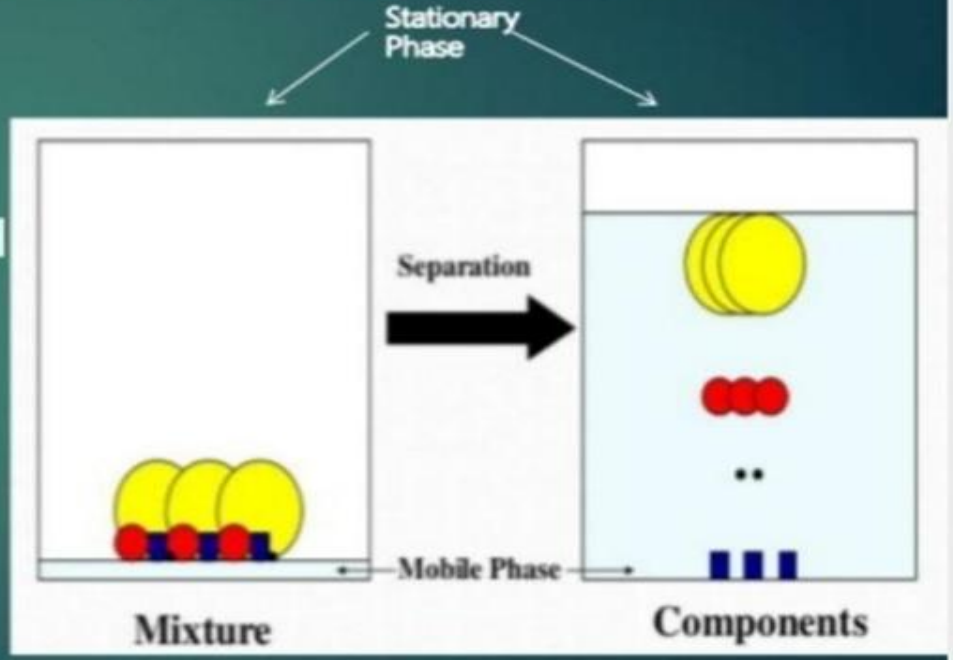
Principle and Application of Chromatography

INTRODUCTION

- Chromatography is a physical process where the components (solutes) of a sample mixture are separated as a result of their differential distribution between stationary and mobile phases.



- Greek *chroma* meaning

HISTORY

- **Tswet**, Russian botanist (referred to *as Father of chromatography*) is credited for the development of chromatography.

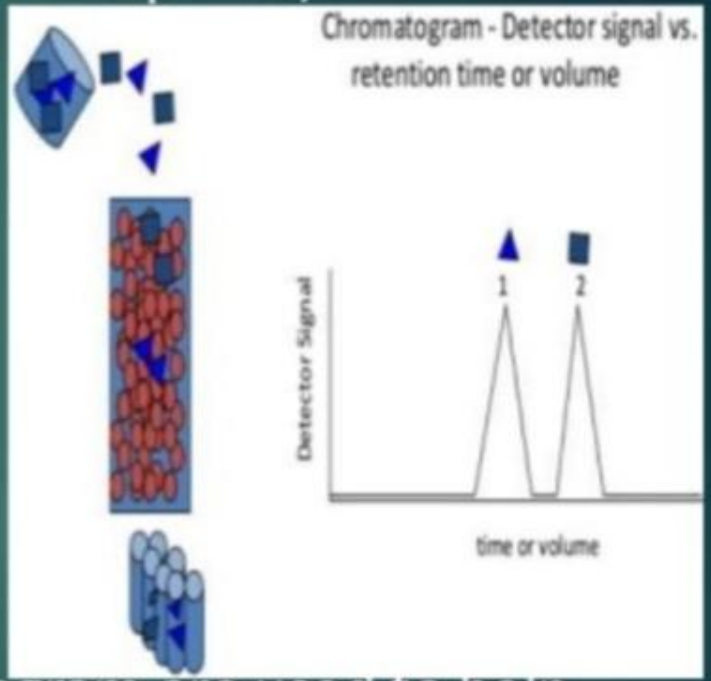
Principle -

- Chromatography is usually based on principle of partition of solute between two phases. It usually consists of a Mobile Phase and a Stationary Phase.

- **The Mobile Phase** usually refers to the mixture of the 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.

CHROMATOGRAM

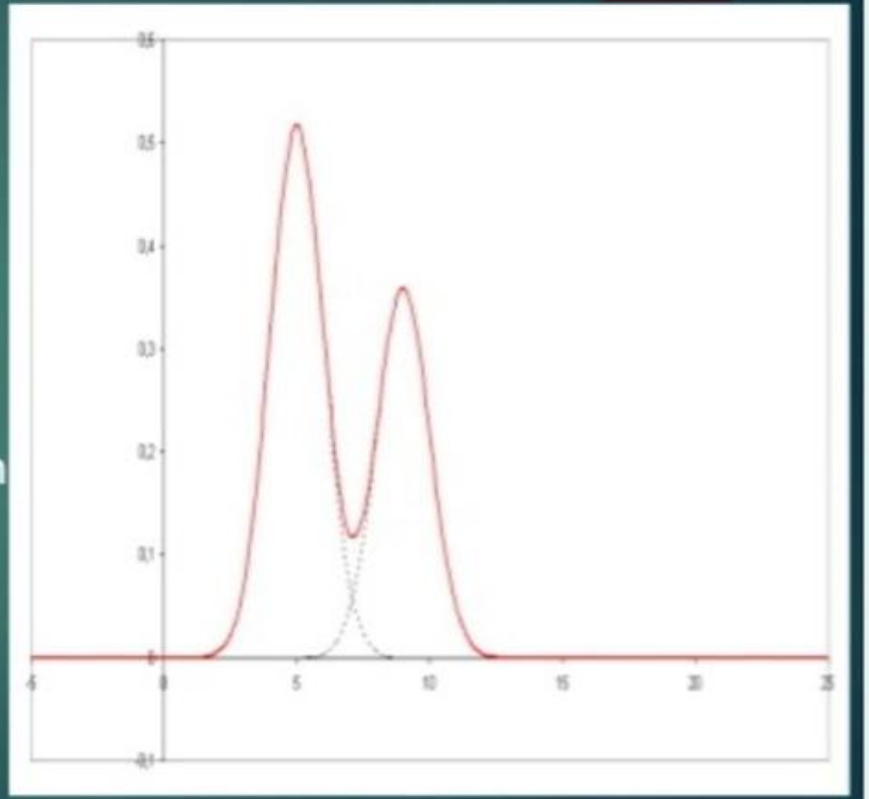
- A graphical presentation of detector response, concentration of analyte in the effluent, or other quantity used as a measure of effluent concentration.



- The retention time or volume is when a solute exits the injector and passes through the column and the detector .

- Data represented by the chromatogram are used to help identify and quantify the solute(s).

- Because eluting solutes are displayed graphically as a series of peaks, they are frequently referred to as chromatographic peaks.
- These Peaks are described in terms of peak (1) width, (2)height, (3)area



CLASSIFICATION

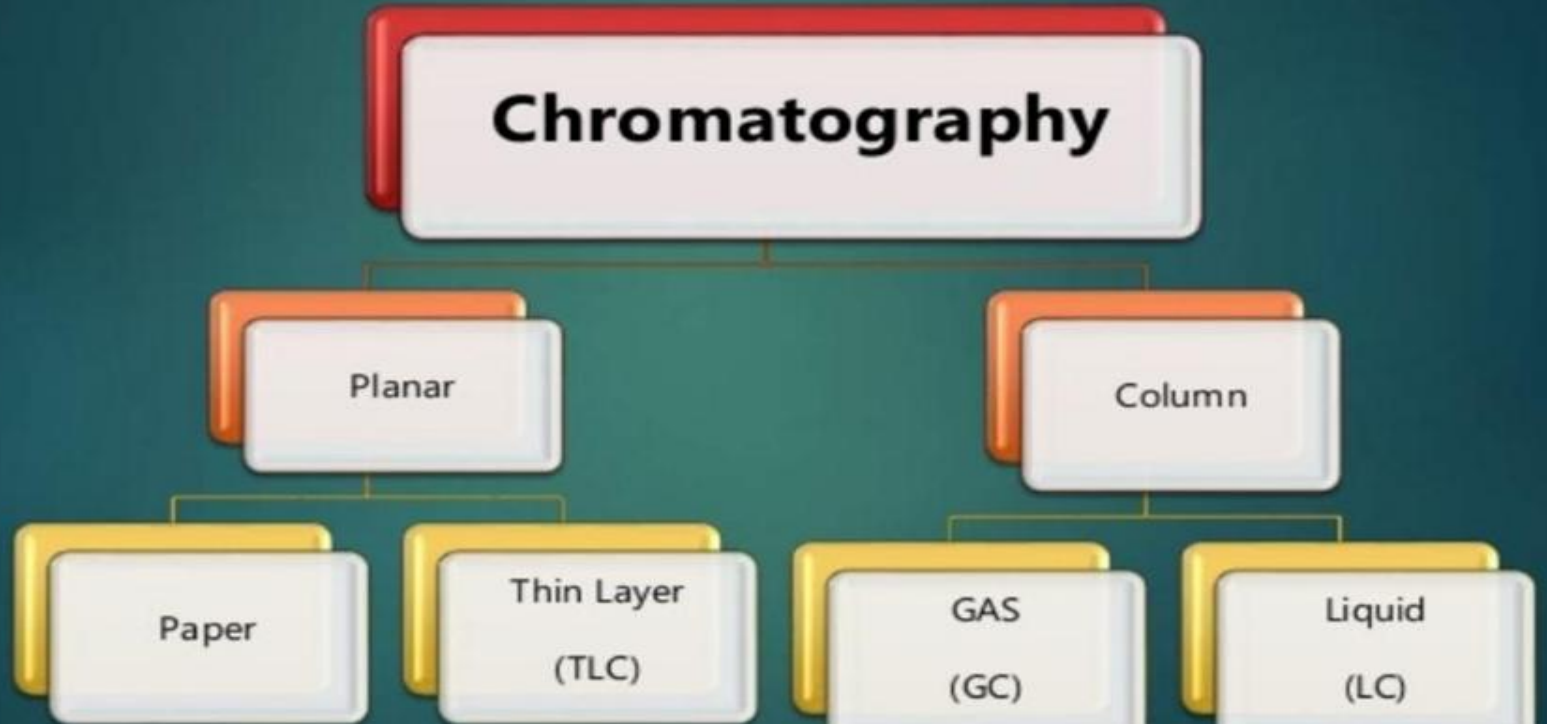
Chromatographic methods can be classified in three different ways :-

a) Based on shape of chromatographic beds .e.g.- Planar and column Chromatography

b) Based on the physical state of mobile and stationary phase. e.g- Gas and liquid chromatography

c) Based on mechanism of separation. e.g.-Ion-exchange chromatography, partition , affinity and adsorption chromatography

Based on shape of chromatographic beds

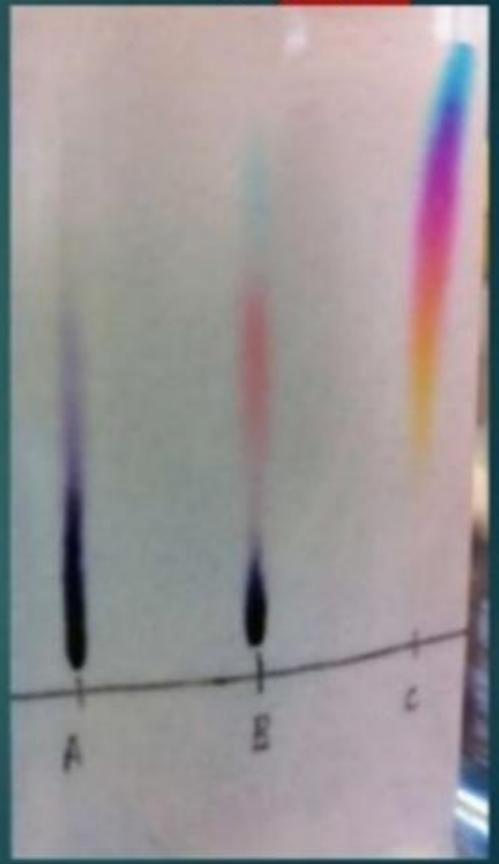


Planar Chromatography

- In Planar Chromatography stationary phase is present on a plane.
- The Plane can be a paper impregnated by a substance acting as a stationary phase- **Paper Chromatography** OR a Thin layer of a substance acting as a stationary phase spread on a glass, metal or plastic plate- **Thin Layer Chromatography.**
- Planar chromatography is also termed as **Open Bed Chromatography.**

Paper Chromatography

- Paper chromatography is a liquid partition chromatography.
- In paper chromatography, the end of the paper is dipped in solvent mixture consisting of aqueous and organic components.
- The solvent soaks in paper by capillary action because of fibrous nature of paper.
- The aqueous component of the solvent binds to the cellulose paper and thereby forms stationary phase with it .
- The organic component of the solvent binds continues migrating, thus forming the mobile phase.

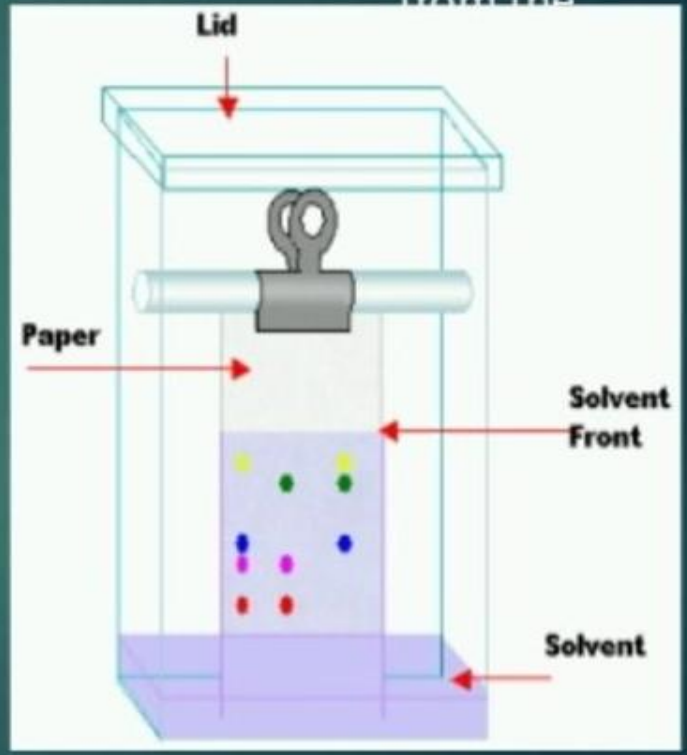


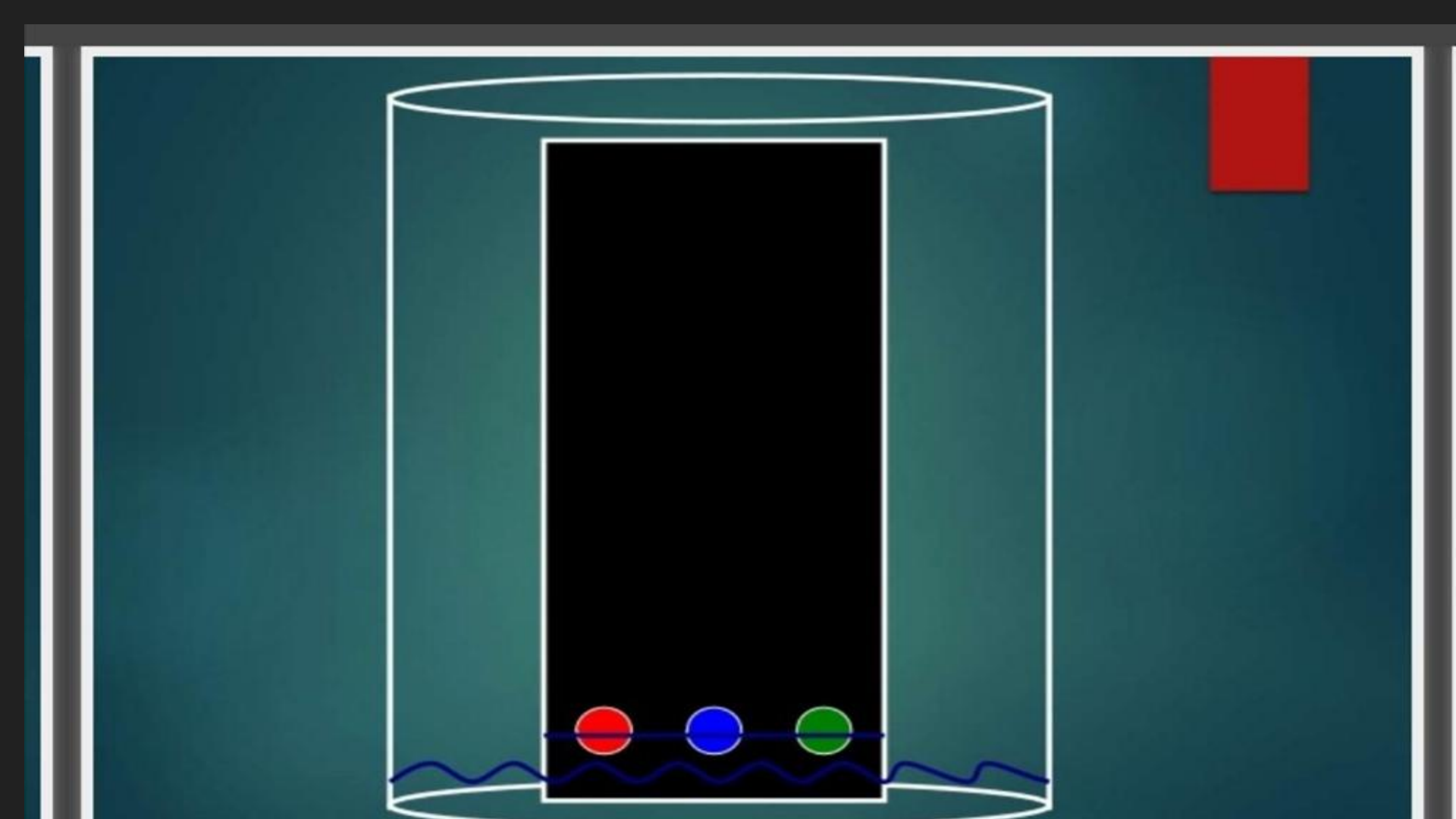
Mechanism of Separation

- Mobile Phase rises up by capillary action.
- Testing sample is concentrated as a minute spot at the bottom of the filter paper.
- Sample mixture gradually rises up with the mobile phase which is liquid.
- Compounds in the mixture will be separated according to their ability of solubility.
- More Polar substances will move slower and less polar substances will travel faster.

Procedure

- A small spot of sample is applied to a strip of chromatography paper about two centimeters away from the base of the plate.
- This sample is absorbed onto the paper and may form interactions with it.
- The paper is then dipped into a solvent, such as ethanol or water, taking care that the spot is above the surface of solvent , and placed in a sealed





- The solvent moves up the paper by capillary action and dissolves the sample mixture, which will then travel up the paper with the solvent solute sample.
- Different compounds in the sample mixture travel at different rates .
- It takes several minutes to several hours.
- **Analysis-** Spots corresponding to different compounds may be located by their color, UV light, Ninhydrin or by treatment with iodine vapors.

Ascending and Descending Paper chromatography

Ascending Chromatography- In this method, the solvent is in pool at the bottom of the vessel in which the paper is supported.

It rises up the paper by capillary action against the force of gravity.

Descending Chromatography- In this method , the solvent is kept in a trough at the top of the chamber and is allowed to flow down the paper .

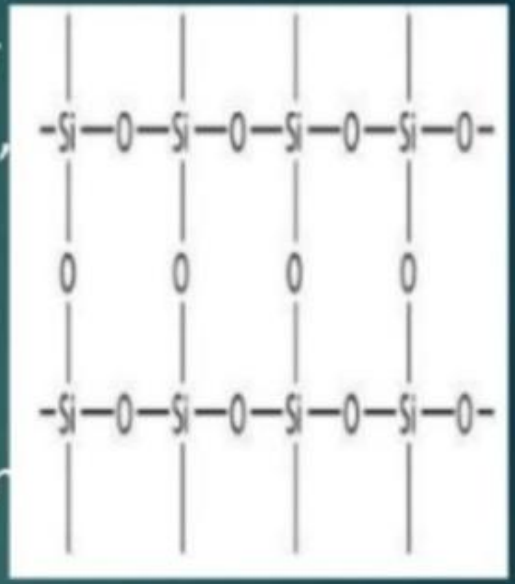
The liquid moves down by capillary action as well as by the gravitational force.

Significance of Paper Chromatography

- It is very easy, simple , rapid and highly efficient method of separation.
- Can be applied in even in micrograms quantities of the sample.
- Can also be used for the separation of a wide variety of material like amino acids , oligosaccharides, glycosides, purines and pyrimidines, steroids, vitamins and alkaloids like penicillin , tetracyclin and streptomycin.

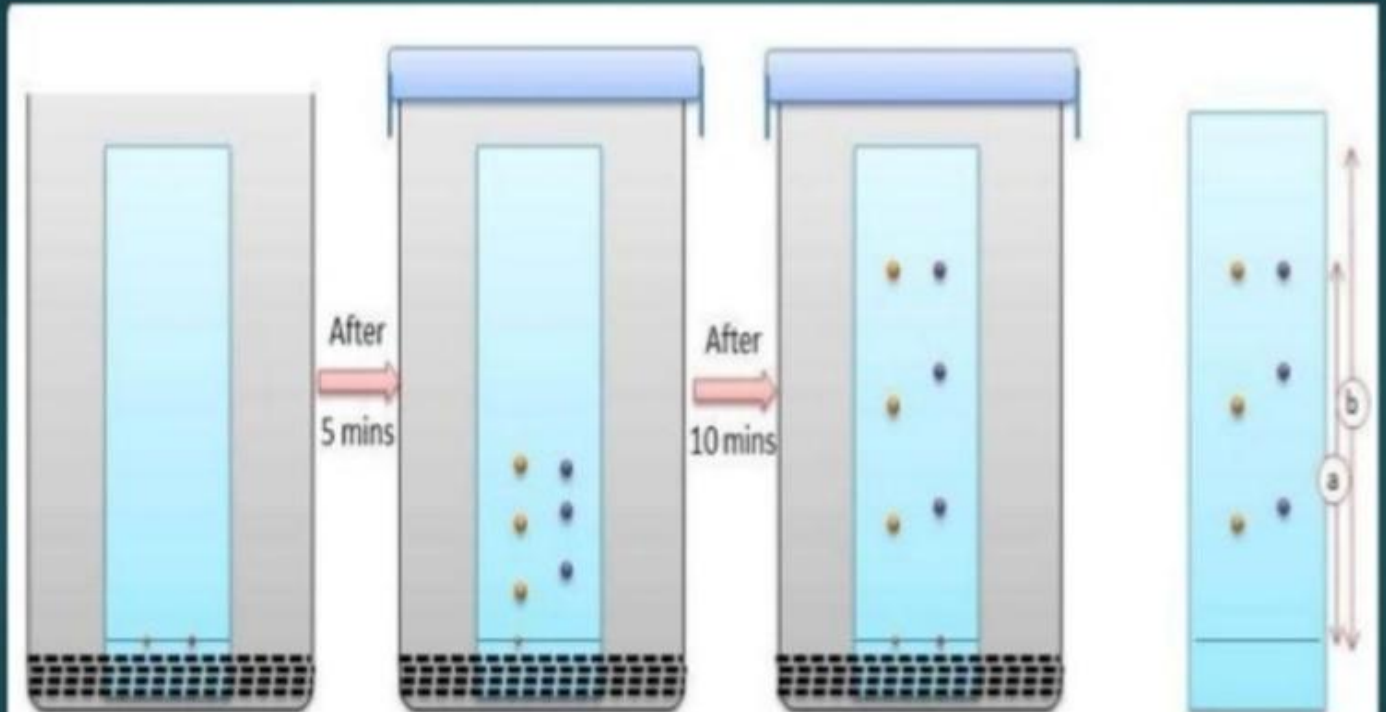
Thin Layer Chromatography (TLC)

- Stationary Phase consists of a thin layer of adsorbent material, usually silica gel , aluminium oxide, or cellulose immobilized onto a flat carrier sheet.



- A Liqiud Phase consisting of the solution to be separated which is dissolved in an appropriate solvent and is drawn up the plate via capillary action , separating the solution based

Steps of TLC



Significance

Its wide range uses include -

- Determination of the pigments a plant contains.
- Detection of pesticides or insecticides in food .
- Identifying compounds present in a given substance.
- Monitoring organic reaction.

Advantages Of TLC over Paper Chromatography

- In case of Paper Chromatography, it takes 14- 16 hrs for the separation of the components, but in TLC , it takes only 3-4 hrs.
- TLC has the advantage that the corrosive reagents like sulphuric acid can also be used which pose a limitation for the paper chromatography.
- It is easier to separate and visualise the components by this method.
- It has capacity to analyse multiple samples in a single run.
- It is relatively a low cost.

R_F value -

- The rate of migration of the various substances being separated are governed by their relative solubilities in the polar stationary phase and non polar mobile phase.
- The migration rate of a substances usually expressed as R_f (relative front).
- R_f = Distance travelled by the substance