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Test for carbohydrates lab report answers

LAB 1 Test organic compounds INITIATION Most of the chemical compounds present in living organisms contain skeletons of molecular carbon atoms (C-C-C-C). These compounds are known as organic compounds because most of these are either present in, or produced by living things. Organic compounds are the main components of cells and tissues. They provide energy for life processes, participate in and regulate metabolic reactions and transmit information. Organic macromolecules in living organisms can be classified as either carbohydrates, proteins, lipids, or nucleic acids, among others. These macromolecules are always made of smaller subunits. The subunits of macromolecules are held together with covalent bindings and have different structures and properties. For example, lipids made of fatty acids have many C-H bonds and relatively little oxygen, while proteins made from amino acids have amino groups (-NH2) and carboxyl (-COOH) groups. These characteristic groups give different chemical properties to macromolecules - for example, monosaccharides such as glucose are polar and soluble in water, while lipids are non-polar and insoluble in water. CARBOHYDRATE CHEMISTRY Carbohydrates are compounds that contain carbon, hydrogen and oxygen. Carbohydrates include a variety of compounds, such as sugar, starch, and cellulose. While sugar and starch serve as energy sources for cells; cellulose are structural components of the walls that surround plant cells. The term carbohydrate literally means hydrated (H20) carbon carbohydrates may contain a sugar molecule (monosaccharides), two sugar molecules (disaccharides), or many sugar units (polysaccharides). In this laboratory we will be concerned about the nature and activities of carbohydrates and their structure. Note: structure dictates how the carbohydrate will react under certain conditions. Since carbohydrates are easily identified by color change in specific reactions, we will examine some of these methods of identification as we perform specific tests for specific carbohydrates. Solutions of the following mono, di- and polysaccharides are available: (a) glucose, (b) fructose, (c) galactose, (d) xylose, e) lactose, (f) maltose, g) sucrose and (h) starch. These solutions are available at both 1% and 6% concentrations (percentage solutions are by weight/volume, thus 1% 1 g in 100 ml solvent). A ninth unknown carbohydrate will also be run through tests with the known sugars. Your challenge is to identify the unknown chemical properties and suggest a possible structure as best you can with its test results. This unknown may or may not be one of the carbohydrates listed above. SUGAR TEST (UNKNOWN SUGAR ANALYSIS) SUGAR REDUCTION TEST (Multiple smoke caps required) Reduction of sugar are those that have a free or free aldehyde or ketone. In a solution of pH 8 or higher the sugar is able to reduce certain weak oxidizing substances such as cupric hydroxide along with a consequent oxidation of the carbonyl group of sugar. Both Benedict's and Barfoed's tests identify reducing sugar. The following reaction is an example: (sugar reduction) glucose + 2Cu (OH)2 --------- > Cu2O + 2H2O + glucose (oxidized cupricions: blue) (reduced cuprous ions: red) Benedictís Test. This test is a test to determine whether the carbohydrate contains a free aldehyde or ketone group (as indicated above). In Benedict's reaction, copper is reduced and the product forms a red precipitate. (Benedict's reagent contains sodium bicarbonate, sodium citrate and copper sulfate.) PROCEDURE: 3 ml of Benedict's testagent shall be used in each of the eight labelled test tubes and 300 µl drops shall be added to 1 % of each known carbohydrate to be tested. Shake each test tube to ensure thorough mixing. 2. Place the pipes in a boiling water bath for 3 minutes. At the end of this time, allow the pipes to cool and record your results on the attached chart. Barfoed's test. This test can distinguish monosaccharides from di- and polysaccharides, because with the conditions of lower pH and shorter incubation time, only monosaccharides can react quickly enough to reduce copper ions. The reagent is similar to Benedict's, except that the pH is lower (about pH 4.5) and the heating time is reduced to 2 minutes. Do not heat for more than 2 minutes. Longer heating can cause hydrolysis of the glycosides connection and thus break disaccharides to monosaccharides. PROCEDURE: 1. Add 3 ml of Barfoed's solution in each of the eight labelled test tubes and add 500 µl drops of the 1 % sugar solution to be tested. 2. Put the pipes in a boiling water bath and observe on 1 minute, but continue to heat until 2 minutes. Record your results after 2 minutes. A rusty or brownish-red color will indicate monosaccharides, no color change or slight change will indicate di and polysaccharides. TESTS BASED ON CARBOHYDRATE DEHYDRATION Mineral acids (HCl and H2SO4) react with carbohydrates to form furfural, hydroxymethyfurfural or levulic acid by dehydration. Pentoses produce furfural acid, while hexoses produce hydroxymethyfurfural. Hydroxymethyfurfural reacts further with the acid to form levulic acid and ant acid. In these tests, polysaccharides and disaccharides are hydrolysed, breaking glycoside links to give monosaccharides. This allows them to react as monosaccharides. Several tests are based on these reactions. We'll investigate three of them. Selivanoff's test. This test is used to distinguish between ketoses and aldoses. The reagent is a solution of resorcinol in concentrated HC1. The acid, when heated together with a sugar, will produce hydroxymethylfurfural, which further responds to the a red color. Ketoses react faster than aldoses and thus reaction time is a means of separation or detection. Ketoses react within 1 minute of warming, while aldoses will require several minutes. Disaccharides containing fructose must react between fructose alone and one of the aldoss. PROCEDURE: 1. 350 µl drops of 1% sugar solution shall be used in each test tube. Add 3 ml of Selivanoffís reagent to each tube. 2. Place the pipes in the hot water bath, time the reactions and begin your observations immediately. Record your results in Table 1 as before. Bials test. This test is used to detect the presence of furans (five-member rings). These sugars, or compounds containing them, react with Bial's reagent to provide a green or olive colored solution. Furan rings contain five carbon, but sugar with furan rings may contain more carbon outside the ring, and any sugar with a furan ring will respond in Bial's test. Pentose furanoses will react with Bial's reagent to form green solution, as hexose furanose will respond to form olive/brown solution. Bial's contains orcinol (5-methyl-1,3 dihydroxybenzene), the parent substance of litmus dyes in concentrated HCl. PROCEDURE: 1. 35 µl decrease in the 1 % sugar solution shall be placed in each test tube. Add 3 ml of Bials test reagent to each test tube. 2. Responsibility each pipe in the hot water bath for 5 minutes. At the end of this time take test tubes out of hot water bath and record your sightings. Mucic Acid Test. This test is one in which concentrated HNO3 is heated together with a aldose sugar to give a dicarboxylic acid. Nitric acid is able to oxidize terminal groups of aldoses, but leaves the secondary hydroxyl groups unchanged. Dicarboxyl acid formed from galactose is called mucic acid and is insoluble in cold aqueous solution. The acids formed by the other common sugars are soluble in H20. The formation of the insoluble precipitate is thus an indication of the presence of galactose. This test requires several hours to complete, and therefore the authorities will do it for you, and you can mark the results in the right place in Table 1. The procedure we will follow will be to place 1ml of 6% carbohydrate solution in each of the test tubes. We will then add 1 ml of concentrated HNO3 and heat in a boiling bath for 1 1/2 hours. We will then remove the pipes, let them stand overnight and read the subsequent results. JOD TEST. (FOR ROLLED-UP POLYSACCHARIDES) Iodine (iodine-potassium iodide, I2KI) dyeing distinguishes starch (a polysaccharid) from monosaccharides, disaccharides and other polysaccharides. The basis of this test is that starch is a coiled polymer of glucose. Iodine interacts with these coiled molecules and turns bluish black. Other non-coiled carbohydrates do not react with iodine. Therefore, a bluish black color is a positive and a yellow-ish brown color (i.e. no color change) is a negative test for starch. Glycogen, the common polysaccharid in animals, has a slight difference in structure and produces only an intermediate color reaction. Test each of the known sugars for the presence of starch. PROCEDURE: 1. 35 µl drops of 1% sugar solution shall be placed in each test tube. Add 35 µl drop of IKI reagent to each tube. 2. Keep the pipes at room temperature. Record your results in Table 1. SUMMARY OF CARBOHYDRATE TEST 1. Benedict's - test to reduce sugar (free or potentially free aldehyde or ketone groups). Aldehyde is oxidized; copper is reduced. Reduced copper precipitate and blue color changes to orange/red (be aware of minor changes in color) 2. Barfoed's - test to reduce sugar that is monosaccharides. Similar to Benedict's but lower pH and shorter time. Only monosaccharides will reduce copper and change color due to the formation of orange/red precipitate. 3. Selivanoff's - test for ketosis vs. aldose. CAUTION: ACID - The color will be red fastest if the sugar is a ketosis. Red < 1 min. (fastest) is a monosaccharid ketosis, Red ~1 min. (slightly slower) is a disaccharide ketosis, Red > 1 min. (longest) is aldose. 4. Bial's - test for furanose ring. CAUTION: ACID - Yellow color becomes greenish if sugar is a furanose (has a five member ring as furan). If sugar is a pentose-furanose: color becomes green / olive; a hexose (or higher)-furanose: color becomes a muddy brown, or if the sugar is a pyranose: no change in color. 5. Mucic Acid - DEMO test for galactose. CAUTION: Acid - aldose + acid forms dicarboxyl acid. Mucic acid is insoluble in H20 and forms white precipitate. 6. Iodine - test for presence of starch. If starch is present, the addition of IKI reversing the fabric will be tested to a blue-black color. IDENTIFICATION OF UNKNOWN CONNECTIONS PROCEDURE: 1. Get an unknown resolution from your laboratory instructor. Record the number in a table. 2. Prepare 6 clean test tubes. 3. Perform each of the carbohydrate diagnostic tests on the unknown. 4. Record your results in Table 1 and describe the chemical properties of your unknown. What do the results tell you about the chemical properties of your unknown? If any of your tests are inconclusive, repeat these tests so that you are sure of the data for your unknown. Table 1: CARBOHYDRATE TEST REPORT Benedict's Barfoed's Selivanoff's Bial's Mucic Acid Iodine Glucose Fructose Galactose Xylose Lactose Maltose Sucrose Starch Unknown