

A Caffeinated Boost on UV Spectrophotometry: A Lab for High School Chemistry or an Introductory University Chemistry Course

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S Supporting Information

ABSTRACT: This laboratory experiment challenges students to identify the country of origin of raw coffee beans from around the world by calculating the percent caffeine and percent water in the supplied samples. The implementation of this experiment enables students in introductory chemistry classes to gain greater understanding of the chemical analysis process and the Beer–Lambert law. Using commercially available raw (green) coffee samples, students extract caffeine from the grounds as an aqueous solution and determine the percent caffeine using UV–vis spectrophotometry. In a second portion of the experiment, raw beans are roasted to determine the percent water content. Using both pieces of data, students are asked to identify the country of origin of given coffee samples in which the percent caffeine ranged from 1.14 to 1.80% (w/w) and the percent water loss ranged from 3.27 to 10.8% (w/w).

KEYWORDS: High School/Introductory Chemistry, First-Year Undergraduate/General, Laboratory Instruction, Physical Chemistry, Hands-On Learning/Manipulatives, Calibration, Food Science, Solutions/Solvents, Quantitative Analysis, UV-Vis Spectroscopy



The inception of this laboratory investigation grew from a professional development workshop, in which a group of high school chemistry and math teachers collaborated with university faculty to develop new laboratory curricula that both engage and challenge students to answer practical questions using evidence and scientific reasoning. To accomplish this task, coffee beans from around the world serve as the central point of investigation in this experiment. The goal for students is to identify the country of origin of raw coffee beans from around the world by calculating the percent caffeine and percent water in the samples. The laboratory lesson was successfully implemented in one teacher's five periods of honors and AP chemistry classes. The concept of this lesson serves as a review of molarity, dilutions, and the relationship between absorbance and concentration as represented in the Beer–Lambert law. Ideally, this experiment should be completed following an introductory experiment that utilizes the absorbance of visible light in FD&C food dyes, colored drinks,¹ or aqueous extracts of colored candies² using a Spectronic 20. Students build on experiences in prior labs by developing a deeper understanding of absorbance and the use of standard solutions in determining the molarity of an unknown sample. This experiment serves as an application of spectrophotometry and its use in the analysis of caffeine in a practical investigation. Numerous procedures highlighting the extraction of caffeine from coffee, tea, soft drinks, and over-the-counter supplements have been published in this *Journal*,^{3–9} but few use coffee beans and are appropriate for the high school classroom curriculum. Several other upper-division organic chemistry and analytical chemistry experiments have been

published in this *Journal* that also feature caffeine as the key analyte.^{10–15}

In this experiment, students are supplied with raw whole coffee beans and ground samples from various countries. The class is challenged to extract and determine the percent caffeine in an aqueous solution using UV–vis spectrophotometry. Caffeine can be extracted from raw coffee beans following the procedure of Souto and co-workers¹⁶ and analyzed using UV energy. (The extract from raw coffee beans does not have the characteristic pigment of roasted coffee beans and helps to deepen a student's understanding of spectroscopy and absorbance of energy outside the visible range.) It is known that the percent caffeine (w/w) in various coffee samples is slightly larger than 1%.¹⁷ In a second portion of this experiment, students determine the percent water content of their supplied coffee samples by performing a cinnamon roast¹⁸ of their supplied raw whole coffee beans. The roasting produces the brown pigment and traditional aromas associated with brewed coffee.

The combination of analyzing the percent caffeine and percent water content in raw coffee beans allows students to determine the country of origin of their supplied sample. This investigation reinforces cross-cutting educational science standards that engage students in scientific questioning, generating evidence, and analyzing data.¹⁹ Ultimately students gain experience of how spectrophotometric techniques and theories presented during lecture apply to solving practical problems and challenges posed to scientists. In a high school setting, this

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experiment can be divided and completed in three 50-min sections.

■ EXPERIMENTAL PROCEDURE

Equipment

Digital heating plate (VWR, VMS-C7), analytical balance (Mettler Toledo, AB54-S/Fact), large capacity centrifuge (Beckman Coulter, Avanti J-25) with a rotor (Beckman Coulter, JA-17), ultraviolet–visible spectrophotometer (Agilent Technologies, 8453), and a coffee grinder.

Coffee Samples

Raw (green) coffee beans were obtained from U-Roast-Em²⁰ and Roastmasters.²¹ The coffee beans purchased from U-Roast-Em included Colombian Supremo, Brazil Sierra Negra, Burundi Bwayi, and Costa Rica Central Valley Café Vida (1 lb quantities). The coffee beans purchased from Roastmasters included Kenya AA, Bolivia Fair Trade/Organic, Guatemala SHB San Agustin Las Minas, Brazil Yellow Bourbon Fazenda Cachoeira, Sumatra Mandheling Organic, and Ethiopia Yrgacheffe (1 lb quantities).

Calibration Curve of Caffeine Absorbance

Caffeine (CAS number: 95789-13-2) was used to make a standard calibration curve to determine the molar absorptivity. A stock solution was prepared by adding 19.4 mg of caffeine (1.00 mmol) to a 100.0 mL volumetric flask and diluting the mark with deionized (DI) water. The stock solution was used to make a set of four standards with the following concentrations: 2.00×10^{-5} M, 5.00×10^{-5} M, 7.99×10^{-5} M, and 9.99×10^{-5} M. (The standards were used to prepare a calibration curve that was supplied to the students and is shown in the Supporting Information).

Extraction and Percent Caffeine Determination

Prior to the first lab session, raw coffee beans from each country (listed in Table 1) were finely ground in a coffee grinder, labeled with an identifier (i.e., sample A), and stored. During the first lab session, students were placed in groups of four and worked collaboratively to weigh approximately 1 g of the coffee grounds (to three significant figures). The sample of coffee grounds was transferred to a coffee filter resting inside a long-stem glass funnel. DI water was heated to 92–98 °C and gently poured over the coffee grounds in three 50.0 mL aliquots and collected in a beaker to extract the caffeine from the grounds.¹⁶ The extract was allowed to cool, capped, and stored to allow a white precipitate to form. (It is believed this precipitate is a protein that is extracted from the raw sample, but the identity of this compound is an ongoing project in our lab.) After one week, the extracts were centrifuged in a large capacity centrifuge for 10 min at 15 000 rpm and 10 °C. Following separation, the supernatant was decanted to a clean beaker or tube. (Caution needs to be taken at this step, as the precipitate is likely to come loose from the bottom of the centrifuge tubes.) In the second lab session, the students transferred 5.00 mL of the extract, using a pipet, to a 100.00 mL volumetric flask that was diluted to the mark with DI water. Following dilution, the samples were analyzed using a UV–vis spectrophotometer at 272 nm.³ In the third lab session, students correlated the absorbance data of their diluted sample to a specific caffeine concentration using the Beer–Lambert law. The students then calculated the molarity of the original caffeine extract and converted this value to percent mass caffeine (w/w) in the respective grounds.

Percent Water Determination

During the second lab session, students were given whole coffee beans (raw) that matched the identity of their grounds from the first lab session. Each group weighed and transferred a sample of raw whole beans having an approximate mass of 2 g (to three significant figures) into a 50 mL beaker. After recording the mass of the raw beans, the students placed the samples in a lab oven with a temperature between 190 and 200 °C. Samples from the entire class were spaced throughout the oven and roasted simultaneously. While the whole bean samples were roasting, the students began diluting the caffeine extract described above. Following the roast time (10 min above 190 °C), the students transferred the beans (and any kernel shells that may have loosened during process) to a coffee filter resting inside a long stem, glass funnel. Then, DI water was poured over the coffee samples to lower the internal temperature of the beans and quench the roasting process.²² The cooled roasted beans were placed in labeled weigh boats and stored in the fume hood for two days. In the third lab session, students reweighed the roasted beans that were allowed to dry. The difference in the pre-roast and post-roast mass of the student samples was attributed to the loss of water during roasting. The change in mass was converted to percent water (w/w) of a given sample.

■ HAZARDS

The use of oven mitts by the instructor and students should occur twice during this experiment. First, when the students are extracting caffeine from the ground samples, the students should use care and protect their hands while pouring 50 mL aliquots of hot water over the ground samples in the coffee filter. Second, while removing the roasted coffee samples from the oven after roasting, the beakers will be extremely hot (Instructors should remove the beakers from the oven and students should wear either oven mitts or hot hands while transferring the beans from the hot beaker to a coffee filter at the benchtop).

■ CALCULATIONS

During the implementation of this lesson in a high school classroom, students were provided with an absorption calibration curve of pure caffeine and known values of percent caffeine and percent water in coffee samples (Table 1). The percent caffeine contained in the student samples was

Table 1. Percent Caffeine and Water Content in Raw Coffee Based on Country of Origin

Origin of Raw Coffee Sample	Average Caffeine (%) ^a	Average Water (%) ^a
Ethiopia Yrgacheffe	1.14 ± 0.03	6.29 ± 0.41
Sumatra Mandheling	1.26 ± 0.00	3.27 ± 0.47
Brazil Sierra Negra	1.31 ± 0.00	7.09 ± 0.01
Burundi Bwayi	1.38 ± 0.02	7.20 ± 0.04
Costa Rica Central Valley Café	1.53 ± 0.03	6.47 ± 0.21
Guatemala SHB	1.55 ± 0.02	10.8 ± 0.35
Bolivia (Fair Trade/Organic)	1.63 ± 0.01	7.17 ± 1.13
Kenya AA	1.63 ± 0.03	7.48 ± 0.23
Columbia Supremo	1.71 ± 0.08	7.57 ± 0.00
Brazil Yellow Bourbon Fozenda	1.80 ± 0.05	7.42 ± 0.06

^aAn undergraduate chemistry major performed an analysis of all the coffee samples listed as part of their capstone research project.

determined by obtaining the quantity of energy absorbed after irradiating the diluted extract samples in a UV–vis spectrophotometer. The quantity of light transmitted through the individual sample was converted to absorbance by the equation $A = \log(I_0/I)$, where I_0 was the initial light intensity and I the intensity of transmitted light.²³ The quantity of energy absorbed was a phenomenon of the molar absorptivity (ϵ) of caffeine. This value was calculated from the slope of the calibration curve of absorbance of light that was supplied to the students, using a series of standard solutions. Lastly, the obtained absorbance value of the diluted caffeine extracts was converted to molar concentration using the Beer–Lambert law: $A = \epsilon bc$, where A equals the absorbance value, ϵ the molar absorptivity of the solution, b the path length of the cuvette cell (i.e., the distance the light travels through the sample), and c the concentration of the solution. Once the concentration of the diluted extract is known, the molarity of the original brew was determined using the dilution formula $M_1V_1 = M_2V_2$. The molarity was converted to mass of caffeine (in grams) and compared to the mass of coffee grounds used in the extraction, resulting in the percent caffeine by mass (w/w). During the investigation it was found that the percent caffeine in the raw coffee beans ranged between 1.14 and 1.80% and agreed with the findings of Belay and co-workers.¹⁷

A cinnamon roast (low temperature) was performed in this experiment to ensure the only change in the beans was the loss of water and not the decomposition of proteins, sugars, phenolic materials that are typically observed during the Maillard reaction (named after Louis Camille Maillard).²² When the samples were placed in a preheated lab oven (between 190 and 200 °C), the temperature of the oven initially dropped due to the opening of the door. It takes approximately 3 min for the system to reheat to 190 °C. After the minimum temperature was reestablished, the beans were heated for an additional 10 min. Once the roast time elapsed, water poured over the roasted samples resting in a filter after they have been removed from the oven quickly lowers the internal temperature and quenches the roasting process. The difference in the pre-roast and post-roast mass of the beans was attributed to a loss of water. This change in mass can be converted to percent water (w/w) in a given sample.

RESULTS

The largest impact of this laboratory was the experience of high school students engaging in scientific inquiry through the collection of data tied to a real-world scenario. Prior to each laboratory session, the students engaged each other and the instructor in questions about science, coffee, and caffeine that extended beyond the scope of percent mass and spectrophotometry. The students were engaged and motivated to perform the task at hand and were appreciative of being challenged as if they were at the college level according to their lab summaries. After reviewing the student data, the calculated percent caffeine in the coffee samples ranged from 1.18 to 2.15% and the percent water ranged from 3.51 to 11.67%. This indicated that high school students obtained results similar to those expected and indicated high school students are equipped to readily accomplish this type of exploration. The unknown samples and the countries listed in the table (of known values) provided to students should however be limited to ensure student data with wider variances can make an accurate identification of the origin of the coffee samples. For example, a student group calculated a percent caffeine value of 1.72% and a percent water content

value of 7.41% for their supplied unknown. The students identified that their coffee sample had been grown in Columbia. Their actual country of origin for their supplied unknown was Brazil (Yellow Bourbon Fojenda). If the supplied table of known origins of samples was limited to a fewer number of choices that had a low, mid, and high value for percent caffeine and water content (i.e., Sumatra Mandheling, Costa Rica Central Valley Café, and Brazil Yellow Bourbon Fojenda), the student experimental data would have been within range to allow the group to make positive identification.

DISCUSSION

A limitation of this experiment for high school instructors is access to a centrifuge and UV–vis spectrophotometer. This is readily addressed if a teacher partners with a neighboring biology, chemistry, or science department at a university to utilize shared resources. If the experiment is to be limited to three 50-min sessions, as written herein, this places a burden on the instructor to actively prepare sample before the lab sessions. During this implementation, the instructor was supported by a chemistry major, who plans to be a chemistry teacher in the future and has applied to a teacher credential program. This support could also be modified in the form of utilizing student classroom aides. The following adjustments would ensure success by a larger number of students during the investigation.

- Have students, working in groups, perform multiple trials so the experimental data can be averaged or eliminated based on erroneous mistakes to improve the likelihood of accuracy.
- Use fresh samples to ensure the percent water content is consistent if the lab is to be run on a yearly basis.
- Limit the number of entries of unknown possibilities supplied to students at the start of the investigation in Table 1.
- Limit the number of various unknown coffee samples used in a particular class period.

SUMMARY

This experiment serves to deepen a student's understanding of percent mass, molarity, dilutions, and the relationship between absorbance and concentration as represented in the Beer–Lambert law. During the investigation, students gather experimental data, perform quantitative analysis, and predict the origin of their coffee samples using scientific inquiry. Instructors can modify the scaffolding in the student handouts to make this challenge an inquiry-based laboratory investigation appropriate for students completing a laboratory at the high school or university general chemistry level.

ASSOCIATED CONTENT

Supporting Information

Instructions for students, student handouts, and instructor notes. This material is available via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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