The Chemistry of Carbohydrates

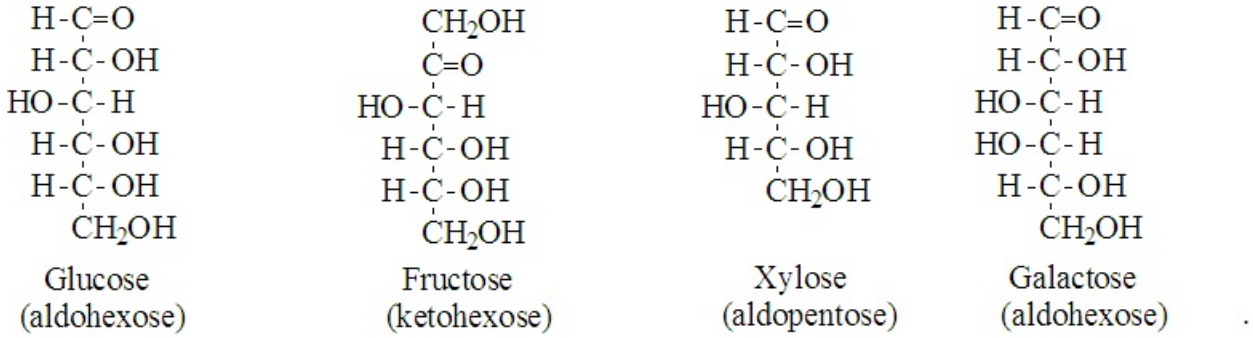
Experiment #5

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.

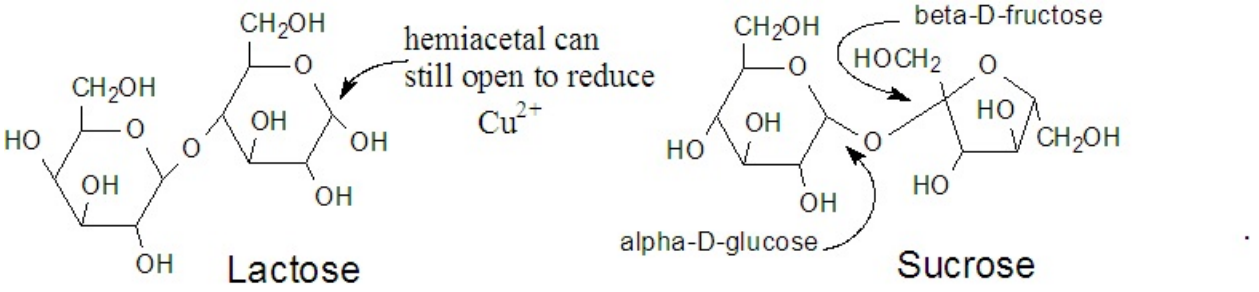
Introduction

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.

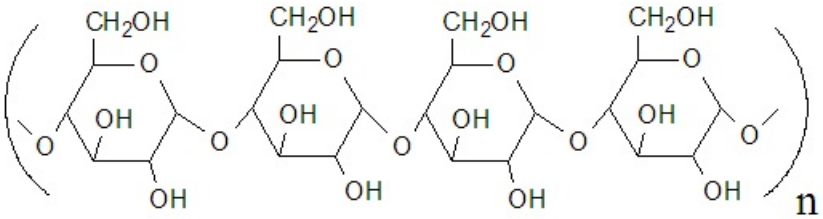


Two common disaccharides are sucrose (table sugar) and lactose (milk sugar); sucrose is a combination of glucose and fructose linked together by their anomeric carbons to produce a nonreducing sugar (it does not reduce Cu^{2+}), whereas lactose is a combination of galactose and glucose linked together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



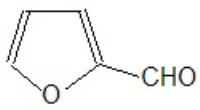
Amylose

Qualitative Tests for Carbohydrates

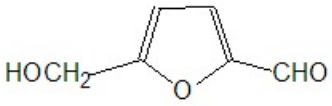
Reducing sugars are usually detected with ***Benedict's reagent***, which contains Cu^{2+} ions in alkaline solution with sodium citrate added to keep the cupric ions in solution. The alkaline conditions of this test causes isomeric transformation of ketoses to aldoses, resulting in all monosaccharides 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.

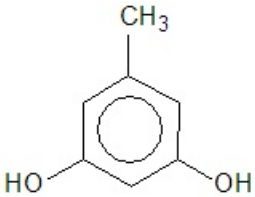
Carbohydrates are dehydrated in the presence of nonoxidizing acids to form furfural and hydroxymethylfurfural.



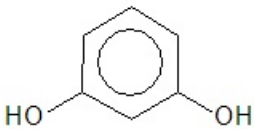
Furfural



Hydroxymethylfurfural



Orcinol



Resorcinol

Seliwanoff's reagent contains resorcinol in 6 M hydrochloric acid. Hexoses 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.

Bial's reagent contains orcinol (5-methylresorcinol) in concentrated HCl with a small amount of $FeCl_3$ catalyst. Pentoses are converted to furfural by this reagent, which form a blue-green color with orcinol. This test is used to distinguish pentoses from hexoses.

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, xylose, sucrose, lactose, starch), Benedict's reagent, Barfoed's reagent, Selinwanoff's reagent, Bial's reagent, iodine in potassium iodide solution, testtubes, beaker for hot water bath, 10% sodium hydroxide solution, bunsen burner.

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

Preliminary Procedures - Do These Before Starting Part A.

- Obtain an unknown carbohydrate 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 his or her own unknown.

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

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

Part A: Enzymatic Digestion of Starch.

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

2. Add 6 mL of neutral (pH 7) phosphate buffer solution to the saliva and mix well.

3. Pour half of the saliva mixture into another clean test tube and label these test tubes S1 and S2 (for saliva)

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

5. Add about 10 drops of starch solution to each of the 4 tubes (2 with saliva and 2 with buffer only) and mix well. Allow the mixtures to stand while you proceed with parts B thru F.

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

7. Add 3 drops of Benedict's reagent to one tube containing saliva (S1) and to one 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 other tube containing saliva (S2) and the other 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 8 (or 9 if you work in pairs) test tubes for each of the 7 test carbohydrates and water blank. Test carbohydrates are: 1-glucose; 2-fructose; 3-xylose; 4-sucrose; 5-lactose; 6-starch; and water blank. Tube number 8 (and 9) is (are) for the solution of your unknown prepared in preliminary procedures. This number scheme is used for parts B, C, D and F.

2. Add about 3 mL of 1% carbohydrate test solution (as listed above) to their respective labeled test tubes. Add 3 mL of deionized water to tube #7 as a blank. If you're working in pairs, you will need an additional tube for the second 3 mL of unknown solution.

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.

6. What conclusions can you make about your unknown?

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-1 above.

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

3. Add 2 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. Do you see a red precipitate at the bottom of the tubes?

6. What conclusions can you make about your unknown now?

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 3 mL of Seliwanoff's reagent 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?

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

Part E: Test for pentoses with Bial's reagent.

CAUTION!!! Be extremely careful when heating these tubes. Bial's reagent contains concentrated hydrochloric acid. Be sure to heat the tube slowly, tilting it slightly and heating along the entire side of the tube. Do not point the tube toward yourself or any of your fellow lab workers.

1. Empty, wash and rinse the test tubes from part D. You will use a Bunsen burner flame to

heat the test tubes directly in this part.

2. Add about 2 mL of 1% xylose, glucose, fructose and your unknown solution to their respective labeled test tube. Be sure to use large test tubes and not the small ones. You will not be analyzing sucrose, lactose, starch nor water for this test.

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

4. Carefully heat each tube (with some agitation) directly over the burner flame. Hold the tube at a diagonal and heat along the sides of the tube rather than at the bottom to prevent eruption of the liquid from the tube. Move the tube diagonally in and out of the flame, until the mixture just begins to boil. Stop heating when the mixture begins to boil.

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

6. What conclusions can you make about your unknown now?

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

Part F: Test for starches with iodine.

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

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 enzyme.

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.

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, xylose, lactose, sucrose, starch) would give furfural when heated with hydrochloric acid.

4. Which of the test carbohydrates would give hydroxymethylfurfural when heated with hydrochloric acid?

5. 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 F. Unknown number _____

Carbo-hydrate	Benedict Test	Barfoed Test	Seliwanoff Test	Bial Test	Iodine Test
Glucose					
Fructose					
Xylose					
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 draw about your unknown after the test with Bial's reagent? How does the color of your unknown in the Bial test support your conclusion?

F-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; pentose or hexose; 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.

A-2. Describe what caused the observed results in the iodine test for the starch/saliva vs. starch/buffer mixtures.

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

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

A-5. What is (are) the product(s) of the reaction of starch with this enzyme?