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## Reagents used in the biomolecules lab answers

At the end of this laboratory, the student should be able to: Identify the functional groups for each of the biomolecules that react in the following biochemical tests: Benedict Test, Iodine Test, Brown Bag Test, Sudan III/IV Test, and Biuret Test. Describe the reaction mechanism for: Benedict Test, Iodine Test, Sudan III/IV Test, and Biuret Test. Interpret the results when presented with data for each biochemistry test. Design experiments to identify biomolecules using biochemical testing. Introductory biological molecules contain specific chemical structures called functional groups, which can be distinguished by biochemical tests, such as Benedict's iodine, and Sudan III/IV. Benedict's test identifies the reduction of sugars (monosaccharides and some disaccharides), which have free ketone or functional groups of aldehyde). The groups reduce  $\text{CuSO}_4$ , a component of Benedict's, resulting in a color change in the Benedict's solution from a turquoise color to a rusty brown brick. Another class of carbohydrates called starches, a polysaccharid, can be detected by iodine testing. Having a high molecular weight, iodine joins the helical structure of starches producing a blue-black precipitate. The Biuret test identifies proteins oxidizing 4-6 peptide bonds of a protein using  $\text{CuSO}_4$  and  $\text{NaOH}$  resulting in a color change in the  $\text{CuSO}_4$  solution from purple to a darker purple color. Finally, the Sudan III/IV test is used to detect lipid hydrocarbon chains. Sudan is a red, non-polar dye that forms hydrophobic interactions with lipid hydrocarbon chains. Alternatively, the Brown Bag test can also be used to identify lipids due to the oily nature of hydrocarbon chains. Carbohydrates: Reducing sugars and starches By reducing sugars Some sugars such as glucose are called reducing sugars because they are able to transfer hydrogens (electrons) to other compounds, a process called reduction. When reducing sugars are mixed with the Benedict's reagent and heated, a reduction reaction causes the Benedict's reagent to change color. The color varies from green to dark red (brick) or rusty brown, depending on the amount and type of sugar. Increasing the amounts of reduction of green orange-brown with sugar In a typical Benedict's test (shown below), approximately 1 ml of sample is placed in a clean test tube along with 10 drops of Benedict reagent ( $\text{CuSO}_4$ ). Reactions are heated in a bath of boiling water for 5 minutes. Below: Benedict's test solutions and reagent are boiled in a water for five minutes. Click the image to see an magnification. Press the Back button to return. Below: Results of several solutions tested with the Benedict test Click on the image to see an extension. Press the Back button to return. Activity #1: Draw a large chart containing four equally divided columns and seven seven In the first row, label the first column Unsated Reaction; the second Color column of the Heated Reaction; the third column positive reaction (+) and negative reaction (-); and the fourth Structure column of the Functional Reaction Group. In the first column, label rows 1-7 with the appropriate samples below: Test tube #1: distilled water + Benedict's #2 test tube: glucose solution + Benedict's test tube #3: solution to remove benedict's #4 test tube: starch solution + Benedict's #5 test tube: onion solution + Benedict's test tube #6: Potato solution + Benedict's In the second column labeled Color of heated reactions, indicate the results of Benedict's test after the reaction warmed up. In the third column, indicate a (+) for benedict's positive reaction and a (-) for Benedict's negative reaction. In the fourth column, draw the reacting functional groups of samples that reacted with the Benedict reagent. The starch iodine solution (IKI) reacts with starch to produce a dark purple or black color. KI Reagent: Iodine is not very soluble in water, therefore, iodine reagent is made by dissolving iodine in water in the presence of potassium iodide. This makes a complex of linear triid ions that is soluble. The triiodide ion slides into the starch coil causing an intense blue-black color. If you had to set up an iodine test, you would do the following: Use a wax marker to mark two test tubes 1 cm from the bottom. Fill one of the tubes up to the 1 cm mark with water and fill the other up to the 1 cm mark with a 1% starch solution. Be sure to remove the starch before filling the tube. Add two drops of IKI solution to each tube and observe any precipitation or color change. Activity #2: Design a chart and record your predictions for the starch test for reaction tubes 3-5. Include in your chart the following headers: Reactants, Expected Color Results, Structure of Reaction Functional Groups. The results of positive and negative control have been included in reaction tubes 1 and 2, respectively, for your comfort. Bottom left: starch solution and IKI - Iodine turns dark in the presence of starch (dark: Positive control) Bottom right: distilled water and IKI (clear: negative control) Click on the image to see an magnification. Press the Back button to return. Test tube #1: starch + IKI Test tube #2: distilled water + IKI Test tube #3: glucose solution + IKI (track: Is glucose a starch or reduce sugar?) Test tube #4: rice solution + IKI (hint: Is rice a starched food or reduces sugar?) Test tube #5: solution (track: see diagram 2b below, then refer to the benedict test results using onion) #6 test tube: Potato solution (track: see diagram 2a below, then refer to the benedict test results using starch) Draw a potato cell in the space provided. Label the cell wall and starch granules. 2a) That are the dark granules inside the potato cells compounds? [Track - What caused iodine to darken?] Click on the images to see an magnification. Press the Back button to return. Potato cells stained with IKI X 100 Potato cells dyed with IKI X 200 2b) Does onion store food as starch? Left: Onion dyed with IKI X 100 - The nuclei of these cells are light brown in this photograph. The numerous starch granules seen in potatoes are absent. Click the image to see an magnification. Press the Back button to return. Lipids Certain types of paper, such as a piece of brown paper bag, can easily absorb lipids and can be used to check for lipids. Below: A drop of vegetable oil was placed in a brown bag (paper) on the left and a drop of water was placed on the paper on the right. The paper was photographed after about a minute. Watch the oil migration. What place do you think will disappear in time? Activity #3: Try this at home, draw 10 circles separated by 2 inches on a piece of brown paper, paper bag or 8 x 5 brown envelope. Gather the materials below. Then do a paper bag test on each item by placing a drop of the sample in the brown bag or use another small piece of bag to crush the solid samples in the circle to release their liquid contents. Allow the paper to dry for 15 minutes, then rotate the paper to the opposite side to review the results. Do you see a stain (oil) for the samples below. Create a chart and record your observations. Record your results on a chart. Include the Sample, Positive Reaction (+), and Negative Reaction (-) headers for the Brown Bag and Functional Reaction Group Structure test on your chart. Circle #1: Circle #2 oil: Circle #3 water: a small piece of Circle #4 chip potato: small piece of circle #5: a small touch of salad with Circle #6 sauce: Circle #7 vinegar: catsup or Circle #8 #7 mustard &t;7gt;.: Your choice Circle #9: Your choice Circle #10: Your choice The copper atoms of the Biuret solution ( $\text{CuSO}_4$  and  $\text{NaOH}$ ) will react with various peptide bonds in polypeptides, producing a color change from blue to a deep violet or blue color. Often, a light pink color can result in the presence of small chains of peptides. Color Indication Blue No Protein or Peptides Violet Protein In the next experiment, a biuret test has been performed on water, egg albumin, a protein found in chicken eggs and starch. The following procedure was used: Three test tubes were labeled at 2 cm as follows: one of the tubes to the 2 cm mark with the second to the brand of 2 cm with albumin solution (a protein), and the third to the brand of 2 cm with starch solution. 5 drops of 3% copper sulfate solution ( $\text{CuSO}_4$ ) were added to each tube. 10 drops of 10% sodium hydroxide solution ( $\text{NaOH}$ ) were added to each tube. Activity #4: Design a chart and record the color of each test tube. Include the Biochemical Test, Color Results, and Reaction Group headings on the chart. Below: Tube 1: Water (control) Tube 2: Albumin (protein) Tube 3: Starch Activity #5: Your grandmother is a 65-year-old diabetic who maintains healthy blood sugar levels by observing her diet. Lately, she's been craving imitation crab meat. She laughs at it all day and adds them to various dishes she prepares for herself. You suspect that as a diabetic, I shouldn't eat this product. Design an experiment to test your hypothesis. You have the following materials at your disposal although you can't use them all: Brown Paper Bag Solution Benedict Biuret KI Sudan III/IV Hot Water Bath A Small Homogenizer Diabetic Glucose Test Strips Blood Pressure Cuff Questions: 1. What is the importance of heating samples when performing the Benedict test? 2. Reagents are joined to the functional group of biological molecules by covalent or non-covalent binding or interactions. Explain this interaction between each reagent and its prospective functional group. 3. What results would you expect if  $\text{KOH}$  or  $\text{NaOH}$  were omitted from the Benedict test? Why? 4. What difference in results would you expect if you performed the Biuret test on a test tube in a protein solution and a glycine solution? 5. Explain why (molecular reason) polysaccharides do not give a positive reaction to reduce sugars? 6. Why is it important to include negative and positive controls when conducting a test? References and credits 20101/Bio%20101%20Laboratory/Chemical%20Composition%20of%20Cells/Chemical%20Composition%20of%20Cells.htm credits 20101/Bio%20101%20Laboratory/Chemical%20Composition%20of%20Cells/Chemical%20Composition%20of%20Cells.htm

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