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# ION EXCHANGE CHROMATOGRAPHY

Presented by  
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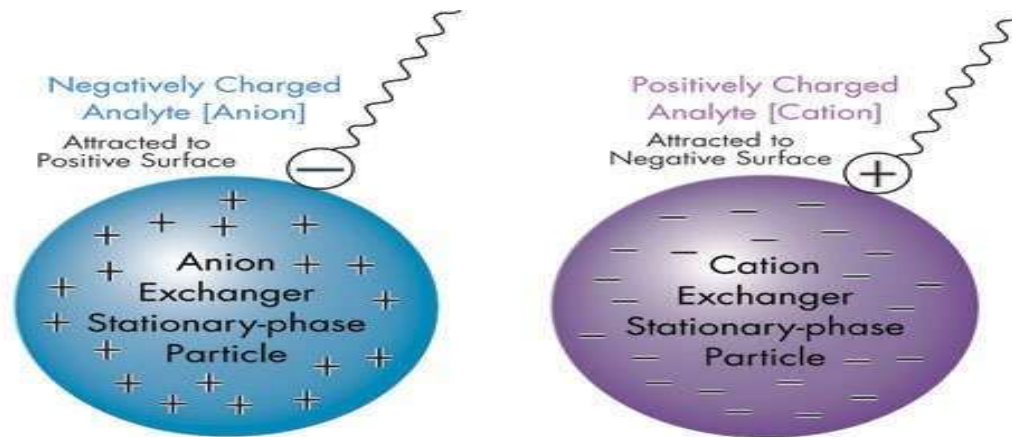
## ❖ INTRODUCTION:

**Ion chromatography** (or **ion-exchange chromatography**) is a chromatography process that separates ions and polar molecules based on their affinity to the ion exchanger. It works on almost any kind of charged molecule including large proteins, small nucleotides, and amino acids

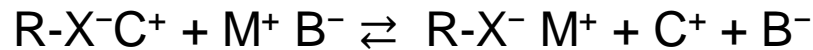
## ❖ PRINCIPLE:

- Adsorption process (Surface phenomenon)

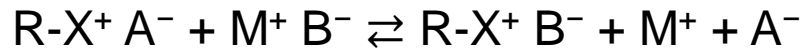
Ion Exchange **chromatography** is used in the separation of charged biomolecules. The crude sample containing charged molecules is used as the liquid phase. When it passes through the **chromatographic** column, molecules bind to oppositely charged sites in the stationary phase.



Cation exchange chromatography retain positively charged **cations** because the stationary phase displays a negatively charged functional group:



Anion exchange chromatography retains **anions** using positively charged functional group:



## CLASSIFICATION OF RESINS:

According to the **chemical nature** they classified as:-

1. Strong cation exchange resin – **Sulphonic acid**
2. Weak cation exchange resin – **Carboxy methy compound**
3. Strong anion exchange resin – **Quaternary ammonium compound**
4. Weak anion exchange resin - **Diethyl aminoethyl compound**

According to the Source they are classified as:

**Natural :**      **Cation** - Zeolytes, Clay

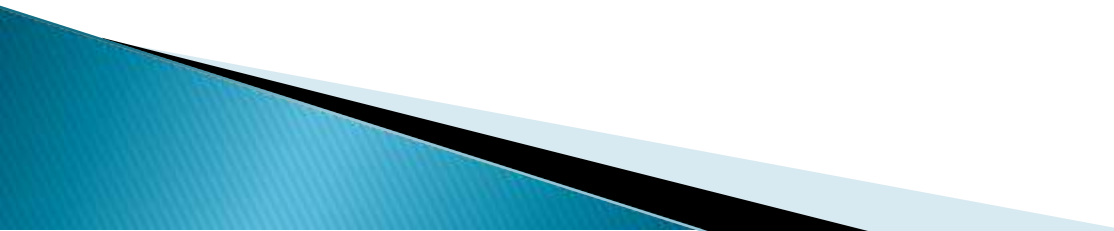
**Anion** - Dolomite

**Synthetic :** Inorganic & Organic resins

Organic resins are **polymeric resin matrix**

The resin composed of – **Polystyrene** (sites for exchangeable functional groups), **Divinyl benzene** (Cross linking agent)- it offers stability

## Properties of Ion exchange resin:

- 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
- 

## Structure of ion exchange resins:

### a) Pellicular type with ion exchange resins:

- 30 - 40 $\mu$  with 1-2 $\mu$  film thickness
- Very low exchange capacity

### b) Porous resin coated with exchanger beads:

- Size 5 - 10 $\mu$
- Porous & highly efficient

### c) Macroreticular resin bead:

- Not highly efficient & low exchange capacity

d) Surface sulfonated & bonded electrostatically with anion exchanger:

- less efficient & low exchange capacity

Physical properties of ion exchange resins:

Cross linking:

- ▶ It affects swelling & strength & solubility

**Swelling:**

- ▶ When resin swells, polymer chain spreads apart

Polar solvents → swelling

Non-polar solvents → contraction

Swelling also affected electrolyte concentration.



## Particle size & Porosity

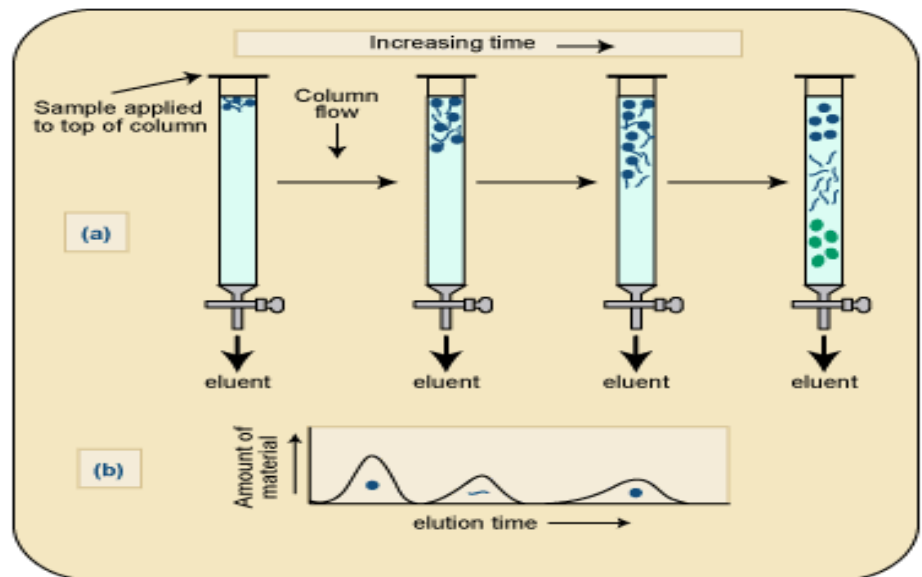
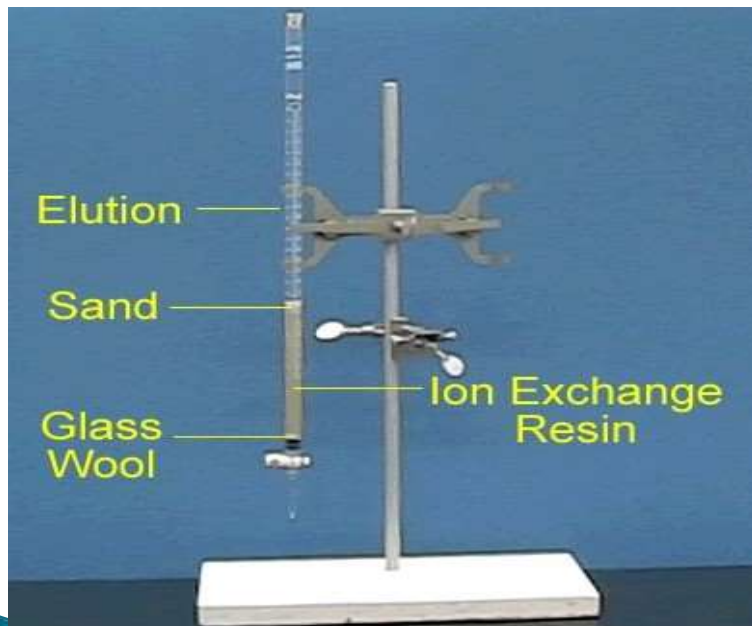
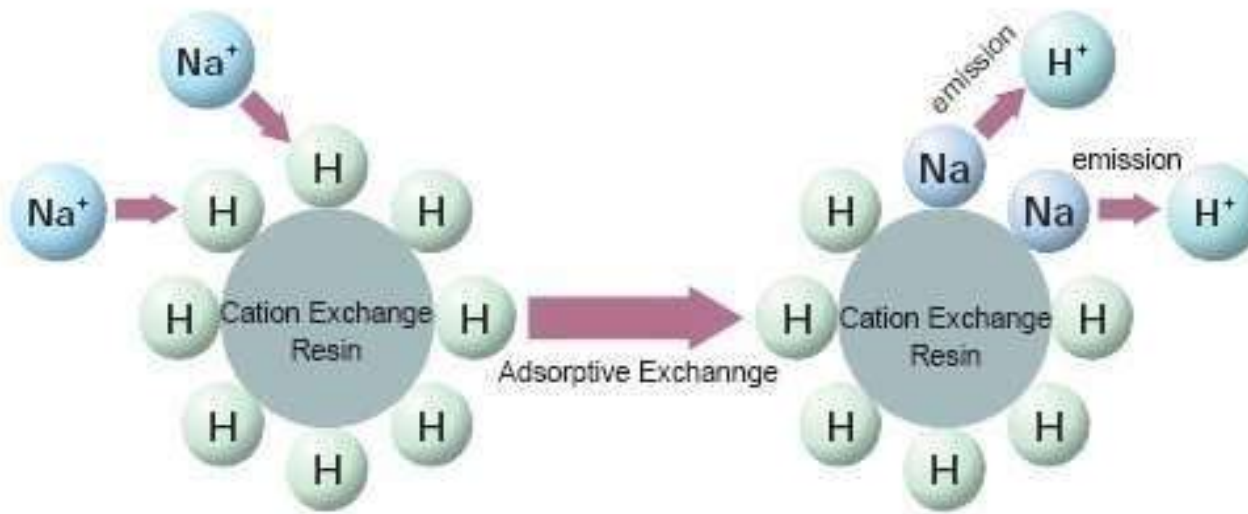
↑ surface area & ↓ particle size will ↑ rate of ion exchange

➤ Particle size 50-100 mesh / 100-200 mesh

## Regeneration

➤ Cation exchange resin are regenerated by treatment with acid, then washing with water

➤ Anion exchange resin are regenerated by treatment with NaOH, then washing with water until neutral



## METHODOLOGY:

### 1. Column

- Column is made up with Glass, stainless steel or polymers  
**Length:** diameter ratio 20:100 to 100:1

### 2. Packing the column

- Wet packing method

### 3. Application of the sample

After packing, sample is added to the top of the column by using syringe or pipette

## **4. Mobile phase**

Acids, alkalis, Buffers

## **5. Elution**

Components of mixture separate & move down the column at different rates depending upon the affinity of the ion for ion exchanger.

- The elutes are collected at different stages

## **6. Analysis of the elute**

- The eluted compound is measured by using spectrophotometry, flame photometry, polarography and conductometry.

## Factors affecting on ion exchange chromatography:


### a. Nature & properties of ion exchange resins

Cross linking & swelling is important

If more cross linking , they are more rigid, but swelling is less

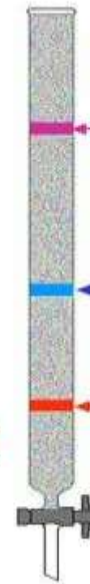
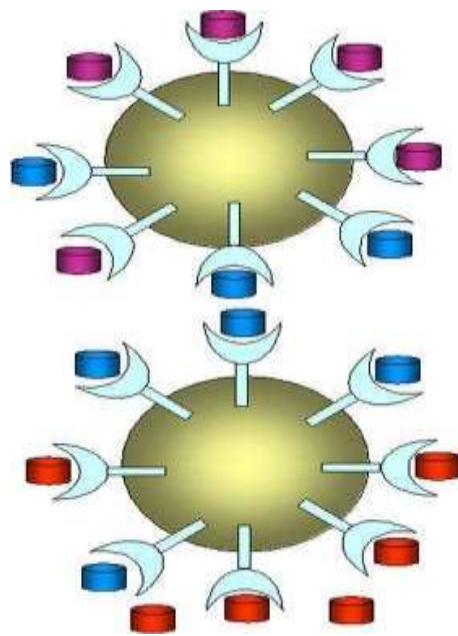
swells less → separation of ions of different sizes is difficult

### b. Nature of exchanging ions

1. Valency of ions
  2. Size of ions
  3. Polarizability
  4. Con. of solution
  5. Concentration & charge of ions
- 

# APPLICATIONS

- Softening of water
- Demineralization of water
- Purification of solutions free from ionic impurities
- Separation of inorganic ions
- Separation of sugars, amino acids
- To separate creatine kinase (CK) isoenzymes from human serum and tissue sourced in autopsy material (mostly CK rich tissues were used such as cardiac muscle and brain)
- Ion Exchange Chromatography is used in different aspects of product development and quality control testing. For example, IC is used to improve stabilities and solubility properties of pharmaceutical active drugs molecules as well as used to detect systems that have higher tolerance for organic solvents.



more highly charged molecules are more tightly bound to the resin, and so travel slowly and are eluted later

moderately charged molecules equilibrating between the resin and the moving buffer more readily

Less charged molecules bind less strongly to the resin, equilibrate with the moving buffer more readily, and so travel rapidly and are eluted sooner



## FILL IN THE BLANKS

1. Hard water is converted into Soft water by \_\_\_\_\_ chromatography
2. Strong cationic exchange resin is \_\_\_\_\_
3. Particle size of resins are increases then rate of ion exchange will be \_\_\_\_\_
4. The principle involved in Ion Exchange chromatography is \_\_\_\_\_
5. The cross linking of ion exchange resin is increases; swelling is \_\_\_\_\_ and exchange of ions will be \_\_\_\_\_

### **KEY:**

1. Ion Exchange chromatography
2. Quaternary ammonium compound
3. Increases
4. Adsorption
5. less & less



