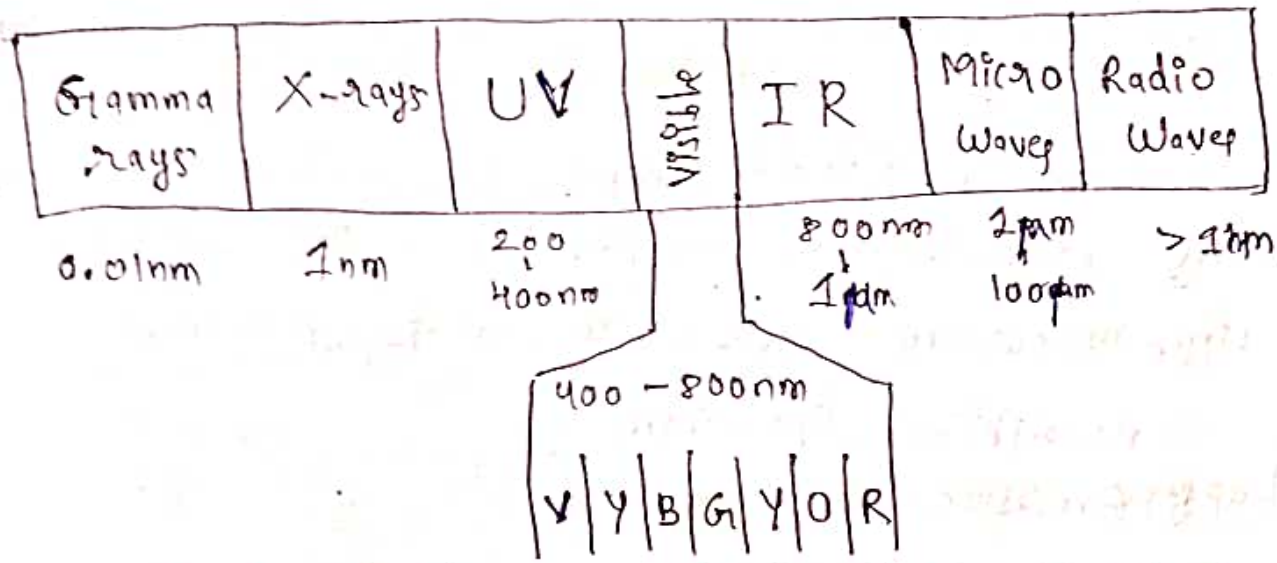
Instrumental Methods

(5). Explain electromagnetic spectrum?

Sol: ← frequency

wavelength →



The range of electromagnetic radiations with different wavelengths and frequencies is known as electromagnetic spectrum.

As the wavelength increases from gamma rays to radio waves, frequency decreases from gamma rays to radio waves. Gamma rays possess short wavelength, high frequency and radio waves possess longer wavelength, low frequency.

Spectroscopy:-

The study of interaction of electromagnetic radiation with the sample (atoms or molecules) is known as spectroscopy.

During the interaction the sample may observs the radiation (or) the sample it self emits the radiation.

After the interaction occurs variation in the intensity of radiation.

The instrument records the variation in the intensity of radiation which is analyzed by detector and the sample information is provided as graphical representation in the spectrum.

The spectrum occurs in two types

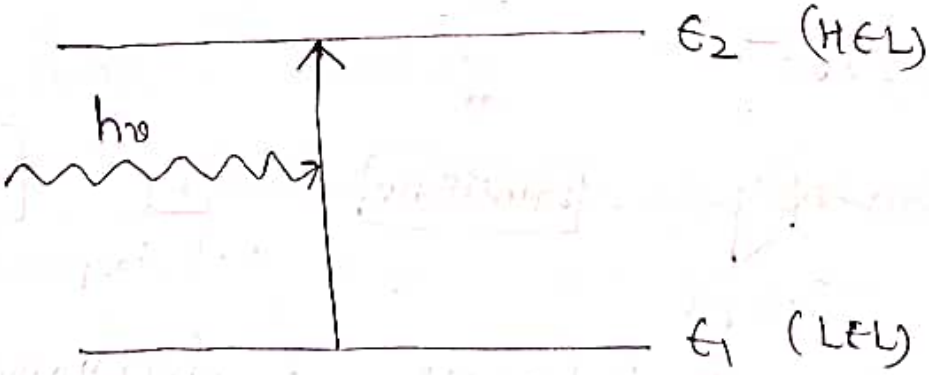
(i) Absorption spectrum

(ii) Emission spectrum.

Absorption Spectrum:-

When the beam of EMR is passing through the sample, if the sample observs the radiation under go electronic ~~transition~~ transission from lower energy level to higher energy level.

The decrease in the intensity of radiation is recorded in the spectrum and the spectrum obtained is known as absorption spectrum.

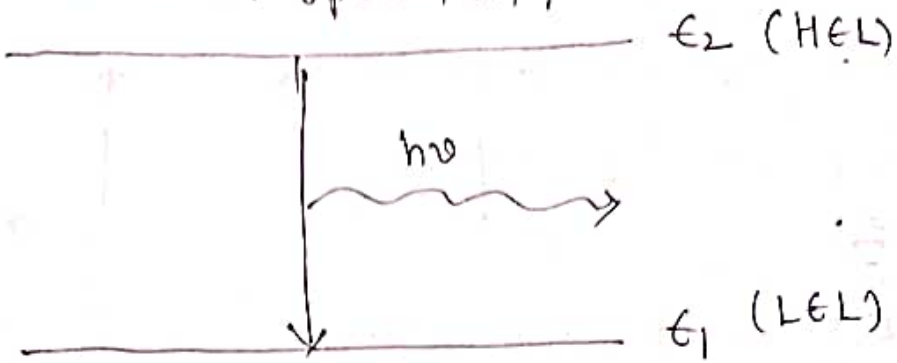


Absorption Spectrum.

Emission Spectrum:-

If the sample it self emits the radiation undergo electronic transitions from higher energy level to lower energy level.

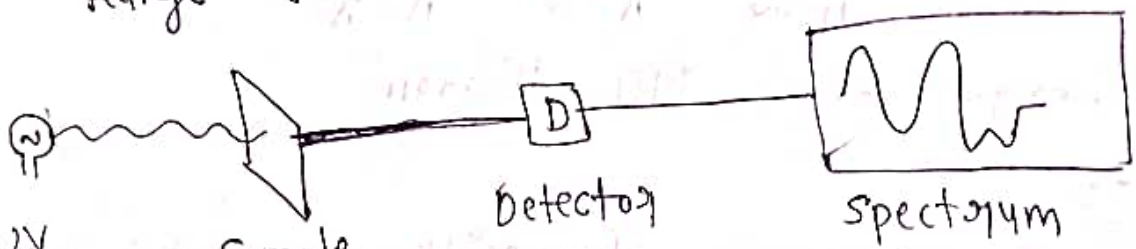
The increase in the intensity of radiation is recorded in the spectrum and the spectrum obtained is known as emission spectrum.



Emission Spectrum

Q. Write the principle and applications of UV spectroscopy.

Sol:- Range of UV = 200nm – 400nm



UV light Sample Detector Spectrum

Principle:-

The interaction of EMR with the sample undergo electronic transitions from lower energy level (ϵ_1) of bonding orbital to higher energy level (ϵ_2) of anti bonding orbital.

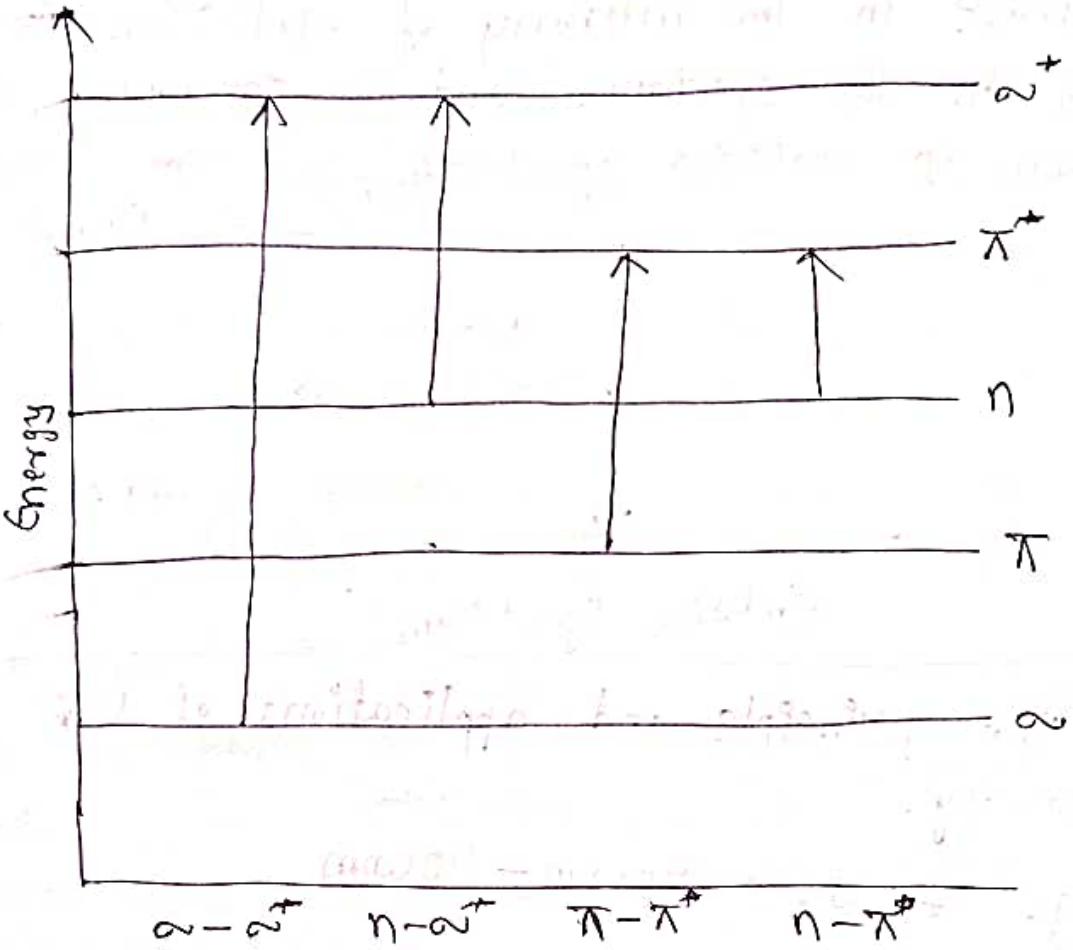
The UV spectroscopy is also known as absorption spectroscopy.

Types of electronic transitions,

(i) $\sigma - \sigma^*$, (ii) $n - \sigma^*$, (iii) $\pi - \pi^*$, (iv) $n - \pi^*$

The energy order of transitions,

$$\sigma - \sigma^* > n - \sigma^* > \pi - \pi^* > n - \pi^*$$

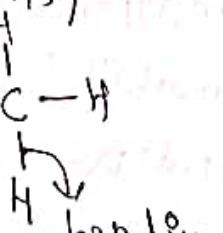


Energy level MO diagram

(i) $\sigma - \sigma^*$:-

The $\sigma - \sigma^*$ electronic transitions occurs in the sample due to the presence of bonding electrons (σ electrons)

ex:- CH_4



(iii) $n-\sigma^*$:-

The $n-\sigma^*$ electronic transitions occurs in the sample due to the presence of non bonding electrons

ex:- $CH_3-\ddot{O}H$

↳ Non bonding electrons

(iii) $\pi-\pi^*$:-

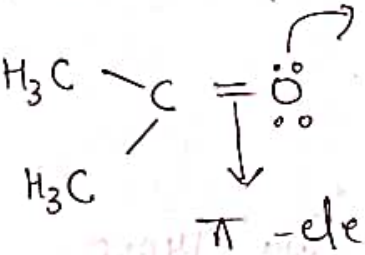
The $\pi-\pi^*$ electronic transitions occurs in the molecule due to the presence of π electrons.

ex:- $CH_2=CH_2$

↳ π electrons

(iv) $n-\pi^*$:-

The $n-\pi^*$ electronic transitions occurs in the molecule due to the presence of non bonding electrons and π electrons.

ex:- 

Applications :-

(i) UV spectroscopy is used for the determination of aromatic and conjugation of compounds.

(ii) It is used for the detection of purity of organic compounds.

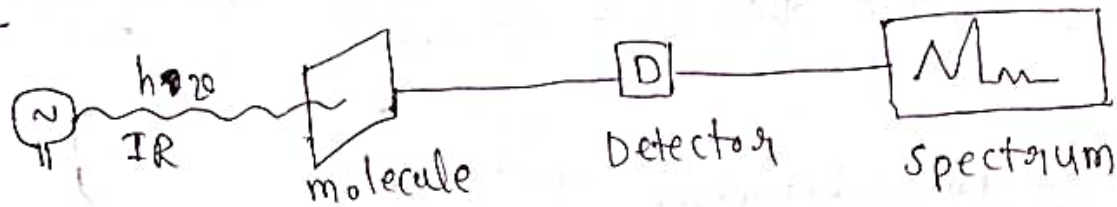
(iii) It is used for the quantitative determination of organic compounds.

(iv) It is used for distinguish cis-trans isomers.

(v) It is used for the detection of toutometric equilibrium.

Q. Write the principle and applications of IR spectroscopy.

Sol:-



Range of IR radiation:-

Near IR : 12500 cm^{-1} – 4000 cm^{-1}

Middle IR : 4000 cm^{-1} – 667 cm^{-1}

Far IR : 667 cm^{-1} – 50 cm^{-1}

Principle:-

The interaction of IR radiation with the sample (molecule) under go vibrational transitions in the molecule. which are

(i) Stretching Vibrations.

(ii) Bending Vibrations.

Stretching Vibrations:-

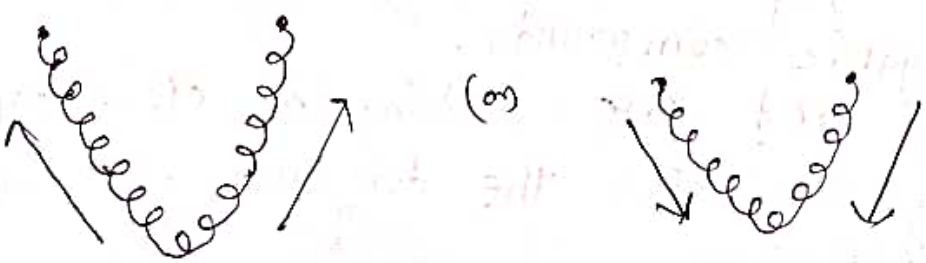
Stretching vibrations are two types.

⇒ Symmetric stretching vibrations

⇒ Asymmetric stretching vibrations

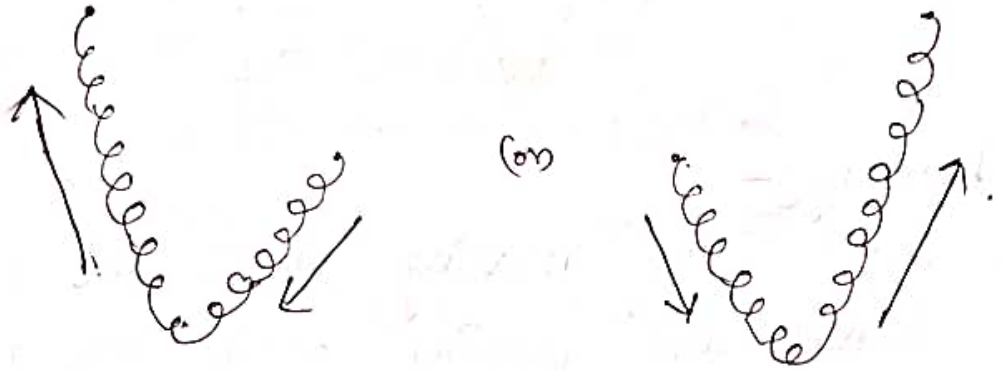
Symmetric Stretching Vibrations:-

During the symmetric stretching vibrations the two bonds are expanding in the two bonds are contracting simultaneously.



Asymmetric Stretching Vibrations :-

During the asymmetric stretching vibrations one bond is expanding and other bond is contracting.



During the stretching vibrations the bond length is changed but the bond angle remains same.

Bending Vibrations :-

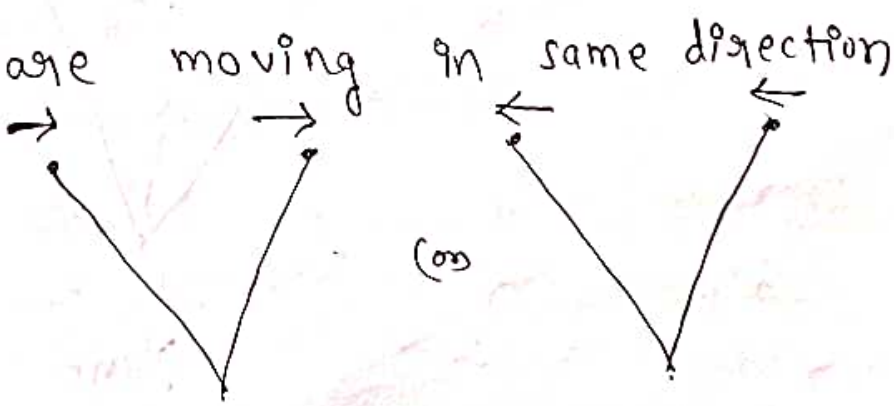
The bending vibrations are 4 types.

(i) Rocking bending } with in the plane
(ii) Scissoring bending }
(iii) Wagging bending } out of the plane.
(iv) Twisting bending }

During the bending vibrations the bond angle is changed but the bond length remains same.

Rocking bending :-

The two bonds are moving in same direction along the axis.

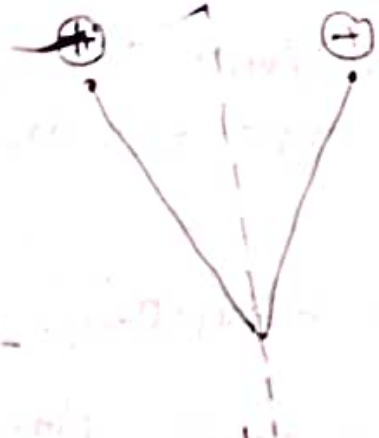


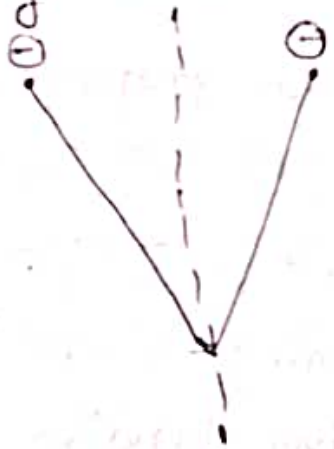
Scissoring bending:-

The two bonds are moving in opposite direction along the axis. → ←

Wagging bending:-

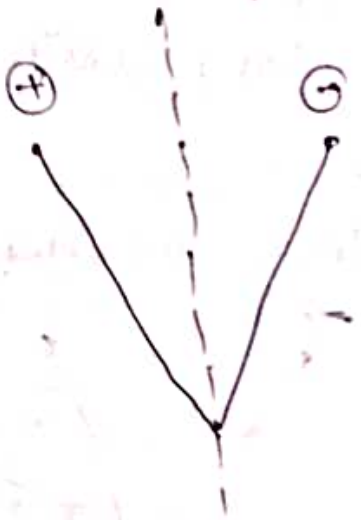
The two bonds are moving above the plane (or)
The two bonds are moving below the plane.





Twisting bending:-

One bond is moving above the plane and another bond is moving below the plane along the axis.



+ = above
− = below

Applications:-

(i) IR spectroscopy is used for the determination of compounds.

(ii) It is used for the identification of functional groups

(iii) It is used for the purity of organic compounds.

(iv) It is used for the detection of symmetric of molecules.

(v) It is used for the bactirial activity of organic compounds.

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(4). Write the Beer-Lambert's law and derive mathematical equation.

A = Absorbance of sample $\left(\log \frac{I_0}{I}\right)$

I_0 = Intensity of incident light

I = Intensity of transmitted light

c = Concentration of the sample.

l = path length of the sample

ε = Molar absorption constant.

Beer law:- Absorbance of the sample (A) is directely proportional to the concentration of the sample.

$A \propto c$

Lambert's law :- Absorbance of the sample (A) is directely proportional to the path length of the sample.

$$A \propto l.$$

Beer-Lambert's law :- Absorbance (A) is directely proportional to the concentration and path length of the sample.

$$A \propto Cl.$$

$$\boxed{A = \varepsilon Cl} \quad \downarrow \varepsilon = \text{Molar}$$

The above equation is Beer-Lambert's law equation.

Mathematical derivation of Beer-Lambert's law :-

When a monochromatic light is passed through a sample the decrease in the intensity of light with respect to the path length of the sample is directely proportional to the concentration of the sample and intensity of light (I).

$$\boxed{-\frac{dI}{dl} \propto CI}$$

$$-\frac{dI}{dl} = KCI$$

$$-\frac{dI}{I} = KC\,dl$$

Limits :-

length $= 0 \Rightarrow$ Intensity $= I_0$

length $= l \Rightarrow$ Intensity $= I$

Integration on both sides.

$$-\int_{I_0}^{I} \frac{dI}{I} = \int_{0}^{l} Kc\,dl$$

$$-\int_{I_0}^{I} \frac{1}{I}\,dI = Kc\int_{0}^{l} dl$$

$$-[\ln]_{I_0}^{I} = Kc[l]_0^l$$

$$-(\ln I - \ln I_0) = Kc(l-0)$$

$$\ln I_0 - \ln I = Kcl \Rightarrow \ln \frac{I_0}{I} = Kcl$$

$\downarrow \log_e$ (or) $\ln = 2.303 \log_{10}$

$$2.303 \log \frac{I_0}{I} = Kcl$$

$$\log \frac{I_0}{I} = \frac{K}{2.303} cl \qquad \downarrow \varepsilon = \frac{K}{2.303}$$

$$\log \frac{I_0}{I} = \varepsilon cl \qquad \downarrow A = \varepsilon cl$$

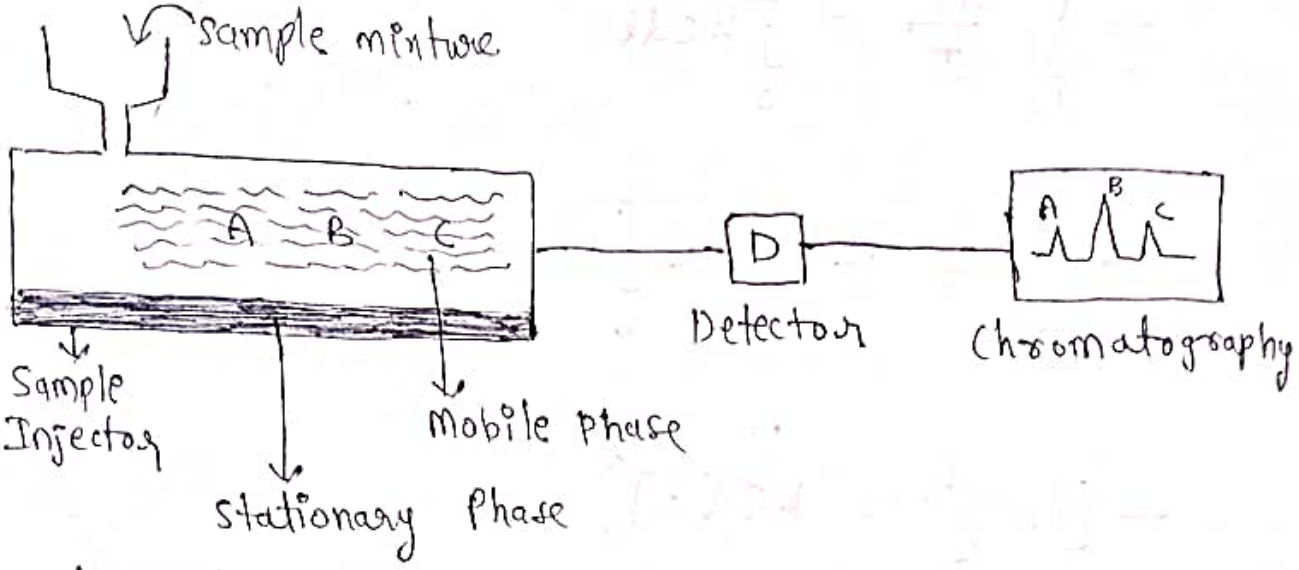
$$\boxed{A = \varepsilon cl}$$

∴ The above equation is known as Beer-Lambert's law equation.

Chromatography :-

Q. What is chromatography?

Sol:-



Chromatography is a seperation technique, the individual colour components from the sample mixture are seperated by the interaction of mobile phase and stationary phase.

Stationary Phase :- Stationary phase is a immobilized phase, it is fixed inside the column. The stationary phase may be solid (or) liquid.

Mobile Phase :- Mobile phase is a mobilized phase, it is moving over the stationary phase carrying with sample mixture. The mobile phase may be liquid (or) gas.

The individual components from the sample mixture are seperated based on the affinities of stationary phase and mobile phase.

Q. Explain the seperation of components from the sample mixture by thin layer chromatography (TLC)

Sol:- Principle :- The seperation of components from the sample mixture is based on the affinities of stationary phase and mobile phase.

The components in the mobile phase moving over the stationary phase. The components which have higher affinity towards stationary phase move slowly while the other components move fast. After the seperation process the individual components from the sample mixture appear has colour spots on the TLC plate.

Procedure :-

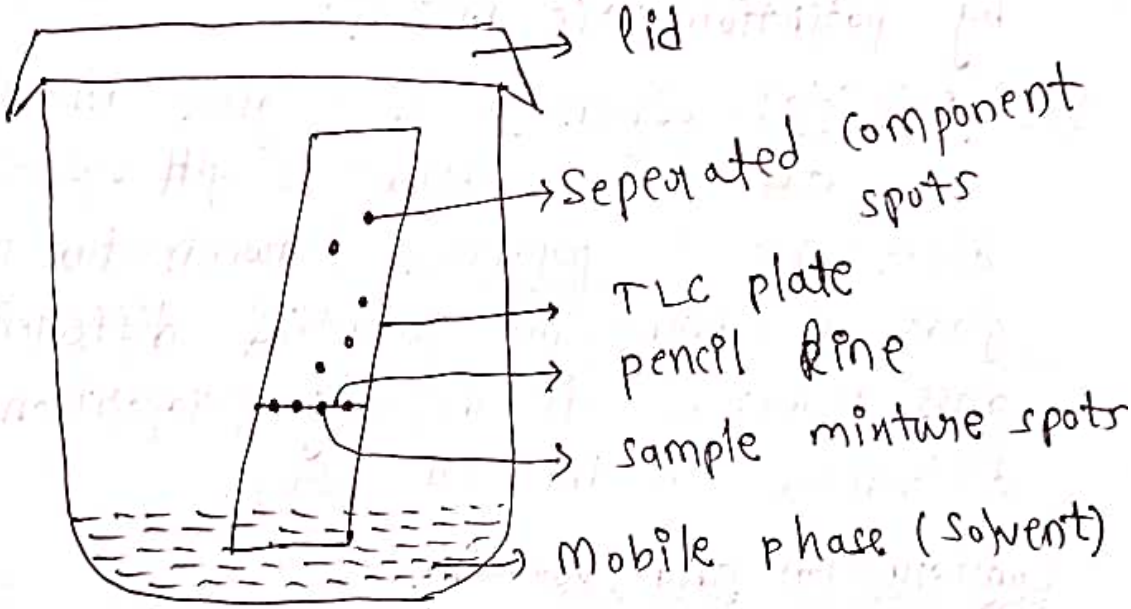
The requirements of thin layer chromatography

(i) Stationary phase - Silica gel (or) cellulose

(ii) Mobile phase - solvent

(iii) TLC plate

(iv) TLC chamber



TLC chamber

The stationary phase is applied on the TLC plate and it is made to dry. At the bottom of the plate a line is marked with pencil and apply the sample mixture spots on the pencil mark.

The mobile phase (solvent) is taken in a TLC

chamber and close the lid. The plate is dipped in the mobile phase and keep the sample spots (pencil mark) one cm above the level of mobile phase. Once the spots are developed, takeout the plate from the chamber and dry it. The sample spots can be identified under UV light chamber

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(Q). What is retention time (T_R).

Sol:- The time between injection and detection of the sample (analyte) is called retention time (T_R). It is expressed in minutes.

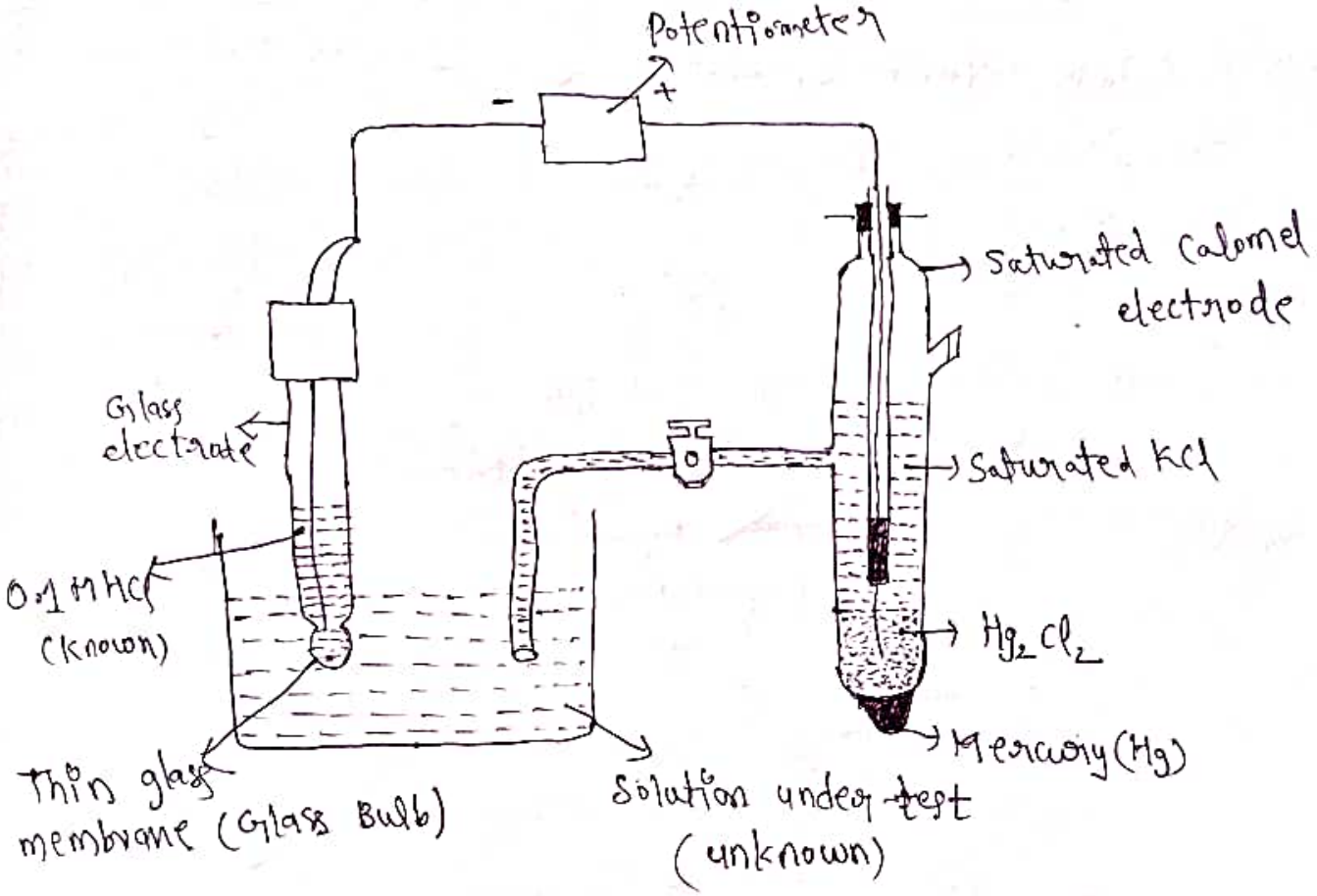
(Q). Write the principle and construction of pH metry by potentiometric method?

Sol:- Principle:- When a thin glass membrane seperates two solutions of pH values, a potential difference is produced between two solutions of glass membrane. The potential difference of the glass membrane is directely proportional to the difference in the pH values.

Construction and Working:-

The unknown pH of the solution can be measured by glass electrode. The glass electrode has bulb which is made up of thin glass membrane. Bulb is filled with 0.1 M HCl known solution. The glass electrode is dipped in the unknown pH of the solution. Whenever two different H^+ ion concentration solutions are seperated by thin glass membrane, a potential

difference is produced between two solutions of the glass membrane. The glass electrode is combined with saturated calomel electrode. Thus a potential difference (emf) of the cell is produced. The unknown pH of the solution is calculated from the emf of cell.



pH determination of solution under-test.

The emf of the cell is calculated by using

$$E_{cell} = E_{calomel} - E_{glass}$$

$$E_{cal} = 0.2422V \;,\; \epsilon_{glass} = \epsilon^{\circ}_{G} + 0.0592V\,pH$$

$$\epsilon_{cell} = 0.2422V - [\epsilon^{\circ}_{G} + 0.0592\,V\,pH]$$

$$\epsilon_{cell} = 0.2422V - \epsilon^{\circ}_{G} - 0.0592\,V\,pH$$

$$0.0592\,V\,pH = 0.2422\,V - E^{\circ}_{G} - E_{cell}$$

$$\therefore pH = \frac{0.2422\,V - \left[E^{\circ}_{G} + E_{cell}\right]}{0.0592\,V.}$$