LABORATORY 5

CARBOHYDRATES, CARBOXYLIC ACIDS, AROMATIC AND HETEROCYCLIC COMPOUNDS

I. Characteristic reactions of carbohydrates

1. Reduction tests of aldehydes

These tests reveal reducing properties of aldehydes and carbohydrates. They are based on the reduction of heavy metal ions and simultaneous oxidation of carbonyl group of aldehydes or monosaccharides to carboxyl group. Ketoses also give positive results in this test because in alkaline environment of the test they isomerize to aldoses. Saccharose (disaccharide composed of α-glucose and β-fructose) is an example of non reducing carbohydrate.

a. Fehling's test

The Fehling reagent is composed of two separate solutions: Fehling I is solution of $CuSO_4$ in diluted H_2SO_4, and Fehling II is sodium potassium tartrate in diluted NaOH. In an alkaline milieu, the insoluble blue sediment $Cu(OH)_2$ is released, however, in the presence of tartrate it remains in solution in a form of tartrate complex compound:

$$\text{glucose} + 2\ \underset{\text{blue}}{CuSO_4} + 4\ NaOH \xrightarrow[\text{sodium-potassium tartrate}]{\text{temp.}} \text{gluconic acid} + \underset{\text{brick-red}}{Cu_2O} + 2\ Na_2SO_4 + 2\ H_2O$$

Procedure: In a test tube, mix 1 ml of Fehling I reagent and 1 ml of Fehling II and heat until boiling. Then divide the solution into two test tubes. Add a few drops of glucose to the first test tube and a few drops of saccharose to the second one. Mix well and prolong the heating in water bath. Observe the precipitation of the orange-red coloured sediment of cuprous oxide Cu_2O in the test tube with glucose.

b. Trommer's test

This test is based on the same reaction, however, sodium potassium tartrate is omitted. To make the test positive without tartrate, addition of $CuSO_4$ solution to alkaline solution of reducing carbohydrate must be performed dropwise. It is important not to exceed a 1:1 carbohydrate to copper salt molar ratio. The final effect is the same as in Fehling's test.

Procedure: To one test tube add 1 ml of glucose, and to the second add 1 ml of saccharose solution. To both test tubes add several drops of 2 M NaOH and mix. Then start adding $CuSO_4$ solution drop-by-drop, to keep the blue precipitate of $Cu(OH)_2$ still soluble. When a trace of precipitate appears, stop $CuSO_4$ adding and heat the test tubes in a boiling water bath. Observe formation of orange-red coloured precipitate of cuprous oxide (Cu_2O) in the test tube with glucose.

c. Tollens' test

In this test, silver ions are reduced to metallic silver, producing a silver mirror on a test tube walls.

$$\text{glucose} + 2\left[Ag(NH_3)_2\right]OH \rightarrow \downarrow 2\,Ag + \text{gluconic acid} + 4\,NH_3 + H_2O$$

Procedure: Clean and dry test tubes should be used in this test. Pour about 1 ml of 0.1 M $AgNO_3$ solution to the test tube and then start adding dropwise 2 M water ammonia solution ($NH_3 \cdot H_2O$). After mixing, initial AgOH precipitate will solubilise, forming $[Ag(NH_3)_2]OH$ complex compound. Add to the tube 5 drops of glucose or formaldehyde solution, mix and heat the tube in a boiling water bath. Observe the appearance of a silver mirror on the tube walls.

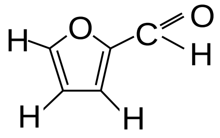
d. Barfoed's test

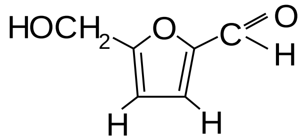
This reducing test is performed to distinguish reducing monosaccharides from reducing disaccharides (e.g. lactose).

Procedure: Add 1 ml of Barfoed's reagent (cupric acetate in an lactic acid solution) (reagent is in the hooded area) to two test tubes. Then add 1 ml of glucose solution to the first test tube and 1 ml of lactose to the second one. Mix and heat both tubes in a boiling water bath. In the case of reducing monosaccharide (glucose), the cuprous oxide (Cu_2O) sediment appears after 3 minutes. For reducing disaccharide (lactose), the same cuprous oxide sediment formation needs 30 minutes of heating.

2. General tests for carbohydrates

Concentrated acids (sulphuric, hydrochloric or acetic) cause dehydration of monosaccharides. Furfural (from pentoses' e.g xylose) or hydroxymethylenefurfural (from hexoses) are formed. They condense with phenol compounds and coloured products are formed.


furfural


hydroxymethylene-furfural

a. Molish's test

Procedure: To 1 ml of carbohydrate solution (glucose) add two drops of α-naphtol solution in ethanol (reagent is in the hooded area). Apply carefully a few drops of concentrated sulphuric acid (under the hood) on the tube wall. Flowing down sulphuric acid slowly underlays the carbohydrate solution, and after a few minutes, at the layers' border, red-violet colour ring appears.

b. Seliwanoff's test for ketoses

Ketoses (e.g. fructose), in the presence of concentrated HCl, undergo dehydration more easily than aldohexoses. Hydroxymethylenefurfural creates red complex with resorcine (1,3-dihydroxybenzene).

Procedure: Add 1 ml of Seliwanoff's reagent (0.05% resorcin in hydrochloric acid) (reagent is in the hooded area) to two test tubes. Then add 10 drops of glucose (aldose) solution to the first test tube and 10 drops of fructose (ketose) to the second one. Mix and heat both tubes in a boiling water bath for 3 min. In the presence of ketose, a red colour will develop.

c. Bial's test for pentoses

Furfural formed under action of concentrated HCl on pentoses condenses with orcin (in the presence of $FeCl_3$) and a green colour of the solution appears.

Procedure: Add 1 ml of Bial's reagent (orcin solution in hydrochloric acid) (reagent is in the hooded area) to two test tubes. Then add 3 drops of xylose (pentose) solution to the first test tube and 3 drops of glucose to the second one. Mix and heat both test tubes in a boiling water bath for 1 min. Then add 3 drops of $FeCl_3$. In the presence of pentose, a green colour of the solution will develop.

II. Characteristic reactions of carboxylic acids

Carboxylic acids are weaker comparing to mineral acids, but stronger than carbonic acid. Specific reactions of carboxylic acids depend on organic part that is connected to carboxylic group or the presence of additional functional group.

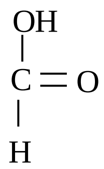
1. Reaction of carboxylic acids with sodium bicarbonate $NaHCO_3$.

Procedure: Add about 1 ml of 5% $NaHCO_3$ solution to 1 ml of short-chain carboxylic acid solution (formic acid - HCOOH). After shaking, observe the release of CO_2 gas bubbles, gathering on the tube walls.

$$HCOOH + NaHCO_3 \rightarrow HCOONa + H_2O + CO_2\uparrow$$

2. Formic acid (methanoic acid) detection

Formic acid is the only one, which possesses both carboxyl and aldehyde groups activities:



The reducing property of formic acid can not be demonstrated using a typical reducing test (in alkaline medium), because of its dissociation in water solution. However, its reducing property can be demonstrated in the following reaction:

$$H\overset{+2}{C}OOH + 2\ \underset{\text{sublimate}}{\overset{+2}{Hg}Cl_2} \rightarrow \downarrow \underset{\text{calomel}}{\overset{+1}{Hg_2}Cl_2} + \overset{+4}{C}O_2\uparrow + 2HCl$$

***Procedure**:* Add a few drops of 5% mercuric chloride $HgCl_2$ (a corrosive sublimate) to 1 ml of formic acid solution. Heat in a water bath to obtain the white precipitate of mercurous chloride (calomel).

3. Lactic acid (α-hydroxypropionic acid) detection

Organic acids with α-hydroxyl group change the color of Uffelmann's reagent to yellow-greenish.

***Procedure**:* Mix a few drops of Uffelmann's reagent (the hooded area) with several drops of lactic acid solution. Violet color of the reagent will be changed to light yellow-greenish.

4. Reactions of fatty acids

a. addition reaction of unsaturated fatty acid with iodine solution

Unsaturated fatty acids react with iodine (and other halogens) discoloring the iodine solution and yielding iodine addition products.

$$R\text{-}CH{=}CH\text{-}COOH + I_2 \rightarrow R\text{-}CHI\text{-}CHI\text{-}COOH$$

***Procedure**:* Add a few drops of Hüble's reagent (iodine in alcoholic solution of $HgCl_2$) (reagent is in the hooded area) to 1 ml of olive oil (oleic acid), and shake. After a few minutes the solution becomes colorless.

b. solubility of fats

Fats are hydrophobic molecules, with a long-chain hydrocarbon component present in fatty acid structures. Presence of this chain results in sparing solubility or insolubility of fats in water. For lipids solubilization we use organic (non-polar) solvents like chloroform, hexane, ether or benzene.

Procedure:

- mix in a test tube a few drops of olive oil and 2 ml of water, and shake for 15 seconds. Observe formation of oil emulsion in water, which is unstable and after a few minutes forms a two phase solvent-system
- mix a few drops of olive oil with 2 ml of chloroform and shake. In the chloroform fat forms a clear solution

c. soap formation

Heating of animal fats (acylglycerols) in alkaline solution leads to hydrolysis of ester bonds, releasing free glycerol and fatty acid salts, known as soaps. Sodium salts of fatty acids form a solid soap, potassium salts form a soft soap, both easily soluble in water. Calcium, magnesium, barium and lead salts are insoluble in water.

Procedure: In a test tube mix 3-5 ml of alcoholic KOH solution with a few drops of olive oil. Heat the tube for a few minutes in a boiling water bath. Add to the tube 10 ml of water and shake well. Observe formation of turbid soap solution with foam on the liquid surface.

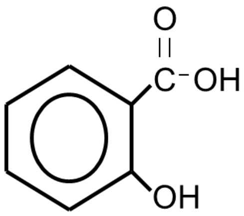
III. Reactions of aromatic and heterocyclic compounds

1. Reaction of phenol with ferric chloride

Ferric chloride reacts with the hydroxyl groups of phenols and phenol derivatives and forms stable-colored complexes: violet, violet-bluish, blue, green and red.

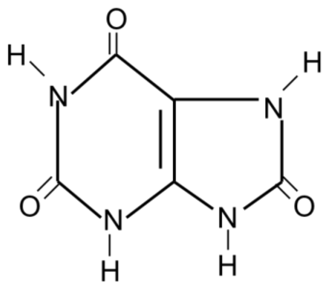
Procedure: Add 1 drop of $FeCl_3$ solution to 1% phenol water solution (0.5 ml). A violet color of this mixture will form. The color disappears after addition of some drops of 1 M sulfuric acid.

2. Salicylic acid detection



Procedure*:* Add to test tube 1 ml of water, a few crystals of salicylic acid and 1 drop of $FeCl_3$ solution. Observe the violet color, similar to previously performed $FeCl_3$ reaction with phenol solution.

3. Uric acid detection (Folin's reaction)



Procedure*:* Dissolve some crystals of uric acid in 1 ml of distilled water, add 1 ml of phosphotungstic acid and some drops of 2 M NaOH. Observe the violet color of the reduced tungsten solution.