

INSTRUMENTAL METHODS AND APPLICATIONS

Introduction

Instrumental methods are Analytical techniques or Spectroscopy methods. Spectroscopy is one of the most powerful tools available for the study of atomic and molecular structure and used in the analysis of a most of the samples.

Spectroscopy deals with the study of interaction of electromagnetic radiation with the matter. During the interaction, the energy is absorbed (or) emitted by the matter. The measurement of this radiation frequency (absorbed (or) emitted) is made using spectroscopy.

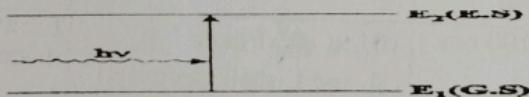
TYPES OF SPECTROSCOPY:- The study of spectroscopy can be carried out under the following two ways,

1. **Atomic spectroscopy:** -It deals with the interaction of the electromagnetic radiation with atoms. During which the atoms absorb radiation and gets excited from the ground state electronic energy level to another.
2. **Molecular spectroscopy-** It deals with the interaction of electromagnetic radiation with molecules. This results in transition between rotational, vibrational and electronic energy levels.

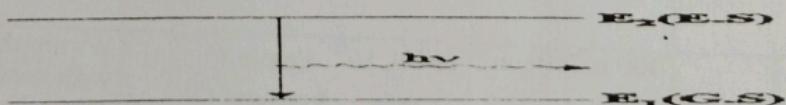
❖ SPECTRUM: - How does a Spectrum arise?

1. **Absorption spectrum:-** When a beam of electromagnetic radiation is allowed to fall on a molecule in the ground state, the molecule absorbs photon of energy $h\nu$ and undergoes a transition from the lower energy level to the higher energy level. The measurement of this decrease in the intensity of radiation is the basis of absorption spectroscopy.

Let us consider a molecule having only two energy levels E_1 and E_2



2. **Emission spectrum:-** If the molecule comes down from the excited state to the ground state with the emission of photons of energy $h\nu$, the spectrum obtained is called *emission*.



ABSORPTION OF RADIATION

- When electromagnetic radiation is passed through a matter, the following changes occur.
- As the photons of electromagnetic radiations are absorbed by the matter, electronic transition, vibrational changes (or) rotational changes may occur. After absorption, molecules get excited from the ground state to excited state. Then they liberate energy quickly in the form of heat (or) re-emit electromagnetic radiation.
- But in some cases, the portion of electromagnetic radiation, which passes into the matter, instead of being absorbed may be scattered (or) reflected (or) re-emitted.
- When the electromagnetic radiation is absorbed (or) scattered, it may undergo changes in polarization (or) orientation.

- In some cases the molecules absorbs radiation and get excited.
- **Fluorescence**:-If the excited molecules re-emits the radiation almost instantaneously (within 10- 8 seconds), it is called fluorescence.
- **Phosphorescence**:-If the excited molecules re-emit the radiation after sometime (slowly), it is called phosphorescence.

Factors affecting the absorbance

The fractions of photons being absorbed by the matter depends on,

1. The nature of the absorbing molecules.
2. The concentration of the molecules. - If the concentration of the molecules is more, the absorbed photons will be more.
3. The length of the path of the radiation through the matter. - If the length of the path is long, the larger number of molecules are exposed and hence greater the photons will be absorbed.

ELECTROMAGNETIC SPECTRUM

The entire range over which electromagnetic radiation exists is known as electromagnetic spectrum. The electromagnetic spectrum covers larger range of wavelength. The major characteristic of various spectral regions are described as follows:

Spectral region & Sources	Wave length range	Energy change involved	Characteristics
Gamma Rays	100 to 1 pm	10^9 to 10^{11} J/mole (Nuclear level)	Shortest wavelength emitted By atomic nuclei.
X-rays	10 to 100 nm	10^7 to 10^9 J/mole (K and L-shell electrons)	Emitted (or) absorbed by movement of electrons close to nuclei.
Ultraviolet	100 to 400 nm	10^7 to 10^6 J/mole (middle and valance shell electrons)	Since air starts absorbing below 180nm, above 180 nm is used for chemical analysis.
Visible	400 to 750 nm	10^6 to 10^5 J/mole (valence electrons)	Within the visible region, a person with normal color vision is able to sense the color.
Infrared	0.75 to 1000 m	10^3 to 10^5 J/mole (molecular, vibration and rotation)	This region corresponds to change in the vibration of the molecules.
Microwave	0.1 to 50 cm	10^1 to 10^3 J/mole (molecular rotation spin orientation)	It corresponds to change in the rotation of molecule.
Radio wave	1 to 30 m	Less than 10^1 J/mole	It corresponds to change the spin of the electron.

Beer – Lambert's Law

It is linear relationship absorbance and concentration of absorbing substances and it is combination of two laws. They are Beer's Law and Lambert's Law.

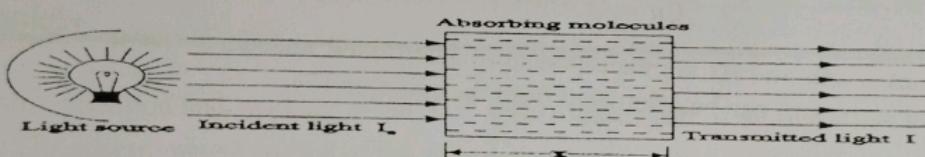
Beer's Law states that, when a monochromatic light is passed through a sample solution, then absorbance of a sample is directly proportional to the concentration of the solution. i.e $A \propto C$

Lambert's Law states that, when a monochromatic light is passed through a sample solution, then absorbance of a sample is directly proportional to the thickness of the solution. i.e $A \propto x$

Beer – Lambert's Law states that, when a monochromatic light is passed through a sample solution, then absorbance of a sample is directly proportional to the concentration and thickness of the solution. i.e $A \propto Cx$ (or) $A = \epsilon Cx$

Derivation of Beer – Lambert's Law Equation

According to **Beer – Lambert's Law**, "when a monochromatic light is passed through a solution of an absorbing substance, the rate of decrease of intensity of light 'dI' with thickness of the absorbing solution 'dx' is proportional to the intensity of incident light 'I' as well as the concentration of the solution 'C'."



- It is mathematically represented as
- $\frac{-dI}{dx} \propto IC$ ----- (1) Since $dI \propto I$
- $\frac{-dI}{dx} = kIC$ ----- (2) $dI \propto c$
- $\frac{dI}{I} = -kCd x$ ----- (3) $dI \propto x$
- where, k = molar absorption coefficient.
- On integrating eq.(3) between limits $I = I_0$ at $x = 0$ and $I = I$ at $x = x$, we get
- $$\int_{I_0}^I \frac{dI}{I} = \int_0^x kCd x$$
- $$-\ln \frac{I}{I_0} = kCx$$
 (or)
- $$\ln \frac{I_0}{I} = kCx$$
 (or)
- $$2.303 \log \frac{I_0}{I} = kCx$$
 (or)

$$\log \frac{I_0}{I} = k/2.303 Cx \text{ (or)}$$

$$A = \epsilon Cx \text{ ----- (4)}$$

Where, $\epsilon = k/2.303$ = molar absorptive coefficient, $\log \frac{I_0}{I} = A$ = Absorbance or Optical density.

- The above equation (4) is called **Beer- Lambert's Law**.
- Thus, the absorbance (A) is directly proportional to molar concentration (C) and thickness (or) path length (x).

Application of Beer-Lambert's law

To determine the unknown concentration of a given solution by using Beer-Lambert's law as follows.

- Let us consider First absorbance of a standard solution = A_s
- standard solution of known concentration= C_s
- absorbance of Unknown solution= A_u
- Solution of Unknown concentration= C_u
- First absorbance ' A_s ' of a standard solution of known concentration ' C_s ' is measured, then
- According to Beer-Lambert's law,

$$A_s = \epsilon C_s x$$

$$A_s / C_s = \epsilon x \quad \dots \dots \dots (5)$$

- Now, absorbance ' A_u ' of a solution of unknown concentration C_u is measured.. Now we have

$$A_u = \epsilon C_u x$$

$$A_u / C_u = \epsilon x \quad \dots \dots \dots (6)$$

From eq. (5) and (6), we get.

$$A_s / C_s = A_u / C_u$$

$$C_u = A_s / C_s * A_u \quad \dots \dots \dots (7)$$

- Since the values of A_u and A_s are experimentally determined and C_s is known. The value C_u (unknown concentration) can be calculated from the equation (7).

Limitations of Lambert-Beer's law

1. This law is not obeyed if the radiation used is not monochromatic.
2. It is applicable only for dilute solutions.
3. The temperature of the system should not be allowed to vary to a large extent.
4. It is not applied to suspensions.
5. Deviation may occur, if the solution contains impurities.
6. Deviation also occurs if the solution undergoes polymerization (or) dissociation.

UV-VISIBLE SPECTROSCOPY

UV spectroscopy is one type of absorption spectroscopy in which light of ultra-violet region (200-400 nm.) and visible region (400-750 nm) is absorbed by the molecule.

Absorption of light in Uv-Vissible region causes Electronic transition from higher energy level to lower energy levels, so it is also known as electronic spectroscopy.

Principle of UV spectroscopy:

Ultraviolet (UV) Visible-spectra arises from the transition of valence electrons within a molecule (or) ion from a lower electronic energy level (ground state E_0) to higher electronic energy level (excited state E_1). This transition occurs due to the absorption of UV (wavelength 100-400 nm) (or) visible (wavelength 400-750 nm) region of the electronic spectrum by a molecule (or) ion.

UV spectroscopy obeys the Beer-Lambert law, which states that,

$$A = \log (I_0/I) = \epsilon c x$$

Where, A = absorbance

I_0 = intensity of light incident upon sample cell

I = intensity of light leaving sample cell

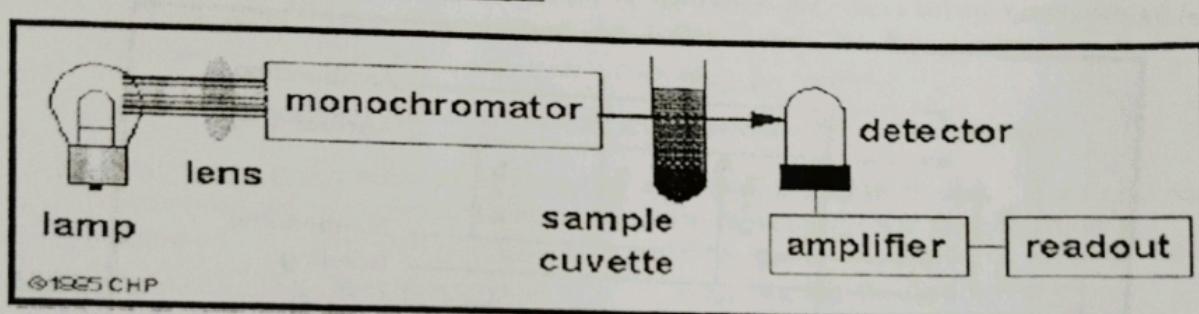
C = molar concentration of solute

x = length of sample cell (cm.)

ϵ = molar absorptivity

From the Beer-Lambert law it is clear that greater the number of molecules capable of absorbing light of a given wavelength, the greater the extent of light absorption. This is the basic principle of UV spectroscopy.

Instrumentation of UV-Vis Spectroscopy:



The Instrumentation and working of the UV spectrometer can be studied simultaneously. Most of the modern UV spectrometers consist of the following parts-

1. Light Source- Tungsten filament lamps and Hydrogen-Deuterium lamps are most widely used and suitable light source as they cover the whole UV region. Tungsten filament lamps are rich in red radiations; more specifically they emit the radiations of 375 nm, while the intensity of Hydrogen, Deuterium lamps falls below 375 nm.

2. Monochromatic- It is generally composed of prisms and slits. The most of the spectrophotometers are double beam spectrophotometers. The radiation emitted from the primary source is dispersed with the help of rotating prisms. The various wavelengths of the light source which are separated by the prism are then selected by the slits such the rotation of the prism results in a series of continuously increasing wavelength to pass through the slits for recording purpose. The beam selected by the slit is monochromatic and further divided into two beams with the help of another prism.

3. Sample and reference cells- One of the two divided beams is passed through the sample solution and second beam is passed through the reference solution. Both sample and reference solution are contained in the cells. These cells are made of either silica or quartz. Glass can't be used for the cells as it also absorbs light in the UV region.

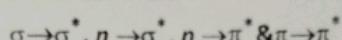
4. Detector- Generally two photocells serve the purpose of detector in UV spectroscopy. One of the photocell receives the beam from sample cell and second detector receives the beam from the reference. The intensity of the radiation from the reference cell is stronger than the beam of sample cell. This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

5. Amplifier- The alternating current generated in the photocells is transferred to the amplifier. The amplifier is coupled to a small servo meter. Generally current generated in the photocells is of very low intensity, the main purpose of amplifier is to amplify the signals many times so we can get clear and recordable signals.

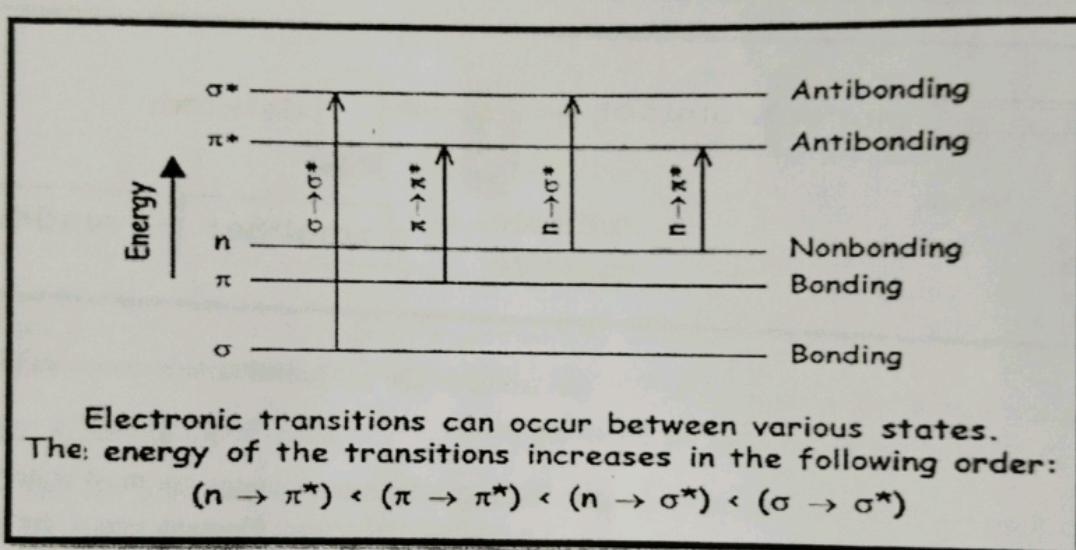
6. Recording devices- Most of the time amplifier is coupled to a pen recorder which is connected to the computer. Computer stores all the data generated and produces the spectrum of the desired compound.

Types of transitions in organic compounds:-

Energy absorbed in the visible and UV region by a molecule causes transitions of valence electrons in the molecule. These transitions are



The energy level diagram for a molecule is shown in the figure,



- $n \rightarrow \pi^*$ transitions:** -These are shown by unsaturated molecule containing hetero atoms like N, O & S. It occurs due to the transition of non-bonding lone pair of electrons to the anti-bonding orbitals. This transition shows a weak band, and occurs in longer wavelength with low intensity.
- $\sigma \rightarrow \sigma^*$ transitions:** -These occur in the compounds, in which all the electrons are involved in single bonds and there are no lone pair of electrons. The energy required is very large. The absorption band occurs in the far UV region (120-136 nm).
- $n \rightarrow \sigma^*$ transitions** -These occur in the saturated compounds containing lone pair (non-bonding) of electrons in addition to $\sigma \rightarrow \sigma^*$ transitions. The energy required for an $n \rightarrow \sigma^*$ transition is less than that required for a $\sigma \rightarrow \sigma^*$ transition. This absorption band occurs at longer wave length in the near UV region (180-200nm).
- $\pi \rightarrow \pi^*$ transitions:** -These occur due to the transition of an electron from a bonding π orbital to an anti-bonding π^* orbital. This transition can occur in any molecule having a π electron system. Selection rule determines whether transitions to a particular π^* orbital are allowed (or) forbidden.

Origin of UV-visible spectroscopy

The actual amount of energy required depends on the difference in energy between the ground state and the excited state of the electrons. $\Delta E = E_1 - E_0 = h\nu$.

Electronic transition depends on the electronic structure of the absorbing medium (sample). The absorption of UV-visible radiation in organic molecule is mainly due to presence of certain functional group.

The two important groups are responsible for absorption and position of absorption in UV-visible spectra. They are
1. Chromophores 2. Auxochromes

1. Chromophores: (The presence of one (or) more unsaturated linkages (π -electrons) in a compound is responsible for the colour of the compound, these linkages are referred to as chromophores) "The substance which is giving colour to the compound".

Examples: $C=C$; $-C\equiv C-$; $-C\equiv N$; $-N=N-$; $C=O$; etc., β -carotene.

Chromophores undergo $\pi \rightarrow \pi^*$ transitions in the short wavelength regions of UV-radiations.

2. Auxochromes: It refers to an atom (or) a group of atoms which does not give rise to absorption band on its own, but when conjugate to chromophore will cause a redshift)

Examples: $-OH$, $-NH_2$, $-Cl$, $-Br$, $-I$, etc., "The substance which is develops intensity of the colour to the chemical compound."

Applications of UV-Vis- Spectroscopy:

1. Detection of functional groups-

UV spectroscopy is used to detect the presence or absence of chromophore in the compound. This is technique is not useful for the detection of chromophore in complex compounds. The absence of a band at a particular band can be seen as an evidence for the absence of a particular group. If the spectrum of a compound comes out to be transparent above 200 nm than it confirms the absence of a) Conjugation b) A carbonyl group c) Benzene or aromatic compound d) Bromo or iodo atoms.

2. Predicting relationship between different groups.

3. Detection of impurities

4. Detection of extent of conjugation

5. Identification of an unknown compound-

6. Determination of molecular weight

7. Determination of Calcium in blood serum

8. Study of tautomeric equilibrium

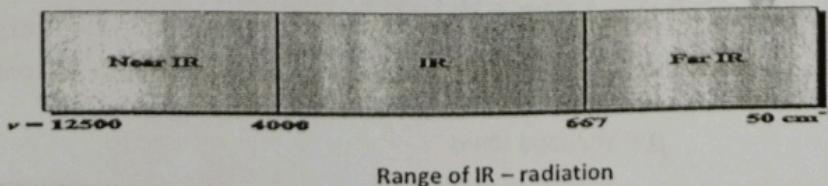
9. Studying kinetics of chemical reactions.

10. Qualitative and Quantitative analysis.

IR-SPECTROSCOPY

IR spectroscopy is one type of Absorption spectroscopy. It deals with the infrared region (50 to 12500 cm^{-1}) of the electromagnetic spectrum. It is used to determine functional groups in molecules.

Absorption of light in IR-region causes transition between vibrational and rotational energy levels so it is also known as vibrational spectroscopy.



Range of IR – radiation

Principle of IR-Spectroscopy: IR-spectra compounds will get IR-spectra gets excited and shows vibrational and rotational spectra, which is characteristics for functional groups and fingerprinting.

Finger print region: The vibrational spectral (IR-spectra) region at $1400 - 700 \text{ cm}^{-1}$ gives very rich and intense absorption bands. This region is termed as finger print region. The region $4000 - 1430 \text{ cm}^{-1}$ is known as Group frequency region.

Uses of finger print region: - Finger print region can be used to detect the presence of functional group and also to identify and characterize the molecule just as a fingerprint can be used to identify a person.

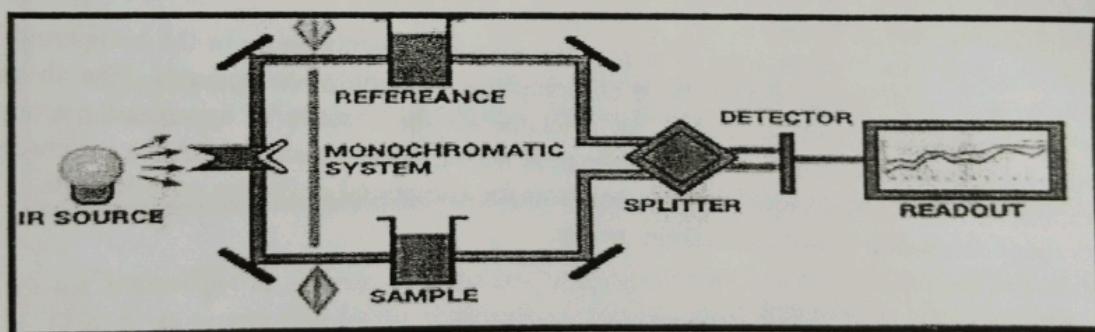
Conditions of IR- spectroscopy: -

1. IR-Spectroscopy gives the information about functional groups present in the sample molecule.
2. IR-Spectroscopy used in organic and In-organic compounds (solids, Liquids, Gases)
3. In general, NaCl, KBr and CaF₂ solutions used in IR-Spectroscopy.
4. During vibration, permanent dipole movement cannot be change.

Instrumentation of IR Spectroscopy

The instrumentation of infrared spectroscopy is illustrated below. First, a beam of IR light from the source is split into two and passed through the reference and the testing sample respectively.

Now, both of these beams are reflected to pass through a splitter and then through a detector. Finally, the required reading is printed out after the processor deciphers the data passed through the detector.



Types of Molecular vibrations

Atoms in a molecule are continuously vibrating, molecules are also vibrating and there are mainly two types of fundamental vibrations,

1. Stretching vibrations -During stretching the distance between two atoms decreases (or) increases, but bond angle remains unaltered. These are two ways occurs, 1. Symmetrical stretching 2. Asymmetrical stretching

2. Bending vibrations (or) deformation vibrations- During bending bond angle increases and decreases but bond distance remains unaltered. These are four ways occurs, 1. Scissoring 2. Rocking 3. Wagging 4. Twisting

$$\text{Vibrational frequency } (\nu) = 1/2\pi c (k/\mu)^{1/2}$$

Where, c = Velocity of radiation

K = Force constant

μ = reduced mass

vibrational changes depend on the masses of the atoms and their spatial arrangement in the molecule. When IR light of the same frequency is incident on the molecule, energy is absorbed resulting in increase of amplitude of vibration. When the molecule returns from the excited state to the original ground state, the absorbed energy is released as heat. Thus, every compound shows characteristic absorption bands in the IR region of the spectrum. Different functional groups produce easily recognizable band at definite positions in the IR spectral range (12500 to 50 cm⁻¹).

The number of fundamental (or) normal vibrational modes of a molecule can be calculated as follows.

1. **For Non-linear molecule:** - A non-linear molecule containing 'n' atoms has $(3n-6)$ fundamental vibrational modes.

Examples: 1.) $CH_4 \rightarrow (3 \times 5 - 6) = 9$ fundamental vibrational modes.
2.) $C_6H_6 \rightarrow (3 \times 12 - 6) = 30$ fundamental vibrational modes.

2. **For Linear molecules:** - A linear molecule containing 'n' atoms has $(3n - 5)$ fundamental vibrational modes.

Example: $CO_2 \rightarrow (3 \times 3 - 5) = 4$ fundamental vibrational modes.

Applications of IR Spectroscopy:

Infrared spectroscopy is widely used in industry as well as in research. It is a simple and reliable technique for measurement, quality control and dynamic measurement. It is also employed in forensic analysis in civil and criminal analysis.

Some of the major applications of IR spectroscopy are as follows.

1. Identification of functional group and structure elucidation.
2. Identification of substances.
3. Studying the progress of a chemical reaction.
4. Detection of impurities.
5. Determination of shape or symmetry of a molecule.
6. Determination of hydrogen bonding in a molecule.
7. Determination of aromaticity.
8. Quantitative analysis.

Chromatography

Chromatography word is obtained from Greek language. Chroma means color and Graphy means writing. That means color writing. It is discovered by **M.Tswett**.

This technique for rapid and separation of colored compound found in plants. This technique has follow three important points,

1. Mobile phase/Stationary phase
2. Characteristics- volatility, polarity
3. Mechanism- column

Stationary phase/ Fixed phase: - It is porous nature (solid or Liquid), For Examples- Alumina, Silica, Limestone, Magnesia, Starch, Activated charcoal etc.

Mobile phase/ Moving phase: - Separating substances either liquid or gas.

~~Chromatography~~ is a technique to separate mixtures of substances into their components on the basis of their molecular structure and molecular composition.

This involves a stationary phase (a solid, or a liquid supported on a solid) and a mobile phase (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it. Sample components that display stronger interactions with the stationary phase will move more slowly through the column than components with weaker interactions. This difference in rates causes the separation of various components. Chromatographic separations can be carried out using a variety of stationary phases, including immobilized silica on glass plates (thin-layer chromatography), volatile gases (Gas chromatography), paper (Paper chromatography) and liquids (Liquid chromatography).

Thin layer chromatography (TLC)

TLC discovered in 1938 by Lsmailov & Schreiber who utilized thin layer(2 mm) of Alumina on glass plates for chromatographic separation while Fagan stanl developed the equipment for reproducible TLC.

TLC is a technique used to identify the components present in a mixture by separating them using a thin stationary phase (silica gel) supported on an inert substrate and a mobile phase (solvent).

- ✓ To study the purity of the compound
- ✓ To study the progress of a reaction
- ✓ To identify the various compounds present in the mixture.
- **Stationary phase-** Polar nature substance can be used, such as silica gel, Alumina, etc. over glass/metal plate, plastic sheet etc.

Silica gel-**Acidic**- steroids, amino acids, hydrocarbons etc. It has polar nature and separation follows adsorption mechanism.

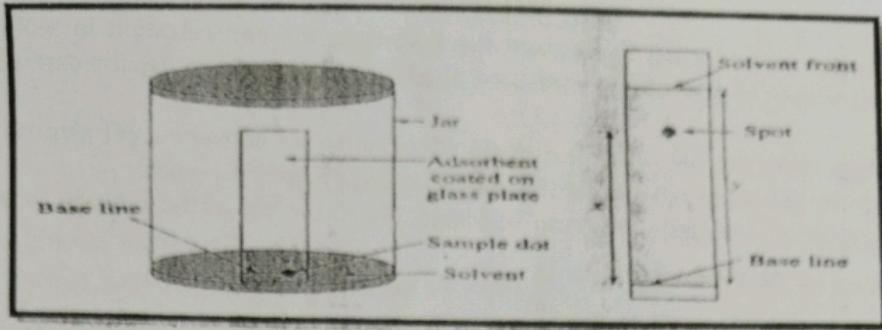
Alumina- **Basic** – Amines, lipids, bile etc.

Cellulose- **Neutral**- carbohydrates, sugar etc.

- **Mobile phase-** Polar/ Non-polar nature substance can be used, such as Isopropyl alcohol, Benzene, Ether for developing solvent/mixture of solvents(reasonably volatile)
- **Sample-** Sample containing mixture of compounds or individual compounds.
- **Requirement materials:** - TLC Chamber (Jar), Silica gel, Solvent (Isopropyl alcohol in water), Glass plate.
- **Principle:** - TLC is an adsorption chromatography based on the principle of differential adsorption of the components of a mixture.

Procedure:-

1. First make a thin layer of silica gel on glass plate. Now dry this layer of silica gel.
2. When the silica gel layer dries up then put and marks of a sample.
3. Now make the 50% solution of Isopropyl alcohol in water.
4. Now put the silica plate with the spot of sample on it in the 50% solution of Isopropyl alcohol in a developing Jar.
5. Now wait until the different composition gets separated.
6. Now measure the distance travelled by the given sample and the distance travelled by solvent.
7. Note down the reading of the Retention factor (R_f) value (0 to 1).



Retention factor (R_f) - distance travelled by sample / distance travelled by solvent = x/y

Advantages:-

- ✓ Sample and purity of samples can be determined.
- ✓ Speed of separation is high
- ✓ Very cheap
- ✓ Solvents can easily be changed

Disadvantages:-

- ✓ Stationary phase is short
- ✓ High detection limit
- ✓ Open system- temperature and humidity can affect the results.

Applications

- ✓ In **organic chemistry**- TLC has been widely purification and Identification of Individual components in a mixture.
- ✓ **Food industries**- Pesticides and fungicides in drinking water, cereals, milk, preservatives, residues and additives in meat, fish etc.
- ✓ **Cosmetology**-Preservatives, fatty acids, surfactants, end products
- ✓ **Pharmaceutical industries**- Ingredients in drugs, preservatives in drugs.
- ✓ **Organic chemistry, Clinical chemistry, Forensic chemistry, Bio-chemistry**- end products and by-products
- ✓ **Environmental science**- Contaminants and pollutants in ground water, soil, dyes used in textiles effluents.

Superiority of TLC:-

TLC is considered to be superior to other chromatographic techniques such as paper and Column chromatography because,

1. Identification and separation by TLC can be carried out quickly.
2. Excellent separation can be obtained with thin layer in 15-50min.
3. It is more sensitive and gives sharper zones diffusion of spot is minimum.
4. This technique is very simple equipment requires is very low.
5. The capillary of thin layer of an adsorbent is higher than that of paper.

pH -METRY

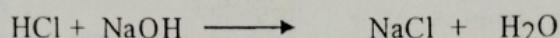
pH - metry is a scientific method used to measure the hydrogen ion concentration in water based solutions, indicating its acidity (or) alkalinity expressed as pH. The pH metry is usually carried out by pH meter.

Principle:- The pH meter measures the difference in electrical potential between a pH electrode and a reference electrode, so the pH meter is also referred to as a potentiometric pH meter.

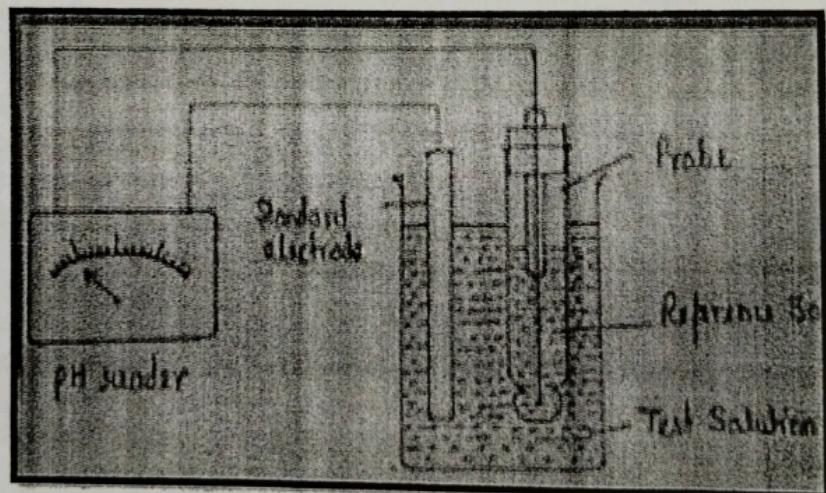
These electrodes are inserted into the solution to be tested. Since the pH of the solution is related to the H^+ ion concentration by the following formula.

$$pH = -\log H^+$$

Measurement of pH gives the concentration of H^+ ions in the solution. When NaOH is added slowly from the burette to the solution of HCl, the fast moving H^+ ions are progressively replaced by slow moving Na^+ ions. As a result pH of the solution increases.



The increase in pH takes place until all the H^+ ions are completely neutralised (upto the end point). After the end point, further addition of NaOH increases the pH sharply as there is an excess of fast moving OH^- ions.



Applications of pH metry:-

1. The rate of chemical reactions, taking place in water, depends on the acidity of water and is therefore useful to know the acidity of water. It is done by using pH meter.
2. It is useful to monitor the pH in agriculture, water quality, swimming pool, environment a remediation.
3. It is useful in healthcare and clinical applications such as blood chemistry
4. It is also useful in direct measurement of pH inside of living cells.
5. Specially designed electrodes are available to measure the pH of semi-solid substances such as foods.