History:

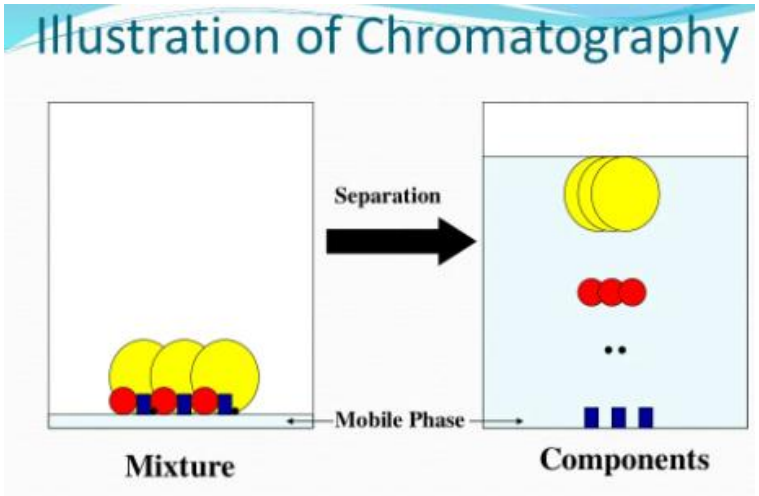
 The subject of Chromatography was introduced into scientific world in a very modest way by M. Tswett in 1906. He employed a technique to separate various pigments such as chlorophylls and xanthophylls by passing the solution of these compounds into the glass column which was packed with finely divided calcium carbonate. After the later, Thompson and Way had realized the Ion Exchange properties of soils. Almost after three decades, in 1935 Adams and Holmes observed the Ion Exchange characteristics in crushed phonograph. This observation opened the field for preparation of Ion Exchanged resins. The concept of Gas-Liquid Chromatography was first introduced by Martin and Synge in 1941.

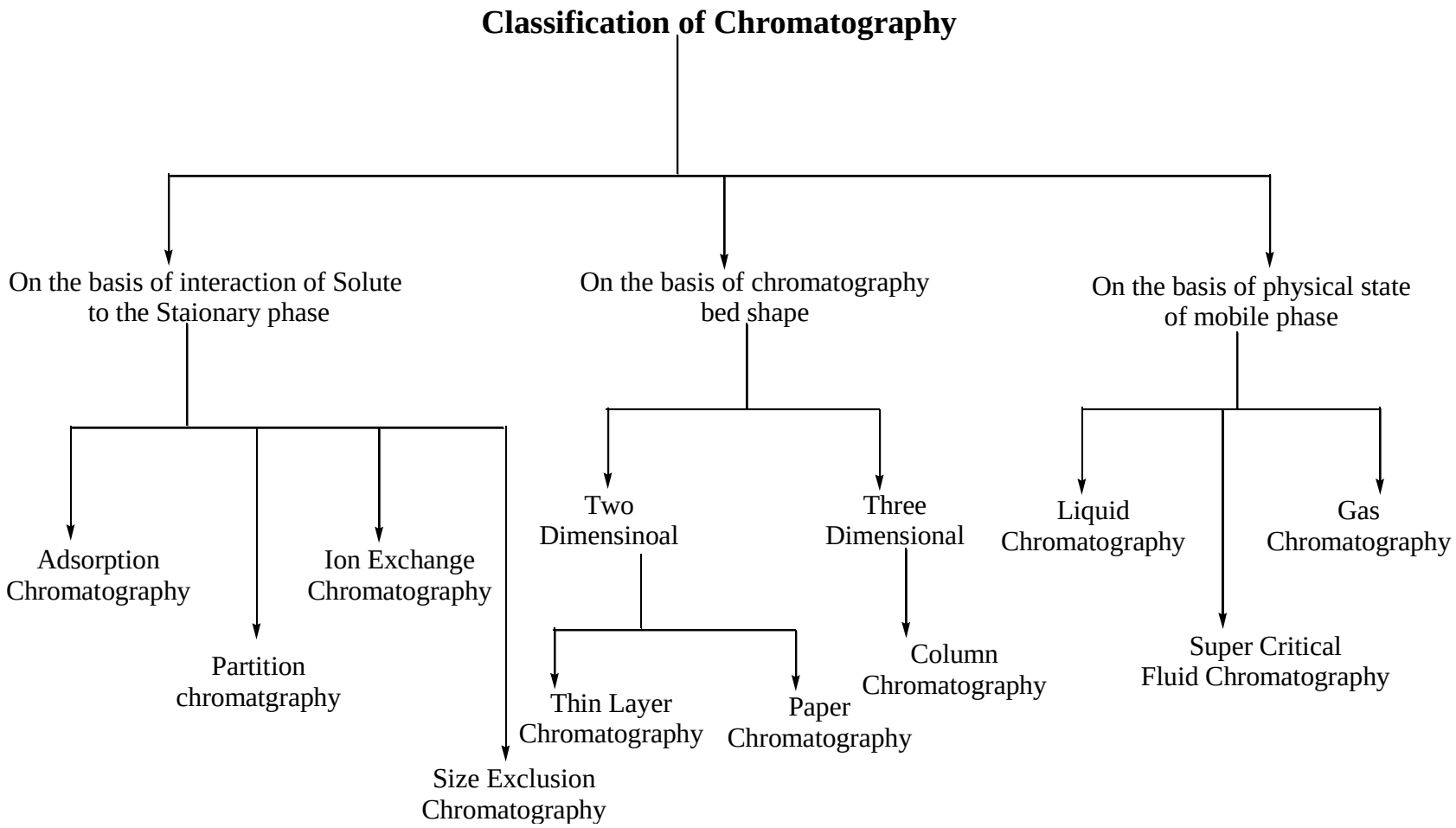
Chromatography:

 Chromatography is a method of separation in which the components to be separated are distributed between two phases, one of these is called a **stationary phase** and the other is a **mobile phase** which moves on stationary phase in a definite direction. The component of the mixture redistribute themselves between two phases by a process which may be adsorption, partition, ion exchange or size exclusion. The stationary phase can be **solid** or a **liquid** and the mobile phase can be **liquid**, **gas** or a **supercritical fluid.**

Introduction: The Term Chromatography (chroma = a colour; graphein = to write) is the collective term for a set of laboratory techniques for the separation of mixtures. Chromatography involves a sample (or sample extract) being dissolved in a mobile phase (which may be a gas, a liquid or a supercritical fluid). The mobile phase is then forced through an immobile, immiscible stationary phase. The phases are chosen such that components of the sample have differing solubilities in each phase. A component which is quite soluble in the stationary phase will take longer to travel through it than a component which is not very soluble in the stationary phase but very soluble in the mobile phase.

 As a result of these differences in mobilities, sample components will become separated from each other as they travel through the stationary phase. Techniques such as H.P.L.C. (High Performance Liquid Chromatography) and G.C. (Gas Chromatography) use columns - narrow tubes packed with stationary phase, through which the mobile phase is forced. The sample is transported through the column by continuous addition of mobile phase. This process is called elution.

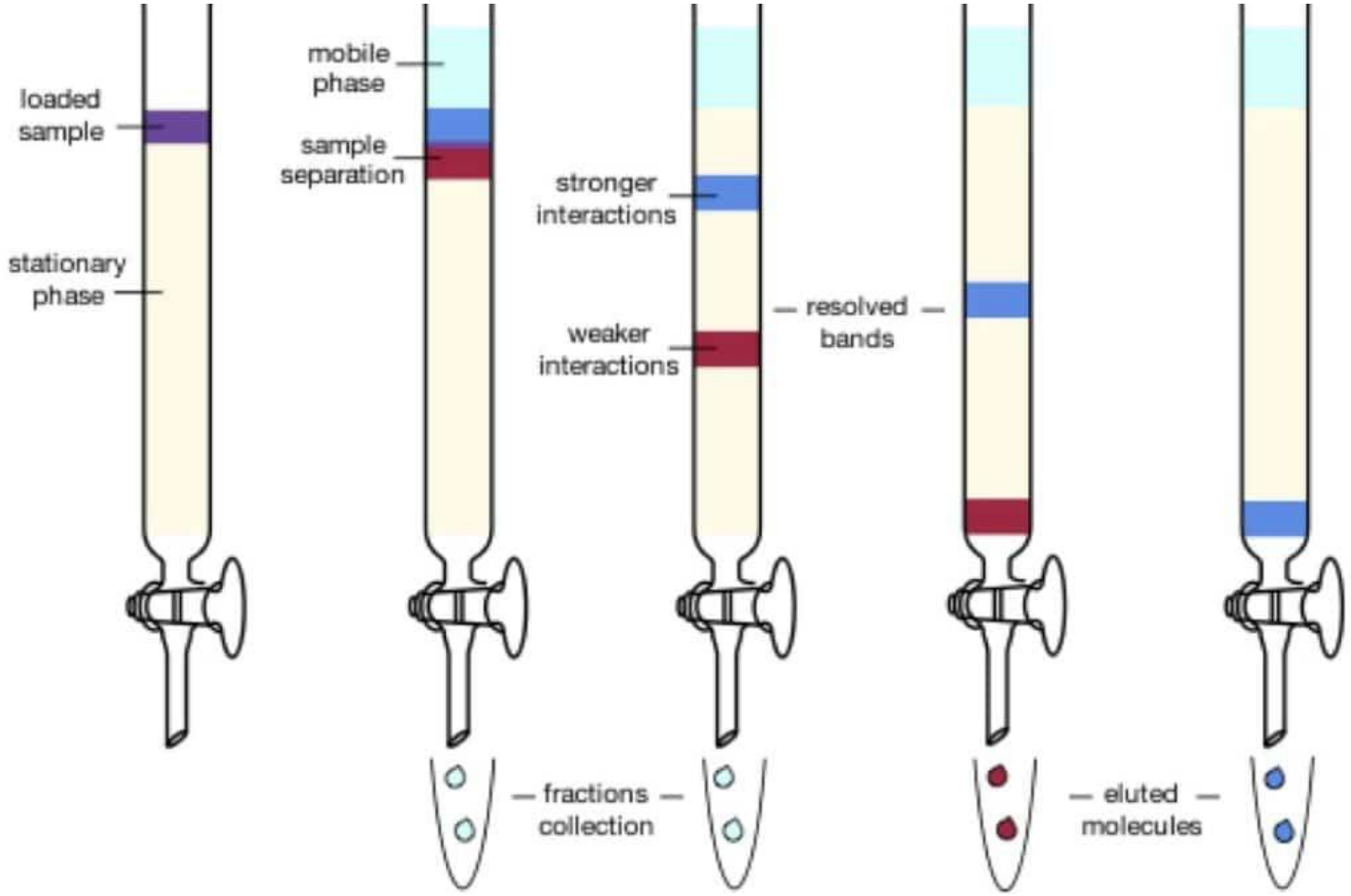



Classification of Chromatography
On the basis of interaction of Solute to the Staionary phase
On the basis of chromatography bed shape
On the basis of physical state of mobile phase
Adsorption Chromatography
Partition chromatgraphy
Ion Exchange Chromatography
Size Exclusion Chromatography
Two Dimensinoal
Three Dimensional
Thin Layer Chromatography
Paper Chromatography
Column Chromatography
Liquid Chromatography
Super Critical Fluid Chromatography
Gas Chromatography

Important terminology in chromatography

Term	Definition
Mobile phase or carrier	solvent moving through the column
Stationary phase or adsorbent	substance that stays fixed inside the column
Eluent	fluid entering the column
Eluate	fluid exiting the column (that is collected in flasks)
Elution	the process of washing out a compound through a column using a suitable solvent
Analyte	mixture whose individual components have to be separated and analyzed

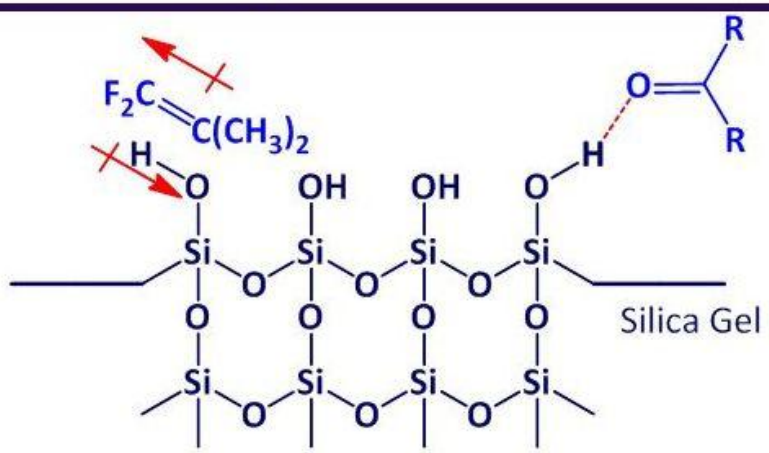
Basic ideas about *stationary phase, mobile phase, eluent, Etc...*

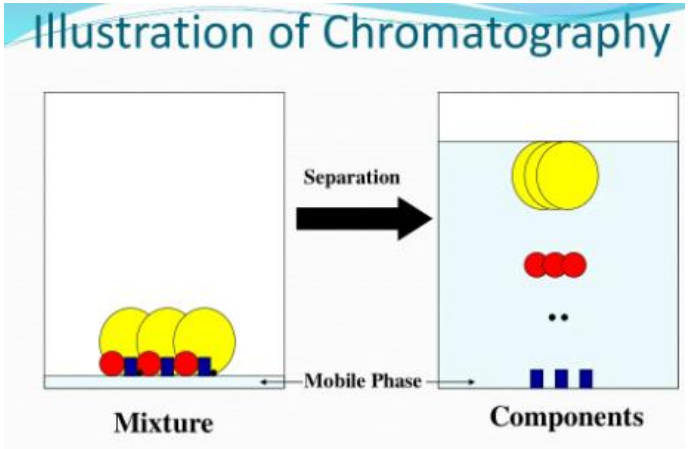


Type of interactions between organic molecule and stationary phase

Stationary Phase

- silica gel (SiO_2) has hydroxyl groups at the surface of the particles
- the surface of silica gel is highly polar
- polar functionality can bind in two ways:
  - through hydrogen bonds
  - through dipole-dipole interactions
- more polar compounds will have greater interactions with the stationary phase, and so will move slower along it





Thin layers chromatography (TLC)

Introduction

TLC is one of the simplest, fastest, easiest and least expensive of several chromatographic techniques used in qualitative and quantitative analysis to separate organic compounds and to test the purity of compounds.

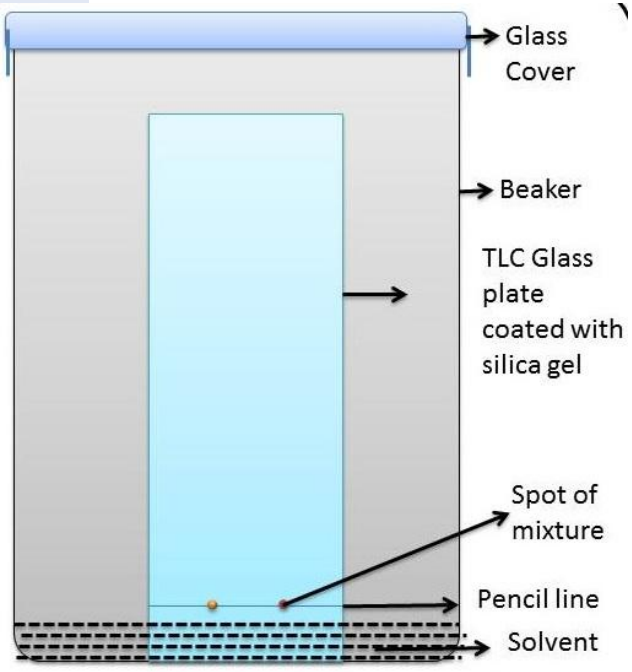
TLC is a form of liquid chromatography consisting of:

- A mobile phase (developing solvent) and
- A stationary phase (a plate or strip coated with a form of silica gel)
- Analysis is performed on a flat surface under atmospheric pressure and room temperature

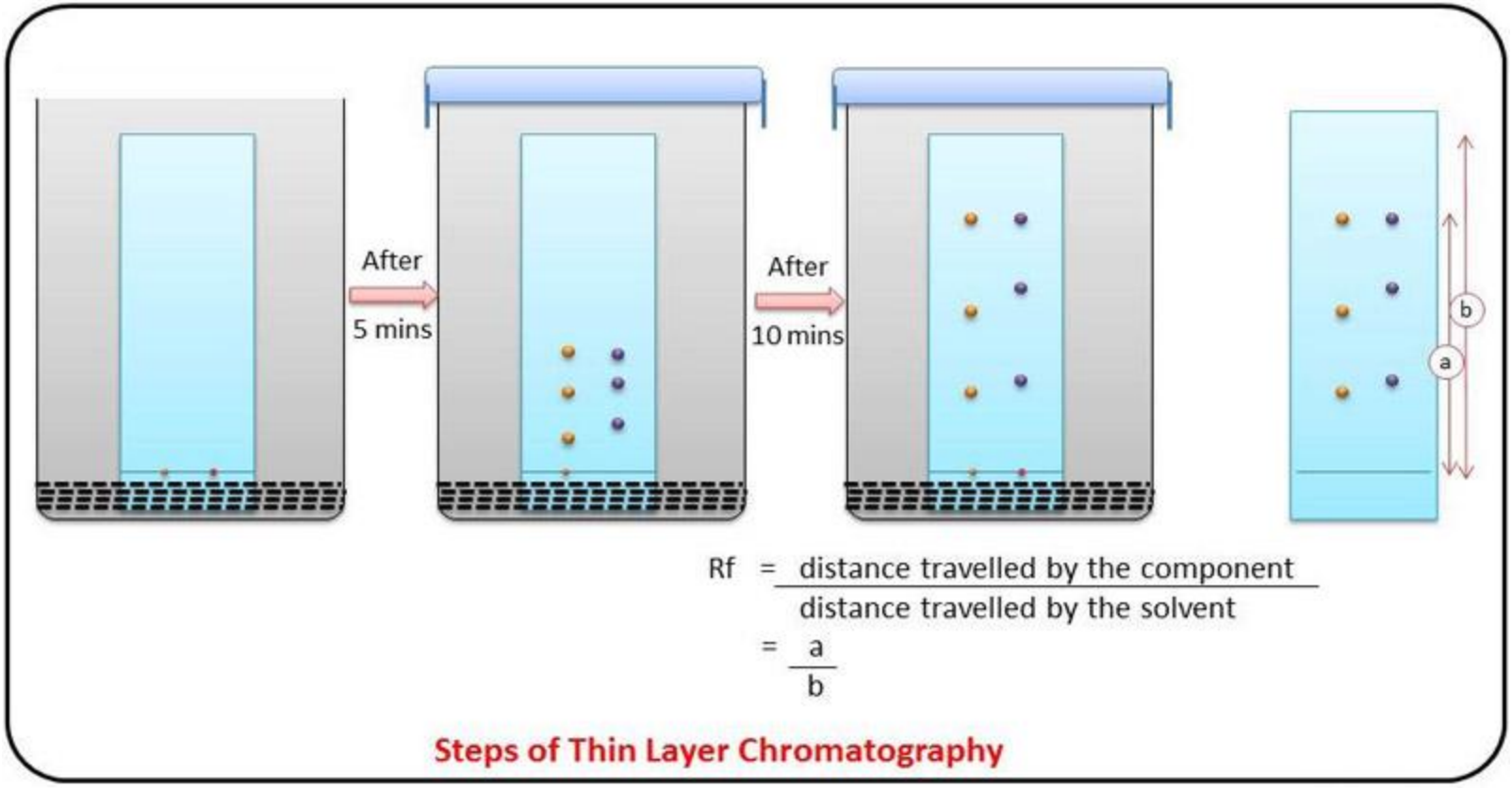
Principle of Thin layer Chromatography

Similar to other chromatographic methods, thin layer chromatography is also based on the principle of separation.

- The separation depends on the relative affinity of compounds towards stationary and the mobile phase.
- The compounds under the influence of the mobile phase (driven by capillary action) travel over the surface of the stationary phase. During this movement, the compounds with higher affinity to stationary phase travel slowly while the others travel faster. Thus, separation of components in the mixture is achieved.
- Once separation occurs, the individual components are visualized as spots at a respective level of travel on the plate. Their nature or character are identified by means of suitable detection techniques.



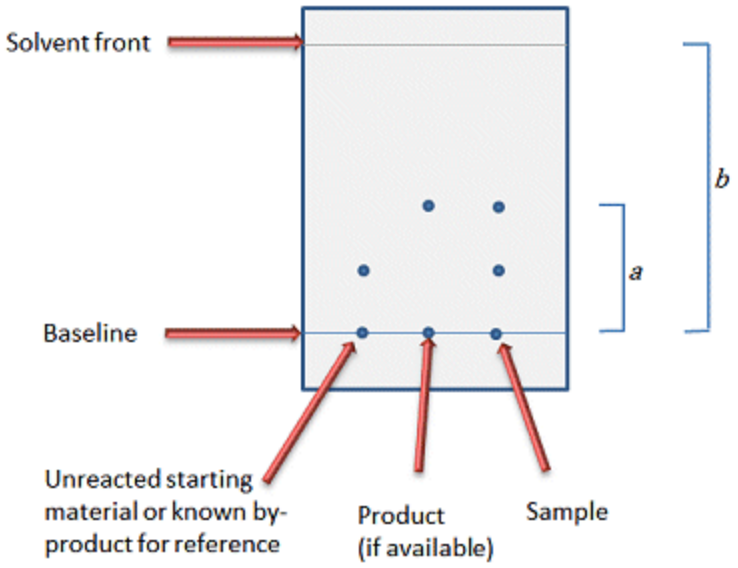
Steps of Thin Layer Chromatography



$$Rf = \frac{\text{distance travelled by the component}}{\text{distance travelled by the solvent}} = \frac{a}{b}$$

Steps of Thin Layer Chromatography

Retention factor

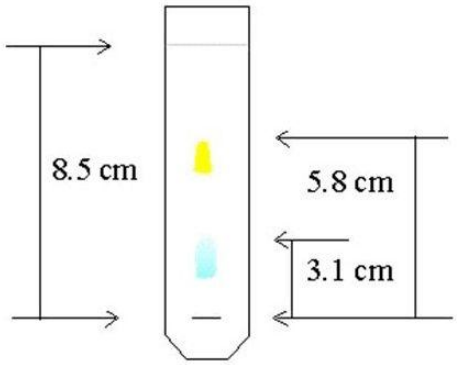


$$R_f = \frac{\textit{Distance from baseline traveled by solute}}{\textit{Distance from baseline traveled by solvent (solvent front)}}$$

$$R_f = \frac{a}{b}$$

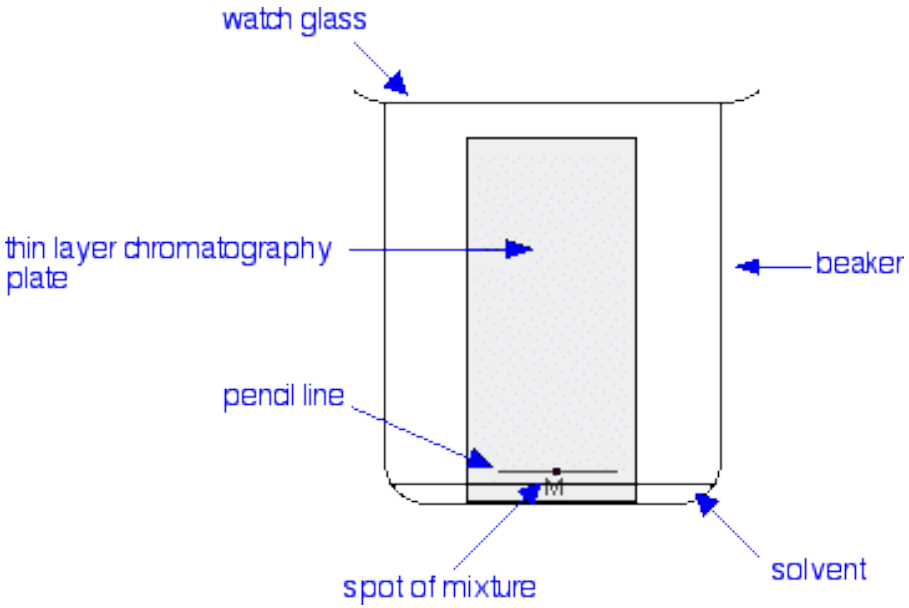
Retention Factor (R_f)

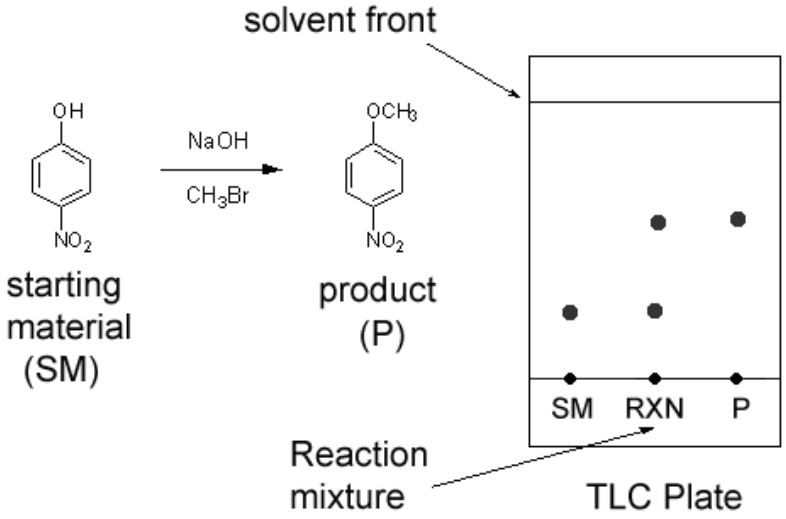
$$R_f = \frac{\text{distance spot moves}}{\text{distance solvent travels}}$$



$$R_f \text{ (yellow)} = \frac{5.8 \text{ cm}}{8.5 \text{ cm}} = 0.72$$

$$R_f \text{ (cyan)} = \frac{3.1 \text{ cm}}{8.5 \text{ cm}} = 0.36$$


watch glass
thin layer chromatography plate
beaker
pencil line
M
spot of mixture
solvent


solvent front
OH
NO2
NaOH
CH3Br
OCH3
NO2
starting material (SM)
product (P)
SM
RXN
P
Reaction mixture
TLC Plate

Paper Chromatography

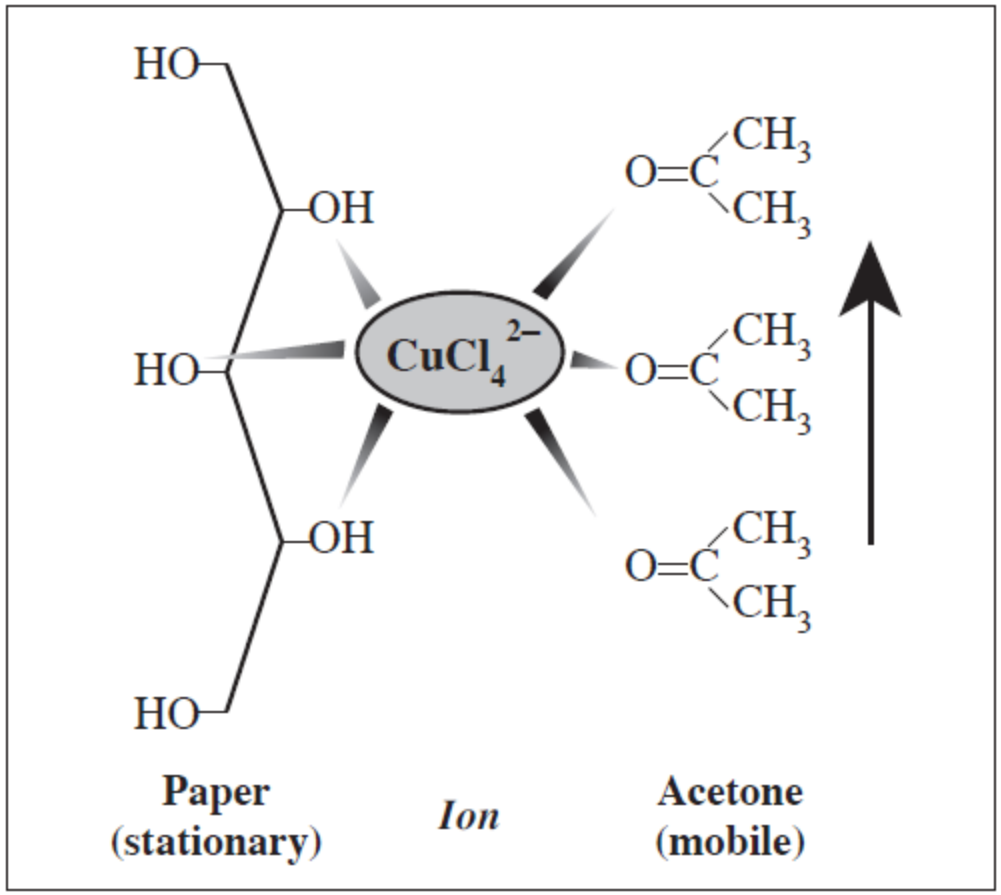
What Is Paper Chromatography?

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. It is an inexpensive method of separating dissolved chemical substances by their different migration rates across the sheets of paper. It is a powerful analytical tool that uses very small quantities of material. Paper chromatography was discovered by Synge and Martin in the year 1943.

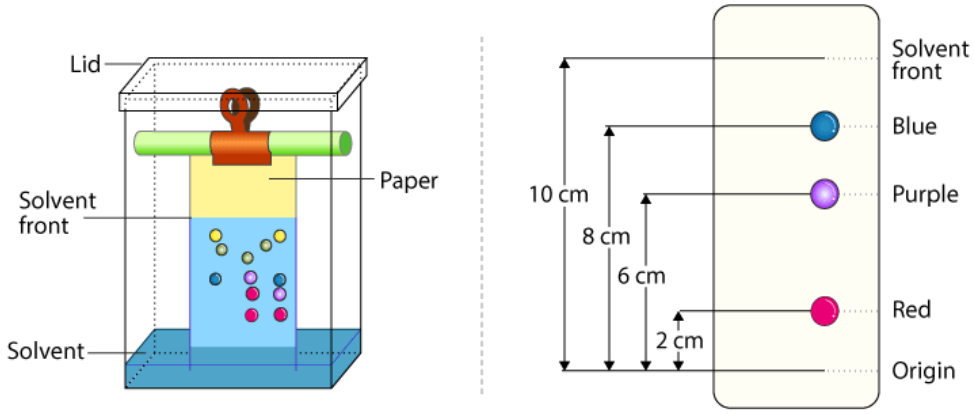
Paper Chromatography Principle

The principle involved can be partition chromatography or adsorption chromatography. Partition chromatography because the substances are partitioned or distributed between liquid phases. The two phases are water held in pores of the filter paper and the other phase is a mobile phase which passes through the paper. When the mobile phase moves, the separation of the mixture takes place. The compounds in the mixture separate themselves based on the differences in their affinity towards stationary and mobile phase solvents under the capillary action of pores in the paper. Adsorption chromatography between solid and liquid phases, wherein the solid surface of the paper is the stationary phase and the liquid phase is the mobile phase.

Type of interactions in paper chromatography



Paper Chromatography



Paper Chromatography Procedure

Below we have explained the procedure to conduct Paper Chromatography Experiment for easy understanding of students.

Selecting a suitable type of development: It is decided based on the complexity of the solvent, paper, mixture, etc. Usually ascending type or radial paper chromatography is used as they are easy to perform. Also, it is easy to handle, the chromatogram obtained is faster and the process is less time-consuming.

Selecting a suitable filter paper: Selection of filter paper is done based on the size of the pores and the sample quality.

Prepare the sample: Sample preparation includes the dissolution of the sample in a suitable solvent (inert with the sample under analysis) used in making the mobile phase.

Spot the sample on the paper: Samples should be spotted at a proper position on the paper by using a capillary tube.

Chromatogram development: Chromatogram development is spotted by immersing the paper in the mobile phase. Due to the capillary action of paper, the mobile phase moves over the sample on the paper.

Paper drying and compound detection: Once the chromatogram is developed, the paper is dried using an air drier. Also, detecting solution can be sprayed on the chromatogram developed paper and dried to identify the sample chromatogram spots.

Applications of Paper Chromatography

There are various applications of paper chromatography. Some of the uses of Paper Chromatography in different fields are discussed below:

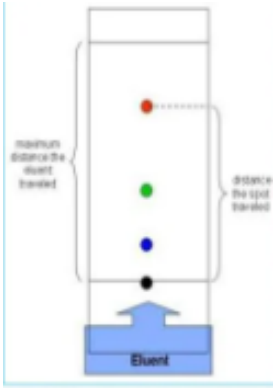
- To study the process of fermentation and ripening.
- To check the purity of pharmaceuticals.
- To inspect cosmetics.
- To detect the adulterants.
- To detect the contaminants in drinks and foods.
- To examine the reaction mixtures in biochemical laboratories.
- To determine dopes and drugs in humans and animals.

Use of TLC

Thin layer chromatography (TLC) *is a technique used to identify the components/compounds present in a mixture by separating them using a thin stationary phase (silica gel) supported on an inert substrate and a mobile phase (solvent).*

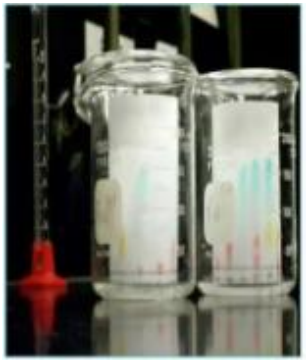
- *To study the purity of the compound*
- *To study the progress of a reaction*
- *To identify the various compounds present in the mixture*

Examples of Chromatography (on the basis of chromatography bed shape**)**:



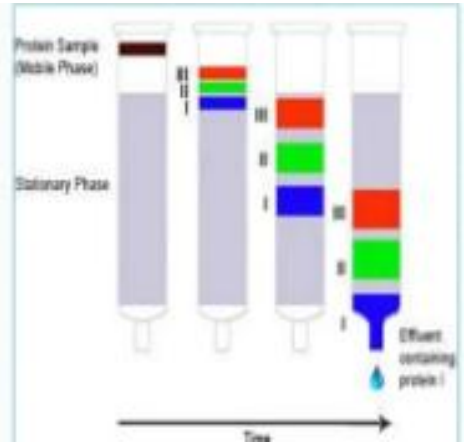
TLC

i). ***Thin Layer Chromatography: (TLC)*** is a chromatography technique used to separate non-volatile mixtures. Thin layer chromatography is performed on a **sheet of glass**, **plastic** or **aluminium foil**, which is coated with the thin layer of **adsorbent material**, usually **silica gel**, **aluminium oxide**, or **cellulose**.



Paper Chromatography

ii). ***Paper Chromatography:*** is an analytical method that is used to separate **coloured chemicals** or substances, **especially pigments**. This can also be used in ink experiments.

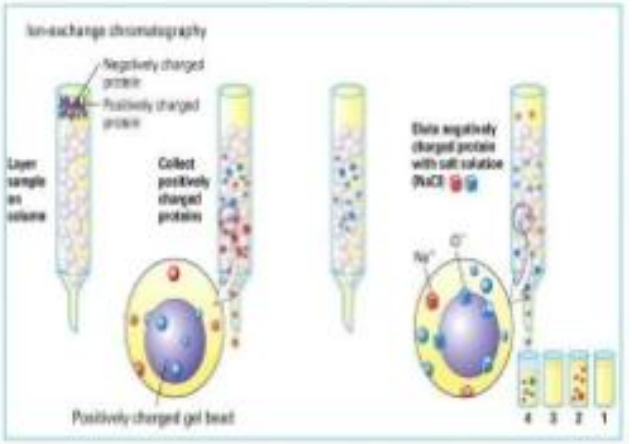


Column Chromatography

ii). ***Column Chromatography:*** in Chemistry is a method use to purify individual chemical compounds from a mixtures of compounds. It is often used for preparative applications on scale from micrograms up to kilograms.

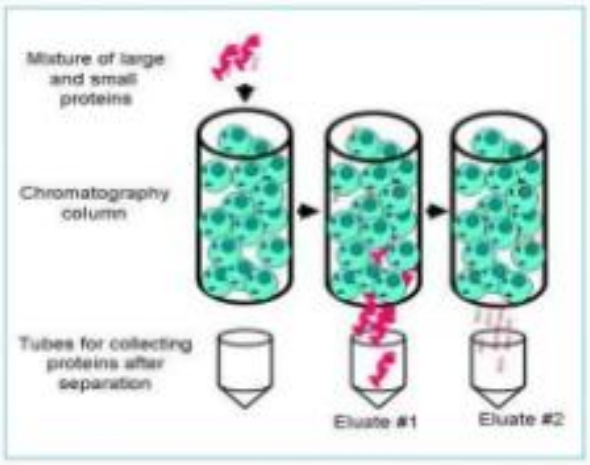
Examples of Chromatography (on the basis of interaction of solute to the stationary phase):

iii). *Ion Exchange Chromatography (Ion Chromatography):* is a process that allows the separation of **ions** and **polar molecules** based on their affinity to the ion exchanger. It can be used for almost any kind of **charged molecules** including **large protein**, **small nucleotide** and **amino acids**. The solution to be injected is called **Sample** and individually separated components are called **analytes**.



Ion Exchange Chromatography (Ion Chromatography) is a process that allows the separation of ions and polar molecules based on their affinity to the ion exchanger. It can be used for almost any kind of charged molecules including large protein, small nucleotide and amino acids. The solution to be injected is called Sample and individually separated components are called analytes.

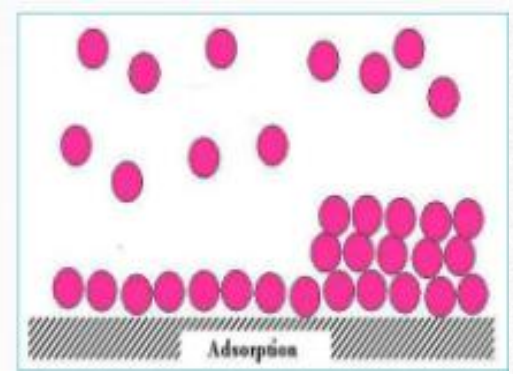
iv). *Size-Exclusion Chromatography (SEC):* is a chromatographic method in which molecules in a solution are separated **by their size**, and in some cases **molecular weight**. It is usually applied to **large molecules** or **macromolecular** complexes such as **proteins** and **industrial polymers**.

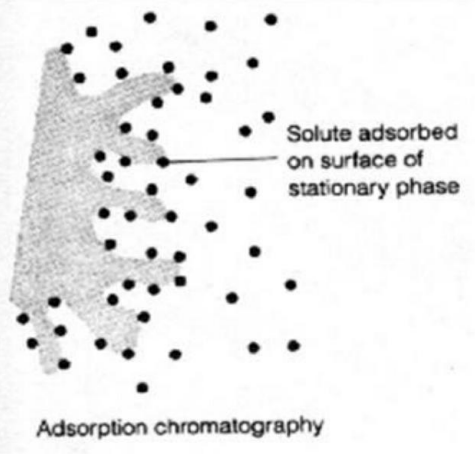


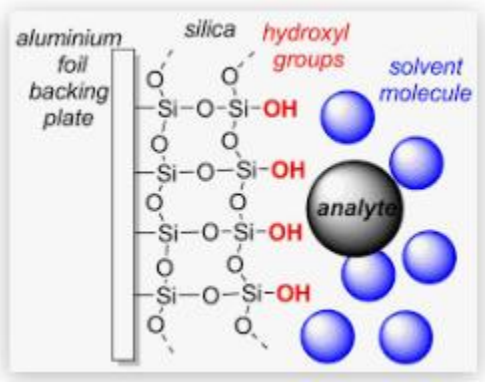
Size-Exclusion Chromatography (SEC) is a chromatographic method in which molecules in a solution are separated by their size, and in some cases molecular weight. It is usually applied to large molecules or macromolecular complexes such as proteins and industrial polymers.

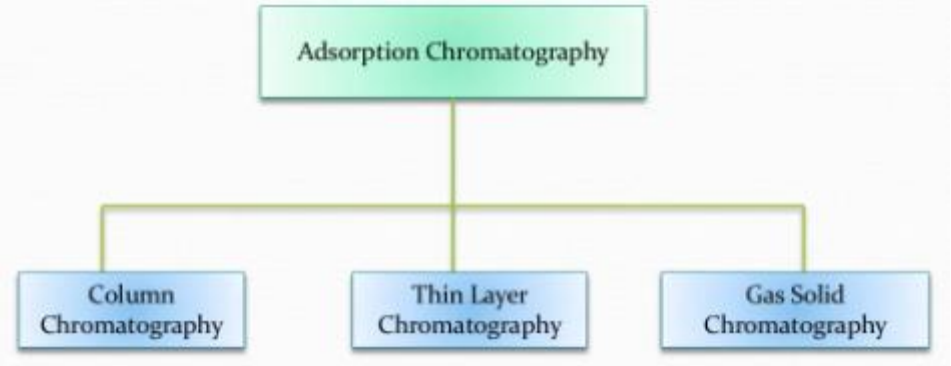
S-13. **Adsorption Chromatography**

 Definition: Adsorption chromatography is probably one of the oldest types of chromatography around. It utilizes a **mobile liquid** or **gaseous phase** that is **adsorbed** onto the **surface of a stationary solid phase**. The **equilibration** between the **mobile** and **stationary phase** accounts for the **separation** of **different solutes**.









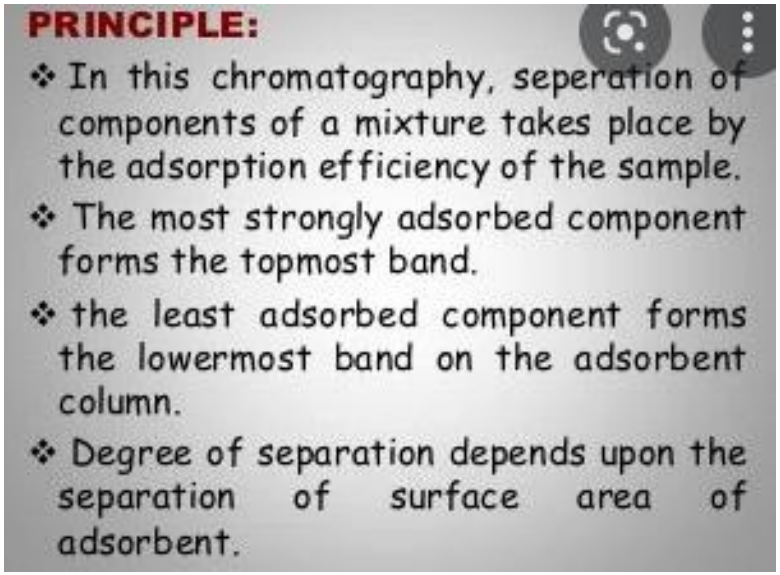
 Principle of Adsorption Chromatography:

Principle of Adsorption Chromatography involves competition of components of sample mixture for **active site** on **adsorbent**. These active sites are formed in molecule due to

 Cracks

Edges

Separation occurs because of the fact that an **equilibrium** is **established** between **molecules adsorbed** on **stationary phase** and those which are **flowing freely in mobile phase**. The more the affinity of the molecule of particular component, less will be its movement.



Type of Adsorption Chromatography: ***i) Column Chromatography***, ***ii) Thin Layer Chromatography***, ***iii) Gas Solid Chromatography*** Types:

Types of Adsorption Chromatography:

Thin Layer Chromatography – It is a chromatography technique where the mobile phase moves over an adsorbent. The adsorbent is a thin layer which is applied to a solid support for the separation of components. The separation takes place through differential migration which occurs when the solvent moves along the powder spread on the glass plates.

Paper chromatography – It is a 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. The solid surface of the paper is the stationary phase and the liquid phase is the mobile phase.

Column chromatography – the technique in which the solutes of a solution are entitled to travel down a column where the individual components are adsorbed by the stationary phase. Based on the affinity towards adsorbent the components take positions on the column. The most strongly adsorbed component is seen at the top of the column.

Gas-Solid chromatography – The principle of separation in GSC is adsorption. It is used for solutes which have less solubility in the stationary phase. This type of chromatography technique has a very limited number of stationary phases available and therefore GSC is not used widely.

S-19. **Ion Exchange Chromatography**

 Definition: Ion Exchange Chromatography (Ion Chromatography) is a process that allows the separation of **ions and polar molecules** based on their **affinity** to the **ion exchanger**. It can be used for almost any kind of **charged molecules** including **large protein**, **small nucleotide** and **amino acids**. The solution to be injected is called **Sample** and individually **separated components** are called **analytes**. It is often used in **protein purification**, **water analysis**, and **quality control**.

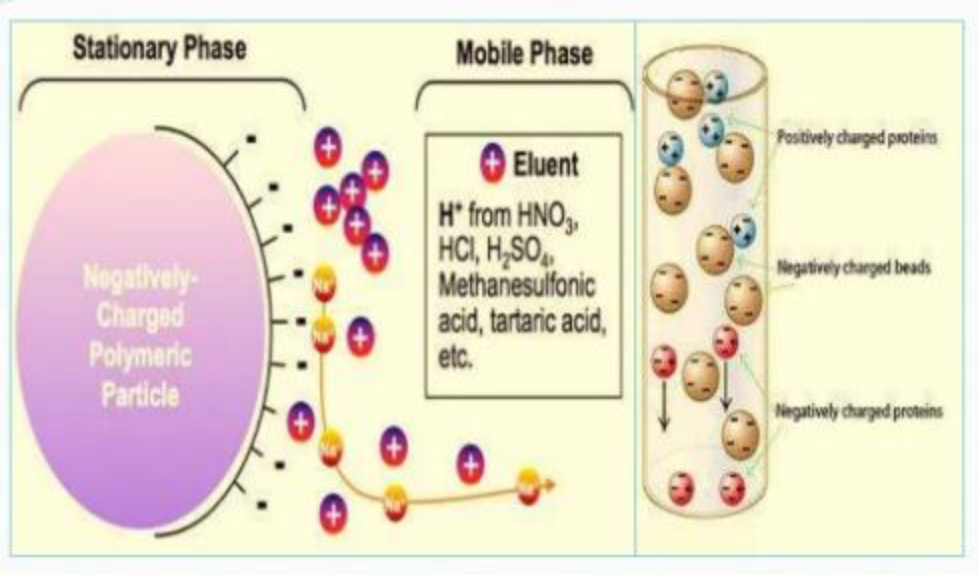


Fig. **ion exchanger**

S-20 **Principle of Ion Exchange Chromatography** :

Ion Exchange Chromatography is based on the **relative retention** of the ions during their progress **through an ion exchange column** which has **functional group** of **opposite charge attached to its surface**. The stronger the charge on the ion, the greater is the retention time in the column.

Ion chromatography is used to separate organic or inorganic charged substances. The stationary phases used are based on typical **ion exchange resins**.

PRINCIPLE OF SEPERATION

This is by reversible exchange of ions between the ions present in the solution and those present in the ion exchange resin.

CATION EXCHANGE:

The separation of cations using cation exchange resin. The cations to be separated are present in solution and exchanges for similar ions present in cation exchange resin, a solid matrix. the exchange can be represented by the following equation:

$$X^+ + R^-K^+ \longrightarrow X^+R^- + K^+ \text{ (solution)}$$

(solution)

- The cations retained by the solid matrix of ion exchange resin can be eluted by using buffers of different strength and hence separation of cations can be effected.

ANION EXCHANGE:

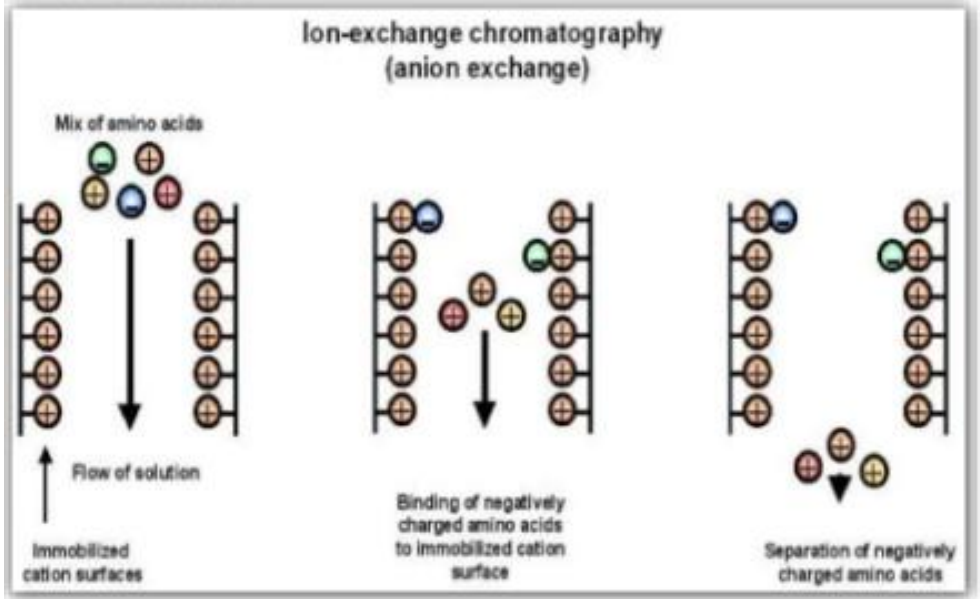
Separation of anion using anion exchange resin can be carried out. The anions to be separated are present in solution and exchanges for similar ions present in anion exchange resin, a solid matrix the exchange can be represented by the following equation:

(solid)

$X^- + R^+Cl^- \longrightarrow X\text{-}R^+ + Cl^-$ (anion exchange)

(solution)

- The anions retained by the solid matrix of ion exchange resin can be eluted by using buffers of different strength and hence separation of anions can be effected.



Ion exchange resin should have following requirements

- It must be chemically stable.
- It should be insoluble in common solvents.
- It should have a sufficient degree of cross linking.
- The swollen resin must be denser than water.
- It must contain sufficient no. of ion exchange groups.

Divided in to two,

1. Natural
   - ✓ cation – Zeolytes, Clay, etc
   - ✓ Anion – Dolomite
2. Synthetic
   - ✓ In organic and Organic resins

2. Synthetic

✓ In organic and Organic resins

Organic resins are **polymeric resin matrix.**

The resin composed of –

- **Polystyrene** (sites for exchangeable functional groups)
- **Divinyl benzene**(Cross linking agent)-offers stability.

2

2. According to chemical nature:

4 types:

1. Strong cation exchange resin
2. weak cation exchange resin
3. Strong anion exchange resin
4. weak anion exchange resin

- **Strongly acidic cation exchanger** ---sulphonic acid groups attached to styrene and di vinyl benzene copolymer.
- **Weakly acidic cation exchanger**---carboxylic acid groups attached to acrylic and divinyl benzene co-polymer
- **Strongly basic anion exchanger**-----quaternary ammonium groups attached to styrene and divinyl benzene co-polymer N+
- **Weakly basic anion exchanger**-----poly alkyl amine groups attached to styrene and divinyl benzene co-polymer

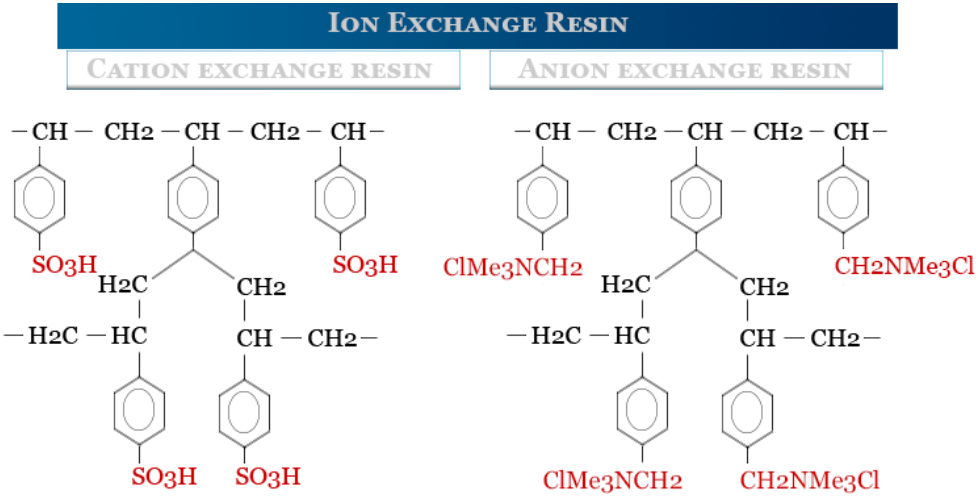
FUNCTIONAL GROUPS PRESENT IN DIFFERENT ION EXCHANGE RESINS:

Strong cation exchange resin- SO_3H

Weak cation exchange resin- COOH,OH,SH,PO_3H_2

Strong anion exchange resin- $N+R_3$,NR_2

Weak anion exchange resin- NHR,NH_2



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   - ✓ In organic and Organic resins

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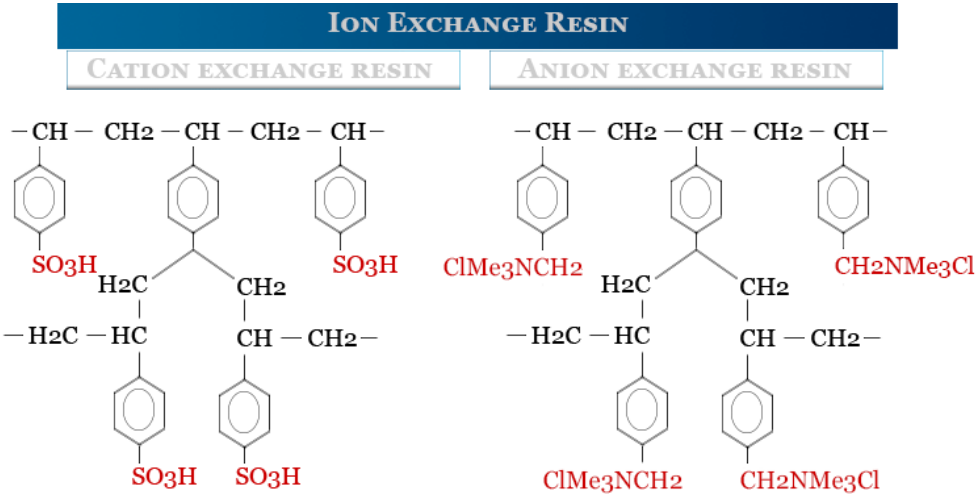
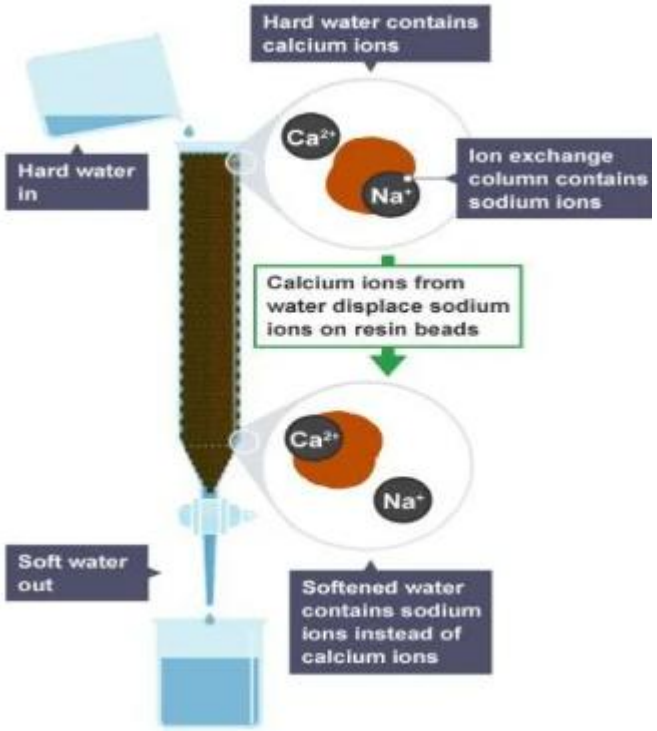
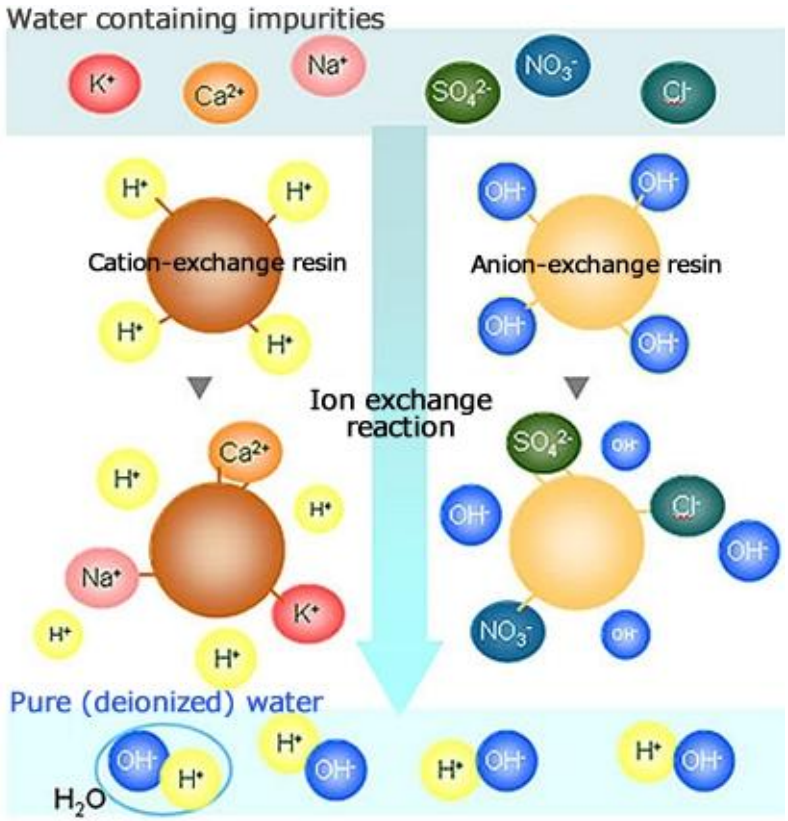
Weak anion exchange resin- NHR,NH_2







ION EXCHANGE TECHNIQUES

 1) Frontal Analysis: ● In Frontal analysis, an incomplete separation of ion is obtained.

► 2) Displacement Devolepment: ● Displacement devolepment of the column is accomplished by means of a substance which has a very strong affinity for the exchanger.

► 3) Elution devolepment: ● When the elution devolepment is performed, the components of a mixture separate and move down the column individually at different rates depending on the affinity of the ion for exchanger.

What is the difference between thin layer chromatography and paper chromatography?

In paper chromatography the separation is take place on the principle of partition. In paper chromatography the mobile phase and stationary phase both are liquid. In this technique filter paper is used in which the cellulose molecules traped the water molecules that act as a stationary phase.

Thin layer chromatography is the separation technique, the principle of TLC may be partition or adsorption of solute. In this technique aluminum foil is used as a support and stationary phase may be silica that is bounded to the surface of aluminum foil. If the solid stationary phase isand used such as silica then separation is takes place on the principle of adsorption chromatography. If liquid molecules as asta stationary phase are bounded on the solid support then the separation will take place on the basis of partition chromatography.

Paper chromatography is only used for qualitative analysis.

TLC is used for qualitative as well as quantitative analysis.