Precipitation Reactions of Protein

By Sandip Kanazariya

PRECIPITATION REACTIONS OF ALBUMIN

- Solubility of protein depends on proportion & distribution of polar hydrophilic end & non - polar hydrophobic end in a protein molecule resulting in a protein dipole moment.
- Ionic polar groups of molecule interact electrostatically both within the same molecule and with the surrounding molecule tending to form aggregates and opposing solubility.

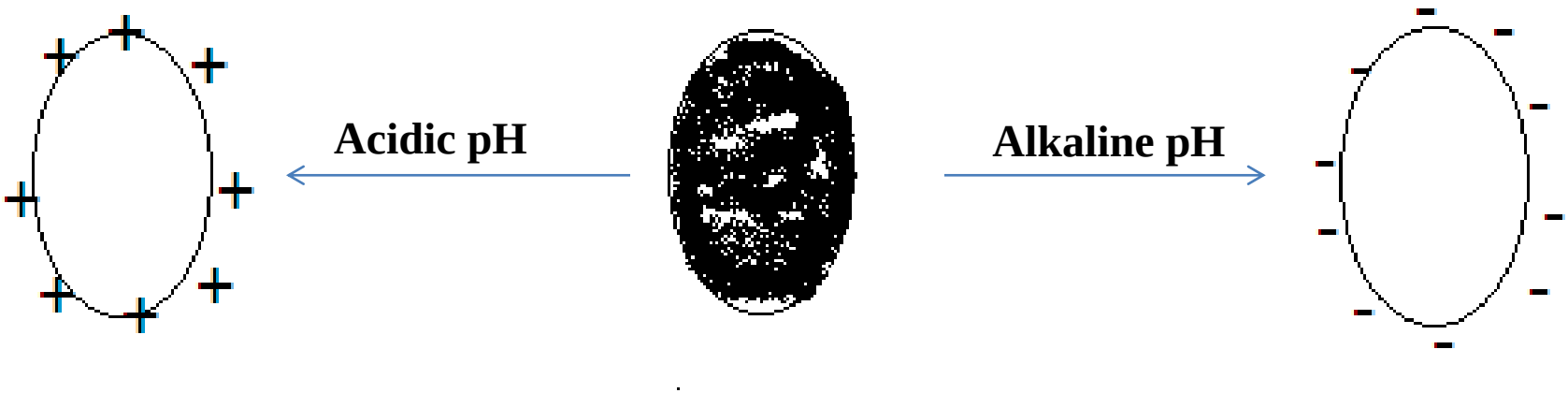
PRECIPITATION REACTIONS OF ALBUMIN

- In solution, the polar water molecule interacts with polar group of protein tending to increase the solubility.
- The solubility of protein molecule is mainly due to change in particle size, shape and hydration.

PRECIPITATION REACTIONS OF ALBUMIN

- Most of the proteins are soluble in dilute acid & alkali.
- Proteins are very reactive & highly specific in behavior due to the presence of active groups in its molecule.
- As they contain both acidic and basic groups in its molecule, they act as **ampholytes** or amphoteric electrolytes.

PRECIPITATION REACTIONS OF ALBUMIN



Cationic Form
Protein +

Dipole ion or
Zwitter ion
+ Protein -

Anionic Form
Protein -

PRECIPITATION REACTIONS OF ALBUMIN

- Isoelectric point is the pH at which protein contains equal number of positive & negative charges & protein can exist as dipolar ion or zwitter ion.
- In dipolar ion total charge is very high & net charge is zero due to presence of equal number of both acidic and basic groups in its molecule.
- They dissociate as acidic or basic depending upon pH of the solution.

PRECIPITATION REACTIONS OF ALBUMIN

- If acid is added it acts as base and if alkali is added it act as an acid. These properties are **maximal at** isoelectric pH.
- Proteins can be precipitated out from its solution by a variety of substances.
- Such reactions which precipitate out protein from their solution are called as **precipitation reaction**.

PRECIPITATION REACTIONS OF ALBUMIN

- Proteins are polymers of alpha amino acids having large molecular weight and form colloidal solutions.
- Proteins can be precipitated either by removal of water layer (Dehydration), denaturation, adjusting the isoelectric pH or by neutralization of charge present on protein molecule.

PRECIPITATION BY STRONG MINERAL ACIDS

1. HELLER'S NITRIC ACID TEST

- **Reagents required-**: Concentrated Nitric acid.
- **Reaction-** When native protein solution is treated with Concentrated HNO_3, white precipitate ring is obtained due to denaturation of protein.

TEST	OBSERVATION	INFERENCE
1. Heller's Nitric acid Test: Take 3ml albumin solution then add 3ml conc. HNO3 test tube take, from side of the test tube and do not mix.	White ppt ring is obtained.	Albumin is precipitated out by strong mineral acid

- **Note:** White precipitate ring is due to denaturation of albumin by strong mineral acid.

2. PRECIPITATION BY HEAVY METAL IONS

- **Reagents Required**: - Lead acetate and silver nitrate.
- **Reaction**: - Proteins are precipitated from their solution by heavy metal ions. These metal ions precipitate the protein from their solution. On the alkaline side of isoelectric pH, Protein dissociates as protein anion(Pr-) which combines with positive metal ion (cation) to form insoluble precipitate of metal proteinate such as lead albuminate and silver albuminate.

TEST	OBSERVATION	INFERENCE
2. Lead acetate test: Take 3 ml of albumin solution in test tube then add 2 drops of 2% Na2NO3 solution and 1 ml of lead acetate solution.	White precipitate is obtained	White precipitate is obtained due to formation of lead albuminate therefore albumin is precipitated by heavy metal ions.
3. Silver nitrate Test Take 3 ml of albumin solution in test tube then add 1 ml of silver nitrate solution.	White precipitate is obtained	White precipitate is obtained due to formation of silver albuminate therefore albumin is precipitated by heavy metal ions

3. PRECIPITATION REACTION OF PROTEIN BY ORGANIC ACID

- **Reagents required:** Sulphosalicylic acid, Trichloroacetic acid, Esbach's reagent and distilled water.
- **Reaction**: These organic acids exist as negative ion i.e. anion. when organic acids are added to albumin solution proteins are precipitated from their solution because on acidic side of isoelectric pH, protein dissociate as cation (protein +ion) which combine with anions (protein - ion) of organic acids to form salt of protein.

TEST	OBSERVATION	INFERENCE
4. Sulphosalicylic acid Test: Take 3 ml of albumin solution in test tube then add 1 ml of Sulphosalicylic acid mix well and observe.	White precipitate is obtained	White precipitate is obtained due to formation of albumin sulphosalicylate therefore albumin is precipitated by organic acid.
5. Trichloroacetic acid Test: Take 3ml of albumin solution in test tube then add 1 ml of Trichloroacetic acid mix well and observe.	White precipitate is obtained	White precipitate is due to formation of albumin trichloroacetate. Therefore albumin is precipitated by alkaloidal reagent.

TEST	OBSERVATION	INFERENCE
6. Esbach's Test: Take 3ml of albumin solution in a test tube add eual volume of Esbach's reagent(1% Picric acid) to it mix well & observe.	Yellow precipitated is obtained	Therefore albumin precipitated from its solution by Alkaloidal reagents.

4. PRECIPITATION OF PROTEIN BY ORGANIC SOLVENTS

- **Reagents Required:** Absolute Alcohol, Acetone & chloroform.
- **Reaction:** Organic solvent such as alcohol, acetone & chloroform when added to albumin solution, it decreases the dielectric constant of solvent & displaces some of the water molecules (dehydration) associated with protein and decreases the concentration of water. These effect tends to decreases the solubility of the protein in solution due to which protein are precipitated out form their solution.
- The mechanism of precipitation in this case is by dehydration, denaturation & removal of charges.

TEST	OBSERVATION	INFERENCE
7. Precipitation by Acetone: Take 3ml of albumin solution in a test tube add 1 ml of acetone to it mix well & observe.	White precipitated is obtained	White precipitated is obtained due to dehydration, denaturation by acetone. Therefore albumin precipitated from its solution by organic solvents

5. PRECIPITATION BY SALT

- **Reagent Required:** Ammonium sulphate.
- **Reaction**: The high molecular weight compound can be precipitated from their solution by addition of highly soluble salt. When protein solution is treated with this salt, the salt molecule takes up water from the protein solution for its own solubility.
- Due to which effective concentration of water required for protein solubility is decreased and proteins are easily precipitated out from their solution at isoelectric pH. This process is called salting out.
- The precipitate formed is apparently due to neutralization & dehydration of molecule & molecule aggregates in solution.

TEST	OBSERVATION	INFERENCE
8. Half Saturation Test: Take 3 ml of albumin solution in a test tube add equal amount (3ml) saturated solution of ammonium sulphate mix it well and observe. Then filter the solution and perform Biuret test with the filtrate	No White precipitate is obtained Filtrate gives violet colour	Albumin is not completely precipitated from its solution by half saturation with ammonium sulphate solution.

TEST	OBSERVATION	INFERENCE
9. Full Saturation Test: Take 5 ml of albumin solution in a test tube saturate it with ammonium sulphate crystals and observe. Then filter the solution and perform Biuret test with the filtrate	White precipitate is obtained Filtrate does not gives violet colour with biuret test	Albumin is completely precipitated from its solution by full saturation with ammonium sulphate crystals.

6. PRECIPITATION OF PROTEIN BY HEAT

Heat Coagulation Test

Heat Coagulation Test

- **Reagents required:** 1% acetic acid
- **Reaction:** When albumin solution is heated, white coagulum is obtained because albumin is denatured by heat (Albumin is a coagulable protein). After addition of 1drop of 1% acetic acid, coagulum increases because pH of albumin solution is shifted towards isoelectric point. At this pH solubility is minimum and more protein is precipitated from its solution.

TEST	OBSERVATION	INFERENCE
10. Heat Coagulation Test : Fill 3/4th of test tube with albumin solution. Heat it at the top and observe. Then Add a drop of acetic acid to it and again observe.	White coagulum or Turbidity or precipitate is obtained which increases after addition of 1 drop of acetic acid.	White coagulum is obtained due to denaturation of albumin by heat i.e. albumin is a coagulable protein. Albumin is precipitated from solution by heat.

Conclusion

1. Albumin is a simple protein.
2. Albumin is denatured by strong mineral acid.
3. Albumin is precipitated by heavy metal ions.
4. Albumin is precipitated by organic acids.
5. Albumin is precipitated by organic solvents i.e. alcohol / acetone.
6. It is completely precipitated from it solution by full saturation with ammonium sulphate but not by half saturation.
7. Albumin is coagulated by heat.

THANK YOU