**Experiment:4**

**Estimation of Proteins**

**Biuret Method:**

\*All proteins contain a large number of peptide bonds.

\*When a solution of protein is treated with an alkaline solution of dilute Copper Sulfate, a colored complex is formed between the cupric ion (Cu+2) and the carbonyl (-C=O) and imine (=N-H) groups of the peptide bonds.

\*An analogous reaction takes place between the Cu+2 ion and the organic compound biuret, therefore the reaction is called biuret reaction.

\*The reaction takes place between the cupric ion and any compound containing at least two NH2CO-, NH2CH2-, NH2CS.

\*Amino acids and dipeptides cannot give the reaction, but tri- and polypeptides and proteins react to give pink to violet products.

\*In the biuret reaction one copper ion is linked between 4 and 6 nearby peptide linkages by coordinate bonds, the more protein present, the more peptide bonds available for reaction.

\*The intensity of the color produced is proportional to the number of peptide bonds undergoing reaction. Thus, the biuret reaction can be used as the basis for a simple and rapid colorimetric method for determining protein.

\*Biuret reagent composed of: Copper Sulfate, Rochelle salt (sodium potassium tartrate) used as complexing agent to keep the copper in solution (not precipitate), NaOH and Potassium iodide to prevent autoreduction of copper.

**Procedure:**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Unknown** | **Std. 5** | **Std. 4** | **Std. 3** | **Std. 2** | **Std. 1** | **Blank (H2O)** |
| Protein Standard 10 mg / ml |
| x | 1.0 ml | 0.80 ml | 0.60 ml | 0.40 ml | 0.20 ml | x |
| 1% NaCL |
| x | x | 0.20 ml | 0.40 ml | 0.60 ml | 0.80 ml | 1.0 ml |
| Unknown |
| 1 ml | x | x | x | x | x | x |
| Biuret reagent |
| 4 ml | 4 ml | 4 ml | 4 ml | 4 ml | 4 ml | 4 ml |

Mix the tubes and incubate all the tubes at R.T for 30 minutes. Read absorbance of standards and unknown at wavelength 545 nm using the spectrophotometer. Plot the standard curve and from the standard curve find out the concentration of protein in the unknown.