Ion exchange chromatography

Ion exchange chromatography (IEC) is a technique that is commonly used in biomolecule purification. It involves the separation of molecules on the basis of their charge. This technique exploits the interaction between charged molecules in a sample and oppositely charged moieties in the stationery phase of the chromatography matrix. Two types of ion exchange separation is possible cation exchange and anion exchange.

In **cation exchange chromatography positively charged molecules** are attracted to a negatively charged solid support. Commonly used **cation exchange resins** are sulfate derivatives**(S-resin)** and carboxylate derived ions **(CM resins**). Conversely,

in **anion exchange chromatography**, **negatively charged molecules** are attracted to a positively charged solid support. Commonly used **anion exchange resins** are, a Quaternary amine **(Q-resin)**; and DiEthyl Amino Ethane **(DEAE)** resin.

The reasons for the success of ion exchange are: its widespread applicability, its high resolving power, its high capacity and the simplicity and controllability of the method.

Principle of ion exchange chromatography:

IEX 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. The molecules separated on the basis of their charge are eluted using a solution of varying ionic strength. By passing such a solution through the column, highly selective separation of molecules according to their different charges takes place.

Pros and Cons of Ion Exchange Chromatography:

IEX Pros	IEX Cons
Permits high flow rate	Sample must be loaded at low ionic strength
Concentrates samples	Clusters of positively charged residues can cause a net-negatively charged protein to bind a cation exchanger, and vice versa
High yield	Small changes in pH can greatly alter binding profile of IEX resin

Buffers are nondenaturing	Particle size greatly influences resolution

Varying pH

A pH gradient can be applied to elute individual proteins on the basis of their isoelectric point (pI) i.e. the point at which the amino acids in a protein carry neutral charge and hence do not migrate in an electric field. As amino acids are zwitter ionic compounds they contain groups having both positive and negative charges.

Based on the pH of the environment, proteins carry a positive, negative, or nil charge. At their isoelectric point, they will not interact with the charged moieties in the column resin and hence are eluted.

A decreasing pH gradient can be used to elute proteins using an anion exchange resin and an increasing pH gradient can be used to elute proteins from cation exchange resins. This is because increasing the buffer pH of the mobile phase causes the protein to become less protonated (less positively charged) so it cannot form an ionic interaction with the negatively charged resin, allowing is elution. Conversely, lowering the pH of the mobile phase will cause the molecule to become more protonated (less negatively charged), allowing its elution.

Resin Selection in Ion Exchange Chromatography:

Ion exchange resins have positively or negatively charged functional groups covalently linked to a solid matrix. Matrices are usually made of cellulose, polystyrene, agarose, and polyacrylamide.

Some of the factors affecting resin choice are :

1-Anion or cation exchanger 2- Flow rate
3- Weak or strong ion exchanger 4- Particle size of the resin
5- Binding capacity.

The Applications of Ion Exchange Chromatography

1- Separation and Purification of blood components such as albumin,recombinant growth factors and enzymes.
2- Biotechnology : Analytical applications such as quality control and process monitoring.
3- Food and clinical research - to study wheat varieties and the correlation of proteinuria with different renal diseases.

4- Fermentation - Cation exchange resins are used to monitor the fermentation process during ß-galactosidase production.

The Technique:

Key steps in the ion exchange chromatography procedure are:

1-A crude protein sample is loaded into the ion exchange chromatography column at a particular pH.

2- Charged proteins will bind to the oppositely charged functional groups in the resin.

3- A salt gradient is used to elute separated proteins. At low salt concentrations, proteins having few charged groups are eluted and at higher salt concentrations, proteins with several charged groups are eluted.

4- Unwanted proteins and impurities are removed by washing the column.

Ion exchange chromatography column

DEAE- Sephadex preparation

DEAE- Sephadex gel have positive charged groups(Anion exchangers)

1- Dissolve (10g) of gel powder in 100 ml D.W

2-Left the powder to settle down, remove the supernatant and repeat dissolving in water for several times until it was become completely clear.

3- Degas the gel by using vacuum pump.

4- Activate DEAE-Sephadex gel with 0.25 M NaCl for 30 min and wash it with D.W .

5- Pour the gel into column and left to package

6- Equilibrate the column with equilibration buffer.

Column preparation

In this technique:

1- Apply the dialysed enzyme to DEAE –sephadex column then equilibrate the column and wash it with an equal volume of 0.01M phosphate buffer solution (pH 7) to wash uncharged and positively charged proteins in the sample.

2- Elution the bound proteins (negatively charged) by using gradient concentrations of sodium chloride ranged between 0.1 and 0.5 M.

3- Detect all protein peaks by measuring the absorbance at 280 nm of each eluted fraction by using UV spectrophotometer .

NaCl (0.25 M):

It was prepared by dissolving 1.461g of NaCl in100ml D.W.

Sodium chloride phosphate Solution

It was prepared at different concentrations of NaCl (0.1, 0.2, 0.3, 0.4, and 0.5 M) in 20mM Potassium phosphate buffer.

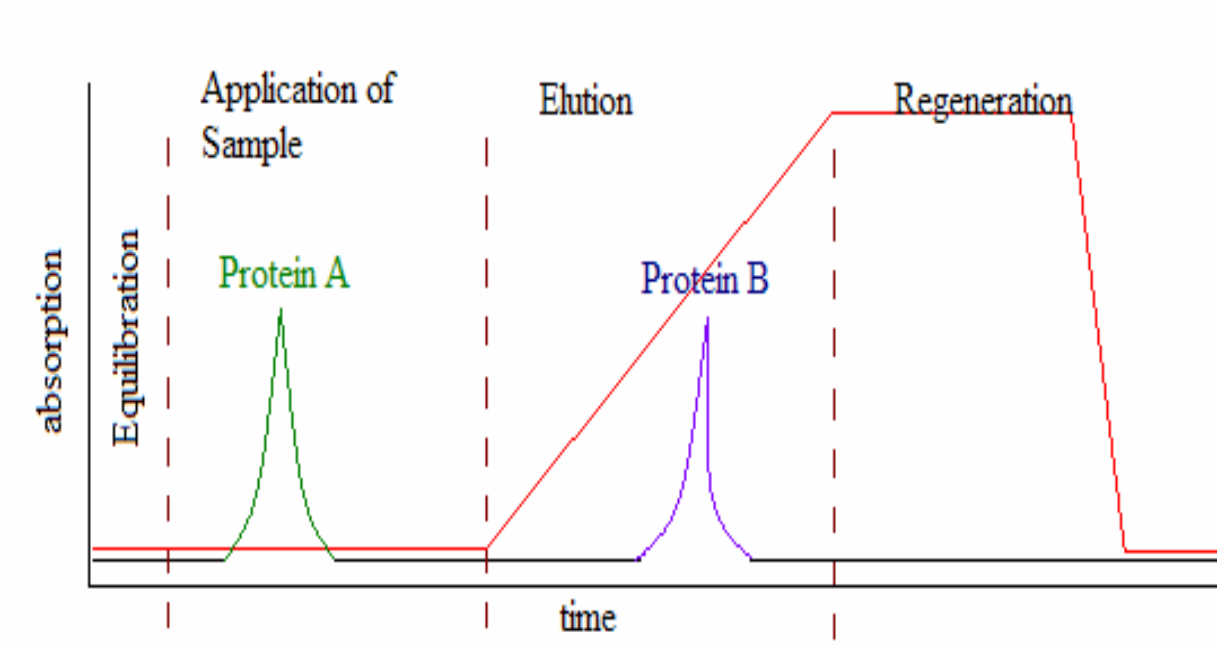
0.1M NaCl =0.5844 gm in 100ml DW

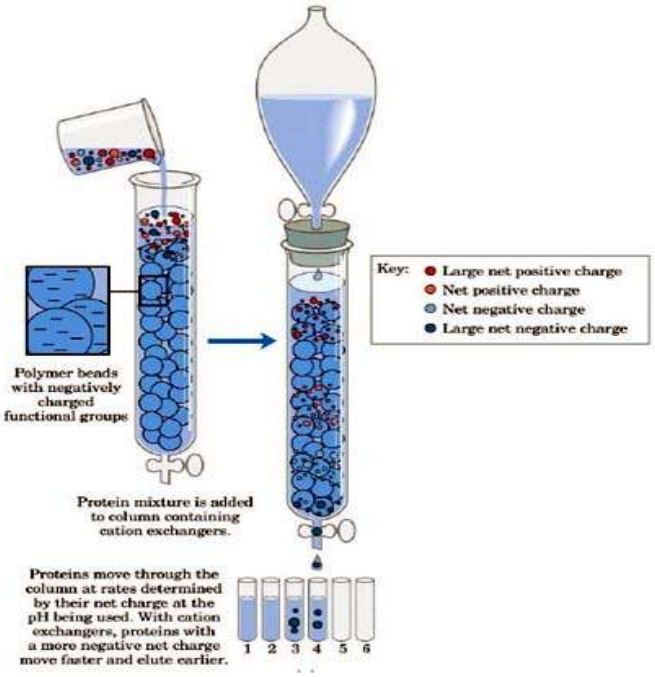
0.2M NaCl =1.1688 gm in 100ml DW

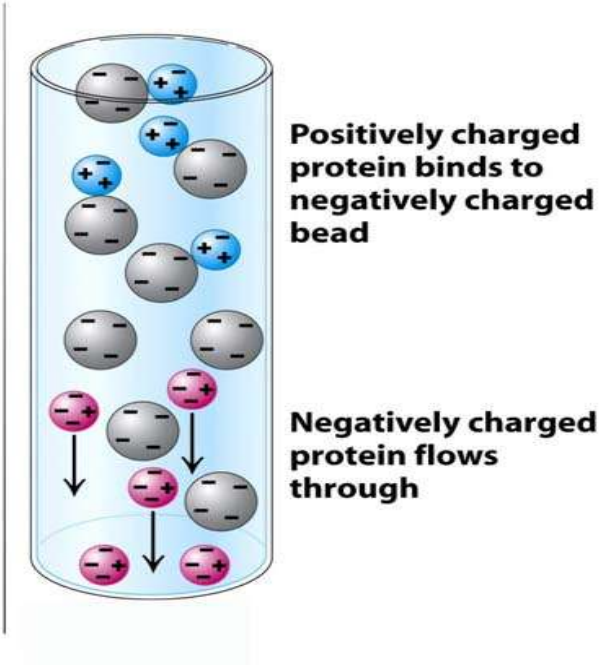
0.3M NaCl =1,7532 gm in 100ml DW

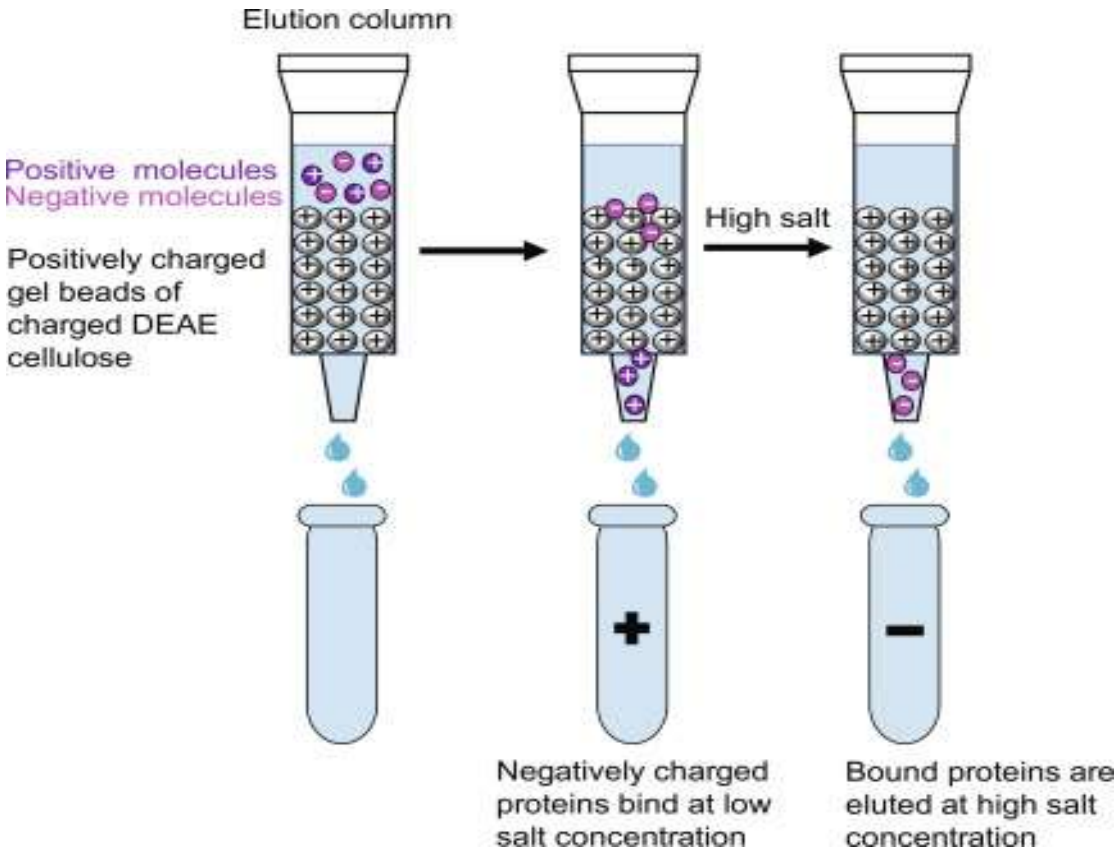
0.4M NaCl =2.3376 gm in 100ml DW

0.5M NaCl =2.922 gm in 100ml DW




Key:
Large net positive charge
Net positive charge
Net negative charge
Large net negative charge
Polymer beads with negatively charged functional groups
Protein mixture is added to column containing cation exchangers.
Proteins move through the column at rates determined by their net charge at the pH being used. With cation exchangers, proteins with a more negative net charge move faster and elute earlier.
1 2 3 4 5 6


Positively charged protein binds to negatively charged bead
Negatively charged protein flows through


Elution column
Positive molecules
Negative molecules
Positively charged gel beads of charged DEAE cellulose
High salt
Negatively charged proteins bind at low salt concentration
Bound proteins are eluted at high salt concentration