Precipitation of proteins

Introduction：

From Columb's law, the force of electrostatic attraction between oppositely charged particles can be expressed as

$$F = e^{+}e^{-} / D\, r^{2}$$

e^{+} and e^{-} are charged particles

D is the dielectric constant of the medium

r is the distance between charges.

Water has a high dielectric constant, thus the force of attraction (F) between the charges is low, protein-protein interaction is small and the protein-water interaction dependent on the structure and composition of the protein and varies from protein to protein.

The solubility of proteins varies tremendously. Many are colloidally soluble in water or salt solutions to some extent, but different reagents greatly affect their solubility.

A. Salting Out:

Large amounts of a very soluble salt added to protein solution cause the effective concentration (activity) of water to decrease and the protein-protein interaction predominates producing a precipitate of the protein. At the high concentration of salt used a rather small change in salt concentration yields as large decrease in protein solubility, and makes this procedure a very useful step in the purification of proteins.

Ammonium sulfate is commonly used for salting out proteins because

it is extremely soluble, and temperature has no marked effect on its solubility. The concentration of ammonium sulfate needed will vary for different proteins. A complex mixture of proteins may be fractionated by stepwise additions of ammonium sulfate.

Most of the animal globulins and very few of the animal albumins are precipitated by half saturation with ammonium sulfate. The albumins are precipitated by complete saturation with ammonium sulfate.

Salting in occurs when a small amount of a neutral salt is added and the protein interacts with the ions, causing less protein-protein interaction and therefore increased solubility.

B. Precipitation by strong acid (Heller's ring test):

The proteins can be precipitated from solution by conc. H_2SO_4. This test is known as Heller's ring test and is used in human medicine to detect albumin in the urine. Other strong acids, such as HCl also give the test with albumins.

C. Organic Solvents:

The addition of various organic solvents, such as ethanol or acetone, increases the tendency of proteins to precipitate. Organic solvents added to aqueous solutions of proteins lower the dielectric constant of water, decreasing the protein-water interaction, thus favoring protein-protein interaction and precipitation.

D. Isoelectric (pI) precipitation:

Protein can be either positively or negatively charged due to the terminal amine, carboxyl groups and the groups on side chain of amino acids, so the ionic form of protein also depends on the pH of the solution.

At the pI, the repulsive electrostatic forces are at a minimum and result in aggreation due to predominating hydrophobic interaction. At this pH, the protein is the least soluble. About 80% of proteins are neatively charged at neutral pH, and enerally have pIs between 4 and 6.

Reagents:

1. Protein: egg albumin solution, casein
2. saturated ammonium sulfate (75 g/100 ml water)
3. solid ammonium sulfate
4. conc. H_2SO_4
5. 95% ethanol
6. acetone
7. 1.0M sodium hydroxide
8. 0.1M, and 1.0M acetate acid

Procedure：

A. Precipitation by Salting Out:

Add ~4 ml of egg albumin solution into centrifuge tube and add ~4 ml of saturated ammonium sulfate solution. Vortex mix~15s , wait~10mins, save the supernatant in test tube. The precipitate is globulins.

To the supernatant which has been half saturated with ammonium sulfate, add solid ammonium sulfate until the solution is completely saturated. Vortex mix~15s ,wait~10mins, then albumin precipitated.

B. Precipitation by strong acid (Heller’s Ring Test):

Add 0.5ml of ovalbumin solution to 2.5ml of deionized water to dilute and mix, then slowly add 0.5ml of concentrated sulfuric acid. A white ring of precipitated ovalbumin forms at the interface of the two liquids.

C. Precipitation by Organic solvents:

Prepare three test tubes containing 0.5ml of egg albumin solution and add ~1.5ml ethanol, ~1.5ml acetone. Mix.

D. pI of protein

Introduce ~0.05g of pure casein into a 10mL volumetric flask. Add~4mL of water and 1mL of 1 M NaOH, swirl until the casein is completely dissolved.

Then add 1mL of 1M acetic acid solution and dilute to 10mL, mix well, this is a 0.5% casein in 0.1M sodium acetate buffer solution.

Set up a series of six test tubes as indicated in the following table. Each tube should contain 4mL of acetic acid solution with different pH. Mix well, then add 1mL of the casein buffer solution to each tube, shake each tube immediately. Note the turbidities just after mix in and record the results after ~10 minutes. The pI of casein is determined by observing the amount of precipitate.

Tube No.	1	2	3	4	5	6
H_2O(mL)	3.9	3.5	3	2	0	2.5
0.1M CH_3COOH(mL)	0.1	0.5	1	2	4	0
1.0M CH_3COOH(mL)	0	0	0	0	0	1.5
0.5% casein in 0.1M CH_3COONa (buffer) (mL)	1	1	1	1	1	1

Results：

A. Salting Out

1. What protein is precipitated at half saturated salt concentration?

   What protein is precipitated at saturated salt concentration?

   Half saturated:_____ Saturated:_____

2. What is the salt used to precipitate the proteins?_____

B. Strong acid

1.Picture

C. Organic solvents

_____>_____

D. pI of protein

Tube No.	1	2	3	4	5	6
H_2O(mL)	3.9	3.5	3	2	0	2.5
0.1M CH_3COOH(mL)	0.1	0.5	1	2	4	0
1.0M CH_3COOH(mL)	0	0	0	0	0	1.5
0.5% casein in 0.1M CH_3COONa (buffer) (mL)	1	1	1	1	1	1
(+:white precipitate, ++:more white precipitate)						
Amount of ppt.	-					
CH_3COO^-(mmole)	0.1					
CH_3COOH (mmole)	0.01					
pH	5.74					

*Take no.1 for example: (pKa of HOAc =4.74 at 25℃)

[CH3COO-]=(0.1×1)/5 [CH3COOH]=(0.1×0.1)/5

$$pH = pKa + \log \frac{[A^-]}{[HA]} = 4.74 + \log \frac{(0.1\times1)}{(0.1\times0.1)}$$

E. Discussions：