Date 18 Dec
Page 01

1) Instrumental
a) UV spectroscopy
b) IR
c) NMR → Proton NMR
→ C-30 NMR
d) Atomic absorption spectroscopy
e) Flame photometer
f) X-ray defraction
g) Flurometry
h) Emission spectroscopy
i) Radio-immuno assay

2) ISO. T&M

Spectroscopy
It is the modern branch of science in which information about the molecule or atom can be obtained by their interaction with electromagnetic radiation.

Primary metabolites:- Carbohydrates. Vitamins. Proteins
Secondary metabolites:- Steroids. flavonoids . terpenes
↓
not more essential for plant but essential for us
↓
their study falls under natural products

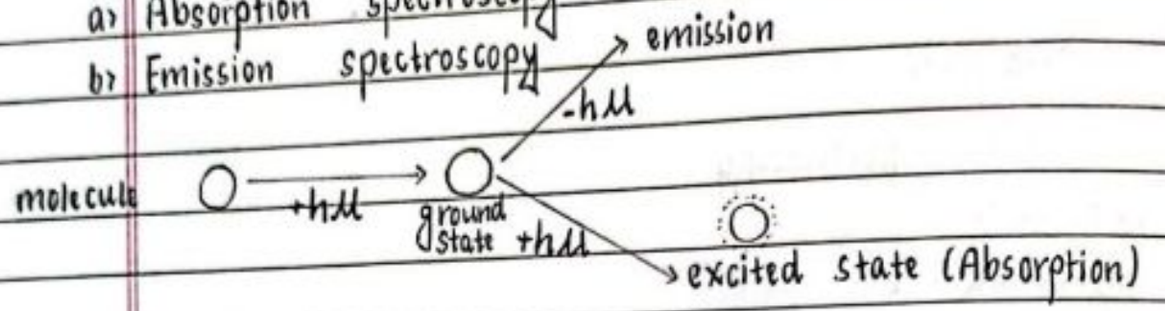
To find out the active chemical constituents from plant there are 2 methods:-
i) Chromatographic
ii) Spectroscopy
Retrospective study for the synthesis.

Process of Spectroscopy

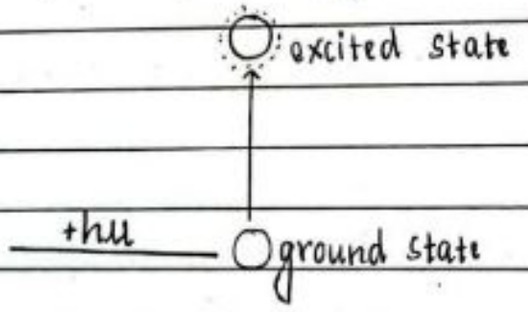
Classified on 2 types:

a) Absorption spectroscopy

b) Emission spectroscopy




a) Absorption Spectroscopy




It is the branch of spectroscopy in which the information about the molecule or atom can be obtained by irradiating them with suitable range of electromagnetic radiation. Molecules or atom absorbs the energy and goes to the excited state, the range and intensity of the absorbed radiation is the basis of the qualitative and quantitative estimation of molecules or atom.

Examples: UV spectroscopy
Infrared spectroscopy
Atomic absorption spectroscopy
Nuclear magnetic Resonance (NMR)



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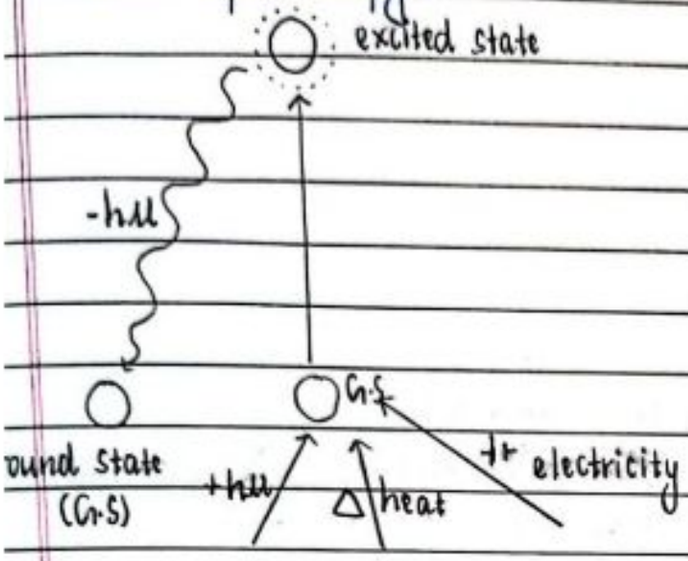
Date
Page 03

## Emission Spectroscopy



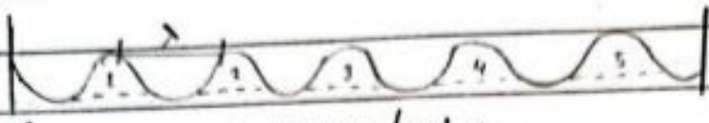

In the emission spectroscopy, the molecule or atom which is to be investigated is first excited to the excited state either by electrical energy, or thermal energy or electromagnetic radiation having high energy the molecule or atom while returning to ground state emits the energy in the form of electromagnetic radiation. The intensity and range of the emitted radiation is the basis of the qualitative and quantitative estimation of molecular atom

Examples:- X-ray emission spectroscopy
Flame photometry
flurometry
Atomic emission spectroscopy

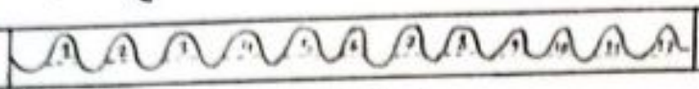
Electromagnetic radiation (EMR)
It is the form of energy which posses both the electric and magnetic properties

- UV ray
- Gamma ray
- Infrared ray
- Microwave
- Radiowave
- Visible wave

Sound:- longitudinal wave  light:- Transverse wave




frequency :. 5 waves/min



frequency :. 12 waves/min

Decrease wavelength. frequency increases

$E = h\upsilon$ $\quad \upsilon = c/\lambda$ $\quad c$ = velocity of light constant

h constant $\quad \upsilon \propto 1/\lambda$

$\therefore E \propto \upsilon$

frequency $\propto \frac{1}{\lambda \text{ (wavelength)}}$

E: high
$\upsilon$: high
$\lambda$: ~~low~~

Gamma ray → X-ray → UV → VIBGYOR → IR → Microwave → Radiowave

E: low
$\upsilon$: low
$\lambda$: ~~high~~

"Cosmic ray"

Gamma radiation → change in nuclear composition
X-ray → ejection of electrons
UV, VIBGYOR → Electronic transition
IR → vibration transition
Microwave → Rotational transition
Radiowave → change in nuclear spin

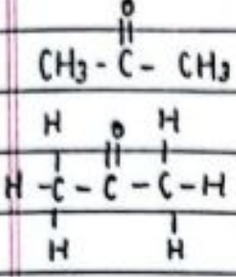
Electromagnetic radiation spectrum

The arrangement of the electromagnetic radiation in increasing order of frequency or wavelength.

Date 19 Dec
Page 05

## UV- Visible spectroscopy

Acetone



No. of $\pi$ elatrons = 2

No. of Sigma ($\sigma$) electrons = 18

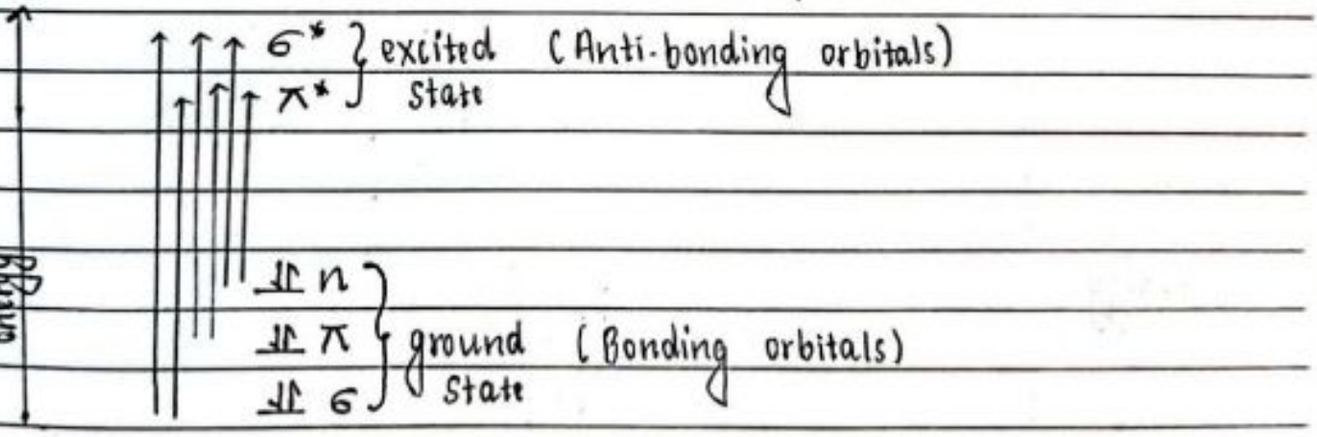
No. of unpaired electrons = 2

## Molecular Orbital theory

The linear combinatiorial of atomic orbital to form equivalent number of molecular orbital.

## Hybridization

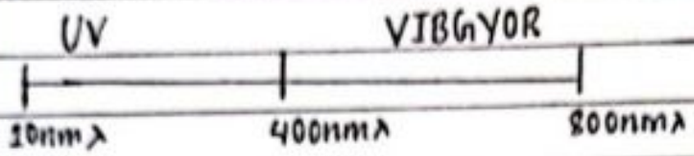
If is the process of mixing of Orbitals having comparable energy to form equivalent number of hybridized orbital.




## Number of Possible transition

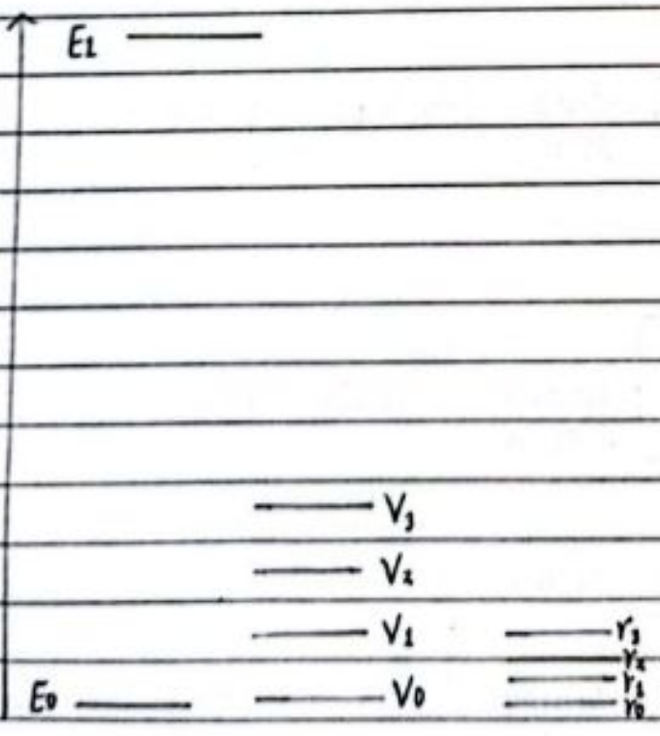
1) $n-\pi^*$
2) $n-\sigma^*$ } requires less energy. So UV- visible focuses on this
3) $\pi-\pi^*$
4) $\pi-\sigma^*$
5) $\sigma-\pi^*$ } requires high energy
6) $\sigma-\sigma^*$

UV- visible starts from 10nm upto 800nm wavelength




10-200nm λ (far UV) high energy
200-400nm λ (near UV) low energy
$\pi$-$\sigma^*$, $\sigma$-$\pi^*$, $\sigma$-$\sigma^*$ transition requires far UV and not useful for us, because it only gives the information of the Pi and sigma bond and sigma bond is present in all molecules.

UV- visible spectrophotometer has range of 200-800nm.




- Within the single electronic transition there are several vibrational transition.
- Within the single vibrational transition there are several rotational transition.



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## Useful transition in UV. visible spectroscopy

$n$-$\pi^*$

$n$-$\sigma^*$

$\pi$-$\pi^*$

## Structure of Pentadiene

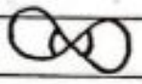
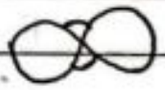
$CH_2=CH-CH=CH-CH_3$ ($C_5H_8$)
conjugated double bond / de-localized

$H_2C=CH-CH_2-CH=CH_2$
Non-conjugated double bond / localized

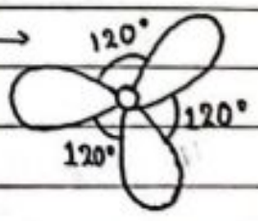
$SP$ = hybridized = 2
Unhybridized = 2

○ ⟶ S-orbital
∞ ⟶ P-orbital

$SP^2$ = hybridized P orbital = 2
Unhybridized P orbital = 3

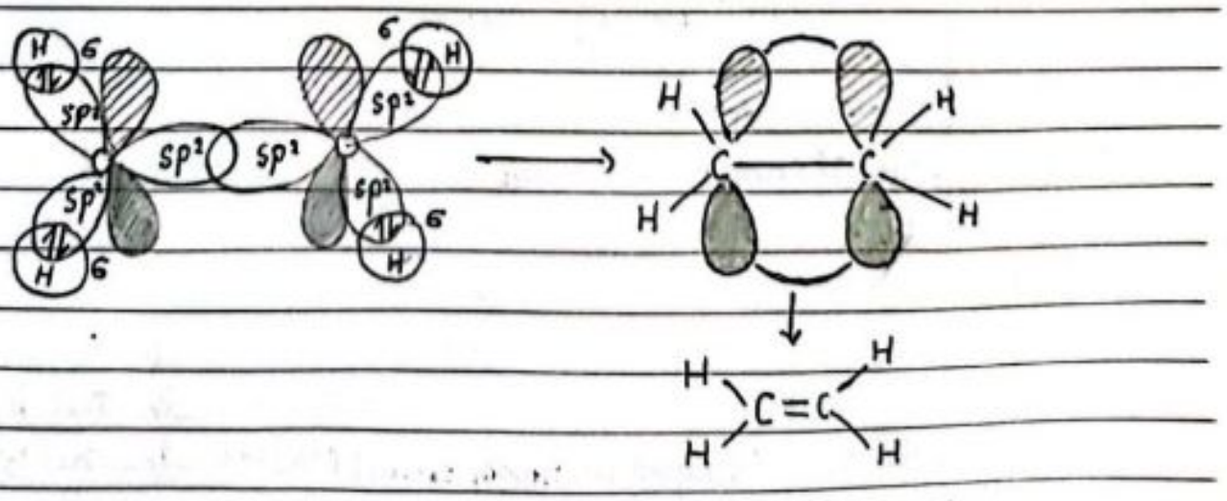
$SP$ orbital ⟶ 
or


$SP^3$ = hybridized P orbital = 4
Unhybridized P orbital = 0

$SP^2$ orbital ⟶ 


## Ethylene

$SP^2$ hybridization $H_2C=CH_2$



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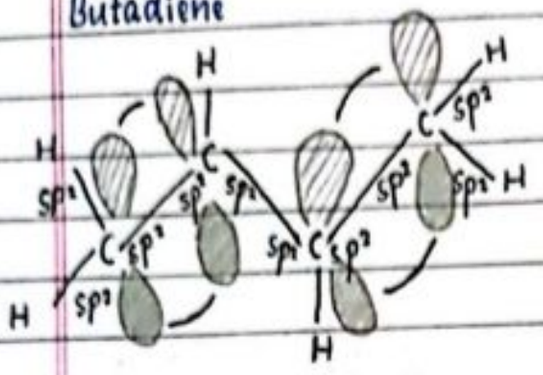
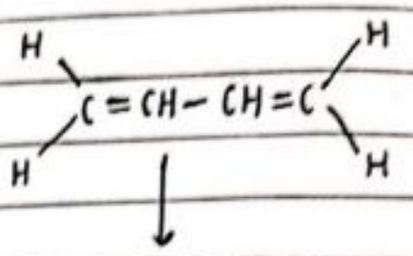
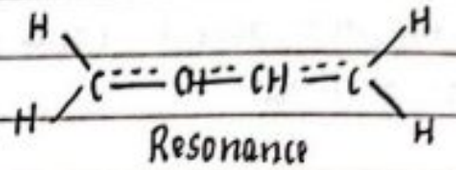
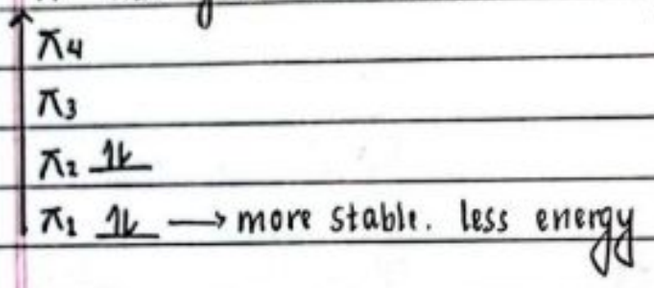
## Butadiene



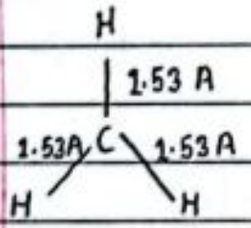




It has four π- orbitals

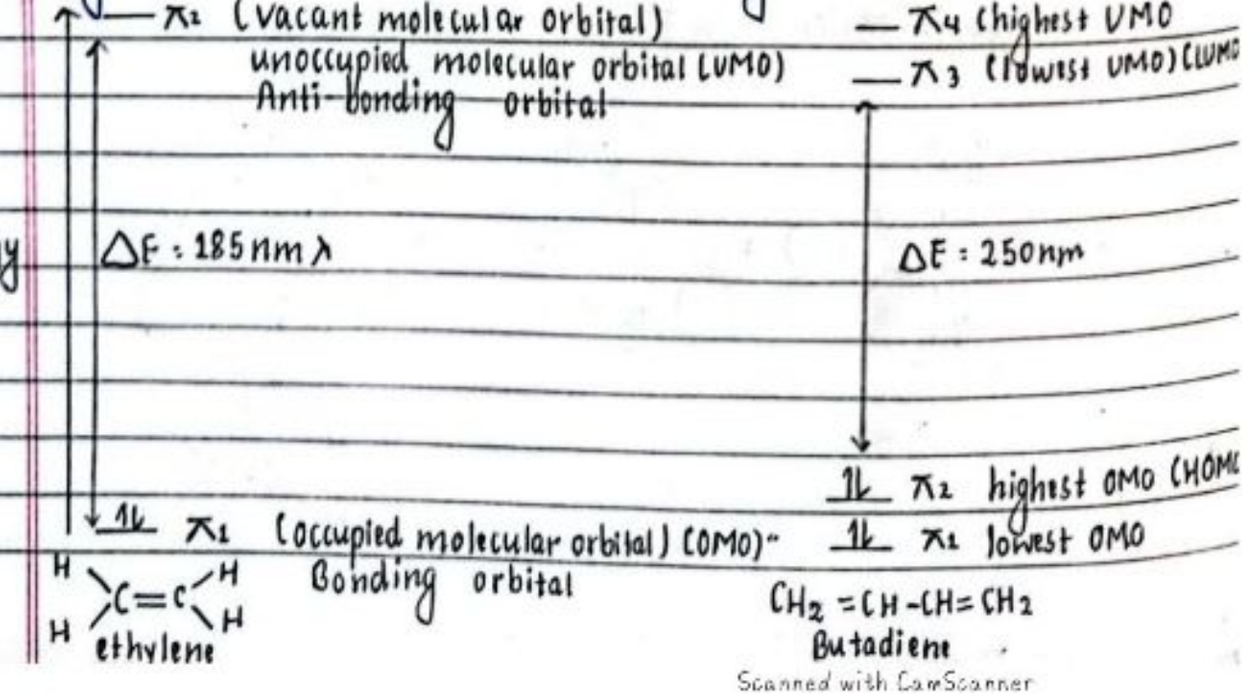



## Methane




Q. Why β- carotene is colured and ethylene is colourless

Soln:-



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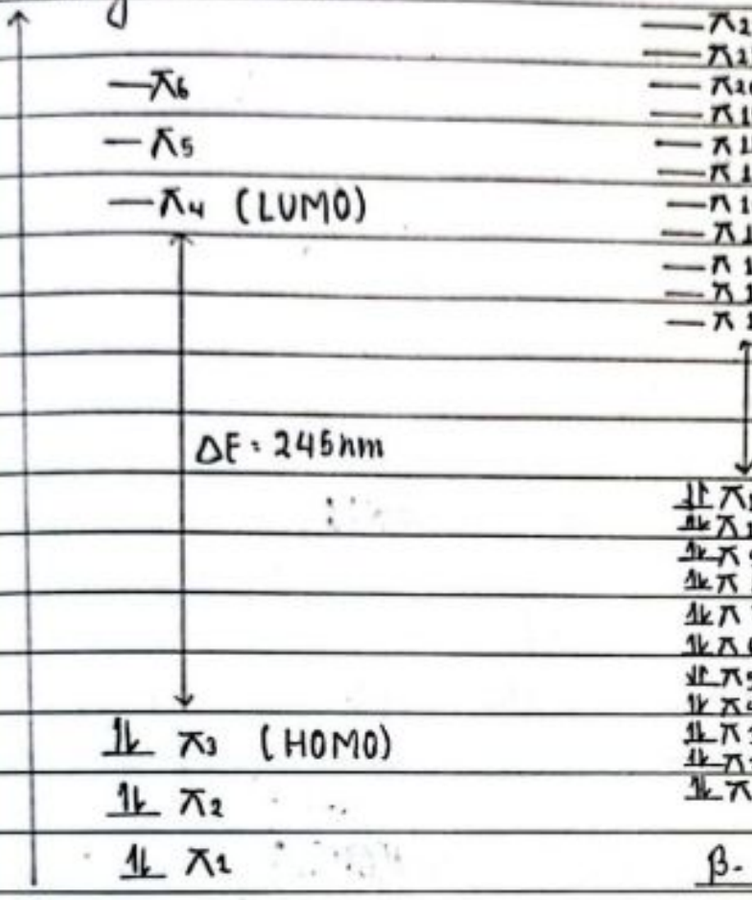
Similarly.

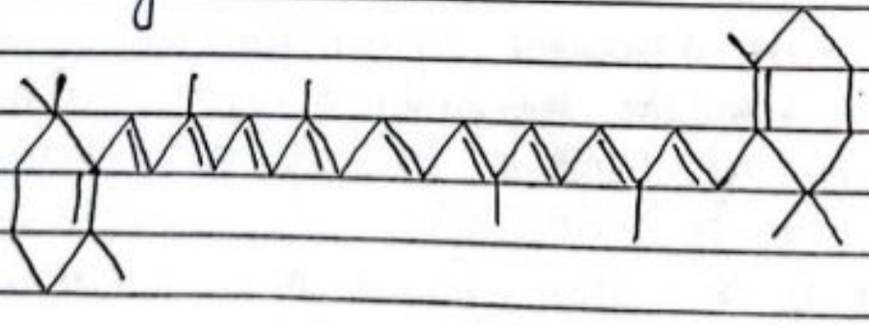



From the above diagramatic representation ethylene has only two Pi-electron and one conjugated double bond. It' requires the higher energy of transition from $\pi_1$ to $\pi_2$, where $\pi_1$ is the occupied molecular orbital and $\pi_2$ is unocupied molecular orbital. The value of $\Delta E$ = 185 nm for ethylene to move from $\pi_1$ to $\pi_2$. whereas β-carotene has 11 conjugated double bond and 22 π-electrons and requires the less energy for transition of $\pi_{11}$ to $\pi_{12}$. $\pi_{11}$ is the highest occupied molecular orbital (HOMO) and $\pi_{12}$ is the lowest unoccupied molecular orbital (LUMO) The value of $\Delta E$ for this is 435 nm. As we know that UV-visible spectrophotometer has range of 200-800nm, the wavelength of β-carotene falls under this and is colured whereas ethylene is colourless. If any molecules it absorbs the colour gives the colour in UV. and hence is visible.

Alternatively,
β-carotene consist of the conjugated chain where there is delocalization. Increase the delocalization decrease the gap between the highest energy Pi anti-bonding orbital and the lowest energy Pi anti-bonding orbital hence takes less energy for transition of electron from HOMO to LUMO. The use of less energy, lower frequency of light get absorbed which means longer wavelength. β carotene gets absorbed within the UV-region, particularly more in the visible region hence. β-carotene molecules gets coloured.

Wheras, in ethylene the gap between the HOMO and LUMO is greater and hard shorter wavelength than β-carotene.. and takes more energy for the exicitation of molecules and the wavelength of the ethylene molecules is 185nm, which dosent falls under the range of UV-visible spectrophotometer and hence colourless

Structure of β-carotene



β-carotene

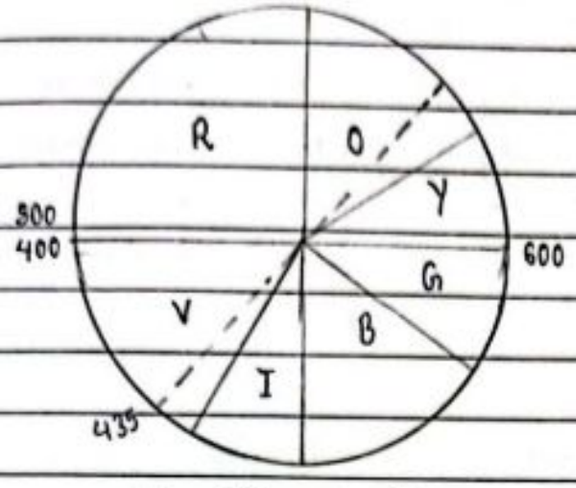



fig:- Munset wheel

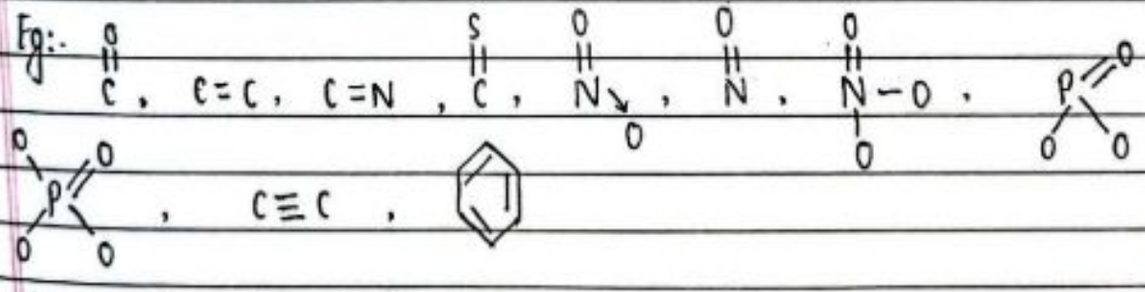
Thus. β-carotene posses yellowish orange colour

## Chromophore

Chromo ⟶ colour

Phore ⟶ bearer

Chromophore is the skeleton. or moiety or functional group within the molecule which is responsible for absorbance of UV- visible radiation.

Eg:- 

It can be classified two types:-

1. Chromophore producing single peaks
2. Chromophore producing double peaks

C=C, C≡C, in these Chromophore, there is only one possible transition i.e. π - π* and produce a single peaks while,



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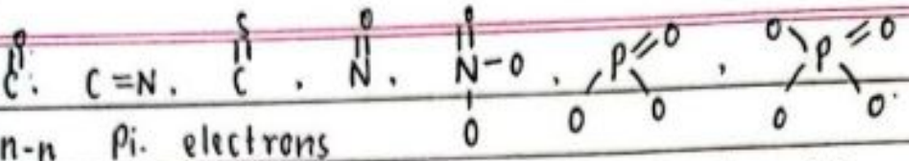
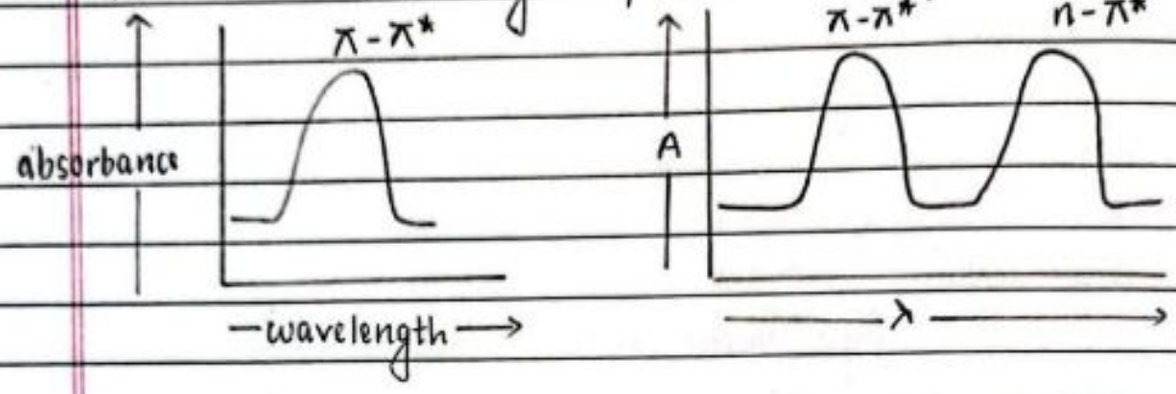



contains both n-n Pi. electrons

So, there is possibility of two transition i.e. $n-\pi^*$ and $\pi-\pi^*$. So, they produce two peaks.




## Auxochrome

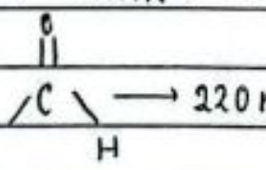
Auxene ⟶ "to enhance"

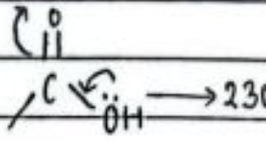
chrome ⟶ colour

Auxochrome is the atom or the group of atom which themselves donot absorbs the UV-radiation but can alter the wavelength absorbed by a particular chromophore

Eg:-

-OH, -NHR, -X, $-NH_2$, -OR, $-NR_2$, -R




## Bathochromic shift

The shift of the peak of the chromophore towards the longer wavelength either due to the presence of the Auxochrome or due to effect of solvent.

Also called "Red shift"



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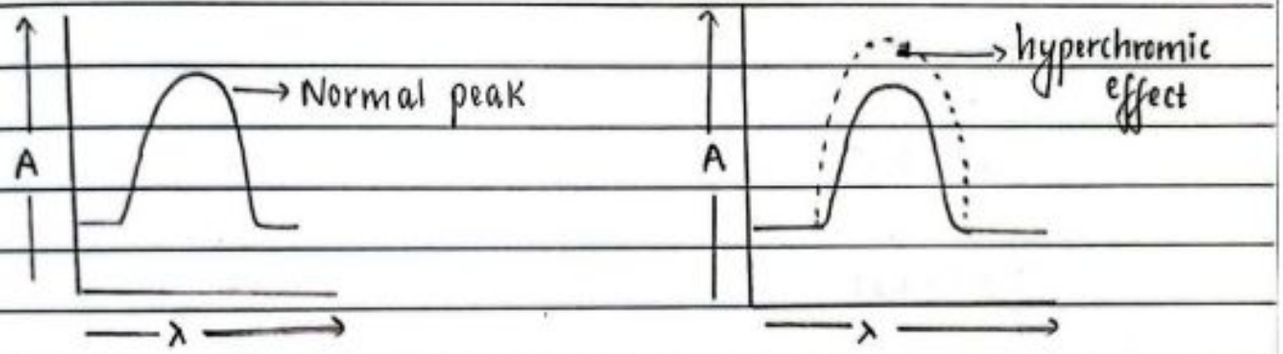
## Hypsochromic effect

The shift of the peak of the chromophore towards the shorter wavelength either due to effect of solvent or due to structural deformity.
Also called "Blue-shift"

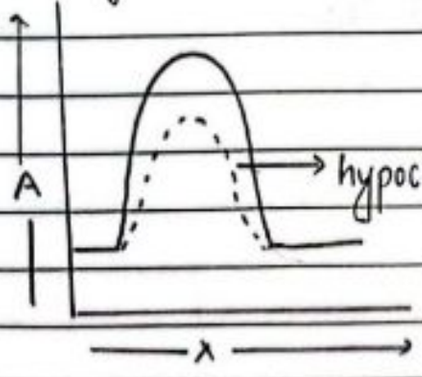
## Hyperchromic effect

Those effects due to which there is increase in intensity of peak of absorbance are called hyperchromic effect.




## Hypochromic effect

Those effects due to which there is decrease in intensity of Peak of absorbance are called hypochromic effects.



Date 21 Dec
Page 14

## Lambert's law

When a monochromatic beam of light is passed through a transparent medium having thickness 't' then, decrease in intensity of light with thickness is directly proportional to the intensity of incident light.

Mathmatically,

$$-\frac{\Delta I}{\Delta t} \propto I$$

$$\text{or, } -\frac{\Delta I}{\Delta t} = KI$$

$$\text{or, } -\frac{\Delta I}{I} = K\Delta T$$

$$\text{or, } -\frac{\delta I}{I} = K\delta T$$

$$\frac{1}{I}\delta I = K\delta t$$

On integration.

$-\ln I = Kt + C$ —— (i) where, C = integration constant

when, $t = 0$ then, I becomes $I_0$

$-\ln I_0 = C$ —— (ii)

Putting the value C in eqn (1). Then.

$\ln I = Kt - \ln I_0$

$-\ln \frac{I}{I_0} = Kt$

$\ln \frac{I_0}{I} = Kt$

$\frac{I}{I_0} = e^{-Kt}$

$\therefore I_t = I_0 e^{-Kt}$ → lambert's law



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## Beer's law

When a monochromatic beam of light is passed through a solution having concentration 'c' then. decrease in intensity of light with concentration is directly proportional to the intensity of incident light.

Mathmatically.

$$-\frac{\Delta I}{\Delta c} \propto I$$

$$-\frac{dI}{dc} = KI$$

$$\text{or, } \frac{-dI}{I} = dc \cdot K$$

On Integration

$$-\ln I = Kc + C' \quad \text{——— (i)}$$

When $c = 0$. then I becomes $I_0$

$$-\ln I_0 = C' \quad \text{——— (ii)}$$

Putting the value of C' in eqn (i) becomes.

$$-\ln I = Kc + (-\ln I_0)$$

$$-\ln \frac{I}{I_0} = Kc$$

$$\ln \frac{I_0}{I} = Kc$$

$$\frac{-I}{I_0} = e^{-Kc}$$

$$\therefore I_t = I_0 e^{-Kc} \longrightarrow \text{Beer's law}$$

## Beer. lambert law

When a monochromatic beam of light is passed through a solution having the concentration 'c' and path length 't'



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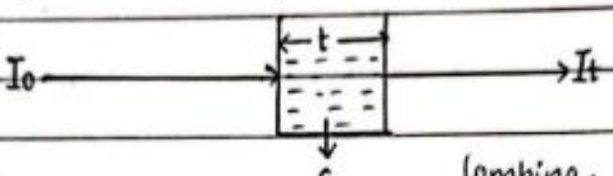
then, decrease in the intensity of light with concentration and thickness is directly proportional with the intensity of incident light.




lombing. the Beer's law and lambert's law

We get. $I_t = I_o e^{-Ktc}$

$$\ln \frac{I_o}{I} = Ktc$$

↓

Absorbance (A)

$\therefore A = Ktc$

Absorbance is directly proportional to thickness of path length and concentration of solution.

$A = Ktc$ Where. c = concentration of solution

$= \varepsilon tc$ t = thickness of pathlength

$= \alpha tc$ $\alpha$. E. K = Molar absorptivity coefficient or Molar extension coefficient

Molar extension coefficient

It is the constant and is a characterstics features of the compound and can be defined as. absorbance of solution of a compound having unit concentration and unit path length.

where. 't' → 1cm. and 'c' → 1%.

Significance

i) It is used to check the purity of compound

ii) It is also used to determine the concentration of the unknown solution.

when, $A = K + C$

↓ ↓

cons. 1

ant

$A = \text{constant} \times C$

$\therefore A \propto C$

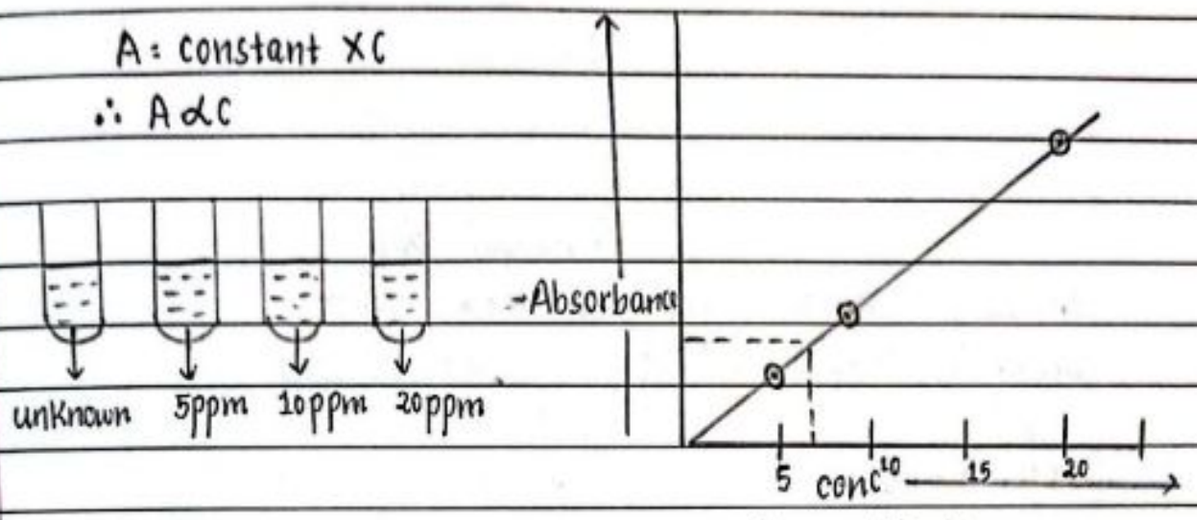



fig:. calibration curve

Limitations

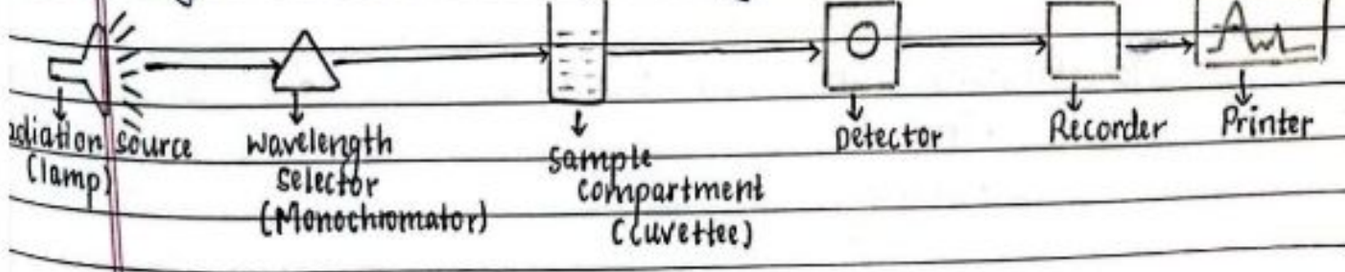
1. It is only suitable for dilute solution.
2. It always requires the monochromatic beam.

Why not suitable for higher concentration?

Because the molecules collides with each others and also the absorbed excited molecules excite the ground state molecules and it individually comes back to ground state.

Instrumentation of UV-visible spectrophotometer

(single beam UV-visible spectroscopy)



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Date 22 Dec
Page 18

1. Radiation Source

Radiation source can be:- Hydrogen discharge lamp
- Mercury Arc lamp
- Deuterium lamp
- Tungsten lamp
- Xenon flash lamp
- Halogen lamp

Nowadays vendors only use these lamps:-

- Deuterium lamp (near UV, 200-400 nm)
- Tungsten or Halogen lamp (visible, 400-800 nm)
- Xenon-flash lamp (UV-visible) (200-800nm)

Criteria for radiation source:-

* It must be continous, stable and must be give the light of desired wavelength.

2. Slit

Purpose:- adjustment of band width.

3. Monochromator / wavelength selector

Purpose:- select the desire wavelength of light.

Wavelength selector are of 2 types:-
i) Monochromators
ii) Filters

Monochromators are more commonly used and are of 2 types
- Prism
- Gratings

4. Sample compartment

Purpose:- Analysis in the liquid form..



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Criteria for selection of solvent to make the solution.

i) The solvent must be transparent and must fall in the required range of light and the solvent must not absorbed the light.

ii) Solvent must be inert. Should not react with sample or cuvettee.

iii) Solvent must dissolved the analyte.

5. Cuvettee

Purpose:- for holding the sample

Criteria for cuvettee:-

Should be made up of the material that should be transparent and inert. They are usually made up of:-

1) Plastic

Cheaper, easily avaiable, use and throw

Used when the sample is sticky and corrosive

2) Glass

used for the visible range

3) Quartz

used for all types of analysis

Expensive

6. Detector

Types of Detector in UV spectroscopy:-

- Barrier layer cell or layer-Barrier cell
- Photo cell
- Photo multiplier tube (PMT)



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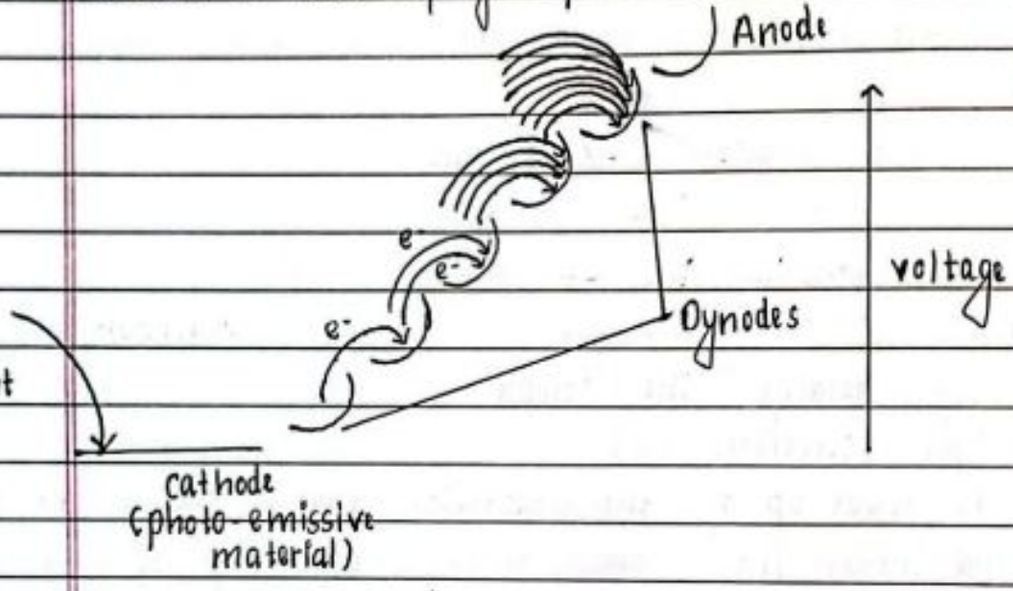
1. Photo multiplier tube (PMT)

PMT generally consist of the two electrodes i.e. cathode and anode.

Cathode is made up of photo emissive material.




- Generally, two photocells serve the purpose of the detector in UV spectroscopy
- One of the photocell receives the beam from the sample cell and the second detector receives the beam from the refrence.
- The intensity of the radiation from the refrence cell is stronger than the beam of the sample cell. This results in the generation of pulsating or alternating currents in the photocell.

2) Barrier-layer cell or layer Barrier cell

- The detector has a thin film metallic layer coated with silver or gold and acts as electrode
- It also has a metal base plate which acts as another electrode.
- These two layers are seperated by a semiconductor layer of selenium.



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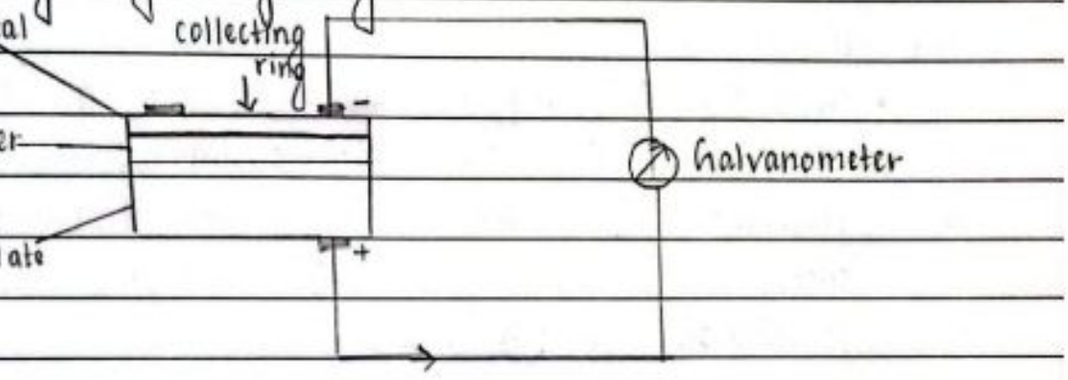
When light radiation falls on selenium layer. electrons become mobile and are taken up by transparent metal layer. This creates a potential difference between two electrodes and cause the flow of current.

- When it is connected to galvanometer, a flow of current observed which is proportional to the intensity and wavelength of light falling on it.




3) Phototubes / Photoemissive tubes

- Consist of a evacuated glass tube with a photocathode and a collector anode.
- The surface of a photocathode is coated with a layer of elements like cesium, silveroxide or mixture of them
- When radiant energy falls on the photosensitive cathode, electrons are emitted which are attracted to anode causing current to flow.
- More sensitive compared to barrier layer cell and therefore widely used.

7. Recorder

It record the electrical signal and transfer it to the graphical representation.



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## Advantages of a Single beam spectroscopy

- Cost-effectiveness: Single beam instruments are less expensive as compared to other alternative.
- Better performance:- High energy throughout due to the non-splitting of the source beam results in high sensitivity of detection

## Disadvantages

- Instability:- This happens due to lack of compensation for disturbances like electronic circuit fluctuations, voltage fluctuations, mechanical component's instability, or drift in the energy of light sources. Such drifts cause abnormal fluctuations in the result.

## Effect of solvent in UV-visible spectroscopy

Solvents can be broadly classified into two categories:

- Polar
- Non-Polar

- The solvent exert a profound influence on the quality and shape of spectrum.
- A drug may absorb a maximum radiation energy at a particular wavelength in one solvent but shall absorbs partially at the same wavelength in another solvents

Eg:- Acetone in n-hexane $\lambda$max at 279nm

Acetone in water $\lambda$ max at 264.5 nm

Most commonly used solvent is 95% ethanol It is best because:-

i) It is cheap

ii) Has good dissolving power

iii) Does not absorbs radiation above 210nm

Criteria for solvent selection:.

- It should not itself adsorbs radiation in the region under investigation.
- It should be less polar so that it has minium interaction with the solute molecules.

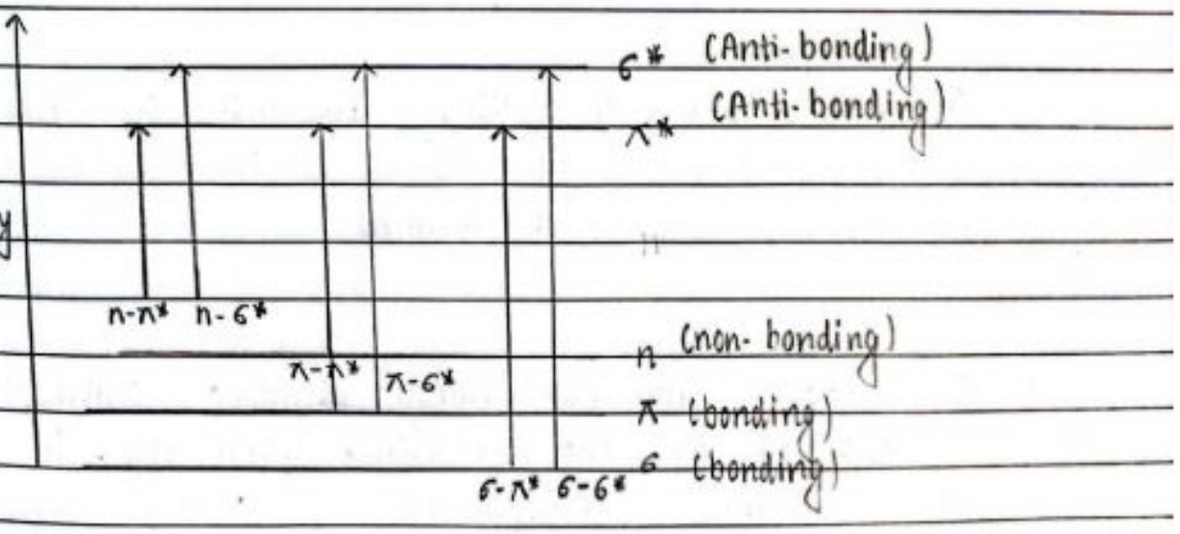
1. Polarity

Polarity plays an important role in the position and intensity of absorption maximum of a particular chromophore.

a) In case of non-polar solvents for eg: Iodine solution (purple colour) the absorption maxima occurs at almost the same wavelength as in iodine vapour (5180 A°)

b) In case of polar solvents. a brownish colour is obtained instead of purple colour, because the absorption occurs at the shorter wavelength.

Colour change polarization of $I_2$ by the electric field of solvent dipole.




1. n-π* transition

a) This band undergoes blue shift "hypsochromic shift" since. the ground state with 2 electrons receives greater stabilization than excited state with only one electron.



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2. $\pi - \pi^*$ transition

a) As solvent polarity is increased this band undergoes red shift.

b) This is so. since excited state is more polar than the ground and hence stabilization is greater relative to ground state in polar solvents.

c) The transition of Polar bands like C=O but not ethylene, are affected by solvent polarity.

2. Purity of solvent

Purified and certified solvents for spectroscopy should be used as we are looking for the 'smooth' absorbance curve of solvent.

3. Resolution and interpretation of Spectrum problems

These are resulted when solvents is used for measurement of near/below it's UV cut off. i.e. approximate wavelength below which they cannot be used because of absorption

4. Dipole moments

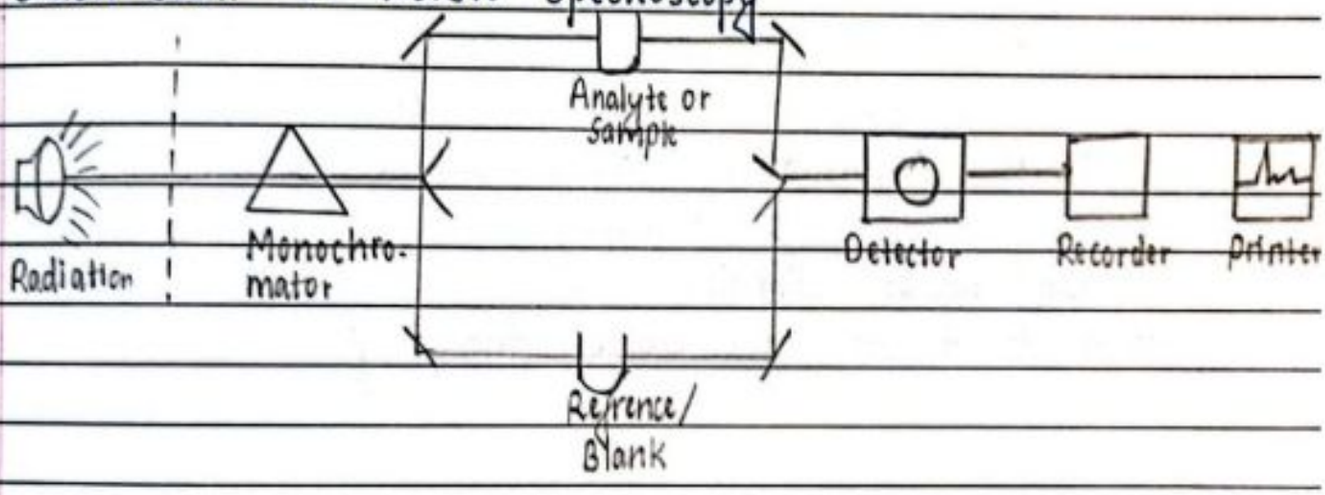
Absorption bands of many substance are relatively sharper and may also exhibit fine structure when measured in solvents of low dipole moment.

* Only those compounds which contains chromophore can be analysed by the UV-visible spectroscopy. In other cases. Chemical transformation by adding chromophore to those compounds also can be analysed.

## Solvent cut off

The solvent cut off is the wavelength below which the solvent itself absorbs all of the light. So, when choosing a solvent be aware of it's absorbance cut off and where the compound under investigation is thought to absorb. If they are close, choose a different solvent and should select the wavelength which is not absorb by solvent and Run blank every time.

## Double beam UV-visible spectroscopy




## Advantages of double beam UV-visible spectroscopy

- Correction of absorbance for solvent blank.
- In single beam design there can be fluctuations in lamp energy between the ratio recording measurements.
- Correction due to fluctuations in stray light, beam intensity variations and electronic noise are applied in real-time.
- High stability because reference and sample are measured virtually at the same moment in time.

## Disadvantages

- Higher cost, lower sensitivity because throughput of light is poorer because of the more complex optics and lower reliability because of greater complexity.



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## Applications of UV- visible spectroscopy

1. To check the purity of compound
2. To determine the concentration of unknown solution.
3. To determine the progress of Reaction
4. To check the authenticity of the product
5. It helps in the structural illucidation of the new compound.
6. It is used for determination of extent of conjugation.

## limitation's

1. It only gives the information about the electrons i.e. limited information compared to IR
2. low sensitivity and selectivity
3. Need regular calibration
4. It dosen't passes the enough details for analysis.

## Calibration of UV- Visible spectrophotometer

1. Control of wavelength

- Dissolve 1 gm of holmium oxide in 1.4M perchloric acid with the aid of heating on water bath and dilute to 25ml with same solvent
- Record the spectrum holmium perchlorate solution from 200nm to 600nm using 1.4M perchloric acid as refrence solution.
- Note down the maxima observed at wavelength against the acceptance criteria given below:.

| S.N | Maximum wavelength (nm) | Tolerance (nm) |
|---|---|---|
| 1 | 241.15 | 240.15 - 242.15 |
| 2 | 287.15 | 286.15 - 288.15 |
| 3 | 361.5 | 360.50 - 362.50 |
| 4 | 536.3 | 533.30 - 539.30 |



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2) Control of Absorbance

Step 1:- Weight 57 to 63 mg of potassium dichromate primary standard and transfer to 100ml volumetric flask. Dissolve in 0.005m $H_2SO_4$ and make up to mark with same acid

Step 2:- Measure the absorbance at 235 nm, 257nm, 313nm and 350nm using 0.005M sulfuric acid as refrence.

Calculation:- Value of A (1%, 1cm)

$$A(1\%, 1cm) = \frac{\text{absorbance} \times 100}{\text{weight in gm} \times 100}$$

| S.N | Wavelength (nm) | Absorbance E (1%, 1cm) | Maximum tolerance |
|---|---|---|---|
| 1 | 235 | 124.5 | 122.9 to 126.2 |
| 2 | 257 | 144.0 | 142.8 to 145.7 |
| 3 | 313 | 48.6 | 47.0 to 50.3 |
| 4 | 350 | 106.6 | 104.9 to 108.2 |
| 5 | 430 | 15.9 | 15.7 to 16.1 |

3) Limit of Stray light

Step 1:- Prepare the solution 1.2% V/v of KCl and dissolved with 50ml of Distilled water.

Step 2:- Determine the absorbance using path length of 1cm at 200nm against purified water as blank

4) Resolution powder

Step 1:- prepare a solution 0.02% V/v toulene in hexane

Step 2:- Record the spectrum of 0.02% V/v toulene in hexane from 250nm using hexane as refrence

Step 3:- Record the absorbance at 269 nm (max) and 266 (min)

Step 4:- Calculate the ratio of absorbance by dividing the absorbance at maxima and minima.

Acceptance criteria:- Absorbance ratio at 269 nm to 266nm is not less than 1.5.



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# Infrared spectroscopy

Date 23 Dec
Page 28

IR spectroscopy is the study of interaction between infrared radiations and matter.
IR is measured in wavenumbers
It is based on absorption spectroscopy.
Wave number is the number of waves per cm. and is represented by $\bar{\nu}$.
It starts from:- 800nm

$800 \times 10^{-9}$ m

$800 \times 10^{-9} \times 100$ cm

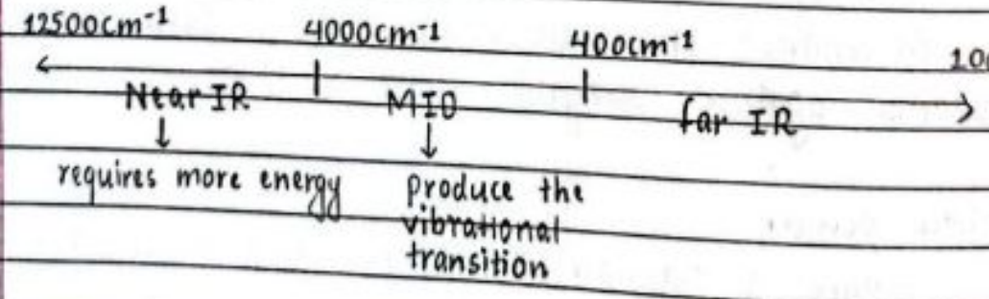
$8 \times 10^{-5}$ cm

Hen. $\bar{\nu} = \frac{1}{\lambda(cm)}$

$= \frac{1}{8 \times 10^{-5} cm}$

$= 12.500$ waves

Range of IR is $12.500\ cm^{-1}$ to $10^{-1}$ cm




## Principle

- The principle of IR spectroccopy is related to the vibrational and rotational energy of a molecule.
- When. the frequency of the IR radiation is equal to the natural frequency, the molecules absorbs the IR radiation.



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- Absorption of IR radiation causes an excitation of molecule from a lower to the higher vibrational level.
- Each vibrational level is associated with a number of closely placed rotational level.
- Therefore the IR spectroscopy is also called 'vibrational-rotational spectroscopy'.
- All the bonds in a molecule are not capable of absorbing IR energy but those bonds which are accompanied by a change in dipole moment will absorb in the IR region and such transition are called IR active transitions.
- The transitions which are not accompanied by a change in dipole moment of the molecule are not directly observed and are considered as IR active.
- In IR spectroscopy the changes in the vibrational energy depends on :-
  i) Mass of the atoms present in a molecule
  ii) Strength of the bonds
  iii) Arrangement of atoms within the molecules.
- No two compounds except the enantiomers can have the similar IR spectra.

## Theory

- When a molecule absorb radiation with a frequency less than 100 $cm^{-1}$. molecular rotation takes and if a molecule absorbs more energetic radiation in the region of $10^4$ to $10^2$ $cm^{-1}$. molecular vibration takes plate.
- A single vibrational energy change is accompanied by a large number of rotational energy changes and thus the vibrational spectra appears as vibrational rotational bands.

## Fate of absorbed Radiation

- There are 3 main process by which a molecule can absorb radiation. each of these route involve an increase of energy which is proportional to the light absorbed.

i) First route occurs when absorption of radiation leads to a higher rotational energy level in a rotational transition

ii) Second occurs when absorption of radiation leads to a higher vibrational energy level in a vibrational transition.

iii) Third occurs when absorption of radiation leads to a higher electronic energy level in it's electronic transitions.

## Applicable for:-

- Only to those molecules which has either permanent dipole moment or dipole moment can be induced by vibration
- Polar component:- has dipole moment
  Eg:- acetone. alcohol. Chloroform. HCl. HF. water
- A polar component:- dipole moment can be induced by the vibrational transition.
  Eg:- $CCl_4$. $CO_2$. methane

## Homonuclear diatomic molecules

They donot have either permanent dipole moment or the dipole moment cannot be induced by vibration.
Eg:- H-H ($H_2$). N≡N ($N_2$) and cannot be studied by IR

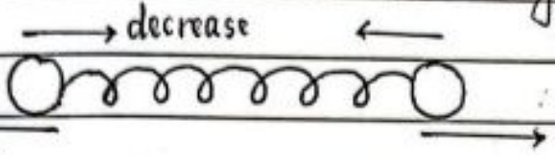
## Vibrational modes in IR

They are of two types:-

1) Stretching vibration
2) Bending vibration

1) Stretching vibration

Stretching vibration are those vibration where there is either increase or decrease in bond lengths takes place during vibration.

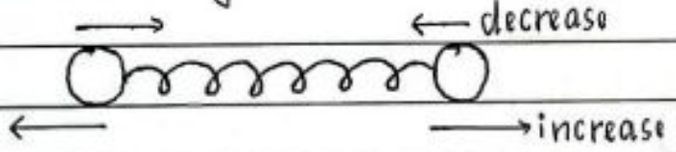



It can be further classified in two types:-

i) Symetrical stretching
ii) Asymmetrical or Unsymmetrical stretching

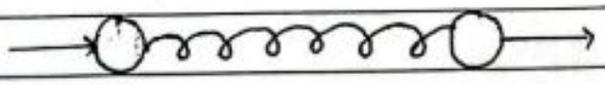
i) Symmetrical Stretching

In these either compress or stretching takes place from both side simultaneously.




ii) Asymmetrical stretching

In these compress from one side. whreas stretch from other side.



2) Bending vibration

Bending vibration are those vibrational modes in which there is either increase or decrease in bond angle takes place during vibration.

It can be of two types:-

i) In-Plane bending
ii) Out-Plane bending



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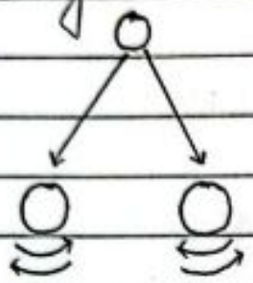
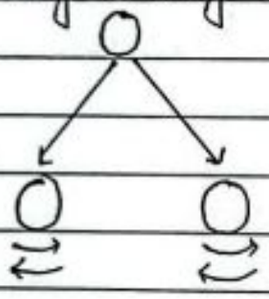
1) In-Plate bending
If the bending takes place within the plane is called in-plane bending.
It can be of two types:-

a) Scissoring bending :- when 2 atoms move away or close towards each other.

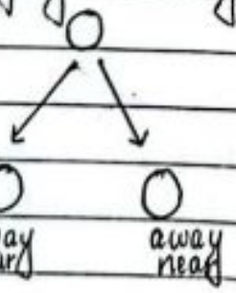


b) Rocking bending :- change in angle between a group of atoms.

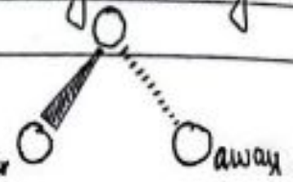


2) Out-Plane bending
If the bending take place outside the plane is called out-Plane bending.
It can be of 2 types:-

a) Wagging bending :- Change in angle between the plane of a group of atom




b) Twisting bending :- change in angle between the plane of 2 groups of atom.



Calculation of vibrational modes in the molecules

Total degree of freedom (TDF) = 3×N (no of atom in molecules)

TDF is the sum of the transitional degree of freedom, vibrational degree of freedom and rotational degree of freedom.

. Transitional degree of freedom is always 3.

. Rotational degree of freedom is 2 for linear molecules and 3 for non-linear molecules

$3N = T+V+R$

$3N = 3+V+2$ (for linear)

$V = 3N-5$

$3N = T+V+R$

$3N = 3+V+3$ (Non-linear)

$V = 3N-6$

Q. Find out the vibrational degree of water ($H_2O$)

$V = 3N-6$

$= 3\times3-6$ where, $N=3$. Since $H_2O$ is non-linear

$= 9-6$

$= 3$

Q. Find out the vibrational degree of freedom of $CO_2$

$CO_2$ is linear and here. $N=3$

Then. $V = 3N-5$

$= 3\times3-5$

$= 9-5$

$= 4$

Q. Find the vibrational degree of freedom of HCl

HCl is linear and here $N=3$

Then. $V = 3N-5$

$= 3\times2-5$

$= 6-5$

$= 1$



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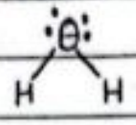
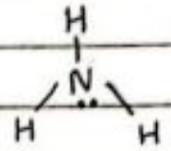
For $H_2O$



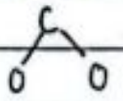
no. of bond of central atom = 2
lone pair = 2
Then. 2+2 = 4 i.e. $sp^3$ hybridization
Hence. $H_2O$ is non-linear

For $NH_3$



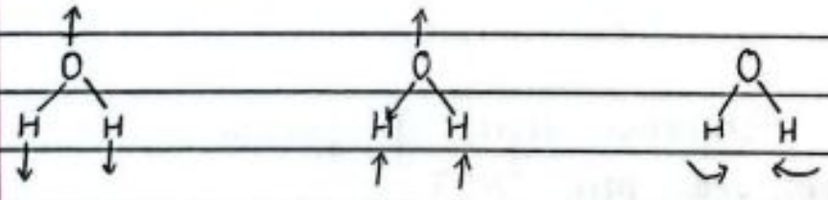
No. of bond of central atom = 3
lone pair = 1
Then 3+1 = 4 i.e. $sp^3$ hybridized
Hence. $NH_3$ is non-linear

For $CO_2$



No. of bond in central atom = 2
lone pair = 0
Then $CO_2$ is SP hybridized and thus linear

Vibrational modes in $H_2O$ molecules



i) symmetrical stretching
ii) Asymmetrical stretching
iii) scissoring

Vibrational modes in $CO_2$ molecules

$\leftarrow O=C=O \rightarrow$
i) Symmetrical stretching

$\rightarrow O=C=O \rightarrow$
ii) Asymmetrical stretching

$\curvearrowright O=C=O \curvearrowleft$
iii) Scissoring in one plane

$\circlearrowleft O=C=O \circlearrowright$
iv) scissoring in other plane

## Sample prepration in IR spectroscopy

Unlike UV-visible spectroscopy, where the sample are analysed only in liquid phase (in the form of solution). In IR spectroscopy sample can be analysed in all phases of matters like in solid, liquid and gas.

Traditionally sample are prepared by following methods

1) For gaseous sample

Pure gaseous sample is filled in 10 cm gaseous sample tube and is subjected for analysis.

Gaseous sample tube must be inert and transparent. It should be inert because it should not react with sample and it should be transparent because it should not absorb the IR radiation.

2) For liquid sample

There are 2 methods for liquid sample.

a) sandwitching

b) Using liquid sample cell

a) Sandwitching

Pure liquid sample is sandwitched between two NaCl, KBr, or $CaF_2$ plates. The liquid which can dissolved these plates are sandwitched between AgCl plates.

b) Using liquid sample cell

In liquid sample cell the pure liquid sample is filled inside the liquid sample cell and subjected for analysis.

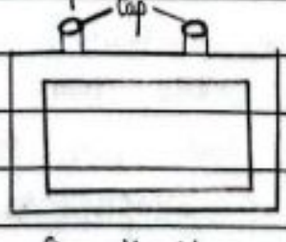



fig:- liquid sample cell



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3) For solid sample

There are 4 methods for solid sample:-

a) Mulling technique
b) Pellete technique
c) Cast-film technique
d) Microtomy

a) Mulling technique

In this method, the pure solid sample is mixed with the mulling agent (nujol, high B.P petroleum) and mixed in mortar with the aid of pestle, the paste formed is sandwitched between NaCl, KBr or $CaF_2$ and subjected for analysis

b) Pellete technique

In this method pure solid sample is mixed with anhydrous KBr in the ratio 1:100 and is grinded in mortar with the aid of pestle, fine powder formed is then pressed in metallic die with high pressure (at about 10 ton) in vacuum. Thin pellete formed is scrapped and put in sample holder and subjected for analysis.

* anhydrous KBr is prepraed by drying the hydrated KBr in oven at 120°C for half hour.

c) Cast-film technique

This technique is mainly applicable for polymers which are difficult to grind and soluble in volatile solvents. The polymer sample is dissolved in suitable volatile solvents. The solution is sprayed over the NaCl plates. After sometimes volatile solvent is evapourated and thin film is formed which is then casted off and put in sample holder and subjected for analysis.



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d) Microtomy

This techniques is only suitable for polymer, which is difficult to grind mainly rubbers. In this technique, a thin section of the sample is cut by using microtome and subjected to analysis.

Modern techniques

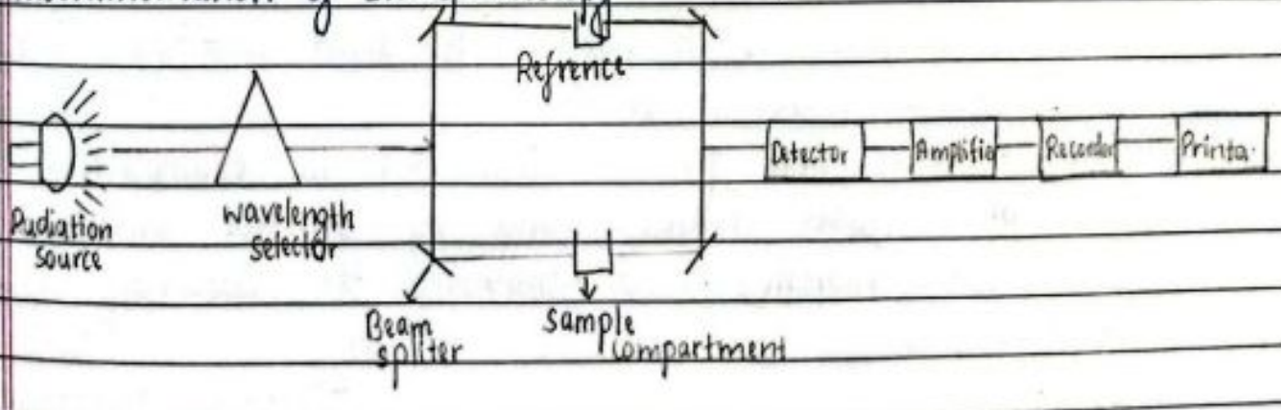
1) DRIFTS (Diffuse. Reflactance. Infrared faurrier transform spectroscopy

- It is mainly for solid sample
- Less sample prepration
- Sample is directly mixed with the anhydrous KBr and subjected for analysis

2) ATR (Attenuted total reflactance)

- It is modern technique and hence. less sample prepration
- It is applicable for both solid and liquid
- Sample is directly put in ATR and subjected for analysis.

Instrumentation of IR spectroscopy




a) Radiation source

Criteria for the radiation source

- It must be continous
- It must provide the radiation of required range
- It must be stable i.e. no fluctuation



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Radiation source can be of 3 types:-

i) Nernst glower

In Nernst glower the evacuated tube is made up of the rare earth metal oxides ( $ZrO_2$, $Y_2O_3$ and thorium oxide ). The tube is of 2mm diameter and 2cm length. Platinum wire is fixed at the 2 sides of tube. when it is heated to 2000 to 2200 Kelvin it emits the IR radiation.

ii) Globar source

The globar source is made up of silicone carbide rod. i.e. 6-8 mm diameter and 5cm length. when it is heated at 1500 Kelvin it emits the IR radiation. The spectral output is comparable with the Nernst glower except at short wavelenghths (less than 5mm) where it's output become larger.

iii) Incandescent

It is made up of Nichrome wire and when heated at 1100 Kelvin it emits the IR radiation.

b) Wavelenghth selector

The main aim is to select the desire wavelenght (i.e. line source) from band source. It can be:-

i) filters:- Glass and gelatin filter are the example of absorption filters which works by selective absorption of unwanted radiation and transmit the radiation which is required.

ii) Prism:- Prism is made from glass, Quartz or fused silica. When white light is passed through glass prism, dispersion of the polychromatic light will occurs. Then by rotation of prism different wavelength of the spectrum can be made to pass

in exit slit on the sample. Narrower slit enables the instrument to distinguish more closely spaced frequencies of radiation and result in better resolution. Wider slit allows more light to reach the detector and provide better systemic sensitivity

iii) Gratings:- Gratings give higher and linear dispersions as compared to prism monochromator. Gratings can be constructed with the materials like alumunium which is resistant to atmospheric moisture. Diffraction gratings consist of large number of parallel lines of 15000 - 30000 per inch or highly polished surface of aluminium.

c) Beam splitters

It splits the radiation light into the two equal halves one goes through the refrence and another through the sample compartment.

d) Sample compartment

We can used the various method for the sample prepration. we can used traditional method or the modern methods such as DRIFTS or ATR.

e) Detector

The main aim of the detector is to detect the range and intensity of light absorbed by analyte. There are different types of detector and there are 3 most common detectors:-

i) Thermal detector

ii) Pyroelectric detector

iii) Photoconducting detector

i) Thermal detector

It consist of thermocouple made up of Bismuth and cadmium junction at different temperature (cold and hot junction). The potential differena (voltage) between the junction changes according to the difference in temperature between the junction IR radiation generally falls on the hot junction. The potential difference between the junction due to the change in temperature consist the flow of current proportional to intensity of IR radiation.

ii) Pyroelectric detector

It consist of the pyroelectric substances i.e. Triglycerin sulphate which coats the electrode. and acts as wafer. When the radiation falls on the pyroelectric substances. the electrodes acts as the capicitor and the capicitance depends on the range and intensity of radiation. It mainly used in FIIR instrume

iii) Photoconducting detector

It is the most commonly used detectors. and is made up of photoconductive substance such as Mercury, cadmium. Telloride. When the light or radiation source falls on these substance they release electrons and emits the conductance. Conductivity depends on the light source falls on it. It has fast response time These detectors have better response than pyroelectric detectors.

Q. How to interpret spectrum in IR spectroscopy

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



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Where. $\bar{\nu}$ = wave number

c = velocity of light

f = force constant i.e. for single bond = $5.0 \times 10^5$ dyne $cm^{-1}$

for double bond (x2) = $10.\times 10^5$ dyne $cm^{-1}$

for triple bond (x3) = $15\times 10^5$ dyne $cm^{-1}$

$m_1$ and $m_2$ are atomic masses of elements between which bond exists.

For C-H

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

$$= \frac{1}{2\times 3.14\times 3\times 10^{10}}\sqrt{\frac{5.0\times 10^5}{\frac{12\times 1}{(6.023\times 10^{23})\,\cancel{2}}}}$$

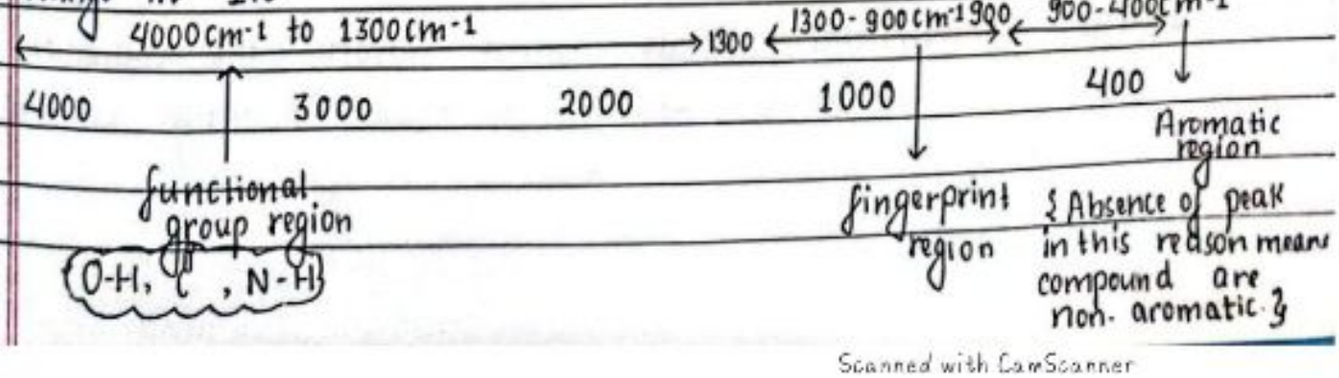
$$= 5.30\times 10^{-12}\sqrt{\frac{5.0\times 10^5}{\frac{12}{6.023\times 10^{23}}}}$$

$$= 5.30\times 10^{-12}\sqrt{\frac{5.0\times 10^5}{1.532\times 10^{-24}}}$$

$$= 5.30\times 10^{-12}\times 5.711\times 10^{14}$$

$$= 3027.31 \approx 3027$$

Range in IR

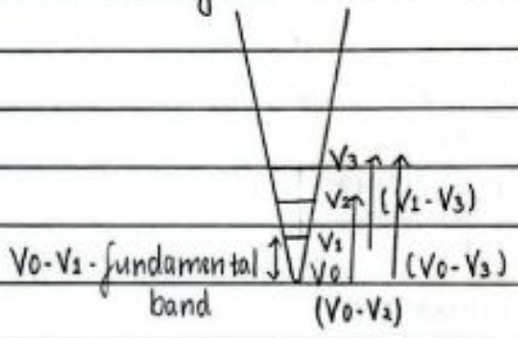


Reasons for showing more peaks in IR then calculated vibrational modes:-

- Overtones
- combination tones
- Fermi resonance

a) Overtones

These tones are appeared on 2nd and 3rd multiple of fundamental bands (i.e. $V_0 - V_1$). These may be due to exicitation from $V_0 - V_2$, $V_0 - V_3$, $V_1 - V_3$, $V_0 - V_3$.




b) combination tones

These tones appears due to the interaction of two fundamental bands.

c) Fermi Resonance

These peaks appeared due to the interaction of fundamental bands with overtones or combination tones.

Reasons for showing less peaks in IR then calculated vibrational modes:-

a) The fundamental bands which absorbs the radiation having wavenumber outside the MID IR range (i.e. 4000 $cm^{-1}$ to 400 $cm^{-1}$)



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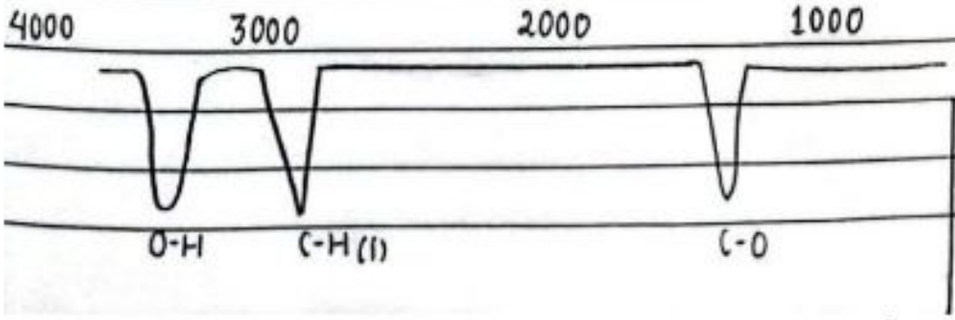
If the two vibrational modes absorbs the radiation of same wave number then they collise with each other.

If the two fundamental bands are too close to each other, they merge with each other.

Some fundamental vibration are very weak that they are unpredictable or difficult to detect.

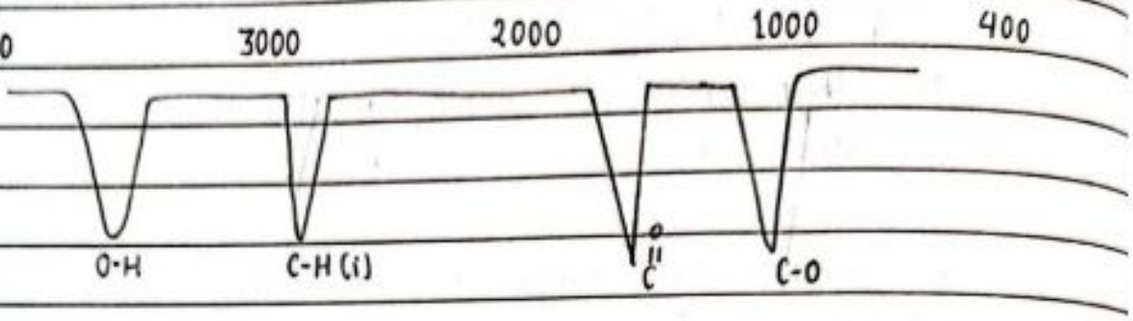
| Bond | functional group | Region ($\bar{\nu}$) |
|---|---|---|
| C-H | Alkane | 3000 - 2850 $cm^{-1}$ |
| C-H | Alkene | 3100 - 3000 $cm^{-1}$ |
| C-H | Aromatic | 3080 - 3020 $cm^{-1}$ |
| C-H | Alkyne | 3300 - 3200 $cm^{-1}$ |
| O-H | Alcohol. phenol. acid | |
| | i) Intermolecular H-bonded | 3600 - 3200 $cm^{-1}$ (broad peak) |
| | ii) Non H-bond | 3640 - 3300 $cm^{-1}$ (strong peak) |
| O<br>C | aldehyde. Ketone. acids. halides. | 1760 - 1690 $cm^{-1}$ (strong peaks) |
| | esters. amide. | |
| C-O | Alcohol. acid. ether. ester | 1250 - 1000 $cm^{-1}$ |
| C=C | Alkene | 1600 - 1500 $cm^{-1}$ |
| C=C | Aromatic | 1580 - 1500 $cm^{-1}$ |
| C≡C | Alkynes | 2300 - 2150 $cm^{-1}$ |
| N-H | Amines, amides | 3500 - 3300 $cm^{-1}$ |
| | | |

For methanol - $CH_3OH$

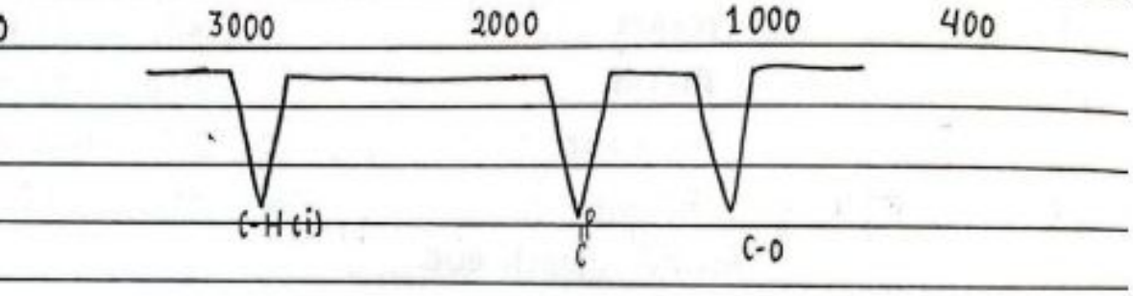



b. broad peak
m. medium peak
V. variable peak
S. Strong peak
W. weak peak

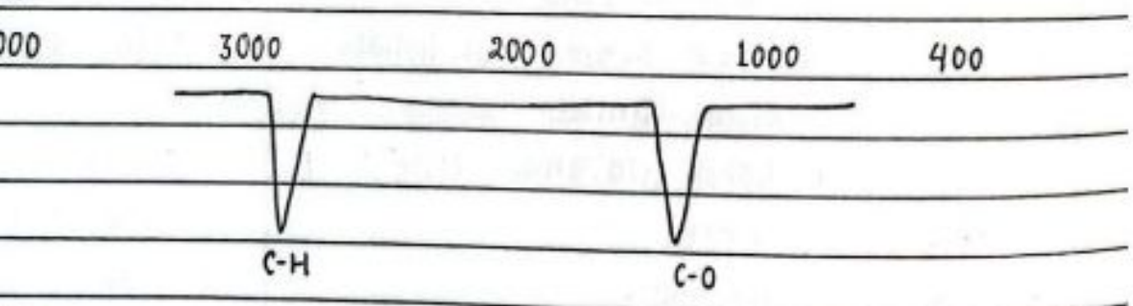
b) Acetic acid ($CH_3-\overset{O}{\overset{\|}{C}}-OH$)



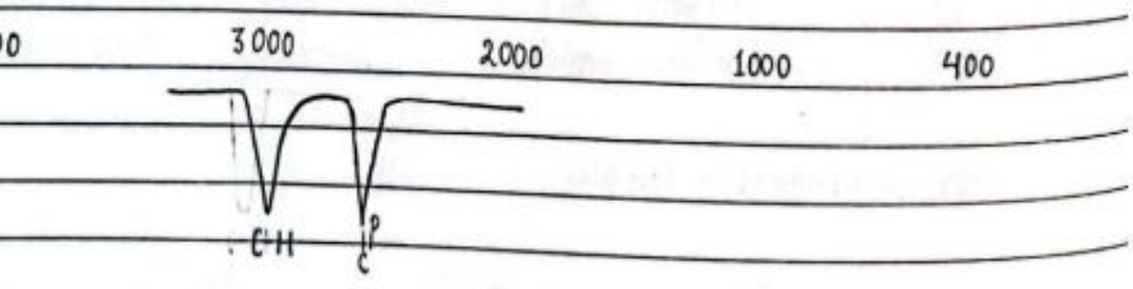

c) Ester ($CH_3-\overset{O}{\overset{\|}{C}}-O-CH_3$)



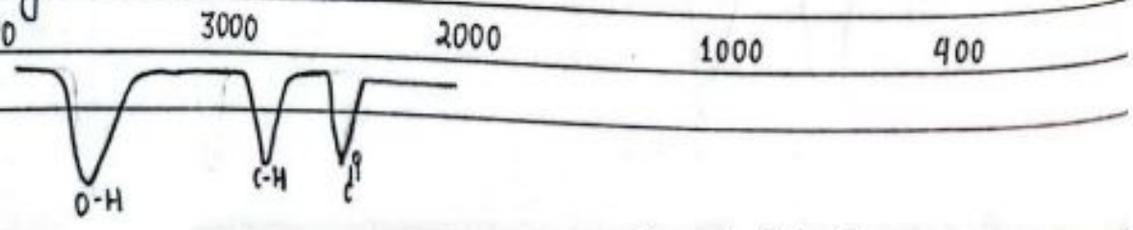

d) ether




e) Ketone




f) Aldehyde



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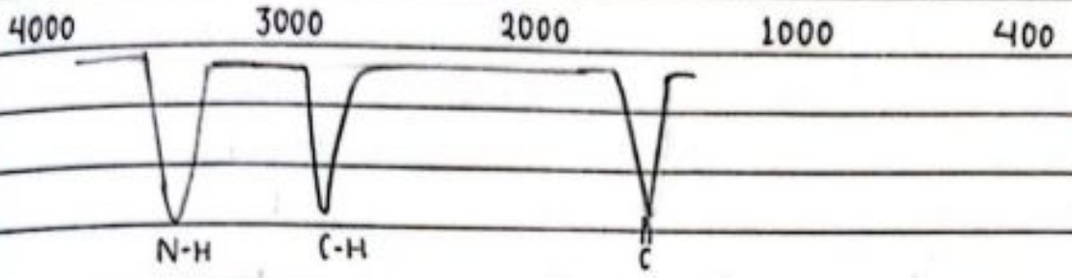
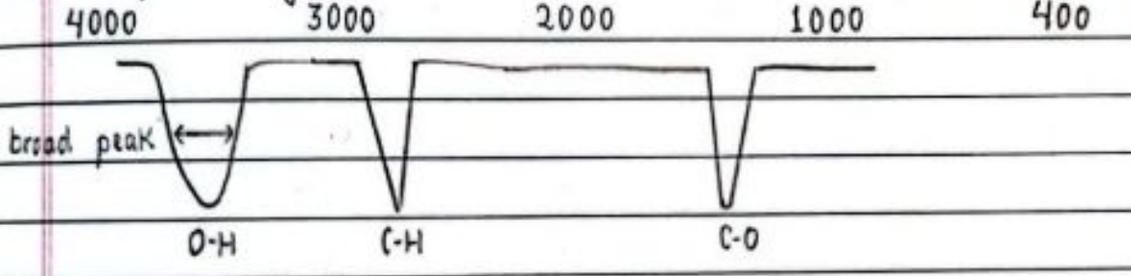
g) Amide

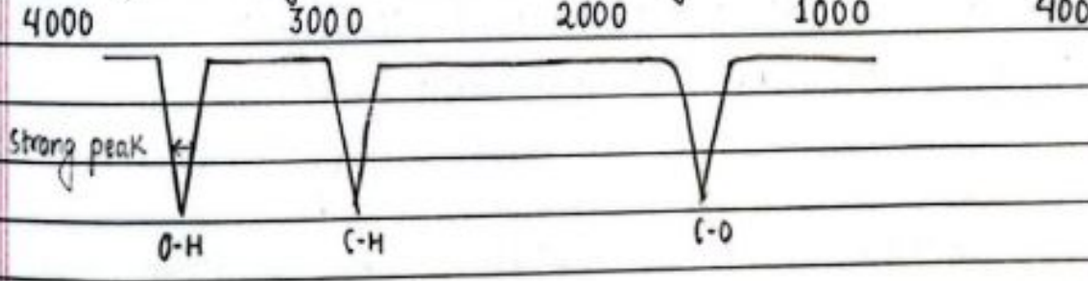



Q. Sketch the IR spectrum for neat alcohol and very dilute solution of alcohol in carbondisulfide and account for the difference in spectrum.

IR spectrum for neat alcohol




IR spectrum for dilute solution of alcohol in carbon disulfide ($CS_2$)




From the above IR spectrum the neat alcohol consist of the intermolecular H-bond, which falls in $3600cm^{-1}$ to $3200cm^{-1}$ that means it gives broad peak., which represent the presence of the exchangeable protons i.e. from alcohol means the greater hydrogen bonding but in dilute solution of alcohol in carbondisulfide consist of non H-bond. which falls in range of $3640cm^{-1}$ to $3300cm^{-1}$ that means it gives strong peak and has lesser hydrogen

bonding.

The sharpness or broadness of the strech in IR spectra depends on extent of hydrogen bonding present in the molecule. Basically, if it undergoes immense intermolecular hydrogen bonding. the peaks tends to be broader and lesser the hydrogen bonding becomes. the sharper the peaks get in the spectra.

Now. if we recall the criteria needed for stronger hydrogen bonding. the more electronegative the atom attach to hydrogen is the better extent of hydrogen bonding as the more electronegative atom will be able to create more δ+ve charge on hydrogen which is better for stronger hydrogen bond.

As we Know. $\bar{\nu} = \frac{1}{2\pi c}\sqrt{\frac{f}{m_1 m_2}}$

$\bar{\nu}$ = wave number
c = velocity of light
f = force constant

$m_1$ and $m_2$ are the atomic masses of elements between which bond exists.

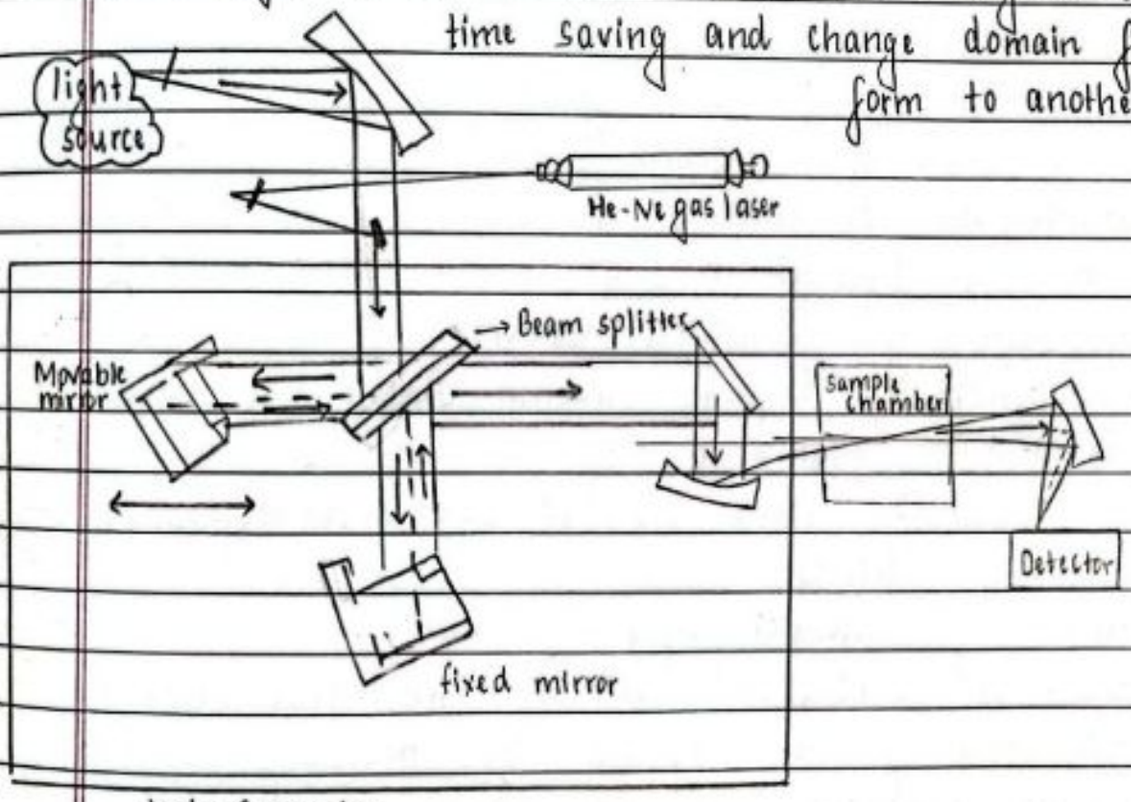
Greater the extent of H- bond, lower the strength of -OH bond i.e. less force constant and hence, less wave number.

Q. Why alcohol is a good solvent for UV- visible spectroscopy but not in IR spectroscopy?

As we Know the alcohol is polar solvent and has the ability to form hydrogen bond, it is inert and doesn't react with the analyte. it is transparent and doesn't adsorb the radiation in the region under the investigation i.e. 200 to 800nm. It has the ability to dissolved most of the analyte. It's UV- visible absorbance cutoff wavelength region is very low (205nm) i.e. all the UV- visible region is

free from cut off and hence widely used in UV-visible spectroscopy
The O-H bond in the alcohol adsorbs at a higher wavenumbers, since it itself adsorbs the radiation in IR and it is actually difficult for the interpretation of the result. Hence, alcohol is the good solvent for UV-visible spectroscopy but not in IR spectroscopy.

Fourier transform Infrared spectroscopy (FTIR)
It is useful because it can scan the whole range at once and is time saving and change domain from one form to another form.




Interferometer
The source energy strikes the beamsplitter and produces two beams of roughly the same intensity. One beam strikes the fixed mirror and returns to the beam splitter. The other beam goes to the moving mirror. The motion of the moving mirror makes the total pathlength variable versus that taken by the fixed mirror beam. When these two beam meet up again at the beam splitter, they recombine, and the difference in their pathlengths create constructive and destructive

interference. an Interferogram.

The recombined beam passes through the sample. The sample absorbs all the wavelengths characterstics of the its spectrum and then substracts specific wavelength from the interferogram. The detector now reports variation in energy-versus-time for all wavelengths simultaneously. A laser beam is superimposed to provide a refrence for the operation of the instrument.

## Applications of IR

1. For detection of functional group
2. Characterization of compound
3. Authentication or checking of purity.
4. Detection of hydrogen bonding
5. Identification of geometrical isomers
6. Determination of polymer structure, plastic and resins
7. It is used as qualitative test.
8. When combined with GC-FTIR (Gas-chromatography) can be used as detector.
9. Detection of molecular impurities.
10. Analysis of formulation such as insecticide and copolymers
11. Identification of the compounds by matching spectrum of unknown compound with the refrence spectrum (fingerprinting)

# Nuclear Magnetic Resonance (NMR)

Date 5 Jan
Page 40

NMR utilizes the radiowave i.e. change in the nuclear spin.
There are two types of mass number

1) Mass number even
2) Mass number odd

1) Nucleus mass no. ever

a) even

Proton (even) Neutron (even)

Examples:- $^{12}C$, $^{24}Mg$, $^{16}O$, Then, the value of $I=0$

b) even

Proton (odd) Neutron (odd)

Examples:- $^{14}N$. $^{26}Al$, Then, the value of $I = 1, 2, 3, 4$

2) Nucleus mass number odd

a) odd

Proton (even) Neutron (odd)

Examples:- $^{1}H$, $^{13}C$, $^{15}N$, $^{19}F$, $^{29}Si$, $^{31}P$ Then. value of $I = 1/2, 3/2, 5/2, 7/2$

Total spin state of a nucleus = $2I+1$
Only those nucleus which have total spin state 2 are suitable for the NMR analysis.

Only those nucleus which have the value of $I = 1/2$ are suitable for NMR analysis and these are $^{1}H$, $^{13}C$, $^{15}N$, $^{19}F$. Among these nuclei, which are mostly used for NMR analysis are $^{1}H$ and $^{30}C$

i.e. $^{1}H$ - NMR
$^{13}C$ - NMR

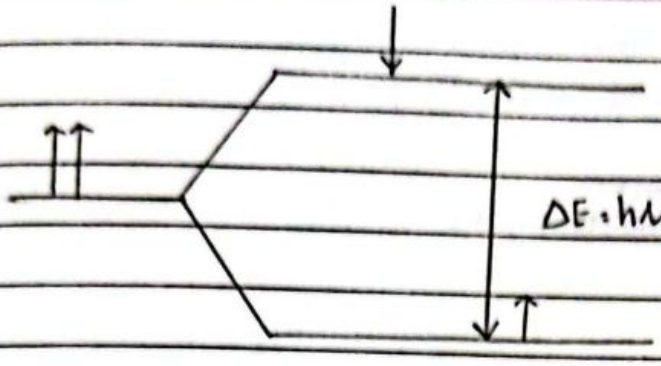
Nuclear magnetic Resonance spectroscopy is the study of spin changes at the nuclear level when a radio frequency energy is absorbed in the presence of magnetic field. When proton is studied then it is called proton NMR. Nuclei with odd mass number only give NMR spectra such as $^{1}H$, $^{13}C$ and $^{19}F$. because they have symmetrical charge distribution. Spin quantum number for such nuclei will be $1/2$, $3/2$ and $5/2$ respectively. Similarly, nuclei $^{2}H$, $^{12}C$, $^{16}S$ and $^{14}N$ do not give NMR spectra because of symmetrical charge distribution and their quantum number is an integral value.

## Principle of NMR

Those nuclei which have the value of $I = 1/2$ i.e. total spin state is equal to 2. In ordinary condition, they have zig-zag orientation and net magnetic moment is zero but when external magnetic field is applied their degeneracy is resolved and they have only 2 orientation either alligned with the external magnetic field (parallel orientation) or opposite to the external magnetic field (anti-parallel orientation) Nucleus which has parallel orientation are said to be ground state and they have low energy and the nucleus which have anti-parrallel orientation are said to be excited state and they contain more energy.

If the nucleus is irradiated with the radio frequency which has the similar energy as the gap between 2 states, then the nucleus started to resonate and the radio-frequency is absorbed.

The range of radio-frequency absorbed provide us the information about the nature of nucleus or atoms.



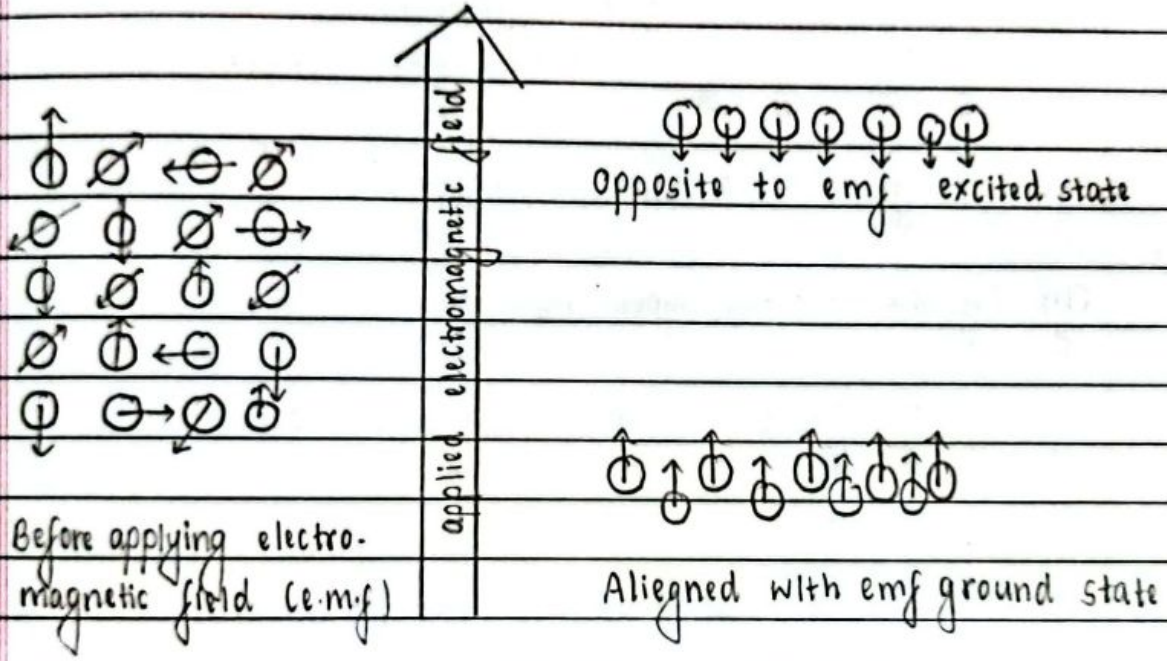
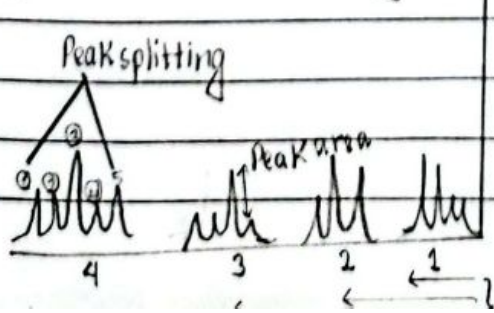




fig:- Principle of NMR

**Information that can be obtained for $^1$H-NMR**

1) Types of hydrogen → No. of signals
2) Environment of hydrogen → Chemical shift
3) Number of hydrogen of each type → Peak area/ Intensity
4) Environment with respect to neighbouring hydrogen / Bond-connectivity / Spin-Spin coupling → Splitting



## Larmor frequency relation

$\mu = \gamma B$ → Applied magnetic field

$\mu$ → larmor frequency

$\gamma$ → gyromagnetic constant

## 17 Types of hydrogen

$CH_3 - CH_2 - CH_3$ → Two types hydrogen

$CH_3 - CH_2 - CH_2 - CH_3$ → Two types hydrogen

$CH_3 - CH_2 - CH_2 - CH_2 - CH_3$ → Three types hydrogen

(a) (b) (c) (b) (a)

$CH_3 - CH(Cl) - CH_3$ → Two types hydrogen

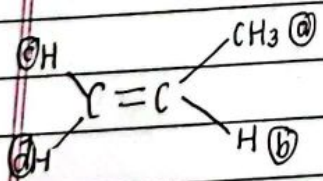
(a) (b) (a)

$CH_3 - CH_2 - Cl$ → Two types hydrogen

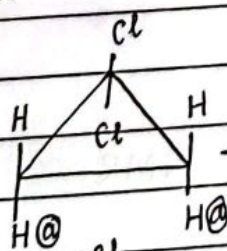
(a) (b)

$CH_3 - CH_2 - CH_2 - CH_2 - Cl$ → four types hydrogen

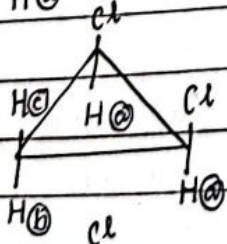
(a) (b) (c) (d)



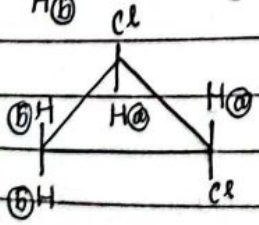

→ four types hydrogen



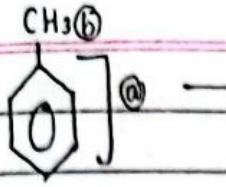

→ One type hydrogen



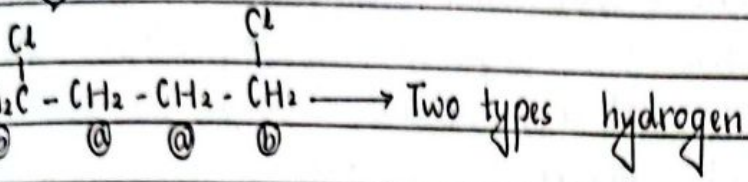

→ Three types hydrogen




→ Two types hydrogen



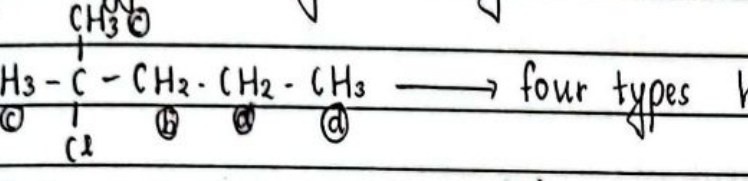

⟶ Two types hydrogen




⟶ Two types hydrogen

## Heterotropic hydrogen

If the hydrogen can be detected just by visualization and they give different signals they are heterotropic hydrogen.

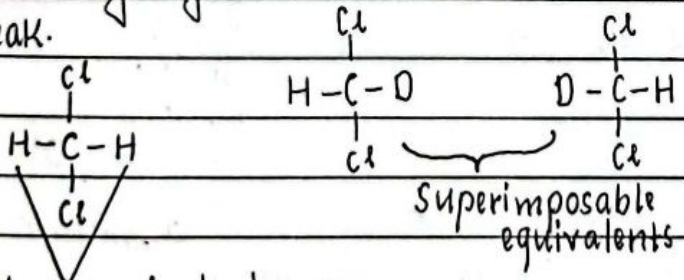



⟶ four types hydrogen

⟶ If the carbon contains 3 hydrogen then these hydrogens are equivalent and always give same signal.

⟶ If the carbon contains 2 hydrogen then there may be 3 different cases:-

Case 1:- Carbon contain 2 hydrogen and 2 other similar groups, Then the hydrogen are said to be homotropic and give the same peak.

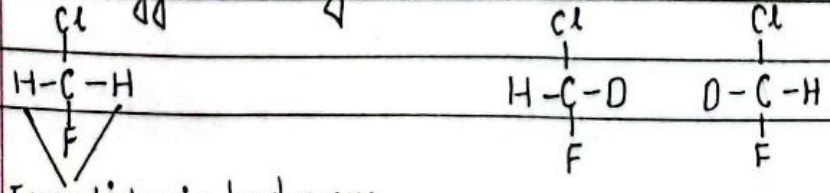



Homotropic hydrogens

↓

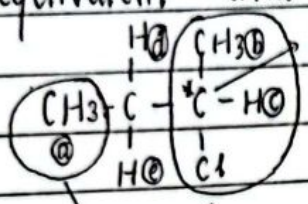
Give same peaks

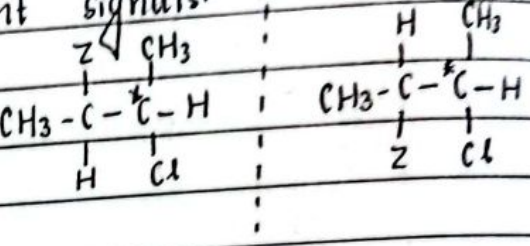
Case 2: If carbon contains two hydrogen and two different groups, the hydrogen are said to be enantiotropic hydrogens and give the same signals in ordinary conditions but in chiral condition give the different signals.




Enantiotropic hydrogens

**Case 3:-** If carbon contains 2 hydrogens and 2 different groups, among them one containing chiral center Then, the models constructed or prepared by replacing hydrogens with dummy are neither mirror image of each other So, they are called Diastropic hydrogens and they are not equivalent and gives different signals.

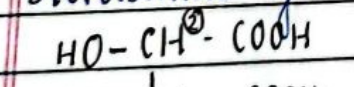


Diastropic hydrogens

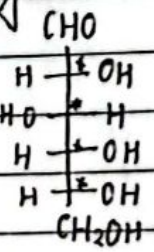
* Steroisomers which are not mirror image of each other are called diastomers. Diastomers have different physical properties like Boiling point, Melting point, solubility. Hence considered as the different compounds.

Steroisomers of tartaric acid

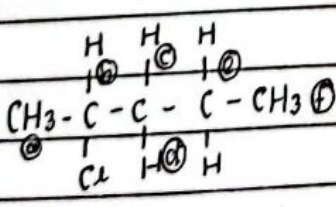


$2^n = 2^2 = 4$

For glucose

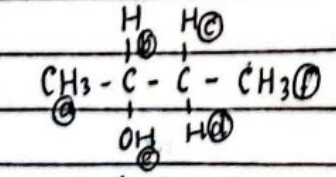


$2^n = 2^4 = 16$



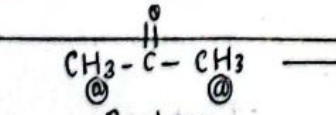
6 types of hydrogen

2. Chloro pentane



6 types of hydrogen

2- butanol



1 types of hydrogen

Acetone

2) Chemical shift

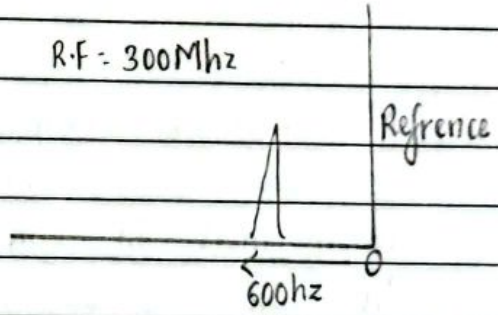
It is the shift of the peak from the refrence peak in NMR. It is denoted by $\mu$ and can be calculated as:

$$\mu = \frac{\text{Observed shift}}{\text{Applied R.F}} \times 10^6$$

$$\mu = \frac{\text{value of frequency of peak} - \text{value of frequeney of refrence}}{\text{Applied R.F}} \times 10^6$$

and the obtained value is called delta ($\delta$) value and is expressed in parts per million.

Q. Refrence value = 0 hertz
sample value = 600 hertz
Applied R.F = 300 Megahertz
Calculate the delta value.




Solution

$$\mu = \frac{600 - 0}{300\text{Mhr}} \times 10^6$$

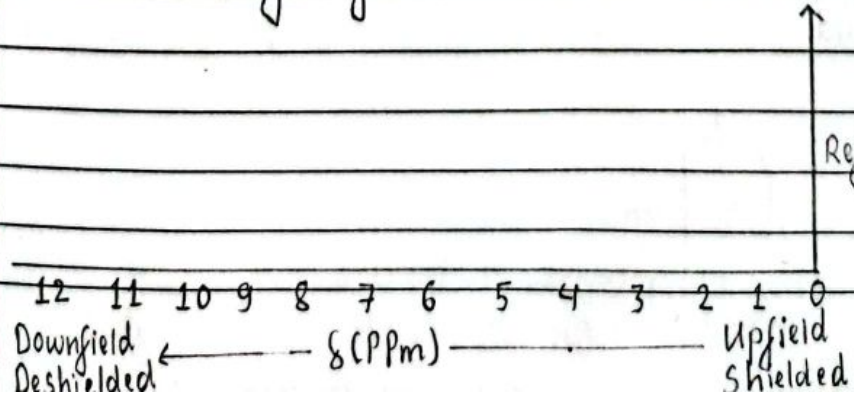
$$= \frac{600}{300 \times 10^6} \times 10^6$$

$$= 2 \text{ ppm}$$

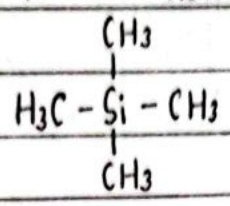
[TCTaw) = 10-$\delta$value]

NMR ranges from 60Mhz to 900Mhz

$\delta$- value is always constant for the particular types of nucleus (hydrogen), no matters what the radiofrequency is.
$\delta$- valde ranges from 0-12.



In NMR, TMS is used as refrence compound.



Q. Why TMS is used as refrence compound in NMR

TMS is used as refrence compound because:-

i) There are 12 equivalent hydrogen in TMS. so it gives single intense peak.

ii) It is inert. it dosen't reacts with analyte or solvent.

iii) It is highly volatile and hence easy to separate from analyte and solvent.

iv) In TMS, methyl groups are attached with more electropositive silicone, so electrodensity around methyl groups are much more in this compound than in other compound. In other words the hydrogens of TMS are highly shielded so must of the compounds are downfielded than TMS.

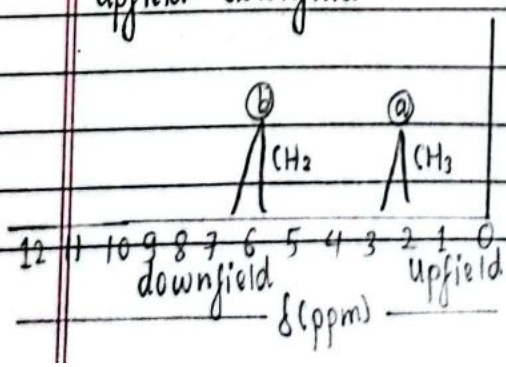
Factors affecting chemical shift

1) Electronegativity

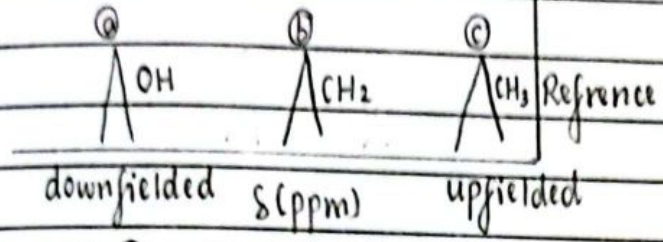
The chemical shift simply increases as the electronegativity of the attached element increases.

$CH_3 - CH_2 - Cl$ ⟶ 2 types of hydrogen

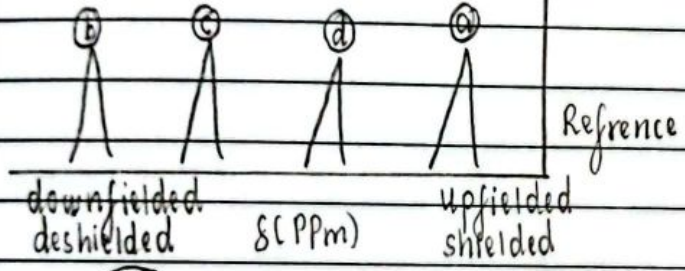
(a) upfield (b) downfield

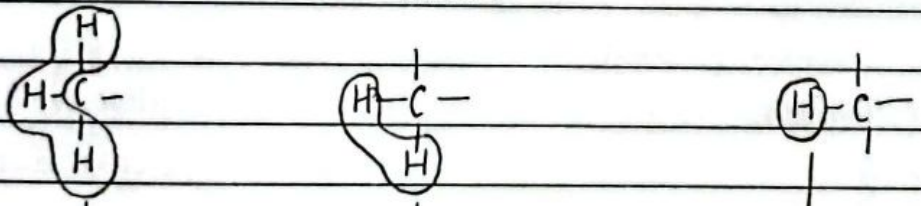


$CH_3 - CH_2 - OH$ (a, b, a) ⟶ Three types of hydrogen




$CH_3(a) - C(c) - CH_2(d) - CH_2(a)$ ⟶ 4 types of hydrogen
H(b)




methyl hydrogen — methylene hydrogen — Methyne hydrogen

downfielded ← methyl < methylene < Methyne → Upfielded

Alkane. $SP^3$ hybridization ⟶ 25% upfielded
Alkene. $SP^2$ hybridization ⟶ 33% upfielded
Alkyne. SP hybridization ⟶ 50% s-characters ⟶ consist of acidic hydrogen
↓
withdrawn electron
↓
downfield

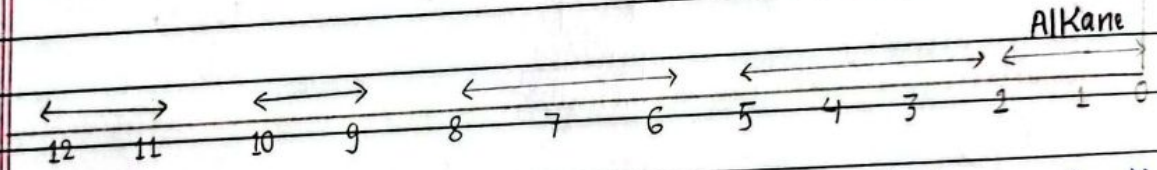
$SP^3$ . $SP^2$ . SP where. s character increases
↓
nearer the nucleus
↓
attract the electrons towards it

But. study shows. alkyne is upfield than Alkene.

2) Magnetic Anisotropy

The unequal feeling of external magnetic field by different part of same molecule due to induction of local magnetic field by revolvement of pi- electrons.

2-5 ⟶ halogenated compound
Alcohol, ether, Alkynes, benzylic hydrogens
6-8 ⟶ Alkenes, aromatic hydrogens
9-10 ⟶ Aldehyde
11-12 ⟶ carboxylic acid

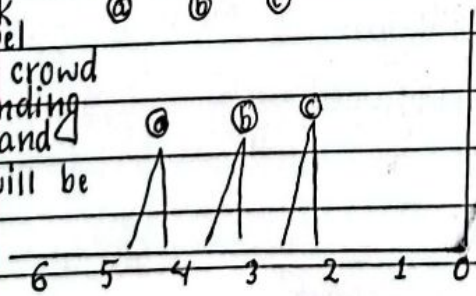



1- chloro propane

4) Vanderwaal's bonding

Electron cloud with the bulk group will repel the electron crowd that surrounding the protons and the protons will be deshielded

$C_1-CH_2-CH_2-CH_3$ ⟶ 3 types of hydrogen
(a) (b) (c)




3) Hydrogen Bonding

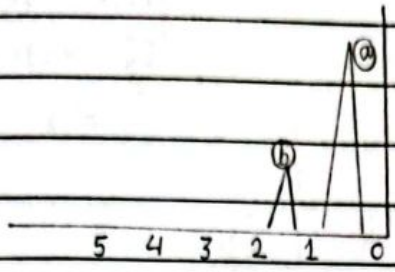
Hydrogen atom exhibit property of hydrogen bonding in a compound then it will get deshielded due to strong electronegative atom attached to it.

3) No. of hydrogen of each type ⟶ Peak Area / Intensity

Peak Integration

The intensity of signal of equivalent hydrogens depends on the number of hydrogens of that type. For eg:- In the compound 2-methyl propane gives 2 signal and the ratio of intensity of peak is 9:1.

$CH_3$@
|
$CH_3$ - CH - $CH_3$@ ⟶ 2 types of hydrogen
@ b



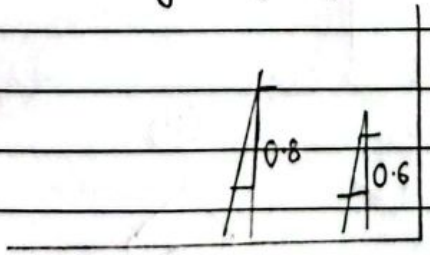

Several steps for calculation..

Step 1:- Measure the intensity of each peak and express the ratio.
For eg:- 5 types of hydrogen a:b:c:d:e

Step 2:- Divide each with the lowest value. If a is lowest then.
$a/a : b/a : c/a : d/a : e/a$

Step 3:- Multiply each value by the lowest whole number. So. that we get nearly whole number value for each peak.

Step 4:- If you know the molecular formula of the compound or the exact number of hydrogen. Then we can calculate the exact number of hydrogen of each type.




Calculate ratio?

0.6 : 0.8

or. $\frac{0.6}{0.6} : \frac{0.8}{0.6}$

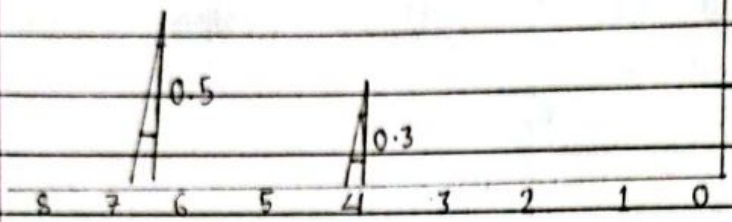
or, 1 : 1.33

or, 1×3 : 1.33×3

or, 3 : 4

Hence. the ratio is 3:4

*




Find out the structure from the given spectrum. If molecular formula is $C_7H_8$.

0.3 : 0.5

or, $\frac{0.3}{0.3} : \frac{0.5}{0.3}$

or, 1 : 1.66

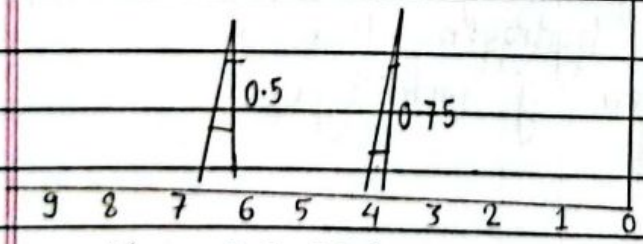
Multiply by 3, or, 1 × 3 : 1.66 × 3

or, 3 : 5


Toulene

*

Find out the structure from the given spectrum if the molecular formula is $C_8H_{10}$.



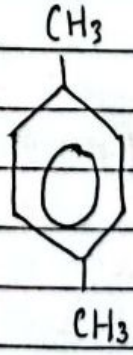

Now, 0.5 : 0.75

$\frac{0.5}{0.5} : \frac{0.75}{0.5}$

1 : 1.5

Multiply by 2 : 1 × 2 : 1.5 × 2

2 : 3



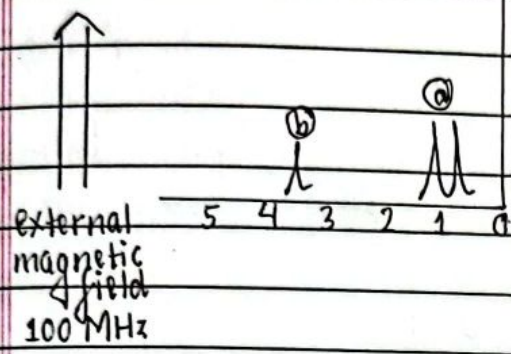
## Spin-Spin Splitting

4) **Spin-Spin Coupling** :- It is a magnetic coupling that causes the Proton to absorb slightly downfield when the external field is reinforced and slightly upfield when the external field is opposed.

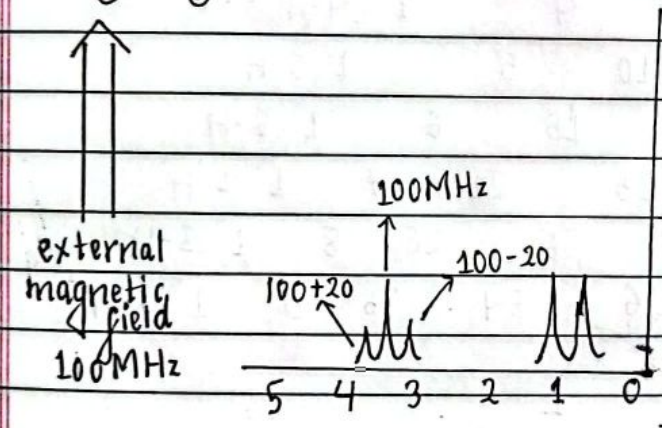
$CH_3-CH-Cl$
ⓐ ⓑ

orientation:- ↑ (+10Hz)
↓ (-10Hz)

→ 100MHz + 10 Hz
→ 100MHz - 10 Hz




$Cl-CH_2-CH_2Cl$
ⓐ ⓑ




Possible orientation:- ↑a ↑b
+10 +10 =+20
* ↑a ↓b
+10. -10 =0
* ↓a ↑b
+10 -10 =0
* ↓a ↓b
-10 -10 = -20

→ 100MHz +20
→ 100MHz
→ 100MHz -20

$CH_3-CHCl_2$
ⓐ ⓑ

| Possible orientation | | | |
|---|---|---|---|
| ↑↑↑ | | | 1 |
| ↑↑↓ | ↑↓↑ | ↓↑↑ | 3 |
| ↓↓↑ | ↓↑↓ | ↑↓↓ | 3 |
| ↓↓↓ | | | 1 |

Ratio for $CH_3$ = 1:3:3:1

**Spin-Spin coupling**
The splitting of the spectral lines into various signals due to interaction between two or more proton is called spin-spin coupling. The magnitude of the spin-coupling interaction between protons is decreases as the number of bonds between the coupled nuclei increases.

For $CH_4$

↑↑↑↑ 1

↑↑↓↓ ↑↓↑↓ ↑↓↓↑ 4

↓↓↑↑ ↓↑↓↑ ↓↑↑↓ 6

↑↑↑↓ ↑↑↓↑ ↑↓↑↑

↓↓↓↑ ↓↓↑↓ ↓↑↓↓ 4

↓↓↓ 1

Ratio 1:4:6:4:1

Formula (n-1)

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | | | | | 0-H |
| | | | | 1 | 1 | | | | 1-H |
| | | | 1 | 2 | 1 | | | | 2-H |
| | | 1 | 3 | 3 | 1 | | | | 3-H |
| | 1 | 4 | 6 | 4 | 1 | | | | 4-H |
| 1 | 5 | 10 | 10 | 5 | 1 | | | | 5-H |
| 1 | 6 | 15 | 20 | 15 | 6 | 1 | | | 6-H |
| 1 | 7 | 21 | 35 | 35 | 21 | 7 | 1 | | 7-H |
| 1 | 8 | 28 | 56 | 70 | 56 | 28 | 8 | 1 | 8-H |
| 1 | 9 | 36 | 84 | 126 | 126 | 84 | 36 | 9 | 1 9-H |

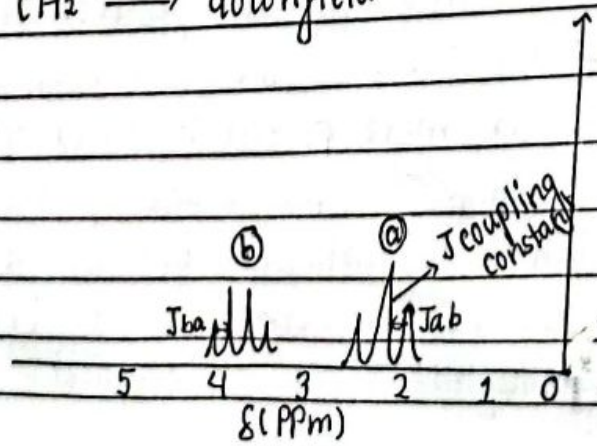
* Ethyl chloride

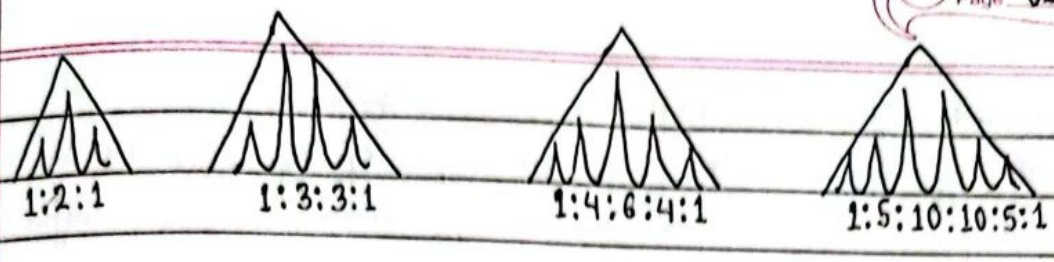
$CH_3 - CH_2 - Cl$ ⟶ 2 types of hydrogen ⟶ gives 2 types of signa

ⓐ ⓑ

$CH_3$ ⟶ Upfielde

$CH_2$ ⟶ downfield





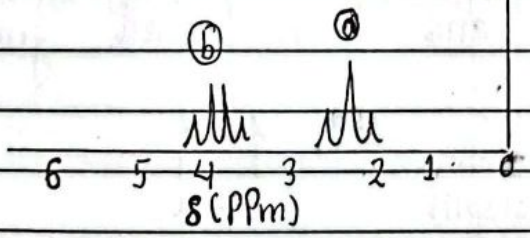

J- coupling constant

The distance between two splitted peaks of same signal is called coupling constant. It is represented by 'J' and is a characterstics features.

$\therefore J_{ab} = J_{ba}$

* $CH_3 - CH_2 - CH_2 - CH_3$ ⟶ 2 types of hydrogen ⟶ gives 2 types of signal
  @ ⓑ ⓑ @

$CH_3$ ⟶ Upfield
$CH_2$ ⟶ Downfield
} ⟶ chemical shift

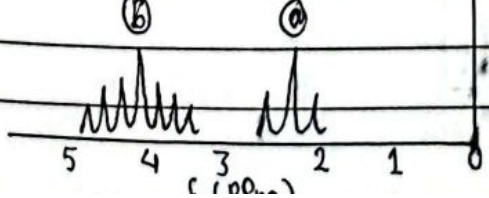



* $CH_3 - CH_2 - CH_3$ ⟶ 2 types of hydrogen ⟶ gives 2 types of signal
  @ ⓑ @

$CH_3$ ⟶ Upfield
$CH_2$ ⟶ Downfield
} ⟶ chemical shift

Peak splitting for $CH_3$ 1:2:1

Peak splitting for $CH_2$ by neighouring hydrogen i.e. $n+1 = 7$



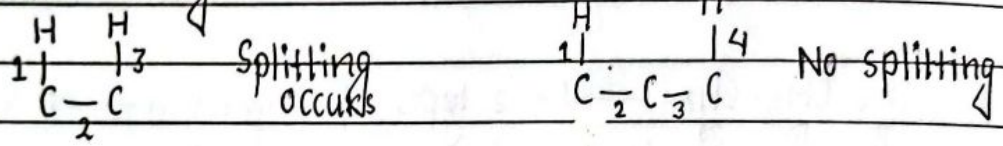
i) Equivalent hydrogens never split each other.
For eg:- $CH_3 - CH_3$ → single types of hydrogen i.e. No splitting

ii) Splitting is only upto 3 bonds, means only adjacent hydrogens split each other.

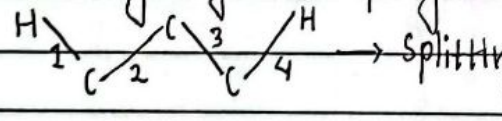
iii) Hydrogen on hetero atoms like -OH, $-NH_2$ do not take part in splitting.

$J_{ab} = J_{ba}$

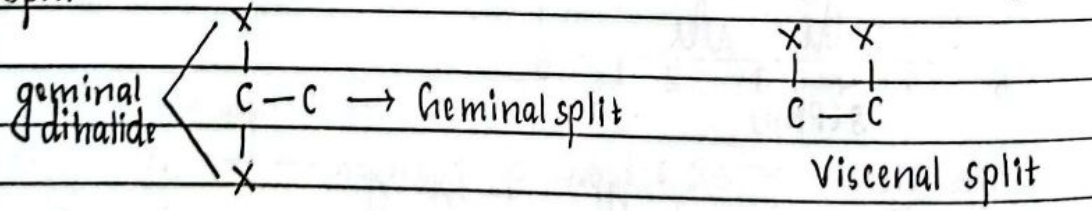
iv) Sometimes long range splitting takes place and is upto 4 bonds away.



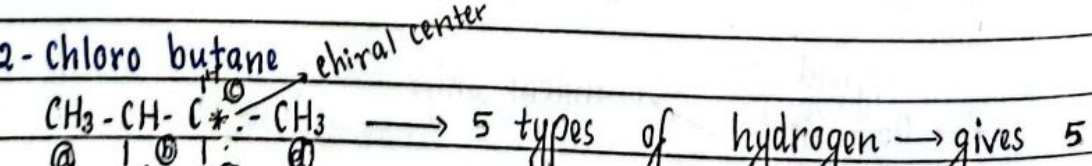

Sometimes long-range coupling splitting may be seen like in

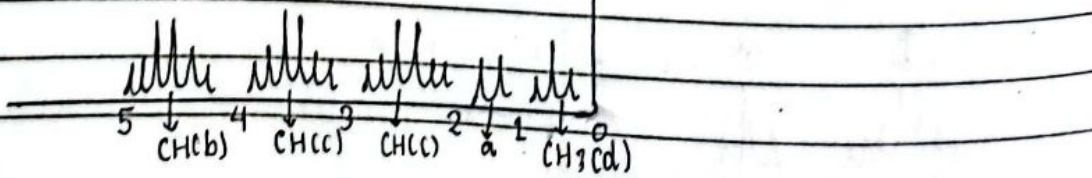



vi) If hydrogens of same hydrogen (Diastopic hydrogen). Then, they split each other and it is called geminal split.

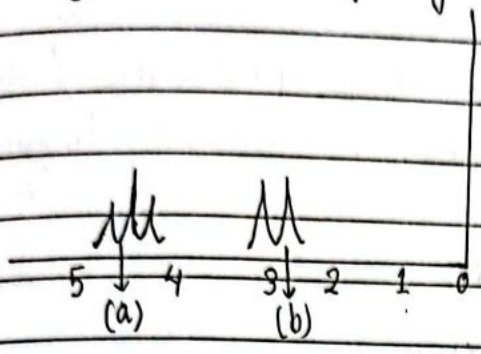



* 2-chloro butane

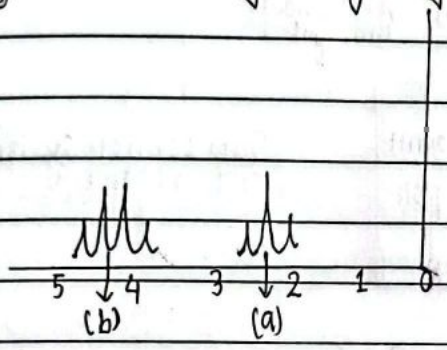


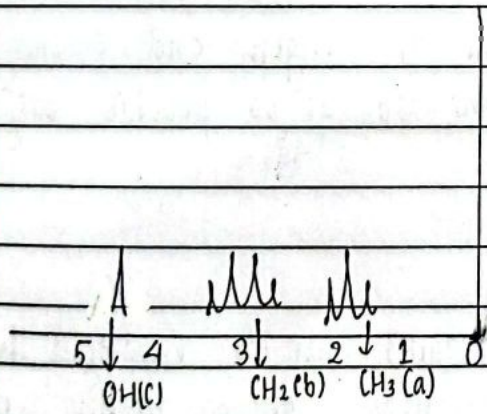
* $CHCl_2-CH_2-Cl$ ⟶ 2 types of hydrogen ⟶ gives two signal
  (a) (b)




* $CH_3-CH_2Cl$ ⟶ 2 types of hydrogen ⟶ gives 2 signal
  (a) (b)




* $CH_3-CH_2-OH$ ⟶ 3 types of hydrogen ⟶ 3 signal
  (a) (b) (c)



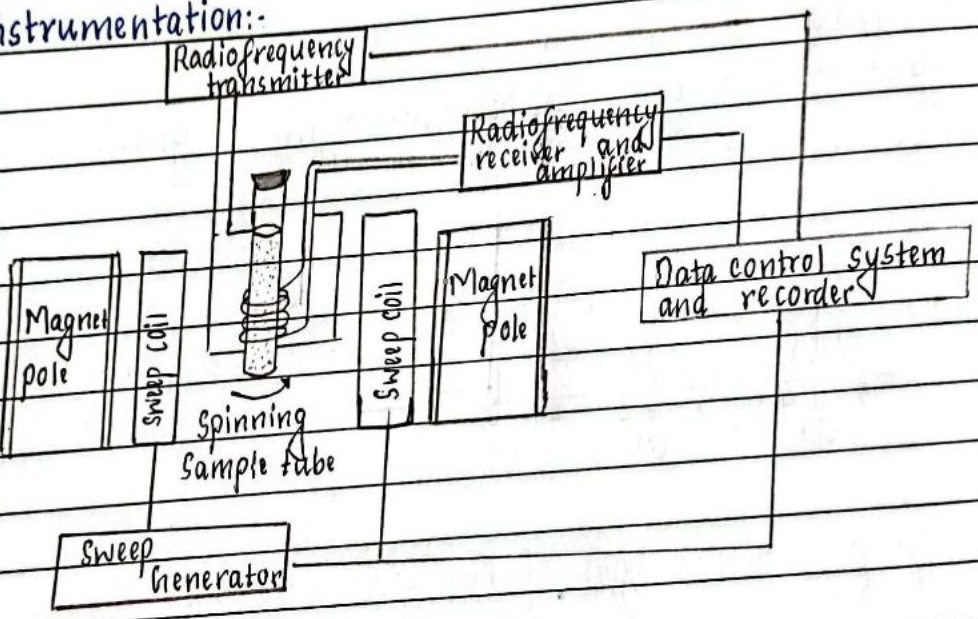

## Application of NMR spectroscopy

1) It is useful to detect total number of Protons present in the molecules.
2) It is useful to distinguish between cis and trans isomers.
3) It is useful to detetting aromaticity of molecules.
4) It is also used to detect hydrogen bonding in the molecules.
5) It is used to detect the purity and composition of sample.

f) It is used to study the dynamic. Kinetic and thermodynamic parameters of chemicals.

g) NMR is also used for measuring physiological functions, Tissue perfusion studies, Angiography, Anatomical imaging and Tumors.

## Instrumentation:-




a) Sample tube
Sample test-tube is about 8.5 cm on length and 0.3 cm on diameter and is spin at 30rpm so as to provide uniform magnetic field.

b) Magnet
Either a permanent or electromagnet can be employed in NMR to supply magnetic field. Currently superconducting magnets cooled in the liquid helium are being used in instrument which require high magnetic strength.

c) Sweep Generator
Sweep generator supplies direct current to sweeps coils so that the total applied magnetic field can be varied over a limited range.

d) Radiofrequency oscillator (Transmitter)

The radiofrequency field is provided by a transmitter coil whose magnetic vector component moves in a plane perpendicular to the direction of magnetic field. The radiofrequency field induces nuclear transition when it's frequency equal to angular precessional velocity.

e) Radiofrequency receiver (Detector)

The flipping of nuclei as a result of irradiation induces a voltage in a receiving coil.

f) Recorder

The voltage from receiving coil is amplified and observed in a recorder. The peaks of an NMR spectrum are result of Plotting intensity of absorption versus frequency or strength.

Notes:-

* Shielding effect

When a proton is present inside the magnetic field or closer to an electropositive atom then more applied magnetic field is required to cause exicitation. Such protons are called as shielded protons and the resulting effects is known as shielding effects.

* Deshielding effects

When a proton is present outside the circulating magnetic field or when it is attached to an electronegative atom then less applied magnetic field is sufficient for excitation. Such protons are called the deshielded protons and the resulting effect is Known as Deshielding effects.

# Atomic Absorption Spectroscopy (AAS)

Date 6 Mar
Page 67

AAS focused on elemental analysis

In Atomic absorption spectroscopy. the sample in which the presence of particular element is to be detected is first aspirated through a nebulizer. fine aerosol formed is then heated either thermally or electrothermally to convert it into gaseous atoms. which are then irradiated with suitable range of electromagnetic radiation the range and intensity of the absorbed radiation is the basis of the qualitative and quantitative estimation of the elements.

## Principle

The liquid sample is aspirated, aerosolized and mixed with combustile gases such as acetylene and air and nitrous oxide in AAS. Then, resulting mixture is ignited into the flame whose temperature ranges from 2100 to 2800°C due to which the metallic elements gets converted into atomic vapour. Then, the resulting unexcited ground state atom absorbs radiation of the particular wavelength from hollow-cathode lamp which is composed of a particular element needed to be analysis. The wavelength of the radiation given off by the source is same as the wavelength of radiation absorbed by the atoms in the flame. The absorbance is directly proportional to the path length in flame and to the concentration of atomic vapour in the flame.

## Detection process

$M^+X^-$ (sample) $\xrightarrow{\text{extraction}}$ $M^+X^-$ (solution) $\xrightarrow{\text{Nebulization}}$ $M^+X^-$ (fine aerosol) $\xrightarrow[\text{Desolvation}]{\text{Drying }\Delta}$ $M^+X^-$ (Residue)

$M^+X^-$ (Residue) $\xrightarrow[\text{Ashing}]{\Delta}$ Evapouratio

$M^+X^-$ (gaseous) $\xrightarrow[\text{Atomization}]{+e^-}$ M (gaseous atom) $\xrightarrow[\text{absorption}]{h\nu}$ $M^*$ (excited state)

9.5

## Instrumentation:-

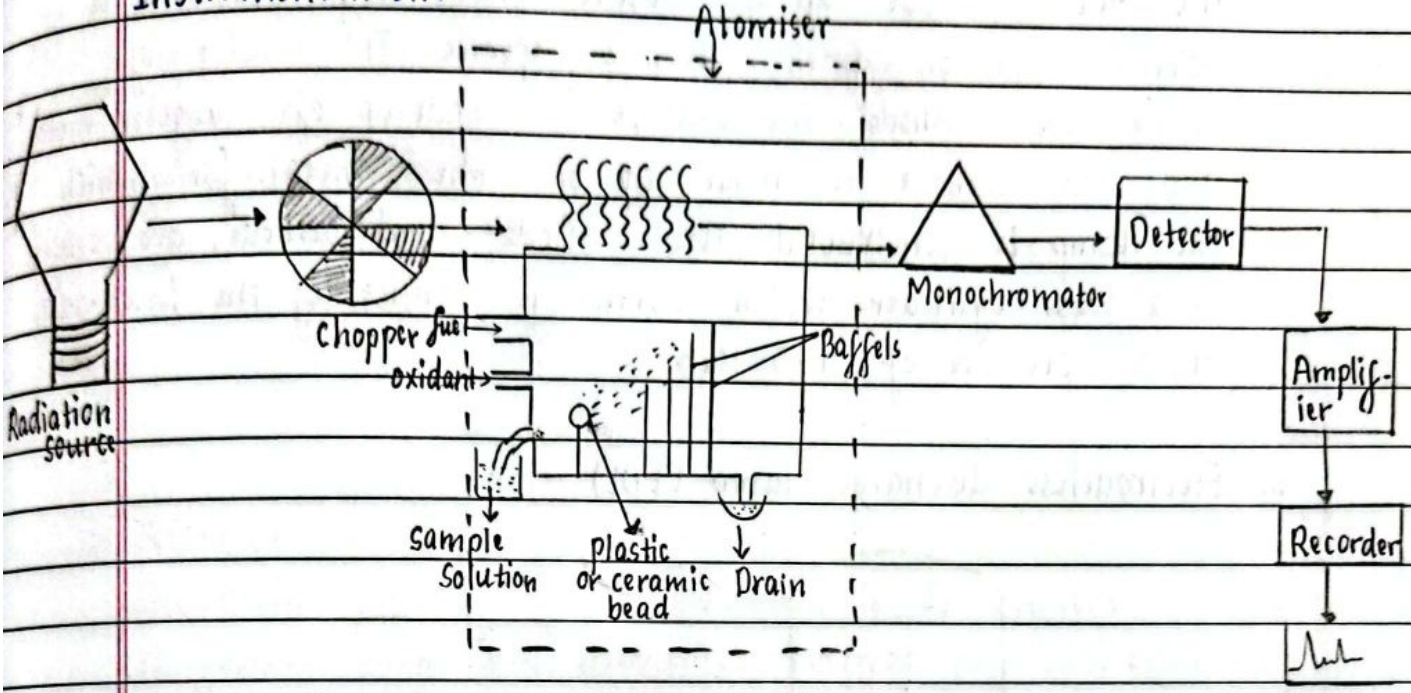



fig:- schematic representation of flame AAS

1) **Radiation Source**

There is seperate radiation source for each elements. The two different types of lamp are used in AAS.

i) HCL (Hollow cathode lamp)

ii) EDL (Electrodeless discharge lamp)

a) **Hollow cathode lamp (HCL)**

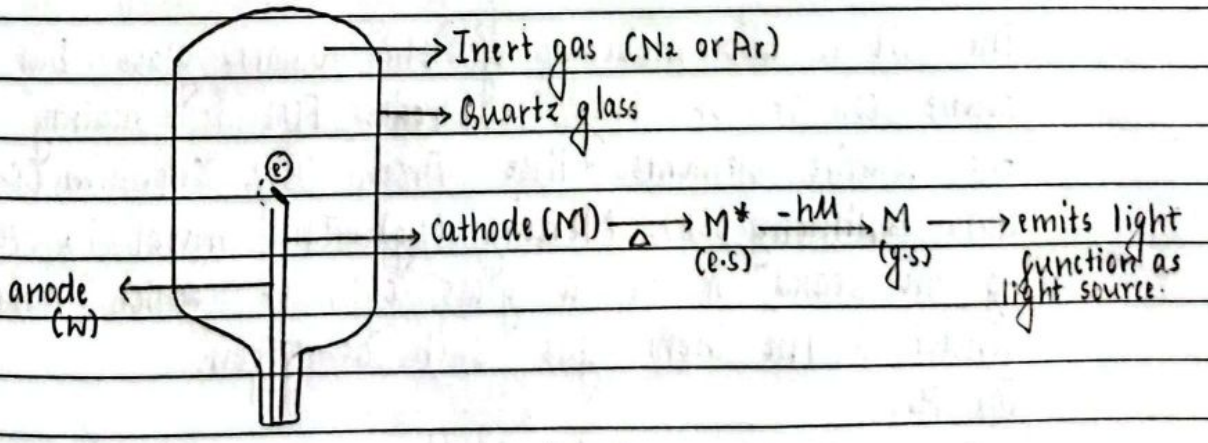



fig:- Hollow cathode lamp

In most of the cases, the hollow cathode lamp (HCl) is used.

The HCL is made up of Quartz glass. They produces a narrow emission from atomic species. It consist of anode and cathode. The anode is made up of Tungsten metal and the cathode is made up of the metal for which the lamp is constructed. The anode and cathode are sealed in a glass cylinder which normally consist of the inert gases at a pressure of 1-5 tarr.

b) Electrodeless discharge lamp (EDL)

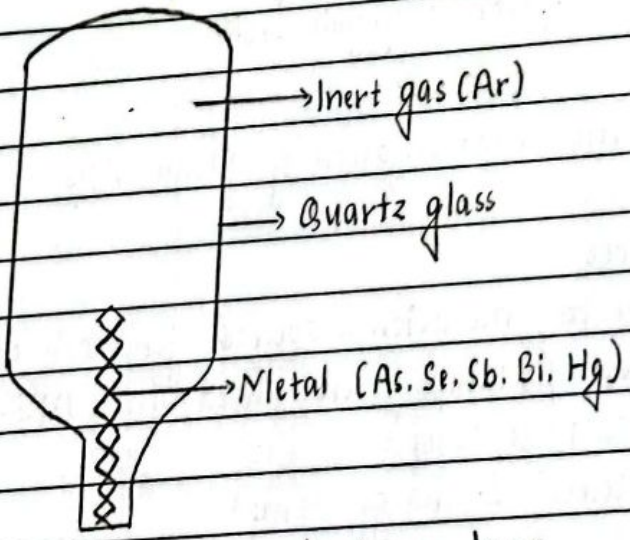



fig:- Electrodeless discharge lamp

The EDL is also made up of the Quartz glass but it dosent consist of any electrodes. EDL is mainly used for the volatile elements like Arsenic (As). Selenium (Se). Bismuth (Bi). Antimony (Sb). Mercury (Hg). The metal i.e. As. Se. Sb. Bi. Hg are sealed inside a glass cylinder which normally consist of the inert gas such as Argon.

For As,

$$As \xrightarrow[\text{acts as a plasma}]{\text{Argon}} As^{*} \xrightarrow[\text{acts as line source}]{-h\nu} As$$

($As^{*}$: e.s; As: g.s)

2) Chopper

The main functions of the chopper is to block the continous light and allows intermittent passing of light from the radiation source.

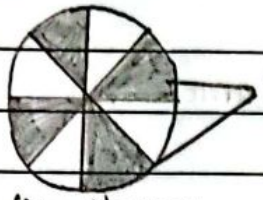



fig:- Chopper

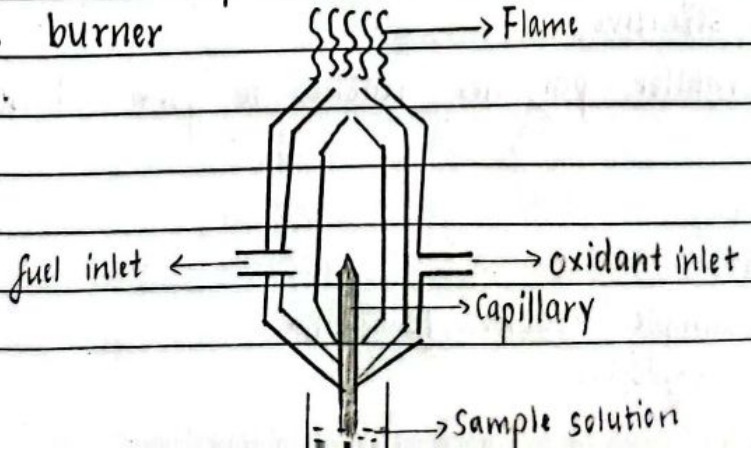
3) Atomiser

The main function of the atomiser is to convert the sample to gaseous atom. It may be thermal or electrothermal atomiser. In the thermal atomiser the flame produced is through the burners. Flame atomizer contains a pneumatic atomiser which converts the sample solution to fine aerosol. Generally, the sample solution is aspirated through a nebulizer and sprayed as a fine aerosols into the mixing chamber. In mixing chamber, the sample aerosols is mixed with fuel and oxidant gases and the sample is carried to burner head where combustion and atomization of sample is occured. and the resulting mixture of solution is converted to atomic vapour state. Fuel gas is introduced through the fuel inlet and oxidant through the nebulizer sidearm. The burners used for flame atomization are:-

i) TCB (Total consumption burner)

ii) Premixed burner

a) TCB



Advantages of TCB

i) 100%. sample reaches to flame.
ii) It is less or non-explosive.

Disadvantages of TCB

i) Larger droplets reaches to flame which reduces the temperature of flame.
ii) It is less sensitive.
iii) It is more noisy.

b) Pre-mixed burner

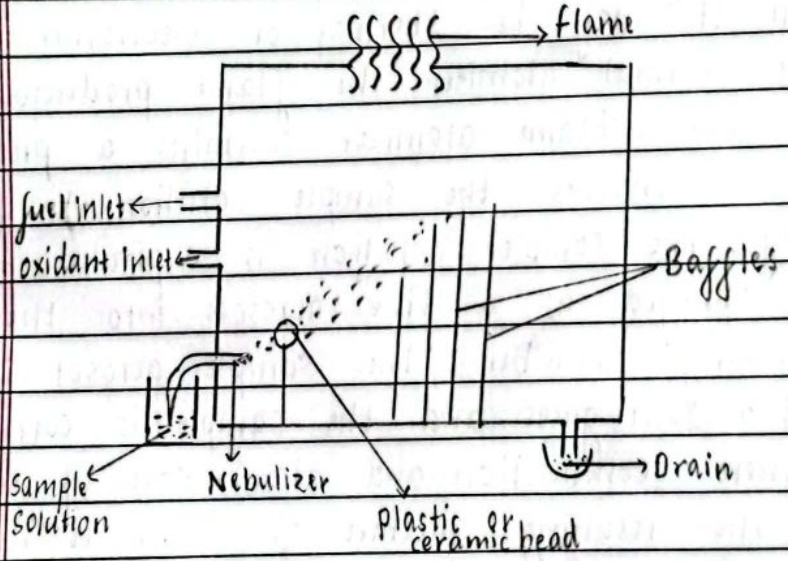



fig:- Pre-mixed burner

Advantages

i) It is less noisy.
ii) It is more sensitive
iii) Only the smaller particles reaches to flame hence no temperatur down.

Disadvantages

i) Only 10%. sample reaches to flame
ii) It can be explosive

The fuel used are:- i) Hydrogen
ii) Liquid petroleum gas (LPG)
iii) Acetylene ($C_2H_2$)

The oxidant used are:- i) Oxygen ($O_2$)
ii) Air
iii) Nitrous oxide ($N_2O$)

The most commonly used fuel is Acetylene ($C_2H_8$) and the most common oxidant are Air and Nitrous oxide ($N_2O$).

| fuel | Oxidant | Temperature (°C) | Max. burning velocity (cm/s) |
|---|---|---|---|
| Natural gas | Air | 1700 - 1900 | 39 - 43 |
| Natural gas | Oxygen | 2700 - 2800 | 370 - 390 |
| Hydrogen | Air | 2000 - 2100 | 300 - 440 |
| Hydrogen | Oxygen | 2550 - 2700 | 900 - 1400 |
| Acetylene | Air | 2100 - 2400 | 158 - 266 |
| Acetylene | Oxygen | 3050 - 3150 | 1100 - 2480 |
| Acetylene | Nitrous oxide | 2600 - 2800 | 285 |

4) Monochromator

The monochromator is used to disperse a broad spectrum of radiation and provide a continous calibrated series of electromagnetic energy bands of the determinable wavelength of frequency range. Light from the source enters the monochromator and is directed to the gratings where dispersion takes place. The diverging wavelengths of light are directed towards the exit slit. A selected emission line from the source can be allowed to pass through the exit slit by adjusting the angle of grating and ultimately fall onto the detector. Diffraction gratings consist of large number of parallel line of 15000 - 30000 per inch on highly polished surface of aluminium.

5) Detector

The detector converts the radiant energy to the electrical signal. It should be sensitive and should have fast response over a considerable range of wavelengths. Generally, the Photomultiplier tube (PMT) used as dectector in AAS. The PMT detector consist of two electrode i.e. anode and cathode. Cathode is made up of photoemissive material. One of the photocell receives the beam from the sample cell and the second receives the beam from refrence. The intensity of the radiation from the refrence cell is stronger than the beam of the sample cell. This results in the generation of Pulsating or alternating currents in photocell.

6) Amplifier

The main function of the amplifier is to increase the amplitude of the signal.

7) Recorder

It records the electrical signal and gives the graphical representation.

8) Printer

It gives the digital prints of the graphical signals.

Date 9 Mar
Page 74

## Instrumentation of Double beam flame AAS:

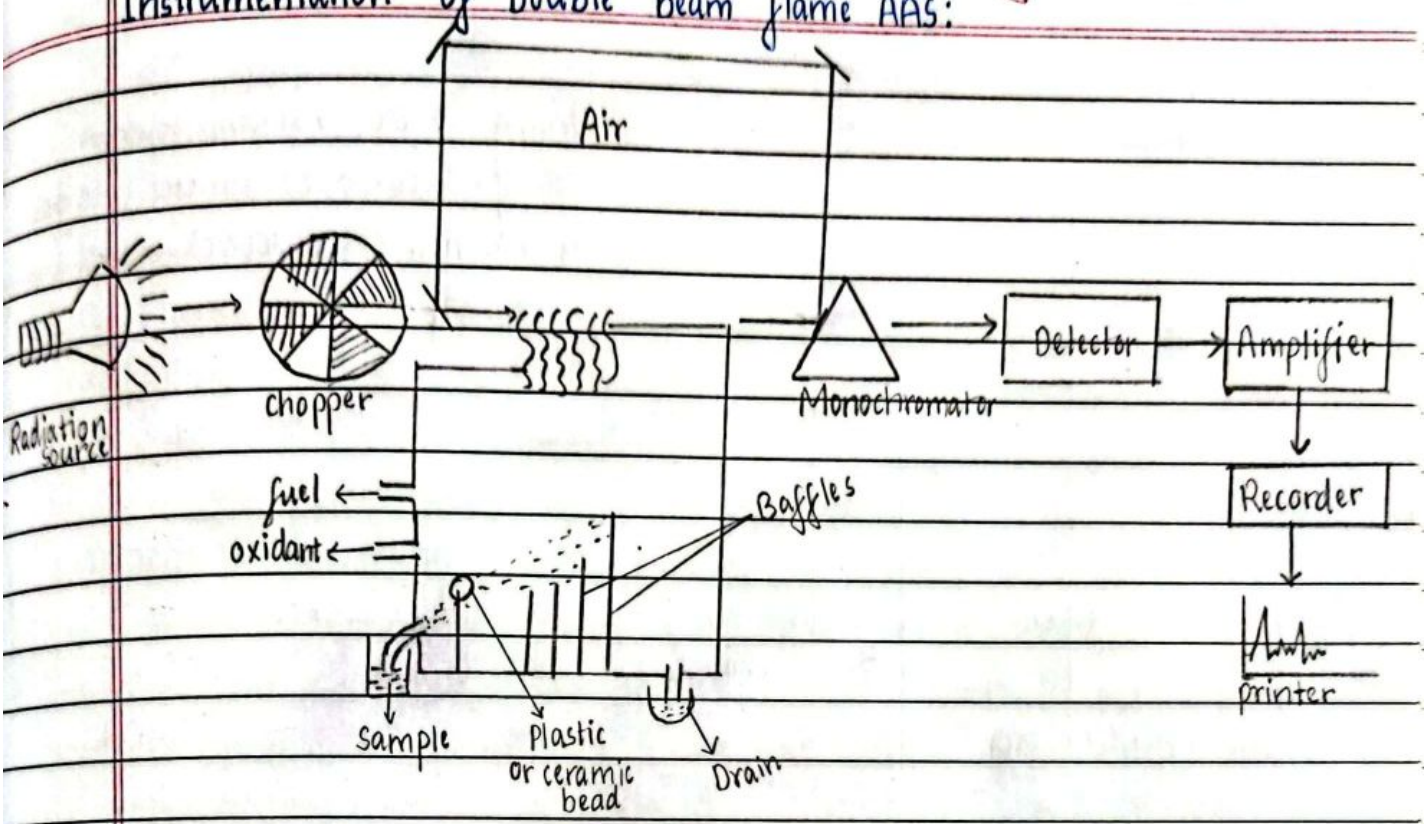



fig:- Double beam flame AAS

## Instrumentation of graphite furnance AAS:

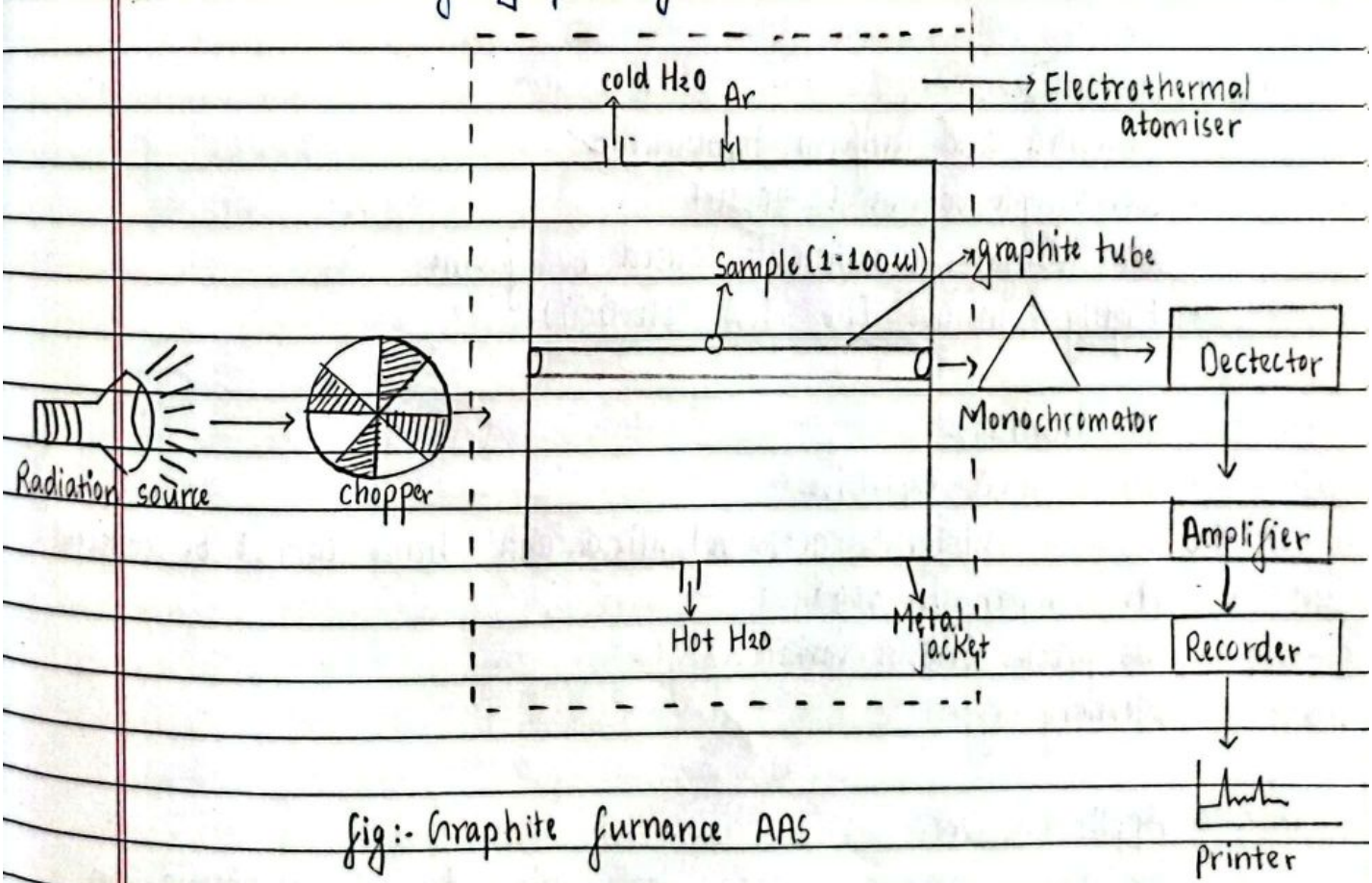
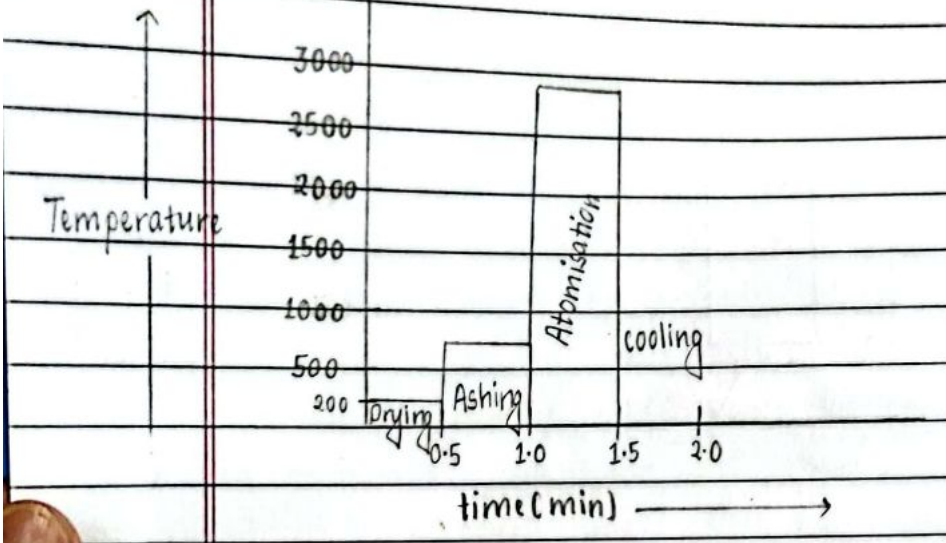



fig:- Graphite furnance AAS

Drying - 200°C → solvent removes
Ashing - (600-800°C) → metal → to gas
Atomisation (2800-3000°C) → metal atom




## Advantages

- Controlled and uniform temperature
- less sample volume (1-100µl)
- No interferance which is caused by flame.
- Highly sensitive (i.e. low detection)

## Disadvantages

- It is more expensive
- Volatiles substances gets volatilized. and hence cannot be detected.
- Autosampler is required.
- less precise. (result variation)
- Memory effect

## Applicable for:-

For those element. which has high transition atomisation except volatile transition element.

## Interferance in AAS

Interferance are the factor which deviate the original result.

1) Spectral Interferances
2) Chemical Interferances
3) Ionization Interferances
4) Solvent Interferances
5) Bulk or matrix Interferances

### 1) Spectral Interferance

- Spectral Interferance is caused mainly by the overlapping of any radiation with radiation of sample.
- The interfering radiation may be due to another element, radical, molecule, unresolved spectra or general background emission from flame or solvent.
- Hence, due to the overlapping of the absorption line of spectrum it shows the more concentrated line than the normal one.
- Every metal has different (i.e. more than one) absorption line of spectrum.

For eg:- Manganese (4031 A°, 4033A°, 4035A°)
Gallium (4033A°)
Potassium (4044A°, 4047A°)

Resolved by:-
It can be resolved by choosing the another absorption line, where there is no interferance of elements in spectrum.

* Spectral interferances also results from the presence of combustion products that exhibit broadband absorption or particulate product that scatter radiation. Both reduce the power of transmitted beam and lead to positive analytical errors.
- When the source of these products is the fuel and oxidant mixture alone, the analytical data can be corrected by making

absorption measurements while a blank is aspirated into the flame.

The correction must be used with both double-beam and single beam instruments because the refrences beam of the double-beam instrument does not pass through the flame.

## Chemical Interferances

These inferances resulting from the chemical processes occuring in flames and electrothermal atomizers and affect the absorption signal. They are categorized in 3 types:-

cation-cation Interferane

cation-anion Interferance

Oxide formation

### cation-cation Interferance

The interferance produce due to both of positive charge (i.e. catio

For eg:-

If we need the analyasis of calcium and magnesium in sample and if there is presence of Alumunium in sample. The Alumunium reacts with calcium and magnesium and form the alloy, which is difficult to atomised and hence gives low spectral absorption than normal.

### esolved by:

The Alumunium has the higher affinity with Lanthanum (La) We can add Lanthanum, then alumunium will reacts with La and make the analyte free (i.e. Ca and Mg)

### cation-anion Interferance

Some cation reacts with the anion and form the refractory material, which are difficult to get atomised

and hence. give the low absorption line than normal.
For eg:- Phosphate ions interfere with determination of Mg and ca. The absorption due to Mg and ca are appreciably weaker in the presence of $PO_4^{---}$ ions. This is due to the formation of stable phosphates of Mg and ca which do not readily split-up into the respective atoms in the flame.

Remedy:-
The addition of excess of strontium (Sr). or lanthamum (la) or thorium (Th) minimizes the interferance of $PO_4^{---}$ ion in the determination of Mg and ca by replacing the analyte in the compound formed with the respective interfering species. In short. these ions combine prefrentially with $PO_4^{---}$.

c) Oxide formation
Some metal reacts with the oxygen and forms the oxide and gives the formation of the refractory material which is difficult to atomised and gives the low absorption than normal

Remedy
Use of the inert atmospheric condition. which is only possible in
Ether increasing the temperature. which segregates the refractory materials.

3) Ionization Interferances
This inferance is mainly seen in case of alkali metal or alkali Earth metal. Those elements which have low ionization potential not only get atomised but also get ionised due to the high temperature of flame and gives the decrease in the absorption lines.

Examples:- The intensity of atomic absorption lines for the alkali metals, such as K, Rb, and Cs is found to be affected by temperature and there is noticeable decrease in absorption in hotter flames.

Remedy:-

i) The resulting effects of ionization equillibrium shift may be eliminated by the addition of ionization suppresor, that promptly gives a compariatively high concentration of electrons to the flame and results in the suppression of analyte ionization.

For eg:- In case of sodium,

$$Na \longrightarrow Na^{+} + e^{\ominus}$$

Add $Cs \longrightarrow Cs + e^{\ominus}$ (gets ionized in low temperature)

$Na + e^{\ominus} \longrightarrow Na$ (gaseous atom)

ii) Low temperature of flame.

4) Solvent- Interferance

Solvent- Interferance i.e. present in AAS is due to the solvent used. The organic solvent increases the intensity and aqueous solvent decreases the intensity of absorption. As, we know the organic solvents burns itself in the flame and increase the temperature of flame and gots the faster atomization.

For eg:-

If we prepare the sample in organic solvent and the standard is prepared in aqueous solvent, the absorption will be more than the normal results and vice-versa.

Remedy

i) It can be overcome by using the same solvent for both standard and sample.

5) Bulk or Matrix Interferance

Matrix interferance is a physical Interferance, and can either supress or enhance absorbance signal of analyte.
It occurs when components of sample matrix other than the analyte react to form molecular species and sample background. The detector picks up the unspecified signals from sample matrix that do not match the absorbance line of the analyte.

Cause:-

1) Characterstics of sample and standards differ in viscosity and surface tension. It results in difference in sample uptake due to changes in nebulization efficiency.
2) Sample and standard prepared in different solvent.
3) Sample and standard measured at different temperature.
4) Sample contains a high concentration of dissolved salts or acid.
5) Organics are present in sample matrix
6) Sample and standards differ in atomization rate in flame.

Remedy

i) Viscosity and the solution problems can be solved by adding methanol to sample and standard. This will enhance nebulization and increase the amount of sample entering the flame to give higher absorbance value.
ii) Incomplete combustion of organics in matrix that produce broad-band signal can be reduced or eliminated by increasing the temperature of the flame to ensure the complete combustion.
iii) Components in matrix react to form molecular species such as oxides and hydroxides which can be prevented by switching to a higher flame atomization temperature.
iv) Use standard addition:. Standard addition means to determine the analyte without eliminating the matrix interferance. Add a

Known component of matrix to both samples and standard so that the interferent becomes insignificant.

## Sample prepration in AAs

i) Dry Ashing
ii) Wet Ashing
iii) Microwave digestion

### 1) Dry ashing

In dry ashing 2gm of sample is placed in platinum crucible and heat in Muffle furnance at 500 - 600°c for 5 to 6 hrs. The Ash produced is dissolved in suitable solvents, filtered and then subjected for analysis.

### 2) Wet ashing

Highly acidic solvents are used in wet ashing. The sequence of solvents used are:- Dil HCl > con HCl > Dil. $HNO_3$ > con. $HNO_3$ > Aquaregia > dilute $HClO_4$ > conc. $HClO_4$. Dissolve the sample in the above solvent in which the sample dissolve and filtered then subjected for analysis.

### 3) Microwave digestion

- It is the fastest method
- It has uniform heating
- There is less chance of volatilization of volatile metals. Here. the sample is placed in sample tube and digested in Microwave.
- The procedure is completed in half. hour and is preferred above two techniques.

## Sample Introduction system in AAS

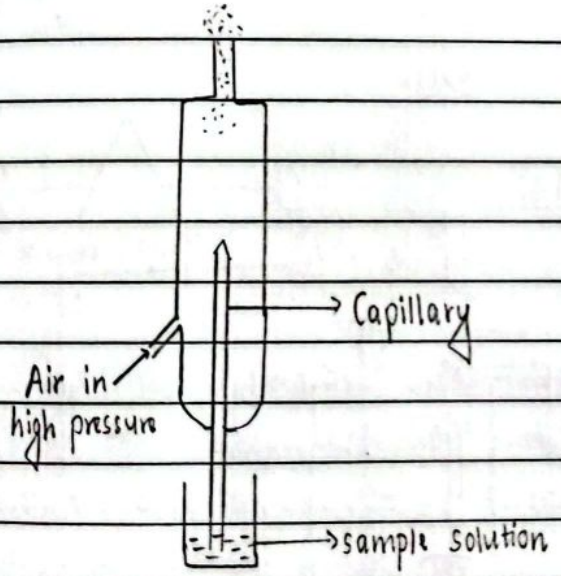
1) Nebulization
2) Electrothermal vapourisation
3) Hydride vapour generation

### 1) Nebulization

It is of two types

i) Pneumatic nebulization
ii) Ultrasonic nebulization

### i) Pneumatic nebulization




### ii) Ultrasonic nebulization

In ultrasonic nebulization, the crystal is added to sample. Due to the vibration of crystals sample is converted to fine aerosols.

### 2) Electrothermal vapourisation

- Used in graphite furnance AAS.
- Here, the sample is heated electrothermally and converted to fine aerosol.

3) Hydride vapour generation

- It is mainly used for the volatile elements like Bi, Se, Sb, As, H
- Here, the sample is mixed with reducing agents and the metals are converted to the metal hydride. which are gases and reaches to quartz tube, where it is heated at about 850-1000°c to atomised them.

$$AsX_3 + NaBH_4 + HCl \longrightarrow AsH_3\uparrow + H_3BO_3 + NaX$$

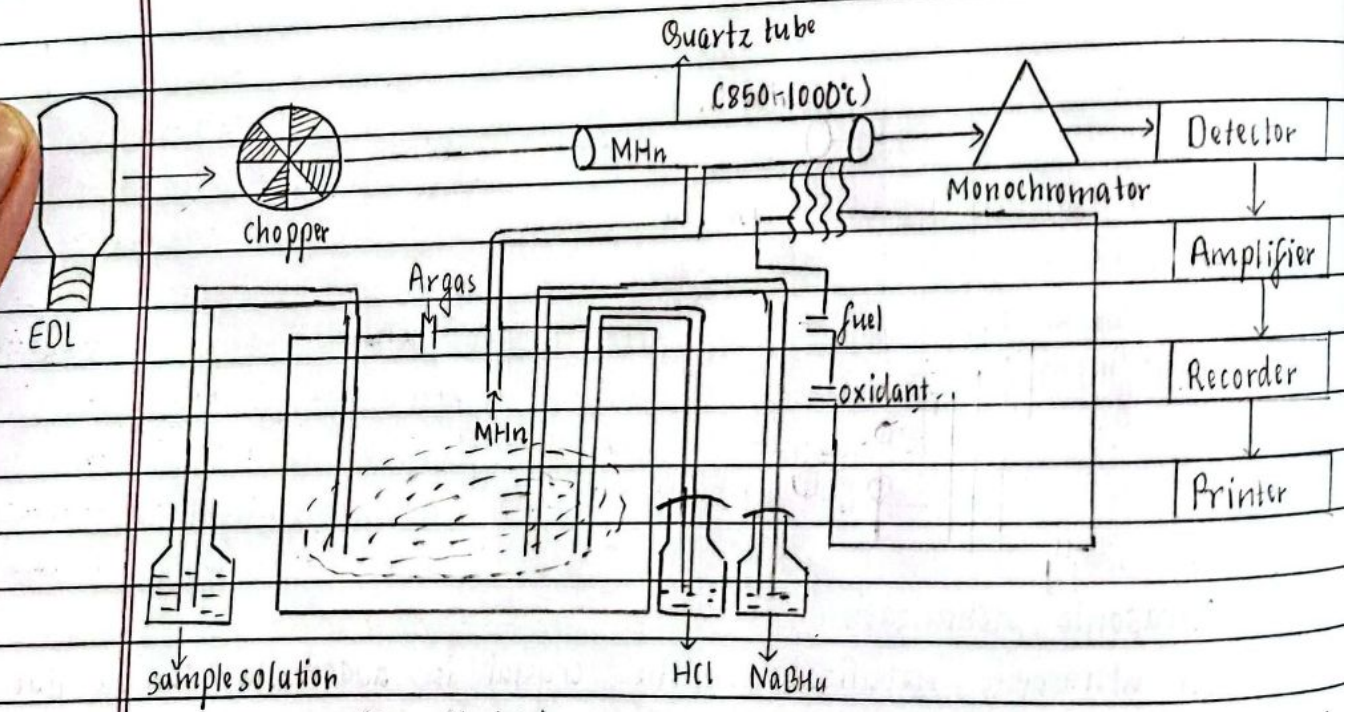



fig:- Hydride vapour generation

Background correction in AAS

Background / Noise in AAS is the absorbance due to solvent or other matrix present in sample solution. So, in order to get the exact result. We have to elliminate or correct background while analysis.

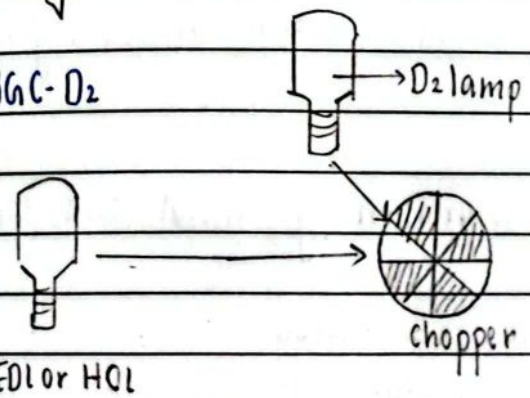
## Background correction in AAS

1) Running of blank
2) Background correction $D_2$ lamp (BGC-$D_2$)
3) Background correction by self-reversal lamp (BGC-SR)
4) Background correction by Zeman techniques.

### 1) Running of blank

Running of blank and setting it zero, gives the environment similar to that of except analyte and hence helps in background correction in AAS.

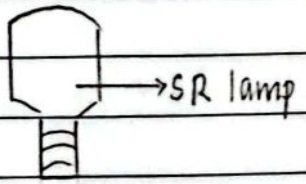
### 2) BGC-$D_2$




$D_2$-lamp absorption is only due to background
HDl or EDl lamp absorption due to both analyte and background.
Absorbance due to analyte = Absorbance of HCl or EDL - Absorbance by $D_2$-lamp
- Used in only flame AAS.

### 3) BGC-SR

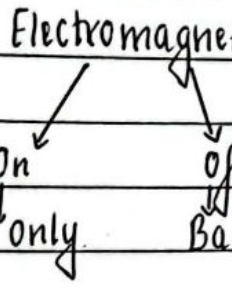
Special type of HCl are used which are self-reversal




It send two types of intensity of light
i) High intensity:- absorbance due to background only
ii) Normal intensity:- absorbance due to analyte and background both
Absorbance due to analyte = Normal intensity - High intensity.
- BGC-SR is used for both flame AAS and Graphite furnance AAS.

4) Zeman- techniques

- It is mainly used in graphite furnance.
- It consist of graphite tube where electromagnet is present which goes on and off.




Absorbance due to analyte = Absorbance when magnetic field is off - Absorbance when magnetic field is on.

Applications of AAS

i) Qualitative and quantitative estimation of metal and some non-metals (67 elements)
ii) AAS is used in mines and minerology.
iii) To find the composition of metal alloys.
iv) Quality assurance of drug containing metals
v) Analysis of micronutrient (Zn, As, Se)
vi) To detect the calcium level in bone AAS is used.
vii) For analysis of copper and iron in Ascorbic acid.
viii) For the analysis of zinc in zinc-Insulin suspension.

Limitations of AAS

- It is expensive.
- It requires Acetylene gas.
- It requires separate lamp for different element.

# Flame Photometry

Date 15 Mar
Page 86

## Principle

In flame photometry the sample solution in which the presence of particular element to be detected is aspirated through a nebulizer then it is heated to excite the elements in excited state and while returning to the groundstate it emits the energy in the formed of electromagnetic radiation. The range and intensity of emitted radiation is the basis of qualitative and quantitative estimation of elements.

$M^+X^-$ (Matrix) $\xrightarrow{\text{Extraction}}$ $M^+X^-$ (solution) $\xrightarrow{\text{Nebulization}}$ $M^+X^\ominus$ (aerosol) $\xrightarrow[\text{Desolvation}]{\text{Drying}}$ $M^+X^-$ (residue)

$M^+X^-$ (residue) $\xrightarrow{\text{Ashing}}$ $M^+X^-$ (gaseous atom) $\xleftarrow{+e}$ $M$ (atomization) $\xleftarrow{\Delta}$ $M^*$ (Excitation) $\xleftarrow{\text{Emission}}$ $M$ (ground state)

$-h\nu$ ↓

range and intensity of emitted radiation is the basis of qualitative and quantitative estimation of elements.

Flame photometry is also known as Atomic Emission spectroscopy. In emission spectroscopy. atoms in the sample absorb thermal energy and causes the outer orbital electrons to become excited from their ground state to higher energy orbital level. After certain time the excited electrons returns to the ground state by emitting electromagnetic radiations normally in the form of light in the UV-visible region. The emitted radiations are analyzed by the means of spectrograph of various wavelength.

Flame emission of various metals in terms of emission wavelength and flame colour.

| Elements | Emission wavelength (nm) | Flame colour | Elements | Emission wavelength (nm) | Flame colour |
|---|---|---|---|---|---|
| Sodium | 589 | Yellow | Calcium | 622 | Orange |
| Potassium | 766 | Violet | Lithium | 670 | Red |

## Emission Spectra

When a substance is heated to a high temperature then the atoms in the vapours get energized. Then, the resultant energized atom return to the ground state by emitting electromagnetic radiation of certain definite wavelength. Thus, the resultant series of bright lines are called as atomic emission spectra.

### Various types of emission spectra

a) Continous spectrum

When white light from sun or any incadescent body or lamp is passed through a prism, it disperses into seven different light bands of colours and a resultant spectrum is known as continous spectrum. A continous spectrum is one in which colours are diffused in one another without any line of demarcation.

b) Line spectrum

When electric current is passed through a gas at low pressure, the atoms of gas are excited and radiate light. When radiate light is passed through the prism the resultant spectrum is known as line spectrum. line spectra are obtained from the atom of luminous gases or vapours when electric discharge passes through them. Study of line spectra help us to know the structure of electron shells of atoms of various elements.

### Various process involved in flame Photometry

a) Desolvation

The metal particles in the flame are dehydrated by the heat of flame and the solvent is evapourated.

b. Vapourization

The heat of flame vapourizes the sample constituents.

c. Atomization

Metal ions present in solvent get reduced into metals atoms during atomization.

d. Excitation

The electrostatic force of attraction between the electrons and nucleus of the atom helps them to adsorb a particular amount of energy. The atoms then jump to the excited energy state.

e. Emission process

Since the higher energy state is unstable so the atoms jump back to the stable low energy state with the emission of energy in the form of radiation of characterstics wavelength which is measured by the photo detector.

Instrumentation:-

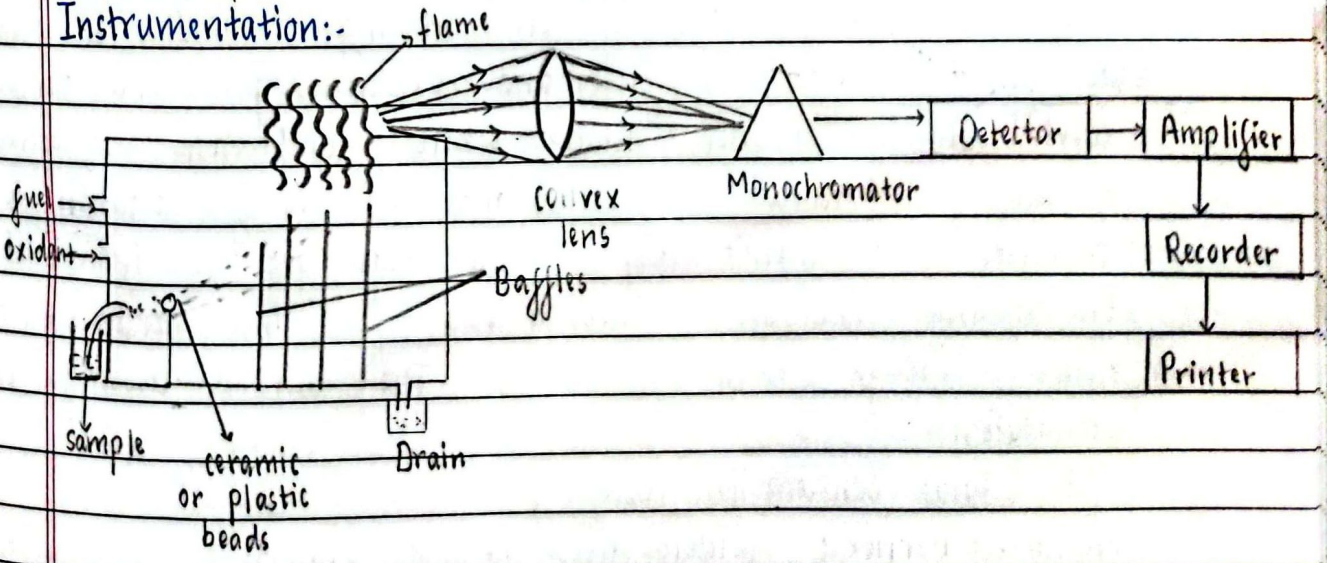



fig:- Instrumentation of flame photometry for convex lens

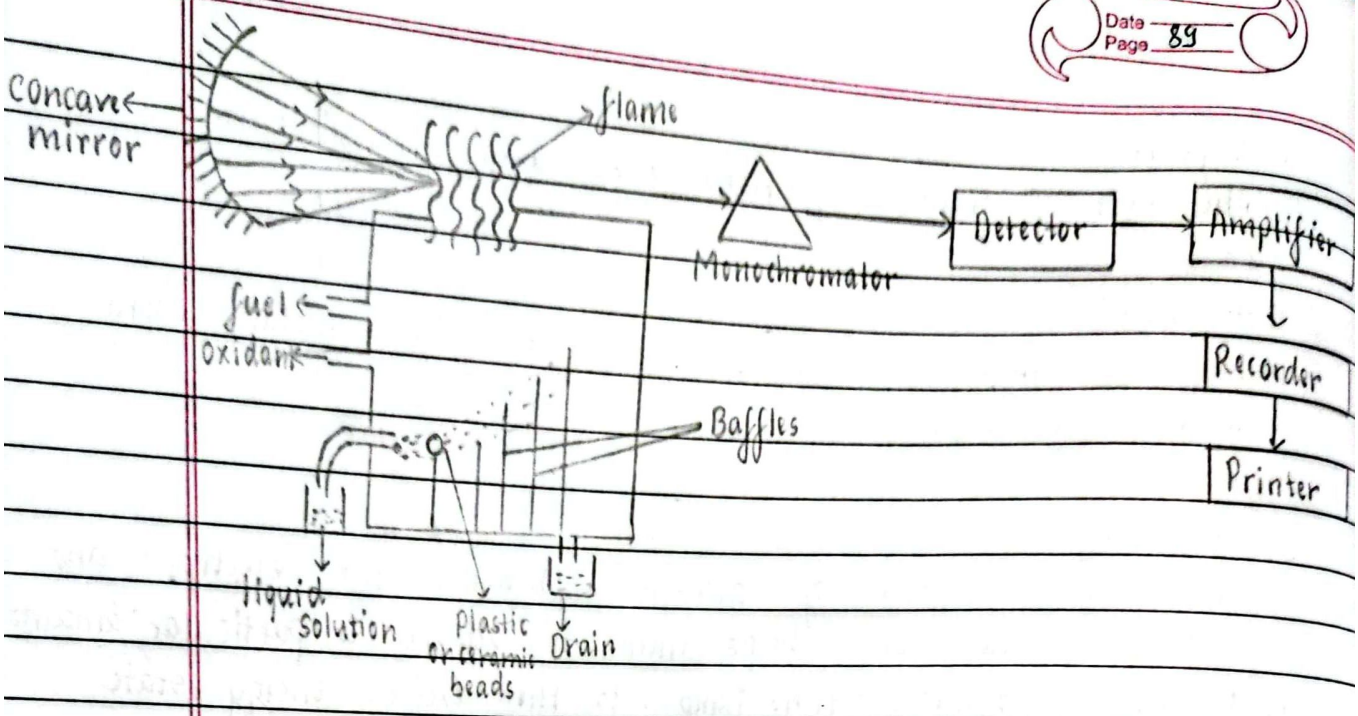



fig:- Instrumentation of flame photometry for concave mirror

a) **Atomiser**

Flame atomiser is generally used in AAS. Flame atomiser contain a pneumatic nebulizer which converts a sample solution into a fine aerosol. Generally, the sample solution is aspirated through a nebulizer and spread (sprayed) as a fine aerosol into the mixing chamber. In mixing chamber, the sample aerosol is mixed with fuel and oxidant gases and sample is carried to the burner head where combustion and atomisation of sample is occured and resultant mixture of solution is converted into atomic vapour state. Fuel gas is introduced into the mixing chamber through the fuel inlet and oxidant enters through nebulizer side-arm. The burners used for flame atomisation:-

a) TCB (Total consumption burner)

b) Pre-mixed burner :- widely used because uniformity in flame intensity. In this energy type of burner, aspirated sample, fuel and oxidant are thoroughly mixed before reaching the burner opening.

| fuel/oxidant | Flame Tempr | fuel / oxidant | Flame Tempr |
|---|---|---|---|
| Hydrogen/Air | 2100°c | Acetylene / oxygen | 3100°c |
| Hydrogen/ oxygen | 2750°c | Propane /Air | 1900°c |
| Acetylene/ Air | 2150°c | Propane /oxygen | 2800°c |
| | | | |

The mainly used fuel is LPG and oxidant is Air because it is mainly used for alkali and alkaline earthmetal and have low ionization potential.

b) Convex lens /concave mirror / optical system

They focuses on the scattering of light and sends to the monochromators. The optical system consist of concave mirror and convex lens. The concave mirror transmits the light emitted from the atoms. Concave mirror also helps to focus the emission to the lens. The lens helps to focus the light on a point or slit.

c) Monochromator

The monochromator is a device used to disperse a broad spectrum of radiation and provide a continous calibrated series of electromagnetic energy bands of determinable wavelength or frequency range. Light from the source enters the monochromator and is directed to the gratings where dispersion takes place. The diverging wavelength of light are directed towards the exit slit. A selected emission line from the source can be allowed to pass through the exit slit by adjusting the angle of gratings and ultimately fall onto the detector. Diffraction gratings consists of large number of parallel lines of 15000- 30000 per inch on highly polished surface of aluminium.

d) **Detectors**

The detectors are devices that convert radiant energy into electrical signal.

Photo detector detects the emitted light and measure the intensity of radiation emitted by the flame. That is, the emitted radiation is converted to an electrical signal with the help of photo detector. The produced electrical signals are directly proportional to the intensity of light.

e) **Amplifier and Recorder**

The output from the detector is suitably amplified and displayed on the digital display and record the spectrograph of various wavelength.

**Boltzman Distribution law**

$$\frac{n^*}{n} = \frac{g^*}{g} e^{-\Delta E/KT}$$

where,

$n^*$ = No. of atoms in excited state

$n$ = No. of atoms in ground state

$g^*/g$ = stastistical factors and constant for particular element

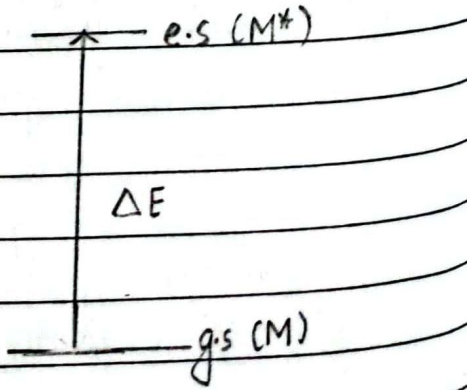
$\Delta E$ = energy gap between the excited state and ground state

$K$ = Boltzmann constant

$T$ = Absolute temperature.

$\frac{n^*}{n} \propto \frac{1}{\Delta E}$ (Inversly proportional to $\Delta E$)

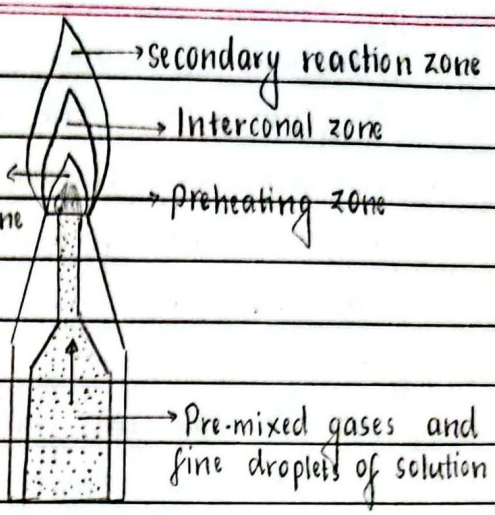
$\frac{n^*}{n} \propto T$ (directly proportional to T)



## Structure of flame

As. seen in figure. the flame may be divided into the following regions or zones.

- Preheating zones
- Primary reaction zone or inner zone
- Internal zone
- Secondary reaction zone




### i) Preheating zone

In this. combustion mixture is heated to the ignition temperature by thermal conduction from the primary reaction zone.

### ii) Primary reaction zone

This zone is about 0.1mm thick at atmospheric pressure.

- There is no thermodynamic equillibrium in this zone and the concentration of ions and free radicals is very high.

### iii) Interconal zone

It can extend up to considerable height. The maximum temperature is acheived just above the tip of inner zone.

-This zone is used for flame photometry.

### iv) Secondary reaction zone

In this zone. the products of the combustion processes are burnt to stable molecular species by the surrounding air.

## Requirements of flame

- It should have proper temperature.
- Temperature should remain constant throughout the operation
- There should not be any fluctuations during burning.

## Functions of flame

To convert the analyte of the liquid sample into vapour state.
To decompose the analyte into atoms and simple molecules
To excite the formed atoms / free atoms / simple molecules to emit radiant energy.

## Interferances in flame photometry

### Spectral interferance

It occurs when the emission lines of two elements cannot be resolved or arises from the background of flame itself. They can be either too close to each other, either ovelapping, or 5-10nm seperation or due to high concentration of salt in sample.

Resolved by:-
They have more than one emission line of spectra for the single element, choose which do not overlap.
Increase the resolution of monochromator.

Molecular emission Interferane : emission from molecule
Flame interferance :- due to change in flame temperature
Remedy:- Running blank.

### Chemical Interferance

The chemical interferences arise out of the reaction between different interferents and the analyte includes :-
cation- anion Interferance:- The presence of certain anions such as oxalate, phosphate, sulfate in a solution may affect the intensity of radiation emitted by an element. For eg:- calcium in present of phosphate ions produces the complex and decrease the intensity of radiation.

6) cation- cation Interferances
These are neither spectral nor ionic in nature. For eg:- Interferance of Aluminium with calcium and magnesium.

3) Ionization Interferances
The sodium ion possess an emission spectrum of it's own with frequencies which are different from those of atomic spectrum of the sodium atom. Thus, ionization decreases the radiant power of atomic emission. The interferance due to ionization can be overcome by adding a large quantity of potassium salts in the unknown and the standard solutions. The addition of potassium prevents the ionization of sodium but itself undergoes ionization.

4) Solvent- Interferance
The organic solvent increases the intensity and the organic solvent decreases the intensity of absorption. If the sample and standard are prepared in two different solvent these type of Interferance can be seen. To resolved this problem. the sample and the standard should be prepare in same solvent.

5) Bulk- Interferances
It may be seen due to the viscosity of sample. For eg:- If the sample is highly viscous less nebulization or aspiration and hence there will be less emission spectra than excepted and vice-versa.
Remedy:-
Addition of methanol to sample and standard enhance the nebulization and increase the emission spectra line.

## Sample prepration in Flame photometry

Same as in AAS by:-

i) Dry- ashing
ii) Wet- ashing
iii) Microwave digestion

## Sample introduction in Flame photometry

Same as in AAS by:-

i) Nebulization
ii) Electrothermal vapourization
iii) Hydride vapour generation

**Applications:-** Method used for samples analyses in Flame photometry

1) Qualitative analysis

Element can be identified by colour of flame.

For eg:-

Sodium ⟶ Yellow  calcium ⟶ orange
Potassium ⟶ Violet  Lithium ⟶ Red

2) Quantitative analysis

i) Standard addition curve
ii) Internal standard method

i) Standard addition curve

Prepare at least five volumetric series of solutions containing equal quantities of the substances to be tested and their signal intensity is determined by aspirating them into flame. The series of refrence solutions containing known concentration of element to be determined is prepare and their intensity is also determined by aspirating them into flame. The concentrations choosen should

be expected to give responses in the linear part of curve. Then the plot of the mean of the reading on a graph against the concentration of the element which is need to be determined then. the concentration of the element in the test solution is determined by extropolating the straight line joining the points on the graph to an extended concentration axis.

For eg:-

If we need to detect sodium in water sample.

Step 1:- Take 5 different volumetric flask, which have capacity of 25 ml.

Step 2:- Add 5 ml of sample to each

Step 3:- Add the standard of sodium (i.e. of 500 ppm) in each vessels in such a way that flask A contains 0ppm of standard. B contains 5ppm of standard, C contains 10 ppm. D contains 20ppm and E contains 50 ppm.

Calculating it by applying formula $N_1V_1 = N_2V_2$

For A = 0 ppm. So, no addition of standard

For B = $N_1V_1 = N_2V_2$

or, $25 \times 5 = 500 \times V_2$

or. $V_2 = 0.25$ ml of standard added

For C = 0.5ml of standard

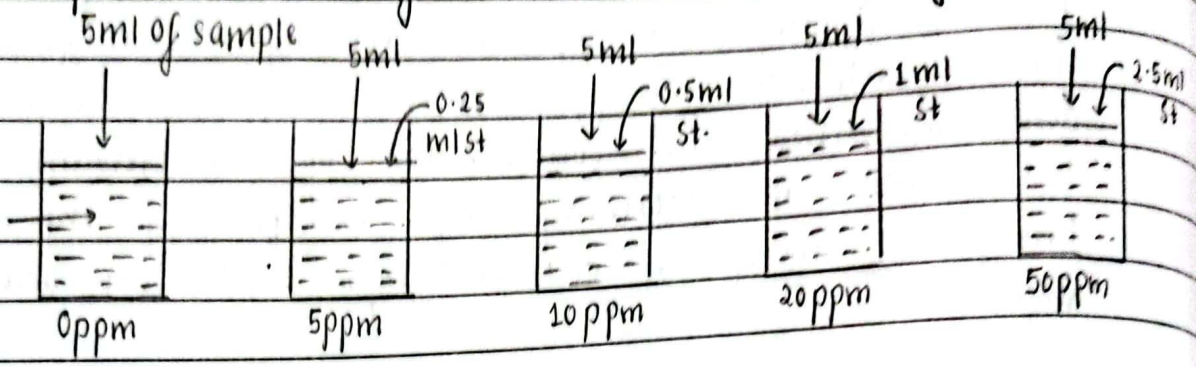
For D = 1 ml of standard

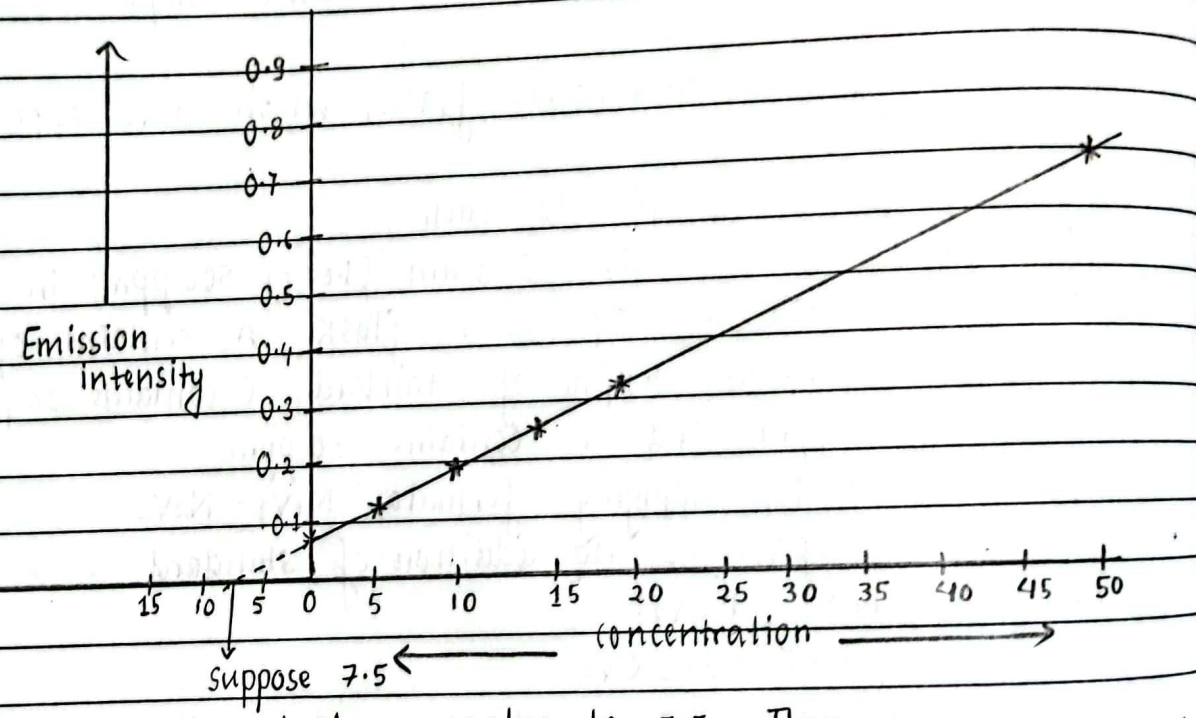
For E = 2.5 ml of standard

Step 4: Make volume by adding water for A → 20ml, for B → 19.75ml for C → 19.5ml, for D → 19 ml and for E → 17.5ml.

Step 5:- Plotting the concentration v/s emission in graph. the flask A doesn't contains the standard but passes through Y- axis not through zero because. the sample itself contains sodium.

Step 6:- Extropolate the line from A to another side of x-axis.




Suppose extrapolation reaches to 7.5. Then,
Concentration of diluted sample = 7.5 ppm
For concentration of original sample = conc of diluted sample x Dilution factor
= 7.5 x 5
= 35.5

Advantages
i) Gives the exact result

Disadvantages
i) Matrix Interferance iii) High expenditure
ii) Time consuming

ii) Internal standard method

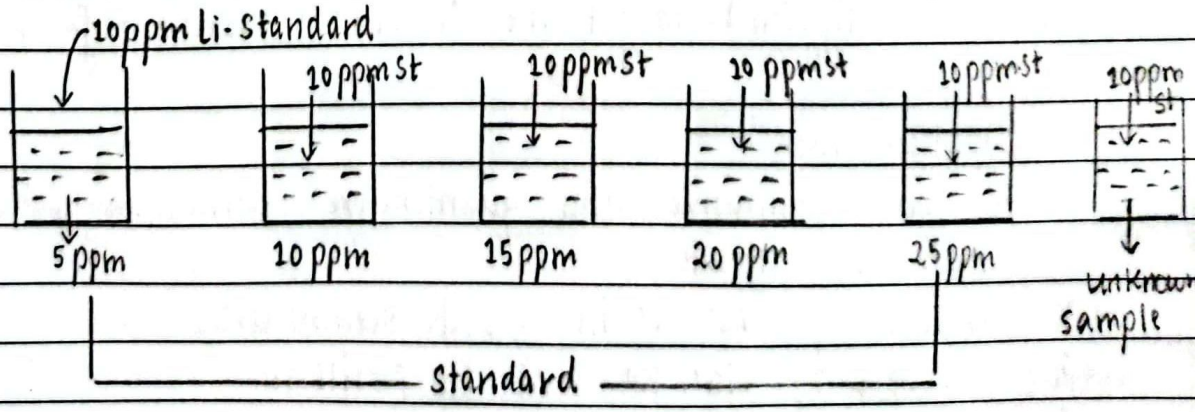
Prepare the standard sample solutions containing known amounts of internal standard elements usually lithium. Then, aspirates these standard samples into the flame and record the signal intensity for each of these elements. Similarly, the sample having unknown concentration also aspirated into flame and record the signal intensities. Then, the ratio of two intensities is calculated and plotted against the concentration of element which is need to be determined The concentration of internal standard element is kept constant. A calibration curve is obtained. Then, the conc of the element present in sample is measured if it's signal intensity is known by aspirating it into the flame.

For eg:-
Analysis of sodium in water sample.

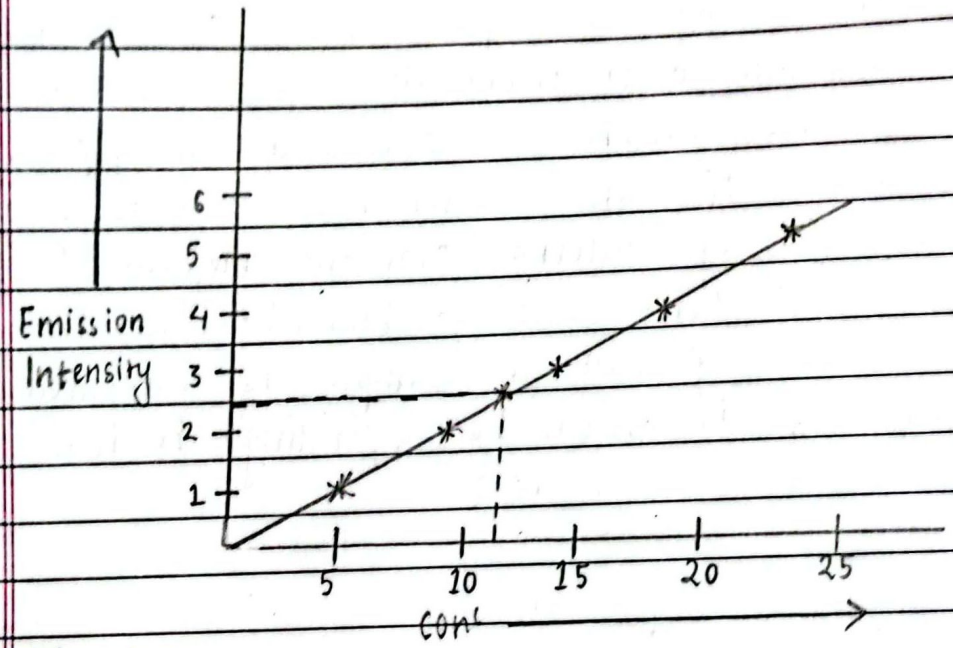
Step 1:- Take the 5 volumetric flask for the standard solution i.e. lithium standard (10ppm). Add fixed amount of internal standard in all flasks.

Step 2:- Take the emission reading for both sodium and lithium.

Step 3:- Plot a graph by obtaining emission intensity i.e. Na/Li in Y-axis and concentration in X-axis.



| | 5ppm | 10ppm | 15 | 20 | 25 | Un known |
|---|---|---|---|---|---|---|
| Na | 50 | 90 | 120 | 240 | 250 | 125 |
| Li | 50 | 45 | 40 | 60 | 50 | 50 |
| Na/Li | 1 | 2 | 3 | 4 | 5 | 2.5 |




## Advantages

* Gives the exact results
* Avoids the fluctuation seen due to temperature

## Disadvantages

* It requires the internal standard
* It is expensive and time-consuming
* It is difficult to get the internal standard for each element

## Applications:-

i) For the qualitative and quantitative estimation of alkali and alkali earth metals.
ii) Detection of $Na^+$, $K^+$ in blood, serum, urine
iii) Detection of $Na^+$, $K^+$ level in fertilizers.
iv) Analysis of $ca^{++}$, $Mg^{++}$, $Na^+$, $K^+$ in drug sample.

vi) Detection of calcium levels in bones
vii) Determination of lead in Petrol.
viii) Measurement of $Na^+$, $K^+$ level in dialysis fluid.

Difference between AAS and AES

| AAS | S.N | AES |
|---|---|---|
| Most of the free gaseous atomic(atomic vapour) are remain in a ground state. | 1. | Most of the free gaseous atoms are excited in higher energy state and emission can be obtained as photon. |
| Absorption will take place in AAS | 2. | Emission will be take place in AES |
| Line source Hollow cathode lamp (HCL) or EDL required in AAS | 3. | Line source is not required in AES |
| AAS requires high temperature to get free gaseous atoms for absorption | 4. | Emission spectra for alkali and alkaline earth metals require low temperature for excitation |
| AAS has superior quality of monochromator and detector | 5. | AES has the inferior quality of monochromator and detector. |
| AAS is more expensive | 6. | AES is less expensive than AAS. |
| More than 60 metals can be estimated in AAS | 7. | Few metals can only be estimated in AES. |
| AAS is more accurate, selective and precise than AES | 8. | AES is less accurate, selective and precise than AAS. |

Limitations of AES

i) limited number of elements that can be analyzed.
ii) The sample requires to be introduced as solution into fine droplets. Many metallic salts, soil, plants and other compounds are insoluble in common solvents. Hence, they

can't be analyzed by this method.

iii) Since. sample is volatilized. if small amount of sample is present. it is tough to analyze by this method. As some of it's gets wasted by vapourisation.

iv) Further during solubilization with solvents. other impurities might mix up with sample and may lead to errors in the spectra observed.

v) The temperature is not high enough to excite transition metals. therefore the method is selective towards detection of alkali and alkaline earth metals.

vi) The relatively low energy avaiable from the flame leads to relatively low intensity of the radiation from the metal atoms.

vii) The low temperature renders to interferance and the stability of the flame and aspiration conditions.

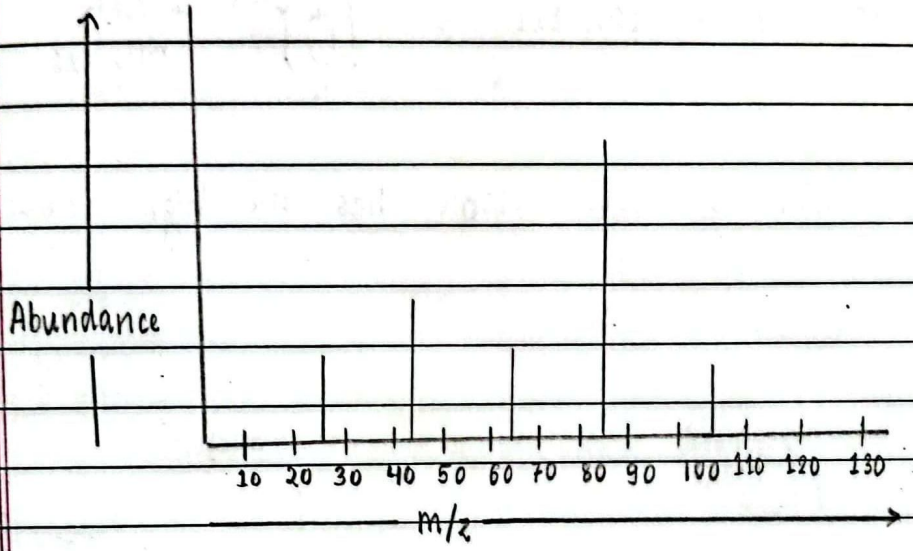
viii) Interferance by other elements is not easy to be eliminated

# Mass spectroscopy

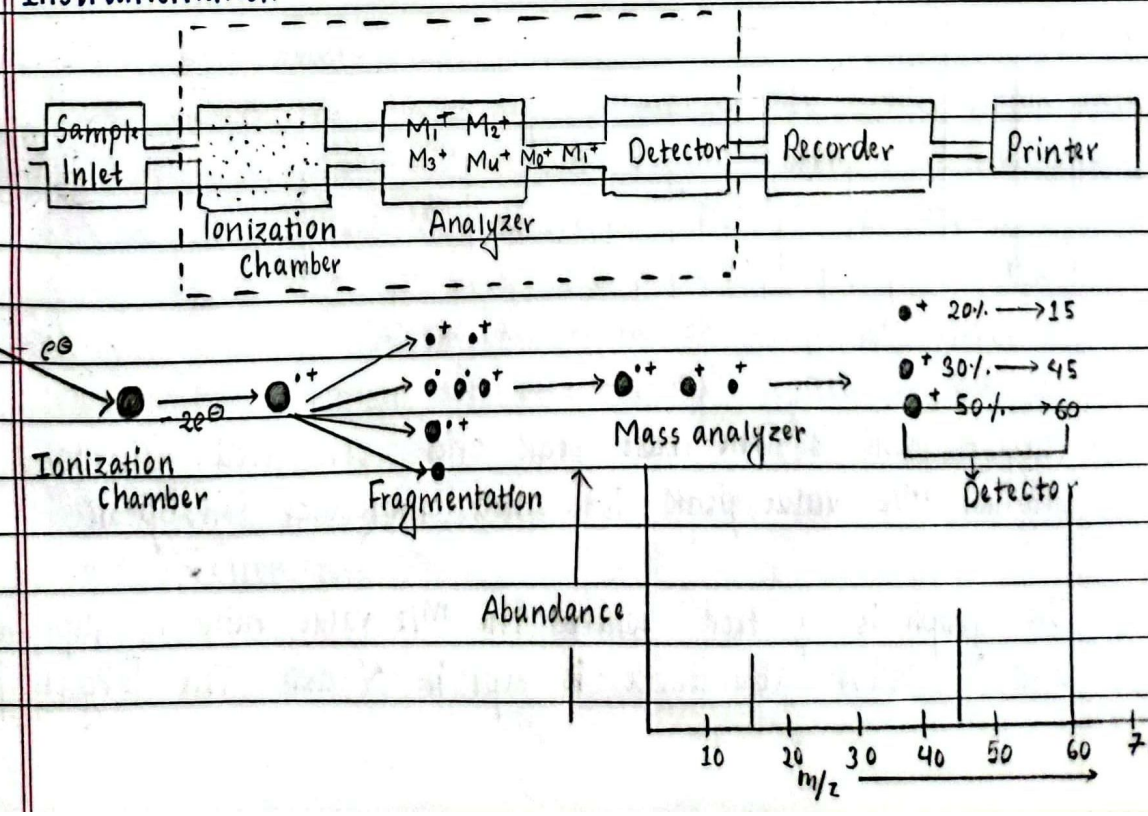
Date 102
Page 2078-12-02

## Principle

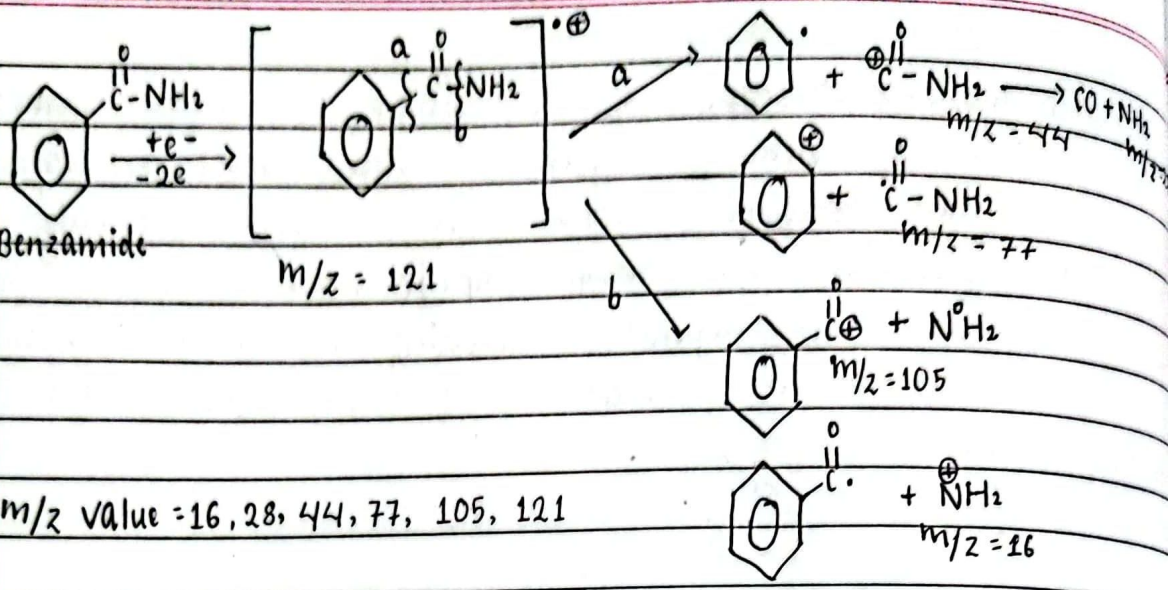
In mass spectroscopy samples which have to be investigated is introduced either in form of fine aerosol or vapour which are then bombarded with electrons. that not only ionizes the molecules but may also fragment them. the fragmented ions are then seperated according to the mass/charge ratio and the relative abundance of ions are detected by detector.




## Instrumentation

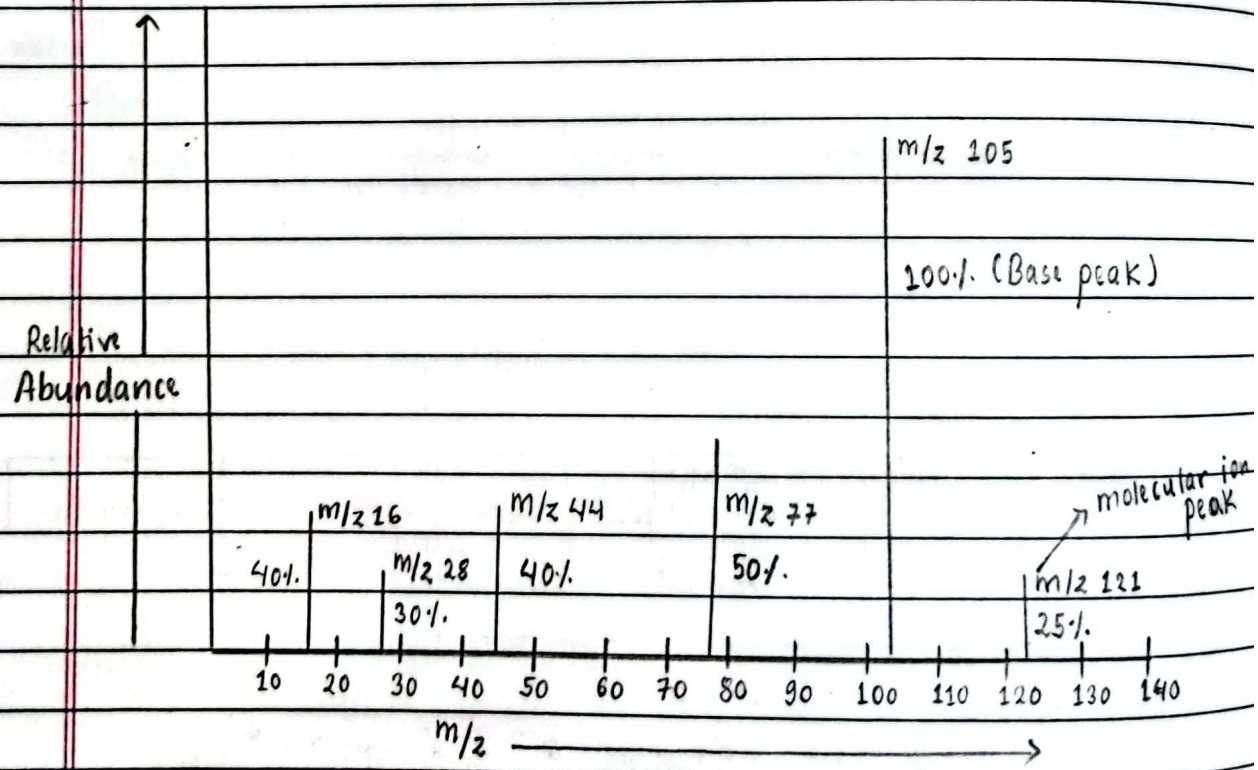


For benzamide




m/z value = 16, 28, 44, 77, 105, 121

- Those ions which is more stable has the high abundance.

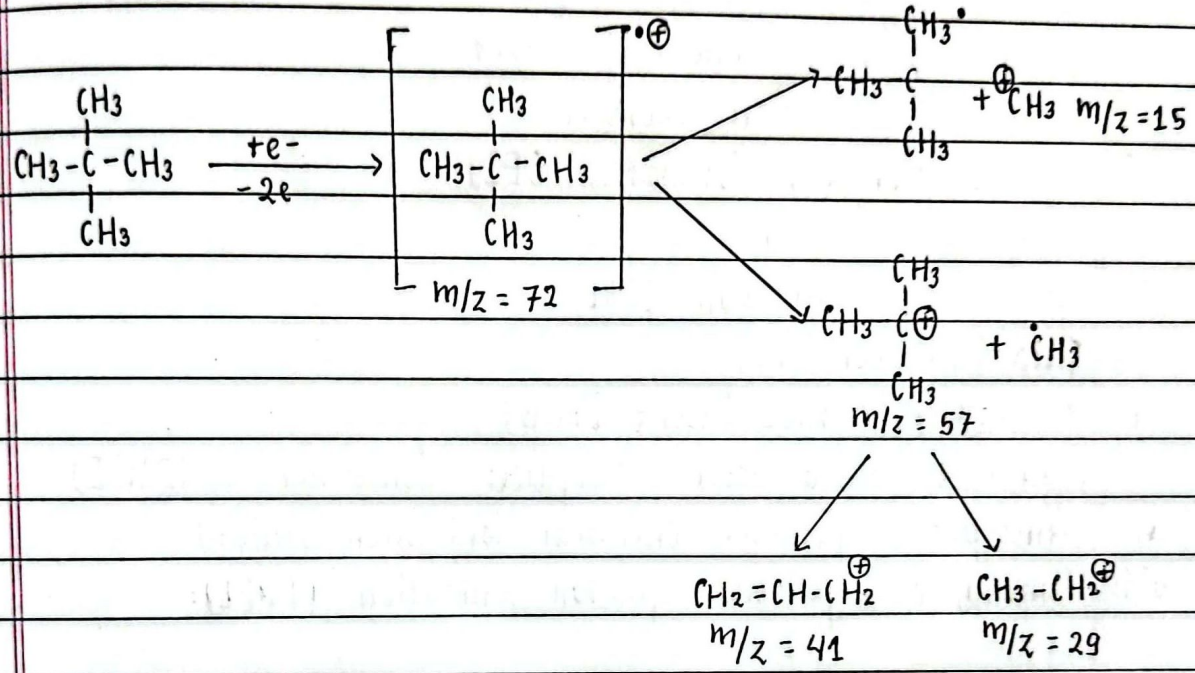



- Largest peak is the base peak and considered as 100%.
- Highest m/z value peak is called molecular ion peak.

The graph is plotted where the m/z value ratio is kept in X-axis and relative abundance is kept in Y-axis. The largest peak

is called base peak and the highest m/z value peak is called molecular ion peak and gives the molecular mass of the compound.

For Neopentane (2-2-dimethyl propane)




## Instrumentation

a) Sample inlet system

Liquid sample are generally introduced by hypodermic needles injection through a silicon rubber diam. A gas phase analyte is introduced directly into source region of the mass spectrometer through the needle valve. The liquid chromatography inlets are used to introduce thermally labile compounds which are not easily separated by gas chromatography. Similarly, direct insertion Probe is widely used to introduce low vapour pressure liquids and solids into the mass spectrometer.

b) Ion source

The Ion source is the part of mass spectrometer that ionize the material under analyte. The ions are then transported by magnetic or electric fields to the mass analyzer. Molecular ions are formed when energy of the electron beam reaches to 10eV. Similarly, fragmentation of the ion reaches only at a higher boambardment energies of 70ev.

Types of Ionization chamber

i) Electron impact ionization (EI)
ii) Chemical ionization
iii) Electrospray ionization (ESI)
iv) Field ionization (FI)
v) Fast atomic boambardment [FAB]
vi) Matrix associated laser desorption ionization (MALDI)
vii) Atmospheric pressure chemical ionization (APCI)
viii) Atmospheric pressure photon ionization (APPI)

i) Electron impact ionization

- Sample inlet in gaseous form
- Mainly used in Gas chromatography MS
- High energy 70 electron volt is used for ionization
- Mainly for volatile and thermally stable molecule.
- Mainly for non-polar compound
- less than 400 dalton
- Fragmentation pattern is similarly for same compound so there is library of existing compound which can be identified by comparision with the compounds in library.
- It is hard ionization technique.

ii) As there is existence of library, it can be used for identification of compound.

Disadvantages

i) Not used for the high molecular weight compound.

ii) Not used for thermally unstable compound because they may get fragmented and molecular mass cannot be determined.

iii) chemical ionization

- Mainly used in GC-MS
- Sample must be introduced in gaseous or vapour phase.
- At first reagent gas is ionised by bombardment of electrons and then ionized gas reacts with the analyte to give $M^{+1}$ or M-1 peak.
- Reagent gas used are methane, Isobutane and ammonia
- Chemical ionization can be positive chemical ionization or negative chemical ionization.

$$CH_4 + e \xrightarrow{2e} CH_4^{\bullet +}$$

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

$$CH_3^{+} + M \longrightarrow [M+H]^{+} + CH_4$$

Sometimes,

$$CH_4^{\bullet} + CH_3^{\bullet} \longrightarrow C_2H_5^{+} + H_2$$

$$C_2H_5^{+} + M \longrightarrow [M+H]^{+} + C_2H_4$$

Sometimes,

$$C_2H_5^{+} + M \longrightarrow [M + C_2H_5]^{+} (M+29)$$

Advantage

- Can get the molecular mass of compound which is difficult to get from Electron Impact technique.

Disadvantages

- Sometimes we get M+ 29 peak
- There is no library so we cant identify compound.

iii) Field ionization

- High electric field is applied
- Due to high voltage molecule get ionized but no fragmentation take place
- There is M+1 and M-1 peak
- Sample introduced is in gaseous phase
- Used in GC-MS.

iv) Electrospray ionization (ESI)

- It is soft ionization technique.
- Mainly used in LC-MS
- Sample in form of fine aerosol is sprayed with electrons as solvent evaporates the charge density on the sample increases and the like charge repeal each other and we can get M+1 and M-1 peak.

Advantages

i) Used for the volatile and thermally unstable compound.
ii) Suitable for the high molecular mass compound

Disadvantages

i) There is no library
ii) Not suitable for a polar compound.

v) Fast atomic bombardment (FAB)
- Medium type of ionization
- Used in LC-Ms
- In this technique inert gaseous atom like Argon, xenon are used to bombard the molecule. At 1st inert gas are ionized and accelerated in high electric field, this inert gaseous atoms strikes the molecules at high speed and molecules get ionized.
- Used for high molecular mass compound
- Sample introduced in form of fine aerosol.

vi) Matrix associated laser desorption ionization (MALDI)
- Used in LC-Ms
- Used for the high molecular mass compound like protein, Nucleic acid.
- Molecule is adsorbed in the surface of matrix and when laser passed because of high energy laser it get desorped while desorption molecules get ionized.
- M+1 or M-1 peak obtained.

vii) Atmospheric pressure chemical ionization (APCI)
- Used for moderately polar to polar compound
- Used in LC-Ms
- Solvent is ionized and ionized solvent reacts with molecule at atmospheric pressure
- M+1 or M-1 peak obtained.

viii) Atmospheric pressure photo ionization (APPI)
- UV-light is used to irradiate the molecule because of high energy of UV light molecule get ionized at

at atmospheric pressure.
- Sample introduced in form of aerosol.
- Used in LC-MS.

Secondary ionization Mass spectroscopy (SIMS)
Solvent ionization → Primary ion → Primary ion may fragment for secondary ion → Secondary ion reacts with molecule (analyte)

Direct analysis at real time (DART)
Used singly not in combination with GC or LC.

c) Mass analyzer
The ions produced in the ionization chamber are accelerated towards the mass analyzer by the application of potential of 2 to 8 Kv. The main function of the mass analyzer is to separate or resolved the ions formed in the ionization source of the mass spectrometer by the deflection of ions towards the magnetic field and on the basis of their mass/charge ratio.

i) Magnetic sector
- Single focusing
- Double focusing

ii) Time of flight (TOF)
iii) Ion trap
iv) Fourier transform ion cyclotron resonance (FT-ICR)
v) Quadruple
vi) Tandem-MS

ii) Magnetic sector mass analyzer

- Single focusing
- It has horse shoe shaped glass tube which is evacutated, consist of sample inlet, electron bombarding source, accelerating plates on one end and collector slit at other end.
- At curvature of tube there is provision to apply etectric/ magnetic field.
- Sample in the form of vapour is allowed through inlet and bombarded with electron beam at 70ev.
- It knocks of one electron from every molecule then, they become +vely charged ions.
- As these molecules become +ve charged, they are accelerated by accelerating plates and travel in straight path.
- By application of electric or magnetic field they travel in curve path and molecular ions are separated according to their masses and collected.
- Different fragments fall on detector then mass spectrum is recorded.

- Double focusing
- It is used differentiate the small mass differences of the fragment.
- These provide the high resolution.
- To acheive better focusing, energy has to be reduced before ions are allowed to enter the magnetic field and increase resolving power can be obtained two mass analyzer in series.
- In a double-focusing mass analyzer beam is first passes radial electrostatic field.

Advantages

- Classical mass spectra
- Very high reproducibility
- High resolution
- High sensitivity
- High dynamic range

Limitations

- Not well suited for pulse ionized method (eg:- MALDI)
- Usually larger and higher cost than other mass analyzers.

Applications:-

- All organic MS analysis method
- Accurate mass measurement
- Isotope ratio measurements

iii) Time of Flight (TOF)

- In this type of analyzer the sorting of ions is done in absence of magnetic field.
- The ions produced are acquiring different velocities depending on their masses.
- Here, the particles reach the detector in the order of increasing order of their masses.
- Here electron multiplier detector is used. The resolution power of this 500-600

Advantages:-

i) Theoritical unlimited mass
ii) Moderate to high resolving power
iii) Moderate cost
iv) Relatively high duty cycle
v) Instrument is not scanning.

iii) Ion trap

Ion trap mass analyzers use a combination of electric or magnetic fields to capture or "trap" ions inside the mass analyzer. Multiple configuration in ion trap

- 3D ion traps
- a linear ion trap (2D trap)
- Electrostatic trap (orbitrap)
- Magnetic field based trap (Ion cyclotron resonance)

The ion traps functions as a mass spectrometer when the trapping field is changed, so that the trajectories of simultaneously trapped ions of consecutive specific mass/charge ratio becomes subquentially unstable, and ions leave the trapping field in order of mass/charge ratio.

- Most commonly used for qualitative work (eg:- metabollic identification, protein identification and screening applications).

iv) Fourier transform ion cyclotron resonance (FT-ICR)

- Ion in a magnetic field move in circular orbits characterstics of their m/z values.
- If energy is provided at a frequency equal to their precession frequency, and in a direction perpendicular to their plane of precession.
- Ions will absorb the energy, enabling them to be detected.

Advantages

- The highest recorded mass resolution of all mass spectrometers.
- Powerful capabilities for ion chemistry and MS/MS experiments.
- Well suited for use with pulse ionized method such as MALDI.
- Non-destructive ion detection.

- Stable mass callibration in superconducing magnet FTICR systems.

Limitations:-

- Limited dynamic range
- Strict low pressure requirements mandate in external source for most analytical applications.
- Subject to space charge effects and ion molecule reactions.

Applications:

- Ion chemistry
- High-resolution electrospray experiments for high-mass analytes.
- laser-desorption for materials and surface characterization.

v) Quadruple

- It consist of 4 voltage carrying rods.
- The ions are passed from one end to another end.
- During this apply the radiofrequency and voltage compkx oscillations will take place.
- Here the single positive charge ions show the stable oscillation and the remaining shows the unstable oscillations
- Mass scanning is carried out by varying each of the radio-frequency and voltage frequencies ratios keeping their ratios constant.
- Quadrupole ion storage (ion trap)
- It store the unstored ions temporarily. they released to the detector by scanning the electric field.

Advantages

- classical mass spectra
- Good reproducibility
- Relatively small and low cost-system.

Limitations

- limited Resolution
- Peak heights variable as a function of mass
- Not well suited for pulse ionization method.

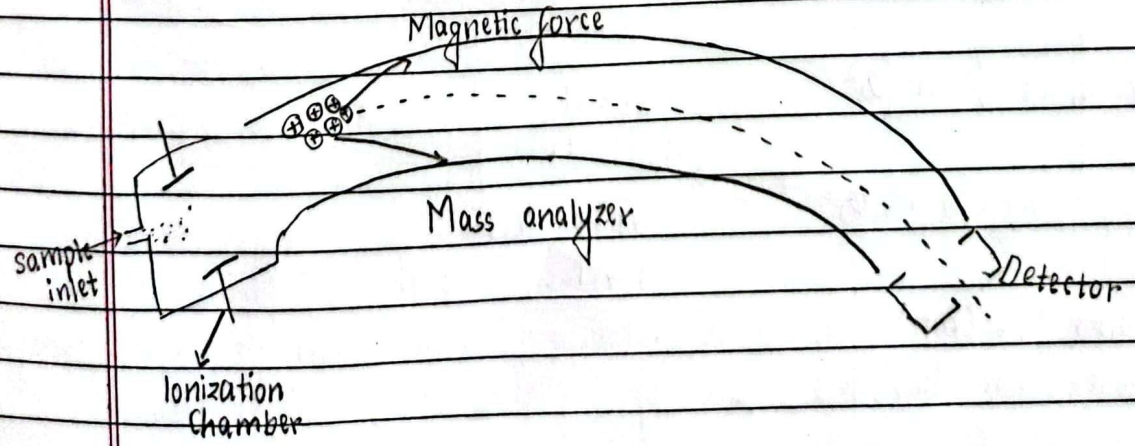
Applications

- In GC/MS and LC/MS system
- Triple quadrupole MS/MS system
- Bimolecular detection

vi) Tandem- MS

It is also known as MS/MS or $MS^2$, is a technique in instrumental analysis where there is two or more mass analyzers are coupled together using an additional reaction step to increase their abilities to analyse chemical sample. A common use of Tandem- MS is the analysis of biomolecules, such as proteins, peptides.

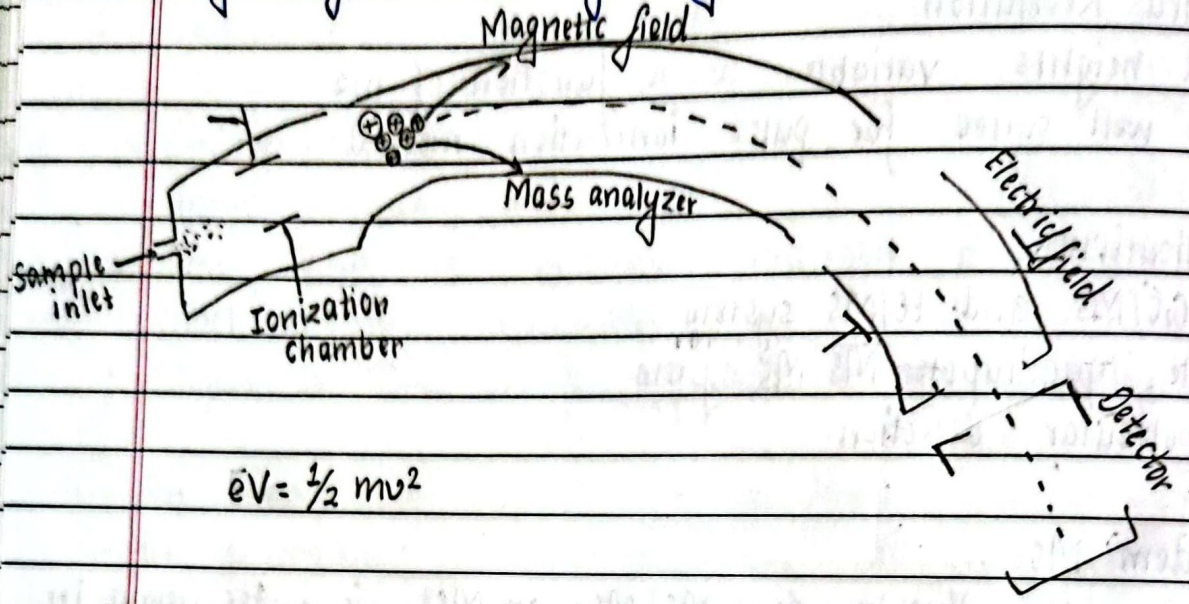
Magnetic sector single focusing




$Bev = \frac{mv^2}{r}$

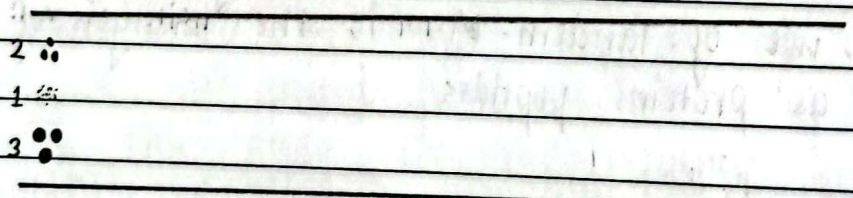
B = Magnetic field
e = Charge
v = velocity

## Magnetic field double focusing




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

## Time of flight (TOF)




K.E = $\frac{1}{2} mv^2$

For 1, K.E = $\frac{1}{2} m_1 v_1^2$

For 2, K.E = $\frac{1}{2} m_2 v_2^2$

For 3, K.E = $\frac{1}{2} m_3 v_3^2$

$V_1 = \frac{d}{t_1}$

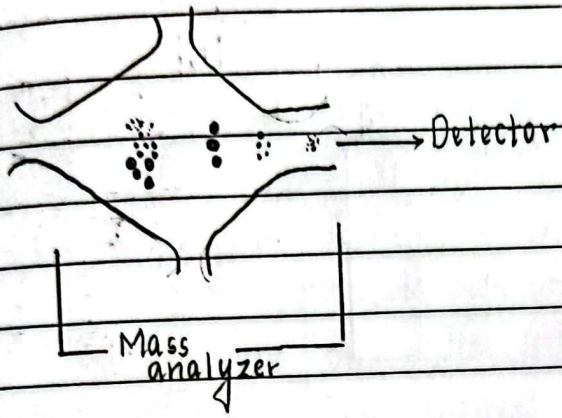
$V_1 t_1 = V_2 t_2$

↑ velocity . ↓ Time of flight

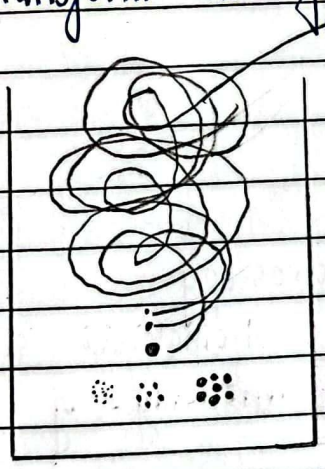
Smaller m/e (ion) has more velocity than larger m/e value ion. So, smaller ion has less time of flight than larger ion.

## Ion trap

In this technique after ionization in ionization chamber all the ions are stored in chamber then, they are released sequentially according to their m/z value.



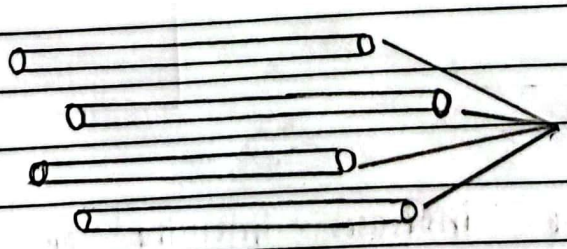

## Fourier transform ion cyclotron resonance



→ smaller ion releases 1st because they are light.

later on larger particles releases

## Quadruple



4 volatage carrying rod

## Tandem MS

$Q_1$ : M/z value separation

$Q_2$ : inert gas (Argon, Nitrogen)

$Q_3$ : fragmentation

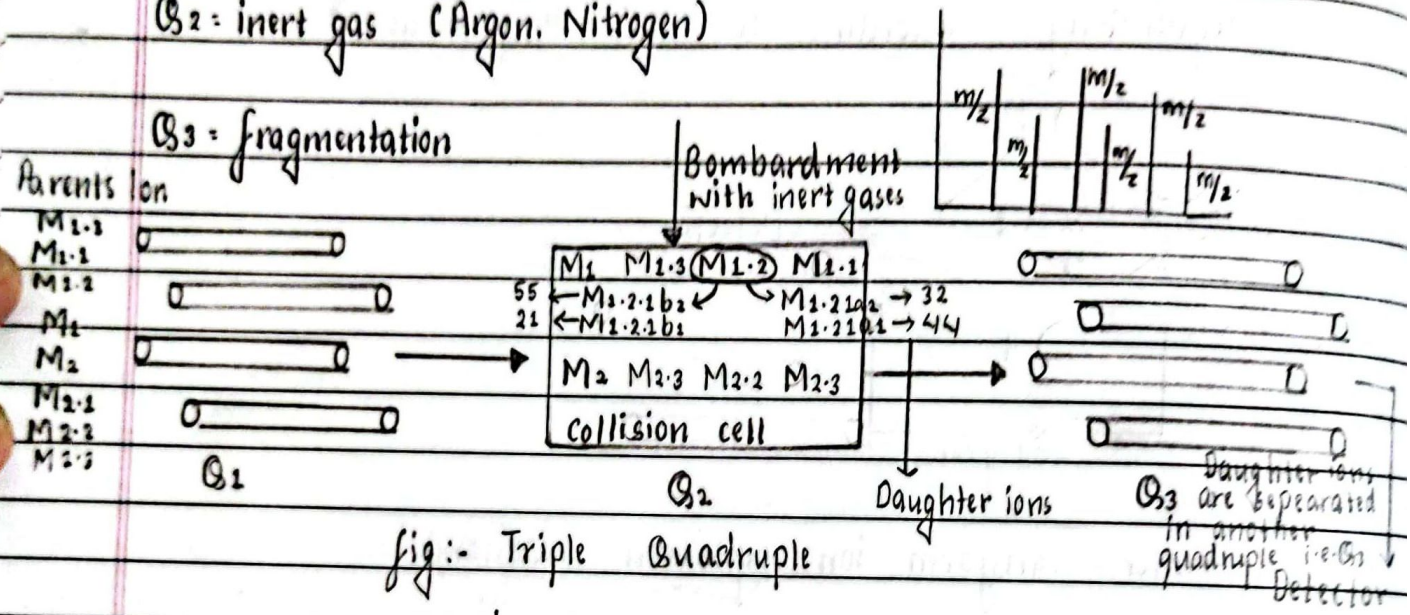


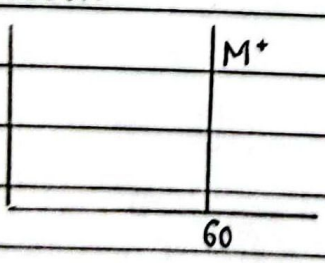

fig :- Triple Quadruple

- Can be detect upto a lower ppb level.
- More ability for analysis.

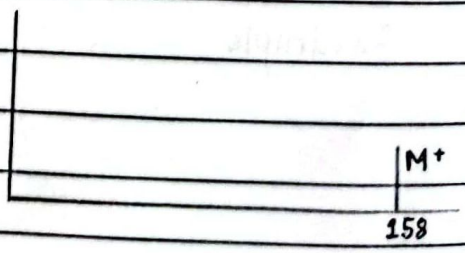
## Fragmentation rules in Mass spectroscopy

1) As the no. of $-CH_2$ group in a homologous series of a particular functional group increases the intensity of molecular ion peak decreases.

$CH_3COOH$

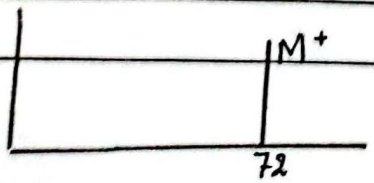



$CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-COOH$

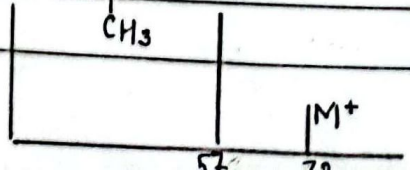



2) As the branching increases intensity of molecular ion peak decreases

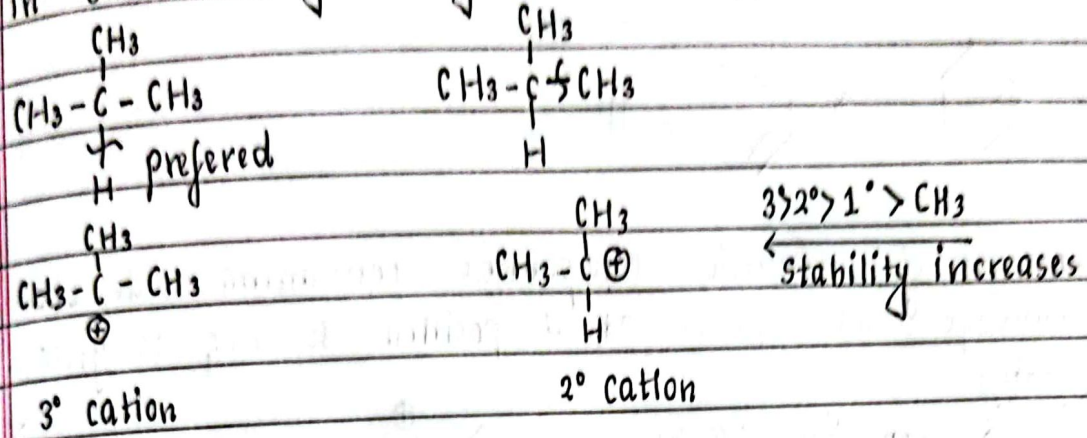
$CH_3-CH_2-CH_2-CH_2-CH_3$



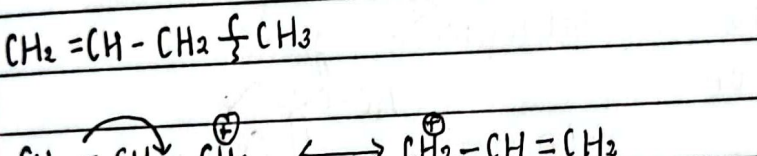

$CH_3-\underset{\underset{CH_3}{|}}{C}-CH_3$



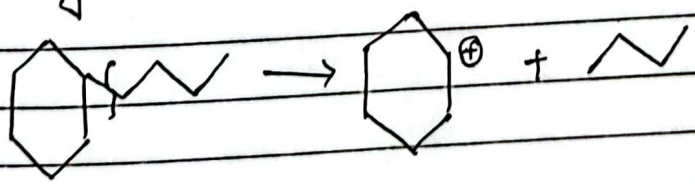
3) While undergoing for fragmentation, fragmentation takes place in such a way to get more stable carbocation.



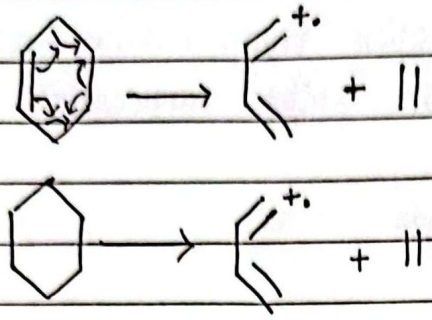

4) In case of open chain double bonded compound cleavage takes place at the carbon next to double bond to yield allylic cation.



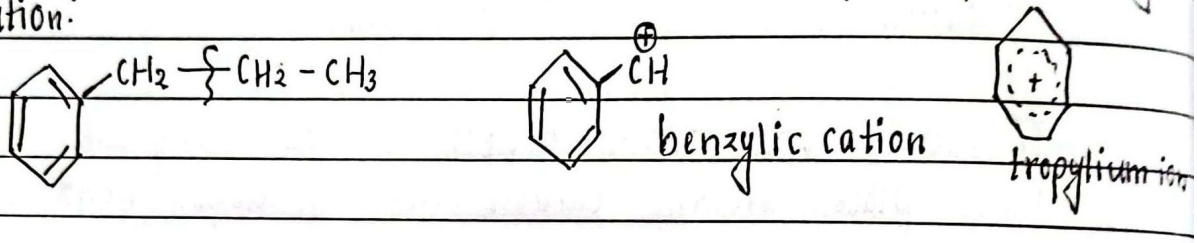

5) In case of cylic aliphatic compounds i.e. cycloalkanes. containing side chain. cleavage takes place of the bond between the ring and side chain and +ve charge remains in ring.



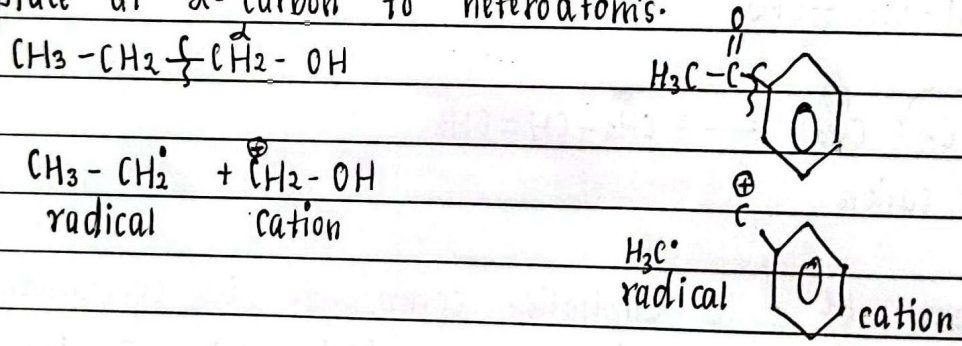

6) Diels - Alder reaction
In case of cyclo hexane cleavage takes place to follow retro Diels - Alder reaction.



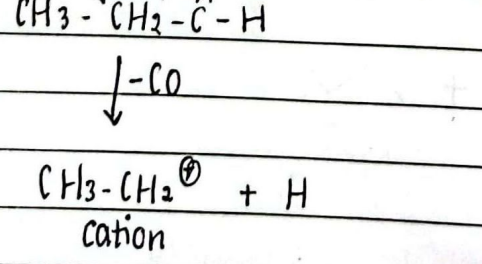
7) In case of aromatic compounds containing side chain cleavage takes place at α- position to ring to yield benzylic cation.




8) In case of compound containing hetero atoms cleavage takes place at α- carbon to heteroatoms.




9) Sometimes cleavage takes place with the elimination of small group such as HX, $H_2O$, $NH_3$, CO etc.

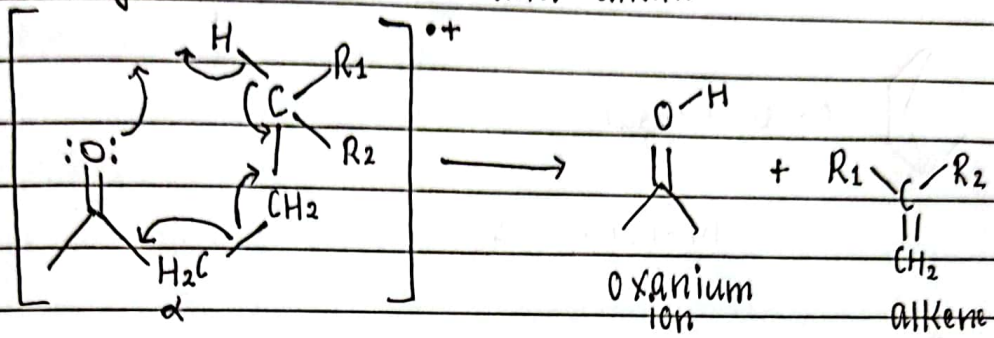



10) Mc Lafferty rearrangement

 → Aromatic compounds

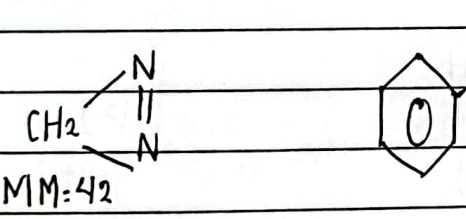


Carbonyl compound containing gamma hydrogen undergoes the special type of rearrangement while fragmentation. formation of oxanium ion and alkene




## Nitrogen rule

If the molecular mass of compound is odd then, there is presence of odd number of nitrogen and if the molecular mass of compound is even then, either it contains no nitrogen or even number of nitrogen atoms.




## Ring rule or molecular formula Index for hydrogen molecule deficiency.

1- There is 1 ring or 1 double bond
2- There is 2 ring or 2 double bond or 1 triple bond.
0- No multiple bond. no ring

$$\text{Molecular hydrogen deficiency (MHO)} = C - \frac{H}{2} - \frac{X}{2} + \frac{N}{2} + 1$$

Dimethyl sulphoxide.

$CH_3-S-CH_3$

$MHO = 2 - \frac{6}{2} + 1$

$= 2 - 2 = 0$

$CH_3-\overset{\overset{O}{\uparrow}}{S}-CH_3$

It means there is no double bond.

$CH_3-\overset{O}{\overset{\|}{C}}-CH_3$ $M.HD = 3 - 6/2 + 1$

$= \frac{6-6+2}{2}$

$= 2/2 = 1$

$M.HD = 7 - \frac{12}{2} + 1$

$= \frac{14-12+2}{2} = 2$

$M.HD = 6 - 6/2 + 1$

$= \frac{12-6+2}{2} = \frac{8}{2} = 4$

Rule of 13

Divide molecular mass by 13.

Quoient represent number of CH.

Reminder represent number of H.

$CH_3-CH_2-CH_2-CH_2-CH_3$ (Pentane)

$C_5H_{12}$

$= 12 \times 5 + 12$

$= 60 + 12$

$= 72$

$13 \overline{)72} (5$

$-65$

$7$

If there is Nitrogen. $N = 14$

$(CH)_x\ Hy + N - CH_2$

If there is oxygen. $O = 16$

$(CH)_x\ Hy + O - CH_4$

$(CH)_x\ Hy$ ← remainder

↑ quotient

$(CH)_3 + H_7 = C_3H_{10} + O - CH_4$

$= C_2H_6O$

$13 \overline{)46} (3$

$-39$

$7$

$CH_3-CH_2-OH$

ethanol

1 = M.H.D.

1 = Type of proton

C30 = 2 type of carbon.

IR = 1690 - 1760

Mass = 58

13 ) 58 ( 4 C-8
-52 H-4
6

$(CH)_6 H_4 + 16 - CH_4$ $C_6H_{60} + O - CH_4$
$C_5H_6O$

$CH_3-\overset{O}{\overset{\|}{C}}-CH_3$

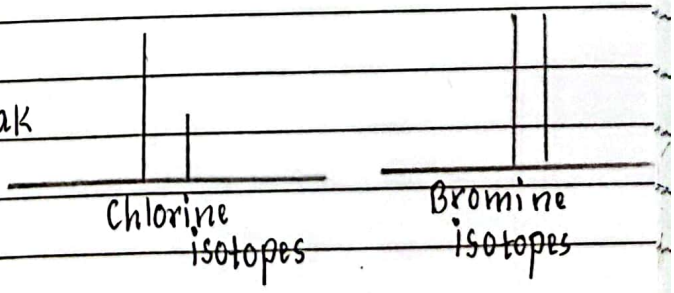
Q. Mass = 60

H-NMR = 4 type of proton
C-30 NMR = 3 type of carbon
IR = 3600 - 3200 $cm^{-1}$
= 1200 - 1000 $cm^{-1}$

13 ) 60 ( 4 C-8
-52 H-4
8

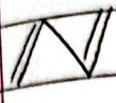
$(CH)_8 H_4 + O - CH_4$ $C_8H_{\cdot}O - CH_4$
$C_7H_8O$

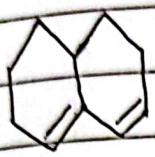
Isotope rule
Chlorine Isotope 1:3 >2 in peak
Bromine 50:50 i.e. 1:1




Applications of Mass spectroscopy
i) It helps to gives the molecular mass.
ii) It helps in structural illucidation.
iii) It helps the interpretation. Quantification. authentication.

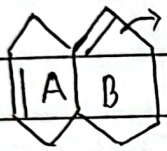
iv) It helps to give the purity of raw materials.
v) It gives the structural illucidation of novel compounds.
vi) Determination of pesticides residue in foods.
vii) Monitoring gases in patient's breath during surgery.
viii) Identification of compounds in TLC.
ix) Identification of drugs and metabolites of drugs in blood, urine, saliva.

 → conjugated dienes

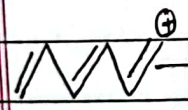
 → heteroannular diene

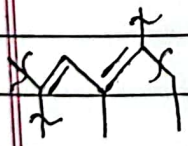
 → Homoannular diene

 → Endocyclic double bond

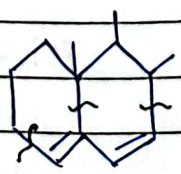


→ Endocyclic for ring B
Exocyclic for ring A

 → Extended conjugated double bond

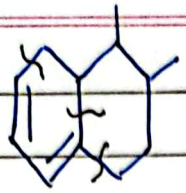
 → Alkyl substituent or Ring residue

Alkyl residue → 5nm
Homoannular → 39 nm
Exocyclic double bond → 5nm
Extended conjugation → 30nm
Parent chromophore → 214 nm

* 

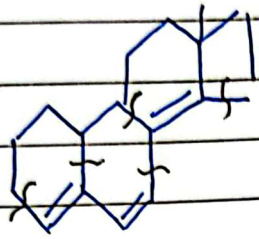
alkyl residue = 3×5 = 15
Parent chromophore = 1 = 214
Exocyclic double bond = 1 = 5
Total = 234
observed = 235

*



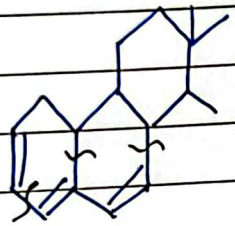
alkyl residue = 3×5 = 15
Parent chromophore = 214
Homoannular diene = 39 Total = 273 nm
Exocyclic double bond = 5

*



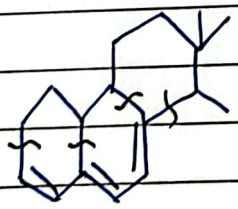
Parent chromophore = 214
alkyl substituent = 5×5 = 25
Exocyclic double bond = 3×5 = 15 Total = 284 nm
Extended conjugation = 1 = 30

*



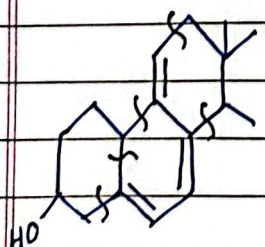
alkyl substituent = 3×5 = 15
Extended conjugation 1 = 30
Parent chromophore = 214 Total = 303 nm
Homoannular diene = 1 = 39
Exocyclic double bond 1 = 5

*

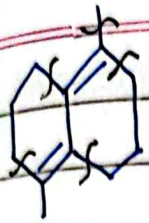


alkyl substituent = 4×5 = 20
Parent chromophore = 214
Exocyclic double bond = 5×2 = 10 Total = 313 nm
Homoannular diene = 1 = 39
Extended conjugation = 1 = 30

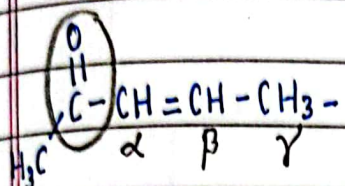
*



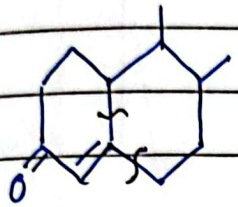

alkyl substituent = 5×5 = 25
Parent chromophore = 214
Extended conjugation = 1 = 30 Total = 323 nm
Homoannular diene = 1 = 39
Exocyclic double bond = 3×5 = 15



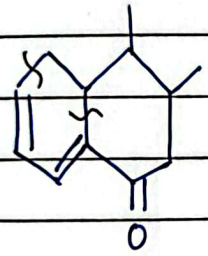
alkyl substituent = 5
Parent chromophore = 214 Total = 254 nm
Exocyclic double bond = 2×5 = 10



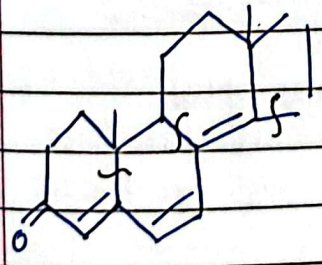

α = 10 β = 12 γ = 18




Parent chromophore = 215
α- alkyl = 0
β- alkyl = 2×12 = 24 Total = 244 nm
Exocyclic double bond = 1×5 = 5



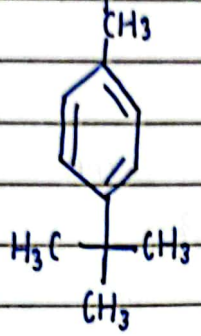

Parent chromophore = 215
α- alkyl = 1 = 10
homo annular diene 1 = 39 Total = 292 nm
exocyclic double bond 1 = 10
γ- alkyl = 1 = 18




Parent chromophore = 215
Exocyclic double bond = 2×5 = 10
γ- alkyl = 3×18 = 54
Extended conjugation 2×30 = 60
β- alkyl = 1 = 12
Total = 351 nm.

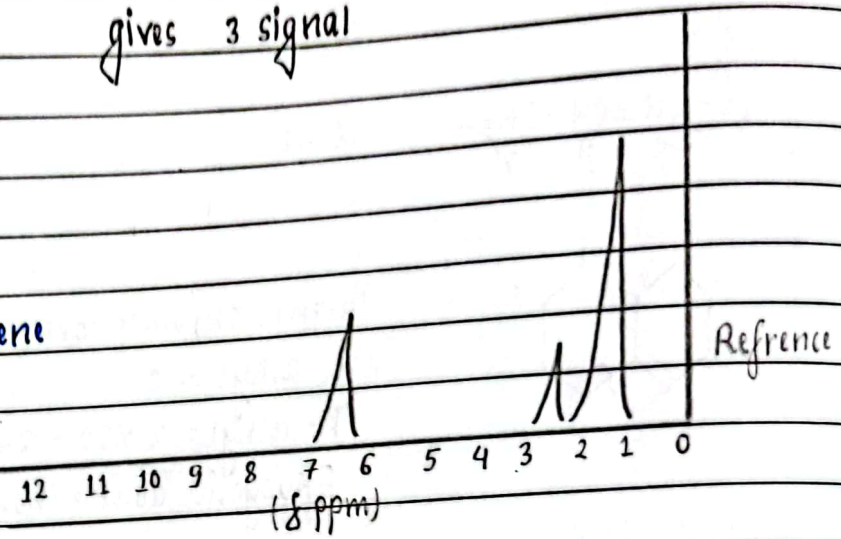
• Write the expected NMR spectra of following compound taking TMS as per the standard refrence

a)




gives 3 signal

P-ter-butyl toulene

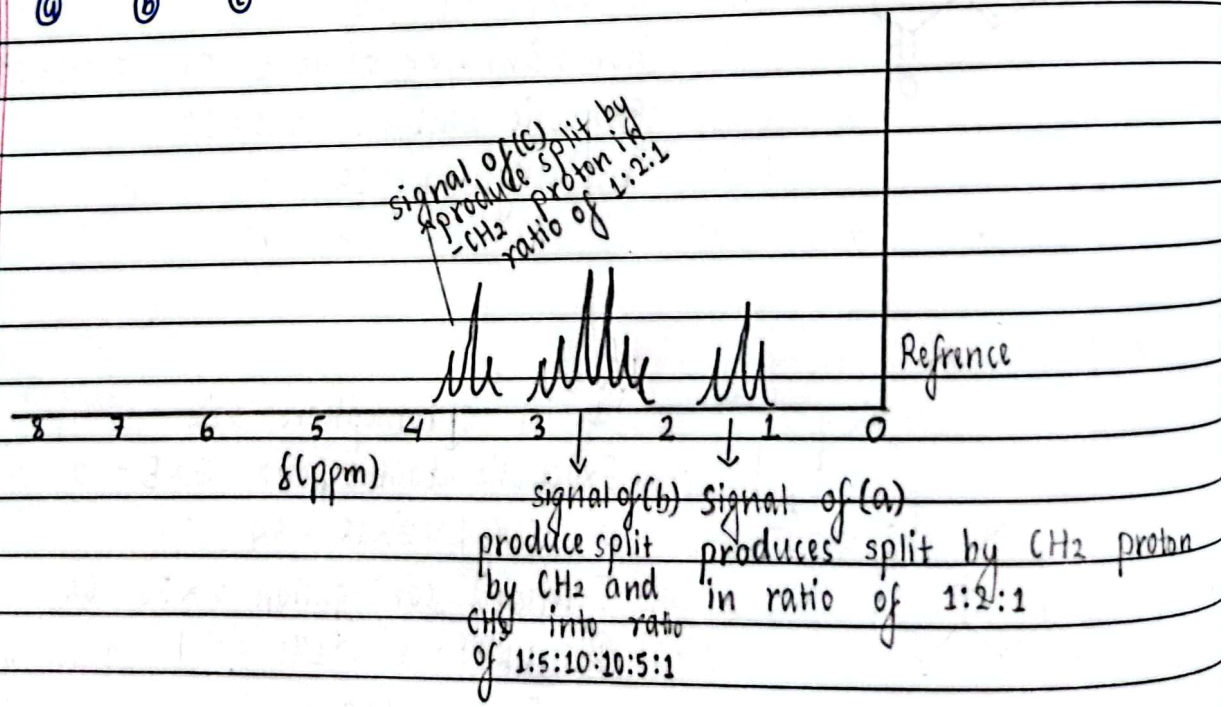



b) 1- bromo propane

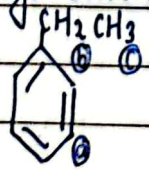
$CH_3 - CH_2 - CH_2 Br$

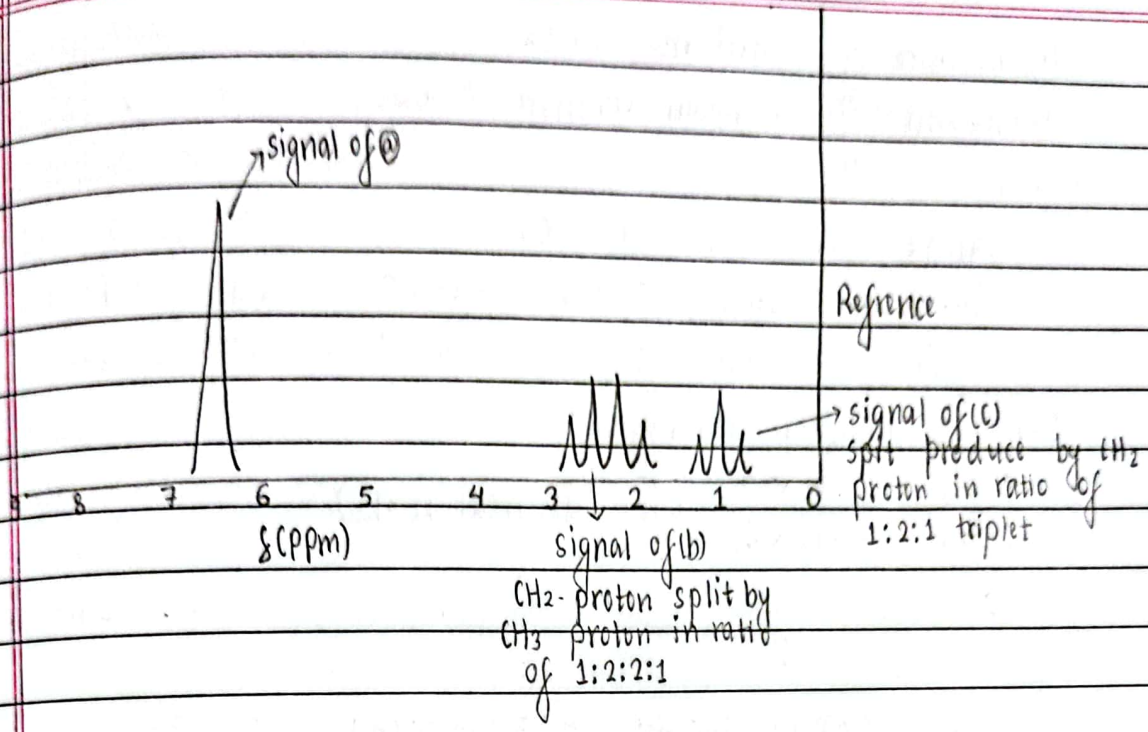
ⓐ ⓑ ⓒ

gives 3 signal

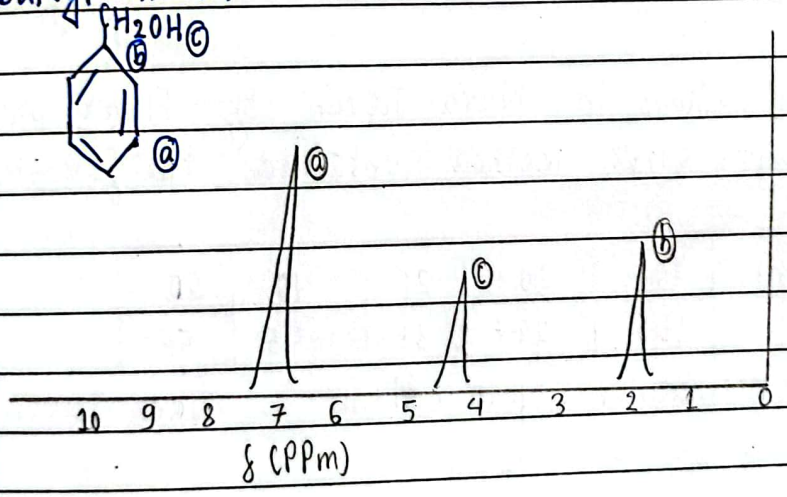



c) Ethyl benzene






d) Benzyl alcohol




2063. The absorption of spectrum for titanium peroxide complex ion in perchloric acid showed at maximum at 400nm. The absorbance of 32.0 mcg/ml solution of titanium gives an absorption of 0.456. An unknown solution treated in an ideal fashion give an absorbance of 0.50. Assuming identical cell, find out the concentration of unknown solution.

Solution:-

$\lambda max = 400 nm$

conc of titanium solution = 32.0 mcg/ml = 0.032 mg/ml
= 3.2 mg/100 ml

Absorbance of titanium = 0.456
Absorbance of unKnown solution = 0.501
From.
Labert beer's law $A = \varepsilon CT$
For titanium solution $0.456 = \varepsilon \times t_1 \times 3.2$ — (i)
For unKnow solution $0.501 = \varepsilon \times t_2 \times C$ — (ii)
Dividing equation (i) by (ii)

$$\frac{0.456}{0.501} = \frac{\varepsilon \times t_1 \times 3.2}{\varepsilon \times t_2 \times C} \quad (\text{for identical } t_1 = t_2)$$

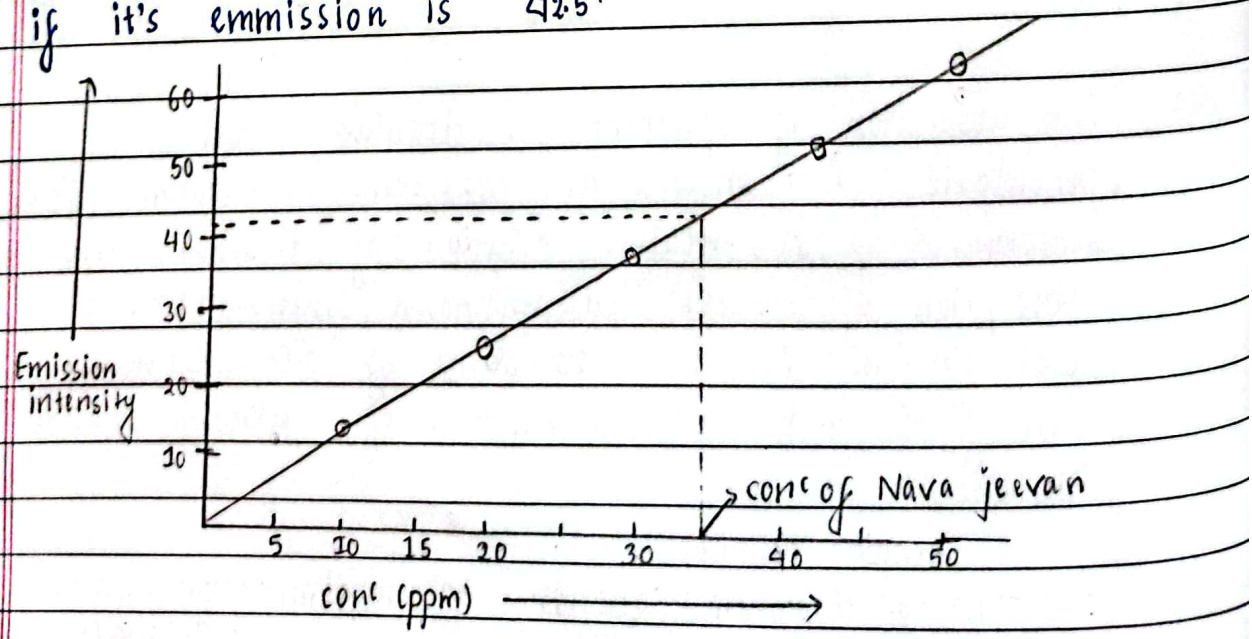
$$\therefore 0.9102 = \frac{3.2}{C}$$

$\therefore C = 3.51$ mg/100 ml $= 35.1$ mcg/ml
∴ The concentration of unKnown solution is 35.1 mcg/ml.

* Estimation of sodium in Nava-Jeevan by Flame photometer using callibration curve method obtained the following observation.

| Conc of Na (ppm) | 10 | 20 | 30 | 40 | 50 |
|---|---|---|---|---|---|
| Emission | 11.5 | 22.7 | 31 | 45 | 55 |

Find the concentration of sodium in Nava-Jeevan sample if it's emmission is 42.5.



* Estimation of sodium in Nava-Jeevan by Flame photometer using standard addition method was done. The standard solution of sodium has concentration of 500ppm. Volume of the sample taken is 10 ml. The observation are as follows:-

| Vol. of standard added (ml) | 0 | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| Emission | 4.5 | 12 | 18.8 | 24.3 | 29.3 | 50.2 |

Find the concentration of sodium in Nava-Jeevan using the plot and using the equation i.e. by extraplotation.

Solution:-

Standard solution of Na = 500 ppm

Volume of sample added = 10 ml

Conc of solution when 0 ml standard added = 0

Conc of solution when 1 ml standard added.

$500 \times 1 = 11 \times x$

$x = 45.45$

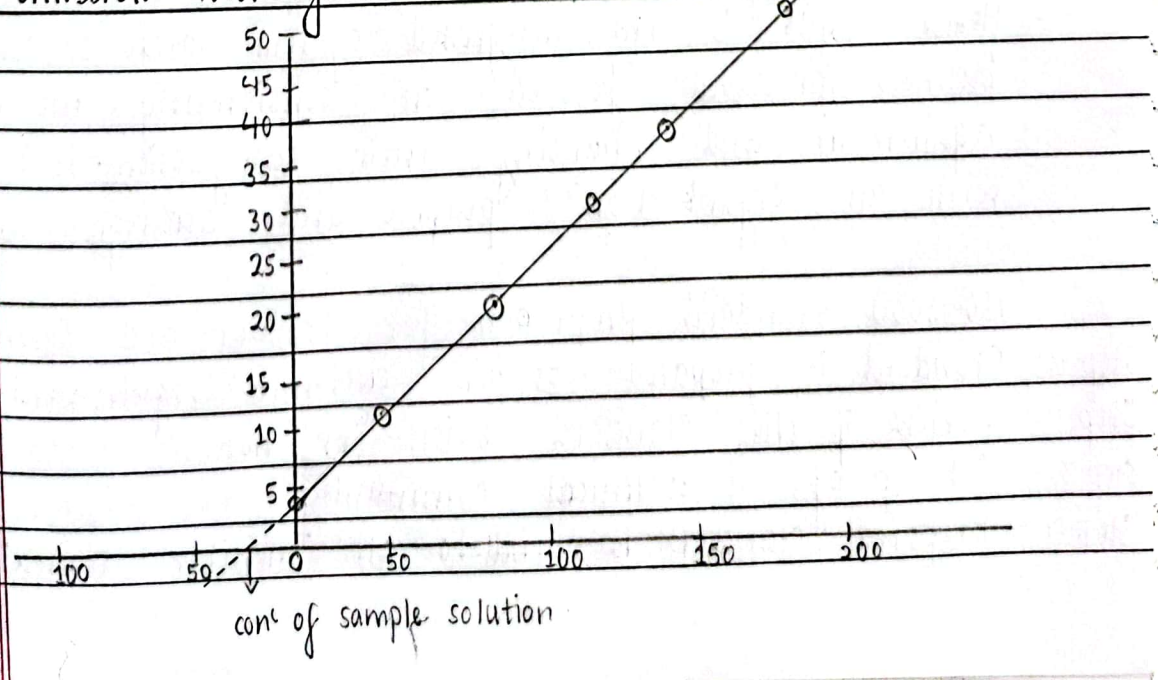
when. 2ml added $500 \times 2 = 12 \times x = 83.33$

when. 3ml added $500 \times 3 = 13 \times x = 115.38$

when. 4ml added $500 \times 4 = 14 \times x = 142.85$

when. 5ml added $500 \times 5 = 15 \times x = 166.66$

| Conc of Navajeevan | 0 | 45.45 | 83.33 | 115.38 | 142.85 | 166.66 |
|---|---|---|---|---|---|---|
| Emission intensity | 4.5 | 12 | 18.8 | 24.3 | 29.3 | 50.2 |



# ISO - 9000



- ISO stands for International organization for standardizat
- The ISO 9000 family of quality management system i a set of standards that helps organizations ensure they customer and other stakeholders needs with statutory and regulatory requirements related to a product or service.
- More than 170 countries are involved in ISO - 9000.
- ISO - 9000 was established in 1987.
- ISO - 9000 describes about the principle of QMS (Quality management system).
- ISO - 9000 includes the vocabulary which gives defination. meaning of terms.
- ISO - 9000 - 2005 includes 8 principle of QMS
- ISO - 9000 - 2015 includes 7 principle of QMS

- Quality means "fit for purpose" "Value of Price paid or meeting the standard specification.
- Supporting services are also included in Quality.
- Psychological criteria is also important means "brand"

- Management is the coordination and adminstration of tasks to acheive a goal.
- Hence, QMS is the integrated system and collection of business processed focused on consistently meeting custom requirement and enhancing their satisfaction. It is alligned with an organization's purpose and strategic direction.

ISO - 9000 standard prepration for Product and Services

Step 1:- Standard is prepared as per customer requirement.
Step 2:- Review if the standard exists or not.
Step 3:- Set up of a technical community.
Step 4:- Prepared standard is send to all member countries.

If there is no objection from any member country. the prepared standard is approved.
If exclaim by certain countries again prepare the standard i.e. suitable for all countries.
Send to the secreterlat
Approved and send to all member countries.

## Principle of ISO-9000-2005

Focus
leadership
Involvement of People
System approach
Process approach
Continous improvement
Fact based decision making
Good relationship with supplier.

## Principle of ISO-9000-2015

### Focus

Customer satisfaction is the most needed when there is launched of any product or services.
Determination of customer need can be performed by:-
• Conference • feedback • Data collection • Exhibition

### leadership

It is the art in which the leader set a goal and objective and motivate the followers so that they willigly acheive the goal and objective of the organization. Leader perform the following activities to acheive the goal
Motivation through incentives. gifts

- Set rules and regulation that everyone need to follow.
- Reward and punishment.

3) Environment of People

leader does create of the environment, trust the followers. recognize and address the grevings. leader also arranged the seminars.

leaders tell that. " Every employee are important to acheive the organization goal.

4) Process approach

- There should be every step wise process for each work which helps to acheive organization goals and objectives.
- Define the roles and responsibilities of each employee.
- Job description of each employees.
- Documentation of every work.
- Coordination between the intra and inter organization.

5) Improvent

- Perfection is never acheived, it is continous and never ending process.
- Correction of mistake can be performed through
  * Customer feedback * Complaints * Product recall

PDSA / PDCA cycle

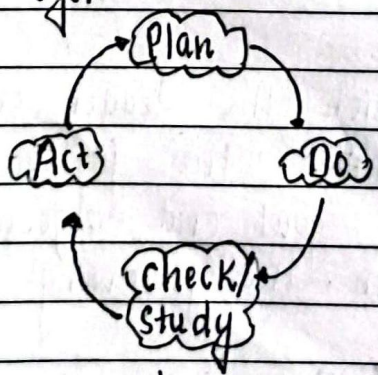



fig:- Improvement cycle

6) Evidence based decision making

- Data should be provided for every process
- Availability of data
- Processed of Raw data
- Prediction of data that may be relevant or irrelevant.

7) Relationship management

- Good relationship for supplier, have the benefits for the short-term or long term.
- Increase efficiency of Product or Services
- Benefits for the organization
- Increase the customer satisfaction
- Increase motivation

There is no guidelines of ISO-9000

## ISO-9001

- ISO-9001 includes the guidelines to certify the organization.
- It is the only standard in the ISO-900 series to which organizations can certify.

| Clauses of ISO-9001, 2008 | Clauses of ISO-9001, 2015 |
|---|---|
| 1) Scope | 1) Scope |
| 2) Normative refrence | 2) Normative refrence |
| 3) Terms and definations | 3) Terms and defination |
| 4) Quality management system | 4) Context of the organization |
| 5) Management responsibility | 5) Leadership |
| 6) Resource management | 6) Planning |
| 7) Product realisation | 7) Support |
| 8) Measurement, analysis and improvement | 8) Operation |
| | 9) Performance evaluation |
| | 10) Improvement |

ISO 9001 standard is based on PDCA concept. therefore having similar structure.

Plan Clause 4:- Context of organization
Clause 5:- leadership
Clause 6:- Planning
Clause 7:- Support

Do:- clause 8:- Operation

Check clause 9 Performance evaluation

Act Clause 10:- Improvement

| | ISO 9001:2008 | ISO 9001:2015 |
|---|---|---|
| 1. | Products | 1. Products and services |
| 2. | Documentation. quality manual. documented procedures, records, instructions | 2. Documented information |
| 3. | Work environment | 3. Environment for operation of process |
| 4. | Monitoring and measuring equipment | 4. Monitoring and measuring resources |
| 5. | Purchased product | 5. Externally provided products and services. |
| 6. | Supplier | 6. External provider |

Clause 4 :- Context of the organization

Sub clause 4.1 :- Understanding the organization and it's context.

Sub clause 4.2 :- Understanding the need and expectations of intrested parties.

Sub clause 4.3 :- Determining the scope of quality management system.

Sub clause 4.4:- Quality management system and it's processes.

## Clause 5:- Leadership

Sub-clause 5.1:- Leadership and commitment
Sub-clause 5.2:- The Quality policy
sub-clause 5.3:- Organization roles, responsibilities

## Clause 6:- Planning

sub-clause 6.1:- Actions to address risks and opportunities
Sub-clause 6.2:- Quality objectives and planning to achelve them.
Sub-clause 6.3:- Planning changes

## Clause 7:- Support

sub-clause 7.1:- Resources
sub-clause 7.2:- Competence
Sub-clause 7.3:- Awareness
sub-clause 7.4:- Communieation
sub-clause 7.5:- Documented information

## Clause 8:- Operation

8.1 Operation planning and control
8.2 Requirements for product and service
8.3 Design and development of product and services
8.4 Control of externally provided processes, product and service
8.5 Production and service provision
8.6 Release of Products and services
8.7 Control of non conforming outputs

## Clause 9:- Performance evaluation

9.1 Monitoring, measurement, analysis and evaluation
9.2 Internal audit
9.3 Management review

## Clause 10:- Improvement

10.1 General
10.2 Non-conformity and corrective action
10.3 Continual improvement

## Benefits of ISO-9001

- Improved consistency of service and product performance
- Higher customer satisfaction level
- Improved customer perception
- Improved productivity and efficiency.
- Cost reductions
- Improved communications, morale and job satisfaction
- Competitive advantage and increased marketing and sales
- Opportunities.

## Clause 1 :- Scope

- Costumer satisfaction
- Continual improvement
- Saitory requirement fulfill

## Clause 2:- Normative refrences

- Document guidelines
- Includes Principle, refrence

## Clause 3 :- Terms and defination

- Various terms are included
- Product indidicates product and services

→ Clause 1, 2 and 3 are similar to ISO-9001-2008 and ISO 9001-2015

# ISO-17025

Date
Page 138

"laboratory management system"
Revised
ISO -17025- 2005
ISO- 17025 - 2017

- Guidelines prepared taking refrence. ISO/ICE -2000, 2005, 2017 → Revised

| ISO- 17025-2005 | ISO- 17025-2017 |
|---|---|
| 5 clauses | 8 clauses |
| Clause 1:- Scope | 1) Scope |
| Clause 2:- Normative refrences | 2) Normative refrences |
| Clause 3:- Terms and Definations | 3) Terms and Definations |
| Includes 9 definations:- | 4) General requirement |
| • Interlaboratory comparision | 5) Structural requirement |
| • Confedentiality | 6) Resource requirement |
| • Verification | 7) Process requirement |
| • Validation | * IEC- International electrotechnical comission |
| • Inpartiality | 8) Management requirement |
| • Internal audit | |
| Clause 4) Management requirement | Clause 4.1 → Impartiality, Confedentiality |
| - Includes 15 sub-clauses | Impartiality :- No biasesness |
| Clause 5) Technical requirement | ↓ |
| - Includes 10 sub-clauses | - By taking oath by employee |
| → General requirement | - Decoding |
| • personnel | - By Public services comission |
| • Instrument | |
| • Method | |
| • Sampling techniques | Showing of confidentiality |
| • Environment factors | - No leak of information |
| • Handling of items | |
| → Assurance of test results | |
| → Test reports | |

| ISO-IEC-17025-2005 | ISO-IEC-17025-2017 |
|---|---|
| • Testing laboratory<br>• callibration laboratory | • Testing<br>• Callibration<br>• sampling laboratory |
| - Doesn't based on risk thinking<br>- Less flexible<br>- 5 clauses | - Risk based thinking<br>- flexibility<br>- 8 clauses |

Clause 5:- General requirement

- legal identity of any organization
- Registration of organization
- legal entity if merged with anothe organization
- Job description of laboratory
- Laboratory head

Clause 5:- Structural requirement

- Human resources / Personeel → Qualifications, Training requirement
- Externally provides product and services
- Enivornment condition and Facility :- Procedure
- Equipments
- Metrological traceability :- should maintain chain of traceability

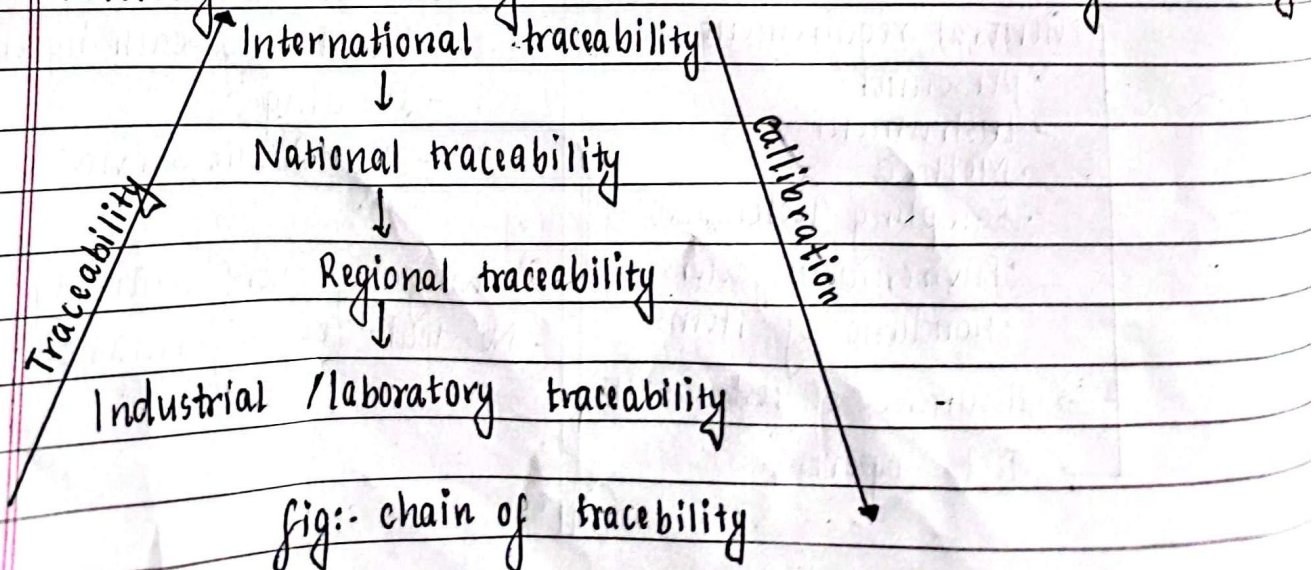



fig:- chain of tracebility

## Accreditation

The action or process of officially recognizing someone as having particular status or being qualified to perform a particular activity.

## Clause 6: Resources requirements

General - all facilities, personnel, equipment system and support system.

Personnel - competant, education, qualification, technical knowledge

Facilities and environment conditions - suitable, radiation, humidity, tempr, sound, microbial contamination free

Equipment :- measuring instrument, software, measuring standard, refrence materials, refrence data, reagents.

Metrological traceability :- document unbroken chain of tracebility of calibrations

Externally provide product and services :- only suitable externally provide product and services are used. Used to support the operation of laboratory.

## Clause 7: Process requirements

Review of requests, tenders and contracts :- Procedure for the review of requests, tenders and contracts.

- requirements are adequately defined, documented and understood.

Selection, verification and validation of methods.

- use of appropriate method for selection
- validation of methods

Sampling - selection of samples or sites
- sampling plan
- refrence of sampling method used.
- date and time of sampling
- identification of equipment used.

7.4. Handling of test and callibration items
- Procedure for transportation, reciept, handling, protection, storage, retention and disposal of return of test or calibration items.

7.5 Technical records
- contains result, report and sufficient information to faeilate the identification of faetors affecting the measuremer

7.6 Evaluation of measurement uncertainability
- From sampling techniques
- From equipment used

7.7. Ensuring the validity of result
- Procedure for monitoring validity of results.
- Use of refrence material or quality control materials.
- Use of alternative instrumentation
- Intermediate ohecks of measurement equipments.
- review of reported results.
- testing of blind sample.

7.8 Reporting of results
- General - results shall be reviewed and authorized prior to release.
- Common requirements for reports (test, callibration or sampling)
  - title
  - Name and adress of laboratory
  - Name and contact information of customer
  - Identification of method used
  - date and issue of report.
- Specific requirements for test reports
- Specific requirements for calibration certificates
- Reporting sampling - Specific requirements
- Reporting statements of conformity
- Reporting opinions and interpretations
- Amendments to reports.

- Complaints
- Non- conforming work
- Control of data and information management

8. Management system requirements

Options :-

option A :- management system documentation
- control of management system documents
- control of records
- Improvement
- corrective actions
- Internal audits
- management rievew

Option B :- laboratory established with ISO 9001 are capable of supporting and demonstrating the consistent fulfillment of requirements of clauses 4 to 7 . also fulfill atleast the intent of management system requirement specified.

- Management system documentation (option A)
- Control of management system documents (option A)
- Control of records (option A)
- Action to adress risk and opportunities (option A)
- Improvement (option A)
- Corrective action (option A)
- Internal audits (option A)
- Management rivews (option A)