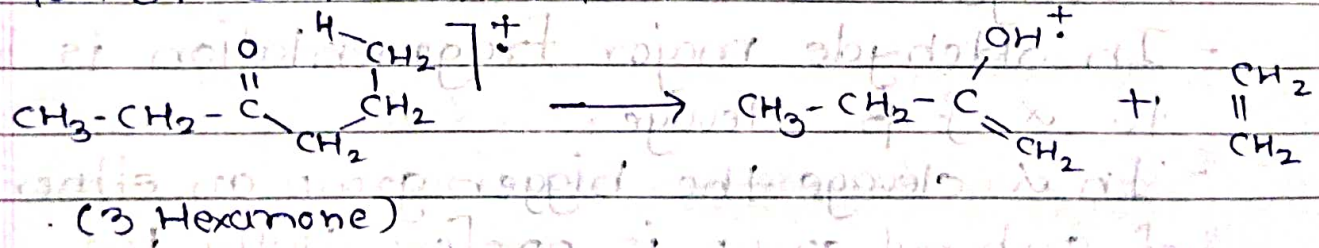
- A variety of carbonyl compound containing hydrogen atom at γ -position to the carbonyl group undergo MC Lafferty rearrangement.

- It involves migration of γ -hydrogen atom followed by β -cleavage.

- Two or more electron mechanism is used to show migration of γ -hydrogen atom leading to homolytic breaking of bond between α -carbon & β -carbon.

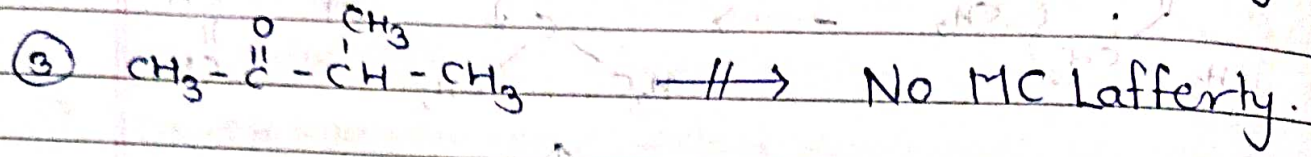
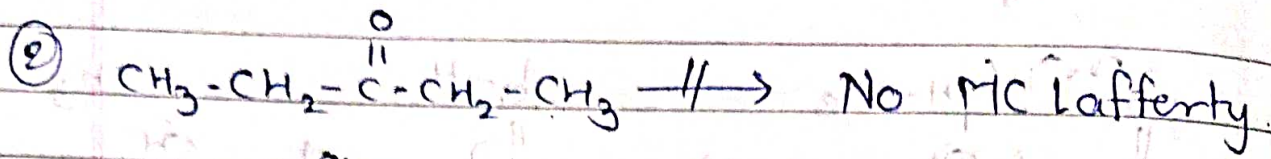
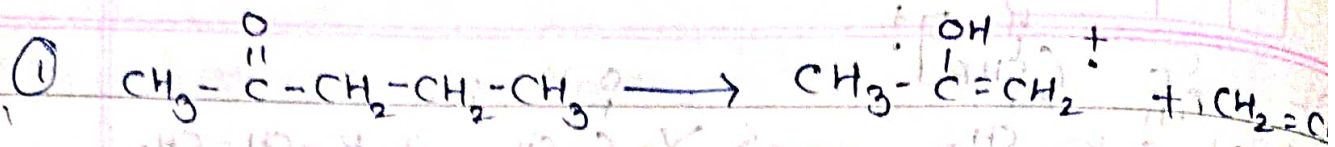


- McLafferty rearrangement can provide useful information about structure of ketone & aldehyde
- Isomeric ketones & aldehyde can be distinguished on the basis of m/e value of McLafferty ion
- For example, 3-hexanone & 4-methyl 2-pentanone can be distinguished as they form McLafferty ion of different m/e value.

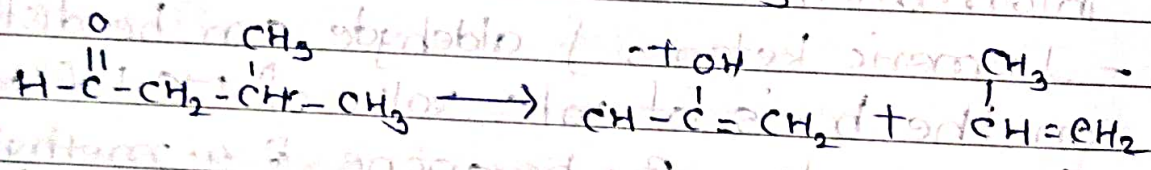
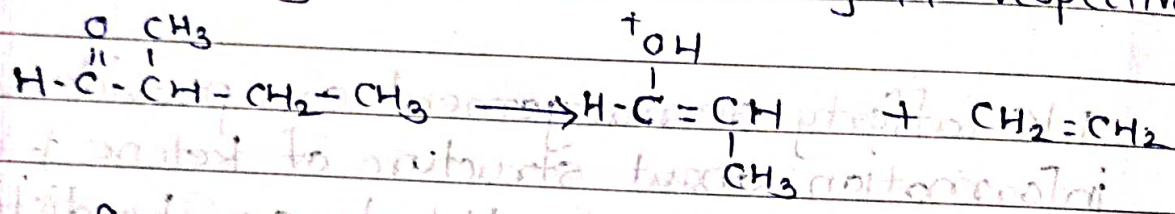


(4 methyl 2 pentanone)

- Further we can distinguish 2-pentanone from 3-pentanone or 3-methyl butanone.

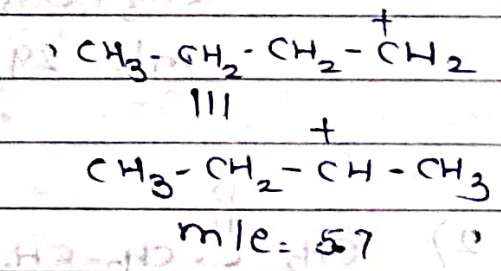
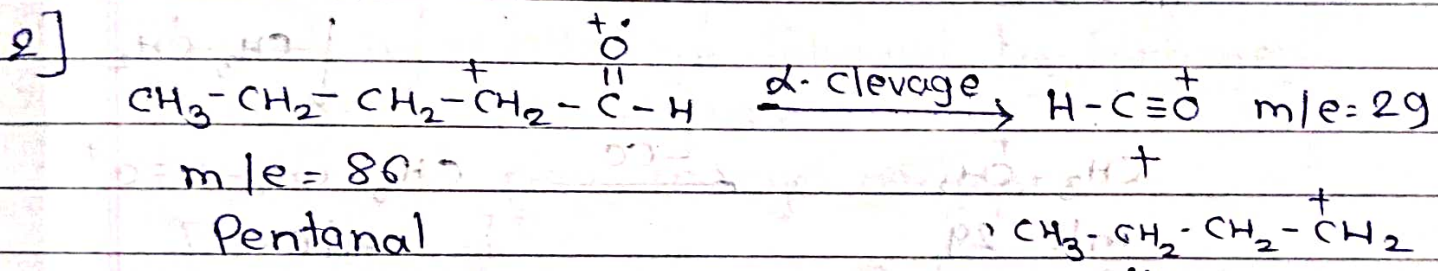
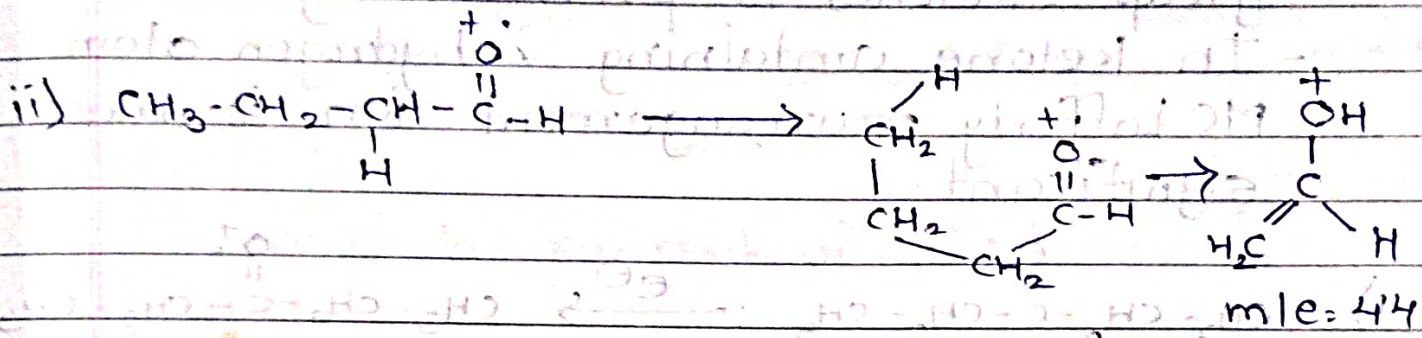
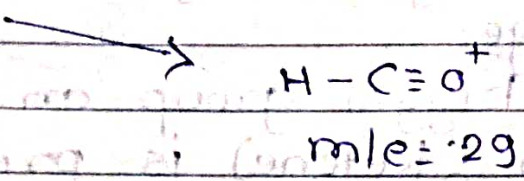
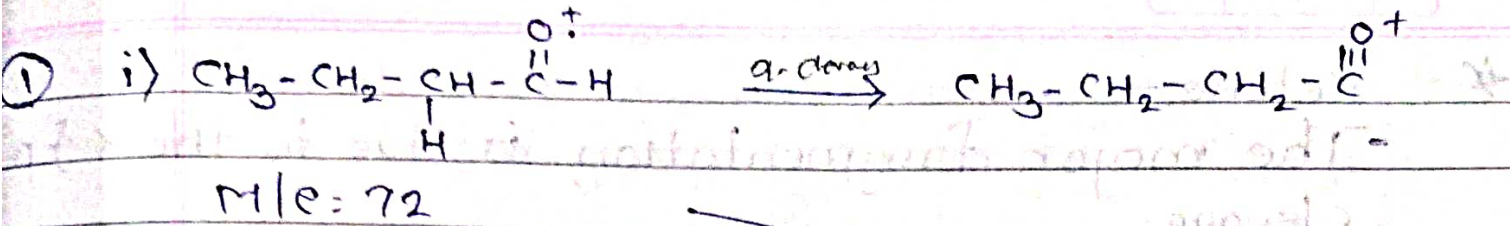


Similarly 2-methyl butanone and 3-methyl butanal can be distinguished as they form McLafferty ion at m/e = 58 & 44 respectively.



★ Aldehyde —

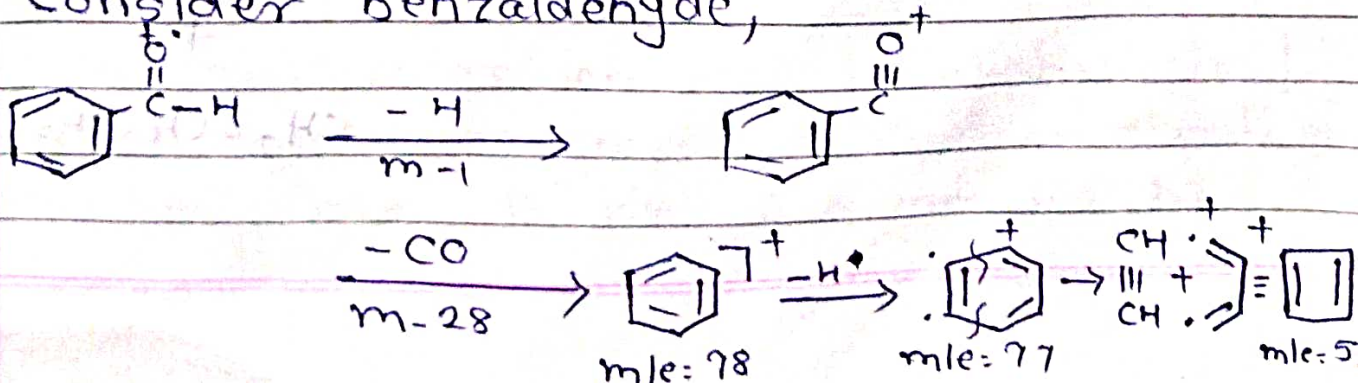
- In aldehyde major fragmentation is fall due to α & β cleavage.
- In α cleavage the bigger group on either side of carbonyl group is preferentially lost.
- In aldehyde containing γ -hydrogen McLafferty rearrangement ion is most significant.
- In aldehyde ~~within~~ which is not α -substituted a peak due to this form at m/e 44 It is base peak.



3) Aromatic aldehyde-

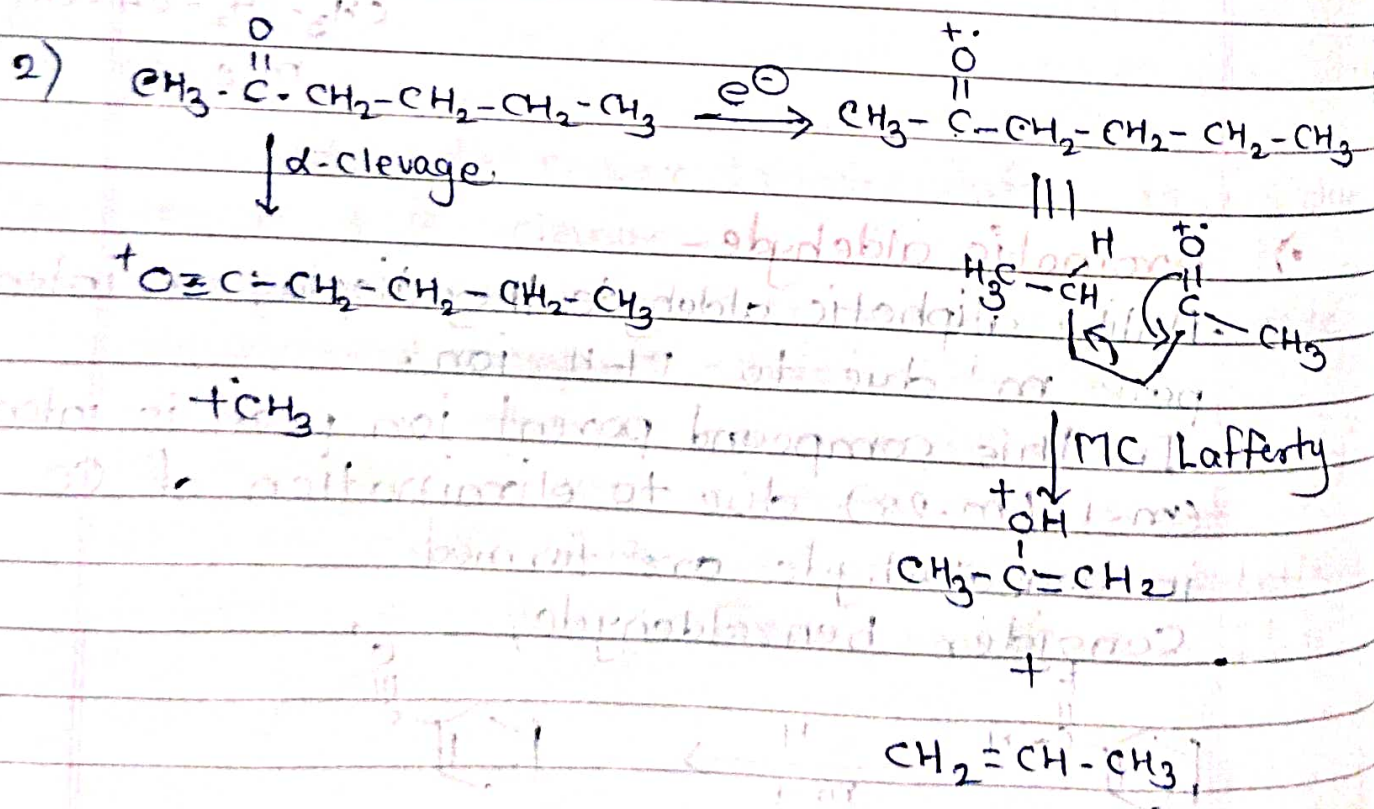
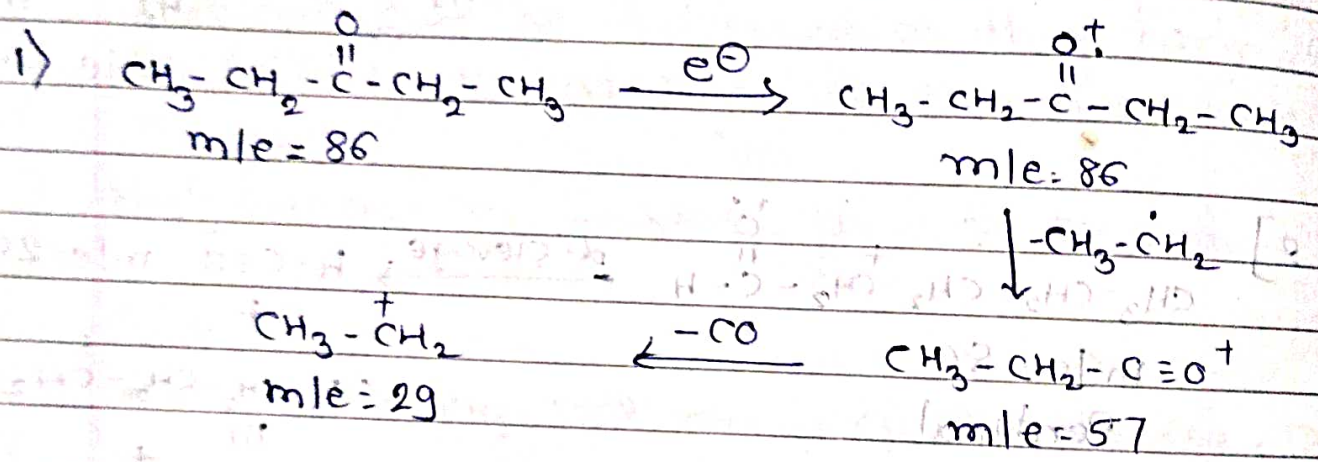
- Unlike aliphatic aldehyde they display an intense peak m^+ due to $\text{M}-\text{H}$ ion.
- In this compound parent ion peak is intense ($m-1$ / $m-28$) due to elimination of CO in benzaldehyde are formed.

Consider benzaldehyde,

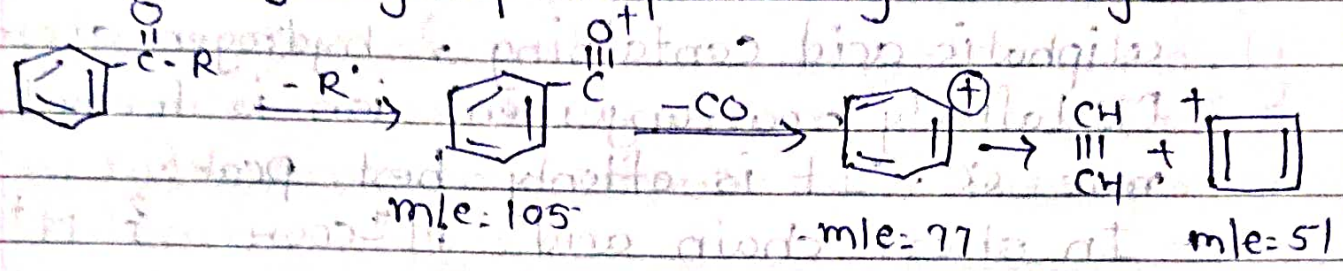


* Ketone -

- The major fragmentation is due to the α cleavage.
- The bigger group on the side of carbonyl group (ketone) is preferentially lost.
- In ketone containing γ -hydrogen atom McLafferty rearrangement ion is most significant.

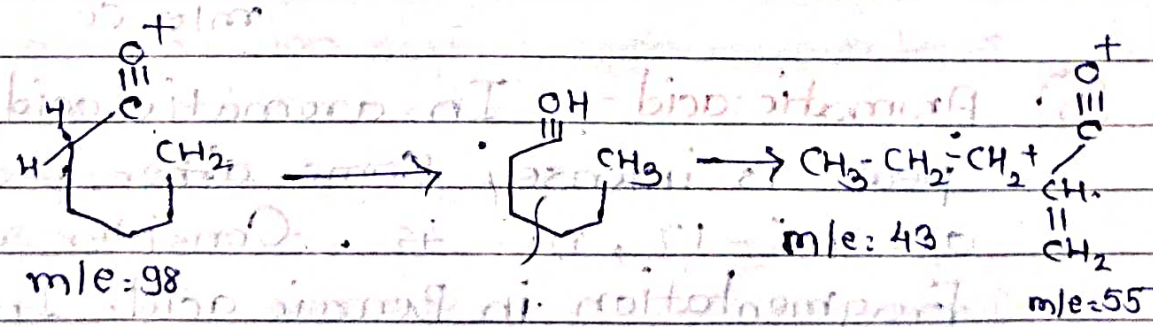
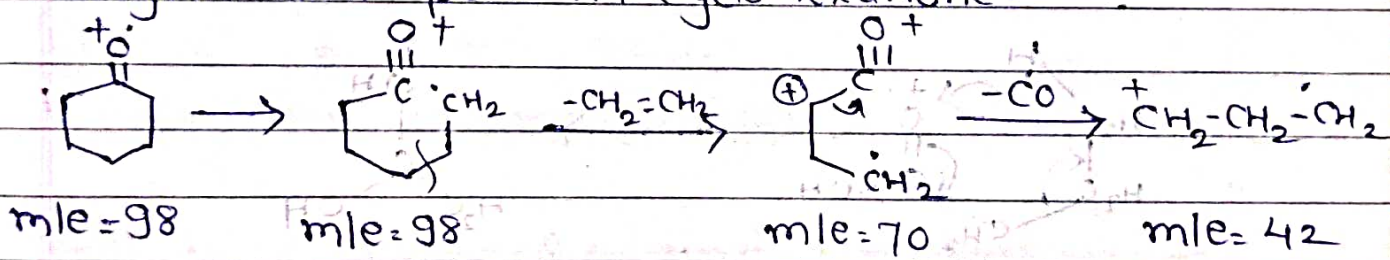


1) **Aromatic ketone** - In aromatic ketone the loss of larger group is prefer by α -cleavage.



2) **Cyclic ketone** -

- The molecular ion peak of cyclic ketone is quite intense.
- The base peak in cyclopentanone or hexanone is formed by α -cleavage followed by hydrogen transport rearrangement.
- Significant peak in cyclohexanone -

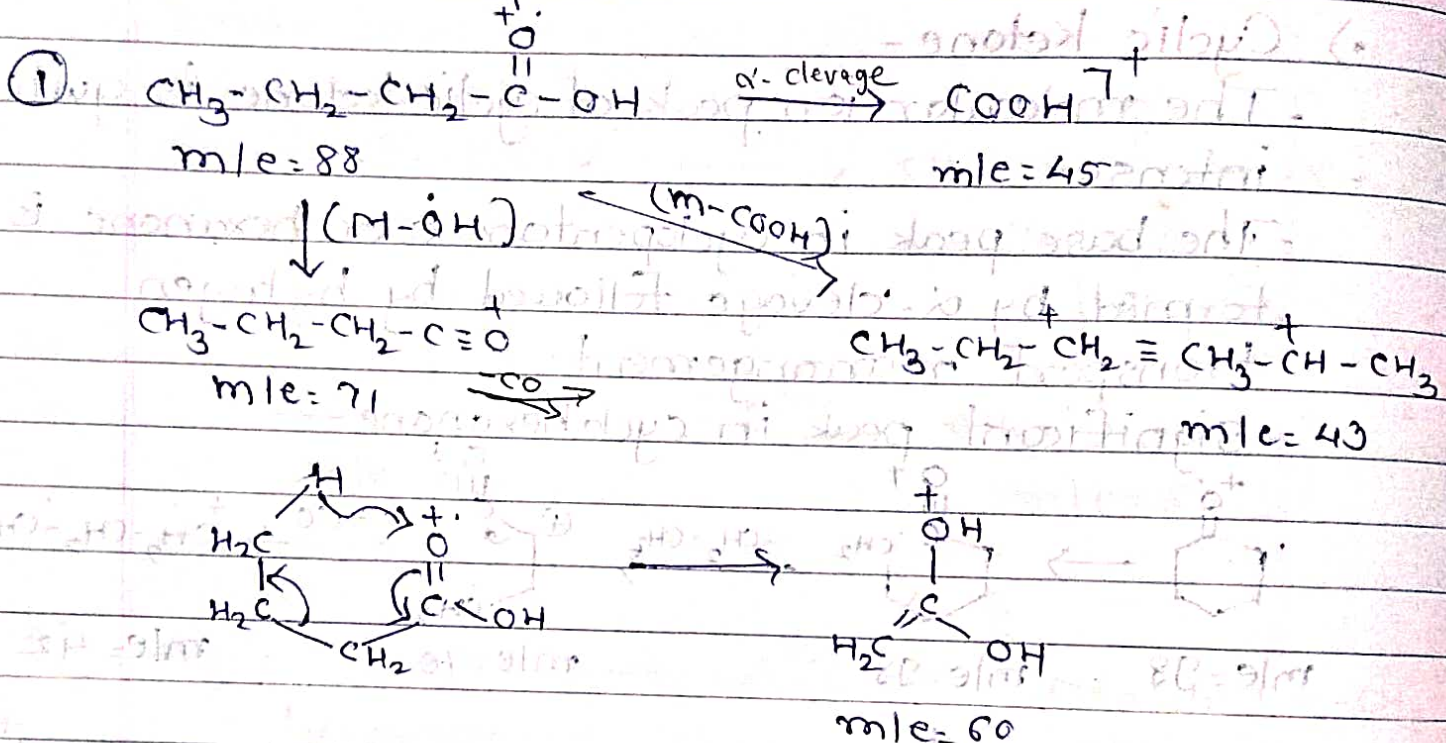


★ **Aromatic & Aliphatic Acid** -

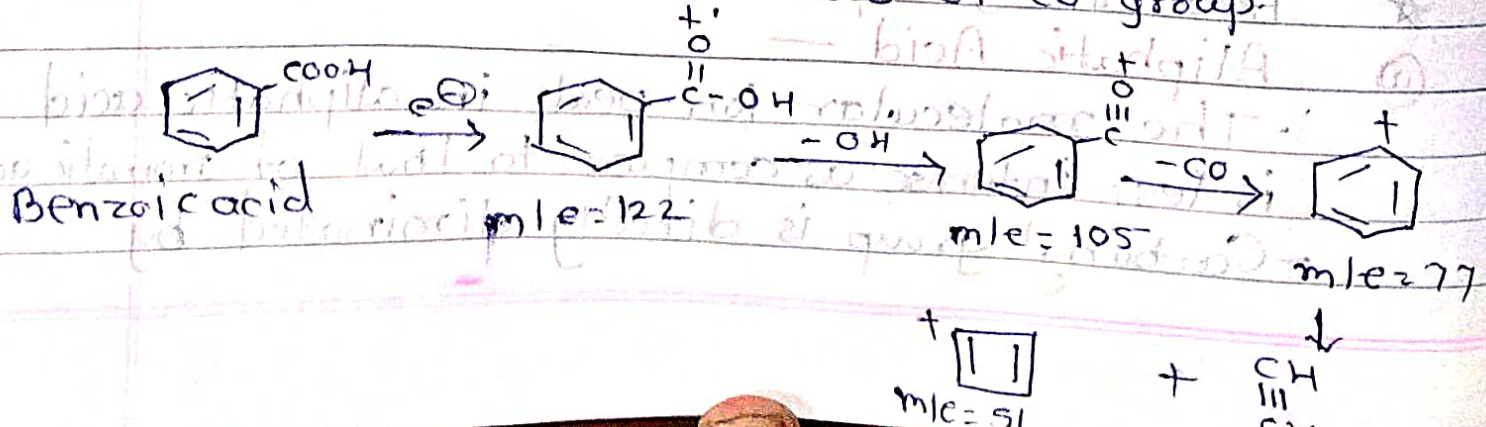
⊙ **Aliphatic Acid** -

- The molecular ion peak in aliphatic acid is less intense as compare to that of aromatic acid.
- Carbonyl group is directly eliminated by

α -cleavage & signal is form at $m/e = 45$
 - If α -carbon atom is not substituted in aliphatic acid containing γ -hydrogen atom a McLafferty rearrangement ion is formed at $m/e = 60$. It is oftenly best peak.
 - In short chain acid M^+COOH & M^+OH peaks are prominent.



② **Aromatic acid** - In aromatic acid parent ion peak is intense. Some other prominent peaks are $m - 17$, $m - 45$. Consider various fragmentation in Benzoic acid. In benzoic acid $M-OH$ peak is prominent, other peak appear at $m/e = 77$ is due to loss of CO group.



* Nitrogen Rule -

4 Marks

According to nitrogen rule, molecule having odd number of molecular weight similarly molecule which having even number of nitrogen atom shows even number of molecular weight.

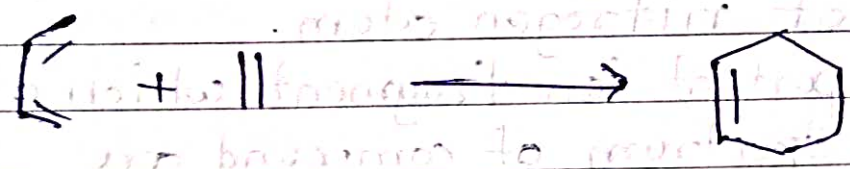
- ① To explain this rule let us consider nitrobenzene -
- The signal for molecular ion appear at $m/e = 123$ (i.e., at odd number molecular mass since compound contains only one (odd) number of nitrogen atom).
- Two important ion fragment which are formed in mass spectrum of compound are.
 - i) NO_2^+ at $m/e = 46$
 - ii) NO^+ at $m/e = 30$Both this fragments appear at even mass no.

- ② Let us consider 2,4 dinitrophenol -
- The molecular ion signal appear at $m/e = 184$.
- Since compound containing two (even) number nitrogen atom.
- Two important fragments appears at,
 - i) $M^+ - H$ at $m/e = 183$
 - ii) $M^+ - CO$ at $m/e = 155$Both this fragments appear at odd mass number.
- It means fragmentation of single bond gives odd number ion fragmentation from even number molecule ion.
- Similarly even number ion fragment result form odd number molecule ion.

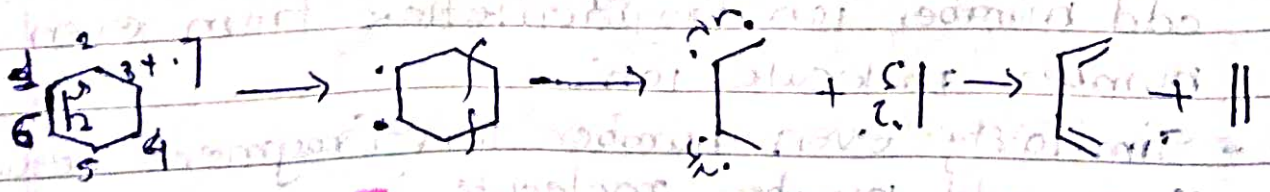
- PAGE NO. _____
DATE: ____/____/____
- ① $\text{CH}_3\text{-NH}_2 = \text{mle} = 31 = \text{Odd (1 N atom)}$
 - ② $\text{CH}_3\text{-CH}_2\text{-NH}_2 = \text{mle} = 45 = \text{Odd (1 N atom)}$
 - ③ $\text{CH}_3\text{-C}\equiv\text{N} = \text{mle} = 41 = \text{Odd (1 N atom)}$
 - ④ $\text{NH}_2\text{-CH}_2\text{-NH}_2 = \text{mle} = 46 = \text{even (2 N atom)}$
 - ⑤ $(\text{NH}_2)_3\text{-CH} = \text{mle} = 61 = \text{Odd (3 N atom)}$

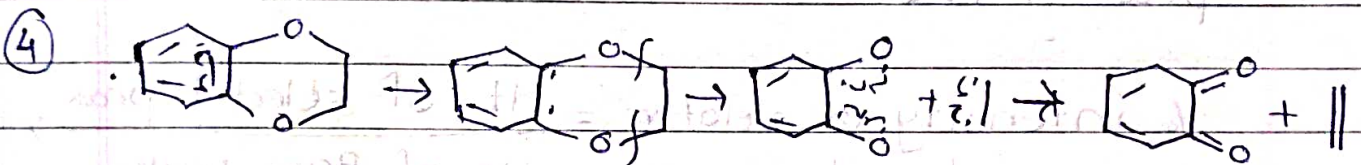
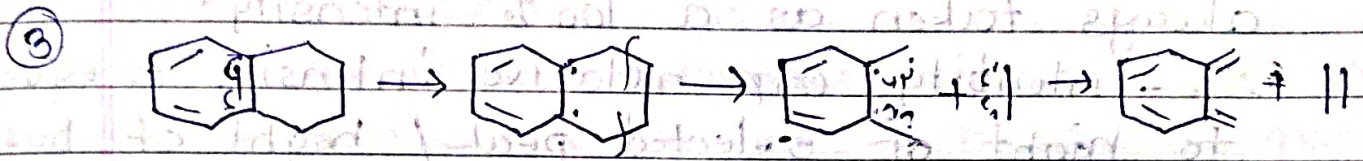
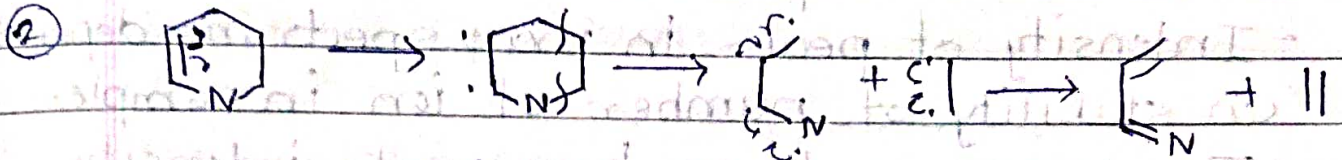
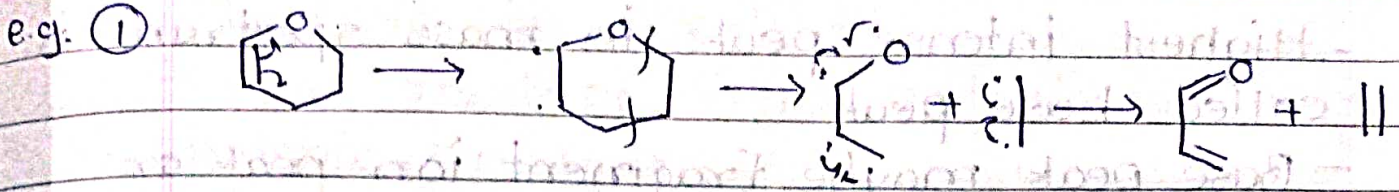
★ Retro-Diels Alder Reaction —

- Generally Diels Alder reaction occurs between dienes & dienophiles gives cyclohexene.



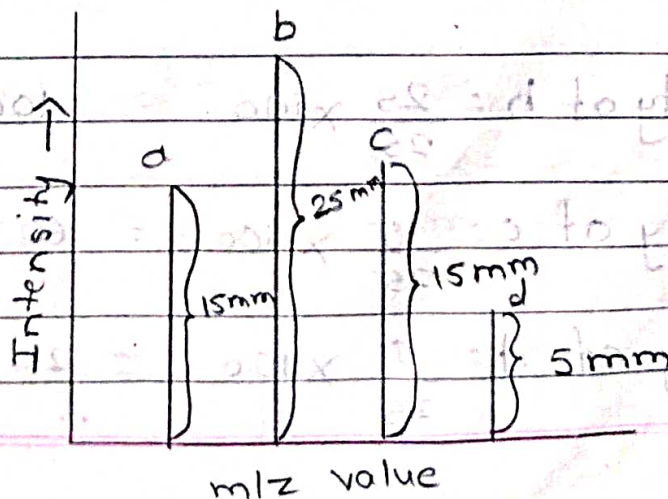
- The retro diels alder reaction is fragmentation of cyclic olefins. The fragmentation occurs in cyclohexene.
- First ionisation is occurs. Both e^- from double bond transfer to the adjacent carbon and e^- from bond between 3,4 & 5,6 transfer to the adjacent atom. The e^- from 6 & 1 carbon atom make a double bond between these & an e^- from 2 & 3 carbon atom make double bond.
- These rearrangement cleave the molecule between 3 & 4 carbon atom & 5 & 6 carbon atom which gives diene & dienophile.





- It is fragmentation mode of cyclic transition
- Fragmentation of olefin involve cleavage of two bond of cyclic system. In this reaction formation of diene & dienophile
- The cyclic olefin which gives unsaturated fragment diene & dienophile called retro diels alder reaction.

* Base Peak \rightarrow



- Highest intense peak in mass spectrum is called base peak.
- Base peak maybe fragment ion peak or molecular ion peak.
- Intensity of peak in mass spectrum depends on stability of number of ion in sample.
- In mass spectrum base peak intensity always taken as a 100% intensity.
- % intensity or relative intensity is equal to height of selected peak / height of base peak $\times 100$.

$$\% \text{ intensity or relative intensity} = \frac{\text{Ht. of selected peak}}{\text{Ht. of Base peak}} \times 100$$

$\text{Stability} = b > a = c > d$

I] For peak 'a' -

i) % intensity of a = $\frac{15}{25} \times 100 = 60\%$

ii) % intensity of b = $\frac{25}{25} \times 100 = 100\%$

iii) % intensity of c = $\frac{15}{25} \times 100 = 60\%$

iv) % intensity of d = $\frac{5}{25} \times 100 = 20\%$

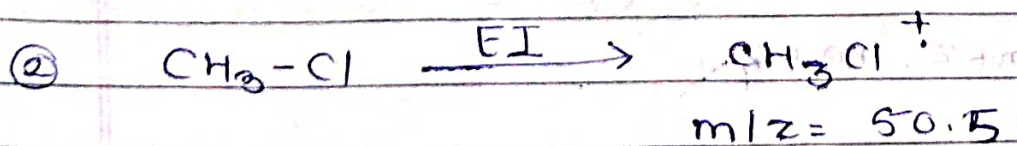
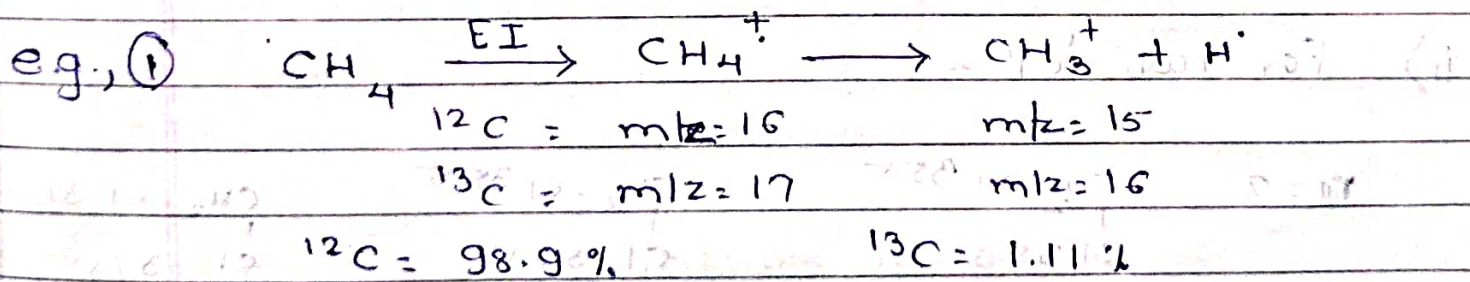
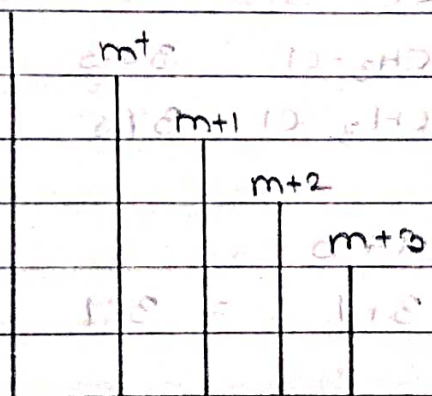
* Isotopic ion peak —

- Peak resulting from isotopic ion causes isotopic ion peak.

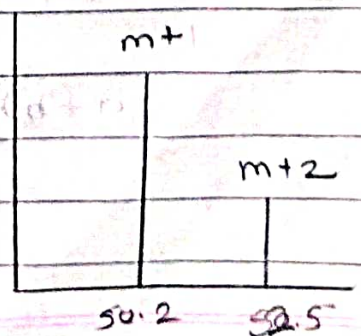
- This peaks appears only in some of the sample in mass spectrum.

- Compound containing Cl, Br, S shows isotopic peaks. and position of peak is at right side of molecular ion.

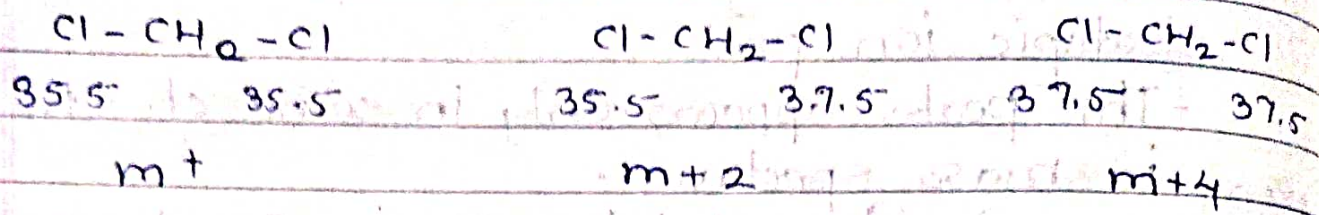
- The appearance of isotopic ion peak $m+1, m+2, m+3$ shows presence of $^{35}\text{Cl}, ^{79}\text{Br}, ^{32}\text{S}$ in mass spectrum.



If Cl is ^{37}Cl then $m/z = 52.5$



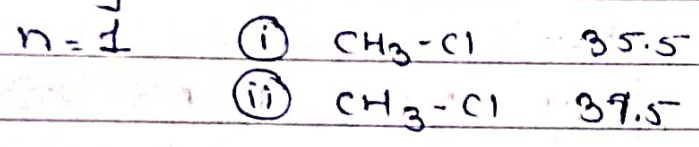
③ If two Cl. → $\text{Cl}-\text{CH}_2-\text{Cl}$



$a:b = 3:1$

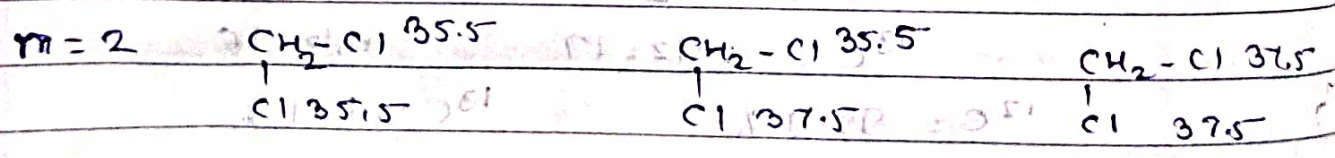
$a = 35.5$ $\text{Cl} = 100\%$
 $b = 37.5$ $\text{Cl} = 32.4\%$

i) Only one 'Cl'.



$(a+b)^2 = a+b$
 $= 3+1 = 3:1 = m+1 : m+2$
 $\frac{35.5}{37.5}$

ii) For two 'Cl' → CH_2-Cl

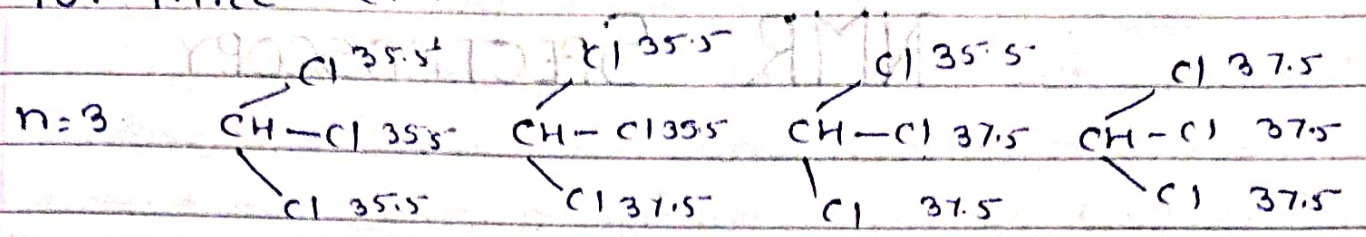


$m+1, m+2, m+4$

$(a+b)^2 = a^2 + 2ab + b^2$
 $= (3)^2 + 2(3 \times 1) + (1)^2$
 $= 9 + 6 + 1$
 $= 9:6:1$

$m+1 : m+2 : m+4$

iii) For three 'Cl' -



m^+ , $m+2$, $m+4$, $m+6$

$(a+b)^3 = a^3 + 3a^2b + 3ab^2 + b^3$
 $= (3)^3 + 3(3)^2(1) + 3(3)(1)^2 + (1)^3$
 $= 27 + 27 + 9 + 1$

$= 27:27:9:1$

one 'Cl' two 'Cl' three 'Cl' 27:27:9:1

$a:1$	m^+	$9:6:1$	m^+	$m+2$
$m+2$		$m+2$		$m+4$
	$m+2$		$m+4$	$m+6$