

Lab 2: Carbohydrate Lab

Name _____

Date _____

Lab safety: Full PPE required for this lab - lab coat, long pants, closed-toe shoes, gloves and safety goggles

Learning Objectives:

1. Describe the basic and chemical structure of a monosaccharide.
2. Differentiate between monosaccharides, disaccharides and polysaccharides.
3. Identify the storage form of carbohydrate in animals and plants.
4. Describe the major functions of carbohydrates.
5. Know the purpose and principle of Benedict's Test.
6. Know how to apply scientific method (formulating hypothesis, designing experiment, collecting data and come up to a conclusion).



Pre-Lab Activity:

Read the introduction for this lab and answer the following questions.

1. Name the most important sugars found as part of nucleic acids.
2. What are the sugars that have 6 carbons called? Name the most important sugars that have six carbons.
3. Name the major storage polysaccharide of:
 - a. Plants: _____
 - b. Animals: _____
4. Why do plants make cellulose?
5. Why can't we digest cellulose?
6. What **macromolecule(s)** does Benedict's reagent identify?

Introduction:

Carbohydrates are also called saccharides, which is derived from the Greek word *sakcharon* meaning sugar. Carbohydrates are composed of Carbon (C), Hydrogen (H) and Oxygen (O). The simplest carbohydrates are called **monosaccharides** and have a generic formula of $(CH_2O)_n$.

Pentoses or monosaccharides with 5 carbons are important in several biochemical pathways, but the two most common pentoses, ribose and deoxyribose, are critical structural components of nucleic acids. Monosaccharides with 6 carbons are **hexoses** and the four most important hexoses are **glucose**, **fructose**, **galactose** and **mannose**. Hexoses have important structural roles in cells, but they also are the preferred energy source of almost all cell types.

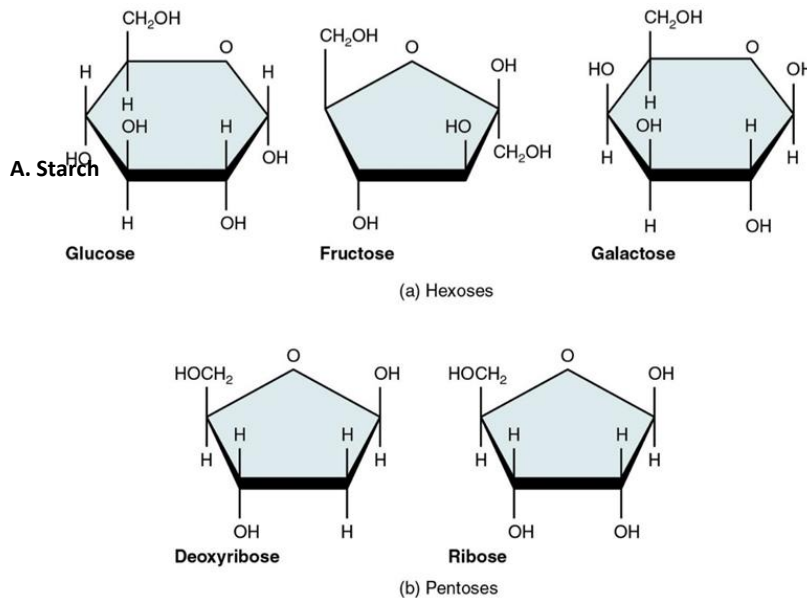


Figure 1: Examples of common monosaccharides.

(Picture source: commons.wikimedia.org)

When two monosaccharides are linked together, they are called **disaccharide** (di=two). Three important disaccharides are **sucrose**, **lactose** and **maltose**.

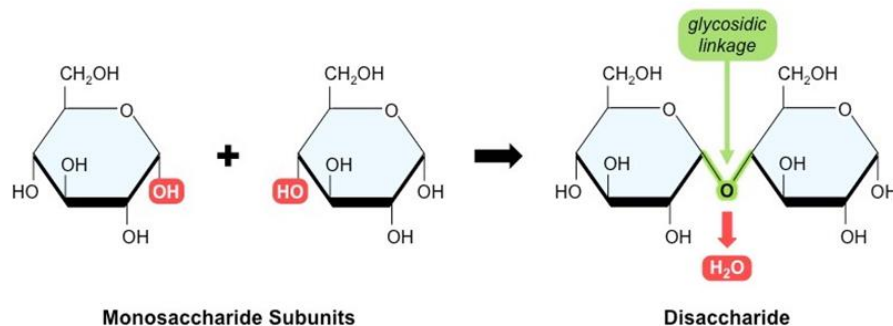


Figure 2: Formation of a disaccharide from two monosaccharides.

(Picture source: <https://images.app.goo.gl/Qu3r4f8iWgKQFK6M7>)

Long chain of monosaccharides form **Polysaccharides**. Large quantities of hexoses are stored most efficiently when they are combined to form much larger polysaccharides. The major storage polysaccharide in plant cells is called **starch**, which is composed entirely of glucose. The major storage polysaccharide in

animal cells is called **glycogen**, and just like starch it is composed entirely of glucose but different from starch due to structural differences (Fig 3). Plants make another important polysaccharide from glucose, but instead of a storage form for future energy needs it is for structural purposes. This polysaccharide is called **cellulose**, and it is composed of a linear chain of thousands of glucose molecules linked between carbons. Animals produce the enzymes necessary to digest (breakdown) starch, and to synthesize and breakdown glycogen, but they do not produce the enzymes necessary to digest cellulose. Cellulose is what we commonly refer to as dietary fiber.

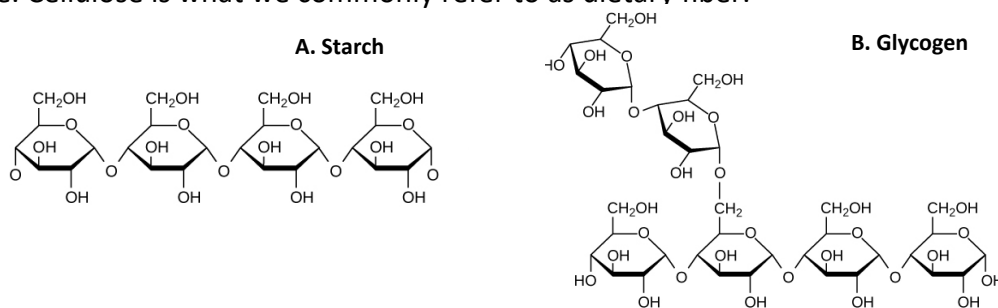


Figure 3: Examples of common polysaccharides.

(Picture source: <https://images.app.goo.gl/GsAnJm6iAicR2gDw9>)

Background of Experiment

A simple biochemical test using **Benedict's reagent** can establish the presence of monosaccharides and most disaccharides (**not sucrose**).

Benedict's reagent is composed of copper and citrate in a buffer with a basic pH. When Benedict's reagent is in the presence of certain **reducing sugars** (like glucose), a reaction occurs (when heated) in which the copper (II) ions are changed to copper (I) ions. The copper (I) ions then form Cu₂O which is a red solid. As the Benedict's reagent is exposed to increasing quantities of sugars, its color changes from blue to green, yellow, orange, or red. Because Benedict's reagent is not specific for any one type of sugar, it cannot be used to make accurate quantitative determinations of the amount or concentration of carbohydrate in a sample. This test is best used for making relative comparisons of the amount of carbohydrate among a group of samples.

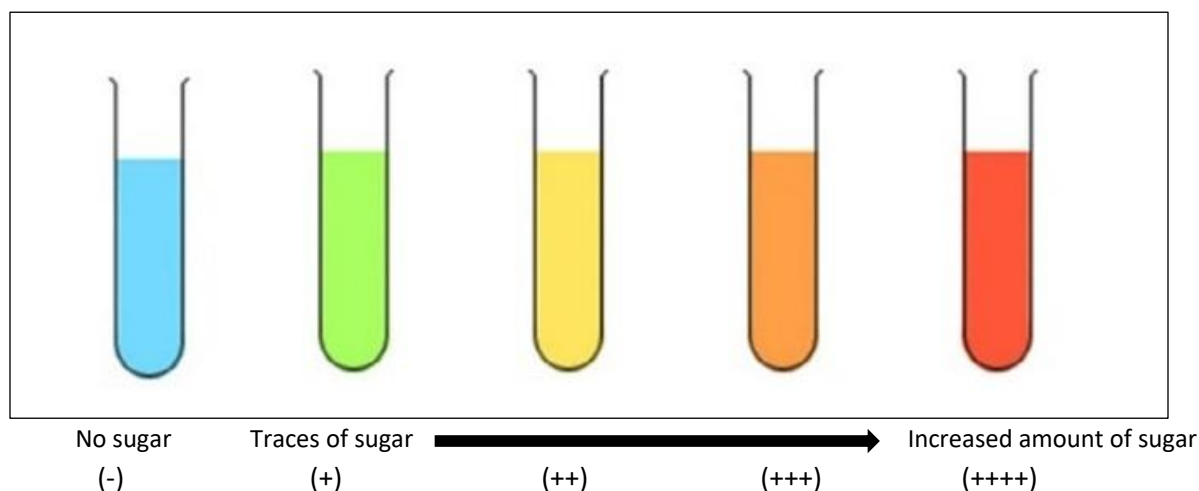


Figure 4: Benedict's Test. Relative concentration of sugar – color indicator.

Quantitative and Qualitative Data

Quantitative data is associated with numbers, and gives information such as how many, or how much. Qualitative data is based on a method of observation on things that cannot be precisely measured. Often this type of measure is represented by a name, symbol or number code. We collect qualitative data using Benedict's Test, where each symbol (-, +) indicates the relative amount of monosaccharides present.

Activity: Benedict's Reagent and Carbohydrates

In this experiment, the sugar levels of different solutions will be tested. Work in a team of four.

Materials Needed

For a team of four students, you need:

- Hot plate, Beaker (400 ml), Test tube holder
- Test tubes (9), marking pencil, measuring cylinder
- Sugar solutions (5% glucose and 5% sucrose) – for control
- 6 drink solutions
- Benedict's reagent
- Distilled water

Controls

A well designed experiment requires controls, to compare the experimental data. Controls could be a positive control or negative control. Make a prediction (color) for the following controls (refer to introduction section) and state whether they would be considered positive or negative.

1) 5% glucose _____

2) 5% sucrose _____

3) Distilled water _____

Hypothesis

Write a hypothesis predicting the results for concentrations of monosaccharides in each solution. This should be a *general* hypothesis relative to the concentration of monosaccharide in each solution (NOT an individual hypothesis for each solution being tested).

Procedure

1. Set up a boiling water bath – Fill the provided beaker approximately half-way with tap water and place it on a hot plate. Set the hot plate on high to get the water boiling, then reduce the temperature to keep the water simmering. The water should be boiling BEFORE you place your tubes in it. DO NOT let the beaker boil dry!

- Label your tubes 1 through 3 and A through F.

SAMPLES

- 5% glucose solution
 - 5% sucrose solution
 - distilled water
 - 6 solutions will be labeled A – F
- Add 2 ml of 5% glucose to tube 1, 5% sucrose to tube 2, and water to tube 3.
 - As a comparison, record the color of each tube in Table 1 before adding Benedict's reagent.
 - Add 2ml of Benedict's solution to test tubes 1-3. Gently shake your tubes so that the sample and Benedict's is well mixed. See instructor for demonstration. Let the tubes sit for 5 minutes.
 - Place the test tubes at the same time in the boiling water bath for 5 minutes.
 - Remove the tubes using the test tube clamp and record the resulting color. Do not mix the tubes again once they have been boiled. If your sample was a solid or contains a precipitate, note the color change in the immediate vicinity of your sample. The rest of the Benedict's may stay blue since a solid cannot mix well.
 - Record the color of your samples 1-3 in the following Table 1.

Table 1. Benedict's Test with Controls

| Results | 5% Glucose | 5% Sucrose | H₂O |
|--|-------------------|-------------------|-----------------------|
| Color before Benedict's Test | | | |
| Color after Benedict's Test | | | |
| Solid or Liquid | | | |
| Type of Control | | | |
| Does the result match your hypothesis | | | |

- Read the ingredients and nutrition facts on the labels for the provided solutions (A-F) and fill out first three rows in the Table 2.
- Make a prediction of your experiment for each provided solution (A - F) in Table 2.
- Add 2 ml of solution A to the test tube labeled A, 2 ml of solution B to test tube labeled B, and so on.
- For comparison, record the color of solutions A-F before beginning the Benedict's test in Table 2.
- Repeat steps 5-7 with the provided solutions. Please place no more than 3 test tubes at a time in the boiling water.
- Record your results in the Table 2.

Table 2. Benedict's Test with Solutions

| Beverage | A: | B: | C: | D: | E: | F: |
|--|----|----|----|----|----|----|
| Grams of sugar/ serving | | | | | | |
| Type of sugar | | | | | | |
| Color before Benedict's Test | | | | | | |
| Predicted result (color) of test | | | | | | |
| <u>Actual result</u> Color? Precipitate? | | | | | | |

15. Compare your results to those of the rest of the class. Are there any differences?

16. Clean up your work area.

- i) Turn off the hot plate, leaving the beaker of hot water sitting on it to cool.
- ii) DO NOT dump any solution with Benedict's Reagent in the sink. The solutions in the test tubes MUST be poured into the waste jug on the instructor's bench.
- iii) Then clean the tubes in the sink with soapy water. If there is any residue or stain clean with brush and return them to the rack upside down to dry over a paper towel.

Reference:

Griner, Richard. *Biological Molecules: Structure and Methods of Analysis*. Department of Biological Sciences, Augusta University, 2003
 Helms, Doris R, et al. *Organic Molecules, Biology in the Laboratory* 3rd Edition, W.H. Freeman 1997

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Post-Lab Activity: Carbohydrate Lab Assignment

Name _____

The following assignment should be printed and submitted to your instructor for a grade.

Answer the following questions.

1. What carbohydrate(s) does Benedict's reagent test for? _____
2. What color indicates a positive result _____
3. What color indicates a negative result _____
4. Tubes 1, 2, and 3 were control tubes. Recall that the purpose of controls is to show that an experiment is working correctly. For example, control tube 3 (water) confirmed that the Benedict's reagent gives a negative result (blue color) when no sugar is present.
In a similar way, state the purposes of control tubes 1 and 2.

Control tube 1: _____

Control tube 2: _____

5. What does a positive test indicate?
6. What is the **chemical basis** for a positive Benedict's test?
7. Write down the name of the drink tested, type of sugar in each drink (e.g., glucose, lactose, sucrose), predicted color, and result color from Table 2.

| Solutions | A | B | C | D | E | F |
|-----------------|---|---|---|---|---|---|
| Drinks Tested | | | | | | |
| Type of Sugar | | | | | | |
| Predicted Color | | | | | | |
| Result Color | | | | | | |

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8. Which solution(s) (A – F) had the most sugar based on the reaction of Benedict's reagent?

9. Using the summary Table in Question 7 above, were the actual results of the experiment same as your predicted results for each solution? Explain. (Try and think about this in terms of the type of sugar in each solution)

10. Are there any solutions where you were surprised by the results? Yes ☐ No ☐
Explain any unexpected results that you had.

11. Rewrite your hypothesis from pg. 4.

12. Based on your hypothesis and experimental result, write down a conclusion that states whether your results support or reject your hypothesis. (Remember, you should have had a *general* hypothesis about monosaccharide content in solutions)