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Development of a Multi-week Drug Delivery Laboratory for Chemical Engineers

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Introduction

Drug delivery is a broad and highly interdisciplinary field that has become a significant area of research in recent decades. Historically, the most common method of drug delivery has been oral administration of small molecule drugs formulated into pills or tablets. Oral administration is favorable for small molecule drugs that are stable in the low pH environment of the gastrointestinal tract and can be rapidly absorbed into the plasma[1]. However, repeated oral doses result in varying drug concentration over time and potential adverse side-effects related to systemic delivery. To enhance efficacy and minimize side-effects, many advanced drug delivery systems are designed to release drugs at a controlled rate or to a specific anatomical location. Further, many modern therapies consist of macromolecules that are not suitable for oral administration and require alternative routes of administration (*e.g.* pulmonary, nasal, transdermal), delivery from implantable devices, or other innovative strategies[2]. The development of drug delivery systems requires insight from many distinct fields, including engineering, medicine, materials science, chemistry and biology. Chemical engineers are positioned to make significant contributions to the field of drug delivery through the application of the fundamental principles of mass balances, transport phenomena and kinetics[3].

Polymer systems have received significant interest as delivery vehicles for releasing therapies at a controlled rate. The rate of drug delivery can be controlled by polymer chemistry and composition, and can be influenced by several mechanisms such as diffusion through polymer matrices, osmosis and polymer degradation[4]. Thus, the design of polymer drug delivery systems requires an understanding of both material properties and fundamental mass transport principles. One polymer that has garnered significant interest in drug delivery applications is alginate, a naturally occurring polysaccharide found in algae. Alginate can be used to encapsulate small molecules and proteins by extruding droplets of alginate/protein solution into a bath of calcium chloride. Divalent calcium cations form bridges with adjacent alginate chains through ionic interactions, which encapsulates the protein in an insoluble alginate matrix with a spherical geometry. The resulting alginate system is highly porous and enables the encapsulated protein to be delivered from the system primarily via diffusion when placed in an aqueous environment[5].

A multi-week laboratory was adapted from previous work to enable students to experimentally measure and analyze controlled release from alginate polymer systems[6-9]. The lab is integrated into an interdisciplinary course that enrolls students from disciplines outside of engineering; therefore, completion of prior chemical engineering courses is not expected. In the first week, a red-dye was used as a model small molecule drug and release from alginate beads into an aqueous solution was quantified with absorption spectroscopy over the course of 60 minutes. The effect of initial dye concentration and alginate composition on the release profile was investigated. In the second week, fluorescently labeled bovine serum albumin (FITC-BSA) was used as a model protein therapy and the release was quantified with fluorescence spectroscopy over the course of several days. A mathematical model for diffusion in spherical coordinates was used to investigate the effect of alginate composition and initial FITC-BSA load on the diffusion coefficient in the polymer system. In the third week, a composite polymer system was fabricated with alginate and chitosan. The relationship between individual and composite polymer properties and BSA-FITC release profiles was investigated. The purpose of this paper

is to outline to the objectives of the lab experience, the materials and methods of the experiments, representative student results, and also provide ideas for future iterations of the lab.

Learning Objectives

The overall objective is to provide a hands-on, experimental laboratory experience related to controlled-release polymers to undergraduate chemical engineering students. This lab is integrated into an upper level technical elective that is focused on mass transport in biological systems and the design and application of diverse drug delivery systems. The lab serves as the primary experimental experience in the course and is designed to build on principles learned in other core curriculum courses, as well as introduce new experimental techniques and analytical equipment. The specific student learning objectives of the lab are provided below.

After completing this laboratory, students should demonstrate the ability to:

- Explain the purpose of controlled-release drug delivery systems and the advantages/limitations relative to conventional oral administration
- Explain the mechanism of alginate crosslinking and subsequent polymer stability in various aqueous environments
- Measure solute concentration in an aqueous solution with spectroscopy-based methods
- Utilize software to manipulate experimental data, construct plots of experimental variables, and implement linear regression analysis
- Determine the effects of drug load and polymer composition on the release profile of model drugs over time
- Construct a calibration curve from a series of standard solutions
- Apply a mathematical model of diffusion to experimental data and predict diffusion coefficients in polymer systems with variable composition
- Apply an ANOVA statistical test to an experimental set of diffusion coefficients
- Compare the experimental drug release profile to a theoretical release profile predicted from estimates of the diffusion coefficient

Experimental Setup

The following reagents are required: sodium alginate, chitosan, 6% calcium chloride in distilled water, 5% sodium citrate/0.9% NaCl in distilled water, pure distilled water, Allura Red (McCormick red food dye), fluorescein-labeled bovine serum albumin (BSA). The total expense of these reagents is approximately $300, most of which is attributed to the fluorescent BSA.

Prior to the lab, sodium alginate is dissolved in distilled water with the model drug and agitated overnight at room temperature. The alginate composition varies from 1-3% alginate by mass and the dye concentration varies from 1-5% by volume. Alternatively, the BSA concentration is 0.5 mg/ml and composite polymer systems were fabricated with 1% alginate/0.25% chitosan dissolved in solution. Alginate/model drug solutions are loaded into glass syringes with Luer-Lock needle tips and inserted into a vertically oriented syringe pump mounted in an aluminum frame. Standard glass beakers are used to collect and sample drug release into distilled water. Beakers are set up on magnetic stir plates and mixed at low speed. Samples are collected with

100-200 µl micropipettes and deposited into 96-well flat-bottomed polystyrene assay plates. Sample absorbance or fluorescence is measured with a multimode plate reader (BioTek Synergy).

Experimental Methods

The experimental protocol is as follows:

a. Load alginate/drug solution into the syringe and remove bubbles. Place syringe into the syringe pump and set to a flow rate of 1 mL/min.

b. Place beaker with 100 mL 6% calcium chloride solution underneath the pump set up. Start the pump and make a batch of 50 spherical beads.

c. Turn off the pump and filter out the beads into a weigh boat.

d. Repeat the process to fabricate a second batch of 50 beads. One of these batches will be for the controlled release experiment and the other will be used to calculate the maximum loading.

e. Dry the excess solution on the surface of the spheres by blotting with a kimwipe and measure the diameter of a distribution of beads with the digital calipers.

f. Place all 50 spheres in a beaker filled with 100 mL of distilled water. Place the water bath on a magnetic stir plate and mix at low speed.

g. Sample the solution concentration over 30 minutes by removing 100 uL of solution with a pipette and transferring to a 96-well plate. Sample at high frequency for the first 5 minutes (every 30 seconds). From 5-10 minutes, sample every minute. After 10 minutes, sample every two minutes.

h. To measure the maximum loading efficiency, place 50 spheres in 100 mL of sodium citrate and swirl until dissolved. Transfer 100 uL of the dissolved solution to a 96-well plate.

i. Measure the absorbance of all dye containing samples at 504 nm using the plate reader (Biotek Synergy). Measure the fluorescence intensity of BSA-containing samples at 488 nm.

j. Export the absorbance and fluorescence intensity data to EXCEL for data analysis.

Data Analysis and Representative Results

Diffusion through the alginate beads can be modeled in spherical coordinates. Fick's second law describes the rate at which concentration is changing at any given point in space[10].

$$\frac{\partial C}{\partial t} = \frac{\mathcal{D}}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial C}{\partial r}\right)$$

where *C* is concentration of the diffusing species (g/ml) and *D* is the diffusion coefficient (cm^2/sec).

Consider the one-dimensional radial diffusion through a sphere of radius *a*, where the sphere is initially maintained at a constant concentration, C_0, and the surface is kept at a constant concentration, C_{sink}. This situation is referred to as a *perfect sink condition*, and is described by the following boundary conditions[11]:

t = 0	0 < r < *a*	$C = C_0$
t > 0	r = *a*	$C = C_{sink}$

The solution to Fick's second law, a second order partial differential equation, under the above conditions is[11]:

$$\frac{M_t}{M_\infty} = 6\left[\frac{\mathcal{D}t}{\pi a^2}\right]^{1/2} - \frac{3\mathcal{D}t}{a^2}$$

Where M_t is the mass released as a function of time and M_∞ is the maximum mass that can be released.

For short timeframes, a plot of M_t/M_∞ vs. $t^{1/2}$ will have a slope equal to $6(\mathcal{D}/\pi a^2)^{1/2}$:

$$\frac{M_t}{M_\infty} = 6\left[\frac{\mathcal{D}t}{\pi a^2}\right]^{1/2}$$

The slope can then be used to calculate the diffusion coefficient from experimental measurements of the mass released over time.

With experimental measurements of solute concentration over time, students are asked to do the following:

- For each set of experiments, plot the absorbance or BSA-concentration as a function of time.
- For each set of experiments, plot the fractional release (M_t/M_∞) versus time. Also plot the fractional release (M_t/M_∞) versus $time^{1/2}$ over a time frame that shows a linear relationship. Calculate the experimental diffusion coefficient.

- Generate a table showing the dependence of the experimental diffusion coefficient on dye concentration and alginate composition.

- Using the solution to Fick's second law, plot the theoretical M_t/M_∞ release profile (M_t/M_∞ vs. $t^{1/2}$) based on the calculated diffusion coefficient and compare it to the experimental fractional release profile.

The figures below represent examples of experimental data collected by students in this lab. Figure 1 shows the absorbance of the distilled water over time while incubating 1% alginate beads loaded with variable red dye content. The figure illustrates an increase in absorbance over time as the red dye diffuses out of the alginate matrix. As expected, the increased dye content leads to a measurable difference in absorbance at all time points. This exercise illustrates the general behavior of small molecule diffusion from the system and is particularly well suited for demonstration during a class since the majority of the dye is released from the alginate within 30 minutes.

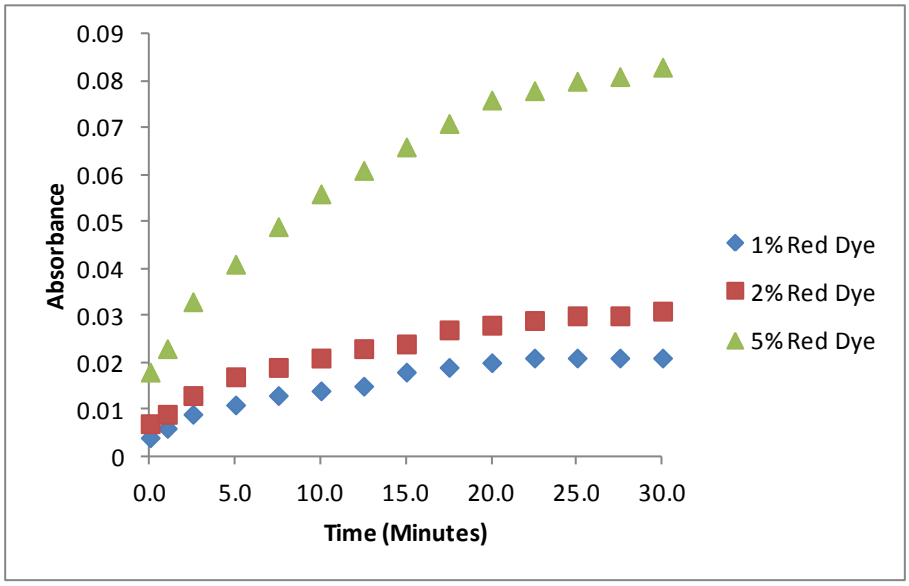

Figure 1. Red dye release profile for variable dye loads in 1% alginate systems.

Figure 2 shows the fractional release (M_t/M_∞) of FITC-BSA from alginate systems with variable composition over time. The fractional release represents the mass of BSA released relative to the total amount of BSA loaded in the population of alginate beads. The maximum release (M_∞) is estimated by dissolution of another population of alginate beads prior to the controlled release experiment. The graph illustrates the significantly longer time frame for drug delivery to the increased size of the protein. In this experiment, the release of BSA takes over four hours and consequently, was completed by students independently outside of class after learning the experimental protocol in the first week with the red dye.

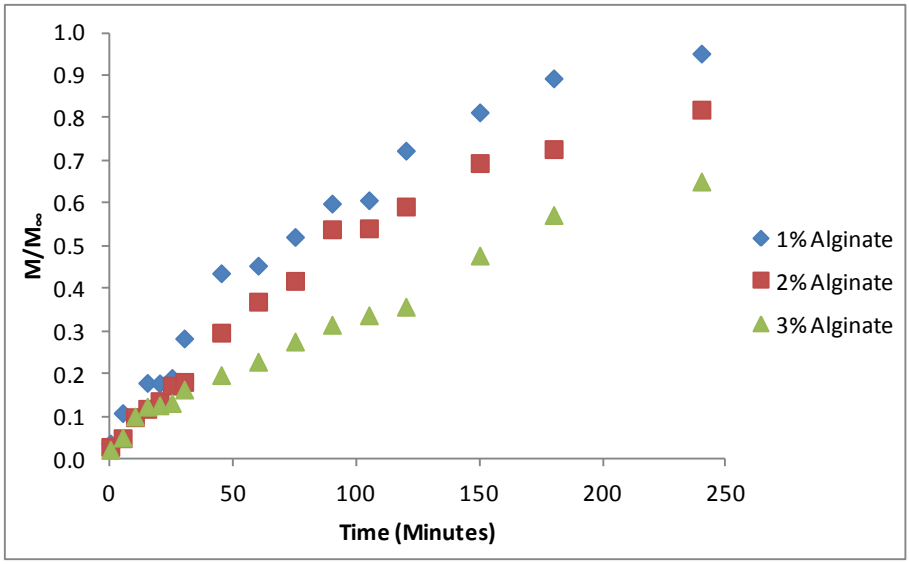

Figure 2. Fractional release of FITC-BSA in alginate systems with variable composition.

Figure 3 shows the linear regression of the fractional release of FITC-BSA from alginate beads as a function of time$^{1/2}$. The graph shows an increase in the slope of the linear fit as the alginate composition is decreased. From the mathematical model, the diffusion coefficient for FITC-BSA in alginate can be calculated from the slope. For beads with an average diameter of 3.05 mm, the diffusion coefficients were calculated in the range of 4.37-1.45x10^{-6} as the alginate composition increased from 1-3% by mass. The decrease in the diffusion coefficient reflects hindered diffusion due to a denser alginate network in the delivery system.

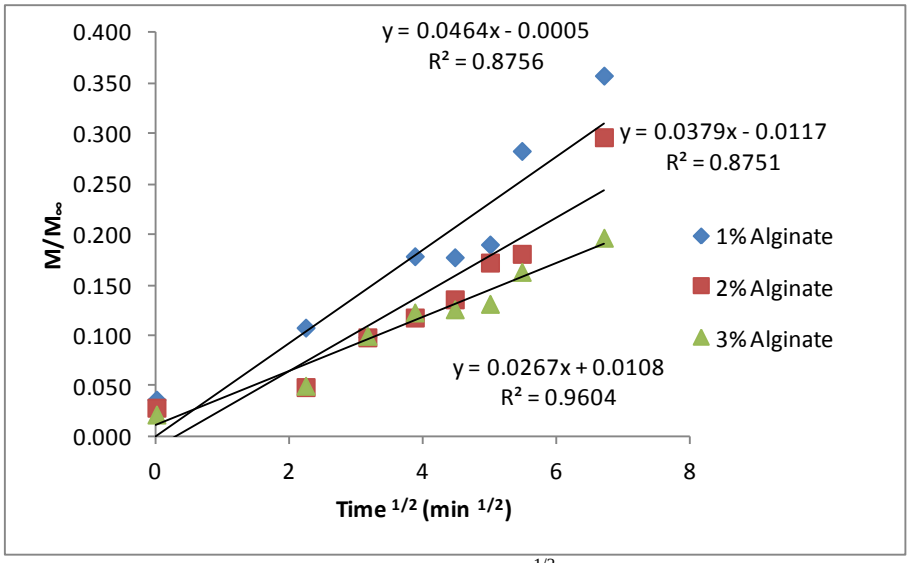

Figure 3. Fractional release as a function of time$^{1/2}$ for initial release.

Figure 4 shows an example of the fractional release over time for the various model drugs and systems studied in the lab. This includes a composite polymer system comprised of 0.25% chitosan and 1% alginate, which is fabricated in the third week. The introduction of chitosan into the alginate matrix further hinders the diffusion of FITC-BSA, as illustrated in Figure 4. In contrast the the release from a pure 1% alginate system, the release of FITC-BSA from the chitosan/alginate system is not complete after four hours. This provides an opportunity to sample the system over a much longer time frame. The figure also shows the release of the red dye from 1% alginate to compare the effect of molecular size on the release profile over time.

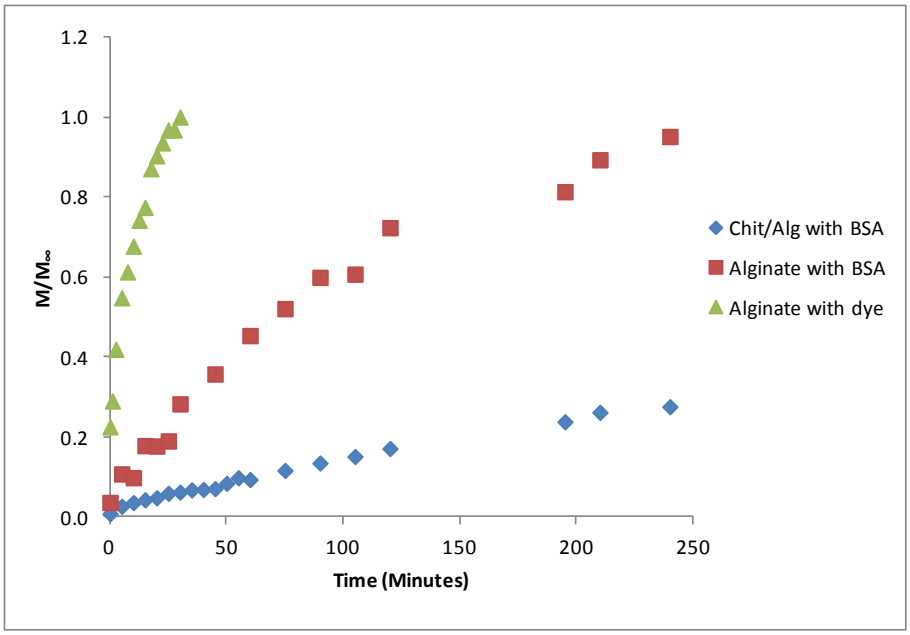

Figure 4. Effect of drug size and polymer composition on fractional release profile

Feedback and Future Directions

This laboratory experience has been integrated into two offerings of the course and is continuing to be developed. In both cases, student feedback indicated enthusiasm for the hands-on experience and the opportunity to fabricate and analyze the effects of real drug delivery system. However, quantitative assessment of specific learning objectives is not available at this time. The long term goal of this laboratory is to provide an in-depth experience that can be continued throughout the semester. Specifically, future iterations of this lab will incorporate simple cell function assays aimed at evaluating the bioactivity of released macromolecules. To this end, the lab can be enhanced by incorporating the following:

- Detailed physical characterization of alginate systems with variable composition, including SEM imaging and measurements of polymer porosity.
- Analysis of the functionality of a biologically active protein released from alginate systems. Growth factor release to cells in culture can be quantified with an ELISA assay. Bioactivity can be evaluated by routine in vitro cell function assays such as proliferation (MTS) and migration (Boyden chamber).

- Analysis of the biocompatibility of the alginate beads. The effect of cellular exposure to alginate systems can be evaluated with routine cytotoxicity assays (LDH) and ELISA assays for inflammatory cytokines.

Conclusion

The multi-week laboratory provides students an opportunity to investigate the effect of molecular size, drug concentration, and polymer composition on the rate of drug delivery from a non-degrading polymer. Additionally, mathematical models are employed to demonstrate the application of fundamental transport phenomena to controlled release drug delivery. In the future, an additional component of the lab will be developed that is focused on the biological effects of protein delivery, including basic cell function assays to evaluate cellular responses to mitogenic and chemotactic stimuli.

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