

Experiment-5: Determination of Proteins using Biuret Protein Assay

Aim: To determine the presence of Proteins in the given sample using Biuret test

Principle:

Under alkaline conditions substances containing two or more peptide bonds form a purple complex with copper salts in the reagent.

Reagent:

A formula for biuret reagent is (per liter final volume): 9 gm Sodium potassium tartrate, 3 gm Copper sulfate, 5 gm Potassium iodide, dissolved in order in 400 mL 0.2 M NaOH before bringing to final volume.

Procedure:

1. Volumes sample, reagent can be scaled up/down and/or volume ratios varied, as with any assay.
2. Warm up the spectrophotometer 15 min. before use.
3. Prepare standards from bovine serum albumin, preferably calibrated using absorbance at 280 nm and the extinction coefficient. Using 5 mL color reagent to 1 mL sample a recommended range is 0.5 to 20 mg protein.
4. Prepare a reference tube with 1 mL buffer.
5. If possible, dilute unknowns to an estimated 1 to 10 mg/mL with buffer; a range of dilutions should be used if the actual concentration cannot be estimated. Tabulate the values in Table 3.1.
6. Use 1 mL sample per assay tube
7. Add 9 mL Biuret reagent to each tube, vortex immediately, and let stand 20 min.
8. Read at 550 nm.

Analysis

Prepare a standard curve of absorbance versus micrograms protein (or vice versa), and determine amounts from the curve. Determine concentrations of original samples from the amount protein, volume/sample, and dilution factor, if any.

Table 3.1: Table for BSA Standard and Test Samples

S. No	Vol of BSA (mL)	Conc. of BSA (mg/mL)	Vol of distilled water (mL)	Vol of Biuret reagent	Duration of Incubation	OD at 550nm
					20min	
Samples						

Result:

The amount protein present in the given sample was.....

Reference:

- 1) Sadasivam S and Balasubramanian T (1985). Practical Manual (Undergraduate), Tamil Nadu Agriculture University, Coimbatore, p.2.
- 2) Sadasivam S, Manickam A (2008). Biochemical Methods, New Age International Publishers, 3rd Edition, ISBN: 978-81-224-2140-8.