Electron Microscope

Scanning Electron Microscope (SEM)

Transmission Electron Microscope (TEM)

Electron microscope

- An electron microscope is a microscope that uses a beam of accelerated electrons as a source of illumination.
- The wavelength of an electron can be up to 100,000 times shorter than that of visible light photons.
- Electron microscopes have a higher resolving power than light microscopes and can reveal the structure of smaller objects

Why do we need Electron Microscope?

- Light microscopes are limited by the physics of light to 500x or 1000x magnification and a resolution of 0.2 micrometers.
- In the early 1930 there was a scientific desire to see the fine details of the interior structures of organic cells(nucleus,mitochondria etc)
- This required 10,000x plus magnification which was just not possible using electron microscopes.
- **It offers unique possibilities to gain insight into**, Structure ,Topology , Morphology And Composition of materials.

There are basic 4 types of Electron Microscope:

1. Analytical Electron Microscopy (AEM)
2. Scanning Transmission Electron Microscope (STEM)
3. Scanning Electron Microscope (SEM)
4. Transmission Electron Microscope (TEM)
5. Mainly 2 types
    - Transmission Electron Microscope (TEM) - allows one the study of the inner structures.
    - Scanning Electron Microscope (SEM) - used to visualize the surface of objects.

- Scanning Electron Microscope (SEM)
- Transmission Electron Microscope (TEM)

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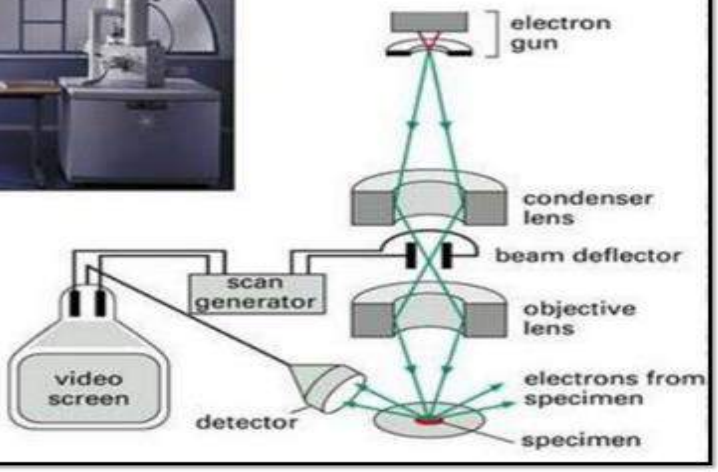


PRINCIPLE OF TEM

- Electrons possess a wave like character.
- Electrons emitted into vacuum from a heated filament with increased accelerating potential will have small wavelength.
- Such higher-energy electrons can penetrate distances of several microns into a solid.
- If these transmitted electrons could be focused - images with much better resolution.
- Focusing relies on the fact that, electrons also behave as negatively charged particles and are therefore deflected by electric or magnetic fields.

Application of TEM

1. TEM is used in a wide varity of fields from Biology, Microbiology, Nanotechnology, Forensic studies etc., some of these application include.
2. To visualize and study cell structures of bacteria, viruses and fungi.
3. To view bacteria flagella and plasmids.
4. To view the shapes and size of microbial cell organelles.
5. To study and differentiate between plant and animal cells
6. It also used in nanotechnology to study nano particles such as ZnO nanoparticles.
7. It is used to detect and identify fractures; damaged micro particles further enable repair mechanism of the particles.

SEM:

The scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. The signals that derive from electron-sample interactions reveal information about the sample.

PRINCIPLE OF SEM:

Accelerated electrons in an SEM carry significant amounts of kinetic energy, and this energy is dissipated as a variety of signals produced by electron-sample interactions when the incident electrons are decelerated in the solid sample. These signals include secondary electrons that produce SEM images.

Application of SEM:

- It is used in varity of fields including Industrial uses nanoscience studies,Biomedical studies,Microbiology.
- Used for spot chemical analysis in energy-Dispersive X-ray spectroscopy.
- Used in the analysis of cosmetic components are very thin in size.

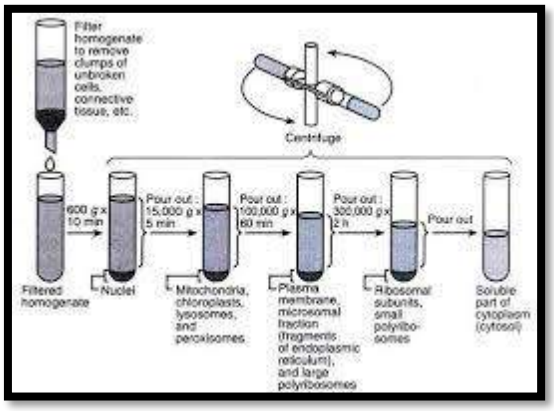
- Used to study the filament structures of microorganisms.
- Used to study the topography of elements used in industries.

CENTRIFUGE

A centrifuge is a laboratory device that is used for the separation of fluids, gas or liquid, based on density. Separation is achieved by spinning a vessel containing material at high speed; the centrifugal force pushes heavier materials to the outside of the vessel.

Definition for Centrifuge and Centrifugation:

- A centrifuge is a device used to separate components of a mixture on the basis of their size, density, the viscosity of the medium, and the rotor speed.
- Centrifugation is the technique of separating components where the centrifugal force/ acceleration causes the denser molecules to move towards the periphery while the less dense particles move to the center



Principle of centrifuge

- In a solution, particles whose density is higher than that of the solvent sink (sediment), and particles that are lighter than it floats to the top.
- The greater the difference in density, the faster they move. If there is no difference in density (isopycnic conditions), the particles stay steady.

- To take advantage of even tiny differences in density to separate various particles in a solution, gravity can be replaced with the much more powerful “centrifugal force” provided by a centrifuge.
- A centrifuge is a piece of equipment that puts an object in rotation around a fixed axis (spins it in a circle), applying a potentially strong force perpendicular to the axis of spin (outward).
- The centrifuge works using the sedimentation principle, where the centripetal acceleration causes denser substances and particles to move outward in the radial direction.
- At the same time, objects that are less dense are displaced and move to the center.
- aboratory centrifuge that uses sample tubes, the radial acceleration causes denser particles to settle to the bottom of the tube, while low- density substances rise to the top.

Relative Centrifugal Force (RCF)

- Relative centrifugal force is the measure of the strength of rotors of different types and sizes.
- This is the force exerted on the contents of the rotor as a result of the rotation.
- RCF is the perpendicular force acting on the sample that is always relative to the gravity of the earth.
- The RCF of the different centrifuge can be used for the comparison of rotors, allowing the selection of the best centrifuge for a particular function.
- The formula to calculate the relative centrifugal force (RCF) can be written as:
- RCF (g Force)= $1.118 \times 10\text{-}5 \times r \times (RPM)2$
- Where r is the radius of the rotor (in centimeters), and RPM is the speed of the rotor in rotation per minute.

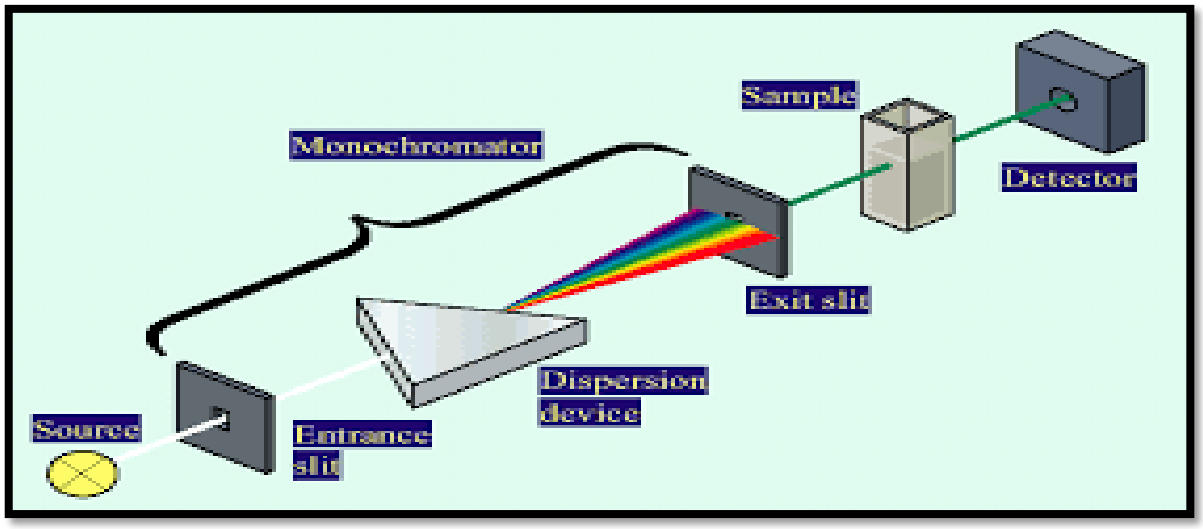
Types of Centrifuge

- Benchtop centrifuge.
- Continuous flow centrifuge.
- Gas centrifuge.
- Hematocrit centrifuge.

- High-speed centrifuge.
- Low-speed centrifuge.
- Micro centrifuge.
- Refrigerated centrifuges.
- **Ultra centrifuge**.
- Vacuum centrifuge

Ultracentrifuge:

- Ultracentrifuges are the centrifuges that operate at extremely high speeds that allow the separation of much smaller molecules like ribosome's, proteins, and viruses.
- It is the most sophisticated type of centrifuge that allows the separation of molecules that cannot be separated with other centrifuges.
- Refrigeration systems are present in such centrifuges that help to balance the heat produced due to the intense spinning.
- The speed of these centrifuges can reach as high as 150,000 rpm.
- It can be used for both preparative and analytical works.
- Ultracentrifuges can separate molecules in large batches and in a continuous flow system.
- In addition to separation, ultracentrifuges can also be used for the determination of properties of macromolecules like the size, shape, and density.



Applications of Centrifugation:

- To separate two miscible substances
- To analyze the hydrodynamic properties of macromolecules
- Purification of mammalian cells
- Fractionation of sub cellular organelles (including membranes/membrane fractions) Fractionation of membrane vesicles
- Separating chalk powder from water
- Removing fat from milk to produce skimmed milk
- Separating particles from an air-flow using cyclonic separation
- The clarification and stabilization of wine

- Separation of urine components and blood components in forensic and research laboratories
- Aids in the separation of proteins using purification techniques such as salting out, e.g. ammonium sulfate precipitation.

Electrophoresis

Electrophoresis is the motion of dispersed particles relative to a fluid under the influence of spatically uniform electric field. Electrophoresis of positively charged particules is sometimes called cataphoresis,electrophoresis of negatively chargedparticles is some time called anophoresis.

SDS PAGE:

SDS PAGE also known as **Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis** is a technique used for separating the proteins based on their molecular weight. It is a widely used technique in forensics, genetics, biotechnology, and molecular biology to separate the protein molecules based on their electrophoretic mobility.

Difference between PAGE and SDS PAGE:

The major difference between native PAGE and SDS-PAGE is that in native PAGE, the protein migration rate is dependent on both the mass and structure, whereas in SDS-PAGE, the migration rate determined only by protein's mass. In native PAGE, protein samples are prepared in a non-denaturing and non-reducing buffer.

SDS PAGE used for:

SDS-PAGE is an electrophoresis method that allows protein separation by mass. The medium (also referred to as 'matrix') is a polyacrylamide- based discontinuous gel. In addition, SDS (Sodium Dodecyl Sulfate) is used. About 1.4 grams of SDS bind to a gram of protein, corresponding to one SDS molecular per two amino acids.

Role of SDS in SDS-PAGE:

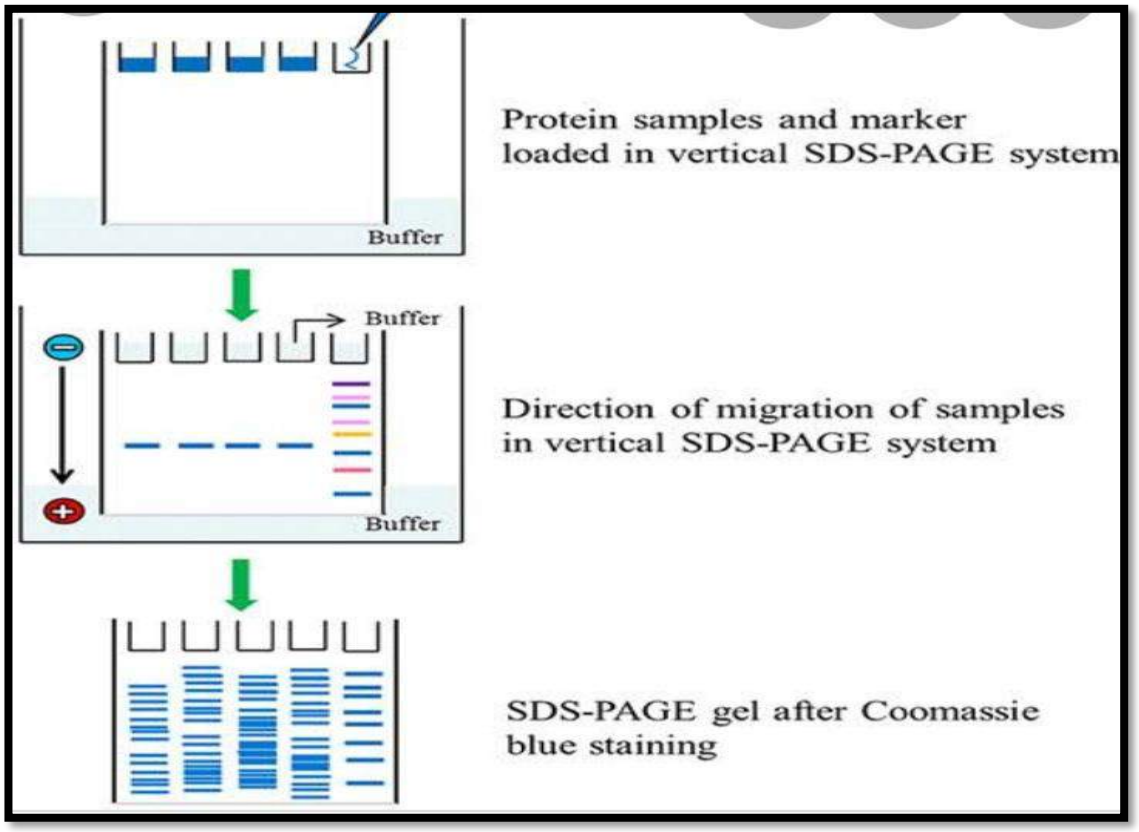
SDS is a detergent present in the SDS-PAGE sample buffer. SDS along with some reducing agents function to break the disulphide bonds of proteins distrusting the territory structure of proteins.

Principle of SDS-PAGE:

The principle of SDS-PAGE states that a charged molecule migrates to the electrode with the opposite sign when placed in an electric field. The migration of charged molecules,

The smaller molecules migrate faster due to less resistance during electrophoresis. The structure and the charge of the proteins also influence the rate of migration.

Sodium Dodecyl Sulphate and Polyacrylamide eliminate the influence of structure and charge of the proteins, and the proteins are separated based on the length of the polypeptide chain.



Applications of SDS-PAGE:

- It is used to measure the molecular weight of the molecules.
- It is used to estimate the size of the protein.
- Used in peptide mapping
- It is used to compare the polypeptide composition different structures.
- It is used to estimate the purity of the proteins.
- It is used in Western Blotting and protein ubiquitination.
- It is used in HIV test to separate the HIV proteins.
- Analysing the size and number of polypeptide subunits.
- To analyse post-translational modifications.

CHROMATOGRAPHY

Definition:

- Chromatography is an important biophysical technique that enables the separation, identification and purification of the components of a mixture far qualitative and quantitative analysis.
- The Russian botanist Mikhail Tsvet coined the term chromatography in 1906.
- It is a powerful separation tool that is used in all branches of science.

Types of Chromatography:

- Thin-Layer Chromatography (TLC)
- Gas Chromatography (GS)
- High Performance Liquid Chromatography (HPLC)

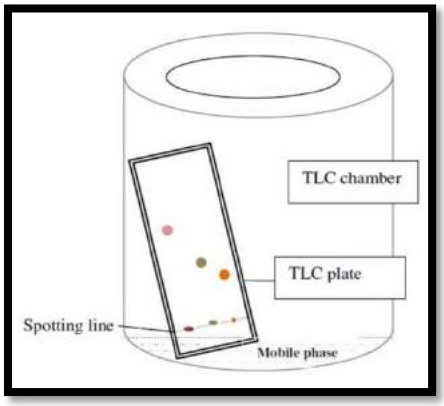
Thin-Layer Chromatography:

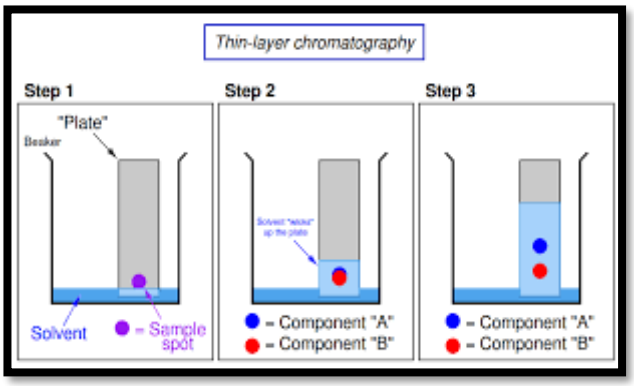
- Thin layer chromatography can be defined as a method of separation or identification of a mixture of compounds into individual components by using

finely divided absorbent solid liquid spread over a plate and liquid as a mobile phase.

Principle of TLC:

- It is based on the principle of absorption chromatography or partition chromatography or combination of both, depending on absorbent, its treatment and nature of solvents employed. The components with more affinity towards stationary phase travels slower components with less affinity towards stationary phase travels faster.
- Once separation occurs the individual components are visualization as spot at a respective level of travel on the plate. Their nature or chamber is identified by means of suitable detection techniques.





Application of TLC:

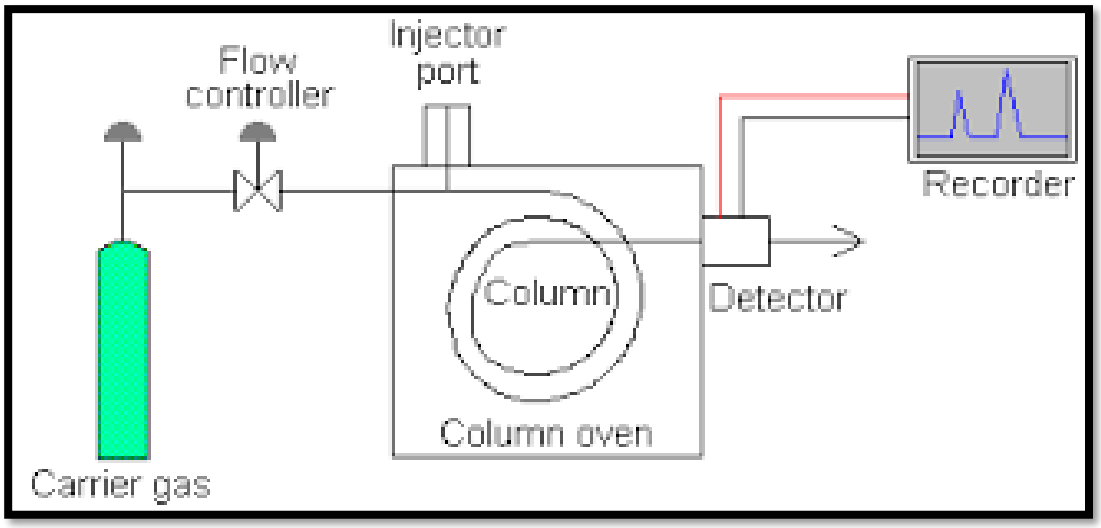
- In monitoring the progress of reactions.
- Identify compounds present in a given mixture.
- Determine the purity of a substance.
- Analysing ceramides and fatty acids.
- Detection of pesticides as insecticides in food and water.
- Analysing the dye composition of fibres in forensics.

Gas Chromatography (GS):

- Gas chromatography differs from others from of chromatography in that the mobile phase is gas and the components are separated as vapors.
- It is thus used to separate and detect small molecular weight compounds in the gas phase.

Principle of Gas Chromatography:

- The equilibrium for gas chromatography is partitioning and the components of the sample will partition between the two phases, the stationary phase and the mobile phase.
- Compounds that have a greater affinity for the stationary phase spend more time in the column and thus elute later and have a longer retention time (Rt) than sample that have a higher affinity for the mobile phase.
- Affinity for the stationary phase is driven mainly by intermolecular interactions and the polarity of the stationary phase can be chosen to maximize interactions and thus the separation.
- Ideal peaks are Gaussian distributions and symmetrical because of the random nature of the analyte interactions with the column.



Application of GS:

- Air borne pollutants.
- Performance enhancing drugs in athlete's urine samples.
- Oil spills.
- Essential oils in perfume preparation.

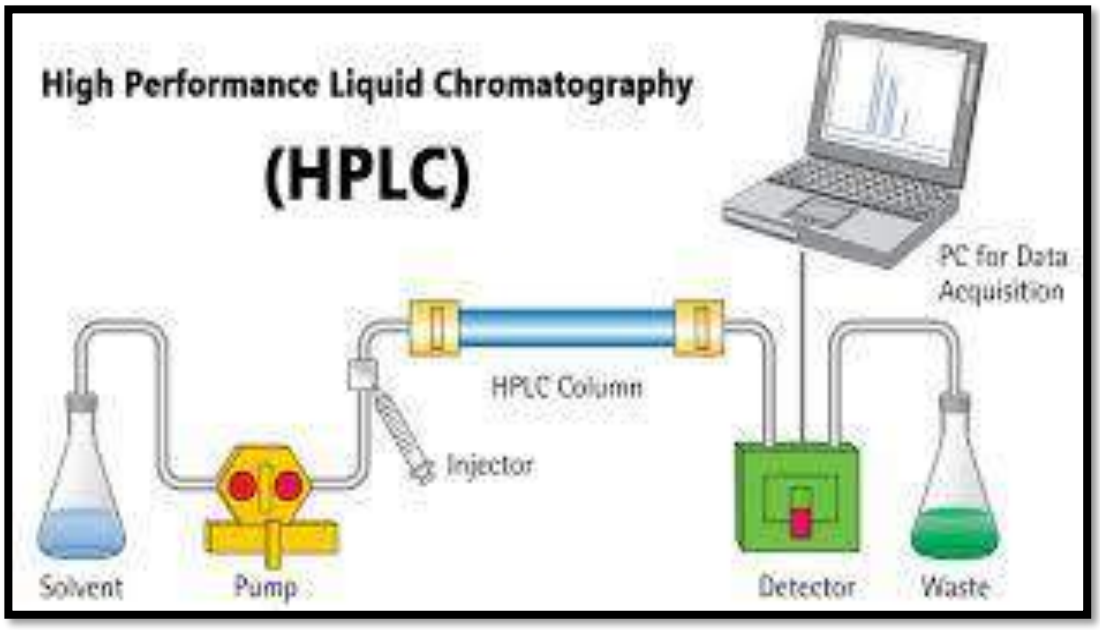
- Gc analysis is used to calculate the content of a chemical product for example in assuring the quality of products in the chemicals industry or measuring toxic substances in soil, air or water.

High Performance Liquid Chromatography (HPLC):

- High performance liquid chromatography or commonly known as HPLC is an analytical technique used to separate, identify or quantify each component in a mixture.
- The mixture is separated using the basic principle of column chromatography and then identify each component and quantified by spectroscopy.

Principle of HPLC:

- The purification takes place in a separation column between a stationary column a stationary and a mobile phase.
- The stationary phase is granular material with very small parous particles in a separation column.
- Via a value with a connected sample loop. i.e., a small tube or a capillary made of stainless steel, the sample is injected into the mobile phase flow from the pump to the separation column using a syringe.
- Subsequently the individual's components of the sample migrate through the column at different rates because they are retained to a varying degree by interaction with the stationary phase.
- At the end of this operation / run a chromatogram in the HPLC software on the computer is obtained.



Applications of HPLC:

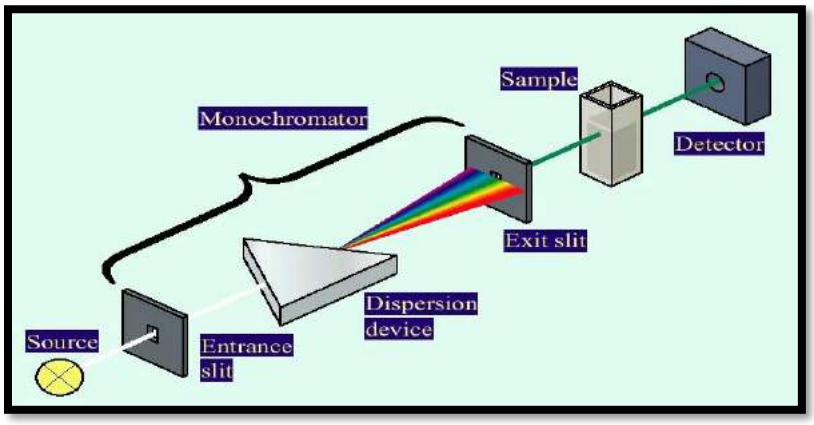
- Analysis of drugs.
- Analysis of synthetic polymers.
- Analysis of pollutants in environments analytics.
- Determination of drugs in biological matrices.
- Isolation of valuable products.
- Product purity and quality control of industrial products and fine chemicals.
- Water purification.
- Pre-concentration of trace components.
- Ligand-exchange chromatography.

SPECTROSCOPY

- A spectroscopy is the study of the interaction between electromagnetic radiation and matter. The matter can be atoms, molecules or ions.
    - Historically, **spectroscopy** originated as the study of the wavelength dependence of the absorption by gas phase matter of visible light dispersed by a prism.

Electromagnetic radiation:

- EM is a form of energy that is all around us.
- EM is a form of energy and has both electrical and magnetic characteristics.
- Electricity and magnetism were once thought to be separate forces.
- James clerk maxwell developed a unified theory of electromagnetism.
- The study of electromagnetism deals with how electrically charged particles interact with each other and with magnetic fields.



Spectrometer:

- Spectrometer is something which can be used to measure the presence of particular compound or particle in a molecule.

Spectrum:

- Spectrum is a plot of the amount of light absorbed by a sample versus the wavelength of the light.
- The amount of light absorbed is called the absorbance.

Spectrophotometer:

- A spectrophotometer is an instrument that measures the amount of light absorbed by a sample.
- Used to measure the concentration of solutes in solution by measuring the amount of the light that is absorbed by the solution in a cuvette placed in the spectroscopy.

Principle of Spectroscopy:

- The basic principle shared by all spectroscopic techniques is to shine a beam of electromagnetic radiation onto a sample, and observe how it responds to such a stimulus. The response is usually recorded as a function of radiation wavelength.

Types of spectroscopy

- UV Spectroscopy(Ultraviolet and visible)
- IR Spectroscopy(Infra-Red)
- NMR Spectroscopy(Nuclear Magnetic Resonance)

1. UV Spectroscopy:

Ultraviolet–visible spectroscopy or ultraviolet–visible spectrophotometry refers to absorption spectroscopy or reflectance spectroscopy in part of the ultraviolet and the full, adjacent visible spectral regions. This means it uses light in the visible and adjacent ranges.

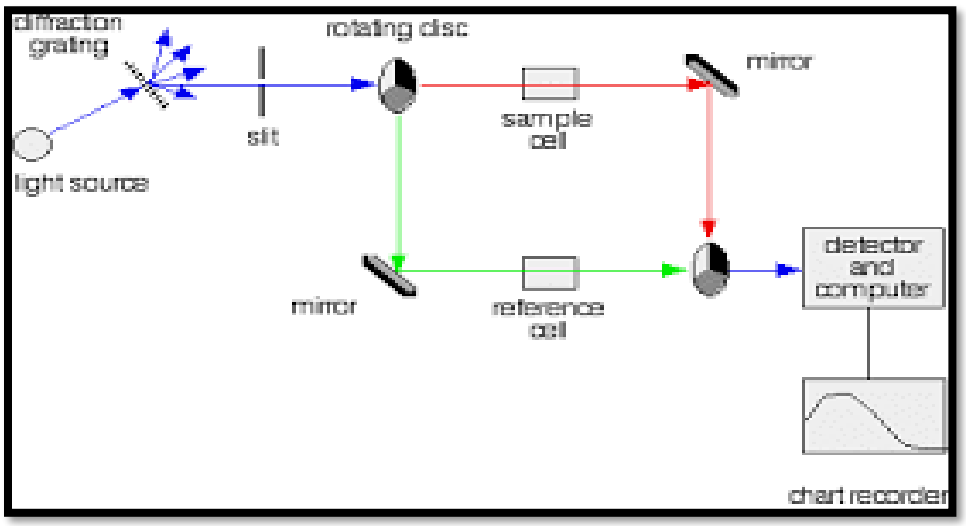
Principle of UV Spectroscopy:

The Principle of UV-Visible Spectroscopy is based on the absorption of **ultraviolet light** or visible light by chemical compounds, which results in the production of distinct spectra. Spectroscopy is based on the interaction between light and matter.

Application of UV Spectroscopy:

- Detection of functional groups.
- Detection of impurities.
- Qualitative analysis, Quantitative analysis.
- Single compound without chromospheres.
- Drugs with chromospheres' reagent.
- It is helps to show the relationship between different groups, it is useful to detect the conjugation of the compounds.

It is used in Traditional Chemistry, Life Science, Microbiology, Food & Agriculture, Material Science, Optical Components, Pharmaceutical Research, Petrochemistry, Cosmetic Industry, Quality Control



2. IR spectroscopy:

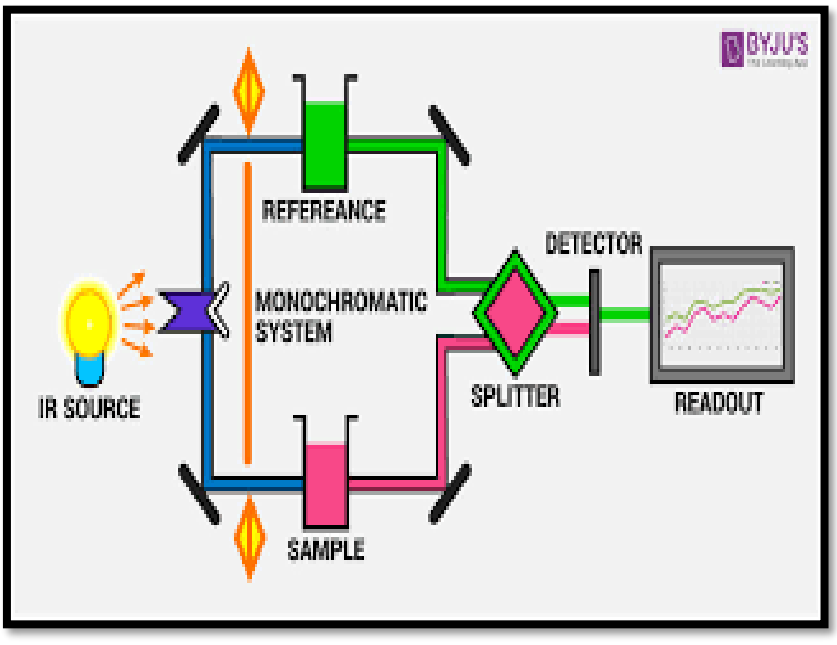
Infrared spectroscopy is the measurement of the interaction of infrared radiation with matter by absorption, emission, or reflection. It is used to study and identify chemical substances or functional groups in solid, liquid, or gaseous forms.

Principle of IR Spectroscopy:

IR spectroscopy detects the absorption of light by a compound, in the IR region of the electromagnetic spectrum. To absorb light a molecule must have a bond within its structure that can exhibit what is referred to as a 'dipole moment' which means electrons within a bond are not shared equally.

Application 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.



NMR spectroscopy:

Nuclear magnetic resonance spectroscopy, most commonly known as NMR spectroscopy or magnetic resonance spectroscopy, is a spectroscopic technique to observe local magnetic fields around atomic nuclei.

Principle of NMR:

The principle behind NMR is that many nuclei have spin and all nuclei are electrically charged. If an external magnetic **field** is applied, an energy transfer is possible between the base energy to a higher energy level (generally a single energy gap).

Application of NMR:

1. Nuclear magnetic resonance spectroscopy is widely used to determine the structure of organic molecules in solution and study molecular physics and crystals as well as non-crystalline materials.
2. NMR is also routinely used in advanced medical imaging techniques, such as in magnetic resonance imaging (MRI).
3. More precise image.
4. Hydrogen bonding of molecules.
5. Drug screening and Designing.
6. In medicine, NMR scans.