

**CELL PERSPECTIVE**

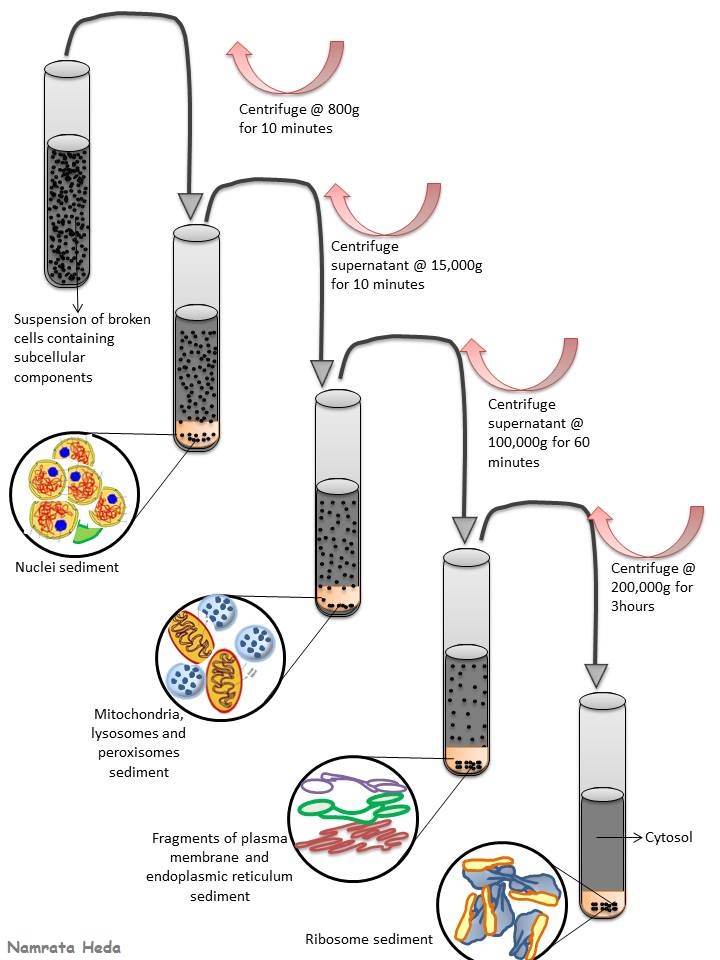
**TOPIC** : ISOLATION OF CELLS AND ORGANELLES,CENTRIFUGATION AND FRACTIONATION,AUTORADIOGRAPHY,ISOTOPIC TRACERS,SPECTROPHOTOMETRY, DIALYSIS,ELECTROPHORESIS,CHROMATOGRAPHY

**SESSION** : 2019-2023

**1. Isolation of cell and organelle**:

**Isolation of organelles** is accomplished by **cell membrane** lysis and density gradient centrifugation to separate **organelles** from contaminating cellular structures. Intact nuclei and **organelles** have distinctivesizes in mammalian **cells**, enabling them to be separated by this methods.

* Centrifugation
* Fractionation



**Uses**

* they can be used to study how cell works, how they change in response to disease, and how they are effected by drugs.
* It used to study how charged particles flow across the cell membrane.
* Cell isolation can also be used as a part of a treatment. Isolation of pancreatic islets cells, followed by their subsequent culture and transplantation, has been used to treat patients with type 1 diabetes.

**Cell separation methods**

There are different ways to isolate cells from complex biological samples. Common characteristics used to isolate cells include cell size, cell density, cell shape and surface protein expression. The most common cell separation methods include:

* Immunomagnetic cell separation
* Fluorescence-activated cell sorting
* Immunodensity cell isolation
* Micro uicic cell sorting

Cell fractionation

It is the process used to separate cellular components while preserving the indivual functions of each component.

Cell **fractionation** is a procedure for rupturing cells, separation and suspension of cell constituents in isotonic medium in order to study their structure, chemical composition and **function**.

Principle of fractionation:

Scientists are able to discern the function of organelles by separating them .It allow us to study the different parts of cells. We can also observe which organelle is making the more energy when we isolate them .

Steps of cell fractionation:

This involves following steps:

* Extraction
* Homogenization
* Centrifugation

1. Extraction:

It is the first step toward isolating any sub-cellular structures. In order to maintain the biological activity of organelles and bio-molecules, they must be extracted in mild conditions called cell-free systems. For these, the cells or tissues are suspended in a solution of appropriate pH and salt content, usually isotonic sucrose (0.25 mol/L) at0-40°C.

1. Homogenization:

The suspended cells are then disrupted by the process of homogenization. It is the process in which a biological sample is brought to a stage where all the fractions of sample are made uniformly small and are evenly distributed .When the process of homogenization is over, the resulting mixture of uniformly divided fractions is called cell homogenate or cell lysate .

Homogenization is made by 4 note able methods:

* Ultrasonic homogenization:

In this process, ultrasonic homogenizer known as sonicators, rupture tissues through a combination of cavitation and ultrasonic waves. This technique is also called as sonification. Sonification is ideally matched for suspended cellular/subcellular structure, as well as for shearing DNA.

However, because it generates a significant amount of heat, ultrasonic homogenization is only appropriate for tissues and molecules that will not be affected by temperature increase.

* Mechanical homogenization:

Mechanical homogenization utilizes direct physical force to bring a biological sample in solution to a state of uniform distribution, such that all fractions’ molecular composition is consistent. Traditionally, mechanical disruption was achieved by freezing tissues and then grinding with a mortar and pestle.

It is easily scaled as well as is it is a quick process with consistent results.

* Freeze thawing:

The **technique** involves **freezing** a cell suspension in a dry

Ice/ethanol bath or freezer and then **thawing** the material at

room temperature or 37°C. This **method** of lysis causes cells to

swell and ultimately break as ice crystals form during

the **freezing** process and then contract during **thawing**.

This process is effectively employed to disrupt bacterial and

Mammalian cells.

* Chemical homogenization:

Chemicals are effectively used to homogenize tissues.

For example, surfactants and detergents targets biological membranes by disrupting the hydrophobic/hydrophilic interface, and are well matched with various bacterial species. Enzyme also tackles the cell membrane and cell wall and can be effectively used in obtaining the extract.

It is prefer able for small sample as the material cost can become overwhelming for industrial sized products.

Centrifugation:

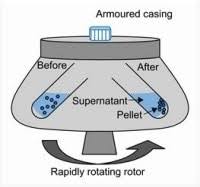
After homogenization, the cell goes into third and last step of fractionation called centrifugation.

Centrifugation is the process completed by applying a centrifugal force, that is, higher than the gravitational force to intensify the separation of the suspension.

**OR** It is a process used to separate or concentrate materials suspended in a liquid medium. The theoretical basis of this technique is the effect of gravity on particles (including macromolecules) in suspension. Two particles of different masses will settle in a tube at different rates in response to gravity.

**Intoduction**

* Centrifugation is a process which involves the use of the centrifugal force for the sedimentation of heterogenous mixtures with a centrifuge, used in industry and in laboratory settings.
* This process is used to separate **two immiscible liquids**.
* **More dense** components of the mixture migrate away from the axis of the centrifuge, while **less dense** components of the mixture migrate towards the axis.



**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 prependicular 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. In a laboratory centrifuge that uses sample tubes, the **radial acceleration** causes denser particles to settle to the bottom to the tube, while low-density substances rise to the top



**Principle**

* A centrifuge is a device for separating particles from a solution according to their size, shape, density, viscocity of the medium and rotor speed.
* In a solution, particles whose density is higher than that of the solvent **sink** (sediments), and particles that are lighter than it float to the top.
* The greater the difference in density, the faster they move. If there is no difference in density (isopyknic conditions), the particles stay steady.

**Instrumentation**

It consist of two components, an electric motor to spin the sample and a rotor to hold tubes.

* Low speed centrifuge
* High speed centrifuge
* Ultra centrifuge

**Centrifuge Rotor**

A centrifuge rotor is the rotating unit of the centrifuge, which has fixed holes drilled at an angle. Test tubes are placed inside these holes and the rotor spins to aid in the separtion of the materials.



A centrifuge Rotor

When the vessel, containing mixture of proteins or other particular matter, such as bacterial cells, is rotated at high speeds, the inertia of each particle yields a force in the direction of the particles velocity that is proportional to its mass.The tendency of a given particle to move through the liquid because of this force is offset by the resistance the liquid exerts on the particle. The net effect of spinning the sample in the centrifuge is that massive, small and dense particle moves outward faster than less massive particles or particles with more drag in liquid. When suspension of particles are spun in a centrifuge, a pallet may form at the bottom of the vessel that is enriched with the most massive particles with low drag in a liquid. Non compacted particles remain mostly in liquid called **supernatant** and can be removed from the vessel thereby separating the supernatant from the pallet.

Advantages:

* Centrifuges have a clean appearance and have little to no odour problems.
* They can be selected for different applications.
* The machine can operate with a higher capacity than smaller machines.
* The device is simple to operate.
* Centrifuge has more process flexibility and higher level of performance.

Disadvantages:

* The machine can be very noisy and can cause vibration.
* The device has a high energy consumption force due to gravitational force.

**4. Autoradiography**

Autoradiography is the bio analytical technique used to visualize the distribution of radioactive labeled substance with radioisotope in a biological sample. In vitro **autoradiography** methods involve the isolation of cellular components such as DNA, RNA, proteins or lipids, followed by labeling with suitable radioisotopes.

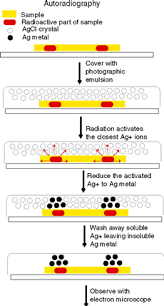
Principle:

Radio isotopes will emit the radiations and emitted radiations will ionize the photographic film and ultimately dark spots will occur.

Photographic film is combination of silver halide with gelatin.

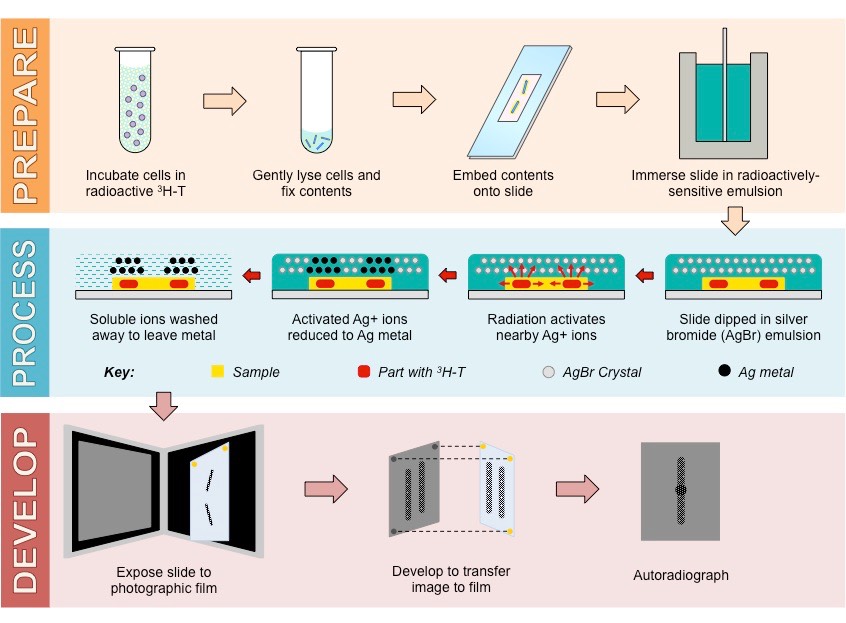
**Introduction**

* It is a method by which a radioactive material can be localized within a particular tissue, cell, cell organellesor even biomolecules.
* It is a very sensitive technique and is being used in a wide variety of biological experiments.
* Autoradiography, although used to locate the radioactive substance, it can also be used for quantitative estimation by using densitometer.



Methodology:

* The radioactive sample is covered with the photographic emulsion.
* The radioactive part of sample activates the silver halide crystals nearby.
* This results in reduction of Ag+ ions to Ag atoms leaving dark color bands.
* The slide is then washed away by fixers to get in soluble Ag atom only.
* The autoradiogram can further be viewed and observed under the microscope.



**Application**

* To find and investigate various properties of DNA.
* To find the location and amount of particular substance within a cell including cell organelle, metabolites etc.
* Tissue localization of radioactive substance.
* To find the site and performance of targeted drug.
* To locate the metabolic activity site in the cell.
* Advantage:
* The use of auto radiographic methods brought significant advances to preclinical research. Classic auto radiographic methods are technically straightforward, and do not require much expertise form the operators, Moreover, the ability to couple radioisotopes with radiotracers that target radioactive decay to specific tissues, made auto radiographic methods highly efficient.
* Disadvantage:
* However, a major limitation of these techniques is the lack of standard methods to evaluate binding specificity of radiotracers to their targets.
* Another limitation of auto radiographic methods is that detection is dependent on radioactive decay of the radioisotopes used. In the case of long-lived radioisotopes, image development may require several hours of exposure, making it very time-consuming.

**5.Isotropic tracers**

**Definition**

“ It can be defined as technique which utilizes a labelled compound to find out or to trace the different intermediates and various steps in biosynthetic pathways in plants, at a given rate and time.”

**OR**

“In this technique different isotopes, mainly the radioactive isotopes which are incorporated into presumed precursor of plant metabolites and are used as marker in biogenic experiments.”

Principle:

The tracer principle states that radioactive isotopes have the same chemical properties as nonradioactive isotopes of the same element. Isotopes of the same element differ only in the number of neutrons in their [**atoms**](https://science.jrank.org/pages/639/Atoms.html), which leads to nuclei with different stabilities. Unstable nuclei gain stability by [**radioactive decay**](https://science.jrank.org/pages/5680/Radioactive-Decay.html) which leads to different types of radioactivity. One type is gamma [**radiation**](https://science.jrank.org/pages/5636/Radiation.html) which is useful in medicine because it penetrates the body without causing damage and can then be detected easily.

**Types of isotopes**

The labelled compound can be prepared by use of two types of isotopes.

* Radioactive isotopes
* Stable isotopes

**Significance of tracer technique**

* **Tracing of biosynthetic pathway**: By incorporation of radioactive isotope of 14C into phenylalanine, the biosynthetic cyanogenetic glycoside prunasin, can be detected.
* **Location and quantity of compound containing tracer**: 14C labelled glucose is used for determination of glucose in biological system.
* Convenient and suitable technique

**Criteria for tracer technique**

* The starting concentration of tracer must be sufficient withstand resistance with dilution in course of metabolism.
* Labelled compound should involve in the synthetic reaction.
* Labelled compound should not damage the system to which it is used.

**Advantages**

* High sensitivity
* Applicable to all living organism
* Wide ranges of isotopes are available
* More reliable, easily administration and isolationprocedure
* Gives accurate result, if proper metabolic time and technique applied

**Limitation**

* Kinetic effect
* Chemical effect
* Radiation effect
* Radiochemical purity
* High concentration distorting the result

**Requirement for tracer technique**

* Preparation of laballed compound
* Introduction of labelled compound into a biological system
* Separation and determination of labelled compound in various biochemical fractions at later time

**Application of tracer technique**

1. Study of squalene cyclization by use of 14C,3H labelled mevalonic acid
2. Study the formation of cinnamic acid in pathway of coumarin from labelled coumarin
3. Study of formation of scopoletin by use of labelled phenylalanine
4. By use of 45Ca as tracer – found that the uptake of calcium by plants from the soil. (CaO and CaCO2)

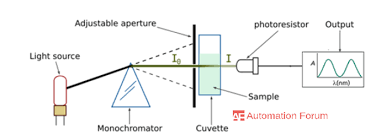
6.**Spectrophotometry**

* A method in which absorption and transmission properties of a material is quantitatively measured as a function of wavelength.

**Principle**

The basic principle behind this method is that:

“Each compound absorbs or transmitslight over a certain range of wavelength”



**Spectrophotometer**

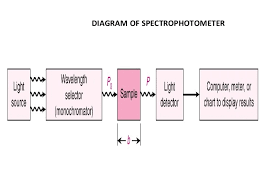
* It is an instrument that measures the amount of photons absorbed by a sample after it is passed through its solution
* With the spectrophotometer, the amount of a known chemical substance can be determined by measuring the absorbance.

**Types**

There are two types of spectrophotometer:

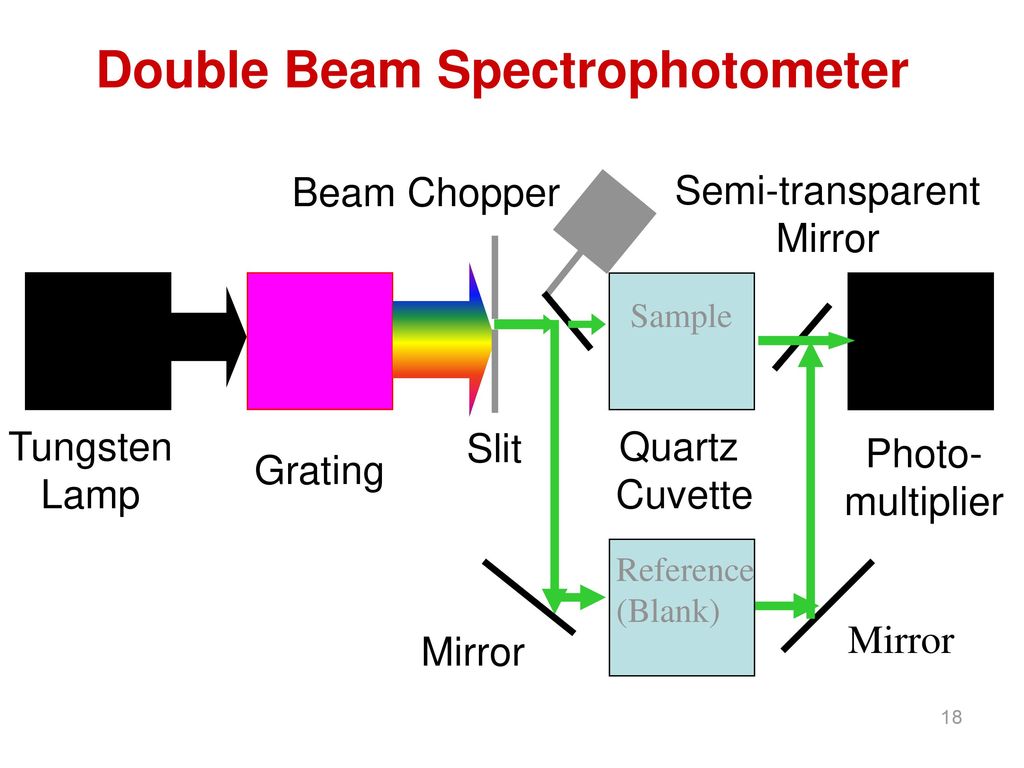
* Single beam spectrophotometer
* Double beam spectrophotometer
* **Single beam spectrometer:**

To measure the intensity of the incident light the sample must be removed so tht the reference can be placed each time. This type of spectrometer is usually less exppensive and less complicated.



* **Double beam spectrometer:**

In this type, before it reaches the sample , the light source is split into two separate beams. From these one passes through the sample and second one is used for reference. This gives an advantage because the reference reading and sample reading can take place at the same time.



**Application**

1. Concentrate measurement
2. Detection of impurities
3. Chemical kinetics
4. Detection of functional group
5. Molecular weight determination
6. Detection of concentration of substances
7. Structure elucidation of organic compounds
8. Monitoring dissolved oxygen content in freshwater and marine ecosystems
9. Characterization of [proteins](https://microbenotes.com/proteins-properties-structure-classification-and-functions/)
10. Detection of functional groups
11. Respiratory gas analysis in hospitals
12. The visible and UV spectrophotometer may be used to identify classes of compounds in both the pure state and in biological preparations.

**7.Dialysis**

**Definition**

* Artificial process that partially replaces renal function
* Removes waste products from blood by diffusion
* Removes excess water by ultrafiltration (maintenance of fluid balance)
* Wastes and water pass into a special liquid – dialysis fluid

**Types**

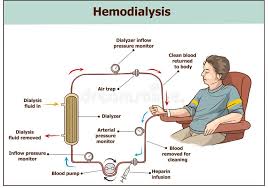
* Haemodialysis
* Peritoneal dialysis

They work on similar principle:

Movemet of solute and water across a semipermeable membrane (dialysis membrane)

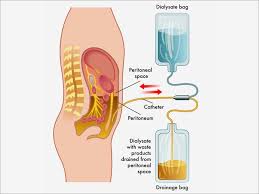
**Haemodialysis**

* Dialysis process occurs outside the body in a machine
* The dialysis member is an artificial one: Dialyser
* The dialyser removes the excess fluid and wastes from the blood to the body
* Haemodialysis needs to pe performed three times a week
* Each session lasts 3-6 hours



**Peritoneal dialysis**

* Uses natural membrane (peritoneum) for dialysis
* Access is by PD catheter, a soft plastic tube
* Catheter and dialysis fluid may be hidden under clothing
* Suitability
* Excludes patients with prior peritoneal scarring e.g. peritonitis, laparotomy
* Excludes patients unable to care for self



**Problem with treatment**

* Hernia :
* aggravation of pre-existing hernia (repair)
* Evolution of new hernia
* Declining effectiveness of the peritoneum:
* e.g. repeated infection
* Effect of glucose in the dialysis fluid

**8.Electrophoresis**

**Introduction**

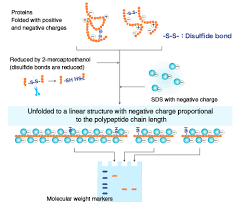
* The term electrophorhesis describes the migration of a charged particle under the influence of electric field
* Many important biological molecules such as amino acids, peptides, proteins, nucleotides, nucleic acid possess ionisable groups, and therefore at any given pH, exists in solution as electrically charged species either as cation or anion
* This is one of the most fundamental processes used in all types of molecular biology and RDT experiments.

**Definition**

* Electrophoresis is migration of charged particles or molecules in a medium under the influence of an applied electric field
* The rate of migration of charged molecules depends upon following:

1. The strength of electric field, size and shape.
2. Relative hydrophobicity of the sample
3. Ionic strength and temperature of the buffer
4. Molecular size of the taken bio molecule
5. Net charge density of the taken bio molecule
6. Shape of the taken bio molecule

* In the process of electrophoresis large molecules have more difficulty in moving through the supporting medium (i.e., gel) whereas the smaller medium has more mobility through it.



**Classification**

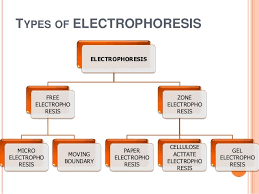
* All modern electrophoretic apparatus have supporting media these days
* A supporting medium is a physical support through which the charged molecules get separated.
* It has two primary functions-absorption and molecular sieving of the taken molecules which are intended to be separated
* Depending upon the presence and absence of supporting media the electrophoresis can be classified as **free electrophoresis** and **zone electrophoresis**

**Applications**

* Peptides, proteins, DNA, viruses, organelles, or bacteria can be separated at resolutions of 3-5% of their electrophoretic mobilities and a throughout of up to 50 mg proteins or 20 million cells per hour may be achieved.
* Highly developed modern machines may be operated continously or at intervals with segmented electrolyte in various modes and with buffers containing up to 60mM ions.

**Types of electrophoresis**

Following table show the types of electrophoresis



**9.Chromatography**

Chromatography ( from greek chroma “color and graphein” to write) is the collective term for a set of **laboratory techniques** for the **separation of mixture**. The mixture is dissolved in a fluid called the **mobile phase**, which carries it through a structure holding another material called the **stationary phase.** The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases.

**History**

Chromatography, literally “color writing” was first employed by Russian scientist **Mikhail Tsvet** in 1900. He continued to work with chromatography in the first decade of the 20th century, primarily for the separation of plant pigments such as chlorophyll, carotene, and xanthophylls. Since these components have different colors ( green, orange and yellow respectively ) they gave the technique its name.

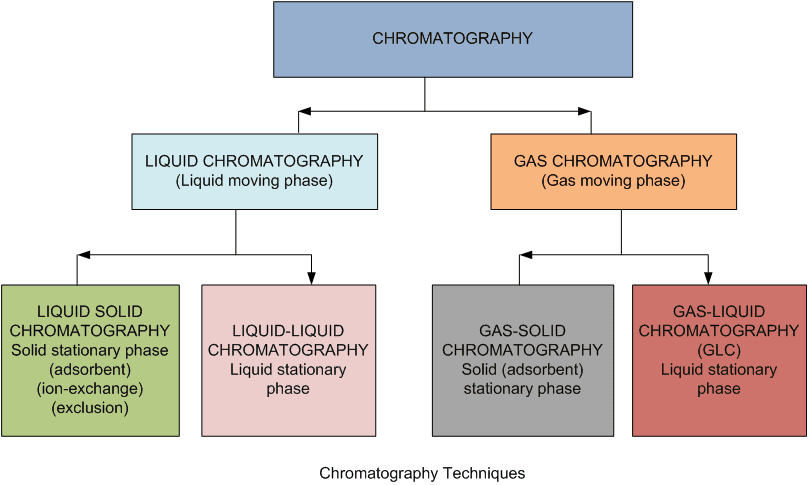
**Principle**

* Chromatography usually consists of mobile phase and stationary phase. the mobile phase refers to the mixture of substances to be separated dissolved in a liquid or a gas. The stationary phase is a porous solid matrix through which the sample contained in the mobile phase percolates. The interaction between the mobile phase and stationary phase results in the separation of the compound from the mixture

**Application of chromatography**

* The chromatographic technique is used for the separation of amino acids, proteins, and carbohydrates.
* It is also used for the analysis of drugs, hormones, vitamins.
* Helpful for the qualitative and quantitative analysis of complex mixtures.
* The technique is also useful for the determination of molecular weight of proteins.

**Types of chromatography**



* **Paper chromatography**

It is a technique that involves placing a small dot or line of sample solution onto a strip of chromatograph paper. The paper is placed in a jar containing a shallow layer of solvent and sealed. As the solvent raises through the paper, it meets the sample mixture, which starts to travel up the paper with the solvent.

* **Thin layer chromatography**

It is a widely employed laboratory technique and is similar to paper chromatograph. However, instead of using a stationary phase of paper, it involves a stationary phase of a thin layer of adsorbent like silica gel, aluminium or cellulose. Compare to paper it has the advantage of faster runs, better separations, and the choice between different adsorbent.

* **Gas-liquid chromatography**

It is separation technique in which the mobile phase is a gas. It is a method of choice for the separation of volantile substances or the volatile derivatives of certain non volatile substances.

Stationary phase is an inert solid material impregnated with a non volatile liquid.

* **Adsorption TLC**

Adsorbents such as activated silica gel, alumina, kieselguhur are used.

* **Adsorption column chromatograph**

It is a separation technique in which the stationary bed is within a tube adsorbents are packed into a column in a glass tube. This service as the stationary phase, living an open unrestricted path for the mobile phase in the middle of the tube.

* **Ion exchange chromatograph**

It reffered to as ion chromatograph uses an ion exchange mechanism separate moleculeson the basis of their electrical charges. Ion exchange chromatograph uses a charge stationary phase to separate charged compound including anions, cations, amino acids, peptides and proteins.

* **Gel filtration chromatograph**

It separates molecules according to their size, shape and molecular weight.

It is also reffered to as molecular sieving or molecular exclusion chromatography.

* **Affinity chromatography**

It is based on selective non covalent interaction between an analyte and specific molecules, reffered to as ligands. The immobilelized ligands act as molecular fish hooks and selectively pickup desired proteins while the remaining proteins pass through the column.

* **HPL chromatography**

It is separation technique in which the mobile phase is a liquid. Liquid chromatograph can be created out either in a column or a plane. Present day liquid chromatograph that generally utilize very small packing particles and a relatively high pressure is reffered high performance liquid chromatography.