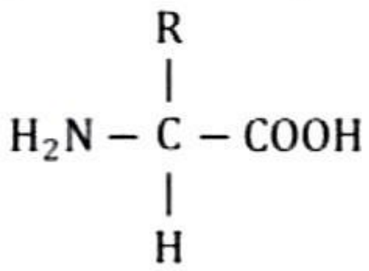
EXPERIMENT

Aim

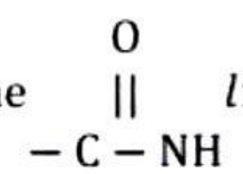
To test the presence of proteins in the given food sample.

Theory

Proteins are high molecular mass complex organic compounds containing nitrogen. Amino acids are molecules that have both $-NH_2$ (amino acid) and $-COOH$ (Carboxylic acid) group.



α − Amino acids

The  linkage that joins the two amino acid units is called peptide linkage. The product formed by the combination of two α-amino acid molecules is called dipeptide and with three α-amino acid molecules is called tripeptide. A polypeptide contains large number of α-amino acid molecules. A polypeptide having molecular mass greater than 10,000 is called protein.

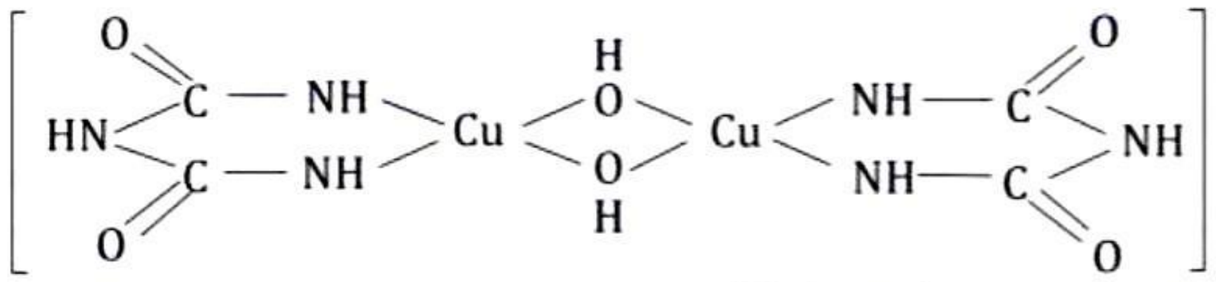
Material Required

Test tubes, burner, Egg albumin dispersion, gelatine dispersion. Millon's reagent and Ninhydrin reagent.

Procedure

1. Biuret Test

To the dispersion of the substance to be tested (say 5% solution of egg albumin) add about 2 ml of NaOH solution. Now add 4-5 drops of 1% $CuSO_4$ solution. Warm the mixture for about five minutes. Bluish violet coloration indicates the presence of protein.



and $[Cu(NHCONHCONH)_2]^{2-}$

Violet color complexes

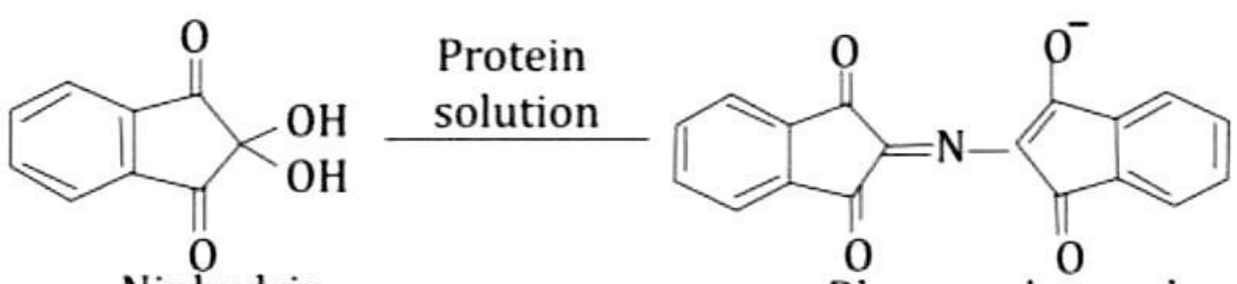
2. Xanthoproteic Test

This test is used for the proteins or amino acids carrying aromatic groups. Aromatic group undergoes nitration with concentrated nitric acid. The product or salt formed after the nitration of aromatic group is either yellow or orange in color.

While performing xanthoproteic test, initially on heating, a yellow color is observed. This yellow-colored compound is xanthoproteic acid which is formed by the nitration of amino acids.
Take about 2 ml of egg albumin dispersion in a test-tube and add a few drops of conc. HNO_3 and heat. A yellow coloration indicates the presence of proteins.

3. Ninhydrin Test

Ninhydrin (2,2-dihydroxyindane-1,3-dione) is a powerful oxidising agent. It gives a characteristic blue-violet coloured compound when reacts with proteins. This characteristic-coloured compound is named as Rhumann's purple. The reaction involved is shown below:



Ninhydrin — Rhumann's purple (Blue -violet)

Take about 2 ml of egg albumin dispersion in a test-tube and add 3-4 drops of Ninhydrin solution. Boil the contents. Intense blue coloration confirms the presence of proteins. Ninhydrin Solution is prepared by dissolving 0.1 g of ninhydrin in about 100 ml of distilled water. This solution is unstable and can be kept only for two days.

4. Millon's Test

This test is given by proteins containing phenolic amino acids. Gelatin does not give this test.
To 1-2 ml of egg albumin, dispersion add 2 drops of Millon's reagent.
White ppt. which changes to brick red on boiling, confirms the presence of proteins.
Millon's Reagent is prepared by dissolving 5 g each of $HgNO_3$ and $Hg(NO_3)_2$ in 100 ml of dil. HNO_3.

Observation

Table.3

S. No.	Experiment	Observation	Inference
1.	**Biuret test** Egg albumin (0.5% w/v) in 0.1M, NaOH (2 ml) +$CuSO_4$ (dropwise)+heat	Violet coloured complex appears.	Appearance of violet colour due to the formation of a complex species of Cu^{2+} ions with – CONH – group confirms the presence of proteins in the sample.
2.	**Ninhydrin Test** Aqueous solution of egg albumin (2-3 ml) +3-4 drops of ninhydrin +heat	Blue coloration appears in the solution.	This blue coloration confirms the presence of protein.
3.	**Xanthoproteic Test** Egg albumin(aq)(1ml) +conc. HNO_3 (few drops) +heat	A yellow colour appears first which on addition of NaOH sol., an orange-coloured solution is obtained.	Appearance of yellow colour indicat es protein may be present. Formation of orange coloured solution confirms the presence of proteins.

4.	**Million's Test** (Given by only those proteins which contains phenolic α amino acids) 1 mL of protein solution + 1 mL of million's reagent	White ppt. is observed which turns red on heating in water bath.	Protein is present.

Results

The protein in the pure form is present in the given sample.

Precautions

1. Freshly prepared reagents should be used while performing the tests.
2. The reagents should be taken in required amount.

VIVA VOCE

Q 1. What are proteins, and what is their role in the human body?

Ans. Proteins are macromolecules made up of amino acids. They play crucial roles in the body, including serving as structural components, enzymes, and contributing to immune function.

Q 2. Explain the Biuret test for detecting the presence of proteins.

Ans. The Biuret test involves adding a solution of copper sulfate and sodium hydroxide to the food sample. A purple coloration indicates the presence of proteins.

Q 3. Can you name some common sources of dietary proteins?

Ans. Common sources of proteins include meat, eggs, dairy products, legumes, nuts, and seeds.

Q 4. Why is it important to include an adequate amount of protein in the diet?

Ans. Proteins are essential for the body's growth, repair of tissues, and the synthesis of enzymes and hormones.

Q 5. Describe an alternative method to the Biuret test for detecting proteins in a food sample.

Ans. The Ninhydrin test involves spraying a solution of ninhydrin on the food sample. A purple or violet color indicates the presence of proteins.

Q 6. How does the structure of proteins differ from carbohydrates and fats?

Ans. Proteins are composed of amino acids linked by peptide bonds, while carbohydrates consist of sugars, and fats are composed of glycerol and fatty acids.

Q 7. Can proteins be detected using the iodine test, which is commonly used for detecting starch?

Ans. No, the iodine test is specific for detecting the presence of starch and does not react with proteins.

Q 8. Why is the Biuret test considered more specific for proteins compared to other tests?

Ans. The Biuret test involves a specific reaction between peptide bonds and copper ions, providing a more accurate indication of protein presence.