



## **Department of Medical Instrumentation Laboratory**

### **Stage\ First**

#### **Separation equipment\ Chromatography apparatus and Types**

#### **Chromatography apparatus**

**Chromatography is an important biophysical technique that enables the separation, identification, and purification of the components of a mixture for qualitative and quantitative analysis.**

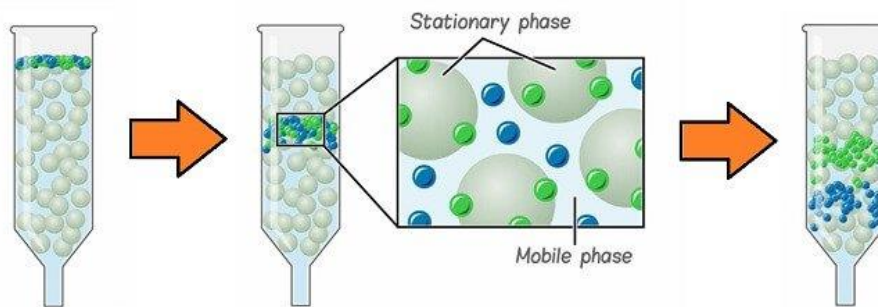
- A wide range of chromatographic procedures makes use of differences in size, binding affinities, charge, and other properties to separate materials.
- It is a powerful separation tool that is used in all branches of science and is often the only means of separating components from complex mixtures.
- Chromatography is a very useful technique as it allows the separation of components of a mixture on the basis of their nature, structure, size, and other properties.
- Chromatography, in general, is based on the principle that components of a mixture are separated when the mixture added to a mobile phase is moved through a stationary phase (which mostly is a solid surface), resulting in some components of the mixture being attached to the stationary phase. At the same time, the rest is passed along with the mobile phase.
- Thus, there are two essential components of all chromatography techniques.

**Two components thus form the basis of the chromatography technique.**

**1-Stationary phase:** in chromatography is the phase that is either a solid or liquid particle attached to a glass or a metal surface on which the components of the mixture to be separated is absorbed selectively.

- The term stationary refers to the fact that this phase remains stationary while the other phase moves.
- Most substances used as stationary phases are porous, thus allowing the attachment of components during chromatography.
- The stationary phase to be selected for a chromatographic process depends on the nature of the components to be separated and the type of chromatography.

- Depending on the type of chromatography gel beads, thin uniform paper, silica, glass, some gases, or even liquid components are used as a stationary phase.



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

- The mobile phase is the solvent that carries the mixture as it moves down the stationary phase.
- The term mobile indicates that the phase is moving down the chromatographic system, whereas the other phase remains stationary.
- Substances used as mobile phases are selected for a chromatographic process depending on the nature of the components to be separated and the type of chromatography.
- Alcohol, water, acetic acid, acetone, or some gases are the commonly used mobile phase in different chromatographic techniques.

## Types of Chromatography

Commonly employed chromatography techniques include:

1. Column chromatography
2. Paper chromatography
3. Thin-layer chromatography(TLC)

### 1- Column chromatography

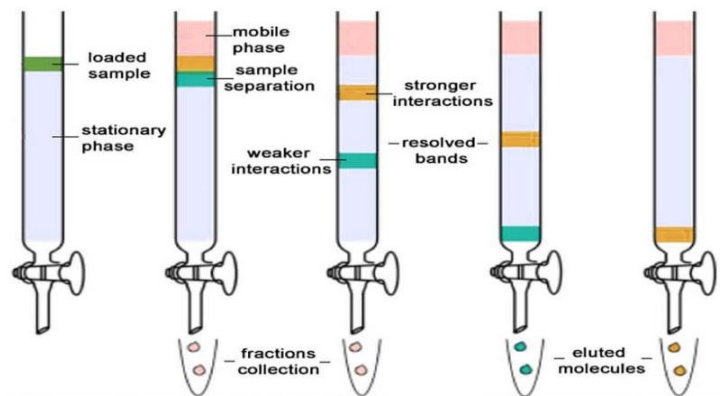
Column chromatography is the separation technique where the components in a mixture are separated on the basis of their differential adsorption with the stationary phase, resulting in them moving at different speeds when passed through a column.

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

### Principle of Column chromatography

- This technique is based on the principle of differential adsorption where different molecules in a mixture have different affinities with the adsorbent present on the stationary phase.

- The molecules having higher affinity remain adsorbed for a longer time decreasing their speed of movement through the column.
- However, the molecules with lower affinity move with a faster movement, thus allowing the molecules to be separated in different fractions.
- Here, the stationary phase in the column chromatography also termed the adsorbent, is a solid (mostly silica) and the mobile phase is a liquid that allows the molecules to move through the column smoothly.



## Uses of Column chromatography

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

## Paper Chromatography

Chromatography technique that uses paper sheets or strips as the adsorbent being the stationary phase through which a solution is made to pass is called paper chromatography.

### Principle of Paper chromatography

- Paper chromatography is of two types based on two different principles.
- The first is the paper adsorption chromatography that is based on the varying degree of interaction between the molecules and the stationary phase.
- The molecules having higher affinity remain adsorbed for a longer time decreasing their speed of movement through the column.
- However, the molecules with lower affinity move with a faster movement, thus allowing the molecules to be separated in different fractions.
- The second type of paper chromatography is the paper partition chromatography. It is based on the principle that the moisture on the cellulose paper acts as a stationary phase for the molecules moving with the mobile phase.

- The separation of the molecules is thus based on how strongly they adsorb onto the stationary phase.
- An additional concept of 'retention factor' is applied during the separation of molecules in the paper chromatography.
- The retention value for a molecule is determined as a ratio of distance traveled by the molecule to the distance traveled by the mobile phase.
- The retention value of different molecules can be used to differentiate those molecules.

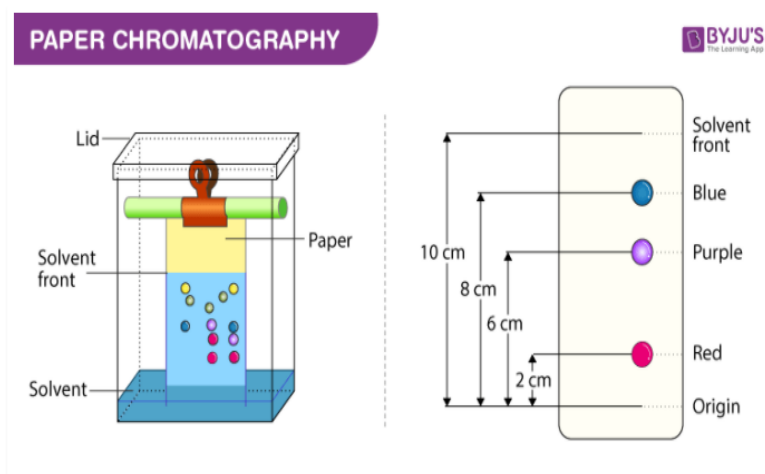
## Uses of Paper chromatography

1-Paper chromatography is performed to detect the purity of various pharmaceutical products.

2-It can also be employed to detect contamination in various samples, like food and beverages.

3-This method can also be used for the separation of impurities from various industrial products.

4-The analysis of the reaction mixtures in chemical labs is also conducted via paper chromatography.



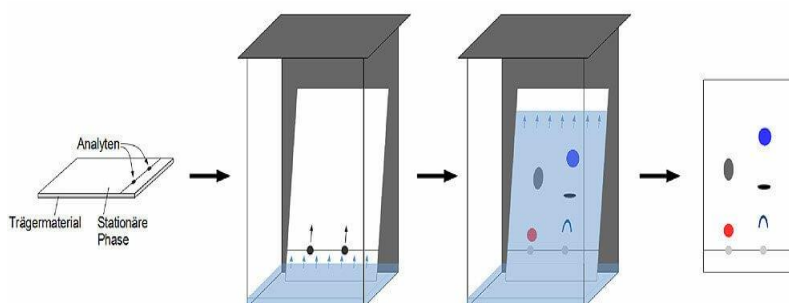
## Thin-Layer Chromatography(TLC)

**Definition:** Thin-Layer chromatography (TLC) is an easy-to-use, fast and highly versatile separation technique for qualitative and quantitative analysis. Thin-Layer Chromatography is performed on a glass, plastic, or aluminum plate, which is coated with a thin layer of adsorbent material.

Thin Layer Chromatography can be defined as a method of separation or identification of a mixture of components into individual components by using finely divided adsorbent solid / (liquid) spread over a glass plate and liquid as a mobile phase.

### Principle of Thin-layer chromatography (TLC)

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



### Uses of Thin-layer chromatography (TLC)

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