

Glucose Assays Revisited: Experimental Determination of the Glucose Concentration in Honey

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Abstract: Routine glucose assays have been incorporated into the biochemistry laboratory curriculum to emphasize the difference between a nonenzymatic assay and an enzymatic assay. Instead of the typical determination of glucose concentration from a random unknown glucose solution, students are asked to determine the glucose concentration in honey. Using honey as the glucose unknown allows students to readily see the difference in the two assays because the nonenzymatic assay reveals all reducing sugars and the enzymatic assay is specific for glucose. In this work the laboratory procedures are described and typical student results are provided.

Glucose assays are important to the biochemistry laboratory curriculum since the sensing of blood glucose levels is critical in the control of diabetes. The colorimetric detection of reducing sugars in solution reflects some of the earliest carbohydrate chemistry available [1]. A dinitrosalicylic acid (DNS) assay has been available since 1955 and is still useful for the quantitative determination of reducing sugars [2]. More recently several enzymatic assays for glucose [3, 4] have been developed. Georgia Southern University has incorporated both a nonenzymatic and enzymatic glucose assay into its biochemistry curriculum. Biochemistry students are asked to generate standard curves for both assays, determine the concentration of glucose in a glucose unknown (honey), and compare the results. The nonenzymatic assay indicates the presence of all reducing sugars whereas the enzymatic assay is specific for D-glucose. Students will get very different results for the two assays because in addition to glucose, honey also contains the reducing sugar fructose in a ratio of 1.2:1 with the glucose [5]. By comparing the two experiments the students are led to the conclusion that the enzymatic assay gives a much more accurate concentration of glucose in honey, but has the disadvantage of being more costly both in terms of supplies (enzymes must be prepared fresh, kept in a buffered solution, and used during a single laboratory period), time frame, and diligence on their part. Before the laboratory begins, students are asked to provide some background information on the carbohydrate content of honey. Honey contains 23 to 41% glucose and 31 to 44% fructose with a total reducing sugar content (includes 4 to 8% maltose and galactose) of 61 to 84% [5, 6]. After determining the number of milligrams of reducing sugar or glucose present in the honey sample from the standard curve, students are asked to provide the percent reducing sugar and percent glucose present in the honey.

Experimental

One three-hour laboratory period is required for each assay. Typically, the nonenzymatic assay is performed in the first laboratory period (the easier of the two laboratories) followed the next week by the enzymatic assay. Both the nonenzymatic and enzymatic assays can be performed using simple bench-top spectrophotometers such as a

Spec-20. Micropipettors are used for preparing the assay samples. All chemicals can be purchased from Sigma.

The DNS reagent can be prepared for the class in advance; however, the preparation of the reagent is a worthwhile exercise for the students, especially at the beginning of the semester after students have returned from a break.

Enzymatic solutions were prepared in advance by the instructor on the day of the laboratory. This was done to save material and minimize waste. It is recommended that the entire enzymatic assay be conducted on the same day with the same solution to minimize any effects of lost enzyme activity. It is instructive for students to prepare their own 0.10 M sodium phosphate (NaH_2PO_4) buffer, pH 7, to use for dilutions in the enzymatic assay.

A standard glucose stock solution is provided to the students for each assay. The students prepare a set of glucose standards ranging from 0.0 to 1.0 mg mL^{-1} (total sample volume 1 mL). Based on the background assignment on sugars in honey, students are also asked to prepare a solution from the honey that should have a concentration in the range of their standard curve for each assay.

Nonenzymatic Assay. After adding the DNS reagent, boiling the samples, and diluting with distilled water, samples containing reducing sugar develop a reddish-brown reduction product. Absorbance is measured at 540 nm.

Enzymatic Assay. Figure 1 provides a reaction scheme for the enzymatic reaction [3]. This involves the oxidation of glucose to δ -gluconolactone using the FAD-dependent enzyme glucose oxidase (eq 1). The glucose oxidase is regenerated in its oxidized form via molecular oxygen to produce hydrogen peroxide (eq 2). The hydrogen peroxide is then used by a horseradish peroxidase to catalyze the oxidation of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), producing a brilliant blue green color (eq 3). The enzymatic solutions are prepared for the students due to cost restrictions. Samples are incubated for 30 minutes in a 37 °C water bath. The absorbance is measured at 725 nm. Students must perform all dilutions using their sodium phosphate buffer instead of the distilled water used in the previous assay.

A standard curve is generated for both assays (absorbance vs. mg reducing sugar or glucose) and the concentration of reducing sugar or glucose is determined for the honey solution.

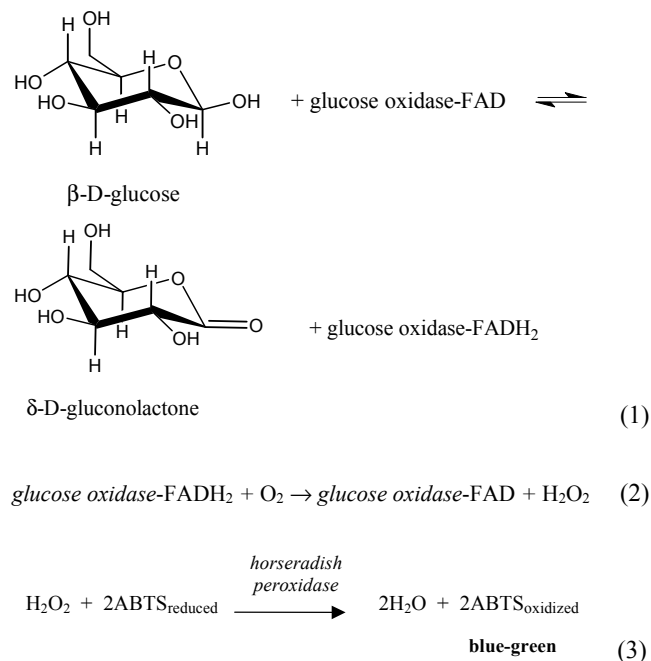
Results and Discussion

A representative set of student standard curves for the nonenzymatic and enzymatic assay are shown in Figure 2.

Table 1. Representative Student Results from the Nonenzymatic and Enzymatic Assays of Glucose^a

Student	Percent reducing sugar (nonenzymatic assay)	Percent glucose (enzymatic assay)
1	85.9	42.8
2	69.8	35.5
3	68.8	37.0
4	81.0	32.1
Average	76.4 ± 8.4	36.8 ± 4.5

^a Percentage values are generated from student-calculated values of mg carbohydrate per mg of honey. Students calculate mg reducing sugar or glucose from their standard curve and then divide by the mg honey in each sample and multiply by 100.

**Figure 1.** Reaction scheme for the enzymatic reaction.

Student results of the percentage of glucose found in the honey solution from both assays are shown in Table 1. Individual student values will vary; however, the averages shown indicate that students can get accurate results. At the worst, students should certainly be able to get a smaller value for % glucose than for % reducing sugar.

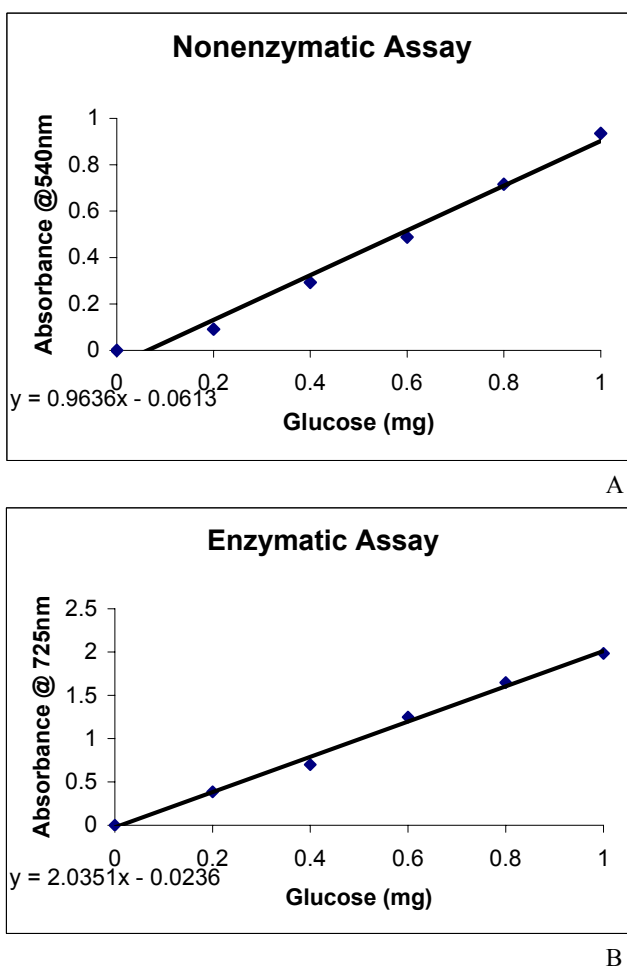
Notes on Student Experimentation

One of the most common sources of student error will occur during the micropipetting of the standard glucose and the honey samples. A review of the use of the micropipette prior to the experiment helps alleviate this problem.

It should be noted that the enzymatic assay requires the preparation of a phosphate buffer. Some students will forget to use the phosphate buffer and use distilled water as they did for the nonenzymatic assay. The enzymes will not perform optimally in a distilled water solution, which will affect the results.

Different floral honeys (e.g., clover, wildflower) can have differing glucose and fructose values. It is suggested that a single brand and variety of honey be used for the entire class.

Students tend to have problems preparing a honey solution. Honey is not a substance that is easy to handle. Deciding how

**Figure 2.** Student data set providing standard curves for the (A) nonenzymatic and (B) enzymatic assay of glucose.

much solvent to add to the solute (honey) instead of how much solute to add to the solvent when preparing solutions was unusual, but a benefit for the students. Preparing the honey solution also allowed the students to work through a number of dilution equations when determining the final concentration of glucose present.

Conclusion

This laboratory exercise illustrates the differences in two glucose assays, both in their specificity and labor intensity. The students generate simple standard curves and assay a honey sample comparing their results to published information on the carbohydrates in honey. The use of honey as the sample for analysis provides a more relevant application of the assay than merely providing the student with a glucose unknown. The materials needed to perform the laboratory are readily available and the equipment is common to most biochemistry laboratories.

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Supporting Materials. Student's Laboratory Exercise: Nonenzymatic and Enzymatic Measurements of Glucose.

These materials can be downloaded in a Zip file (<http://dx.doi.org/10.1333/s00897040802a>).

References and Notes

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