	YILDIZ TECHNICAL UNIVERSITY **BIOMEDICAL ENGINEERING DEPARTMENT** **BME2901- BIOCHEMISTRY COURSE** **2020-2021 FALL SEMESTER**

EXPERIMENT 7
REACTIONS SPECIFIC TO CARBOHYDRATES

7.1. PURPOSE OF THE EXPERIMENT

To identify the presence of sugar in different solutions by taking advantage of the reductive properties of free ketone or aldehyde groups in the structure of sugars.

7.2. THEORETICAL KNOWLEDGE

Carbohydrates, which are one of the energy-providing compounds in the body, are found free or combined with other substances.

Glucose, fructose, ribose, deoxyribose, xylose, glyceraldehyde, dihydroxy acetone, lactose in milk, blood glucose, fructose in seminal fluid, and glycogen in liver and muscle cells are examples of free carbohydrates.

Ribose and deoxyribose in the structure of nucleic acids; galactose in the structure of cerebrosides found in muscle and nerve cell membranes; galactose found as prosthetic groups in glycoproteins; amino sugars, such as glycosamine and galactosamine which are incorporated into the structure of heteropolysaccharides, are examples of compound carbohydrates.

Carbohydrates are defined as polyhydroxy alcohols containing potentially active aldehyde or ketone groups in their structure, or as substances which, when hydrolysed, give these products.

<u>Carbohydrates can be classified into four main groups;</u>

a) Monosaccharides

They are the most simple and non-hydrolysable sugars. They are named according to their carbon numbers and the functional group (aldehyde or ketone) in the composition.

b) Disaccharides

They are clinically important sugars formed by the coupling of two monosaccharide units to each other with glycosidic bonds. When disaccharides hydrolyzed, they are separated into monosaccharides that form them.

c) Oligosaccharides

They consist of short chains (3-10 monosaccharide units) of monosaccharide units, or residues, joined by characteristic linkages called glycosidic bonds. The most abundant are the disaccharides, with two monosaccharide units.

d) Polysaccharides

Polysaccharides are carbohydrates (e.g. starch, cellulose, or glycogen) whose molecules consist of a number of sugar molecules bonded together. Homopolysaccharides contain only a single type of monomer such as glycogen, starch, cellulose; heteropolysaccharides such as heparin, hyaluronic acid, chondroitin sulphate contain two or more different kinds of monomers. When polysaccharides are hydrolysed, they are broken down into the monosaccharide units that form them.

Carbohydrates present in a solution can be easily identified by performing certain tests in the laboratory. The important tests for carbohydrate detection are shown in Table 7.1.

All monosaccharides, some disaccharides, oligosaccharides and polysaccharides, having the free aldehyde or ketone groups in their structures as a reducing group. Carbohydrates with free or potentially free reducing groups easily reduce metal like copper (Cu), Ba, Hg (mercury), Iron (Fe) & silver (Ag) in alkaline solution when blue alkaline cupric oxide or hydroxide suspended in alkaline medium is heated it forms blue precipitate of cupric oxide (CuO) but in presence of reducing substances, e.g reducing sugars having free or potentially free aldehyde or ketonic group upon heating blue cupric hydroxide converted into insoluble brownish red cuprous oxide (Cu_2O) suspensions of metal hydroxide, used in metal reduction test and to precipitate in alkaline medium to check that organic compound having more than one alcoholic groups are added to give free metals.

All monosaccharides are reducing sugars as they all have a free reactive carbonyl group. Some disaccharides like maltose have exposed carbonyl groups and are also reducing sugars but less reactive than monosaccharides.

Test	Procedure	Observation	Inference	Reaction
Molisch's Test	2-3 drops of betanaphthol solution is added to 2ml of the test solution. Very gently added 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 betanaphthol with furfural (produced by the dehydration of the carbohydrate).
Iodine test	4-5 drops of iodine solution is added to 1ml of the test solution and mixed the contents gently.	Blue colour is observed.	Presence of polysaccharide	Iodine forms coloured adsorption complexes with polysaccharides.
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.
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 monosaccharide. 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.
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 minute is obtained.	Presence of ketoses [Sucrose gives a positive ketohexose test	When reacted with Seliwanoff reagent, ketoses react within 2 minutes forming a cherry red condensation product.
		A faint red colour produced.	Presence of aldoses	Aldopentoses react slowly forming the coloured condensation product.
Bials 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.	Presence of pentoses	The furfurals formed produces condensation products with specific colour.
		A muddy brown to gray product.	Presence of hexoses,	

Table 7.1. Some examples of test for carbohydrate detection.

One of the most popular tests used for the estimation or detection of reducing sugars and non-reducing sugars is the Fehling's test. The test developed by German chemist H.C. Von Fehling is also used to differentiate between ketone functional groups and water-soluble carbohydrates.

The working principles of Fehling, Benedict and Barfoed tests based on the reducing properties of carbohydrates are based on the same principle. The only difference is that the medium is alkaline in the first two experiments, while the medium is slightly acidic in the Barfoed experiment.

The common principle of experiments is sugars with common aldehyde or ketone groups show the ability to reduce heavy metal hydrates (Cu, Bi, Ag) in an alkaline environment with the effect of heat. During the experiment, sugars are oxidized and give off sugar acids (aldonic acids).

7.3. MATERIALS AND METHODS

Fehling Test

Fehling test is a method used in the determination of compounds having reducing properties. Carbohydrates containing free aldehyde and ketone groups have reducing properties in alkali solutions. In addition, monosaccharides act as reducing agents in weak acid solutions.

7.3.1. Materials

$CuSO_4$ (Copper Sulphate), Na-K tartrate, NaOH (Sodium Hydroxide), Glucose, Distilled water, Fruit Juice, BSA, Water Bath, Test Tube, Pipette.

Preparation of Markers

Fehling 1 solution:

The compound dissolved in 7% $CuSO_4$ (34.6 crystalline copper sulfate) by gently heating in 300 ml of distilled water. Then, 200 ml of distilled water are added to complete the solution to a volume of 500 ml.

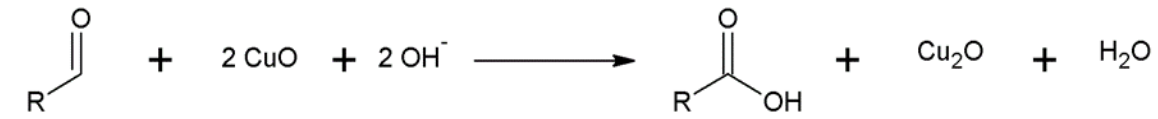
Fehling 2 solution:

Mixture of 35 g Na-K tartrate and 10 g NaOH is dissolved with some distilled water. After that, the mixture volume is complete to 100 ml with distilled water.

7.3.2. Experimental Method:

1. Equal volumes (1 ml) of Fehling I and Fehling II solution are transferred in a test tube.
2. The mixture is shaken. A dark blue colour solution appears. This solution is called “Fehling reagent”.
3. The Fehling reagent is placed in a water bath at 60 ^{o}C until boiling starts. If the colour of the solution does not change, it means that the reagent is clean and does not contain reducing agents.
4. After this control, 1 mL unknown concentrations of glucose are added to Fehling reagent while boiling is continued. If there is excess sugar in the solutions, turbidity with yellow colour or precipitate is seen.

5. If the amount of sugar in the tested solution is small, an amount of solutions equal to the volume of the Fehling reagent is added. If a yellow or red colour precipitate occurs, there is sugar. If no precipitate is formed, it is understood that there is no sugar in the examined solutions.



Aldehyde Fehling's solution Carboxylic acid Red precipitate