The Chemistry of Carbohydrates

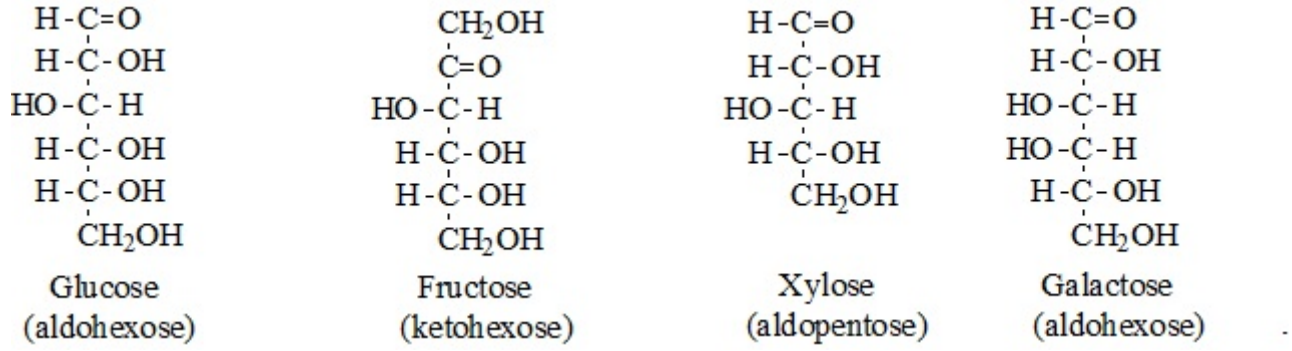
Experiment #5

Objective: To determine the carbohydrate class of an unknown by carrying out a series of chemical reactions with the unknown and known compounds in each class of carbohydrates.

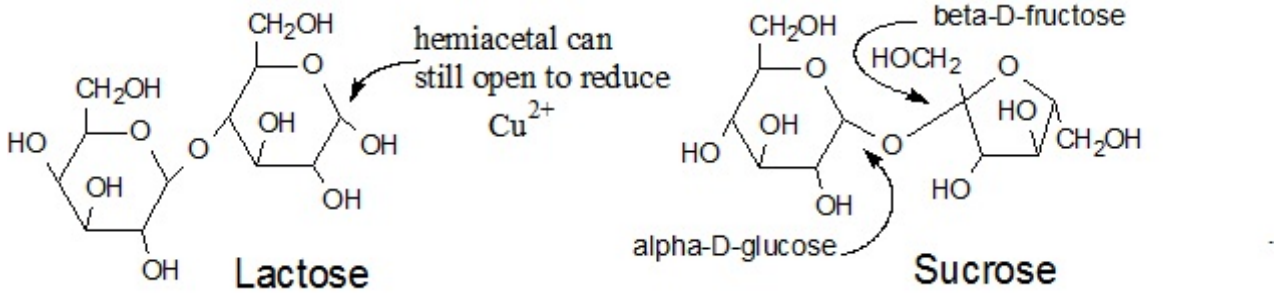
Introduction

Simple sugars, starches and cellulose are organic compounds that have the approximate formula $C(H_2O)_n$, which accounts for the name carbohydrate (or hydrate of carbon) that is usually applied to this group of compounds. They are not truly hydrates of carbon but are polyhydroxy (alcohol) compounds that contain an aldehyde or ketone functional group. These functional groups give the carbohydrates some of their chemical properties that will be studied in this lab.

Simple sugars are called monosaccharides (one sugar), or disaccharides (2 sugars). Some monosaccharides are glucose, fructose, galactose, and xylose. Note that xylose is a pentose and fructose is a ketose.

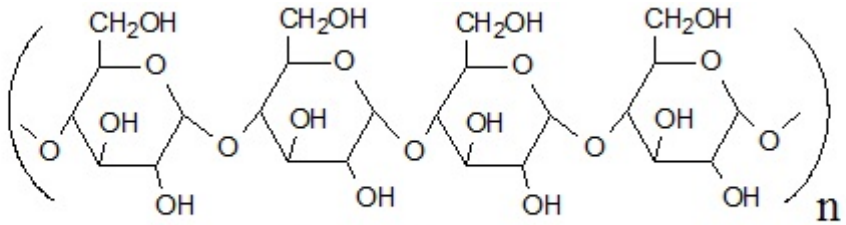


Two common disaccharides are sucrose (table sugar) and lactose (milk sugar); sucrose is a combination of glucose and fructose linked together by their anomeric carbons to produce a nonreducing sugar (it does not reduce Cu^{2+}), whereas lactose is a combination of galactose and glucose linked together by a β-1,4-glycosidic bond to produce a reducing disaccharide.



When many sugar molecules are linked together into a polymer, the resulting compound is called a polysaccharide. Starches and celluloses are polysaccharides. Amylose is a linear chain polymer of glucose, whereas amylopectin (a plant starch) and glycogen (an animal starch) are branched polymers of glucose (see the text book for structures of the branched starches). Starches are

broken down in the body by enzymes, such as amylase, to produce disaccharides and monosaccharides.

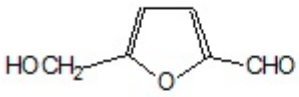


Amylose

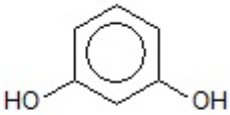
Qualitative Tests for Carbohydrates

Reducing sugars are usually detected with ***Benedict's reagent***, which contains Cu^{2+} ions in alkaline solution with sodium citrate added to keep the cupric ions in solution. The alkaline conditions of this test causes isomeric transformation of ketoses to aldoses, resulting in all monosaccharides and most disaccharides reducing the blue Cu^{2+} ion to cuprous oxide (Cu_2O), a brick red-orange precipitate. This solution has been used in clinical laboratories for testing urine.

Barfoed's solution contains cupric ions in an acidic medium. The milder condition allows oxidation of *mono*saccharides but does not oxidize *di*saccharides. If the time of heating is carefully controlled, disaccharides do not react while reducing monosaccharides give the positive result (red Cu_2O precipitate). Ketoses do not isomerize with this reagent. Hexoses are dehydrated in the presence of nonoxidizing acids to form 5-hydroxymethylfurfural.



Hydroxymethylfurfural



Resorcinol

Seliwanoff's reagent contains resorcinol in 6 M hydrochloric acid. Ketohexoses undergo dehydration when heated in this reagent to form hydroxymethylfurfural, that condenses with resorcinol to give a red product. Ketohexoses (such as fructose) and disaccharides containing a ketohexose (such as sucrose) form a cherry-red condensation product. Other sugars may produce yellow to faint pink colors.

Iodine forms a deep blue color in the presence of starch. Potassium iodide is added to the reagent solution in order to make the iodine more soluble in water. Some forms of starch may yield a greenish color. Simple carbohydrates (mono- and disaccharides) and cellulose do not cause any change in the orange-brown color of the iodine reagent.

Reagents and Materials

Packet of carbohydrate unknown, 1% solutions of carbohydrate standards (glucose, fructose, sucrose, lactose, starch), Benedict's reagent, Barfoed's reagent, Selinwanoff's reagent, iodine in potassium iodide solution, testtubes, beaker for hot water bath, 10% sodium hydroxide solution.

Preliminary Procedures - Do These Before Starting Part A.

- Obtain an unknown carbohydrate (solid) and prepare a 1% solution of your unknown by dissolving 0.25 g in 25 mL of deionized water. Test your unknown carbohydrate solution in parallel with the known carbohydrate solutions in each of the following tests. If you work in pairs, each student in the group must have her or his own unknown in solution.

- Set up a hot water bath using a 400 mL beaker with less than 100 mL water (about 80 mL) and heat to near boiling on a hot plate. Do not fill more than one-quarter full.

- The purpose of this experiment is to demonstrate that different classes of carbohydrates can be distinguished from one another by specific chemical tests.

***** Begin by setting up tubes for the enzymatic digestion of starch in Part A, steps 1-5.**

Part A: Enzymatic Digestion of Starch.

1. Collect about 2 mL of your saliva in a clean large test tube.

2. Add 4 mL of neutral (pH 7) phosphate buffer solution to the saliva and mix well.

3. Pour half of the saliva mixture into a clean small test tube and label these test tubes S1 and S2 (for saliva). The large test tube is S1 and the small tube is S2.an be used for other parts.

4. Add about 3 mL of neutral buffer (no saliva) to each of 2 other clean test tubes and label these B1 and B2 (for buffer). Use a large test tube for B1 and a small test tube for B2.

5. Add about 6 drops of starch solution to each of the 4 tubes (2 with saliva and 2 with buffer only) and mix well. Be sure to mix well, especially the small tubes. Allow the mixtures to stand while you proceed with parts B thru E.

6. After allowing the solutions to stand for at least 30 min, test large test tubes for glucose with Benedict's reagent and test the small test tubes for starch with iodine as described below.

7. Add 3 drops of Benedict's reagent to the large tube containing saliva (S1) and to the large tube containing buffer (B1). Add 1.0 mL of 10% sodium hydroxide solution to these 2 tubes, mix well and place both tubes in the hot water bath for 3 minutes.

8. Add 1 drop of iodine solution to the small tube containing saliva (S2) and to the small tube containing buffer (B2) and mix well. It is not necessary to heat these 2 tubes.

9. Record your observations for these 4 tubes on the report sheet and answer the questions for this part.

Part B: Test for reducing sugars with Benedict's reagent.

1. Label 7 (or 8 if you work in pairs) test tubes for each of the 6 test carbohydrates and water blank. Test carbohydrates are: 1-glucose; 2-fructose; 3-sucrose; 4-lactose; 5-starch; and 6-water blank. Tube number 7 (and 8) is (are) for the solution of your unknown prepared in preliminary procedures. Do not use the solid unknown in these tests, only the solution of unknown in water. This number scheme is used for parts B, C, and D.

2. Add about 2 mL of 1% carbohydrate test solution (as listed above, *i.e.* 2 mL of 1% glucose in tube 1, 2 mL of 1% fructose in tube 2 and so on) to their respective labeled test tubes (see step 1 above). Add 2 mL of deionized water to tube #6 as a blank. Add 2 mL of your unknown carbohydrate solution to tube 7. If you're working in pairs, add 2 mL of your partner's unknown carbohydrate solution to tube 8.

Caution: Benedict's reagent is caustic, rinse thoroughly with water if you get this solution on your skin or clothing.

3. Add 3 drops of Benedict's solution to each tube and mix thoroughly.

4. Place the test tubes in the hot water bath and note the color changes within 2 min as they are heated (the water bath should be hot, but not necessarily boiling, heat the tubes no more than 3 min).

5. Record observations on your report sheet, such as color and whether there is any solid.

6. What conclusions can you make about your unknown? Is it a reducing sugar?

Discard these solutions in the carbohydrate hazardous waste container in the hood.

Part C: Test for mono- vs disaccharides with Barfoed's reagent.

1. Empty, wash and rinse the test tubes from part B and bring the hot water bath to a boil.

2. Add about 2 mL of 1% carbohydrate solutions to the respective labeled test tubes from the previous part, including the water blank, as described in B-2 above.

Caution: Barfoed's reagent is caustic, rinse thoroughly with water if you get this solution on your skin or clothing.

3. Add 1 mL of Barfoed's reagent to each tube and mix well.

4. Place all the tubes in the boiling water bath at the same time and heat for 2 minutes after the water begins to boil again.

5. Record observations on the report sheet. Note any red precipitate at the bottom of the tubes.

6. What conclusions can you make about your unknown now? Is it a monosaccharide or disaccharide?

Discard these solutions in the carbohydrate hazardous waste container in the hood.

Part D: Test for ketohexoses with Seliwanoff's reagent.

1. Empty, wash and rinse the test tubes from part C and make sure you have sufficient water in your boiling water bath.

Caution: Seliwanoff's reagent is caustic, rinse thoroughly with water if you get this solution on your skin or clothing.

2. Add about 2 mL of Seliwanoff's reagent (about 2 droppers full) to each labeled test tube.

3. Add 1 drop of the respective carbohydrate solution and 1 drop of water blank to the appropriate test tubes as described in part B-1 above and mix well.

4. Place all the test tubes in the boiling water bath at the same time and heat for 3 min after the water begins to boil again.

5. Record observations on the report sheet. Do you see any color changes?

6. What conclusions can you make about your unknown now? Is it a ketose?

Discard these solutions in the carbohydrate hazardous waste container in the hood.

Part E: Test for starches with iodine.

1. Empty, wash and rinse the test tubes from part D.

2. Add about 2 mL of each carbohydrate test solution or deionized water to the respective labeled test tube as described in part B-1.

3. Add 1 drop of iodine in potassium iodide reagent to each tube and mix well.

4. Record observations on the report sheet. Does the iodine solution change color?

Discard these solutions in the carbohydrate hazardous waste container in the hood.

Complete part A to determine what happened with the starch in the presence of saliva enzymes or in buffer solution with no saliva.

Summarize your conclusion about the identity of your unknown carbohydrate on the Report Sheet indicating the support for this conclusion as a result of each of the tests you performed.

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Name _____ Chemistry 4X, Sec _____

The Chemistry of Carbohydrates

Experiment #5 Pre-lab Exercise

1. Show the chemical reaction that takes place when Benedict's reagent (Cu^{2+}) reacts with glucose (use open chain Fisher projection).

2. Do you expect fructose to give a positive reaction with Barfoed's reagent? Explain why or why not.

3. Indicate which of the test carbohydrates (glucose, fructose, lactose, sucrose, starch) would give 5-hydroxymethylfurfural when heated with hydrochloric acid.

4. Describe the reaction that takes place when starch is hydrolyzed by an enzyme? What is the product of starch hydrolysis? What is the name of the enzyme in saliva that would hydrolyze starch?

Name _____ Section _____

The Chemistry of Carbohydrates

Experiment #5 Data & Report Sheet

Observations for Parts B thru E. Unknown number _____

Carbohydrate	Benedict Test	Barfoed Test	Seliwanoff Test	Iodine Test
Glucose				
Fructose				
Lactose				
Sucrose				
Starch				
Water				
Unknown #				

B-1. What conclusions can you make regarding your unknown after the test with Benedict's reagent? Is your unknown a reducing sugar?

C-1. What conclusions can you make about your unknown after the test with Barfoed's reagent? Is your unknown a monosaccharide? An aldose?

D-1. What conclusions can you make about your unknown after the test with Seliwanoff's reagent? How does the final color of your unknown in the Seliwanoff test support your conclusion?

E-1. What conclusions can you make about your unknown after the test with iodine reagent?

Summarize your conclusions. What is the identity of your unknown (aldose or ketose; monosaccharide, disaccharide or starch)?

Name _____ Section _____

Part A. Observations. Indicate whether each test was positive or negative and indicate what color each solution was after performing the tests.

Starch mixture	Benedict's Test	Iodine Test
Starch/Saliva	S1	S2
Starch/Buffer	B1	B2

A-1. Describe what caused the observed results in the Benedict's Test for starch/saliva vs. starch/buffer mixtures. Does either solution contain a reducing sugar (such as glucose)?

A-2. Describe what caused the observed results in the iodine test for the starch/saliva vs. starch/buffer mixtures. Does either solution still contain starch?

A-3. What conclusions can you make regarding the action of saliva on starch? What is the product of this reaction?

A-4. What enzyme is involved? You may want to consult the text book for this.