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Laboratory Experiment

Multivariate Polymer Laboratory on Synthesis of Alginate Hydrogel Beads and Analysis of Dye Loading and Release

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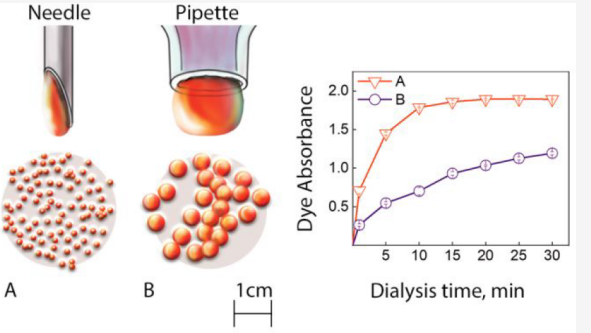
ABSTRACT: We have developed a multivariate and multidisciplinary lab experiment on the synthesis of alginate hydrogel beads of various sizes and dye encapsulation for upper-division undergraduate and graduate students. This experiment introduces students to several common concepts in polymer chemistry, materials science, and drug delivery. The experiment series runs over two 3-h laboratories where students first synthesize alginate beads by dropwise addition of an alginate solution into aqueous $CaCl_2$. The alginate bead size is regulated physically via the dropper opening and chemically via cross-linking ion variation, cross-linking time, and surfactant. During the second lab session, students explore the effects of bead size and solution environment on the gel's loading capacity, encapsulation efficiency, and controlled release using a model dye. Finally, students present their results, methodology, and data analysis to the class. A report and an exam allow the students to demonstrate their knowledge. Students learn critical drug delivery concepts throughout the project, including controlling particle size, loading capacity, and encapsulation efficiency. They also acquire valuable insight into the applications of gel particles and standard techniques used in industrial and academic research. The developed lab experience includes experiments of varying difficulty and can be adapted to first- and second-year undergraduate students by simplifying the suggested laboratory procedures.



KEYWORDS: *Upper-Division Undergraduate, Hands-On Learning/Manipulatives, Polymer Chemistry, Colloids, UV−Vis Spectroscopy*

INTRODUCTION

Polymer chemistry is a vast discipline with growing branches in many different fields of science and engineering that frequently blend throughout a science, technology, engineering, and mathematics (STEM) student's education.[1] Polymers have recently experienced a rise in popularity due to their integration into the fields of biomedicine and therapeutics.[2,3] Biopolymers are especially prevalent in recent works due to their use in the production of materials with customizable properties, including biocompatibility, biodegradability, sustainability, affordability, and accessibility.[2] The aforementioned properties are essential in the development of materials for drug delivery, vaccines, skin grafting, artificial tissues, and medicinal chemistry applications.[4−11] Thus, introducing undergraduate students to the research and applications of polymer biomaterials early in their education can be vital for sparking interest in this highly relevant subject.

One of the most recognizable biopolymers is alginate, which is derived from brown algae. Alginate is biocompatible and biodegradable and has been used for wound dressings and bioprinting due to its natural resemblance to human soft tissues.[12,13] Owing to its nontoxicity, low cost, and abundant potential, alginic acid has been used to introduce students to cross-linking,[14,15] ionic bonds,[16] rheology,[17] and mechanical properties, among other topics.[18] Aqueous solutions of alginate can be used to produce hydrogels (insoluble networks containing large amounts of water) via ionic cross-linking with divalent cations and have been featured in studies on tissue engineering, wound healing, and drug delivery.[7,19−21] A prime example is that of Floreani and colleagues, who employed alginate-based hydrogels loaded with doxorubicin as controlled drug delivery vehicles to treat epithelial carcinoma (A549s) in human lung tissue.[22] Introducing the concept of functional hydrogels from the perspective of their structure−property relationships as part of cross-curriculum learning is crucial for allowing students to develop their analytical skills and connect various disciplines, such as polymer chemistry, engineering, and biomedical sciences.

The increasing relevance of polymer chemistry has resulted in a growing number of undergraduate lab experiences that cover concepts such as cross-linking, gelation, self-assembly, nano-

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technology, and drug encapsulation/release.[23] For instance, Hurst has designed an experiment that allows students to study a pH-sensitive chitosan−poly(vinyl alcohol) copolymer based hydrogel for use as a drug delivery carrier.[24] Students studied the effect of gelation time on swelling behavior and paracetamol release in a simulated gastric fluid environment from pH-sensitive chitosan and poly(vinyl alcohol) cross-linked hydrogel.[24] Similarly, Ward et al. have incorporated an experiment on the release of blue food coloring dye from chitosan−alginate bioplastics in a semester-long research project.[25] While prior studies have focused on the impact of hydrogel swelling and the environment on dye release from hydrogel films,[24−26] the use of hydrogel particles and the role of hydrogel size in dye release have been overlooked. Drug delivery systems are prominent in emerging nanomedical technology and, when scaled appropriately, can help students master critical scientific concepts while completing a laboratory experiment with biomedical relevance.

Most laboratory experiments on drug delivery designed for upper-division undergraduate students use centimeter-sized bulk hydrogel films.[24−26] In contrast, alginate gel beads could be a more relevant example of drug delivery due to their clinical use cases,[12,22] cost-effectiveness, ease of synthesis, and ability to be viewed and analyzed with the naked eye. Equally important, students would also benefit from an integrated learning approach that combines synthesis with materials characterization and potential applications. For example, Pignolet et al. designed a demonstration experiment of alginate cross-linking with calcium ions.[14] Later, Wong and colleagues introduced a visual analysis to demonstrate drug release from alginate beads with and without chitosan shells for K−12 students.[27] While previous works on drug release from alginate beads included only qualitative analysis of the released drug,[14,27] the current work introduces students to quantitative characterization of encapsulation efficiency and loading capacity, two crucial drug carrier parameters. Introducing quantitative characterization through the dye release from alginate beads with varying surface area to volume ratios (SVRs) would allow students to apply cross-curriculum knowledge and enhance analytical skills.

This work is set up as a two-module research project using an easy-to-make alginate gel as a platform to study the structure−property relationships relevant to drug delivery, with students working in groups of two to three to produce a presentation that details their results. During the first module, students study the effect of alginate gel synthesis conditions on gel bead size and explore related background information. For the second module, students investigate how the size of the alginate gel and solution environment affect the dye encapsulation efficiency and release rates. Students strengthen their problem-solving, communication, and critical thinking skills through project-based learning. Furthermore, the presentation allows students to improve collaborative work and literature search skills and their capacity to articulate the significance of their discoveries. Students are introduced to the interdisciplinary topic of hydrogels as drug delivery vehicles, which allows them to connect knowledge from the diverse fields of chemistry while also introducing them to a wide range of methods for polymer material characterization. The designed experiment combines the hands-on experience of regulating alginate gel fabrication with the use of basic instrumentation techniques and thus creates a stimulating environment to learn structure−property relationships and associated hydrogel applications. These experiments allow students to explore a broad spectrum of concepts in polymer and colloidal sciences, including size monodispersity, loading capacity and efficiency, release quantification, physical cross-linking, and ion chelation. Since hydrogels are a facile yet comprehensive topic, students from chemistry, materials, biomedical engineering, and related disciplines would benefit from solving the real-life scientific problems described in our study.

EXPERIMENTAL OVERVIEW

This multidisciplinary experiment aims to introduce and enhance students' knowledge about the synthesis of drug delivery vehicles and the therapeutics of encapsulation and release. The total duration of the experiment is approximately 4−6 h, and it can be completed as a single continuous lab experiment or divided into multiple sessions. The experiment starts with a presentation of the experimental overview by an instructor. The detailed procedure of the experiment and complementary materials are uploaded online through the The University of Alabama at Birmingham (UAB) learning management system (LMS), Canvas, in advance and serve as a part of the prelaboratory assignment. While working in a group of three was determined to be the most effective method for conducting the experiment with undergraduate students, specific modifications can be made. Graduate students, for example, worked in pairs and were able to finish the experiment without the assistance of the instructors. On the other hand, undergraduate students who had to work in pairs had no trouble with the initial half of the laboratory experiment; nevertheless, additional assistance from the instructors was required during the drug release portion of the experiment. After completing the laboratory experiments, students report their findings through a presentation with the analyzed data, followed by questions from the instructor and peers. Despite the provided report outline, students are free to select their presentation format. During the first session, students learn how to regulate the size of alginate beads by varying dropper diameter. Students produce alginate beads by extrusion dripping of alginate solution (0.5 wt %) into a curing solution of $CaCl_2$ (1 wt %) using a plastic pipet and syringes with 16- and 27-gauge needles (Figure 1). Erythrosine B, a red coloring dye, was added to the polymer solution at a concentration of 1 mg mL^{-1} for improved visualization and precise determination of the size of alginate beads. When the polymer drop enters the $CaCl_2$ solution, positively charged Ca^{2+} ions interact with the negatively charged carboxylic groups (COO^-) of alginic acid, resulting in ionic cross-linking of the alginate polymer in a fashion commonly referred to as the "egg-box model" (Figure S1).[28,29] Once cross-linked, calcium-cross-linked hydrogels have been proven to have no or minimal deleterious effect on cell viability and proliferation.[30] Students retrieve the alginate beads from the solution via filtration and take a picture of the beads adjacent to a ruler. Quantitative analysis of the average sizes of the alginate beads produced using different droppers is performed using ImageJ software provided by the NIH website.[31] The average diameter of the particles calculated for at least 20 distinct beads was used to describe the bead size.

Furthermore, each group of students was assigned an additional experiment regarding the chemical regulation of the bead size:

- Regulation of bead size by varying cross-linking time: Rather than filtering the alginate beads from the solution immediately as in the first section, students let the beads sit in the $CaCl_2$ solution for 1 h.

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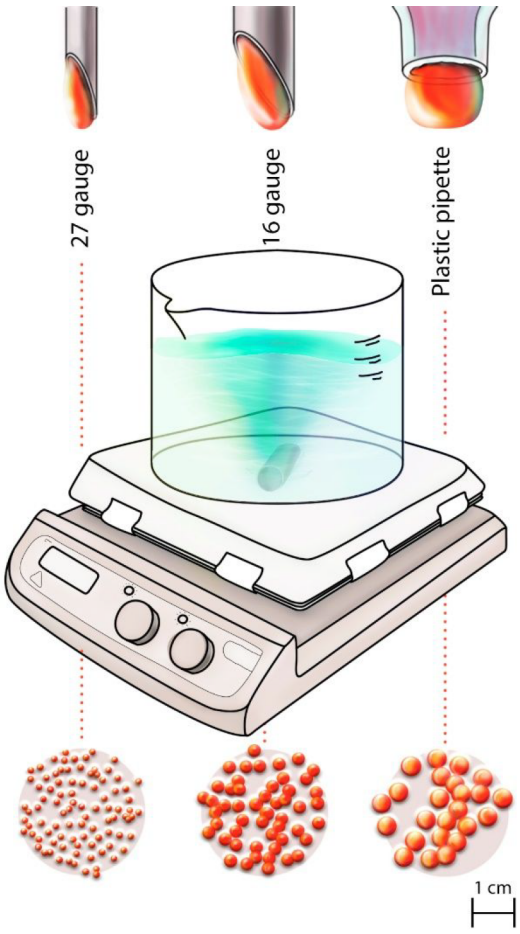

Figure 1. Schematic representation of the alginate bead synthesis using droppers with various opening sizes (27- and 16-gauge syringe needles and a pipet tip).

- Regulation of bead size by using a surfactant: Students prepare both the alginate solution and the $CaCl_2$ solution containing 0.1 wt % surfactant Tween 80.
- Regulation of bead size by varying cross-linking ions: Instead of using Ca^{2+} as the cross-linking ion, students make 1 wt % $BaCl_2$ curing solution using Ba^{2+} as the cross-linking ion.

In this portion of the lab experiment, students explore the effect of cross-linking conditions and surfactant on the alginate beads' size, rigidity, and sphericity.

The second session is focused on erythrosine B dye encapsulation and its controlled release from alginate beads. The students begin by creating an erythrosine B calibration curve. Briefly, students prepare a series of solutions with concentrations of 0.02, 0.015, 0.01, 0.008, 0.005, and 0.00025 mg mL^{-1} via stepwise dilution of erythrosine B stock solution (0.1 mg mL^{-1}) followed by UV−vis spectroscopic analysis of dye solutions of known concentrations. By recording the maximum absorbance for each solution, an absorbance versus concentration curve is plotted using graphing and data analysis software to generate a calibration curve.

To quantitatively study the effect of the bead's size and solution environment on the dye release, students dialyze dye-loaded alginate beads (1 g per batch) of various sizes (synthesized using a 27-gauge needle, a 16-gauge needle, and a plastic pipet) in DI water and 1 wt % ethylenediaminetetraacetic acid (EDTA) solution (40 mL). Students monitor the dye release by gathering UV−vis spectroscopic measurements of the bead solution after different dialysis times and plotting the maximum absorbance of the released dye as a function of time. Using the calibration curve equation, students use the maximum absorbances of the released dye to obtain the dye concentration and plot it as a function of time. Knowing the solvent volume used for dialysis and the concentration of the released dye, students calculate the amount of the encapsulated dye and the gel loading capacity. Additionally, students determine the dye loading efficiency by soaking "empty" alginate beads in the stock dye solution and performing UV−vis analysis of the stock and bead solutions. Initially, students combine 5 mL of the dye solution (0.15 mg mL^{-1}) and 3 g of the alginate beads in a 50 mL Falcon tube and allow it to sit for 1 h. During this time, the dye diffuses inside the beads, resulting in a decrease of dye concentration in the solution. After 1 h, students take 1 mL of the solution from the Falcon tubes containing the beads and dilute it to 10 mL. The same dilution procedure is performed for the stock dye solution. Afterward, students collect UV−vis spectroscopic measurements of the obtained solutions to observe the decrease in absorbance upon dye encapsulation within the alginate beads. Using the corresponding maximum absorbances, students calculate the initial and final dye concentrations in solution using the calibration curve and determine dye loading efficiency (see the Supporting Information).

Upon completion of the laboratory experiment, students present their data creatively while explaining their observations related to the current experiments. Following each presentation, the presenting group answers several questions from their peers and the instructor, which constitutes one part of the evaluation of the students' understanding of the subject. The main evaluation criteria include (1) students' understanding of the topic of cross-linked hydrogels and their properties, (2) understanding of the chemical purpose and use, and (3) student's understanding of changes in the physical properties of the gel. The final exam includes questions on the fundamental and experimental knowledge acquired during this experiment. Detailed experimental procedures, instrumentation, materials, and calculations; figures with chemical structures and SEM images; notes for an instructor and a table of student observations; a step-by-step ImageJ guide; and example questions from the final exam are available in the Supporting Information.

HAZARDS

A 1 N NaOH solution can cause eye damage and skin burns and must be handled with care. EDTA, $CaCl_2$, $BaCl_2$, Tween 80, and erythrosine B are listed as irritants that can cause a burning feeling or a rash when contacting the skin, can cause eye irritation, and can cause harm after ingestion. Tartrazine can result in an allergic reaction when inhaled or ingested, or upon contacting the skin. All chemicals must be carefully handled and used with proper personal protective equipment (PPE), including lab coats, gloves, and safety goggles. For additional information, the material safety data sheets (MSDSs) can be requested from a vendor.

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RESULTS AND DISCUSSION

Regulating the Alginate Bead Size by Varying Dropper Opening

Figure 1 schematically shows the alginate bead synthesis using different sizes of the dropper opening. Figure 2a–c shows the

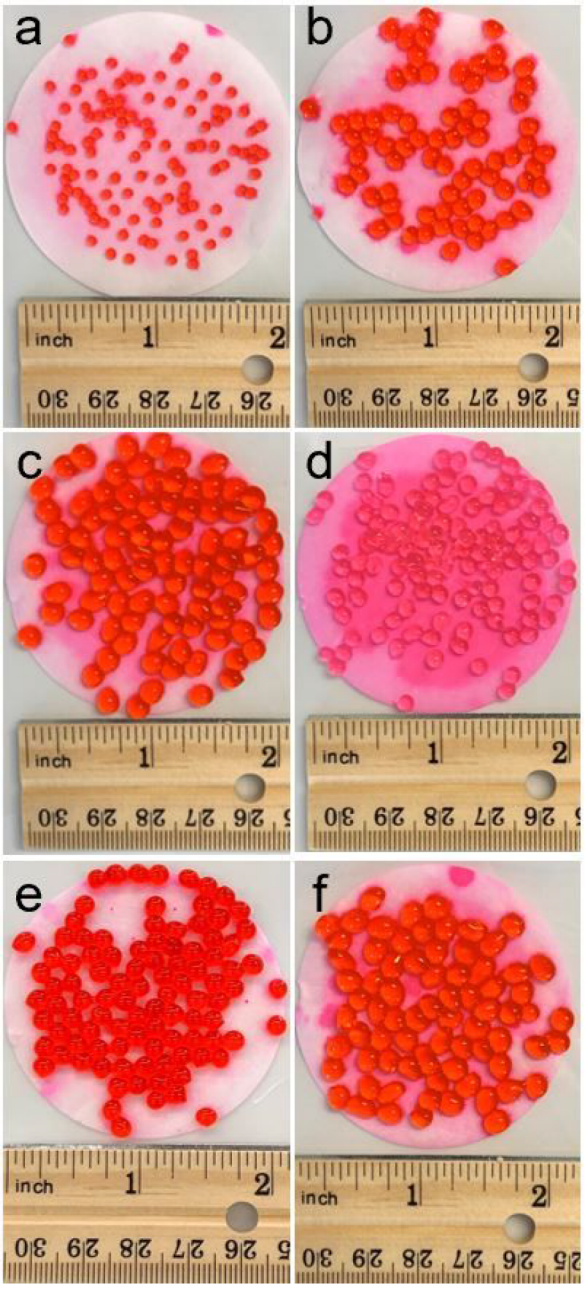

Figure 2. Effect of dropper opening size on alginate bead size: (a) 27- and (b) 16-gauge needles and (c) a pipet tip. The alginate beads obtained with a pipet tip using (d) increased cross-linking time, (e) surfactant, and (f) Ba^{2+} ions. The bottom scale is in centimeters.

alginate beads obtained using 27- and 16-gauge needles and a pipet tip, with the size analysis summarized in Table 1. Scanning electron microscopy (SEM) images in Figure S2 show the different dropper openings, which help students to visualize the dependence of gel bead size on opening size. In addition, Figure S2d shows the surface morphology of a dry alginate bead obtained with the plastic pipet tip.

Table 1. Effect of Dropper Opening Size on Alginate Bead Size

dropper	opening size, mm	av bead diam, cm
27-gauge needle	0.210[a]	0.21 ± 0.01
16-gauge needle	1.194[a]	0.36 ± 0.02
pipet tip	2.3 ± 0.2[b]	0.48 ± 0.04

[a]Inner needle diameters were obtained from vendor specifications. [b]Inner diameter measured using SEM images.

Chemical Control of Alginate Bead Size

For chemical control of the bead size, each student group carried out one of three additional experiments, which included increasing the time allowed for physical cross-linking to 1 h, using a surfactant with the alginate/dye and using Ba^{2+} ions as ionic cross-links. The representative optical images of the obtained alginate beads are shown in Figure 2d–f, and the average bead diameters for each condition are summarized in Table 2. The student observations from the most recent

Table 2. Effect of Cross-Linking Time, Cross-Linking Ions, and Surfactant Presence on Average Size of Alginate Beads

	reaction conditions		
sample	cross-linker	time, min	av diam, cm
pipet tip	$CaCl_2$	5	0.48 ± 0.04
	$CaCl_2$	60	0.33 ± 0.02
	$CaCl_2$[a]	5	0.36 ± 0.03
	$BaCl_2$	5	0.44 ± 0.02
	$BaCl_2$	60	0.36 ± 0.02

[a]A 0.1% surfactant is used.

semester are provided in Table S1. The students noticed that longer cross-linking time positively correlates with increased Ca^{2+} ion diffusion into the gel network, resulting in 30% shrinkage of the beads (Table 2). Students were encouraged to compare the rigidities of alginate beads fabricated at different cross-linking times by squashing the beads in their hands. Alginate beads soaked in calcium solutions for more prolonged periods were ultimately more firm, as the extended time allowed calcium ions to diffuse further into the alginate matrix, resulting in higher cross-linking density. On the other hand, adding a surfactant to the polymer mixture reduces the surface tension between the alginate solution and the tip of the dropper, ultimately producing smaller beads.[32] In the current experiment, the addition of 0.1 wt % Tween 80 to the alginate/dye mixture decreased the size of the alginate beads by 25%. The surface tension of the cross-linking solution deforms the initially round alginate droplet, creating a deformative barrier for the alginate bead to overcome. This impact force can potentially misshape the alginate bead, which is why surfactants are sometimes added to the alginate or cross-linking solution.[33,34] Thus, students observed the effect of the inclusion of the Tween 80 on the bead sphericity, as the surfactant helped to maximize the preservation of the spherical alginate droplet due to the reduced collision tensions between the entering alginate droplets and the surface of the cross-linking solution. Finally, students observed an approximately 10% decrease in bead size when using Ba^{2+} as a counterion instead of Ca^{2+}. The smaller size of the Ba^{2+}-cross-linked beads is due to a higher affinity between the metal ion and the polymer network.[35] Furthermore, when Ba^{2+} was used as a cross-linking ion, the beads shrank by only 20% after 1 h of cross-linking, compared to a 30% size decrease when Ca^{2+} was used.

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The difference in bead size after 1 h of cross-linking can be due to the alginate's greater affinity to larger divalent cations or alginate's greater affinity for Ba^{2+} than for Ca^{2+}.[36] The Supporting Information contains a more thorough explanation of the effects of cross-linking duration, surfactant, and cross-linking ions on the size of alginate beads.

Student data and general trends are summarized in Figure 3. While most drug delivery vehicles have nano- and micrometer

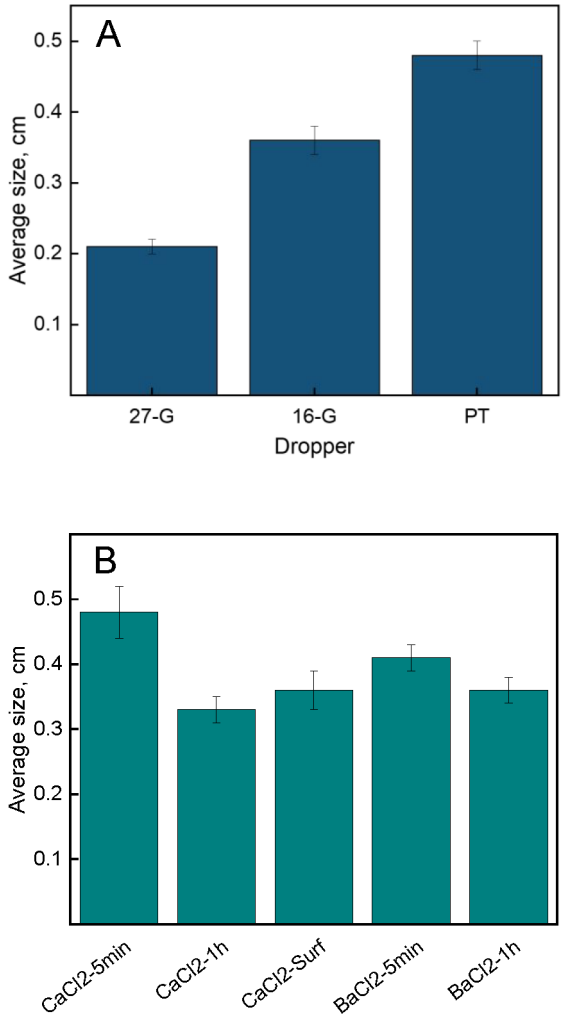

Figure 3. (A) Effect of dropper opening size on alginate bead size. (B) Control of bead size via cross-linking time, surfactant, and cross-linking ions. 27-G, 16-G, and PT stand for 27-gauge needle, 16-gauge needle, and pipet tip, respectively.

sizes, this experiment is based on beads of millimeter size, allowing students to observe the size difference with the naked eye. Adding dye during synthesis helps students' visualization of the alginate bead dimensions. While erythrosine B was the primary dye in this experiment, other procurable dyes, including tartrazine and food coloring dyes, can also be employed (Figure S3). Notably, using ImageJ to evaluate alginate bead sizes allows students to familiarize themselves with the frequently employed scientific tool (see the Supporting Information). While the presented experiment did not require students to obtain scanning electron microscopy (SEM) images of their samples, the lab experiment can easily be modified to do so. Instead, students used SEM images obtained by the instructor, which provided a visual aid for understanding the effect of dropper size opening on alginate bead size and morphology in general, and it demonstrated the instrument's capabilities. Additional assigned experiments encourage students' critical thinking about materials' structure−property relationships. For example, students were encouraged to squeeze alginate beads produced at 5 and 60 min cross-linking times to observe enhanced mechanical robustness between beads due to tighter networks at a higher cross-linking time. In addition, learning about the interaction of alginic acid groups with different cross-linking ions and the cross-linking kinetics leads to a greater understanding of alginate chemistry. Students were attentive to their labmates' experiments on the effects of cross-linking time, cross-linking ions, and surfactant presence and actively participated in the question-and-answer part during the final report. The additional experiments inspire students to conduct their own literature-based research in order to understand the observed size changes and explain to their peers the reasoning behind these changes during the final report.

Dye Loading and Release Analysis

Drug delivery systems with controlled-release capabilities serve as a stimulating topic that engages students and facilitates the teaching of important chemistry topics such as molecular diffusion, absorbance, and chelating reactions. In this experiment, we introduce students to the biomedical applications of drug delivery systems through a facile experiment that involves alginate beads loaded with a dye as a model drug. Figure 4 schematically summarizes the dye release experiment from alginate beads. Upon placing dye-containing alginate beads into an aqueous solution, the dye starts diffusing from the beads to the solution. While dye diffusion can be readily observed with the naked eye, the release is quantified with the use of UV−vis spectroscopy. The structure of the model "drug" erythrosine B,

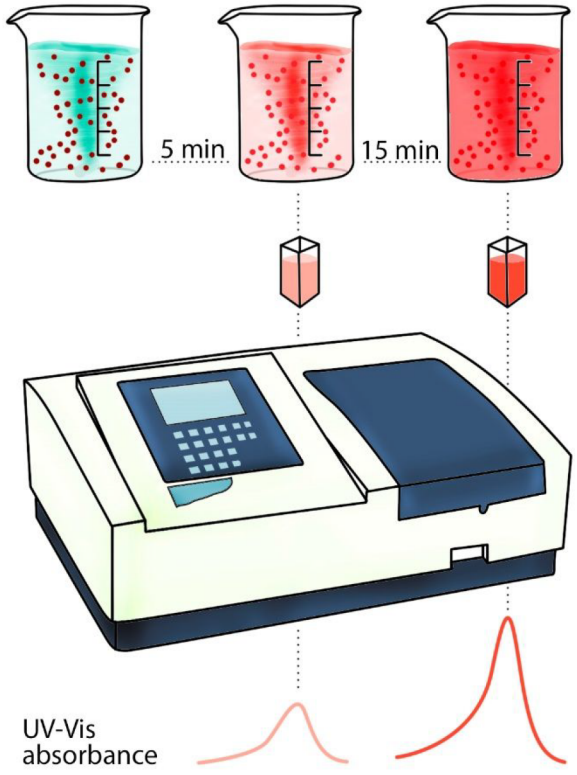

Figure 4. Illustration of a dye release experiment measured by UV−vis spectroscopy.

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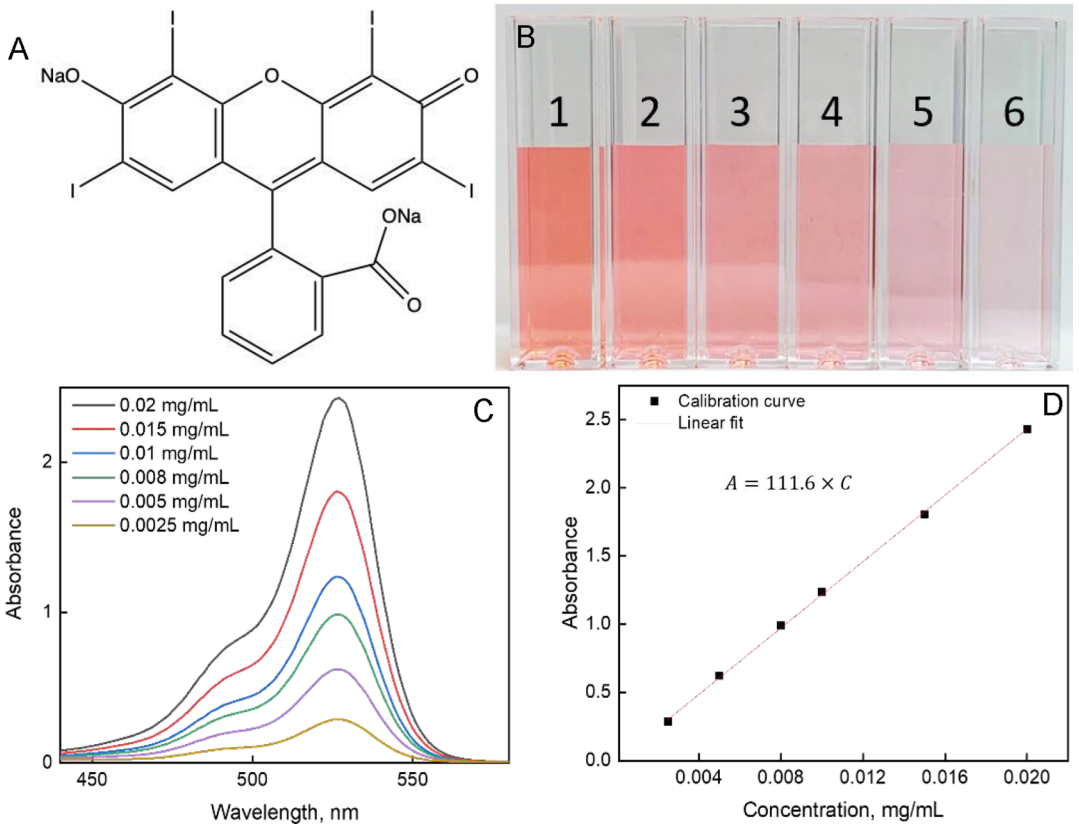

Figure 5. (A) Chemical structure of erythrosine B. (B) Solutions of the dye prepared at concentrations of 0.02 (1), 0.015 (2), 0.01 (3), 0.008 (4), 0.005 (5), and 0.0025 (6) mg/mL. (C) UV−vis absorbance spectra of different dye concentrations. (D) Calibration curve of light absorbance against different concentrations of the dye.

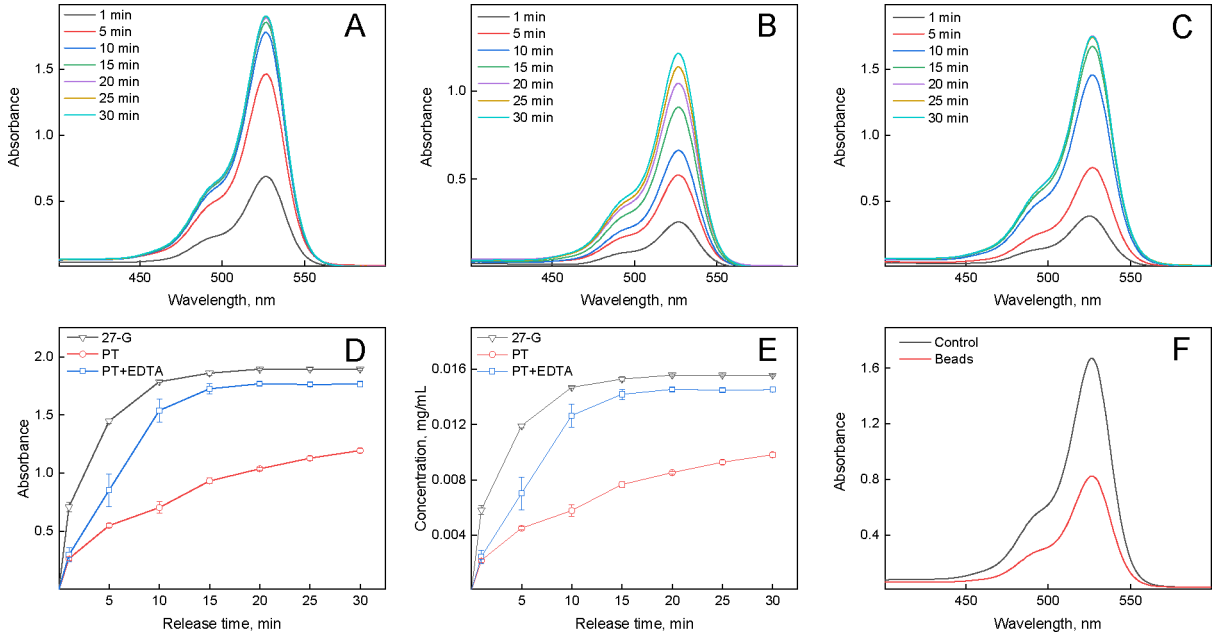

Figure 6. UV−vis absorbance spectra of released erythrosine B dye from beads produced with a (A) 27-gauge needle, (B) pipet tip, and (C) pipet tip followed by transferal to 1% EDTA solution. (D) Maximum absorbance of the released dye as a function of time and (E) corresponding concentration of the released dye. (F) Drug loading efficiency of alginate beads obtained with a pipet tip. 27-G and PT stand for 27-gauge needle and pipet tip, respectively.

optical images of its standard solutions with the corresponding absorbances, and the obtained calibration curve are shown in Figure 5. Students produce the released dye absorbance graphs as shown in Figure 6A−C. Taking the maximum absorbance at every time point (Figure 6A−C), students plot the maximum absorbance of released dye as a function of time (Figure 6D),

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convert the dye absorbance to dye concentration, and plot it as a function of time (Figure 6E). The Supporting Information provides a detailed discussion about the effect of the bead size on the dye release. Briefly, the faster release of dye from smaller beads can be explained by the higher surface area to volume ratio (SVR). The larger SVR provides more points of contact with the outer surface per unit of dye encapsulated, which results in more opportunities for the dye to diffuse out.[37,38] When the big beads produced with the pipet tip are dialyzed in EDTA solution, students can notice a prompt dye release within the first 10 min, reaching a plateau afterward (Figure 6C−E). Faster dye release in EDTA solution compared to DI water is due to the bead dissolution. The EDTA chelates Ca^{2+} from the alginate matrix, causing breakage of ionic cross-links within the polymer network (Figure S4). This leads to the dissolution of the alginate matrix, promoting faster dye release from the beads.[39−41]

When engineering a particle for drug encapsulation, having increased therapeutic effects and decreased side effects directly correlate with the amount of drug encased in the particle. On account of this observation, it becomes critical to educate students about loading capacity and loading efficiency when exploring the properties of drug delivery vehicles. While the loading capacity determines how much drug/dye per unit mass of a particle is incorporated into a drug vehicle, loading efficiency represents the percent of drug encapsulated to the total added drug. The loading capacity of alginate beads was determined by using the maximal absorbance of the released dye in the presence of EDTA, taking into account that EDTA breaks down the beads and releases all encapsulated dye. The loading capacity of alginate beads was calculated as 0.064 ± 0.001 mass % basis for beads produced with the use of a pipet tip. The Supporting Information presents the detailed calculation of loading capacity. The same calculations can be used to determine the effective loading capacity, i.e., the total amount of dye released under particular conditions, for samples without EDTA by using the corresponding sample's maximum absorbance. When evaluating loading efficiency, students can see a drastic decrease in absorbance when comparing the maximum absorbances of the stock solution to the bead-containing solution. This observation is a result of dye diffusion into the beads, resulting in a decrease of dye concentration in the surrounding solution. Students convert the absorbance of erythrosine B dye solution containing beads synthesized by using a plastic tip to the concentration (0.07 mg/mL), which corresponds to the encapsulation efficiency (EE%) of 50 ± 5% (see the Supporting Information for detailed calculations). While students used one dye concentration to assess loading efficiency, the experiment can be expanded to employ a series of dye solutions with increasing concentrations to determine the maximum loading efficiency of the gel.

This experiment provides students with an excellent opportunity to refresh and enhance their hands-on experience in general and analytical chemistry as each group of students needs to obtain their own calibration curve. Plotting a calibration curve also requires basic knowledge of graphing software such as Microsoft Excel, Origin, or Prism. Dialysis of the "drug" from the beads and quantifying the released amounts by UV−vis spectroscopy helps students refresh their analytical skills and introduces them to the biomedical applications of gels. The versatility of this experiment exposes students to experiments that an actual research scientist would perform. Furthermore, we found that introducing students to the use of a metal-chelating agent was a significant learning experience that helped acquaint them with the concept of reversible physical cross-links using chelating agents. Students learned how to apply the acquired knowledge in practice through the experiment and presentation report. Encapsulation and controlled release are other key topics for hydrogels which students are introduced to through the determination of loading capacity and encapsulation efficiency of alginate beads.

Evaluation of Students' Performance

For the final report presentation, students had a week to create the slides based on their cumulative data from both laboratory sessions. The presentation was in the form of a scientific presentation that included an introduction, experimental details, hypotheses, results, and conclusions. The introduction helped students connect the experiment with their chemistry backgrounds and real-world applications, while experimental details assisted in the analysis of the experimental procedure and aided students in formulating experimental hypotheses. Students had complete flexibility to present their findings as they best saw fit with the results. Students exhibited creativity when presenting the data, using tables, linear graphs, and simple comparisons. Students' reports were graded based on their data, explanation of the results, and answers to the instructor and lab peers' questions. The majority of questions concerned the following topics:

- hydrogels and their applications
- physical and chemical cross-links
- chelating agents and their applications
- loading efficiency and loading capacity
- basics of absorbance spectroscopy

Similar questions were included in the final exam. The example questions of the final exam are given in the Supporting Information. Upon concluding the work, students demonstrated strong knowledge of the synthesis of hydrogels as drug delivery vehicles, drug release characterization, drug loading capacity, and drug loading efficiency. This experiment has been implemented for the past three semesters as a part of the undergraduate/graduate polymer chemistry laboratory course. The average grade of the presentations was an "A" for the cohort, and the average exam grade was 82 ± 10% (n = 45). The presentation was used as a practice tool to allow students to deliver their scientific results clearly and logically—a crucial component of a successful scientific career—and to prepare students for related exam content. The presentation was graded on its content, which included a clear introduction, findings, and conclusion, as well as students' understanding of the material and ability to explain the project's main ideas. The exam was compiled based on the questions in the Supporting Informtion to assess the knowledge gained from the described experiment.

CONCLUSIONS

We created a multivariate polymer laboratory experiment on the synthesis of alginate hydrogel beads of various sizes and dye encapsulation for upper-division undergraduate and graduate students. The designed experiment has various stages of difficulty and can be divided into different laboratory levels. This experiment is accompanied by a detailed procedure and can be adapted for first-year undergraduate students or graduate students as an independent course project. Upon completion, students acquire crucial knowledge in the fast-growing field of drug delivery systems and biomedicine and broaden their polymer, analytical, and general chemistry knowledge. This experiment helps students understand how to control the size of

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drug delivery systems through physical (the size of the dropper opening) or chemical (cross-linking time, surfactant, or cross-linking ions) approaches. Moreover, students learn how to carry out and quantify drug encapsulation and release. The designed experiment is facile and highly reproducible. Finally, this laboratory experiment enhances students' hands-on experience with UV−vis spectroscopy and introduces a new technique—scanning electron microscopy.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.2c00649.

Materials, detailed procedures, and calculations for all experimental steps (PDF, DOCX)

Example questions from a final exam (PDF, DOCX)

Additional figures with chemical structures and SEM images (PDF, DOCX)

Step-by-step ImageJ guide (PDF, DOCX)

Notes for instructors and a table of student observations (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

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