Types of Chromatography (IEC, SEC, AC, HPLC)

Ion exchange chromatography (IEX)

- "Ion exchange chromatography may be defined as the reversible exchange of ions in the solution with ions electrostatically bound to some sort of insoluble matrix or a stationary phase."

- This technique is extremely useful in the separation of charge compounds like proteins differing by only one charged amino acid.

- In Ion exchange chromatography technique one can choose whether to bind the substance of interest and allow the contamination to pass through the column and vice versa.

- This technique has been developing since 19th century which was firstly used for purifying the drinking water.

- Ion exchange chromatography relies on the attraction between oppositely charged stationary phase, known as an ion exchanger, and analyte.

- The ion exchanger consists of an inert support medium coupled covalently to positive (anion exchanger) or negative (cation exchanger) functional groups.

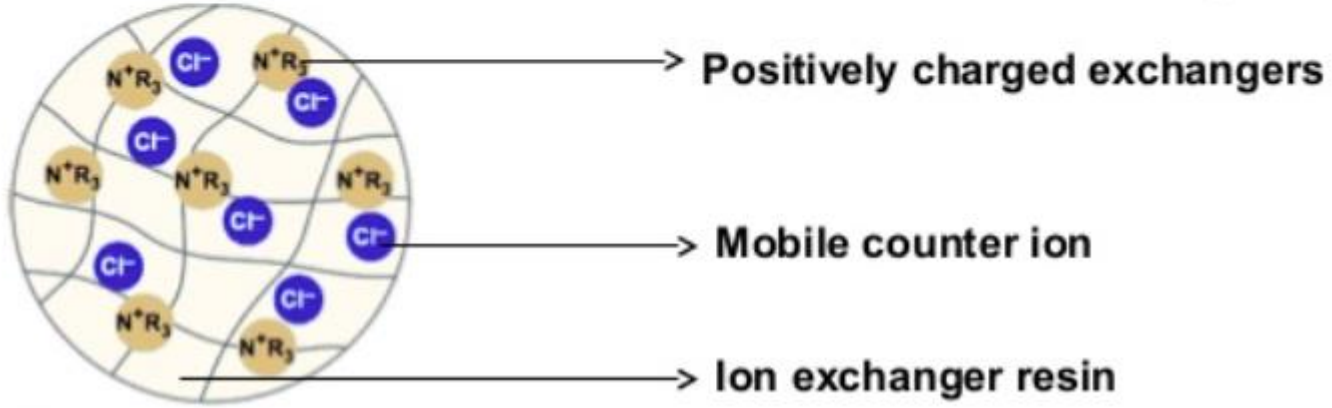
- To these covalently bound functional groups the oppositely charged ions are bounded (mobile counter ion), which will be exchanged with like charge ions in the sample having charge magnitude more than the ions bounded to the matrix.

- Thus if anion exchange chromatography is performed, negatively charged sample components will interact more with the stationary phase and will be exchanged for like charged ions already bounded to the matrix.

Anion exchangers

- The anion exchangers have positively charged exchanger with negatively charged mobile counter ion available for exchange.
- If the basic functional groups are introduced, the resin becomes anion exchanger.
- Tertiary amines ⟹ Strong anion exchangers

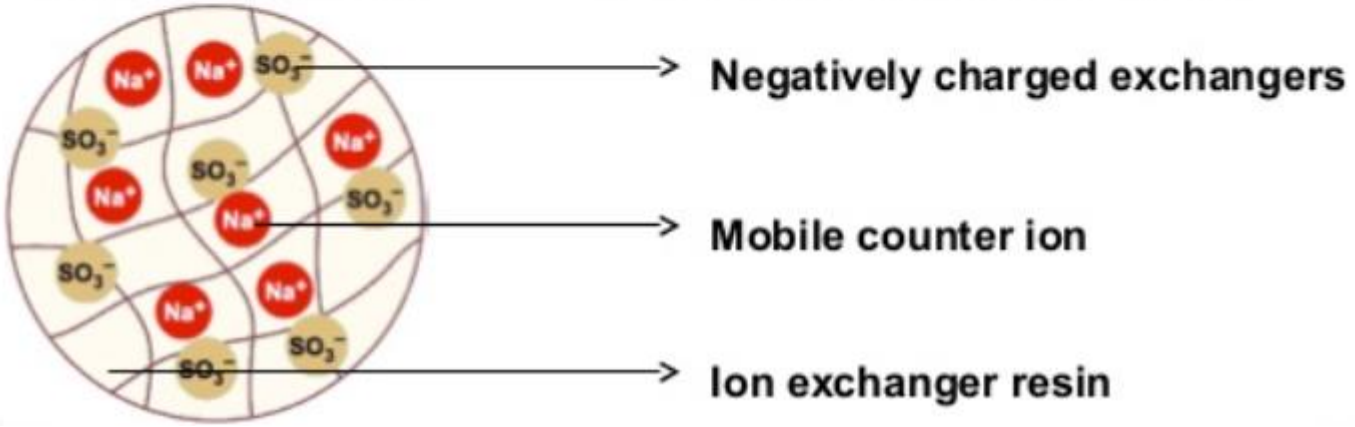
  Secondary amines ⟹ Weak anion exchangers



Cation exchangers

- The cation exchangers have negatively charged exchanger with positively charged mobile counter ion available for exchange.
- If acidic functional group are introduced, then the resin becomes cation exchangers.
- Sulphonic acid ⟹ Strong cation exchangers

  Carboxylic acid ⟹ Weak cation exchangers



Size exclusion chromatography (SEC)

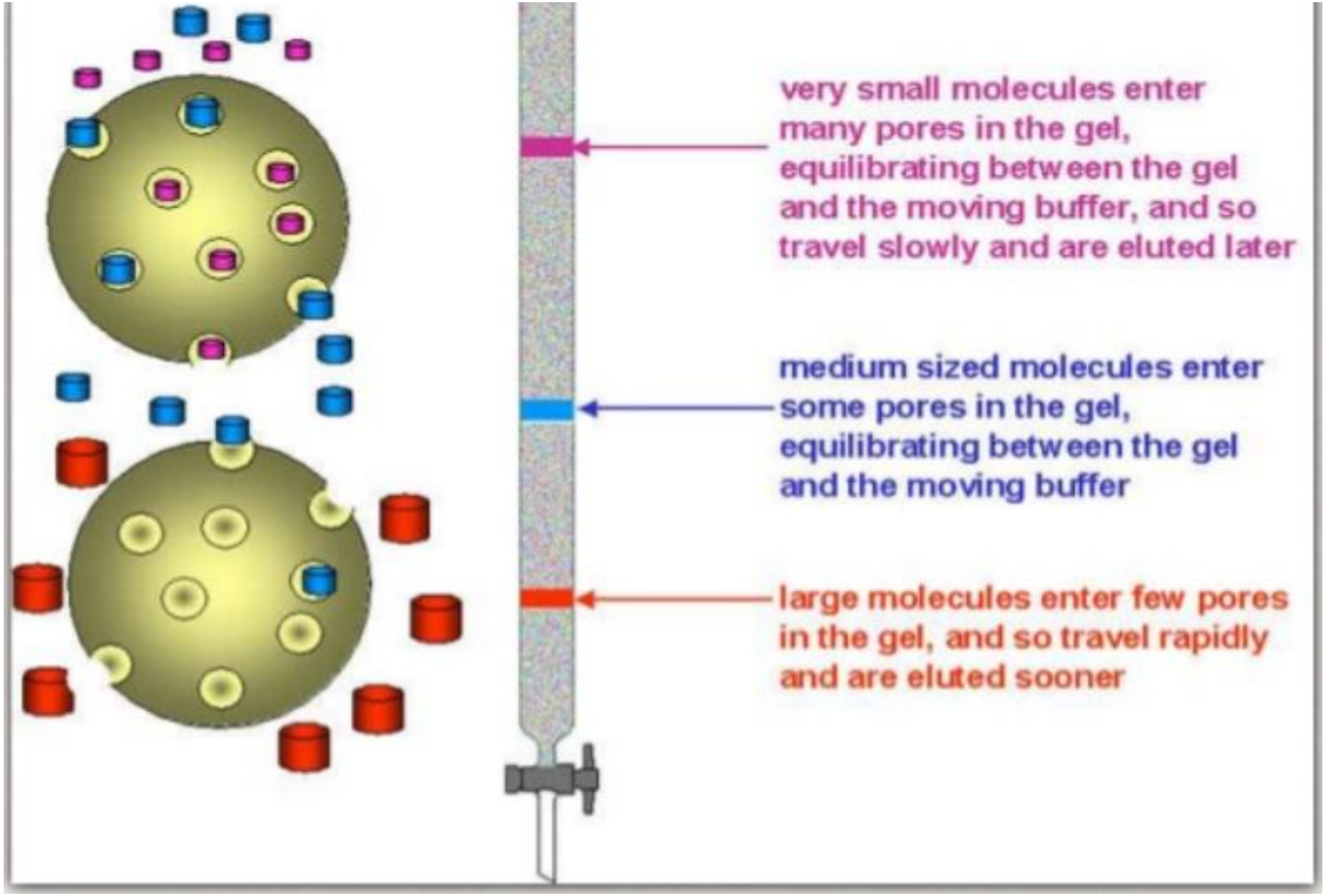
- Size exclusion chromatography is a mechanical sorting of molecules based on the size of molecules in solution.

- Small molecules are able to permeate more pores and are retained longer than larger molecules.

TYPES OF SIZE EXCLUSION CHROMATOGRAPHY

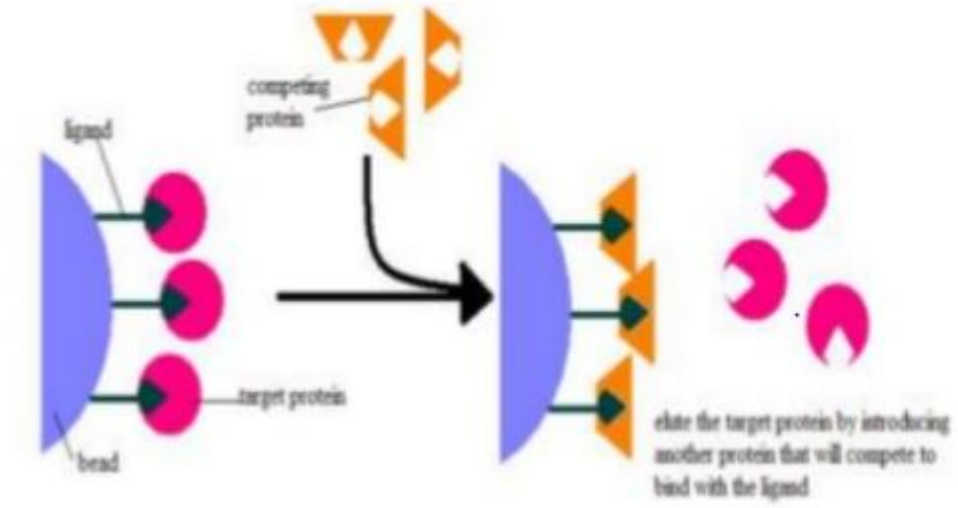
Two basic types of SEC are:-

- **GEL PERMEATION CHROMATOGRAPHY (GPC)**
  - Uses a hydrophobic column packing material and a non-aqueous mobile phase (organic solvent) to measure the molecular weight distribution of synthetic polymers.
- **GEL FILTERATION CHROMATOGRAPHY(GFC)**
  - Uses a hydrophilic packing material and an aqueous mobile phase to separate, fractionate, or measure the molecular weight distribution of molecules soluble in water, such as polysaccharides and proteins.


very small molecules enter many pores in the gel, equilibrating between the gel and the moving buffer, and so travel slowly and are eluted later
medium sized molecules enter some pores in the gel, equilibrating between the gel and the moving buffer
large molecules enter few pores in the gel, and so travel rapidly and are eluted sooner

Affinity chromatography

- **Affinity Chromatography** is essentially a sample purification technique, used primarily for biological molecules such as proteins.
- It is a method of separating a mixture of proteins or nucleic acids (molecules) by specific interactions of those molecules with a component known as a ligand, which is immobilized on a support. If a solution of, say, a mixture of proteins is passed over (through) the column, one of the proteins binds to the ligand on the basis of specificity and high affinity (they fit together like a lock and key).
- The other proteins in the solution wash through the column because they were not able to bind to the ligand.



Principle

- Affinity chromatography is one of the most diverse and powerful chromatographic methods for purification of a specific molecule or a group of molecules from complex mixtures
- It is based on highly specific biological interactions between two molecules such as interactions between enzyme and substrate,receptor and ligand,or antibody and antigen.
- These interactions which are typically revesible are used for purification by placing one of the interacting molecules referred to as affinity ligand onto a solid matrix to create a stationary phase while a target molecule is in the mobile phase.
- Many of the commonly used ligands coupled to affinity matrices are now commercially available and are ready to use.

HPLC

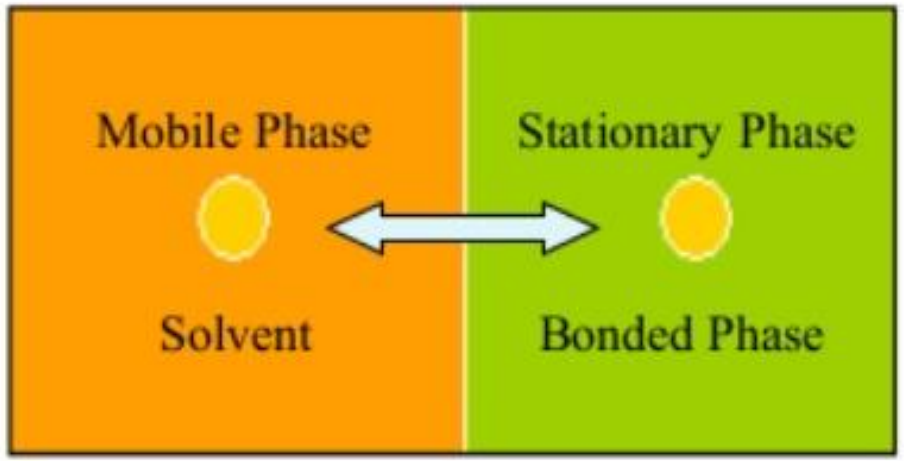
- HPLC is a form of liquid chromatography used to separate compounds that are dissolved in solution.
- HPLC is characterized by the use of high pressure to push a *mobile phase* solution through a column of *stationary phase* allowing separation of complex mixtures with high resolution.
- HPLC instruments consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector.
- Compounds are separated by injecting a sample mixture onto the column.
- The different component in the mixture pass through the column at differentiates due to differences in their partition behavior between the mobile phase and the stationary phase.

Principle

- The principle of separation in normal phase mode and reverse phase mode is adsorption.
- When a mixture of components are introduced into a HPLC column, they travel according to their relative affinities towards the stationary phase.
- The component which has more affinity towards the adsorbent, travels slower.
- The component which has less affinity towards the stationary phase travels faster. Since no 2 components have the same affinity towards the stationary phase, the components are separated

Partitioning

- Separation is based on the analyte's relative solubility between two liquid phases



Types of HPLC

Normal Phase.

-Polar-Stationary phase

-Nonpolar- Solvent(Mobile phase)

Reverse Phase.

- Non-polar- Stationary phase
- Polar - Mobile phase (solvent).

	Normal Phase	Reversed Phase
Stationary phase	Polar (silica gel)	Non-polar (C18)
Mobile phase	Non-polar (organic solvents)	Polar (aqueous/organic)
Sample movement	Non-polar fastest	Polar fastest
Separation based on	Different polarities (functionality)	Different hydrocarbon content