

## **EXPERIMENT 1- QUALITATIVE ANALYSIS OF CARBOHYDRATES**

A **carbohydrate** is an organic compound with the general formula  $C_m(H_2O)_n$ , that is, consists only of carbon, hydrogen and oxygen, with the last two in the 2:1 atom ratio. Carbohydrates make up the bulk of organic substances on earth and perform numerous roles in living things.

The carbohydrates (saccharides) are divided into four chemical groups: **monosaccharides, disaccharides, oligosaccharides and polysaccharides**. Polysaccharides serve for the storage of energy (e.g., starch in plants and glycogen in animals) and as structural components (e.g., cellulose in plants and chitin in arthropods). Structural polysaccharides are frequently found in combination with proteins (glycoproteins or mucoproteins) or lipids (lipopolysaccharides). The 5-carbon monosaccharide ribose is an important component of coenzymes (e.g., ATP, FAD and NAD) and the backbone of the genetic molecule known as RNA. The related deoxyribose is a component of DNA. Saccharides and their derivatives include many other important biomolecules that play key roles in the immune system, fertilization, preventing pathogenesis, blood clotting and development [1].

This experiment aims to introduce you with the identification of unknown carbohydrates. To gain maximum benefit, **observations should be related, as far as possible, to the structure of the substances examined.**

Some important points:

1. Most of the tests and reactions described are not quantitative and volumes are approximate, despite these facts some tests do not work if quantities greatly in excess of those stated are used.
2. **DO NOT** place your pipettes in reagent bottles as this leads to contamination.
3. In most tests, it is important to apply a control test using water instead of the solution under examination. If you are in doubt about the result of a test, perform the reaction with a suitable known compound.
4. In this experiment, sugar samples are given in their solid state. To perform each procedure, you should prepare your own sugar solution by taking very small amounts of solid sugars.
5. When you need to boil your sample in a test tube, prepare a hot water in a large beaker and put your test tube inside the beaker. **DO NOT** forget to put boiling chips in the beaker.

## TESTS ON CARBOHYDRATES:

### 1) Molisch's Test:

Molisch's Test is a sensitive chemical test for all carbohydrates, and some compounds containing carbohydrates in a combined form, based on the dehydration of the carbohydrate by sulfuric acid to produce an aldehyde (either furfural or a derivative), which then condenses with the phenolic structure resulting in a red or purple-colored compound.

#### Procedure:

- Apply this test two different carbohydrate solutions of your own choice, preferably to one monosaccharide and one polysaccharide.
- Place 2 mL of a known carbohydrate solution in a test tube, add 1 drop of Molisch's reagent (10%  $\alpha$ -naphthol in ethanol).
- Pour 1-2 mL of conc.  $H_2SO_4$  down the side of the test tube, so that it forms a layer at the bottom of the tube.
- Observe the color at the interface between two layers and compare your result with a control test.
- **A brown color due to charring must be ignored and the test should be repeated with a more dilute sugar solution.**

#### Questions:

- ✓ Write the reaction step(s) involved in this test?
- ✓ Give an example of a protein structure that would give positive test with Molisch's Reagent.

### 2) Carbohydrates as Reducing Sugars:

A **reducing sugar** is any sugar that, in a solution, has an aldehyde or a ketone group. The enolization of sugars under alkaline conditions is an important consideration in reduction tests. The ability of a sugar to reduce alkaline test reagents depends on the availability of an aldehyde or keto group for reduction reactions. A number of sugars especially disaccharides or polysaccharides have **glycosidic linkages** which involve bonding a carbohydrate (sugar) molecule to another one, and hence there is no reducing group on the sugar; like in the case of sucrose, glycogen, starch and dextrin. In the case of reducing sugars, the presence of alkali causes extensive enolization especially at high pH and temperature. This leads to a higher susceptibility to oxidation reactions than at neutral or acidic pH. These sugars, therefore, become potential agents capable of reducing  $Cu^{+2}$  to  $Cu^+$ ,  $Ag^+$  to  $Ag$  and so fort. Most commonly used tests for detection of reducing sugars are **Fehling's Test, Benedict's Test and Barfoed's Test**.

### a) Fehling's Test:

Fehling's Solution (deep blue colored) is used to determine the presence of reducing sugars and aldehydes. Perform this test with **fructose, glucose, maltose and sucrose.**

#### Procedure:

- To 1 mL of Fehling's solution A (aqueous solution of  $\text{CuSO}_4$ ) add 1 mL of Fehling solution B (solution of potassium tartrate).
- Add 2 mL of the sugar solution, mix well and boil.
- Try to see **the red precipitate of cuprous oxide** that forms at the end of the reaction.

#### Questions:

- ✓ Write the reaction(s) involved in Fehling's Test.
- ✓ What is the function of tartrate?
- ✓ Some disaccharides such as maltose are reducing agents, whereas others, such as sucrose are not. Explain briefly by including the structures of the sugars.

### b) Barfoed's Test:

Barfoed's reagent, cupric acetate in acetic acid, is slightly acidic and is balanced so that it can only be reduced by monosaccharides but not less powerful reducing sugars. Disaccharides may also react with this reagent, but the reaction is much slower when compared to monosaccharides. Perform this test with **glucose, maltose and sucrose.**

#### Procedure:

- To 1-2 mL of Barfoed's reagent, add an equal volume of sugar solution.
- Boil for 5 min. in a water bath and allow to stand.
- You will observe **a brick-red cuprous oxide precipitate** if reduction has taken place.

#### Questions:

- ✓ Write the reaction(s) involved in the Barfoed's Test.
- ✓ When you test starch with Barfoed's reagent, what would be the answer, positive or negative? Explain your answer by giving reasons and structures.

### c) Seliwanoff's Test:

Seliwanoff's Test distinguishes between aldose and ketose sugars. Ketoses are distinguished from aldoses via their ketone/aldehyde functionality. If the sugar contains a ketone group, it is a ketose and if it contains an aldehyde group, it is an aldose. This test is based on the fact that, when heated, ketoses are more rapidly dehydrated than aldoses. Perform this test with **glucose, fructose, maltose and sucrose.**

### **Procedure:**

- Heat 1 mL of sugar solution with 3 mL Seliwanoff's reagent (0.5 g resorcinol per liter 10% HCl) in boiling water.
- In less than 30 seconds, a red color must appear for ketoses.
- Upon prolonged heating, glucose will also give an appreciable color.

### **Questions:**

- ✓ Write the reaction(s) involved in Seliwanoff's Test.
- ✓ What is the function of resorcinol?
- ✓ What is the aim of using a strong acid?
- ✓ What is the result of testing sucrose with Seliwanoff's reagent? Explain your answers by giving reasons and structures.

### **d) Bial's Test:**

Bial's Test is to determine the presence of pentoses (5C sugars). The components of this reagent are resorcinol, HCl, and ferric chloride. In this test, the pentose is dehydrated to form furfural and the solution turns bluish and a precipitate may form. Perform this test with **ribose and glucose.**

### **Procedure:**

- To 5 mL of Bial's reagent, add 2-3 drops of sugar solution and boil.
- Upon boiling, note the green-blue color formed.

### **Questions:**

- ✓ Write the reaction(s) involved in Bial's Test.
- ✓ Is it possible to distinguish DNA and RNA structures by using Bial's Test?
- ✓ The boiling step is common for each test for the reducing sugars. Why boiling is necessary for the reduction to take place?

### **3) Action of Alkali on Sugars:**

#### **Procedure:**

- Heat 1 mL glucose solution with 1 mL 40% NaOH for 1 min.
- Cool and apply test for reducing sugars (e.g.; Fehling's Test).
- Apply a control test with glucose solution to observe the difference.

### Questions:

- ✓ Explain the reaction of glucose in the alkali medium by giving your reasons and related structures.
- ✓ Explain the difference between glucose solution and alkali treated glucose solution when a test for reducing sugars is applied.

#### 4) The Inversion of Sucrose:

Sucrose is a disaccharide, which means that it is a molecule that is derived from two simple sugars (monosaccharides). In the case of sucrose, these simple sugars are glucose and fructose. Inverted sugar is a mixture of glucose and fructose. It is obtained by splitting sucrose into these two components. The splitting of sucrose is a hydrolysis reaction which can be induced simply by heating an aqueous solution of sucrose. Acid also accelerates the conversion of sucrose to invert.

### Procedure:

- Add 5 mL of sucrose solution to two test tubes.
- Add 5 drops of conc. HCl to one test tube.
- Heat both tubes in boiling water bath for 10 min.
- Cool and neutralize with diluted NaOH (use litmus paper).
- Test both solutions for the presence of reducing sugar with Fehling's Test.

### Questions:

- ✓ Explain the result by giving the reasons and related reactions and structures.
- ✓ What would you expect from a similar reaction with starch?

#### 5) Iodine Test:

Iodine test is an indicator for the presence of starch. Iodine solution (iodine dissolved in an aqueous solution of potassium iodide) reacts with starch producing a **blue-black color**. Apply this test to all the polysaccharides provided.

### Procedure:

- To 2-3 mL of polysaccharide solution, add 1-2 drops of iodine solution.
- Observe the different colors obtained for each of the polysaccharide solutions.

### Questions:

- ✓ Explain the reaction between iodine solution and polysaccharides by giving the structures of related compounds.
- ✓ Each polysaccharide tested gives different color results with the iodine test. Explain the reason briefly.

6) **Unknown Part:**

- Take an unknown solid from your assistants and please **DO NOT forget to write your unknown number in your lab reports.**
- Carry out the carbohydrate tests in a reasonable sequence to determine your unknown (**Please DO NOT trust on your solubility observations and physical appearances of your unknown**).