

Ex. No. 3: Estimation of Mulberry Leaf Proteins.

Aim: To estimate the mulberry leaf protein using Biuret method.

Principle: The –CO-NH- bond (peptide) in polypeptide chain reacts with copper sulphate in an alkaline medium to give a purple colour which can be measured at 540 nm.

Reagents Required:

1. Biuret Reagent: Dissolve 3 g of copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) and 9 g of sodium potassium tartarate in 500 ml of 0.2 mol/liter sodium hydroxide; add 5 g of potassium iodide and make up to 1 liter with 0.2 mol/liter sodium hydroxide.

2. Protein Standard: Dissolve 250 mg Bovine Serum Albumin (BSA) in 50 ml of 0.1 N NaOH in a volumetric flask. One ml of this solution contains 5 mg BSA.

3. Mulberry Leaf Extract: Prepare 0.5% homogenate in 0.1 N NaOH / Distilled water using mortar and pestle, centrifuge at 3000 rpm for 10 min, collect the clear supernatant and use as sample.

Apparatus and Glass wares required: Test tubes, Pipettes, Colorimeter, etc.,

Procedure:

1. Pipette out 0.0, 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard protein solution in to the series of labeled test tubes.
2. Pipette out 1 ml of the given sample (mulberry leaf extract) in another test tube.
3. Make up the volume to 1 ml in all the test tubes with distilled water. A tube with 1 ml of distilled water serves as the blank.
4. Now add 3 ml of Biuret reagent to all the test tubes including the test tubes labeled 'blank' and 'sample' (mulberry leaf homogenate).
5. Mix the contents of the tubes by vortexing / shaking the tubes and warm at 37 °C for 10 min.
6. Now cool the contents and record the absorbance at 540 nm against blank.
7. Then plot the standard curve by taking concentration of protein along X-axis and absorbance at 540 nm along Y-axis.

For drawing Standard Graph please refer figure 1.

8. Then from this standard curve calculate the concentration of protein in the given sample.

Observations and Calculations:

Volume of BSA Solution (ml)	Volume of distilled water (ml)	Concentration of standard Protein (mg)	Volume of Biuret reagent (ml)	Incubate at 37°C for 10 min & cool to room temperature	A ₅₄₀
0.0	1.0	00	3		0.00
0.2	0.8	1	3		
0.4	0.6	2	3		
0.6	0.4	3	3		
0.8	0.2	4	3		
1.0	0.0	5	3		
1.0 Sample	0.0	To be estimated	3		

Result: The given unknown sample contains ----- mg protein/ml.

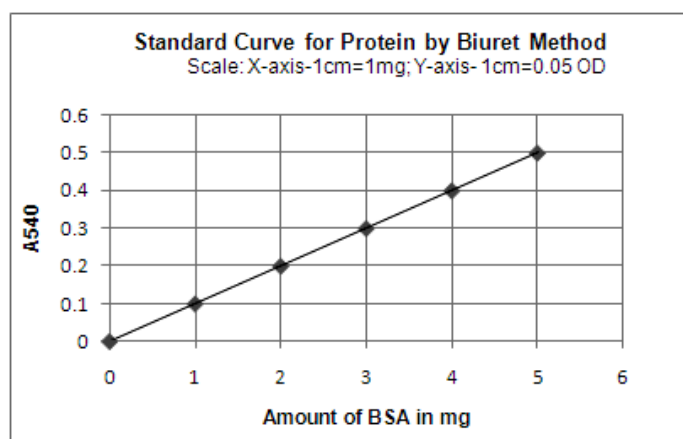


Figure 1