

UNIT-V**Instrumental Methods of analysis and its Applications****Electromagnetic spectrum:**

The arrangement of all types of electromagnetic radiations in order of their increasing wavelengths or decreasing frequency is known as complete electromagnetic spectrum. The visible spectrum (from violet to red through rainbow colors) represents only a small portion of the electromagnetic spectrum. If we arrange all types of electromagnetic radiations in order of their increasing wavelengths, then the portion above the visible region is called infra-red while that below it is the ultraviolet region.

Infrared radiations have longer wavelengths and are thus, less energetic. Microwaves have larger wavelengths and are used in telephone transmission.

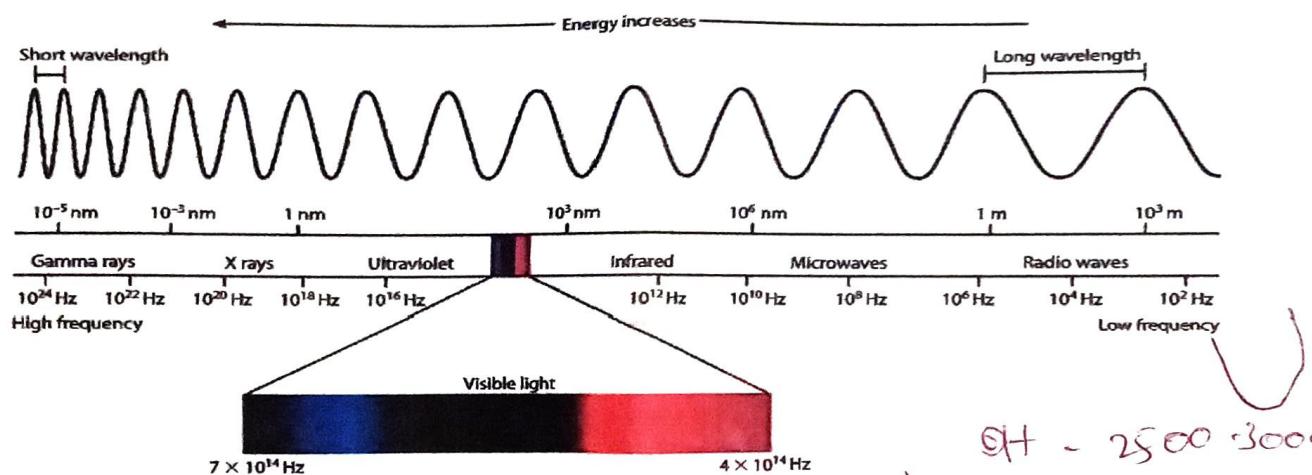


Figure: Electromagnetic spectrum

Although, all types of radiations travel as waves with the same velocity, yet they differ from one another in certain properties.

Absorption of Radiations: electromagnetic radiation (light) can pass through a sample of organic molecule, and then some of the light is absorbed at certain wavelengths, while remaining light is unaffected.

A molecule can only absorb the radiation of certain frequency, if there exists within a molecule an energy transition of magnitude, ΔE which is equal to $h\nu$.

When electromagnetic radiations are passed through an organic compound, then electrons of the component atoms are excited. In addition, the vibrational and the rotational energies of the molecules as a whole are quantized. Thus any wavelength of light that a particular molecule will absorb will be due to the changes in the electric, vibrational and the rotational energy levels permissible for its atoms. The wavelengths absorbed are measured with the help of a spectrometer.

If we plot the changes in absorption against wavelengths, we get certain absorption bands which are highly characteristic of a compound and the technique provides an excellent tool to ascertain the molecular structure of an unknown substance.

In an electromagnetic spectrum, we may note that:

Radiation absorbed	Effect on the molecule of a substance and information obtained
Ultra-violet (190-400)nm And visible (400-800)nm	Changes in electronic energy levels within molecule, conjugated unsaturation, conjugation with non-bonding electrons, extent of π -electron system.
Infra-red (667-4000) cm⁻¹	Changes in the rotational and vibrational energy (or) moments of the molecule. Detection of almost all functional groups in organic chemistry Which have specific vibrational frequencies such, C=O, O—H, NH ₂ , C≡C etc.
Radio-frequency (60-300)MHz	Nuclear magnetic resonance induces changes in the magnetic properties of certain atomic nuclei, notably that of hydrogen (hydrogen atoms in different environments can be detected, counted and analyzed for structure determination).
Electron beam impact (70cV, 6000 kJ mol⁻¹)	Ionization and fragmentation of the molecule into a spectrum of fragment ions (determination of molecular weight and deduction of molecular structure from the fragments obtained).

UV-Visible spectrophotometry

Introduction:

UV-Visible spectroscopy is a study of electronic transition taking place in a molecule on irradiation with UV-Visible range of radiation. Hence this technique also called as electronic spectroscopy. Since it involves the change of electrons (σ , π and n) from ground state to higher energy state. It is very useful to measure the number of conjugated double bonds and also aromatic conjugation within the various molecules. It also distinguishes between the conjugated and non conjugated system; α , β unsaturated carbonyl compounds from β , γ -analogues; homoannular and heteroannular conjugated dienes etc.

For visible and ultra-violet spectrum, electronic excitations occur in the range 200-800nm or 200-800m μ and involve the promotion of electrons to the higher energy molecular orbital. Since the energy levels of molecules are quantized, ($E=h\nu$) the energy required to bring about the excitation is a fixed quantity.

RANGE:

UV-Visible wave length Range 200-800nm or m μ .

UV wave length range 200-400nm and visible wavelength 400-800nm. shown figure1

A record of the amount of light absorbed by the sample as a function of the wave length of light in m μ (milli microns) or nm(nanometer) is called absorption spectrum which generally consists of absorption

bands. The far ultra-violet region ($< 200\text{nm}$) is not much studied due to absorption by oxygen and nitrogen. Moreover studies in these regions require vacuum instruments.

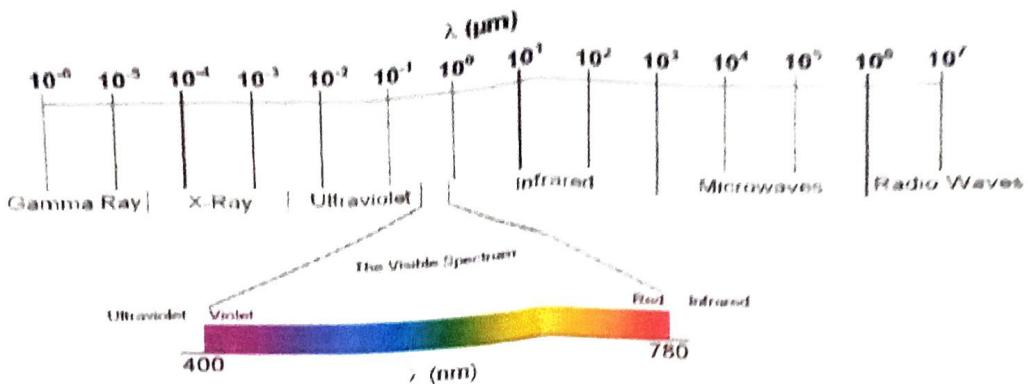


Figure 1: the range of visible region

Absorption laws:

In the Ultra-violet and visible spectroscopy involves in two absorptions laws. These two laws which govern the absorption of light by the molecules.

These are: 1. Lambert's law

2. Beer's law

Lambert's law: This law states that when a beam of monochromatic radiation passes through a homogeneous absorbing medium, the rate of decrease of intensity of radiation with thickness of absorbing medium is proportional to the intensity of the incident radiation.

Mathematically the law is expressed as:

$$-\frac{dI}{dx} = kI$$

Where I = intensity of radiation after passing through a thickness x of the medium

dI = small decreases in the intensity of radiation on passing through infinitesimally small thickness, dx of the medium

$-\frac{dI}{dx}$ = rate of decrease of intensity of radiation with thickness of the absorbing medium

k = proportionally constant or absorption coefficient, its value depends upon the nature of the absorbing medium

Let I_0 be the intensity of radiation before entering the absorbing medium ($x = 0$)

Then I intensity of radiation after passing through any thickness, say x of the medium can be calculated as:

$$\int_{I_0}^I \frac{dI}{I} = - \int_{x=0}^{x=x} k dx$$

$$\ln \frac{I}{I_o} = -kx$$

$$= \frac{I}{I_o} = e^{-kx}$$

It is taken in exponential form.

The above Lambert's law equation can also be written by changing the natural logarithm to the base 10

log apply.

$$\log \frac{I}{I_o} = 10^{-kx}$$

$$I = I_o 10^{-ax}$$

log removed

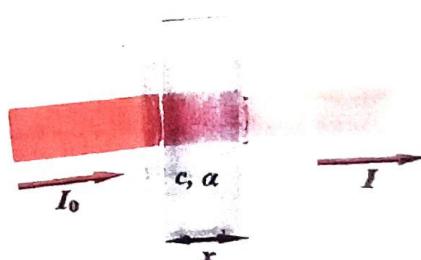
Where a = extinction coefficient of the absorbing medium

$$(a = \frac{k}{2.303})$$

Where $a = k/2.303$. Where k = absorption coefficient, this value depend upon the nature of the absorbing medium.

Beer's law: This law states that when a beam of monochromatic radiation passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with the thickness of the absorbing solution is proportional to the intensity of the incident radiation as well as the concentration of the solution.

Mathematically the law is expressed as



*The Deeper the Glass, the Darker the view,
the lesser of the incident Light that gets
through*

$$-\frac{dI}{dx} = k'Ic$$

Where c = concentration of the solution in moles/lit

K' = molar absorption coefficient and its value depends upon the nature of the absorbing solution

Suppose I_0 be the intensity of the radiation before entering the absorbing solution (when $x=0$), then the intensity of radiation, I , after passing through the thickness x , of the medium can be calculated as:

$$\int_{I_0}^I \frac{dl}{l} = - \int_{x=0}^{x=x} k' c dx$$

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

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

exponential form applied.

Or the above equation can also written by changing the nature of lagraitham to the base 10.

$$\log \frac{I}{I_0} = -kcx$$

$$I = I_0 10^{-\alpha cx}$$

(where α' = molar extinction coefficient of the absorbing medium)

Where $\alpha' = K'/2.303$. Where α' = molar absorption coefficient of the absorbing solution. this value depend upon the nature of the absorbing substance.

Beers law also can be states as:

Alternative expression: on combing the two laws, the Beers- Lamberts law can be formulated as below:

$$\log \frac{I_0}{I} = \epsilon \cdot c \cdot l = A$$

(Or)

$$A = \epsilon \cdot c \cdot l$$

I_0 = Intensity of Incident light

I =intensity of transmitted light

C = concentration of solution in moles/lit

l =path length of the sample (usually 1cm)

ϵ = molar absorption coefficient (or Molar absorptivity)

A =aborbance

Limitations of Beer and lamberts' laws:

The linearity of the Beer-Lambert law is limited by chemical and instrumental factors. Causes of nonlinearity include:

- Deviations in absorptivity coefficients at high concentrations ($>0.01M$) due to electrostatic interactions between molecules in close proximity
- scattering of light due to particulates in the sample
- fluorescencce or phosphorescence of the sample

- changes in refractive index at high analyte concentration
- shifts in chemical equilibria as a function of concentration
- non-monochromatic radiation, deviations can be minimized by using a relatively flat part of the absorption spectrum such as the maximum of an absorption band .
- stray light

Basic principle of UV-Visible spectrophotometry:

UV spectrophotometer principle follows the Beer-Lambert Law. This law states that whenever a beam of monochromatic light is passed through a solution, the rate of intensity of incident radiation decreases with thickness as well as concentration of the solution

This law is expressed through this equation:

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

A= stands for the absorbance,

I_0 =refers to the intensity of light upon a sample cell,

I =refers to the intensity of light departing the sample cell,

C= stands for the concentration of the solute,

l = stands for the length of the sample cell and

ϵ = refers to the molar absorptivity.

From the Beer-Lambert law, it has been established that the greater the number of the molecules that are capable of absorbing light at a certain wavelength, the greater the extent of the absorption of light.

A molecule absorbs UV radiation of frequency (v), the electron in the molecules undergoes transitions from lower energy state to higher energy state shown below.

$$E = h\nu$$

$$E_1 - E_2 = h\nu \quad (\text{Where } \nu = \text{frequency}; h = \text{plank constant})$$

Three types of electronic transitions observed in molecules or of analyte absorbing UV or visible radiation.

1. The transitions involving sigma (σ) Pi (π) and nonbonding electrons.
2. The transitions involving charged transfer electrons
3. The transitions involving d and f electrons.

The increasing order of electronic energy levels: $\sigma \rightarrow \sigma^* > n \rightarrow n^* > \pi \rightarrow \pi^* > n \rightarrow \pi^*$

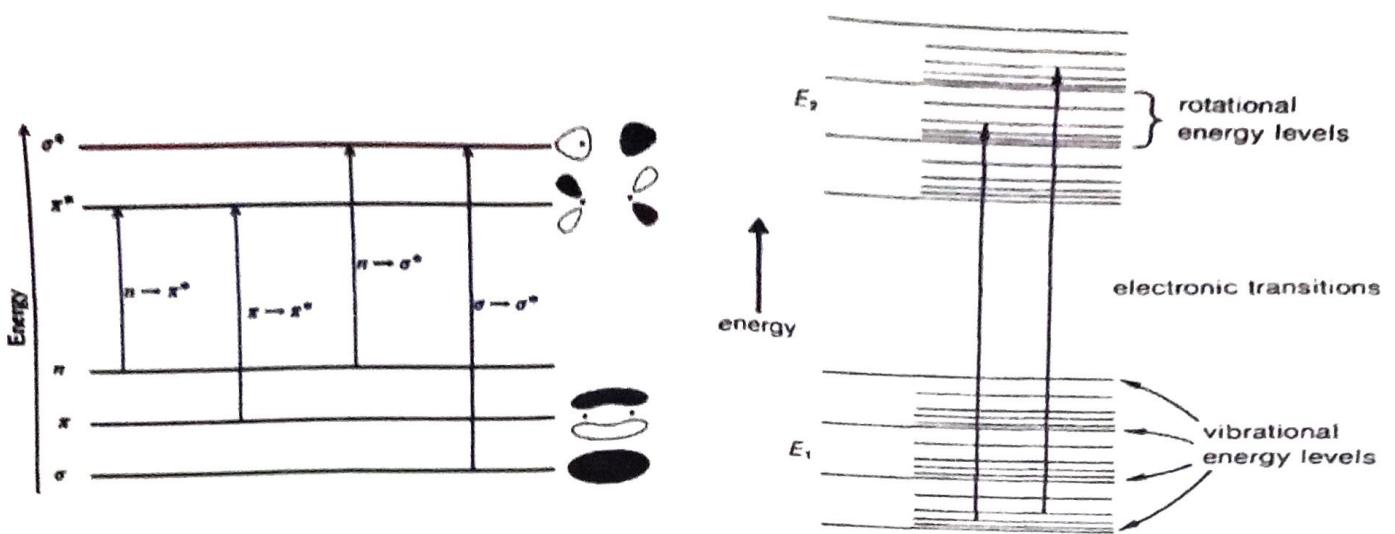


Figure: Change electronic transitions of atom or molecule

TYPES OF ELECTRONIC TRANSITIONS:

According to the molecular orbital theory, when a molecule is excited by the absorption of energy (UV or Visible light), its electrons transfer from bonding orbital to an antibonding orbital.

1. The antibonding orbital which is associated with the excitation of σ (sigma)electron is called σ^* antibonding orbital. So σ to σ^* transitions takes place when σ electron is promoted to antibonding orbital. It is represented as $\sigma \rightarrow \sigma^*$ transition.
2. When a nonbonding electron(n) gets promoted to an antibonding sigma orbital (σ^*), than it represents $n \rightarrow \sigma^*$ transition.
3. Similarly $\pi \rightarrow \pi^*$ transition represents the transfer of π electrons to antibonding π orbital i.e., π^* orbital. Similarly when an n-electron (non-bonding) is promoted to antibonding π orbital, it represents $n \rightarrow \pi^*$ transition. The energy required for various transitions obey the following order.

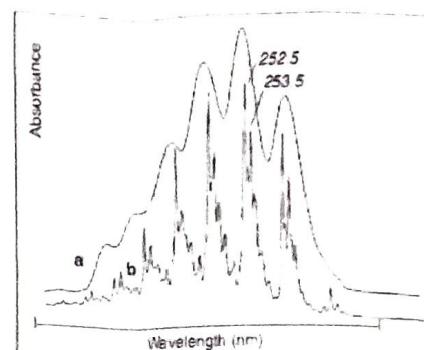
$$\sigma \rightarrow \sigma^* > n \rightarrow \sigma^* > \pi \rightarrow \pi^* > n \rightarrow \pi^*$$

Electronic transitions of organic compounds: transitions involve electrons engaged in σ or π or non-bonding electron orbitals of light atoms such as H,C,N,O.

- **$\sigma \rightarrow \sigma^*$ transition:** Examples: Alkanes, and hydrocarbons.. etc,....
- **$n \rightarrow \sigma^*$ transition:** Examples: Alcohols, Amines, Ethers, carbonyl compounds, ethers, esters, etc.....
- **$n \rightarrow \pi^*$ transition:** Examples: Carbonyl compounds(Aldehydes and ketones), Acids, Esters, etc.....

- $\pi \rightarrow \pi^*$ transition: Examples: Alkenes, Alkynes, Carbonyl compounds, Cyanides, Azo compounds etc.....

UV-Visible absorption Graph: the graph between absorbance or transmittance vs wavelength (λ_{max}). The graph is shown below.



APPLICATIONS OF UV-VIS SPECTROSCOPY

1. Detection of functional groups : ex: C=C, N=N, C=O etc.
2. Extent of conjugation ex: C=C-C=C, C=C-C=C-C=C, C=C-C=C-C=C-C=C etc.
3. Distinction in conjugated and non-conjugated compounds ex: 1,3 butadiene shows highest wavelength when compared with 2-butene
4. Identification of unknown compound
5. Examination of polynuclear hydrocarbons ex: Benzene, Naphthalene, Anthracene etc.
6. Elucidation of the structure of vitamins A and K
7. Preference over two tautomeric forms
8. Determinations of configurations of geometrical isomers ex: Trans molecule shows highest wavelength (λ) when compared with Cis molecules.
9. Distinguish between equatorial and axial conformations
10. It is most widely used technique for quantitative trace analysis, using beers lamberts law is applied
11. It is used food colors and quality of wines
12. It is used to determine the quantitative analysis of transition metals and biological macromolecules
13. It is used to determine the kinetics or rate constant of chemical reaction.
14. It is used in life sciences, to estimate DNA/protein, High level multicomponent quantification.
15. This spectrophotometry used as detector in HPLC
16. It is also used in cement industries; determine the metal ions such as Fe, Mn, etc.

Infrared Spectroscopy (or) IR spectroscopy

Introduction:

IR Spectrum is an important record which gives sufficient information about the structure of a compound. This technique provides a spectrum containing a large number of absorption bands from which a wealth of information can be derived about the structure of an organic compound. The absorption of IR radiation (quantized) causes the various bands in a molecule to stretch and bend with respect to each other.

The most important region for organic chemist is $2.5\text{-}15\mu$ in which molecular vibrations can be detected and measured in an IR spectrum and in a Raman spectrum. The range of infrared region is $0.8\text{-}200\mu$ (or) $50\text{-}12500\text{cm}^{-1}$.

For near IR region 0.8 to 2.5μ or 12500 to 4000 cm^{-1}

For IR region 2.5 to 15μ or 4000 to 667 cm^{-1}

For far IR region 15 to 200μ or 667 to 50 cm^{-1}

The absorption of IR radiations can be expressed either in terms of wavelength (λ) or wavenumber.

Mostly IR spectra of organic compounds are plotted as percentage transmittance against wavenumber.

The relationship of wavelength and wavenumber is as follows:

Wave number = $1/\text{wave length in centimeters}$

If wavelength (λ) is $2.5\mu = 2.5 \times 10^{-4}\text{ cm}$, then wave number = $1/2.5 \times 10^{-4} = 4000\text{cm}^{-1}$ ($1\mu = 10^{-4}\text{ cm}$).

The wavelength 15μ corresponds to wavenumber equal to 667 cm^{-1} . Thus in terms of wavenumber, the ordinary infrared region covers 4000 to 667cm^{-1} . Band intensity can be expressed in terms of absorbance (A) or transmittance (T).

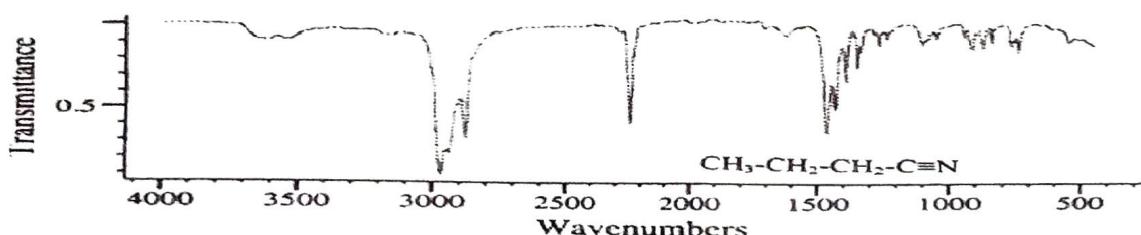


Figure: Infrared spectrum between transmittance and wavenumber

Principle of Infrared spectroscopy:

The absorption of IR radiation causes an excitation of molecule from lower to the higher vibrational level. We know that each vibrational level is associated with a number of closely spaced rotational levels. Clearly the IR spectra considered as vibrational and rotational spectra. All the bands in molecule are not capable of absorbing IR energy but only those bands which are accompanied by a change of dipole moment will absorb in the IR region. Such vibrational transitions which are accompanied by a change in the dipole moment of the molecule are called IR active transitions. Thus these are responsible for absorption of energy in the IR region. On the other hand vibrational transitions which are not accompanied by a change in the dipole moment of the molecule are called IR inactive transitions.

Separation of charge
Separation of plane 9

Stretching:



Bending or Deformation:

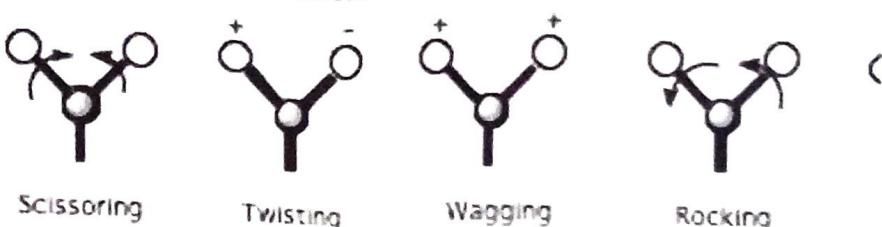


Figure: Types of Vibrations in IR spectroscopy

Ex: C=O, N-H, O-H etc. groups having permanent dipole moment and thus absorbs strongly in the ordinary IR region. But transitions in the C-C symmetrical alkenes and alkynes are not having permanent dipole moment and hence do not absorb in the ordinary IR region.

Applications of IR Spectroscopy:

1. Identification of organic compounds such as , natural products, polymers, synthesized compounds
2. Study of structure elucidation,
3. Study of chemical reaction,
4. Study of keto-enol tautomerism,
5. Study of complex molecules,
6. Study of conformational isomers,
7. Study of geometrical isomers,
8. Study of rotational isomers
9. It is used to find the food contaminants in the food items
10. Identification of bacterial and fungal in biology
11. Qualitative analysis of functional groups,
12. Distinction between two types of hydrogen bonding,
13. Quantitative analysis,
14. Detection of impurity in a compound.
15. Identification of pharmaceutical and pharmaceutical compounds
16. It is used to identify the paint pigment using FT-IR
17. It is used to determine the agricultural impurities like Humic acid or humus in soil

CHROMATOGRAPHY (Separation methods)

Thin layer chromatography (TLC) (Solid-Liquid Chromatography):

Principle of Thin layer chromatography (TLC):

The principle of separation is adsorption. One or more compounds are spotted on a thin layer of adsorbent coated on a chromatographic plate. The mobile phase solvent flows through because of capillary action (against to gravitational force). The components move according to their affinities towards the adsorbent.

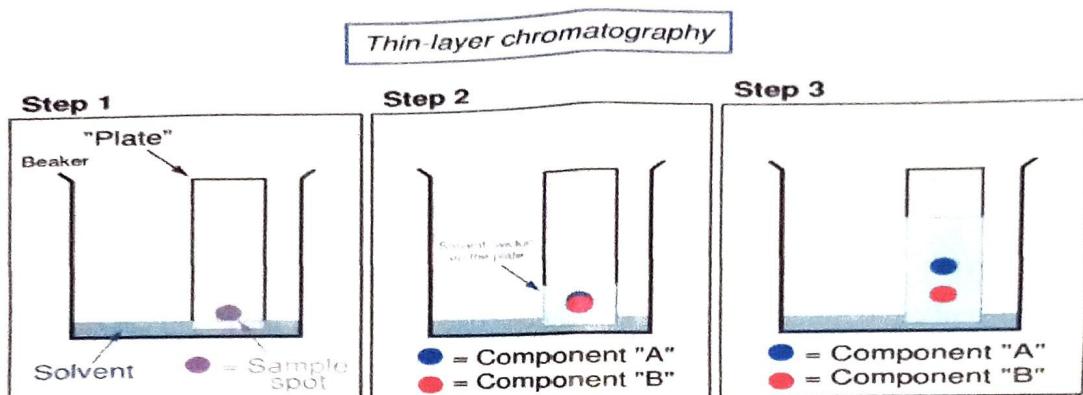


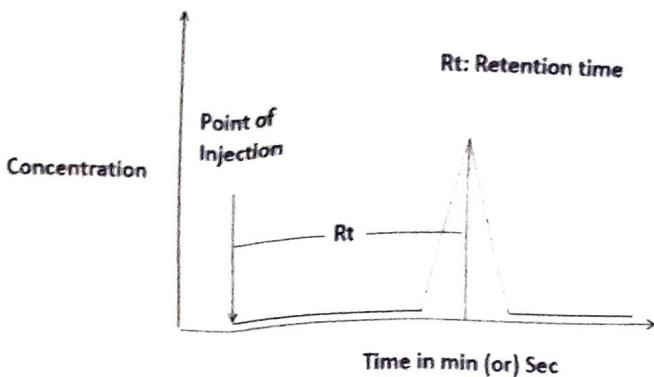
Figure: Schematic representation of TLC principle.

- The component with **more affinity towards the stationary phase** travels slower.
- The component with **lesser affinity towards the stationary phase** travels faster.

Thus the components are separated on a thin layer chromatographic plate based on the affinity of the components towards the stationary phase. Since no two components have the same affinity towards the stationary phase, the components are separated.

Retention Time (R_t):

- Retention time (R_t) is the difference in the time between point of injection and appearance of peak maxima is called retention time (R_t).
- Retention time is represented with "R_t".
- Retention time is the time required for 50% of a component to be eluted from a column also called retention time.
- Retention time is measured in minutes or seconds.



Applications of Thin layer Chromatography (TLC)

The applications of Thin layer chromatography are wider no limitations to the compounds that can be analyzed

1. Separation of mixtures of drugs or chemicals, biological origins and plant extracts (natural products).
2. Separation of carbohydrates, vitamins, antibiotics, proteins, alkaloids and glycosides etc...
3. Identification of drugs
4. Identification of related compounds in drugs
5. to detect the presence of foreign substances in drugs
6. to detect the decomposition of drugs
7. to check the purity of sample
8. to examine the chemical reaction

pH Metry

Introduction:

The acidity and alkalinity of aqueous media, where most reactions in nature occur, depend (according to the Arrhenius definition of acids and bases) on the concentration of the hydronium (H_3O^+) and hydroxyl (OH^-) ions. The former is quantified by the more general concept of pH, initially proposed by the Danish chemist Søren Peder Lauritz Sørensen in 1909 and revised in 1924 to adjust definitions in relation to the electrochemical cells used for its measurement. The pH is notionally defined for any medium as the decimal logarithm of the reciprocal of the hydrogen ion activity.

The pH scale: The values for pH make more sense when compared with that of known substances. Note that the pH scale is logarithmic and that each next value contains ten times less hydrogen ions. A pH=0 contains the most, and is highly acidic.

pH meter Principle and working Procedure:

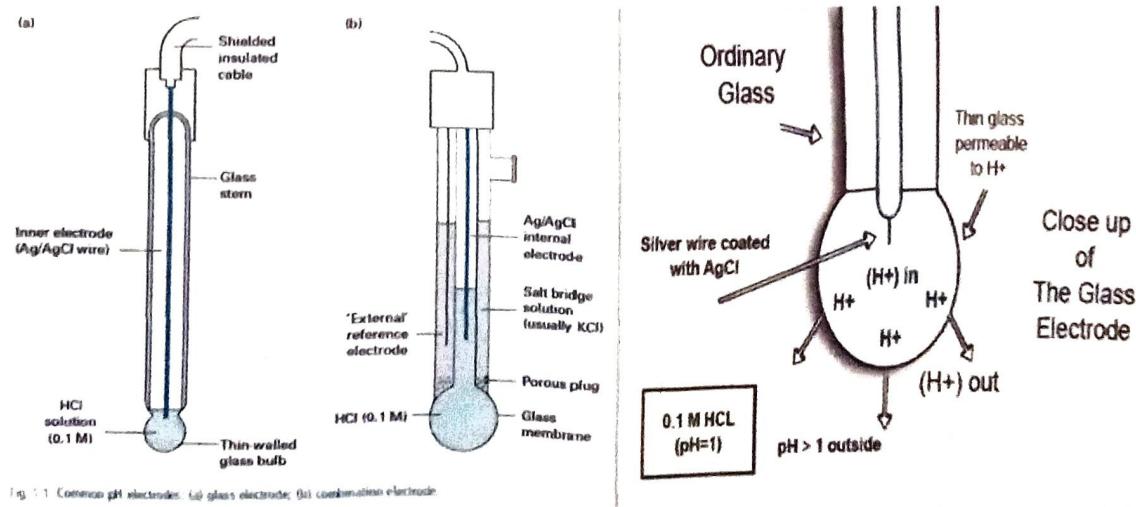
A pH meter is used to determine the acidity or alkalinity of the solution. pH is the concentration of hydrogen ions in the solution. A solution containing more H^+ ions remains acidic while the solution containing more OH^- ions remains alkaline. pH Scale of solutions ranges from 1 to 14.

The solution having pH value 1 will be the highly acidic and with pH value 14 will be highly basic. The acidity and alkalinity of any solution depends on the concentration of hydrogen ions (H^+) and Hydroxyl ion (OH^-) respectively. A neutral solution as pure water has pH 7.0.

pH meter is used to determine the pH of different solutions in Chemical, pharmaceuticals, medical and food industry etc... it is more accurate method than the pH strip. A pH meter contains a pH probe that passes the electrical signals to the pH meter and pH meter displays the pH value of the solution.

The glass pH probe contains two electrodes, a sensor electrode and reference electrode. These electrodes are in the form of glass tubes one contains pH 7 buffer and other contains saturated potassium chloride solution. The sensor electrode bulb is made up of porous glass or permeable glass membrane coated with silica and metal salts.

A silver wire coated with silver chloride is measured in pH 7 buffer in the bulb. Another silver wire coated with silver chloride is immersed in the saturated potassium chloride solution in reference electrode as shown in the figure.



When the probe placed in a solution to measure the pH, hydrogen ions accumulate around the bulb and replace the metal ions from the bulb. The exchange of ions generates some electric flow that is captured by the silver wire.

The voltage of the electric flow is measured by the pH meter by converting it into pH value by comparing the generated voltage with reference electrode.

Increase in acidity of the solution has greater concentration of hydrogen ions that increases the voltage. The increased voltage decreases the pH reading in pH meter.

In the same manner, an increase in alkalinity decrease the hydrogen ions or increases in hydroxyl ions concentration also decreases the voltage and increase the pH value in pH meter.

$$E = E^{\circ} - \frac{2.303RT}{F} \log[H^+]$$

$$E = E^{\circ} + 0.0591 \text{ pH}$$

Or

$$pH = \frac{E - E^{\circ}}{0.059 F} \text{ at } 25^{\circ}$$

The overall working principle of pH sensor and pH meter depends upon the exchange of ions from sample solution to the inner solution (pH 7 buffer) of glass electrode through the glass membrane. The porosity of the glass membrane decreases with the continuous use that decreases the performance of the probe.

Application of pH meter:

The measurement of pH is important for many applications in medicine, biology, chemistry, agriculture, forestry, environmental science, oceanography, civil engineering, chemical engineering, water treatment and water purification, food science, and nutrition.

