JOURNAL OF CHEMICAL EDUCATION

Laboratory Experiment

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A Laboratory Experiment for Rapid Determination of the Stability of Vitamin C

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Supporting Information

ABSTRACT: Experiments in laboratory manuals intended for general, organic, and biological (GOB) chemistry laboratories include few opportunities for students to engage in instrumental methods of analysis. Many of these students seek careers in modern health-related fields where experience in spectroscopic techniques would be beneficial. A simple, rapid, easily implemented experiment was developed to measure vitamin C levels in solutions using visible spectroscopy. When vitamin C is added to a mixed reagent containing Fe^{3+} and 1,10-phenanthroline, the vitamin C reduces the Fe^{3+} to Fe^{2+} which subsequently combines with the 1,10-phenanthroline to form a reddish-orange colored complex that is measured by visible spectroscopy at 510 nm. The experiment includes measurement of the stability of aqueous vitamin C solutions when exposed to long-term storage and to heat. Students in GOB laboratory classes were able to produce results with good precision. The experiment was also extended to include the measurement of vitamin C in nutrition supplements.

KEYWORDS: *First-Year Undergraduate/General, Analytical Chemistry, Hands-On Learning/Manipulatives, Instrumental Methods, UV−Vis Spectroscopy, Applications of Chemistry, Laboratory Instruction*

INTRODUCTION

The determination of vitamin C (ascorbic acid) in undergraduate laboratories is a fairly common practice because students are eager to practice new skills on substances that are relatively familiar to them. A large number of these procedures are based on teaching the students by means of the classic iodimetric or iodometric titration of vitamin C.[1−7] Another, less popular titration method involves the use of 2,6-dichlorophenol indophenol.[8,9] Instrumental methods of analysis, especially electrochemistry and HPLC, are also popular, but perhaps in more advanced undergraduate and graduate lab courses.[2,10−15] Because of their relative complexity and need for more advanced laboratory skills none of these techniques seemed suitable for our first-year general, organic, and biological (GOB) chemistry laboratory course. Therefore, we developed a precise, engaging laboratory experiment that could involve relatively simple laboratory instrumentation and skills appropriate for GOB students.

The basic principles of spectrophotometry and the preparation and use of standard calibration curves are important concepts and skills commonly covered in introductory chemistry laboratory experiments. These skills are essential to chemistry majors but are also important to prehealthcare students, due to the ever-increasing breadth of technologies and instrumental methods of analysis in the modern healthcare environment. As with much of the introductory coursework for prehealth students, these experiments are the foundations upon which additional, more field-related knowledge is built. Unfortunately, laboratory manuals intended for prehealthcare students in general, organic, and biological chemistry (GOB) laboratory courses offer few engaging experiments involving the application of scientific instrumentation. Therefore, many students may not use or learn spectroscopic analysis until they are actually expected to use advanced instrumentation in a job-related setting. An added challenge of the GOB chemistry laboratory is to provide experiments that demonstrate the relevance of chemistry to these students, many of which are required to take the course by nursing schools, and their future careers.[16] In an effort to combine the introduction of instrumental analytical techniques with relevant health-related content into the GOB laboratory, we developed and implemented laboratory exercises involving the determination of vitamin C stability under various conditions using visible absorption spectroscopy.

In addition to the above-mentioned learning outcomes, our goal is to develop a simple, rapid, and accurate test method that involved the use of skill sets appropriate for students in the GOB chemistry laboratory. This experiment is designed, similar to other GOB experiments, to use small graduated cylinders and disposable squeeze bulb plastic pipets that are calibrated for volume instead of conventional volumetric pipets. As long as the students take care in their measurements, the precision of their measurements is not compromised. Continuing with the theme of speed without losing quality, we use a detection reagent that rapidly forms a stable colored complex that is directly proportional to the concentration of the analyte. The students create a standard curve and then determine the concentration of vitamin C remaining in a solution that is prepared 1 week in advance and stored under different conditions; open to air and closed to air. Students also measure the vitamin C remaining in freshly prepared vitamin C solutions exposed to heat for varying lengths of time. The measured concentration of the unknown solutions are used to calculate the vitamin C degraded in solution over time.

Received: May 11, 2016
Revised: August 13, 2016
Published: September 13, 2016

DOI: 10.1021/acs.jchemed.6b00344
J. Chem. Educ. 2016, 93, 1766−1769

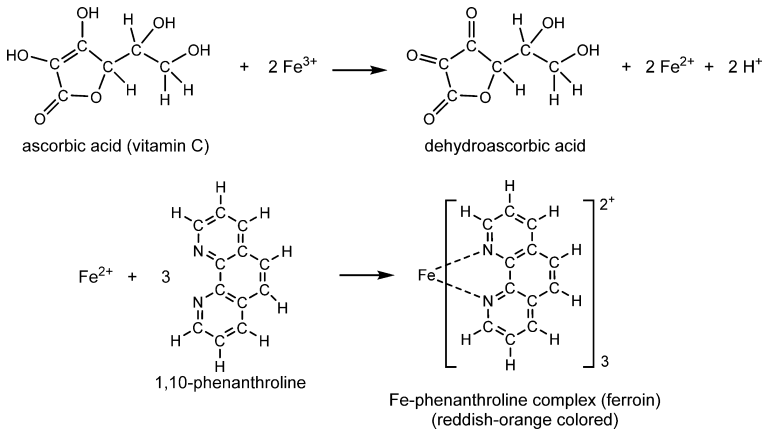

Figure 1. Reaction of vitamin C and Fe^{3+} and the formation of Fe−phenanthroline complex.

Questions posed to the students as part of their results writeup can lead them to thinking about what happens to the vitamin C in fruit juice or vitamin supplement tablets, thus making the experiment more meaningful and increasing learning outcomes overall.

To achieve our goal, we reviewed a variety of spectrophotometric techniques available for the determination of vitamin C, including the reaction with 4-chloro-7-nitrobenzofurazane,[17] the iron(III)−ferronate complex formation,[18] the copper(II)−neocuproine reagent technique,[19] the oxidation of vitamin C with 2,6-dichlorophenollindophenol,[20] the FRAP assay method (reduction of ferric tripryidyltriazine to the ferrous form),[21] and the reduction of iron(III) to iron(II) by vitamin C and the subsequent complex formation with 1,10-phenanthroline (Figure 1).[22] The latter technique fit our needs the best, providing both stability, speed, and reproducibility. Although some of the other spectrophotometric techniques cited may offer greater accuracy where interferences are a problem, the 1,10-phenanthroline−iron complex procedure offers us greater advantages of simpler waste disposal and fit our original intent to analyze solutions containing only vitamin C. Other advantages include the color reagent is stable for many weeks, the color development is achieved within one to 2 min, and the complex solutions are stable for at least 24 h. The reaction conditions are slightly acidic with a working pH range of 1.5−6.5.[22] Maintaining the pH is easily attained by adding acid to the combined iron(III)−1,10-phenanthroline color reagent solution.

EXPERIMENTAL PROCEDURE

In this experiment, the stability of aqueous vitamin C is determined for differently treated solutions; stored for 1 week (both open and closed environments) or freshly prepared and exposed to heat (50 °C). Also, the stability of solid vitamin C is determined by leaving it exposed to air for a week. The students' results demonstrate that vitamin C aqueous solutions are not "shelf stable" or heat-stable, however solid samples are much more stable and there is little change in vitamin C concentration upon exposure to air for 1 week.

Standard solutions of vitamin C for the shelf life stability determinations are prepared 1 week ahead of the experiment. However, it is important that the standard solutions used for calibration and the solution made from the solid vitamin C that was exposed to air for 1 week be prepared on the day of the experiment by the instructor or by the stockroom assistants who normally prepare solutions.

Students set up measured aliquots of a freshly prepared standard vitamin C solution and then combine them with a mixed Color Reagent, containing Fe^{3+} and 1,10-phenanthroline in acidic medium. The vitamin C reacts with the reagent, as discussed above, to produce a reddish-orange colored complex that absorbs an amount of light at 510 nm that is directly proportional to the concentration of vitamin C in the solution. Students then measure and record the absorbance using a visible spectrophotometer and prepare a standard calibration curve from these results. Our students produced precise linear standard calibration curves (see data below).

Students then heat an aliquot of the standard vitamin C solution at 50 °C, for 1 min, and a second aliquot for 15 min. After heating, the solutions are placed in an ice bath to cool, then mixed with the Color Reagent and the absorbance of the resulting solution is measured as above. From these results and the standard curve, the students determine the vitamin C remaining in the solution and calculate the percentage decrease in vitamin C due to heating. The students learn that the longer the solution is heated, the greater the loss of vitamin C.

HAZARDS

Eye protection should be worn at all times while in the laboratory and care must be taken when handling laboratory glassware. The mixed color reagent used in this experiment is prepared with 1 M nitric acid and has a pH below 2. Gloves should be worn while handling these reagents. Solid 1,10-phenanthroline is hazardous if ingested or if it contacts the skin or eyes. Handle the solid with gloves in a well-ventilated area.

RESULTS AND DISCUSSION

Students enrolled in our GOB laboratories (13 separate groups of 2−3 students) performed this experiment for the past three semesters and obtained reproducible results. The students measured and mixed the various solutions, read the absorbance, and prepared their own standard calibration curves. The students subsequently used their standard curves to obtain vitamin C concentrations for different test solutions. A point of emphasis in this experiment is the direct proportionality of the concentration of vitamin C with absorbance in spectrophotometry. The standard calibration curves should follow the

DOI: 10.1021/acs.jchemed.6b00344
J. Chem. Educ. 2016, 93, 1766−1769

Beer–Lambert Law and instructors should emphasize goodness-of-fit for each set of data.

In Figure 2 below, we show a plot of the average results of the standard calibration curves for the 13 groups of students.

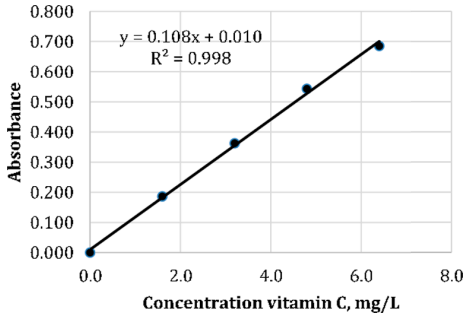

Figure 2. Standard calibration curve for student-generated data. Average of 13 groups of students.

The students calibrated their instruments by setting the absorbance reading to 0.000 for their blank solutions. The data yielded a straight line with a slope of 0.108/mg/L and R^2 = 0.998. This linearity and goodness-of-fit are typical of our individual student group data.

The vitamin C stability data collected from each student group closely matched the expected results; vitamin C concentration decreased over time and with heat. However, unlike the standard curve data, there was more variability among the student groups in their measurements over three semesters.

In vitamin C solutions prepared and then heated at 50 °C for 1 min, the concentration of vitamin C decreased by an average of 12% with a standard deviation of 15. A longer heating period (15 min) resulted in an average 41% decrease with a standard deviation of 27. As expected, the longer heating time resulted in decreasing the vitamin C concentration. Also as expected, both the opened and closed vitamin C solutions that were held at lab temperatures for 1 week showed significant average decreases in vitamin C content. The opened vitamin C solution showed an average 87% decrease in vitamin C with a standard deviation of 11. The closed solution showed an average 93% decrease in vitamin C with a standard deviation of 6. These two results, including standard deviations, were too close to draw any conclusions regarding differences in vitamin C stability between "open" storage and "closed" storage. Finally, the students reported an average 4% decrease in vitamin C for the solid that was stored 1 week exposed to air with a standard deviation of 16. Reagent vitamin C (L-ascorbic acid) is, according to one manufacturer, "stable at room temperature in closed containers under normal handling conditions. Stable to air when dry; aqueous solutions are rapidly oxidized by air".[23]

Most of our students were careful in their lab technique and obtained results similar to those of the authors. We find that because measurements are one of the main focuses of the GOB laboratory, it is necessary to take more time (either prelab or postlab) to discuss proper measuring technique and how slight variations can affect precision.

During the most recent semester, we also asked our GOB laboratory students to test a vitamin C tablet from a commercial nutritional supplement. The goal was to compare the measured vitamin C quantity to the reported nutrition label quantity as well as test the stability of a sample of a commercial product. The students seemed to connect this part of the experiment to a type of sample they may encounter outside the lab. It serves as an example of why nutrition labeling is important and how nutritional information may be measured by government agencies. Half of the student groups this semester obtained results that agreed reasonably well with the nutrition label, typically 525 to 600 mg vitamin C per tablet (data not shown). However, the authors did not check the variability of vitamin C among the tablets within the bottle, which could explain their differences.

CONCLUSIONS

We propose a simple, rapid, and precise method to determine vitamin C content in various solutions. This experiment is easy to implement and was successfully carried out with satisfactory results in three separate semesters of GOB laboratories taught by different instructors at Washburn University. Learning outcomes gained by the students include the importance of careful solution preparation, the use of a spectrophotometer, analysis of spectroscopy results, and the preparation and use of standard calibration curves. In addition, students gained an experimental sense of the stability of vitamin C and its aqueous solutions. Discussions and questions can be designed to lead students to further explore health-related implications of the stability of vitamin C solutions and supplements.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.6b00344.

Contains handouts given to the students at the beginning of the semester, which includes a brief explanation of the significance of vitamin C, the chemistry of the experiment, detailed description of the experimental procedure, data treatment, report sheets, and a series of questions to ensure the students understand the concepts. (PDF)

Contains handouts given to the students at the beginning of the semester, which includes a brief explanation of the significance of vitamin C, the chemistry of the experiment, detailed description of the experimental procedure, data treatment, report sheets, and a series of questions to ensure the students understand the concepts. (DOCX)

Notes to instructors, which provides useful information on waste disposal, laboratory equipment, reagent preparation, examples of calculations, and an example of a typical set of data from a group of students. (PDF)

Notes to instructors, which provides useful information on waste disposal, laboratory equipment, reagent preparation, examples of calculations, and an example of a typical set of data from a group of students. (DOCX)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors acknowledge the students in the GOB laboratory classes for conducting the experiment and for their feedback.

DOI: 10.1021/acs.jchemed.6b00344
J. Chem. Educ. 2016, 93, 1766–1769

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DOI: 10.1021/acs.jchemed.6b00344
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