5. Instrumental Methods and Applications

Spectroscopy:

Spectroscopy is the branch of science dealing with the study of interaction of electromagnetic radiation with matter. Spectroscopy is the most powerful tool available for the study of atomic and molecular structure and is used in the analysis of a wide range of samples.

The study of spectroscopy can be carried out under the following two heads:

1. Atomic spectroscopy.

This spectroscopy is concerned with the interaction of electromagnetic radiation with atoms which are commonly in their lowest energy state, called the ground state.

2. Molecular spectroscopy.

This spectroscopy deals with the interaction of electromagnetic radiation with molecules. This results in transition between rotational and vibrational energy levels in addition to electronic transitions.

The spectra of molecules are much more complicated than those of atoms. Molecular spectroscopy is of great importance now days, because the numbers of molecules are extremely large as compared with free atoms.

Differences between molecular spectra and atomic spectra:

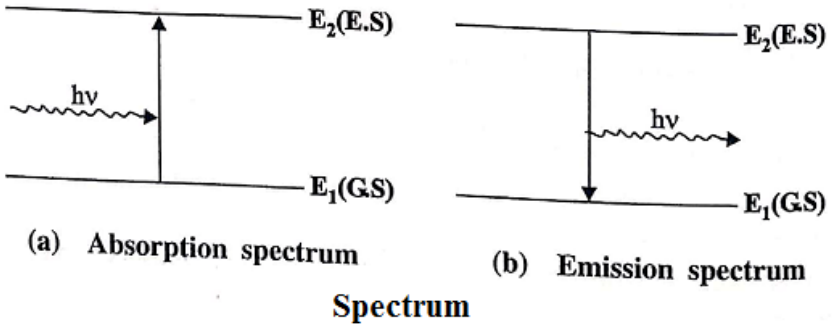
Atomic spectra	Molecular spectra
1. It occurs from the interaction of atoms + electromagnetic radiation 2. Atomic spectra is a line spectra. 3. It is due to electronic transition in an element.	1. It occurs from the interaction of molecules + electromagnetic radiation. 2. Molecular spectra is a complicated spectra. 3. It is due to virbrational, rotational and electronic transition in a molecule.

Spectrum:

Spectrums are two types. They are

1. Absorption spectrum

Consider a molecule having only two energy levels E_1 and E_2 as shown in the figure below.



Spectrum

When a beam of electromagnetic radiation is allowed to fall on a molecule in the ground state, the molecule absorbs photon of energy **hυ**, 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. The spectrum thus obtained is called the **absorption spectrum** (Fig. a).

Emission spectrum:

If the molecule comes down from the excited state to the ground state with the emission of photons of energy **hυ**, the spectrum obtained is called emission spectrum, (Fig. b).

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 polarisation (or) orientation.
- In some cases the molecules absorbs radiation and get excited.

1. Fluorescence

If the excited molecules re-emits the radiation almost instantaneously (within 10^{-8} seconds), it is called **fluorescence**.

2. Phosphorescence

If the excited molecules re-emits the radiation sometime (slowly), it is called **phosphorescence**

Factors Affecting 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 concentrations of the molecules are 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:

An electromagnetic spectrum is obtained when all types of electromagnetic radiations are arranged in the order of increasing wavelength or decreasing frequencies (Fig.).

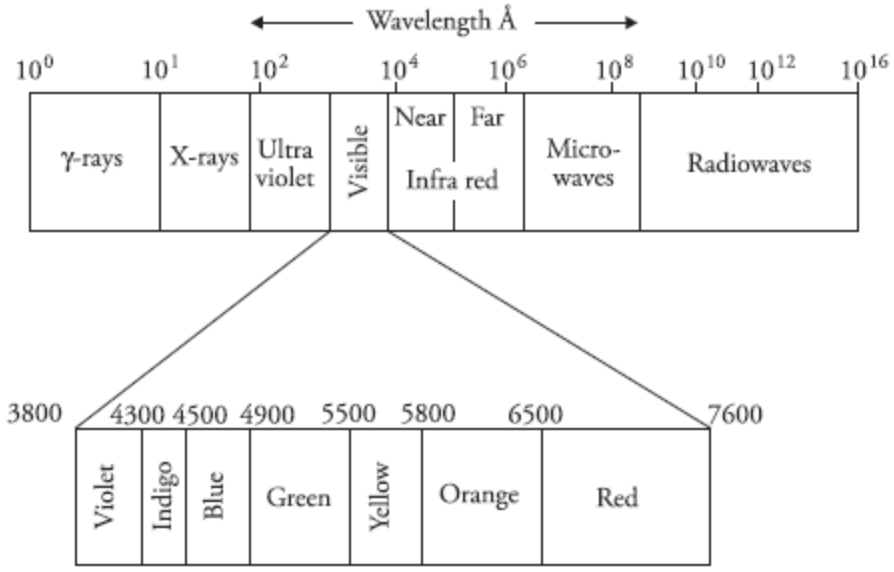
Visible light lies in the wavelength range 3800–7600 Å. The region of 3800 Å corresponds to violet colour and that of 7600 Å corresponds to red colour. If the wavelength is less than 3800 Å, the radiation is called ultraviolet radiation and if it is greater than 7600 Å, it is called infrared radiations. Both infrared and ultraviolet radiations are not visible to the human eye. Beyond infrared region, on the side of increasing wavelength are microwaves. Beyond microwaves, lie the radio waves which have the highest wavelength.

The arrangement of different types of electromagnetic radiation in increasing order of their wavelength is

Cosmic rays < g rays < X rays < Ultraviolet rays < Visible rays < Infrared rays < Microwaves < Radiowaves

Since $V=\frac{C}{\lambda}$, increasing wavelength means decreasing frequency. Thus, cosmic rays have the highest frequencies and radio waves, the least frequencies.

All types of electromagnetic radiations travel with the same speed, the velocity of light, but their wavelength (or frequency) differ from each other. Radio waves are least energetic (maximum wavelength, minimum frequency) and cosmic rays have maximum energy (minimum wavelength $E=\frac{hC}{\lambda}$,)



Electromagnetic spectrum

Beer Lambert Law:

Lambert's Law:

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 = infinitesimally small decrease 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 = proportionality 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, the intensity of radiation after passing through any thickness, say x of the medium can be calculated as:

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

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

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

$$I = I_0 e^{-kx}$$

The intensity of the radiation absorbed, I_{abs} is given by:

$$I_{abs} = I_0 - I = I_0(1 - e^{-kx})$$

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

$$I = I_0 10^{-ax}$$

Where a = extinction coefficient of the absorbing medium ($a = \frac{k}{2.303}$)

Beer's Law:

When a beam of monochromatic radiation is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with thickness of the absorbing solution is proportional to the intensity of incident radiation as well as the concentration of the solution.

Mathematically, this law is stated as

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

Where c = conc. of the solution in moles litre^{-1}.

K= molar absorption coefficient and its value depends upon the nature of the absorbing substance.

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

$$\int_{I_0}^{I} \frac{dI}{I} = \int_{x=0}^{x=x} k'cdx$$

$$\ln \frac{I}{I_0} = -k'cx$$

$$\frac{I}{I_0} = e^{-k'cx}$$

$$I = I_0 e^{-k'cx}$$

The above equation can also be written by changing the nature of logarithm to the base 10.

$$I = I_0 10^{-a'cx}$$

Here $a' = \frac{k}{2.303}$ where a' = molar extinction coefficient of the absorbing solution.

Beer's law can also be stated as:

When a monochromatic light is passed through a solution of an absorbing substance, its absorption remains constant when the conc (c) and the thickness of the absorption layer (x) are changed in the inverse ratio.

Alternative expression: On combining the two laws, the Beer-Lambert Law can be formulated as below

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

Where I_0 = Intensity of incident light

I = Intensity of transmitted light

c = Concentration of solution in moles /litre

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

ε = Molar extinction coefficient (or molar absorptivity) A = Absorbance

Limitations of Beer Lambert Law:

- Non-linearity arises at high concentration.
- Only applicable to monochromatic radiations.
- When fluorescent compounds are present.
- When solute and solvent for complexes through some sort of association.

pH Metry or pH Meter:

The pH scale is a series of numbers. These numbers express the degree of acidity or alkalinity of a solution. The term pH was introduced by Sorensen, and defined it as

pH= —log[H^+]

In other words, it is the negative logarithm of hydrogen ion concentration expressed in molarity.

Principle

A glass electrode is used to determine pH. The electrode consists glass membrane which separates two solutions varying in pH, a potential difference is found to exist between the difference pHof the **t**wo surfaces of the glass which varies the difference of pH of the two solutions. The glass bulb is filled with normal hydrochloric and saturated with quinhydrone or in contact with Ag/AgCl electrode. The platinum wire dipping in the electrolytic passes out of the glass tube (G) .The glass bulb is kept in the solution of which pH is to be measured which is contained in the beaker (B).The potential differences are measured against a standard calomel electrode(SCE) shown in Fig. The cell to be used is represented as

Glass/ H^+ // SCE

The relation by which the EMF of a suitable pH measuring electrode is related to the hydrogen ion concentration based on Nernst equation

$$E = E^0 - \frac{2.303RT}{F} \log(H^+)$$

$$E = E^0 + 0.0591 \text{ pH}$$

$$pH = \frac{E - E^0}{0.0591}$$

The standard value of E° is evaluated by calibrating the apparatus with the help of a buffer solution of known pH value.

Since the potential of standard calomel electrode is known, that of the glass electrode can easily be calculated from the assumed emf of the complete cell and the values of pH evaluated with the help of the above relationship. The glass gives a very good response to pH values ranging from 0 to 9. The accuracy is not good for solutions to high alkalinity.

Applications

- The measurement of pH reflects the effective concentration and activity of H^+ and other ions in solution.
- For chemical reactors and scrubbers, they provide indications of the solution used being acidic or basic qualitatively.
- These meters find major application to correct the hypochlorite concentration for an Oxidation--Reduction Potential (ORP).
- Water treatment plants, micro-electronic laboratories and pharmaceutical laboratories are in constant need of pH level monitoring and control of their very accurate and precise applications.
- In the agricultural field, it is used to determine the soil pH.
- The cosmetic products like shampoo and soaps testing with pH meter to know the acidic nature or basic nature of products.
- It is also used in the chemical and food industry, manufactures of paints and textile industry.

UV-Visible Spectroscopy

Principle

Ultraviolet (UV) Visible-spectra arises from the transition of valency 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.

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.

$$\Delta E = E_1 - E_0 = h\upsilon$$

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 groups.

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

Instrumentation

A spectrophotometer is used to detect the percentage transmittance or absorbance of light radiation when light of a certain intensity or frequency range is passed through the sample. The instrument compares the intensity of the transmitted light with that of the incident light. It consists of the following.

(i) **An intense source of radiant energy**

The most common source of visible radiation is tungsten filament lamp. Hydrogen/deuterium discharge lamps are used for the ultraviolet region.

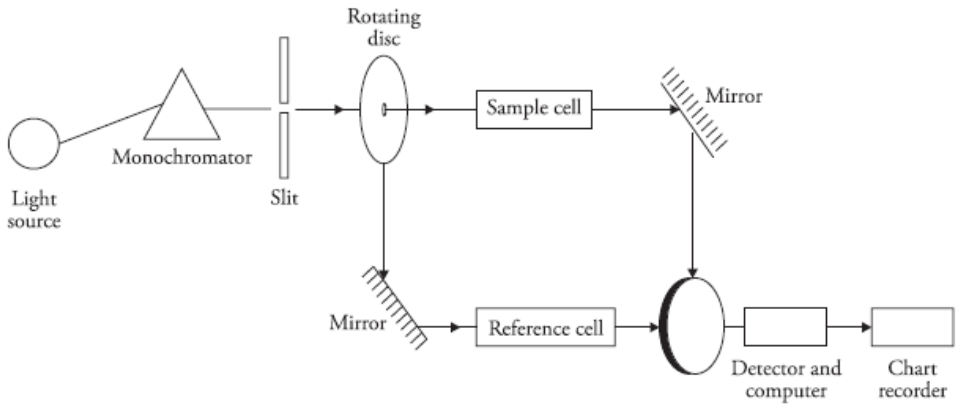
(ii) **A filter or monochromator** to isolate the desired wavelength region.

(iii) **Sample holder**

A pair of cuvettes, one for the sample and the other for the blank or reference solution is used. The thickness of the cuvette is generally 5 cm.

(iv) **Radiation detectors**

These are photoelectric devices which convert radiant energy into electric signals



Ordinary spectrometers cover a range 220–800 nm. Spectroscopic techniques are not very useful below 200 nm since oxygen is strongly absorbed at 200 nm and below. To study absorption below 200 nm, the instrument has to be evacuated. it is then termed as vacuum UV spectroscopy.

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**.

Examples: — C=C—; — C=N—; —N=N—; — C=0 etc

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

Auxochromes:

It is a group which itself does not act as a chromophore but when attached to a chromophore, it shifts the adsorption towards longer wavelength along with an increase in the intensity of absorption.

Examples; -OH, $-NH_2$, -OR, -NHR, and $-NR_2$

Bathochromic shift:

It is also known as red shift. It shifts to higher wave length or shift to lower frequencies.

Hypsochromic shift:

It is also known as blue shift. It shifts to lower wavelength or shift to higher frequencies.

Hyperchromic effect:

It is an increase in intensity of the peak in UV - visible spectra.

Hypochrornic effect:

It is a decrease in intensity of the peak in UV - visible spectra.

Difference between Chromophore and Auxochrome

Chromophore	Auxochrome
1. This group is responsible for the colour of the compound 2. It does not form salt. 3. It contains at least one multiple bond 4. Examples: — C=C—; — C=N—; —N=N—; — C=0 etc	1. It does not impact colour, but when conjugate to chromophore produce colour. 2. But it forms salt. 3. It contains lone pair of electrons. 4 Example: — OH, — NH_2, — NR_2.

Types of transitions involved in organic molecules:

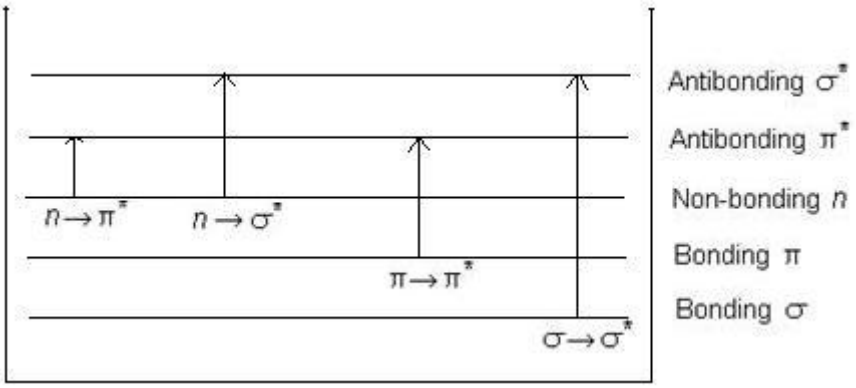
Energy absorbed in the visible and UV region by a molecule causes transitions of valence electrons in the molecule. These transitions are

$$n\rightarrow\pi^*,\ \pi\rightarrow\pi^*,\ n\rightarrow\sigma^*\text{ and }\sigma\rightarrow\sigma^*$$

The energy requirement order for excitation for different transitions is as follows.

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

$n\rightarrow\pi^*$ transition requires lowest energy while $\sigma\rightarrow\sigma^*$ requires highest amount of energy.



Energy levels of electronic transitions

1. n→π* transition

$n\rightarrow\pi^*$ transition requires lowest energy due to longer wavelength. So they are forbidden and corresponding bands are characterized by low molar absorptivity. $\varepsilon_{max} < 100$. It is also known as R- band. They are further characterized by hypsochromic shift or blue shift observed with an increase in solvent polarity.

2. π→π* transition

It is due to the promotion of an electron from a bonding π orbital to an anti-bonding π* orbital. Energy requirement is between n→ π* and $n\rightarrow\sigma^*$. But the extended conjugation and alkyl substituent shifts the λ_{max} towards longer wavelength (Bathochromic shift).

3. n→σ* transition

Saturated compounds with lone pair of electrons undergo $n\rightarrow\sigma^*$ transition in addition to $\sigma\rightarrow\sigma^*$ transition. Corresponding absorption bands appear at longer wavelengths in near UV region.

4. σ→σ* transition

These transitions can occur in such compounds in which all the electrons are involved in single bonds and there are no lone pair of electrons. Energy required for $\sigma\rightarrow\sigma^*$ transition is

very large so the absorption band occurs in the far UV region. So this transition can't normally be observed.

Applications of UV and Visible Spectroscopy:

- Detection of Impurities
- Structure elucidation of organic compounds
- Quantitative analysis
- Qualitative analysis
- Dissociation constants of acids and bases
- Kinetics of reaction can also be studied using UV spectroscopy
- Quantitative analysis of pharmaceutical substances
- Molecular weight determination

Infrared Spectroscopy (IR Spectroscopy):

The termed infrared covers the range of electromagnetic spectrum between 0.78 and 1000 mm. In infrared spectroscopy, the wavelength is measured in "wave numbers" which have the unit cm^{-1}.

$$\text{Wave number} = \frac{1}{\text{wave length (in cm)}}$$

IR is mainly divided into three sections such as near, middle and far IR

Region	Wave number range (cm^{-1})
Near IR	12800-4000
Middle IR	4000--200
Far IR	200-10

Principle

IR radiation does not have enough energy to induce electronic transitions as seen with UV. Absorption of IR is restricted to compounds with small energy differences in the possible vibrational and rotational states. For a molecule to absorb IR, the vibrations or rotations within a molecule must cause a net change in the dipole moment of the molecule. The alternating electrical field of the radiation interacts with fluctuations in the dipole moment of the molecule. If the frequency of the radiation matches the vibrational frequency of the molecule then radiation will be absorbed, causing a change in the amplitude of molecular vibration.

Instrumentation

The instrument consists of the following.

(i) **Light source** Infrared radiations are produced by electrically heating Globar or a Nernst filament to 1000–1800 °C. Globar is a rod of silicon carbide, whereas the Nernst filament is a high resistance element composed of sintered oxides of zirconium, cerium and thorium.

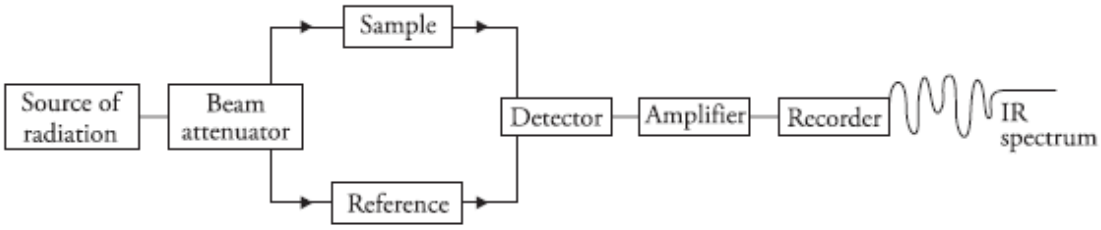
(ii) **Filter or monochromator** Optical prisms or gratings are used to obtain monochromatic light. Glass or quartz cannot be used as the prism material because they absorb in the infrared region. Prisms are made of sodium chloride or alkali metal halides.

(iii) **Sample holder** Cells are made of NaCl or alkali metal halides as they do not absorb IR radiations. Solid samples are ground with KBr and made into a disc. The sample should be absolutely dry as water absorbs strongly in the IR region. Solvents used for IR are chloroform,

carbon tetrachloride, carbon disulphide, etc.

(iv) **Detectors** Thermocouple based detectors are used. Light from the source is split into beams, one of which passes through the sample and the other through the reference. The beam on passing through the sample becomes less intense. There is a difference in intensity of the two beams. This is detected and recorded and an IR spectrum is obtained.

The schematic diagram of an IR spectrophotometer is shown in Figure



Molecular rotations

Rotational levels are quantized and absorption of IR by gases yields line spectra, however, in liquids or solids, these lines broaden into a continuum due to molecular collisions and other interactions.

Molecular Vibration

Fundamental modes of molecular vibrations can be classified into two types: Stretching and bending vibrations.

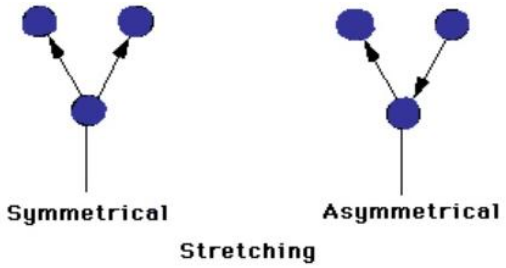
Stretching Vibration

They involve movement of atom within the same bond axis such that the bond length changes without any change in bond angle in regular interval.

Types of stretching vibrations

Symmetrical stretching: The atoms of a molecule either move away or towards the central atom, but in the same direction.

Asymmetric Stretching: One atom approach towards the central atom while other departs from it.



Bending or Deforming Vibrations

- They involve movement of atoms which are attached to a common central atom, such that there is change in bond axis and bond angle of each individual atom without change in their bond lengths.
- Bending vibrations generally requires less energy and occur at longer wavelengths (lower cm^{-1}) than stretching vibrations.

Types of bending vibrations

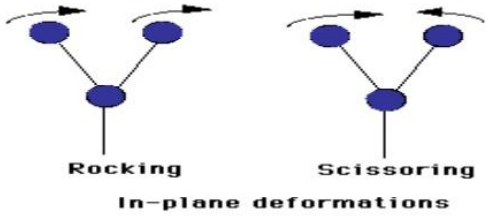
1. In-plane vibrations

a. Rocking:

In-plane bending of atoms occurs wherein they move backward and forward with respect to the central atom.

b. Scissoring:

In-plane bending of atoms occurs wherein they move back and forward. i.e., they approach to each other.



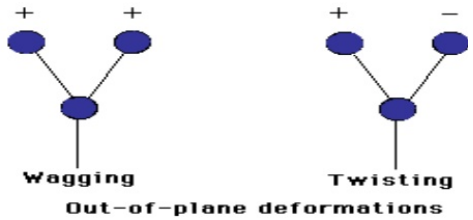
2. Out-plane vibrations

a. Wagging:

Two atoms oscillate up and below the plane with respect to the central atom.

b. Twisting:

One of atom moved up the plane while other downs the plane with respect to central atom.



Infrared spectrum is usually studied under two regions

(a) Functional group region (4000 – 1500 cm^{-1})

(b) The finger print region (1500 – 667 cm^{-1})

The higher frequency region 4000 – 1500 cm^{-1} is called the functional group region. The characteristic stretching frequencies of all the important functional groups such as OH, NH_2, C=O, etc., lie in this region. The region below 1500 cm^{-1} (1500–667 cm^{-1}) is rich in absorptions caused by bending vibrations and those resulting from the stretching vibrations of C—C, C—O and C—N bonds. These bands are unique to an organic compound just like finger prints are characteristic to an individual. Hence, this region is termed as the fingerprint region

Table 1 Characteristic IR absorption frequencies of important functional groups that help in their identification.

Group	Type of vibration	Region (in cm^{-1}) and intensity
alkane	C-H str C-C str C-H def	2960-2850(m, s) 1300-800(w) 1485-1440(m)
alkenes	>C=C-H str C=C str	3100-3000 (m) 3090-3075 (m) ~1675 (m)
non conjugate diene	C=C str	1650-1600 (v) variable
conjugate diene	C=C str	-1600 (w) ~1650 (w)
alkynes	C≡C-H str C-H def	3300 (s) 650-610 (s)
aromatic	Ar-H str C=C str C-H def	3050-3000 (v) variable 1600 (v) 1580 (v) 1500 (m_ 1450 (m) 900-700 (m)

Applications of Infrared Spectroscopy:

- The identification and structural analysis of organic compounds such as natural products, polymers and synthesized products.
- It is used to find the food contaminates in food items.
- In biology, it is used to find bacterial and fungal identification.
- It is used in pharmaceutical drugs in pharmaceutical companies.
- The paint pigment is also detected by using FT-IR.
- In agricultural fields, it is used to detect the humic acid or humus in soil.
- The organic solvents purity is also detected by IR

Chromatography:

Chromatography is a separation technique used to separate the different components in a liquid mixture. The mobile phase may be a gas or liquid. The mobile phase is then passed through another phase called stationary phase. The stationary phase may be a solid packed in a glass plate or a piece of chromatography paper.

The various components of the mixture travel at different speeds, causing them to separate. There are different types of chromatographic techniques such as column chromatography, TLC, paper chromatography, and gas chromatography.

Stationary Phase

The stationary phase in chromatography is the phase that is either a solid or liquid particle attached to a glass or a metal surface on which the components of the mixture to be separated is absorbed selectively.

Mobile Phase

The mobile phase in chromatography is the phase that is either liquid or gas that is passed through a chromatographic system where the components of the mixture are separated at different raters by adsorbing them to the stationary phase.

Solid Liquid Chromatography or Column chromatography

Column chromatography is the separation technique where the components in a mixture are separated on the basis of their differential adsorption with the stationary phase, resulting in them moving at different speeds when passed through a column.

It is a solid liquid chromatography technique in which the stationary phase is a solid & mobile phase is a liquid or gas.

Principle of Column chromatography

- This technique is based on the principle of differential adsorption where different molecules in a mixture have different affinities with the absorbent present on the stationary phase.
- The molecules having higher affinity remain adsorbed for a longer time decreasing their speed of movement through the column.
- However, the molecules with lower affinity move with a faster movement, thus allowing the molecules to be separated in different fractions.
- Here, the stationary phase in the column chromatography also termed the absorbent, is a solid (mostly silica) and the mobile phase is a liquid that allows the molecules to move through the column smoothly.

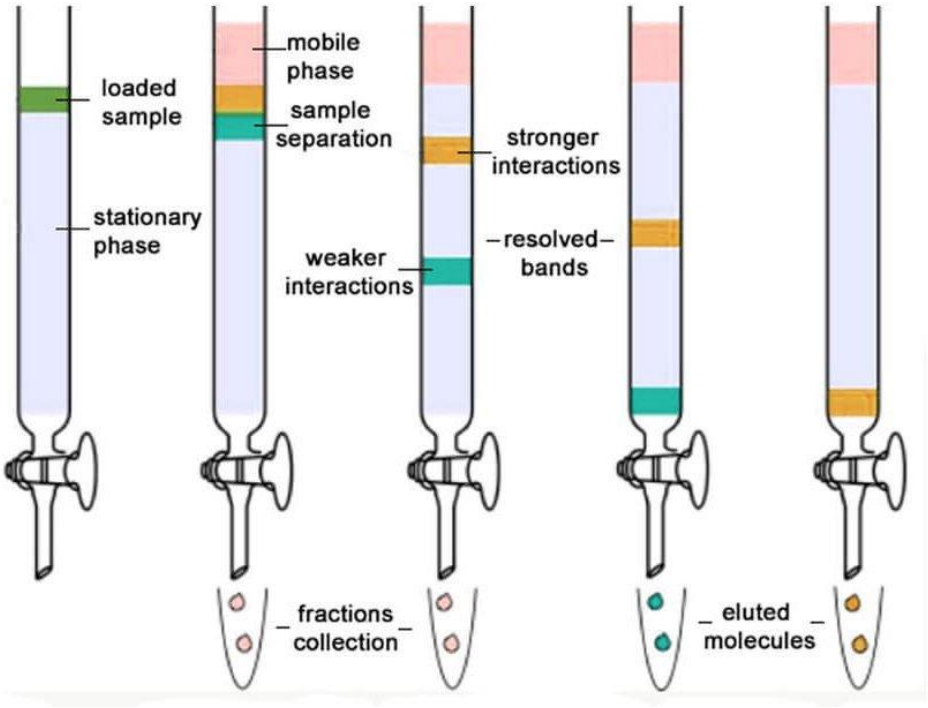


Figure: Column chromatography

Steps of Column chromatography

- The column is prepared by taking a glass tube that is dried and coated with a thin, uniform layer of stationary phase (cellulose, silica).
- Then the sample is prepared by adding the mixture to the mobile phase. The sample is introduced into the column from the top and is allowed to pass the sample under the influence of gravity.

- The molecules bound to the column are separated by elution technique where either solution of the same polarity is used (isocratic technique), or different samples with different polarities are used (gradient technique).
- The separated molecules can further be analyzed for various purposes.

Uses of Column chromatography

- Column chromatography is routinely used for the separation of impurities and purification of various biological mixtures.
- This technique can also be used for the isolation of active molecules and metabolites from various samples.
- Column chromatography is increasingly used for the detection of drugs in crude extracts.

Thin Layer Chromatography (TLC)

Thin layer chromatography is a separation technique where the stationary phase is applied as a thin layer on a solid support plate with a liquid mobile phase.

Principle of Thin-layer chromatography (TLC)

- This chromatography technique is based on the principle that components of a mixture are separated when the component having an affinity towards the stationary phase binds to the stationary phase. In contrast, other components are eluted with the mobile phase.
- The substrate/ ligand is bound to the stationary phase so that the reactive sites for the binding of components are exposed.
- Now, the mixture is passed through the mobile phase where the components with binding sites for the substrate bind to the substrate on the stationary phase while the rest of the components are eluted out with the mobile phase.
- After separation, the molecules are seen as spots at a different location throughout the stationary phase.
- The detection of molecules is performed by various techniques.

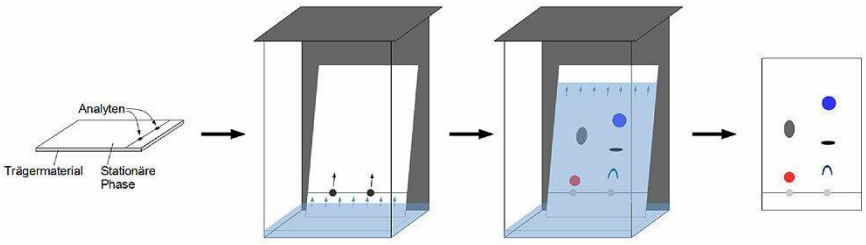


Figure: Thin-layer chromatography (TLC).

Steps of Thin-layer chromatography (TLC)

- The stationary phase is uniformly applied on the solid support (glass, thin plate or aluminum foil) and dried.
- The sample is injected as spots on the stationary phase about 1 cm above the edge of the plate.
- The sample loaded plate is then carefully dipped into the mobile phase not more than the height of 1 cm.
- After the mobile phase reaches near the edge of the plate, the plate is taken out.

- The retention factor is calculated as in paper chromatography, and the separated components are detected by different techniques.

Uses of Thin-layer chromatography (TLC)

- Thin-layer chromatography is routinely performed in laboratories to identify different substances present in a mixture.
- This technique helps in the analysis of fibers in forensics.
- TLC also allows the assay of various pharmaceutical products.
- It aids in the identification of medicinal plants and their composition.

Retention Time

The amount of time between the injection of a sample and its elution from the column is known as the retention time. it is given the symbol t_R. The retention time is the sum of time a sample component spends in the mobile phase and the amount of time it spends in the stationary phase. The latter is called the net or adjusted retention time (t_s). The fundamental relationship describing retention in chromatography (both gas and liquid) is

Retention Time $t_R = t_M + t_s$