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Article

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Fabrication of Chromatographic Devices for Screening Cosmetics for Hydroquinone and Retinoic Acid as a Chemistry Project To Connect with the Community

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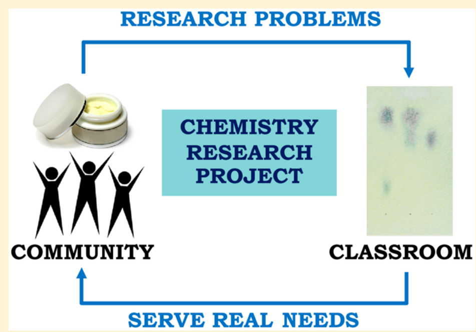
Supporting Information

ABSTRACT: This article demonstrates how a student research project could connect classroom and community. Using local citizens' concerns about the adulteration of cosmetics by prohibited substances as a research problem, fifth-year pharmaceutical chemistry students were challenged to use their knowledge to create cost-effective and environmentally friendly chromatographic devices from easily obtainable materials. After validation of the procedure, the devices were utilized for screening skin-whitening and antiwrinkle cosmetic samples submitted to the faculty through the community service program for the screening of hydroquinone and retinoic acid. Coactivities such as delivery of health education to the community and program evaluation were also conducted by students to promote their community engagement and work experience in a real-world context. Furthermore, the knowledge gained from the project was passed on to younger students to illustrate in lectures and stimulate their interest in chemistry. The project was well-received and considered useful by the community since it addressed the real needs of the people, thereby helping to cross the line between the academic world and the real world and foster relationships between the university and the community.



KEYWORDS: *Upper-Division Undergraduate, Analytical Chemistry, Problem Solving/Decision Making, Chromatography*

The incorporation of research projects into undergraduate curricula is one of the most effective pedagogical strategies for strengthening students' knowledge integration as well as systematic and creative thinking.[1−3] Students have opportunities to experience and work with almost all aspects of the design process to solve problems. Furthermore, if a research topic is taken from the community, it not only addresses the real needs of the people but also offers a chance to incorporate community-based learning into the course. As shown in many studies, implementing this type of pedagogy into the teaching of chemistry as well as other disciplines promotes students' learning in real-world contexts and fosters the skills of community engagement and social contribution.[4−9] This article presents a project undertaken at the Faculty of Pharmacy, Silpakorn University, Thailand. Not only was it a senior chemistry project, but it was also integrated into the faculty's service program held for the community on and off campus. Finally, the knowledge and experience gained from the in-lab and outreach activities were passed on to younger students to enhance their understanding of chromatography and to stimulate students' engagement and motivation in chemistry.

■ PROBLEM ORIGIN

Hydroquinone (HQ) is used for the treatment of hyperpigmentation, e.g., melasma, freckles and age spots.[10] However, long-term use may cause exogenous ochronosis, hypersensitivity to light, severe skin irritation, blistering, and scaling as well as possible cancer risks.[11,12] Retinoic acid (RA) is approved for use in treating severe acne and certain keratinization disorders and used off-label to treat wrinkling and irregular pigmentation of photoaged skin. However, it can cause an irritating reaction that manifests itself as erythema and scaling and enhances the skin's susceptibility to sunburn. Furthermore, RA has been reported to cause adverse effects on fetuses in animal studies, and there are no adequate controlled studies in humans; thus, using RA during pregnancy should be avoided or considered with extreme caution.[13−16] Because of these side effects and risks, products intended for medical use containing HQ and RA are classified as drugs, and the use of these compounds in cosmetics is not allowed in many countries, including Thailand.

Despite these legal regulations, cosmetics adulterated with HQ and RA are still available in Thailand. For this reason, screening for these compounds hidden in illegal products helps to ensure consumers' safety. Nowadays, HQ and RA can be identified by using colorimetric test kits. However, false positive results may be obtained if the samples contain certain ingredients such as vitamin C or sodium metabisulfite. Thin-

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layer chromatography (TLC) carried out on silica gel plates has also been employed.[17,18] This method is more specific than the test kits, but one of the drawbacks is the use of unsafe organic mobile phases such as chloroform and hexane. To overcome this problem, reversed-phase TLC is an interesting choice since it offers the possibility of using polar mobile phases such as mixtures of water and alcohol, which are considered safer and greener. Nevertheless, TLC analysis in reversed-phase mode is currently limited by the high price of commercial C_8 or C_{18} bonded silica plates.

In a community survey about health-related problems and concerns from the previous year, people residing around the campus stated a need to ascertain the safety of the cosmetic products they used. As a result, an investigation into screening methods for prohibited HQ and RA in cosmetics collected from the local area was assigned as a research topic to senior pharmaceutical chemistry students. In order to compete with the commercial TLC plates and other current methods, it was necessary for the devices to be prepared using cheap materials available in the country and for the designers of the test to be concerned about the safety to the analysts and the effects on the environment. The students were asked to review any relevant literature and then propose and discuss a concept and set of experiments with their instructors. Finally, they came up with the idea of preparing reversed-phase stationary phases by impregnating laboratory filter papers with vegetable oil (Figure 1). For this purpose, suitable types of filter papers and

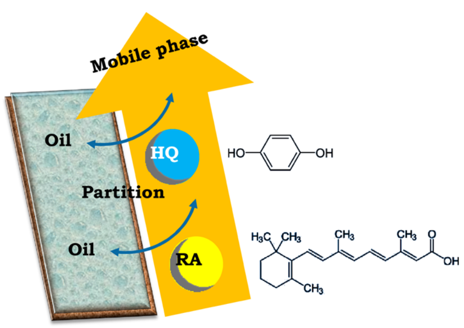

Figure 1. Reversed-phase separation of HQ and RA on vegetable-oil-impregnated filter paper.

concentrations of oil for the dipping solution were investigated. Subsequently, the oil-impregnated papers were tested for their separation ability using mobile phases composed of ethanol and water. Finally, the optimized method was validated for specificity and sensitivity prior to its application for the analysis of real samples.

■ LABORATORY EXPERIMENTS

Fabrication of Oil-Impregnated Papers

Whatman No. 1 and No. 4 filter papers were cut into rectangles and dipped in oil solutions containing various percentages of coconut oil (5−20% v/v) in 2-propanol at room temperature (25 °C) for 2 min (Figure 2). Subsequently, they were removed from the oil solutions and left in a fume hood for the solvent to evaporate for 1 h. The resulting papers were kept in a closed plastic bag until used.

Preparation of Standard Solutions and Samples

Standard solutions (1 mg/mL) of HQ and RA were prepared in ethanol. To analyze the samples, cosmetic creams were mixed with ethanol and vortexed for 5 min. The mixtures were then placed on ice for 15 min and filtered through filter papers to remove any wax. The collected filtrate was used for the analysis.

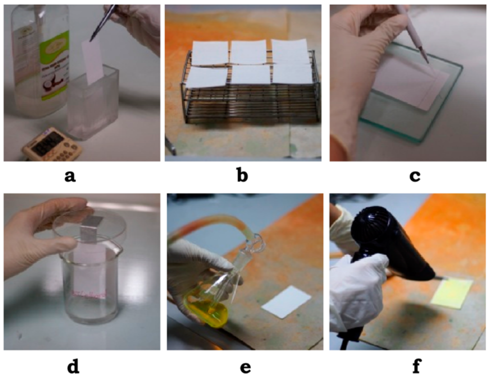

Figure 2. Schematic illustration of the fabrication of chromatographic devices and separation of HQ and RA: (a) oil dipping; (b) solvent evaporation; (c) spotting of standards and samples; (d) development in the mobile phase; (e) drying then spraying of visualization reagent; (f) heating.

Chromatographic Procedures

A 1 μL aliquot of standard or sample solution was spotted onto the oil-impregnated paper 1 cm from the lower edge. The paper was then placed in a closed chamber containing the mobile phase, with which it was saturated. To investigate the optimal mobile phase, various ratios of ethanol to water (50:50, 60:40, 70:30, 80:20, 90:10, and 100:0) were tested. The separation was conducted at room temperature until the mobile phase reached a distance of 5 cm from the starting point. The paper was then removed from the chamber and dried at room temperature. Visualization of the spots was done by spraying the paper with 5% phosphomolybdic acid in ethanol and blowing hot air at it from a household hair dryer. Under visible light, HQ and RA are seen as blue and bluish green spots, respectively.

Validation of the Screening Method

The limit of detection (LOD), defined as the lowest quantity of analyte that could be visualized after the chromatographic separation, was determined by spotting varied amounts of standard HQ and RA solutions on the papers. The specificity was verified by examining the interference effects from common ingredients used in cosmetic creams, i.e., vitamin C, vitamin E, α-arbutin, and sodium metabisulfite. Finally, the screening results were confirmed by HPLC using an ODS column (5 μm, 4.6 mm i.d., 150 mm), water/methanol (45:55) as the mobile phase at a flow rate of 1 mL/min, an injection volume of 20 μL, and a detector wavelength of 295 nm.[17,18]

■ HAZARDS

Procedures involving the use of organic solvents, i.e., oil dipping and chromatography, should be performed in a fume hood, and suitable gloves and eye protection should be worn. Ethanol and 2-propanol are flammable solvents and should never be used near open flames. They are harmful if ingested and inhaled and cause dryness of the skin. Phosphomolybdic acid is an oxidizing agent and causes skin burns and eye damage. To prevent accidents, students should be provided with material safety data sheets for all of the chemicals prior to the experiments.

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RESULTS AND DISCUSSION

Optimal Conditions for the Fabrication of Oil-Impregnated Papers

The use of different types of filter paper did not significantly change the ability to separate HQ and RA. However, Whatman No. 4 papers gave the fastest migration of the mobile phase, probably because of their higher porosity compared with No. 1 papers, so they were chosen for use in order to minimize the run time. It was found that a higher percentage of oil in the dipping solution improved the resolution of HQ and RA but increased the run time required to develop a 5 cm migration distance (Figure 3). Obviously, filter papers without oil impregnation (0% oil) had no reversed-phase characteristics and thus could not separate HQ and RA. In this study, 15% coconut oil in 2-propanol was chosen for immersion of the papers because it gave devices that satisfactorily separated the analytes within a reasonable run time.

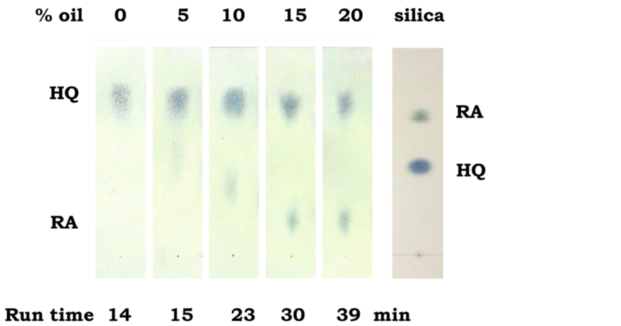

Figure 3. (left) Effects of the percentage of coconut oil on the separation and run time required to develop a 5 cm migration distance and (right) the migration order of HQ and RA on a commercial silica gel plate. The mobile phase used was 70% ethanol in water.

Optimal Chromatographic Conditions

Because the filter papers were impregnated with low-polarity liquid, i.e., coconut oil, a reversed-phase characteristic was created, and the use of polar mobile phases such as safer and greener mixtures of water and alcohol was possible. From the optimization experiments, 70% ethanol in water was found to be the most suitable mobile phase since it gave good resolution of HQ and RA (Figure 4). Mobile phases containing less than 70% ethanol had an inadequate ability to elute RA from the initial spot. On the other hand, the use of ethanol content higher than 70% was not economical. Under this optimized condition, the analysis was accomplished within 30 min, and the R_f values of HQ and RA were 0.9 and 0.2, respectively.

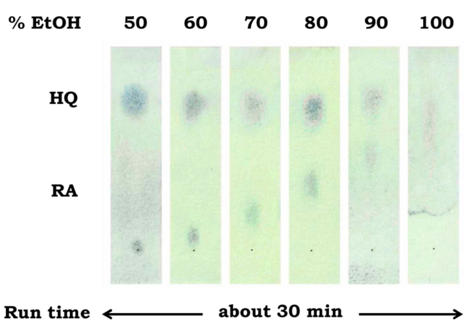

Figure 4. Effect of ethanol content in the mobile phase on the separation.

Method Validation Results and Applications on Real Samples

The validation results revealed that while colorimetric test kits gave false positive results with some ingredients, i.e., vitamin E, vitamin C, and sodium metabisulfite, no interference from these substances was found in the students' proposed method. Vitamin E, sodium metabisulfite, and α-arbutin did not form colored spots with the visualization reagent, whereas vitamin C appeared as a blue spot with an R_f value of 0.7, which differed from those of HQ and RA. The method had LODs of 0.10 and 0.125 μg for HQ and RA, respectively. When the method was applied to the analysis of 15 skin-whitening and antiwrinkle cosmetic samples obtained from the community, two of them were found to contain HQ. The results from the screening complied with the confirmatory tests done by HPLC, and thus, the reversed-phase paper chromatography method proposed in this study was reliable.

ASPECTS OF COURSE SETTING AND KEY LEARNING

At Silpakorn University, a senior project is compulsory for all fifth-year pharmacy students and takes place over two consecutive semesters for a total of three credits. The students enrolling in these courses work in groups of three to five members and openly select research topics relevant to their specialty tracks and areas of interest, e.g., pharmacology, clinical pharmacy, or industrial pharmacy. From about 160 students, a group of three students who were interested in pharmaceutical analysis chose to conduct this project. As shown in Figure 5, the Senior Project I course was devoted to giving students some background on the principles and steps of the research process. The students were then assigned to develop the proposal under the supervision of senior project advisors. The activities involved included a review of relevant literature and a discussion of concept as well as planning a timeline and experiment design. This in turn covered selection of oil type, filter paper, and solvent as well as variation of parameters, e.g., oil content in the dipping solution. The proposal was submitted to the faculty and presented to the class at the end of the course. After the proposal was approved by the academic committees, laboratory work was carried out throughout the Senior Project II course in the second semester. In the last week of the course, the results were reported and presented. In each course, the students received a grade that was evaluated by the advisors and members of the academic staff. The assessment criteria involved the quality of work (proposal, lab work, and final report), group participation, problem-solving ability, responsibility, and presentation.

In key learning areas related to chemistry, the students conducting this project rigorously practiced the techniques of planar chromatography, including preparation and application of samples, development of plates, visualization of spots, and interpretation of results. Rather than working with ready-to-use plates, the students needed to create alternatives using their knowledge of separation mechanisms. By impregnating filter papers with vegetable oil, which is immiscible with the mobile phase, the students gained a greater insight into "partition chromatography", with the separation taking place via the differential partition of the solutes between the two liquid phases, namely, the moving liquid (mobile phase) and the

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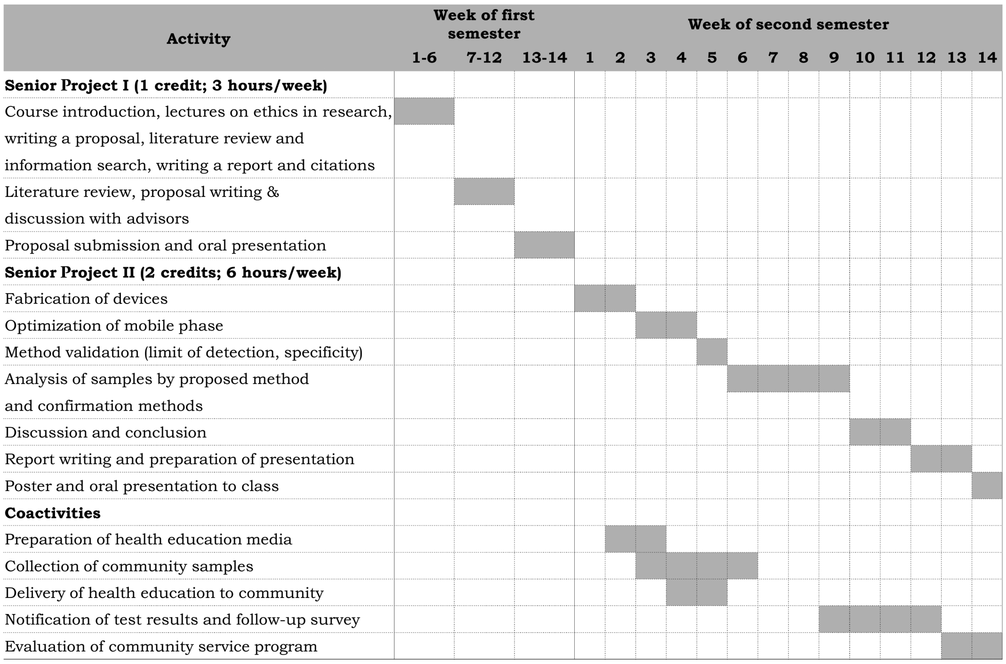

Figure 5. Timeline of senior project courses and coactivities.

liquid held on the support (stationary phase). Additionally, they learned to turn "normal phase" into "reversed phase" by replacing water adsorbed on the paper with nonpolar vegetable oil. In this area, the students could observe the influence of the reversed-phase degree of the stationary phase on the solute retention by varying the oil concentration in the dipping solution (Figure 3). To test their understanding of polarity of organic compounds related to chemical structures, the students were asked prior to the experiments to predict the migration order of HQ and RA on oil-impregnated papers. They were later able to perform experiments to explore the sequence of spots on their reversed-phase device, which was the opposite of that on normal-phase silica gel plates (Figure 3). Apart from optimization of the stationary phase, the students had a chance to study the effects of the polarity of the mobile phase on the solute elution (R_f) and resolution by varying the ratio of water to ethanol. This experiment presented a practical way to investigate the optimal mobile phase for the chromatographic separation. Finally, once the method was optimized, the students learned about the pivotal step, i.e., method validation prior to utilization. Furthermore, they had to consider the confirmation of the screening results by analyzing the samples with a more reliable method, i.e., HPLC.

Besides analytical chemistry, the students considered the 12 principles of green chemistry[19,20] and determined that the use of safer solvents was the principle most relevant to this project. By the use of two mobile apps, namely, "Green Solvents" and "Lab Solvents",[21] to search for information on the environmental impacts and hazards of common solvents, a mixture of water and ethanol was deliberately selected for this project as a green mobile phase. Additionally, hexane, which was previously used by other workers as the solvent in the oil-dipping solution,[22] was replaced by safer 2-propanol. Overall, not only were eco-friendlier methods successfully developed, but the students were also stimulated to think about the environmental impacts of their experimental designs.

■ IMPLEMENTATIONS BEYOND THE CLASS

To demonstrate the applicability of the method and to address the community's needs, the student-fabricated devices and methods were employed as analytical tools in the faculty's community service program. This activity allowed the local people to send any cosmetics about which they were worried to be screened for HQ and RA within a defined period. At sample collecting kiosks located on and off campus (i.e., at the faculty office, the university drug store, and the dispensing unit in the community hospital), student-designed submission forms inquiring about the details of senders and samples were provided (see the Supporting Information). Once the in-lab analysis was complete, the results were posted to the applicants within 1−2 weeks. Follow-up questionnaires were also attached to inquire about their satisfaction and benefit gained as well as comments and suggestions (see the Supporting Information). This information was beneficial for an analysis of the effectiveness of the project. It therefore allowed the students to learn, in addition to the chemistry context, how to improve their activity through process management.

In parallel with the screening service, the students reached out to the community to inform and educate the consumers about the harmful effects of HQ and RA contained in illegal cosmetics. Since this activity was an extra load and did not count toward their evaluation or grade, the students were asked beforehand if they could do it voluntarily. With a very positive response, all three students were willing and happy to comply. In addition, since all fifth-year pharmacy students at Silpakorn University had previously taken and passed pharmacology and toxicology as well as art and pharmaceutical design courses, this activity was not a difficult task for them. The public health education was achieved by creating posters and displaying them at the sample collecting points. In addition, the students orally educated people while they were waiting in the pharmacy unit after dropping off their prescriptions. To achieve this goal, students had to prepare attractive educational media using plain language that had to be easily and clearly understood by their

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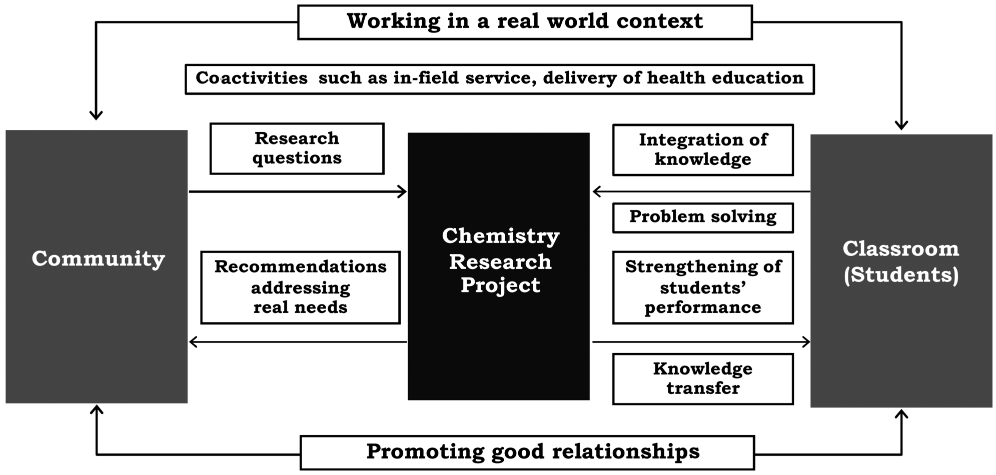

Figure 6. Summary of the connection between classroom and community via a chemistry research project.

audience. These actions taught the students how to communicate effectively with people from different backgrounds in the real world.

KNOWLEDGE TRANSFER

At the end of the course, the students were assigned to present their work to classmates and members of the academic staff by word and posters on the senior project presentation day. As observed by the advisors and other attendees, the students performed well and were proud to present their innovative and practicable work to the general public. Apart from the chemistry work in the laboratory, the students shared their experience gained from the community activities with other students. The feedback from the audience indicated that this experiment and its related activities were interesting and capable of firing the imagination of some students to conduct further experiments with other vegetable oils or target analytes. Apart from that, the knowledge and experience gained from the project were transferred by the instructor into the compulsory pharmaceutical analysis course for third-year students. According to 139 students' feedback from the lecture evaluation questionnaires (see the Supporting Information) asking about their attitudes, satisfaction, and knowledge gain, they were strongly satisfied with and motivated by such a real case (4.66/5). They claimed that it helped them to understand not only the content relating to planar reversed-phase chromatography (4.48/5) but also the importance of pharmaceutical analysis in real contexts such as for consumer protection (4.67/5). Moreover, some students stated that the chemistry project strongly stimulated their interest in pursuing a chemistry major in the future since they realized that chemistry could not only be a career but also a tool that they could use to help people in society. In summary, these findings reflected that, quite apart from the senior students with direct involvement, the project was useful for other students if the knowledge and experience were appropriately passed on to them.

CONCLUSION

It is clear that this chemistry project brought benefits in several ways. Even though this particular project has now been completed, similar types of classroom and community connected projects with different topics are still being implemented at the Faculty of Pharmacy, Silpakorn University, each year if and when the community's needs meet the students' interests. As summarized in Figure 6, the community acted as a good source of research questions. One of these was used to set up the project, effectively helping to strengthen students' skills of knowledge integration and creativity as well as laboratory and research skills. Furthermore, these voluntary activities performed in the community encouraged students to express their social contributions and learn within a real-world context. By its use as an illustrated case, the knowledge and experience gained from this project could be passed into the classroom to enhance the understanding of the content and stimulate younger students' motivation and interest in chemistry. Finally, the project responded to the real needs of people, thus promoting good relationships between the university and the community.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.6b00272.

Sample submission form (PDF, DOC)
Questionnaires for service program with follow-up results (PDF, DOC)
Evaluation results of lecture in the Pharmaceutical Quality Control course (PDF, DOC)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Iimoto, D. S.; Frederick, K. A. Incorporating Student-Designed Research Projects in the Chemistry Curriculum. *J. Chem. Educ.* **2011**, *88* (8), 1069−1073.

(2) Dintzner, M. R.; Maresh, J. J.; Kinzie, C. R.; Arena, A. F.; Speltz, T. A Research-Based Undergraduate Organic Laboratory Project: Investigation of a One-Pot, Multicomponent, Environmentally

DOI: 10.1021/acs.jchemed.6b00272
J. Chem. Educ. XXXX, XXX, XXX−XXX

Friendly Prins−Friedel−Crafts-Type Reaction. *J. Chem. Educ.* **2012**, *89* (2), 265−267.
(3) Carriazo, J. G. Laboratory projects using inquiry-based learning: an application to a practical inorganic course. *Quim. Nova* **2011**, *34*, 1085−1088.
(4) Karukstis, K. K. Community-based research. A new paradigm for undergraduate research in the sciences. *J. Chem. Educ.* **2005**, *82* (1), 15.
(5) Coe, J.; Best, A.; Warren, J.; McQuistan, M.; Kolker, J.; Isringhausen, K. Service-learning's impact on dental students' attitude towards community service. *European Journal of Dental Education* **2015**, *19* (3), 131−139.
(6) Donaghy, K. J.; Saxton, K. J. Service Learning Track in General Chemistry: Giving Students a Choice. *J. Chem. Educ.* **2012**, *89* (11), 1378−1383.
(7) Sutheimer, S. Strategies To Simplify Service-Learning Efforts in Chemistry. *J. Chem. Educ.* **2008**, *85* (2), 231.
(8) Marcus, M. T.; Taylor, W. C.; Hormann, M. D.; Walker, T.; Carroll, D. Linking service-learning with community-based participatory research: An interprofessional course for health professional students. *Nursing Outlook* **2011**, *59* (1), 47−54.
(9) Guerra, D. V. Service-learning in physics: The consultant model. *J. Higher Educ. Outreach Engagement* **2005**, *10* (3), 143−151.
(10) Sarkar, R.; Arora, P.; Garg, K. V. Cosmeceuticals for hyperpigmentation: What is available? *Journal of cutaneous and aesthetic surgery* **2013**, *6* (1), 4.
(11) U.S. Food & Drug Administration, Department of Health and Human Services. Hydroquinone [CAS 123-31-9]; Supporting Information for Toxicological Evaluation by the National Toxicology Program. https://ntp.niehs.nih.gov/ntp/noms/support_docs/hydroquinone_may2009.pdf (accessed August 2016).
(12) Westerhof, W.; Kooyers, T. J. Hydroquinone and its analogues in dermatology − a potential health risk. *Journal of Cosmetic Dermatology* **2005**, *4* (2), 55−59.
(13) Ramos-e-Silva, M.; Celem, L. R.; Ramos-e-Silva, S.; Fucci-da-Costa, A. P. Anti-aging cosmetics: Facts and controversies. *Clinics in dermatology* **2013**, *31* (6), 750−758.
(14) Mukherjee, S.; Date, A.; Patravale, V.; Korting, H. C.; Roeder, A.; Weindl, G. Retinoids in the treatment of skin aging: an overview of clinical efficacy and safety. *Clinical interventions in aging* **2006**, *1* (4), 327−48.
(15) Camera, G.; Pregliasco, P. Ear malformation in baby born to mother using tretinoin cream. *Lancet* **1992**, *339* (8794), 687.
(16) Torras, H. Retinoids in aging. *Clinics in dermatology* **1996**, *14* (2), 207−215.
(17) Association of Southeast Asian Nations (ASEAN). Identification and Determination of Hydroquinone in Cosmetic Products by TLC and HPLC. http://www.asean.org/storage/images/archive/MRA-Cosmetic/Doc-4.pdf (accessed August 2016).
(18) Association of Southeast Asian Nations (ASEAN). Identification and Determination of Retinoic Acid (Tretinoin) in Cosmetic Products by TLC and HPLC. http://www.asean.org/storage/images/archive/MRA-Cosmetic/Doc-1.pdf (accessed August 2016).
(19) U.S. Environmental Protection Agency. Basics of Green Chemistry. https://www.epa.gov/greenchemistry/basics-green-chemistry (accessed August 2016).
(20) de la Guardia, M.; Garrigues, S. The Concept of Green Analytical Chemistry. In *Handbook of Green Analytical Chemistry*; John Wiley & Sons: Chichester, U.K., 2012; pp 1−16.
(21) Ekins, S.; Clark, A. M.; Williams, A. J. Incorporating Green Chemistry Concepts into Mobile Chemistry Applications and Their Potential Uses. *ACS Sustainable Chem. Eng.* **2013**, *1* (1), 8−13.
(22) Du Toit, M. H.; Eggen, P.-O.; Kvittingen, L.; Partali, V.; Schmid, R. Normal- and Reverse-Phase Paper Chromatography of Leaf Extracts of Dandelions. *J. Chem. Educ.* **2012**, *89* (10), 1295−1296.

DOI: 10.1021/acs.jchemed.6b00272
J. Chem. Educ. XXXX, XXX, XXX−XXX