Experiment 12

Sugar!

The Task

The goal of this experiment is to identify the organic functional group responsible for the reaction of sugars with Fehling's reagent.

Skills

At the end of the laboratory session you should be able to:

- carry out Fehling's test on carbohydrates,
- construct models of simple organic compounds.

Other Outcomes

At the end of the laboratory session you will have:

- become familiar with the use of stick structures to represent organic molecules,
- become familiar with the common organic functional groups.

The Assessment

You will be assessed on your understanding of stick notation and your ability to build molecular models. See Skill 13.

Introduction

Until the middle of the 19^{th} Century sugar was a luxury item, only affordable by the richest members of society. Nowadays, sugar pervades our entire popular culture and it is hard to imagine a world without it. For example, sugar is considered to be one of the chief components that little girls are made of, and who could forget the advice of Lou Reed, "Hey, sugar, take a walk on the wild side."

In this experiment we will take a close look at a specific chemical reaction, the reaction of Fehling's reagent with different sugars (carbohydrates). This chemical test was developed in 1849 by the German chemist Hermann von Fehling. One of its uses is to screen for the presence of glucose in urine, and thus to detect diabetes. But what is the basis of the test? What part of a sugar molecule reacts with Fehling's reagent? That is what we want to determine in this experiment.

Functional Groups

See Skill 13 for an explanation on the use of stick structures to describe the molecular structure of organic compounds. Understanding this notation is essential for this experiment. A functional group in organic chemistry is a group of atoms in a molecule that give rise to the characteristic chemical reactions of that molecule. A simple example is –OH, the alcohol functional group, *i.e.* an oxygen atom with a single covalent bond to a hydrogen atom. Any molecule containing this group is classified as an alcohol, *e.g.* methanol (CH_3OH) or ethanol (C_2H_5OH). Other common functional groups are the ketone, aldehyde, carboxylic acid and amine groups. (See Figure 1.)

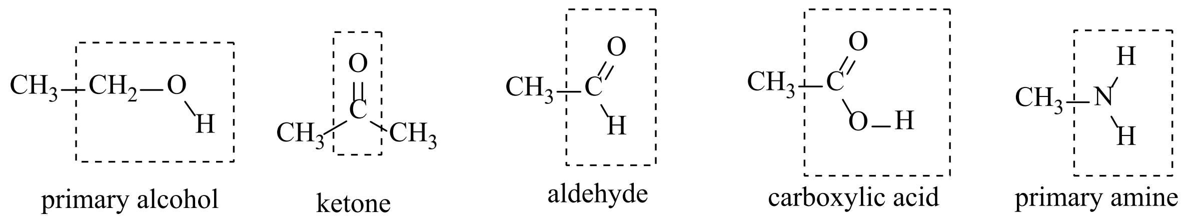


Figure 1. Examples of some common functional groups

Fehling's test is based on the reaction of a functional group of sugar molecules with Fehling's reagent. In this experiment you will first determine which sugars give a positive test with Fehling's reagent and then, by testing the reaction of some simple organic molecules containing only a single functional group, you should be able to deduce which functional group of the sugars it is that Fehling's reagent reacts with. At this stage, you may not be familiar with many organic functional groups or even any at all, but, never mind, you will become familiar with them in the course of doing the experiment.

Safety

Chemical Hazard Identification

glucose – non-hazardous.

maltose – non-hazardous.

sucrose – non-hazardous.

lactose – non-hazardous.

starch – non-hazardous.

α-**amylase** – non-hazardous.

4 M HCl - hazardous. Causes burns; irritating to respiratory system, eyes and skin.

0.2 M NaCl – non-hazardous.

iodine solution - non-hazardous.

acetaldehyde – hazardous. Irritating to skin, eyes and respiratory system.

3-pentanone – hazardous. Highly flammable, irritating to respiratory system.

ethanol – hazardous. Highly flammable, low to moderate toxicity, irritant.

ethyl acetate – hazardous. Highly flammable, irritating to eyes.

acetamide – hazardous. Low to moderate toxicity.

Fehling's reagent A – non-hazardous.

Fehling's reagent B – hazardous. Causes burns, avoid contact with skin.

Risk Assessment and Control

Moderate risk.

Care needs to be taken to avoid burns or scalds from hotplates and boiling water baths.

Avoid skin contact with hazardous chemicals.

Waste Disposal

All solutions contaminated with Fehling's reagent should be disposed of in the marked Heavy Metal Disposal containers located in a fume hood.

Experimental

This experiment is to be carried out in pairs.

Part A The reaction of sugars with Fehling's reagent

In this part of the experiment you will observe the reaction of several common sugars with Fehling's reagent. Because Fehling's reagent is unstable, it is always prepared fresh. This is done by mixing two separate solutions, known as Fehling's solution A and B.

(A1) Take 4 macro test-tubes from your locker. To the first test-tube add approximately 0.1 g of glucose, to the second add approximately 0.1 g of maltose, to the third 0.1 g of sucrose and to the last 0.1 g of lactose. Make sure you have labelled the test-tubes so that you know which is which.

(A2) Add 2 mL of water to each test-tube and dissolve the sugar.

(A3) To each test-tube add 2 mL of both Fehling's solution A and Fehling's solution B.

(A4) Place each test-tube in a boiling water bath (a 250 mL beaker on a hotplate stirrer). Some of the reactions take time to occur, so proceed to Part B and come back to this part later to record your observations in your logbook.

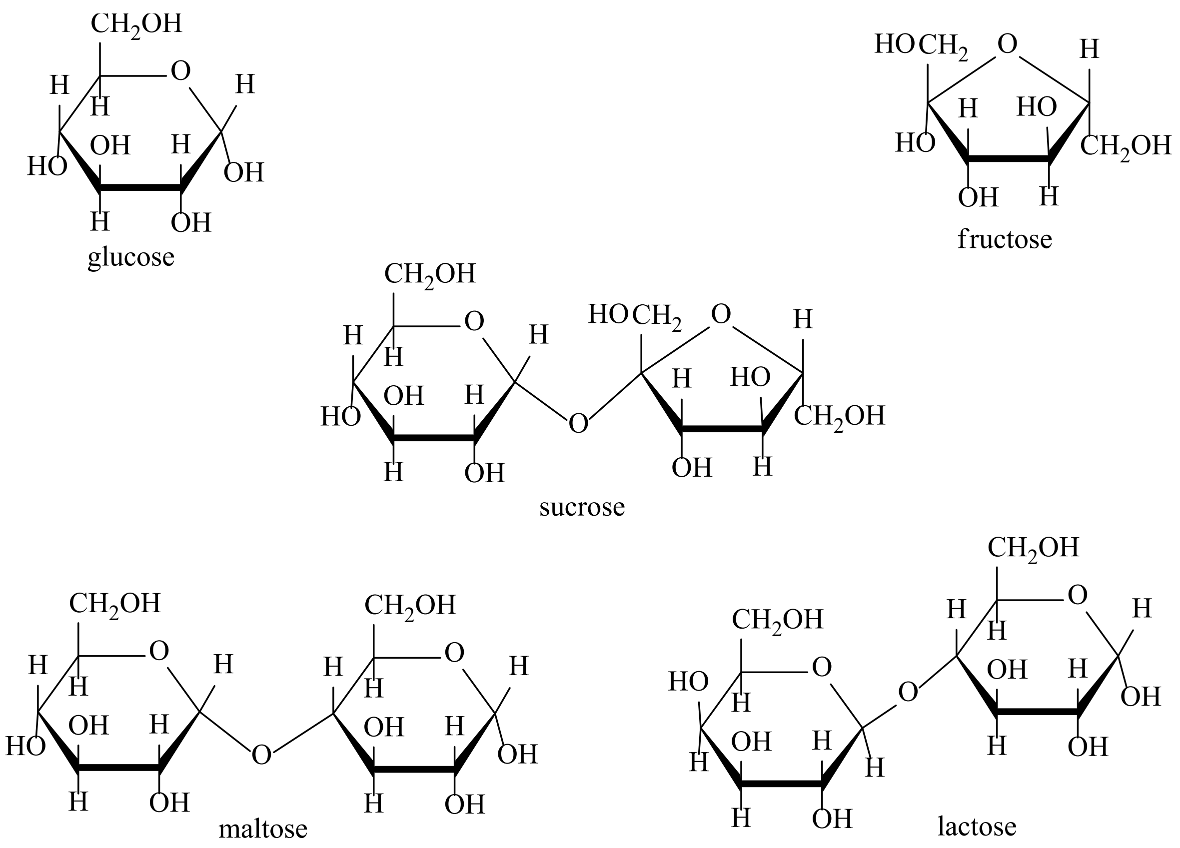


Figure 2. Structures of various sugars

For your logbook:

From your observations and the structures of the sugars given above, make an initial hypothesis of which functional group in the sugar molecules reacts with Fehling's reagent.

The subsequent parts of the experiment will allow you to test your hypothesis.

Part B Model building

To understand the mechanism of how a reaction is occurring at an atomic level it is often useful to construct a model of the molecule involved. In the model kits provided a carbon atom is represented by a black ball with four holes (maximum 4 covalent bonds), an oxygen atom by a red ball and a hydrogen atom by a white ball. The little white adaptors are used for C–H and O–H bonds, whilst the short grey connectors are used for C–C and C–O bonds.

(B1) Using the model kits provided, construct a model of the glucose molecule based on the cyclic structure shown in Figure 2. Re-read Skill 13 (or ask your demonstrator for help) if you are having trouble interpreting the structure of glucose.

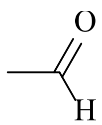
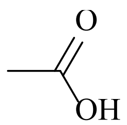
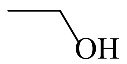
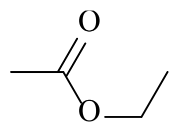
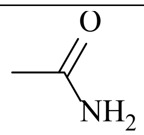
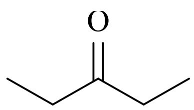
(B2) Once you have constructed your glucose molecule, keep it in a safe place on the bench. You will need it later, during the Group Discussion at the latest.

For your logbook:

Draw the structure of glucose in stick notation in your logbook.

Part C Reaction of simple organic molecules with Fehling's reagent

The sugars you have been using in Part A are relatively complex organic molecules. To determine which functional group in the sugars reacts with Fehling's reagent, in this part of the experiment you will test a number of simpler organic compounds, each with only one functional group. The names and structures of the organic compounds are given below.

Compound	stick structure	Functional group	
acetaldehyde		CHO	aldehyde
acetic acid		COOH	carboxylic acid
ethanol		OH	alcohol
ethyl acetate		COOR	ester
acetamide		$CONH_2$	amide
3-pentanone		CO	ketone

(C1) Collect 6 macro test-tubes from your locker. In each test-tube mix 2 mL of Fehling's solution A and 2 mL of Fehling's solution B.

(C2) Appropriately label each test-tube and then add 2-3 drops of one of each of the test substances, *i.e.* acetaldehyde, acetic acid, ethanol, ethyl acetate, acetamide and 3-pentanone.

(C3) Place the test-tubes in a boiling water bath and observe the result. **Record your observations in your logbook**. Again the reactions may take some time, so proceed to part D, perform the first iodine test (steps (D1) and (D2)), and then come back to this section and complete your logbook.

For your logbook:

Based on your observations, which organic functional group would you conclude reacts with Fehling's reagent?

Part D Hydrolysis of starch

Hydrolysis is the reaction of a compound with water. In the laboratory, such reactions are often catalysed by the presence of acids or bases.

Starch is a polymer, consisting of many units of glucose covalently linked together. In this part of the experiment you carry out two tests, Fehling's test as well as an iodine test.

Iodine Test

(D1) To 10 drops of 0.5 % starch solution add 2 drops of the dilute iodine solution. **Record your observations in your logbook**.

(D2) Prepare a solution of 0.1 g of glucose in 2 mL of water and then add 2 drops of the dilute iodine solution. **Record your observations in your logbook.**

For your logbook:

Based on your observations, does iodine react with a single glucose unit or a polymer of glucose (starch)?

Iodine Test of Starch Hydrolysis

(D3) Set up 11 macro test-tubes, each containing 2 drops of dilute iodine solution and 2 drops of water.

(D4) Place a 250 mL beaker containing 50 mL of water on a wire gauze mat on a tripod with a heat mat under it, and heat to boiling with a Bunsen burner. Whilst waiting for the water to boil, add 25 mL of 0.5 % starch solution to a small beaker. Once the water bath is boiling, and with a stopwatch at the ready, add 2 mL of 4 M HCl to the 25 mL of starch solution, immediately immerse it in the beaker of boiling water and start timing.

(D5) At intervals of 1 minute, remove 10 drops from the solution and add to individual test-tubes in the set prepared in (D3). **Record your observations in your logbook.** Stop recording after 10 samplings or when you observe no further changes.

(D6) Add 5 mL of 0.5 % starch solution to a macro test-tube. To this solution add 0.5 mL of α-amylase (a salivary enzyme). Add 3 drops of 0.2 M NaCl solution (to provide Cl^- ions as a co-catalyst) and stir briefly. Retain this solution for use in steps (D7) and (D9).

(D7) Remove 10 drops from the solution and add to the last test-tube set up in (D3). **Record your observations in your logbook.**

For your logbook:

Is starch broken down more rapidly by α-amylase at room temperature or by dilute HCl at 100 ºC?

Fehling's Test

(D8) To a macro test-tube add 2 mL of 0.5 % starch solution. To this solution add 2 mL of Fehling's solution A and 2 mL of Fehling's solution B. **Record your observations in your logbook**.

(D9) After you have completed step (D7), carry out a Fehling's test (again with heating) on the remaining solution from step (D6). **Record your observations in your logbook**.

For your logbook:

Does Fehling's reagent react with a glucose monomer or a glucose polymer (starch)?

Group Discussion

Based on your observations from Parts A and C, can you explain the reaction of Fehling's reagent with sugars? Examine your model of glucose and the cyclic structure of glucose shown in the notes. Is the functional group you identified as the cause of the Fehling reaction present in the structure of glucose? If not, can you imagine how the glucose molecule might rearrange its structure to produce the necessary functional group? Hint: Playing with your model may help here.

Sucrose is a disaccharide consisting of glucose and fructose linked together (see the structures in Figure 2). Based on your observations in this experiment, would you expect sucrose to show a positive Fehling's test result when broken down into its individual sugar units (*e.g.* either via acid hydrolysis or enzymatically)?

The basis of the iodine-starch reaction is the formation of a blue iodine-starch complex? Why do you think that iodine doesn't react with sugar monomers? What might be responsible for the blue colour of the iodine-starch complex?