

For Teachers 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.





Biuret reaction

Biuret is a chemical compound resulting from the condensation of two molecules of urea. If biuret is placed into an alkaline solution containing cupric ions, the formation of a violet complex occurs. This is called biuret reaction. It is not specific to the biuret, but cupric ions in an alkaline environment react with any compound containing two or more CONH_2 , CH_2NH or CSNH_2 groups.

Therefore the reaction is negative with amino acids and dipeptides whereas it is positive with polypeptides, since there are several CONH_2 groups. The colour intensity is proportional to the number of peptide bonds involved in the reaction.

Necessities



Reagents	Formula		Quantity (g) or Concentration (M)
Sodium Hydroxide	NaOH	 	$\text{NaOH}_{(aq)}$ 10%
Copper (II) Sulphate	CuSO_4		$\text{CuSO}_{4(aq)}$ 1%
Keratin powder			
Distilled Water			
Milk			
Wool flock			

List of materials/tools

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

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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 some water.
- 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

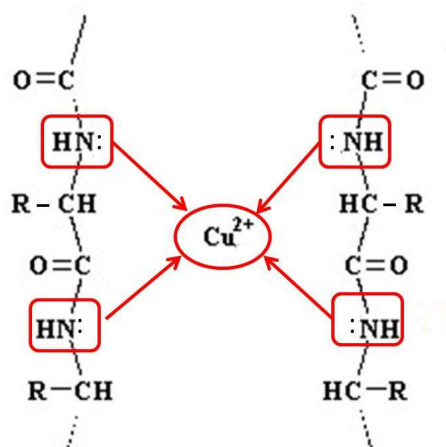


Results:

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

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

If there are peptide bonds in the solution, Cu²⁺ ions form a coordination complex with 4 atoms of nitrogen engaged in the peptide bond.



The greater the number of peptide bonds, the darker the violet



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Substances	Colour with Biuret reagent*
Distilled water	<i>Light blue</i>
Milk	<i>Violet</i>
Keratin	<i>Violet</i>
Wool flock	<i>Violet</i> <i>The colour is darker</i>

* Copper II (cupric ion) alkaline solution, which interacts with the peptide bond to form a coloured adduct is called Biuret reagent, generally used for measuring total protein concentration.



Questions/Quiz

1. What is the function of positive and negative controls?

The colour is darker.

They are used to verify the correct execution of the experiment.

2. Why was milk used as positive control? Do you think that it could be replaced by other substances?

Milk certainly contains proteins; you could have used any other substance rich in proteins, preferably soluble in water and colourless, in order not to alter the test result.

3. After the assay, can we say that keratin is a protein?

Since the biuret assay is specific for proteins, we can definitely state 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?

The colour is much more evident in the test tube containing keratin. Good results are also obtained with the wool flock, though the colour is darker because of the presence of other substances. It is not worth using other types of wool (including coarse wool) because when they are in contact with NaOH the background colour becomes brownish and this alters the results.