

Aim 7

To Estimate the Protein Content in the given sample by Biuret Method

Introduction

A compound containing two or more peptide bond react with alkaline CuSO_4 of the biuret reagent to give a violet coloured complex. The number of peptide bonds present in the protein complex determines the intensity of the colour obtained.

Reagents

1. Biological sample (soaked gram seed)
2. Standard protein solution (bovine serum albumin – 10 mg/ml)
3. **Biuret reagent** – 9 g of Sodium potassium tartrate is dissolved in 500 ml of 0.2N NaOH. In this solution, 3 g of $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ is added and dissolved. Then, add 5 g of KI and raise the volume to 1.0 litre with 0.2N NaOH (Sodium potassium tartrate stabilizes the Cu^{2+} ions and KI acts as antioxidant)

Procedure

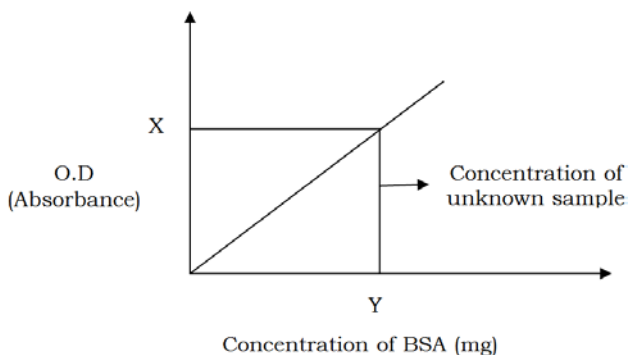
1. Different concentration of BSA solution is pipetted out into series of test tubes.
2. Take one tube as blank
3. Add distilled water in each test tube to make final volume to 2 ml.
4. 3 ml of biuret reagent is added in each test tube.
5. Mix properly and the test tubes are incubated at 37°C for 10 minutes.

6. Measure the intensity of colour at 540 nm. Draw the standard curve and calculate the quantity of unknown sample.
7. 5 g biological sample is taken for extraction of protein and homogenized in pestle mortar by adding nearly 25 ml of 10 mM Phosphate buffer(pH 7.0) containing 0.1 M NaCl. The homogenized suspension is centrifuged at 10,000 rpm for 5 minutes. Take different volume of the supernatant and estimate the protein content after suitable dilution.

Observation Table

S. No.	BSA volume (ml)	BSA Conc. (mg)	Distilled water volume (ml)	Biuret reagent (ml)		O.D. 540 nm
Blank	-	-	2.0	↑ 3 ↓	Mix properly and incubate the tubes at 37°C for 10 min.	
1	0.1	1	1.9			
2	0.2	2	1.8			
3	0.3	3	1.7			
4	0.4	4	1.6			
5	0.5	5	1.5			
6	0.6	6	1.4			
7	0.7	7	1.3			
8	0.8	8	1.2			
9	0.9	9	1.1			
10	1.0	10	1.0			
Unkonon	1.0		1.0			
	2.0		-			

Standard curve



Calculations

O.D. of the test sample- x

From standard curve

Suppose x O.D. corresponds to y mg of protein.

Unknown sample taken - 1.0 ml

So 1 ml of unknown sample contains y mg of protein

Protein (mg %) = $yx100= 100y$ mg

Alternatively, concentration can be calculated by the formula

O.D test-

O.D. standard-

Protein (mg %) = $\{(O.D. \text{ test}/O.D. \text{ standard}) \times (\text{concentration of standard in mg} / \text{Volume of sample in ml})\} \times 100$

Precautions

1. Glassware should be clean and dry.
2. Use distilled water.
3. Pipetting should be accurate.
4. BSA should be weighed properly for preparing a standard curve.
5. BSA solution should be prepared gently and frothing should be avoided.