Carbohydrates are classified as polyhydroxy aldehydes or polyhydroxy ketones. Therefore, they will exhibit chemical properties associated with both alcohols and carbonyl compounds. In the following series of analyses you will be examining the reactivity of some monosaccharides, disaccharides and a polysaccharide.

In the Benedict’s Test a reducing sugar (a sugar with a free or potentially free, i.e., a cyclic hemiacetal, aldehyde group) reacts with the blue-colored Cu$^{2+}$ ion in the presence of base. The copper (II) ion is reduced to a red-orange Cu$_2$O precipitate whereas the aldehyde group is oxidized to the carboxylic acid functional group. In addition to all aldose monosaccharides giving a positive Benedict’s Test, ketose monosaccharides, though lacking an aldehyde group, react due to the presence of an hydroxyl group next to the ketone group. Thus α-hydroxy ketones give positive tests. If there is no potential free aldehyde group, i.e., the aldehyde group is tied up in a glycosidic bond (an acetal bond), the sugar is referred to as a non-reducing sugar.

The Barfoed’s Test is a variation of the redox reaction mentioned previously. Copper (II) acetate in acetic acid is not as reactive as the Cu$^{2+}$ Benedict’s reagent. Thus, one can distinguish monosaccharides from disaccharides based on how fast the red-orange precipitate forms. Typically, monosaccharides react within 2-3 minutes, whereas disaccharides take longer.

The Seliwanoff Test is used to distinguish ketoses from aldoses using the aromatic alcohol in the presence of concentrated hydrochloric acid. This is useful for both monosaccharide ketoses as well as disaccharide ketoses. A positive test is noted by a red colored solution; a yellowish straw or apricot color is not indicative of a positive test.

To distinguish pentoses from hexoses on can use the Bial’s Test. Pentoses react with orcinol in the presence of FeCl$_3$ and concentrated HCl to give a characteristic blue-green color. Non-reacting sugars may produce a brown precipitate but the solution usually remains the yellow color of the FeCl$_3$.

Starch is composed of two fractions; the linear, helical fraction and the branched amylopectin fraction. When I$_2$ is inserted into the interior of the amylose fraction, a dark blue color is observed.

Procedures:

Carbohydrate Test Solutions:
Glucose, galactose, fructose, arabinose, maltose, lactose, sucrose, and starch

Benedict’s Test

1. Prepare a boiling water bath and label eight clean small test tubes.
2. In separate test tubes add 1 mL of the Benedict’s reagent. To each test tube add 5 drops of the test carbohydrate solution. Mix the samples.
3. Place all of the test tubes at the same time into the boiling water bath.
4. Note and record how long it takes for the red Cu₂O precipitate to form; also note if the blue Benedict’s reagent color disappears.
5. After 10 minutes remove all the tubes. Keep the boiling water bath going for the remaining three experiments. Did any sugars not produce the red precipitate? Which are reducing sugars? Which are not?

Barfoed’s Test

1. Use the boiling water bath from before and label a new set of 8 clean small test tubes.
2. In separate test tubes add 1 mL of the Barfoed’s reagent. To each test tube add 10 drops of the test carbohydrate solution. Mix the samples.
3. Place all of the test tubes at the same time into the boiling water bath.
4. Note and record how long it takes for the red Cu₂O precipitate to form.
5. After 10 minutes remove all the tubes. Based on your results which are monosaccharides? Which are disaccharides?

Seliwanoff Test

1. Use the boiling water bath from before and label a new set of 8 clean small test tubes.
2. In separate test tubes add 1 mL of the Seliwanoff’s reagent. To each test tube add 3 drops of the test carbohydrate solution. Mix the samples.
3. Place all of the test tubes at the same time into the boiling water bath.
4. Note and record how long it takes for the first clear red colored solution to form.
5. Remove all the tubes as soon as the first positive test is seen as prolonged heating (in excess of 5 minutes) may cause spurious results. Which sugar solution(s) contain a ketose?

Bial’s Test

1. Use the boiling water bath from before and label a new set of clean small test tubes
2. In separate test tubes add 1 mL of the Bial’s reagent. To each test tube add 10 drops of the test carbohydrate solution. Mix the samples.
3. Place all of the test tubes at the same time into the boiling water bath.
4. Note and record how long it takes for the first clear blue-green solution to form.
5. Remove all the tubes as soon as the first positive test is seen as prolonged heating may cause spurious results. Which sugar solution(s) contain a pentose?

Iodine test

1. Place 3 drops of each test carbohydrate solution in separate wells of a clean spot plate.
2. Add 1 drop of the iodine solution to each test carbohydrate solution.
3. Note and record the color of each sample.
4. Did any other solutions besides the starch solution give a positive test?
Suppose you saw no sign of a color change in the Benedict’s test, no sign of a red precipitate after 10 minutes with the Barfoed’s test, and a dark red colored solution with the Seliwanoff test.

Indicate which sugar(s) you could have: _____________________________________________

Suppose you saw a red precipitate with the Benedict’s test, a red precipitate after 2 minutes with the Barfoed’s test, and a straw-colored solution after more than 5 minutes with the Seliwanoff’s test.

Indicate which sugar(s) you could have: _____________________________________________

Suppose you saw a red precipitate with the Benedict’s test, no sign of a red precipitate after 10 minutes with the Barfoed’s test, and a straw-colored solution after more than 5 minutes with the Seliwanoff’s test.

Indicate which sugar(s) you could have: _____________________________________________