

Lab.4: Concentration and partial purification of a protein

Choice of a starting material is key to the design of a purification process. In bulk protein purification, a common first step to isolate proteins is precipitation with ammonium sulfate $(\text{NH}_4)_2\text{SO}_4$.

Critical factors that affect the concentration at which a particular protein will

precipitate include:

1. The number and position of polar groups.
2. Molecular weight of the protein.
3. pH of the solution.
4. Temperature at which the precipitation is performed.

Ammonium sulfate is widely used for precipitation and fractionation of proteins as well as for the crystallization of proteins and protein-nucleic acid complexes. Ammonium sulfate is also utilized in hydrophobic interaction chromatography and antibody purification.

Advantages of using ammonium sulphate:

1. High concentrations of ammonium sulfate inhibit microbial growth and maintain the protein in a folded state.
2. The low density of saturated solutions (1.25 g/cm^3) allows pelleting of proteins by centrifugation.
3. A low heat of solubilization avoids the risk of protein denaturation that can occur when the sample temperature increases.
4. Ammonium sulfate is readily available, and is relatively inexpensive.

The disadvantages of using ammonium sulphate:

1. In this method a high amount of salt is used which must be removed from the precipitate.
2. Salt must be removed from the protein sample, and both dialysis and gel filtration are used.

Ammonium sulfate precipitation is one of the most commonly used methods for large and laboratory scale protein purification and fractionation that can be used to separate proteins by altering their solubility in the presence of a high salt concentration. The hydrophobic groups on the proteins get exposed to the atmosphere, attract other protein hydrophobic groups and get aggregated. Protein precipitated will be large enough to be visible.

Principle:

The experiment is based on the fact that ammonium sulfate neutralizes the charge on the protein molecules, and induces their dehydration—resulting in a protein precipitation (**salting out**). Ammonium sulfate acts by pulling water molecules away from the non-polar units of proteins. The decrease in available water molecules increases the surface tension and enhances hydrophobic interactions, thus allowing the protein to precipitate from a solution or bind to a hydrophobic column. The solubility of proteins varies according to the ionic strength of the solution, thus according to the salt concentration. At low ion concentrations ($<0.5 \text{ M}$), the solubility of proteins increases with increasing salt concentration, the presence of salt stabilizes the various charged groups on a

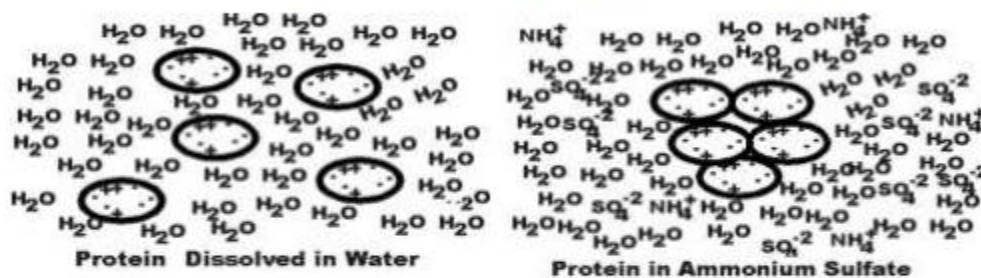
protein molecule, thus attracting protein into the solution and enhancing the solubility of protein. this is commonly known as salting-in.

At a high ionic strength, the salt concentration is increased; a point of maximum protein solubility is usually reached. Further increase in the salt concentration that there is less and less water available to solubilize protein. Finally, protein starts to precipitate when there are not sufficient water molecules to interact with protein molecules. This phenomenon of protein precipitation in the presence of excess salt is known as salting-out.

Fractional Precipitation (salting out)

- Proteins require H₂O molecules interacting with surface groups, in order to stay in aqueous solution (hydration).
- Salting out usually uses increasing concentrations of ammonium sulfate [(NH₄)₂SO₄] to compete with the protein groups for the available H₂O.
- Every protein in the solution has its own solubility limits in ammonium sulfate, independent of the other proteins in the mixture.

Protein Precipitation



- "Salting Out" when enough salt has been added, proteins precipitate
- cold prevents denaturation
- collect by filtration or centrifugation
- redissolved in solution using a buffer with low salt content.
- works best with divalent anions like sulfate, especially ammonium sulfate which is highly soluble at ice temperatures

⌋ The ammonium sulfate concentration is increased stepwise, and the precipitated protein is recovered at each stage. This is usually done by adding solid ammonium sulfate.

⌋ The ammonium sulfate concentration added should be increased to a value that will precipitate most of the protein of interest whilst leaving the maximum amount of protein contaminants still in the solution. ⌋ The precipitated protein of interest can subsequently be recovered by centrifugation and dissolved in standard buffer to prepare the sample for the next stage of purification.

Ammonium sulfate precipitation is a useful technique as an initial step in protein purification because it enables quick, bulk precipitation of cellular proteins. It is also often employed during the later stages of purification to concentrate protein from dilute solution following procedures such as gel filtration.

Preparation of saturated (NH₄)₂SO₄ solution Procedure:

1. Transfer sample of protein solution to beaker containing a stir bar and place in a container containing ice, then put it on magnetic stirrer.

