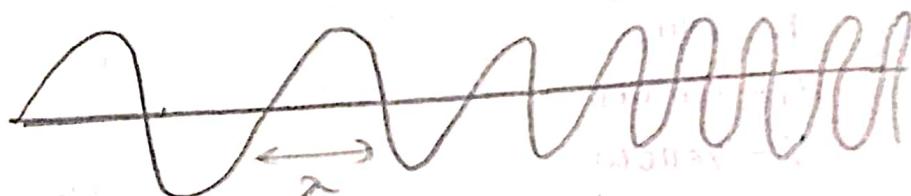


## UNIT-V

### Instrumental Methods & Applications

In this unit we explain about electromagnetic radiation spectroscopy, chromatography and pH-metry concepts.

Electromagnetic radiation (or) spectrum:-  
The radiation rays are arranged in increasing order of the frequency and decreasing order of wave length is known as electromagnetic radiation.



on the basis upon frequency and wave length divided into seven types.

Radiation waves are used extra Gold sand all  
RICH MAN IN VIZAG

1. Radiowaves:-  
The wave length of the radiowaves is greater than 0.1mm ( $>0.1\text{ mm}$ )

1. The radiowaves are used in broadcasting, signaling like Tele communication, satellite communication, radar communication.

2. Microwaves:-  
The wave length of the microwaves is 0.1mm to 1mm ( $0.1\text{ mm} - 1\text{ mm}$ )

1. The microwaves are used in cooking purpose in Bakery's.

3. Infrared waves:-  
1. The wave length of the infrared waves 1mm to 700nm ( $1\text{ mm} - 700\text{ nm}$ )

2. Infrared rays produce overheat to the object (or) body which causes skin cancer & rashes.

### 3. The intensity of EMR radiations and function of Sun

layer. It can drift back and forth from one layer to another.

**Visible light :-** The range of visible light is  $700\text{nm} - 400\text{nm}$ .

1. The wavelength of the visible light is  $700\text{nm} - 400\text{nm}$ .

2. Visible light is used to visualize the color of the object in VIBGYOR Manner.

V - violet      R - red

I - Indigo

B - Blue

G - Green

Y - Yellow

O - Orange

R - Red



$\Rightarrow$  In case the object absorbs violet colour which emits yellow colour as a complementary colour.

### 5. Ultra violet rays:-

1. The wavelength of the ultraviolet waves

is  $400\text{nm}$  to  $1\text{nm}$  ( $400\text{nm} - 1\text{nm}$ )

2. The UV radiation is used to zoom up the object and also used fluorescent lamps.

### 6. X-rays :-

1. The wavelength of the X-rays  $1\text{nm}$  to  $10^{-3}\text{nm}$

2. X-rays are used to identify structures of

bones and skeleton in medical purpose.

### 7. Gamma-rays:-

1. The wavelength of the  $\gamma$ -rays is less than

$10^{-3}\text{nm}$  ( $\approx 10^{-3}\text{nm}$ )

2. The  $\gamma$ -rays are used in to kill cancerous cells in Radiotherapy treatment.

Absorption of Radiation:- According to Bohr's atomic theory we will explain absorption and emission process.

Absorption Spectra:-

when ever electron jumps from lower energy level to high energy level which requires energy from the electromagnetic radiation:



Emission spectra:-

when ever electron jumps from higher energy levels to lower energy level which releases energy to the electromagnetic radiation is known as emission.



~~Beer-Lambert's LAW:-~~

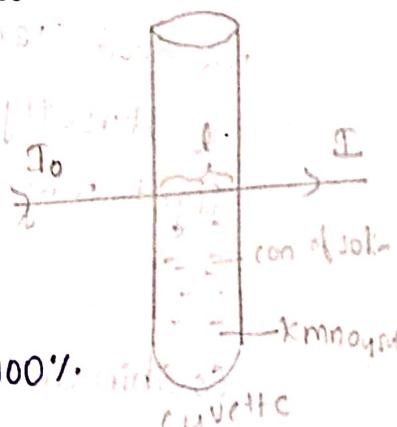
When ever monochromatic light radiation incident towards the sample solution Absorbance is directly proportional to concentration of the solution and path length of the light ray which move through the solution is known as Beer - LAMBERT's LAW.

case (i) :- NO absorption medium

Transperancy

~~and thus~~ absorption is 0%,

$I_0 = I_{\text{abs}}$  Transperancy is 100%.



case (ii) absorbing Medium opaque properties

$$I_0 = A \quad I = 0 \text{ intensity of radiation}$$

Absorbance = 100%, That is, the medium

Transmission = 0%.

According to absorption of radiation & Absorbance

(A)

$$A = \frac{I_0}{I}$$

$$\text{Transmission (T)} = \frac{I}{I_0}$$

$$A = \log_{10} \left[ \frac{1}{T} \right]$$

$$A = \log_{10} \left[ \frac{I_0}{I} \right]$$

Beers law:-

when ever monochromatic light radiation incident towards the sample solution Absorbance is directly proportional to concentrate solution

$$A \propto C$$

Lambert's law:-

when ever monochromatic light radiation incident towards the sample solution Absorbance is directly proportional to path length of the light ray

$$A \propto l$$

Combination of Beer's law and Lambert's law

$$A \propto Cl$$

$$A = \epsilon Cl \quad (2)$$

from Eq(1) and (2) finally we get Beer's Lambert's formula

$$A = \log_{10} \left[ \frac{I_0}{I} \right] = \epsilon cl$$

$$A = \log_{10} \left[ \frac{I_0}{I} \right] = \text{ecl}$$

where

A = Absorbanie

$I_0$  = Intensity of the incident light

$I_0$  = Intensity of the Transmitted light  
 $I$  = Intensity of the Transmitted light

$\epsilon = \text{molar absorptivity}$  Constant

Molecular extensions co-efficient

$c$  = concentration of the solution

$c$  = concentration of the solution  
to fit the light rays

$f$  = path length of the light rays

$l$  = path length of  $\sigma$

Limitations :- It is only for colored solution.

1. It is preferable only for colored monochromatic light.

1. It is preferable to use monochromatic light.
2. In this we use only one wavelength.

It is preferable only for dilute solutions.

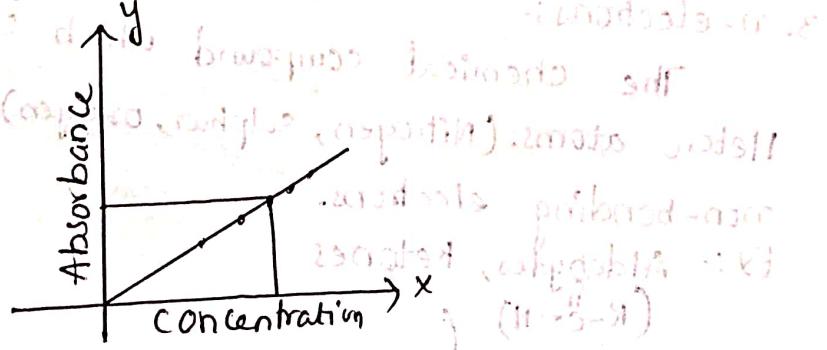
3. It is preferable only for experiments where there is no restriction

4. During the experiment we maintain constant

5. In this experiment we measured the rate of photosynthesis.

temperature. This shift has long and wide

1. By using Beer-Lambert's law of absorption, we can determine concentration of unknown solution using graphical representation.



2. In Beer Lambert's law we calculate molar absorptivity constant with the help of  $A = \epsilon cl$ .

3. In Beer Lambert's law we identify color of the compound with wavelength value. ( $\lambda_{max}$ )

UV-visible Spectroscopy:

It is an spectroscopy technique which having far UV region is 180nm to 200nm. and near UV region is 200nm to 400nm. Visible region is 400nm to 700nm. is known as UV-visible Spectroscopy.

Types of electrons present in organic compound:-

In organic chemistry we have three types of electron

on the basis upon bonds formation.

1.  $\sigma$  electrons:-

The chemical compound which consisting of single bond in its structure which produces ' $\sigma$ ' electrons.

Ex:- Alkanes ( $C \equiv C$ )

2.  $\pi$  electrons:-

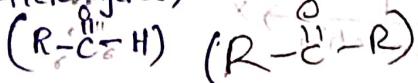
The chemical compound which consisting of double bond and triple bond in its structure which produce ' $\pi$ ' electrons.

Ex:- Alkenes ( $C \equiv C$ ), Alkynes ( $C \equiv C$ )

3.  $n$ -electrons:-

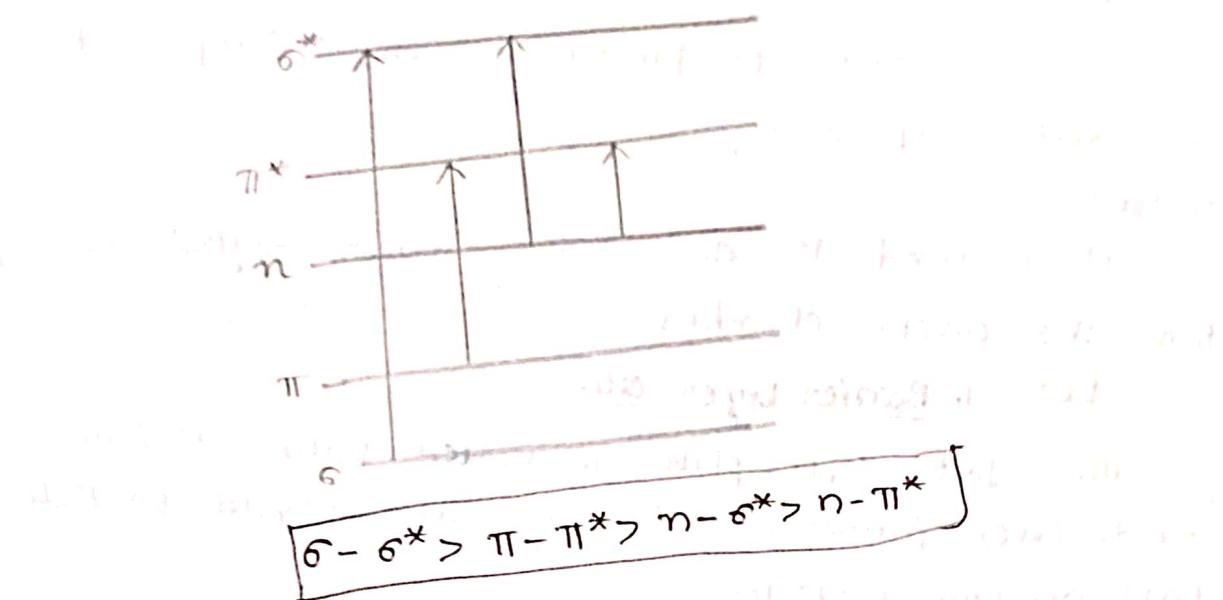
The chemical compound which consisting of Hetero atoms. (Nitrogen, sulphur, oxygen) which produce non-bonding electrons.

Ex:- Aldehydes, Ketones

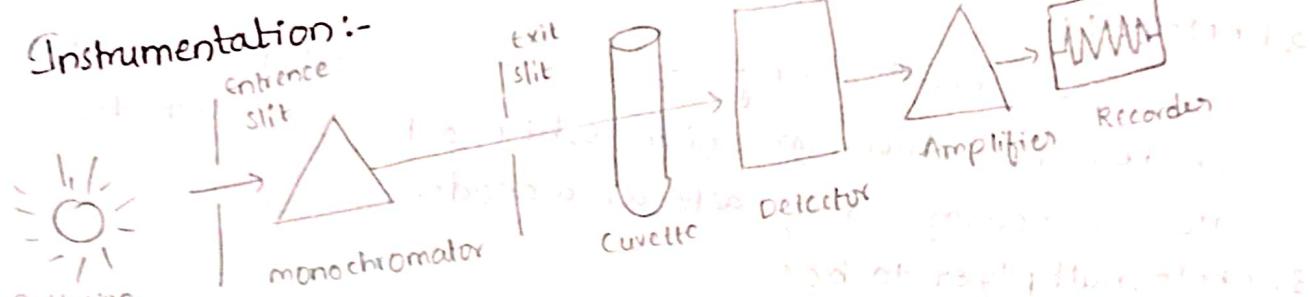


## Principle:-

In UV-visible spectroscopy our working principle is absorption (or) Transition (or) excitation. In this spectroscopy  $\sigma$  electrons,  $\pi$  electrons and  $n$ -electrons produce four types of transition. In this four transition  $\sigma$  to  $\sigma^*$  transition is higher energy transition and  $n-\pi^*$  transition is lower energy transition.



## Instrumentation:-



## Radiation source:-

The purpose of radiation source is to produce electric magnetic radiation.

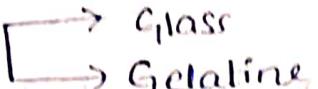
Ex:- 1. Xenon discharge lamp

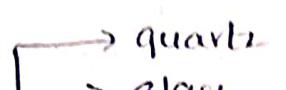
2. Deuteronium lamp

3. Tungsten halogen

## Monochromator

It is used to separate polychromatic radiation into monochromatic radiation.

Ex :- (1) filter 

(2) prism 

(3) gratings 

The equi spaced mechanic lines which are coated with aluminium foil which acts as monochromatic

### Cuvette :-

It is a small test tube made up of a quartz (ox) silicon o is used to full fill the sample component in our visible spectroscopic.

### Detector :-

It is used to detect the sample signal coming from the cuvette chamber

Ex :- 1. Barrier layer cell:-

The flat copper plate is coated with selenium oxide ( $SeO_2$ ) further coated with silver or gold particle ( $Ag$ ) or ( $Au$ ) particles.

### 2. Photo cell :-

The metallic ring  $\frac{1}{3}$  portion is coated with silver oxide ( $Ag_2O$ ) chromium oxide which acts as a cathode the remaining ring acts as a anode.

### 3. Photomultiplier tube :-

which allow light and electrons.

### 4. P-n junction diode :-

The combination of P-type semiconductor and n-type conductor to produce P-N Junction diode.

## Applications

### 1. Qualitative analysis:-

In this analysis we identify the names of anions and cations present in sample solution.

### 2. Quantitative analysis:-

In this analysis we detect the amount of

the substance by Spectrophotometric titration.

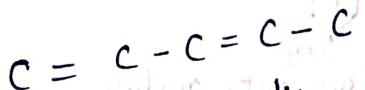
### 3. It is used to Identify

(or) unsaturation. the compound is in conjugation

### 4. It is used to Identify

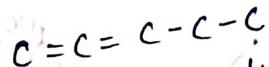
(or) non-conjugation.

(i)



1,3 pentadiene

(longer wavelength)



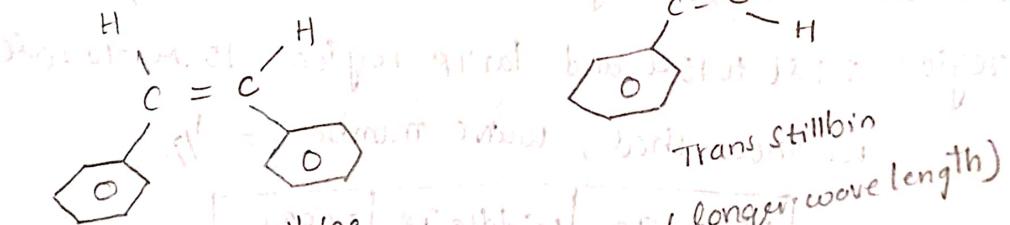
1,2 pentadiene

(shorter wavelength)

### 5. It is used to analyse geometrical isomerism means

the compound is principle Trans manner.

the compound is principle cis manner.



cis stillbene (shorter wavelength)

trans stillbene (longer wavelength)

no. of chromophore units present

### 6. It is used to detect

in sample compounds.

1. Potentiometry / Redox

2. TLC (detail)

2 Marks

### \* Chromophores:-

The chemical substance which is give ~~in its~~ colour to the compound is known as chromophore.

Example:- Beta ( $\beta$ ) - carotin - orange colour to the carrots

Lycopene - Red colour to the Tomatos.

pH indicator.

### \* Auxochrome:-

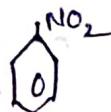
The chemical substance which is used to develop

Intensity of the colour.

Example:-  $-OH$ ,  $-NH_2$ ,  $-NO_2$  etc



Benzene  
(colourless)



Nitrobenzene  
(Pale yellow)



Para nitro aniline  
(Pure Yellow)

### IR Spectroscopy (or) vibrational Spectroscopy:-

It is one of the spectroscopy technique which consists near IR region is  $1.8\text{ }\mu\text{m}$  to  $2.5\text{ }\mu\text{m}$ , middle IR region  $2.5\text{ }\mu\text{m}$  to  $15\text{ }\mu\text{m}$  and far IR region  $15\text{ }\mu\text{m}$  to  $20\text{ }\mu\text{m}$ .

We know that, wave number  $\nu = \frac{1}{\lambda}$

Near IR	Middle IR	Far IR
$1.8\text{ }\mu\text{m}$	$2.5\text{ }\mu\text{m}$	$15\text{ }\mu\text{m}$ to $20\text{ }\mu\text{m}$

$$1\text{ }\mu\text{m} = 10^4\text{ cm}^{-1}$$

$$1.8\text{ }\mu\text{m} \Rightarrow \frac{1}{\lambda} = \frac{1}{1.8\text{ }\mu\text{m}} = \frac{1}{1.8 \times 10^{-4}} = \frac{10^4}{1.8} = 5555.5\text{ cm}^{-1}$$

$$2.5\text{ }\mu\text{m} = \frac{1}{\lambda} = \frac{1}{2.5\text{ }\mu\text{m}} = \frac{1}{2.5 \times 10^{-4}} = \frac{10^4}{2.5} = 4000\text{ cm}^{-1}$$

$$15\text{ }\mu\text{m} = \frac{1}{\lambda} = \frac{1}{15\text{ }\mu\text{m}} = \frac{1}{15 \times 10^{-4}} = \frac{10^4}{15} = 666.6\text{ cm}^{-1}$$

$$20\text{cm} = \frac{1}{\lambda} = \frac{1}{20\mu} = \frac{1}{20 \times 10^{-4}} = \frac{10^4}{20} = 500\text{cm}^{-1}$$

Principle :-

In IR Spectroscopy we have vibrational forces in two categories.

1. Bending vibrations

2. Stretching vibrations

### Bending vibrations

The vibrational forces which having molecule positions displacement in bending manner.

1. scissoring

2. Rocking

3. Wagging

4. Twisting

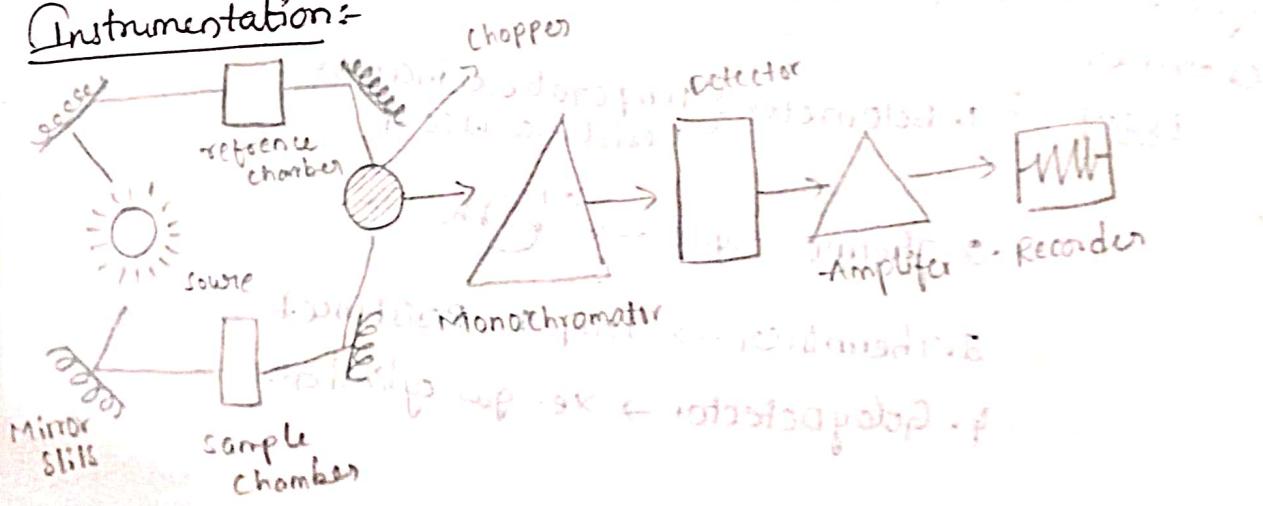
### Stretching forces :-

The vibrational forces which having extension and compression of the molecule known as stretching vibrations

1. Symmetrical

2. Asymmetrical

### Instrumentation :-



## Radiation Sources:-

The purpose of Radiation source which is used to

produce electromagnetic radiation for IR Spectroscopy.

Example:- 1. Nernst glove :- The combination of Yttrium,



Erbium, zirconium with oxygen gas at 1600 - 1800°C

2. Globar radiation source :- The sintered  $\text{Pa}_2\text{SiC}_2$  having temperature range 1360 - 1600°C which acts as radiation source.

## Sampling :-

- 1) solid
- 2) liquid
- 3) Gas
- 4) solution

## Mono chromator :-

The purpose of Mono chromator is which separates

Poly chromatic radiation into Monochromatic radiation.

### Example :-

1. filters → Glass
- Gelatine

2. prisms → Quartz
- Glass

3. Grating - equi spaced metallic lines are coated with aluminium film.

## Detector :-

The purpose of Detector which is used to detect signals like Sample, reference coming from monochromator chamber.

Example :- 1. Bolometer { Temperature increase  
Resistance also ↑ }

2. Thermo couple → 

3. Thermister → Temp ↑ Resistance ↓

4. Golay Detector → x-e-gas cylinders.

## Applications :-

1. Detection of impurities
2. Detection of functional groups.
3. Quality of Drugs.
4. Identification of carbonyl compound present in sample (c)
5. Analysis of fertilizers and pesticides
6. Identification of aldehydes and ketones
7. Detection of contaminant material present in alcohols and fruit juices.

chromatography :-

The word chromatography is derived from greek language chromo colour, graph means writing

⇒ Michael Swift proposed chromatography in 1900 to analysis of plant pigments by column chromatography.

Definition :-  
Chromatography is an separation technique which is used to separation of mixture of components by colour writing technique.

Solid liquid chromatography :-  
It is an chromatography technique in which stationary phase is solid state and mobile phase is liquid state known as solid liquid chromatography.

is liquid state (High performance liquid chromatography)

Example:- TLC, HPLC, etc

Thin layer chromatography :-  
It is an solid liquid chromatography in which stationary phase is silica gel substance and mobile phase is Solvent mixture known as TLC.

## Stationary Phase (silica and slurry)

### TLC plate:-

Weighting accurately 5gm of

Silica gel slurry on the <sup>20 ml</sup> watch <sup>TLC</sup> Plate

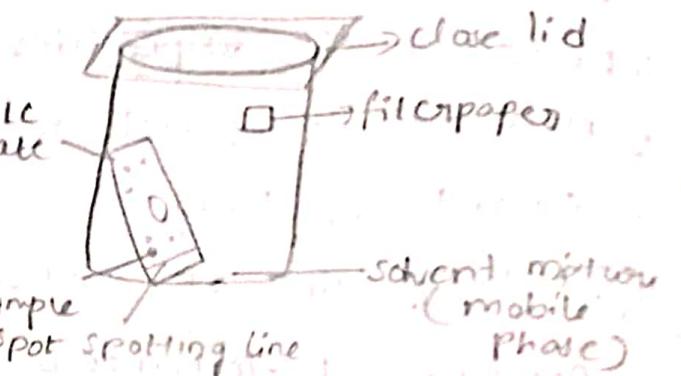
(glass) - to this add distilled water

- to prepare slurry state then

Pour the slurry over the glass

Plate and dry it 20 minutes in the

presence of air.



### Solvent Mixture:-

The combination of 12ml of normal butanol 3ml of acetic acid 5ml of distilled water acts as a mobile phase in TLC chamber.

### Stationary Phase:-

In chromatography the phase which having steady state (or) fixed state is known as stationary phase.

### Mobile phase:-

In chromatography the phase which having movable manner is known as mobile phase.

### Sample:-

Take one drop of Amino acid mixture on the

spotting line in TLC plate.

### Filter paper:-

The absorption of unwanted moisture present in the TLC chamber.

### Retention time (or) Retardation factor:-

$R_f$  is defined as the ratio of distance travelled by sample and distance travelled by solvent.

$$R_f = \frac{\text{Distance travelled by sample}}{\text{Distance travelled by solvent}}$$

### Applications :-

1. WADA - World Anti Doping Agency  
The chromatography is used to identify traces of drug molecules present in athlete's body in doping test.
2. Ebola virus (Ebola) Ebola virus:- The chromatography which is used to control extension of Ebola virus nucleus. The chromatography is used to detect contaminant of alcohol.
3. Chromatography is used to detect fruit juice and food samples.
4. Horse meat issue:- Horse meat is one of the Global issues. In USA 2003 Horse meat was mixed with horse meat which means the mixing of traces of horse meat like pig meat in beef.
5. Chromatography is used to extract plasma from blood samples that means we separate plasma contents like white blood cells, red blood cells, platelets.
6. Chromatography is used to separate mixture of components by  $R_f$  value.

No. of vibrational nodes:

Non-linear molecule:- for molecule with  $(N)$  atoms  $= 3N - 6$

Translational Nodes  $= 3$

Rotational Nodes  $= 3$

Vibrational Nodes  $= \frac{3N-6}{2}$

Linear molecule :- for molecule with  $(N)$  atoms  $= 3N$

Translational Nodes = 3

Rotational Nodes = 2

vibrational nodes =  $3N - 5$

Example:-  $\text{H}_2\text{O} \rightarrow 3N - 6 = 3(3) - 6 = 9 - 6 = 3$

$\text{CHCl}_3 \rightarrow 3N - 6 = 3(4) - 6 = 12 - 6 = 6$

$\text{CO}_2 \rightarrow 3N - 6 = 3(2) - 6 = 6 - 6 = 0$

Potentiometry :-

Principle :- Works on potentiometric principle

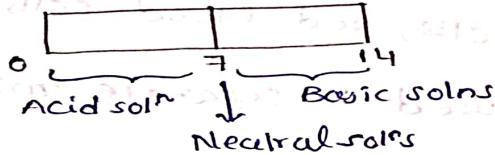
The word  $\text{pH}$  is derived from french language "P" mean pouvoir means concentration  $\text{H}^+$  means Hydrogen. So  $\text{pH}$  is nothing but concentration of  $\text{H}^+$  ions.

Scientist details :-

The Danish biochemist S.P.L. Sorenson proposed  $\text{pH}$  concept in Jan 6, 1909 to explain analysis of plant pigments.

pH scale :-

The scale which explains the nature of aqueous solution that is water as a solvent that type of solutions are aqueous solutions.



Definition :-

$\text{pH}$  is defined as the negative logarithm of hydrogen ion concentrations is known as  $\text{pH}$ .

\*  $\text{pH}$  has no units.

Formula :-  $\text{pH} = -\log [\text{H}^+]$

$$\text{pH} = \log \frac{1}{[\text{H}^+]}$$

$$\text{pOH} = -\log [\text{OH}^-]$$

$$\text{pOH} = \log \frac{1}{[\text{OH}^-]}$$

\* The relationship between  $pH$  and  $pOH$  is always equal to 14

$$pH + pOH = 14$$

$$pOH = 14 - pH$$

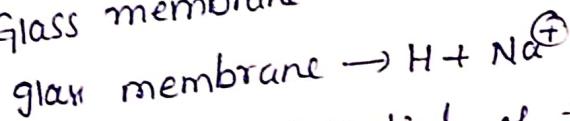
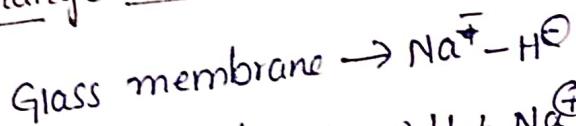
$$pH = 14 - pOH$$

construction and working process:-

Part A :- Explain Glass membrane electrode:-

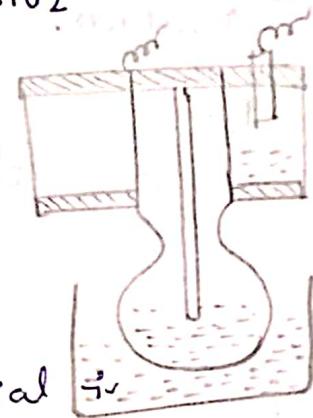
Glass membrane composition is 72%  $SiO_2$ , 22%  $Na_2O$  + 6%  $CaO$

Exchange reaction:-



electrode potential of this material

$$E_{\text{glass}} = E_{\text{glass}}^\circ + 0.0591 \cdot \log [Cl^-]$$



$Ag, AgCl(s), 0.1M HCl/Glass$  / sample solution  $pH = sc$

$$E_{\text{cell}} = E_{\text{cell}}^{\circ CS} - E_{\text{cell}}^{\circ \text{glass}}$$

$$E_{\text{cell}} = E_{\text{sc}}^{\circ} - E_{\text{G}}^{\circ} + 0.0592 \cdot pH$$

Part B :-

Calibration of  $pH$  meter:-

The glass membrane electrode is calibrated with the 3 types of buffer solution to get accuracy  $pH$  value.

The buffer solutions are like

1. Acidic buffer solution ( $pH = 4.2$ )

2. Basic buffer solution ( $pH = 9.0$ )

3. Neutral buffer solution ( $pH = 7.0$ )

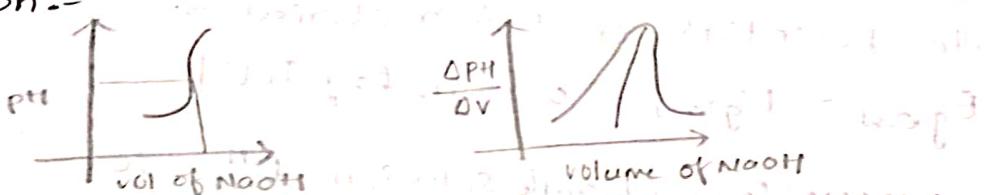
Point C :-

Take it 20ml of HCl solution into a 100ml beaker. To this add 20ml of distilled water, burette is filled with NaOH solution and add the NaOH solution in drop wise manner to the HCl solution to take 20 (or) more readings. Then draw the graph and final we should calculate the strength of HCl present in the given solution.

Table :-

S.NO	volume of NaOH	pH	$\Delta pH$	$\Delta V$	$\frac{\Delta pH}{\Delta V}$

Graph :-



Calculation :-

$$\frac{NaOH}{N_1} = ?$$

$$V_1 = \text{from graph}$$

$$\frac{HCl}{N_2} = ?$$

$$N_2 = 0.1N$$

$$V_2 = 20ml$$

$$\text{The strength of HCl soln} = N_1 \times \text{GEW of HCl}$$

$$= N_1 \times 36.5$$

$$= \frac{?}{20} \text{ grms.}$$

Applications :-

1. pHmetry is used in blood analysis, The pH value of the blood sample is 7.35 - 7.42
2. pHmetry is used to prevent tooth decay infections in dental treatment. preferable tooth paste value above's 8

3. pHmetry is used in agriculture sector. The pH value of the best soil is close to 7.

4. In laboratory diagnostics Urine pH value is 6.8 to 7.5

5. Rain water inner mouth atmospheric pH value is 5.6

6. Chicken pH value is 5.8 mutton, fish pH value is 6.8 Egg white, milk & pH value is 7.2

### Finger print region:-

The region b/w  $400\text{cm}^{-1}$  and  $1500\text{cm}^{-1}$  is on IR

The region b/w  $400\text{cm}^{-1}$  and  $1500\text{cm}^{-1}$  is on IR

Spectrum is known as the Finger print region. → The importance of the finger print region is that each different compound produce different pattern through in this part of the spectrum.

### Conductometric titration:

By Conductometric titration technique, only a few specific redox titration can be carried out.

It shows less accurate results when the total electrolytic concentration is high in solution. It makes it less satisfactory.