Concentration and partial purification of a protein

Choice of a starting material is key to the design of a purification process. In a plant or animal, a particular protein usually isn't distributed homogeneously throughout the body; different organs or tissues have higher or lower concentrations of the protein. Use of only the tissues or organs with the highest concentration decreases the volumes needed to produce a given amount of purified protein. In bulk protein purification, a common first step to isolate proteins is **precipitation** with **ammonium sulfate** $(NH_4)_2SO_4$.

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/cm3) 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:

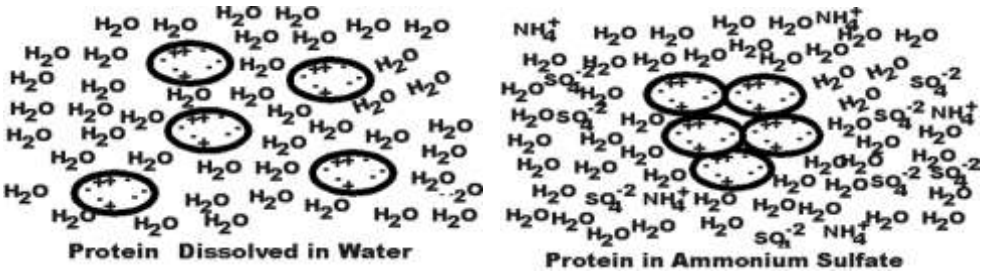
The experiment is based on the fact that ammonium sulfate neutralizes the charge on the protein molecules, and induces their dehydration-resulting in a protein precipitation (salting-out). Ammonium sulfate acts by pulling water molecules away from the non-polar units of proteins. The decrease in available 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 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 H_2O molecules interacting with surface groups, in order to stay in aqueous solution (hydration).
- Salting out usually uses increasing concentrations of ammonium sulfate $[(NH_4)_2SO_4]$ to compete with the protein groups for the available H_2O.
- Every protein in the solution has its own solubility limits in ammonium sulfate, independent of the other proteins in the mixture.

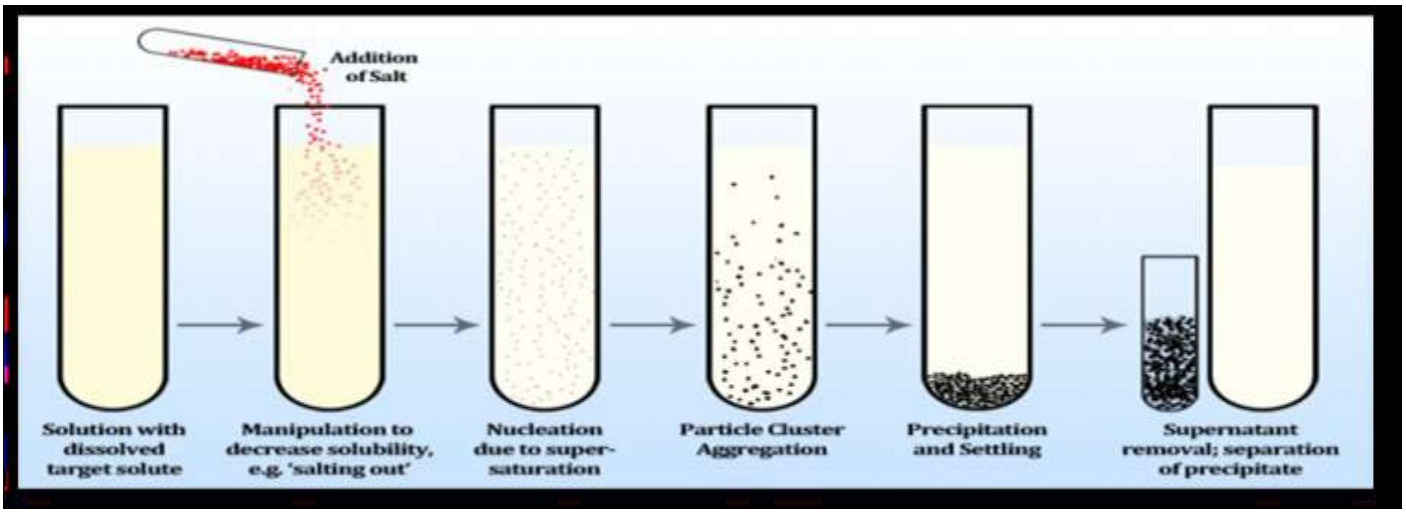
Protein Precipitation



- "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 $(NH_4)_2SO_4$ 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.)

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.
2. While sample is stirring (for about 30 minutes), slowly add ammonium sulfate to bring final concentrations (20-90%) 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°C for long term storage.

Table A.1 Amount of Ammonium sulfate required for protein precipitation.

Initial concentration of ammonium sulfate	Percentage saturation at 0°																
	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
	Solid ammonium sulfate (grams) to be added to 1 liter of solution																
0	106	134	164	194	226	258	291	326	361	398	436	476	516	559	603	650	697
5	79	108	137	166	197	229	262	296	331	368	405	444	484	526	570	615	662
10	53	81	109	139	169	200	233	266	301	337	374	412	452	493	536	581	627
15	26	54	82	111	141	172	204	237	271	306	343	381	420	460	503	547	592
20	0	27	55	83	113	143	175	207	241	276	312	349	387	427	469	512	557
25		0	27	56	84	115	146	179	211	245	280	317	355	395	436	478	522
30			0	28	56	86	117	148	181	214	249	285	323	362	402	445	488
35				0	28	57	87	118	151	184	218	254	291	329	369	410	453
40					0	29	58	89	120	153	187	222	258	296	335	376	418
45						0	29	59	90	123	156	190	226	263	302	342	383
50							0	30	60	92	125	159	194	230	268	308	348
55								0	30	61	93	127	161	197	235	273	313
60									0	31	62	95	129	164	201	239	279
65										0	31	63	97	132	168	205	244
70											0	32	65	99	134	171	209
75												0	32	66	101	137	174
80													0	33	67	103	139
85														0	34	68	105
90															0	34	70
95																0	35
100																	0