Using Colourimetric Tests in Identification of Macromolecules From Solutions

Jamie Kang

Student ID: 20956456

Lab Partner: Tolani Basorun

TAs: Sarah Mae Sparkes & Caleb Ryan

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Introduction

Carbohydrates, lipids, proteins and nucleic acids are the major classes of biological macromolecules that make up biological systems. In this experiment, colourimetric tests were used to identify some types of macromolecules present in a variety of sample compound solutions. The macromolecules identified in this experiment include protein, carbohydrates such as starch and glycogen, as well as reducing sugars which are present in carbohydrate macromolecules.

Starch is used by plants as a storage for carbohydrates. It is composed of two different structures of glucose polymers; amylase and amylopectin. Amylose exhibits a helical coiled structure, primarily attributed to its α-1,4-glycosidic linkages connecting glucose monomers. In contrast, amylopectin, while also featuring α-1,4-glycosidic linkages, incorporates α-1,6-glycosidic linkages that introduce branching within the polymer. This branching pattern, occurring approximately every 25 to 30 glucose units, disrupts the helical coils seen in amylose. Interestingly, glycogen, a molecule employed by animals for energy storage, closely resembles amylopectin in structure of the glucose polyer that surrounds glycogenin. However, glycogen displays a higher density of α-1,6-glycosidic linkages, occurring at intervals of roughly 8 to 14 glucose units. This increased frequency of branching serves to further reduce the prevalence of helical coils within the glycogen structure.

The unique helical structures present in the previously mentioned polysaccharides are crucial in the mechanism of iodine tests, one of the colourimetric tests used in this experiment. A key material used in the iodine testing process is Lugol's iodine, which is a solution of 5% elemental iodine, 10% potassium iodide and distilled water. Elemental iodine I_2 is non-polar and thus has limited solubility in water. However, when potassium iodide salts are added to the solution, I_2 reacts with the dissociated iodine ions I^- to form polyiodide ions such as I_3^- which are soluble in water (Calabrese & Khan, 2000). The helical structures provide space within their cores for the polyiodide ions to fit, resulting in the formation of amylose-iodine complexes, which belong to a category known as charge transfer (CT) complexes (Goedecke, 2016).

CT complexes are formed when two or more molecules are stabilized by electrostatic attraction, with one molecule acting as an electron donor and the other as an electron acceptor (Aly & Hassan, 2014). During the electronic transition from donor to acceptor molecules, light is absorbed, producing intense color visible to the human eye corresponding to the complementary shade of the absorbed wavelength (Calatayud & Zamora, 2013; Goedecke, 2016). In the case of the amylose-iodine complex, the length of the helical coils determine the colour produced; longer coils undistrupted by braching absorb more wavelengths of light (Brust et al., 2020). Thus, amylose gives a deep blue-violet colour due to its lack of branching while amylopectin and glycogen gives a reddish brown colouration (Ball et al., 2011). While starch contains more amylopectin than amylose, the colour produced from amylose is far more intense; the colour produced from amylopectin is mostly overshadowed and results in the signature blue-black colouration.

In this experiment's iodine test, the 1% glycogen solution and the 1% starch solution was used as positive controls, with each solution expected to produce reddish brown and blue-black colouration respectively.

Another colourimetric test used in this experiment is Benedict's test, which uses alkaline copper (II)-citrate complex (Benedict's reagent) to identify reducing sugars in solution (Markina et al., 2016). Reducing sugars contain hemiacetal/hemiketal groups (aldehyde/ketone in straight-chain form) that act as a reducing agent. Redox reactions between the reducing agent of sugars and the cuprous ion from the Benedict's reagent results in the formation of Cu_2O particles, which vary in resulting size depending on the reaction conditions such as pH (Markina et al., 2016).

The Cu_2O particles absorb different wavelengths of light depending on its size; bigger Cu_2O particles produced in reaction lead to brick-red colouration; smaller particles lead to yellow colouration (Markina et al., 2016). Due to the blue colour of the Benedict's reagent, positive results of the Bendict's test will display a variety of hues; small concentrations of smaller particles will produce green colouration due to yellow and blue mixing, while large concentrations of bigger particles will be brick-red.

In the samples provided, 1& glucose, 1% maltose, and 1% lactose solutions will be used as the

positve control for Benedict's test as they are solutions of reducing sugars.

The final test utilized in this experiment is the Biuret test, which identify the prescence of peptide bonds, and in turn proteins. Cu^{2+} ions in solution are reduced by peptide bonds in alkaline conditions, and the reaction forms a coordination complex that absorbs light; producing a violet colour (Bhagavan, 2002). The intensity of the colouration is determined by the concentration of coordination complexes formed. 1% copper sulfate and sodium hydroxide solutions were used in this experiment; and the 1% protein sample was used as the postive control.

In all three colourimetric tests, distilled water was used as the negative control; pure H_2O does not react and produce a colour with any chemicals used in this experiment for identification.

Materials and Methods

Procedures outlined in the *BME 285L Labortatory Manual* (2023, p. 16–24) were followed to complete the experiment. Tests were conducted in the order of iodine, Benedict's and Biuret. After cleaning and drying the test tubes between Benedict's and Biuret tests, trace amounts of residual water were still present on the inner surface of the tubes. Due to safety reasons, test tube clamps were omitted from the procedure. Instead, the test tubes were handled with bare hands to provide better grip as the tubes did not reach high enough temperatures to cause harm to the skin.

Results

In the experiment, three solutions/reagents were used; pale yellow, light blue, and no colour was observed for Lugol's iodine, Benedict's reagent, and Biuret reagent respectively.

The sample solutions were all colourless before being tested on except for 5% honey and 1% protein solutions which had a subtle yellow tint, and beer which had a orange tint.

Table 1

Observations From Colourimetric Tests per Sample

	Observations		
Sample	Iodine Test	Benedict's Test	Biuret Test
1% glucose	–	brick-red precipitate	–
0.3% glucose-1-phosphate	–	–	–
1% maltose	–	brick-red precipitate	–
5% honey	–	brown precipitate	–
1% sucrose	–	–	–
1% lactose	–	brick-red precipitate	–
1% glycogen	red-brown colouration	orange precipitate	–
1% starch	blue-black colouration	–	–
1% protein	–	–	violet tint
beer	–	yellow-orange precipitate	–
distilled water	–	–	–
unknown solution #39	–	brick-red precipitate	–

In Table 1, solutiuons that did not react to colourimetric tests were marked with a minus sign, and changes in colour from solutions that tested positve were recorded.

Additional observations were as follows. The solution of 1% glycogen under Benedict's testing displayed a green hue and a minor presence of an orange precipitate, which, when mixed with the green solution, resulted in a brownish appearance. 1% protein under iodine testing exhibited a deeper shade of blue in comparison to the other negative results. Beer under iodine testing exhibited a yellow-orange coloration as opposed to the pale yellow color observed in other negative results, and under Biuret testing, it exhibited an olive coloration as opposed to the expected light blue hue observed in other negative results.

Discussion

Under iodine testing, 1% glycogen solution produced a red-brown colouration indicating presence of glycogen, and 1% starch solution produced a blue-black colouration indicating presence of starch.

Under Benedect's test, 1% glucose, 1% maltose, 1% lactose, and unknown solution #39 produced a brick-red precipitate, indicating high concentrations of reducing sugars. 5% honey pro-

duced a brown colour, 1% glycogen produced a minor amount of orange precipitate, and beer produced orange precipitate; all indicating some concentration of reducing sugar in solution.

Under Biuret testing, only 1% protein produced a violet colouration indicating presence of protein.

As expected, all the postive controls produced a positive result in their respective tests. Most sample solutions with known chemical species behaved accordingly; however, there were some unexpected results. Notable observations will be discussed.

The Biuret test did not indicate the presence of protein in the 1% glycogen solution, which was unexpected as glycogen contains a protein called glycogenin in its core. It may have been that the thick branching of glucose chains surrounding glycogenin prevented the interaction of the Biuret reagent with the protein.

1% glycogen testing slightly positive under Benedict's test was also unexpected; although glycogen has one reducing end per molecule, it is covalently bonded to glycogenin, preventing the redox reaction in theory. It may have been that during the boiling process, some hydrolysis may have taken place that created reducing sugars.

The unknown solution #39 tested positive to Benedict's test only; the brick-red colouration indicates that this solution contains reducing sugars, such as glucose, maltose, and lactose.

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