History:

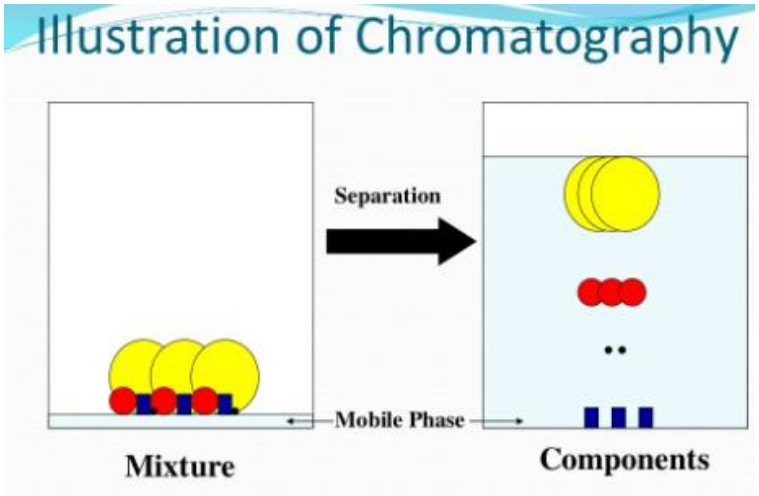
 The subject of Chromatography was introduced into scientific world in a very modest way by M. Tswett in 1906. He employed a technique to separate various pigments such as chlorophylls and xanthophylls by passing the solution of these compounds into the glass column which was packed with finely divided 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.

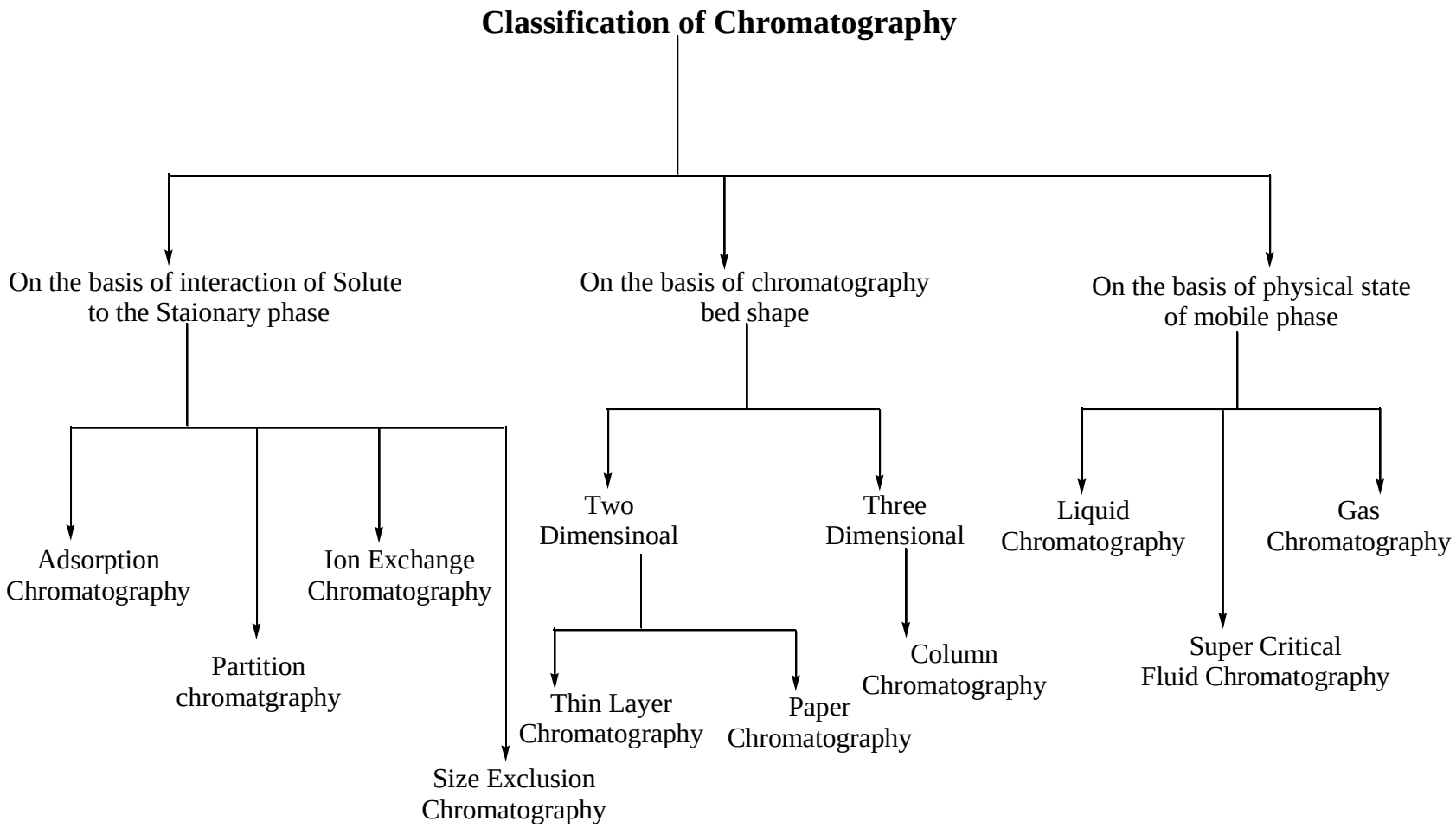
Chromatography:

 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.**

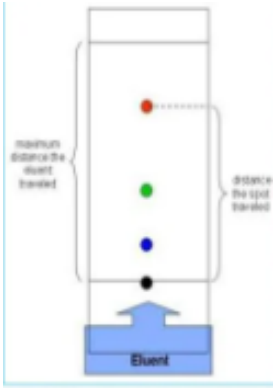
Introduction: The Term Chromatography (chroma = a colour; graphein = to write) is the collective term for a set of laboratory techniques for the separation of mixtures. Chromatography involves a sample (or sample extract) being dissolved in a mobile phase (which may be a gas, a liquid or a supercritical 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.



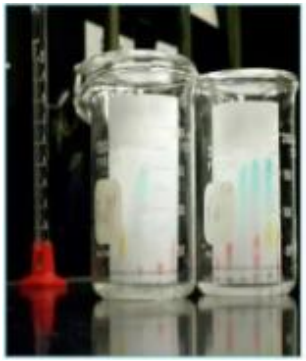

Classification of Chromatography
On the basis of interaction of Solute to the Staionary phase
On the basis of chromatography bed shape
On the basis of physical state of mobile phase
Adsorption Chromatography
Partition chromatgraphy
Ion Exchange Chromatography
Size Exclusion Chromatography
Two Dimensinoal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

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



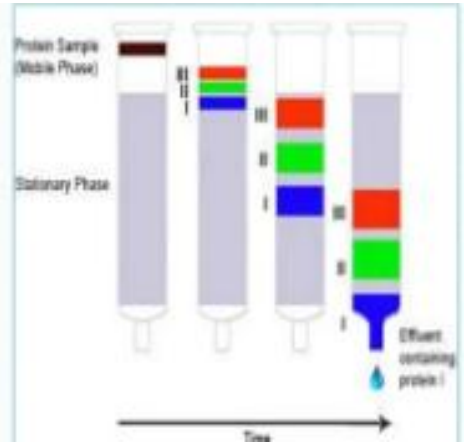
TLC

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.

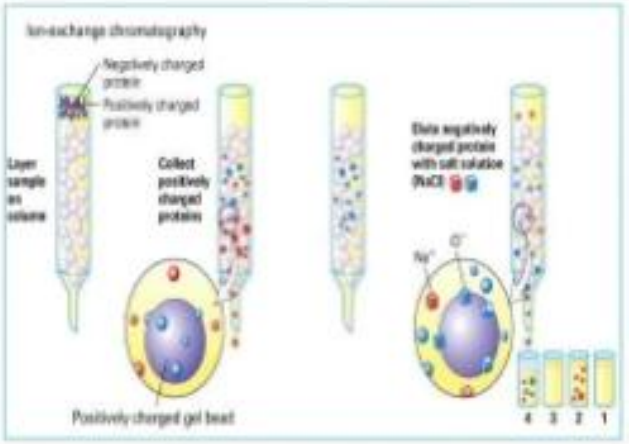


Column Chromatography

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**.



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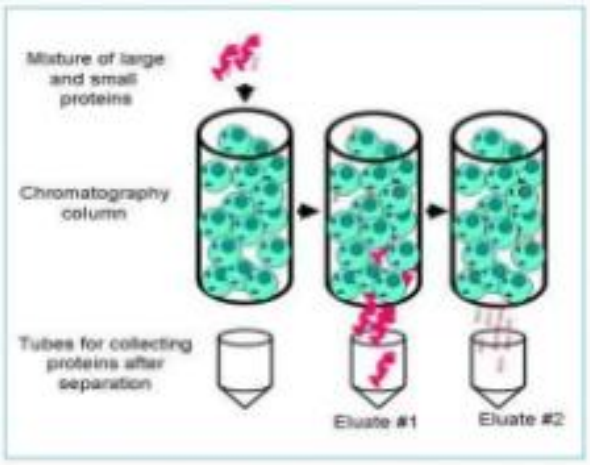
iv). *Size-Exclusion Chromatography (SEC):* is a chromatographic method in which molecules in a solution are separated **by their size**, and in some cases **molecular weight**. It is usually applied to **large molecules** or **macromolecular** complexes such as **proteins** and **industrial polymers**.



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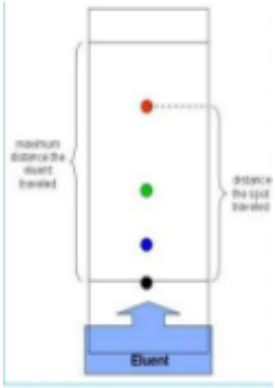

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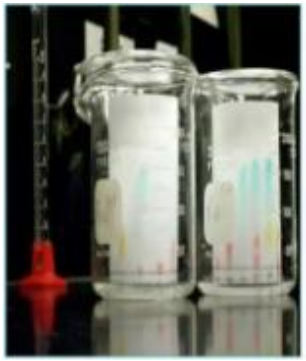

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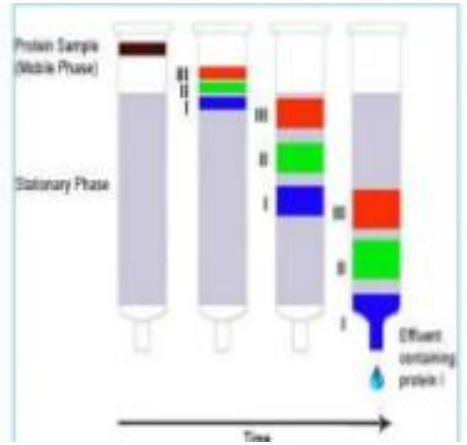

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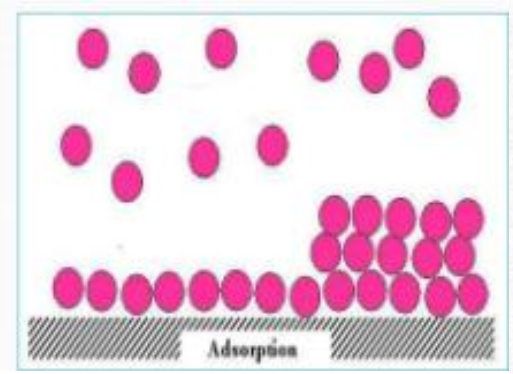
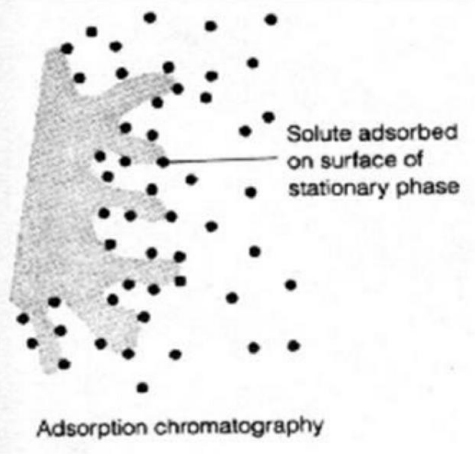
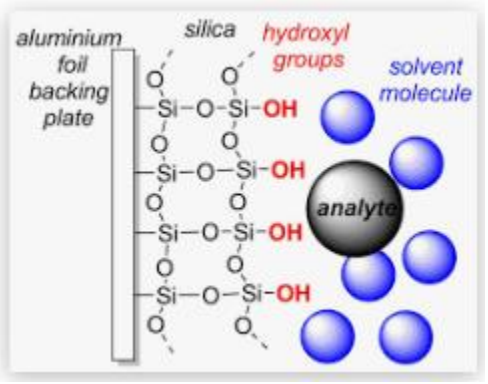
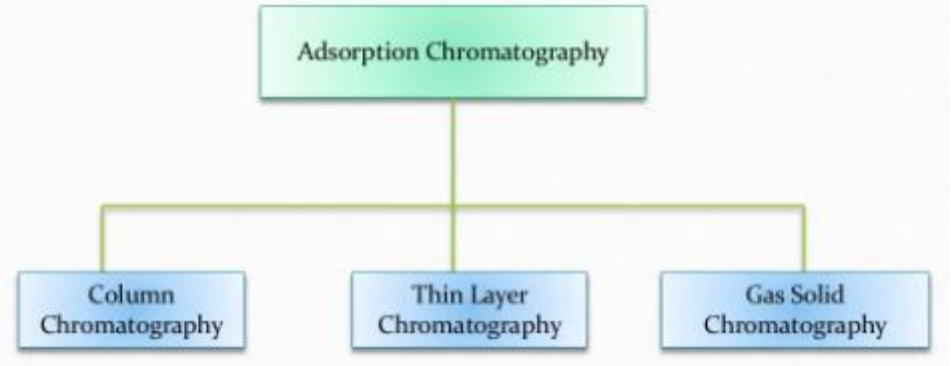
S-13. **Adsorption Chromatography**

 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**.









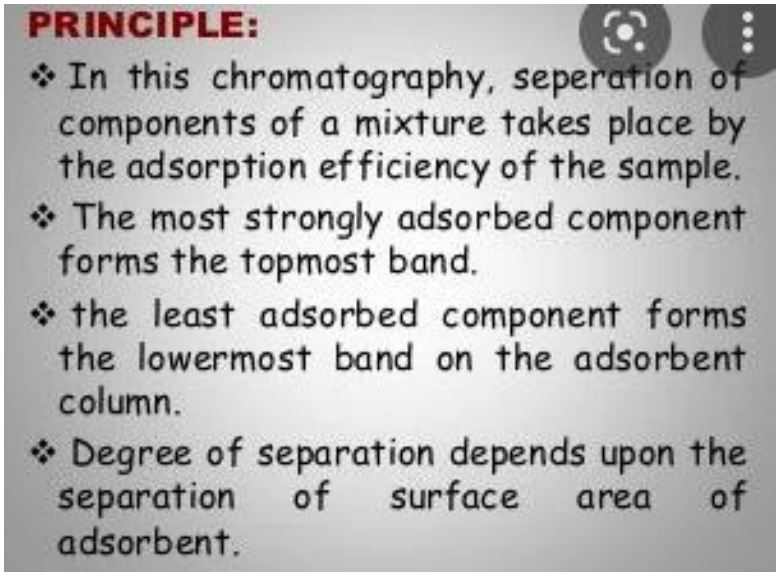
 Principle of Adsorption Chromatography:

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:

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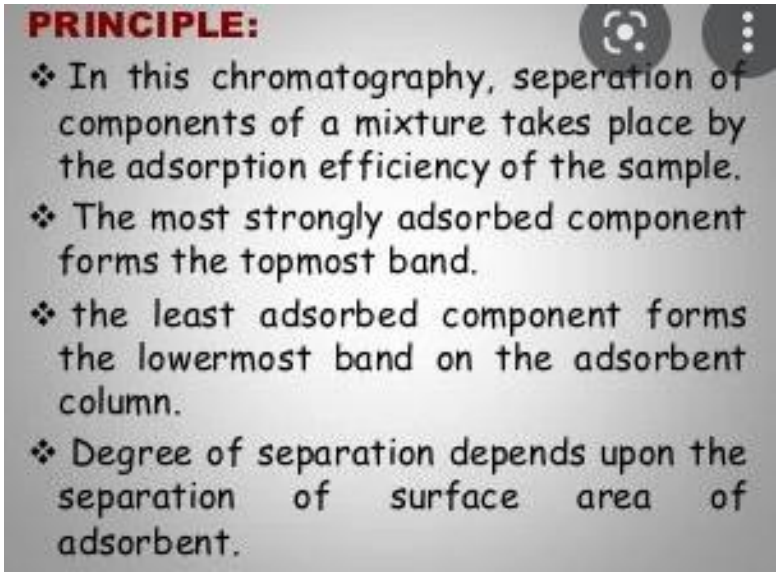


Type of Adsorption Chromatography: ***i) Column Chromatography***, ***ii) Thin Layer Chromatography***, ***iii) Gas Solid Chromatography*** Types:

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.

Applications of Adsorption Chromatography

1. Separation of aromatic or aliphatic non-polar compounds such as lipids.
2. Separation of high molecular weight compounds.
3. Separation / purification of Nucleic acids.
4. Analysis of plant pigments, Fat soluble vitamins.

Advantages of Adsorption Chromatography -

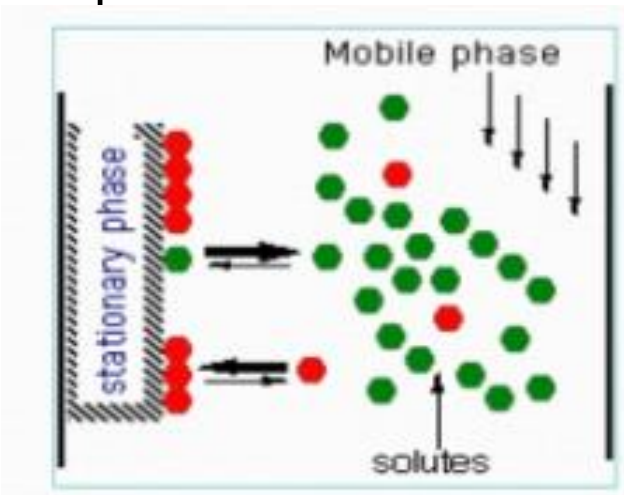
- It has a wide range of mobile phases for the separation of compounds.
- The complex sample mixtures can be easily separated by this method.
- Simple and low cost technique.

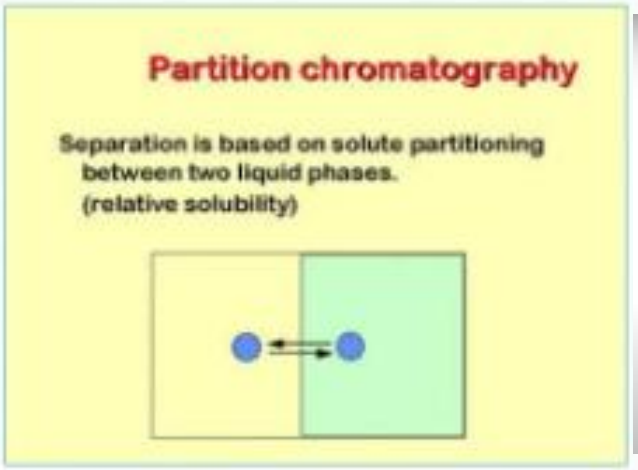
Disadvantages of Adsorption Chromatography –

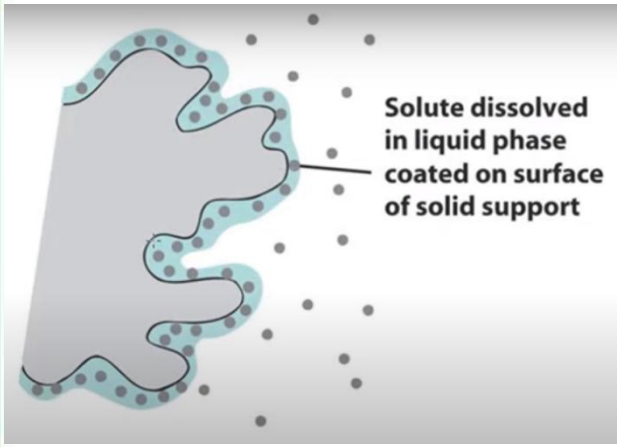
- Results obtained are not reproducible.
- Some compounds may permanently retain on the stationary phase.

S-16. **Partition Chromatography**

 Definition: This form of chromatography is based on a thin film formed on the surface of a solid support by a liquid stationary phase. Solute equilibrates between the mobile phase and the stationary liquid.







Partition coefficient K_D

- Based on thermodynamic equilibrium

Ratio of Analyte

$$K_D = \frac{C_s}{C_m}$$

C_s: concentration in stationary phase

C_m: concentration in mobile phase

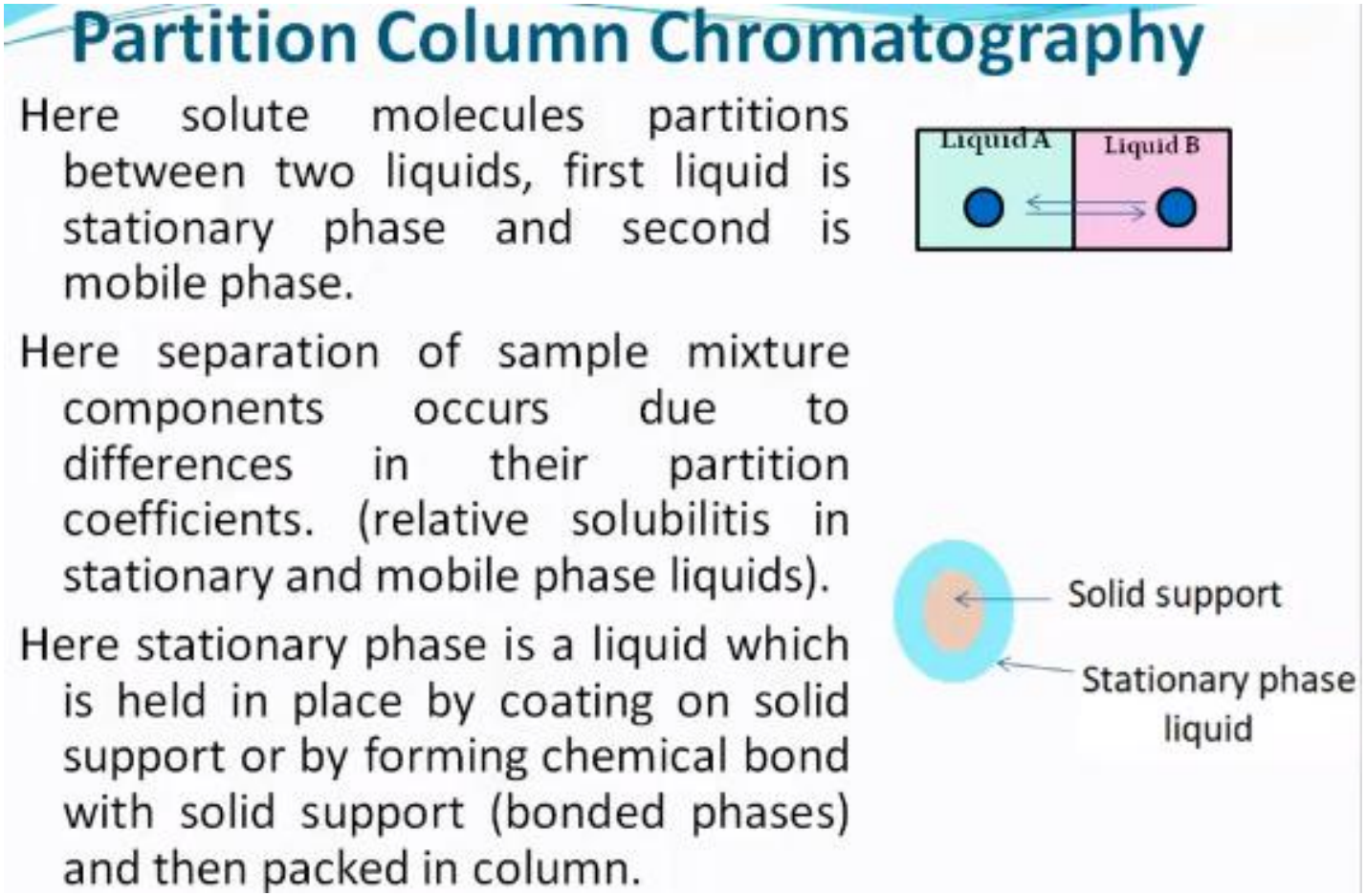
The same principle as Liquid Liquid Extraction

Partition Chromatography types

i) Liquid-liquid Chromatography ii) Gas-liquid Chromatography

Partition Chromatography:

Separation is due to difference in solubility of components in two immiscible liquids. The stationary phase is a liquid thin film on an inert solid support. The **stationary liquid** is usually **more polar** than the **mobile phase**. **Cellulose powder** and **wetted silica gel** are examples of **supports** in partition chromatography that carry film of water act as stationary phase. Solid Support Film of the liqiud stationary Phase



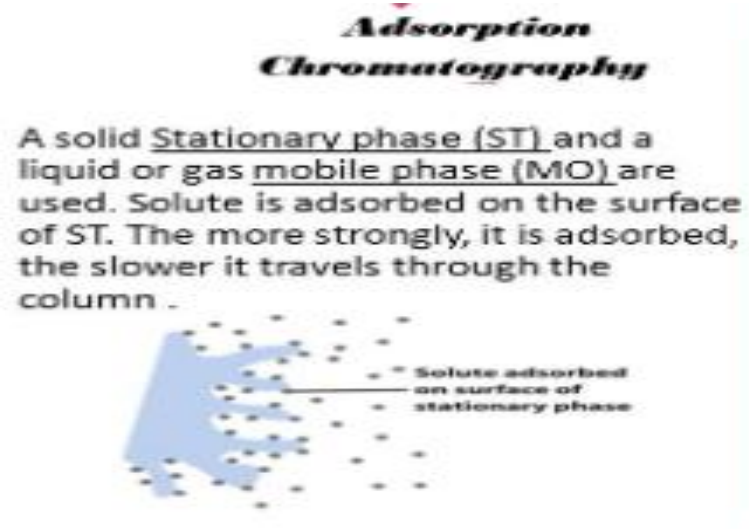
S-17 **Principle of Partition Chromatography**:

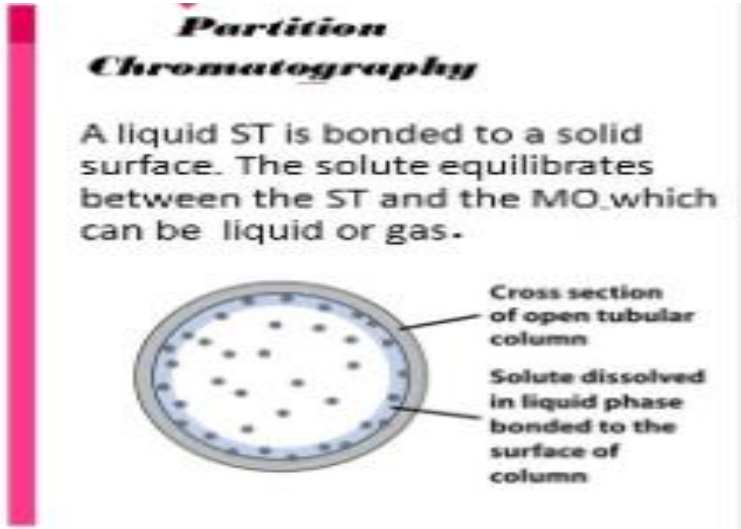
Separation of components of a sample mixture occurs because of partition. Stationary phase is coated with a liquid which is immiscible in mobile phase.

Partition of component of sample between sample and liquid/ gas stationary phase retard some components of sample more as compared to others. This gives basis for separation.

The stationary phase immobilizes the liquid surface layer, which becomes stationary phase. Mobile phase passes over the coated adsorbent and depending upon relative solubility in the coated liquid, separation occurs. The component of sample mixture appear separated because of differences in their partition coefficient.

Difference Between Adsorption and Partition Chromatography?





Adsorption chromatography is defined as a type of chromatography in which separation occurs based on adsorption.	Partition chromatography is a type of chromatography in which separation is based on partition.
Extraction	
Adsorption chromatography is a liquid-solid extraction.	Partition chromatography is a liquid-liquid extraction.
Stationary Phase	
The stationary phase is in the solid state of adsorption chromatography.	The stationary phase is a liquid state in partition chromatography.
Developments	
Adsorption chromatography was not further developed.	Partition chromatography leads to the development of other types of chromatography.

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**.

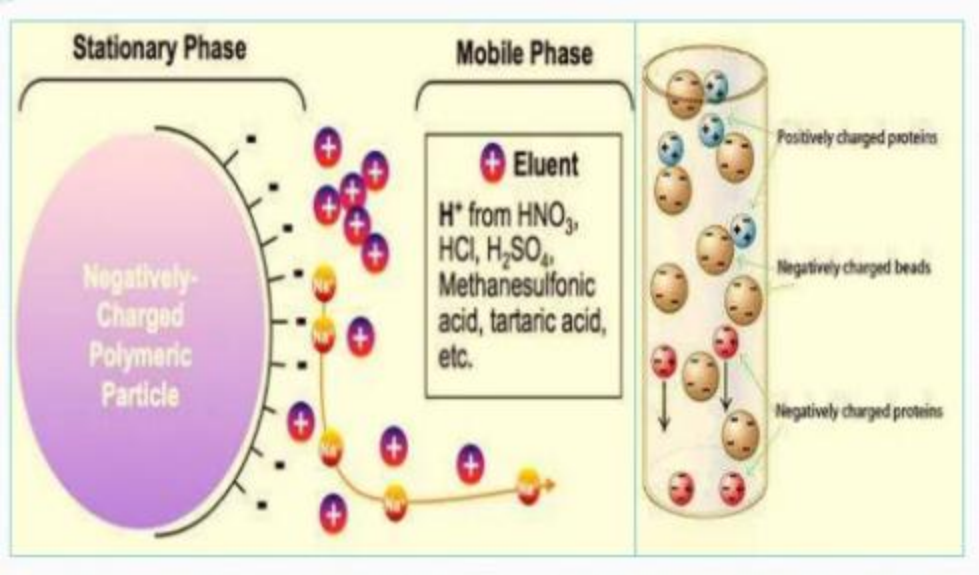


Fig. **ion exchanger**

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)

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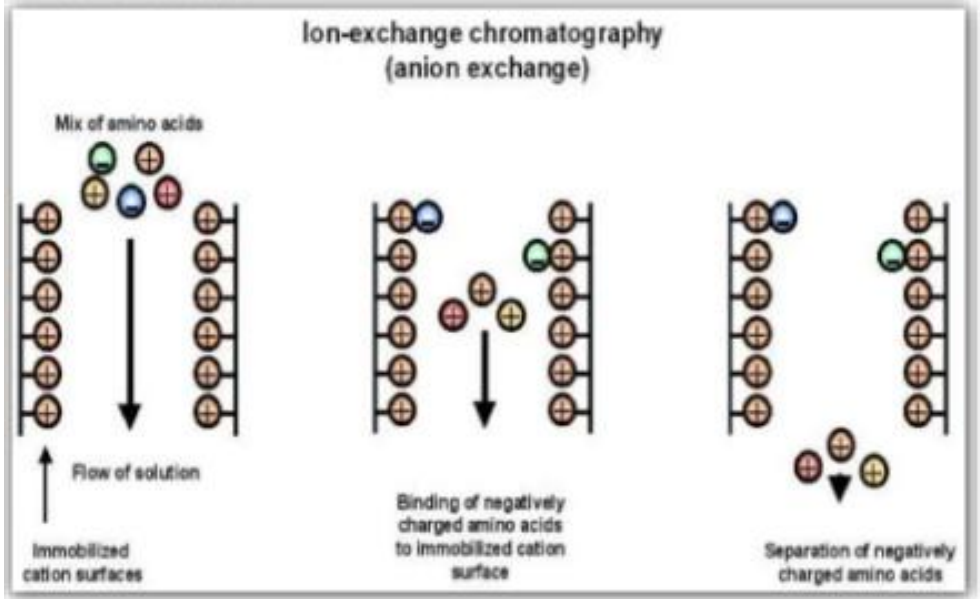


Ion exchange resin should have following requirements

- 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

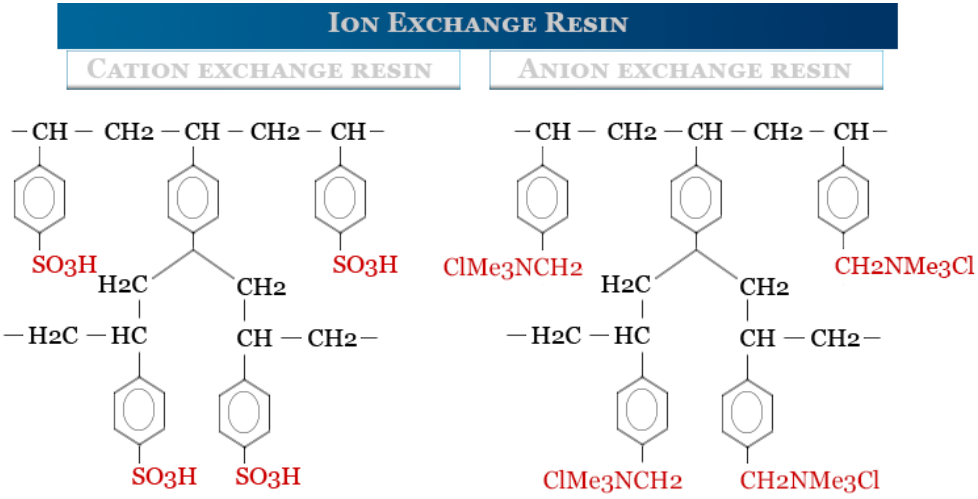
FUNCTIONAL GROUPS PRESENT IN DIFFERENT ION EXCHANGE RESINS:

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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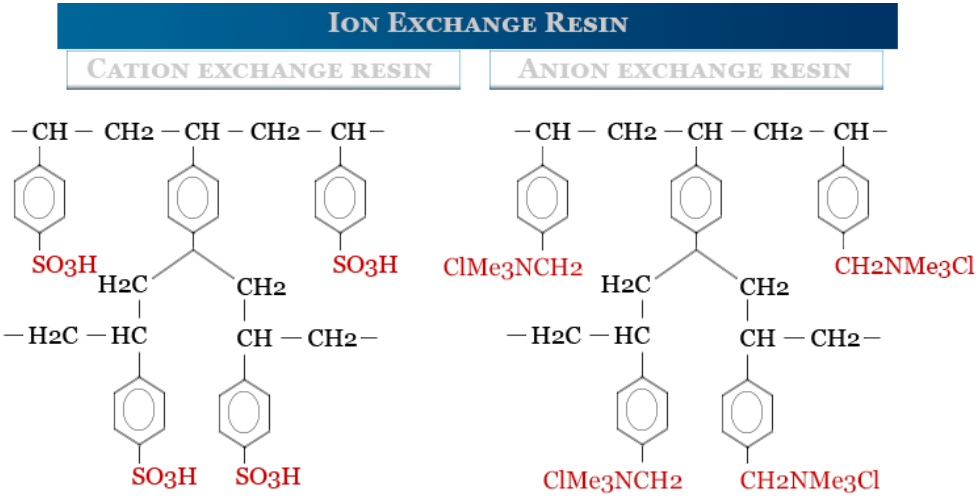

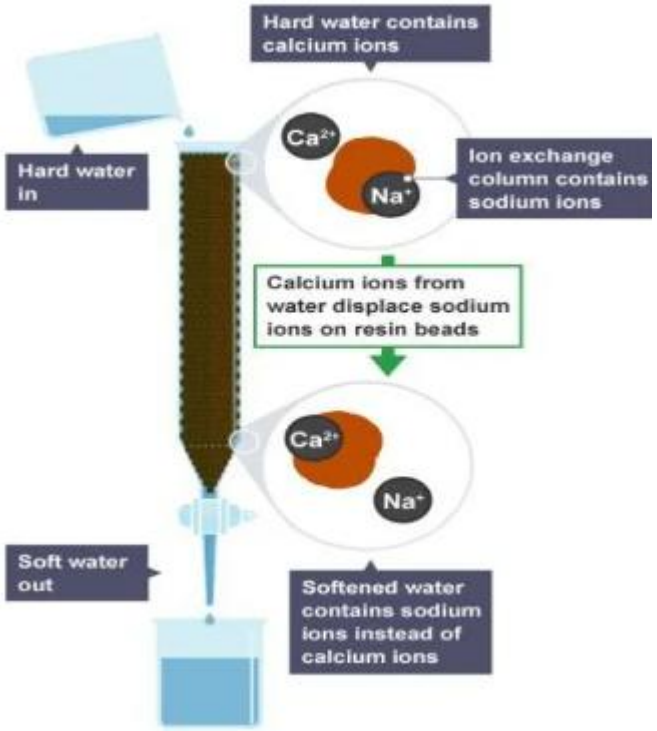

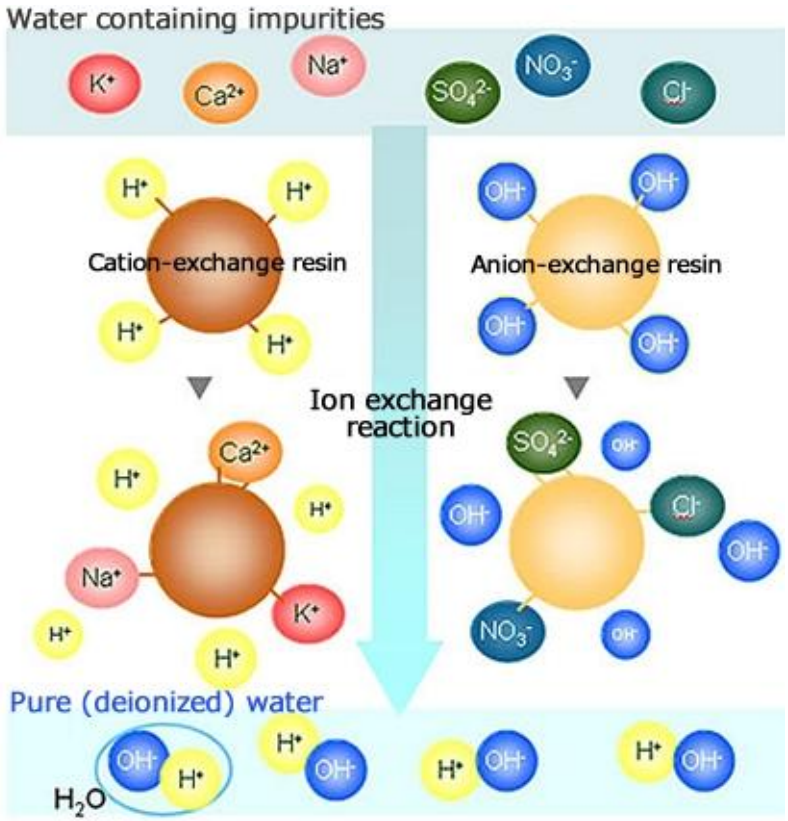

ION EXCHANGE TECHNIQUES

 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.