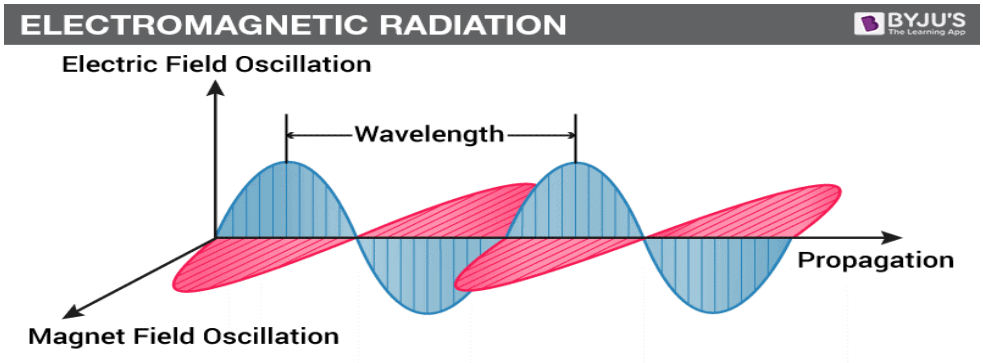
UNIT 3: INSTRUMENTAL METHODS AND APPLICATIONS

Electromagnetic Spectrum. Absorption of radiation: Beer-Lambert's law - Principles of UV-Visible, Infrared (IR) and Nuclear Magnetic Resonance (NMR) spectroscopy.

Basic concepts of Thin Layer Chromatography (TLC), Gas Chromatography (GC) and High-Performance Liquid Chromatography (HPLC), Separation and purification of mixture of compounds.

Electromagnetic radiation

Electromagnetic radiation is a form of radiant energy which has both particle as well as wave nature. In vacuum, it normally travels in straight line with the speed of light 2.99×10^8 m/s. It has both electric and magnetic field components which are coplanar and oscillate perpendicular to each other and perpendicular to the direction of wave propagation.



Properties of Electromagnetic radiation

The Properties of Electromagnetic radiation can be described easily by ascribing wave nature to these radiations.

Definition of 'Wavelength'

Definition: Wavelength can be defined as the distance between two successive crests or troughs of a wave. It is measured in the direction of the wave.
The wave length of visible light ranges from 3800A0 (violet end) to 7600 A0 (red end)

Unit	Symbol	Value (m)	How many in 1 meter?
meter	m	$10^0=1$	1
decimeter	dm	10^{-1}	10
centimeter	cm	10^{-2}	10^2
millimeter	mm	10^{-3}	10^3
micrometer	µm	10^{-6}	10^6
nanometer	nm	10^{-9}	10^9
Angstrom*	Å	10^{-10}	10^{10}

Frequency

The number of waves which can pass through a point in one second.

One wave per second is also called a Hertz (Hz)(Hz) and in SI units is a reciprocal second (s−1)(s−1).

$c=\lambda\nu$

Velocity

Velocity of a wave is denoted by c, and is defined as the distance travelled by a wave in one second.

Electromagnetic radiation travel with the speed of light,hence the value of c is 2.99×10^8 m/s.

Frequency

The number of cycles per second is defined as Frequency. It is defined as Hertz (Hz) or sec^{-1}. If 'E' is the energy, 'h' is Planck's constant which is equal to 6.62607×10^{-34} and '*v*' is the frequency we can derive the relation given below.

E = hv

Thus, we can see that frequency is directly proportional to energy.

Period

Period is commonly characterised by the symbol 'T'. It is the total time which a wave takes to travel 1 wavelength.

wave number

The total number of waves in a length of one centimetre.

Wave number is the reciprocal of wavelength and is expressed in per centimetre or cm-1

Electromagnetic Spectrum

Electromagnetic spectrum in simple terms is defined as the range of all types of electromagnetic radiation. We shall learn about the concept in detail and understand all its underlying aspects in this lesson.

What are Electromagnetic Spectrum?

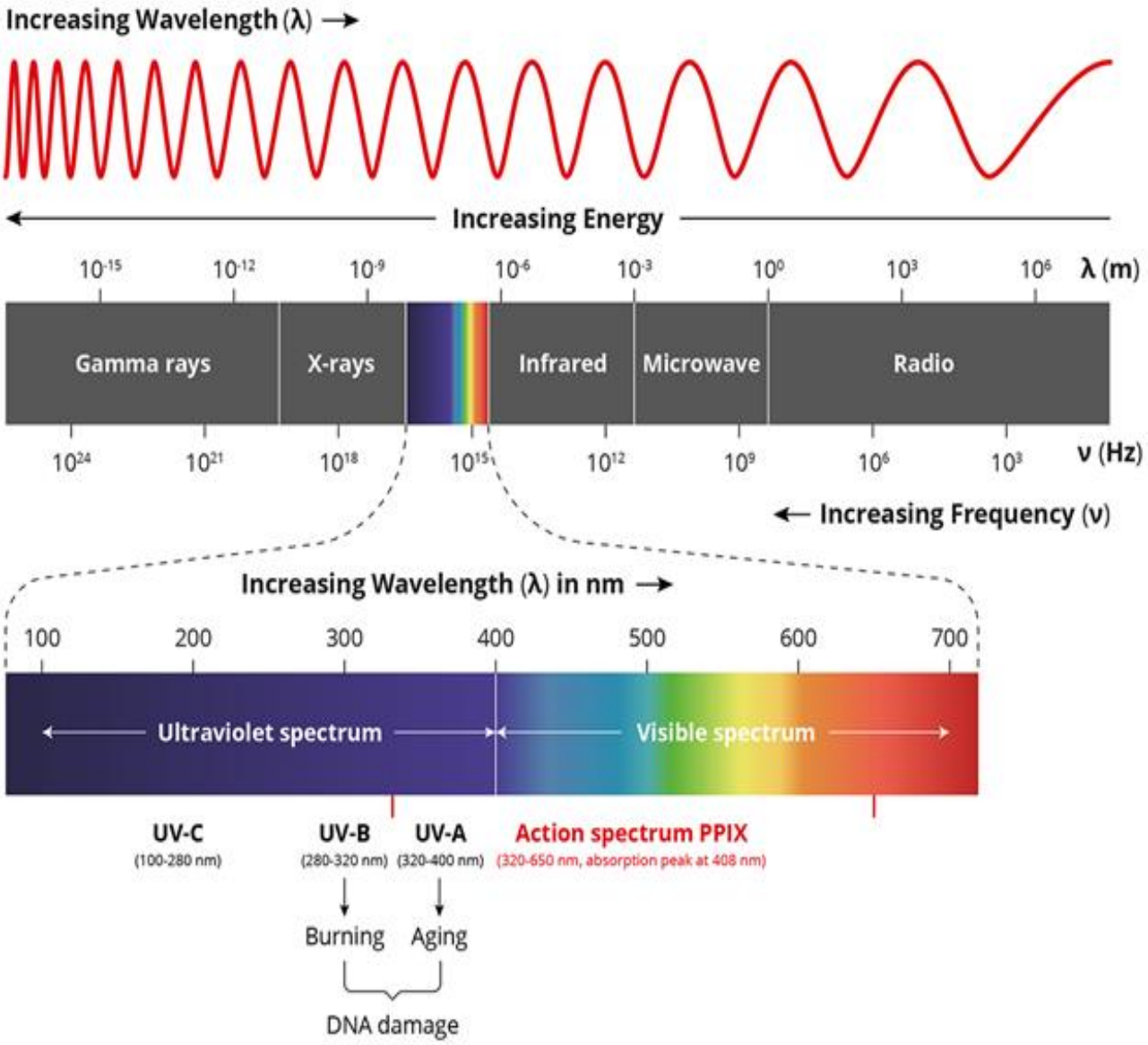
The electromagnetic spectrum is a range of frequencies, wavelengths and photon energies covering frequencies from below 1 hertz to above 10^{25} Hz corresponding to wavelengths which are a few kilometres to a fraction of the size of an atomic nucleus in the spectrum of electromagnetic waves. Generally, in a vacuum electromagnetic waves tend to travel at speeds which is similar to that of light. However, they do so at a wide range of wavelengths, frequencies, and photon energies.

The electromagnetic spectrum consists of a span of all electromagnetic radiation which further contains many subranges which are commonly referred to as portions. These can be further classified as infra-red radiation, visible light or ultraviolet radiation.

Electromagnetic Waves in Electromagnetic Spectrum

The entire range (electromagnetic spectrum) is given by radio waves, microwaves, infrared radiation, visible light, ultra-violet radiation, X-rays, gamma rays and cosmic rays in the increasing order of

frequency and decreasing order of wavelength. The type of radiation and their frequency and wavelength ranges are as follows:



Type of Radiation	Frequency Range (Hz)	Wavelength Range
gamma-rays	$10^{20} – 10^{24}$	$< 10^{-12}$ m
x-rays	$10^{17} – 10^{20}$	1 nm – 1 pm
ultraviolet	$10^{15} – 10^{17}$	400 nm – 1 nm
visible	$4 – 7.5*10^{14}$	750 nm – 400 nm
near-infrared	$1*10^{14} – 4*10^{14}$	2.5 µm – 750 nm
infrared	$10^{13} – 10^{14}$	25 µm – 2.5 µm
microwaves	$3*10^{11} – 10^{13}$	1 mm – 25 µm
radio waves	$< 3*10^{11}$	> 1 mm

We see the uses of the electromagnetic waves in our daily life as :

Radio: A radio basically captures radio waves that are transmitted by radio stations. Radio waves can also be emitted by gases and stars in space. Radio waves are mainly used for TV/mobile communication.

Microwave: This type of radiation is found in microwaves and helps in cooking at home/office. It is also used by astronomers to determine and understand the structure of nearby galaxies and stars.

Infrared: It is used widely in night vision goggles. These devices can read and capture the infrared light emitted by our skin and objects with heat. In space, infrared light helps to map the interstellar dust.

X-ray: X-rays can be used in many instances. For example, a doctor can use an x-ray machine to take an image of our bone or teeth. Airport security personnel use it to see through and check bags. X-rays are also given out by hot gases in the universe.

Gamma-ray: It has a wide application in the medical field. Gamma-ray imaging is used to see inside our bodies. Interestingly, the universe is the biggest gamma-ray generator of all.

Ultraviolet: Sun is the main source of ultraviolet radiation. It causes skin tanning and burns. Hot materials that are in space also emit UV radiations.

Visible: Visible light can be detected by our eyes. Light bulbs, stars, etc. emit visible light.

Spectroscopy

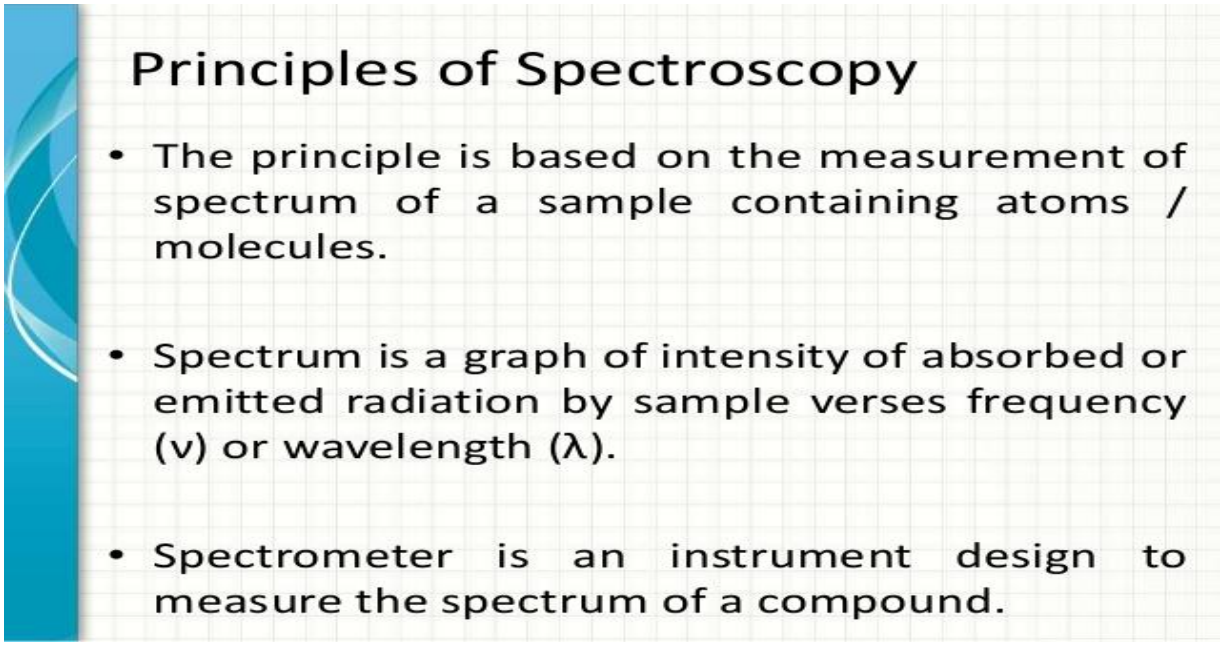
Spectroscopy is the branch of science which deals with the study of the interaction of electromagnetic radiation with matter.

1.Nuclear Magnetic Resonance (NMR) – Excitation of the nucleus of atoms through radiofrequency irradiation. Provides extensive information about molecular structure and atom connectivity

2. Infrared Spectroscopy (IR) – Triggering molecular vibrations through irradiation with infrared light. Provides mostly information about the presence or absence of certain functional groups.

3. Mass spectrometry – Bombardment of the sample with electrons and detection of resulting molecular fragments. Provides information about molecular mass and atom connectivity.

4. Ultraviolet spectroscopy (UV) – Promotion of electrons to higher energy levels through irradiation of the molecule with ultraviolet light. Provides mostly information about the presence of conjugated π systems and the presence of double and triple bonds.



Basic principle of UV spectroscopy

- All atoms and molecules are capable of absorbing energy in accordance with their own structure variation and so the kind and amount of radiation absorbed by a molecule depend upon:
- The structure of the molecule.
- The number of molecules interacting with the radiation.
- When electromagnetic radiation is absorbed by a molecule, it undergoes transition from a state of lower to state of higher energy. If the molecule is monatomic, the energy absorbed can only be used to raise the energy levels of electrons. If the molecule consists of more than one atom, the radiation absorbed may bring about changes in electronic, rotational, vibrational or translational energy.

Table 10.5 Electronic Transitions Involving *n*, σ, and π Molecular Orbitals

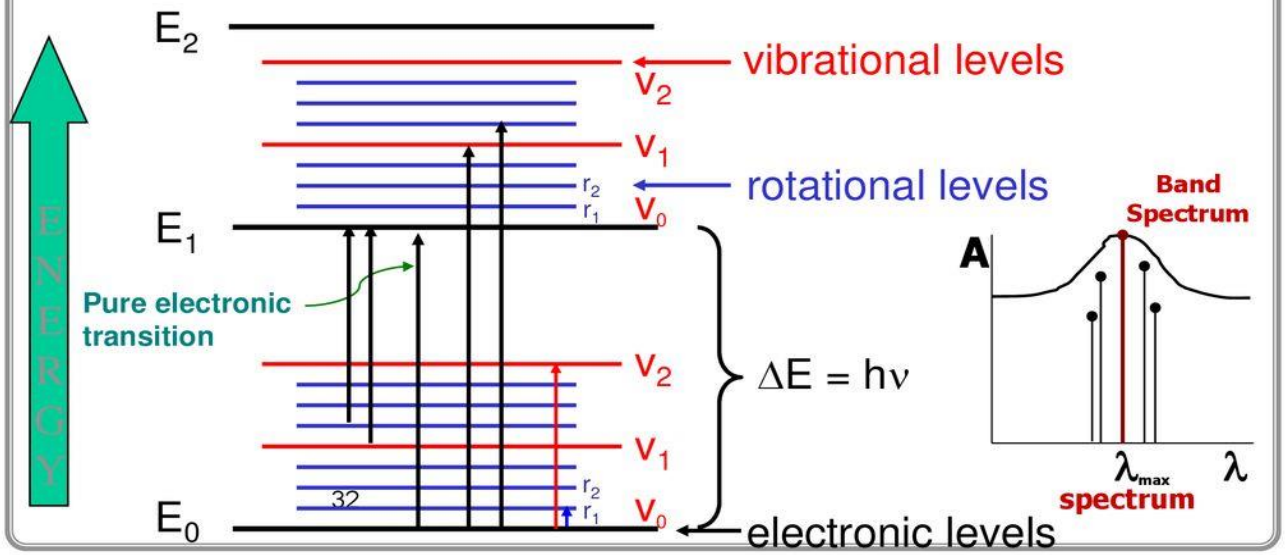
Transition	Wavelength Range (nm)	Examples
$\sigma \rightarrow \sigma^*$	< 200	C—C, C—H
$n \rightarrow \sigma^*$	160–260	H_2O, CH_3OH, CH_3Cl
$\pi \rightarrow \pi^*$	200–500	C=C, C=O, C=N, C≡C
$n \rightarrow \pi^*$	250–600	C=O, C=N, N=N, N=O

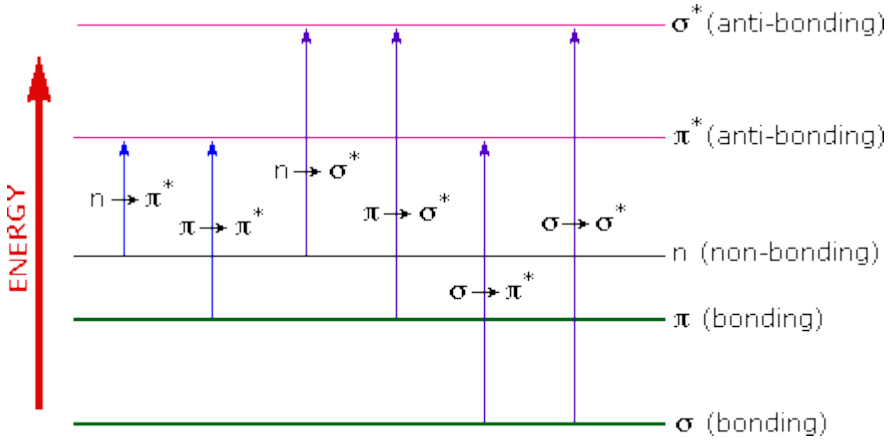
Principle and Basic concept

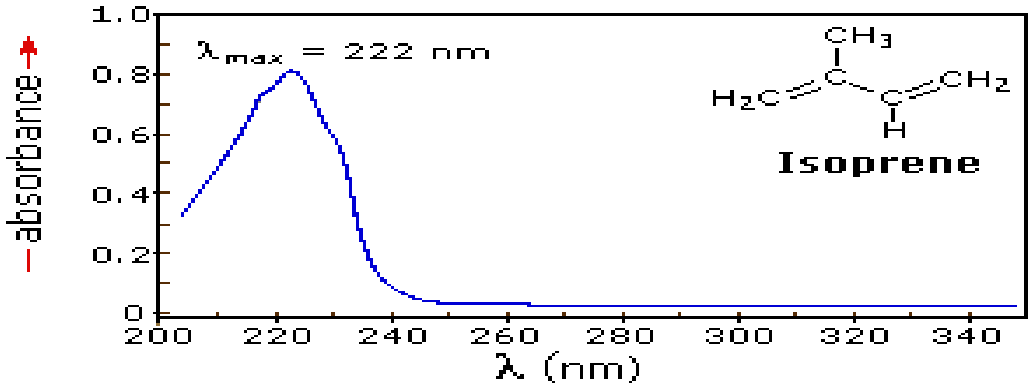
- Ultraviolet – visible spectroscopy (λ 200 - 800 nm) studies the changes in electronic energy levels within the molecule arising due to transfer of electrons from π- or non-bonding orbital's.
- It commonly provides the knowledge about π-electron systems, conjugated unsaturations, aromatic compounds and conjugated non-bonding electron systems etc
- UV- Visible is divided into the ultraviolet (UV, 190-400 nm) and visible (VIS, 400-800 nm) regions. Since the absorption of ultraviolet or visible radiation by a molecule leads transition among electronic energy levels of the molecule, it is also often called as **electronic spectroscopy**.

Why we get a broad band spectrum in UV-Visible Spectroscopy?

- When a molecule absorbs light having sufficient energy (e.g. UV-Vis radiation) to cause an electronic transitions, additional vibration and rotation transitions also occur
- Molecule can absorb one photon of just the right energy to cause the following simultaneous changes:







Applications of UV spectroscopy

1. Detection of functional groups

2. Detection of extent of conjugation
3. Identification of an unknown compound
4. Determination of configurations of geometrical isomers

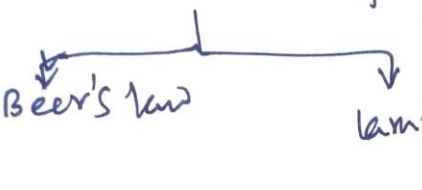
5. Determination of the purity of a substance.

- Traditional Chemistry
- Life Science
- Microbiology
- Food & Agriculture
- Material Science
- Optical Components
- Pharmaceutical Research
- Petrochemistry
- Cosmetic Industry
- Quality Control

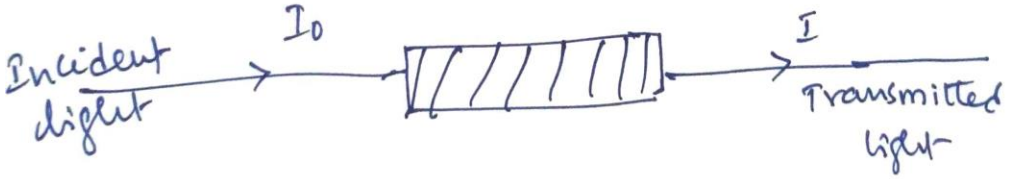
BEER - LAMBERT law

The Absorbance of light is directly proportional to the concentration of the Sample and path length of the Sample/solution.

It is combination of two laws



measuring the Absorbance of the Sample/solution.



Absorbance: $A = I_0 / I$

Transmission: $T = I / I_0$.

Beer's law

Statement: Absorption of light in sample/solution is directly proportional to the concentration of solution in which light travels.

$A = \log_{10}(I_0/I) \propto c$

$A = \varepsilon c$; c = Concentration of Sample

Lambert's law:

Statement: Absorbance of light in Homogeneous solution is directly proportional to the path length of sample in which the light passes.

$A = \log_{10}(I_0/I) \propto l$

$A = \varepsilon l$; ε = molar extinction coefficient

l = path length of sample

Combining Both laws

For absorbance: $A = \log_{10}(I_0/I) = \varepsilon \cdot c \cdot l$

$$\boxed{A = \varepsilon \cdot c \cdot l}$$

c = concentration

l = length.

- A is the amount of light absorbed for a particular wavelength by the sample
- ε is the molar extinction coefficient
- L is the distance covered by the light through the solution
- c is the concentration of the absorbing species

Principle of Infra-red Spectroscopy

The absorption of Infra-redv radiations causes an excitations 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 Infra-red spectra is considered as **Vibrational-rotational spectra**.

All the bonds in a molecule are not capable of absorbing infra-red energy but only those bonds which are accompanied by a change in dipole moment will absorb in the infra-red region.

Such vibrational transitions which are accompanied by a change in the dipole- moment of the molecule are called infra-red active transitions. Thus, these are responsible for absorption of energy in the Infra-red region.

On the other hand, the vibrational transitions which are not accompanied by a change in dipole moment of the molecule are not directly observed and these are Infra -red inactive.

For example, vibrational transitions of C=O, N-H, O-H etc. bonds are accompanied by a change in dipole-moment and thus , absorb strongly in the Infra-red region. But transitions in carbon -carbon (C-C) bonds in symmetrical alkenes and alkynes are not accompanied by the change in dipole-moment and hence do not absorb in the infra-red region .A molecule of the Organic compound will show a number of peaks in the infra-red region.

Region of Spectrum (cm^{-1})	**Absorption**
2500-4000	N−H, O−H, C−H
2000-2500	C≡C, C≡N
1500-2000	C=O, C=N, C=C
below 1500	**Fingerprint region**

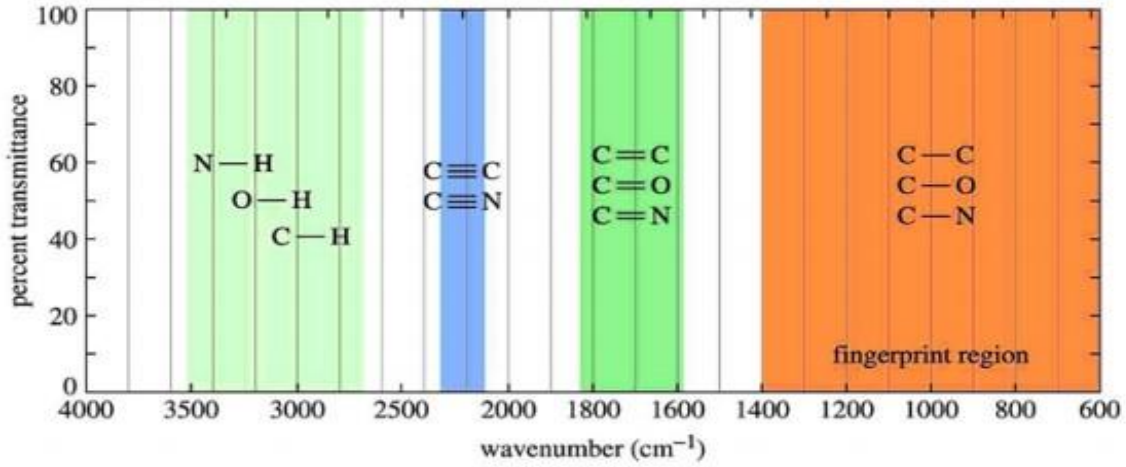
Finger Print Region

One of the important functions of IR spectroscopy is to determine the identity of two compounds. Two identical compounds have exactly the same spectra when run in the same medium under similar conditions. The region below 1500cm-1 is rich in many absorptions which are caused by bending vibrations and those resulting from the stretching vibrations of C-C, C-O, and C-N bonds. In a spectrum, the number of bending vibrations is usually more than the number of stretching vibrations. The said region is usually rich in absorption bands. It is called Finger Print Region.
Some molecules containing the same functional group show similar absorptions above 1500cm-1 but their spectra differ in finger print region. The identity of an unknown compound can also be revealed by comparing its Infra-red spectrum with a set of spectra of known compounds under identical conditions. Finger print region can be subdivided into three regions as follows

i) 1500-1350cm-1
ii) 1350-1000cm-1
iii) Below 1000cm-1

IR ABSORPTION RANGE

The typical IR absorption range for covalent bonds is **600 - 4000 cm^{-1}**. The graph shows the regions of the spectrum where the following types of bonds normally absorb. For example a sharp band around 2200-2400 cm^{-1} would indicate the possible presence of a C-N or a C-C triple bond.



APPLICATIONS OF IR SPECTROSCOPY

- Identification of functional group and structure elucidation. Entire **IR** region is divided into group frequency region and fingerprint region. ...
- Identification of substances. ...
- Studying the progress of the reaction. ...
- Detection of impurities. ...
- Quantitative analysis.

NMR Spectroscopy Principle

- All nuclei are electrically charged and many have spin.
- Transfer of energy is possible from base energy to higher energy levels when an external magnetic field is applied.
- The transfer of energy occurs at a wavelength that coincides with the radio frequency.
- Also, energy is emitted at the same frequency when the spin comes back to its base level.
- Therefore, by measuring the signal which matches this transfer the processing of the NMR spectrum for the concerned nucleus is yield.

NMR Spectroscopy Working

- Place the sample in a magnetic field.
- Excite the nuclei sample into nuclear magnetic resonance with the help of radio waves to produce NMR signals.
- These NMR signals are detected with sensitive radio receivers.
- The resonance frequency of an atom in a molecule is changed by the intramolecular magnetic field surrounding it.
- This gives details of a molecule's individual functional groups and its electronic structure.
- Nuclear magnetic resonance spectroscopy is a conclusive method of identifying monomolecular organic compounds.
- This method provides details of the reaction state, structure, chemical environment and dynamics of a molecule.

NMR Spectroscopy Applications

1. NMR spectroscopy is a Spectroscopy technique used by chemists and biochemists to investigate the properties of organic molecules, although it is applicable to any kind of sample that contains nuclei possessing spin.
2. For example, the NMR can quantitatively analyze mixtures containing known compounds. NMR can either be used to match against spectral libraries or to infer the basic structure directly for unknown compounds.
3. Once the basic structure is known, NMR can be used to determine molecular conformation in solutions as well as in studying physical properties at the molecular level such as conformational exchange, phase changes, solubility, and diffusion.
4. NMR spectroscopy is a Spectroscopy technique used by chemists and biochemists to investigate the properties of organic molecules, although it is applicable to any kind of sample that contains nuclei possessing spin.
5. For example, the NMR can quantitatively analyze mixtures containing known compounds. NMR can either be used to match against spectral libraries or to infer the basic structure directly for unknown compounds.

What is Chromatography?

Chromatography is the technique for the separation, purification, and testing of compounds.

The term ***"chromatography"*** is derived from Greek, chroma meaning, ***"colour,"*** and graphein meaning "***to write."***

In this process, we apply the mixture to be separated on a stationary phase (solid or liquid) and a pure solvent such as water or any gas is allowed to move slowly over the stationary phase, carrying the components separately as per their solubility in the pure solvent.

Principles of Chromatography

Chromatography is a separation method where the analyte is combined within a liquid or gaseous mobile phase., which is pumped through a stationary phase.

Usually one phase is hydrophilic and the other lipophilic. The components of the analyte interact differently with these two phases. Depending on of their polarity they spend more or less time interacting with the stationary phase and are thus retarded to a greater or lesser extent.

This leads to the separation of the different components present in the sample. Each sample component elutes from the stationary phase at a specific time, its retention time. As the components pass through the detector their signal is recorded and plotted in the form of a chromatogram.

Thin Layer Chromatography (TLC): Principle, Procedure & Applications

Thin layer chromatography is a kind of chromatography used to separate and isolate mixtures that are non-volatile in nature. Just like other chromatography processes, this one consists of a mobile phase and a stationary phase.

The latter one here is a thin layer of absorbent material, such as aluminium oxide, silica gel, or cellulose. This layer is applied to plastic, glass, or aluminium foil sheets called an inert substrate. The mobile phase in the TLC procedure is a solvent or a mixture of it.

TLC principle.

Thin Layer Chromatography Principle

The separation principle of the TLC procedure is based on the given compound's relative affinity towards the mobile and the stationary phase. The process begins here by moving the mobile phase over the stationary phase's surface. During this movement, the higher affinity compounds gain less speed as compared to the lower affinity compounds. This results in their separation.

Once the procedure gets completed, different spots can be found on the stationary surface at distinct levels, reflecting various elements of the mixture.

Basically, the compounds that are more attracted towards the stationary phase secure their position at lower levels while others move towards the higher levels of the surface. So their spots can be seen accordingly.

After the TLC principle, now let's move on to its procedure.

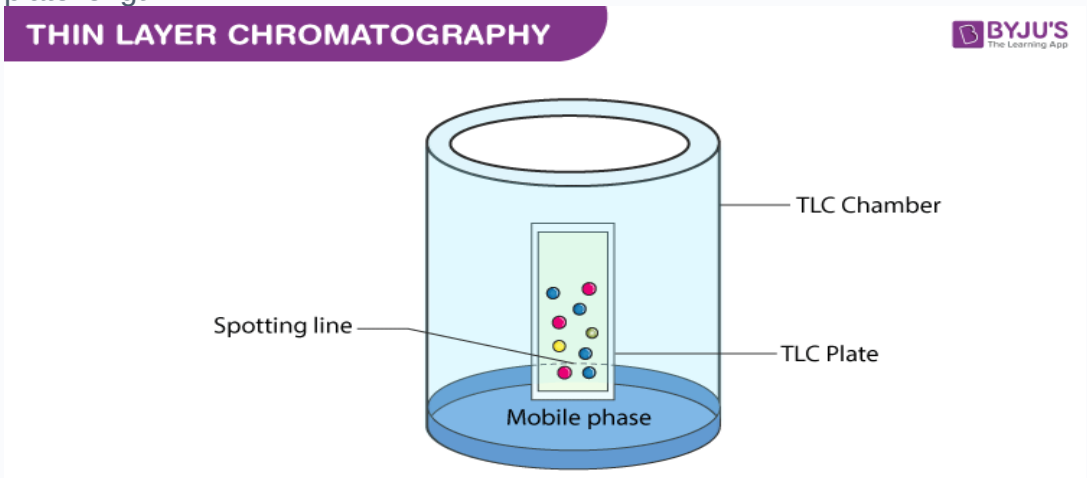
Thin Layer Chromatography Procedure
. A few components involved in the TLC procedure are as follows.

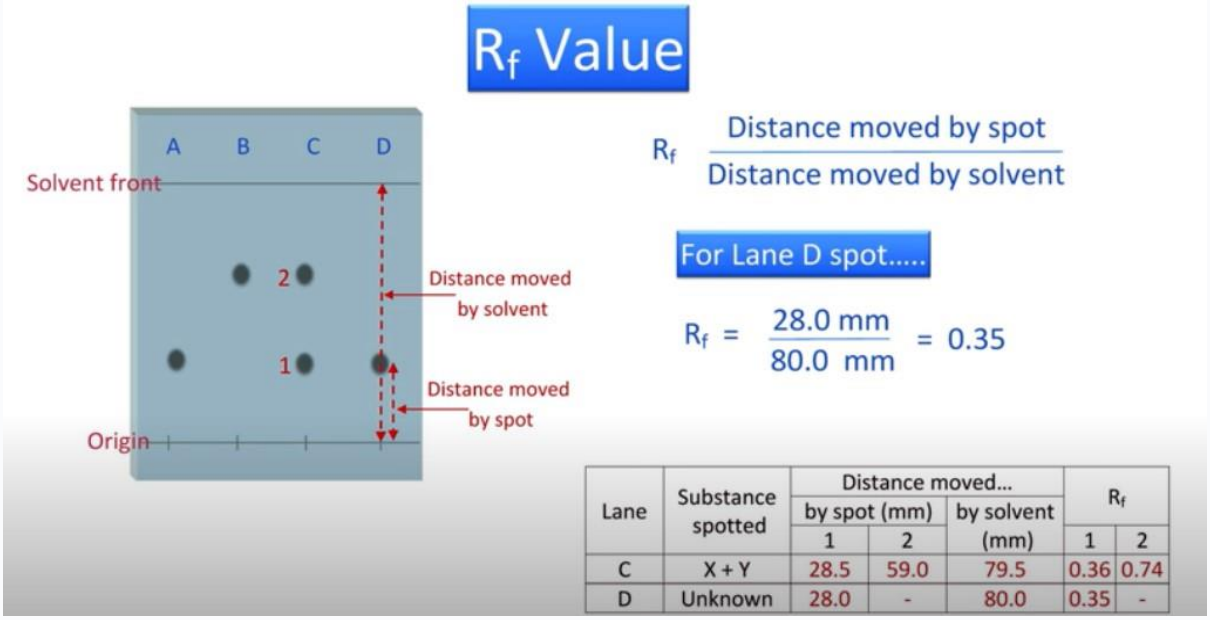
TLC Plates: These are used for applying the thin layer of stationary phase. They are inert or stable in nature. Usually, ready-to-use plates are preferred by the people conducting experiments.

Mobile Phase: This comprises a solvent (or solvent mixture). The taken solvent needs to be chemically inert, of the highest possible purity, and particulate-free. Only then can the TLC spots be able to develop.

TLC Chamber: This is where the thin layer chromatography procedure takes place. It keeps the dust particles away from the process and does not let the solvent evaporate. In order to develop the spots appropriately, a uniform environment is maintained inside this chamber.

Filter Paper: This gets placed inside the chamber after being moistened with the mobile phase solution. It ensures that the mobile phase rises uniformly throughout the TLC plate length.





After collecting all these components, the process begins. Here are the steps followed in it:

- The process starts by making a thin mark on the TLC plate's bottom with a pencil. It helps in the application of sample spots. These spots are kept at equal distances.
- The sample is then applied to these spots made on the line.
- Then the TLC chamber is filled with the mobile phase up to a few centimetres of its bottom.
- After pouring the mobile phase, the moistened filter paper is placed along with the inside of the chamber wall. This helps to avoid the edge effect by maintaining equal humidity.
- Finally, the prepared stationary phase plate is put inside the chamber. At this point, the sample spots are kept on the mobile phase's side.
- The chamber is then closed after placing the plate into it.
- Once enough time has elapsed for the process, the plate is taken out and allowed to dry.
- At last, the sample spots get analyzed through a suitable method for the sample, such as UV light, KMnO4 stain, and iodine staining.

This way, the TLC procedure gets completed. After analyzing the compound, it gets described in its relative mobility's terms, i.e., its Rf value is calculated. This value changes for each compound, even under the same circumstances.

Rf = (distance covered by the sample) / (distance covered by the solvent)

Thin Layer Chromatography Applications

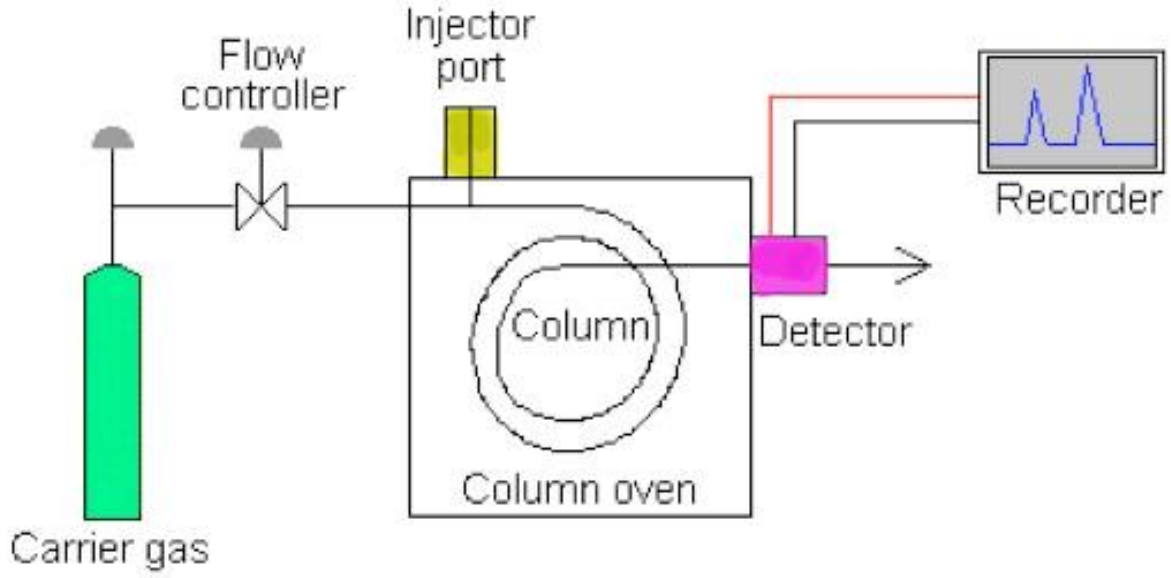
. Some standard TLC applications include:

- Being a separation process, TLC proves to be highly effective for separating pharmaceutical formulations that consist of multiple components.
- The process can be used to examine a given product's purity.
- Medicines like local anaesthetics, analgesics, sedatives, hypnotics, anticonvulsant tranquilizers, and steroids go through the TLC procedure for their qualitative testing.
- The cosmetic industry also uses TLC for checking the presence of preservatives in the products.
- A given compound can be purified using TLC and then compared with a standard sample.
- TLC also finds its use in Biochemical analysis. Here, it can be used for biochemical metabolites' separation from urine, blood plasma, serum, and body fluids.
- Just like the cosmetic industry, the food industry also utilizes TLC for the detection of preservatives, artificial colours, and sweetening agents.
- A reaction's progress can also get tracked with TLC to see whether it is complete or not.

- **What is gas chromatography ?**

- Chromatography is a technique that separates components in a mixture by the difference in partitioning behavior between mobile and stationary phases. Gas chromatography (GC) is one of the popular chromatography techniques to separate volatile compounds or substances. The mobile phase is a gas such as helium, and the stationary phase is a high-boiling liquid that is adsorbed on a solid.

- ***Which are the most common mobile phases in gas chromatography?***
-
- The most common **mobile phases** (**carrier gases**) for gas chromatography (GC) are **He, H_2 and N_2** which have the advantage of being chemically inert toward both the sample and the stationary phase. However, other gases such as Ar and CO_2 have also been used though much less frequently.



- **The principle of gas chromatography**
- Components in the mixture are distributed between two phases, one of which is a stationary phase, and the other is a mobile phase gas, or carrier gas, that carries the mixture through the stationary phase. Compounds in the mobile phase interact with the stationary phase as they pass through. Due to the differences in properties and structures of each component, the size and affinity of each interaction with the stationary phase are different. Therefore, under the same driving force, the retention time of different components differs in the column, thus moving out of the column in different orders
- **application of gas chromatography**

  1.Gas **chromatography** is widely used for the separation and analysis of mixtures of many compounds at very low concentrations . ...

  2.The main areas of **application of gas chromatography** are

  3.Analysis of toxic compounds, solvents, hydrocarbons

  4. forensic field,

  5. Pollution studies, environmental analysis.

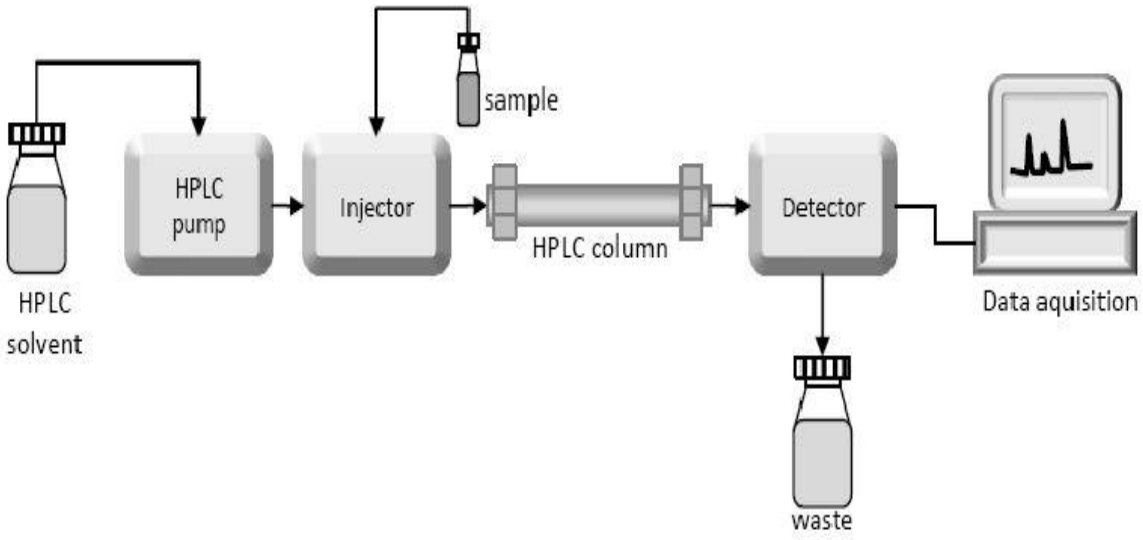
HPLC

- **HPLC stands for High Performance Liquid Chromatography.**
- Its earlier name was High Pressure Liquid Chromatography because it involved use of liquid mobile phase requiring higher pressures than gases used in Gas Chromatography. The technique has found immense scope of applications in both academic and industrial laboratories requiring identification and quantification of mixtures of organic compounds.

HPLC

- High-Performance Liquid Chromatography (HPLC) is a popular and versatile technique that provides affordable solutions on separation, identification, and quantification of constituents of complex organic samples.
- **Different combinations of these parts are:**
- Pumps
- Detectors
- Injectors yield an infinite number of configurations based on the application
- **Mobile Phase**
- The mobile phase serves to transport the sample to the system. Essential criteria of the mobile phase are inertness to the sample components. Pure solvents or buffer combinations are commonly used. The mobile phase should be free of particulate impurities and degassed before use.
- **Pumps**
- Variations in flow rates of the mobile phase affect the elution time of sample components and result in errors. Pumps provide a constant flow of the mobile phase to the column under constant pressure.
- **Injectors**
- Injectors are used to provide constant volume injection of the sample into the mobile phase stream. Inertness and reproducibility of injection are necessary to maintain a high level of accuracy.
- **Column**
- A column is a stainless-steel tube packed with a stationary phase. It is a vital component and should be maintained properly as per supplier instructions for getting reproducibility and separation efficiency run after run.

- **Column Oven**
- Variation of temperature during the analytical run can result in changes in the retention time of the separated eluting components. A column oven maintains a constant column temperature using air circulation. This ensures a constant flow rate of the mobile phase through the column.
- **Detector**
- A detector gives a specific response for the components separated by the column and also provides the required sensitivity. It has to be independent of any changes in mobile phase composition. The majority of the applications require UV-VIS detection, though detectors based on other detection techniques are also popular these days.
- **Data Acquisition & Control**
- Modern HPLC systems are computer-based and software controls operational parameters such as mobile phase composition, temperature, flow rate, injection volume and sequence, and also acquisition and treatment of output.



Applications of HPLC

- Water purification.
- Detection of impurities in pharmaceutical industries.
- Pre-concentration of trace components.
- Ligand-exchange **chromatography**.
- Ion-exchange **chromatography** of proteins.
- High-pH anion-exchange **chromatography** of carbohydrates and oligosaccharides.

What Is Column Chromatography?

In chemistry, Column chromatography is a technique which is used to separate a single chemical compound from a mixture dissolved in a fluid.

It separates substances based on differential adsorption of compounds to the adsorbent as the compounds move through the column at different rates which allow them to get separated in fractions.

This technique can be used on a small scale as well as large scale to purify materials that can be used in future experiments. This method is a type of adsorption chromatography technique.

Column Chromatography Principle

When the mobile phase along with the mixture that needs to be separated is introduced from the top of the column, the movement of the individual components of the mixture is at different rates.

The components with lower adsorption and affinity to stationary phase travel faster when compared to the greater adsorption and affinity with the stationary phase. The components that move fast are removed first whereas the components that move slowly are eluted out last.

The adsorption of solute molecules to the column occurs in a reversible manner. The rate of the movement of the components is expressed as:

R_f = the distance travelled by solute/ the distance travelled by the solvent

R_f is the retardation factor

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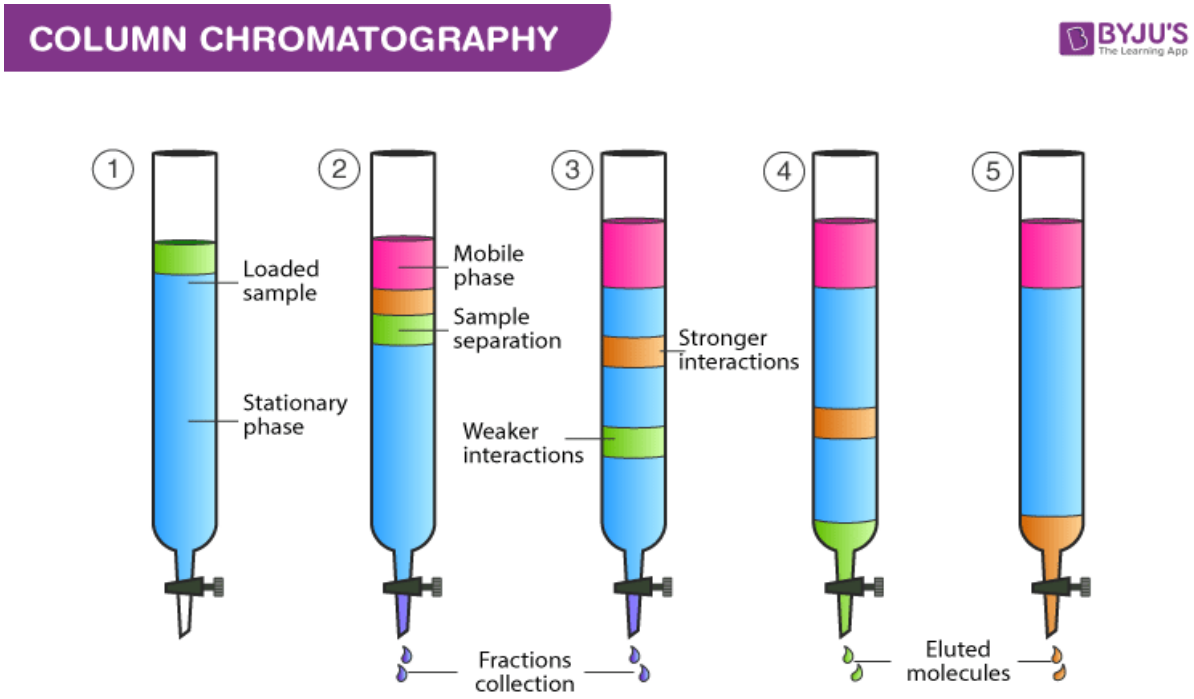
Column Chromatography Diagram



Column Chromatography Experiment

- The stationary phase is made wet with the help of solvent as the upper level of the mobile phase and the stationary phase should match. The mobile phase or eluent is either solvent or mixture of solvents. In the first step the compound mixture that needs to be separated, is added from the top of the column without disturbing the top level. The tap is turned on and the adsorption process on the surface of silica begins.
- Without disturbing the stationary phase solvent mixture is added slowly by touching the sides of the glass column. The solvent is added throughout the experiment as per the requirement.
- The tap is turned on to initiate the movement of compounds in the mixture. The movement is based on the polarity of molecules in the sample. The non-polar components move at a greater speed when compared to the polar components.
- For example, a compound mixture consists of three different compounds viz red, blue, green then their order based on polarity will be as follows blue>red>green
- As the polarity of the green compound is less, it will move first. When it arrives at the end of the column it is collected in a clean test tube. After this, the red compound is collected and at last blue compound is collected. All these are collected in separate test tubes.

Column Chromatography Applications

- Column Chromatography is used to isolate active ingredients.
- It is very helpful in Separating compound mixtures.
- It is used to determine drug estimation from drug formulations
- It is used to remove impurities.
- Used to isolation metabolites from biological fluids.