Qualitative tests of carbohydrates

QUALITATIVE TESTS FOR CARBOHYDRATES

Preliminary Procedure

Obtain an unknown carbohydrate and prepare a 1% solution by dissolving 0.25 g of carbohydrate in 25 mL of deionized water.

1. MOLISCH TEST

Principle: Carbohydrates when treated with concentrated sulphuric acid undergo dehydration to give furfural derivatives. These compounds condense with Alpha naphthol to form colored products. Pentoses yield furfural while Hexoses yield 5-Hydroxy methyl furfurals.

Procedure:

Take 2 ml of carbohydrate solution in a clean and dry test tube. Add 2 drops of ethanolic Alpha Naphthol (Molisch reagent) and mix. Incline the test tube and add carefully 2 ml of concentrated sulphuric acid along the side of the test tube so as to form 2 layers.

Interpretation: This is a sensitive but a non-specific test and is given positive by all types of carbohydrates. If the oligosaccharides or polysaccharides are present they are first hydrolysed to mono saccharides which are then dehydrated to give the test positive.

An appearance of reddish violet or purple colored ring at the junction of two liquids is observed in a positive Molisch test.

2) BENEDICT'S TEST

Principle:

- Carbohydrates with free aldehyde or ketone groups have the ability to reduce solutions of various metallic ions.
- Reducing sugars under alkaline conditions tautomerise and form enediols.
- Enediols are powerful reducing agents.
- They reduce cupric ions to cuprous form and are themselves converted to sugar acids.
- The cuprous ions combine with OH- ions to form yellow cuprous hydroxide which upon heating is converted to red cuprous oxide.

Procedure

- Take 5 ml of Benedict's reagent.
- Add 8 drops of carbohydrate solution.
- Boil over a flame or in a boiling water bath for 2 minutes.
- Let the solution cool down.

Interpretation:

- Benedict's test is a semi quantitative test. The color of the precipitate gives a rough estimate of a reducing sugar present in the sample.
- **Green color - Up to 0.5 G% (+)**
- **Green precipitate - 0.5-1.0 G% (++)**
- **Yellow precipitate -1.0-1.5 G% (+++)**
- **Orange precipitate- 1.5-2.0 G% (++++)**
- **Brick red precipitate- > 2.0 G %(+++++)**

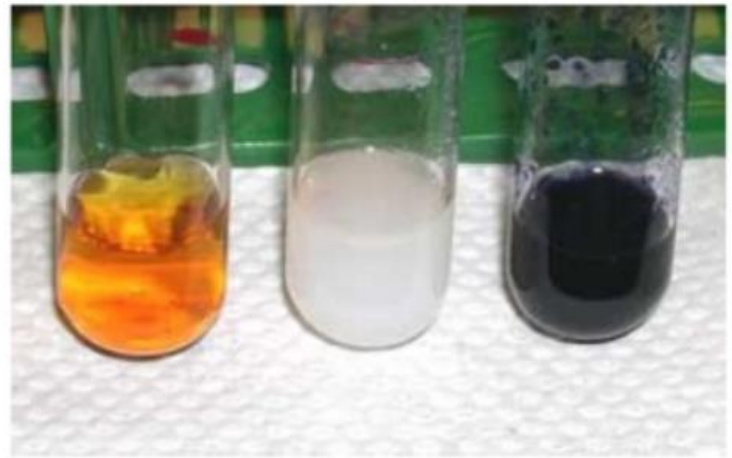
Benedict's test is a semi quantitative test. The color formed depends upon the amount of reducing sugar present in the mixture.

IODINE REACTION

- This is a test for polysaccharides

Principle :

Iodine forms a coordinate complex between the helically coiled polysaccharide chain and iodine centrally located within the helix due to adsorption. The color obtained depends upon the length of the unbranched or linear chain available for complex formation



Left to right: Lugol's iodine, starch solution, starch solution with iodine.

Interpretation

Amylose- A linear chain component of starch, gives a deep blue color

Amylopectin- A branched chain component of starch, gives a purple color

Glycogen- Gives a reddish brown color

Dextrins- Amylo, Eryhthro and Achrodextrins, formed as intermediates during hydrolysis of starch give violet, red and no color with iodine respectively.

Fehlings test

In this test the presence of aldehydes but not ketones is detected by reduction of the deep blue solution of copper(II) to a red precipitate of insoluble copper oxide. The test is commonly used for reducing sugars but is known to be NOT specific for aldehydes. For example, fructose gives a positive test with Fehling's solution as does acetoin.

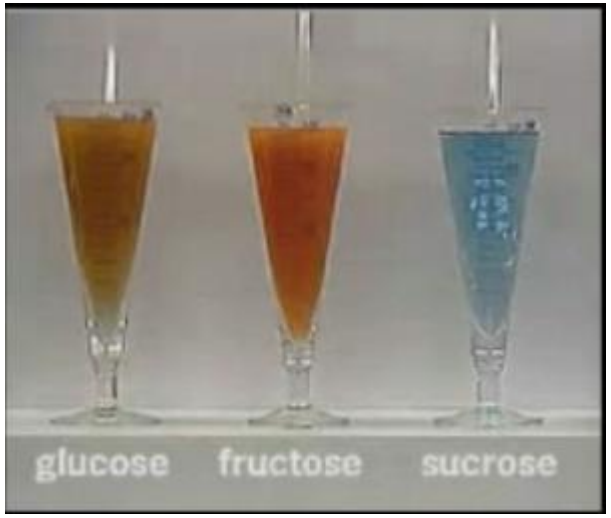
Two solutions are required:

Fehling's "A" uses 7 g $CuSO_4.5H_2O$ dissolved in distilled water containing 2 drops of dilute sulfuric acid.
Fehling's "B" uses 35g of potassium tartrate and 12g of NaOH in 100 ml of distilled water.

These two solutions should be stoppered and stored until needed.

For the test:
Mix 15 ml of solution-"A" with 15 ml of solution-"B"
Add 2 ml of this mixture to an empty test tube.
Add 3 drops of the compound to be tested to the tube.
Place the tube in a water-bath at 60° C.

A **positive** test is indicated by a green suspension and a red precipitate.



The test is sensitive enough that even 1 mg of glucose will produce the characteristic red colour of the compound.

Tollens test

This test is given by reducing sugars. Carbohydrates reacts with Tollens reagent forms a silver mirror on the inner walls of the test tube. This confirms the presence of reducing sugars. Silver ions are reduced to metallic silver.

The chemical reaction is given below.

$$NH_4OH + AgNO_3 \longrightarrow NH_4NO_3 + Ag(OH)$$

$$2Ag(OH) \longrightarrow Ag_2O + H_2O$$

$$\begin{array}{l} CH_2OH \\ | \\ (CHOH)_4 + Ag_2O \\ | \\ CHO \\ \text{Glucose} \end{array} \longrightarrow \begin{array}{l} CH_2OH \\ | \\ (CHOH)_4 + 2Ag \\ | \qquad \text{Silver mirror} \\ COOH \end{array}$$

Note: *The appearance of silver mirror confirms the presence of reducing sugars.*