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Laboratory Experiment
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Design, Implementation, and Evaluation of Paper-Based Devices for the Detection of Acetaminophen and Phenacetin in an Advanced Undergraduate Laboratory

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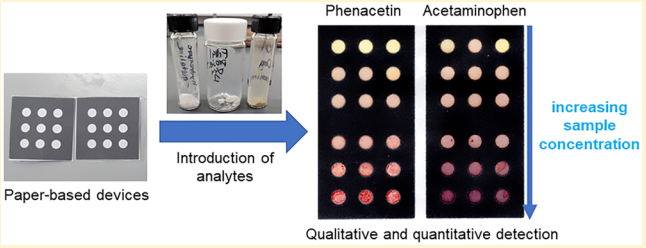
Supporting Information

ABSTRACT: Reported herein is a multidisciplinary experiment for senior-level undergraduate teaching laboratories in the synthesis of the analytes acetaminophen and phenacetin; the fabrication of paper-based devices, using eyeliner, acrylic spray paint, or wax-printing, for sensing of those analytes; and the use of the newly fabricated devices for successful qualitative and quantitative analyte detection. This experiment includes elements of organic, analytical, and materials chemistry, as well as device engineering, and provides a strong pedagogical experience for the undergraduate student participants. The experiment was tested over two years in the Advanced Organic Laboratory, and 90% of students over the two years successfully completed all experimental objectives. The modular nature of the reported experiments and inexpensive costs of materials and instrumentation significantly enhance the practical applicability of this experiment and the likelihood of widespread adaptation.



KEYWORDS: *Hands-On Learning/Manipulatives, Upper-Division Undergraduate, Analytical Chemistry, Interdisciplinary/Multidisciplinary, Organic Chemistry, Applications of Chemistry, Calibration, Dyes/Pigments, Qualitative Analysis, Quantitative Analysis*

INTRODUCTION

The development and implementation of solid-state detection systems for broad varieties of analytes is useful in a number of real-world scenarios, including in public health,[1] national security,[2] and environmental remediation applications.[3] Moreover, it requires significant training in a number of subdisciplines, including analytical chemistry, materials chemistry, and organic and/or inorganic synthesis. The topic of solid-state detection is not often taught at the undergraduate level, likely because of the complexity of the topic and because this topic does not fall into one of the traditional chemistry subdisciplines (organic, inorganic, physical, or analytical chemistry). While interdisciplinary chemistry research, including in the area of chemical detection[4] (and especially solid-state detection),[5] has experienced a resurgence in recent decades, interdisciplinary chemistry education has been slower to follow suit.[6−9] The lack of interdisciplinary education means that graduates of traditional chemistry programs may not have the skills necessary to engage in interdisciplinary chemistry research and will be ill-prepared for professional employment and/or for a graduate career in such areas.

To address this gap between significant interdisciplinary work in the chemical enterprise and less interdisciplinary undergraduate chemistry education, our laboratory has developed a number of new experiments for the undergraduate teaching laboratory that have a strong interdisciplinary focus and substantial pedagogical advantages. In particular, we have previously reported the synthesis and analysis of a near-infrared emitting squaraine fluorophore;[10] the synthesis and characterization of a fluorescent conjugated polymer and the subsequent fabrication of nanoparticles and thin films;[11] and the synthesis, analysis, and practical applications of a cyclodextrin-containing metal−organic framework for environmental pollutant removal.[12]

In continuation of our goals to create relevant and interdisciplinary experiments for undergraduate teaching laboratories, we focused next on the area of colorimetric chemical detection, an active research topic in our own group.[13−15] Chemical detection, while a highly active research area in general,[16−18] has only been reported for undergraduate teaching laboratories in isolated cases,[19−24] and the scope of analytes and detection methods have been fairly limited. Colorimetric detection, or detection based on the changes in color of a transducing signaling element, has a number of advantages in practical detection applications due to the ability to observe color changes using naked eye detection.[25] This also has been introduced, in limited examples, in the undergraduate[26] (and even high school)[27] laboratories, usually in

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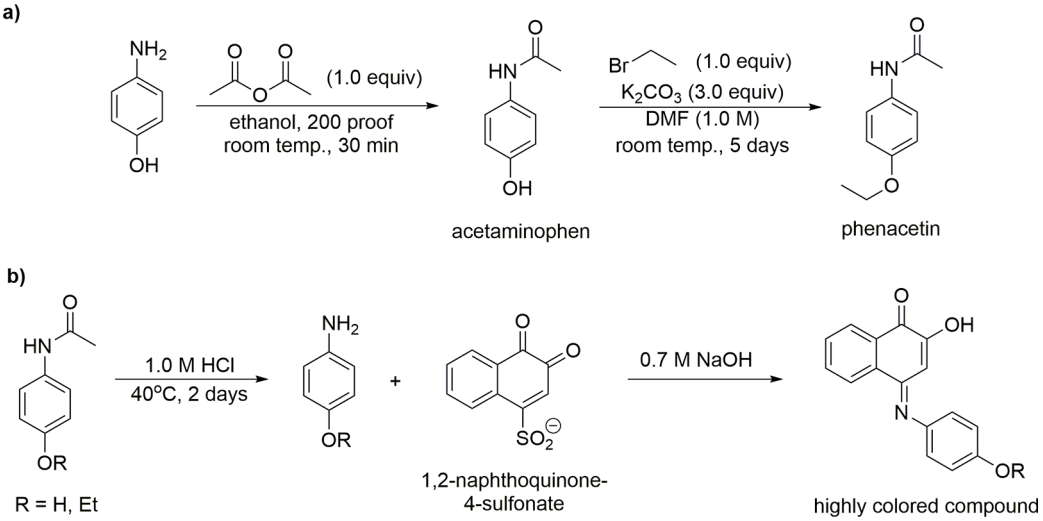
Scheme 1. (a) Synthesis of Acetaminophen and Phenacetin and (b) Detection of Acetaminophen and Phenacetin Using the Chromophore 1,2-Naphthoquinone-4-sulfonate



analytical chemistry settings, for the detection of a variety of high impact analytes. Moreover, paper-based devices have become widely used materials for qualitative and quantitative colorimetric chemical detection in the industry.[28−33] Therefore, this area was deemed important for inclusion in an undergraduate curriculum, with the goal of introducing students to state-of-the-art technologies that could be applicable to their future careers.

Reported herein is the design, implementation, and evaluation of an interdisciplinary experiment for the undergraduate chemistry laboratory focused on paper-based devices. This integrated experiment includes two topics that are of substantial interest in both the chemistry education and chemistry research communities: solid-state (in particular paper-based) device fabrication[34] and colorimetric detection.[35] As part of the experiment, students synthesize and purify acetaminophen and phenacetin (Scheme 1a), two compounds that are of interest in forensic analyses because they can be used to adulterate illicit materials, that are subsequently detected using the chromophore 1,2-naphthoquinone 4-sulfonate (Scheme 1b).[36] To achieve detection, students fabricate paper-based devices using an inexpensive filter paper medium with hydrophilic barriers formed from wax-based eyeliner,[35,37] acrylic spray paint,[38,39] or wax-printing[40] (three technically easy and robust methods for doing so); then introduce a color-changing chromophore to the paper-based device; and use the colorimetric response of the chromophore as a way to monitor and detect the presence of the newly synthesized analytes. This experiment has elements of organic chemistry (in the synthesis and purification of analytes), analytical chemistry (in the color-based analyte detection system), materials chemistry (in the device fabrication), and colorimetric analysis in the design and use of the color-changing chromophore, which together provide a truly unique, interdisciplinary educational experience for undergraduate students. This combination of paper-based devices, colorimetric detection, and analyte synthesis is relatively unique in the chemical literature and has robust pedagogical advantages associated with this interdisciplinary approach.

EXPERIMENTAL PROCEDURES

Materials and Methods

All chemicals were received from Sigma-Aldrich or from Fisher Scientific and were used as received. IR spectra were acquired using a Shimadzu IRAffinity-1S FTIR spectrometer with Miracle 10 single reflection ATR accessory. NMR spectra were acquired using a Bruker Ascend 400 MHz spectrometer. Eyeliner (Wet n Wild H2O Proof Liquid Eyeliner), 6 mm diameter stickers (hole punched from a larger sticker using a 6 mm hole puncher), clear packing tape, and black Valspar High-Gloss Lacquer were obtained from a local CVS Pharmacy or hardware store. Whatman #4 filter paper was obtained from Fisher Scientific both as 55 mm circles, for the spray-painting procedure, and as 460 mm × 570 mm sheets that were cut into 8.5 in. × 11 in. sheets for use in the wax printer or 2 in. × 2 in. sheets for use in the eyeliner procedure. A Xerox Color Qube 8580 was used for wax-printing. A free trial of Adobe Illustrator was downloaded for use in the wax-printing part of this experiment, and the free program ImageJ was downloaded for the color analysis part of this experiment. Micropipettes were purchased from Fisher Scientific and used as received.

Analyte Synthesis

A 1.00 g portion of the assigned aminophenol (*ortho*, *meta*, or *para*) and an equimolar amount of acetic anhydride were combined in absolute (200 proof) ethanol and allowed to mix at room temperature for 30 min to form acetaminophen or an acetaminophen structural isomer. Details of the reaction workup varied depending on the aminophenol starting material but included rotary evaporation of the ethanol solvent and recrystallization of the crude reaction product to obtain pure material. A small amount of this material (25−50 mg) was set aside for later detection. The phenacetin analyte was formed by combining the remaining acetaminophen, or acetaminophen analogue, with an equimolar amount of bromoethane and 3 equiv of potassium carbonate in dimethylformamide. The solution was left to stir at room temperature for 5 days (until the next lab period), when it was extracted into ethyl acetate and isolated via rotary evaporation. All intermediates and final products were characterized by ^{1}H NMR and ^{13}C NMR (both in DMSO-d_6) and IR spectroscopy, and copies of student-

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acquired spectra are included in the Supporting Information. The samples were then treated with 1.0 M HCl at 40 °C (at a concentration of 1 mg analyte/mL 1.0 M HCl) for 2 days in order to deprotect the amine moiety in preparation for the reaction with sodium 1,2-naphthoquinone-4-sulfonate.

Fabrication of Paper-Based Devices

The fabrication of paper-based devices was testing using three distinct procedures in the first year of implementation, with the wax-printing method (#3) used exclusively in the second year.

(1) Using eyeliner: Nine 6 mM diameter stickers were placed on a 2 in. × 2 in. piece of Whatman #4 filter paper in a 3 × 3 array, and liquid eyeliner was used to draw borders (approximately 2 mm thick) around the edge of each sticker. After removal of the stickers via tweezers, the colored filter paper was heated in an oven at 125 °C for 10 s to partially melt the wax in the eyeliner and fix the position of the circles. Clear packing tape was used to coat the back of the device so that the solution would not bleed through.

(2) Using acrylic lacquer: Nine 6 mm diameter stickers were placed in a 3 × 3 array on a 55 mm circle of Whatman #4 filter paper, with space between each sticker. The filter paper was flipped over, and nine additional stickers were placed on the opposite side of the paper in exactly the same positions as the first 9 stickers. The filter paper was then placed on a Buchner filter funnel that was large enough to fit the full paper without bending the edges. The vacuum was turned on fully, and acrylic lacquer was used to spray each side of the paper. After full drying of the lacquer, all stickers were removed. Clear tape was again placed on the back of the device to ensure that the solution would not bleed through.

(3) Using a wax printer (2017 semester): Adobe Illustrator was used to pattern a wax-printed device with a 3 × 3 pattern of 6 mm diameter circles, and a wax printer that was connected to Illustrator was used to print the wax device as designed on the program. Once printed, the wax device was heated at 125 °C for 2 min in the oven to ensure that all barriers were fully melted and that the pattern was set on the filter paper. Clear packing tape was placed on the back of the device to ensure that solution would not bleed through.

(4) Using a wax printer (2018 semester): Adobe Illustrator was used to pattern two wax-printed devices with a 3 × 6 pattern of 6 mm diameter circles (for the calibration curves), and one device with a 3 × 3 pattern of 6 mm diameter circles (for the unknown analysis), and a wax printer that was connected to Illustrator was used to print the wax device as designed on the program. Once printed, the wax device was heated at 125 °C for 2 min in the oven to ensure that all barriers were fully melted and that the pattern was set on the filter paper. Clear packing tape was placed on the back of the device to ensure that solution would not bleed through.

Use of Paper-Based Devices

Each patterned device was treated with 6 μL of the chromophore solution (2.0 mg/mL of sodium 1,2-naphthoquinone 4-sulfonate in deionized (DI) water) and 6 μL of sodium hydroxide solution (27.2 mg/mL in DI water). Without allowing the device to dry, 4 μL portions of the phenol analytes (1 mg/mL solution in 1.0 M HCl) were added to each of the chromophore spots, following the general schematic shown in Figure 1, with the three circles per row

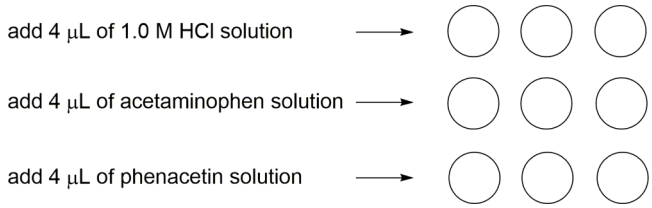

Figure 1. Placement of blank and analyte solutions on the paper-based devices for analysis.

used for triplicate measurements of the same sample. Finally, 2 μL of cetyltrimethylammonium bromide (2.0 mg/mL in DI water) was added as a color stabilizer. After drying of those analytes and comparison to a control sample of 4 μL of 1.0 M HCl (lacking any phenol analyte but using otherwise identical conditions), a scan of the filter paper-based device was taken, using an EPSON V19 Perfection flatbed scanner, and the colors in the photo were used to indicate the presence of the target analyte. This procedure was also used for unknown analysis (vide supra).

Generation of Calibration Curves

Analyte solutions of the concentrations 0.5, 0.25, 0.125, and 0.0625 mg/mL in 1.0 M HCl were created by serial dilution from the previously prepared 1 mg/mL solution for both acetaminophen and phenacetin. The first 3 × 6 patterned device was treated with 6 μL of the chromophore solution (2.0 mg/mL of sodium 1,2-naphthoquinone-4-sulfonate in DI water) and 6 μL of sodium hydroxide solution (27.2 mg/mL in DI water). Without allowing the device to dry, 4 μL of the acetaminophen solutions, or 4 μL of 1.0 M HCl (for the control), was added to each of the hydrophilic zones, following the general schematic shown in Figure 2, with the three circles

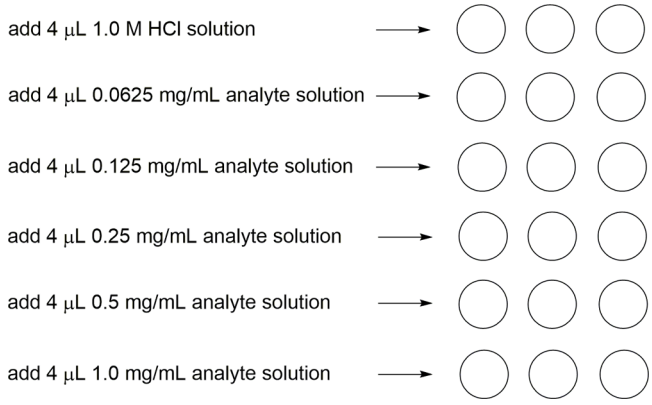

Figure 2. Placement of blank and analyte solutions on the paper-based devices for calibration curve.

per row used for triplicate measurements of each sample. Finally, 2 μL of cetyltrimethylammonium bromide (2.0 mg/mL in DI water) was added as a color stabilizer to each hydrophilic zone. This procedure was repeated on the second 3 × 6 patterned device for the phenacetin solutions. After the devices had dried completely, scans were taken using an EPSON V19 Perfection flatbed scanner, and ImageJ was used to collect the mean gray value (MGV) for each individual zone. The average values for each trial were used to create a

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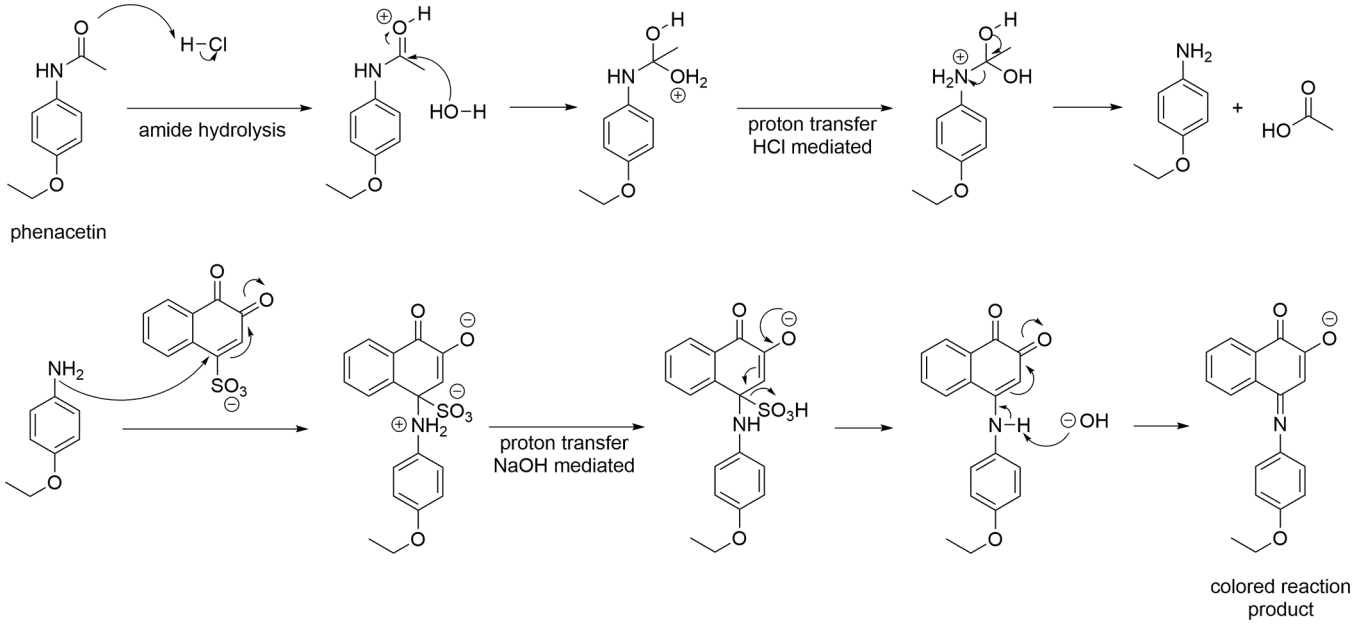
Scheme 2. Mechanism for the Formation of the Colored Product from the Reaction of Phenacetin with 1,2-Naphthoquinone-4-sulfonate



calibration curve, and the trendline of the curve, found using Excel, was used for unknown quantification.

HAZARDS

Personal protective equipment, including laboratory goggles, gloves, and a laboratory coat, was worn at all times during this experiment. Chemical hazards include the following: acetic anhydride is flammable, can cause severe burns to skin and eyes, and is harmful if ingested; *p*-aminophenol is harmful if ingested and is very toxic to aquatic life; *m*-aminophenol is harmful if ingested and is very toxic to aquatic life; *o*-aminophenol is harmful if ingested; ethanol is very flammable and can damage organs if swallowed; acetaminophen is a skin, eye, and respiratory irritant and is toxic in large doses; *N,N*-dimethylformamide is flammable and is a skin, eye, and respiratory irritant; bromoethane is highly flammable, harmful if ingested, and a suspected carcinogen; potassium carbonate can cause skin and eye irritation; phenacetin is a suspected carcinogen and toxic in large doses; cetyltrimethylammonium bromide is a skin, eye, and respiratory irritant, is harmful if ingested, and is toxic to aquatic life; 1,2-naphthoquinone-4-sulfonic acid sodium salt is an inhalation hazard; and sodium hydroxide is corrosive. Hazards from heat and hot ovens include the possibility of burns. Hazards from spray-painting include danger from inhalation. NMR instruments produce a strong magnetic field, and people with pacemakers (or other similar devices) are advised to consult with their physician before entering a place that houses an NMR spectrometer.

RESULTS AND DISCUSSION

Pedagogical Goals

This experiment teaches a number of important laboratory techniques and requires student knowledge in a number of key chemistry subject areas, including organic, materials, and analytical chemistry, leading to a high pedagogical impact of this experiment for upper-level chemistry majors who are preparing for future employment or graduate school, where they will likely use many of these interdisciplinary techniques. The introduction of the chromophoric (i.e., color-changing) element required discussion around the mechanism of the reaction between the analyte and the chromophore, and how the electronic and chemical structure of the reaction product relates to its coloration. The students were also introduced to paper-based devices and microscale reactions for the first time, prompting discussions regarding the immobilization of the microscale reaction on a paper substrate using simple hydrophobic barriers, the ease and cost-effectiveness of such devices, and their usefulness in situations where more expensive, instrumentation-based analyses cannot be implemented. Finally, the students were asked to create serial dilutions of their synthesized analytes in order to create calibration curves for their detection with 1,2-naphthoquinone-4-sulfonate. Through this, the students realized how analytical techniques are typically used for unknown identification

The detection of phenacetin and acetaminophen in basic conditions using sodium 1,2-naphthoquinone-4-sulfonate chromophore on a paper-based device has been reported[36] and was modified by the authors for use in this undergraduate laboratory experiment. In particular, the first part of the experiment requires chemical synthesis of the analytes (Scheme 1a). Acetaminophen and acetaminophen isomers were formed from reacting the appropriate isomer of aminophenol (*o*-, *m*-, or *p*-) with acetic anhydride in 200 proof ethanol.[41] After stirring at room temperature, the crude products were isolated and recrystallized. In the same lab period, the students synthesized phenacetin and phenacetin isomers by placing their acetaminophen product into a round-bottom flask with *N,N*-dimethylformamide, bromoethane, and potassium carbonate. While this reaction is reportedly conducted at elevated temperatures for several hours,[42] it was found to be efficient at room temperature over 5−7 days. Typically, the majority of acetylation and alkylation reactions gave 80−90% yields, with the acetylation reaction to produce *o*-acetaminophen and the alkylation reaction to produce *m*-phenacetin producing slightly lower yields between 40% and

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60%. The students collected ^{1}H and ^{13}C NMR spectra and IR spectra of their products, examples of which can be found in the Supporting Information. All students were able to determine the purity of the products using their NMR spectra by identifying all relevant peaks in both the ^{1}H and ^{13}C spectra. Isomers could clearly be differentiated by differences in the aromatic regions of the ^{1}H and ^{13}C NMR spectra, and the full conversion of acetaminophen to phenacetin was evident due to the disappearance of the alcohol peaks in the IR spectrum and the appearance of ethyl proton peaks in the ^{1}H spectrum.

This synthesis provides an opportunity for the instructor to review important concepts in organic synthesis, including the reactivity of all starting materials compared to products; the stoichiometry, solvent choice, and reaction conditions used under the optimized conditions; and the purification techniques used, including rotary evaporation and recrystallization. Many of the students collected ^{13}C NMR spectra for the first time in this portion of the experiment and were asked to analyze their products for purity before continuing on to the detection portion of this experiment, an important practice to instill in teaching laboratories. All students in the laboratory were able to accomplish the synthesis successfully, although some struggled with the purification of phenacetin when residual *N,N*-dimethylformamide solvent resulted in an oily crude product, an issue which can be solved by taking the phenacetin up in ethyl acetate and washing several times with a large amount of water. This yielded product that was sufficiently pure for the next step of the project.

The formation of acetaminophen and acetaminophen derivatives by an amine acylation and the formation of phenacetin and phenacetin derivatives by a nucleophilic addition of the phenol alcohol with bromoethane are mechanisms that the students will have learned in sophomore organic chemistry. The mechanism for the formation of the colored product, however, is more complex (Scheme 2) and first involves the acid-promoted deprotection of the acyl amine of either of the analytes to form the primary amine. Following this, in basic conditions, the primary amine acts as a nucleophile in a Michael-type addition to 1,2-naphthoquinone-4-sulfonate. The sulfonate group is then removed in the re-forming of the α,β-unsaturated ketone. The basicity of the solution then promotes the formation of an imine in conjugation with an enolate and thus the final product. It is important for students to realize the highly conjugated nature of the product and the connection between conjugation and visible color. The reported reaction products of acetaminophen and phenacetin with sodium naphthoquinone-4-sulfonate are dark blue/purple and red, respectively.[36] Using modified versions of these analytes (i.e., with *ortho* and *meta* substitution rather than *para*) resulted in differently colored products (Figure 3) which introduced students to the relationship between electronic structure in conjugated small molecules and observable colors. Students realized that differences in the electronic structure of an analyte change the electronic structure, and therefore the color, of the product.

The fabrication of devices introduced students to important concepts in hydrophobic and hydrophilic surfaces, including the fact that the Whatman filter paper is hydrophilic, that the eyeliner, spray paint, and wax are all hydrophobic, and that simple heating of the hydrophobic barriers is generally sufficient to fix the barriers in place, immobilize the reagents, and prevent mixing between sampling sites. Students were intrigued by the fact that materials they had seen previously

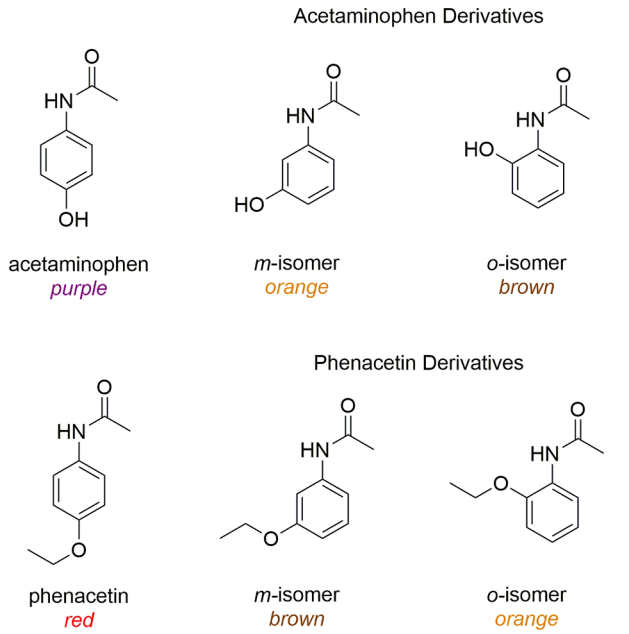

Figure 3. Differences in product color upon reaction of analytes with 1,2-naphthoquinone-4-sulfonate.

(especially the eyeliner) could be used to accomplish an important scientific objective. Pictures of devices created by students are shown in Figure 4. Although many of these techniques for device fabrication and hydrophobically driven immobilization are standard in materials science and solid-state chemistry, it was the first time the students had fabricated such devices, and the pedagogical opportunities around solid-state chemistry were abundant. The use of the wax printer and Adobe Illustrator to make devices and ImageJ to analyze devices provided additional pedagogical opportunities for the instructor to introduce students, often for the first time, to technologies that are common in solid-state detection.

The final component of this laboratory, the colorimetric detection of analytes, included significant amounts of information pertaining to analytical chemistry and colorimetric detection methods, including the use of mean gray values (MGVs), obtained from RGB analysis, and the application of calibration curves for quantitative colorimetric detection. The students were introduced to the rigorous analytical techniques required to create serial dilutions, the methods for application of those serial dilutions to the creation of a calibration curve, and finally, the use of that calibration curve for the quantitative analysis of an unknown sample provided by the teaching assistant. Using ImageJ, the MGV of each of the hydrophilic zones was obtained. Graphing the average MGV against the known analyte concentrations provided linear curves for which trendlines were found using Excel (examples of which can be found in the SI). The trendlines were then used to find the concentration of unknown samples based on the MGV. This quantitative analysis was compared against the student's initial, qualitative assessment of the unknown concentration, reaffirming the concept that pairing a color-changing detection scheme with computer-based analysis leads to more accurate quantitation. An example of student calibration curves and unknown comparison is shown in Figure 5.

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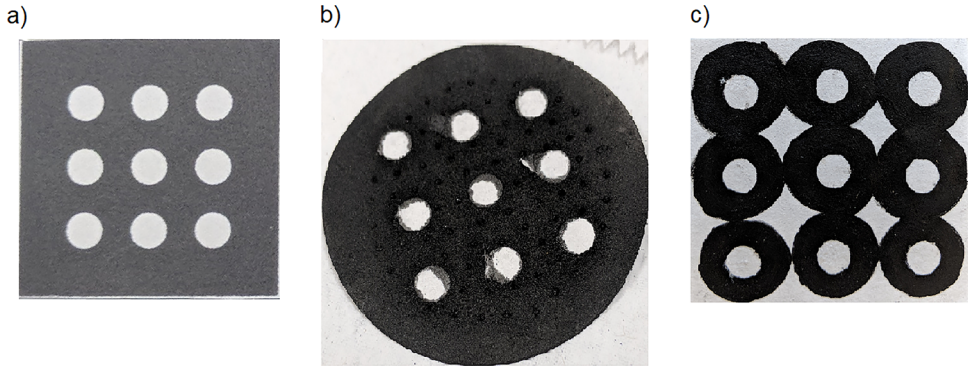

Figure 4. Student-created paper-based devices made from (a) wax-printing, (b) spray-painting, and (c) eyeliner.

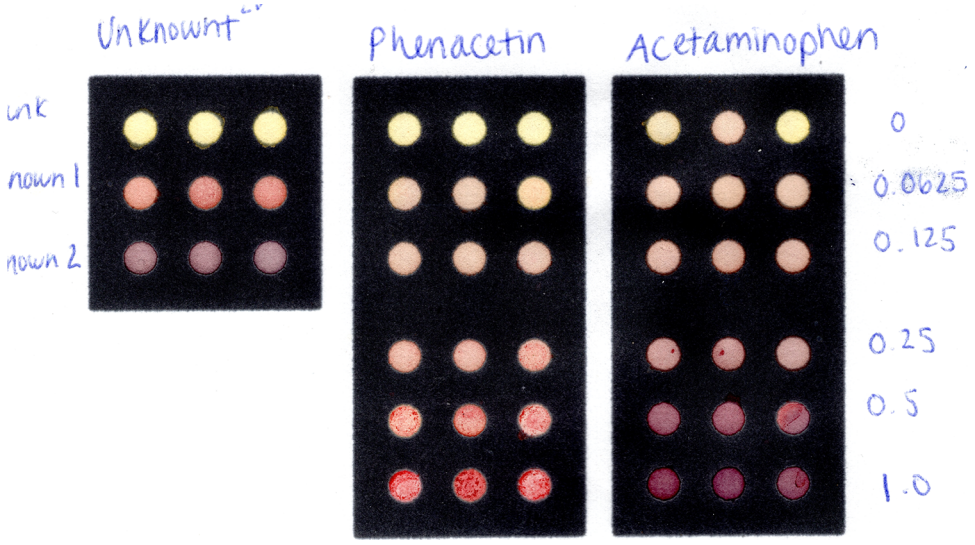

Figure 5. Student-created *p*-acetaminophen and *p*-phenacetin calibration curves, and an unknown comparison scanned using a flatbed scanner.

Student Responses

This experiment was first tested as a final, collaborative experiment in the Advanced Organic Chemistry Laboratory at the University of Rhode Island in the Fall of 2017, where various methods for the fabrication of the wax barriers on paper-based devices were tested, including the use of wax-based eyeliner, acrylic spray paint, and a wax printer. The nine students in the class were split into three groups, with each student in the group beginning with a different starting material (*o*-, *m*-, or *p*-aminophenol), and using a different method for wax barrier formation (wax-based eyeliner, spray paint, or wax-printing). The experiment was summarized in a formal lab report in which the grouped students share results to answer a series of in-depth discussion questions, provided in the Supporting Information, on the outcome of using different analytes and different methods for paper-based device fabrication. For the students in the Fall 2017 laboratory, the following results were observed: 100% students successfully fabricated the devices; 100% made and purified the analytes; and 77% were able to detect the analytes using the devices they had made. Issues with the analyte detection component stemmed from student error in formulating correct concentrations of the chromophore, which we plan to address with the use of more accurate balances in the laboratory. More accurate balances could not be incorporated into the Fall 2018 semester, however, and thus correctly prepared solutions of the chromophore were provided by the teaching assistant (T.L.M.).

In the Fall 2018 Advanced Organic Chemistry Laboratory, the experiment was incorporated as a regular experiment module. On the basis of Fall 2017 student feedback and input from the teaching assistant (T.L.M.), wax-printing was identified as the optimal method of device fabrication, and *p*-aminophenol as the optimal starting material for organic syntheses, as the *para* isomers generally led to higher product yields and darker colors upon reacting with NQS; these were used for the entire student population in the Fall 2018 laboratory. For the students in the Fall 2018 laboratory, the following results were observed: 100% students successfully fabricated the devices; 100% made and purified acetaminophen; 50% made and purified phenacetin (with the relatively low percentage attributed to technical difficulties in the laboratory not associated with the experimental procedure); and 92% were able to detect the analytes using the devices they had made, with errors here stemming from students using micropipettes incorrectly. In this second iteration, a calibration curve and unknown concentration analysis were implemented with the goal of achieving quantitative detection, and 50% of students accurately determined the concentration of their unknown solution. Overall, student feedback from both years was highly positive and included positive statements such as, "I enjoyed seeing the color change." 100% of the respondents across both years of implementation agreed or strongly agreed

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with the statement, "I think the instructor should use the solid-state sensing experiment in future classes."

CONCLUSION

This experiment is highly modular, in that it can be adapted for laboratories that wish to focus more specifically on one area. For example, an analytical laboratory could purchase the required analytes, thereby skipping the synthetic component, and focus entirely on colorimetric analysis and detection. A materials science laboratory can focus more on the optimization of device fabrication methods, and less on both synthesis and colorimetric analysis. Significant cost savings are also accessible through the use of inexpensive eyeliner-based devices and would accomplish largely the same pedagogical objectives.

In conclusion, the highly interdisciplinary experiment reported herein contains important aspects of a variety of areas of chemistry, including synthetic organic chemistry, materials science, and analytical chemistry, and fills a pedagogical need for teaching truly interdisciplinary science to undergraduate chemistry students. On the basis of our implementation of this experiment over two years in the advanced undergraduate organic laboratory in our department, we have demonstrated high modularity of the experiment; a variety of experimental options for fitting time, budgetary, and pedagogical constraints; and strong positive feedback from students who participated in the experiment. Current efforts in our laboratory are focused on ways to continue to develop interdisciplinary undergraduate experiments, and the results of these and other investigations will be reported in due course.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.9b00028.

Student handouts, discussion questions and answers, copies of student-acquired spectra, photographs of student-fabricated devices, and student-derived calibration curves (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Song, J.; Mauk, M. G.; Hackett, B. A.; Cherry, S.; Bau, H. H.; Liu, C. Instrument-Free Point-of-Care Molecular Detection of Zika Virus. *Anal. Chem.* **2016**, *88* (14), 7289−7294.

(2) Thomas, S. W., III; Amara, J. P.; Bjork, R. E.; Swager, T. M. Amplifying Fluorescent Polymer Sensors for the Explosives Taggant 2,3-Dimethyl-2,3-Dinitrobutane (DMNB). *Chem. Commun.* **2005**, No. 36, 4572−4574.

(3) Khairy, M.; El-Safty, S. A.; Shenashen, M. A. Environmental Remediation and Monitoring of Cadmium. *TrAC, Trends Anal. Chem.* **2014**, *62*, 56−68.

(4) Mako, T. L.; Racicot, J. M.; Levine, M. Supramolecular Luminescent Sensors. *Chem. Rev.* **2019**, *119* (1), 322−477.

(5) Farrell, M. E.; Pellegrino, P. M. Army Relevant Biological Hazards Detection with Commercial SERS Substrates. *Proc. SPIE* **2012**, *8460*, 84600J.

(6) Hooker, P. D.; Deutschman, W. A.; Avery, B. J. The Biology and Chemistry of Brewing: An Interdisciplinary Course. *J. Chem. Educ.* **2014**, *91* (3), 336−339.

(7) Rapp, T. L.; Phillips, S. R.; Dmochowski, I. J. Kinetics and Photochemistry of Ruthenium Bisbipyridine Diacetonitrile Complexes: An Interdisciplinary Inorganic and Physical Chemistry Laboratory Exercise. *J. Chem. Educ.* **2016**, *93* (12), 2101−2105.

(8) Cowden, C. D.; Santiago, M. F. Interdisciplinary Explorations: Promoting Critical Thinking via Problem-Based Learning in an Advanced Biochemistry Class. *J. Chem. Educ.* **2016**, *93* (3), 464−469.

(9) Ramalho, M. J.; Pereira, M. C. Preparation and Characterization of Polymeric Nanoparticles: An Interdisciplinary Experiment. *J. Chem. Educ.* **2016**, *93* (8), 1446−1451.

(10) Marks, P.; Levine, M. Synthesis of a Near-Infrared Emitting Squaraine Dye in an Undergraduate Organic Laboratory. *J. Chem. Educ.* **2012**, *89* (9), 1186−1189.

(11) Mako, T.; Levine, M. Synthesis of a Fluorescent Conjugated Polymer in the Undergraduate Organic Teaching Laboratory. *J. Chem. Educ.* **2013**, *90* (10), 1376−1379.

(12) Jones, D. R.; DiScenza, D. J.; Mako, T. L.; Levine, M. Environmental Application of Cyclodextrin Metal-Organic Frameworks in an Undergraduate Teaching Laboratory. *J. Chem. Educ.* **2018**, *95* (9), 1636−1641.

(13) Serio, N.; Moyano, D. F.; Rotello, V. M.; Levine, M. Array-Based Detection of Persistent Organic Pollutants via Cyclodextrin Promoted Energy Transfer. *Chem. Commun.* **2015**, *51* (58), 11615−11618.

(14) Chaudhuri, S.; DiScenza, D. J.; Smith, B.; Yocum, R.; Levine, M. Array-Based Detection of Isomeric and Analogous Analytes Employing Synthetically Modified Fluorophore Attached β-Cyclodextrin Derivatives. *New J. Chem.* **2017**, *41* (23), 14431−14437.

(15) DiScenza, D. J.; Lynch, J.; Miller, J.; Verderame, M.; Levine, M. Detection of Organochlorine Pesticides in Contaminated Marine Environments via Cyclodextrin-Promoted Fluorescence Modulation. *ACS Omega* **2017**, *2* (12), 8591−8599.

(16) McEwen, C. N.; Ligler, F. S.; Swager, T. M. Chemical and Biological Detection. *Chem. Soc. Rev.* **2013**, *42* (22), 8581−8583.

(17) Miranda, O. R.; Creran, B.; Rotello, V. M. Array-Based Sensing with Nanoparticles: 'Chemical Noses' for Sensing Biomolecules and Cell Surfaces. *Curr. Opin. Chem. Biol.* **2010**, *14* (6), 728−736.

(18) Diehl, K. L.; Anslyn, E. V. Array Sensing Using Optical Methods for Detection of Chemical and Biological Hazards. *Chem. Soc. Rev.* **2013**, *42* (22), 8596−8611.

(19) Smith, M. K.; Martin-Peralta, D. G.; Pivak, P. A.; Mirica, K. A. Fabrication of Solid-State Gas Sensors by Drawing: An Undergraduate and High School Introduction to Functional Nanomaterials and Chemical Detection. *J. Chem. Educ.* **2017**, *94*, 1933−1938.

(20) Hobbs, J. M.; Patel, N. N.; Kim, D. W.; Rugutt, J. K.; Wanekaya, A. K. Glucose Determination in Beverages Using Carbon Nanotube Modified Biosensor: An Experiment for the Undergraduate Laboratory. *J. Chem. Educ.* **2013**, *90*, 1222−1226.

(21) Rosu, C.; Cueto, R.; Veillon, L.; David, C.; Laine, R. A.; Russo, P. S. Discovering Volatile Chemicals from Window Weatherstripping

DOI: 10.1021/acs.jchemed.9b00028
J. Chem. Educ. 2019, 96, 1719−1726

through Solid-Phase Microextraction/Gas Chromatography-Mass Spectrometry. *J. Chem. Educ.* **2017**, *94* (11), 1784−1789.
(22) He, P.; Colon, L. A.; Aga, D. S. Determination of Total Arsenic and Speciation in Apple Juice by Liquid Chromatography-Inductively Coupled Plasma Mass Spectrometry: An Experiment for the Analytical Chemistry Laboratory. *J. Chem. Educ.* **2016**, *93* (11), 1939−1944.
(23) Spradlin, C.; Sullivan, M.; Tolias, S.; Lunsford, S. Differential Pulse Voltammetry: A Versatile Approach to Detection of Phenol-A Common Ecosystem Contaminant. *Chem. Educator* **2015**, *20*, 39−41.
(24) Nievas, F. L.; Bogino, P. C.; Giordano, W. Programmed Lab Experiments for Biochemical Investigation of Quorum-Sensing Signal Molecules in Rhizospheric Soil Bacteria. *Biochem. Mol. Biol. Educ.* **2016**, *44* (3), 256−262.
(25) Tang, L.; Li, J. Plasmon-Based Colorimetric Nanosensors for Ultrasensitive Molecular Diagnostics. *ACS Sensors* **2017**, *2* (7), 857−875.
(26) Prabpal, J.; Vilaivan, T.; Praneenararat, T. Paper-Based Heavy Metal Sensors from the Concise Synthesis of an Anionic Porphyrin: A Practical Application of Organic Synthesis to Environmental Chemistry. *J. Chem. Educ.* **2017**, *94* (8), 1137−1142.
(27) Mendez, J. D. An Inexpensive 3D Printed Colorimeter. *Chem. Educator* **2015**, *20*, 224−226.
(28) Martinez, A. W.; Phillips, S. T.; Carrilho, E.; Thomas, S. W., III; Sindi, H.; Whitesides, G. M. Simple Telemedicine for Developing Regions: Camera Phones and Paper-Based Microfluidic Devices for Real-Time, Off-Site Diagnostics. *Anal. Chem.* **2008**, *80*, 3699−3707.
(29) Carrilho, E.; Martinez, A. W.; Whitesides, G. M. Understanding Wax Printing: A Simple Micropatterning Process for Paper-Based Microfluidics. *Anal. Chem.* **2009**, *81*, 7091−7095.
(30) Vella, S. J.; Beattie, P.; Cademartiri, R.; Laromaine, A.; Martinez, A. W.; Phillips, S. T.; Mirica, K. A.; Whitesides, G. M. Measuring Markers of Liver Function Using a Micropatterned Paper Device Designed for Blood from a Fingerstick. *Anal. Chem.* **2012**, *84*, 2883−2891.
(31) Hofstetter, J. C.; Wydallis, J. B.; Neymark, G.; Reilly, T. H., III; Harrington, J.; Henry, C. S. Quantitative Colorimetric Paper Analytical Devices Based on Radial Distance Measurements for Aqueous Metal Determination. *Analyst* **2018**, *143*, 3085−3090.
(32) Nilghaz, A.; Lu, X. Detection of Antibiotic Residues in Pork using Paper-Based Microfluidic Device Coupled with Filtration and Concentration. *Anal. Chim. Acta* **2019**, *1046*, 163−169.
(33) de Oliveira, T. R.; Fonseca, W. T.; de Oliveira Setti, G.; Faria, R. C. Fast and Flexible Strategy to Produce Electrochemical Paper-Based Analytical Devices using a Craft Cutter Printer to Create Wax Barrier and Screen-Printed Electrodes. *Talanta* **2019**, *195*, 480−489.
(34) Fernandes, S. C.; Walz, J. A.; Wilson, D. J.; Brooks, J. C.; Mace, C. R. Beyond Wicking: Expanding the Role of Patterned Paper as the Foundation for an Analytical Platform. *Anal. Chem.* **2017**, *89* (11), 5654−5664.
(35) Sriram, G.; Bhat, M. P.; Patil, P.; Uthappa, U. T.; Jung, H.-Y.; Altalhi, T.; Kumeria, T.; Aminabhavi, T. M.; Pai, R. K.; Madhuprasad; Kurkuri, M. D. Paper-Based Microfluidic Analytical Devices for Colorimetric Detection of Toxic Ions: A Review. *TrAC, Trends Anal. Chem.* **2017**, *93*, 212−227.
(36) da Silva, G. O.; de Araujo, W. R.; Paixão, T. R. L. C. Portable and Low-Cost Colorimetric Office Paper-Based Device for Phenacetin Detection in Seized Cocaine Samples. *Talanta* **2018**, *176*, 674−678.
(37) Veerubhotla, R.; Das, D.; Pradhan, D. A Flexible and Disposable Battery Powered by Bacteria Using Eyeliner Coated Paper Electrodes. *Biosens. Bioelectron.* **2017**, *94*, 464−470.
(38) Chen, B.; Kwong, P.; Gupta, M. Patterned Fluoropolymer Barriers for Containment of Organic Solvents within Paper-Based Microfluidic Devices. *ACS Appl. Mater. Interfaces* **2013**, *5* (23), 12701−12707.
(39) Nurak, T.; Praphairaksit, N.; Chailapakul, O. Fabrication of Paper-Based Devices by Lacquer Spraying Method for the Determination of Nickel(II) Ion in Waste Water. *Talanta* **2013**, *114*, 291−296.
(40) Altundemir, S.; Uguz, A. K.; Ulgen, K. A Review on Wax Printed Microfluidic Paper-Based Devices for International Health. *Biomicrofluidics* **2017**, *11* (4), 041501.
(41) Bernhard, Y.; Winckler, P.; Chassagnon, R.; Richard, P.; Gigot, E.; Perrier-Cornet, J.-M.; Decreau, R. A. Subphthalocyanines: Addressing Water-Solubility, Nano-Encapsulation, and Activation for Optical Imaging of B16 Melanoma Cells. *Chem. Commun.* **2014**, *50*, 13975−13978.
(42) Reddy, M. G.; Varathan, E.; Lobo, N. P.; Easwaramoorthi, S.; Narasimhaswamy, T.; Mandal, A. B. Three-Ring-Based Thermotropic Mesogens with a Dimethylamino Group: Structural Characterization, Photophysical Properties, and Molecular Order. *J. Phys. Chem. C* **2015**, *119*, 9477−9487.

DOI: 10.1021/acs.jchemed.9b00028
J. Chem. Educ. 2019, 96, 1719−1726