Biuret Test

The biuret test is a chemical test that can be used to check for the presence of peptide bonds in a given analyte. Therefore, the biuret test can be also be used to gauge the amount of protein present in the analyte. In this test, the presence of peptides results in the formation of pale purple coloured coordination compounds of the copper (II) ion (when the solution is sufficiently alkaline).

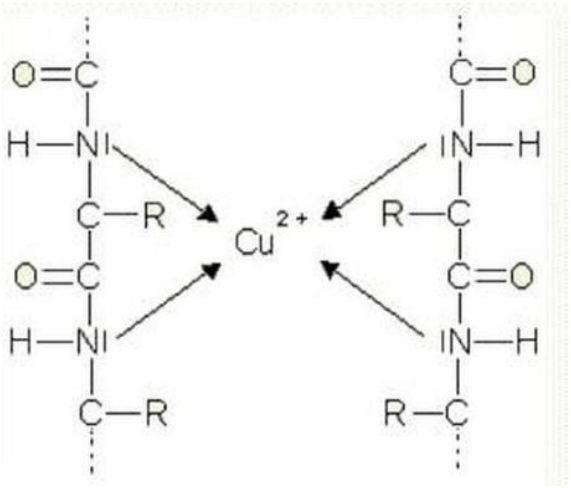
It can also be noted that the intensity of the purple colour and, therefore, the absorption at 540 nm is directly proportional to the concentration of proteins in the given analyte (as a consequence of the Beer-Lambert law). A positive reaction for this test is also received when the analyte contains biuret molecules ($[H_2N\text{-}CO]_2NH$) since the bonds in this molecule are similar to peptide bonds.

The Biuret reagent is a solution composed of sodium hydroxide (NaOH), hydrated copper (II) sulfate, and potassium sodium tartrate. Sodium hydroxide provide the alkaline medium and potassium sodium tartrate is added to chelate and thus stabilize the cupric ions in the solution or to maintain their solubility in alkaline solution.

Biuret Test Principle

The copper (II) present in the reaction binds itself to the nitrogen atoms that are present in the protein peptides. Since this test is not greatly disturbed by the presence of amino acids in the sample, it can be used to gauge the concentration of proteins in whole tissue samples. However, the samples of proteins that are purified via ammonium sulfate ($(NH_4)_2SO_4$) precipitation are not ideal for this test since buffers like ammonia interfere with it.

The reaction between the copper (II) ions and the nitrogens belonging to the peptide bonds results in the displacement of peptide hydrogens (as long as the environment is sufficiently alkaline).



Now, four nitrogen atoms donate lone pairs to form coordinate covalent bonds with the cupric ion (illustrated above), resulting in the formation of a chelate complex. This chelate complex has the ability to absorb light with a wavelength of 540nm, which imparts a purple colour to it. Therefore, the formation of a purple coloured complex indicates the presence of proteins in the

analyte. Note that the concentration of peptide bonds in the analyte contribute to the intensity of the purple colour.

Biuret Test Procedure

The procedure that can be followed to conduct a biuret test is provided below.

- Take 3 clean and dry test tubes.
- Add 1-2 ml of the test solution, egg albumin, and deionized water in the respective test tubes.
- Add 1-2 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.

Biuret Test Results

Observation	Interpretation
No color change, i.e., the solution remains blue	Proteins are absent (negative biuret test)
The solution turns from blue to deep purple	Proteins are present (positive biuret test)

Application

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.