

## EXPERIMENT 12

# The Chemistry 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 often 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 unit), or **disaccharides** (2 sugar units). When several sugar molecules are linked together into a polymer, the resulting compound is called a **polysaccharide**. Starches and celluloses are polysaccharides. The most common monosaccharides are glucose, fructose, galactose, ribose and mannose, with all but fructose in this list classified as **reducing sugars** because they will reduce some metal ions, such as  $Cu^{2+}$  to  $Cu^+$ . The most common disaccharides are sucrose (table sugar) and lactose (milk sugar). Sucrose is a combination of glucose and fructose that are linked together to produce a nonreducing sugar (it does not reduce  $Cu^{2+}$ ). The nature of the glycosidic bond in lactose gives a reducing sugar.

### MATERIALS NEEDED

Carbohydrate test solutions, 1% each (glucose, fructose, sucrose, starch) and glucose solutions (0.1 mM, 0.3 mM, 1 mM, 3 mM, and 10 mM), glucose test strips, Benedict's solution, iodine solution, phosphate buffer solution (pH 7), foods to test for carbohydrates (should include table sugar, honey, corn syrup, corn starch, and powdered confectionary sugar).

### PROCEDURE

#### Part A. Glucose Test Strips

**Note: The instructor may arrange one set of 5 test strips with a drop of the appropriate glucose test solution; each student should do the remainder of this part individually.**

Simple test strips are used by diabetics to test glucose in the urine. Place one drop of the following glucose solutions on separate test strips: 10 mM, 3 mM, 1 mM, 0.3 mM and 0.1 mM. Match the colors with those on the test strip container and compare with the sugar content of the test solution. The molecular weight of glucose is 180 g/mol; therefore a 10 mM solution would contain 1.8 g/L, or would be approximately 0.18% glucose. You should check this calculation and determine the percent concentration for the other four test solutions. Make a table in your notebook with mM concentration and % concentration of each test solution in the first two columns and the experimental % concentration by comparison of color in the third column.

### Part B: Test for Reducing Sugars

Sugars that have a free aldehyde group will react with  $\text{Cu}^{2+}$  (blue) to form  $\text{Cu}^+$  (brick red,  $\text{Cu}_2\text{O}$ ). Many monosaccharides (e.g., glucose, galactose, mannose) and disaccharides (e.g., maltose and lactose) have free aldehyde groups that give positive tests. Fructose is a keto sugar that can undergo isomerization in alkaline solution to produce glucose or mannose. Fructose will give a positive test with the  $\text{Cu}^{2+}$  (Benedict's) solution.

Set up a hot water bath using a 400 mL beaker that is about half filled with water and heat to near boiling. While the water is heating, add about 3 mL of 1% solution of the carbohydrates to 4 labeled test tubes: glucose, fructose, sucrose and starch. Add 3 mL of deionized water to a fifth tube as a blank. Add 5 drops of Benedict's solution to each tube and mix thoroughly. Place the test tubes in the water bath and note the color changes as they are heated. You should make a table in your notebook with names of the sugars in the first column and any color change within 5 min for each solution tested with Benedict's solution in the second column, including the water blank. Have a third column in your table for the iodine test in part C.

### Part C. Iodine Test for Starch

Iodine ( $\text{I}_2$ ) will react with starches to produce a complex with a color that depends on the type of starch. Amylose, a linear plant starch, gives a deep blue iodine complex. Amylopectin, a branched plant starch, gives a purple complex. And glycogen, a branched animal starch, gives a reddish-brown complex with iodine. All of these will look nearly black at high concentrations.

Add about 2 mL of a 1% solution of each of the carbohydrates to separate clean test tubes: glucose, fructose, sucrose, and starch. Add 2 mL of deionized water to another tube as a blank. Add 3 drops of iodine solution to each test tube. Record the color in your notebook. Indicate which carbohydrates reacted with the iodine in solution.

#### Part D. Enzymatic Digestion of Starch

Collect about 1 mL (or more) of saliva in a clean test tube and add 3 mL of pH 7 buffer and mix well. Pour half of the saliva mixture into another clean test tube. Add 2 mL of pH 7 buffer (no saliva) to 2 other clean test tubes. Label each tube to avoid confusing them (e.g., label 2 tubes saliva and 2 tubes buffer only). Add 1 mL of 1% starch solution to each of the four tubes. Allow the solutions to stand for 20 to 30 min and test for glucose and starch. While these solutions are standing for the enzyme reaction to proceed, continue with part E below.

To test for glucose and starch, add 5 drops of Benedict's solution to one test tube with the starch-saliva mixture and to one tube with the starch-buffer mixture. Place these 2 test tubes in the hot water bath and determine which of the tubes contain glucose (see part B). Add 3 drops of iodine solution to the remaining 2 test tubes to see which test positive for starch (see part C). Is it necessary to heat the iodine test solutions? Record your observations in your notebook and draw conclusions regarding the action of saliva on starch. See questions at the end.

#### Part E. Testing Foods

**Note: The instructor may arrange for each group to prepare one of the following solutions and have students share these. Solutions to be tested are: table sugar, honey, corn syrup, corn starch and powdered sugar. Add a small amount (about 1 g or 1/4 tsp) of the following food carbohydrates to about 100 mL of deionized water. Keep each carbohydrate separate, do not mix them together and label them properly.**

Add about 3 mL of each of these solutions to separate test tubes and test with Benedict's solution as in part B. Be sure to heat the solutions for the Benedict's test. Add 3 mL of each of the carbohydrate solutions to 5 other test tubes and test with iodine solution as in part C. Is it

necessary to heat the iodine test solutions? Record your results in your notebook, indicating whether each food carbohydrate contains reducing sugars or starch. What carbohydrate would be present if the test is negative for both reducing sugar and starch? See results in parts B and C.

Questions to answer in your notebook:

1. What is the color that results if there is no reaction in the Benedict's test (part B)? What is the purpose of the water blank in this test?
2. Which of these carbohydrates gives a positive reaction with the Benedict's solution? Why does it give a positive test?
3. What color do you observe for the distilled water blank with the iodine reagent?
4. Which of these carbohydrates gives a positive reaction with the iodine reagent? Why?
5. From the color you observe when the starch solution is mixed with the iodine solution, what kind of starch do you think is in the solution?
- 6.\* Was there a positive reaction to Benedict's solution for the starch mixed with saliva? For the starch mixed with buffer? Was there a positive reaction to iodine for the starch mixed with saliva? For the starch mixed with buffer? What do these results mean? What happens to the starch in the presence of saliva? Is all of the starch completely broken down by the saliva in the time you have allowed the reaction to proceed? What is in saliva to cause this to happen?
7. From your observations for the tests on food carbohydrates, how would you describe each of these food items in terms of the sugars you tested in parts B and C? Record in your notebook your best guess for the carbohydrate content of each food from your observations. (Might it contain glucose or fructose? Sucrose? Starch?)