

## **Proteins:**

The reactions of proteins include color and physical reactions follows:

**A. Color Reaction:** These depend upon the presence of certain amino acid Residues or specific groupings in the protein molecules.

**B. Physical Reaction:** these include:

1. Solubility.

2. Precipitation reaction:

a. By complex acid (alkaloidal reagent). In acid solution the protein becomes

Positively charges and is precipitated by complex anions.

b. By heavy metals. In alkaline solution the protein is negatively charged and is precipitated by heavy metal cations.

### **A- Colour Reaction:**

#### **Experiment 1 Biuret reaction Test:**

TO 2 ml of protein solution add an equal volume of 10% NaOH, mix thoroughly and add slowly 2 drops of 1% Copper solution. A pink of violet color is formed depending upon the amount of protein present. Carry out blank using water instead of the protein solution.

All proteins give a positive Biuret test but dipeptides and a free amino acid do not give a positive result.

This test is given by any substance containing at least two CO-NH- groups joined together or through a single atom of carbon or nitrogen. Biuret is a compound obtained by heating solid urea; therefore crystalline urea could also give this test.

### **Experiment 2 Ninhydrin reaction Test:**

TO 2 ml of protein solution add 2 ml of 1% solution of Ninhydrin. Heat to boiling for one or two min. and allow cooling. A blue color develops if the test is positive. The addition of  $\text{NH}_4\text{OH}$  even aids in the development of color. This test is given by all protein and all protein hydrolysis product including amino acids and other primary amines.

Notes:

1. The solution to be tested must be between pH5 and pH7.
2. This test forms the base of several methods for the quantitative estimation of amino acid.

### **Experiment 3 Xanthoproteic Test:**

To 2 ml of protein solution add 1 ml of conc. Nitric acid. A white precipitate forms. Heat carefully and upon heating it turns yellow and finally dissolves. Cool and carefully add excess Sodium hydroxide Solution. Note that the yellow color deepens into an orange. This Reaction is due to the presence of Phenyl group  $\text{C}_6\text{H}_5$ .

This test is only given by protein or amino acid containing the aromatic benzene ring.