INTRODUCTORY BIOCHEMISTRY

Chapter: 4(iv)
Protein Purification and Characterization
Lecture - 17

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Protein Enrichment

❖Isoelectric precipitation

The solubility depends on the pH of the solution. It is positively charged at ↓pH and negatively charged at ↑pH.

The intermediate pH at which a protein molecule has a net charge of zero is called the isoelectric point of that protein.

due to the net charge, the protein can interact with water molecules to dissociate itself from other protein molecules, thus, more soluble.

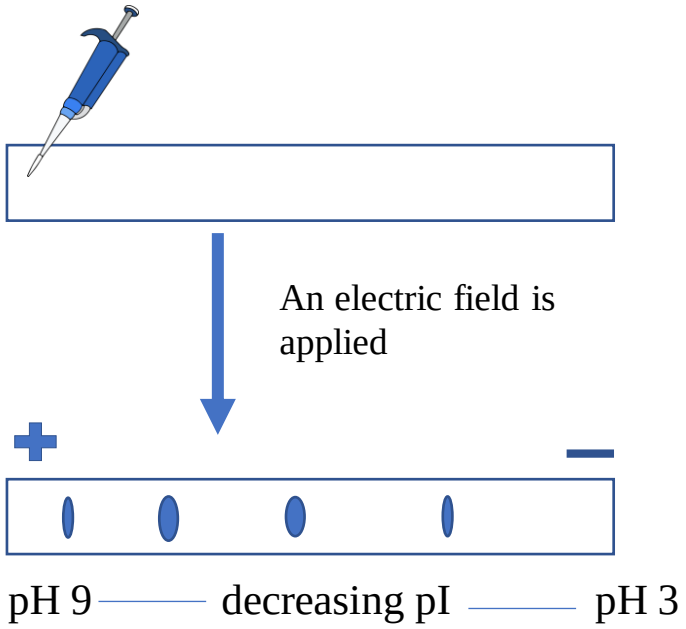
So protein is the least soluble when the pH of the solution is at its isoelectric point (pI) .

❖Isoelectric precipitation

A pH gradient is established by allowing a mixture of low molecular weight organic ampholytes to distribute themselves in an electric field generated cross the gel.

A protein that is in a pH region below its pI will be positively charged and so will migrate toward the cathode

it migrates through a gradient of increasing pH, until it reaches the pH that matches its pI so migration ceases.



❖ Solvent fractionation

-the process of isolating components of any mixture based on their different solubilities in a solvent or mixture of solvents.

The solubility of a protein depends on the dielectric constant of the solution.
A substance with a high dielectric constant is easily polarized.

As the dielectric constant of a solution decreases the magnitude of the electrostatic interactions between charged species increases.

This tends to decrease the solubility of proteins in solution because they are less ionized, and therefore the electrostatic repulsion between them is not sufficient to prevent them from aggregating.

❖Solvent fractionation

The dielectric constant of aqueous solutions can be lowered by adding water-soluble organic solvents, such as ethanol or acetone.

The amount of organic solvent required to cause precipitation depends on the protein and therefore proteins can be separated on this basis. The optimum quantity of organic solvent required to precipitate a protein varies from about 5 to 60%.

Protein Purification

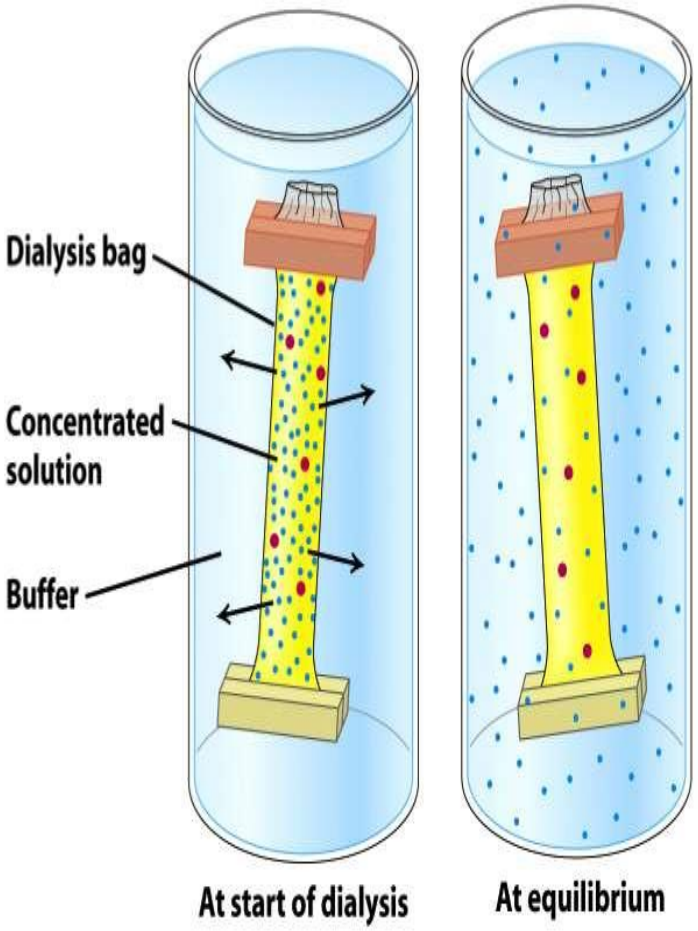
❖ Dialysis

A procedure that separates proteins (Pr.) from small solutes by taking advantage of the Pr.' larger size.

partially purified pr. extract placed inside a semi-permeable membrane (dialysis bag) which is suspended in a larger volume of buffered solution.

the membrane allows the exchange of salt and buffer but not protein

Thus buffers & salts exchange until an equilibrium is established between the inside & outside of the membrane.



❖ Ultrafiltration

A protein solution is placed in a cell containing a semipermeable membrane, and pressure is applied.

Smaller molecules pass through the membrane, whereas the larger molecules remain in the solution.

The separation principle of this technique is therefore similar to dialysis, but because pressure is applied separation is much quicker.

The portion retained by the cell (large molecules) is called the retentate, whilst the part passes through the membrane is called the ultrafiltrate.