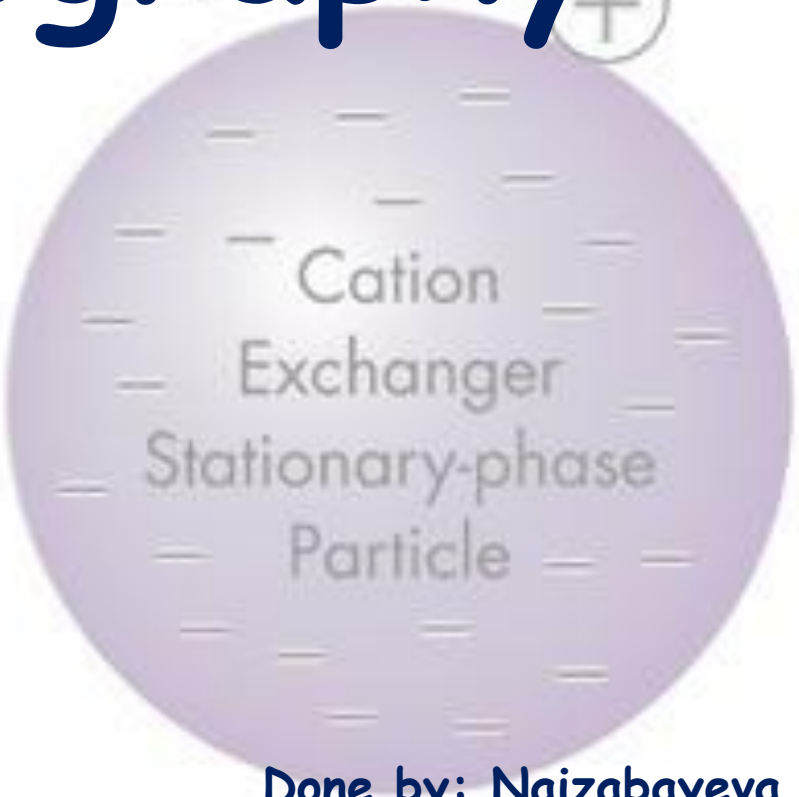
Ion exchange chromatography



Done by: Naizabayeva D.
Accepted by: Kenzhebayeva S.S.

Content

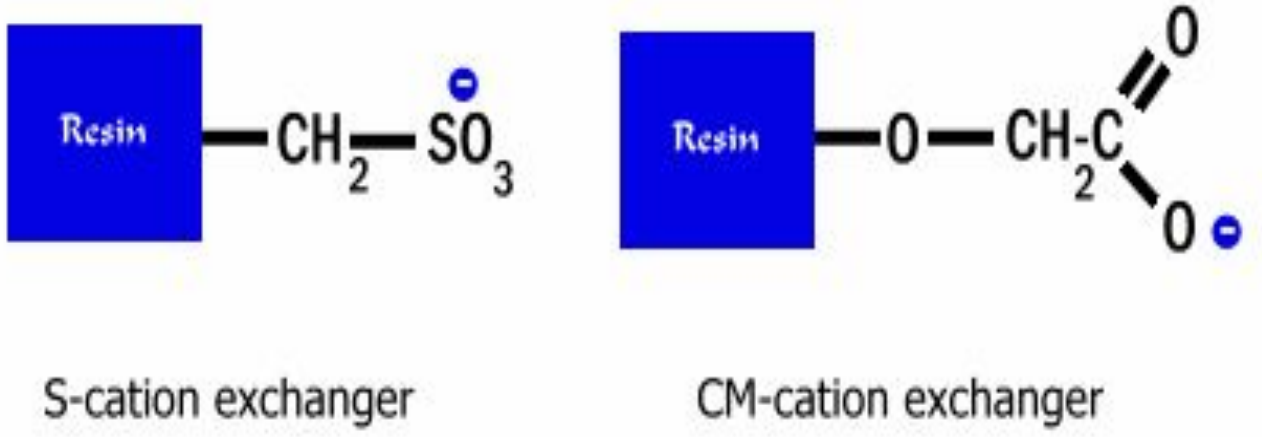
1. Introduction
2. Procedure
3. Advantages/ disadvantages

1. Introduction

Ion exchange chromatography occurs due to electrostatic attraction between buffer-dissolved charged proteins and oppositely charged binding sites on a solid ion exchange adsorbent. An ion exchange adsorbent (also called media, resin, gel, or matrix) usually consists of spherical porous inert beads with charged groups (functional groups) densely grafted onto the beads' surfaces; the charges of functional groups are neutralized by free counter-ions.

Cation exchange chromatography

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

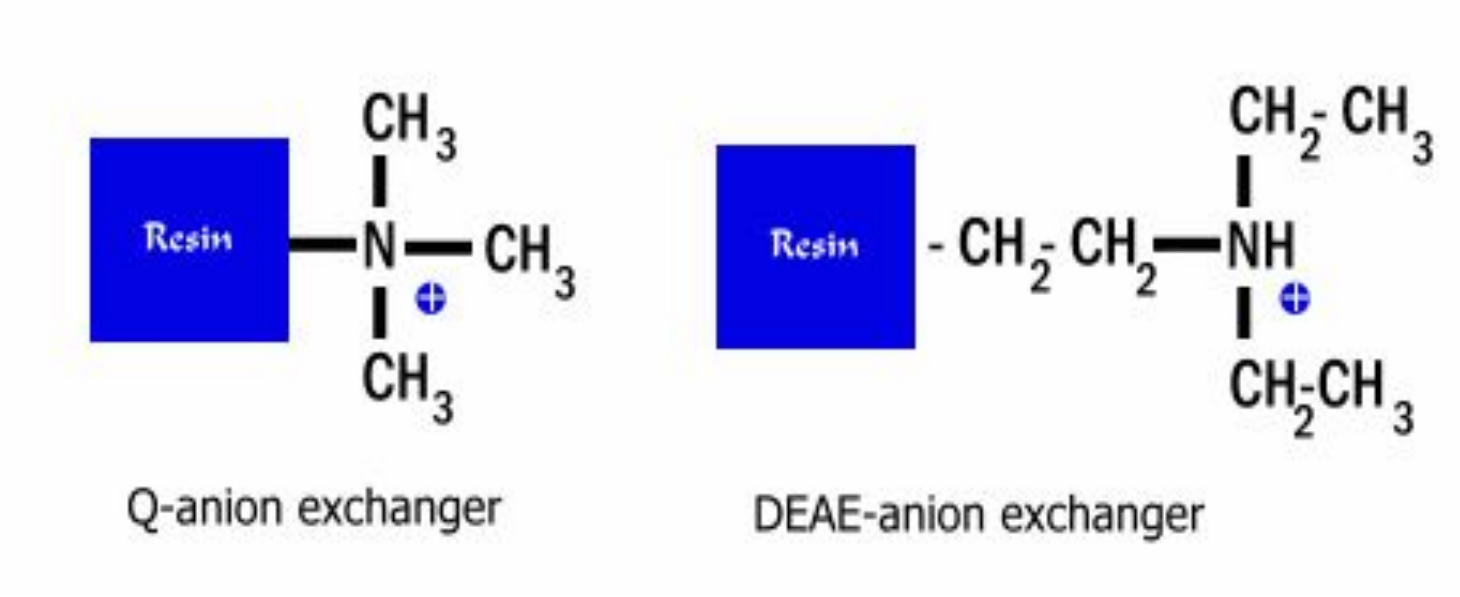


S-cation exchanger

CM-cation exchanger

Anion exchange chromatography

*negatively charged molecules is attracted to a positively charged solid support. Commonly used anion exchange resins are Q-resin, a Quaternary amine; and DEAE resin, DiEthylAminoEthane



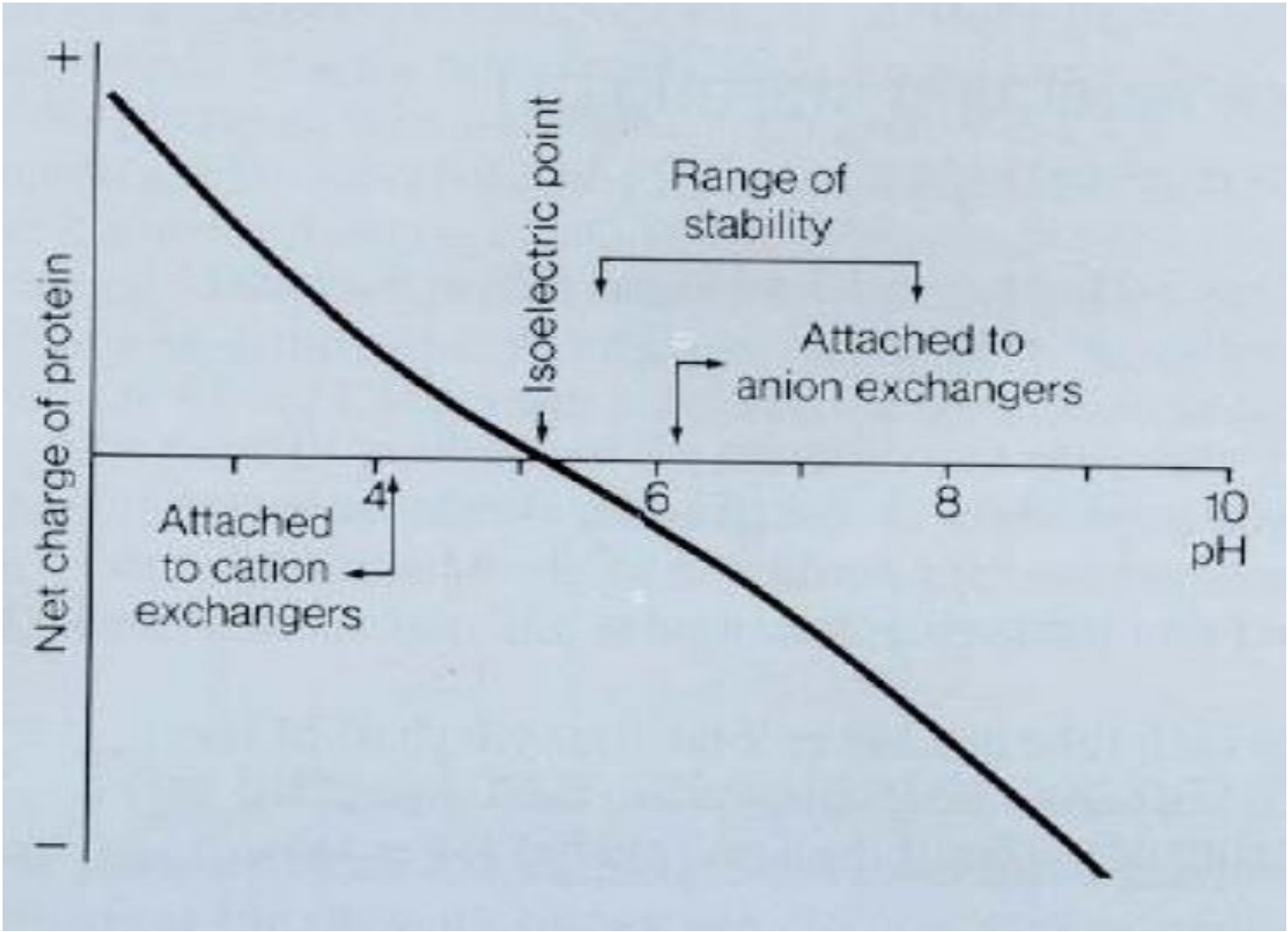
Procedure

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

1. 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 bound with exchangeable counterions, such as chloride or sodium. The pH and ionic strength of the start buffer are selected to ensure that, when sample is loaded, proteins of interest bind to the medium and as many impurities as possible do not bind.

Important to consider the stability of proteins in choice of ion exchangers. Isoelectric focusing can be used to identify suitable ion-exchanger type



Recommended Buffers for Polypeptide Ion-Exchange Chromatography

A wide range of buffers are available for use with ion-exchange chromatography. Recommended buffers for various ranges of pH are listed below.

Anion-Exchange Chromatography Buffers

Buffers for anion exchange are generally basic amines.

Buffer	Concentration	Anion	pKa	Buffering Region
L-histidine	20 mM	Cl-	6.15	5.5 - 6.8
bis-Tris	20 mM	Cl-	6.50	5.8 - 7.0
bis-Tris propane	20 mM	Cl-	6.80	6.4 - 7.3
Triethanolamine	20 mM	Cl-	7.77	7.3 - 8.2
Tris	20 mM	Cl-	8.16	7.5 - 8.8
diethanolamine	20 mM	Cl-	8.88	8.4 - 9.4

Cation Exchange Chromatography Buffers

Buffers for cation-exchange chromatography are acids.

Buffer	Concentration	Cation	pKa	Buffering Region
formate	20 mM	Na+	3.75	3.3 - 4.3
acetate	20 mM	Na+	4.76	4.2 - 5.2
MES	20 mM	Na+	6.15	5.5 - 6.7
phosphate	20 mM	Na+	2.1/7.2	2.0 - 7.6
HEPES	20 mM	Na+	7.55	7.6 - 8.2

2. 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 start buffer in order to bind all charged target proteins. Oppositely charged proteins bind to ionic groups of the IEX medium, becoming concentrated on the column. Uncharged proteins, or those with the same charge as the ionic group, pass through the column at the same speed as the flow of buffer, eluting during or just after sample application, depending on the total volume of sample loaded.

3. Elution

Elution with salt gradient. Addition of salt increases the number of ions competing with proteins for functional groups on the stationary phase. Proteins spend more time in the solution, the rate of their movement down the column increases dramatically, and proteins begin to elute from the column, usually in order of increasing charge. Most proteins are eluted at NaCl concentrations < 1M.

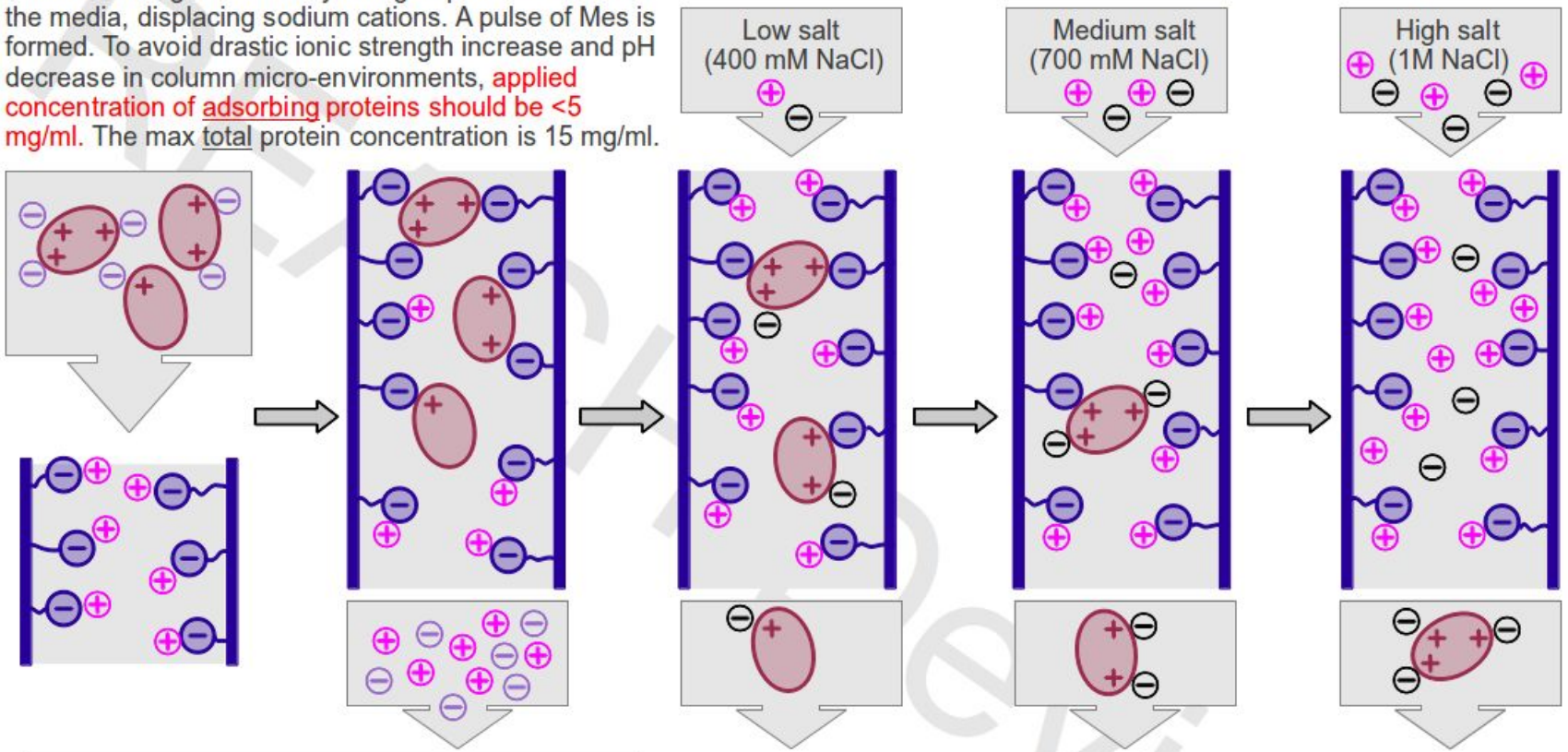
Elution by pH change. Change of pH in the column can be aimed to decrease the net absolute value of the charges of adsorbed proteins, decrease their attraction to the stationary phase, and accelerate the elution. In practice, pH changes in the column are difficult to control, as they do not reliably correspond to pH changes of the applied eluting buffer. This happens because of the buffering power of proteins adsorbed to the column and, for weak ion exchangers (see below), buffering power of the adsorbent functional groups themselves. Resolution of proteins by pH elution is achieved in a separate technique called "Chromatofocusing."

Elution by affinity. Affinity elution can be achieved for a specific protein if and only if an oppositely charged ligand that will strongly bind to this protein is known and available. Addition of such a ligand to the eluting buffer will produce a protein+ligand species with a smaller absolute value of the net charge, and therefore the targeted protein will bind less to the stationary phase. Affinity elution is often useful in enzyme purifications.

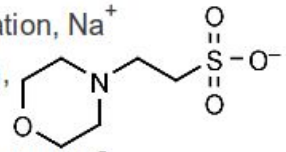
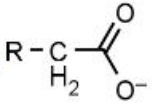
Mechanism

A mixture of proteins in Mes buffer is loaded into the cation exchanger. Positively charged proteins adsorb to the media, displacing sodium cations. A pulse of Mes is formed. To avoid drastic ionic strength increase and pH decrease in column micro-environments, applied concentration of adsorbing proteins should be <5 mg/ml. The max total protein concentration is 15 mg/ml.

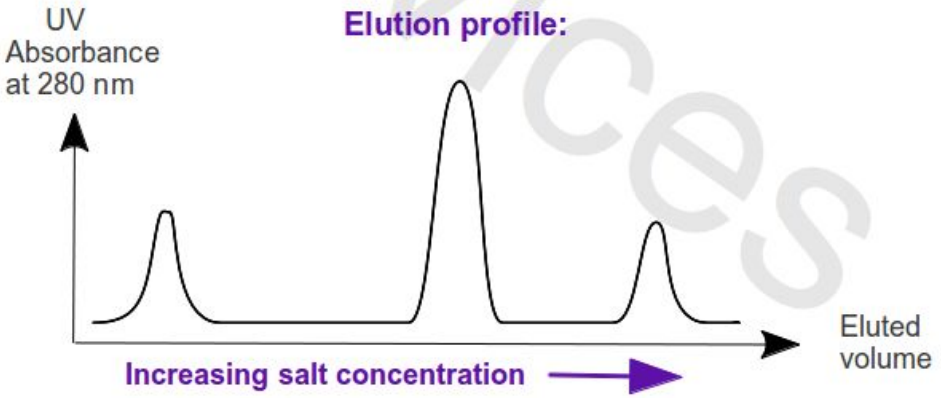
Proteins are eluted with increasing salt (NaCl) gradient



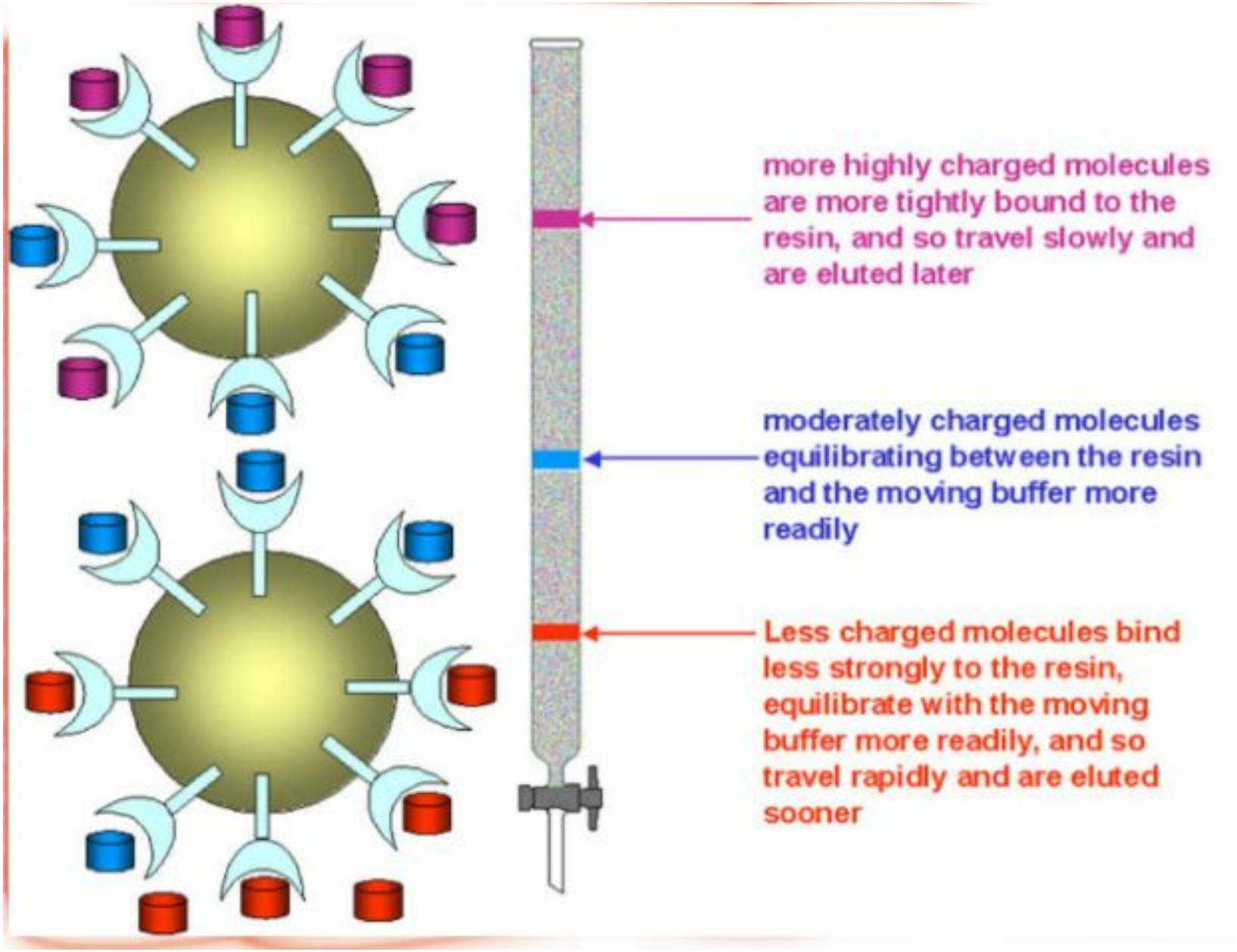
Legend:

- ⊕ – Sodium cation, Na^+
- ⊖ – Mes anion, 
- ⊖ – Chloride anion, Cl^-
- ⊖ – Carboxymethyl anion (CM), 
- – Protein bearing a number of positive charges (as marked)

Elution profile:



Mechanism


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

4. Regeneration

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

Anion exchange resin is regenerated by treatment with NaOH, then washing with water

Advantages VS Disadvantage

It is a non-denaturing technique. It can be used at all stages and scales of purification

- ✔An IEX separation can be controlled by changing pH, salt concentration and/or the ion exchange media
- ✔It can serve as a concentrating step. A large volume of dilute sample can be applied to a media, and the adsorbed protein subsequently eluted in a smaller volume
- ✔It offers high selectivity; it can resolve molecules with small differences in charge.

- ✔costly equipment and more expensive chemicals
- ✔ Mass transport is provided in quite time referred to be longer than other methods
- ✔ Require huge amounts of solutions

Thanks for attention!