

Lab.4: Concentration and partial purification of a protein  
Ammonium sulfate precipitation (The salting-out technique)

The salting-out technique is one of the most commonly used methods for protein purification from a solution, it offers a simple and rapid method. In solution, proteins form hydrogen bonds with water molecules through their exposed polar and ionic groups. When high concentrations of small, highly charged ions such as ammonium sulfate are added, these groups compete with the proteins to bind to the water molecules. This removes the water molecules from the protein and decreases its solubility, resulting in precipitation (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**.

In general, higher molecular weight proteins will precipitate out at lower salt concentrations.

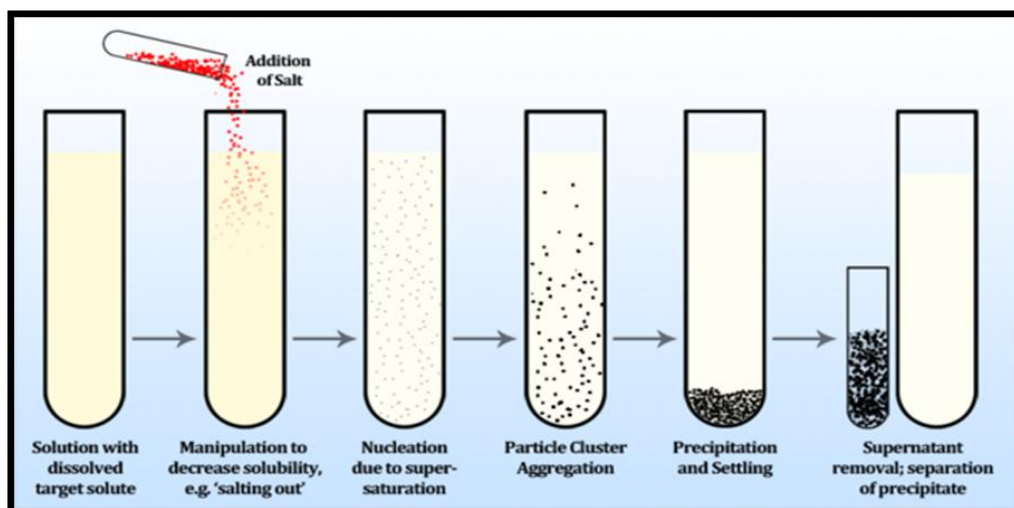
**The most commonly used salt for this procedure is  $(\text{NH}_4)_2\text{SO}_4$  due to:**

- Its high solubility
- Lack of buffering capacity
- Minimal cost
- The low density of the resulting solution relative to other salts, which aids in centrifugation separation

**Critical factors that affect the concentration at which a particular protein will precipitate include:**

- The number and position of polar groups
- Molecular weight of the protein
- pH of the solution
- Temperature at which the precipitation is performed

A disadvantage of this method is the high amount of salt that must be removed from the precipitate. To remove the salt from the protein sample, both dialysis and gel filtration chromatography are used.



Ammonium sulfate is added to the protein solution either as solid form or as saturated solution.

### **Preparation of saturated $(\text{NH}_4)_2\text{SO}_4$ solution**

Add 750 g of ammonium sulfate to 1000 ml of water in a beaker or flask. Simply stir the solution at room temperature with a magnetic stirrer for 15 minutes or until saturation. Gently decant the clear supernatant solution after the undissolved solids settle on the bottom of the flask. (Filtration is not really necessary.)

### **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.
2. While sample is stirring (for about 30 minutes), slowly add ammonium sulfate to bring final concentrations (50%, 55%, 60%, and 70%) saturation (see the table).
3. Transfer to tubes and centrifuge at 6000 rpm for 30 minutes.
4. Carefully remove and discard supernatant into the waste container.
5. Resuspend pellet in 1 ml of distilled water.
6. Transfer protein solution to dialysis tubing and dialyze versus the distilled water.
7. Remove protein solution from the tubing and centrifuge to remove any remaining debris.
8. Determine the concentration and store at  $-80^\circ\text{C}$  for long term storage.