

Student's Card 3 Renewable Keratin wastes for use in metal mining

Module 3

Objective: KERATIN IDENTIFICATION

Introduction

To identify the presence of keratin by means of the Biuret assay.

The Biuret assay is a chemical test used for detecting the presence of peptide bonds. It is considered as a general test for compounds (proteins and peptides) having two or more peptide (CO-NH) bonds.





Cupric ions in an alkaline environment react with any compound containing two or more peptide (CO-NH) bonds by producing a violet colour. Therefore, the reaction is negative with amino acids and dipeptides whereas it is positive with polypeptides, since there are several CO-NH groups. The colour intensity is proportional to the number of peptide bonds involved in the reaction.

Necessities



List of materials/tools

- Pipettes
- Test tubes
- Test tubes rack
- Vetro graphic pen
- Glass Rods
- Gloves
- Safety Glasses

Reagents	Formula		Quantity (g) or Concentration (M)
Sodium Hydroxide	NaOH	 	NaOH _(aq) 10%
Copper (II) sulphate	CuSO ₄		CuSO _{4(aq)} 1%
Keratin powder			
Distilled Water			
Milk			
Wool flock			

Lab Procedure

- Mark 4 test tubes; in the first test tube pour about 3-4 cm³ of water, in the second one, the same amount of milk, so you have both positive and negative control tests. In the third tube dissolve keratin powder in 3-4 cm³ of water, in the fourth test tube put some wool flock and pour some water.

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- Then add about 2 cm³ of NaOH into each test tube and mix, particularly in the fourth test tube.
- Then put about 2 cm³ of CuSO₄ solution in each test tube.
- Shake it, let it sit for a few seconds and observe the colour change.
- Make a note in the table if the reaction is positive or negative.

Additional Safety Notes



Using NaOH, wear gloves and safety glasses

Results:

If the colour remains light blue, the result is **NEGATIVE**

If the colour changes into violet, the result is **POSITIVE**

Substances	Colour with biuret reagent
Distilled water	
Milk	
Keratin	
Wool flock	



Questions/Quiz

1. What is the function of positive and negative controls?
2. Why was milk used as positive control? Do you think that it could be replaced by other substances?
3. After the assay, can we say that keratin is a protein?
4. Did the test tubes containing respectively keratin and wool flock have the same colour after the assay? How can you explain the difference between them?