Aim - Qualitative Determination for Carbohydrates

Carbohydrates are the most abundant biomolecules on the earth presents in the form of sugars, starch, cellulose and other complex substances and are therefore, defined as sugars and their derivatives. Monosaccharides are the simplest carbohydrates and the most common is glucose which is the most important one for living organisms. An aldehyde contains one carbonyl group at an end carbon. A carbonyl group in the middle would be ketone. They contain carbon, hydrogen and oxygen in the ratio of 1:2:1 and its empirical formula for many carbohydrates is $C_x(H_2O)_y$. Glucose is the major source of energy whereas starch and glycogen function as storage polysaccharides in plants and animals respectively. The carbohydrates are also structural components of cell walls, connective tissues in animals and exoskeletons of invertebrates.

The carbohydrates are classified as follows: i) Monosaccharides: a single sugar unit of polyhydroxy aldehydes or ketones and cannot be hydrolysed into simpler compounds. Examples- glucose, fructose, galactose etc. ii) Oligosaccharides: they are composed of 2-10 units of monosaccharides linked to each other by glycosidic bonds. Examples- sucrose, lactose, maltose, etc. iii) Polysaccharides: they are made of more than ten units of monosaccharides linked by glycosidic bonds. Examples- starch, cellulose, glycogen, etc.

Some of the different types of tests for detection of carbohydrates in the sample are as follows:

Name of the tests	**Application**
Molisch's test	General test for carbohydrates
Anthrone test	General test for carbohydrates
Iodine test	For starch and Glycans
Seliwanoff's test	For ketones
Fehling's test	For reducing sugars
Benedict's test	For reducing sugars
Picric acid test	For reducing sugars
Mucic acid test	For galactose
Bial's test	For pentoses
Osazone test	For reducing sugars

Molish's Test

Principle: Molisch's test is a general test for the identification of all carbohydrates (monosaccharide, disaccharide, and polysaccharide) and glycoprotein. The glycosidic linkage in the sugar molecules are hydrolysed by conc. Sulphuric added to the solution to yield monosaccharides, where in the presence of an acid the monosaccharides are dehydrated to form furfural and its derivatives. This is very reactive and condenses with α-napthol to give a purple or violet coloured product.

Materials:

- Concentrated H_2SO_4
- α-napthol: prepare 5% (w/v) α-napthol in ethanol (to be prepared fresh)

Method:

- Add 2-3 drops of α-napthol reagent to 2ml of the test solution in a test tube.
- Inclined the test tubes and add one ml of conc. H_2SO_4 very gently along the side of the test tube, so that the two distinct layers are formed.
- A purple colour ring appearing in the junction of two layers indicates the presence of carbohydrates in the sample (test solution)

Anthrone Test

Principle: This is another test for all carbohydrates. On acid hydrolysis the carbohydrates reacts with acids released glucose and is dehydrated to form furfural which reacts with anthrone to give bluish green coloured complex.

Reaction:

Glucose Hydroxymethyl furfural Bluish green complex

Materials:

- Boiling water
- Concentrated H_2SO_4
- 0.2% (w/v) anthrone solution in conc. H_2SO_4 (ice-cold)

Method:

- Take 0.5-1ml of the test solution in a test tube.
- Add about 2ml of anthrone reagent to it
- Mix thoroughly all the content
- Observe for a colour change to bluish green.
- In case of no colour development, keep the tubes in boiling water for about 10 minutes and examine.

Iodine Test:

Principle: Starch when reacts with iodine a coloured adsorption complex is formed which is blue in colour and a reddish brown complex while reacts with glycogen. It is a test for amylase, amylopectin and glycogen. On heating or on an addition of alkali like NaOH or KIH, the coloured disappears. This reaction is only physically association where Iodine traps in the coiled structure of polysaccharide becomes linear and the Iodine molecules are released and the colour disappears on heating or on addition of alkali. The test will be answered by fructose, sucrose and other keto containing carbohydrates.

Materials:

- Iodine solution: 0.005N iodine solution in 3% (w/v) potassium iodide solution.
- 1% test solution of glucose, sucrose, starch, glycogen, cellulose etc.
- Water bath
- Dry test tubes
- Pipettes

Method:

- Take 1-2 ml of the test solution in a test tube.
- Add 5 drops of iodine solution to it and mix gently.
- Look for the development of blue colour
- Heat the solution, the blue colour will disappear and on cooling the colour reappears.

Fehling's Test

Principle: Fehling's Test is a sensitive test for reducing sugars. Fehling's solution (A) copper sulphate and Fehling's solution B is alkaline sodium potassium tartarise (sodium potassium titrate) acts as a chelating agent in this reaction, cupric oxide is reduced by the reducing sugar and cuprous oxide is precipitated on heating which give reddish brown precipitate indicating the presence of reducing sugar.

Materials:

- Fehling's solution A: 35g of $CuSO_4$. $5H_2O$ is dissolved in water and the volume is made to 500 ml.
- Fehling's solution B: 120g KOH and 173g of Na-potassium tartarate (Rochelle salt) is dissolved in water and volume is made to 500 ml.
- Fehling's reagent: Equal volume of Fehling's solution A and B is mixed immediately prior to use.
- Dry test tubes

Method:

- Take 1ml of the test solution in a test tube.
- Add 1ml of Fehling's reagent to it.
- Mix thoroughly and place the test tubes in vigorously boiling water bath.
- Presence of red precipitate of cuprous oxide indicating the presence of reducing sugar in the test solution.

Benedict's test:

Principle: This test is used to detect reducing sugar (carbohydrate having free aldehyde or ketone functional group). The Reducing sugar under alkaline condition form enediols. Benedict's solution contains milder alkali Na_2CO_3. Enediols are powerful reducing agents. They can reduce cupric ions to cuprous ions which is the basis for Benedict's reaction. The cuprous hydroxide during the process of heating is converted to red cuprous oxide, which indicates the presence of reducing sugar.

Reaction:

$Na_2CO_3 + 2H_2O \rightarrow 2NaOH + H_2CO_3$

$2NaOH + CuSO_4 \rightarrow Cu(OH)_2 + Na_2SO_4$

$Cu(OH)_2 \rightarrow CuO + H_2O$

D-Glucose $+ 2CuO \rightarrow$ D- Gluconic acid $+ Cu_2O$

Materials:

- Boiling water bath
- Benedict's reagent A: 173g of sodium citrate and 100g of anhydrous Na_2CO_3 are dissolved in 600ml of hot distilled water and then diluted to 800 ml with distilled water.
- Benedict's reagent B: 17.3g of $CuSO_4$, $5H_2O$ is dissolved in 100ml of hot distilled water.
- Benedict's reagent: Reagent A is added to Reagent B slowly with constant stirring and the final volume is made of 1L.
- Dry test tubes.

Method:

- Take 1 ml of the test sample in dry test tube.
- Add about 2 ml of Benedict's reagent to it and mix well.
- Keep the test tubes in boiling water bath for 5 minutes.
- Observe for the formation of brick red precipitates as positive results.

Seliwanoff's test:

Principle: Seliwanoff's test is used to distinguish aldoses from ketoses (fructose and sucrose,etc.). Aldoses give negative results in this test. On treatment with conc. Acid, ketoses are dehydrated more rapidly to give furfural derivatives and on condensation with resorcinol give cherry red complex. This test is responded positively by sucrose as it gets hydrolyzed to give glucose and fructose due to the action of conc. HCl present in the reagent. Fructose being a keto sugar gives red color complex when react with Seliwanoff's reagent.

Materials and Reagents:

- Test solution: 5 % Glucose, 5 % Sucrose, 5 % Fructose.
- Seliwanoff's reagent (0.5% resorcinol in 3N HCl)s
- Water bath
- Pipettes
- Dry test tubes

Method:

- Take 1 ml of sample in test tube and take and 1 ml of distilled water in another tube as control.
- Add 3 ml of Seliwanoff's reagents in both test tube
- Keep the test tubes in water bath for 1-2 minutes.
- Look for the development of red color.

Note: If the reaction is allowed for longer time, aldoses also produce positive results.

Osazone test:

Principle: This test is for reducing sugars which have a free aldehyde or keto group that form osazone crystals. A non-reducing sugar like sucrose donot form an osazone. When phenyl hydrazine reacts with reducing sugars at a boiling temperature, osazone is formed. Phenyl hydrazine reacts with carbonyl compound in neutral or slightly acidic condition to give phenyl hydrazone, which is highly soluble. When hydrazone reacts with further phenyl hydrazine molecules, the condensation products formed are insoluble which precipitate out as crystals.

Materials and Reagents:

- Test solutions (1% of sugar solution)
- Phenyl hydrazine hydrochloride
- Sodium acetate
- Glacial acetic acid
- Clean and dry Test tubes

Method:

- Take 3 ml of test solution and distilled water (blank) in separate test tubes.
- Add 2g of Phenyl hydrazine hydrochloride with 3g of sodium acetate and add 0.3g of this mixture to the test solution.
- Add 2 to 3 drops of glacial acetic acid in each test tubes.
- Mix well and keep the test tubes in boiling water bath for 20 minutes.
- Cool and observed test tubes for the formation of yellow crystals.

  For further identification: Take out the crystals and mount onto a glass slide using cover slip and observe under both low and high power in the microscope. Glucose forms glucazone and fructose forms fructosazone, both give broomstick/needle shape/ feather-like yellow coloured crystals. Maltose gives sunflower shaped maltosazone crystals. Lactose gives hedgehog or cotton ball shaped lactosazone crystals. Glucose, fructose, and mannose form identical osazones as these sugars differ only at carbon numbers 1 and 2, osazone formation involves C_1 and C_2 are masked during osazone formation. Disaccharide, namely, lactose and maltose yield osazone crystals which are soluble in hot water, and are therefore separated out as crystal only on cooling. No osazone formation for sucrose.

  Bial's test:

  Principle: This test is used to differentiate pentoses sugar from hexoses sugars. Pentosses (such as ribose sugar) when treated with acidic medium gets dehydrates and form furfural which condense with orcinol in presence of ferric ion to give blue green colored complex which is soluble in butyl alcohol.

Materials:

- Test reagent
- Bial's reagent (0.4g is added to the 200 ml of hydrochloric acid and 0.5 ml of ferric chloride solution)
- Water bath
- Dry test tubes
- Pipettes

Method:

- Take 2ml of Bial's reagent in a clean and dried test tube.
- Add 4-5 drops of test solution to into the reagent test tubes.
- Keep in hot water bath for 30 seconds.
- Observe for the development of bluish green color.
- This bluish green color indicates a positive test for pentoses, all other colour indicates a negative results for pentoses. Hexoses generally react and produce green, red, or brown products.