EXPERIMENT 6

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

Materials Needed

About 3-5 g each of Glucose, Fructose, Maltose, Sucrose, Starch

sodium bicarbonate, $NaHCO_3(s)$	15 mL 5% sucrose
25 mL 1% cooked starch suspension	5 mL 6 M HCl
50 mL Benedict's reagent	5 mL dilute iodine solution
0.15 g/mL glucose solution	0.20 g/mL fructose solution
polarimeter, spot plates, Parafilm, test tubes, hot plate	molecular model kit

Additional Reading Assignment

Denniston, Chapter 17 and Appendix D.

Background

Carbohydrates are named as such because they generally contain C, H, and O in the ratio $C_n(H_2O)_n$. 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 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 2).

A ***polarimeter*** is an instrument designed to detect and measure the rotation of plane-polarized light. Only chiral molecules are capable of rotating light in this way or ***optically active***. Chiral molecules will be explored using molecular models in part III of this lab. 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 ℓ 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 α-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 1. Open-chain and cyclic structures of glucose.

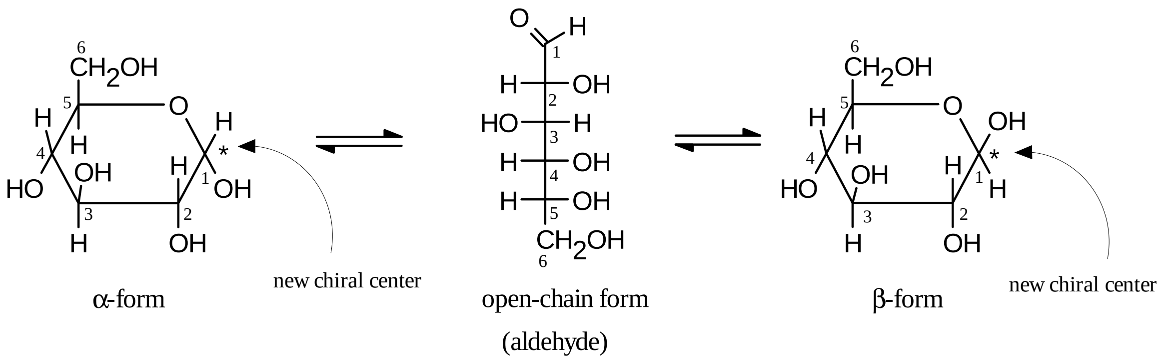
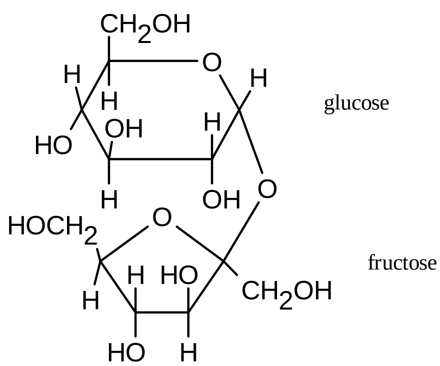
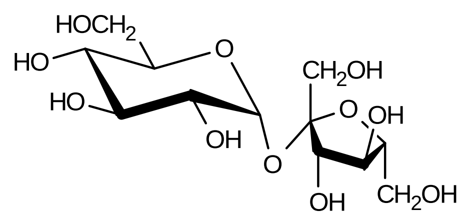


Figure 5. Sucrose: a disaccharide that is a combination of α-glucose and fructose.





Haworth formula
more realistic structural formula

Procedure

Part I. Physical Tests

1. **Solubility**.

Label five 15-cm test tubes as follows: glucose, fructose, maltose, 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 #5) 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 four 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° (-1.0° to +1.0° 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.

Part 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 $NaHCO_3$. Label as "hydrolyzed sucrose," and save for later testing.

3. **Reducing Ability**. Label eight clean 15-cm test tubes as follows: glucose, fructose, maltose, sucrose, starch, hydrolyzed starch, hydrolyzed sucrose, and blank. Place 2-3 mL of Benedict's solution in each test tube then add 10 drops of the corresponding carbohydrate solutions from the solubility study in Part I.1 and the hydrolyzed solutions from parts II.1 and II.2. To the eighth tube, add distilled water. Heat the tubes in a boiling water bath for 5-10 minutes. Record your observations on the report sheet.

Part III. Molecular Models of Chiral Compounds

Chiral Carbons and Planes of Symmetry. ***(****Please answer all questions on a separate sheet of paper.)*

Definitions *- A carbon atom that has four different groups bonded to it is called a* ***chiral carbon****. If an object can be visually divided into halves that are mirror images of each other then the object possesses a* ***plane of symmetry.*** *(Biologists describe such objects as having "bilateral symmetry" see* http://www.shelterpub.com/_symmetry_online/sym1_mirror_symmetry.html *for a nice description.)*

Make a model in which a tetrahedral carbon atom has four different color balls bonded to it. For purposes of consistency, please use orange, green, red, and white as the four colors.

1. Does the model possess a plane of symmetry?

This model can be used to represent any molecule that contains one chiral carbon. As an example consider 3-methylhexane.

2. Show the structure of 3-methylhexane and use an asterisk to label the chiral carbon.
3. List the four different groups attached to the chiral carbon in 3-methylhexane.

Note that the model with four different color balls can be used to represent 3-methylhexane by simply stipulating that each ball represents one of the four groups you listed in #3.

4. Does 3-methylhexane possess a plane of symmetry?
5. For further practice in finding chiral carbons, draw structures of each of the following and mark any chiral carbons present with an asterisk: 1-butanol, 2-butanol, 1,2-butanediol, 1,3-butanediol, 1,4-butanediol, 2,3-butanediol, glucose (open-chain).

Replace the red ball on your model with a second white ball so that now you have a model of a tetrahedral carbon atom with two identical groups attached.

6. Does the model possess a plane of symmetry?

Chirality and Enantiomers

As you have just seen, the presence of one chiral carbon in a molecule causes means it cannot have a plane of symmetry. Objects that lack a plane of symmetry are usually ***chiral****. (The only exceptions are when they possess other symmetry elements such as a center or axis of symmetry.) This means that they possess a "handedness" and can exist as two different forms, a "right-handed" and a "left-handed" form.*

7. Which of the following objects are chiral: a baseball hat, a baseball glove, a baseball bat, a baseball, a baseball pitcher, a baseball diamond? (Ignore the presence of logos or other writing on these objects)
8. Which of the following molecules are chiral: 1-chlorohexane, 2-chlorohexane, 3-chlorohexane, 1-chloropentane, 2-chloropentane, 3-chloropentane?

Go back to your original model (4 different colors) and make a second identical model of it. Make sure the two models are identical by trying to superimpose them; all of the atoms on one should superimpose on atoms of the same color in the other.

Superimposability is a foolproof way to test whether two molecules are identical or not. Superimposable molecules are identical to each other.

Now switch any two balls on one of the models.

9. Are the models still superimposable?
10. Are they identical?
11. What is the word for non-identical structures that have the same molecular formula?

Place the two models side-by-side on the desktop so that they both have the white ball pointing up. Now rotate them (keeping the white ball up) so that the red ball of each is pointing at the other model.

*You should now be able to observe that the models are **mirror images** of each other. At the same time, they are clearly not identical. Hence, the two models represent mirror-image isomers of each other. Mirror-image isomers are called **enantiomers**. To resummarize all of your previous work, **all molecules that possess one chiral carbon and, therefore, lack a plane of symmetry are chiral and exist as a pair of enantiomers.***

Again, switch any two balls on one of the models.

12. Are the models still mirror images of each other?
13. Does either possess a plane of symmetry?
14. Are the models superimposable?
15. Do the models represent identical or different structures?
16. Do the models represent enantiomers? Why or why not?

Note that switching any two groups on a chiral carbon creates the enantiomer of the molecule you started with.

Molecules that are not Chiral

Now replace the red ball on each of the models with a second white ball so that each now could be representing a CH_2 group with two different things attached to it.

17. Are the models mirror images of each other?
18. Does either possess a plane of symmetry?
19. Are the models superimposable?
20. Do the models represent identical or different structures?
21. Do the models represent enantiomers? Why or why not?

Now switch any two balls on one of the models.

22. Does that change anything in terms of the answers to questions #17-21?
23. Objects that possess a plane of symmetry are never chiral. Explain why.

When a tetrahedral atom has two identical groups attached there will always be a plane of symmetry cutting between the two identical groups. (The only exception would be if one of the two non-identical groups contained a chiral carbon or was in some other way chiral.) Molecules represented by the models used in this part of the lab are, therefore, not capable of existing as enantiomers and are, thus, not chiral. Such molecules have mirror images that are identical to themselves.

Representing Molecules with Chiral Carbons

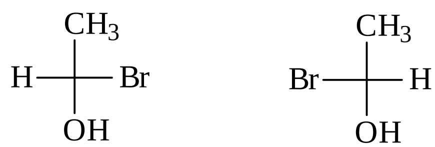
The great German chemist, Emil Fischer, did a great deal of work with carbohydrate molecules such as those being examined in parts I and II of this lab. These compounds have multiple chiral carbons. In order to make representing these structures on paper easier, he devised the structural convention known as a Fischer projection.

Go back to a simple model with a central carbon atom and four different colors attached. Hold the model do that the bonds to two of the attached balls are parallel to the floor and pointing straight at you. The other two bonds should be pointing away from you and be perpendicular to the floor. Verify that the molecule in this orientation is represented by wedge/dash representation shown at left below.

wedge/dash formula   Fischer projection

A Fischer projection (or "cross formula") uses a simple cross to represent this orientation of a chirality center.

Make models of each pair of molecules before answering the corresponding question.



24. Are the structures above enantiomers or identical structures?

25. Are the structures above enantiomers or identical structures?

26. Are the structures above enantiomers or identical structures?

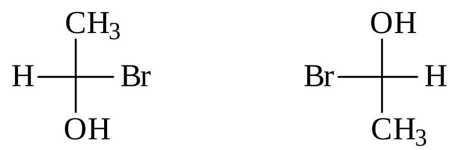
27. Are the structures above enantiomers or identical structures?

Note that all of the above examples(#24-27) involve switching two groups on a Fischer projection.

28. When the only difference between two Fischer projections is that two groups have been switched then the relationship between those two structures is what?

Make models of each pair of molecules before answering the corresponding question.

29. Are the structures above enantiomers or identical structures?



30. Are the structures above enantiomers or identical structures?

31. Are the structures above enantiomers or identical structures?

Base your answer to questions #32-34 on the above three examples (i.e., #28-30).

32. When the only difference between two Fischer projections is that the projection appears to have rotated 90° then the relationship between those two structures is what?

33. When the only difference between two Fischer projections is that the projection appears to have rotated 180° then the relationship between those two structures is what?

34. When the only difference between two Fischer projections is that three of the attached groups have rotated then the relationship between those two structures is what?

PRE-LABORATORY QUESTIONS

EXPERIMENT 6: 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 AND OBSERVATIONS SHEET

EXPERIMENT 6: CARBOHYDRATES

Names ____ Section ____________ Date ____________

I. Physical Tests

1. Water Solubility

	Color	Crystalline Form (describe solid)	Solubility
Glucose			
Fructose			
Maltose			
Sucrose			
Starch*			

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

____

____

2. Observed Rotation

	Trial 1	Trial 2	Trial 3	Average Rotation
Blank				
Glucose				

(a) Calculate the specific rotation of glucose. Show your calculation.

EXPERIMENT 6: DATA AND OBSERVATIONS (continued)

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_2Test Color																

3. Reducing Ability - Benedict's test observations

Glucose	
Fructose	
Maltose	
Sucrose	
Starch	
Hydrolyzed starch	
Hydrolyzed sucrose	
Blank	

REPORT - EXPERIMENT 6

Results Table **<u>EXPT 6 - Properties of Carbohydrates : Solubility, Reactivity, Chirality and Specific Rotation</u>**

Names____Date ___________

Compound Name **and Structure**	Appearance	Results of Solubility/Reactivity Tests			expt'l $[\alpha]_D$	lit $[\alpha]_D$	lit mp (°C)[a]
		H_2O solubility	Iodine test	Benedict's test			
glucose			NA				
fructose			NA		NA	NA	
maltose			NA		NA	NA	
sucrose			NA		NA	NA	
starch					NA	NA	NA
hydrolyzed starch					NA	NA	NA
hydrolyzed sucrose			NA		NA	NA	NA

[a]Reference used for literature values ____

Questions

1. Use the space below to show structures of glucose, fructose, maltose, and sucrose. Which are monosaccharides and which are disaccharides? Which of the monosaccharides are aldoses and which are ketoses? Are any of these pentoses?

2. Which carbohydrates gave a positive Benedict's test? What functional groups are necessary for a carbohydrate to give a positive test? Explain.

3. Using structural formulas, write a complete, balanced equation for the hydrolysis of sucrose. Explain why hydrolyzed sucrose gave the result in the Benedict’s test that it did.

4. Write an equation for the reduction of Cu^{2+} by a sugar showing a positive Benedict's test.

5. Why are all sugars solids with high melting points? Explain in terms of intermolecular forces.

6. How precise were the specific rotation measurements? How accurate? Identify at least two sources of error in the measurement.