

I. Protein precipitation by neutral salt

1. Principle.

This method is used to precipitate the protein. Neutral salts like ammonium sulfate precipitate proteins by neutralization of charges on the proteins and dehydration. The precipitation of a protein by neutral salt is commonly known as salting-out method. As the salt concentration of a solution is increased, more of the bulk water becomes associated with the ions leading to increase the protein-protein interaction. The amount of neutral salt required to cause protein precipitation varies with the nature of the protein and the p^H of the solution.

2. Procedure.

1. In clean dry test tube add 3 ml of protein solution.
2. Add 3 ml of saturated ammonium sulfate.
3. Mix gently and allow to stand.
4. Observe the appearance of white precipitate which indicates the presence of globulin.
5. Filter the precipitate and then dissolve it with saline and keep the supernatant to precipitate the albumin.
6. Check up the solution with biuret test to make sure that the precipitate is protein.
7. For the supernatant from step 5 add fine powder of ammonium sulfate with continuous shaking until the solution is saturated.
8. Allow to stand.
9. Observe the appearance of turbidity then the formation of white precipitate of albumin.
10. You can also make sure by using biuret test from the precipitate.

J. Protein precipitation by salts of Heavy Metals

1. Principle.

Heavy metal salts usually contain Hg^{2+} , Pb^{2+} , Ag^{1+} , Tl^{1+} , Cd^{2+} and other metals with high atomic weights. The reaction of a heavy metal salt with a protein usually leads to an insoluble metal protein salt. In alkaline medium proteins have negative charges. The positive charge of heavy metal salt neutralizes the negative charge of protein to iso electric point at which the proteins are more liable to precipitate.

2. Procedure.

1. In clean dry test tube add 3 ml of protein solution.
2. Add few drops of 25% mercuric nitrate.
3. Observe the formation of white precipitate.