ABSTRACT

Title of Thesis: **SOLUTE RELEASE FROM POLYMER CAPSULES LOADED WITH LIPOSOMES**

Priyanka Acharya, Master of Science, 2017

Directed by: Prof. Srinivasa R. Raghavan
Department of Chemical & Biomolecular Engineering

Polymer capsules and lipid vesicles (liposomes) are two types of containers that have been extensively studied for their applications in drug delivery. In this thesis, we explore a hybrid of these two structures (i.e. polymer capsules bearing encapsulated liposomes) and study the release of solute from these structures. The capsules are made by contacting the anionic biopolymer alginate with multivalent cations such as calcium (Ca^{2+}) or holmium (Ho^{3+}), which crosslink the alginate chains. Liposomes prepared from conventional phospholipids are loaded with a model solute (an aromatic dye) and then encapsulated in the alginate capsules. We study the effects of different variables on solute release, including the capsule size and architecture, crosslinking ion type and concentration, and crosslinking time. In addition, we compare release from the bare capsules (not containing liposomes) with that from capsules containing liposomes. A key finding is that the latter releases solute over a much longer time compared to the former. Overall, the results from this study will guide the design of new structures for drug delivery applications.

SOLUTE RELEASE FROM POLYMER CAPSULES LOADED WITH LIPOSOMES

Priyanka Acharya

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Advisory Committee:

Prof. Srinivasa R. Raghavan, Dept. of Chemical & Biomolecular Engineering, Chair

Prof. Panagiotis Dimitrakopoulos, Dept. of Chemical & Biomolecular Engineering

Prof. Amy Karlsson, Dept. of Chemical & Biomolecular Engineering

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Chapter 1: INTRODUCTION & OVERVIEW

Vesicles and biopolymer capsules have been widely studied as drug carriers for many years [1-5]. Vesicles are a type of nanocontainer enveloped by a bilayer of lipid or surfactant molecules (lipid vesicles are called liposomes). The aqueous core of liposomes can be used to encapsulate many different types of solutes. However, liposomes are very sensitive structures and are often destabilized by changes in the external environment such as temperature or pH [6]. Capsules can be defined as structures that consist of a solid shell and an inner core. These types of containers are much more mechanically stable than liposomes and can also hold a variety of solutes in their aqueous core [7]. However, a disadvantage of capsules is their tendency to release solute rather quickly, i.e., over a few hours.

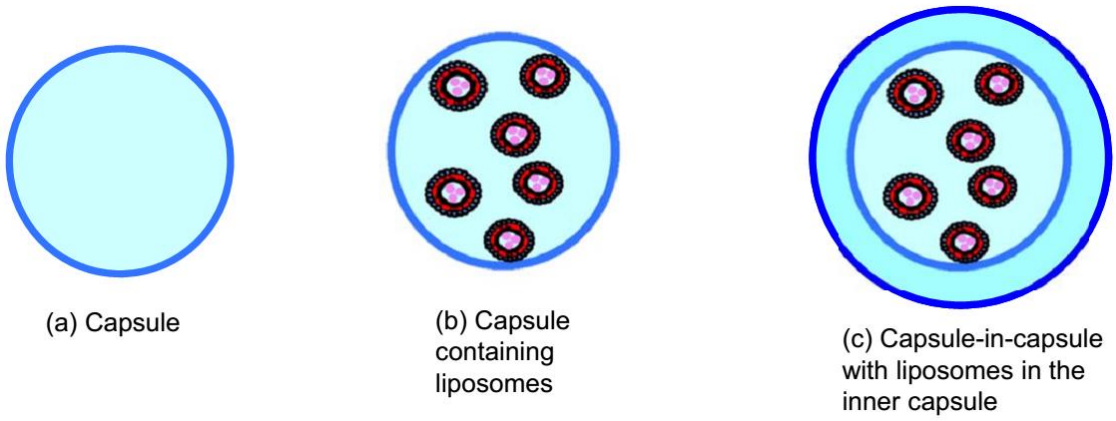

Figure 1.1. Three different structures of polymer capsules. (a) Bare capsules; (b) Capsules containing liposomes; and (c) Capsule-in-capsule, with the inner capsule containing liposomes.

In this thesis, we explore the release of solutes from different types of containers. We focus on hybrid structures that integrate polymer capsules with liposomes, as shown

in Figure 1.1. More specifically, we wish to see if we can extend the release of solute by varying the architecture of the container. The first container investigated is a bare polymer capsule (Figure 1.1a). Our capsules are made by contacting the anionic biopolymer alginate with multivalent cations such as calcium (Ca^{2+}) or holmium (Ho^{3+}), which crosslink the alginate chains. The second container investigated (Figure 1.1b) is the above polymer capsules with encapsulated liposomes. The third type of structure studied (Figure 1.1c) is a capsule-in-capsule, where the inner capsule contains liposomes. Controlled release experiments conducted with these three types of containers are described in Chapter 3. We find that the combination of transport resistances from the liposomal bilayer and the capsule allow for an extended release of solutes encapsulated in the liposomes. This concept is illustrated in Figure 1.2.

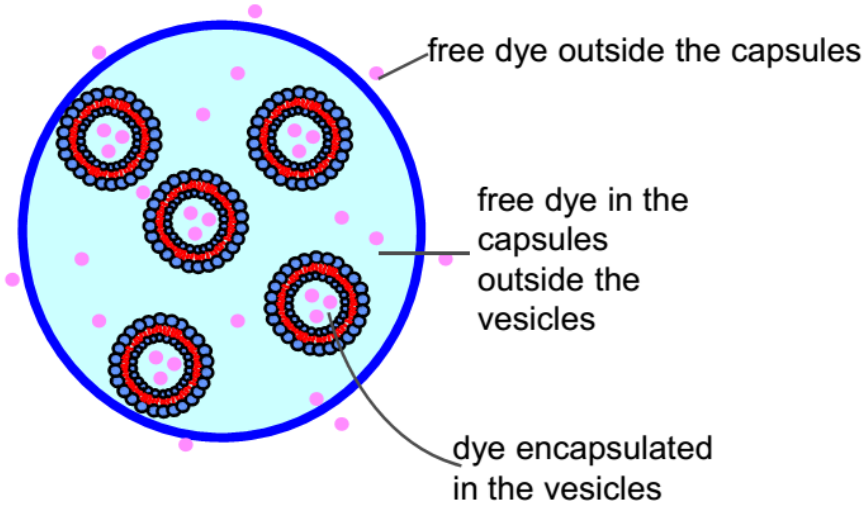

Figure 1.2. Solute release from a polymer capsule bearing encapsulated liposomes. At time zero, all the solute is in the liposomes. As time progresses, the dye leaks out of the liposomes, then through the capsule lumen and shell, and finally into the external medium. The combination of transport resistances from the liposomal bilayer and the capsule allow the solute release to occur over an extended period.

To our knowledge, there are few systematic studies on solute release from biopolymer capsules or from capsule-liposome hybrid structures such as those shown in Figure 1.1. This provides the rationale for this thesis. We have systematically varied several properties of our liposome-loaded capsules to see if there was any effect on the kinetics and extent of solute release. These properties include:

- Capsule crosslinking time
- Crosslinking cation type
- Concentration of crosslinking solution
- Capsule size (microcapsules vs macrocapsules)
- Capsule architecture (as noted in Figure 1.1)

The results from our studies provide many insights into the ability of capsules and liposomes to hold and release solutes. One key finding is that having encapsulated liposomes inside polymer capsules enables a much slower, extended release of solute as compared to a bare capsule loaded with the solute.

Chapter 2: BACKGROUND

In this chapter, we describe the basic properties of vesicles and biopolymers used in this study. The main components of the vesicle loaded capsules are alginate, holmium, and lipid vesicles. We will talk about capsule and vesicle preparation. Then, we will briefly describe the techniques used in this study, specifically UV-Vis Spectroscopy.

2.1. Vesicles and Liposomes

Vesicles are self-assembled containers that are formed spontaneously in water by the organization of lipid or surfactant molecules into closed spherical bilayer structures. The typical structure of a vesicle is shown in figure 2.1. The molecules that make up vesicles are usually amphiphilic in nature, with a hydrophilic head that prefers to be in contact with water and hydrophobic tail(s) that wants to be shielded from water. The shell of a vesicle is a bilayer of these lipid or surfactant molecules, with the hydrophilic heads facing the water on both sides while the hydrophobic tails are buried inside the bilayer away from the water. The formation of vesicles is driven by the hydrophobic effect and the geometry of the amphiphilic molecules [6]. The hydrophobic effect refers to the desire of hydrophobic molecules or molecules with hydrophobic domains to minimize their contact with water. Thus, the bilayer has a tendency to fold into a closed spherical structure in order to minimize the contact of the hydrophobes with the water at the bilayer ends. Vesicles with a single bilayer are called unilamellar vesicles (ULVs) while vesicles with several concentric bilayers separated by narrow water regions are called multi-lamellar vesicles (MLVs) [6].

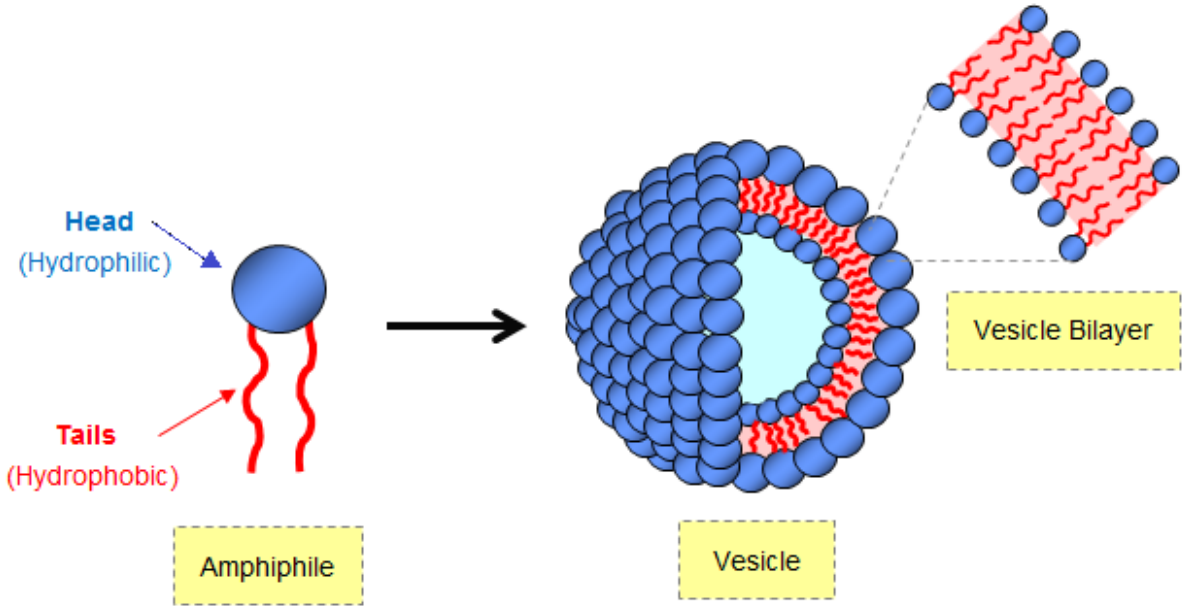

Figure 2.1. Structure of vesicles formed by the self-assembly of amphiphiles. The vesicle is formed by the folding of an amphiphilic bilayer 5-10 nm in thickness.

Vesicles formed from lipids are referred to as liposomes. Lipids are amphiphilic biomolecules that have a low solubility in water because they have two hydrophobic tails. Thus, the preparation of lipid vesicles usually involves the use of an organic solvent [8].

2.1.1 Vesicle Preparation from Lipids:

The process of obtaining liposomes or lipid vesicles is depicted in Figure 2.2. First, the lipids are dissolved in an organic solvent such as chloroform. Then, the solvent evaporated out under a nitrogen stream to yield a dry lipid film. This film is then hydrated in water or buffer solution at a temperature above the gel to liquid crystal transition temperature of the lipid[8]. This solution is stirred during this process and MLVs are formed. A couple of freeze thaw cycles are then applied to the MLV suspension and

then the solution is either sonicated or extruded through a polycarbonate filter of a given pore size[6,9].

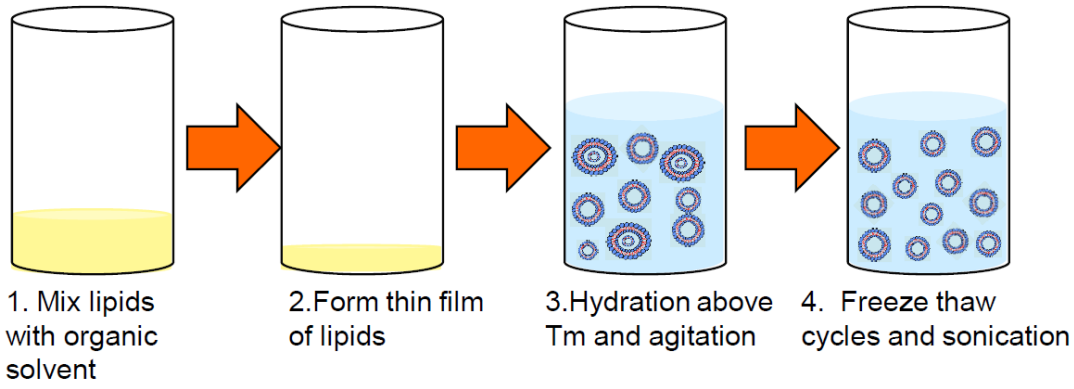

Figure 2.2. Preparation of liposomes (lipid vesicles)

2.2 Biopolymers

Biopolymers are macromolecules of biological origin that consist of long chains of repeating, covalently bonded units. They can be classified into three categories: polypeptides or proteins, polynucleotides, and polysaccharides. For our studies, we will focus on polysaccharides[6]. The polysaccharide that is of special interest to us is alginate and we will describe its properties below in more detail.

2.2.1 Alginate

Sodium alginate is a water soluble, anionic polysaccharide that is obtained from brown seaweed. It is used for a variety of biomedical applications due to its low cost, biocompatibility, low toxicity, and its ability to easily crosslink with multivalent cations. It is a linear unbranched polymer consisting of 1,4-linked β-D-mannuronic (M) and α-L-guluronic (G) residues (Figure 2.3). The G blocks of alginate can interact with specific

multivalent ions (e.g. Ca^{2+}, Ho^{3+}, Ba^{2+}). The crosslinking of polymer chains occurs via exchange of the monovalent sodium ions from the G blocks with the multivalent cations thus forming "egg box" junctions, which results in the conversion of an alginate solution into a crosslinked gel network[10-13].

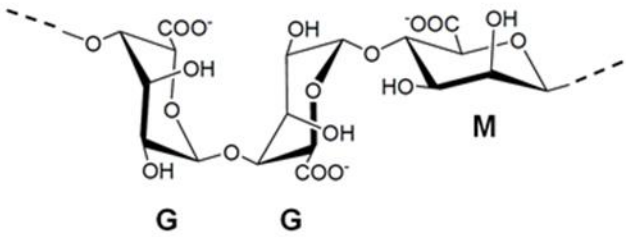

Figure 2.3. Structure of alginate biopolymer.

2.3. Polymer Capsules

Capsules are defined as structures having an inner core enclosed within a solid shell. They can be prepared using various techniques such as emulsification, layer-by-layer assembly, electrostatic complexation, ionic crosslinking, and coacervation [2].

2.3.1. Capsule Formation

For this study, we prepare the capsules via the ionic crosslinking process. This method uses electrostatic interactions between polymers and multivalent cations. To form capsules, solution of an anionic biopolymer (in our case, alginate) is dropped into a solution of holmium, which is a multivalent cation. Crosslinking occurs at the interface of the drop, thus forming an interfacial shell as show in Figure 2.4. Note that this method helps facilitate encapsulation of solutes within the capsules. Any solutes or structures that are inside the generating drop are preserved in the capsule[6,14].

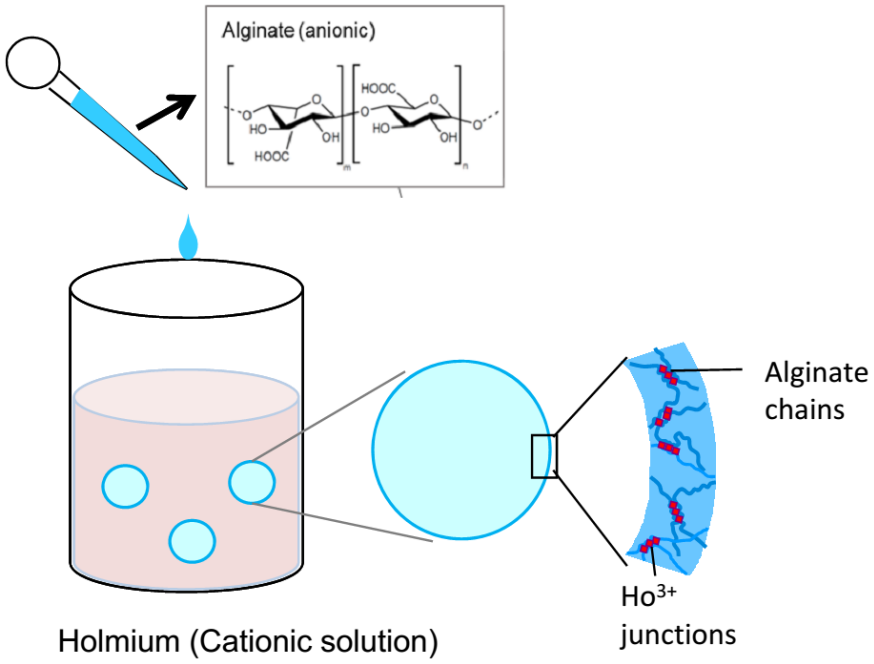

Figure 2.4. Creation of Alg/ Ho^{3+} capsules. A drop of alginate is added to a solution of holmium (multivalent cation).

2.3.2. Polymer Capsules Bearing Encapsulated Liposomes

Polymer capsules bearing encapsulated capsules are prepared using a modified process of the ionic crosslinking technique outlined in Figure 2.5 below. Briefly, encapsulated liposomes are mixed with the anionic alginate solution as shown in step one. This solution is then added drop wise to a bath of cationic holmium solution. The capsules are then allowed to crosslink for a certain amount of time, and are subsequently washed and stored in a solution of deionized water. The electrostatic interactions that occur between the negatively charged alginate and positively charged holmium stabilize the capsule shell. In addition, these capsules are stable under stirring.

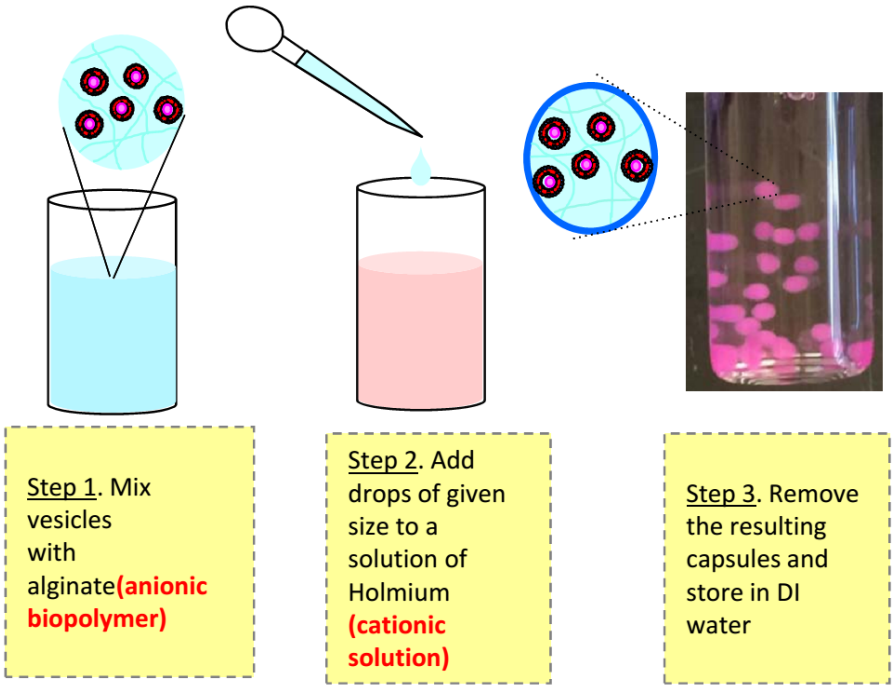

Figure 2.5. Creation of Alg/Ho^{3+} capsules bearing encapsulated lipid vesicles (liposomes).

2.4 Characterization Techniques: UV-VIS Spectroscopy

UV-Vis absorption spectroscopy is an analytical technique used to study molecules that adsorb radiation in the ultraviolet (200 to 400 nm) and visible (400 to 800 nm) regions of the electromagnetic spectrum. When a molecule absorbs radiation in the UV-Vis range, it generally excites the electrons into higher energy levels. Since this absorbed energy is quantized, the molecule does not absorb energy continuously throughout the UV and visible regions. This means that the molecule will absorb radiation only at wavelengths that provide the exact amount of energy to excite the electrons to higher energy levels. Each compound will thus have a unique UV-Vis spectrum.

A typical UV-Vis experiment is done with a solution of low solute concentration which is then placed in a cuvette into the sample cell of a UV-Vis spectrometer. Light is broken down into its component wavelengths in the spectrometer and passed through the sample. The absorption intensity is measured for each wavelength and a UV-Vis spectrum (plot of absorbance vs. wavelength) is produced for the sample.

UV-Vis spectroscopy can be used to determine the concentration of the solute. This is done using the Beer–Lambert law.

$$A = \varepsilon \cdot c \cdot \ell \tag{2.1}$$

where A is the measured absorbance at a particular wavelength, c is the concentration of the solute in mol/L, ℓ is the path length of the sample, and ε is the molar extinction coefficient or molar absorptivity at that wavelength[14]. UV-Vis spectroscopy plays an important role in controlled release studies. For example, as will be discussed in Chapter 3, the amount of acid red 52 dye released from the three containers studies is quantified by measuring the absorbance of the external solution over time.

Chapter 3: POLYMER CAPSULES LOADED WITH LIPOSOMES: CONTROLLED RELEASE STUDIES

3.1. Introduction

In this chapter, we synthesize polymer capsules loaded with liposomes and study the release of solute from these structures. As mentioned in Chapter 1, our motivation for studying this hybrid structure is its potential to be used for controlled release applications. Liposomes have the ability to encapsulate hydrophilic solutes in their aqueous core. We hypothesize that the advantage of this capsule/liposome hybrid structure will be that it can prolong the release of solute because of the presence of two transport barriers (the liposomal bilayer and the capsule shell). Furthermore, the capsule will protect the encapsulated liposomes from the external environment such as changes in pH and temperature.

Polymer capsules containing nanoscale liposomes have been studied for the past couple of decades [4,12,15-22]. For example, the release of small solutes (e.g., dyes) from liposomes loaded into alginate capsules has been studied by Takegi et al. and Ullrich et al. The release kinetics was found to be dependent on temperature and lipid composition [22]. Dhoot et al. studied release of a fluorescent protein from liposomes loaded into alginate capsules. They found that the release rate of the protein was dependent on the crosslinking ions used to make the capsule as well as the lipid composition of the liposomes [21]. These studies have mostly not considered other factors that could influence the release of solute such as the capsule structure or architecture itself.

In our work, we focus on studying release of a hydrophilic dye (acid red 52) from three different containers: capsules, capsules loaded with liposomes, and capsule-in-capsules loaded with liposomes. The liposome composition is kept constant while various properties of the capsules are varied. These properties include capsule size, capsule crosslinking time, type of crosslinking cation, and concentration of the crosslinking cations. Our data reveal several systematic trends in solute release, and these are discussed in detail in this chapter.

3.2. Experimental Section

Materials and Chemicals

Alginate (medium molecular weight), cholesterol, calcein, sephadex G-50, calcium chloride dihydrate, and holmium (III) hexahydrate chloride were obtained from Sigma-Aldrich. The phospholipid dipalmitoylphosphatidyl-choline (DPPC) was purchased from Avanti Polar Lipids. The dye acid red 52 was obtained from TCI America. Deionized water purified by a reverse osmosis system was used in all experiments.

Preparation and Characterization of Liposomes

Liposomes were prepared by the thin film hydration method. Briefly, dried films of the lipids were hydrated under moderate stirring. This hydration step was done with a 25 mM aqueous solution of acid red 52. The liposomal solution was freeze-thawed five times, and then sonicated for 3 hours. This gave liposomal sizes of around 200 nm, as measured by dynamic light scattering (DLS). The solution was then passed through a Sephadex G50 size exclusion column to separate out the free dye from the dye-filled liposomes.

Dynamic Light Scattering (DLS)

Dynamic light scattering was used to analyze the size of the liposomes. The experimental setup consists of a laser source that is focused through a lens on a sample and the scattering light from the sample is recorded by a detector that is typically placed 90° to the source. Here, a Photocor-FC instrument with a 5 mW laser light source at 633 nm was used to analyze the size of the liposomes. Studies were done at 25°C with the scattering angle being 90°. A logarithmic correlator was used to measure the intensity autocorrelation function. From this, a diffusion coefficient for the liposomes was obtained. The apparent hydrodynamic radius of the liposomes was extracted from the diffusion coefficient through the Stokes-Einstein relationship.

Preparation of Alginate Capsules and Capsule-in-Capsules

Alginate macrocapsules were prepared by dropping 1 wt % alginate through a 22-gauge needle into a solution of a multivalent cation, typically holmium (Ho^{3+}). The capsules were crosslinked for 30 minutes before being washed and stored in DI water. Capsules prepared by this method were typically 2.5 mm in size. To prepare the capsule-in-capsules, the 2.5 mm capsules were added to a 1 wt% alginate solution. This was then dropped into a solution of holmium through a transfer pipette, and upon crosslinking for 30 min, a 5 mm outer capsule was formed with a smaller capsule in it. The larger capsule was then washed and stored in DI water.

To prepare liposome-containing capsules and capsule-in-capsules, the above procedure was modified. Instead of using just an alginate solution, an alginate solution mixed with dye-filled liposomes was used. The rest of the steps were the same as above.

The setup used to prepare to liposome-loaded alginate microcapsules is shown in Figure 3.1. Briefly, a feed solution of alginate and liposomes was passed through a syringe pump into a glass capillary and pulses of nitrogen gas were used to shear off the droplets from the capillary tip. The gas flowed as a casing around the tip of the capillary; so for every pulse of gas, an aqueous droplet was released from the capillary tip. A function generator that was connected to a gas flow controller generated these pulses of gas [23].

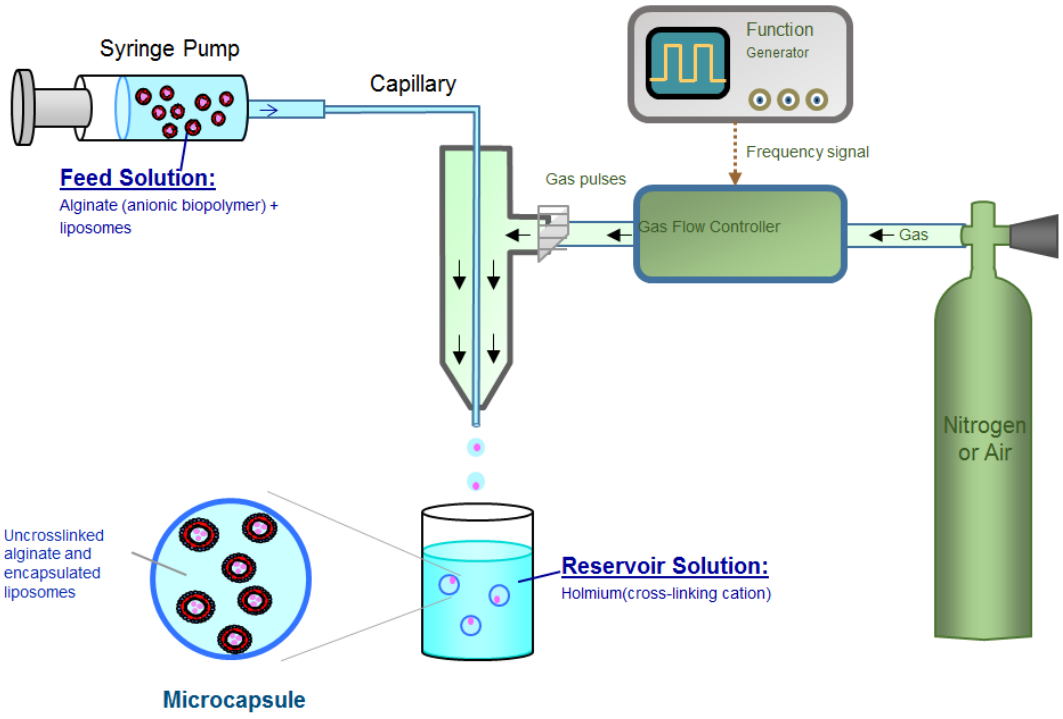

Figure 3.1 Preparation of alginate microcapsules loaded with liposomes.

Dye Release Experiments

Release experiments were conducted via two setups, as shown in Figure 3.2. In the first setup, two capsules were placed inside a vial of water that was continuously stirred (Figure 3.2a), and the dye concentration around the capsules was measured. In the second setup, capsules were placed in a dialysis bag, which was then placed in a reservoir filled with water that was continuously stirred (Figure 3.2b). In both cases, the stirring was achieved by placing the vials on a magnetic stir plate and the dye concentration was measured via UV-Vis spectroscopy on a Varian Cary 50 spectrophotometer.

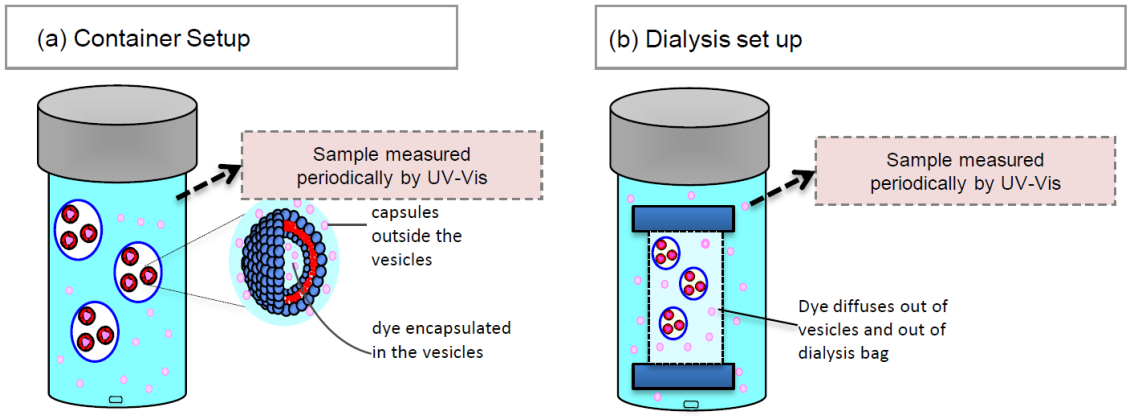

Figure 3.2. Experimental setups for dye release studies. (a) Capsules in a vial of continuously stirred DI water. (b) Capsules in a dialysis bag that is placed in a reservoir of continuously stirred DI water.

Microscopy

A Zeiss Axiovert 135 TV inverted microscope equipped with Motic Image Plus imaging system was used for optical microscopy. Capsules were imaged with a 2.5X objective.

3.3. Results and Discussion

3.3.1. Dye Release from Alg/Ho^{3+} Capsules

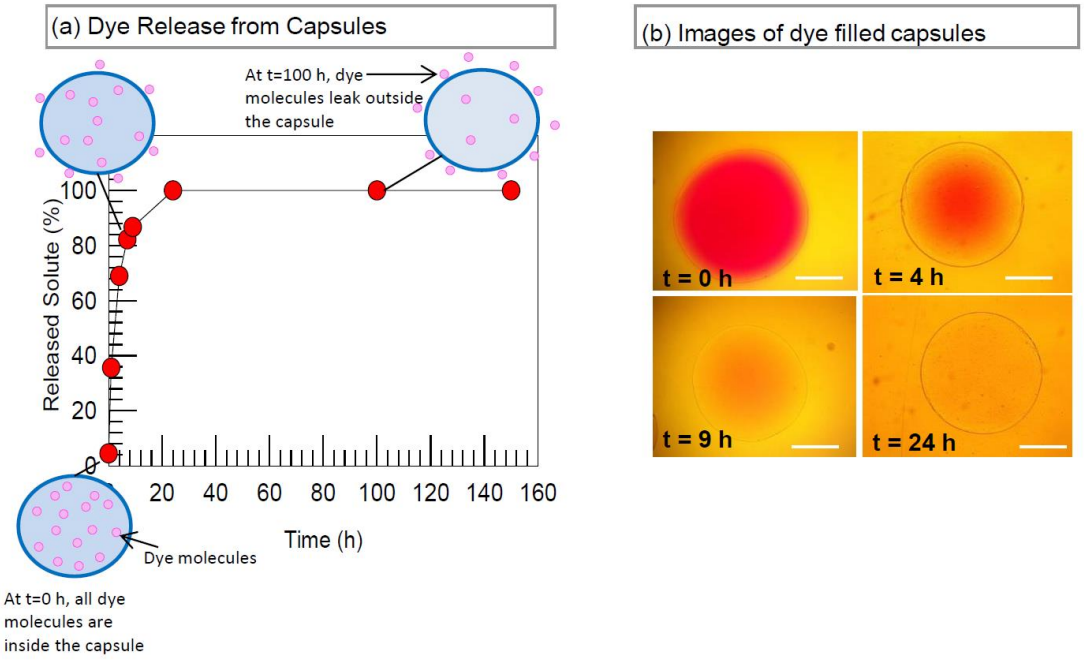

Figure 3.3. Release of acid red 52 dye from Alg/Ho^{3+} capsules. (a) Release curve. Schematics of the capsule at various times are also shown. (b) Images of the capsule from optical microscopy. Scale bars are 1 mm.

First, we discuss dye release from capsules loaded with dye (i.e., with no liposomes present). The capsules studied here are formed by the ionic crosslinking of alginate (Alg), which is an anionic biopolymer, using Ho^{3+} cations. We added a 1 wt% Alg solution dropwise through a 22-gauge needle into a solution of 100 mM Ho^{3+}, whereupon the alginate droplets were converted into capsules. The thickness of the capsule shell increases with incubation time, and we left the droplets to incubate for 30 min. For the release experiments, the capsules were loaded with 25 mM of acid red 52 dye and then placed in DI water. Dye release was monitored in the external solution, as shown in Figure 3.2a. Once the dye concentration in the solution had plateaued, this

value was used to normalize the data and thereby construct a plot for the cumulative % of dye release vs. time, as shown in Figure 3.3a. Correspondingly, we also took images of the capsule using optical microscopy, and these are shown in Figure 3.3b.

From the dye release curve in Figure 3.3a, we see that almost 80% of the dye is released within 10 h and almost 100% within 24 h. This result is also visually seen from the images in Figure 3.3b. At the start of the experiment, the dye is contained in the capsule, which is why the capsule appears a bright orange color under the microscope. As time progresses, the dye is rapidly released and the capsule loses that color. By the 24 h mark, the capsule is indistinguishable in its color from the external solution.

3.3.2. Dye Release from Alg/Ho^{3+} Capsules Loaded with Liposomes

Next, alginate-holmium capsules with encapsulated liposomes were prepared. The liposomes were composed of the phospholipid DPPC combined with cholesterol in a 70:30 molar ratio. Liposomes were prepared by the thin film hydration method, as described previously, using a 25 mM solution of the acid red 52 dye. Following sonication, the liposome diameter was measured to be 200 nm by DLS. The liposomes were then passed through a size-exclusion column to separate out the free dye from the dye-filled liposomes. The latter was then mixed with 1 wt% alginate and the mixture was added to 100 mM Ho^{3+} and incubated for 30 min. The resulting capsules with liposomes in them were then placed in DI water. Dye release was then monitored in the external solution, as shown in Figure 3.2a. A normalized release curve for this case is shown in

Figure 3.4. Correspondingly, we also took images of the capsule using optical microscopy, and these are shown in the figure as well.

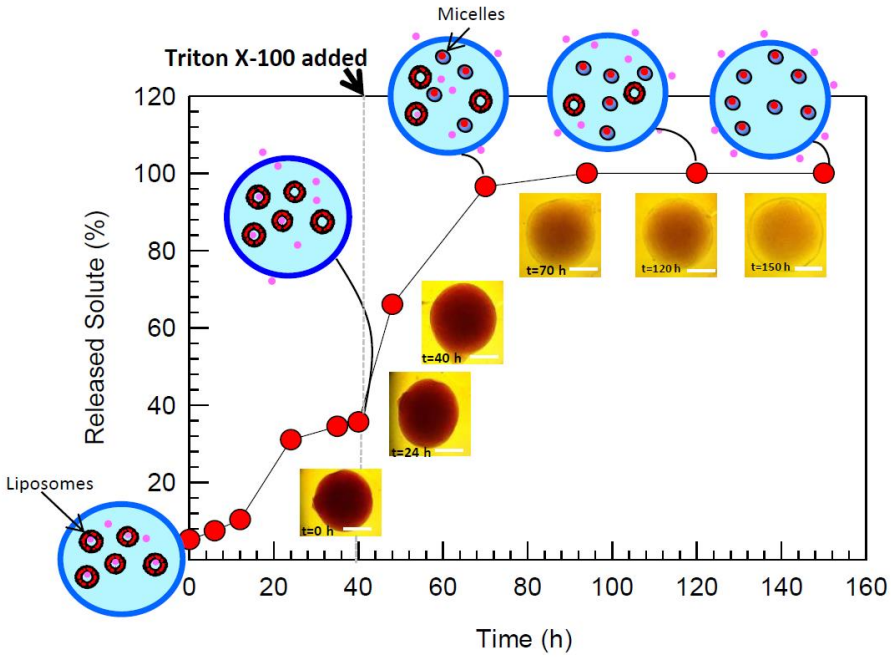

Figure 3.4. Release of acid red 52 dye from liposome-loaded Alg/Ho^{3+} capsules. The detergent Triton X-100 was added at the 40 h mark (indicated by the arrow). Schematics and images of the capsule from optical microscopy at various times are also shown. Scale bars in the images are 1 mm. Note from the schematics that beyond the 40 h mark, the liposomes in the capsule are converted to micelles.

Figure 3.4 shows that only about 35% of the dye is released in the first 40 h, which is considerably less than in the case of the bare capsules from Figure 3.3. This means that the presence of liposomes in the capsules slows down the release of dye. Note that, at time zero, most of the dye is contained *inside* the liposomes, as shown by the schematics in Figure 3.4. As time progresses, the dye leaks out of the liposomal bilayer, and then out of the capsule shell into the external solution. In contrast, the dye in the bare capsules simply diffuses out through the shell. Thus, we attribute the slow release of dye to the presence of multiple transport barriers. Note also from the images that the capsule with liposomes appears dark, i.e., does not transmit light. This is because the liposomes

scatter light and render the capsule turbid. For the full 40 h period, the capsule continues to appear dark, indicating that the liposomes are intact in the capsule lumen.

At 40 h, the detergent Triton X-100 is added to the external solution. Triton X-100 is a detergent that is known for its ability to destroy liposomes and convert them into micelles (~ 5 nm in diameter). This molecule diffuses through the capsule shell into the interior of the capsules, whereupon the liposomes in the capsule are turned into micelles. In the process, the hydrophilic dye in the liposomes will be released into the capsule lumen and therefore it will get released more easily than in the case when it was inside the liposomes. We therefore expect that the introduction of Triton X-100 should result in an increase in the dye concentration in the external solution. This is indeed what we see in Figure 3.4. A spike in dye concentration is seen at the 40 h mark, and correspondingly, the images show that the capsule becomes less turbid, which reflects the internal conversion of liposomes into much smaller micelles. By 100 h, full dye release is achieved. Note that the curve is normalized relative to this dye concentration.

Figure 3.5 shows a comparison of the release curves from bare capsules (i.e., the data in Figure 3.3; black symbols) and that from liposome-loaded capsules (i.e., the data in Figure 3.4; red symbols). In addition, a third release curve is shown for liposome-loaded capsules where the Triton X-100 was added at a different time point (72 h; green symbols). In all these experiments, the capsule is the same, i.e., it is Alg/Ho^{3+} with the same concentration of Alg and Ho^{3+} and crosslinked for the same amount of time. The dye concentration is the same for the red and green curves (liposome-loaded capsules)

and this is approximately the same as that in the bare capsules. Figure 3.5, clearly shows that the liposome-loaded capsules extend the dye release over a much longer time compared to the bare capsules. The release saturates around 35% for both the red and green curves between 20 and 40 h. In both cases, a spike in dye release is observed when Triton X-100 is added, suggesting that the added Triton X-100 disrupts the liposomes. This in turn implies that the liposomes were intact before the Triton X-100 was added.

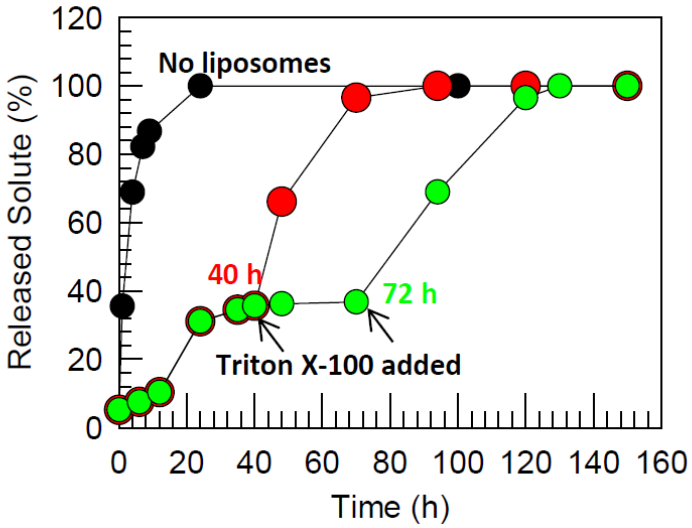

Figure 3.5. Release of acid red 52 dye from bare Alg/Ho^{3+} capsules (black curve) and from liposome-loaded Alg/Ho^{3+} capsules (red and green curves). In the case of the red curve, Triton X-100 was added at the 40 h mark (indicated by the arrow) while in the case of the green curve, Triton X-100 was added at the 72 h mark (indicated by the arrow).

3.3.3. Effect of Alg/Ho^{3+} Crosslinking Time on Dye Release

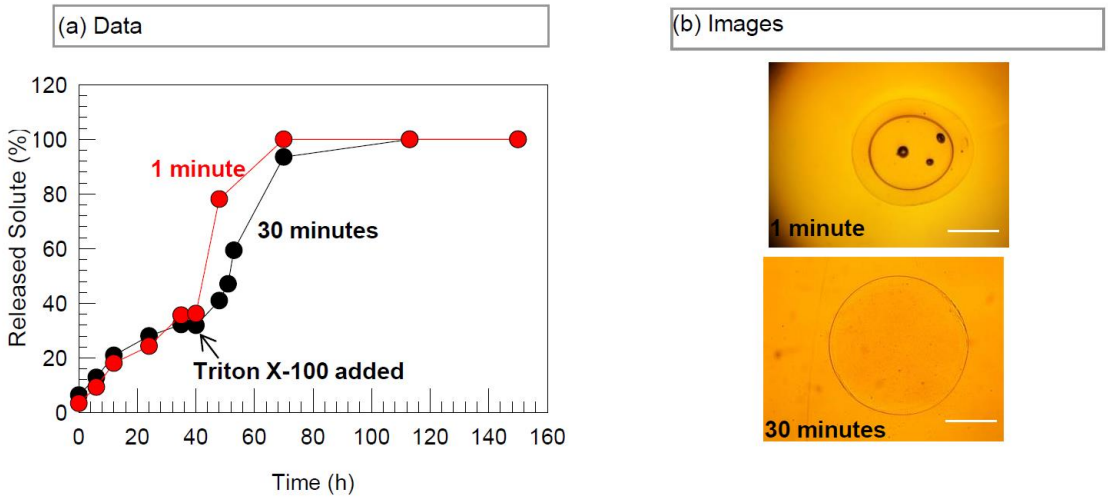

Figure 3.6. Effect of Alg/Ho^{3+} crosslinking time on the release of acid red 52 dye from liposome-loaded Alg/Ho^{3+} capsules. (a) The red curve corresponds to capsules crosslinked for 1 min whereas the black curve is for capsules crosslinked for 30 min. In both cases, Triton X-100 was added at the 40 h mark (indicated by the arrow), which causes a spike in dye release. (b) Images of bare Alg/Ho^{3+} capsules. Scale bars are 1 mm. Note that when capsules are crosslinked for just 1 min, there is a clear distinction between a shell and an inner core. For capsules crosslinked for 30 min, no such distinction exists.

Next, we studied the effect of capsule crosslinking time. The capsules were made by dropwise addition of 1% Alg (bearing liposomes) into a 100 mM Ho^{3+} solution, and crosslinking (incubation) times of 1 min and 30 min were used. Figure 3.6 shows that the dye-release curves are similar for both cases. This suggests that dye release from liposome-loaded capsules is independent of crosslinking time. Generally, in the case of Alg/Ho^{3+} capsules, we expect the Ho^{3+} ions to diffuse into the droplet and crosslink the Alg chains. With greater crosslinking time, we expect a higher extent of ion-induced crosslinking in the capsule. Interestingly, we observe that Alg/Ho^{3+} capsules crosslinked for 1 min have a distinct outer shell and a core (Figure 3.6b). This suggests that the Ho^{3+}

ions have only diffused partially into the droplet in 1 min, leaving the core as an uncrosslinked aqueous fluid. In contrast, capsules crosslinked for 30 min appear uniform (Figure 3.6b), i.e., the shell has grown inward with time and enveloped the entire capsule. In other words, the 1 min case corresponds to a core-shell morphology whereas the 30 min case is a uniform gel. Despite these morphological differences, the release curves are practically the same. This is likely to be the case because the Alg/Ho^{3+} network is highly porous, with the pores being much larger than the size of the solute (dye) molecules. Thus, the dye can freely diffuse through the Alg/Ho^{3+} network with a diffusivity similar to that in water.

3.3.4. Effect of Ho^{3+} Concentration on Dye Release

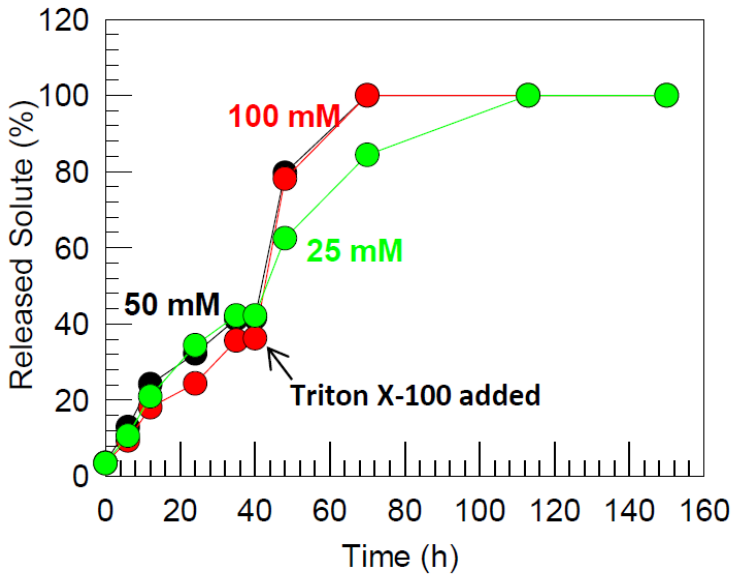

Figure 3.7. Effect of Ho^{3+} concentration on the release of acid red 52 dye from liposome-loaded Alg/Ho^{3+} capsules. Data are shown for three Ho^{3+} concentrations, as indicated. In all cases, Triton X-100 was added at the 40 h mark (indicated by the arrow), which causes a spike in dye release.

Next, we varied the concentration of Ho^{3+}. Liposome-loaded Alg/Ho^{3+} capsules were prepared using Ho^{3+} solutions of 25 mM, 50 mM, and 100 mM (the crosslinking time was 30 min in all cases). Figure 3.7 shows dye release curves for the three cases, and again the curves are all similar. Increasing the Ho^{3+} should increase the crosslinking of the Alg in the capsule, i.e., lead to a denser network. Nevertheless, a similar release is observed from both less and more dense networks. This again suggests that all the Alg/Ho^{3+} networks have pores that are much larger than the dye molecules, allowing the dye to freely diffuse through the networks, much like through water.

3.3.5. Effect of Crosslinking Cation (Ho^{3+} vs. Ca^{2+}) on Dye Release

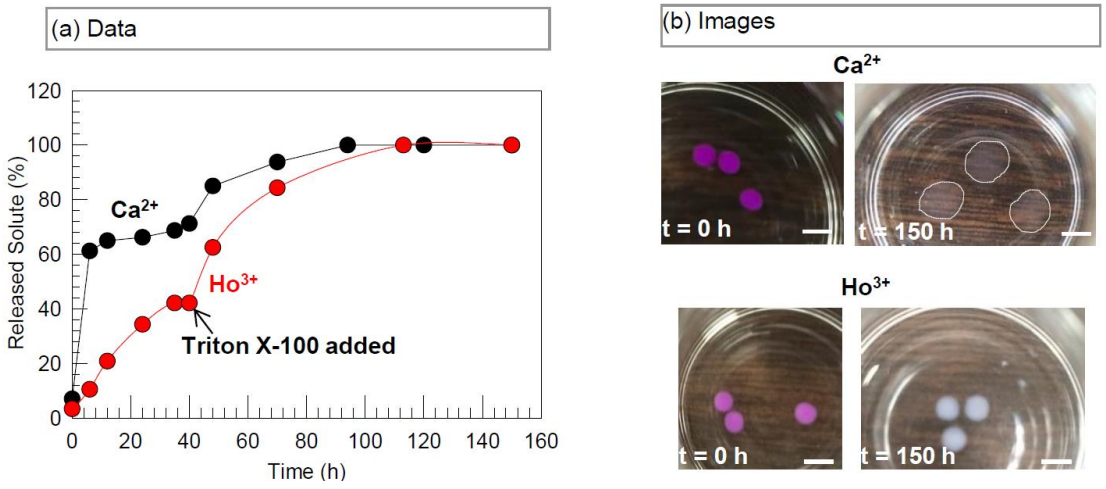

Figure 3.8. Effect of crosslinking cation (Ho^{3+} vs. Ca^{2+}) on the release of acid red 52 dye from liposome-loaded Alg capsules. (a) Release curves (Triton X-100 was added at the 40 h mark in both cases). (b) Photos of the capsules at t = 0 and 150 h. In the Ca^{2+} case, the capsules swell a lot more and become nearly transparent. The swollen capsules are circled for clarity. Scale bars are 3 mm.

We move on to the effect of the crosslinking cation. Liposome-loaded Alg capsules were prepared using either Ho^{3+} or Ca^{2+} at 100 mM, with a 30 min crosslinking time. Figure 3.8a compares the release curves and it is clear that the crosslinking cation

does affect dye release. At 40 h, 35% of the dye is released from the Alg/Ho^{3+} capsules while 69% of the dye is released from the Alg/Ca^{2+} capsules. Photos of both capsules are shown in Figure 3.8b at the start and the end of the experiment. Both capsules start off with a bright purple hue due to the dye being inside the liposomes, which are in the capsule. As time goes by, both capsules lose their color as the dye is released into the solution. However, the Alg/Ho^{3+} capsules appear milky white at the end of the experiment (150 h). The Alg/Ca^{2+} capsules, on the other hand, appear less turbid (bluish) at 150 h. This is because the Alg/Ca^{2+} capsules swell significantly compared to the Alg/Ho^{3+} capsules. This swelling is observed as quickly as 24 h after the start of the experiment. We believe this swelling explains the faster dye release from the Alg/Ca^{2+} capsules. Capsule swelling generally implies an increase in the pore size of the alginate network [24]. Although pore size on its own may not be a factor (see Figures 3.6 and 3.7), the Alg/Ca^{2+} network does seem more permeable than the Alg/Ho^{3+} one.

3.3.6. Effect of Experimental Technique on Dye Release

We also varied the technique used to do the dye release experiment. The data in Figures 3.3 to 3.8 were collected using the technique illustrated in Figure 3.2a, where the capsules were placed in a container and stirred, with the supernatant being analyzed periodically. As an alternative, we used the dialysis technique illustrated in Figure 3.2b. For this, we placed liposome-loaded capsules in a water-filled dialysis bag, which was then placed in reservoir of water that was continuously stirred. With the dialysis setup, the release of dye is due to diffusion only as the capsules are contained in the dialysis bag, which is not stirred. In the container setup on the other hand, there is both diffusion

and convection. Figure 3.9 compares the release curves from the two experiments on a normalized scale. The capsules in both cases were identical: liposome-loaded Alg/Ho^{3+} capsules prepared using 100 mM Ho^{3+} with a 30 min crosslinking time. The experiments were done such that the volume of fluid into which dye releases was kept about the same, so that the dye concentrations between the two experiments were also comparable on an absolute scale. Figure 3.9 shows that the curves are very similar. About 40% of dye is released within the first 40 h in both cases. The rest gets released once the Triton X-100 is added. For the dialysis experiment, the Triton X-100 is added to the external fluid and it has to diffuse into the dialysis bag and then into the capsules in order to disrupt the liposomes. Thus, there is a lag time before the spike in dye release occurs. Figure 3.9 confirms that our results are independent of the method used to measure dye release.

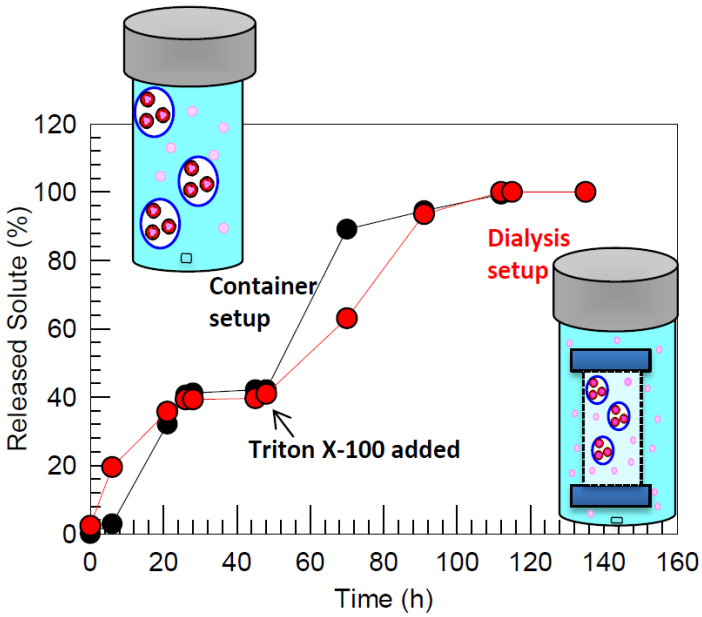

Figure 3.9. Release of acid red 52 dye from liposome-loaded Alg/Ho^{3+} capsules measured by two techniques: capsules in container (black symbols) and capsules in dialysis bag (red symbols). Schematics of the two techniques are shown. In both cases, Triton X-100 was added at the 40 h mark.

3.3.7. Effect of Different Dyes on Release

We also varied the type of dye used in the release experiments. For these experiments we compared the release with the dyes acid red 52 and calcein. Acid red 52 and calcein have molecular weights of 580.6 g/mol and 622.5 g/mol, respectively, as specified by the manufacturer. Liposome-loaded Alg/Ho^{3+} capsules were prepared with a 100 mM Ho^{3+} solution with a 30 min crosslinking time. Dye concentrations of 25 mM were used and the experiments were done using the container setup to compare the effect of the different dyes on release. We expected that the release experiments done with the acid red 52 dye would release faster as it is a lighter molecule than calcein and thus can diffuse out faster. However, as we can see in figure 3.10a, the release curves are similar. This seems to suggest once again that the pores of the Alg/Ho^{3+} networks are much larger than both the acid red 52 and calcein dye molecules, allowing the different dyes to freely diffuse through the networks at similar rates.

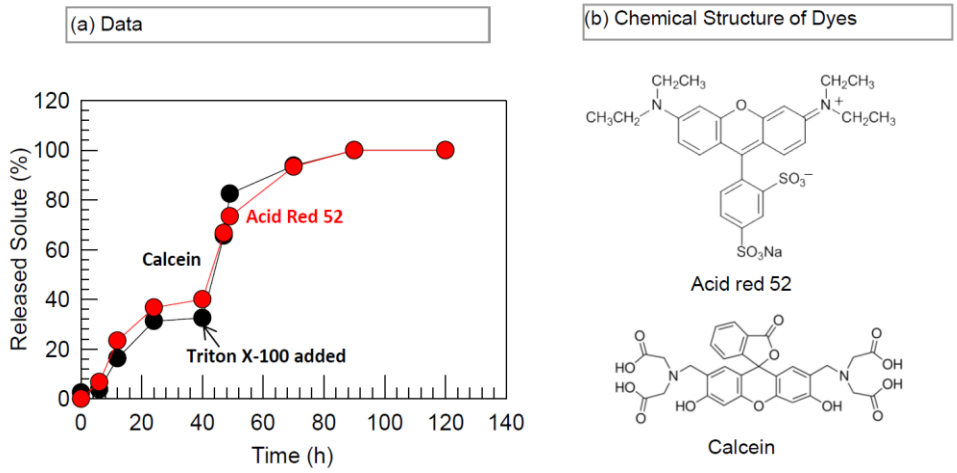

Figure 3.10. Release of acid red 52 and calcein dye from liposome-loaded Alg/Ho^{3+} capsules. (a) Release curves on a normalized scale. The red curve corresponds to the release experiment being done with acid red 52 and the black curve corresponds to the release experiment being done with calcein. In both cases, Triton X-100 was added at the 40 h mark (indicated by the arrow), causing a spike in dye release. (b) Chemical structures of the dyes used: acid red 52 and calcein.

3.3.8. Effect of Capsule Size on Dye Release

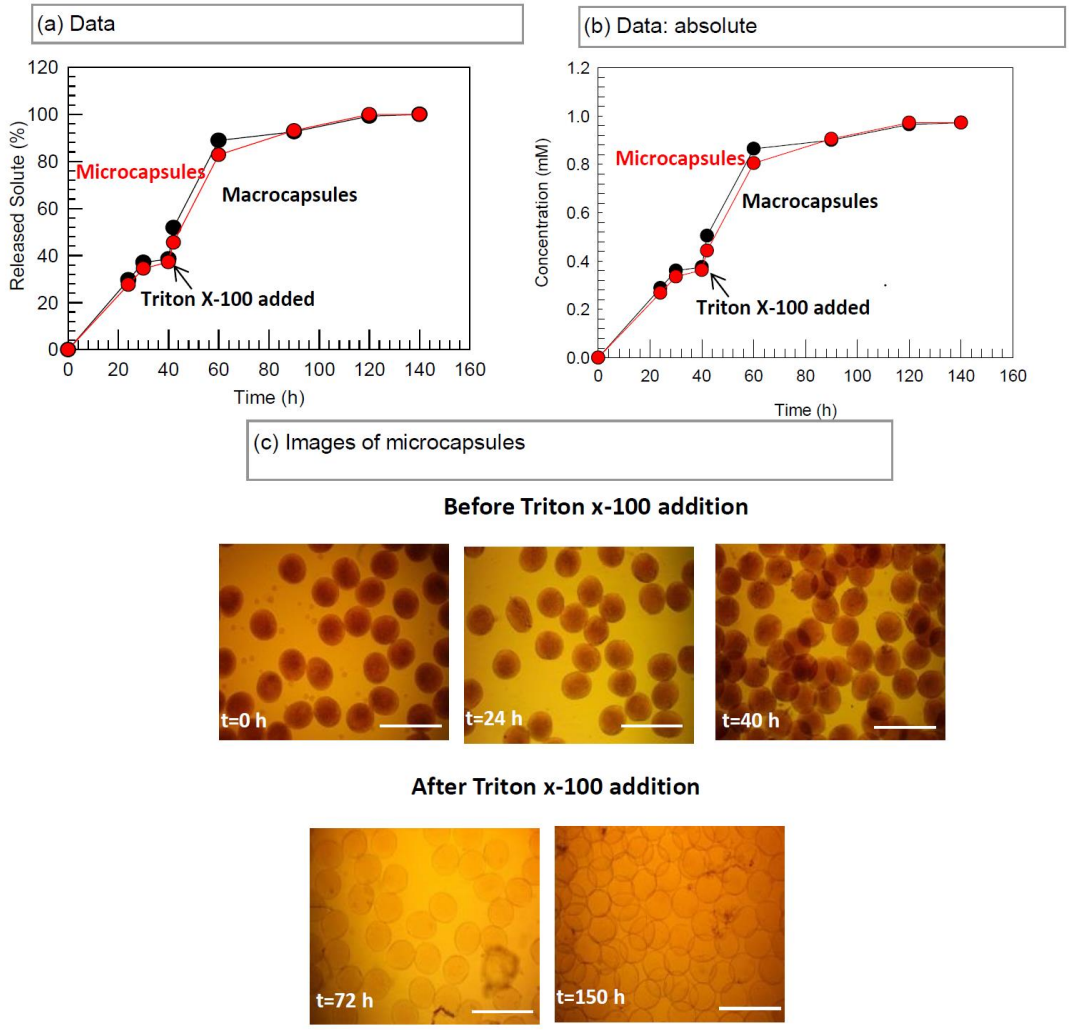

Figure 3.11. Release of acid red 52 dye from liposome-loaded Alg/Ho^{3+} capsules of two sizes: macrocapsules (2.5 mm diameter) (black curve) and microcapsules (400 µm diameter) (red curve). In both cases, Triton X-100 was added at the 40 h mark. (a) Release curves on a normalized scale. (b) Release curves on an absolute scale. (c) Images of the microcapsules from optical microscopy at various times. Scale bars in the images are 1 mm.

The next variable we looked at was the capsule size. For this, we used the same capsule composition: liposome-loaded Alg/Ho^{3+} capsules prepared using 100 mM Ho^{3+}

with a 30 min crosslinking time. First, we prepared macrocapsules (2.5 mm diameter), which are the same ones studied in Figures 3.4 to 3.9. Next, we prepared microcapsules (400 μm diameter) using the microfluidic technique described in the Experimental Section. We used the dialysis setup to compare the two. Equal weights of the two sets of capsules were placed in separate dialysis bags, which were each placed in a reservoir of water. Figure 3.11a and b show that both macro- and microcapsules exhibit similar release curves. This suggests that dye release is independent of capsule size. The absolute (non-normalized) data in Figure 3.11b indicate that both sets of capsules hold the same amount of dye, which validates the size comparison. We also collected images of the microcapsules at various time points, as shown in Figure 3.11c. The images are strikingly similar to those in Figure 3.4. That is, for the first 40 h, the microcapsules appear dark because the liposomes are intact in the capsule lumen. At 40 h, Triton X-100 is added and correspondingly, the microcapsules become less turbid, which reflects the internal conversion of liposomes into much smaller micelles. By 150 h, full dye release is achieved and the microcapsules are completely transparent.

3.3.9. Dye Release from Capsule-in-Capsules

Finally, we investigate a different architecture for the Alg/Ho^{3+} capsules, i.e., a capsule-in-capsule, with the internal capsule having liposomes in them. First, we prepared liposome-loaded Alg/Ho^{3+} capsules (2.5 mm diameter), using 100 mM Ho^{3+} with a 30 min crosslinking time. Then we mixed these with Alg and used that mixture as a feed to make the capsule-in-capsules, which had an overall diameter of 5 mm (see Experimental Section). The two architectures were compared in a conventional

vial/container setup (Figure 3.2a), with the capsules placed in water at t = 0 and the vial being stirred.

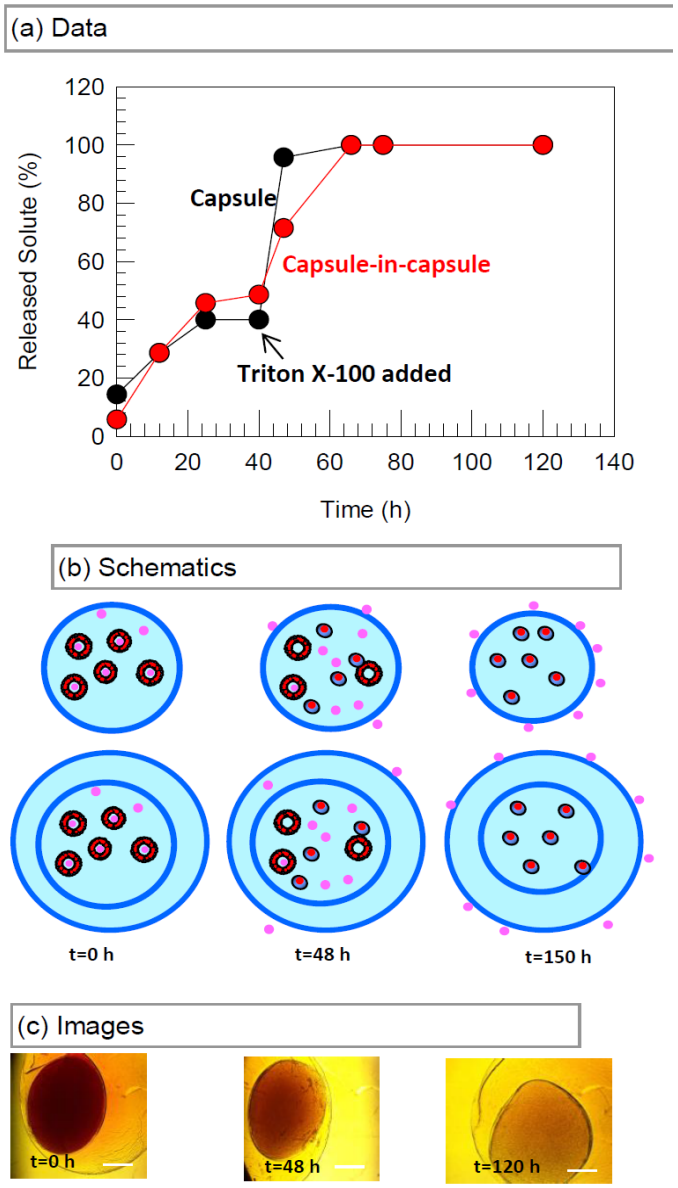

Figure 3.12. Release of acid red 52 dye from liposome-loaded Alg/Ho^{3+} capsules of two architectures: conventional capsule (black curve) and capsule-in-capsule, with liposomes in the inner capsule (red curve). In both cases, Triton X-100 was added at the 40 h mark. (a) Release curves. (b) Schematics of the two architectures at various times. (c) Images of the capsule-in capsule from optical microscopy at various times. Scale bars in the images are 1 mm.

The point of this experiment was to see whether placing liposome-loaded capsules inside a larger capsule would prolong the dye release as the dye would have to diffuse out of the liposomes and then out of both the inner and outer capsule into the release medium (as illustrated by the schematics in Figure 3.12b). However, Figure 3.12a shows the release profile of dye from the two types of capsules, and they are nearly the same. The reason is that both experiments were done while the sample was being stirred. Thus, both diffusion and convection are involved during the mass transfer of dye out of the capsules. To observe the extended dye release with the capsule-in-capsule, we need to re-do this experiment in a setup that emphasizes the role of diffusion, such as the dialysis setup. Microscope images of the capsule-in-capsule are shown in Figure 3.12c. (These images are difficult to acquire because the structures are large and therefore the whole structure cannot be seen.) Still, the images do show that the inner capsule is dark due to the liposomes inside it. Once the Triton X-100 is added, the inner capsule becomes much lighter, indicating that the liposomes are broken down into micelles.

3.4. Conclusions

We have studied the release of dye from hybrid structures that combine two commonly used drug carriers: capsules and liposomes. Our results show that dye release from liposome loaded capsules is much slower than dye release of capsules alone. This result is promising as it has potential utility in drug delivery applications. By varying various properties of the alginate capsules such as crosslinking time, crosslinking ion, holmium concentration, and capsule size, we found that the dye release from the liposome loaded capsules is only dependent on the crosslinking ion. We also observed

that solute release from a capsule-in-capsule with liposomes architecture is no different than release from liposome-loaded capsules. In all our experiments, we used the detergent Triton X-100 at some point to lyse the liposomes and observed an acceleration in dye release shortly thereafter. This is indication that prior to the detergent addition, the liposomes were still intact.

These studies show systematic trends on solute release from different containers and may be useful in guiding the design for new structures in drug delivery.

Chapter 4: CONCLUSIONS & RECOMMENDATIONS

4.1. Conclusions

In this thesis, dye release was studied for three different structures: polymer capsules, polymer capsules with encapsulated liposomes, and capsule-in-capsule with liposomes. We have shown that loading liposomes within polymer capsules can extend dye release. The properties of the capsule were varied such as capsule crosslinking time, concentration of crosslinking solution, crosslinking ion, and capsule size to observe its effect on dye release from these liposome loaded capsules. From our studies, we have found that only the type of crosslinking ion used to make the capsules changed the release rate of dye from the liposome-loaded capsules. Furthermore, we provide indirect proof that the liposomes remain intact during the dye release experiment. We show that there is a noticeable increase in dye release after the addition of the liposome destabilizing detergent, Triton X-100. This dye release after the Triton addition suggests that the liposomes were previously intact. Finally, we were able to study the release from a capsule-in-capsule structure with the inner capsule being loaded with liposomes. We found that this type of structure has similar release characteristics as liposome-loaded capsules. The trends observed in this thesis give us insight into solute release behavior from the different structures studied and may be useful in controlled release applications.

4.2. Future directions

For future studies, all experiments should be repeated with microcapsules. Since we have shown that capsule size does not change the release rate of dye, we should expect similar release profiles for all the variables tested. In addition, there are new ink jet technologies that are able of producing capsules sizes below 100 microns[11]. Decreasing the size of the polymer capsule-liposome system is useful if *in vitro* or *in vivo* studies were to be conducted in the future.

Another property of the liposome loaded capsules that could be investigated in the future is the lipid composition. In particular, the ratios of DPPC and cholesterol could be varied and its effect on release from the alginate capsules could be observed[25]. This would provide us with insight as to the optimal ratio of lipids to use for these studies. In addition, since DPPC is a temperature sensitive lipid, we could study the effect on temperature on dye release from the capsules[19]. More specifically, we could see if we could trigger release of dye when we increase the temperature to above the T_m of the lipids. This could be useful in control release applications where a triggered release is desired.

Studying the interactions between the alginate and the liposomes would also be useful to understand the structural changes that may occur in the vesicles when the polymer is added[18,24]. For this, techniques like SANS and cryo-TEM can be used. Cryo-TEM may be useful in seeing how the size and morphology of the liposomes change when mixed with alginate. SANS would help to verify the presence of liposomes in the alginate solution and detect any interactions that may occur between the alginate and liposomes.

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Publication that is planning to be submitted after defense:

1. Acharya, P. and Raghavan, S.R. Solute Release From Polymer Capsules bearing Liposomes. *Manuscript in preparation*