# An Inquiry-Based Investigation of Controlled-Release Drug Delivery from Hydrogels: An Experiment for High School Chemistry and Biology

Joanna L. Sylman and Keith B. Neeves*

Department of Chemical and Biological Engineering, Colorado School of Mines, Golden, Colorado 80401, United States

S *Supporting Information*

ABSTRACT: A laboratory experiment on controlled-release drug delivery systems is presented. The intended audience is high school chemistry and biology students. The exercise is meant to complement curriculum on diffusion, enzyme reactions, and polymerization. In the experiment, students used a gelatin hydrogel as the drug delivery system and food dye as a drug. Bromelain, a protease found in pineapples, was used to mimic the enzyme-rich environment of the human body. Bromelain cleaved the gelatin fibers, thus increasing the rate of release. The release of the food dye from the gelatin was measured by spectrophotometry. Students generated and tested hypotheses regarding the effect of gelatin and enzyme concentrations on release. Students were then asked to analyze the release data and to determine if their conclusions matched their hypotheses. The experiment can be adapted as structured, guided, or open inquiry-based instruction.

Hydrogel Loaded with Drug

Diffusion

Diffusion + Degradation
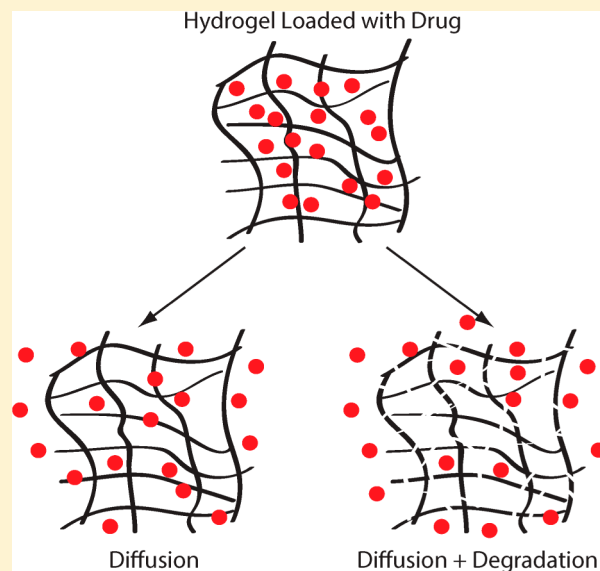
KEYWORDS: *High School/Introductory Chemistry, Analytical Chemistry, Chemical Engineering, Laboratory Instruction, Hands-On Learning/Manipulatives, Inquiry-Based/Discovery Learning, Bioanalytical Chemistry, Biotechnology, Drugs/Pharmaceuticals, Enzymes*

Controlled-release drug delivery is a topic that is useful for engaging students in fundamental concepts of chemistry, namely, molecular diffusion, enzymatic reactions, and polymerization reactions. Controlled-release drug delivery refers to systems that release drugs into the body at a well-defined rate in response to a stimuli (e.g., pH, temperature) or time.[1] Controlled-release formulations have become common for over-the-counter drugs taken orally, such as antihistamines and anti-inflammatory drugs. Controlled-release systems are also used as implants to slowly deliver drugs directly to the tissue of interest, such as a tumor. Often these controlled-release systems are made of a porous polymer matrix in which drugs are embedded. The rate of drug release is dictated by either diffusion of the drug out of the matrix or by a combination of diffusion and degradation of the matrix.[2]

A laboratory exercise focused on the design of a controlled-release drug delivery system is described. The "drug" is food coloring, and the controlled-release system is gelatin. Gelatin has been used for controlled release of drugs for treating tumors.[3,4] Students explore the effect of gelatin concentration and enzymatic degradation of the gelatin on the drug release

profile. The exercise was developed to appeal to different learning styles and to be integrated into curricula on diffusion, enzyme kinetics, and polymerization.[5] For auditory learners, there is a background lecture and discussion of the major topics. For visual learners, there is observation of the dye diffusing from the gelatin and graphing of the data. For tactile learners, there is the making and handling of the gelatin drug delivery system.

The exercise is presented as a structured inquiry-based investigation, where the instructor primarily directs the students in testing the parameters that affect drug release from gelatin.[6] First, students are invited to inquire about the mechanisms of drug delivery in a structured discussion. Second, students perform a laboratory experiment with the help of the instructor. This experiment has also been implemented as both guided and open inquiry. In guided inquiry, the instructor presents the question for investigation, and the students take a more central role in deciding how to proceed with the investigation. In open inquiry, students ask their own questions and develop their own

experimental procedure. The structured inquiry-based investigation takes a minimum of two class periods, whereas the guided and open inquiry can be a project that lasts several weeks.

EXPERIMENTAL OVERVIEW

Students generated hypotheses about the effects of the gelatin and bromelain concentration on drug release. Food dye, acting as the drug, was incorporated into the gelatin drug delivery system. Gelatin cubes were placed into a test tube with either tap water or enzyme solutions to mimic physiological-like conditions. The rate of drug release was recorded by spectrophotometry over time, typically a few days. A minimum of two class periods (50 min) was required to complete the lab experiment. In the first laboratory session, the instructor led a discussion on drug delivery and controlled release. The students then made hypotheses and prepared the gels and enzyme solutions. In the next laboratory session, the gelatin cubes were placed in test tubes with and without enzyme solutions and the release of food dye to the surrounding solutions was measured by spectrophotometry. Following the second class, measurements can be made less frequently to determine the full drug release profile. The students analyzed their results and determined the drug release rate for each condition tested. A group of four students was optimal for completing all the required tasks.

EXPERIMENTAL DETAILS

Prelab Discussion

Prior to starting the lab work, the major concepts critical to understanding the experiment were stated. (A brief presentation that provides the general structure for going over the material is included in the Supporting Information.) First, the students were asked about the various routes of drug delivery. Second, the concept of localized drug delivery was introduced where the objective was to deliver drugs directly to the afflicted tissue. Third, new terms were defined, such as hydrogel, gelatin, bromelain, release rate, and degradation reaction rate. (Background information and teaching tips are available in the Supporting Information.) Students made hypotheses related to how the gel concentration and enzyme concentration would affect the rate of drug release by diffusion.

Gelatin Drug Delivery Systems

To make drug delivery systems, students calculated and weighed the appropriate quantity of gelatin (Knox Unflavored Gelatin) to make 5%, 10%, and 15% (m/v) gels. The gelatin was dissolved in tap water heated to 90 °C in a graduated beaker. Once the gelatin was completely dissolved, more water was added, if needed, to replace any loss of volume due to evaporation. Next, 15 drops of food dye (McCormick Food Coloring) was added to the dissolved gelatin solution. Red, blue, and green dyes give the most visually appealing results. The total volume of gelatin solution should yield a 5 mm high gel in the container. For example, 40 mL of solution was used for a 10 cm Petri dish (height = volume/area = 40 cm^3/π*(5 cm)^2 $\sim$ 0.5 cm = 5 mm). Finally, the gelatin solutions were placed in a refrigerator overnight to form a solid gel.

Enzyme Solutions

To simulate enzymatic degradation of the matrix, bromelain was used, which is found in many meat tenderizer powders including McCormick Meat Tenderizer. Bromelain is an enzyme found in pineapples, which digests the gelatin polymers. The presence of bromelain in pineapples is why you cannot make a Jell-O salad with pineapples, at least not without heating the pineapple to 60 °C to denature the protein. Meat tenderizer solutions of 1 and 5 mg/mL in water were used in this experiment. About 100 mL of each enzyme solution was sufficient.

Drug Release Profiles

The students cut 5 mm × 5 mm × 5 mm cubes of gelatin with a razor blade or used a coring tool (Harris Uni-Core, Prod #15081, Ted Pella, Inc.) to cut cylinders with a diameter of 5 mm and a thickness of 5 mm. The gelatin cube or cylinder was then placed in a test tube containing 5 mL of either a water or enzyme solution. Each group of students had 9 test tubes (Table 1); 3 gel concentrations (5%, 10%, and 15%) in each of

Table 1. Summary of Experimental Conditions

Samples	Gelatin (% m/v)	Bromelain/(mg/mL)
1	5	0
2	5	1
3	5	5
4	10	0
5	10	1
6	10	5
7	15	0
8	15	1
9	15	5

the 3 solutions (water, 1 mg/mL bromelain, 5 mg/mL bromelain). Next, the students took absorbance data using a spectrophotometer (Vernier LLC, SpectroVis Plus). The students used a plastic transfer pipet to mix the dye into solution by pipetting up and down. Using the same pipet, a couple milliliters were removed from the test tube and transferred to the cuvette for spectrophotometry measurements. The absorption maxima for McCormick food dyes are 500 nm for red, 610 nm for blue, and 625 nm for green. After the measurement was performed, the students replaced the solution from the cuvette into the test tube. A change in absorbance was expected over the first 30−60 min, which allowed for a few measurements during a class period. The majority of the release of the dye is complete after 2 days. The gelatin maintained its original structure for the duration of the measurements, unless it was immersed in the highly concentrated meat tenderizer solution, in which case it degrades.

HAZARDS

The raw materials for this exercise are all nontoxic. Safety glasses should be worn at all times while in the laboratory. Students are encouraged to wear lab coats because the food dye can stain clothing.

RESULTS AND DISCUSSION

Students formulated hypotheses on how gel concentration and enzyme concentration would affect the rate of drug release by diffusion using nine experimental conditions (Table 1). The most common hypotheses were "the higher the gel concentration, the longer it will take for the drug to release" and "higher enzyme concentration will give faster drug release". Other hypotheses included "the higher gel concentration has
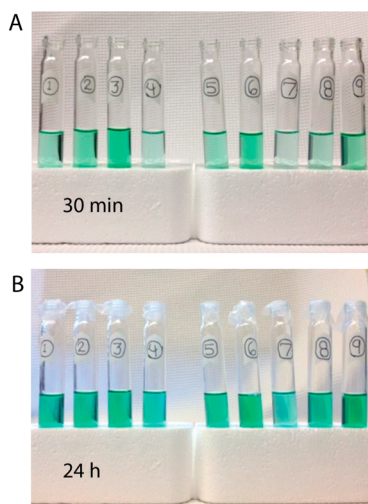
Figure 1. Release of green food dye from gelatin in bromelain solutions after 30 min (A) and 24 h (B). The numbers on the test tubes refer to the samples in Table 1.

more drug so the drug release will be faster" and "the enzyme will degrade the drug and slow drug release". These latter two hypotheses resulted from misconceptions that the gelatin was the drug, rather than the drug delivery vehicle and that drug concentration was correlated to the gelatin concentration. Students tested their hypothesis by measuring drug release from gelatin by spectrophotometry. The data collected during the experiment were absorbance as a function of time. Students analyzed these data in terms of either the absorbance or the percent food dye released (see Calculation Spreadsheet in Supporting Information).

Visual observation showed that the release of the food dye varied with both gel concentration and bromelain concen-

tration (Figure 1) . After only 30 min (Figures 1 and 2), there was clearly more dye released from the 5% gelatin (test tube 1) than the 15% gelatin (test tube 7). The differences between the 5% and 10% gelatin were modest and not discernible visually. After 48 h, the percent release of the food dye with no enzyme added, as measured by absorbance, was 57% for the 5% gelatin, 53% for the 10% gelatin, and 38% for the 15% gelatin (Figure 2). The interpretation of these results was that as the gel concentration increases, there were more gelatin fibers per unit volume to impede the diffusion of the food dye. In other words, the path that the food dye has to travel becomes more tortuous with increasing gel concentration. A measurable quantity of food dye was released within 30 min, so students could make measurements in the first class period. Ideally, students or the instructor would make additional measurements over 2 weeks, which was the time for 100% release from the 15% gelatin. However, the overall trend of slower release for higher concentration gels was captured with measurements at 30 min, 24 h, and 48 h.

In the first 30 min, the rate of drug release increased with increasing bromelain concentration (Figure 2). After 30 min, the rate of release was essentially independent of enzyme concentration. This observation is characteristic of a surface erosion mechanism,[1] where the enzyme cannot penetrate into the bulk of the gel due to hindered diffusion owing to the relatively large size of the enzyme compared to the small size of the gelatin pores. The effect of the enzyme was greater for the higher gel concentrations. For example, for a 5% gel, the difference in percent release after 5 h was 20% and 39% for no enzyme and 5 mg/mL, respectively (Figure 2). For a 15% gel the difference in percent release after 5 h was 8% and 35% for no enzyme and 5 mg/mL bromelain, respectively. The bromelain conditions were meant to mimic the in vivo process of enzymatic degradation of a drug delivery system. One area of confusion for students was the incorrect conclusion that a
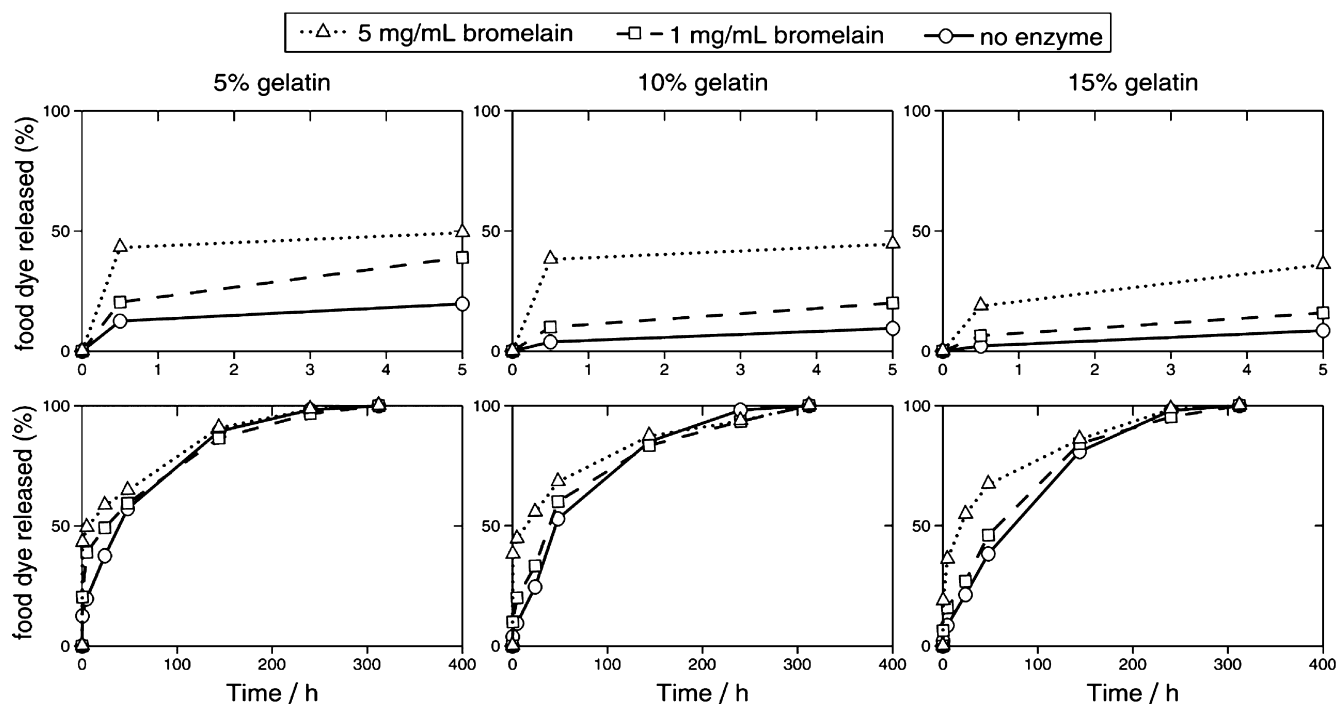

Figure 2. Percent release of food dye as a function of gelatin and bromelain concentration. Graphs on the top show release over the first 5 h and graphs on the bottom show release over two weeks.

higher concentration of the enzyme was equivalent to increasing the degradation reaction rate of gelatin fibers. The degradation reaction rate was independent of the enzyme concentration. However the drug release rate was faster because higher enzyme concentration meant that more gelatin fibers were simultaneously being cleaved, leading to a looser gel and faster diffusion.

In piloting this experiment, several sources of error have been noted that can lead to unexpected results. One source of error is nonuniform sizes of the gelatin cubes or cylinders. This nonuniformity can be the result of not leveling the gelatin solution in the refrigerator. If the solution is placed at an angle, the thickness of the gelatin cubes will vary. Another source of variability is if the gelatin is not completely dissolved when making the drug delivery systems, which results in clumps of gelatin aggregates. Improper calibration of the spectrophotometer leads to skewed results. Students must use the correct wavelength for their dye. For the earliest measurements there is rapid release of the food dye from the gelatin. It takes about 10−20 min to measure all the samples; therefore, the order in which the nine samples are measured matters. It is recommended that students record the time to the minute for all measurements due to the nature of the fast initial release.

This learning exercise offers a simple and inexpensive method to teach students strategies for producing drug delivery systems that are meant to release drugs in a controlled manner. This exercise also provides a qualitative demonstration of diffusion without the specialized equipment needed to measure diffusion coefficients.[7,8] This exercise is concluded with a discussion of why controlled release of drugs could be advantageous for treating different types of disease. For example, for chronic diseases such as diabetes, it would be desirable to have a long-term release of insulin. For more acute applications, such as treating a headache, a fast burst of drug would be best.

The Student and Instructor Guides in the Supporting Information are written for a structured-inquiry experiment. A significant amount of background information is provided to make this experiment accessible to students at several different levels. To extend this experiment to guided inquiry, the instructor can edit the Student Guide to contain less background material and allow students to develop their own experimental procedure, rather than giving explicit instructions. This exercise has also been extended to open inquiry approaches where students generate their own parameters to test. Such parameters have included temperature (20 °C versus 37 °C), salt concentration (0, 50, 100, 150 mM NaCl), and pH (4, 5, 6, 7, 8).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

A guide for the instructor, a guide for the students; slides for discussion, and a Microsoft Excel spreadsheet for analyzing absorbance data. This material is available via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: kneeves@mines.edu.

Notes

The authors declare no competing financial interest.

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