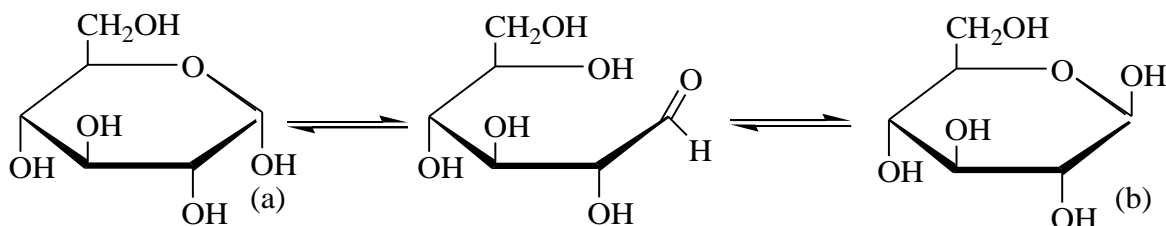
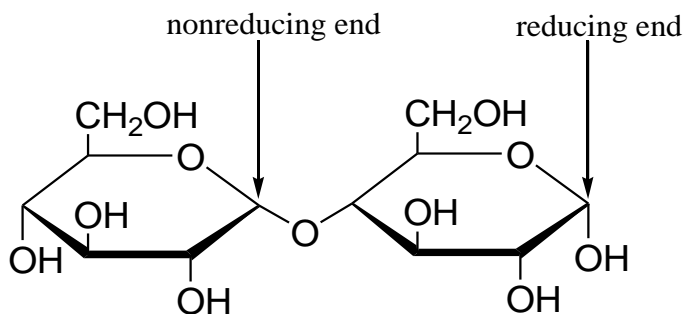

Experiment 15 – Carbohydrates

Discussion

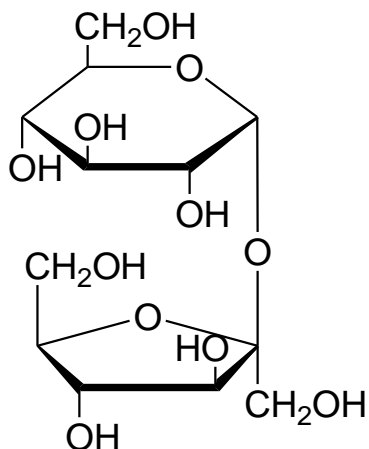
Carbohydrates are a major food source. Rice, potatoes, bread, corn, candy, and fruits are rich in carbohydrates. A carbohydrate can be classified as a monosaccharide (for example, glucose or fructose), a disaccharide (sucrose or lactose), which consists of two joined monosaccharides, or a polysaccharide (starch or cellulose), which consists of thousands of monosaccharide units linked together. If you look at the functional groups present, carbohydrates are polyhydroxy aldehydes or ketones or compounds that yield polyhydroxy aldehydes or ketones upon hydrolysis (i.e., recall in biochemistry, we refer to these as aldoses and ketoses, respectively). Monosaccharides exist mostly as cyclic structures containing hemiacetal (or hemiketal) groups. These structures are in equilibrium in solutions with the corresponding open-chain structures bearing aldehyde or ketone groups. Glucose, blood sugar, is an example of a polyhydroxy aldehyde as shown below:



Disaccharides and polysaccharides exist as cyclic structures containing functional groups such as hydroxyl groups, acetal (or ketal) groups, and hemiacetal (or hemiketal) groups. Most of the di-, oligo-, and polysaccharides have two distinct ends. The end that has a hemiacetal (or hemiketal) on its terminal is called the *reducing end*, and the one that does not contain a hemiacetal (or hemiketal) terminal is the *nonreducing end*. The name “reducing” is given because hemiacetals (and to a lesser extent hemiketals) can reduce an oxidizing agent such as Fehling’s reagent. Consider the following disaccharide:



Please note that not all disaccharides or polysaccharides contain a reducing end. An example is sucrose (shown below), which does not have a hemiacetal (or hemiketal) group on either of its ends:



Polysaccharides, such as amylose or amylopectin, do have a hemiacetal group on one of their terminal ends, but they are mainly nonreducing substances because there is only one reducing group present for every 2,000–10,000 monosaccharidic units. In such a low concentration, the reducing group does NOT give a positive test with Benedict's or Fehling's reagent.

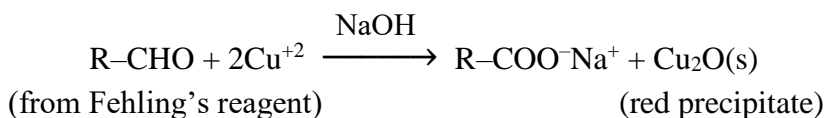
On the other hand, when a nonreducing disaccharide (sucrose) or a polysaccharide such as amylose is hydrolyzed, the glycosidic linkages (acetal) are broken and reducing ends are created. Hydrolyzed sucrose (a mixture of D-glucose and D-fructose) will give a positive test with Benedict's or Fehling's reagent as well as hydrolyzed amylose (a mixture of glucose and glucose-containing oligosaccharides). The hydrolysis of sucrose or amylose can be achieved by using a strong acid such as HCl or with the aid of biological catalysts (i.e., enzymes).

Starch can form an intense, brilliant, dark blue or violet colored complex with iodine. The straight chain component of starch (or amylose) gives a blue color, while the branched component (or amylopectin) yields a purple color. In the presence of iodine, amylose forms helixes, where the iodine molecules assemble as long polyiodide chains. The helix-forming branches of amylopectin are much shorter than those of amylose. Therefore, the polyiodide chains are also much shorter in the amylopectin-iodine complex than in the amylose-iodine complex. The result is a different color (purple). When starch is hydrolyzed and broken down to small carbohydrate units, the iodine will not give a dark blue (or purple) color. The iodine test is used in this experiment to indicate the completion of the hydrolysis.

In this experiment you will investigate some chemical properties of carbohydrates in terms of their functional groups.

Reducing and Nonreducing Properties of Carbohydrates

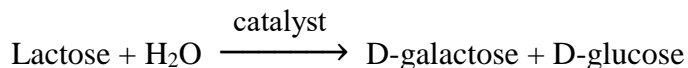
1. *Aldoses (polyhydroxy aldehydes)*. All aldoses are reducing sugars because they contain free aldehyde functional groups. The aldehydes are oxidized by mild oxidizing agents (e.g., Benedict's or Fehling's reagent) to the corresponding carboxylates. For example:



2. *Ketoses (polyhydroxy ketones)*. All ketoses are reducing sugars because they have a ketone functional group next to an alcohol functional group.
3. *Hemiacetal functional group (potential aldehydes)*. Carbohydrates with hemiacetal functional groups can reduce mild oxidizing agents such as Fehling's reagent because hemiacetals can easily form aldehydes through mutarotation.

Hydrolysis of Acetal Groups

Disaccharides and polysaccharides can be converted into monosaccharides by hydrolysis. For example:



Procedure

Reducing or Nonreducing Carbohydrates

1. Place approximately 2 mL (approximately 40 drops) of Fehling's solution (20 drops each of solution part A and solution part B) into each of five labeled test tubes.
2. Add 10 drops of each of the following carbohydrates to the corresponding test tubes as shown in the following table.

<i>Test Tube No</i>	<i>Name of Carbohydrate</i>
1	Glucose
2	Fructose
3	Sucrose
4	Lactose
5	Starch

3. Place the test tubes in a boiling water bath for 5 min. A 600 mL (or any available large) beaker containing about 200 mL of tap water and a few boiling stones is used as the bath.

Record your results on your data sheet. Which of those carbohydrates are reducing carbohydrates? **Caution:** Remember to use boiling stones; they prevent bumping. Handle the hot test tubes with a test tube holder and the hot beaker with beaker tongs.

Hydrolysis of Carbohydrates

Hydrolysis of Sucrose (Acid versus Base)

1. Place 3 mL of 2% sucrose solution in each of two labeled test tubes. To the first test tube (#1), add 3 mL of water and 3 drops of dilute sulfuric acid solution (3 M H₂SO₄). To the second test tube (#2), add 3 mL of water and 3 drops of dilute sodium hydroxide solution (3 M NaOH). **Caution:** To avoid burns from the acid or the base, use gloves when dispensing these reagents.
2. Heat the test tubes in a boiling water bath for about 5 min. Then allow both solutions to cool to room temperature by carefully placing in a test tube rack.
3. To the contents of test tube #1, add dilute sodium hydroxide solution (3 M NaOH) (about ten drops) until red litmus paper turns blue. When using litmus paper, do NOT place the litmus into your solution; instead, use your glass stirring rod, dipped into the test tube solution, to spot the litmus.
4. Test a few drops of each of the two solutions (test tubes #1 and #2) with Fehling's reagent following the procedure that is described for carbohydrates above. Record your results on your data sheet.

Acid-Catalyzed Hydrolysis of Starch

1. Place 5.0 mL of starch solution in a 150 x 15 – mm test tube and add 1.0 mL of dilute sulfuric acid (3 M H₂SO₄). Mix it by gently shaking the test tube. Heat the solution in a boiling water bath for about 5 min.
2. Using a clean medicine dropper, transfer about 3 drops of the starch solution into a white spot plate and then add 2 drops of iodine solution. Observe the color of the solution. If the solution gives a positive test with iodine solution (the solution should turn blue), the hydrolysis is not complete and you should continue heating.
3. Transfer about 3 drops of the boiling solution at 5 min. intervals for an iodine test (*Note: Rinse the medicine dropper very thoroughly before each test*). When the solution no longer gives the characteristic blue color with iodine solution, stop heating and record the time needed for the completion of hydrolysis on the data sheet.

Name: _____

Section: _____

Data and Calculations for Experiment 15**Reducing or Nonreducing Carbohydrates**

<i>Test Tube No.</i>	<i>Substance</i>	<i>Color Observation</i>	<i>Reducing or Nonreducing Carbohydrates</i>
1	Glucose		
2	Fructose		
3	Sucrose		
4	Lactose		
5	Starch		

Hydrolysis of Carbohydrates***Hydrolysis of Sucrose (Acid versus Base Catalysis)***

<i>Sample</i>	<i>Condition of Hydrolysis</i>	<i>Color Observation</i>	<i>Fehling's Test (positive or negative)</i>
1	Acidic (H ₂ SO ₄)		
2	Basic (NaOH)		

Acid-Catalyzed Hydrolysis of Starch

<i>Sample</i>	<i>Heating Time (min)</i>	<i>Color Observation</i>	<i>Iodine Test (positive or negative)</i>
1	5		
2	10		
3	15		
4	20		
5	25		
6	30		

Name: _____

Section: _____

Questions

1. How does the iodine test distinguish between amylose and amylopectin?
2. Why is sucrose a nonreducing sugar? Identify the glycosidic linkage present.
3. How can you tell when the hydrolysis of starch is complete? Why does the test work this way? What is the monosaccharide that results at the end?
4. Why does amylose give a negative test with Fehling's solution?
5. In your own words, write a logical, coherent conclusion on the back of this page which demonstrates a thorough working knowledge and understanding of important concepts and underlying chemical principles pertinent to this experiment, forms appropriate conclusions based on interpretations of results, includes applications of and improvements in the experiment, and demonstrates accountability by providing justification for any errors. If additional space is needed, please use additional paper. (For additional guidelines on writing this conclusion, please refer to the **Moorpark College Chemistry Department Laboratory Report Rubric** found in the lab manual and department website.)