DOÑA REMEDIOS TRINIDAD ROMUALDEZ MEDICAL FOUNDATION, INC.
*Calanipawan Road, Tacloban City, Philippines 6500*

# COLLEGE OF MEDICAL TECHNOLOGY

## LABORATORY EXERCISE 6: QUALITATIVE ANALYSIS OF CARBOHYDRATES

**Yr./Sec:____________ Group No.: ________ Date: _________________** **DISCUSSION:**

A **carbohydrate** is a polyhydroxy aldehyde, a polyhydroxy ketone or a compound that yields these compounds hydrolysis. They are the most abundant bioorganic molecules in Earth, comprising about 60-90% of the total weight of plants in the form of cellulose and starch. Cellulose serves the structural support and starch provides source of energy for plants.

Carbohydrates are classified according to its molecular size; these are monosaccharides, disaccharides, oligosaccharides and polysaccharides. **Monosaccharides** are the most basic unit of carbohydrates and they cannot be broken down into simpler forms upon hydrolysis. It exists as single polyhydroxy aldehyde or a polyhydroxy ketone with 3-7 carbon atoms. Most common form of monosaccharide exists at 6-carbon atom. Physical characteristics of a monosaccharide are; it exists as white, crystalline solids that are water-soluble.

Only a few classes of carbohydrates are of special biological importance. This includes the glucose, galactose, fructose and ribose. **Glucose** is the most important monosaccharide; it is a normal constituent of blood and tissue fluids and is the primary substrate for ATP production. **Galactose** also known as brain sugar is found as cerebroside in the brain and as ganglioside in the nerve tissues. It is also the sugar of galactolipids. Pentoses like ribose and deoxyribose are constituent of nucleic acids and also in some enzymes.

**Oligosaccharide** is composed of 2-10 monosaccharide units that are bonded to each other. Disaccharides are a combination of 2 monosaccharide unit that are covalently bonded to each other via glycosidic linkage formation following a removal of water. Like monosaccharide; they exist as white crystalline solids that are water-soluble. **Polysaccharide** is a polymeric form of carbohydrates that is composed of many covalently bonded monosaccharides. They exist in linear form or in branch type with homogenous or heterogeneous monomers. They are known to perform different types of biological function but most of the time they are broken down for energy production.

The two important components of carbohydrate — the **carbonyl group (aldehyde and ketone** and **alcohol** are responsible for most of the interesting reactions they exhibit.

## OBJECTIVES:

**At the end of the exercise, the students must be able to:**

**1.** Identify the various chemical reactions of carbohydrates according to their classification. **2.** Characterize and differentiate the different carbohydrates present in the unknown sample. **3.** Correlate the different chemical reactions of carbohydrates to its biochemical roles in the living organisms.

## MATERIALS/APPARATUS:

APPARATUS

**1** Test tubes

2. Graduated cylinder

3. 50 mL beaker

4. 250 mL beaker 5. Bunsen burner 6. Micoscope

REAGENTS

1. Molisch Reagent 8. Conc. $H_2SO_4$ 15. 1% conc. of 2. Fehling's Rgt. 9. 10% NaOH Glucose, Fructose, 3. Benedict's Rgt. 10. $Na_2CO_3$ Galactose, Sucrose, 4. Barfoeds Rgt. 11. Conc. $HNO_3$ Lactose, Maltose, 5. Seliwanoff's Rgt. 12. Phenylhydrazine Xylose and Starch. 6. Bial's Rgt 13. $Ba(OH)_2$
7. Sat. Picric Acid 14. Lead acetate

**PROCEDURE:**

## PART I: PREPARATION OF THE UNKNOWN

You will be given an unknown carbohydrate to characterize using the tests describe in the succeeding experiment. Make a schematic diagram of the sequence of tests you have performed to identify your unknown sugar. (Draw schematic diagram in a separate sheet of paper). At the end of the experiment, you should be able to give the identification of your unknown carbohydrate.

Dissolve about 0.5g in 150 mL of water. Use this solution for the tests to characterize the unknown carbohydrate.

❖ **RESULTS:**

**1. Physical Properties of the unknown carbohydrate:**
**ANSWER: CLEAR SOLUTION**

**2. Solubility of the unknown CHO in water:**

**ANSWER: SOLUBLE IN WATER**

## PART I: COMMON REACTIONS OF CARBOHYDRATES

**A. MOLISCH TEST (α-naphthol reaction)**

**Principle:** Molisch Test is a general test for carbohydrates and monosaccharaides will give positive results rapidly. This is based on the dehydration of carbohydrates by concentrated $H_2SO_4$, in the presence of heat to form furfural derivatives. Hexoses for example form hydroxymethyl furfuraldehyde. It condenses with α-naphthol to produce a purple-colored complex.

**Procedure:**

Label 8 micro-test tube with number 1-8. Place 1 mL of each of the following solutions into the test tubes. Add distilled water to the eight-test tube to serve as control.

**(1): 1% Xylose solution (5): 1% Lactose Solution**
**(2): 1% Glucose solution (6): 1% Starch solution**
**(3): 1% Fructose solution (7): Unknown Solution**
**(4): 1% Sucrose solution (8): Distilled water**

To each test tube, add 3 drops of Molisch reagent. Shake each test tube to ensure mixing. With the test tube held at 45$^o$ angle, carefully and slowly run 10 drops of conc. $H_2SO_4$ down the wall of the test tube so that two layers form. Note the color at the point where the 2 layers meet. Record your observations in table 1.

| TABLE 1: RESULTS FOR MOLISCH TEST | |
|---|---|
| **CARBOHYRATE** | **OBSERVATIONS** |
| 1% Xylose solution | + 3 DROPS |
| 1% Glucose solution | + 9 DROPS |
| 1% Fructose solution | + 4 DROPS |
| 1% Sucrose solution | + 3 DROPS |
| 1% Lactose Solution | + 5 DROPS |

| | |
|---|---|
| 1% Starch solution | + 5 DROPS |
| Unknown Solution | + 5 DROPS |
| Distilled water | - |



**1. Which of the carbohydrate(s) require an extended time to give positive result? Explain why.**

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**2. Why do all give a positive result Molisch Test?**

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**B. MOORE'S TEST**

**Principle:** Carbohydrates containing free aldehyde group are being liberated with the influence of concentrated alkali in the presence of heat. This will subsequently polymerize forming a resinous substance — a caramel.

**Procedure:**

In separate test tubes, place 12 drops each of 1% solutions of glucose, fructose and galactose. In each test tube add 12 drops of 10% NaOH solution. Put the tubes in boiling water bath for 2-3 mins. Note the color and the odor produced.

Repeat the test using the same sugar solutions but use 1% $Ba(OH)_2$ solution as the alkali. Note the results and compare them with results obtained with NaOH. Record your observations in Table 2.

**TABLE 2. RESULTS FOR MOORE'S TEST**

| SUGARS | OBSERVATION | | | |
|---|---|---|---|---|
| | 10% NaOH | | 10% KOH | |
| | ODOR | COLOR | ODOR | COLOR |
| 1% Glucose | | | | |
| 1% Fructose | | | | |
| 1% Galactose | | | | |
| Unknown Solution | | | | |

**3. What is the basis for the results obtained?**

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## C. CARBOHYDRATE REDUCTION TEST

Sugars containing free aldehyde or ketone group readily reduce alkaline solution of oxide metals like copper, bismuth and silver. All carbohydrates that has free aldehyde group reacts with $Cu^{2+}$ complex ion to form a red-orange precipitate of copper(I) oxide ($Cu_2O$).

There are four tests that are commonly performed under this reduction test. **Fehling's test** is the reaction of carbohydrates with alkaline copper-tartrate complex producing brick-red precipitate. **Benedicts test** uses alkaline copper-citrate complex as oxidizing agent producing a red-orange or occasionally yellow precipitate**. Barfoed's Test,** this is a special type of reducing test because it



distinguishes reducing monosaccharide and reducing disaccharide in an acidic medium using copper acetate.

In addition to that, **Picric Acid test** is also used to detect the presence of reducing sugars. They react with Picric Acid (toxic yellow crystalline solid) also chemically known as 2,4,6-trinitrophenol (TNP) to form a mahogany red colored — **picramic acid** in the presence of alkaline environment.

### a. FEHLING'S TEST & BENEDICT'S TEST

**Procedure:** Prepare 24 test tubes (8 for each set/procedure). Label the test tube with number 1-8 with corresponding code (***F for Fehlings; Be for Benedicts & Ba for Barfoeds)***. Place 1 mL of each of the following solutions into eight test tubes.

**(1): 1% Xylose solution (5): 1% Lactose Solution**
**(2): 1% Glucose solution (6): 1% Maltose Solution**
**(3): 1% Fructose solution (7): 1% Starch solution**
**(4): 1% Sucrose solution (8): Unknown Solution**

**FEHLINGS TEST:** To each test tube, add 2 mL Fehling's reagents (a mixture of 1mL Fehling's A and 1 mL Fehling's B). Shake the solution and place the test tube in a boiling water bath at the same time. Remove the test tubes after 5 minutes and allow them to cool. After 10 minutes observe any changes that occurred in each test tube. Record your observations on table 3.

**BENEDICT'S TEST:** Add 2 mL of Benedict's reagent to each test tube. Shake the solution and place the test tube in a boiling water bath at the same time. Remove the test tubes after 5 minutes and allow them to cool. After 10 minutes observe any changes that occurred in each test tube. Record your observations on table 3.

**BARFOED'S TEST:** Add 2 mL of Barfoed's reagent to each test tube. Shake the solution and place the test tube in a boiling water bath at the same time. Remove the test tubes after 5 minutes and allow them to cool. After 10 mins, observe any changes that occurred in each test tube. Record your observations on table 3.

| TABLE 3: RESULTS FOR FEHLING'S, BENEDICT'S TEST & BARFOEDS TEST | | | |
|---|---|---|---|
| | OBSERVATIONS | | |
| | | | |

| CARBOHYRATE | FEHLING'S TEST | BENEDICT'S TEST | BARFOED'S TEST |
|---|---|---|---|
| 1% Xylose solution | | | |
| 1% Glucose solution | | | |
| 1% Fructose solution | | | |
| 1% Sucrose solution | | | |
| 1% Lactose Solution | | | |
| 1% Maltose Solution | | | |
| 1% Starch solution | | | |
| Unknown Solution | | | |
| Distilled water | | | |



**4. Arrange the sugars according to the degree of reducing power.**

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**5. What can you conclude from those results with regards to the reaction of monosaccharides and disaccharides to Barfoed's Test?**

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## PART II: SPECIFIC REACTIONS OF CARBOHYDRATES

### A. SELIWANOFF'S TEST

**Principle:** This test will distinguish between ketohexoses and aldohexoses. Ketoses will give cherry red color within a few minutes (about 2 mins.), while aldoses require larger time. Concentrated HCl will dehydrate the ketoses more rapidly than aldoses forming 5-hydroxymethylfurfural derivatives. These derivatives will react with resorcinol to produce red product.

**Procedure:** Prepare 7 test tubes and label them accordingly. Place in each test tube 1 mL of the following sugar solution:

**(1): 1% Xylose Solution (4): 1% Sucrose solution**
**(2): 1% Glucose solution (5): 1% Lactose Solution**
**(3): 1% Fructose solution (6): 1% Starch solution**
**(7): Unknown Solution**

To each test tube add 2 mL of the Seliwanoff reagent and shake. Place all tubes into boiling water bath at the same time. Observe the changes in each test tube during the first 15 minutes. Record the color and time of its formation in each solution tested. Record your observations in Table 4.

| TABLE 4: RESULTS FOR SELIWANOFF TEST | | |
|---|---|---|
| **CARBOHYRATE** | **OBSERVATIONS** | |
| | **REACTION** | **TIME** |
| **1% Xylose solution** | | |
| **1% Glucose solution** | | |
| **1% Fructose solution** | | |
| **1% Sucrose solution** | | |
| **1% Lactose Solution** | | |
| **1% Starch solution** | | |
| **Unknown Solution** | | |

**6. Why does fructose react more rapidly than glucose?**

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**7. What other substance/s can be used in place of resorcinol?**

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**B. RUBNER'S TEST**

**Principle:** This test differentiates glucose and lactose in the urine. Lactose will give a red color if treated with lead acetate and heated in the presence of ammonia.

**Procedure:** Prepare 3 test tubes and label them accordingly. Place in each test tube 3 ml of the following sugar solution:

**(1): 1% Glucose solution (2): 1% Lactose Solution (3): Unknown Solution**

To each test tube add 0.3-0.5 grams of lead acetate, shake, and boil for 5 minutes. Cool the solution in running water. Once cooled, add 2 mL of conc. $NH_4OH$, and the boil for another 5 minutes. Set aside for 5-10 minutes until color change is observed. Record the results in Table 5.

| TABLE 5: RESULTS FOR RUBNERS TEST |
|---|

| CARBOHYRATE | OBSERVATIONS |
|---|---|
| 1% Glucose solution | |
| 1% Lactose Solution | |
| Unknown Solution | |

**C. BIAL-ORCIN TEST**

**Principle:** This test will differentiate pentoses and hexoses. Under strongly acidic conditions of the Bial's test, both pentoses and hexoses will dehydrate and react with orcinol in the presence of $FeCl_3$ to produce specific colors. A blue or green color is positive for pentoses.

**Procedure:** Prepare 6 test tubes and label them accordingly. Place in each test tube 1 mL of the following sugar solution:

**(1): 1% Xylose solution (4): 1% Fructose Solution**
**(2): 1% Arabinose solution (5): 1% Sucrose solution**
**(3) 1% Glucose solution (6): Unknown Solution**

To each test tube add 2 mL of Bial's reagent, shake each test tube. Place all test tubes at the same time in boiling water bath. Observe carefully the changes that will occur in each test tube. Record your observations in table 6.

| TABLE 6: RESULTS FOR BIAL-ORCIN TEST | | |
|---|---|---|
| CARBOHYRATE | OBSERVATIONS | |
| | COLOR | TIME |
| **1% Xylose solution** | | |
| **1% Arabinose solution** | | |
| **1% Glucose solution** | | |
| **1% Fructose solution** | | |
| **1% Sucrose solution** | | |
| **Unknown Solution** | | |

**D. IODINE TEST**

**Principle:** This test will detect the presence of polysaccharides. Polysaccharides will combine with iodine to form a blue, red or purple color as a sign that the test is positive. Iodine is adsorbed onto the surface of the polysaccharide, forming a colored complex.



**Procedure:** Prepare 6 test tubes and label them accordingly. Place in each test tube 1 mL of the following sugar solution:

(1): 1% Glucose solution (4): 1% Starch Solution
(2): 1% Maltose solution (5): 1% Glycogen Solution

(3): 1% Sucrose solution (6): Unknown Solution

To each test tube add 1-2 drops of Iodine solution. Note the color formed. Record your observations in Table 7.

| TABLE 7: RESULTS FOR IODINE TEST | |
|---|---|
| **CARBOHYRATE** | **OBSERVATIONS** |
| **1% Glucose solution** | |
| **1% Maltose solution** | |
| **1% Sucrose Solution** | |
| **1% Starch solution** | |
| **1% Glycogen Solution** | |
| **Unknown Solution** | |

**8. What is responsible for the intense blue color formed in the Iodine test?**

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**E. <u>REACTIONS WITH PHENYLHYDRAZINE</u>**

**Principle:** When reducing sugars are treated with phenylhydrazine crystals, formation of phenylhydrazone is observed. On heating the hydrazine will be reduced to osazone thus forming crystals of different forms according to what carbohydrate is reacted.

**Procedure:** Prepare 6 test tubes and label them accordingly. Place 1ml in each test tube: **(1): 1% Glucose solution (4): 1% Sucrose Solution**

**(2): 1% Fructose solution (5): 1% Maltose Solution**

**(3): 1% Galactose solution (6): Unknown Solution**

Add a pinch of phenylhydrazine to each test tube. Mix thoroughly and place a cotton plug on the mouth of the tube. Place them in boiling water bath for 45 mins to 1 hr. Allow to cool slowly at room temperature. Examine the crystals under LPO and attach your photos or draw an illustration at the back part of this page.

**9. Which sugar for characteristic of osazone crystals? How do crystals differ?**

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## STUDY GUIDE EXERCISES:

1. **What are the differences between Fehling's and Benedict's test? State them in terms of reagent used, stability and other factors.**

**2. What test for carbohydrate is clinically relevant in quantitative detection of the presence of CHO in the urine? Explain the principle of the test.**

**3. What factors may cause false-negative color reactions?**

**4. Cite the physiological relevance of knowing the specific classes of carbohydrates?**

**5. What are stereoisomers? Do all isomeric sugars have the same physical and chemical properties?**

**6. In a separate sheet of paper, attach a photo or illustration of the positive results of all qualitative tests of CHO.**