JOURNAL OF CHEMICAL EDUCATION
Laboratory Experiment
Cite This: J. Chem. Educ. 2018, 95, 1626–1630
pubs.acs.org/jchemeduc

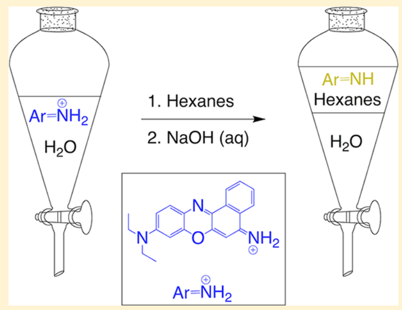
From Observation to Prediction to Application: A Guided Exercise for Liquid–Liquid Extraction

Tevye C. Celius,* Ronald C. Peterson, Amelia M. Anderson-Wile, and Mitchell Kraweic-Thayer

Donald J. Bettinger Department of Chemistry and Biochemistry, Ohio Northern University, Ada, Ohio 45810, United States

Supporting Information

ABSTRACT: In this organic chemistry laboratory experiment, students are guided through a series of exercises to understand the behavior of compounds during liquid–liquid extraction. Aspects of density, structure, solubility, acid–base theory, and pK_a are all incorporated into activities that students complete in groups. After using their initial observations to make predictions about the two colored compounds, nile blue and 2,6-dichloroindophenol, students check the reliability of their predictions by carrying out liquid–liquid extractions of these compounds in the laboratory. The set of exercises culminates in the application of the procedures to a real-world situation.



KEYWORDS: *Second-Year Undergraduate, Organic Chemistry, Laboratory Instruction, Inquiry-Based/Discovery Learning, Hands-On Learning/Manipulatives, Acid/Bases, Dyes/Pigments, Physical Properties, Separation Science*

INTRODUCTION

One of the most challenging laboratory techniques for organic chemistry students to master is separation by liquid–liquid extraction. This ubiquitous technique challenges students at several levels because it involves the integration of multiple concepts, such as structure, solubility, acid–base theory, and pK_a, in order to analyze the process.[1] To further complicate matters, in a typical organic laboratory application there are no visible clues for when compounds move from one layer to another. To introduce and demonstrate the process of liquid–liquid extraction, the use of dyes has been proposed.[2−8] Herein, a two-week, guided inquiry laboratory experiment is described in which students collaborate in groups on activities and conduct experiments that explore the underlying physical and chemical processes involved in a liquid–liquid extraction. The pedagogical goals of the experiment are listed in Table 1.

Table 1. Pedagogical Goals

Questions To Be Answered in the Experiment	Experiment Part
Which layer is which in the separatory funnel?	I
Are various compounds soluble under acidic, basic, or neutral conditions?	II
What acid–base reactions occur?	Prelab, III
What are the major species present in the equilibrium mixture of a given solution?	Prelab, III
What are the solubility properties of the major species?	III, IV
How do you extract a dye molecule between aqueous and organic layers?	IV
How do you plan and execute the separation of two organic molecules using extraction?	I–V

Most students can perform adequately when faced with these questions individually, but struggle when they must combine them in the design and execution of an extraction. We have designed the experiment so that in the first week students work together in the laboratory as groups of four composed of two pairs to explore which layer is which in a bilayer and to determine the effect of pH on the solubilities of compounds with different functional groups. The groups of students meet in the classroom to discuss the results of their experiments and collaborate to complete a series of worksheets that are designed to guide students in how to combine the pertinent information and to make predictions about the extraction of two different organic dyes. The groups return to the lab to test their predictions about the dyes. The use of highly colored molecules allows students to directly observe which layer contains their compound. During the second week of the experiment, the groups work together to develop a plan for applying the knowledge and skills gained in the first week to a real-world problem.[9−11] This series of exercises provides students multiple opportunities to practice the use of the fundamental concepts of acid–base theory, pK_a, and solubility in the context of liquid–liquid extraction and guides students in their applications of these parameters. Students progress from a manipulation that they can easily observe (the presence of the dyes) to one where the presence of the materials is not readily observable but must be substantiated by additional analytical techniques.

Received: October 12, 2017
Revised: June 18, 2018
Published: July 16, 2018

DOI: 10.1021/acs.jchemed.7b00779
J. Chem. Educ. 2018, 95, 1626–1630

Nile blue and 2,6-dichloroindophenol were chosen for this experiment on the basis of their acid−base characteristics and their availability. It was desirable for one dye to become water-soluble upon deprotonation and the other upon protonation. In addition these dyes were chosen because they both require multiple extraction steps to move the compounds between layers. To our knowledge, nile blue has not previously been reported in this type of experiment, but 2,6-dichloroindophenol has been used as a demonstration.[5]

■ EXPERIMENT

This experiment is typically carried out during the middle of first-semester organic chemistry, which is several weeks after students have completed their initial studies of acid−base chemistry. Over the past seven years, the experiment has been carried out with approximately 1300 students. The experiment requires two 3 h laboratory periods and works best if there is adequate space to break into discussion groups. Over the course of the laboratory periods, students transition between carrying out experiments in the laboratory and working on guided worksheets in the classroom several times. A detailed procedure is in the Supporting Information.

During the first week, a brief prelab discussion is presented, students form into groups of four, and group jobs are assigned (manager, recorder, technician, and presenter). Either students are allowed to choose groups on their own or the instructor assigns groups ahead of time. Students break into pairs and enter the laboratory to investigate which layer is which in a bilayer and to determine the effect of pH on the solubilities of compounds with different functional groups (Parts I and II). Upon completing their experimental work, students return to the classroom to compare their results within their group. The groups then share their results with the class. This group of four students works together to complete a series of guided activities (Part III) designed to lead them through mapping out the extraction process. Students finish week one by returning to the laboratory in pairs to test their predictions by carrying out the extraction of a colored dye. To ensure that all students receive adequate practice with the separatory funnel, each student carries out an extraction on one of the two dye molecules. Students begin week two in the classroom where they develop their own procedure for separating a mixture of two compounds. Once students have mapped out an instructor approved method, they move into the laboratory to carry out the extraction and subsequent TLC analysis of the mixture.

■ HAZARDS

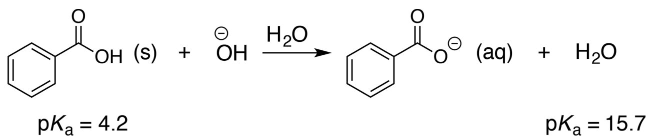
Gloves and googles should be worn at all times during this experiment. All organic solvents and reagents should be treated as flammable and harmful if inhaled, swallowed, or absorbed through the skin. Dichloromethane is a carcinogen, and should only be handled in a ventilated fume hood. Due to the potential presence of organic peroxides, naphthalene is considered a type B explosive and should be handled with care. Napthalene is a suspected carcinogen. *n*-Hexane is a neurotoxin and may be replaced with *n*-pentane, *n*-heptane, or cyclohexane with minor procedural modifications. Sulfuric acid, hydrochloric acid, benzoic acid, and sodium hydroxide are corrosive. Ethyl 4-aminobenzoate is a category 1 skin sensitizer and may cause skin or eye irritation.

■ RESULTS AND DISCUSSION

Prelaboratory Assignment

Before tackling a topic as difficult as extraction, students must have a strong foundation in acid−base chemistry. Prior to coming to the laboratory, students write out balanced acid−base reactions using pK_a values as in Scheme 1 to assist with their predictions. The ability to draw conclusions about the reactivity of a compound based on its structural similarity to a known species is one of the most challenging skills for any organic chemistry student to master. The molecules in this experiment (Figure 1) increase in complexity to help students master this skill.

Scheme 1. Balanced Acid−Base Reaction



Parts I and II: Laboratory Experiments Investigating Solvent Layering and Solubility

Students begin the experiment by investigating the fundamental concepts of density and solubility. The instructor provides minimal prelaboratory discussion to facilitate students making observations and conclusions about these extraction principles. In part I, students evaluate water/hexane and water/dichloromethane mixtures and draw conclusions about which layer is on the top based on experimental observations. Hexanes and dichloromethane were chosen due to their different densities when compared with that of water. Additionally, these solvent combinations are used for the extraction in part IV. This portion of the exercise allows students to explore the phenomenon of solvent layering independent of the extraction process. In part II of the experiment, students evaluate the solubility of three compounds in water, aqueous acid, aqueous base, and organic solvent. The molecules that are investigated in part II share key structural moieties with the molecules from the prelaboratory exercise (Figure 1), which allows students to begin relating the acid−base properties of the compounds with their solubilities. For both parts I and II, students generally come to the appropriate conclusions with minimal assistance from the instructor. Problems may arise if students use too much material (>50 mg/mL) or if they do not allow enough time for materials that are slow to dissolve to reach equilibrium.

Part III: Guided Classroom Activities

At this point in the experiment, students meet in a classroom to discuss the results of part II (Figure 1). Each group comes to the classroom with two complete data sets that allow students to discuss any discrepancies in the data within the group, and they report their results to the larger laboratory section. Students are allowed to return to the laboratory to rerun any necessary experiments. Using the prelaboratory assignments as a guide, students write balanced acid−base reactions, make predictions about the equilibrium mixture based on pK_a values, and use these predictions to explain their observations about solubility (Scheme 1). Making conclusions about the structure of a molecule and its solubility based on structurally similar compounds is a useful tool for students to have in their extraction toolboxes. The complexity of the

DOI: 10.1021/acs.jchemed.7b00779
J. Chem. Educ. 2018, 95, 1626−1630

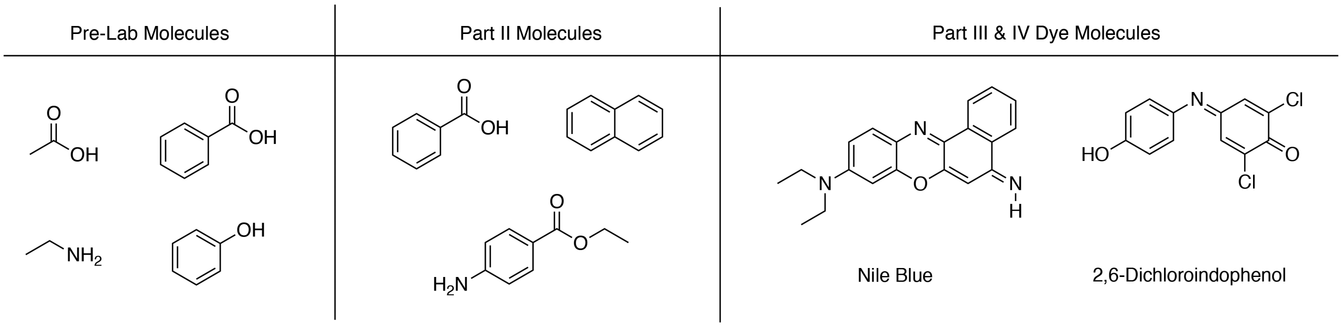
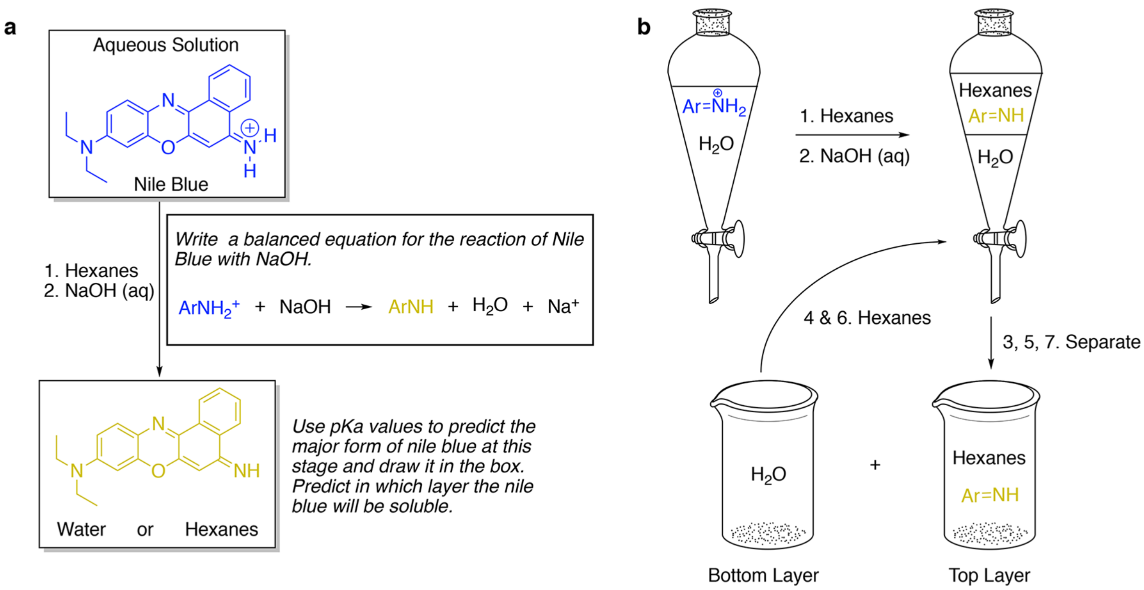


Figure 1. Structure of Molecules used in Prelab, Part II, Part III, and Part IV.

Scheme 2. Portion of the Guided Student Worksheets Showing (a) the Balanced Acid–Base Reaction for Nile Blue[a] and (b) the Separatory Funnel Scheme[b]



[a](1) Add 10 mL of hexanes to 30 mL of aq nile blue. (2) Add 10 mL of aq NaOH. [b](1) Add 10 mL of hexanes to 30 mL of aq nile blue. (2) Add 10 mL of aq NaOH. (3) Shake and separate the layers. (4) Add 10 mL of hexanes to the aqueous layer. (5) Shake and separate the layers. (6) Add 10 mL of hexanes to the aqueous layer. (7) Shake and separate the layers.

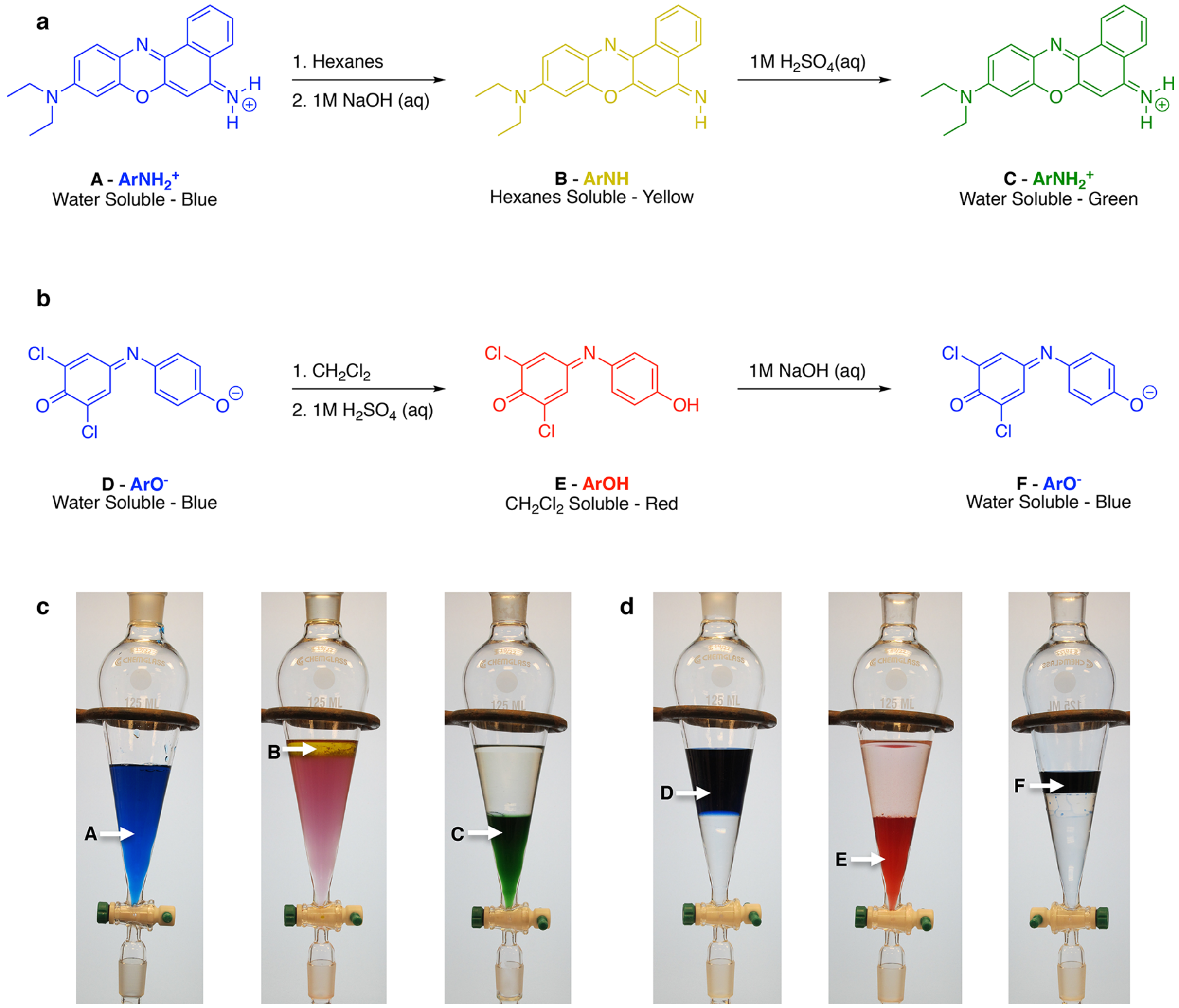
molecules was purposefully increased over the course of the experiment to allow students to focus on basic concepts before tackling more complex molecules such as nile blue and 2,6-dichloroindophenol (Figure 1).

Following the discussion of part II, students make predictions about the solubility of nile blue and 2,6-dichloroindophenol (Figure 1). With assistance, students can typically see that acid–base chemistry happens at the exocyclic, iminium nitrogen in nile blue and at the phenolic oxygen in 2,6-dichloroindophenol. These more complex molecules challenge students to think critically about the acid–base chemistry they already know. Working in groups of four, students complete extraction flowcharts for both nile blue and 2,6-dichloroindophenol (Scheme 2 and Supporting Information). Students explicitly write out balanced acid–base reactions for both dyes, use pK_a values to predict the equilibrium mixture after the reaction, and predict the solubility properties of the major component in the equilibrium mixture. For example, Scheme 2a shows the balanced reaction between protonated nile blue ($ArNH_2^+$) and aqueous sodium hydroxide. Students also look at the extraction of nile blue under acidic conditions and complete a similar worksheet for 2,6-dichloroindophenol (Supporting Information). The result of these exercises is that students are required to consider extraction of two different compounds under both acidic and basic conditions. Students were not allowed to continue to the next part until this exercise had been successfully completed, which may require some guided assistance from the instructor.

With solubility predictions in hand, students read through the extraction procedure and complete the separatory funnel scheme (Scheme 2b). Even when students understand the chemistry behind what they are doing, they often find the physical manipulations of the separatory funnel difficult. In Scheme 2b, students follow the movement of nile blue from the aqueous layer to the hexanes layer in the separatory funnel. Scheme 2b was designed to challenge students to think about the location of the dye and which layer they will be collecting at each stage of the separation. In addition to tracking the movement of the dye in the separatory funnel, students must show the physical separation of the mixture into beakers and think about which layer to return to the separatory funnel for the second and third extractions. A separatory funnel scheme for 2,6-dichloroindophenol (Supporting Information), which

DOI: 10.1021/acs.jchemed.7b00779
J. Chem. Educ. 2018, 95, 1626–1630

Scheme 3. Acid−Base Reactions for (a) Nile Blue and (b) 2,6-Dichloroindophenol and the Laboratory Extraction of (c) Nile Blue and (d) 2,6-Dichloroindophenol



relies on dichloromethane as the organic solvent, is also completed by students. The diagrams compel students to think about which layer is on the top and which one is on the bottom in an extraction rather than just memorizing a trend. To complete the separatory funnel schemes, students will rely on the insights they gained in parts I and II to assist in making their predictions. After the instructor has checked that students have completed the separatory funnel schemes, students return to the laboratory to test their proposed extractions. Most students in a typical class make good predictions which allows the instructor to focus on guiding students who are struggling.

Part IV: Testing Predictions with Dye Solutions

Students often carry out extractions between two, clear, colorless solutions, which can make it difficult for students to perform confidently. Nile blue and 2,6-dichloroindophenol were selected for this extraction due to their color changes as a function of protonation state (Scheme 3). The color for the protonated form of nile blue does not match its original color because its color is very sensitive to pH. The original color may be reobtained, but it requires more careful control of the pH than is typical in an extraction.[12] 2,6-Dichloroindophenol was observed to change from blue (**D**) to red (**E**) and finally back to blue (**F**) upon extracting with 1 M H_2SO_4/dichloromethane followed by 1 M NaOH/dichloromethane. Using these dyes provides students with a visual confirmation of whether or not their predictions were correct. In a typical section most students correctly predict the partitioning of the dyes and therefore see a positive correlation between their predictions and experimental results.

Parts I−IV Postlaboratory Questions

Students complete postlaboratory questions involving the separation of benzoic acid, ethyl-4-aminobenzoate, and naphthalene, which were the compounds they used for solubility tests in part II. By working through a guided separation scheme, students are prepared to develop their own extraction procedure during the following laboratory period.

Part V: Applications of Knowledge and Skills Gained to a Real-World Problem

Without the necessity of introducing extraction for the first time, the instructor has the opportunity to work with students to propose an extraction procedure instead of following a "cookbook" style procedure. Clove bud oil has been used as a mixture of natural products that students separate by extraction.[13] The separation of simulated methylenedioxypyrovalerone (MDPV) from cutting agents[7] has also been used. Both of these are published experiments, and in principle, the

DOI: 10.1021/acs.jchemed.7b00779
J. Chem. Educ. 2018, 95, 1626−1630

guided inquiry portion of this experiment could be coupled with any traditional extraction laboratory experiment. In each case, students develop an extraction procedure which they are required to explain to the instructor. The instructor requires the students to rework the procedure until they come up with something that makes sense and is safe. Therefore, most students are successful with the physical extraction.

Student Results

The average score for students in 22 sections of the laboratory over 7 years (average 15.9 students per section) was 91.4% for the worksheets at the end of the laboratory exercise (Supporting Information). The exercise was deemed successful because at the end of the semester the average score for the extraction question (based on Postlab Question 2, See Supporting Information) on the final exam was 85.5%.

■ CONCLUSION

Students are guided through several principles of extraction including acid–base reactions, solubility, and solvent density with the ultimate goal for students to be able to plan and execute the separation of a mixture based on knowledge of the molecular structures of the components in the mixture. Colored dyes were used to show movement between layers. Students completed several worksheets designed to map out the process before physically carrying out an extraction. The inclusion of the guided activities and worksheets in this experiment challenged students to think critically about what was actually happening in their separatory funnels.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.7b00779.

Student handouts/worksheets and instructor notes (PDF, DOCX)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: t-celius@onu.edu.

ORCID

Tevye C. Celius: 0000-0002-9302-935X
Ronald C. Peterson: 0000-0001-5998-6116
Amelia M. Anderson-Wile: 0000-0002-8442-0007

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to thank Kelly Hall for help in implementing this lab in our curriculum and the Donald J. Bettinger Department of Chemistry and Biochemistry for supporting the development of this experiment.

■ REFERENCES

(1) Mistry, N.; Fitzpatrick, C.; Gorman, S. Design Your Own Workup: A Guided-Inquiry Experiment for Introductory Organic Laboratory Courses. *J. Chem. Educ.* **2016**, *93* (6), 1091–1095.
(2) Elder, J. W. Microscale Acid-Base Extraction: A Colorful Introduction. *J. Chem. Educ.* **1990**, *67* (3), 266.
(3) Hobbs, G. D.; Woodyard, J. D. A Colorful Extraction Experiment. *J. Chem. Educ.* **1982**, *59* (5), 386.
(4) Horowitz, G. A Discovery Approach to Three Organic Laboratory Techniques: Extraction, Recrystallization, and Distillation. *J. Chem. Educ.* **2003**, *80* (9), 1039–1041.
(5) Kelly, T. R. A Simple, Colorful Demonstration of Solubility and Acid/Base Extraction Using a Separatory Funnel. *J. Chem. Educ.* **1993**, *70* (10), 848–849.
(6) Love, B. Extraction of Organic Compounds from Aqueous Solutions. *J. Chem. Educ.* **1994**, *71* (6), 517–518.
(7) Raydo, M. L.; Church, M. S.; Taylor, Z. W.; Taylor, C. E.; Danowitz, A. M. A Guided Inquiry Liquid/Liquid Extractions Laboratory for Introductory Organic Chemistry. *J. Chem. Educ.* **2015**, *92* (1), 139–142.
(8) Silversmith, E. F. Extraction of Acid with Aqueous Base. *J. Chem. Educ.* **1972**, *49* (12), A694.
(9) Just, J.; Bunton, G. L.; Deans, B. J.; Murray, N. L.; Bissember, A. C.; Smith, J. A. Extraction of Eugenol from Cloves Using an Unmodified Household Espresso Machine: An Alternative to Traditional Steam-Distillation. *J. Chem. Educ.* **2016**, *93* (1), 213–216.
(10) Ntamila, M. S.; Hassanali, A. Isolation of Oil of Clove and Separation of Eugenol and Acetyl Eugenol. An Instructive Experiment for Beginning Chemistry Undergraduates. *J. Chem. Educ.* **1976**, *53* (4), 263.
(11) O'Shea, S. K.; Von Riesen, D. D.; Rossi, L. L. Isolation and Analysis of Essential Oils from Spices. *J. Chem. Educ.* **2012**, *89* (5), 665–668.
(12) Wang, X.-L.; Sun, R.; Zhu, W.-J.; Sha, X.-L.; Ge, J.-F. Reversible Absorption and Emission Responses of Nile Blue and Azure a Derivatives in Extreme Acidic and Basic Conditions. *J. Fluoresc.* **2017**, *27* (3), 819–827.
(13) Williamson, K. L.; Minard, R.; Masters, K. M. *Macroscale and Microscale Organic Experiments*, 5th ed.; Houghton Mifflin: Boston, 2007; pp 115–118.

DOI: 10.1021/acs.jchemed.7b00779
J. Chem. Educ. 2018, 95, 1626–1630