Qualitative Analysis of Carbohydrates

Materials Required:

1) Glassware
2) Test tubes
3) Test tube holder
4) Water bath
5) Spatula
6) Dropper

Reagents Required:

1) Molisch's Reagent
2) Iodine solution
3) Fehling's reagent A
4) Fehling's reagent B
5) Benedict's qualitative reagent
6) Barfoed's reagent
7) Seliwanoff 's reagent
8) Bial's reagent
9) Phenylhydrazine hydrochloride
10) Sodium acetate
11) Glacial acetic acid
12) Glucose, fructose
13) Microscope

Procedure:

1) Molisch's Test:

In a test tube, add 2 ml of the test carbohydrate solution and 2 drops of α-naphthol solution. Carefully incline the tube and pour dropwise conc. H_2SO_4, using a dropper,along the sides of the tube. Observe the violet colour at the junction of the two liquids.

2) Fehling's Test:

In a test tube, add 2 ml of the test carbohydrate solution and add equal volumes of Fehling A & Fehling B and place it in a boiling water bath for few minutes.. When the contenst of the test tube comes to boiling, mix them together and observe any change in color or precipitate formation. The production of yellow 'or brownish-red precipitate of cuprous oxide indicates the presence of reducing sugars in the given sample.

3) Benedict's Test:

In the test tube with 2 ml of Benedict's reagent, add 5-6 drops of the test carbohydrate solution and mix well. Place the test tube in a boiling water bath for 5 minutes and observe any change in color or precipitate formation. Cool the solution. Observe the colour change from blue to green, yellow, orange or red depending upon the amount of reducing sugar present in the test sample.

4) Barfoed's Test:

To 2 mL of the test solution add about 2-3 mL of Barfoed's reagent. Mix it well and boil it for one minute in the water bath and allow to stand for a few minutes. Formation of a red precipitate of cuprous oxide in the bottom and along the sides of the test tube immediately, only monosaccharides answer this test. Since Barfoed's reagent is slightly acidic, This test is specific for monosaccharides.

5) Seliwanoff's Test:

To 2 mL of Seliwanoff 's reagent, add two drops of test solution. The mixure is heated to just boiling. A cherry red condensation product will be observed indicating the presence of ketoses in the test sample. There will be no significant change in colour produced for aldose sugar.

6) Bial's Test:

To 5 mL of Bial's reagent add 2–3 mL of test solution and warm gently in a hot water bath for 2minutes . The formation of a bluish green product is indicative of pentoses. Hexoses generally react to form muddy brown products.

7) Iodine Test:

Add 2 drops of iodine solution to about 2 mL of the carbohydrate containing test solution. A blue-black colour is observed which is indicative of presence of polysaccharides.

8) Osazone Test:

To 0.5 g of phenylhydrazine hydrochloride add 0.1 gram of sodium acetate and ten drops of glacial acetic acid. Add 5 mL of test solution to this mixture and heat under boiling water bath for about half an hour. Cool the solution slowly and examine the crystals under a microscope. Needle-shaped yellow osazone crystals will be observed for glucose and fructose, whereas lactosazone shows mushroom shaped and maltose produces flower-shaped crystals.

No.	Test	Observation	Inference	Reaction
1	**Molisch's Test** 2-3 drops of beta-naphthol solution are added to 2ml of the test solution. Very gently add 1ml of Conc. H_2SO_4 along the side of the test tube..	A deep violet coloration is produced at the junction of two layers.	Presence of carbohydrates.	This is due to the formation of an unstable condensation product of beta-naphthol with furfural (produced by the dehydration of the carbohydrate).
2	**Iodine test** 4-5 drops of iodine solution are added to 1ml of the test solution and contents are mixed gently.	Blue colour is observed.	Presence of polysaccharide.	Iodine forms coloured adsorption complexes with polysaccharides.
3	**Fehling's test** About 2 ml of sugar solution is added to about 2 ml of Fehling's solution taken in a test-tube. It is then boiled for 10 min	A red precipitate is formed	Presence of reducing sugar	This is due to the formation of cuprous oxide by the reducing action of the sugar.
4	**Benedict's test** To 5 ml of Benedict's solution, add 1ml of the test solution and shake each tube. Place the tube in a boiling water bath and heat for 3 minutes. Remove the tubes from the heat and allow them to cool.	Formation of a green, red, or yellow precipitate	Presence of reducing sugars	If the saccharide is a reducing sugar it will reduce Copper [Cu] (11) ions to Cu(1) oxide, a red precipitate
5	**Barfoed's test** To 2 ml of the solution to be tested added 2 ml of freshly prepared Barfoed's reagent. Place test tubes into a boiling water bath and heat for 3 minutes. Allow to cool.	A deep blue colour is formed with a red ppt. settling down at the bottom or sides of the test tube.	Presence of reducing sugars. Appearance of a red ppt as a thin film at the bottom of the test tube within 3-5 min. is indicative of reducing mono-saccharide. If the ppt formation takes more time, then it is a reducing disaccharide.	If the saccharide is a reducing sugar it will reduce Cu (11) ions to Cu(1) oxide

6	**Seliwanoff test** To 3ml of of Seliwanoff's reagent, add 1ml of the test solution. Boil in water bath for 2 minutes.	A cherry red colored precipitate within 5 minutes is obtained. A faint red colour produced	Presence of ketoses [Sucrose gives a positive ketohexose test] Presence of aldoses	When reacted with Seliwanoff reagent, ketoses react within 2 minutes forming a cherry red condensation product Aldopentoses react slowly, forming the coloured condensation product.
7	**Bial's test** Add 3ml of Bial's reagent to 0.2ml of the test solution. Heat the solution in a boiling water bath for 2 minutes.	A blue-green product A muddy brown to gray product	Presence of pentoses. Presence of hexoses.	The furfurals formed produces condensation products with specific colour.
8	**Osazone Test** Two two ml of the test solution, add 3ml of phenyl hydrazine hydrochloride solution and mix. Keep in a boiling water bath for 30mts. Cool the solution and observe the crystals under microscope.	Formation of beautiful yellow crystals of osazone Needle shaped crystals Hedgehog crystals Sunflower shaped crystals	Glucose/fructose Presence of lactose Presence of maltose	Reducing sugars forms ozazone on treating with phenylhydrazine

Differences Encountered in a Real Laboratory:

In an actual laboratory setting, there are certain important steps that are not necessarily applicable in a virtual lab:

1. Always wear lab coat and gloves when you are in the lab. When you enter the lab,switch on the exhaust fan and make sure that all the reagents required for the experiment are available. If it is not available, prepare the reagents using the components for reagent preparation.
2. Care should be taken while handling caustic acids like Conc. Sulphuric acid [H_2SO_4], nitric acid [HNO_3], Hydrochloric acid [HCl]. These acids should be opened and used in FUMEHOOD only. Accidental spill of these acids will cause severe burns and itching. Wash the spilled area with cold water and inform the lab assistant immediately.
3. When Sodium hydroxide is prepared, make sure that it is handled with care as the sodium hydroxide solution is caustic in nature.
4. Always check the water level in the water bath and if it is up to the level [nearly half the volume], switch on the water bath and adjust to the required temperature. Take care while using the water bath for the boiling step in the experiment. Hold the test tube using a test tube holder.
5. There should be a proportion between the reagents added and the test solution to obtain good result within the time mentioned. The droppers used should not be mixed between the reagents, always use individual droppers for each reagent.
6. The color formed will depend upon the quality of the reagents. So care should be taken while preparing the reagents. If commercially available reagents are used assure that it is not kept open for long time.
7. Clean the test tubes and glass wares with soap and distilled water. Recap the reagent bottles once the experiment is completed. The water bath and the exhaust fan should be switched off .