Tests for Carbohydrates

Goals

- Observe physical and chemical properties of some common carbohydrates.
- Use physical and chemical tests to distinguish between monosaccharides, disaccharides, and polysaccharides.
- Identify an unknown carbohydrate.
- Relate the process of digestion to the hydrolysis of carbohydrates.

Discussion

A. Benedict’s Test for Reducing Sugars

All of the monosaccharides and most of the disaccharides can be oxidized. When the cyclic structure opens, the aldehyde group is available for oxidation. Benedict’s reagent contains Cu$^{2+}$ ion that is reduced. Therefore, all the sugars that react with Benedict’s reagent are called reducing sugars. Ketoses also act as reducing sugars because the ketone group on carbon 2 isomerizes to give an aldehyde group on carbon 1.

![Chemical structure of ketose and aldose](image)

When oxidation of a sugar occurs, the Cu$^{2+}$ is reduced to Cu$^+$, which forms a red precipitate of cuprous oxide, Cu$_2$O(s). The color of the precipitate varies from green to gold to red depending on the concentration of the reducing sugar.

![Chemical reaction](image)

Sucrose is not a reducing sugar because it cannot revert to the open-chain form that would provide the aldehyde group needed to reduce the cupric ion.

![Chemical structure of sucrose](image)
B. Seliwanoff’s Test for Ketoses

Seliwanoff’s test is used to distinguish between hexoses with a ketone group and hexoses that are aldehydes. With ketoses, a deep red color is formed rapidly. Aldoses give a light pink color that takes a longer time to develop. The test is most sensitive for fructose, which is a ketose.

C. Fermentation Test

Most monosaccharides and disaccharides undergo fermentation in the presence of yeast. The products of fermentation are ethyl alcohol (CH$_3$CH$_2$OH) and carbon dioxide (CO$_2$). The formation of bubbles of carbon dioxide is used to confirm the fermentation process.

\[
\text{C}_6\text{H}_{12}\text{O}_6 \xrightarrow{\text{yeast}} 2\text{C}_2\text{H}_5\text{OH} + 2\text{CO}_2(\text{g})
\]

Although enzymes are present for the hydrolysis of most disaccharides, they are not available for lactose. The enzymes needed for the fermentation of galactose are not present in yeast. Lactose and galactose give negative results with the fermentation test.

D. Iodine Test for Polysaccharides

When iodine (I$_2$) is added to amylose, the helical shape of the unbranched polysaccharide traps iodine molecules, producing a deep blue-black complex. Amylopectin, cellulose, and glycogen react with iodine to give red to brown colors. Glycogen produces a reddish-purple color. Monosaccharides and disaccharides are too small to trap iodine molecules and do not form dark colors with iodine.

E. Hydrolysis of Disaccharides and Polysaccharides

Disaccharides hydrolyze in the presence of an acid to give the individual monosaccharides.

\[
\text{Sucrose} + \text{H}_2\text{O} \xrightarrow{\text{H}^+} \text{Glucose} + \text{Fructose}
\]

In the laboratory, we use water and acid to hydrolyze starches, which produce smaller saccharides such as maltose. Eventually, the hydrolysis reaction converts maltose to glucose molecules. In the body, enzymes in our saliva and from the pancreas carry out the hydrolysis. Complete hydrolysis produces glucose, which provides about 50% of our nutritional calories.

F. Testing Foods for Carbohydrates

Several of the tests such as the iodine test can be carried out with food products such as cereals, bread, crackers, and pasta. Some of the carbohydrates we have discussed can be identified.

Lab Information

Time: 3 hr
Comments: Tear out the report sheets and place them next to the matching procedures.
Related Topics: Carbohydrates, hemiacetals, aldohexoses, ketohexoses, reducing sugars, fermentation
**Experimental Procedures**

**A. Benedict’s Test for Reducing Sugars**

**Materials:** Test tubes, 400-mL beaker, droppers, hot plate or Bunsen burner, 5- or 10-mL graduated cylinder, Benedict’s reagent, 2% carbohydrate solutions: glucose, fructose, sucrose, lactose, starch, and an unknown

Place 10 drops of solutions of glucose, fructose, sucrose, lactose, starch, water, and unknown in separate test tubes. Label each test tube. Add 2 mL of Benedict’s reagent to each sample. Place the test tubes in a boiling water bath for 3–4 minutes. The formation of a greenish to reddish-orange color indicates the presence of a reducing sugar. If the solution is the same color as the Benedict’s reagent in water (the control), there has been no oxidation reaction. Record your observations. Classify each as a reducing or nonreducing sugar.

**B. Seliwanoff’s Test for Ketoses**

**Materials:** Test tubes, 400-mL beaker, droppers, hot plate or Bunsen burner, 5- or 10-mL graduated cylinder, Seliwanoff’s reagent, 2% carbohydrate solutions: glucose, fructose, sucrose, lactose, starch, and an unknown

Place 10 drops of solutions of glucose, fructose, sucrose, lactose, starch, water, and unknown in separate test tubes. Add 2 mL of Seliwanoff’s reagent to each. *The reagent contains concentrated HCl. Use carefully.*

Place the test tubes in a boiling hot water bath and note the time. After 1 minute, observe the colors in the test tubes. A rapid formation of a deep red color indicates the presence of a ketose. Record your results as a fast color change, slow change, or no change.

**C. Fermentation Test**

**Materials:** Fermentation tubes (or small and large test tubes), baker’s yeast, 2% carbohydrate solutions: glucose, fructose, sucrose, lactose, starch, and an unknown

Fill fermentation tubes with a solution of glucose, fructose, sucrose, lactose, starch, water, and unknown. Add 0.2 g of yeast to each and mix well. See Figure 1.

![Fermentation tube filled with a carbohydrate solution](image)
If fermentation tubes are not available, use small test tubes placed upside down in larger test tubes. Cover the mouth of the large test tube with filter paper or cardboard. Place your hand firmly over the paper cover and invert. When the small test tube inside has completely filled with the mixture, return the larger test tube to an upright position. See Figure 2.

![Figure 2 Test tubes used as fermentation tubes](image1)

Set the tubes aside. At the end of the laboratory period, and again at the next laboratory period, look for gas bubbles in the fermentation tubes or inside the small tubes. Record your observations. See Figure 3.

![Figure 3 Fermentation tubes with CO2 bubbles](image2)

### D. Iodine Test for Polysaccharides

**Materials:** Spot plate or test tubes, droppers, iodine reagent, 2% carbohydrate solutions in dropper bottles: glucose, fructose, sucrose, lactose, starch, and an unknown

Using a spot plate, place 5 drops of each solution of glucose, fructose, sucrose, lactose, starch, water, and unknown in the wells. (If you do not have a spot plate, use small test tubes.) Add 1 drop of iodine solution to each sample. A dark blue-black color is a positive test for amylose in starch. A red or brown color indicates the presence of other polysaccharides. Record your results. Complete the table to identify your unknown.

### E. Hydrolysis of Disaccharides and Polysaccharides

**Materials:** Test tubes, 10-mL graduated cylinder, 400-mL beaker (boiling water bath), hot plate or Bunsen burner, spot plate or watch glass, 10% HCl, 10% NaOH, red litmus paper, iodine reagent, Benedict’s reagent, 2% starch and sucrose solutions in dropper bottles

Place 3 mL of 2% starch in two test tubes and 3 mL of 2% sucrose solution in two more test tubes. To one sample each of sucrose and starch, add 20 drops of 10% HCl. To the other samples of sucrose and starch, add 20 drops of H₂O. Label the test tubes and heat in a boiling water bath for 10 minutes.
Remove the test tubes from the water bath and let them cool. To the samples containing HCl, add 10% NaOH (about 20 drops) until one drop of the mixture turns litmus paper blue, indicating the HCl has been neutralized. Test the samples for hydrolysis as follows:

**Iodine Test**  Place 5 drops of each solution on a spot plate or watch glass. Add 1 drop of iodine reagent to each. Record observations. Determine if hydrolysis has occurred in each.

**Benedict’s Test**  Add 2 mL of Benedict’s reagent to each of the samples and heat in a boiling water bath for 3–4 minutes. Determine if hydrolysis has occurred in each.

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**F. Testing Foods for Carbohydrates**

**Materials:**  Sugar samples (refined, brown, “natural,” powdered), honey, syrups (corn, maple, fruit), foods with starches: cereals, pasta, bread, crackers, potato, Benedict’s solution, Seliwanoff’s reagent, iodine reagent

Obtain two carbohydrate samples to test. Perform the Benedict’s, Seliwanoff’s, and iodine tests on each. Describe the kinds of carbohydrates you identify in each sample.
**Pre-Lab Study Questions**

1. What happens to glucose or galactose when the Cu$^{2+}$ in Benedict’s is reduced?

2. Would you expect fructose or glucose to form a red color rapidly with Seliwanoff’s reagent?

3. Why don’t all the disaccharides undergo fermentation with yeast?

4. How can the iodine test be used to distinguish between amylose and glycogen?

**Results of Carbohydrate Tests**

<table>
<thead>
<tr>
<th></th>
<th>A. Benedict’s Test</th>
<th>B. Seliwanoff’s Test</th>
<th>C. Fermentation Test</th>
<th>D. Iodine Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
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<tr>
<td>Fructose</td>
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<tr>
<td>Sucrose</td>
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<td></td>
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<tr>
<td>Lactose</td>
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<tr>
<td>Starch</td>
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<tr>
<td>Water</td>
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<tr>
<td>Unknown</td>
<td>#_______</td>
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</table>
Questions and Problems

Q.1 From the results in part A, list the sugars that are reducing sugars and those that are not.

Reducing sugars

Nonreducing sugars

Q.2 What sugars are ketoses?

Q.3 What sugars give a positive fermentation test?

Q.4 Which carbohydrates give a blue-black color in the iodine test?

Identifying an Unknown Carbohydrate

<table>
<thead>
<tr>
<th>Unknown No.__________</th>
<th>Results with Unknown</th>
<th>Possible Sugars Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benedict’s (A)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seliwanoff’s (B)</td>
<td></td>
<td></td>
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<tr>
<td>Fermentation (C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iodine (D)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

What carbohydrate(s) is/are in your unknown?
Questions and Problems

Q.5 What carbohydrate(s) would have the following test results?
   a. Produces a reddish-orange solid with Benedict’s and a red color with Seliwanoff’s reagent in 1 minute
   b. Gives a color change with Benedict’s test, a light orange color with Seliwanoff’s reagent after 5 minutes, and produces no bubbles during fermentation
   c. Gives no color change with Benedict’s or Seliwanoff’s test, but turns a blue-black color with iodine reagent

E. Hydrolysis of Disaccharides and Polysaccharides

<table>
<thead>
<tr>
<th>Results</th>
<th>Sucrose + H₂O</th>
<th>Sucrose + HCl</th>
<th>Starch + H₂O</th>
<th>Starch + HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benedict’s test</td>
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<td></td>
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<td></td>
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<tr>
<td>Hydrolysis products</td>
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<tr>
<td>present</td>
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</tr>
</tbody>
</table>

Questions and Problems

Q.6 How do the results of the Benedict’s test indicate that hydrolysis of sucrose and starch occurred?

Q.7 How do the results of the iodine test indicate that hydrolysis of starch occurred?
Tests for Carbohydrates

Report Sheet

Q.8 Indicate whether the following carbohydrates will give a positive (+) or a negative (−) result in each type of test listed below:

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Benedict’s Test</th>
<th>Seliwanoff’s Test</th>
<th>Fermentation Test</th>
<th>Iodine Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
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<tr>
<td>Galactose</td>
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<tr>
<td>Sucrose</td>
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<tr>
<td>Lactose</td>
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<tr>
<td>Maltose</td>
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</tr>
<tr>
<td>Amylose</td>
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<td></td>
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</tr>
<tr>
<td>Amylopectin</td>
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F. Testing Foods for Carbohydrates

<table>
<thead>
<tr>
<th>Test</th>
<th>Food Item 1</th>
<th>Food Item 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benedict’s test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seliwanoff’s test</td>
<td></td>
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<tr>
<td>Iodine test</td>
<td></td>
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</tr>
<tr>
<td>Possible carbohydrates present</td>
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<td></td>
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</tbody>
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