

Exercise 21

Aim: To detect the presence of proteins.

Principle: Proteins respond to some colour reactions due to the presence of one or more radicals or groups of the complex protein molecule. All proteins do not contain the same amino acids, and hence they do not respond to all colour reactions. Nitrogen atoms in the peptide chain form a complex (violet colour) with copper ions in the Biuret test. (Biuret test is for peptide bond in the molecule of a protein.) Xanthoproteic test is specific for protein containing aromatic amino acids. The benzene ring in the amino acids is nitrated by heating with nitric acid and forms yellow nitro-compounds which turns to orange colour with alkali.

Requirement: Glasswares: test tube, spirit lamp; Chemicals: 40% NaOH, 1% CuSO₄ solution, Concentrated HNO₃, 20% NaOH solution; Miscellaneous: test tube holder, test tube stand.

Procedure

(a) Biuret test

- Take 2 mL of protein solution (milk, albumin of egg or gram seed extract) in a test tube.
- Add 1 mL of 40% NaOH solution and 1 or 2 drops of 1% CuSO₄ solution.
- A violet colour indicates the presence of proteins. Care must be taken that excess of copper sulphate is not added otherwise there will be blue colour instead of violet colour.

(b) Xanthoproteic test

- Add carefully 1 mL of concentrated HNO₃ to 2 mL of protein solution (albumin of egg, milk or gram seed extract).
- A white precipitate is formed.
- Boil the solution and the colour changes to yellow.
- Cool the test tube and add 2 mL of 20% NaOH (or ammonia solution) to make it alkaline.
- The colour changes to orange indicating the presence of proteins.

Discussion

A yellow stain is often observed on skin when it comes in contact with nitric acid. The reason of yellow stain is xanthoproteic reaction.

Questions

1. Why does the skin turn yellow when it inadvertently comes in contact with HNO_3 ?
2. Why are only few drops of CuSO_4 solution added during the biuret test?

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