Deep Science Publishing
https://doi.org/10.70593/978-93-49307-52-0_32

Chapter 32

# Biuret assay for protein estimation: Principles, methodology, and analytical applications

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## 1. Introduction:

The Biuret reagent, made of sodium potassium tartrate and alkaline $CuSO_4$ solution, is used to detect compounds with adjacent peptide bonds, forming a distinctive violet complex (Voet, et al., 2018). The intensity of this color directly indicates protein concentration, measured at 540 nm using a green filter. The Biuret method is highly sensitive, detecting protein concentrations as low as 1-2 mg/mL (González et al., 2008; Berg et al.,2015).

## 2. Materials Required:

1. Standard flasks (100 mL, 500 mL, 1000 mL)
2. Test tubes
3. Beaker
4. Dropper
5. Graduated pipette
6. 400 mg Bovine serum albumin (BSA)
7. Sodium hydroxide (10%w/v)
8. Cupric sulphate (0.15% w/v)
9. Sodium potassium tartrate solution (0.6% w/v)
10. Potassium iodide (5 g)
11. Spectrophotometer
12. (Spectronic-20D$^+$)

## 3. Procedure:

1. Pipette 0.5 to 2.5 mL of standard bovine serum albumin (BSA) into test tubes labeled **S1 to S5**, corresponding to concentrations of 2 to 10 mg.

2. Dilute each solution with distilled water to a final volume of 2.5 mL.
3. To each test tube, add 2.5 mL of Biuret reagent and incubate the tubes at room temperature for 10 minutes.
4. Prepare the unknown solution to a total volume of 100 mL using distilled water. Transfer 1 mL and 2 mL of the unknown solution into separate test tubes and treat them in the same way as the standard solutions.
5. Measure the intensity of the violet coloration at 540 nm using a spectrophotometer. Construct a standard graph with optical density on the Y-axis and concentration on the X-axis. Use this graph to determine the protein content in the unknown solution.

## 4. Observations:

| S. No | Particulars | Blank | Working Standard | Unknown | Sample |
|---|---|---|---|---|---|
| 1 | Bovine Serum Albumin (mL) | 0 | 2.5 | 2.5 | 0 |
| 2 | Concentration (µg) | 0.5 | 2.0 | 2.0 | 2.5 |
| 3 | Volume of Unknown (mL) | 1.0 | 1.5 | 1.5 | 2.5 |
| 4 | Volume of water (mL) | 1.5 | 1.0 | 1.0 | 2.5 |
| 5 | Biuret reagent (mL) | 2.0 | 0.5 | 0.5 | 2.5 |
| 6 | Optical density at 540 nm | 2.5 | 0 | - | - |

## 5. Precautions:

1. The complex formed may undergo structural degradation if incubated for more than 10 minutes.
2. Given the high sensitivity of serum albumin, all samples and glassware must be pure and sterilized to ensure accurate and reproducible results.
3. Since the sample becomes hygroscopic, it is recommended to use salinized glassware to prevent the loss of the protein's natural state.

## 6. References:


1. Voet, D., Voet, J.G., & Pratt, C.W. (2018). *Fundamentals of Biochemistry: Life at the Molecular Level* (5th ed.). Wiley.
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