Experiment
Identification tests for Proteins

Aim

To identify proteins in the given samples and to differentiate **Albumin** and **Casein** using standard qualitative protein tests.

References

1. S.P. Singh, Practical manual of Biochemistry, CBS Publishers and Distributors, New Delhi, First Edition, 31-50.
2. R. Chawla, Practical Clinical Biochemistry, Methods and Interpretations, Jaypee Brothers Medical Publishers (P) LTD New Delhi, Third Edition, 39-44.

Requirements

- **Glassware Requirement:-** Test tube, Test tubes holder, Water bath, Glass road.
- **Chemical Requirement:-** Protein sample (egg/milk)**,** Sodium hydroxide (NaOH)**,** Copper sulfate ($CuSO_4$) solution, Acetic acid.

Principle

Proteins are polymers of amino acids joined by peptide bonds.

- **General protein tests** (Biuret Test) confirm presence of protein.
- **Specific behavior tests** such as heat coagulation and acid precipitation help differentiate proteins.

Albumin is heat coagulable, while **Casein** precipitates at its isoelectric point in acidic conditions.

1. Biuret Test (General test for proteins)

Procedure

1. Take **2 mL** of the given **protein solution** in a clean, dry test tube.
2. Add **2 mL of 10% sodium hydroxide (NaOH)** and mix well to make the solution alkaline.
3. Add **2–3 drops (≈0.1 mL)** of **1% copper sulfate ($CuSO_4$) solution** dropwise.
4. Shake the test tube gently and allow it to stand for **1 minute**.
5. Observe the color change.

Observation

- Violet or purple color appears.

Inference

- Presence of proteins (peptide bonds)

2. Heat Coagulation Test (Specific for Albumin)

Procedure

1. Take **2.0 mL** of the given test solution in a clean, dry **test tube**.
2. Hold the test tube in a **slanting position** with a test tube holder.
3. Gently heat **only the upper one-third** of the solution over a flame for **1–2 minutes**.
4. Observe the heated portion and compare it with the unheated lower portion.
5. If turbidity or precipitate appears, add **2–3 drops (≈0.1 mL)** of **1% dilute acetic acid**.
6. Mix gently and note the final observation.

Observation

- A **white turbidity or coagulum** appears in the heated portion of the solution.
- The coagulum **persists after addition of dilute acetic acid**.

Inference

- **Positive test**: Persistent white coagulum indicates the **presence of heat-coagulable protein (albumin)**.
- **Negative test**: No coagulum on heating indicates **absence of heat-coagulable proteins.**

3. Acid Precipitation Test (Specific for Casein)

Procedure

1. Take **2 mL of milk sample** in a clean test tube.
2. Add **2 mL of distilled water** to dilute the sample; mix gently.
3. Add **1% acetic acid dropwise (about 0.5–1.0 mL)** with gentle shaking.
4. Continue adding acid until the solution becomes **slightly acidic** (pH ≈ 4.6).
5. Allow the test tube to stand undisturbed for **2–3 minutes**.
6. Observe the formation of any precipitate.

Observation

- Formation of a **white, curdy precipitate**.

Inference

- The appearance of a **white precipitate** confirms the **presence of casein protein** in the given sample.
- Absence of precipitate indicates **casein is not present**.

Result

The given sample shows positive protein reactions.

- **Albumin** is identified by **heat coagulation**.
- **Casein** is identified by **acid precipitation with acetic acid**.