

Experiment

Qualitative analysis of Carbohydrates

Aim

To detect and identify carbohydrates present in the given sample using standard qualitative tests.

References

1. S.P. Singh, Practical manual of Biochemistry, CBS Publishers and Distributors, New Delhi, First Edition, 19-37.
2. R. Chawla, Practical Clinical Biochemistry, Methods and Interpretations, Jaypee Brothers Medical Publishers (P) LTD New Delhi, Third Edition, 32-38.

Requirements

- **Glassware Requirement:-** Test tube, Test tubes holder, Water bath, Glass rod.
- **Chemical Requirement:-** Sample Solution, Molisch reagent, concentrated sulfuric acid, Benedict's reagent, Fehling's solution A/B, Barfoed's reagent, Iodine solution

Principle

Carbohydrates undergo characteristic chemical reactions due to the presence of aldehyde or ketone groups and multiple hydroxyl groups. Based on dehydration, reducing properties, monosaccharide behavior, and iodine complex formation, different qualitative tests help identify carbohydrates.

1. Molisch Test (General Test for Carbohydrates)

Procedure

1. Take **2 mL** of the given test solution in a clean, dry test tube.
2. Add **2–3 drops (≈0.1 mL)** of **Molisch reagent (α -naphthol in alcohol)**.
3. Mix gently without shaking.
4. Incline the test tube and **slowly add 1 mL of concentrated sulfuric acid** along the side of the test tube using a dropper.
5. Do **not mix or shake** the contents.
6. Allow the test tube to stand undisturbed for **1–2 minutes**

Observation

- Formation of a **violet / purple ring** at the **junction of two layers** (acid layer and aqueous layer).

Inference

- Violet or purple ring present → Carbohydrates are present **in the given sample** (Positive Molisch test).
- No colored ring → Carbohydrates are absent (Negative Molisch test).

2. Benedict's Test (Test for Reducing Sugars)

Procedure

1. Take **2 mL** of **Benedict's reagent** in a clean, dry test tube.
2. Add **0.5 mL** of the given **test solution** (aqueous sample).
3. Mix the contents gently.
4. Place the test tube in a **boiling water bath** for **2–3 minutes**.
5. Remove the test tube and allow it to cool.

Observation

Green, yellow, orange, or brick-red precipitate.

Inference

- Green / Yellow / Orange / Brick-red precipitate → Reducing sugar present (Positive Benedict's test).
- No color change (blue solution) → Reducing sugar absent (Negative Benedict's test).

3. Fehling's Test (Confirmation of Reducing Sugars)

Procedure

1. Take **1 mL** of **Fehling's solution A** in a clean test tube.
2. Add **1 mL** of **Fehling's solution B** to the same test tube.
3. Mix well to obtain a deep blue solution.
4. Add **1 mL** of the given **test solution** (aqueous sample).
5. Mix gently.
6. Heat the mixture in a **boiling water bath** for **2–3 minutes**.
7. Observe the color and precipitate formation.

Observation

- Formation of a **brick-red precipitate** at the bottom of the test tube.
- Sometimes intermediate colors (green → yellow → orange) may appear before brick-red.

Inference

- Brick-red precipitate present → Reducing sugar is present (Positive Fehling's test).
- No precipitate; solution remains blue → Reducing sugar is absent (Negative Fehling's test).

4. Barfoed's Test (Test for Monosaccharides)

Procedure

1. Take **2 mL** of **Barfoed's reagent** in a clean, dry test tube.
2. Add **1 mL** of the given **test solution** (aqueous carbohydrate sample).
3. Mix gently.
4. Heat the test tube in a **boiling water bath** for **1–2 minutes only**.
5. Remove and allow the test tube to cool.

6. Observe for precipitate formation.

Observation

- Formation of a **brick-red precipitate** within **1–2 minutes**.
- No immediate precipitate or only faint turbidity if disaccharides are present.

Inference

- Brick-red precipitate within 1–2 minutes → Monosaccharide present (Positive Barfoed's test).
- No precipitate within 2 minutes → Monosaccharide absent (**may indicate disaccharide or non-reducing sugar**).

5. Iodine Test (Starch)

Procedure

1. Take **2 mL** of the given **test solution** in a clean, dry test tube.
2. Add **2–3 drops** (≈ 0.1 mL) of **iodine solution** (I_2 in KI).
3. Mix gently.
4. Observe the color change immediately.

Observation

Polysaccharide	Color Produced
Starch	Blue-black
Glycogen	Reddish-brown
Dextrin	Yellow to brown
Cellulose	No color change

Inference

- Blue-black color → Starch present
- Reddish-brown color → Glycogen present
- No color change → Polysaccharide absent or cellulose present

Result

The given sample shows characteristic reactions indicating the presence of carbohydrate(s). The nature of carbohydrate (reducing sugar, monosaccharide, or polysaccharide) is identified based on positive tests.