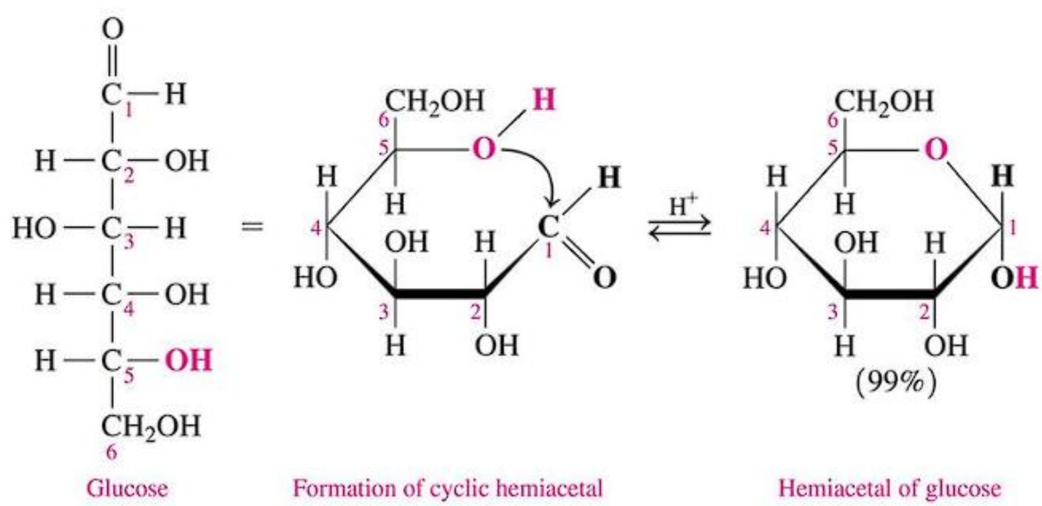
III. Effect of alkali on carbohydrates:

Benidict's, Fehling's and Barfoed's Tests:

These tests are based on the most important chemical property of sugar, the reducing property. Benidicts and Fehlings tests are used to determine the presence of reducing sugars while Barfoed's test is used more specifically to distinguish monosaccharides from disaccharides. The ability of sugars to reduce oxidizing agent depends on the availability of free aldehyde or ketone group for this reaction. All monosaccharides are reducing sugars. Now you may ask how it is possible since you have learned that, in solution, the higher monosaccharides (pentoses and hexoses) exist primarily in the cyclic hemlacetal forms in which the carbonyl group is tied up.

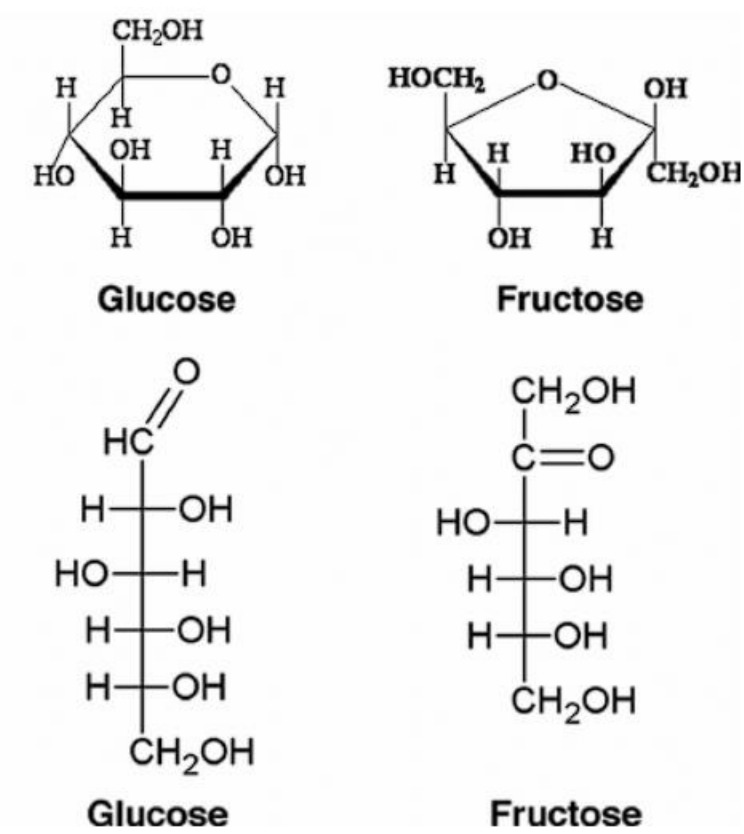
Similarly, an aldehyde group reacts with an alcohol group forming a hemiacetal.



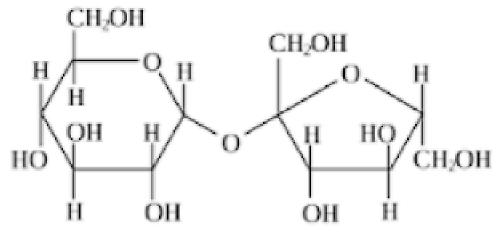
Even if the carbonyl group is tied up, these pentoses and hexoses are easily oxidized due to the fact that the cyclic forms are in equilibrium with a small amount of open chain form.

When solutions of pentoses and/or hexoses are treated with an oxidizing agent, the open-chain aldehyde form is oxidized and removed from the equilibrium mixture. Some of the α-and β-forms are converted to more open chain form to re-establish the equilibrium which in turn is further oxidized. Disaccharides have glycosidic linkages which in some cases involve bonding between the free aldehyde or ketone groups. In such cases there is no reducing in the sugar for example sucrose is a non-reducing sugar for this particular reason, sucrose is composed of α-glucose and β-fructose.

The glycosidic linkage is formed by the loss of water between the hemiacetal OH-group of a-glucose and the corresponding hydroxyl group of fructose.



Sucrose:



There is no possibility for the presence of open-chain form in sucrose. Maltose on the other hand is composed of two molecules of glucose. In solution it exists in an equilibrium mixture of α-, β- and the open chain form. Again, because of the presence of small amount of open-chain form in the second glucose unite, maltose is a reducing sugar which gives a positive Benidict's and Fehling's tests.

Those disaccharides which contain a free hemlacetal group are reducing sugars although milder than the monosaccharides. Polysaccharides are long molecules and have so very few of these open-chain "endings", that none of the polysaccharides have reducing properties. Reducing sugars can react with many different oxidizing agents, Benidict's and Barfoed's tests both have cupric ion (Cu) as oxidizing agent, so does the widely used Fehling's test. The alkaline solution favours the aldehyde form of sugar. In these reactions, the Cu^2 oxidizes the aldehyde group to a carboxyl group whereas the metal ion is reduced to cuprous (Cu) ion which forms a red cuprous oxide precipitate.

IV. Iodine Test:

lodine test is used to distinguish polysaccharides from other carbohydrates and starch from other polysaccharides. Polysaccharides such as amylase, amylopectin, glycogen, starch and dextrin from characteristic color complexes when treated with iodine. The amylase in starch is responsible for the Intense blue color with lodine. The linear chains of glucose molecules in amylose may be colled in a helix that contains six glucose units per turn. The inner space of the helix accommodates are iodine molecule, which is thought to be held in position by hydrogen atoms of the glycopyranose rings. Polysaccharides with branched chains of glucose molecules (i.e amylopectin and glycogen) do not readily form linear helixes and yield less intensely colored lodine complexes. So dextrin gives a red color, glycogen produces a red-brown color. Cellulose, monosaccharides and disaccharides give no color with iodine.

Experimental Procedure

Effect of alkali:

A. **Benedict's Test**:

1. To a series of five test tubes, add 5 ml of Benedict's reagent.

2. To each tube add 8 drops of 1% solution of glucose, sucrose, lactose, xylose and starch respectively.

3. Mix the contents of each tube and place them in a boiling water bath for 3 minutes.

4. Cool the tubes under tap water. A positive test is denoted by the formation of a yellow, green or red precipitate.

5. Report your observations and compare their appearance: the size of the particles and the amount of precipitate.

B. **Fehling's Test**:

1. Mix 1 ml of Fehling's solution A with 1 ml of Fehling's solution B in each of three test tubes.

2. Add equal amounts of 1% solution of glucose, sucrose and lactose to each test tube respectively.

3. Mix the contents of each tube and place the tubes in a boiling water bath for 5 minutes.

4. Report your observations and explain your results.

C. **Barfoed's Test**:

1. Add 3 ml of Barfoed's reagent into three separate test tubes.

2. Add 2 ml of 1% solutions of glucose, lactose and starch to the test tubes respectively.

3. Mix the contents of the tubes and place them in a boiling water bath.

4. Observe the tubes during the first five minutes and remove any tube that become cloudy or changes color.

5. Continue heating the tubes for another 5 to 10 minutes.

6. Report your observations and notice the time of positive reaction in each tube.

Iodine Test:

1. Into six test tubes add about 2 ml of 1% solutions of glucose, sucrose, starch, glycogen, dextrin and distilled water (serve as blank), each solution in a separate tube.

2. Add few drops of iodine (1% lodine in 2% potassium iodide) solution into each tube.

3. Mix the contents of each tube and compare the color of the blank to that of the other tubes,

4. Report the color which you observed in each tube.

5. Heat the tubes containing starch and lodine in a boiling water bath for 10 to 15 minutes. If nothing happens, add few milliliters of water to dilute the solution and continue heating for another five minutes.

6. Report your observations and explain your results.

Reagent: How to prepare

1. **Benedict's Reagent**: dissolve 17.3 g of CuSO4.5H2O in 100 ml of hot H_2O. Dissolve separately with heating 173 g of sodium citrate and 100 g of Na_2CO_3 in

100 ml of H_2O. Allow to cool, add the citrate carbonate solution with mixing to the copper sulfate solution. Dilute to 1L with water.

2. **Fehling's Solution A**: dissolve 125 g potassium hydroxide (KOH) and 173 g sodium potassium tartarate ($NaKC_2H_4O.4H2O$) in distilled water and dilute to 500 ml with water.

Fehling's Solution B: dissolve 34.65 g copper sulfate ($CuSO_4$) in distilled water and dilute to 500 ml with water.

3. **Barfoed's Reagent**: dissolve 13.3 g of cupric acetate in 200 ml of distilled water then add 1.9 ml of glacial acetic acid, CH3COOH.

4. **Iodine Solution**: dissolve 25 mg of jodine in 100 ml of 2% potassium iodide.