

Chromatography

Ion Exchange Chromatography

Definition

- **Ion-exchange chromatography** (or *ion chromatography*)
- It is a process that allows the separation of **ions** and **polar molecules** based on the charge properties of the molecules.



ION-EXCHANGE CHROMATOGRAPHY

- It is the process by which similar charged ions such as cations, anions can be separated.
- **PRINCIPLE**
- **The principle of separation is by reversible exchange of ions**
- By using the suitable ion exchange resin it can be separated.
- It exchanges the ions according to their **relative affinities**.
- The exchange takes place in a reversible manner between the **ions of the solution** and the ions present in the **ion exchange resin**.

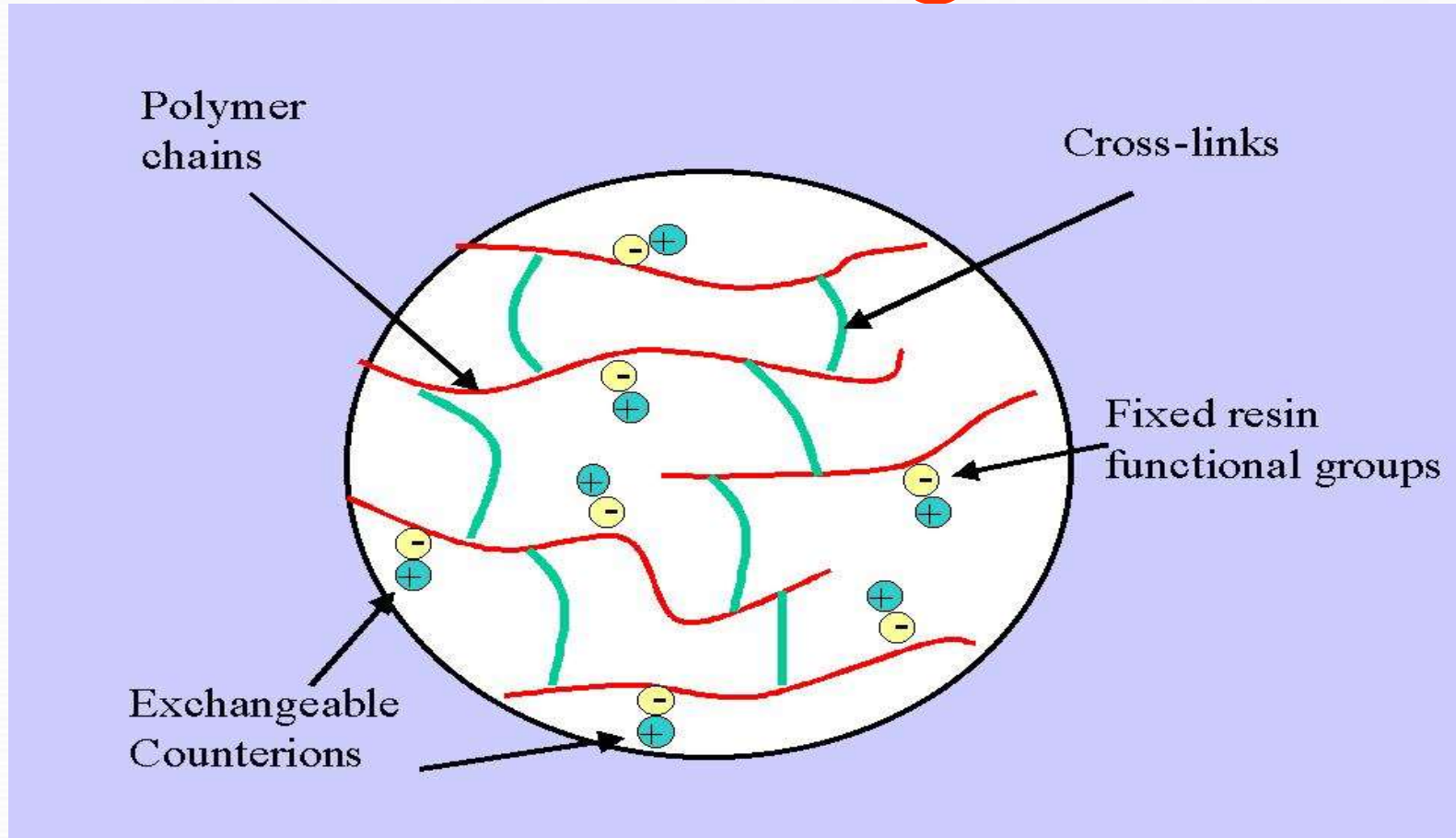
Ion-exchange chromatography

- The solution to be injected is usually called a *sample*, and the individually separated components are called *analytes*
- It can be used for almost any kind of charged molecule including *large proteins*, *small nucleotides* and *amino acids*.
- It is often used in *protein purification*, *water analysis*.

Principle

- Ion exchange chromatography retains analyte molecules based on ionic interactions.
- The stationary phase surface displays **ionic functional groups (R-X)** that interact with analyte ions of opposite charge.
- This type of chromatography is further subdivided into:
 1. **cation exchange chromatography**
 2. **anion exchange chromatography.**

Ion Exchangers



Ion exchangers – Functional groups

Anion exchanger

- Aminoethyl (AE-)
- Diethylaminoethyl (DEAE-)
- Quaternary aminoethyl (QAE-)

Cation exchanger

- Carboxymethyl (CM-)
- Phospho
- Sulphopropyl (SP-)

Cation (+vely) exchange chromatography

- Cation exchange chromatography retains positively charged cations because the stationary phase displays a negatively charged functional group



Anion (-vely) exchange chromatography

- Anion exchange chromatography retains anions using positively charged functional group:



Procedure

1. **A sample** is introduced, either manually or with an autosampler, into a sample loop of known volume.
2. **The mobile phase** (buffered aqueous solution) carries the sample from the loop onto a column that contains some form of stationary phase material.
3. **Stationary phase material** is a resin or gel matrix consisting of agarose or cellulose beads with covalently bonded charged functional groups.

Procedure

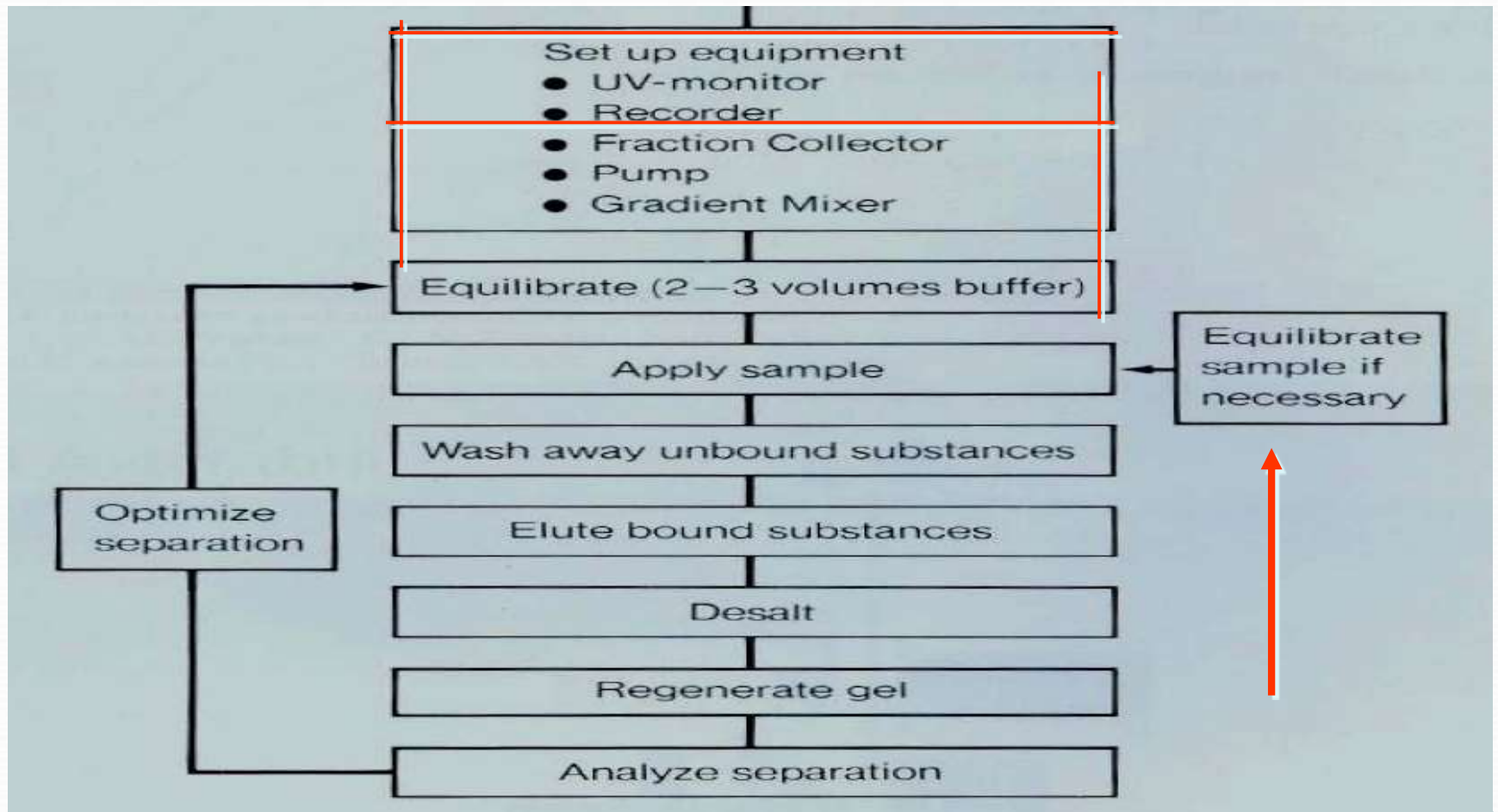
4. **The target** analytes (anions or cations) are retained on the **stationary phase** but can be eluted by increasing the concentration of a similarly charged species that will displace the analyte ions from the stationary phase.

For example, in cation exchange chromatography, the **positively charged** analyte could be displaced by the addition of **positively charged sodium ions**.

Procedure

5. **The analytes** of interest must then be detected by some means, typically by conductivity or UV/Visible light absorbance.
6. **A chromatography data system** (CDS) is usually needed to control an IC.

Procedure



Separating proteins

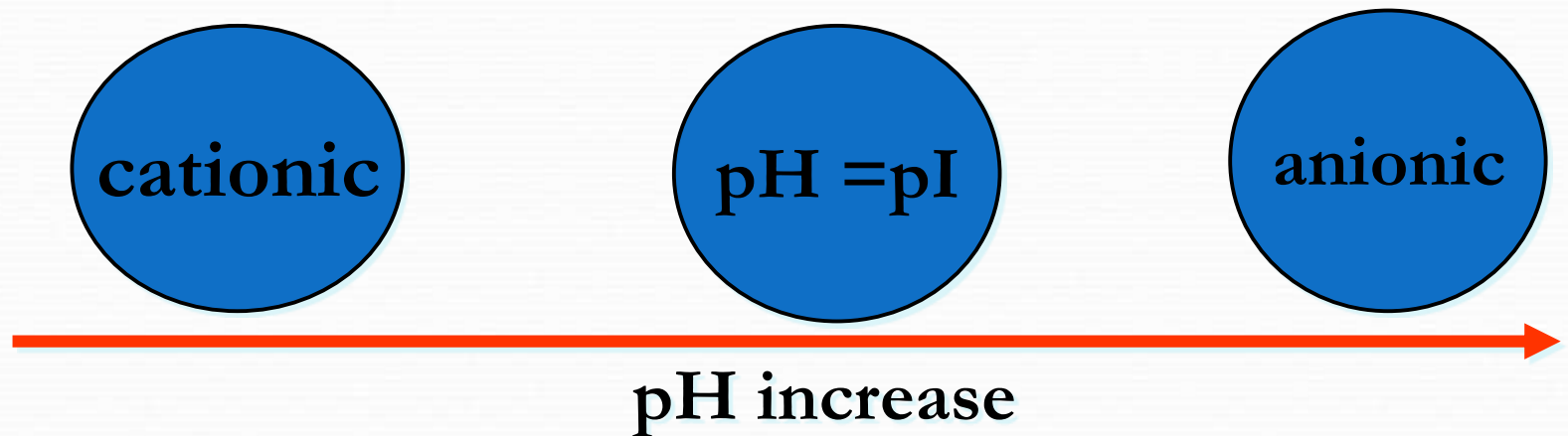
- Proteins have numerous functional groups that can have both positive and negative charges.
- Ion exchange chromatography separates proteins according to their net charge, which is dependent on the composition of the mobile phase.

Affect of pH in the separation of proteins

- By adjusting the pH or the ionic concentration of the mobile phase, various protein molecules can be separated.
- For example, if a protein has a net positive charge at pH 7, then it will bind to a column of negatively-charged beads, where as a negatively charged protein would not.

Effect of pH in the separation of proteins

- Proteins are charged molecules. At specific pH, it can exist in **anionic** (-), **cationic** (+) or **zwitterion** (no net charge) stage.



*pI isoelectric point

Choosing your ion-exchanger: know your proteins

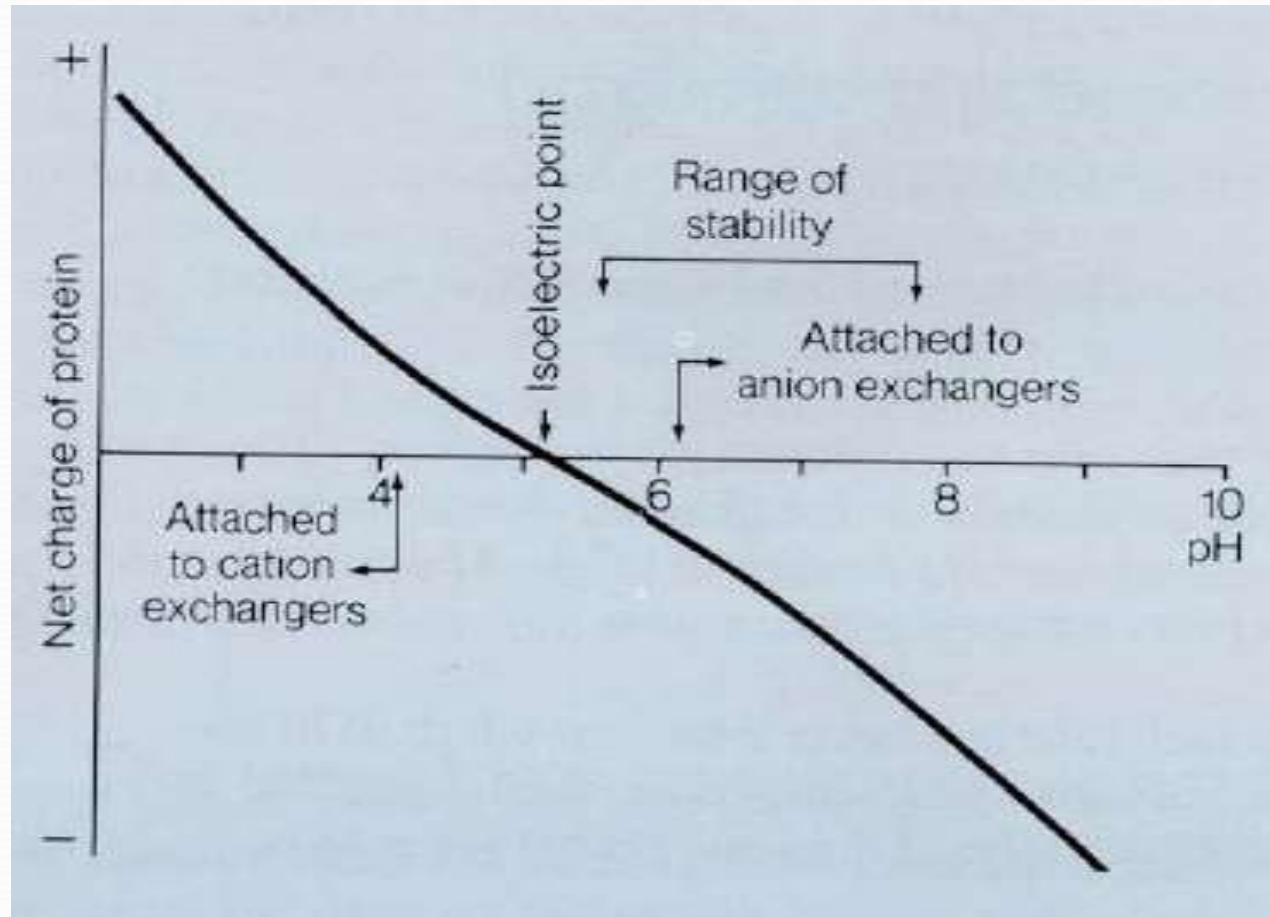
1. Stability of proteins

- stable below pH value, use **cation-exchanger**
- stable above pH value, use **anion-exchanger**

2. Molecular size of proteins

- <10,000 mw, use matrix of small pore size
- 10,000-100,000 mw, use Sepharose equivalent grade

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



Advantages

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

Advantages

- **Softening of water** : removal of monovalent and divalent ions like sodium, potassium, calcium etc
- Demineralisation or deionisation of water
- Separation of inorganic ions (anions & Cations)
- Organic separations : amino acids, proteins, antibiotics, vitamins, fatty acids etc are separated.
- Biochemical separations : like isolation of some drugs or metabolites from blood, urine etc.

Advantages


- Concentration of ionic solutions
- Ion exchange column in HPLC : separations of compounds of mixed nature are used in HPLC.

Disadvantages

- ✓ costly equipment and more expensive chemicals
- ✓ turbidity should be below 10ppm

Factors affecting ION EXCHANGE RESINS

- Nature and properties of ion exchange resins.
crosslinking and swelling is important factor which depends on the proportion of cross linking agent and polystyrene.
If more then more rigid, but swells less and separation of ions of different sizes is difficult as the can't pass through the pores present and it becomes selective to ions of different sizes.



Less cross linking agent is present, they are less rigid but swell more. It more separation will not be efficient as exchange of functional groups does not take place due to wide pore.

Optimum quantity of cross linking agent should be added to the polymeric ion exchange resins for the separation to be effective.

- Nature of exchanging ions :

valency of ions - at low conc. And at ordinary temperatures, extent of exchanges with increase in valency

size of ions – for similar charged ions, exchange increases with decrease in the size of hydrated ions.

polarizability – exchange is preferred for greater polarizable ion.



concentration of solution-
concentration and charge of ions-