Lab #7: CARBOHYDRATES LAB

INTRODUCTION

In this experiment we will become acquainted with some of the common carbohydrate reactions of fructose, sucrose, cellulose, lactose, and starch. We will use these reactions to determine which of the five is present in an unknown.

PROCEDURE

Part I: Benedict's Test

Benedict’s reagent is a copper compound that will oxidize only aldehyde groups (aldoses) and not alcohols. If you consider cyclic forms of carbohydrates, hemiacetals give positive tests while acetals give negative tests. The reason for this is that the cyclic form interconverts (is in equilibrium) with the linear form that contains an aldehyde, for example:

1) Add about 1-2 inches of water and 3 boiling chips to a 600 mL beaker (it is the largest beaker in your drawer). Place the beaker and water on a hot plate, turn the hot plate on, and begin to boil the water.

2) Label 7 medium size test tubes (from your drawer) for each of the 5 sugars, your unknown, and your partner’s unknown. DO NOT ADD THE SUGAR SOLUTIONS UNTIL LATER

3) Using the metering dispenser, place 2 mL of Benedict's solution in EACH OF THE 7 labeled, medium size test tubes and heat in gently-boiling water bath for at least 2 minutes. NOTE: groups of 3 students will have 8 tubes.

4) Remove the tube that is labeled for the fructose solution. Add 10 drops of the 5% fructose solution to the Benedict's solution, mix thoroughly, and set the tube in the boiling water bath for 60 more seconds. Remove the tube from the water bath and check for a color change. A color change from clear blue to cloudy green, yellow or to brick red indicates the carbohydrate is a reducing sugar. The brick-red color is copper(I) oxide. Record your results in the DATA TABLE.

5) Repeat this Benedict's test for your other 6 sample test tubes as follows:

   a) Remove the tube labeled for sucrose from the boiling water bath. Repeat step 4, but use the sucrose solution instead of the fructose solution. Record your results in the DATA TABLE.

   b) Remove the tube labeled for cellulose from the boiling water bath. Repeat step 4, but use the cellulose solution instead of the fructose solution. Record your results in the DATA TABLE.
c) Remove the tube labeled for **starch** from the boiling water bath. Repeat step 4, but use the **starch** solution *instead of the fructose solution*. Record your results in the DATA TABLE.

d) Remove the tube labeled for **lactose** from the boiling water bath. Repeat step 4, but use the **lactose** solution *instead of the fructose solution*. Record your results in the DATA TABLE.

e) Remove the tube labeled for **partner 1's unknown** from the boiling water bath. Repeat step 4, but use **partner 1's unknown** *instead of the fructose solution*. Record your results in the DATA TABLE.

f) Remove the tube labeled for **partner 2's unknown** from the boiling water bath. Repeat step 4, but use the **partner 2's unknown** *instead of the fructose solution*. Record your results in the DATA TABLE.

6) Empty the contents of *all of the test tubes* into the liquid waste container that is located in the hood.

**Part II: Seliwanoff’s Test**

A positive Seliwanoff's test is indicative of a **ketohexose**, and is observed as a change in color from **colorless** to **red**. **Fructose** is a **ketohexose**; therefore it will give a positive Seliwanoff's test. Oligosaccharides and polysaccharides that contain **fructose residues** will also give a positive test.

![Fructose and Seliwanoff's reagent reaction](image)

1) Maintain the boiling water bath used in *Part I*, you may need to add more water because of evaporation.

2) Put 5 mL of freshly prepared Seliwanoff’s reagent in a **medium test tube** labeled for **fructose**. Then add 10 drops of 5% fructose solution and place the tube in a boiling water bath. (Caution: This is a strongly acidic solution.) A red color within two minutes is indicative of a ketohexose. **Record your results in the DATA TABLE.**

3) Repeat this Seliwanoff’s test for your other 6 sample test tubes as follows:

   a) Using a test tube labeled for **sucrose**, repeat step 2 (above), but use the **sucrose** solution *instead of the fructose solution*. **Record your results in the DATA TABLE.**

   b) Using a test tube labeled for **cellulose**, repeat step 2, but use the **cellulose** solution *instead of the fructose solution*. **Record your results in the DATA TABLE.**

   c) Using a test tube labeled for **starch**, repeat step 2, but use the **starch** solution *instead of the fructose solution*. **Record your results in the DATA TABLE.**
d) Using a test tube labeled for lactose, repeat step 2, but use the lactose solution instead of the fructose solution. Record your results in the DATA TABLE.

e) Using a test tube labeled for partner 1's unknown, repeat step 2, but use partner 1's unknown instead of the fructose solution. Record your results in the DATA TABLE.

f) Using a test tube labeled for partner 2's unknown, repeat step 2, but use partner 2's unknown instead of the fructose solution. Record your results in the DATA TABLE.

4) Empty the contents of all of the test tubes into the liquid waste container that is located in the hood.

**Part III: Iodine test**

Some of the starch molecules are shaped like very long spiral staircases, inside of which is just enough space to accommodate iodine molecules. The blue color arises when the electrons of the entrapped iodine molecules interact with the electrons of the starch molecule and the resulting complex absorbs visible light (appears dark). Be sure to rinse your graduated cylinder between sugar additions so you do not cross contaminate your samples!

1) Add 2 mL of the 5% fructose solution to a small test tube labeled for fructose, then add two drops of iodine solution to the tube.
   - A deep blue/black color is a positive test for starch.
   - Record your results in the DATA TABLE.

2) Repeat for the other 6 carbohydrate solutions as follows:

   g) Using a test tube labeled for sucrose, repeat step 1 (above), but use the sucrose solution instead of the fructose solution. Record your results in the DATA TABLE.

   h) Using a test tube labeled for cellulose, repeat step 1, but use the cellulose solution instead of the fructose solution. Record your results in the DATA TABLE.

   i) Using a test tube labeled for starch, repeat step 1, but use the starch solution instead of the fructose solution. Record your results in the DATA TABLE.

   j) Using a test tube labeled for lactose, repeat step 1, but use the lactose solution instead of the fructose solution. Record your results in the DATA TABLE.

   k) Using a test tube labeled for partner 1's unknown, repeat step 1, but use partner 1's unknown instead of the fructose solution. Record your results in the DATA TABLE.

   l) Using a test tube labeled for partner 2's unknown, repeat step 1, but use partner 2's unknown instead of the fructose solution. Record your results in the DATA TABLE.

3) Empty the contents of all of the test tubes into the liquid waste container that is located in the hood.
DATA TABLE (write (+) for a positive test and (–) for a negative test)

<table>
<thead>
<tr>
<th></th>
<th>Benedict’s Test</th>
<th>Seliwanoff’s Test</th>
<th>Iodine Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Sucrose</td>
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<tr>
<td>Cellulose</td>
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<tr>
<td>Starch</td>
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<td>Lactose</td>
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<tr>
<td>Unknown #</td>
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</tbody>
</table>

**Conclusion**: Unknown Number ________________ is ________________________.
Part IV: Representing 3D structures with two-dimensional drawings.

(a) Fischer projection formulas of open-chain monosaccharides:

- Select model #1 of an open-chain monosaccharide. If the model comes apart when you are manipulating it and you are not sure how to put it back together, bring it to the instructor!

Does the model represent an aldose or ketose? ______________

Does the model represent a triose, tetrose, etc.? ______________

Does the model represent a L- or a D- sugar? ______________

Classify this monosaccharide as a combination of the previous 3 answers ______________.

Determine and draw the Fischer projection for this model below, being sure to view each C atom so that the –H and –OH groups are facing up towards you (above the plane).

Replace model #1 and repeat the same procedure for models #2-4.

<table>
<thead>
<tr>
<th>MODEL# 2</th>
<th>MODEL #3</th>
<th>MODEL#4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classification:</td>
<td>Fischer Projection</td>
<td>Fischer Projection</td>
</tr>
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</tbody>
</table>

(b) Haworth projection formulas of cyclic monosaccharides.

View model #5 of the cyclic monosaccharide and answer the following:

- Is the ring structure a pyranose (6 sides) or a furanose (5 sides): ______________

- Is the structure α or β? ______________

Draw the Haworth projection for the compound represented by this model.