**Experiment:3**

**Precipitation of Protein**

**Introduction:**

\*When the structures of native proteins are altered by chemical or physical means, the protein molecules tend to agglomerate and precipitate and the protein becomes denaturated.

\*Denaturation can be either irreversible (excessive heating or extreme PH changes) or reversible (treatment with ammonium sulfate).

\*Denaturation accompanied by loss of protein biological activity.

\*Protein precipitation is due to a disruption of hydrogen bonds, ionic bonds and sometimes the stronger covalent disulfide bonds.

**1/Influence of Strong Mineral Acids**

\*Proteins are amphiprotic substance (can accept or give protons).

\*When strong acids are added to a neutral protein solution, the carboxylate groups become undissociated carboxyl groups, and the nitrogen atoms become protonated, resulting a positively charged protein molecule.

\*The protein will precipitate because the ionic bonds and hydrogen bonds are disrupted.

**Procedure:**

1- To three separate test tubes, add 3 ml of clear 1% egg albumin to each tube.

2- To one, add 2 ml concentrated HCL, to another 2 ml concentrated HNO3 and to the third 2 ml concentrated H2SO4.

Note and describe the changes that occur.

|  |  |  |  |
| --- | --- | --- | --- |
| **3** | **2** | **1** | **Test Tube No.** |
| 3 ml | 3 ml | 3 ml | 1% egg albumin |
| x | x | 2 ml | Con. HCL |
| x | 2 ml | x | Con. HNO3 |
| 2 ml | x | x | Con. H2SO4 |

Note and describe the changes that occure.

**2/Precipitation by Alkaloidal Reagent**

\*Alkaloidal reagents are acids that can combine with alkaloids (Alkaloids are organic bases from plants).

\*Certain acidic reagents (alkaloidal reagents) e.g : Trichloroacetic acid, Tannic acid, Phosphotungstic acid, Picric acid, Sulfosalicylic acid combine with protein to form insoluble protein salts (e.g Protein tannate).

**Procedure:**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **6** | **5** | **4** | **3** | **2** | **1** | **Test Tube No.** |
| x | x | 3 ml | 3 ml | 3 ml | 3 ml | 1%egg albumin |
| x | 3 ml | x | x | x | x | 1% urea |
| 3 ml | x | x | x | x | x | 1%alanine |
| 5 drops | 5 drops | x | x | x | 5 drops | 10%Trichloroacetic acid |
| x | x | x | x | 5 drops | x | 5%aqueous tannic acid |
| x | x | x | 5 drops | x | x | 20%phosphotungstic acid |
| x | x | 5 drops | x | x | x | 20%sulfosalicylic acid |

 **3/Precipitation by Metallic Salts**

When heavy metal cations (e.g: Pb, Cu, Hg, Ag) are added to protein solution, the metal ions combine with the negatively charged groups to form insoluble metal ion proteinate.

**Procedure:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **4** | **3** | **2** | **1** | **Test Tube No.** |
| x | 2 ml | 2 ml | 2 ml | 1%egg albumin |
| 2 ml | x | x | x | 1%urea |
| 5 drops | x | x | 5 drops | 0.2M copper sulfate |
| x | x | 5 drops | x | 0.2M lead acetate |
| x | 5 drops | x | x | 0.2M mercuric chloride |

Mix well, and describe the result