Carbohydrates are polyhydroxy compounds (1) that are either aldehyde or ketones containing Carbon, Hydrogen and oxygen. They are important to understand as there are large no. of carbohydrates varying from small sugar molecule i.e. simple glucose to polymers such as glycogen, cellulose. Qualitative analysis of carbohydrates refers to finding out which specific sugar is present in the unknown solution.

KEYWORDS: Qualitative tests for carbohydrates are Molisch’s test, Iodine test, Barfoed’s test, Benedict’s test, Seliwanoff’s test, Bial’s test, Fehling’s tests and Osazone test. Carbohydrates are polyhydroxy compounds (1) that are either aldehyde or ketones containing Carbon, Hydrogen and oxygen. They are important to understand as there are large no. of carbohydrates varying from small sugar molecule i.e. simple glucose to polymers such as glycogen, cellulose. Qualitative analysis of carbohydrates refers to finding out which specific sugar is present in the unknown solution.
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**INTRODUCTION**

a carbohydrate\(^{(1)}\) is a biological molecule consisting of carbon(C), hydrogen(H) and Oxygen (O) atoms, usually with a H:O ratio of 2:1 with the empirical formula C\(_m\)(H2O)\(_n\). Carbohydrates are actually polyhydroxyaldehydes and polyhydroxyketones, often but not always with general formula of \((\text{CH}_2\text{O})_n\), where \(n\) equals to 3 or more. Carbohydrates are divided into 3 general classes, depending on the number of carbohydrates molecules they contain\(^{(1)}\):

1) **Monosaccharide:**
   Monosaccharides are classified by the no. of carbon atoms they contain. For example, monosachharide containing 5 carbons= pentoses, 6 carbons=hexoses.

2) **Oligosaccharides:**
   They are carbohydrates containing few monosaccharide units, can be further classified as: a) Disaccharides (2 monosaccharide units) e.g. sucrose (glucose-fructose) b) Trisaccharides (3 monosaccharide units) e.g. maltose (glucose-glucose)

3) **Polysaccharides:**
   They contain large number of monosaccharide units.
   e.g. starch, cellulose and glycogen are most important.
   Many carbohydrates can be identified using condensation reagents, which react with the carbohydrates to produce highly coloured products.

- Carbohydrates are the compounds of great biological importance\(^{(4)}\):
  - They are the main source of energy\(^{(2)(5)}\), body begins to digest carbohydrates first among all three main sources of energy namely- proteins, fats and carbohydrates.
  - They serve as a form of stored chemical energy.
- They form a part of structure of cells and tissues of plants as well as animals.

**METHODS & OBSERVATIONS**

**Tests for identification of unknown carbohydrates:**

![Diagram of carbohydrate tests]

Table-1.1

(1)
(2) Molisch’s Test

(8,16)
**Principle:**
Carbohydrates when treated with concentrated H$_2$SO$_4$, undergo dehydration to give furfural derivatives which condense with Alpha naphthol to form coloured products.

**Procedure:**
In a test tube, add 2 ml of the test carbohydrate solution and 2 drops of ethanolic $\alpha$-naphthol solution (Molisch’s reagent). Carefully incline the tube and pour dropwise conc. H$_2$SO$_4$, using a dropper, along the sides of the tube. Observe the violet colour at the junction of the two liquids.

**Interpretation:**
This is a sensitive but a non-specific test is given positive by all types of carbohydrates. If oligosaccharides or polysaccharides are present, they are first hydrolysed to mono saccharides which are then dehydrated to give the test positive.

An appearance of *reddish violet* or *purple coloured ring* at the junction of two liquids is observed.
(2) Iodine test$^{(9,16)}$

**Principle:** Iodine forms a coordinate complex between the helically coiled polysaccharide chain and iodine centrally located within the helix due to adsorption. The colour combined depends upon the length of the unbranched or linear chain available for complex formation.

**Procedure:**
Add 2 drops of iodine solution to about 2 mL of the carbohydrate containing test solution. A blue-black colour is observed which is indicative of presence of polysaccharides.

**Interpretation:** This is a test for polysaccharides which is indicated by formation of blue/brown/red colour. No change in colour is indicative of presence of mono or disaccharide.

3) Barfoed’s Test$^{(10,16)}$

**Principle:**
Aldoses and ketoses can reduce cupric ions even in the acidic conditions. This test is used to distinguish reducing monosaccharides by controlling pH and time of heating. Monosaccharides react very fast whereas disaccharides react very slowly.

**Procedure:**
To 2 mL of the test solution add about 2-3 mL of Barfoed’s reagent. Mix it well and boil it for one minute in the water bath allow to stand for a few minutes. Formation of a red precipitate of cuprous oxide in the bottom and along the sides of the test tube immediately, only monosaccharides answer this test. Since Barfoed’s reagent is slightly acidic, This test is specific for monosaccharides.

![Figures showing test results](image)

**Interpretation:**
Positive reaction indicates the presence of a reducing mono saccharide. On prolonged heating, disaccharides can also give this test positive.

4) **BENEDICT’S TEST:** \(^{(11,16)}\)

**Principle:** Carbohydrates with free aldehyde or ketone groups have the ability to reduce solutions of various metallic ions.

- Reducing sugars under alkaline conditions tautomerise and form enediols.
- Enediols are powerful reducing agents.
They reduce cupric ions (Cu\(^{3+}\)) to cuprous (Cu\(^{2+}\)) form and are themselves converted to sugar acids.

The cuprous ions combine with OH\(^-\) ions to form yellow cuprous hydroxide which upon heating is converted to red cuprous oxide.

**Procedure:** In the test tube with 2 ml of Benedict's reagent, add 5-6 drops of the test carbohydrate solution and mix well. Place the test tube in a boiling water bath for 5 minutes and observe any change in colour or precipitate formation. Cool the solution. Observe the colour change from blue to green, yellow, orange or red depending upon the amount of reducing sugar present in the test sample.

**Interpretation:** It is semi quantitative test. The colour of the precipitate gives a rough estimate of a reducing sugar present in the sample:

- **Blue:** none
- **Green:** up to 0.5gm% (+)
- **Green precipitates:** 0.5-1.0gm% (++)
- **Yellow precipitates:** 1.0-1.5gm% (+++)
- **Orange precipitates:** 1.5-2.0gm% (++++)
- **Brick red precipitates:** >2.0gm% (+++++)
5) Seliwanoff’s Test: \(^{(12,16)}\)

**Principle:**

Keto hexoses on treatment with Hydrochloric acid (HCl) form 5-hydroxy methyl furfurals which on condensation with resorcinol gives a cherry red coloured complex.

**Procedure:**

To 2 mL of Seliwanoff’s reagent, add two drops of test solution. The mixture is heated to just boiling. A cherry red condensation product will be observed indicating the presence of ketoses in the test sample. There will be no significant change in colour produced for aldose sugar.

![fig.5](image)

**Interpretation:**

- This test is given positive by ketohexoses so it is answered by fructose, sucrose and other fructose containing carbohydrates.
- This test distinguishes between glucose and fructose.
- Overheating of the solution should be avoided.
- Upon continuous boiling, aldoses get converted to ketoses and give a positive reaction with seliwanoff reagent.
(6) **Bial’s test:** \(^{(13,16)}\)

**Principle:**

The test reagent dehydrates pentoses to form furfural. Furfural further reacts with orcinol and the iron ion present in the test reagent to produce a bluish product.

**Procedure:**

To 5 mL of Bial’s reagent add 2–3 mL of test solution and warm gently in a hot water bath for 2 minutes. The formation of a bluish green product is indicative of pentoses. Hexoses generally react to form muddy brown products.

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**Interpretation:**
This test is specific for pentoses indicated by formation of bluish product. All other colors indicate negative result for pentoses. Hexoses generally react to form green, red or brown products.

Some other tests are:

**FEHLING’S TEST:** (14,16)

**Principle:**
This test is used to differentiate between reducing and non-reducing sugars. A reducing sugar reacts with fehling’s reagent in alkaline medium to form an orange to red precipitates.

**Fehling’s reagent:**
Fehling’s solution is composed of equal parts of two solutions:

1. **Fehling’s solution A:**
   69.28 gms copper sulfate pentahydrate dissolved in 1 litre of distilled water.

2. **Fehling’s solution B:**
   346 gms potassium sodium tartrate and 120 grams sodium hydroxide in 1 litre of distilled water.

**Procedure:**
0.5 ml of test solution, mix with 2.0 ml of fehling’s solution in a test tube and heat the mixture.
Interpretation:

Positive result is detected by reduction of the deep blue solution of cupric (II) to a red precipitate of insoluble cuprous oxide (Cu₂O).

Osazone Test: (15,16)

Principle:

A solution of reducing sugar when heated with phenyl hydrazine, characteristic yellow crystalline compounds called osazone are formed. These crystals have definite crystalline structure, precipitation time and melting point for different reducing sugars.

Procedure:

To 0.5 g of phenylhydrazine hydrochloride add 0.1 gram of sodium acetate and ten drops of glacial acetic acid. Add 5 mL of test solution to this mixture and heat under boiling water bath for about half an hour. Cool the solution slowly and examine the crystals under a microscope. Needle-shaped yellow osazone crystals will be observed for glucose and fructose, whereas lactosazone shows mushroom shaped and maltose produces flower-shaped crystals.

Interpretation:
<table>
<thead>
<tr>
<th>No.</th>
<th>Test</th>
<th>Observation</th>
<th>Inference</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Molisch’s test:</td>
<td>Very gently add 1ml of Concent.</td>
<td>Presence of carbohydrates.</td>
<td>This is due to the formation of an unstable condensation product of beta-naphthol with furfural (produced</td>
</tr>
<tr>
<td></td>
<td>2-3 drops of beta-</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>naphthol solution</td>
<td></td>
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<tr>
<td></td>
<td>are added to 2ml of</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>the test solution.</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>A deep violet coloration is produced at the junction of two layers.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1) Glucose

**Needle** shaped glucosazine crystals.

2) Maltose:

**Sunflower** shaped maltosazine crystals.

3) Galactose:

**Rhombic plates**- galactosazine 12 crystals

4) Lactose:

**Powder puff** hog shaped lactosazine crystals.
<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H$_2$SO$_4$ along the side of the test tube.</td>
<td>by the dehydration of the carbohydrate.</td>
</tr>
<tr>
<td>2</td>
<td><strong>Iodine test:</strong>&lt;br&gt;4-5 drops of iodine solution are added to 1ml of the test solution and contents are mixed gently.</td>
<td>Presence of polysaccharide.</td>
</tr>
<tr>
<td></td>
<td><strong>Blue colour is observed.</strong></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><strong>Fehling’s test:</strong>&lt;br&gt;About 2 ml of sugar solution is added to about 2 ml of Fehling’s solution taken in a test-tube. It is then boiled for 10 min</td>
<td>Presence of reducing sugar</td>
</tr>
<tr>
<td></td>
<td>A red precipitate is formed</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><strong>Benedict’s test:</strong>&lt;br&gt;To 5 ml of Benedict’s solution, add 1ml of the test solution and shake each</td>
<td>Presence of reducing sugars</td>
</tr>
<tr>
<td></td>
<td>Formation of a green, red, or yellow precipitate</td>
<td></td>
</tr>
</tbody>
</table>
| Page 5 | **Barfoed’s test:**
|        | To 2 ml of the solution to be tested added 2 ml of freshly prepared Barfoed’s reagent. Place test tubes into a boiling water bath and heat for 3 minutes. Allow to cool. |
|        | A **deep blue colour** is formed with a red ppt. settling down at the bottom or sides of the test tube. |
|        | Presence of reducing sugars. Appearance of a red ppt as a thin film at the bottom of the test tube within 3-5 min. is indicative of reducing mono-saccharide. If the ppt formation takes more time, then it is a reducing disaccharide. |
|        | If the saccharide is a reducing sugar it will reduce Cu (11) ions to Cu(1) oxide |

| Page 6 | **Seliwanoff’s test:**
<p>|        | To 3ml of of Seliwanoff’s reagent, add 1ml of the test solution. Boil in |
|        | A <strong>cherry red colored precipitate</strong> within 5 minutes is obtained. |
|        | Presence of ketoses [Sucrose gives a positive ketohexose test ] |
|        | When reacted with Seliwanoff reagent, ketoses react within 2 minutes forming a cherry red |</p>
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>7</td>
<td><strong>Bial’s test</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Add 3ml of Bial’s reagent to 0.2ml of the test solution. Heat the solution in a boiling water bath for 2 minutes.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a) A <strong>blue-green</strong> product</td>
<td>Presence of pentoses</td>
</tr>
<tr>
<td></td>
<td>b) A <strong>muddy brown</strong> to gray product</td>
<td>Presence of hexoses.</td>
</tr>
<tr>
<td></td>
<td><strong>The furfurals formed produces bluish products.</strong></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td><strong>Osazone Test:</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 ml of the test solution, add 3ml of phenyl hydrazine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Formation of beautiful <strong>yellow crystals</strong> of osazone</td>
<td>Presence of Glucose/fructose</td>
</tr>
<tr>
<td></td>
<td>Reducing sugars forms ozazone on treating with phenylhydrazine</td>
<td></td>
</tr>
<tr>
<td>Chemical Reagent</td>
<td>Needle shaped crystals.</td>
<td>Presence of lactose</td>
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<td>------------------</td>
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</tr>
<tr>
<td>Hydrochloride solution and mix. Keep in a boiling water bath for 30 mts. Cool the solution and observe the crystals under microscope. <strong>Hedgehog crystals</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sunflower shaped crystals</strong></td>
<td>Presence of maltose</td>
<td></td>
</tr>
</tbody>
</table>

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