Practical Work N°03 : Protein quantification using the Biuret Method

1. Objectives

- To rapidly quantify the protein content in various food samples
- To apply a colorimetric method for biochemical analysis
- To understand and utilize spectrophotometry principles
- To construct and utilize a standard calibration curve

2. Principle

1. In alkaline medium, copper ions (cu^{2+}) from the biuret reagent form a violet-colored complex with peptide bonds (-co-nh-) in proteins. The intensity of this violet coloration, measured by absorbance at 540 nm, is directly proportional to the protein concentration in the sample.

3. Experimental protocol

Step 1: solution preparation

A. Biuret reagent preparation (250 ml)

Component	Quantity	Purpose
Copper (ii) sulfate pentahydrate ($CUSO_4 \cdot 5H_2O$)	0.75 g	Source of Cu^{2+} ions
Sodium potassium tartrate ($KNAC_4H_4O_6 \cdot 4H_2O$)	3.0 g	Stabilizes copper ions
Potassium iodide (KI)	0.5 g	Prevents auto-reduction of copper
Sodium hydroxide (NAOH)	5.0 g	Creates alkaline medium

❖ **Preparation steps:**

1. Dissolution a: dissolve tartrate + KI + NAOH in 150 ml distilled water
2. Dissolution b: dissolve $CuSO_4$ separately in 50 ml distilled water
3. Mixing: slowly add solution b to solution a under constant agitation
4. Final volume: transfer to 250 ml volumetric flask, complete to mark
5. Storage: store in labeled amber bottle at room temperature

B. Sample preparation

- Standard: BSA 10 mg/ml
- Milk: diluted 1:10 with distilled water
- Tomato juice: diluted 1:2 with distilled water

Step 2: tube preparation

Tube	Standard (ml)	Sample (ml)	Distilled water (ml)	Biuret reagent (ml)	Final volume (ml)
Blank	0.0	0.0	1.0	2.0	3.0
Std 1	0.2	0.0	0.8	2.0	3.0
Std 2	0.4	0.0	0.6	2.0	3.0
Std 3	0.6	0.0	0.4	2.0	3.0
Std 4	0.8	0.0	0.2	2.0	3.0
Sample 1 (milk)	0.0	0.5	0.5	2.0	3.0
Sample 2 (tomato)	0.0	0.5	0.5	2.0	3.0

Step 3: spectrophotometric reading

- Incubation: 20 minutes at room temperature
- Wavelength: 540 nm
- Reference: blank tube
- Measurement: record absorbance for all tubes

4. Calculations & data analysis

A. Standard curve construction

- Plot absorbance (y-axis) vs. Concentration (x-axis)
- Determine regression equation: $y = mx + b$
- Calculate correlation coefficient (r^2)

B. Protein concentration calculation

$$C_{prot} = C_{read} \times \text{dilution factor}$$

Where:

- C_prot = actual protein concentration in original sample
- C_read = concentration obtained from standard curve
- Dilution factor = sample dilution factor (10 for milk, 2 for tomato juice)

5. Expected results & interpretation

Sample	Absorbance (540 nm)	Concentration from curve (mg/ml)	Final concentration (mg/ml)
Milk			
Tomato juice			

6. Safety precautions

- Wear appropriate ppe (lab coat, gloves, safety goggles)
- Handle naoh with extreme care (corrosive)
- Dispose of chemical waste properly
- Clean all equipment after use

7. Discussion questions

1. Which food sample shows the highest protein content ? Explain based on your results.
2. Why is milk diluted 1:10 before analysis?
3. What is the purpose of the blank tube in this experiment?
4. How does the biuret method specifically detect proteins?
5. What are the limitations of this method?