Separation And Isolation Of Constituents

Chromatography

1. **Paper Chromatography**
2. **Thin layer chromatography TLC**
3. **Ion exchange chromatography**
4. **Gel – Filtration Chromatography**
5. **Column Chromatography**
6. **Gas Chromatography GLC**
7. **High performance liquid Chromatography HPLC**
8. **Electro chromatography**
9. **Affinity Chromatography**

Chromatography

Chromatography is widely used for the separation and identification of components of a mixture. these components can be separated by dissolving them in an appropriate liquid **(mobile phase)** and allowing them to move through an absorbent matrix **(stationary phase)** .

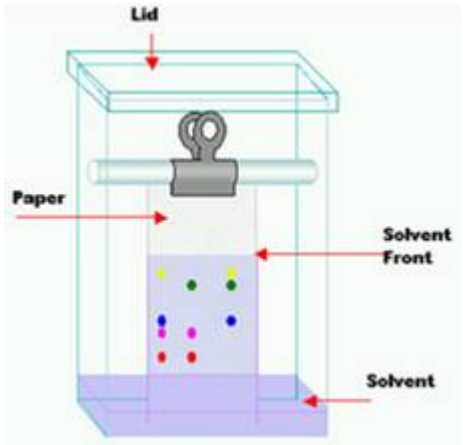
Chromatography is used in many different ways:

- It is used to **determine the unknown substances**.
- The Police, and other detectives use chromatography when trying to **solve a crime.**
- It is also used to determine the presence of **cocaine in urine, alcohol in blood and lead in water.**
- Adsorption chromatography has proved particularly valuable in the **isolation and purification of vitamins, hormones, many alkaloids, cardiac glycosides, anthraquinones , etc.**

Different types of chromatographic techniques

1- Paper Chromatography

This is a useful techniques for the separation and identification of a components of a mixture.

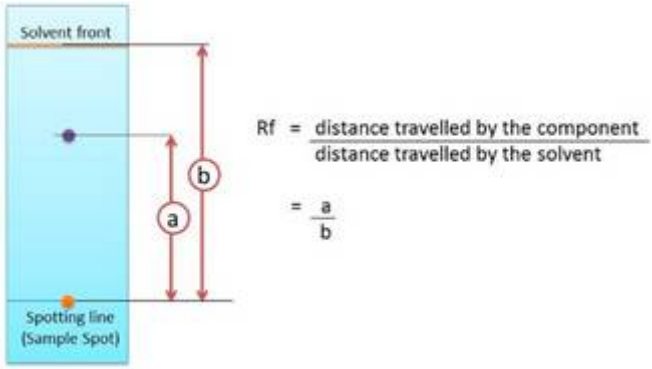


Principle Of Paper Chromatography

The principle involved is partition chromatography wherein the substances are distributed or partitioned between liquid phases. One phase is the water, which is held in the pores of the filter paper used; and other is the mobile phase which moves over the paper. The compounds in the mixture get separated due to differences in their affinity towards water (in stationary phase) and mobile phase solvents during the movement of mobile phase under the capillary action of pores in the paper.

retention factor (R*f*) value

The retention factor (R*f*) may be defined as the ratio of the distance traveled by the solute to the distance traveled by the solvent.



Those chemical components with lower affinity and adsorption toward the stationary phase move or travel faster, while those with higher affinity adsorption towards the stationary phase move or travel slower. Each chemical components has a definite Rf value. Hence it is used as an identification value.

- If R*f* value of a solution is zero, the solute remains in the stationary phase and thus it is immobile.
- If R*f* value = 1 then the solute has no affinity for the stationary phase and travels with the solvent front.

The Stationary Phase

The stationary phase can be a cellulose filter paper on which the separation of compounds occurs.

The Mobile Phase

The mobile phase consist of a volatile organic solvent or a mixture of solvent. Example of solvent used include: water, methanol, propanol, acetone, acetic acid etc.

Procedure Of Paper Chromatography

a) **Selection of suitable filter paper :** Filter paper is selected based on pore size, and the quality of the sample to be separated.
b) **Preparation of sample:** involves dissolution of sample in suitable solvent used in making mobile phase. The solvent used should be inert with the sample under analysis.
c) **Spotting of sample on the paper:** Samples are to be spotted at proper position on the paper preferably using a capillary tube.

d) **Development of chromatogram:** Sample spotted paper is subjected to development by immersing it in the mobile phase. The mobile phase moves over the sample on the paper under the capillary action of paper.

Advantages Of Paper Chromatography

1. Paper Chromatography requires very less quantitative material.

2. Paper Chromatography is Cheaper compared to other chromatography methods.

3. Both unknown inorganic as well as organic compounds can be identified by paper chromatography method.

4. Paper Chromatography do not occupy much space compared to other analytical methods or equipment.

Disadvantages Of Paper Chromatography

1. Large quantity of sample cannot be applied on paper chromatography.

2. In quantitative analysis paper chromatography is not effective.

3. Complex mixture cannot be separated by paper chromatography.

4. Less Accurate compared to HPLC or HPTLC

Uses And Applications Of Paper Chromatography

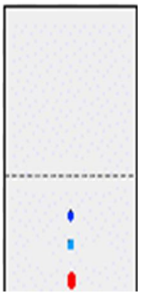
Paper chromatography is specially used for separation of amino acids, for determination of biochemicals in urine.

2- Thin Layer Chromatography

Thin Layer Chromatography is a technique used to isolate mixtures. The experiment is conducted on a sheet of **aluminium foil, plastic, or glass which is coated with a thin layer of adsorbent material**. The material usually used is **aluminium oxide, cellulose, or silica gel**.

Thin Layer Chromatography Principle

Thin layer chromatography (TLC) depends on the principle of separation by adsorption. The separation relies on the relative affinity of compounds towards both the phases while the compounds in the mobile phase move over the surface of the stationary phase. The movement occurs in such a way that the compounds which have a higher affinity to the stationary phase move slowly while the other compounds travel fast. On completion of the separation process, the individual components from the mixture appear as spots at respective levels on the plates.

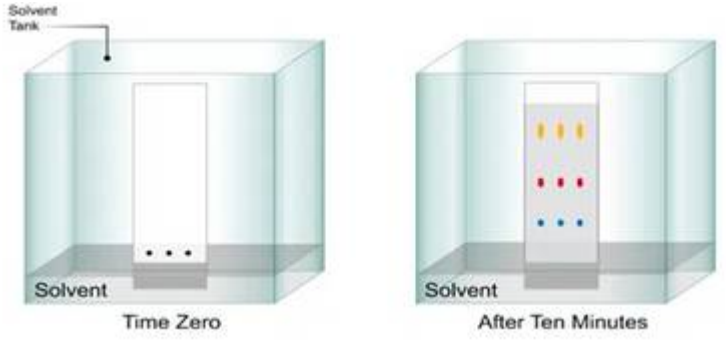


Thin Layer Chromatography requires the followings

1) Thin Layer Chromatography: which are **chemically inert and stable**. The stationary phase is applied on its surface in the form of a thin layer.
2) Thin Layer Chromatography Chamber – Chamber **prevents the solvent evaporation and keeps the entire process dust-free.**
3) Thin Layer Chromatography Mobile phase – **Mobile phase is the one that moves and consists of a solvent.** This phase should be pure. The higher the quality the development of spots is better. The most common solvents are methanol, ethanol and other alcohols, chloroform, ether and ethyl acetate

Thin Layer Chromatography Experiment

- To apply sample spots, thin marks are made at the bottom of the plate with the help of a pencil.
- Apply sample solutions to the marked spots using capillary tubes.
- Pour the mobile phase into the TLC chamber.
- Place the plate in the TLC chamber and close it with a lid.
- Immerse the plate for development. The sample spots should be above the level of the mobile phase, don't be immersed in the solvent.
- Wait till the development of spots. Once the spots are developed, take out the plates and dry them. The sample spots can be observed under a UV light chamber.



Each spot has a retention factor (R_f) expressed as:

R_f = dist. travelled by sample / dist. travelled by solvent

The factors affecting R_f are the solvent system, amount of material spotted, and temperature. TLC is one of the fastest, least expensive, simplest and easiest chromatography technique.

Thin Layer Chromatography applications

- TLC is extremely useful in Biochemical analysis such as separation or isolation of biochemical metabolites from its blood plasma, urine, serum, etc.
- Thin layer chromatography can be used to identify natural products like fixed oil, waxes, glycosides, etc

Disadvantages Of Thin Layer Chromatography:

1. Some factors such as humidity and temperature can affect the final outcome of the chromatogram.
2. It is only a qualitative analysis technique and not quantitative.

3- Ion exchange chromatography

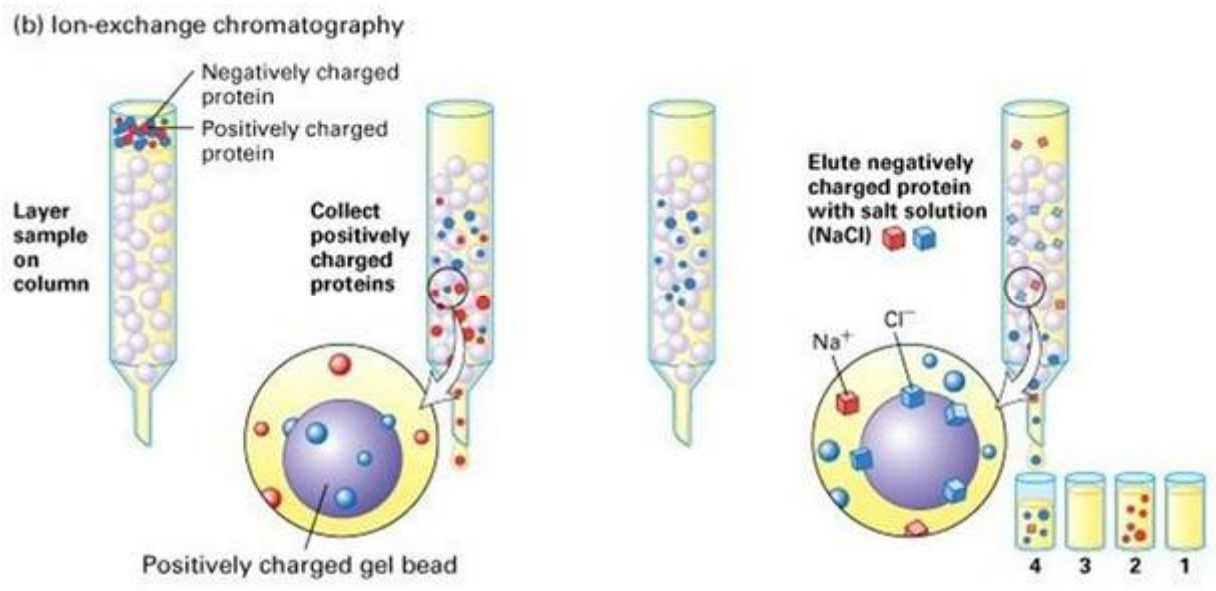
Ion exchange (IEX) chromatography is a technique that is commonly used in biomolecule purification. It involves the separation of molecules on the basis of their charge. This technique exploits the interaction between charged molecules in a sample and oppositely charged moieties in the stationery phase of the chromatography matrix.

There are two types of ion exchange separation : **cation** exchange and **anion** exchange. **In anion exchange the stationary phase is positively charged while in cation exchange it is negatively charged**.

Principle of Ion Exchange Chromatography

IEX chromatography is used in the separation of charged biomolecules. The crude sample containing charged molecules is used as the liquid phase. When it passes through the chromatographic column, molecules bind to oppositely charged sites in the stationary phase.

The molecules separated on the basis of their charge are eluted using a solution of varying ionic strength.



The Applications of Ion Exchange Chromatography

Ion exchange is the most widely used chromatographic method for the separation and purification of charged biomolecules such as polypeptides, proteins, polynucleotides, and nucleic acids, and for Separation and Purification of blood components such as albumin,]recombinant growth factors and enzymes.

Its widespread applicability, **high capacity and simplicity, and its high resolution are the key reasons for its success as a separation method.**