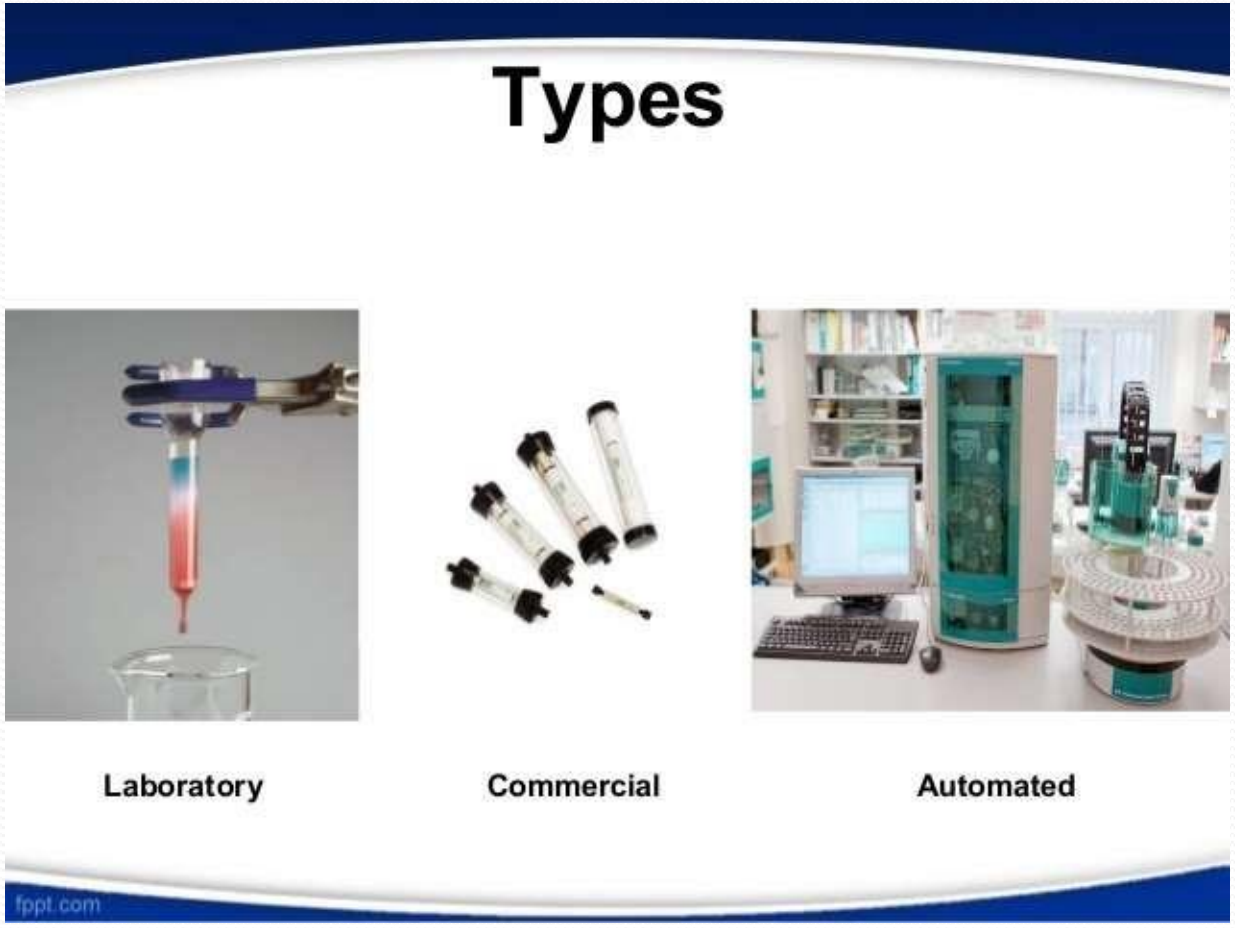
ION-EXCHANGE CHROMATOGRAPHY

Introduction:-

- "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 is a distinct principle of chromatography performed in the column

Types Laboratory Commercial Automated:-



Principle:-

- 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

Working:-

- Consider a column having $E^- Y^+$ cation exchanger in which E^- is negative charged exchanger and Y^+ is the mobile counter ion.
- Let X^+ be the cation in the sample having charge greater than Y^+.
- The X^+ ion can exchange sites with the counter ion Y^+ with satisfying the following relationship;

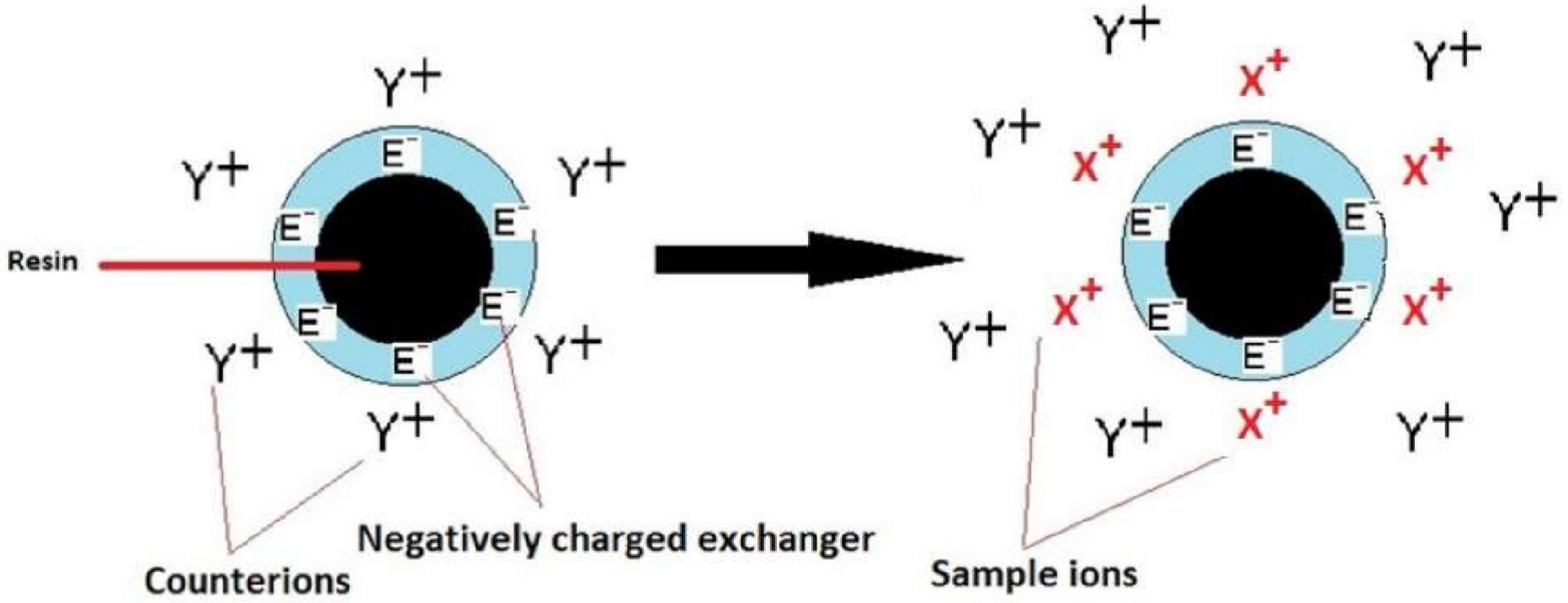
$$E^- Y^+ X^+ \rightarrow E^- X^+ Y^+ + \text{The remaining neutral and negatively charged particles}$$

- Desired bounded cation (X+) can now be eluted by either of the two ways;

1. By adding a component M+ having magnitude of charge more than that of X+ so that M+ will replace X+ and X+ will be eluting out.
2. By changing pH of the solvent (mobile phase so that X+ have no charge and is then unbounded from the matrix and can be eluted out.

Working

Ion Exchange Chromatography (Cation Exchange)



Exchange Between Y^+ and X^+ occurs

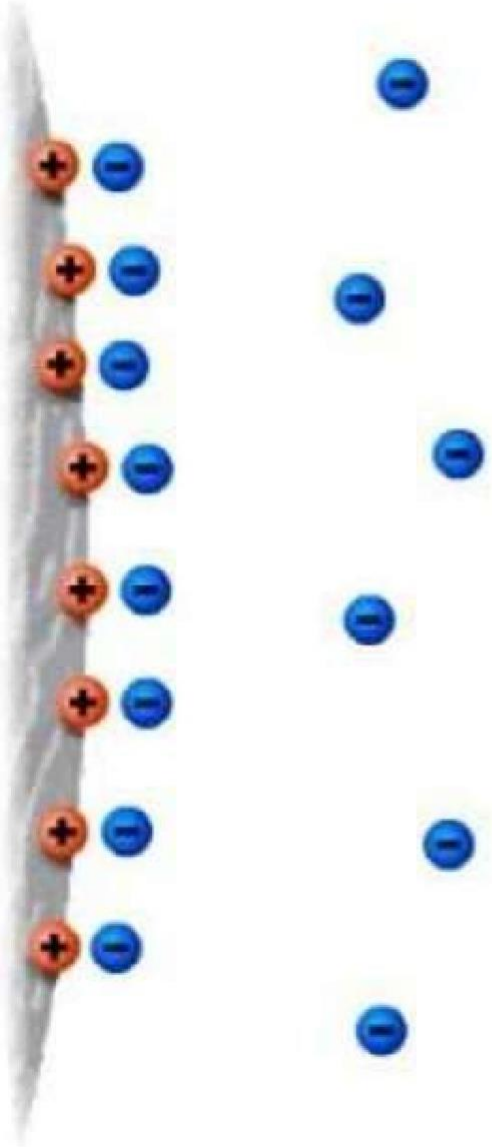
Working Principle:-

The 4 basic steps of ion exchange chromatography:-

1. Equilibration
2. Sample application and wash
3. Elution
4. Regeneration

Ion Exchange Chromatography

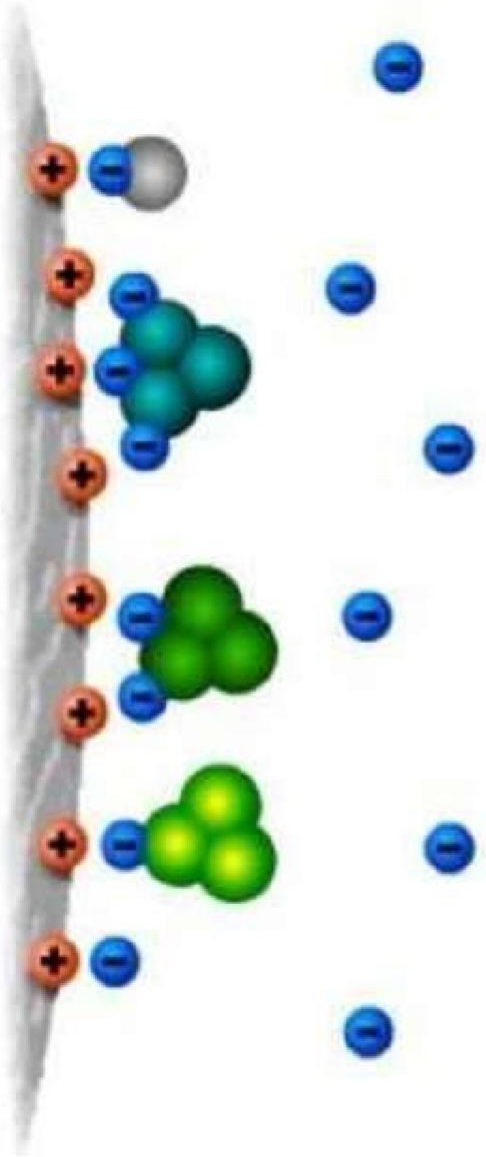
EQUILIBRATION



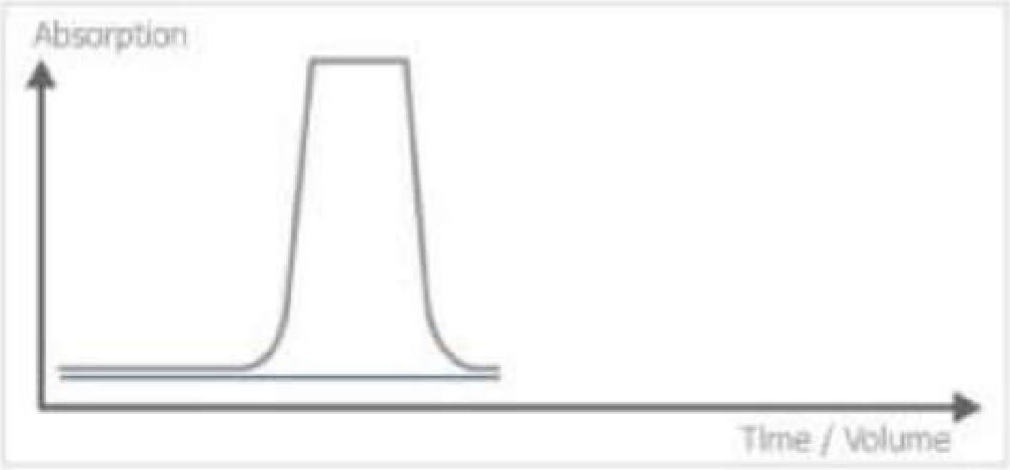
The first step is the equilibration of the stationary phase to the desired start conditions. When equilibrium is reached, all stationary phase charged groups are associated with exchangeable counter-ions, such as chloride or sodium, as shown by the blue (counter ions) and red (stationary phase charged groups) ions.

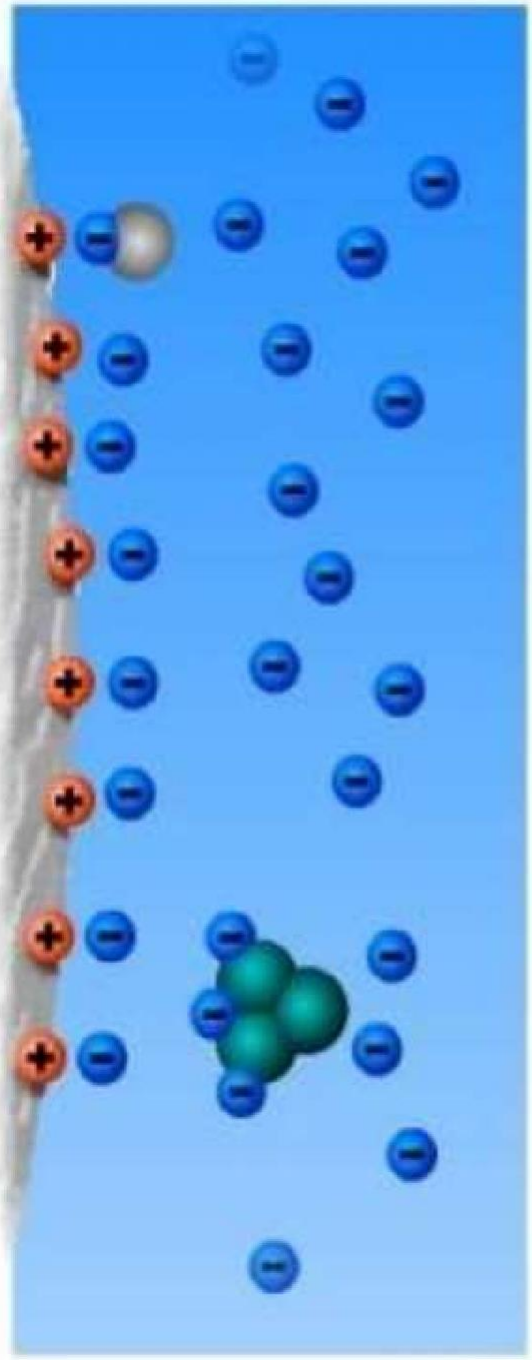
Ion Exchange Chromatography

SAMPLE APPLICATION AND WASH



The second step is sample application and wash. The goal in this step is to bind the target molecule/s and wash out all unbound material. The sample buffer should have the same pH and ionic strength as the starting buffer in order to bind all apropriately charged proteins.

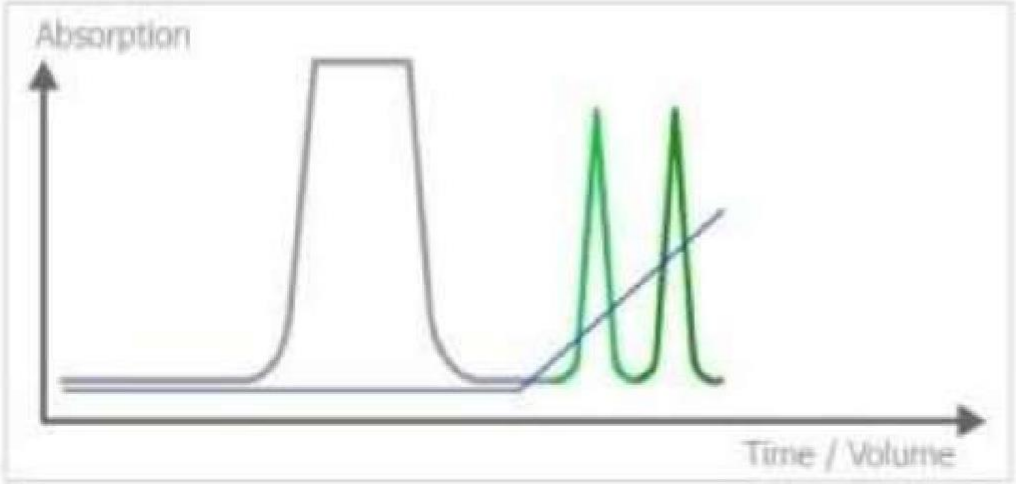


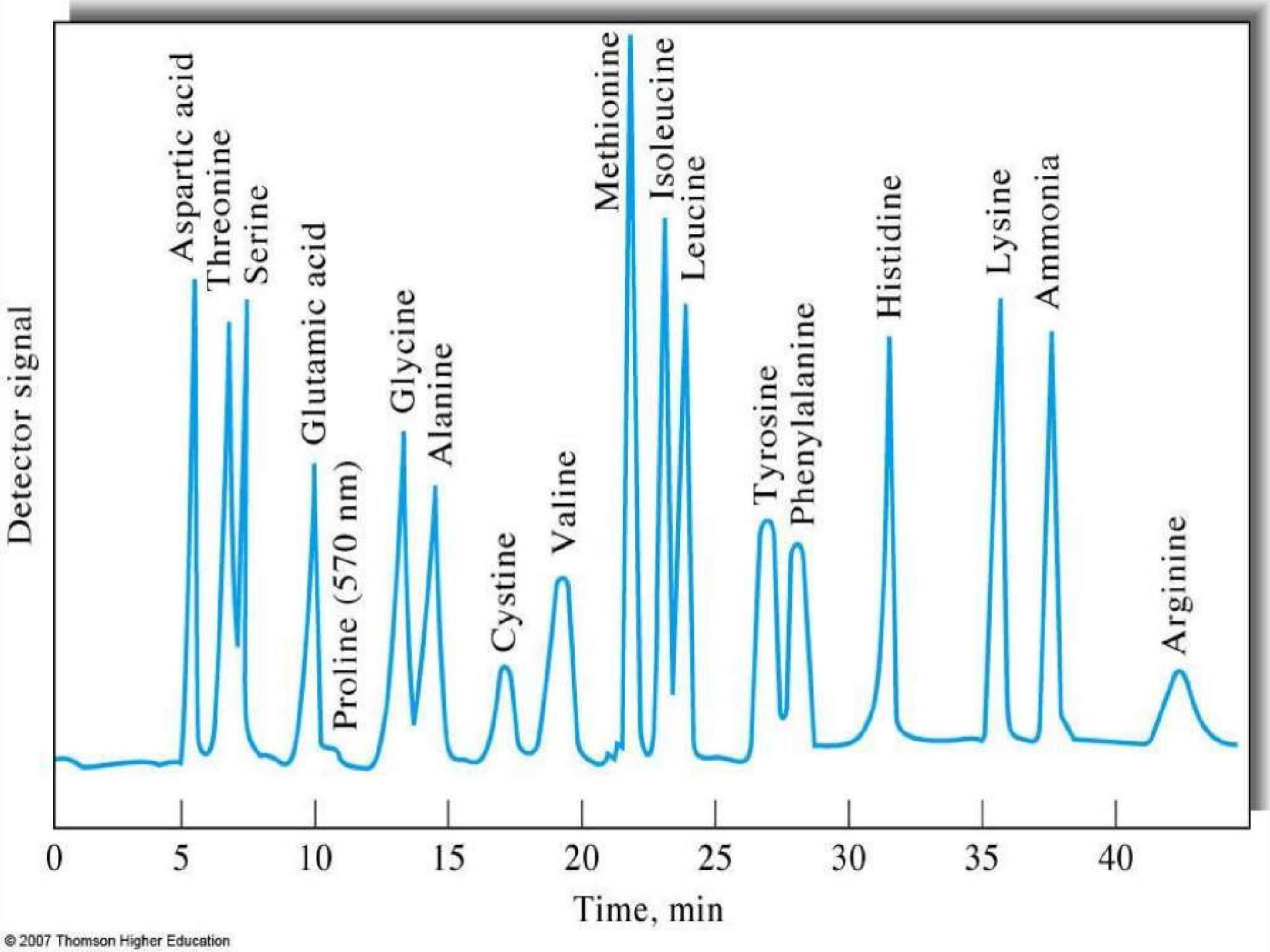


Ion Exchange Chromatography

ELUTION

In the third step, elution, biomolecules are released from the ion exchanger by a change in the buffer composition. A common way is to increase the ionic strength with sodium chloride, or another simple salt, in order to desorb the bound proteins. Proteins are desorbed relative to the number of charged groups on their surface.





Requirement:-

1.Column :-

glass, stainless steel or polymers

2.Packing the column :-

Wet packing method: A slurry is prepared of the eluent with the stationary phase powder and then carefully poured into the column. Care must be taken to avoid air bubbles.

3.Application of the sample:-

After packing, sample is added to the top of the stationary phase, use syringe or pipette.

This layer is usually topped with a small layer of sand or with cotton or glass wool to protect the shape of the organic layer from the velocity of newly added eluent.

Continue...

4. Mobile phase:-

Acids, alkalis, buffers.

5. Stationary phase:-

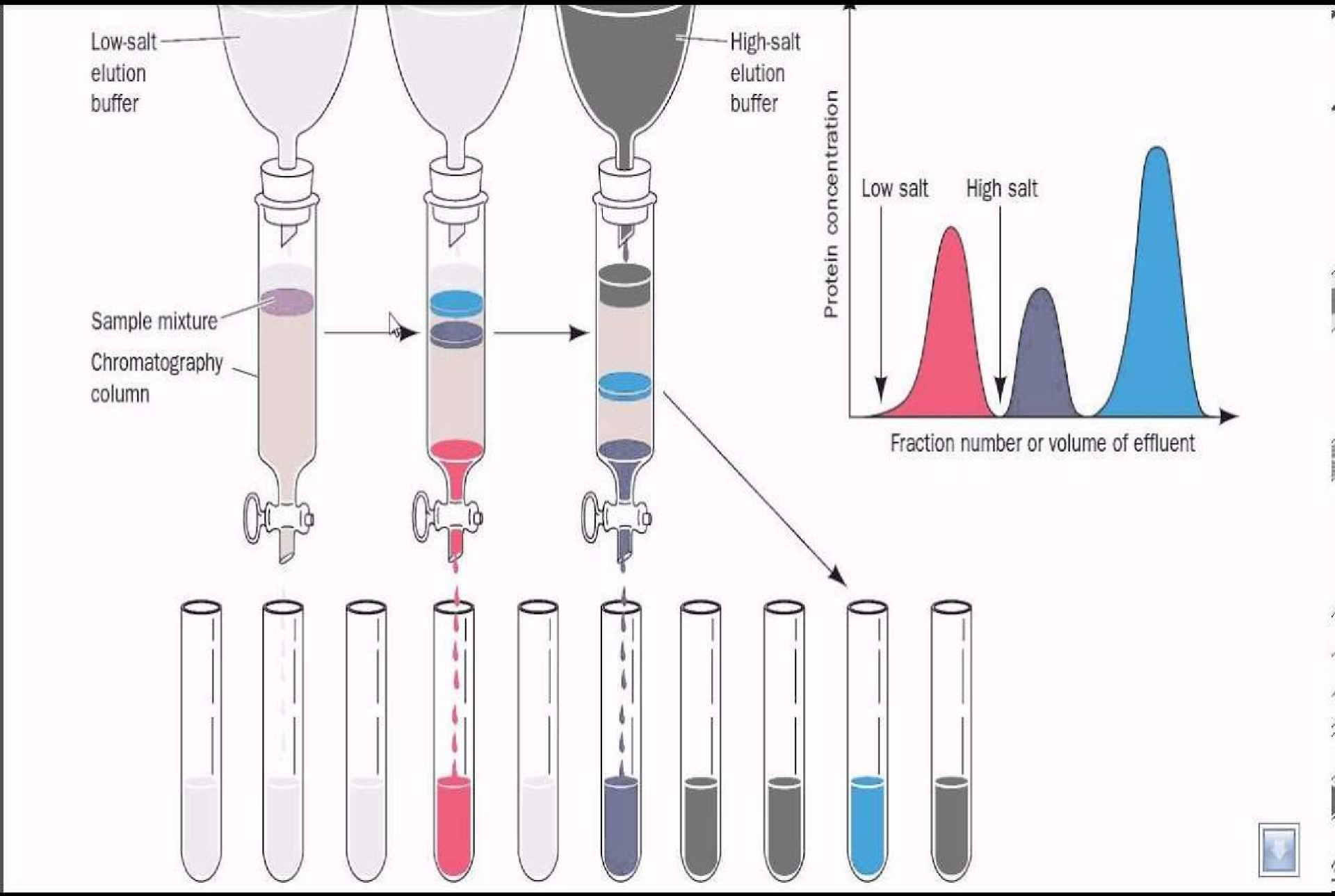
The ionic compound consisting of the cationic species (M+) and the anionic species (B-)

6. Elution:-

- Components of mixture separate & move down the column at different rates depending upon the affinity of the ion for ion exchanger.
- The eluates are collected at different stages

7. Analysis of the eluate:-

Spectrophotometric, flame photometry polarographic, conductometri.


Low-salt elution buffer
High-salt elution buffer
Sample mixture
Chromatography column
Protein concentration
Low salt
High salt
Fraction number or volume of effluent

Factors Affecting ion-exchange Separation:-

A . Ion Exchange Resin:

The swelling factor and cross linking is important for the effective separation. The cross linking should be controlled as its affects the exchanger's capacity. Swelling helps in proper exposure of charged functional groups for exchange of ions.

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. Concentration of solution.
5. Concentration & charge of ions

C . pH of the mobile phase

D . Ionic strength

E . Mobile phase modifiers

F . Temperature

G. Buffer: The pH of the buffer should impart the same charge to the sample ions as present in the Column. Anionic Exchange Chromatography should be carried out with cationic buffers and vice versa because buffer ion will indulge in ion exchange, which will be of no use.

Ion Exchangers:-

There are three classes of ion exchangers

1. Resins:- Ion exchange resins are used for the separation of small molecules

2. Gels:- Ion exchange gels are used for the separation of large molecules like proteins ,nucleic acids

3. Inorganic exchangers:- Separations involving harsh chemical conditions(high temperature , high radiation levels, strongly basic solutions or powerful oxidizing agents) employ inorganic ion exchangers

Classification of ion exchange resins:-

According to the chemical nature they classified as-

- Strongly acidic cation exchanger:-

sulphonic acid groups attached to styrene and divinyl-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.

- Weakly basic anion exchanger:-

poly alkyl amine groups attached to styrene and divinyl benzene co- polymer.

According to the source:-

- Natural resins :

  Cation - Zeolytes, Clay

  Anion - Dolomite

- Synthetic resins: Inorganic & Organic resins

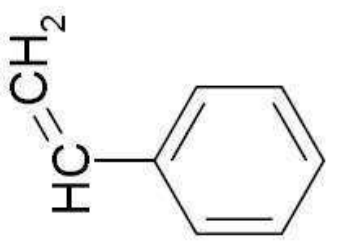
- Organic resins:- are polymeric resin matrix.

Ion Exchange Resin:-

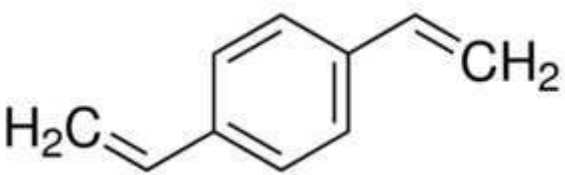
- Resins are amorphous particles of organic materials
- Polystyrene resins for ion exchange are made by co- polymerization of styrene and divinyl-benzene.
- Divinyl-benzene content is varied from 1 to 16 percent to increase the extent of cross linking.
- Benzene groups are modified to produce cation exchange resin and anion exchange resin
- Resin made from both of these materials differ in their flow properties, ion accessibility, and chemical and mechanical stability.
- Selection of one or the other type of resin is done on the basis of compounds being separated

Polystyrene resins:-

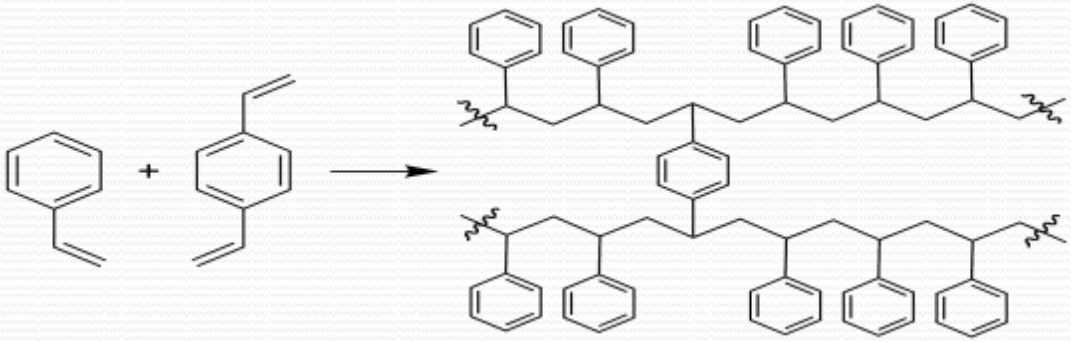
- Polystyrene resins are prepared by polymerisation reaction of styrene and divinyl-benzene.
- Higher concentration of divinyl-benzene produces higher cross linkages.
- Polystyrene resin are very useful for separating small molecular weight compounds, however, unsatisfactory for the separation of macromolecules



styrene



divinylbenzene



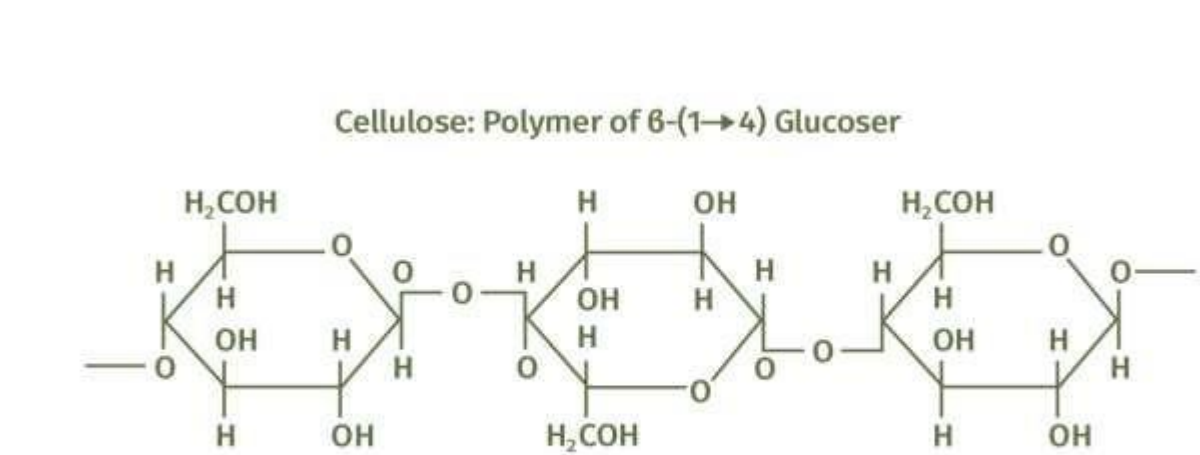
Ion exchange gels:-

- Cellulose and dextran ion exchangers , which are polymers of the sugar glucose , posses larger pore sizes and lower charge densities.

- Because they are much softer than polystyrene resins , dextran and its relatives are called gels .

Cellulose :-

- Cellulose is a high molecular weight compound which can be readily obtained in a high pure state.
- Cellulose has much greater permeability to macromolecules



Exchange Medium

- The choice of ion exchangers depends upon the stability, molecular weight, and ionic strength of the sample components.
- The volume of exchanger used for separation is usually 2.5 fold greater than to exchange with the ion in the sample.
- The ion exchanger are packed in column having suitable buffer.
- The ion exchangers are of two types;

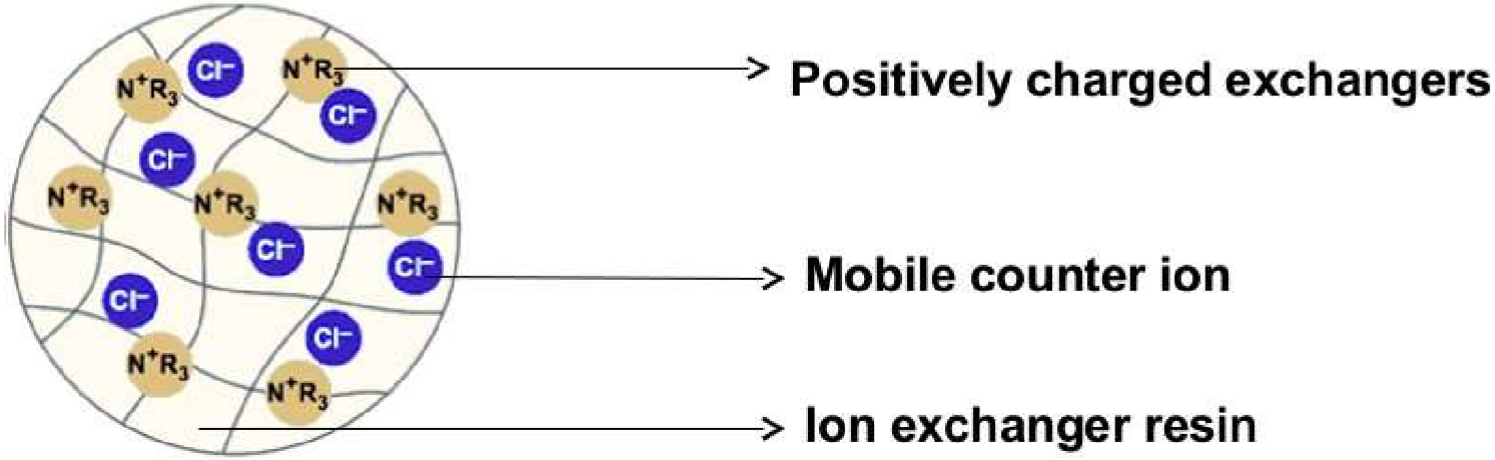
  Cation Exchangers

  Anion Exchangers

1. 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

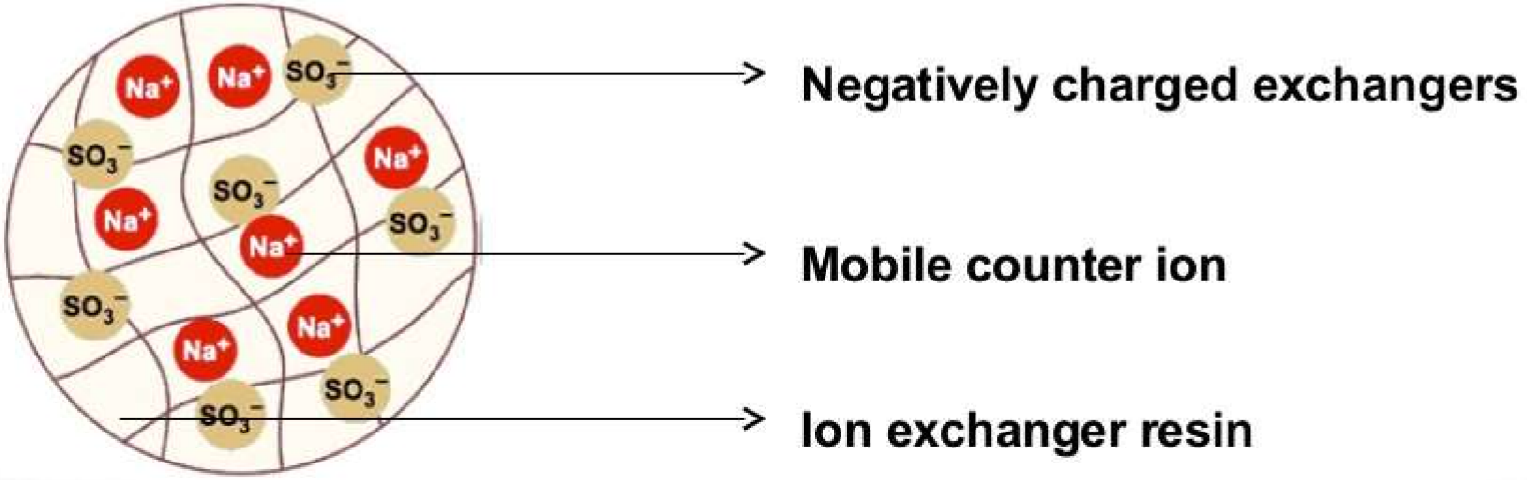
  Secondary amines ⟹ Weak anion exchangers



2. 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



Anion and cation Exchangers

Examples

Strong anion exchangers		
$-CH_2N^+(CH_3)_3$	trimethylaminoethyl	TAM
$-C_2H_4N^+(C_2H_5)_3$	triethylaminoethyl	TEAE
Weak anion exchangers		
$-C_2H_4N^+H_3$	aminoethyl	AE
$-C_2H_4N^+(C_2H_5)_2$	diethylaminoethyl	DEAE
Strong cation exchangers		
$-SO_3^-$	sulpho	S
$-CH_2SO_3^-$	sulphomethyl	SM
Weak cation exchangers		
$-CH_2COO^-$	carboxymethyl	CM

adapted from N & B Table 5-2

Advantages

- Cost effective
- Re-usable
- Easily collectable
- Low maintenance cost
- Efficient technique
- Quick separation

APPLICATIONS:-

- Softening of hard water
- Demineralization of water
- To analyze base composition of nucleic acid
- To concentrate the metal ions in the sample
- To measure the additives in food and drug sample
- To separate protein mixtures
- For extraction of enzymes from tissues.
- Purification of solutions free from ionic impurities.
- Separation of inorganic ions.
- Separation of sugars, amino acids and proteins.
- Ion exchange column in HPLC.