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Thermal Responsive Release of a Model Drug, Rhoadmine B, from Alginate Bead System

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Executive Summary

Stimuli responsive drug release vehicles have become of increased interest in recent years. Common stimuli that have been investigated include light, pH, and temperature. The goal of this project was to investigate the drug release profiles of alginate beads that have PNIPAAm, a thermal responsive polymer, incorporated into the matrix. The purpose of doing this was to create a drug delivery system that can be tuned to have a specific release profile or specific release time by varying the amount of PNIPAAm in the delivery matrix. The drug used for delivery was rhodamine B.

The project was designed to investigate the release speed and mechanism of beads made with differing ratios of alginate to PNIPAAm. After obtaining data, the percent of drug released versus time can be fitted to a power law curve. This power law curve and its corresponding constant and exponent can be classified using a simplified form of Fick's 2nd Law of Diffusion. The constant k describes the speed of the diffusion and is a function of bead thickness, while the exponent n describes the diffusion case, which has four distinct regions: zero-order constant diffusion, anomalous, Fickian, and pseudo-Fickian.

After analysis, it was determined that the mixed PNIPAAm beads had significantly higher k values than the pure alginate beads. This shows that the PNIPAAm speeds the drug release. This is due to the hydrophobic behavior displayed by PNIPAAm above its lower critical solution temperature (LCST), which causes the pores of the network to collapse and expel the drug. The n values for the different systems showed that the release changes from a zero order release for pure alginate to a Fickian diffusion case for the 50/50 mixture of alginate and PNIPAAm.

One of the largest impacts of my work was on my personal and career goals. In the fall, I will be attending the University of Michigan to begin my PhD in Chemical Engineering. I plan to pursue a career in academia upon completion of my doctorate. Completing this project has increased my confidence in my ability to conduct, analyze, and report original research. I have also gained experience in using literature surveys to trouble shoot, explain results, and provide supporting evidence. I also had the opportunity to refine my technical skills through using laboratory equipment, practicing safe laboratory procedures, and learning to use specific equipment, such as the microscope and the plate reader.

This project will contribute to the body of work being done by researchers around the world to create stimuli responsive drug delivery systems to overcome obstacles and improve the efficiency of release systems. One application of using thermal responsive drug delivery systems can lead to advances in topical delivery systems that respond to the body's temperature change, creating a consistent release profile. This could lead to a reduction in required dosages. This would increase patient compliance to the recommended treatment procedures, which could improve his or her quality of life.

The next step for this project would be to confirm the result by obtaining additional data sets at the same experimental conditions. If concise results are obtained that show an appreciable difference between the n and k values for each ratio of PNIPAAm to Alginate, then it would be recommended to continue investigation into tuning the release profile by varying the overall % of polymer in the beads. Also, it would be beneficial to test the release profile in PBS to give an initial confirmation of the viability of using these beads in vivo. The biggest

improvements that are needed for success in this project are consistent morphology and an improved sampling procedure that ensures the samples obtained are not only of the localized concentration.

As an undergraduate, I have had the opportunity to explore three different research projects. Through these experiences, I would advise other students to choose their advisor carefully. An advisor that is highly supportive, easily accessible, and sufficiently able to answer questions makes beginning researcher less daunting and more enjoyable. Choose a project that excites you, because you are quickly going to become an expert in your project topic! When your hard work returns faulty or meaningless results, and you start to get discouraged, remind yourself that failure is a key portion of the research process, and often more is learned through failure than success. I wish students the best as they embark on these endeavors!

Introduction

Controlled and targeted drug release has become a topic of great interest among the academic and medical community. Conventional drug release systems can be inadequate and ineffective for special circumstances. For example, research in administering drugs orally that are normally injected necessitates the design of a "smart" release vehicle.[1] Insulin, a protein based drug that is typically administered to diabetic patients through a pump or an injection, is one such drug. Oral delivery would be preferred to injection to increase patient compliance and comfort,[1] but bypassing the stomach and releasing in the intestines for uptake into the blood stream can prove to be a difficult task. During the digestive process, proteins are broken down into their constituents in the highly acidic stomach environment, which would prove fatal for the needed insulin proteins. This type of delivery quandary would warrant the use of a "smart" release system that can be activated by environmental stimuli, such as the pH change between the stomach and the intestines.

Another medical opportunity that can be addressed with smart polymers is topical applications of drugs. Obtaining the correct distribution of medication in a topical application can be particularly challenging if the drug seeps through the skin into the blood stream.[2] The negative outcomes of this scenario can range in severity. In the best case, the quickly leaching drug would necessitate frequent applications of the medication, decreasing effectiveness of the treatment. More severe cases would include local toxicity in the blood because of the leached drug. To approach this issue, research has been done into infusing electrospun PVA mats with PNIPAAm, a thermal responsive polymer, to allow for the topical applicator to respond to the temperature change on the body surface.[2] The study found this to prolong the release and

promote even distribution of the drug in the affected area while decreasing leaching. Advances in using “smart” materials for topical applications will increase treatment effectiveness and promote patient compliance.

Hydrogels are excellent candidates for environmentally sensitive releases because they have properties that allow them to achieve rapid transition between swelling and shrinking or gel and solid, depending on the type of polymer used.[3] The transition can be initiated by relatively small changes in physical or chemical stimuli. Some of the common stimuli that are investigated for use in drug delivery include temperature, electric field, light, and pH. The following report will introduce investigations into temperature stimulated drug delivery.

The purpose of this project was to design and test experimental methods to determine the impact that incorporating a thermal responsive polymer, PNIPAAm, into alginate beads would have on the release of a small molecule model drug, rhodamine B. It was hypothesized that the release profile, which is evaluated by fitting the time versus total drug released (up to 60%)[4] to a Fickian model, can be tuned by varying the ratio of alginate to PNIPAAm in the drug delivery matrix. Release behavior was recorded using a modified Franz diffusion cell setup.

This investigation into incorporating thermal responsive materials into bead carriers for targeted drug release is part of a larger investigation into controlled drug release for wound healing applications. Work has been conducted on polyelectrolyte beads, which produce pH responsive behavior. Better bead synthesis methods are also being developed under this project heading to improve the consistency and customizability of bead size and morphology.

During this project, many recommendations for further investigation were created. Future studies should focus on varying the overall weight percent of polymer, as well as evaluating the

efficiency of the release profile for wet beads at 37 °C and the release profile of wet and dry beads at room temperature. The beads should also be released into PBS. A better drug loading determination method should be investigated, preferably one that degrades the polymer matrices, allowing for complete release of incorporated rhodamine B.

Background

To exploit the temperature responsive nature of hydrogels as drug delivery vehicles, poly(N-isopropylacrylamide) (PNIPAAm) is a commonly used polymer.[5] The structure of PNIPAAm is shown in Figure 1. Synthesized via free radical polymerization from N-isopropylacrylamide (NIPAAm) monomer, PNIPAAm exhibits swelling-shrinking response when heated above its lower critical solution temperature (LCST), which occurs at 32 °C.[6] Since this is close to body temperature (37 ^{o}C), PNIPAAm is used in many biomedical investigations. PNIPAAm can be easily dissolved into an aqueous solution at temperatures below the LCST. If a crosslinking agent is added, PNIPAAM can exist as a loose, transparent gel at room temperature that becomes hard and opaque when the LCST is exceeded (Figure 2). This transitioning behavior is due to the unique constituents of the PNIPAAM molecule, which consist of both hydrophilic and hydrophobic components. When the temperature is lower, the hydrogen bonds in the side-chain amide group interact strongly with the surrounding water molecules. As the temperature is increased, the hydrophobic segments increase intramolecular interactions between the polymer chain and other polymer chains. The increase in temperature

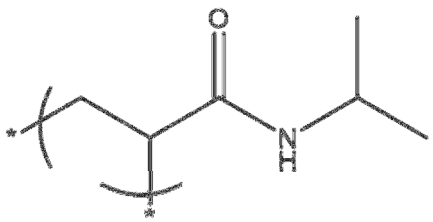

Figure 1: Structure of pNIPAAm

also weakens the hydrogen bonding between the water and the polymer chains.[3] These two phenomena cause the network to tighten, expel any interspersed water molecules, and decrease in volume. The collapsed network can be observed by a visual change from transparent to opaque (See Figure 2). The transition is considered completely reversible and almost instantaneous when the material temperature crosses the 32 °C LCST.

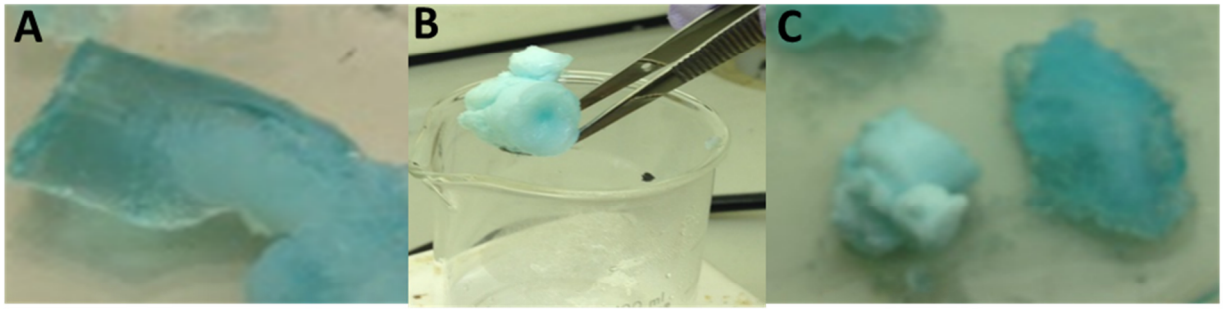

Figure 2: Crosslinked PNIPAAM gel before heating (A), during heating (B), and immediately following heating (L) as compared to original (R) (C). Gel is dyed with blue food coloring for ease of visualization.

One of the downsides of using PNIPAAm in drug release systems is that it is not biodegradable.[5] Although it is not biodegradable, a cursory study done by Malonne, Eeckman, and Fontaine *et al.* showed that PNIPAAm and its common copolymers do not appear to cause acute or subacute toxicity in mice. The research group asserted that further toxicity studies should be conducted prior to approving PNIPAAm for use in human drug delivery systems. Despite the possibility of negative effects on the body and the lack of biodegradability, PNIPAAm is used frequently in biomedical investigations.

Because of the biodegradability issues, it is beneficial to combine PNIPAAm with a known biodegradable matrix, such as alginic acid (alginate). Alginate is a natural polymer extracted from brown seaweed.[8] It is commonly used in the medical field because it is non-toxic, biocompatible, and biodegradable. One interesting application of alginate in the medical field is

the creation of molds for orthodontic applications. Aqueous alginate solutions can be easily crosslinked by addition to a dilute calcium chloride solution. The resulting hydrogel is commonly used in orally delivered drugs, and can be combined with other polymers and chemicals to create non-gelatinous soft capsules.[9] Based off of the work done by Shi, Alves, and Mano, it was decided to investigate if controllable drug release profiles could be achieved by incorporating temperature responsive PNIPAAm into alginate beads. Expanding off of their original investigation, it was determined that a standard 3 wt.% polymer solution would be used and that the ratio of alginate to PNIPAAm would be varied to research the feasibility of creating a tunable release profile. The chosen ratios of polymer weight percent of alginate to PNIPAAm included 90/10, 75/25, 66/33, and 50/50. Pure alginate beads were used as a control for every drug release conducted.

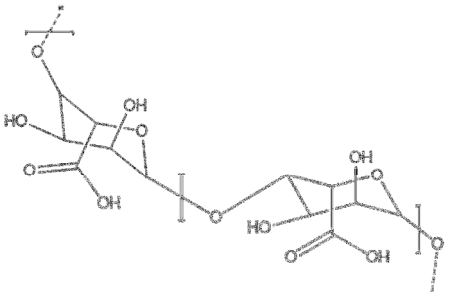

Figure 3: Structure of alginate

In order to economically research the effectiveness of drug release matrices, a model drug is commonly used. Rhodamine B was selected as the model drug for this investigation. This small molecule is assumed to accurately mimic the release of actual small molecule medications. Rhodamine B is fluorescent magenta, which allows for facile absorbance readings and convenient visual representation of amount released with respect to time. For rhodamine B at low concentrations, the Beer-Lambert law can be used to model the absorbance as a function of concentration.[11] This relationship

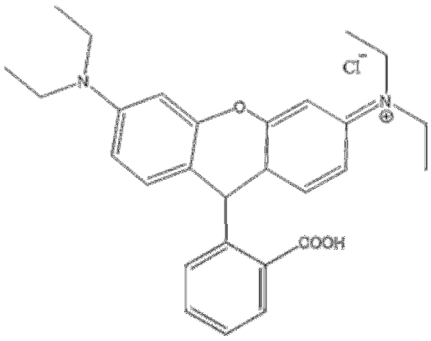

Figure 4: Structure of rhodamine B

can be utilized to create a connection between absorbance of a sample and the amount of drug released in the solution. This relationship can be used to model the drug release behavior over time.

Drug release from polymeric beads is a well investigated field. These delivery vehicles can be comprised of a degradable matrix or a non-degradable matrix. The kinetics and transport mechanisms of the release system are unique to the type of matrix. The alginate and PNIPAAm delivery system used for this study is considered to be non-degradable during the chosen release period of up to 3 days and in the chosen release medium, DI water (pH 5.5). To model the unsteady state small molecule drug release behavior of a non-biodegradable system, Fick's 2nd Law can be used. To utilize Fick's 2nd Law of Diffusion for a one-dimensional system (x-direction shown), the concentration (*c*) is found with respect to time as shown in Equation 1 below.

Equation 1: $$\frac{\partial c}{\partial t} = \frac{\partial}{\partial x}\left(D\frac{\partial c}{\partial x}\right)$$

Upon substitution and approximations, an alternate form of Fick's 2nd Law can be derived that relates released mass at time *t* (M_t) to the equilibrium mass (M_∞). This equation makes use of the constant *k*, which represents the diffusion coefficient (*D*) and the film thickness (L) (i.e., $k = \frac{2}{l}\left(\frac{D}{\pi}\right)^{.5}$).[12] The *k* constant can be used as a metric to understand how fast the drug will release in the system. The exponent *n* can be used to determine the main mechanism for diffusion that the system is following. Descriptions of the possible values for *n* can be found in Table 1.

Equation 2: $$\frac{M_t}{M_\infty} = kt^n$$

Table 1: Exponential values for modeling systems using Fick's 2nd Law of Diffusion.[12]

n	**Case**
$n <= 1$	Zero Order diffusion (Constant rate of diffusion after initial penetration of drug)[8]
$1 > n > 0.5$	Anomalous diffusion
$0.5 > n$	Pseudo-Fickian Diffusion
$n = 0.5$	Fickian Diffusion

Experimental Methods

Materials

Materials used for this project included PNIPAAm (Polysciences, Inc- M_n 40,000), rhodamine B (Sigma- ≥95% (HPLC)), alginic acid sodium salt from brown algae (Sigma), and calcium chloride (EMD Millipore- anhydrous). DI water used was purified in house and has a conductivity of < 1 μS/cm.

Method

To test the drug release profile, beads were synthesized. To begin, stock solutions of aqueous alginate (3 wt. %) and aqueous PNIPAAm (10 wt. %) were created by dissolving the respective polymers in DI water. A stock solution of dissolved rhodamine B was also created. Because rhodamine B has limited solubility in water (8 mg/ml),[13] it was important to create an aqueous solution of rhodamine B prior to introducing it to the viscous alginate solution. This step aids in creating a more uniform drug loading in the beads. Rhodamine B was added on top of the measured water, and then stirred magnetically to create a 1.4 wt. % solution. These solutions were then combined to create a bead stock solution for each of the four desired ratios of alginate to PNIPAAm: 90/10, 75/25, 66/33, and 50/50. Each solution contained a total of 2 wt. % polymer. After the solutions were combined, each beads solution was vortexed for 10

seconds to ensure uniform mixing before making the beads. A bath of weak $CaCl_2$ (3 wt. %) and rhodamine B (1 wt. %) solution was prepared by dissolving 3 g of $CaCl_2$ in 100 g of water. After the $CaCl_2$ was dissolved, additional rhodamine B (1 g) was dissolved in the bath. The addition of rhodamine B was used to decrease the likelihood of rhodamine B leaching out of the beads during preparation.

Beads were formed by adding the alginate, PNIPAAm, and rhodamine B solution dropwise to the $CaCl_2$ bath, which was being magnetically stirred rapidly. The bead solution was added to a 3 CC syringe, and then extruded by hand with a consistent pressure at a rate of two drops per second through a 20 G surgical needle. The needle was held approximately 2 cm from the surface of the liquid.

After exhausting the solution in the syringe, beads were immediately removed from the solution by filtering through a wire mesh. The beads were then rinsed with ~5 mL of DI water to remove excess $CaCl_2$. The mesh was set on KimWipes to absorb excess moisture before photographing the beads. Bead photographs were taken prior to drying so that the morphology could be observed (Figure 7). Beads were then transferred to a fume hood, where they were allowed to dry for approximately 1 week. Beads were photographed again post drying (Figure 8).

The drug release was done using a modified Franz diffusion cell release chamber (Fig. 5 & 6). The chamber consists of a 50 mL centrifuge tube with a modified lid that accepts a G14 surgical needle. A second hole in the lid minimizes pressure issues during sampling. This chamber was created by Dr. Bi-min Newby and Eric

Figure 5: Modified Franz Diffusion Cell setup.

Brink (The University of Akron, Department of Chemical and Biomolecular Engineering). To begin the release, a water bath was prepared at 37 °C using an incubator (PolyScience). The incubator was filled with water deep enough to submerge the centrifuge tubes up to their 45 mL mark. Needle tips were wrapped in cotton gauze to provide a physical barrier to prevent uptake of beads during sampling. Beads were weighed and recorded, in groups of 5, and then added to the bottom of the chamber. Once all chambers were filled with 5 beads, the first chamber was filled with DI water to the 45 mL mark, then immediately placed in the water bath. To maintain accuracy of timing between sampling periods, one chamber was filled per minute. In order to obtain a standard deviation and ensure the accuracy of results, each different alginate to PNIPAAm ratio was tested in triplicate and tested at the same time as a pure alginate control.

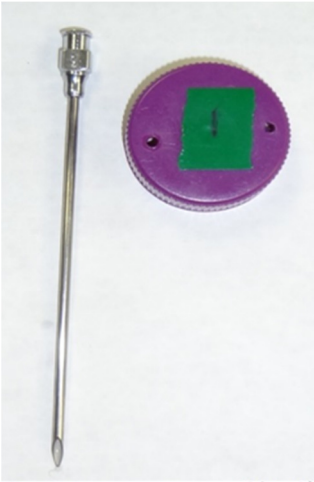

Figure 6: Detail of port and diffusion cell cap

Once the chambers were filled, samples of the release environment were taken at regular intervals. For the first hour, samples were taken every 15 minutes. For the second hour, samples were taken every 30 minutes. For the next four hours, the samples were taken every hour. Longer time samples were taken to use as the drug loading measurement. For each sample, a 1 mL syringe was inserted into the chamber's port, the syringe was pumped 5 times, and approximately 0.5 mL of solution was removed. The solution-filled syringe was set on a scale, which was then tared. The solution was emptied into a microcentrifuge tube, then the syringe was placed back on the scale and the difference in weight was recorded. To prevent contamination between sampling, the sampling syringe was rinsed with DI water between

samples. To maintain a constant volume release environment, 0.5 mL of DI water was added to the chamber with a clean syringe after each sample was taken.

After completing the sampling cycle, with the final measurement after 120 hours to determine drug loading, the samples were analyzed for drug content. Any sample that had a rhodamine B concentration higher than 5 ppm was diluted with DI water to maintain the accuracy of the Beer-Lambert law calibration.[11] The weight of added water was recorded. For accurate readings, the concentration of rhodamine B in aqueous solution also had to remain at a level where the absorbance would not exceed 1. For each sample, 200 µL of solution were removed from each centrifuge vial and placed in a 96-well plate. The Tecan Infinite M200 Plate Reader was used to read the absorbance of each well at a wavelength of 554 nm.[11] These absorbance readings, along with the sample weights, dilution weights, and sampling times, were used to calculate the amount of drug released at each sample time. The amount released at each sample time was converted into a percentage by using the total amount of drug released.

As mentioned previously, rhodamine B can be analyzed via absorbance measurements, and at low concentrations (5 ppm or less), can be evaluated using Beer-Lambert law. The Beer-Lambert law is shown in Equation 3, where A is the absorbance (no units), c is the concentration in ppm, ε is the molar absorptivity in $L*mol^{-1}*cm^{-1}$, and b is the path length in cm.

Equation 3: $$A = \varepsilon bc$$

The Beer-Lambert law is only valid in linear regions, with the εb term representing the slope of the line. This constant was previously determined for rhodamine B in water by creating

a serial dilution of samples, taking absorbance readings at 554 nm wavelength light, then performing a linear regression on that data. The value of the calibration curve slope (εb) was found to be 0.1338. The concentration of rhodamine B in each sample was obtained via Equation 4, which is a rearrangement of Equation 3, using the calibration curve slope for εb.

Equation 4: $$[rhodamine\ B] = \frac{absorbance\ @\ \lambda_{554}}{0.1338}$$

The path length, *l*, was assumed to be constant, and was thus reported analyzed data was assumed to be independent of path length. To find the gram amount of drug in each representative sample, Equation 5 was used. To determine the gram/gram concentration of rhodamine in the representative sample, Equation 6 was used. This g/g concentration was then multiplied by the amount of solution in the sampling environment (45 mL) to find the total gram amount of rhodamine B in the sampling environment at the time of sampling. These values, along with the drug loading, were used to find a percentage release at each sampling time

Equation 5: $$Drug\ in\ sample = \frac{[rhodamine\ B] * mass\ solution\ sampled}{1{,}000{,}000}$$

After calculations, the percent drug released was then plotted as a function of time. The graph was truncated after 60% drug release. For a polymeric matrix, the simplified version of Fick' law shown in Equation 2 is only applicable up to 60% release from a polymeric matrix.[4] The data was fitted to a power law curve, and the coefficients and exponents were recorded in Table 2. Correct safety and waste disposal procedures were followed to dispose of chamber liquid and remaining beads.

Data and Results

The synthesis of the drug release beads was quantified by taking photographs of the wet beads (Figure 7) and dry beads (Figure 8). From these pictures, assessment of the size and surface morphology can be made.

The release behavior of the beads was analyzed using Excel. The percent drug released with respect to time was calculated via the method outlined above. The percent drug released was graphed versus time, and then fitted to a power law trend line. (Figure 9) The equation for the trend line was used to determine the *n* and *k* values, as shown in Equation 2. As previously mentioned, the constant *k* represents the speed of diffusion, and the exponent *n* explains the release behavior. Values for *k*, *n*, and the assumed release case for each type of bead can be found in Table 2.

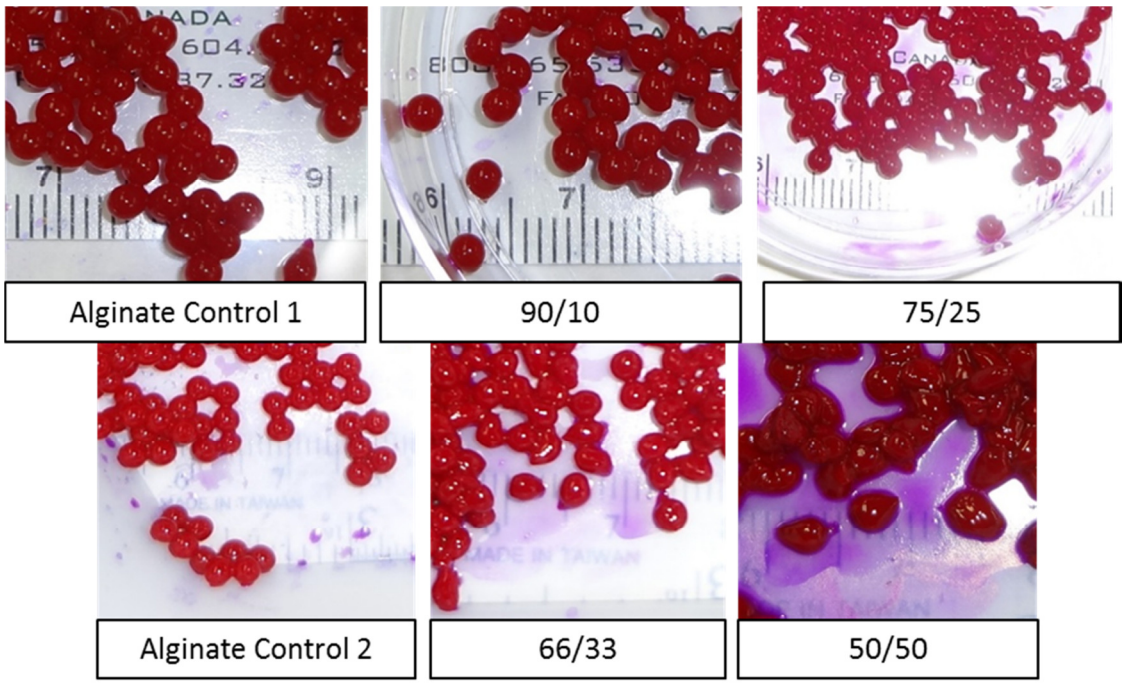

Figure 7: Photographs of wet beads with centimeter ruler behind to provide scale information.

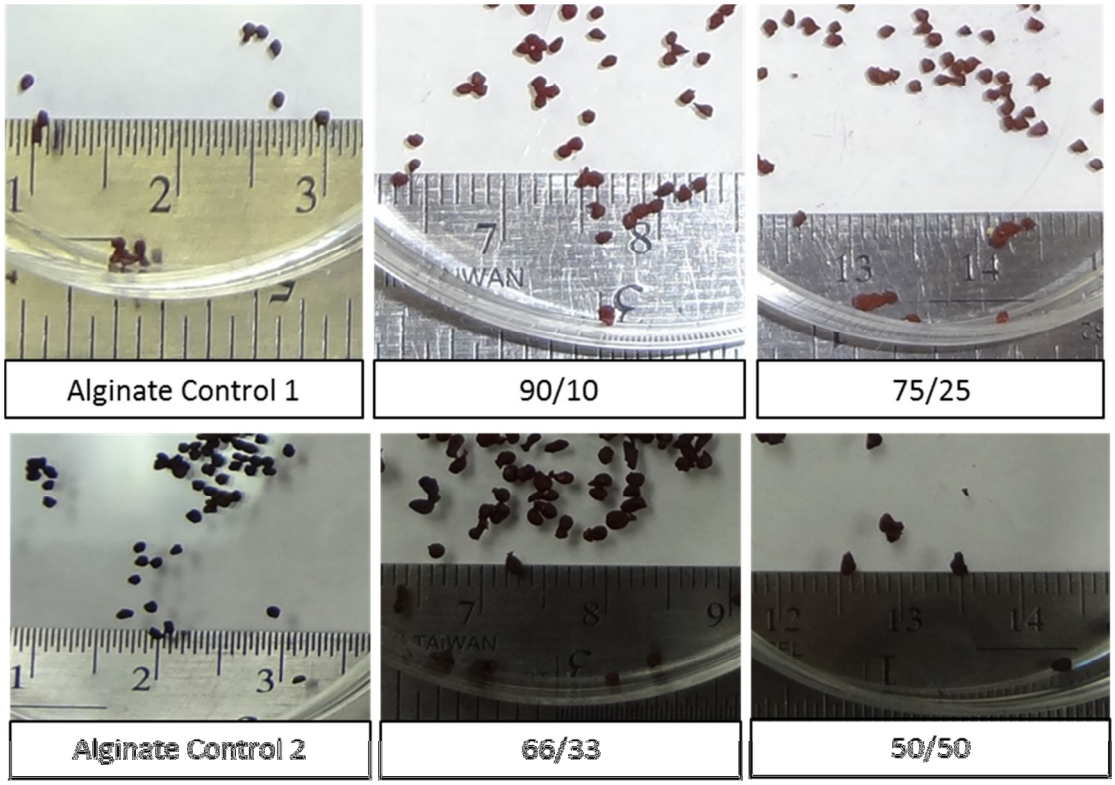

Figure 8: Photographs of dried beads with centimeter ruler behind to provide scale information.

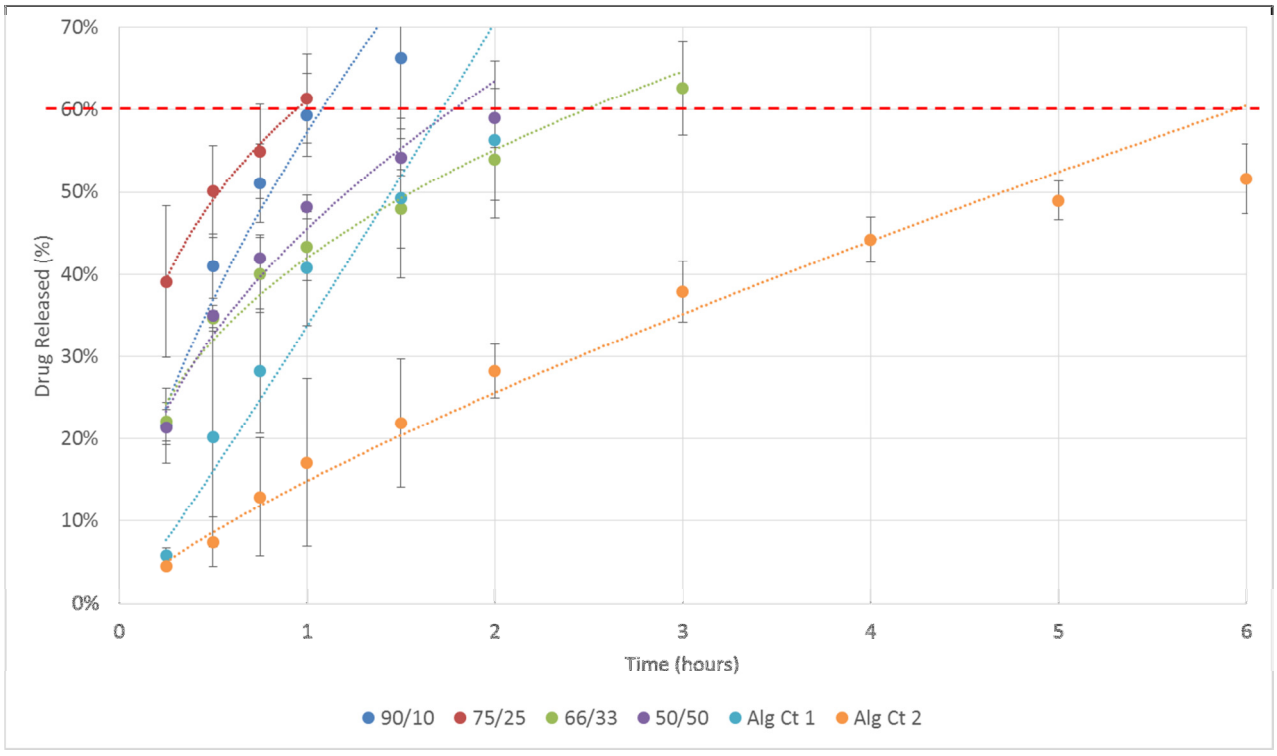

Figure 9: Graph of drug released versus time for all 6 types of beads. Error bars are shown to show standard error for each data point. Each point is the average of three data readings. The

trend lines shown were obtained using a power law fit. The dotted red line represents the 60% release threshold for modeling diffusion using Equation 2.

Table 2: A summary of the coefficient *k* and exponent *n* for each type of bead. The case, based off of the exponent *n* as defined in Table 1, is also listed.

	k	***n***	**Case**
Alg Ct1	0.3370	1.07	Constant Rate of Diffusion
Alg Ct2	0.1488	0.78	Anomalous / Constant Rate of Diffusion
90/10	0.5728	0.63	Anomalous Diffusion
75/25	0.6121	0.32	Pseudo-Fickian Diffusion
66/33	0.4201	0.39	Pseudo-Fickian Diffusion
50/50	0.4554	0.48	Fickian Diffusion

Table 3: Summary of estimated times for 60% release and drug loading for each type of bead.

	60% release time (h)	**Std Dev**	**Drug Loading (mg)**	**Std Dev**
Alg Ct1	2	9.5%	0.2334	3.0%
Alg Ct2	7	4.2%	0.3498	2.4%
90/10	1	5.1%	0.1883	3.1%
75/25	0.9	5.4%	0.1613	1.2%
66/33	3	5.7%	0.4242	2.2%
50/50	2	3.6%	0.5188	7.0%

Discussion / Analysis

An analysis of the efficiency and behavior of a drug release system can be evaluated from the *n* and *k* constants produced from fitting the experimental data to a Fickian diffusion model. Based off of the *n* and *k* values, it can be seen that the addition of PNIPAAm to the system can significantly alter the release profile. In general, the addition of PNIPAAm speeds the release, as seen by the significantly larger *k* values for the mixed beads as compared to the pure alginate beads. This behavior can be attributed to the LCST behavior of PNIPAAm. Since the PNIPAAm is fully incorporated into the crosslinked alginate network, the pores of the network will collapse

when the LCST is reached, promoting the expulsion of the rhodamine D molecules and accelerating the drug release. Previous investigations into drug release from PNIPAAM and alginate beads has shown similar behavior, with the drug releasing more rapidly when the beads were brought above the LCST of the PNIPAAm.[10]

It can also be observed that the addition of PNIPAAm quickly moves the release system from exhibiting a constant diffusion profile when no PNIPAAm is added towards exhibiting Fickian diffusion for the highest percentage of PNIPAAm (50/50). With increasing polymer percentage of PNIPAAm, the release profile starts to behave according to a Fickian model. It would be of interest to determine if pure PNIPAAm beads exhibit Fickian diffusion.

The 60% release threshold for modeling shows the upper end of the range over which the Fickian diffusion model holds.[4] There was not a discernable trend to relate the 60% release time with the amount of PNIPAAm added, which could be attributed to experimental errors. Since both alginate control bead data sets were created using the same batch of beads, the significantly variable *k* values and 60% release times may be due to environmental variation between the two release recordings. One such investigation variable could be non-uniform temperature in the incubator. The release environment was kept open for the duration of sampling, which could allow for non-uniform change in heat in the water bath.

During further investigations, a better way to sample the release environment should be developed. Because the tip of the needle in the modified diffusion cell is close to where the beads are located (~2 cm above), the local concentration during sampling may be higher than the overall environment, despite attempts to distribute the drug by pumping the syringe prior to removing sample. Evidence of this being an issue could be observed during absorbance

readings, where an unexpected spike in absorbance would be seen early in the sampling process. A possible remedy could be inducing agitation during the release period, perhaps through addition of magnetic stirring or a shaker plate.

For future investigation into the usefulness of alginate and PNIPAAm matrices for drug release, it is necessary to go beyond investigating the release profile at only body temperature and incorporate a study of the release at other body conditions, such as pH. Release studies must be conducted in phosphate-buffered saline (PBS) since PBS mimics the body environment through providing a pH similar to blood. Also, alginate responds to pH stimulation, and it would be important to understand how the incorporation of PNIPAAM influences its behavior in a modified pH.

Further investigations and improvements should be done to confirm and reinforce the behavior observed. Many of the discrepancies may be remedied by incorporating additional data. One of the biggest improvements to the experimental method that can be made is increasing the number of samples taken during the diffusion modeling stage (before 60% release is achieved). For example, the 75/25 bead system has only four data points that were measured prior to the 60% release point. More data points in this region will increase the accuracy of the model, perhaps clarifying some of the cases where the n is near an expected case, such as the difference in *n* values seen between the two alginate controls.

To increase the accuracy of drug loading measurement, a matrix degradation technique should be used to measure the total amount of drug in each bead. Because release profile can be influenced by morphology, investigations should be made into techniques that will create a uniform bead morphology for every bead solution.

During observations of the release environment, it was noted that the dry beads often released rhodamine immediately upon addition of water. This may be attributed to residual rhodamine B being on the surface of the beads. Beads should be rinsed thoroughly prior to drying to promote uniform drug loading and prevent spikes in release profile due to initial drug loading.

In conclusion, the addition of PNIPAAm to alginate beads tends to decrease the release time, therefore speeding up the drug delivery of the small molecule model drug, rhodamine B, into a body-temperature aqueous release environment. Further investigations should include the collection of more data to confirm the behavior observed in this experiment. Further investigations should also include improvements to the consistency of bead morphology and to the release procedure, as well as modeling in PBS as a proof-of-concept for viability as a drug release matrix.

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