UNIT –V

Ion exchange chromatography- Introduction, classification, ion exchange resins, properties, mechanism of ion exchange process, factors affecting ion exchange, methodology and applications

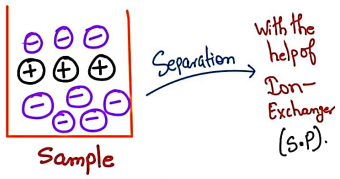
Gel chromatography- Introduction, theory, instrumentation and applications

Affinity chromatography- Introduction, theory, instrumentation and applications

* Ion Exchange Chromatography.

Introduction → (Purification is done on the Basis on Ion).

- IEC it is a technique that allows the Separation of Ions based on their affinity to Ion-Exchanger.
- Stationary Phase → Ion Exchanger.



☆ It is a Useful & Powerful method for Separation of Mixture of Charge Substance.

Types of Ion Exchanger (Stationary Phase) → ⓐ Cationic Ion Exchanger.
ⓑ Anionic Ion Exchanger.

ⓐ Cationic Ion Exchanger is ⊖ vely Charged that attract ⊕ve ions.

ⓑ Anionic Ion Exchanger is ⊕ vely Charged that attract ⊖ve ions.

2.

⇒ The **Principle of Separation of ions. Reversible exchange of ions b/w the target ion ⊕nt in Sample to the Ions ⊕nt in Ion Exchanger. (Stationary Phase).

- These ion Exchanger Contains Covalently linked Charged group (+ or −) ⇒ Called Counter Ion.
- These Counter Ions are Capable of exchanging with Ion Present in Sample.

#Classification of Ion-Exchange Resin

(a) Cationic →

(i) Inorganic
- Natural
  - Clay
  - Modified Greensand.
- Synthetic
  - Zeolite.

(ii) Organic
- Natural
  - Lignite
- Semi Synth.
  - Zeocarb
- Synthetic
  - Acrylic acid Copolymer

(b) Anionic →

(i) Inorganic
- Natural
  - Dolomite
- Synthetic
  - Heavy Metal Silicates.

(ii) Organic
- Semi-Synthetic
  - Sephadex
- Synthetic
  - Amine formaldehyde resin.

Properties of Ion Exchange Resin →

(1) Ion Exchange Resin must be Chemically Stable.

(2) Insoluble in Common Solvent.

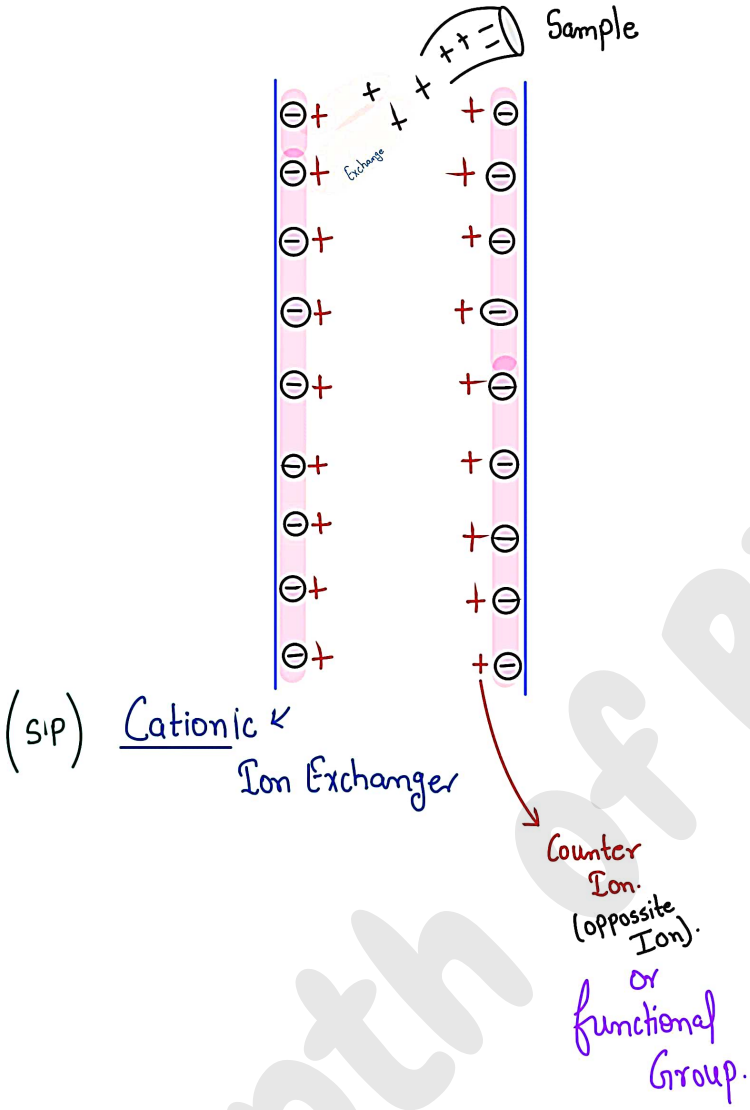
(3) Sufficient Hydrophilic (for proper swelling of Resin).
↓
Beads (swell Properly)

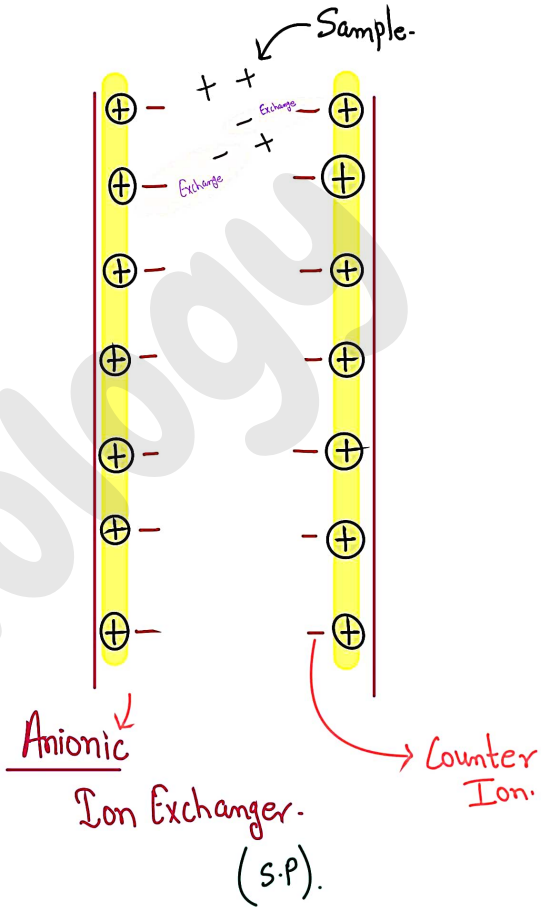
(4) Sufficient number of Ion Exchange group.

(5) Sufficient degree of Cross linking.

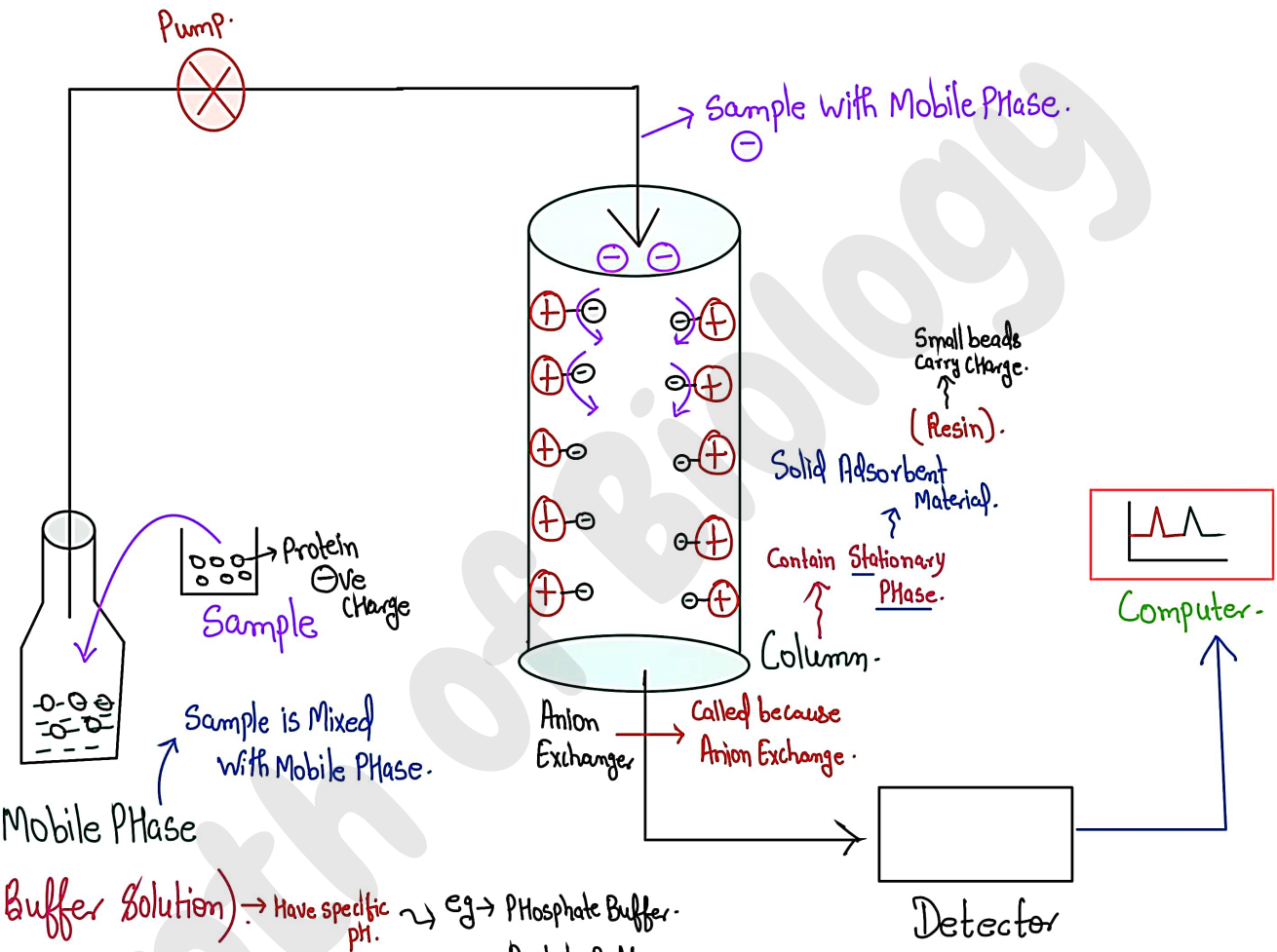
Mechanism of Ion-Exchange Resin

☆ Same as Principle.





Instrumentation



- Sample movement is done with the Help of Mobile Phase.
- Wet Packing Method is Used (Buffer + Sample) ↓ Then Introduce ← Slurry. (Packing of Column).

☆ Column → Made up of Glass. (In Lab).
↓
Made up of Stainless steel. (In Industries).

Steps Involved in Ion Exchange Chromatography → 5.

(a) Equilibration →
(b) Sample Loading →
(c) Washing.
(d) Elution.

(a) Equilibration → It is a process of establishing a stable Baseline or establishing a Consistent enviroment within the Chromatographic Column before Sample Injection. ← Baseline

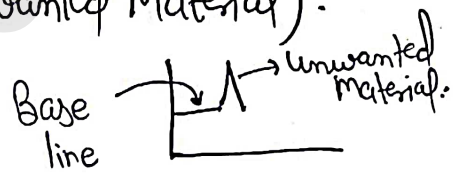
Computer

☆ Base line indicate that Column is properly Packed

(b) Sample loading → Sample is mixed with Mobile Phase or Buffer Solution. & then run on the Column during loading the sample in Column the flow rate of system is very slow. So, sample have sufficient time to replace resin & bind itself it is called Sample Loading.

6.

(3) Washing → Sample molecule which is not bind with Stationary Phase (freely available in the Column) need to washout or remove from the Column. Via running buffer in the Column. So, Unwanted material remove from Column & system show a peak (which Indicate removal of Unwanted Material).

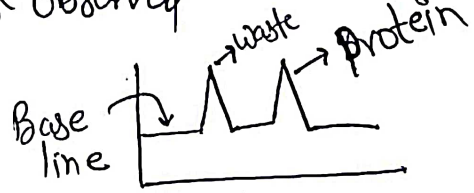


↓

& we Collect Washout Material.

(4) Elution →

Elution buffer is load in Column, So it break the bond b/w Stationary Phase & Sample (Protein) & our sample (Protein) now remove from Column. & again peak observed



↓

Collect purified Sample.

⇒ In Elution first we run the Low Salt Concentration Solution.

Low Salt Conc. Soln. ⟶ Stationary Phase — Protein

Weak bond break.

Then we Increase Salt Concentration.

High Salt Conc. Solution ⟶ SP = Protein (bond)

Strong Bond break.

- We determine the Concentration of Sample in Mixture by Calculating the area of Peak.

4. Factors affecting Ion Exchange

1. Nature & property of Ion Exchange resin. ⇒

☆ Depends on the Conc. of Cross Linking →

If, Cross Linking ↑ ⇒ Rigid ↑ ⇒ Swelling ↓ → affinity for Exchange ↓

- So, the Separation is difficult.

2. Nature of Exchanging Ions →

(a) Valency of Ion → Valency ↑ → Ion Exchange Property ↑

eg → $Na^{+} < Ca^{+2} < Al^{+3} < Th^{+4}$.

(b) Size of Ion → (↓)se Size of Hydrateion = Exchange ↑

(c) Polarizability → Highly Polarion = Greater Exchange

(d) Conc. & Charge on Ions →

Applications of Ion Exchange Chromatography

1. **Softening of hard water:** Hardness of water is due to the presences of Ca2+, Mg2+ and other divalent ions may be removed by passing the hard water through the cation exchanger charged with Na + ions

2.Complete demineralization of water: This requires complete removal of ions i.e., both cations and anions. For this, water is passed through an acidic cation exchanger then metallic cations are exchanged with H+ ions.

3. Purification of organic compounds: Many natural products extracted in water have been found to contain ions originally present in water. Those ions can be removed by using ion exchange process.

4.Separation of amino acids: Ion exchange methods can be used to separate the complex mixture of 18 amino acids obtained by the acid hydrolysis of proteins.

5. Purification and recovery of pharmaceuticals: The process is used for purification and recovery of antibiotics, vitamins, alkaloids, hormones and other chemicals of pharmaceutical importance during their manufacturing process.

6 **Biochemical separations:** Used for biochemical separations like some drugs or metabolites from blood, urine or other biological fluids.

7 **Other applications:** For the measurement of various active ingredients in medicinal formulations. For the measurement of drugs and their metabolites in serum and urine, for residue analysis in food raw materials.

➤ For the measurement of additives such as vitamins and preservatives in food and beverages