ABSTRACT

Title of Thesis: **Delayed Release of Hydrophilic Solutes from Capsules**
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Hydrophilic small-molecule solutes (e.g., drugs) can be loaded into hydrogels, but the solutes leak out rapidly (in minutes) when the gels are placed in water. Solute release can be completely stopped if the gel is covered by a thin shell of paraffin wax (melting point T_m ~ 57°C); in such 'capsules', the wax shell is a hydrophobic solid. Here, we vary the design of our capsules to achieve '*delayed release*' of solutes into water: i.e., no release for a delay period (6 to 72 h) followed by a slow and sustained release thereafter. The key is to include an additive in the shell that is miscible with the wax but is weakly hydrophilic and has a lower T_m. Examples are fatty acid esters, notably isopropyl palmitate (IPP, T_m ~ 11°C). For example, when a solute-loaded gel is covered by a 80/20 wax/IPP shell, we find a 3-day delay at 25°C before any release, followed by a near-constant release rate (i.e., 'zero-order' release) for the next 20 h. The time delay and release rate can be tuned via the concentration and type of additive in the shell. The delayed release occurs because the wax/IPP shell is an inhomogeneous crystal (as wax and IPP are only partially miscible). Our approach can be used to delay the release of dyes, drugs (e.g., diclofenac), and reactive agents (e.g., H_2O_2) out of the core gel. The simplicity and generality of our approach should make it useful for controlled release applications in the pharmaceutical, agrochemical, and cosmetics industries.

Delayed Release of Hydrophilic Solutes from Capsules

By

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Chapter 1: Introduction and Overview

This thesis deals with aqueous container structures that can encapsulate solutes and subsequently release these solutes to the external medium. Controlled release of solutes out of such containers is an important problem that is encountered in various industrial applications, including pharmacy and medicine (where the solutes are drugs), agriculture (where the solutes are fertilizers or hormones to help plants grow), cosmetics (where the solutes are moisturizers, vitamins, or sunblock agents), foods, and various consumer products. The scenario that is most relevant for this thesis is where the solutes are small, hydrophilic molecules, with molecular weights below about 1000. Such solutes are water-soluble, and they can hence be packaged in containers with an aqueous core.

Typical examples of aqueous containers are hydrogels and capsules. The term 'hydrogel' refers to a water-swollen solid in which polymer chains are crosslinked into a 3-dimensional (3-D) network across the volume.[3] The crosslinks between polymer chains can be covalent (chemical) bonds or weaker physical bonds (e.g., ionic or hydrogen-bonds). [4] Common examples of physically crosslinked gels are those of gelatin and alginate. [5] Gelatin is a denatured form of the protein collagen, and it forms gels due to H-bonding interactions between the chains. Alginate is an anionic polysaccharide derived from brown algae and it forms gels upon contact with divalent ions like calcium (Ca^{2+}).[6,7]

The term 'capsule' refers to a spherical structure with a core surrounded by a thin shell. For example, if the aforementioned alginate is combined with a cationic

polysaccharide like chitosan, the result is a capsule in which the core is surrounded by an alginate/chitosan shell (where the anionic and cationic chains are complexed together). [8,9] We typically work with capsules and gels in the form of spheres with diameters ranging from 100 μm to a few millimeters. These can be easily prepared in the lab and can be loaded with small-molecule solutes. As model solutes, we work with water-soluble dyes, which can be easily analyzed by absorption spectroscopy.

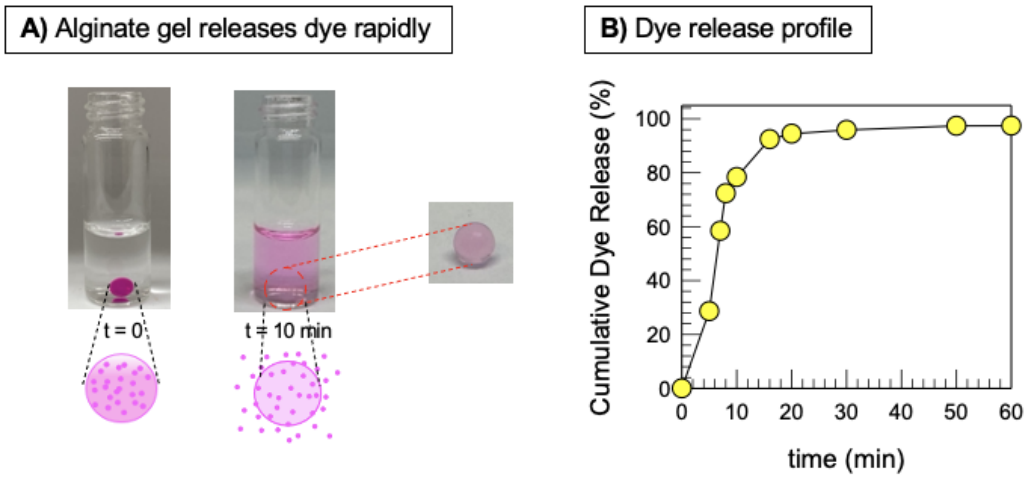


Figure 1.1 Rapid release of small, hydrophilic solutes out of typical hydrogels. (A) A spherical alginate gel loaded with Rhodamine B (water-soluble dye) is placed in water at $t = 0$. Within minutes, the dye leaks out of the gel into the external solution. As a result, the solution appears pink. When the gel is taken out of the vial, it is seen to be depleted in the dye (i.e., it is nearly colorless). (B) Plot of cumulative dye release (normalized by the final concentration) vs. time. Most of the dye is released within 20 min.

One issue with using the above structures for controlled release applications is that solutes tend to leak out rapidly from them. For example, a spherical alginate gel loaded with a pink dye is shown in Figure 1.1A. When this gel is placed in water, within minutes, the dye leaks out of the gel and the water assumes a pink color. Within an hour, the dye is completely released (plot in Figure 1.1B). This time-window for the release is

far too short for many applications. Thus, some questions that motivate our work, are the following. Can we keep solutes encapsulated (with no release) for much longer times — even a month or longer? Can we release solutes in a slow and sustained manner over a long time (up to days)? And can we achieve a *delayed release*, where there is no release for a period of time, followed by a slow and sustained release?

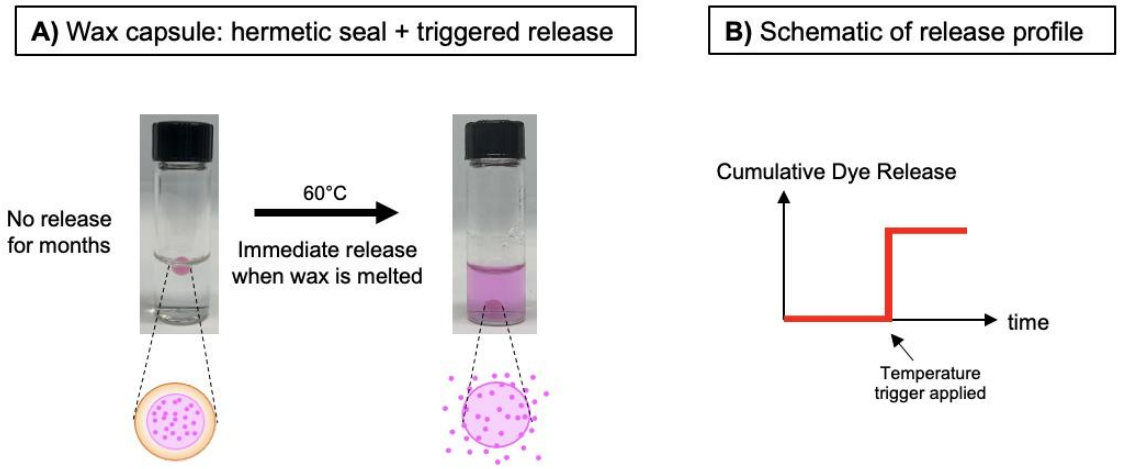
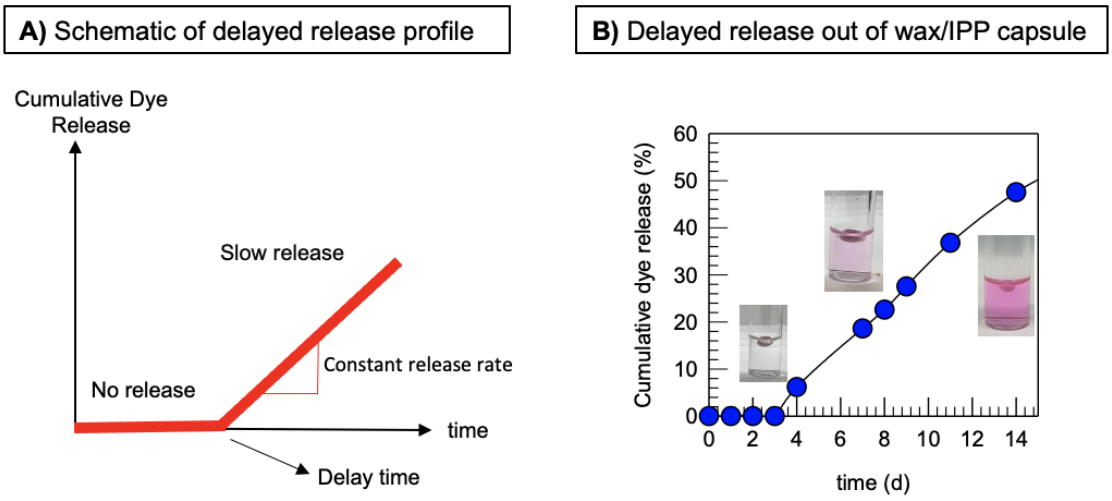


Figure 1.2 Wax-shelled capsules enabling hermetic sealing and triggered release of solutes. (A) The capsule has a core of alginate gel loaded with Rhodamine B and a shell of paraffin wax (T_m = 57°C). When placed in water at 25°C, there is no release of dye out of the capsule for months, indicating a 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.

Recent studies from our lab have addressed the first of the above questions: i.e., how to keep solutes encapsulated in an aqueous gel for a very long time (months). The key to such long-term storage is to cover the gel with an extremely hydrophobic layer that would be impermeable to water. Different hydrophobic layers are possible, but the one most relevant to the present work is a layer made out of paraffin wax (Figure 1.2A). [1] We showed that capsules with a gel core and a wax shell could be easily made — simply

by dropping a cold gel into a molten wax solution for a few seconds. For example, a dye-loaded alginate gel enveloped in a wax shell is shown in Figure 1.2A. When this wax-shelled capsule is placed in water, no leakage of dye into the surrounding water is seen at room temperature for months. In other words, the wax shell serves as a *hermetic seal* for the solute, and the reason for the impermeability is because wax is a hydrophobic solid. Moreover, paraffin wax has a melting point $T_m \sim 57°C$. [10] Thus, when the capsule is placed in water at a temperature $T > T_m$, the wax shell melts, and in turn the solute is released. Release out of wax-shelled capsules can therefore be triggered by heat (Figure 1.2B), but this approach cannot release any solute at room temperature.



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In this thesis, we focus on the challenge of *delayed release* out of gels or capsules, which is sketched in Figure 1.3A. Here, we would like to ensure no release (i.e., the solute remains hermetically sealed) for a period of time, which is termed the 'delay time'.

Following this time, we would like the solute to be released, preferably at a constant rate. To our knowledge, this combination of properties, has never been reported before. We will show in Chapter 3 that such delayed release can indeed be achieved by making a variation of our wax-shelled capsule. Instead of a shell of pure paraffin wax, we combine the wax with another molecule with a specific set of properties. An example is a fatty acid ester such as isopropyl palmitate (IPP), which is an ester of palmitic (C_{16}) acid. IPP has a T_m = 11°C and is thus a liquid at room temperature (whereas wax is solid). [11] But like wax, IPP is immiscible with water, indicating that it is rather hydrophobic, and indeed, it is miscible with wax as well. However, IPP is slightly hydrophilic, as indicated by the fact that it is soluble in ethanol whereas wax is not.

A typical release profile from a capsule is shown in Figure 3B. The core of this capsule is an alginate gel, which is loaded with the same pink dye as in previous figures. The shell is a 80/20 mixture of wax/IPP. We find that there is zero release of solute for about 3 days (72 h), and a linear release over the next 10 days. The delay time as well as the release rate (slope in the linear region) can both be tuned by varying the concentration of IPP in the shell. In addition to IPP, many other additives, including other fatty acid esters can give rise to such a release profile. Other parameters that influence the release profile include the shell thickness, temperature, solute concentration, solute type etc. All these are discussed in Chapter 3. The significance of this work is that we can now design or 'program' capsules exhibiting delayed release. These could be useful in applications ranging from medicine to agriculture to cosmetics to consumer products.

Chapter 2: Background

In this chapter, we describe the basic properties of capsules, with specific focus on capsules with a gel core and a wax shell, which are the ones examined in our study. Then, we will describe the techniques used in this study, specifically UV-Vis Absorption Spectroscopy (UV-Vis) and Differential Scanning Calorimetry (DSC).

2.1. Capsules in Industrial Applications

Capsules are commonly utilized as delivery vehicles in the cosmetic, pharmaceutical, agrochemical, and food industries. The purpose of adding capsules to various products is to contain, protect, and deliver active ingredients, such as drugs, [12] cosmetics,[13] food supplements,[14] fragrances, [15] agrochemicals, [16] and chemical reagents.[17] Capsules embedded in a product can extend the shelf life of active ingredients in their core by preventing their degradation or their contact with external media. One example in this regard is capsules loaded with probiotic bacteria in yogurt. [18]

In common parlance (especially in medicine), the term capsule is used for any container-like structure. Thus, the terms ‘gel’, ‘gel-bead’, and ‘gel-capsule’ are often used interchangeably in the context of pharmaceutical products. More precisely, capsules can be defined as structures having an *inner core* enclosed by a *distinct shell*. The core can be liquid or a gelled solid or even hollow. The shell has to be a solid to enclose the core, but it too can be a gel (solid swollen with water), a soft water-free solid (e.g., an elastomer), or a hard solid. Examples of hard shells include crystalline or glassy

polymers; other organic materials (e.g., wax); or inorganic materials (like silica or gold). Capsules can be prepared using various techniques such as emulsification, layer-bilayer assembly, electrostatic complexation, ionic crosslinking, or coacervation.

Capsules that can release their encapsulated solutes over long periods of time are especially useful in the pharmaceutical industry. [19] For example, if a capsule can release drug slowly, a patient may be able to take a capsule just once a day instead of three times a day. [20] In this regard, many drug products are touted to have 'extended-release' capabilities. [21,22] To extend drug release, a pill or capsule is often given an 'enteric coating', which is a polymeric coating that prevents the capsule from dissolving or disintegrating in the stomach. [23] Because the stomach is acidic (pH ~ 2) whereas the intestine is neutral (pH ~ 7), enteric coatings are polymers that are insoluble in acid but soluble under neutral conditions. Capsules loaded with probiotic bacteria can also be enteric-coated to protect the live bacteria from being destroyed by stomach acid, allowing them to be delivered to the intestines where they can colonize the gut and provide beneficial effects.

2.2 Capsules with a Wax Shell

Paraffin wax is a versatile substance derived from petroleum. It is a mixture of hydrocarbons with chain lengths ranging from C_{17} to C_{35}. The main components are straight-chain alkanes, especially *n*-docosane ($C_{22}H_{46}$) and these constitute > 95% by weight. Side components include branched alkanes and monocyclic cycloalkanes. Paraffin wax is a colorless, odorless, crystalline solid at room temperature and its melting

temperature T_m is around 57°C. It is used in candles, crayons, lubrication, electrical insulation, foods, and cosmetics. [24,25,26,27,28]

Previously, our lab devised a route to making capsules with a gel or liquid core and a shell of paraffin wax. [1] The procedure for synthesizing such capsules is shown in Figure 2.1A. A gel bead is cooled and then dropped into a solution of molten wax. After incubation for a few seconds, the structure is removed, whereupon the wax cools and forms a shell around the gel core. Figure 2.1B shows that the shell is around 0.7 mm thick around a core with a diameter around 3 mm.

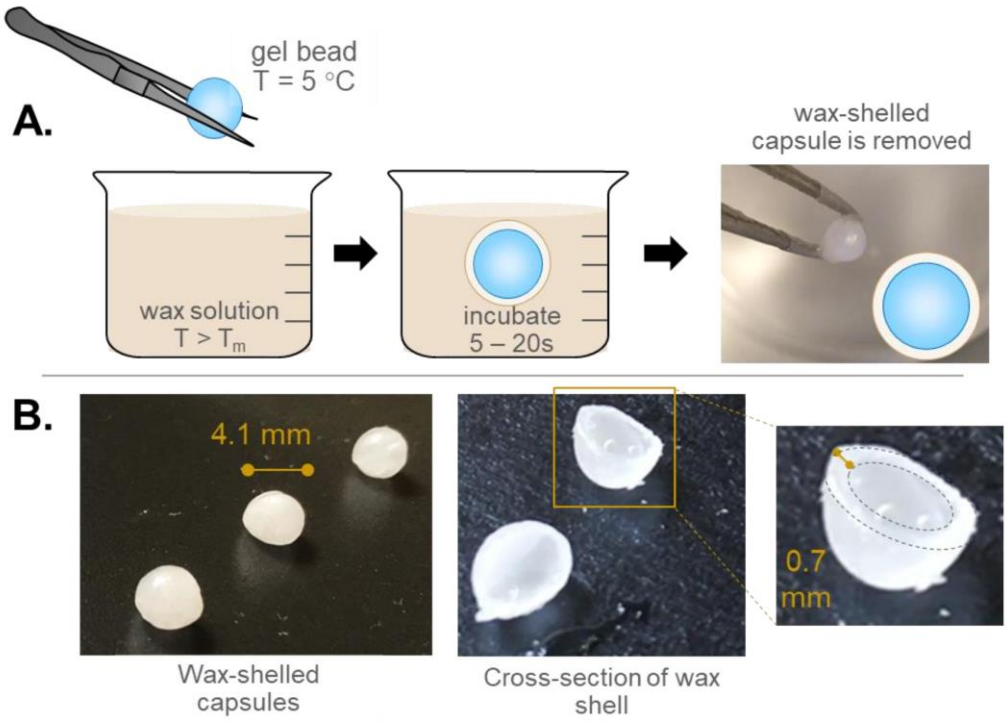

Figure 2.1. Synthesis of wax-shelled capsules. A) A cold gel-bead (at 5°C) is dropped into molten wax (at a temperature $T > T_m$ of the wax). A wax shell forms within 5 to 20 s. The capsule is then removed using tweezers. B) Cross-sections of cut capsules showing their average diameter (4.1 ± 0.1 mm) and shell thickness (0.7 ± 0.2 mm). [1]

Such wax-shelled capsules can encapsulate hydrophilic solutes in the gel core. When these capsules are placed in water at room temperature (or at any $T < T_m$), the solutes do not leak out for months (see Figure 1.2). In other words, the wax shell serves as a *hermetic seal* for the solute, and this is because wax is a hydrophobic solid. However, when the capsule in water is heated to $T > T_m$, the wax shell melts, and in turn the solute diffuses out of the gel core. Our research in Chapter 3 builds on these findings and alters the capsule design to enable delayed release (after a few days) at room temperature.

2.3 Behavior of Waxes and Their Mixtures

All waxes are crystalline solids, but the precise crystal structure depends on their composition. When a wax is heated, the solid crystal melts into an amorphous liquid at the melting temperature T_m. Paraffin wax ($T_m \approx 57°C$) mainly consists of straight *n*-alkanes. Other waxes with higher T_m include bees wax (composed mostly of C_{45} alkyl esters and a minor fraction of *n*-alkanes, $T_m \approx 63°C$), rice bran wax (mostly alkyl acids and esters, $T_m \approx 80°C$) and sunflower wax (C_{40} to C_{60} esters, $T_m \approx 76°C$). Waxes are classified as phase-change materials (PCMs), i.e., materials that absorb considerable energy as they undergo their solid-to-liquid phase transition. [29,30,31,32] This energy can be released during the reverse (liquid-to-solid) transition. PCMs are also referred to as latent heat storage (LHS) materials.

Generally, the higher the number of carbon atoms in the constituent molecules of a wax, the higher the T_m. Also, waxes tend to have broad melting transitions around their

T_m. In contrast, purified *n*-alkanes or other hydrocarbons have sharp melting transitions. For example, *n*-docosane ($C_{22}H_{46}$), the main component in paraffin wax, has a T_m = 42°C, while *n*-eicosane ($C_{20}H_{42}$) has a T_m = 37°C. Other organic compounds with different functional groups are also crystalline solids with sharp melting transitions. For example, palmitic acid is a saturated fatty acid with a C_{16} tail attached to a –COOH group and it has a T_m = 63°C. Isopropyl palmitate (IPP) is the isopropyl ester of palmitic acid. IPP has a T_m = 11°C, which means that it is a liquid at room temperature. [11]

When two different waxes are mixed together in the molten state and cooled, the resulting solid can be a homogeneous 'solid solution' in which molecules of the two components co-crystallize together. [29] This tends to be the case when the waxes are chemically quite similar, such as for mixtures of sunflower wax and rice bran wax. [33] These mixtures can thereby exhibit a single T_m. Alternatively, if the waxes are chemically distinct, the solid state can have distinct crystals of A and B, and accordingly the mixture could exhibit two distinct T_ms. [29] This is the case for mixtures of rice bran wax and paraffin wax, for example. In a similar vein, mixing a wax with a second component such as a purified fatty acid (like palmitic acid) or an ester (like IPP) can affect the phase behavior in the solid state.

2.4 Characterization Techniques: Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) is a technique used to study the thermal properties of materials. The principle of DSC is based on measuring the heat flow associated with changes in the physical and chemical properties of a sample as it is

heated or cooled. First, a small amount of the sample is placed in a sample pan along with an empty reference pan. The two pans are then heated or cooled together in a controlled manner. As the temperature changes, the sample undergoes physical and chemical changes that either absorb or release heat, which in turn changes the heat flow between the sample and the reference pan. The difference in heat flow between the sample and reference pan is measured by a thermocouple and recorded as a function of temperature.

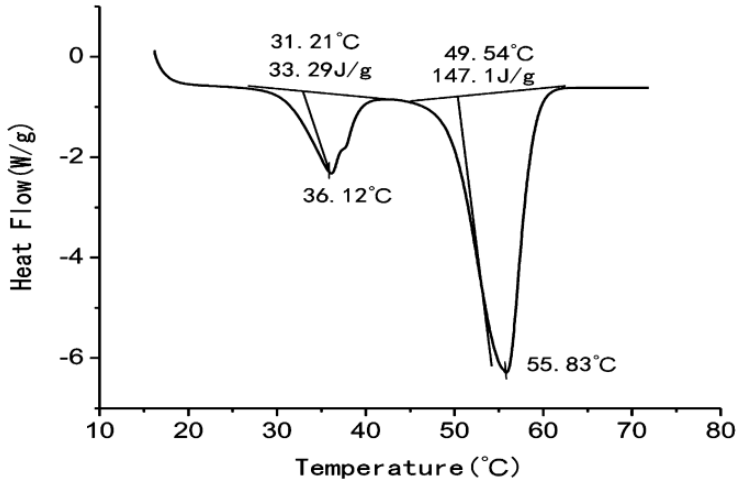

Figure 2.2. DSC data for paraffin wax. Two endothermic peaks are seen around 36 and 56°C. [2]

We used DSC in this thesis to examine the melting behavior of mixtures containing wax and other components. A DSC thermogram of paraffin wax as it is heated is shown in Figure 2.2. A first small endothermic peak arises at 36°C and this is attributed to a solid-solid phase transition of the wax. (That is, when paraffin wax is heated above 36°C, it still remains a solid.) [2] The latter peak is the primary peak and it occurs at ≈ 56°C. This corresponds to the melting temperature T_m, i.e., the solid-liquid phase change. The areas under these peaks can be computed by integration and are shown

in the figure. The sums of the two areas totals to 180.4 J/g and this is the latent heat storage (LHS) of the wax. [2]

2.5 Characterization Techniques: UV-Visible Spectroscopy

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 molecule in solution. This relationship is described by the Beer-Lambert Law: [34]

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

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 thus UV-Vis spectroscopy plays a vital role in studying their release from carriers.

Chapter 3: Delayed Release from Capsules

3.1 Introduction

In this Chapter, we will present our results on delayed release capsules. The system we have created is one based on a hydrogel core and a shell composed of paraffin wax + another hydrophobic additive. For the hydrogel core, we use alginate. Alginate gels can be easily created at the millimeter scale by dropping a solution of sodium alginate into a reservoir of a divalent cation like Ca^{2+}. During this gel formation, the gels can be loaded with various hydrophilic solutes. We then create a wax shell around the alginate core by a procedure modified from that used earlier (see Figure 2.1).

The key to delayed release is the additive mixed into the paraffin wax. After experimenting with many additives, we found that fatty-acid esters are ideal candidates for our purpose. Esters are products of reactions between an alcohol and an acid. For example, one of the main esters in this work is isopropyl palmitate (IPP), which is an ester of isopropanol and a fatty acid, *viz*. palmitic (saturated C_{16}) acid. We will show that a shell composed of wax/IPP gives the delayed release profile at room temperature shown in Figure 1.3. We can tune both the delay time and the subsequent release rate. Why do esters like IPP induce such a release profile? To find this out, we have conducted a series of experiments using techniques such as differential scanning calorimetry (DSC). Based on these results, we will present our hypothesis that the crystal structure of the wax shell at room temperature is altered by esters like IPP. As a result, the solute is able to slowly escape out of the capsule core, possibly by diffusion between the crystallites in the shell.

3.2 Experimental

Materials. Isopropyl palmitate (> 95% purity, molecular weight: 298.5 g/mol) was from TCI America. Hydrogen peroxide (30% solution) was purchased from BDH Chemicals. Isopropyl myristate (96% purity, molecular weight: 270.4 g/mol) and isopropyl laurate (> 97% purity, molecular weight: 298.5 g/mol) were purchased from Thermo Scientific. All other chemicals were from Sigma-Aldrich. The biopolymer used was alginate, medium viscosity alginic acid, sodium salt from brown algae. The dyes used were Rhodamine B and fluorescein sodium salt. Other chemicals included calcium chloride dihydrate ($CaCl_2$) salt, potassium permanganate (97% purity), diclofenac sodium salt and paraffin wax (ASTM D 87, T_m 53 – 58 ºC). A heating tape (288 W, 1" width x 4 ft length) was purchased from Zoro. Deionized (DI) water was used in all experiments.

Preparation of Solute-Loaded Gels. A 2% alginate solution in DI water was loaded into a pipette and added dropwise to a reservoir solution containing 0.5 M of $CaCl_2$ in DI water. The Ca^{2+} ions crosslink the alginate chains, thus converting the droplet into a gelled bead. After 20 min of incubation in the reservoir, the alginate gels were washed and stored in DI water. The size of the gels could be controlled by changing the diameter at the end of the pipette or using a needle at the end of a syringe. Typical gel diameters were 3-4 mm. A given gel was then placed in a solution of the solute of interest (dye or drug, typically at a concentration of 1 mM) for 10 min to allow the solute to diffuse into the gel.

Preparation of Capsules. The technique used for preparing capsules with a waxy shell is further discussed under Figure 3.1 in the next section.

Solute Release Experiments. Solute-loaded capsules were placed in vials containing 2 mL of DI water at ambient conditions. The solute concentration in the water was determined by taking a sample of 1 mL and analyzing it using a Cary 50 UV-Vis spectrophotometer. Absorbance was measured at the absorption peak of the solute. The sample was then returned to the vial after measurement. Cumulative release (%) was calculated by normalizing the measured solute concentration with that at infinite time.

Optical Microscopy. Optical images of the capsules were taken using a handheld DinoLite USB digital microscope purchased from Amazon. Images were generally taken at a magnification of 230 x.

Differential Scanning Calorimetry (DSC). A Discovery DSC 25 (TA Instruments) was used to measure the thermal properties of various samples. 10 mg of the sample was placed in aluminum pans and sealed using a compressor (Tzero Press, TA Instruments). Sample were equilibrated at 0°C for 5 min and then heated to 90°C at 1°C/min. The samples were kept at 90°C for 5 min and then cooled back to 25°C at 5°C/min.

3.3 Results and Discussion

3.3.1 Capsule Synthesis

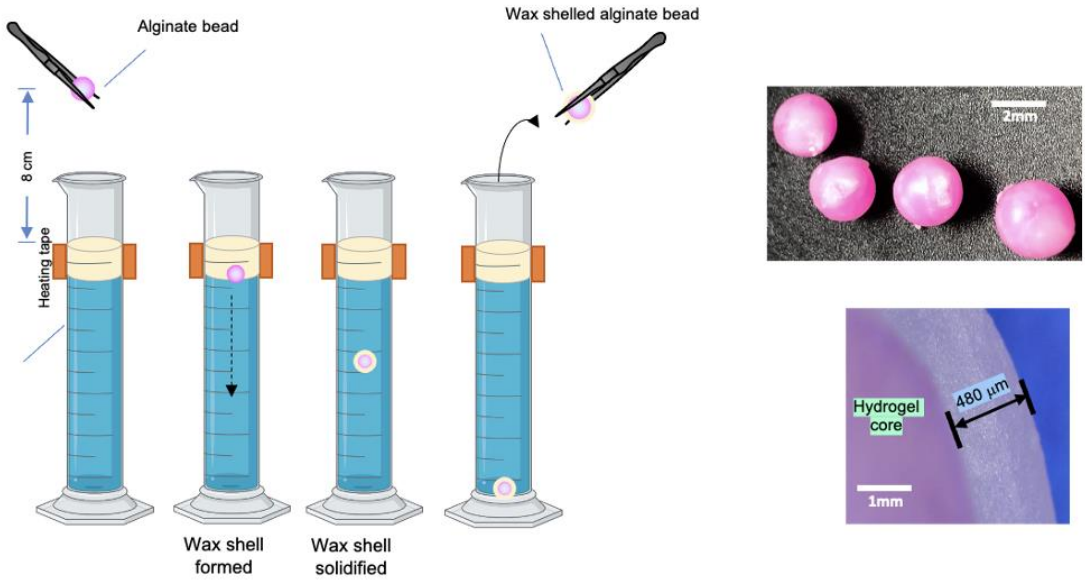

Figure 3.1 Synthesis of capsules with a waxy shell around a gel core. A gel-bead cooled to ~ 5°C is dropped into a graduated cylinder. The cylinder has a layer of molten waxy liquid floating atop a column of water/ethanol (30/70). Heating tape is used to melt the wax. As the cold bead passes vertically downwards through the molten wax, a thin wax shell forms around the bead. As the structure settles due to gravity, it gets cooled by the water/ethanol, which is kept cold by immersion in an ice bath. The shell solidifies, giving a robust capsule (gel core surrounded by a waxy shell), which settles to the bottom of the cylinder.

Figure 3.1A illustrates the process of creating capsules with a waxy shell. The procedure begins with the creation of gels by dropping a solution of 2% sodium alginate into 0.5 M $CaCl_2$. Droplets of alginate (an anionic biopolymer) are converted into gel-beads due to the crosslinking of alginate chains by Ca^{2+} cations. After 20 min of incubation, we obtain spherical beads with ~ 4 mm diameter. These beads are then cooled to 5°C (by keeping in the fridge) and the cool beads are dropped into molten (liquid) wax that is kept at a temperature slightly above its melting point (T_m). For example, paraffin

wax has a T_m of 57°C and therefore the molten wax is maintained at ≈ 60°C. The temperature difference between the wax and the cool bead is crucial in forming a wax shell around the gel. The shell forms in ~ 5 s, after which the structure has to be removed out of the molten wax and cooled to room temperature. Upon cooling, the wax shell solidifies, giving a capsule with a gel core and a wax shell.

In our original procedure, which was used earlier in the lab (Figure 2.1), the capsule was removed manually out of the molten wax after ~ 5 s using tweezers. But the use of tweezers sometimes damaged the capsule at that early stage. Therefore, we modified the procedure, as shown in Figure 3.1. Here, a cold bead is dropped vertically through a graduated cylinder and moves downward by gravity. The cylinder has ~ 40 mL of water/ethanol (30/70) at the bottom and above this is a 2 mL layer of molten wax (roughly 2 cm). The wax is kept molten by wrapping heating tape around that top section of the cylinder (see photo). The temperature of the molten wax is maintained at ≈ 60°C by adjusting the temperature of the heating tape. Also, the cylinder is immersed in an ice bath to keep the water/ethanol solution cool towards the bottom.

With this arrangement, as the cold bead travels through the molten wax layer, a wax shell forms around the bead. But as the structure travels further down the cylinder, the wax shell cools and solidifies. Ultimately, we obtain a capsule with a gel core surrounded by a wax shell, just as in our earlier procedure. The capsule settles at the bottom of the cylinder, and they can be taken out and stored in DI water. The advantages of this new procedure are that the capsule shells are more uniform, and moreover, we can

generate a large number of capsules more quickly by this method. Figure 3.1 shows that the thickness of the waxy shell around the gel-core is typically around 480 μm.

There are some key considerations for the success of this procedure. First, the temperature of the molten wax must be just a bit higher than the T_m of the wax — if it is too high, the shell does not form. Second, the residence time of the cold gel bead in the liquid wax has to be in the range of 2 to 5 s. If this time is longer or shorter, a stable shell does not form around the gel bead. This consideration dictates the height of the molten wax layer in our setup (Figure 3.1) – it is maintained around 2 cm (using 2 mL of the wax). The third point is the density of the aqueous liquid below the wax layer. If pure water is used, the density is too high for the wax-shelled capsule to travel smoothly to the bottom of the cylinder (it would instead get stuck at the bottom of the wax layer). By using a water/ethanol 30/70 mixture, the density (≈ 0.853 g/cm^3) was sufficiently lower than that of the capsule, allowing the capsule to travel downward.

3.3.2 Delayed Release from Wax/IPP Capsules

We used the procedure shown in Figure 3.1 to prepare capsules with an alginate gel core and a waxy shell. As a model solute, we used the zwitterionic dye Rhodamine B, which was present within the gel core. We placed the dye-loaded capsule in DI water and monitored the dye concentration in the external solution over time. If pure wax (i.e., paraffin wax, with a T_m of 56°C) is used to form the shell, the dye remains hermetically sealed in the capsule at room temperature (i.e., there is zero release of dye even for months).

To achieve delayed release at room temperature, we experimented with a range of additives to the paraffin wax. First, we show results for the fatty-acid ester, isopropyl palmitate (IPP). Shells of wax/IPP were made exactly as in Figure 3.1, with the only difference being that the wax layer was replaced by a mixture of wax/IPP. Note that IPP is a liquid at room temperature. Molten paraffin wax is completely miscible with liquid IPP. When such a wax/IPP solution is cooled, it forms a solid provided the IPP content is 60% or below. Thus, we were able to make capsules with an alginate gel core surrounded by shells of 80/20, 60/40, and 40/60 wax/IPP. All shells were ~ 480 μm in thickness. Dye release was studied from these capsules by UV-Vis spectroscopy.

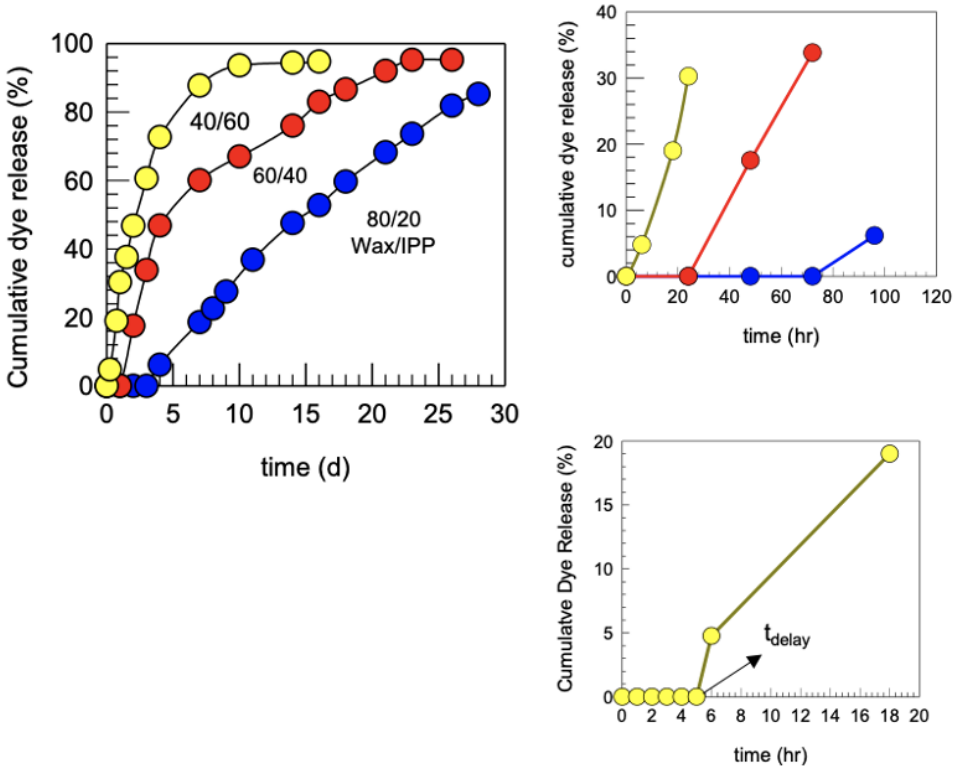

Figure 3.2 Delayed release curves for capsules with wax/IPP shells. The capsules each have an alginate gel core loaded with 1 mM of the dye Rhodamine B. Their shells are mixtures of wax/IPP in ratios of 80/20, 60/40, and 40/60. At $t = 0$, the capsules are placed in DI water and the dye concentration is monitored. The plots show the cumulative dye release (normalized by the final concentration) vs. time.

Data for dye release from these capsules are shown in Figure 3.2. The data are plotted in a normalized fashion, where 100% refers to complete release of dye out of the capsule. The key result from Figure 3.2 is that the release of dye shows a delay followed by subsequent release. That is, there is zero release of dye for a considerable time, which we denote as the *delay time* t_{delay}. In the case of an 80/20 wax/IPP shell, t_{delay} is 3 days, whereas for 60/40 wax/IPP, t_{delay} is 1 day, and finally for 40/60 wax/IPP, t_{delay} is 6 h. Thus, the lower the IPP content, the longer the delay. Moreover, in the 80/20 case, after this delay, the dye is released in a slow and sustained manner for the next 28 days (4 weeks). That is, the slope of the release curve, which is the *release rate*, is nearly constant over a long time. Such a linear plot, with a constant release rate, is termed 'zero-order release' in the literature. For 60/40 and 40/60 wax/IPP, the curves do deviate from linearity. However, the release rate from the initial linear portion of the curve is higher (i.e., the slope is steeper) with increasing IPP fraction.

3.3.3 Delayed Release from Wax/Ester Capsules

Next, we evaluated other fatty-acid esters apart from IPP for their effects on inducing delayed release. IPP is an ester of isopropanol and palmitic (saturated C_{16}) acid. We decided to try isopropyl esters of myristic (C_{14}) acid and lauric (C_{12}) acid, i.e., isopropyl myristate (IPM) and isopropyl laurate (IPL). We maintained the same configuration for the capsules: alginate gel-cores with a shell of 80/20 wax/ester, and with Rhodamine B as the solute. Figure 3.3 shows a delayed-release profile for all three cases and t_{delay} is about 3 days with all of them. After the delay, near-linear plots are seen in all cases, with the release rates increasing in the order wax/IPP < wax/IPL < wax/IPM.

The trend does not perfectly follow the order of the carbon-chain length in the esters. Nevertheless, the fact that a delayed release is seen with different esters suggests that there is indeed a systematic nature to these results.

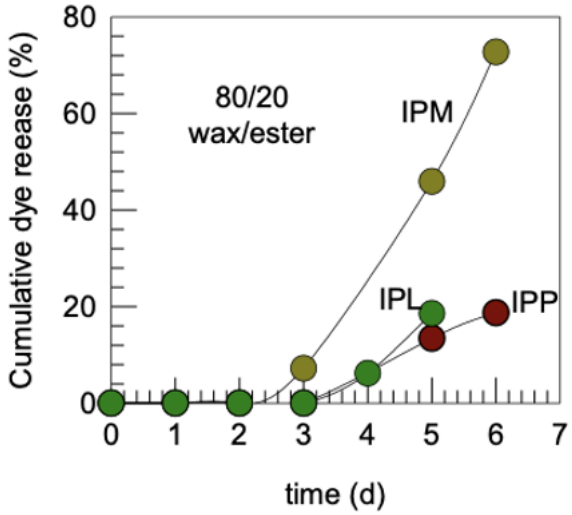

Figure 3.3 Delayed release curves for capsules with wax/ester shells. The capsules each have an alginate gel core loaded with 1 mM of the dye Rhodamine B. Their shells are mixtures of wax/ester in a 80/20 ratio, with the esters being IPP (ester of C_{16} fatty acid), IPM (C_{14}) and IPL (C_{12}). At $t = 0$, the capsules are placed in DI water and the dye concentration is monitored. The plots show the cumulative dye release (normalized by the final concentration) vs. time.

3.3.4 Delayed Release of Various Solutes

Does the delayed-release occur with all kinds of solutes? To test this, we first examined a second dye, fluorescein, which is an anionic molecule. 1 mM of this dye was loaded into an alginate gel and covered by a shell of 80/20 wax/IPP. The resulting capsule was placed in DI water and the dye concentration was monitored over time. Delayed release is again observed with fluorescein (Figure 3.4A). The delay time t_{delay} is the same at ≈ 3 days as in Figure 3.2 and this is followed by a slow release. Similar

results were also obtained with another anionic dye, Acid Red 52 (data not shown). We also studied the release of an anionic drug, diclofenac sodium which is a non-steroidal anti-inflammatory drug that is commonly used to reduce pain in the body. 1% of diclofenac was again placed in the core of a capsule with a shell of 80/20 wax/IPP. The release profile again shows a 3-day delay and thereafter a slow and sustained release.

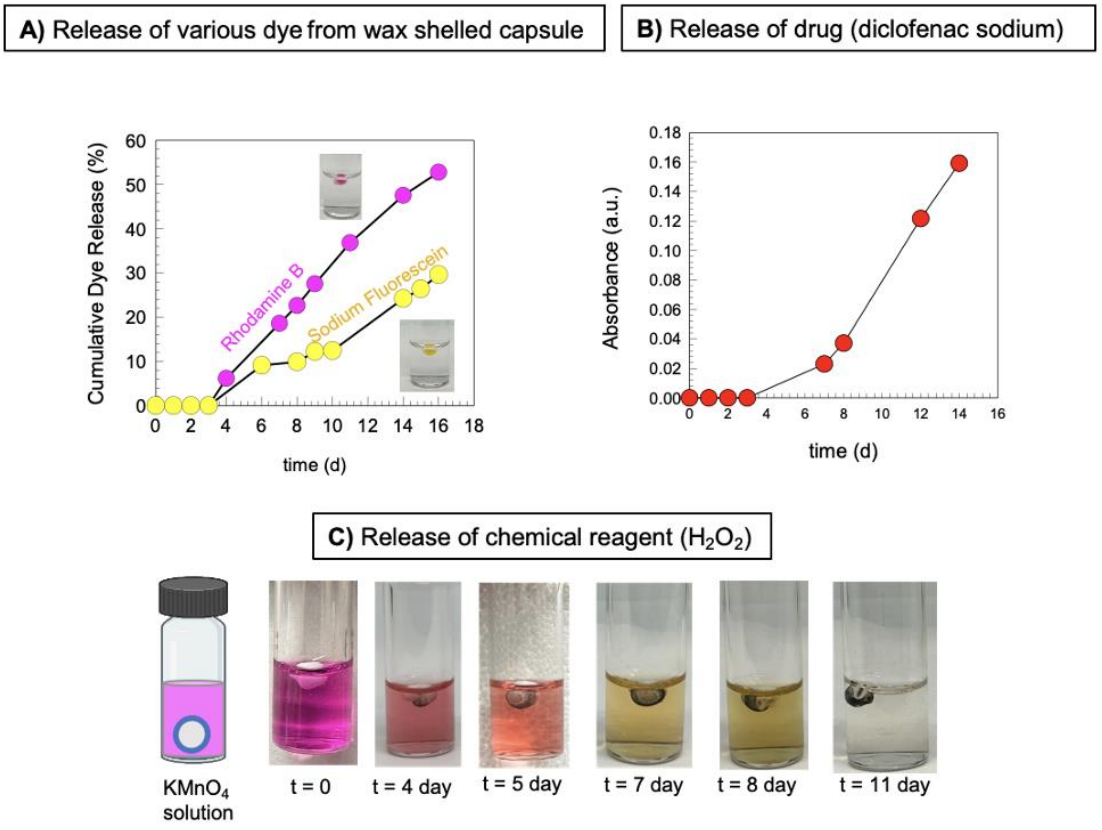

Figure 3.4 Delayed release of different solutes. In all cases, the solutes are released out of capsules with a wax/IPP 80/20 shell (A) Data for a zwitterionic dye, Rhodamine B and an anionic dye, fluorescein. (B) Data for an anionic drug (diclofenac sodium). (C) Data for H_2O_2 released out of a capsule placed in dilute $KMnO_4$ solution. The color change over 11 days shows the slow release of H_2O_2 into the solution.

Additionally, the encapsulation of hydrogen peroxide (H_2O_2) was also tested. H_2O_2 is a strong, oxidizing and reducing agent that is frequently used in chemical reactions. We encapsulated a 30% H_2O_2 solution in a capsule with a 80/20 wax/IPP shell.

The release of H_2O_2 can be visually detected using a colorimetric indicator, potassium permanganate ($KMnO_4$). At $t = 0$, the capsule with H_2O_2 is placed in a 0.01 mM $KMnO_4$ solution, which has a purple color. If H_2O_2 is released, the color of the solution will change due to the reduction of $KMnO_4$. No change is observed for the first 3 days, indicating a delay in H_2O_2 release. On Day 4, the solution turns from pink to a light red and on Day 7, it turns yellow. Finally, by Day 11, the solution turns colorless. The results again indicate a delayed release of H_2O_2, followed by a slow and sustained release.

3.3.5 Impact of Shell Thickness on Delayed Release

The thickness of the shell in the capsule can also have an impact on the release of contents from the core. In our typical capsules with a 80/20 wax/IPP shell, the shell thickness was 480 μm. We tried to make a thinner shell in one of two ways. First, we lowered the amount of molten wax in the cylinder in Figure 3.1. This decreases the residence time for the gel-bead as it travels downward. Alternatively, instead of using a cold bead taken right out of the fridge ($T \sim 5$°C), we allowed the bead to thaw a bit to a temperature around 13°C before dropping the bead through the cylinder. Both methods gave rise to thinner shells, with a shell thickness around 280 μm (Figure 3.5).

Figure 3.5 shows the effect of shell thickness on the release data. In both the thin- and thick-shelled capsule, 1 mM of Rhodamine B is present in the core, and the shell is composed of 80/20 wax/IPP. The data show the same time delay (≈ 3 days) before release starts to occur. The release rate is a bit higher in the case of the thin-shelled capsule. This makes sense because a thinner shell presents less of a transport barrier to the release of

the solute from the core to the external solution. We conclude that the shell thickness has a minor, but still important effect on the release profile.

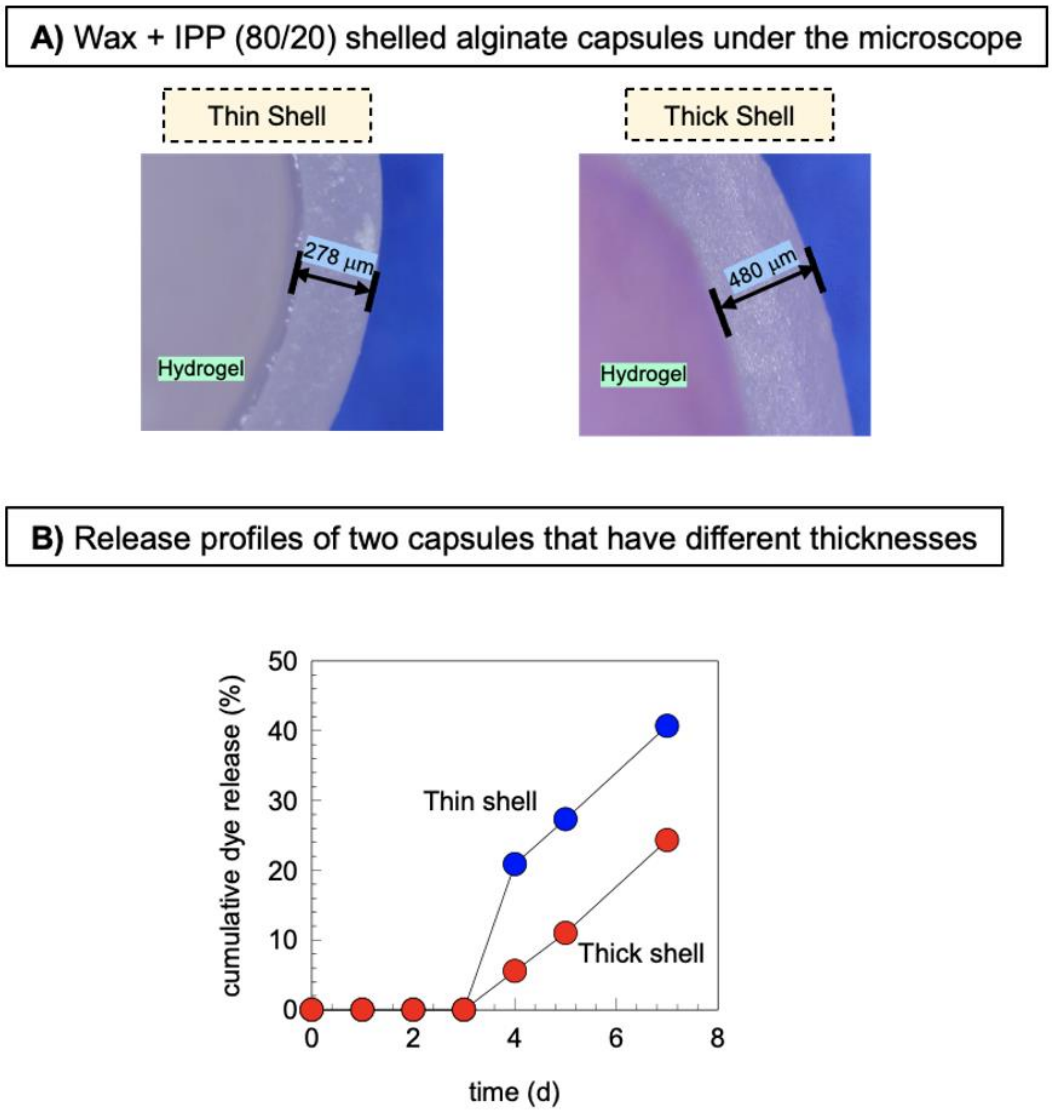

Figure 3.5 Delayed release out of capsules with thick and thin shells. The data are for release of Rhodamine B out of capsules with a wax/IPP 80/20 shell. The typical shell is 480 µm thick and the data are identical to those in Figure 3.2. For comparison, data are shown for a capsule with a thinner shell (280 µm thick). Both capsules release after the same time delay, but at different rates.

3.3.6 Thermal Characterization of Wax/IPP Mixtures

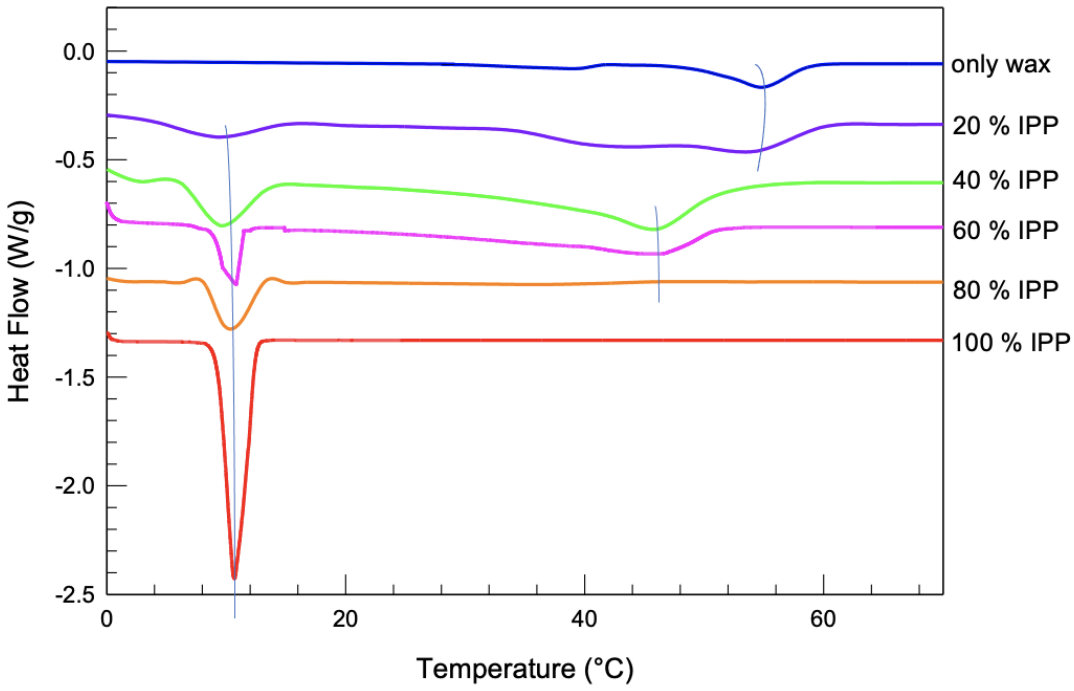

Figure 3.6 DSC thermograms for wax/IPP mixtures. The data were collected as the samples were heated. The endotherms exhibit one or more peaks, as discussed in the text.

Figure 3.6 shows data from DSC on wax/IPP mixtures. Paraffin wax shows two melting peaks — a sharp one at 57°C (i.e., the T_m^W of wax) and a shallow one at T_2 = 39°C. This thermal behavior is consistent with data from the literature (see Figure 2.2). It indicates that paraffin wax is a mixture of various components. For comparison, a sample of IPP alone shows a single and sharp melting peak at T_m^{IPP} = 11°C, which is consistent with the thermal response of purified waxy materials. Note that IPP is a liquid at room temperature.

When wax and IPP are combined, the mixtures show thermal characteristics of both the components. The 20/80 wax/IPP sample shows a sharp peak at T_1 = 11°C = T_m^{IPP} peak and a very small second peak at T_2 = 43°C, which is the T_m of the mixture. At room temperature, this sample is a solid, although it is quite soft compared to wax alone. The 40/60 and 60/40 wax/IPP samples have similar responses to the 20/80 one: one peak at T_1 = 11°C = T_m^{IPP} and a second peak at T_2 = 45°C, which represents the T_m of the sample. Finally, in the case of the 80/20 wax/IPP sample, the peak at T_1 = 11°C is very shallow and we observe two additional peaks at T_2 and T_3, the last of which is the T_m of the sample. Note that the highest temperature peak (corresponding to the T_m) shifts downward from 57°C for wax alone to 43°C for 20/80 wax/IPP.

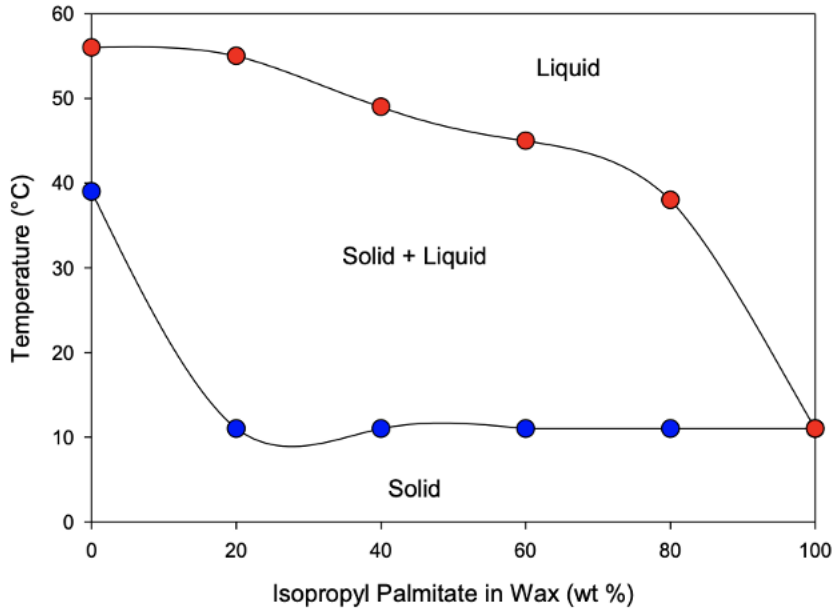

Figure 3.7 Pseudo-phase diagram for wax/IPP mixtures. The top curve corresponds to T_m values, and above this curve the samples exist in a liquid state. The bottom curve corresponds to T_1 values and it indicates the threshold below which the samples are solid. Between these two curves, both solid and liquid states co-exist.

The above data are used to construct a pseudo-phase diagram for wax/IPP in Figure 3.7. This is a 'pseudo' phase diagram because the wax is a mixture, not a pure component. The lower points (in blue) in the figure correspond to the first peaks (i.e., T_1 or the onset melting peak). The upper points (in red) correspond to the last peaks (i.e., the melting temperatures T_m). Above the red curve, the sample is liquid, while below the blue curve, it is solid. In the region between the red and blue curves, there is a co-existence of solid and liquid states.

We conclude a few important points from Figures 3.6 and 3.7. First, the shift in T_m from 57°C to 43°C (along the red curve) shows that the IPP incorporates into the wax crystallites to some extent, i.e., the two components co-crystallize. At the same time, the IPP also exists as free molecules, i.e., IPP liquid surrounds the crystallites in a wax/IPP shell. This is why the peak corresponding to T_m IPP is still seen at a temperature close to 11°C in all the wax/IPP mixtures. We believe the presence of free IPP can explain the delayed-release seen with our capsules.

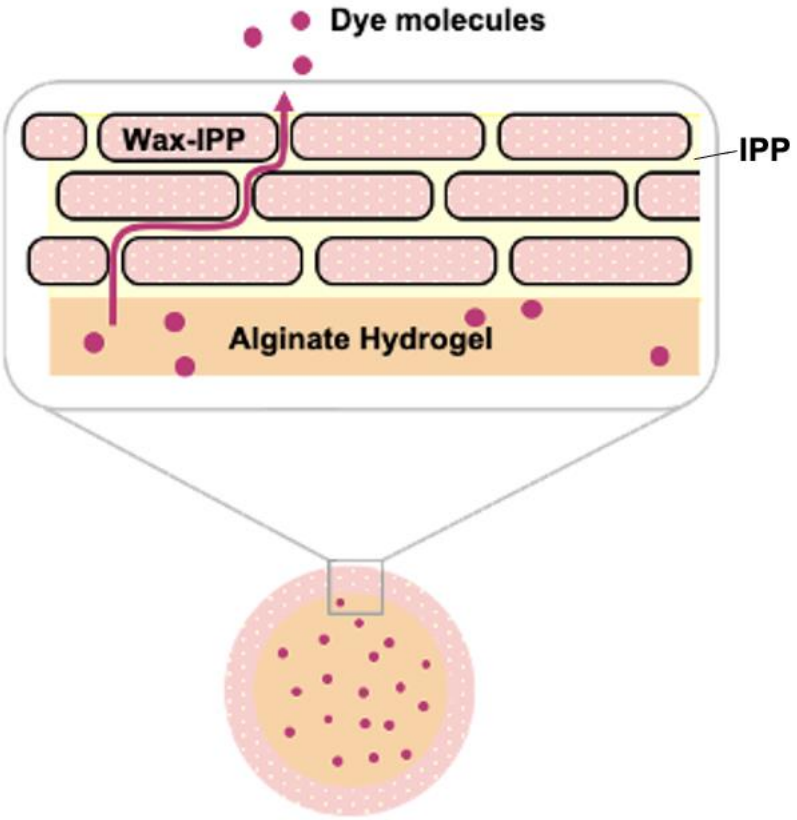

Figure 3.8 Schematic of mechanism. The schematic represents the release of hydrophilic dye molecules from alginate core through the co-crystallized wax/IPP (80/20) shell.

Figure 3.8 shows a schematic of the postulated structure in the shell of a capsule with a composition like 80/20 wax/IPP. The wax and IPP will co-crystallize to form crystalline domains ("crystallites") in the shell. Around these crystallites, free IPP molecules will also exist in the shell. Both wax and IPP are hydrophobic and thus their presence in the shell will create a barrier to the release of hydrophilic solutes in the gel core. Indeed, it is worth noting that a dye like Rhodamine B is completely insoluble in both molten wax as well as IPP liquid. This explains why no release of dye across the shell is seen for several days from such a capsule.

However, while wax is completely hydrophobic, IPP has a weak hydrophilic character. For example, wax is insoluble in polar solvents like ethanol whereas IPP is soluble in ethanol. Thus, there is a very weak affinity of dyes and other solutes to IPP. Given enough time, the dye will diffuse slowly through the IPP and thus release into the water. The release will be mediated by the very low solubility of the dye in the wax/IPP shell, which explains why a constant-rate (zero-order) release is seen.

3.3.7 Conclusions

We have discovered a delayed release of solutes from hydrogel capsules. This means that there is no release of the solutes for a certain period, followed by a gradual and consistent release afterwards. Previously, we found that solute release can be completely stopped if the gel is covered by a thin shell of paraffin wax (melting point T_m ~ 57°C); in such 'capsules', the wax shell is a hydrophobic solid. We found a new and faster way of making these wax shelled capsules. Using the new method, we were able to make more capsules compared to the preexisting method in the same amount of time. To achieve delayed release, we mixed an additive with the molten wax, which is compatible with the wax but has a weak hydrophilic nature and a lower melting temperature (T_m). One example of such an additive is isopropyl palmitate (IPP), which has a T_m of approximately 11°C and belongs to the group of fatty acid esters. The wax/IPP shell keeps hydrophilic solutes sealed perfectly and doesn't release the solutes for several days. We also found that when the IPP amount in wax/IPP mixture was increased, the delay time of hydrophilic solute was decreased, therefore, we achieved that delay time can be tuned by adding more IPP. Moreover, we tried to combine wax with different esters other than IPP such as isopropyl laurate (IPL) and isopropyl myristate (IPM). The results for

the delay time were similar to IPP, they both delayed the release for 2 days. In addition, different types of solutes were loaded in the core and shelled with wax/IPP (80/20). For the different types of solutes such as sodium fluorescein salt, acid red, anti-inflammatory drug and H_2O_2, and the results have shown that the release was delayed for three days in each case. By doing DSC analysis, we concluded that the delayed release is caused by the presence of crystalline domains when wax and IPP co-crystallizes. The delayed release phenomenon is attributed to the fact that the wax/IPP shell forms an uneven crystal structure since wax and IPP are only partially miscible. Therefore, the uneven crystal structure of the wax/IPP shell, resulting from their partial miscibility, was related to "bricks" consisting of wax+IPP, with liquid IPP present between the bricks. The solute molecules were able to flow between these brick-like structures, thereby causing the delayed release. These studies show that this system may be useful for many applications such as cosmetics, pharmaceuticals and delivering agricultural chemicals.

Chapter 4: Conclusions and Recommendations

4.1 Conclusions

In this thesis, we presented a design for a biopolymer hydrogel capsule system that utilizes wax to delay the release of its solute (such as hormones and therapeutics) in several days. The wax shell acts as a barrier, preventing the solutes from escaping. In order to introduce the desired delayed release behavior, we have combined an additive with the wax shell. To achieve the delayed release effect, an additive with weak hydrophilic properties and a lower melting temperature (T_m) was mixed with the molten wax. This additive, such as isopropyl palmitate (IPP), a fatty acid ester with a Tm of approximately 11°C, effectively sealed the hydrophilic solutes within the wax shell, preventing their release for several days. We observed that this system then slowly releases the solute when stored at room temperature. Moreover, a new and more efficient method for making these wax/IPP shelled capsules has been discovered, resulting in an increased production rate compared to the previous method within the same timeframe. Our method to create wax/IPP shelled capsules are extremely simple, fast, and easy. Additionally, we have shown that the system works well with a drug.

Subsequently, the release rate becomes nearly constant, exhibiting a "zero-order" release profile for the following 20 hours. Importantly, the delay period and release rate can be adjusted by varying the concentration and type of additive present in the shell. The delayed release phenomenon can be attributed to the fact that the wax/IPP shell forms an inhomogeneous crystal structure due to the partial miscibility of wax and IPP.

Our approach holds great promise for delaying the release of various substances, including dyes, drugs like diclofenac, and reactive agents like H_2O_2, from the core gel. This simple yet versatile approach has potential applications in controlled release systems within the pharmaceutical, agrochemical, and cosmetics industries.

4.2 Future Directions

There are multiple ways that this research can be expanded upon. For instance, our approach can be extended to create wax/IPP shelled capsules with different types of hydrogels such as chitosan. For these types of capsules, the release rate of hydrophilic solutes could be observed. Moreover, to understand the crystalline structure of wax/IPP shells better, different characterization techniques could be used such as X-ray spectroscopy and polarized optical microscopy.

Another area of importance that needs to be studied further is whether it is possible to delay the release when the hydrogel core is loaded with larger molecules. Since the wax/IPP shelled capsules are delaying the release of small hydrophilic drugs, there is a need to try larger molecules. These further studies could help with understanding the mechanism of our system. In addition, other types of waxes that are biodegradable such as beeswax and soy wax could be tested in the system.

These are just a few examples of future research that could build on the research described in this paper. Currently, there is nothing in the literature wax/additive shelled

capsules in the literature. Therefore, based on the work that we have presented, there is a lot of potential for future research.

Abbreviations

IPP	Isopropyl Palmitate
IPL	Isopropyl Laurate
IPL	Isopropyl Myristate
DI water	Deionized Water

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