

# EXPERIMENT 5

## Properties of Carbohydrates : Solubility, Reactivity, and Specific Rotation

### Materials Needed

About 3-5 g each of Glucose, Fructose, Maltose, Ribose, Galactose, Lactose, Sucrose, Starch	
sodium bicarbonate, $\text{NaHCO}_3(\text{s})$	15 mL 5% sucrose
25 mL 1% cooked starch suspension	5 mL 6 M HCl
50 mL Benedict's solution	5 mL dilute iodine solution
0.150 g/mL glucose solution	unknown fructose solution
polarimeter, spot plates, Parafilm, extra test tubes	

### Additional Reading Assignment

Timberlake, Chapter 16.

### Background

Carbohydrates are named as such because they generally contain C, H, and O in the ratio  $\text{C}_n(\text{H}_2\text{O})_n$ . The smallest carbohydrate molecules are called *monosaccharides* and these are the basic building blocks for larger carbohydrate molecules, the *disaccharides* and *polysaccharides*. Monosaccharides and disaccharides are collectively referred to as *sugars* because of their often-sweet taste. Polysaccharides include the *starches* and *cellulose*.

Most monosaccharides can exist as either an open-chain or a cyclic structure (Figure 5-1), with these two forms being in equilibrium with each other. The cyclic structure contains a new chiral carbon not present in the open-chain form. Monosaccharides are classified as *aldoses* if they contain an aldehyde group in their open-chain form, or as *ketoses* if they contain a ketone group. Glucose is an example of an aldose and fructose is a ketose. The most common disaccharide, sucrose or table sugar, is a combination of glucose and fructose (Figure 5-2).

A *polarimeter* is an instrument designed to detect and measure the rotation of plane-polarized light. The rotation is directly proportional to the number of optically active molecules in the path of the light. If the sample tube is long, there will be many molecules, and the rotation will be large. Similarly, if the concentration of the sample is high, there will also be many molecules, and the rotation will be large.

Mathematically the relationship for *optical rotation* is

$$\text{Rotation (degrees)} = [\alpha]_D^{20} \times \ell \times d$$

where  $\ell$  is the length of the tube in decimeters (dm) and  $d$  is the concentration of the solution in g/mL. The *specific rotation*,  $[\alpha]_D^{20}$ , is the rotation experienced by a sample in a 1.0-dm tube at a 1.0 g/mL sample concentration. The 20 refers to the temperature (in °C) normally used, and the D refers to the wavelength of light used.

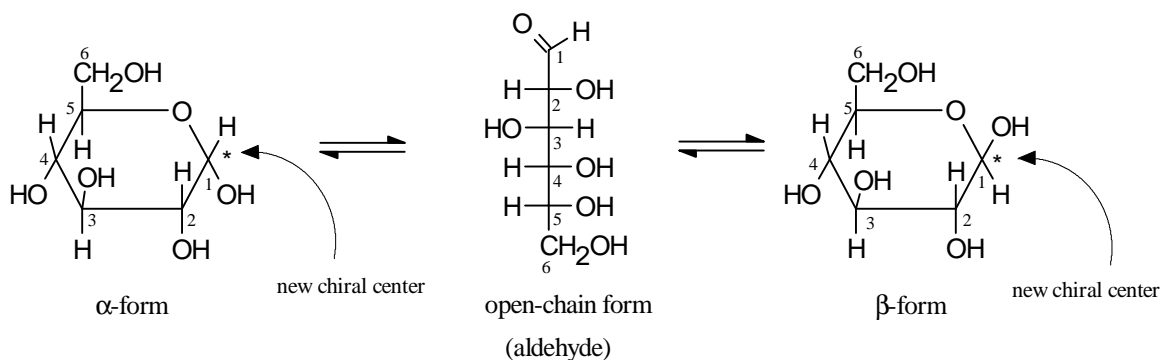
Chemical reactions of carbohydrates take place principally at the aldehyde and ketone sites. Aldoses contain the easily oxidizable aldehyde functional group and, therefore, give positive results when tested with Benedict's solution (just as simple aldehydes do as we saw in expt #4). The  $\alpha$ -hydroxy ketone group of ketoses is also easily oxidized. Therefore, all monosaccharides give a positive Benedict's test.

Another example of a specific test for carbohydrates is the *iodine test*, which detects the presence of

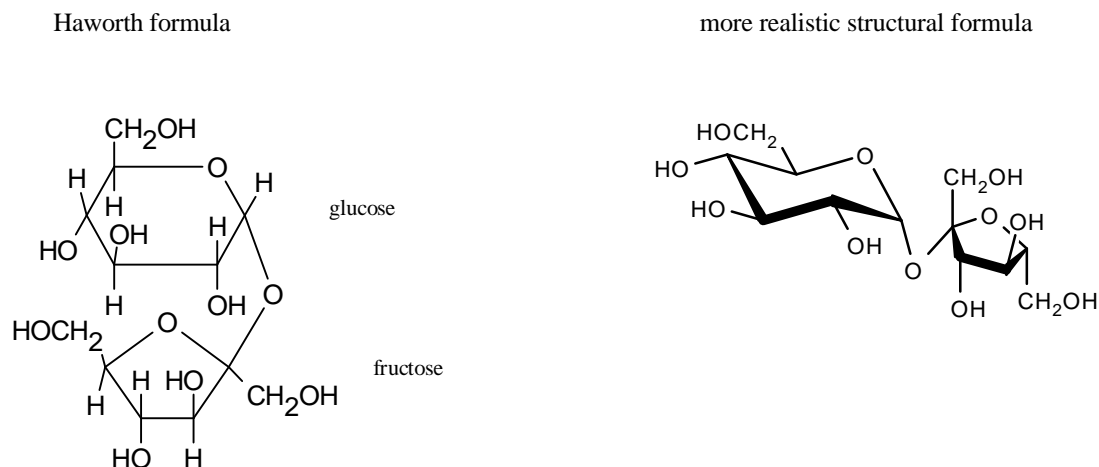
polysaccharides. Iodine is adsorbed onto the surface of the polysaccharide, forming a deeply colored complex.

**All di- and polysaccharides may be hydrolyzed by reaction with water** (a reaction which is catalyzed by acid) **to produce monosaccharides**. Enzymes may also be used instead of acid, but they are much more specific for the particular carbohydrate.

**Figure 5-1. Open-chain and cyclic structures of glucose.**



**Figure 5-2. Sucrose: a disaccharide that is a combination of  $\alpha$ -glucose and fructose.**



## **Procedure**

### **I. Physical Tests**

#### **1. Solubility.**

Label eight 15-cm test tubes as follows: glucose, fructose, galactose, lactose, maltose, ribose, sucrose, and starch. Add a small amount of each carbohydrate to the appropriate tube, enough to just barely cover the curved portion of the bottom. Note the color and physical form of each. Add 10 mL of distilled water to each tube, cover with a square of Parafilm, hold with your thumb, and shake vigorously. Shake each tube several times before comparing solubilities. Be sure not to mistake a slow rate of solution for insolubility.

To decide if starch (tube #8) has formed a true solution or a suspension, filter one-quarter of the liquid through a folded filter paper using a small funnel. Add 2 drops of dilute iodine solution ( $I_2$  plus starch gives a purple color) both to the filtrate and to another one-quarter of the unfiltered solution. Observe the resulting mixtures carefully and note your observations on the report sheet. If starch is completely soluble both tests should look exactly the same. If the filtered solution gives a lighter color than the unfiltered, then the starch is only partly soluble in water and the unfiltered "solution" is really a suspension. In addition, a suspension, by definition, contains solid particles that reflect and scatter light and often this can be discerned as an opalescent appearance to the liquid. This is known as the Tyndall effect.

**Save the seven sugar solutions and the untreated starch solution (one-half of the original) for future tests (part II).**

#### **2. Light Rotation.**

Procedures for the use of the polarimeter will be written on the black board and demonstrated by the instructor. In addition, the T.A. can assist you with its use.

a. **"Blank Solution"** During the first part of the lab the polarimeter tube will be filled with distilled water. Practice taking readings with the blank - the rotation should be about  $0^\circ$  ( $-1.0^\circ$  to  $+1.0^\circ$  would be acceptable results). Once you have gained confidence, take three readings, note them on the report sheet, and calculate the average.

b. **0.15-g/mL Glucose Solution.** During the second part of the lab the polarimeter tube will be filled with a 0.15-g/mL glucose solution. Take three readings of the rotation of this solution. From the average rotation, the length of the tube in decimeters, and the concentration of the solution, determine the specific rotation  $[\alpha]_D^{20}$  for glucose.

c. **Unknown Fructose Solution.** During the final part of the lab the polarimeter tube will be filled with a fructose solution of unknown concentration. Take three readings of the rotation of this solution. From the average rotation, the length of the tube, and the known specific rotation of fructose,  $[\alpha]_D^{20} = -92^\circ$ , you can determine the fructose concentration in the tube.

### **II. Chemical Tests**

1. **Hydrolysis of Starch.** On a spot plate, place 1 drop of dilute iodine solution in each depression. In a large test tube, place 20 mL of 1% cooked starch suspension. Heat in a boiling water bath, and when hot, add 2 mL of 6 M hydrochloric acid (HCl). Then replace the tube in the water bath, note the time and immediately remove 1 drop of the hot mixture with a clean stirring rod and transfer to the first iodine sample in the spot plate. Clean the stirring rod, and repeat the test at 1-minute intervals. Stop testing when the starch-iodine color is no longer produced or after 15 minutes, whichever is shorter. Continue heating the starch solution in the water bath for an additional 10 minutes. Transfer the solution to a small beaker and neutralize it by carefully adding solid sodium bicarbonate ( $NaHCO_3$ ) with stirring until no more  $CO_2$  bubbles are evolved or until blue litmus paper no longer indicates an acidic solution. Label the beaker "hydrolyzed starch," and save for later testing.

2. **Hydrolysis of Sucrose.** Mix 10 mL of a 5% solution of sucrose with 2 mL of 6 M HCl in a large test tube. Heat in a boiling water bath for 15 minutes. Neutralize as above with NaHCO<sub>3</sub>. Label as "hydrolyzed sucrose," and save for later testing.

3. **Reducing Ability.** Label 11 clean 15-cm test tubes as follows: glucose, fructose, galactose, lactose, maltose, ribose, sucrose, starch, hydrolyzed starch, hydrolyzed sucrose, and blank. Place 2-3 mL of Benedict's solution in each test tube, and heat the tubes in a boiling water bath for 2-3 minutes. Then add 10 drops of the corresponding carbohydrate solutions from the solubility study in Part I.1.b and the hydrolyses in II.1 and II.2, above. To the eleventh tube, add distilled water. Continue heating, and observe the results for the next few minutes. Record the results of the tests on the report sheet. Clean the test tubes leaving the labels in place for use in part 4.

## PRE-LABORATORY QUESTIONS

## EXPERIMENT 5: CARBOHYDRATES

Name \_\_\_\_\_ Section \_\_\_\_\_ Date \_\_\_\_\_

1. What is the ratio of C, H, and O in most carbohydrates?
2. List two types of tests that can be used for the identification of sugars. Briefly describe how each is carried out and what type of experimental observations are obtained and what these results indicate about the sugar tested.

# DATA, OBSERVATIONS AND REPORT

## EXPERIMENT 5: CARBOHYDRATES

Name \_\_\_\_\_ Section \_\_\_\_\_ Date \_\_\_\_\_

Partner(s) \_\_\_\_\_

### I. Physical Tests

#### 1. Solubilities

Indicate form: crystalline solid, amorphous solid (powder), or liquid

Indicate solubility: "+" = soluble; "+/-" = slightly soluble; "-" = insoluble.

	Color	Form	Solubility in water
Glucose			
Fructose			
Galactose			
Lactose			
Maltose			
Ribose			
Sucrose			
Starch*			

\*Describe the results of the iodine tests on the filtered and unfiltered starch "solutions" below.

#### 2. Light Rotation

	Trial 1	Trial 2	Trial 3	Average Rotation
Blank				
Glucose				
Fructose				

- (a) Calculate the specific rotation for glucose. (The concentration was 0.15 g/mL). Show your calculation.

$$[\alpha]_D = \text{_____}^\circ$$

- (b) Calculate the concentration of fructose. (The specific rotation of fructose is  $-92^\circ$ .) Show your calculation.

$$\text{concentration} = \text{_____ g/mL}$$

## II. Chemical Tests

1. **Hydrolysis of Starch.** Using the scale below, indicate the intensity of the color by placing lines in the blocks. Thus a deep color might be indicated as \_\_\_\_\_, and a weak color as \_\_\_\_\_.

Min.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
I <sub>2</sub> Test Color																

### 3. **Reducing Ability**

Record a positive test result as "+", a negative test as "-", and if in doubt use a "+/-".

	Benedict's test	
	Observations	Result
Glucose		
Fructose		
Galactose		
Lactose		
Maltose		
Ribose		
Sucrose		
Starch		
Hydrolyzed starch		
Hydrolyzed sucrose		
Blank		

## Questions

1. Use this page to show structures of glucose, fructose, galactose, lactose, maltose, ribose, and sucrose. Which are monosaccharides and which are disaccharides? Which of the monosaccharides are aldoses and which are ketoses? Are any of these pentoses?



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