

Identifying macromolecules using iodine's test, benedict's test, and biuret test

[Science](#), [Biology](#)



Introduction

The main objective of this experiment was to test for traces of starch, glycogen, reducing sugars, and protein in twelve substances- 1% glucose, 0.3% glucose1phosphate, 1% maltose, 5% honey, 1% sucrose, 1% lactose, 1% glycogen, 1% starch, 1% protein, beer, distilled water, and an unknown solution. The tests that were used to identify the macromolecules in the substances were, the iodine test to identify polysaccharides, Benedict's test to detect the presence of simple carbohydrates, and biuret test to find the presence of protein. To identify the presence of a macromolecule the predicted color change in the substances, which will differ in each test. The colour change helped to determine positive controls and negative controls indicating the presence or absence of a macromolecule.

Another purpose of the experiment was to take the results of the eleven known samples and compare them to the unknown solution's results to help identify the unknown solution. Iodine test was used to identify polysaccharides, specifically starch and glycogen. Starch is made up of two components, one of them is amylopectin which consists of approximately 70-80% of the starch granule. Amylopectin has a highly branched structure and changes to a violet-red or brown-red colouring when mixed with iodine, depending on its type. A substance mixed with iodine produces a blue colour only in the component called amylose which has glucose with bonds α -1, 4 and is unramified (Hollo & Szejtli, 1957). Another possible outcome of the iodine test is to have no starch or glycogen present and keep the solution yellow.

Benedict's test turns the substance blue, green, yellow, or orange-red when heated up. The colours depend on the level of carbohydrates in the substance (Sur, Shukla, & Agashe, 1972). The resultant that is blue to blue-green or yellow-green is negative (negative control/no reducing sugars), yellowish to bright yellow is a moderate positive (some reducing sugars), and bright orange is a very strong positive (positive control). Benedict's reagent is used as a simple test for reducing sugars. A reducing sugar is a carbohydrate consisting either a free aldehyde or free ketone functional group as part of its molecular structure. A single molecule can have more than one functional group as part of its structure. When a molecule with multiple functional groups is involved in a reaction all, some or none of the functional groups may be involved (University, 2006).

The solution used in the Biuret Test is a solution of copper sulfate (CuSO_4) and potassium hydroxide (KOH). The KOH is present to increase the pH of the solution to alkaline levels, the main part is the copper (II) ion from the CuSO_4 . When peptide bonds are present in this alkaline solution, the copper (II) ions will form a coordination complex with four nitrogen atoms connected in peptide bonds. Proteins also contain amide groups. When an amino group and a carboxyl group join to form a peptide bond, the amino group ($-\text{NH}_2$) becomes an amide group ($-\text{NH}$). Therefore, proteins also bond with copper ions at a basic pH. The copper ions will bond with the amide groups in the proteins to form a blue color that is measured using a spectrophotometer. The amount of blue color that is formed compared to the quantity of protein in your samples. (Biuret Protein, 2010).

Materials and Methods

All procedures were carried out as outlined in Identification of Some Macromolecules, BIOL130L lab manual, pages 15-20. (Department of Biology, 2018). No deviations were made to these protocols.

Results

Beaker 1

Beaker 1 consisted of 1% glucose solution. For the Iodine testing, the glucose was mixed with iodine solution and the resultant solution remained yellow. For the Benedict's testing, the glucose was mixed with the benedict solution and heated, and the resultant solution turned to orange. For the Biuret's testing, the glucose was mixed with 10% NaOH and then with 1% CuSO₄, the resultant solution remained clear.

Beaker 2

Beaker 2 consisted of 0.3% glucose1phosphate solution. For the Iodine testing, the glucose1phosphate was mixed with iodine solution and the resultant solution remained yellow. For the Benedict's testing, the glucose1phosphate was mixed with the benedict solution and heated, and the resultant solution remained blue. For the Biuret's testing, the glucose1phosphate was mixed with 10% NaOH and then with 1% CuSO₄, the resultant solution remained clear.

Beaker

3 Beaker 3 consisted of 1% maltose solution. For the Iodine testing, the maltose was mixed with iodine solution and the resultant solution remained yellow. For the Benedict's testing, the maltose was mixed with the benedict

solution and heated, and the resultant solution turned to orange. For the Biuret's testing, the maltose was mixed with 10% NaOH and then with 1% CuSO₄, the resultant solution remained clear.

Beaker 4

Beaker 4 consisted of 5% honey solution. For the Iodine testing, the honey was mixed with iodine solution and the resultant solution remained yellow. For the Benedict's testing, the honey was mixed with the benedict solution and heated, and the resultant solution turned to orange. For the Biuret's testing, the honey was mixed with 10% NaOH and then with 1% CuSO₄, the resultant solution turned yellow.

Beaker 5

Beaker 5 consisted of 1% sucrose solution. For the Iodine testing, the sucrose was mixed with iodine solution and the resultant solution remained yellow. For the Benedict's testing, the sucrose was mixed with the benedict solution and heated, and the resultant solution remained blue. For the Biuret's testing, the sucrose was mixed with 10% NaOH and then with 1% CuSO₄, the resultant solution remained clear.

Beaker 6

Beaker 6 consisted of 1% lactose solution. For the Iodine testing, the lactose was mixed with iodine solution and the resultant solution remained yellow. For the Benedict's testing, the lactose was mixed with the benedict solution and heated, and the resultant solution turned to orange. For the Biuret's testing, the lactose was mixed with 10% NaOH and then with 1% CuSO₄, the resultant solution remained clear.

Beaker 7

Beaker 7 consisted of 1% glycogen solution. For the Iodine testing, the glycogen was mixed with iodine solution and the resultant solution turned reddish-brown. For the Benedict's testing, the glycogen was mixed with the benedict solution and heated, and the resultant solution remained blue. For the Biuret's testing, the glycogen was mixed with 10% NaOH and then with 1% CuSO₄, the resultant solution remained clear.

Beaker 8

Beaker 8 consisted of 1% starch solution. For the Iodine testing, the starch was mixed with iodine solution and the resultant solution turned black. For the Benedict's testing, the starch was mixed with the benedict solution and heated, and the resultant solution remained blue. For the Biuret's testing, the starch was mixed with 10% NaOH and then with 1% CuSO₄, the resultant solution remained clear.

Beaker 9

Beaker 9 consisted of 1% protein solution. For the Iodine testing, the protein was mixed with iodine solution and the resultant solution remained yellow. For the Benedict's testing, the protein was mixed with the benedict solution and heated, and the resultant solution turned to dark blue. For the Biuret's testing, the protein was mixed with 10% NaOH and then with 1% CuSO₄, the resultant solution turned purple.

Beaker 10

Beaker 10 consisted of beer. For the Iodine testing, the beer was mixed with iodine solution and the resultant solution remained yellow. For the Benedict's

testing, the beer was mixed with the benedict solution and heated, and the resultant solution turned to greenish-yellow. For the Biuret's testing, the beer was mixed with 10% NaOH and then with 1% CuSO₄, the resultant solution remained yellow.

Beaker 11

Beaker 11 consisted of distilled water. For the Iodine testing, the water was mixed with iodine solution and the resultant solution remained yellow. For the Benedict's testing, the glucose was mixed with the benedict solution and heated, and the resultant solution remained blue. For the Biuret's testing, the glucose was mixed with 10% NaOH and then with 1% CuSO₄, the resultant solution remained clear.

Beaker 12

Beaker 12 consisted of an unknown solution #11. For the Iodine testing, the unknown was mixed with iodine solution and the resultant solution remained yellow. For the Benedict's testing, the glucose was mixed with the benedict solution and heated, and the resultant solution turned to a dirty orange. For the Biuret's testing, the glucose was mixed with 10% NaOH and then with 1% CuSO₄, the resultant solution remained clear.

Discussion

Result 1

The iodine test for the 1% glucose turned out to match the negative control due to lack of starch and glycogen. The Benedict's test changed the solution into orange once it was heated, confirming it to be a positive control. This showed that the glucose solution had reducing sugars. Sugars that contain

aldehyde groups that are oxidised to carboxylic acids are classified as reducing sugars (Hunt, n. d.). The Biuret's test agreed to the negative control due to the lack of protein in the substance.

Result 2

The iodine test for the 0.3% glucose1phosphate turned out to match the negative control due to lack of starch and glycogen. The Benedict's test also turned out to be a negative control once it was heated. This showed that the glucose1phosphate solution did not have reducing sugars. The Biuret's test agreed to the negative control due to the lack of protein in the substance.

Result 3

The iodine test for the 1% maltose turned out to match the negative control due to lack of starch and glycogen. The Benedict's test changed the solution into orange once it was heated, confirming it to be a positive control, and presence of simple sugars. The Biuret's test agreed to the negative control due to the lack of protein in the substance.

Result 4

The iodine test for the 5% honey turned out to match the negative control due to lack of starch and glycogen. The Benedict's test changed the solution into orange once it was heated, confirming it to be a positive control. The Biuret's test was not positive neither negative control. According to USDA, honey has 0.3g of protein for every 100g of honey (Food, n. d.).

Result 5

The iodine test for the 1% sucrose turned out to match the negative control due to lack of starch and glycogen. The Benedict's test also turned out to be

a negative control once it was heated. This showed that the sucrose solution did not have reducing sugars. The Biuret's test agreed to the negative control due to the lack of protein in the substance.

Result 6

The iodine test for the 1% lactose turned out to match the negative control due to lack of starch and glycogen. The Benedict's test changed the solution into orange once it was heated, confirming it to be a positive control. The Biuret's test agreed to the negative control due to the lack of protein in the substance.

Result 7

The iodine test for the 1% glycogen changed the solution to reddish brown which matched the positive control due to the presence of glycogen. The Benedict's test also turned out to be a negative control once it was heated. This showed that the glycogen solution did not have reducing sugars. The Biuret's test agreed to the negative control due to the lack of protein in the substance.

Result 8

The iodine test for the 1% starch changed the solution to reddish brown which matched the positive control due to the presence of starch. The Benedict's test also turned out to be a negative control once it was heated. This showed that the starch solution did not have reducing sugars. The Biuret's test agreed to the negative control due to the lack of protein in the substance.

Result 9

The iodine test for the 1% protein turned out to match the negative control due to lack of starch and glycogen. The Benedict's test also turned out to be a negative control once it was heated. This showed that the protein solution did not have reducing sugars. The Biuret's test agreed to the positive control due to the presence of protein in the substance.

Result 10

The iodine test for the beer turned out to match the negative control due to lack of starch and glycogen. The Benedict's test changed the solution into greenish-yellow once it was heated, confirming it to be a negative control. This showed that the glucose solution did not have reducing sugars. The Biuret's test agreed to the negative control due to the lack of protein in the substance.

Result 11

The iodine test for the distilled water turned out to match the negative control due to lack of starch and glycogen. The Benedict's test also turned out to be a negative control once it was heated. This showed that the water solution did not have reducing sugars. The Biuret's test agreed to the negative control due to the lack of protein in the substance.

Result 12

The iodine test for the unknown solution turned out to match the negative control due to lack of starch and glycogen. The Benedict's test changed the solution into a dirty orange once it was heated, confirming it to be a positive control. This showed that the glucose solution had reducing sugars. The

Biuret's test agreed to the negative control due to the lack of protein in the substance.

References:

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