ABSTRACT

Title of Thesis: **Slow and Sustained Release of Hydrophilic Solutes from Capsules**
Hannah Cho, Master of Science, 2024

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Capsules can be used to deliver solutes encapsulated in their aqueous core, including drugs. However, small, hydrophilic solutes tend to leak out of typical capsules in a matter of minutes. Solute release can be completely prevented if the capsule shell is made of a hydrophobic solid like paraffin wax. The goal of this study is to achieve release profiles between these extremes — i.e., release of solutes in a *slow and sustained manner* over a period of days. For this, we modify the wax-shell design above and include a nonionic surfactant (from the Brij series) along with the wax. Most of our studies have been done with Brij-C10, which has a polyethylene glycol (PEG) head and a hexadecyl (C_{16}) tail. We show that capsules with a shell of 80/20 wax/Brij can release model dyes from the aqueous core over 20+ days. Such slow release has not been reported previously. The release rate can be tuned via the concentration of Brij in the shell (the higher the Brij, the faster the release) as well as the chemistry of the Brij (i.e., the size of the surfactant head or tail). The sustained release occurs because the Brij-bearing shell has microchannels through which the solute can permeate. Our approach can be used to slowly release many solutes, including dyes, drugs, and reactive reagents (such as H_2O_2). The simplicity and versatility of this approach make it highly suitable for controlled release applications across the pharmaceutical, agrochemical, and cosmetics industries.

Slow and Sustained Release of Hydrophilic Solutes from Capsules

By

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Chapter 1: Introduction and Overview

Aqueous container-like structures play a crucial role in diverse industrial applications. These containers encapsulate a wide variety of solutes and are used not only in pharmaceutical or medicinal products but also in food, agriculture, cosmetics, agrochemicals, etc.[1-3] The core of a given container could either be water or a hydrogel. The solutes loaded in the core are usually small, hydrophilic molecules with molecular weights less than 1000 Da. Some examples of solutes that could be encapsulated in these containers are drugs, nutrients, agrochemicals, or fragrances.[1] However, achieving controlled release of solutes out of these containers remains a persistent challenge in various applications.

Capsules and hydrogels are the two kinds of aqueous containers that are typically used to encapsulate solutes. The term 'capsule' refers to a spherical structure with a core surrounded by a thin shell. The core of the capsule can be a hydrogel or simply water. Hydrogels are three-dimensional networks of crosslinked polymer chains, which entrap large amounts of water. The polymer chain networks in hydrogels are formed through either physical or chemical bonds. Physically crosslinked hydrogels are formed by weak, physical interactions between the chains such as ionic or hydrogen bonds. In contrast, chemically crosslinked hydrogels are formed by stronger covalent bonds between the chains.[4] Some of the most used natural polymers for physically crosslinked hydrogels are alginate and gelatin. Alginate, an anionic polysaccharide extracted from brown-algae, can be crosslinked by multivalent cations (such as Ca^{2+}) through ionic bonding.[5-7] Gelatin,

obtained by denaturing the protein collagen, forms a gel through hydrogel bonding between its chains.[8] Capsules or gels fabricated in the lab range in size from 100 μm to a few millimeters. As model solutes, we work with water soluble dyes, which can be easily analyzed by absorption spectroscopy.

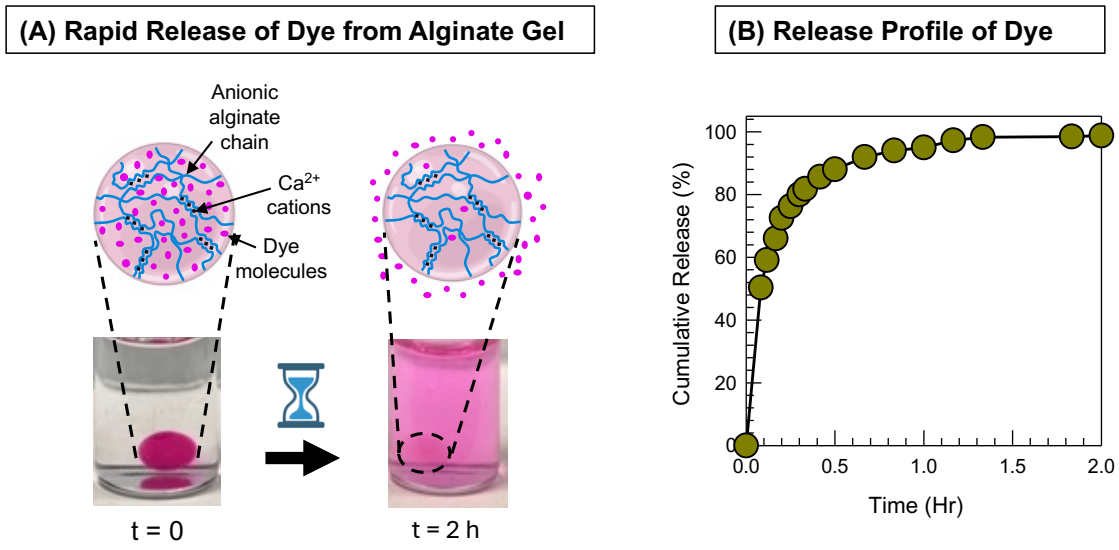

Figure 1.1 Rapid release of a small, hydrophilic solute out of a hydrogel. (A) An alginate gel loaded with Rhodamine B dye is placed in water at $t = 0$. Within 2 h, most of the dye leaks out of the gel into the water. At the 2 h mark, the gel is barely visible in the vial, while the external solution has turned pink. (B) Release profile of the dye, showing cumulative release (i.e., concentration of dye in the external solution normalized by the final concentration) vs. time. More than 80% of the dye is released within 30 min.

One issue with using hydrogels for controlled release applications is that the hydrophilic solutes encapsulated within them are often much smaller than the mesh or pore size of the gel. This results in rapid release of the solute via diffusion. For example, a spherical alginate gel loaded with a dye is shown in Figure 1.1. The gel almost instantly starts to release the dye when placed in water. Most of the dye contained in the hydrogel is

released in minutes and the release is completed within 2 h. This time window for the release of small molecule solutes is far too short for many applications.

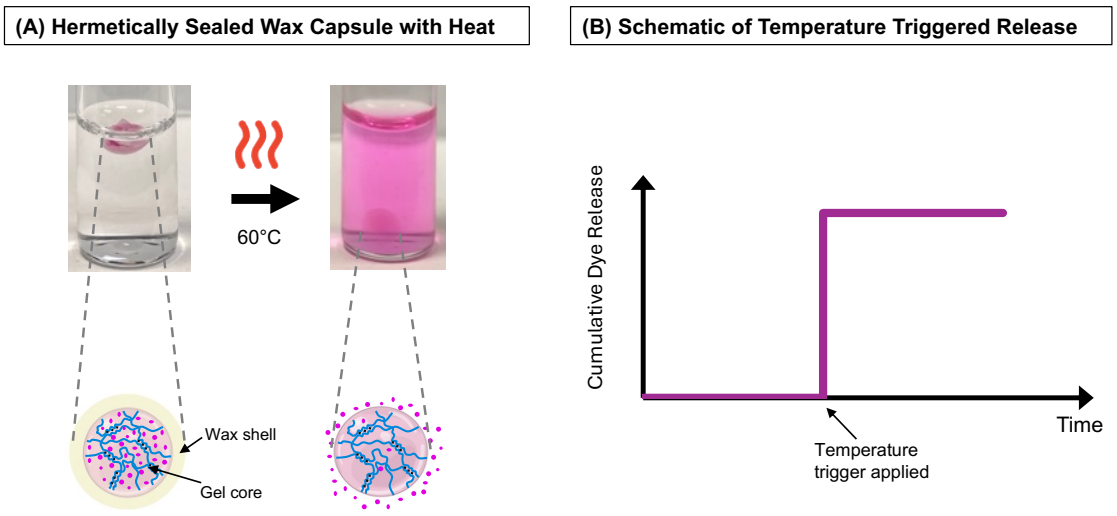

Figure 1.2 Wax-shelled capsules that can be triggered by heat. (A) The capsule has a core of alginate gel loaded with Rhodamine B and a shell of paraffin wax (T_m = 57°C). When placed in water at 25°C, there is no release of dye out of the capsule for long times, indicating a hermetic seal. When heated to 60°C ($T > T_m$), the wax shell melts and dye is rapidly released. (B) Schematic plot of cumulative dye release vs. time showing the step-change in release at temperatures above T_m.

Our lab previously reported a way to prevent leakage of solutes from a hydrogel for a very long time (Figure 1.2).[1] For this, we created capsules with the hydrogel as the core and a shell made of paraffin wax. Such capsules were made by dipping a cold hydrogel into molten wax for a few seconds. When a dye-loaded alginate gel is covered with a wax shell, the dye will no longer leak into water for months at room temperature. The shell creates a *hermetic seal* because wax is hydrophobic. The only way for solute to be released is by melting the shell, i.e., by heating to the melting temperature T_m of the wax, which is 57°C.

In this thesis, we will discuss our approach to obtaining slow and sustained release of solutes from capsules at room temperature. As shown by Figures 1.1 and 1.2, current designs for containers (gels or capsules) fall into *two limiting cases* when it comes to solute release *at room temperature*: very quick release (Figure 1.1) or no release at all (Figure 1.2). Our goal instead is to achieve the release profiles shown in Figure 1.3A, where release of solute occurs over an extended period (e.g. days of weeks) instead of minutes or hours.

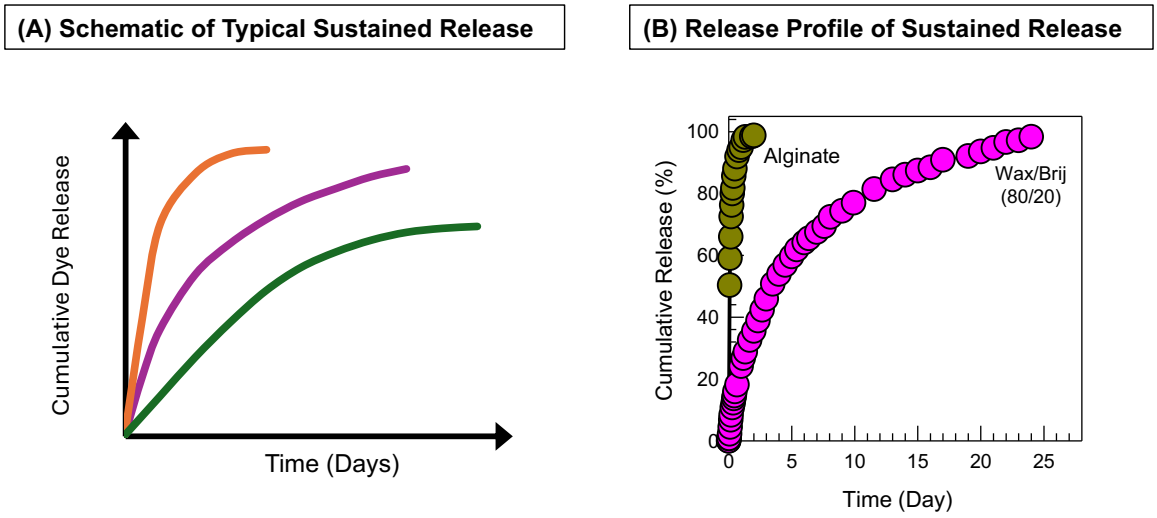

Figure 1.3 Sustained release of solutes at room temperature. (A) Schematics of sustained release profiles targeted in this study. The solute should be released over a period of days. (B) Sustained release profile for a capsule with a wax/Brij (80/20) shell. For comparison, the release profile from the alginate gel in Figure 1.1 is also shown. In both cases, the solute is Rhodamine B. The capsule releases the solute in a slow and sustained manner over 24 days.

We will show in Chapter 3 that the above release profiles can be achieved by modifying the wax-shell design shown in Figure 1.2. Specifically, we incorporate a nonionic surfactant (Brij) along with paraffin wax into the shell. This surfactant is a waxy solid with a T_m of 32°C. The data in Figure 1.3B are for a capsule with a liquid core and a shell of wax/Brij in a 80/20 weight ratio. The same dye, Rhodamine B, is loaded into the

liquid core and its cumulative release into water is monitored. A slow release of the solute is observed over a time scale of days and all the dye is released over 24 days. We will study various ways to adjust the release rate, i.e., make it either faster or slower. These include varying the shell composition (wax/Brij ratio) and the type (chemistry) of the Brij surfactant. We will show in Chapter 3 that slow release can be achieved for a variety of solutes, including dyes, drugs, and reactive reagents (such as H_2O_2). The mechanism for the slow release will be discussed, with emphasis on the role of the surfactant in the shell. The significance of this approach is that it is versatile and simple and allows for release profiles that are difficult to achieve with any alternative gel or capsule design. Thus, our approach should be attractive for controlled release applications across the pharmaceutical, agrochemical, and cosmetics industries.

Chapter 2: Background

2.1. Industrial Applications of Capsules

Capsules are commonly utilized as delivery vehicles in diverse industries such as food, agricultural, cosmetics, pharmaceutical, marine, and chemical.[1, 2, 9, 10] Active ingredients such as drugs, cells, nutrients, fragrances, or agrochemicals are encapsulated for a variety of reasons. Encapsulation can enhance the product by extending shelf life, controlling active component delivery or preventing side effects. In food and pharmaceutical industries, encapsulation can also mask odors and tastes.[11-13] For instance, in chewing gums, sweeteners and flavors are encapsulated to prevent degradation and control the release rate.[13, 14]

A capsule is essentially a small container composed of an inner core and an outer shell. The inner core typically holds the material of interest while the outer shell, made of a secondary material, encases it. The core within the capsule could exist in a range of physical states spanning from liquid, solid or gelled-solid, and gas, to even hollow compositions. To protect and enclose the core, the outer shell is usually a solid. The solid can be a liquid-swollen gel, a liquid-free soft solid, or a hard solid. Some examples of hard shells range from natural to synthetic polymers with plasticizers, resins, or wax to change the chemical or physical properties. There are different methods to synthesize capsules. These include physical methods like coacervation, ionic gelation, and layer-by-layer assembly or chemical methods such as emulsification or interfacial polymerization.[15]

Many terms are used interchangeably to describe patterns of solute release from capsules: sustained release, controlled release, prolonged release, or extended release. Extended release of drugs has been popular to allow patients to take fewer doses.[16-18] This increases patient compliance, i.e., patients will be more likely to take their medicine and not miss doses.[19] Extended release is achieved with a pH-responsive polymeric coating around the solid drug.[20] The pH in the gastrointestinal track ranges from a very acidic pH of 1.6 in the stomach to a slightly basic pH of 8 in the intestine. The polymeric coating will dissolve in the intestine, whereas it will remain undissolved in the stomach.

2.2 Wax Shelled Capsules

Waxes are hydrophobic materials. Paraffin wax, for example, is a type of wax that is synthesized from petroleum and used in various products such as food, candles, polish, and cosmetics.[21, 22] Paraffin wax is composed of saturated hydrocarbons, mainly straight-chain n-alkanes with chain lengths ranging from C_{18} to C_{45}.[23] There are also small amounts of other hydrocarbons like branched chains, cyclic chains, and other functional groups.[24-26] Paraffin wax at room temperature is a colorless, odorless, crystalline hard solid. It melts above a melting temperature T_m of around 57°C.[1, 23]

In our lab, paraffin wax was previously utilized as a shell for capsules with a liquid-core or gel-core. These capsules were synthesized as shown in Figure 2.1. For the gel-core (Figure 2.1A), a cold gel bead is dropped into a molten wax solution, incubated for a few seconds, and then removed with a tweezer. Because of the temperature difference between the cold gel and the hot solution, the wax cools and solidifies, creating a shell around the

gel. For the liquid-core capsule (Figure 2.1B), a cold solution is dropped with a pipette into the hot wax. Here again, the hot wax will solidify around the cold liquid droplet, creating a wax-shelled capsule. Typically, the core diameter is 1 to 5 mm and the wax shell is 0.5 to 2 mm in thickness.

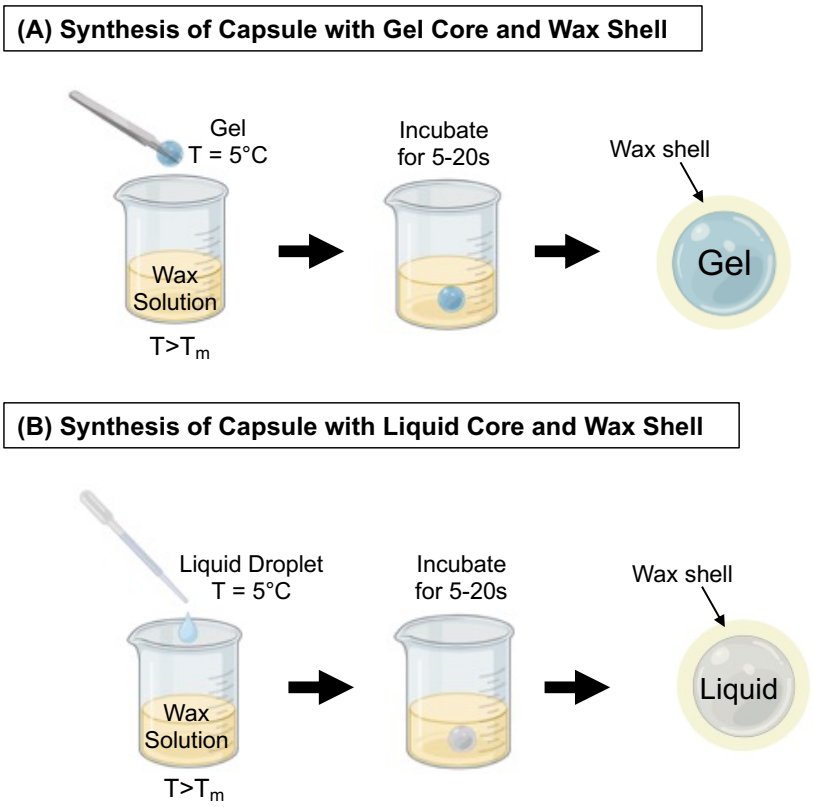

Figure 2.1 Synthesis of wax-shelled capsules. (A) Gel-core capsules. A cold gel bead is dropped into molten wax. A wax shell forms in 5 to 20 seconds, after which the capsule is removed with a tweezer. (B) Liquid-core capsules. Using a pipette, a cold liquid is dropped into molten wax. Within a few seconds, a wax shell forms around the liquid droplet, after which the resulting capsule is removed with a tweezer.

Wax-shelled capsules can encapsulate hydrophilic solutes in their gel or liquid cores. As shown earlier in Figure 1.2, when these capsules are placed in water at room temperature (or at any $T < T_m$), the solutes do not leak out for months or longer. Thus, the

hydrophobic wax shell serves as a *hermetic seal* for hydrophilic solutes. However, when a given capsule in water is heated to $T > T_m$, the wax shell will melt, and the solute will be released. In Chapter 3, the outer shell is modified to incorporate hydrophilic domains, enabling the sustained release of solutes.

2.3 Crystallization of Wax

Above its T_m, paraffin wax is a viscous liquid. When cooled below T_m, wax will crystallize into a solid.[27] Crystallization involves nucleation, growth, and agglomeration. The nucleation stage begins with molecules bonding to form solid clusters or nuclei. Then, during the growth stage, additional molecules adhere to these nuclei, which leads to the growth of crystals. In the final agglomeration stage, the crystals clump together. Crystallization is influenced by various factors such as temperature, impurities, or other additives.[24, 26, 27]

Crystallization of wax is constrained by inhibitors such as surfactants and polymers.[26, 27] There are three modes of inhibition: nucleation, co-crystallization, and adsorption. During nucleation inhibition, the inhibitor and wax nucleate together, but the inhibitor prevents formation of large nuclei by generating multiple nucleation sites. Co-crystallization means that the inhibitor disturbs the crystallization process by co-crystallizing with the wax. This modifies the morphology of the crystals and weakens their agglomerates. During adsorption inhibition, the inhibitor adsorbs on the crystals and decreases crystal-crystal adhesion, thereby hindering crystal growth.

Surfactants are widely used as additives to alter wax crystallization. The tail of a surfactant is hydrophobic while its head is hydrophilic. The addition of a surfactant to wax initiates the co-crystallization inhibition mode. The hydrophobic tail of the surfactant co-crystallizes with the wax crystals. However, the hydrophilic head of the surfactant prevents their agglomeration. This will modify the morphology of wax crystals as the three-dimensional structure will be hindered.[26, 27] An example of surfactants that could modulate wax crystallization are those from the Brij class. Brij is the trade name for a class of nonionic surfactants with polyoxyethylene heads and n-alkyl tails.[28] We will use Brij surfactants in Chapter 3.

2.4 Characterization Techniques: UV-Visible Spectroscopy

Ultraviolet-visible (UV-Vis) 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) range. When a sample is exposed to light within the UV-Vis range, molecules in the sample absorb the radiation and use this energy to promote electrons into higher energy states. Each molecule has a distinct UV-Vis absorption spectrum, as it selectively absorbs energy only at specific wavelengths. The absorption peak in the spectrum occurs at a wavelength where the most energy is absorbed. The absorbance A at a given wavelength is directly proportional to the concentration c of the molecules in solution. This relationship is described by the Beer-Lambert Law:[29]

$$A = \log_{10}\left(\frac{I_0}{I}\right) = \varepsilon \cdot c \cdot l \tag{2.1}$$

Here, I_0 is the intensity of the incident light, I is the transmitted intensity, ε is the molar absorptivity or extinction coefficient, c is the concentration of the molecule in mol/L, and l is the path length through the sample. Most commonly used dyes, drugs, and cosmetic agents have absorbances in the UV-Vis region and therefore UV-Vis spectroscopy plays a vital role in studying their release from carriers.

Chapter 3: Slow and Sustained Release from Capsules

3.1 Introduction

The goal of this study is to achieve a sustained release of small molecule solutes from capsules. In this Chapter, we design capsules that enable this release profile. The starting point for our work is our earlier design of a capsule with a shell of paraffin wax. As discussed in Chapter 1, such a shell is completely hydrophobic and does not permit any release of hydrophilic solutes, i.e., it is a hermetic seal. Here, we modify the wax shell by including an additive that is miscible with the wax. By judicious choice of this additive, we have been able to achieve the desired release profile. We have also focused solely on liquid-core rather than gel-core capsules. That is, the core is simply water with added solute. A waxy shell is formed around such an aqueous droplet by the procedure illustrated earlier in Figure 2.1B in Chapter 2.

What kind of additive in a paraffin wax shell could give rise to sustained release? We hypothesized that a surfactant would be the ideal choice of additive. Surfactants are amphiphilic molecules with a hydrophilic head and a hydrophobic tail. If surfactant molecules were present in the wax shell, we further hypothesized that there could be new transport pathways for solute molecules to traverse the hydrophobic shell. We will show that certain nonionic surfactants from the Brij family do indeed work for our purpose. Then, we will discuss if the mechanism for solute transport through the shell is indeed in line with our above hypothesis.

3.2 Experimental

Materials. The following were obtained from Sigma-Aldrich: alginate (medium viscosity alginic acid, sodium salt obtained from brown algae); the surfactants Brij C10 and Brij S10; the dyes Rhodamine B, sodium fluorescein, and Acid Red 52; and other chemicals calcium chloride dihydrate salt ($CaCl_2$), potassium permanganate, diclofenac sodium salt, and paraffin wax. Hydrogen peroxide (30% solution) was obtained from BDH Chemicals. Deionized (DI) water was used in all experiments.

Preparation of Liquid Core Capsules. A droplet of cold water with dissolved solute is dropped into a hot wax solution with a micropipette. The droplet is left in the wax solution for around 5 s to create a wax shell. The capsule is then removed with a tweezer.

Solute Release Experiments. Capsules loaded with solutes were placed in vials with 2 mL of DI water at room temperature. The solute concentration in water was analyzed using a Cary 50 UV-Vis spectrophotometer by taking 1 mL of a sample into a cuvette. The absorbance of the solute was measured at the peak absorbance of the solute which differed depending on the solute. The sample that was removed was returned to the vial after measurement. The cumulative release curve was determined by normalizing the measured solute concentration against the concentration observed at infinite time.

Optical Microscopy. Capsules were cut in half using a razor blade. Images were then captured using a DinoLite USB digital microscope at a 200x magnification.

Scanning Electron Microscopy (SEM). Molten wax/Brij C10 solution was cast into a thin solid film. After solidifying, the film was immersed in water for couple weeks and removed to be dried for another couple weeks. A sample of the dried tablet was analyzed before and after being placed in water after dropping ionic liquid (HILEM IL 1000) using a Hitachi SU-70 field-emission SEM.

In the rest of the chapter, we will show how we were able to achieve a sustained release with the wax/Brij C10 capsules and how we can control the total time of release. Moreover, from the SEM images of the wax/Brij C10 tablet, we can observe the morphology of the wax/Brij C10 as to why we are able to have sustained release from our capsule and how the surfactant acts as an inhibitor during the wax crystallization process.

3.3 Results and Discussion

3.3.1 Selection of Surfactant Additive

Our hypothesis was that surfactants could be used as additives to paraffin wax in order to modulate solute release across the waxy shell in capsules. Which surfactants to use? In this regard, we homed in on certain requirements. First, the surfactant had to form a homogeneous mixture with the wax. This would ensure that the wax shells could be reproducibly formed and that the transport properties across the shell would be systematically altered. Second, the mixture of wax and surfactant needed to have strong mechanical properties, ideally indistinguishable from those of the wax alone.

We set out to investigate both these aspects. For this, we first combined 10% of a surfactant with molten wax. The melting temperature T_m of wax is 57°C and therefore the experiment was done above this T_m at a temperature $T \sim 60$°C. We first examined if the wax and surfactant formed a homogeneous, clear solution. If it did, we cooled the solution to room temperature and examined the integrity of the waxy solid. That is, we compared this solid with a control layer of solid paraffin wax.

With these considerations, we studied a number of common surfactants. These included sodium dodecyl sulfate (SDS), an anionic surfactant, and cetyl trimethyl ammonium bromide (CTAB), a cationic surfactant. Both SDS and CTAB are insoluble in molten wax (Figure 3.1A) and were hence ruled out. We then tried nonionic surfactants like Triton X-100 or Tween 80, which are liquid at room temperature. These liquids form two phases when mixed with wax at 60°C (Figure 3.1A). We also tried the zwitterionic

phospholipid, lecithin (from soy). Lecithin is soluble in molten wax. When the solution is cooled, the result is a solid waxy layer (Figure 3.1B). However, this layer is noticeably softer and more malleable than the control wax layer (Figure 3.1B). Therefore, lecithin was also ruled out because the shell in the capsule would be too weak.

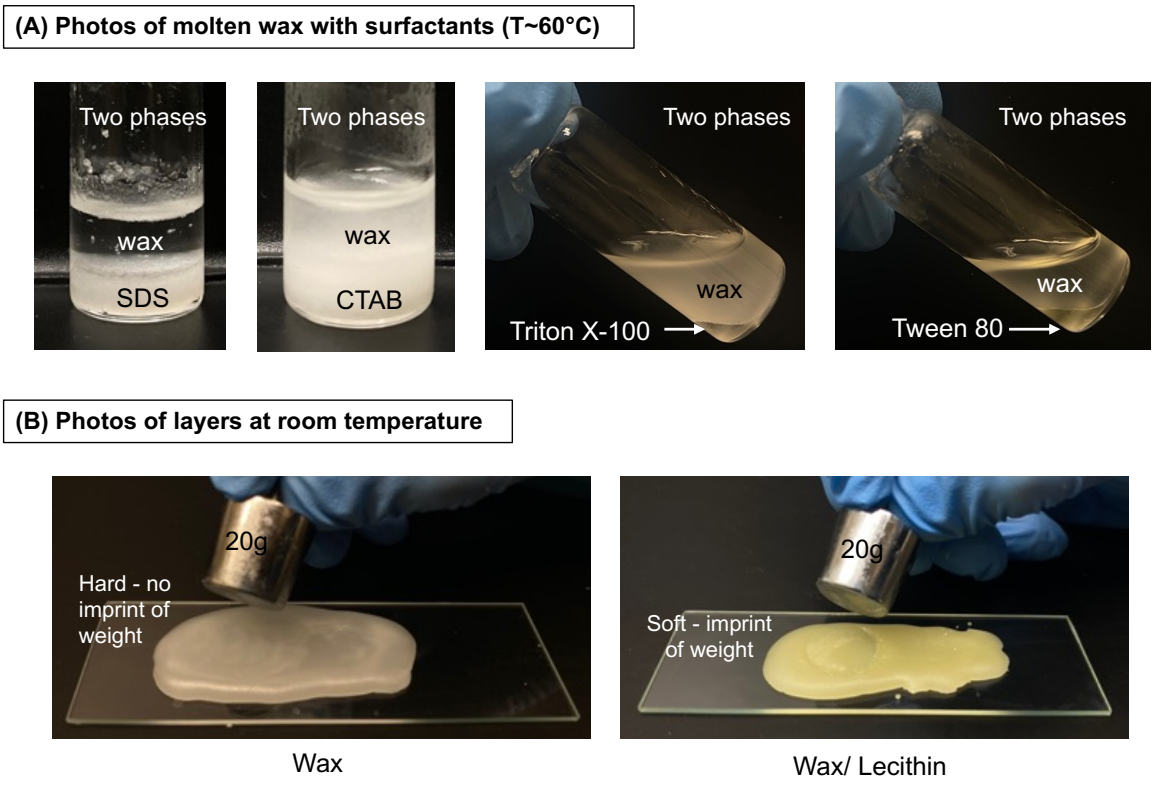

Figure 3.1 Results for mixtures of various surfactants with paraffin wax. (A) Photos of samples with 10% surfactant in molten wax at 60°C (above the T_m of the wax). All the surfactants form multiphase mixtures. (B) Photos of solid layers after cooling to room temperature. A 20 g weight is pressed into the layers. The control (wax) layer is rigid and thus the weight does not leave an imprint on it. A layer of 10% lecithin in wax, however, is soft and the weight leaves an imprint on it.

Finally, we come to the nonionic surfactants from the Brij series. These all have a head that is an oligomer of ethylene oxide (EO) units and an alkyl tail. For example, the main Brij we have used has 10 EO units in its head and a hexadecyl (C_{16}) tail. We denote

this as Brij1. It is a waxy solid at room temperature and its T_m is 32°C. When 10% Brij1 is added to molten wax at 60°C, it forms a homogeneous solution, indicating that Brij1 is rather hydrophobic and thus miscible with the nonpolar molecules of wax. When this solution is cooled, the resulting solid forms a robust layer, which was nearly identical in its mechanical properties to a control wax layer. Thus, Brij1 has the desired characteristics to be tried as an additive to wax for the capsule shell.

3.3.2 Capsule Synthesis

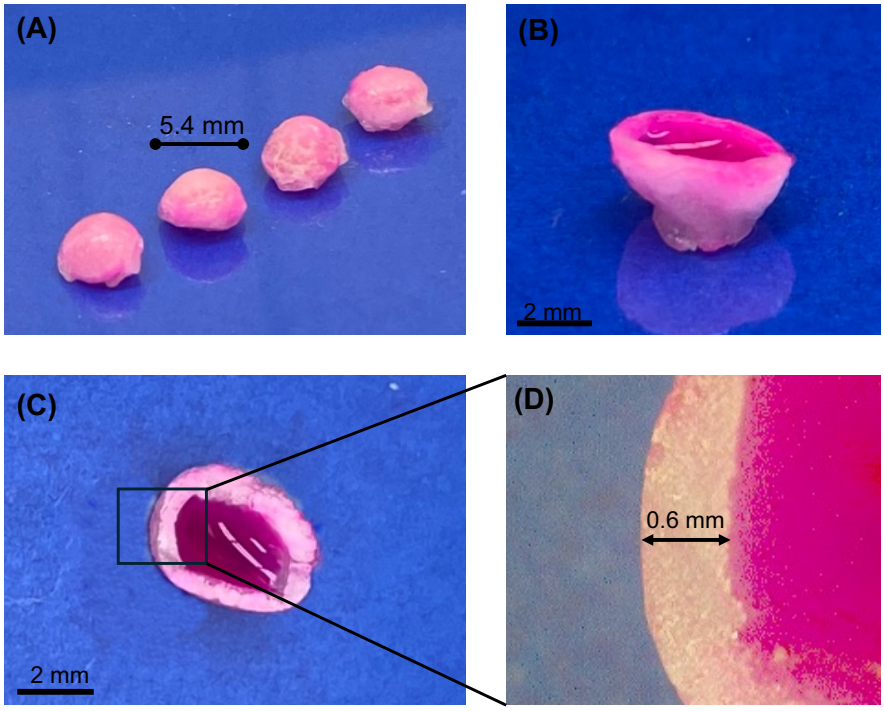

Figure 3.2 Liquid-core capsules with wax/Brij1 shells. (A) Series of capsules (B, C) Cross-section of a capsule cut in half, showing a liquid core containing the solute (dye). (D) Close-up showing the capsule shell.

Capsules with a liquid core are synthesized by the procedure shown in Figure 2.1B in Chapter 2. The capsule shell is either wax alone (for the control capsules) or wax with

Brij. First, the mixture of wax and Brij is heated to a temperature T of 60°C in a vial. It is important that T is above the T_m of the wax, but not much higher. Water with the dissolved solute of interest is cooled to 5°C. Using a micropipette, droplets (20 µL volume) of this cold aqueous solution are introduced into the vial of molten wax and incubated for 5 to 20 s. The temperature difference between the hot wax and the cold droplet is key to forming a wax shell around the droplet. The shell forms within 5 to 15 s, and seconds later, the wax-shelled capsules are removed with a tweezer. Figure 3.2 shows photos of several capsules with wax/Brij1 shells and diameters around 5 mm. The liquid core in the capsules can be seen from Figure 3.2B. A close-up image of the shell in Figure 3.2D shows its thickness to be ~ 0.6 mm.

Synthesizing liquid-core capsules is a meticulous process, requiring careful attention to many details. The three main variables are: the temperature of the molten wax, the temperature of the liquid droplet, and the incubation time of the droplet in the molten wax. If the molten wax is too hot or if the liquid droplet is not cold enough, the wax shell may not form at all or it may form and then re-melt. Likewise, even if a robust shell forms, if the capsule is left to incubate in the vial for > 10 s thereafter, the shell will re-melt. Also, when taking out the capsules with tweezers, the capsules must be gripped gently because the shell has not fully solidified.

3.3.3 Sustained Release from Wax/Brij Capsules

We now discuss release experiments with liquid-core capsules having an 80/20 wax/Brij1 shell (i.e., there is 20 wt% of Brij1 in the shell). As a standard solute, we used the zwitterionic dye Rhodamine B: 1 mM of this dye was encapsulated in the liquid core of the capsules. We placed the dye-loaded capsules in DI water and monitored the dye concentration in the external solution over time. For comparison, we did the same experiment with an alginate gel and with capsules having a shell of wax alone. Figure 3.3 plots the cumulative release, which is the % dye released into the external solution normalized by that at infinite time. The dye is completely released within 1 h from the alginate gel, whereas there is no release at all from capsules with a wax shell. With the wax/Brij1 capsule, however, we see a slow and sustained release, with about 50% of the dye being released over 5 days. Thus, these data are consistent with our hypothesis that surfactants in the wax shell can assist in slowly releasing solutes from the core.

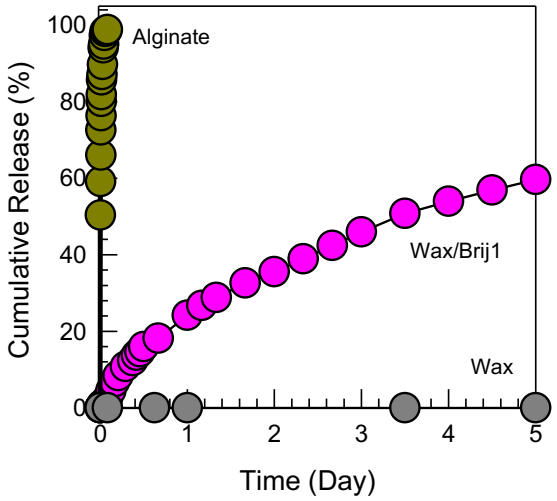

Figure 3.3 Release curves for different container structures. All contain 1 mM of the dye, Rhodamine B. The alginate gel releases the dye rapidly, while a liquid-core capsule with a shell of pure wax does not release the dye at all. The liquid-core capsule with a shell of 80/20 wax/Brij1 releases the dye in a slow and sustained manner over days.

Figure 3.4 shows data for three sets of capsules with different shell compositions: the wax/Brij1 weight ratio is altered from 70/30 to 80/20 to 90/10. The cumulative release of the same Rhodamine B dye is plotted over a 30 day period for all these cases. All these capsules show sustained release of dye and there is a consistent trend: the more the Brij1 in the shell, the faster the release. For instance, by day 3 (Figure 3.4B), there is a 20% higher release with 70/30 compared to 90/10. The bar graph in Figure 3.4C shows the time to reach 99% release and it varies from 20 days for 70/30 to 28 days for 90/10. Thus, the presence of the surfactant Brij1 in the shell clearly speeds up the release, and the more the Brij1, the greater this effect.

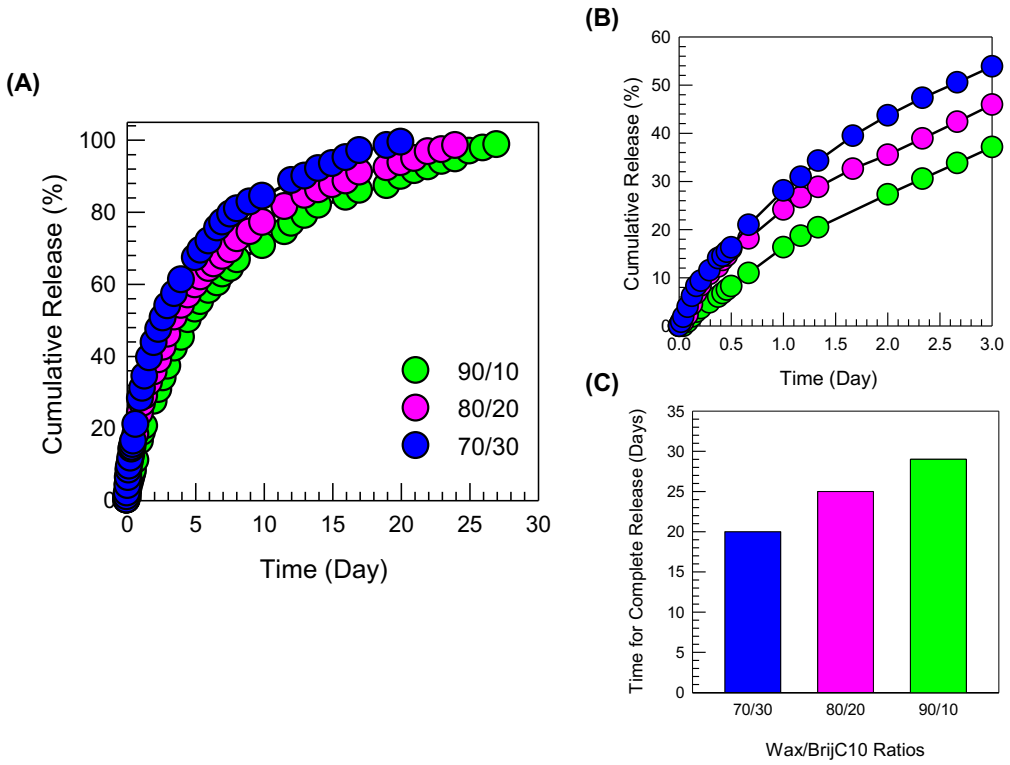

Figure 3.4 Sustained release profiles for capsules with wax/Brij1 shells. Results are shown for three sets of capsules with wax/Brij1 ratios of 70/30, 80/20, and 90/10. All are loaded with 1 mM Rhodamine B. (A) Plots over the entire duration; (B) Close-ups of data from the first 3 days; (C) The number of days taken to reach 99% release.

3.3.4 Results with Different Brij Surfactants

To verify the generality of our result, we experimented with a second Brij surfactant (Figure 3.5). The results in Figures 3.3 and 3.4 are with Brij1, which has 10 EO units in its head and a C_{16} tail. For comparison, we studied another Brij surfactant, Brij2, which also has 10 EO units in its head, but a longer stearyl (C_{18}) tail. Structures of both surfactants are provided in Figure 3.5. Because Brij2 has a longer tail, it is more hydrophobic than Brij1. Note that Brij2 is also a solid at room temperature and due to its longer tail, it has a higher T_m of 38°C. The cumulative release of Rhodamine B from capsules with Brij1 and Brij2 is compared in Figure 3.5. In both cases, the shells had 80/20 ratios of wax/Brij. Sustained release of solute is seen also with Brij2, and it is slower than with Brij1. Specifically, on day 5, there is 10% lower release with Brij2 than with Brij1. These results suggest that the presence of more hydrophobic surfactants in the shell leads to slower release.

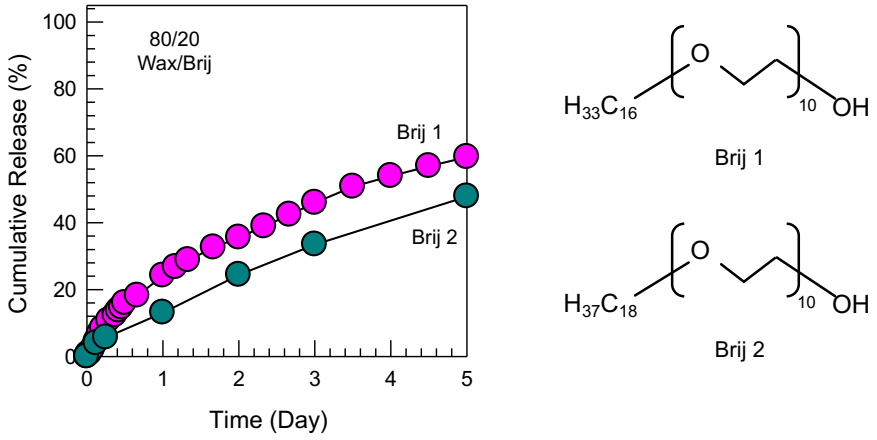

Figure 3.5 Sustained release profiles for capsules with two different surfactants in the shell. The structures of the two surfactants, Brij1 and Brij2, are shown on the right. The capsule shells had 80/20 ratios of wax/Brij. Their cores were loaded with 1 mM of Rhodamine B. Sustained release is seen in both cases, with the more hydrophobic Brij2 giving a slower release.

3.3.5 Results with Different Solutes

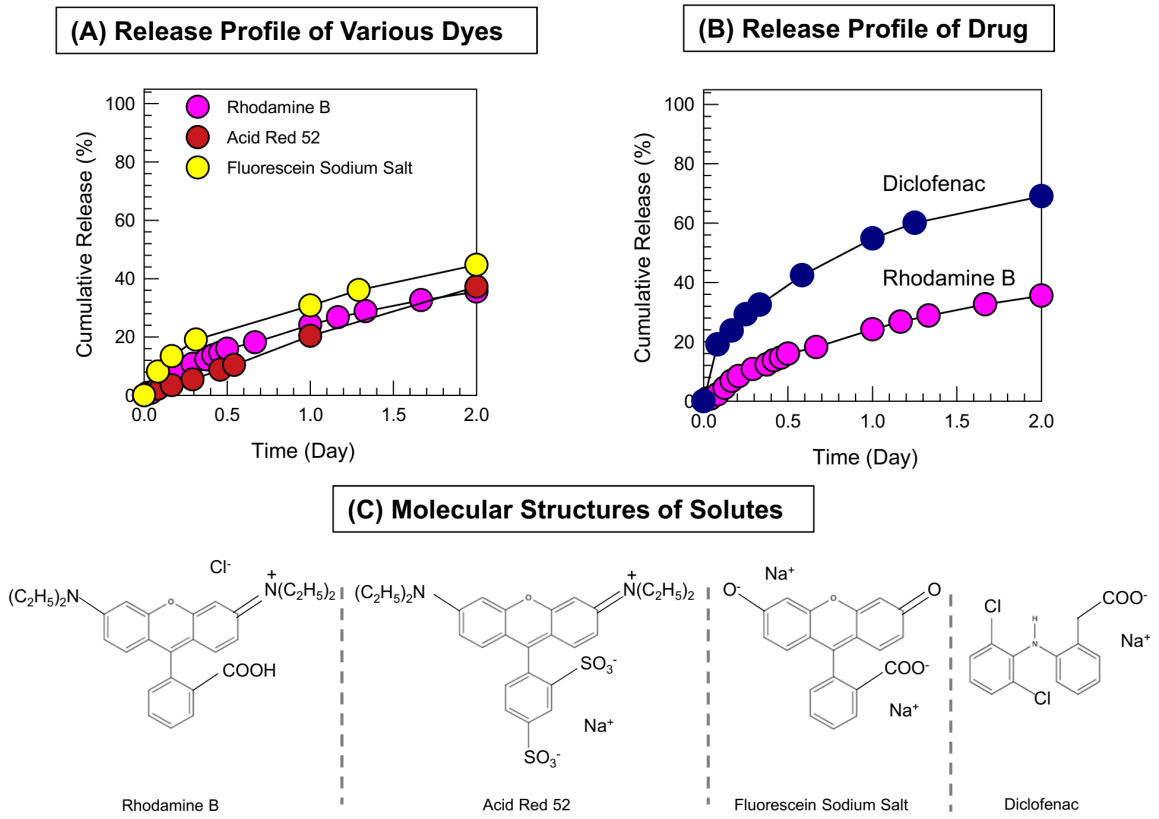

Figure 3.6 Sustained release of a variety of solutes. In all cases, the capsule shell had 80/20 wax/Brij1 and the core contained 1 mM of the solute. (A) Release profiles for three dyes. (B) Release profile of a drug. (C) The structures of the various solute molecules.

Next, we proceeded to examine if sustained release from our capsules occurred with all kinds of solutes. Our solute thus far is Rhodamine B, which is a zwitterionic dye, as seen from its structure in Figure 3.6C. We also tested two other dyes: Acid Red 52 and sodium fluorescein, which are both anionic molecules (Figure 3.6C). 1 mM of each dye was loaded into capsules with their shells being 80/20 wax/Brij1. The release profiles in Figure 3.6A show that all three dyes get released in a slow and sustained manner, with the curves being nearly identical. In addition to dyes, we also tested diclofenac, a non-steroidal

anti-inflammatory drug (NSAID) that is typically used to reduce pain and inflammation (structure also shown in Figure 3.6C). The release of diclofenac is similar, but faster than that of the dyes (Figure 3.6B).

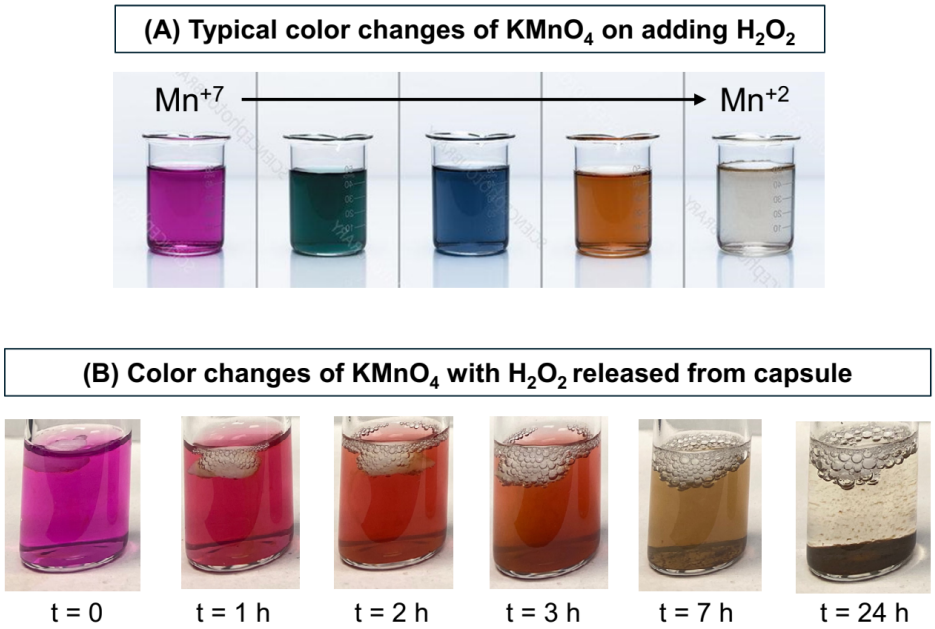

Figure 3.7 Slow release of hydrogen peroxide (H_2O_2) from a capsule. Release of H_2O_2 from the core of a capsule with a shell of 80/20 wax/Brij1 is shown over time in (B). The vial contains $KMnO_4$, which is a colorimetric indicator for H_2O_2 and its typical color changes are shown in (A). The color changes in the vial with the capsule occur over 24 h.

Next, we studied the release of a small molecule, hydrogen peroxide (H_2O_2), which is a well-known reactive chemical. Instead of using UV-Vis spectroscopy to monitor the release like with other solutes, we used a colorimetric indicator that allows for visual detection. The indicator is potassium permanganate ($KMnO_4$), which changes color from purple to red to orange to yellow to clear upon adding increasing concentrations of H_2O_2 (Figure 3.7A). Note that if a given H_2O_2 solution is added to $KMnO_4$, the color change occurs instantly (within seconds). We loaded 30% H_2O_2 into a capsule with a 80/20

wax/Brij1 shell and placed the capsule in a vial of 0.5 M $KMnO_4$. Figure 3.7 shows a series of photos of this vial, taken over a day. An initial change in color is observed only after 1 h, and the transition to a yellow color occurs after 7 h. The transition to a clear state takes 24 h. This result again shows that even small-molecule solutes can be released in a slow and sustained manner from our capsules. Sustained release of H_2O_2 could be useful to support plant growth: H_2O_2 provides extra oxygen to roots for growth and germination.[30]

3.3.6 Morphology of Wax/Brij Layers

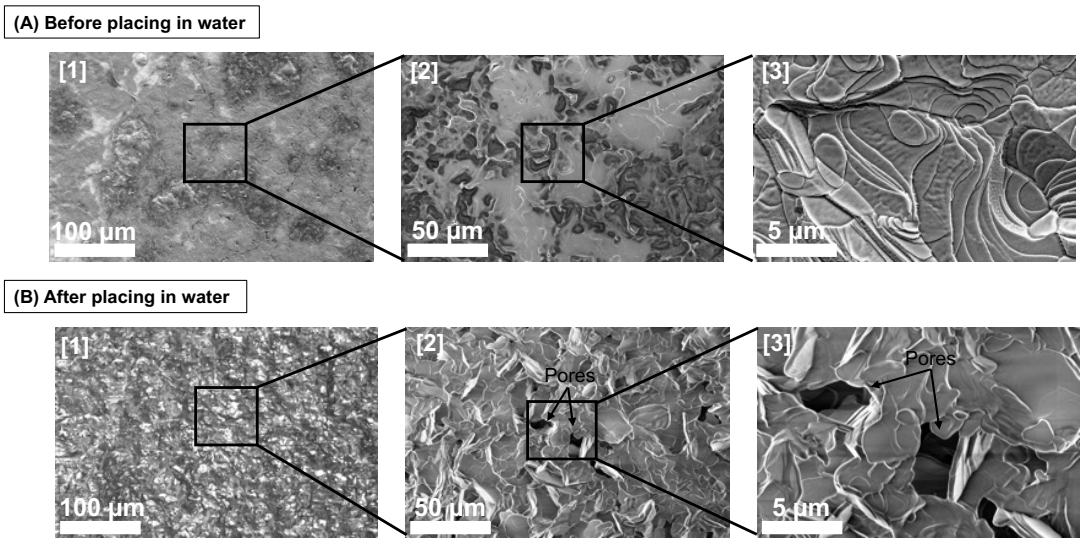

Figure 3.8 SEM of a wax/Brij1 layer. A 70/30 wax/Brij1 layer is imaged at different magnifications. (A) Initial layer. (B) The layer was left in water for two weeks and then imaged again.

Why does the presence of Brij surfactants in the wax layer allow for solute to pass through? To understand this, we studied a layer of 70/30 wax/Brij1 by SEM. That is, we cast a thin film from a molten wax/Brij1 mixture and allowed it to solidify into a layer. We then imaged this solid layer (Figure 3.8A). Then this layer was placed in water for two

weeks, then removed and allowed to dry, and then imaged again (Figure 3.8B). The structures in both sets of images appear to be crystallites of wax/Brij. But the striking point in the lower set is the presence of holes (pores) with sizes of several μm (these are clearly seen in Image B3). No such pores are present in the initial layer. In other words, when the wax/Brij1 layer is placed in water, there is some erosion of the layer, which results in these micropores. It is likely that the eroding structures are crystallites rich in Brij1. With prolonged contact with water, these crystallites partially dissolve, i.e., some of the Brij1 moves from the layer to the water, which explains the micropores. For comparison, we also placed a layer of pure wax (no Brij) in water for two weeks and did SEM on it. No micropores were seen in this case.

The results of Figure 3.8 suggest the mechanism by which surfactants like Brij affect solute transport, which is schematically shown in Figure 3.9. We expect that the wax and the Brij will co-crystallize in the solid capsule shell. But because the wax is in significant excess (e.g., 80/20 weight ratio), most of the crystallites will be enriched in wax molecules. However, some of the crystallites will be enriched in the Brij. Because Brij has a hydrophilic portion and is water-soluble, the hydrophilic solute in the capsule core will have an affinity for the Brij-rich crystallites. When the capsule is placed in water, the solute can then take a path from the core to the external water through the Brij-rich crystallites. In effect, we hypothesize that the crystallites create microchannels that facilitate solute transport. After prolonged contact with water, these microchannels could suffer significant erosion so that they manifest as micropores.

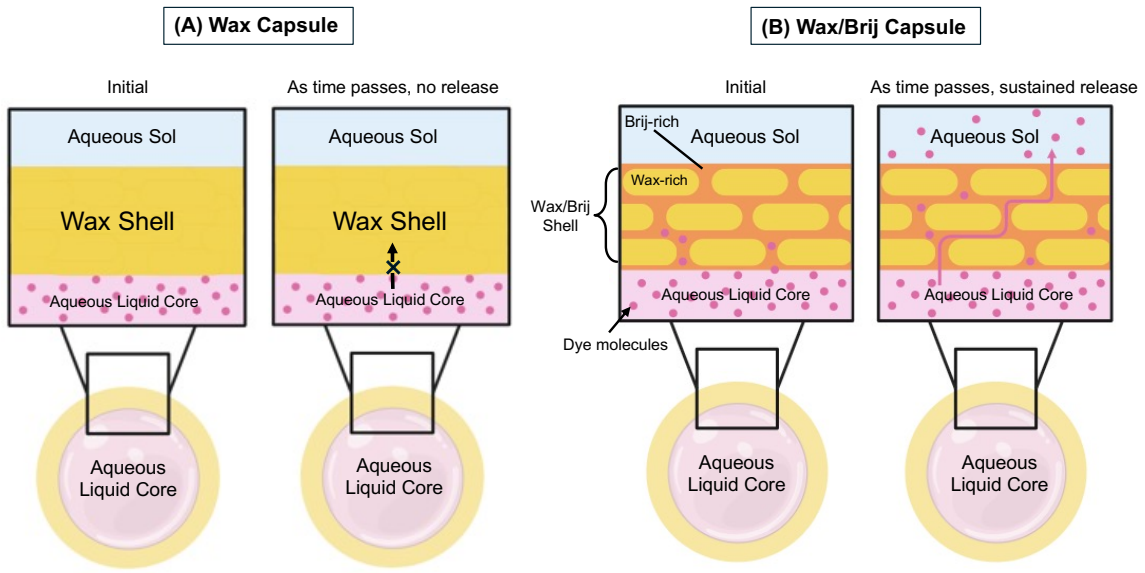

Figure 3.9 Schematic of mechanism. The schematic describes how the hydrophilic solutes are being released from wax and wax/Brij capsules in aqueous solution. (A) Wax capsules initially and as time passes with no release. (B) Wax/Brij capsules initially and as time passes with sustained release.

The permeability P of a solute across a shell or membrane can be expressed by $P = D \cdot S$, where D is the diffusivity and S the solubility of the solute. If we compare a shell of wax alone (Figure 3.9A) with that of wax/Brij (Figure 3.9B), D of the solute will be the same in both cases (D is dictated mainly by solute size). Thus, an increase in P in the case of wax/Brij *must be due to an increase in the solubility S* of the solute in the shell. In other words, $S = 0$ for hydrophilic solutes in a hydrophobic wax shell, which is why the wax capsule shows no release. However, $S > 0$ (but not very high) for the same solutes in a wax/Brij shell, which explains the sustained release we see in the latter case.

3.4 Conclusions

With the new wax shell, we were able to achieve slow and sustained release of solutes from liquid core capsules. The capsule releases the solutes from the beginning and

continue to release for days or weeks instead of minutes or hours. Previously, we found paraffin wax shell with solutes in the gel core can be used to hermetically seal the solutes where there was no release for a period, due to the hydrophobic nature of paraffin wax (T_m = 57°C), until it was triggered by heat where $T>T_m$. By modifying the wax shell with an additive, we managed to accomplish a sustained release of solutes over days or weeks. An additive that was used for this thesis was a surfactant, Brij. We wanted a surfactant that forms a homogeneous mixture with wax and have strong mechanical properties. The common surfactants used such as SDS, CTAB, Tween 80, Triton X-100, and Lecithin did not meet these requirements. The surfactant Brij1, with 10 EO units in its head and a C_{16} tail, becomes a miscible solution with wax with strong mechanical properties. By varying the ratios of wax and Brij1, we were able to adjust the total days to release, depending on its specific application, while following the same sustained release profile. In addition to changing ratio, we have also studied different types of surfactants, such as Brij1 and Brij2, which can vary in their degrees of hydrophobicity. Brij2 has same 10 EO units in its head like Brij1 but it has a longer C_{18} tail. Because Brij2 is more hydrophobic than Brij1, the sustained release was slower than Brij1. The standard solute used for all the experiments was Rhodamine B. Other solutes such as anionic dyes (Acid Red 52 and sodium fluorescein), a non-steroidal anti-inflammatory drug (diclofenac), and reactive reagent (H_2O_2) were loaded in the liquid core 80/20 wax/Brij1 capsule, showing similar sustained release as Rhodamine B. In order to understand the mechanism of the solutes releasing from our wax/Brij shell, a morphological study was done with SEM. Brij co-crystalizes with paraffin wax where most of the crystallites are enriched in wax molecules with some crystallites enriched with Brij. When placed in water, the solute molecules can travel from

core through the Brij-rich crystallites to the external water, creating microchannels. After prolonged exposure to water, the microchannels are eroded away, creating micropores. The sustained release of solutes from the wax/Brij1 capsule can be utilized in various industries such as pharmaceuticals, cosmetics, and medical.

Chapter 4: Conclusions and Recommendations

4.1 Conclusions

In this thesis, we have demonstrated a liquid core structured capsule encased in a wax mixture shell, effectively achieving our objective of slow and sustained release. We have synthesized wax mixture shell that could be modified to change the release rate with various solutes which can be useful in different industries.

In order to release hydrophilic solutes from a completely hydrophobic paraffin wax, an additive was added to change the hydrophobic properties of the wax shell. We wanted the additive to be a surfactant but not all of them met our requirements of being able to be a homogeneous solution with paraffin wax and have strong mechanical properties. Common surfactants like SDS, CTAB, Tween 80, Triton X-100, and Lecithin did not meet these requirements. SDS, CTAB, Tween 80, and Triton X-100 were insoluble in molten wax. Lecithin was soluble in molten wax but softer than wax once cooled. Finally, a solid surfactant at room temperature, specifically Brij, was used for the purpose of this thesis as it is both soluble in wax and give strong mechanical properties. The Brij1 has 10 EO units in its head and a hexadecyl C_{16} tail. The Brij co-crystallizes with paraffin wax where most of the crystallites are wax enriched with some crystallites that are Brij enriched. The Brij-rich crystallites create microchannels for the solutes to transport through from the core to the external water. The microchannels eventually erodes after prolonged period in water, creating micropores.

With the wax/Brij1 capsule, sustained release of solutes is achieved for weeks. Similar sustained release was observed with different dyes, drug, and chemical reagent. The release rate of the capsules can be tuned by using different ratios of Brij1 with wax. Moreover, the use of other Brij variants also contributes to the modification of the release rate from the capsules. Thus, the introduction of our innovative wax/Brij1 capsule, engineered for sustained release, demonstrates its versatility across pharmaceutical, medical, cosmetic, and agrochemical industries.

4.2 Future Directions

This study could also be further improved by utilizing more different Brij, other than Brij1 and Brij2. Since only two variants of Brij were studied in this thesis, conducting further research with additional Brij variants would strengthen our findings.

Furthermore, our approach can be extended to be utilized as a membrane-like layer in a larger container such as a test tube or a petri dish. With larger containers, we are able to load larger amounts of solutes. Having large amounts of solutes with the wax/Brij allows larger concentration of solutes to be released to the external environment in a sustained manner. Reactive solutes such as peroxides, reacted with a catalyst, can produce dissolved oxygen in the surrounding environment to be used in cell culturing or tissue engineering.

Wax/Brij capsule or membrane can be further utilized to produce dissolved oxygen. Reactive solutes such as peroxides when reacted with a catalyst produce oxygen. The capsule with a catalyst loaded alginate gel around the wax/Brij shell with peroxide solute

such as H_2O_2 in the core would produce dissolved oxygen when placed in water. This new capsule or membrane could be utilized in cell culturing or tissue engineering to produce dissolved oxygen for cells to survive. Other than the examples listed, there are many more areas that could be expanded up on with this wax/Brij capsule.

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