Diffusion Through Hydrospheres: Drug Release Model

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Abstract

Hydrogels are commonly used as a drug delivery model due to their biocompatibility and mechanical properties. It is important to model drug delivery to gain accurate predictions of how a drug will release into the body. These models help to maximize drug efficacy and safety. The drug release rate is primarily dependent on the rate at which the drug will diffuse out of its encapsulation. Several factors affect the drug release rate of a drug such as a temperature, pH, drug molecule size, surface area of the tablet, etc. To test some of these factors, in this experiment, alginate hydrophores of various sizes are loaded with being loaded with two different molecules. Each size of alginate hydrospheres will be loaded with either BSA or methylene blue. The factors affecting release rate are the surface area to volume ratio of the alginate hydrospheres, and the size of the molecule itself. As the small hydrospheres have a larger surface area to volume ratio they had a faster diffusion rate than the larger hydrospheres. In addition to this, BSA diffused slower than methylene blue as it had a larger molecular weight.

Keywords: drug release, diffusion rate, hydrogels, hydrospheres

Introduction

Hydrogels are hydrophilic, three-dimensional networks with multifaceted biomedical applications. They have mechanical strength while also bioactive, making them suitable for use in the body. They are composed of crosslinked molecules such as polymers enabling them to hold large amounts of water [1]. Water retention is essential for materials to be bioactive as the body is composed mainly of water, meaning that biomaterials must also be compatible with water. Therefore, crosslinking is advantageous in hydrogels offering moisture without loss of structure. Hydrogels have been utilized for several biomedical applications, such as wound dressings, scaffolds, and drug delivery [2]. When utilizing hydrogels for drug delivery, several properties such as safety, biodegradability, loading capacity, and drug release must be optimized. In a recent cancer study, drug loading was optimized by loading the drugs to the hydrogel in vitro, injecting and forming the hydrogel in situ, and allowing the gel to degrade in vivo. The drugs being loaded onto the hydrogel were Cisplatin and doxorubicin (DOX), chemotherapeutic drugs, where incorporation of the drugs in vitro into a hydrogel allowed for more control on dosing and release rate [3]. The sequence of this procedure also allowed several drugs to be loaded and effectively delivered on the hydrogel. In addition to these properties, hydrogel behavior can vary based on temperature and pH. For example, a previous study mentioned that it was found through in vitro testing that the hydrogel showed a faster drug release rate at a pH of 7 than a pH of 7.4 [3]. The release rate is critical in drug delivery as it is vital for proper dosing. This contributes to both the safety and efficacy of a drug. The drug needs to be released at a rate that the particle's potency is not lost and can still carry out its desired effect. However, this also needs to be balanced with a release rate and nontoxic dosage for the patient. This experiment aims to demonstrate the importance of these factors.

In this experiment, alginate hydrospheres of various sizes will be utilized as drug delivery models. The hydrospheres will be loaded with BSA or methylene blue with the diffusion rate out of the beads modeling drug release behavior. The factors affecting the release of BSA and methylene blue will depend on both the ratio of surface area to volume and particle size. It is hypothesized that as the surface area to volume ratio increases, the diffusion rate will also increase. Furthermore, it is also hypothesized that the diffusion rate will decrease as the particle size increases.

Methodology

Experimental Design

This experiment utilized alginate hydrospheres to model drug delivery. Alginate was being used as a versatile biopolymer with easily controllable thickening, gel-forming, and stabilizing properties [4]. Different factors of drug delivery were modeled by varying both the size of the alginate beads and the particle size of the substance loaded onto the beads. Beads using 900µL and 100µL of alginate blue were made, some loaded with BSA and the remaining loaded with methylene blue.

Experimental Setup

Methylene Blue Hydrospheres:

20 mL of water was added to 0.25g of alginate powder and placed in a water bath. The mixture was vortexed and heated until homogeneous, and then 3mL of 0.5% methylene blue was added and vortexed. A 1000µL pipette was used with the tip cut at an angle and set to 300µL. Three large hydrogels were made by quickly dropping 300µL of the alginate solution in 0.27M calcium chloride solution. The process was repeated with 100µL of alginate solution to create nine small hydrospheres.

BSA hydrospheres:

20 mL of water and 3mL of 0.5 mg/mL BSA were added to 0.3g of alginate powder and vortexed until homogeneous. A 1000µL pipette was used with the tip cut at an angle and set to 300µL. Three large hydrogels were made by quickly dropping 300µL of the BSA-alginate solution in 0.27M calcium chloride. The process was repeated with 100µL of BSA-alginate solution to create nine small hydrospheres.

Standard Curves:

Standard curves were created for both methylene blue and BSA. For methylene, blue concentrations were made according to the table below:

Table 1: Standard Curve Methylene Blue Concentrations

Standard	The volume of 0.5% Methylene Blue (µL)	Volume Water (µL)
stock	220	0
1	110	110
2	55	165
3	22	198
4	11	209
5	5.5	214.5
6	2.2	218
7	1.1	219
8	0.5	219.5
blank	0	220

Each concentration was vortex and disrupted to the well plate in 100µL aliquots. The BSA standard curve was made similarly. However, a serial dilution was utilized and a working reagent (WR) to give the BSA a deep purple hue. The dilution is shown in Table 2, and the working reagent was calculated as follows:

(# standards + # unknowns) × (# replicates) × (volume of WR per sample) = total volume WR required

(7+7) x (2) x(200µL)= 5.6mL

Table 2: Standard Curve BSA Concentrations

Standard	Volume Water (µL)	The volume of BSA (µL)	Final Concentration (µg/mL)
stock	0	300 stock	2000
1	125	375 stock	1500
2	325	325 stock	1000
3	175	175 from vial 1	750
4	325	325 from vial 2	500
5	325	325 from vial 4	250
6	325	325 from vial 5	125
7	400	100 from vial 6	25
blank	400	0	0

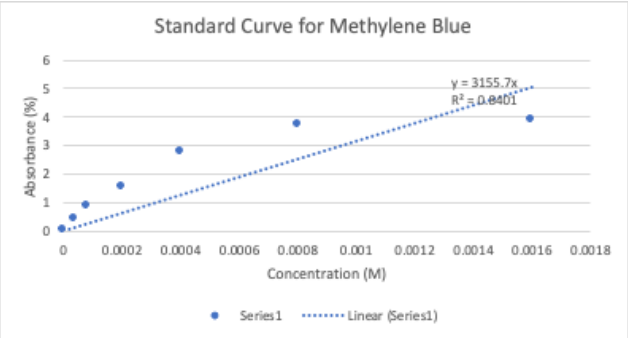
Diffusion:

For the diffusion portion of the experiment, each type of bead was placed in a small beaker with 10mL of water and a magnetic stir rod. While being stirred, two 100µL samples were taken from each methylene blue beaker, and 75µL samples were taken from the BSA beakers. Samples were taken starting at time 0 and then at 10-minute intervals until the time reached 60minutes. The samples were stored in well plates, and 200 µL of working reagent was added to each BSA sample. The BSA plate was then incubated at 37°C for 30 minutes, and then both plates were read in the BioTek reader. The BSA plate was read at 562 nm, and the methylene blue was read at 664nm.

Measurement and Analysis:

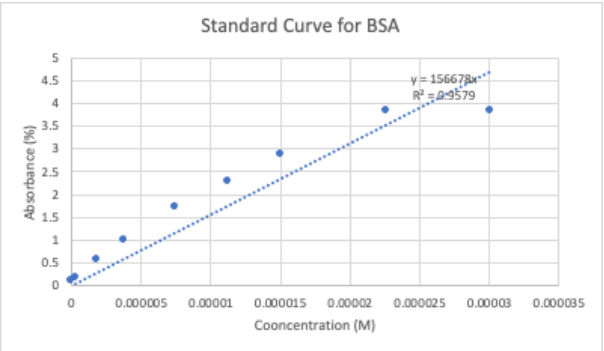
Data were collected every 10 minutes with 100 µL samples because this interval provided sufficient data with minimal impact on the remaining concentration. The standard curves were created using the absorbances from the BioTek reader and concentrations shown in Tables 1 and 2. These standard curves were then used to identify the unknown concentrations of each experimental condition. The standard curves are shown below:

Figure 1



Shows the linear regression curve created by averaging the absorbances for known concentrations of methylene blue

Figure 2



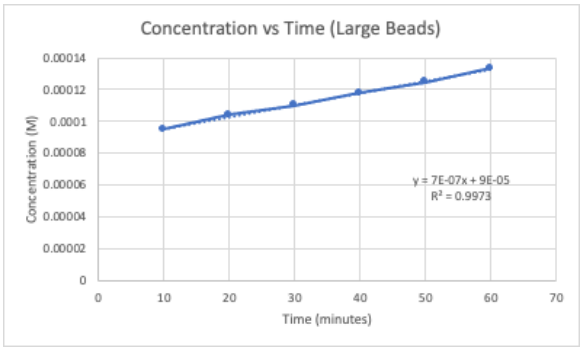
Shows the standard curve made by averaging the absorbance for known concentrations of BSA

Using the linear equation of the standard curves, the concentrations of the dilution samples were calculated and averaged. Then in excel, the data of concentration vs. time averages for each experimental group was formatted into a linear regression with a trend line that resulted in the best data fit. The slope of these lines represented the diffusion rates. Finally, the unaveraged diffusion

rates were used in a paired t-test using prism software to evaluate statistical significance between experimental conditions.

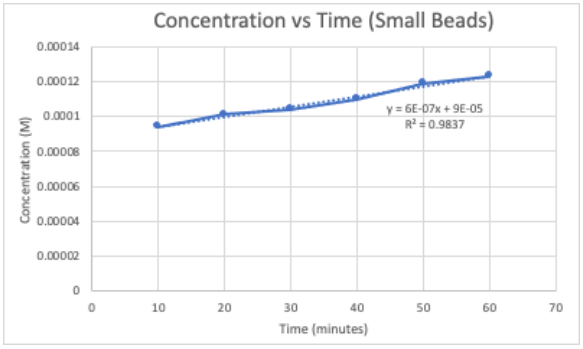
Results

Figure 3



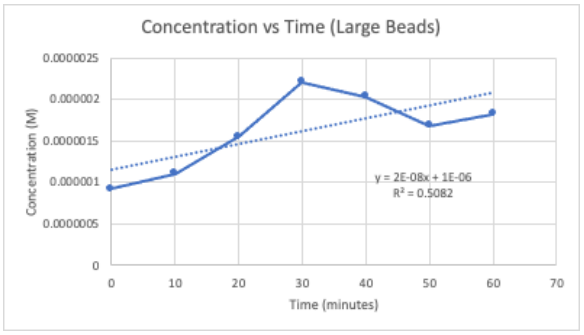
Linear regression on concentration vs. time graph showing the diffusion rate of three 300 µL methylene blue hydrospheres

Figure 4



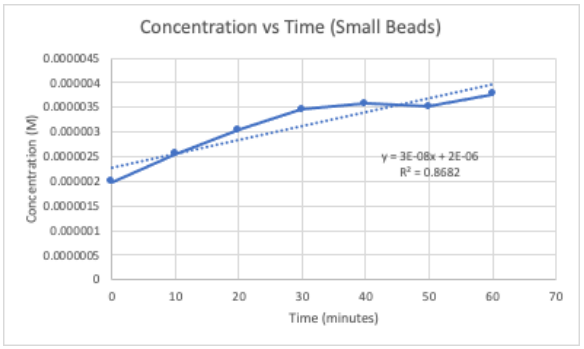
Linear regression on concentration vs. time graph showing the diffusion rate of nine 100 µL methylene blue hydrospheres

Figure 5



Linear regression on concentration vs. time graph showing the diffusion rate of three 300 µL BSA hydrospheres

Figure 6



Linear regression on concentration vs. time graph showing the diffusion rate of three 100 µL BSA hydrospheres

Table 3: Unaveraged diffusion Rates for Each Experimental Condition

Experimental Group	Diffusion Rate 1 (M/min)	Diffusion Rate 2 (M/min)
Large Methylene Blue	7.424×10^{-7}	7.379×10^{-7}
Small Methylene Blue	5.532×10^{-7}	6.003×10^{-7}
Large BSA	1.536×10^{-8}	1.564×10^{-8}
Small BSA	2.774×10^{-8}	2.822×10^{-8}

Table 3: Unaveraged diffusion Rates for Each Experimental Condition. The table depicts the difference in diffusion rates for each experimental condition. The large BSA beads have a slower diffusion than the small beads. The methylene blue hydrospheres have faster diffusion rates than the BSA hydrospheres. These values were used to calculate the statistical significance between experimental conditions in a paired t-test.

Statistical Results:

Table 4: Statistical Significance Results for Experimental Condition Comparison

Conditions	Large vs. Small Beads Methylene Blue	Large vs. Small Beads BSA	Small Beads BSA vs. Methylene Blue	Large Beads BSA vs. Methylene Blue
P-Value	0.0825	0.0051	0.0276	0.0021
Significance	no	yes	yes	yes
Mean Difference	1.634×10^{-7}	1.248×10^{-8}	-5.488×10^{-7}	-7.247×10^{-7}
Standard Deviation of Differences	3.012×10^{-8}	-1.414×10^{-10}	3.364×10^{-8}	3.380×10^{-9}

Table 4: Statistical Significance Results for Experimental Condition Comparison. The table shows the statistical calculation between the experimental conditions and the determination of the significance of the results. Statistical significance was found between 3 out of 4 of the statistical conditions. There was no statistical significance between the large and small methylene blue hydrospheres.

Two different types of molecules were used to make the hydrospheres: methylene blue and BSA. In addition to this, each molecule was used to make two different-sized hydrospheres: 100µL (n=9) and 300µL hydrospheres (n=3). Figures 1 and 2 show the standard curves for methylene blue and BSA, respectively. These served as a control and a baseline comparison to identify absorbances at unknown concentrations. Using this data, the diffusion rates of each experimental condition were calculated and analyzed. In figures 3-6, the over trend of concentration increasing with time can be seen. This data was fitted with linear regression with the slope of the line representing the diffusion rates. Figures 3-6 depict each experimental condition's averaged concentrations and absorbances, while Table 3 shows the averaged diffusion rates.

Discussions

Diffusion through hydrospheres can serve as a drug release model with the diffusion rate mimicking the release rate of a drug. Several factors can affect this rate, such as the surface area of the hydrosphere itself and the molecular weight of the molecule that the hydrospheres are composed of. This experiment investigated these factors through several experimental conditions. The data showed statistical significance between these conditions for 3 out of the 4 conditions, where statistical significance was not seen in the large vs. small methylene blue hydrospheres (seen in table 4). Diffusion is affected by the object's surface area from which diffusion is taking place. This can be qualitatively seen between the methylene blue small vs. large beads (figures 3 and 4) and the BSA small vs. large beads (figures 5 and 6). In figure 5a steeper slope, indicating a faster diffusion rate can be seen in the trend line in comparison to figure 6. This is confirmed in the statistical testing in Table 4, where there is a statistically significant P-value of 0.0051 for the BSA small vs. large beads. This shows that diffusion is affected by the surface area, where when the surface area increases, the diffusion rate will also increase. This is seen where the smaller beads have a higher surface area because there were 9 100 μL hydrospheres versus the large beads where there were only 3 300 μL hydrospheres. Even though these beads had the same total volume of 900 μL, the increased surface area of the small beads provided a larger area for which diffusion could take place, therefore increasing its rate. This is important to take into consideration for drug design as the surface area can primarily affect how fast the drug is released in the body. This trend was also observed in a study investigating the effect of tablet surface area to volume on tablets containing hydroxypropylmethylcellulose. In that study, tablets of various geometries and surface area to volume ratio were tested. It was found that tablets with a larger surface area to volume ratio had faster release profiles [5]. This aligns with the results in this experiment for the BSA beads but not the methylene blue beads. There was no statistical significance between the methylene blue small hydrosphere and large hydrosphere. This could have been a result of an error in the experiment. When the beads were initially placed in the water, they had some methylene blue on them which would offset the amount that was diffused from the spheres. This could skew the results as there would not have been an equal amount of methylene blue coating the small beads, and there would have been on the large beads.

Diffusion rates are also affected by the size of the molecule itself. The BSA is a much larger molecule than methylene blue. BSA has a molecular weight of 66,400 Da [6], and methylene blue has a molecular weight of 319.84 Da [7]. BSA having a much larger molecular weight will cause diffusion to occur at a much slower rate. This trend can be observed by comparing the large bead diffusion rates of the molecules and the small bead diffusion rates. A qualitative comparison can be seen in Figure 3, showing the large beads for methylene blues, and Figure 5, showing the large bead diffusion trend for BSA. A much steeper slope can be seen on the trendline for methylene blue, and this difference is confirmed as statically significant in Table 4, where the P-value for this comparison is 0.0021. Similar results can be seen in Figure 4 and Figure 6 for the small beads, and the P-value for this comparison is again statistically significant

with a value of 0.0276. This trend is observed because the bigger the molecule, the less room for diffusion. This trend was also observed in a study investigating different sizing of Lidocaine diffusing through poly(lactic-co-glycolic acid) (PLGA)-based microparticles. Here, when porosity was held constant, as the size of lidocaine increased, the diffusion rate decreased [8]. This is important because it indicates that the drug's size can significantly impact how fast the drug is released into the body. This means there needs to be additional testing when formatting drug tablets that consider the size of the molecule during its diffusion from its encapsulation.

These findings support the original hypothesis where it can be seen that the surface area of the hydrosphere (drug encapsulate) has a significant effect on how quickly a drug will diffuse. In addition to this, the second hypothesis was also supported, showing that the size of the drug (modeled by BSA and methylene blue) affects the diffusion rate. The larger the molecule, the slower the diffusion rate, shown by the statistically significantly slower diffusion rate for BSA.

Conclusions

The hydrospheres in this experiment model drug release rates and how these rates are affected by the surface area of the drug encapsulation and drug molecule size. Both hypotheses were either entirely or partially supported, as seen through paired t-tests showing a statistical significance between experimental groups. An increase in the surface area of the drug encapsulation leads to an increased diffusion rate, and the bigger the molecule diffusing, the slower the diffusion rate. This was seen as the small hydropheres had a faster diffusion rate than the large hydrospheres and the BSA diffused significantly slower than the methylene blue. These findings are important when considering drug design as it shows that the drug tablet and the structure of the drug itself can have a significant effect on how the drug is released into the body. These factors are of great concern when evaluating the safety and efficacy of a drug.

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