

Color reactions of proteins

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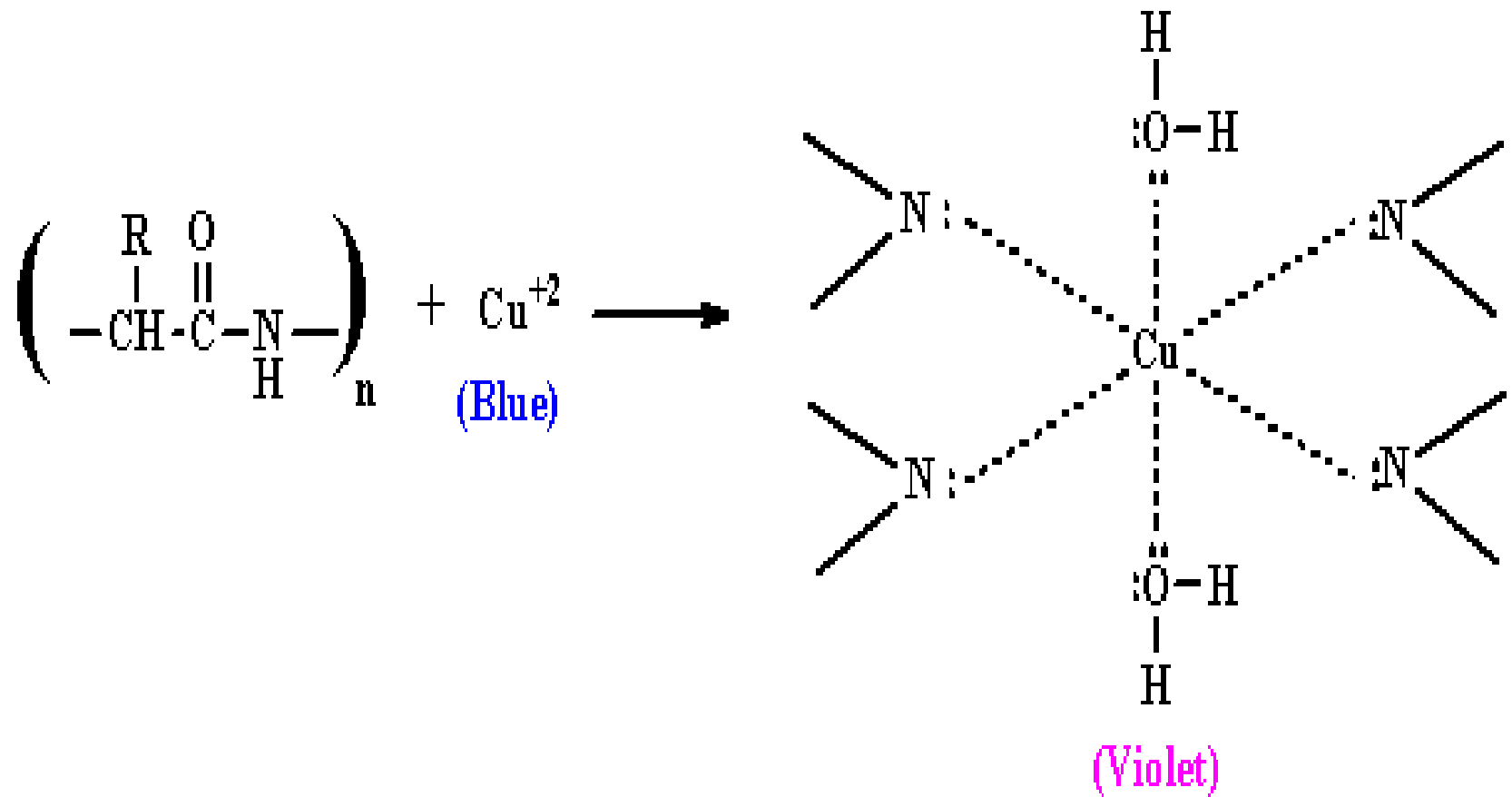
Biuret test

- The biuret test is often general test used to determine the presence of peptide bond in proteins that contain two or more peptides bond, (peptide must have at least 3 amino acids).

The principle of the test:

- The biuret test depends upon the reaction of cupric ions Cu^{+2} in an alkaline solution with peptide linkage of the protein to produce a **chelate complex** with violet-purple color.
- Lone electron pairs from 4 nitrogen atoms in the peptide bond coordinate a copper ion Cu(II) ion to form chelate complex.
- The greater the concentration of peptide bonds, the greater color intensity.

Color caused by the coordination complex of the copper atom and nitrogen atoms of tow peptide chain .



Why we called this reaction as a biuret test ?

- Biuret is also the result of the condensation of 2 molecules of urea in a hot water. The reagent is so named because the peptide bonds in urea give a positive result for the test.

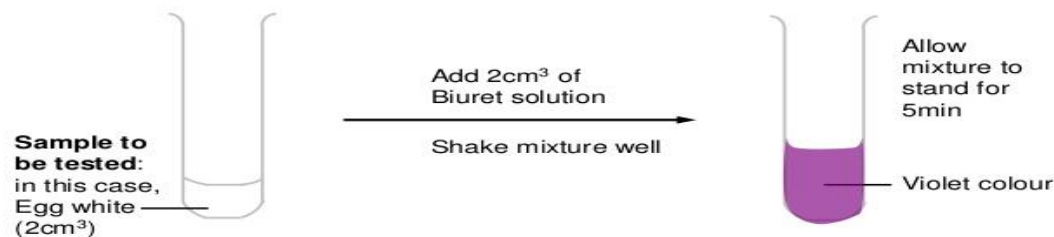
Reagents :

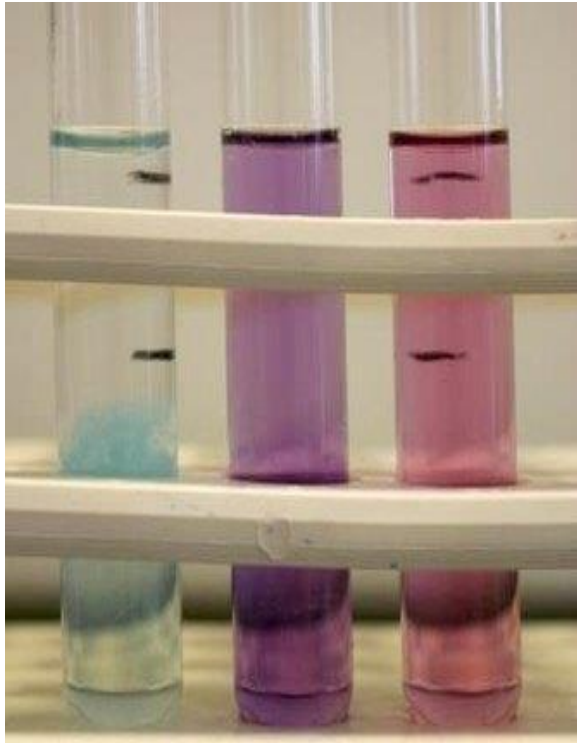
- Copper sulphate (CuSO_4) 0.5% solution in water →
give blue to violet color
- Sodium hydroxide(NaOH) 10% solution in water →
provide alkaline medium, does not participate in the reaction.

Procedure

- Into two separate test tubes, add 2ml of the following solution 2% albumin, and 0.5% ??
- Add 2ml of 10% NaOH into each test tube and mix the contents.
- Add 5 to 10 drops of 0.5% CuSO₄ into each tube.

Test for Proteins (Biuret test) – 2nd method





OBSERVATIONS

No change (solution remains blue)

The solution turns from blue to violet (deep purple)

The solution turns from blue to pink

INTERPRETATION

Proteins are not present

Proteins are present

Peptides are present
(Peptides or peptones are short chains of amino acid residues)

In your report :

- Can a biuret assay gives a positive reaction with amino acids ? Why
- Give the functions of Tyrosine ?