Experiment 6

Carbohydrates

Objectives

During this experiment you will look at some of the physical and chemical properties of carbohydrates. Many of the carbohydrates, especially the mono-saccharides, are classified as reducing sugars. The term reducing refers to redox types of reactions, specifically reduction (the gaining of electrons) and oxidation (the loss of electrons). Since many of the mono-saccharides are aldoses (aldehyde containing sugars), they can be easily oxidized to a carboxylic acid. On the other hand, what about ketoses, such as fructose, which is a ketone sugar. Do ketoses function as reducing sugars like aldoses? You will take advantage of this chemical property to assess which saccharides (carbohydrates) are reducing sugars and which are not. In addition, your instructor will demonstrate a simple way of seeing why these compounds are called "carbohydrates."

Background

Carbohydrates are probably the single largest type of organic compounds in the biosphere. If you look at the different types of organic compounds around you, you will see plants which contain as their principal structure component the chemical compound called cellulose (a polymer of glucose containing b-1,4 glycoside bonds). Many plants also produce starches (containing principally a-1,4 glycoside bonds) which are also polymers of glucose. As you are probably aware, we cannot metabolize cellulose but can metabolize starch, and this is related to the type of glycosidic bonds in the polymer. We also have many different mono- and di-saccharides (e.g., glucose, galactose, fructose, sucrose and lactose). The other principal classes of biological polymers such as proteins and lipids are far and away much smaller in quantity.

Using the space shown below, draw the Fischer projections in the first row for the carbohydrates listed. Then, if the carbohydrate is an aldose (aldehyde-containing sugar), in the next row down, show the Fischer projection for the carboxylic acid derived from the carbohydrate when the aldose gets oxidized. The only functional group that gets oxidized is an aldehyde, not an alcohol or ketone.

Glucose	Galactose	Ribose	Fructose	Glyceraldehyde		

It is not necessary to memorize the names of the new carboxylic acids derived from the carbohydrates, but the common name for the carboxylic acid derived from glucose is actually called gluconic acid.

Procedure

Instructor Demo: Is Carbon In Carbohydrates?

The term "carbohydrate" is derived from the empirical formula for a mono-saccharide, such as glucose. The molecular formula of glucose is $C_{6}H_{12}O_{6}$, but the empirical formula is $CH_{2}O$. What this means, is the for each carbon atom there will be two hydrogen atoms and one oxygen atom. This is the empirical formula for other carbohydrates as well. Someone might ask it is possible to remove the water, and be left basically with only carbon. This is possible. Using concentrated sulfuric acid, which is a strongly dehydrating acid, you can effectively remove the water leaving only carbon.

This demonstration which is to be performed by your instructor is easy to do. Using a 100-mL or a 150-mL beaker, add enough sucrose to the beaker so that it is about half filled. Then, very carefully pour concentrated sulfuric ($18 M H_2SO_4$) to just barely come to the top of the sucrose. You may have to wait for a few moments but very soon you will be able to see a reaction taking place. Describe what you observe during this demonstration experiment in your laboratory notebooks.

Tollen's Reaction

This particular reaction was one of the reactions which you have already performed during the experiment characterizing aldehydes and ketones. It is sometimes called the "Silver Mirror" test because it produces metallic silver as a byproduct when an aldehyde is oxidized into a carboxylic acid. It does not typically work for ketones or for certain disaccharides which are referred to as "non-reducing sugars." Remember that oxidation is defined as the "loss of electrons" and reduction is "the gain of electrons." These are in essence redox (reduction and oxidation) reactions.

This test is based on the ability of an aldehyde (which is easily oxidized) to reduce silver ions in solution, forming either a black deposit of free silver or a silver mirror within the test tube. The aldehyde group is oxidized to an acid during this reaction. Tollens reagent is made by reacting silver nitrate solution with dilute ammonium hydroxide. Rinse all glass equipment with distilled water before use.

The NH4OH used in this experiment must be prepared within one hour of use, it cannot

be made before the day of the experiment.

In a positive test, the diamminesilver(I) complex oxidizes the aldehyde to a carboxylate ion and in the process is reduced to elemental silver and aqueous ammonia. The elemental silver precipitates out of solution, occasionally onto the inner surface of the reaction vessel, giving a characteristic "silver mirror". The carboxylate ion on acidification will give its corresponding carboxylic acid. The carboxylic acid is not directly formed in the first place as the reaction takes place under alkaline conditions. The ionic equations for the overall reaction are shown below; R refers to an alkyl group.

 $\left[\operatorname{Ag(NH_3)_2}\right]^+ (\operatorname{aq}) + e^- \to \operatorname{Ag}(\operatorname{s}) + 2 \operatorname{NH_3}(\operatorname{aq})$ RCHO (aq) + 3 OH⁻ \to RCOO⁻ + 2 H₂O + 2 e⁻

The negative result for the test is no precipitate of silver formed when the carbonyl to be tested is added. A ketone will give a negative result because it cannot be oxidized easily. A ketone has no available hydrogen atom attached to the carbonyl carbon, meaning it can't be so easily oxidized - unlike an aldehyde, which has this hydrogen atom.

Using the freshly prepared Tollens Reagent, add about 5 mL to four very clean test tubes. To each of these tubes, containing the Tollens Reagent, add one of the following:

- Tube 1: 5 drops of 10% lactose
- Tube 2: 5 drops of 10% fructose
- Tube 3: 5 drops of 10% glucose
- Tube 4: 5 drops of 10% sucrose

Allow the tubes to stand undisturbed and note the results. The solution containing the glucose may take 10-15 minutes to react. Do not shake or agitate.

Based on your observations, which of the carbohydrate shown above would you classify as being a reducing carbohydrate (sugar)? Remember, a reducing sugar is one which gets oxidized (electrons

are taken away) and those electrons are given to the oxidizing agent (which in this case is the Ag^+ ion). When the Ag+ ion acquires a single electron it is converted into the Ag element (metal) which then plates out on the glass. This is an indication of a reaction taking place and the Ag is deposited on the glass. Hence, the Silver Mirror test, since this is the process by which mirrors are actually made but using other aldehydes.

Carbohydrate	Observations and Explanations
lactose	
fructose	
glucose	

an a a a a	
sucrose	

Barfoed's Reagent

This chemical test of different carbohydrates is designed to be a positive test for monosaccharides. However, what happens if other carbohydrates, such as disaccharides are analyzed? Do they also give a positve test?

Using your text book, draw the structures for the four carbohydrates (plus galactose) being used in both the Tollen's and this test. Based on the structures of these carbohydrates, you should be able to determine why some of them react and some do not.

Glucose	Sucrose	Fructose	Lactose	Galactose		

To perform this test, add 1 mL of Barfoed's reagent to each of four different test tubes (make certain they are labeled so that the hot water does not remove the tape label.

- Tube 1: 5 drops of 10% lactose
- Tube 2: 5 drops of 10% fructose
- Tube 3: 5 drops of 10% glucose
- Tube 4: 5 drops of 10% sucrose

After you add the carbohydrates solutions to each of the test tubes containing the 1-mL of Barfoed's reagent, place the test tubes in a boiling water bath, a positive test for monosaccharides is the formation of a brick-red precipitate within five minutes. Disaccharides generally don't give any reaction even for ten minutes. The precipitate isn't nearly as voluminous as that seen with Benedict's test and tends to adhere to the walls of the test tube. The main thing you should be looking for is a change in color. Remember that one of the indicators of a chemical reaction is a change in color or formation of a solid (precipiate).

Carbohydrate	Observations and Explanations				
lactose					
fructose					

glucose	
sucrose	

Conclusions

While there may not be a large number of different experiments performed in this protocol, they are sufficient to give you an understanding of the different chemical and physical properties of carbohydrates.

Compound	MW	Amount	mmol	mp	bp	Density	ηd	msds
glucose (10%)	180.16	1.0 grams in 10 mL water						<u>msds</u>
sucrose (10%)	342.32	1.0 grams in 10 mL water						<u>msds</u>
lactose (10%)	342.32	1.0 grams in 10 mL water						<u>msds</u>
starch	polymer	1.0 grams in 10 mL water						<u>msds</u>
fructose (10%)	180.16	1.0 grams in 10 mL water						<u>msds</u>
Compound	g/mol	grams or mL	10 ⁻³ M	°C	°C	g/mL	ηD	msds

Chemicals, Reagents, and Supplies

Reagent Synthesis:

- Tollens Reagent (prepared by each student group, but it is convenient to let the Instructor add the ammonium hydroxide): For each group, add 8 mL of 0.1 M silver nitrate to a beaker or flask (measure in a 10-mL graduated cylinder). Add dilute ammonium hydroxide (the NH4OH must be prepared within one hour of being used, it cannot be prepared on a previous day) 1 drop at a time until the brown precipitate of silver oxide that is formed just dissolves (mix by swirling after each drop is added). (The liquid may remain slightly brownish in color, but it cannot still have a precipitate.) Now, add 7 mL of distilled water and mix. Use about 5 mL of Tollens reagent for each of the three tubes used in the experiment.
- **Barfoed's Reagent** looks like Benedict's solution but differs somewhat. The reagent is prepared by dissolving 70 g copper acetate monohydrate and 9 mL glacial acetic acid in water to a final volume of one liter. The reagent is stable for years.

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