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Detection of protein (Biuret test)

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Proteins

Proteins are the complex compound formed by thousands of **amino acids**.

Amino acids

Amino acids are building block of proteins and among the best-known components of living organisms. They vary in size, structure, electric charge and solubility in water because of the variation in their side chains (R groups).

Objectives

- To detect the protein in the given solution.
- To demonstrate the presence of the peptide bond.

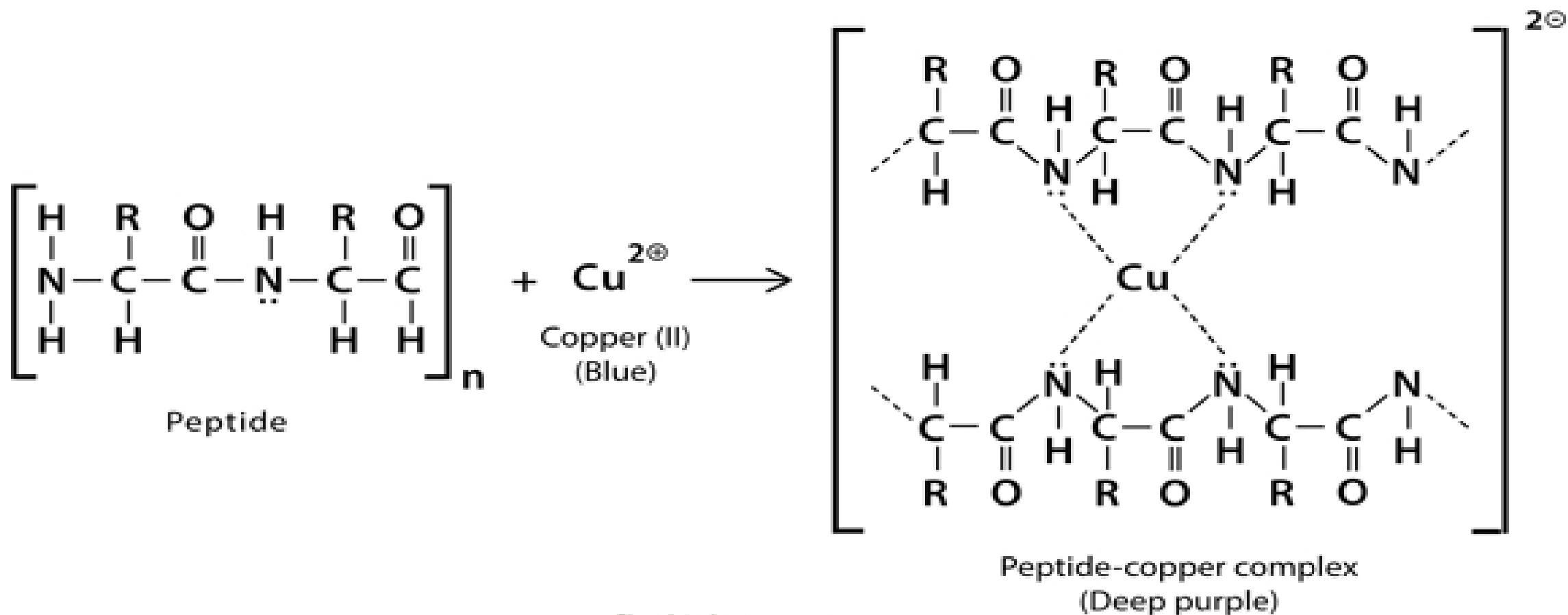
Biuret Test Principle (How does the biuret test work?)

A Biuret test is a chemical test used to determine the presence of a peptide bond in a substance. It is based on the biuret reaction in which a peptide structure containing at least two peptide links produces a violet color when treated with alkaline copper sulfate.

In presence of an alkaline solution, blue-colored copper II ion can form a complex with the peptide bonds since the peptide has unshared electron pairs in nitrogen and oxygen of water.

The colored coordination complex is formed between Cu^{2+} ion and carbonyl oxygen ($>\text{C}=\text{O}$) and amide nitrogen ($=\text{NH}$) of the peptide bond. Once this complex has been formed, the solution turns from blue to purple. The deeper the purple color, the higher is the number of peptide-copper complexes.

Biuret Test



Biuret Test Procedure

Requirements:

- Protein solution
- Biuret reagent
- Test tubes
- Pipettes

Procedure

- Take clean and dry test tubes.
- Add 1 ml of the test solutions in the respective test tubes.
- Add 1 ml of Biuret reagent to all the test tubes.
- Shake well and allow the mixtures to stand for 5 minutes.
- Observe for any color change.

Result Interpretation:

Negative test: Proteins are not present if there is no color change (i.e. solution remains blue)

Positive test: Protein or peptides are present if the solution turns from blue to violet (i.e. deep purple)

Biuret Test Uses

1. It can be used to detect the amount of protein in the urine.
2. Biuret reaction with protein is applicable to the quantitative determination of total protein by spectrophotometric analysis.