Quantitative Assay for Starch by Colorimetry Using a Desktop Scanner

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A recent *Journal* article by Wright *(1)* described dramatic color-change demonstrations using solutions of household chemicals, including that of starch–iodine complex formation. It occurred to us that starch–iodine solution colorimetry might provide a suitable means of introducing quantitative data analysis in high school chemistry laboratories. The procedure to produce a standard curve for starch concentration measurement by image analysis using a color scanner and computer for data acquisition and color analysis is described. Color analysis is performed by a Visual Basic computer program that measures red, green, and blue (RGB) color intensities for pixels within the scanner image. The red component of the image of the reaction solution is used to quantify the starch concentration. The computer program is available in Supplemental Material.[W] The chemicals used in the demonstration are inexpensive and readily available in stores. An experiment to measure starch from a potato is described.

Procedures

Preparation of Starch Stock Solution

A stock starch solution was prepared according to instructions given by Wright *(1)* using cornstarch. A starch slurry (1 g cornstarch plus water) was slowly poured and mixed into approximately 75 mL of water that had just been boiled. This solution was brought to 100-mL volume by further addition of water to form a stock solution of [starch] = 1g/dL. Successive dilutions of this starch stock solution were prepared using water.

Mixture with Iodine Indicator Solution

A dilute iodine indicator solution was prepared in water by addition of 50 μL of tincture of iodine per 3 mL of water (approximately 1 part tincture of iodine per 60 parts water). The starch–iodine reaction, producing a color change of the dilute iodine indicator solution from yellow–red to deep blue, took place in a standard clear-plastic 96-well microtitre plate using 200 μL of the iodine solution followed by addition of 50 μL of starch solution.

Isolation of Starch from a Potato

A raw Russet potato was weighed (155.0 g), skinned (140.5 g, potato without skin), and then processed in an electric "juicer" to produce potato juice (96.8 g or 68.9% recovery of the skinned potato mass). The juice was collected in two 50-mL conical-bottom screw-cap plastic centrifuge tubes. The juice had high solids content and readily separated into liquid and solids upon standing. Solids formed in two layers: a dense white solid (starch) and a brown layer (likely to be cell wall components) of lesser density. The dense white solid became tightly packed upon standing. After 2 hours standing, the fuliginous liquid portion of the juice was separated from the solid components by decanting. The brown solids were effectively removed from the mixture by two cycles of a wash procedure: the combined solids were resuspended in cold tap water by vigorous shaking of the tube; after allowing the mixture to settle for an additional 2 hours, the less dense brown solids were carefully resuspended in the liquid fraction by slow rotation and inversion of the tube, whereupon the liquid suspension including the brown solids was discarded by release of the tube cap, leaving the white solid in the bottom of the tube. There was apparently only incremental loss of starch via the wash procedure as judged by the unchanged height of the white solid in the tube after the wash; correspondingly, the water content of the white solids fraction was apparently unaffected by the wash procedure.

This procedure left a bright white solid paste (47.4 g) of homogeneous appearance. After thorough stirring of the wet paste, 1 g of wet paste was used to form 100 mL of a potato starch solution according to the procedure used for the cornstarch stock solution. Successive dilutions in water of this solution were prepared and reactions with the iodine indicator solution were analyzed by color image analysis according to the procedures given for the standard curve.

Color Image Analysis

A color JPEG image (150 dpi) of the microtitre plate was obtained using a desktop scanner, with a bright white piece of paper placed between the top of the microtitre plate and the scanner cover. Color analysis of the image for each well (to obtain red, green, and blue component data) was performed using a color data reduction program, COLORS.EXE, written in Visual Basic. This program is available in Supplemental Material.[W] Briefly, upon designation of an area within the image (that is, a portion of the microtitre plate well), the program returns an average value for the red, green, and blue color components of the pixels within that area. Note that the same RGB data can also be obtained (pixel by pixel) using many commercial image analysis programs such as those typically included with scanner software. It was found that a standard curve for starch concentration of the diluted starch solutions could be obtained by plotting the red component of the well images versus the starch solution concentration.

Hazards

Tincture of iodine (2% USP, sold as a skin antiseptic) contains ethanol and is flammable. It is also poisonous and should not be ingested. Care should be taken to avoid staining of clothing. Protective eye cover should be worn at all times.

Results

Analysis of the Stock Solution Curve

An example of a JPEG image of a microtitre plate used to generate a standard curve for starch concentration is shown in Figure 1. From the RGB image analysis, it was found that the blue coloration of solutions that developed with increasing starch content correlated with an increased absorbance of red (Figure 2).

The intensity of the red component of the well solutions as a function of starch concentration was well characterized by a conventional four parameter logistic dose–response curve (also shown in Figure 2). Although a fine dark precipitate was seen in the wells at starch concentrations above 50 mg/dL, this did not adversely affect the regularity of the starch concentration curve as determined by average color analysis. The analytical response range spanned more than one order of magnitude of dilutions of the starch solution, with a sensitivity (lowest detectable concentration) of the order of 10 mg/dL. The data derived from use of a stock solution produce a standard curve by which measurements of starch concentrations could be made from unknown solutions.

Analysis of Yield of Starch from a Potato

The quantity of starch contained in a Russet potato was measured. The results are shown in Figure 2. By comparison to the standard curve, the starch concentration in the solution made from the potato-derived starch paste (1 g paste/100 mL water) gave a value of 0.40 g/dL; thus, 1 g of starch paste contained 0.4 g starch. Correspondingly, 47.4 g of recovered starch paste was calculated to contain 19.0 g starch, a yield of 14% of the skinned potato mass of 140.5 g.

The liquid portion of the potato juice gave no color change when mixed with the iodine indicator solution. Assuming no loss of starch in processing after acquisition of the potato juice, the total starch content of the potato may be estimated to be 18%, given 69% potato juice recovery of the skinned potato mass and assuming that the unrecovered mass was of the same composition as the juice. This result is within the expected (albeit broad) range of 13–23% *(2)* for potato total starch content.

Discussion

The experiment to measure starch concentration is a means to introduce of the concept of the standard curve in quantitative analytical chemistry. For a laboratory exercise, the concentration of starch in an unknown solution could be determined, or, as in the example given, the starch yield from a potato could be measured. The mass balance gives a likely upper limit for the starch content of the entire potato using certain assumptions. If a sufficiently precise and accurate means of determining the density of the potato is available, the relationship between yield and recovery could be examined further using an established correlation between potato density and starch content *(2)*. One obvious follow-up question from the potato experiment is whether the measured starch content of the starch paste is simply a measure of its dry weight (i.e., whether the paste is of pure starch in water).

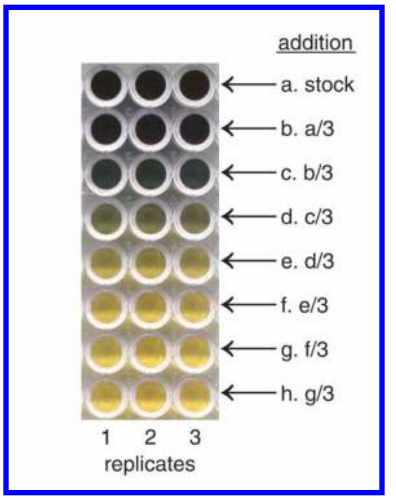

Figure 1. Scanner image (JPEG, 150 dpi) of solution mixtures in a microtitre plate. Color scanning was performed in 24-bit color data mode, in which red, green, and blue intensities are each represented by 8 bits (integer values of 0–255). Because of some degree of shadowing, apparently due to the depth of the plate and the meniscus, color data acquisition was made within a uniformly positioned, bright area within each well; specifically, data were obtained from the middle of the lower half of each well. The color JPEG image of this figure is available in the Supplemental Material.[w]

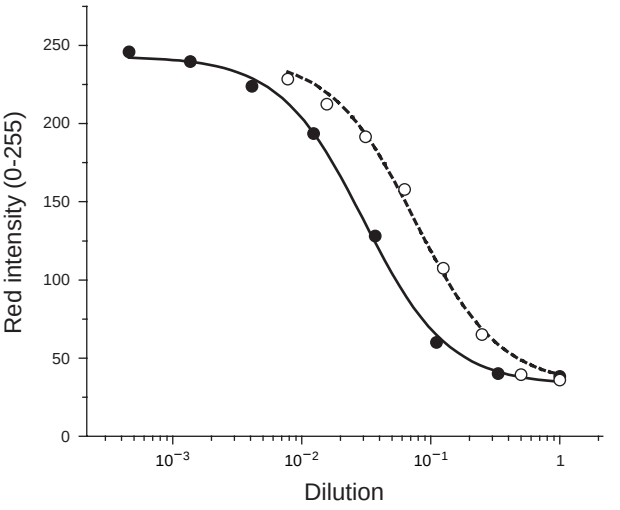

Figure 2. Red component intensity data from color image analysis for iodine indicator solution mixed with dilutions of the cornstarch stock solution (solid circles) and of the potato paste solution (open circles). The data for the dilutions of the cornstarch stock solution (1 g/dL) comprise the standard curve for starch concentration measurement by colorimetry. For the standard curve, data shown are means for triplicate well measurements. The intraassay coefficient of variation (standard deviation/mean) for triplicate measurements ranged from 1 to 6%. The standard curve data are well characterized by a fitted "four parameter logistic" curve (solid line): $y = y_{min} + (y_{max} - y_{min})/(1 + (x/x_c)^p)$. For the data shown: $y_{min} = 32.9$, $y_{max} = 243.0$, $x_c = 0.030$; $p = 1.33$. For the potato starch solutions data, $x_c = 0.076$ (dashed line). By comparison to the standard curve, the calculated concentration of starch in the potato paste solution was 0.40 g/dL (= 1g/dL x 0.030/0.076).

Discussion could include that of the appropriate strategies for obtaining accurate results for an unknown using this type of standard curve (namely, finding a dilution resulting in data within the highest resolution portion of the curve). Further experimentation might be undertaken to determine whether a standard curve of greater sensitivity (lowest analyzable concentration) might be obtained by manipulation of the iodine content or acid concentration of the reaction mixture *(1)*. Additionally, more extensive experimental procedures might be developed to measure starch in grain *(3)* or fruit (e.g., to produce serial measurements of starch content of apples during ripening) *(4)*. Numerous government agency resources on the Internet (e.g., ref *2–4*) from around the world exemplify the importance of starch measurement in commercial agriculture.

[W]Supplemental Material

Notes for the instructor, a color image of the microtitre plate containing the stock solutions, and a Visual Basic computer program (COLORS.EXE) for RGB data analysis from a computer image file are all available in this issue of *JCE Online.*

Literature Cited

1. Wright, S. W. *J. Chem. Educ.* **2002,** *79,* 44–46.
2. International Starch Institute: Technical Memorandum on Production of Potato Starch. *http://home3.inet.tele.dk/starch/isi/starch/tm5www-potato.htm* (accessed Feb 2004).
3. Juliano, B. O. *Cereal Sci. Today* **1971,** *16,* 334–336, 338, 360. Information also cited on Research Impact on USDA Rice Quality Evaluation Program Home Page. *http://usda-ars-beaumont.tamu.edu/qual8.html* (accessed Feb 2004). This U.S. Department of Agriculture site also gives a history of methods used for determination of starch in rice.
4. Two agricultural agency Web sites discussing the rationale and methods for grading of starch content in apples are *http://tfpg.cas.psu.edu/part6/part61a.htm* and *http://www.gov.on.ca/OMAFRA/english/crops/facts/00-025.htm* (accessed Feb 2004).