

Estimation of Protein by Biuret Method

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Aim: To estimate the 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: 5 mg BSA/ml.

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

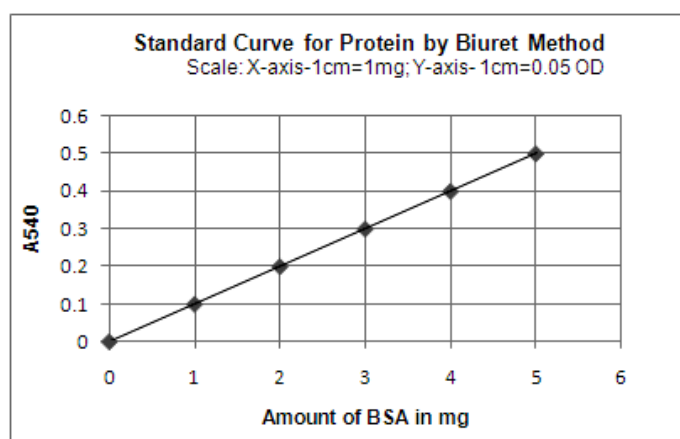
Procedure:

1. Pipette out 0.0, 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard in to the series of labeled test tubes.
2. Pipette out 1 ml of the given sample in another test tube.
3. Make up the volume to 1 ml in all the test tubes. 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 'unknown'.
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 to room temperature 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.
8. Then from this standard curve calculate the concentration of protein in the given sample.

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

Observations and Calculations

Volume of standard BSA (ml)	Volume of distilled water (ml)	Concentration of Protein (mg)	Volume of Biuret reagent (ml)	Incubate At 37°C for 10 Min & Cool	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 UK	0.0	To be estimated	3		



References

1. A Manual of Laboratory Techniques, 1983. Editors: Raghuramulu N., Nair, M K and Kalyanasundaram, S., National Institute of Nutrition, Hyderabad, India.
2. Plummer D.T., 1988. An introduction Practical Biochemistry, 3rd edition, Tata Mc Graw-Hill publishing Company, New Delhi, pp 159.
