ABSTRACT

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Polymer capsules and beads can be easily created by combining either two oppositely charged polymers or one polymer and a multivalent salt. These structures have been mainly investigated until now for their controlled release properties. Here, we study the ability to impart new functionalities to capsules by embedding a second kind of colloidal or nano-structure in their interior. Three different concepts are explored in this regard. First, we demonstrate capsules that are responsive to pH. For this purpose, we entrap vesicles made from a diacetylene surfactant in chitosan capsules. The resulting capsules change their color blue blue to red as the pH of the solution is increased. The next concept involves capsules with the ability to sense and separate cations from solution. In this case, nanoscale particles of synthetic clay (laponite) are localized in alginate beads. The resulting hybrid beads can effectively separate a cationic dye from a mixture of cationic and anionic dyes. The third concept involves linking of magnetic chitosan-gelatin capsules in a chain to create an *artificial earthworm*, i.e., a structure that can undergo guided motion in a fluid environment under an external magnetic field.

Polymer Capsules: Fundamental Studies and New Concepts

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Dedication

This thesis is dedicated to my Mom and Dad for stressing right from the beginning the importance I should pay to education, being enthusiastic and encouraging with all endeavors I undertook in this regard.

Acknowledgements

A number of people deserve not just thanks, but direct credit for some of the work this thesis represents.

Firstly, I would like to acknowledge the support of my thesis advisor Dr Srinivasa Raghavan. He has constantly provided excellent guidance and encouragement on my research work and academic matters. His attitude towards my shortcomings or occasions when I turned up with half baked ideas in the course of this research has been extremely constructive with prompt suggestions about what would be a way to improve or better way to proceed. Finally, I would like to thank him for patiently listening and providing advice on problems I occasionally brought before him as a graduate student that were related to both non academic as well as research matters. Dr Raghavan‘s clear principles and empirical approach to creating and validating new technologies will continue to guide me and provide an example that I hope to emulate throughout my career.

I would also like to thank my committee members, Dr. Joseph Schork and Dr. Sheryl Ehrman from the Department of Chemical and Biomolecular Engineering Department for their immense support and guidance during the course of this thesis.

Given the stereotype of graduate students as introverted and antisocial, it seems amazing that I have not had to work with a single one. I am grateful to my all group colleagues – Bani Cipriano, George Chacko, Matt Dowling, Aimee Ketner, Rakesh Kumar, Hee-Young Lee, Kunshan Sun, Shih-Huang Tung, Dr Tosin Oluwatosin Ogunsola, Dr Chao

Zhu and Dr Kho who are truly dedicated, bright, and fun loving people. No one could ask for a better group of people to work with.

And finally, I want to mention “everybody” back home, my family and my friends, who didn't forget me even though I ran off to study thousands of miles away from them ... thanks!

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Chapter 1: INTRODUCTION & OVERVIEW

This thesis revolves around the theme of *capsules*. Capsules are spherical structures with a thin shell enclosing a solvent-filled interior. In particular, our main interest will be on polymer capsules, where the shell is composed of a single polymer or a mixture of polymers. Such capsules ranging in size from a few microns to several millimeters can be prepared by several methods. Chapter 2 gives a detailed description of capsule preparation and structure.

Traditionally, capsules have been investigated from the point of view of controlled release and drug delivery. That is, small molecules are encapsulated within the capsule (the shell protects the molecules against various environmental factors) and these molecules are then slowly released from the capsule at a controlled rate. Indeed, controlled release from capsules has also been an ongoing aspect of investigation within our laboratory. However, the focus of this thesis is not on controlled release but instead on the range of other functionalities that can be imparted to capsules. Recent work in our laboratory has shown that a variety of colloidal or nanoscale structures, including vesicles and nanoparticles, can be encapsulated quite easily in the interior of polymer capsules and beads. *The goals for this thesis are to exploit this ability to alter the capsule nanostructure as a way to impart new functionalities to the capsules*. We thus view capsules from a different viewpoint – not simply as containers that deliver an encapsulated drug or molecule, but as a platform for a variety of applications.

It is worth reiterating the key aspects of our approach. Consider colloidal structures having a given property or function. By encapsulating these structures within a polymer capsule, we ensure that they remain localized – i.e., the colloids are too big to be able to diffuse through the capsule shell into the external fluid. At the same time, the *properties or functionalities of the colloids are also imparted to the hybrid capsules*. This idea will be explored by means of three separate studies as part of this thesis:

(a) *Vesicle-Loaded Capsules with pH-Responsive Properties*

(b) *Nanoclay-Loaded Beads with Ion-Sensing Capabilities*

(c) *Magnetic Particle-Filled Capsules as a Way to Create Artificial Earthworms*

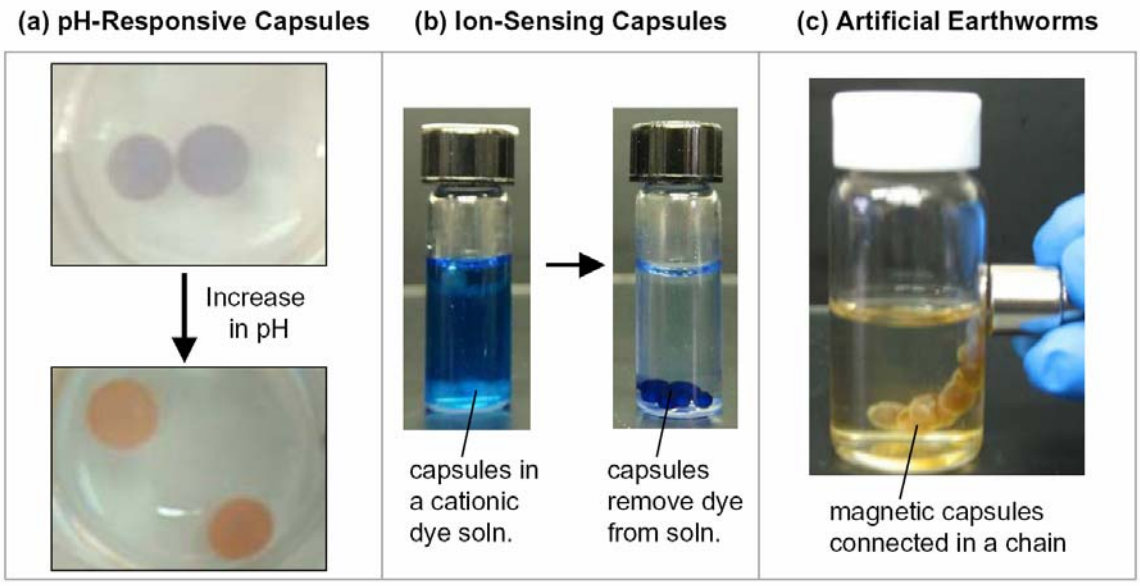

FIGURE 1.1. Schematics of the three types of hybrid systems studied: (a) vesicle-loaded capsules with pH-sensitive properties; (b) nanoclay-loaded beads with the ability to sense and separate cations; and (c) artificial earthworms based on capsules loaded with magnetic particles.

Figure 1.1 shows photographs representing the three concepts explored in this thesis. First, *pH-responsive capsules* are shown in Figure 1.1a the capsules exhibit different colors depending on the pH of the external fluid. This ability to respond to pH is conferred to the capsules by entrapping a certain kind of vesicles in them. The vesicles are made from a diacetylene surfactant and undergo a chromatic transition in response to pH. We will show that this pH-responsive behavior is retained when the vesicles are localized within capsules. Interestingly, the use of capsules offers certain advantages for pH sensing (in terms of stability) compared to the use of vesicle solutions.

The next set of photographs, shown in Figure 1.1b demonstrate *ion-sensing beads*. Here, nanoscale particles of a synthetic clay (laponite) are localized in polymer beads. The laponite particles have a strong affinity for cationic species over anionic ones. This property is conferred to the beads, which can thus be used for sensing and separating a cationic dye from a solution (this can be done selectively from a mixture of cationic and anionic dyes as well). Here again, the use of beads is much more convenient than the direct use of laponite particles because the particles tend to aggregate in solution.

Finally, Figure 1.1c shows a chain of magnetic capsules, which together represent an *artificial earthworm*. This is an effort to create a biomimetic structure out of polymer capsules. Individual capsules, each filled with magnetic nanoparticles, are chemically fused together. The resulting "earthworm" can undergo guided motion through the use of a magnetic field. Note that the concentration of magnetic particles is low enough that we can easily match the density of the earthworm with its surrounding fluid. That is, the

earthworm floats in the fluid, but when required it can be made to “swim” to its final location. Such a design may have uses in terms of understanding the locomotion of small worms and other animals. The same design can be easily implemented with smaller structures, and such miniature earthworms could be useful in microfluidic devices or even in certain medical applications.

Chapter 2: BACKGROUND

In this chapter, we discuss basic aspects pertaining to the components of the hybrid capsules and beads to be studied in Chapter 3. The main components are the biopolymers, chitosan, gelatin, and alginate and the structures we will place inside the capsules/beads include vesicles, clay nanoparticles and magnetic nanoparticles. Thereafter, we provide a brief description of the formation of capsules and beads. We then briefly describe the techniques to be used in this study, specifically UV-Vis spectroscopy.

2.1. BIOPOLYMERS

Macromolecules of biological origin fall broadly under three classes: polypeptides or proteins; polynucleotides; and polysaccharides.[1] For the purposes of this study, we will focus on biopolymers that have the ability to render viscosity to water by forming entangled networks or gels. These include chitosan, gelatin, and alginate which are each discussed in more detail below. Other water-soluble polysaccharides of interest include xanthan gum, gellan gum, guar gum, locust bean gum and gum arabic.

Chitosan

Chitosan is a linear polysaccharide obtained by the deacetylation of chitin.[2] Chitin, in turn, is a natural polysaccharide that constitutes the hard exterior shell of insects and crustaceans. Among biological polymers, chitin is next only to cellulose in abundance. However, while chitin is insoluble in water, its deacetylated derivative, chitosan, is water-soluble under acidic conditions (pH < 6.5). Under these conditions, the

amine groups along the chitosan backbone are ionized and chitosan acts as a cationic polyelectrolyte. Note that chitosan is strictly a copolymer of mostly D-glucosamine (β-(1, 4)-2-deoxy-2-amino-D-glucopyranose) sugars and a small fraction of the N-acetyl-D-glucosamine (β- (1, 4)-2-deoxy-2-acetamido-D-glucopyranose) sugars from the parent chitin. The structures of these sugars are shown in Figure 2.1.

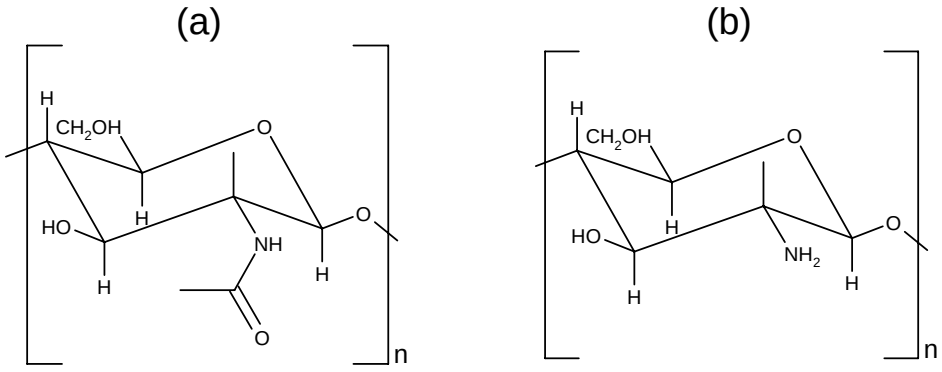

Figure 2.1. Structures of the parent sugars in (a) chitin and (b) chitosan. The N-acetyl-D-glucosamine sugar in chitin is deacetylated to give the D-glucosamine sugar in chitosan.

Gelatin

Gelatin is produced by partial hydrolysis of the protein, collagen, which is extracted from the bones and connective tissues of animals such as cattle, pigs and horses. When gelatin is added as a powder to water and heated, it forms a solution of moderate viscosity. This solution sets to form an elastic gel upon cooling. The same triple helical Gly-X-Y domains abundant in collagen fibers (typically X = proline and Y = hydroxyproline), also form the physical crosslinks in the gel.[3] The gel melts when heated above a characteristic temperature of 32°C and this melting transition is reversible. It should be noted that gelatin under ambient conditions is weakly anionic. At higher pH, however, gelatin is strongly anionic.

Alginate

Sodium alginate is linear, unbranched polysaccharide composed of 1,4-linked β-D-mannuronic (M) and α-L-guluronic (G) residues (Figure 2.2a). It is extracted from the cell walls of marine brown algae (Phaeophyta). In water, alginate chains can be ionically crosslinked by divalent cations (e.g. Ca^{2+}, Ba^{2+}, Sr^{2+}) to form a gel.[4] The crosslinking occurs via the exchange of monovalent Na^{+} ions on G residues with the divalent cations (Figure 2.2b). This is a co-operative process, with several divalent ions occupying cavities between adjacent chains (like eggs in an egg-box)[5] (Figure 2.2c). Note that alginate gels are irreversible and stable to heat.

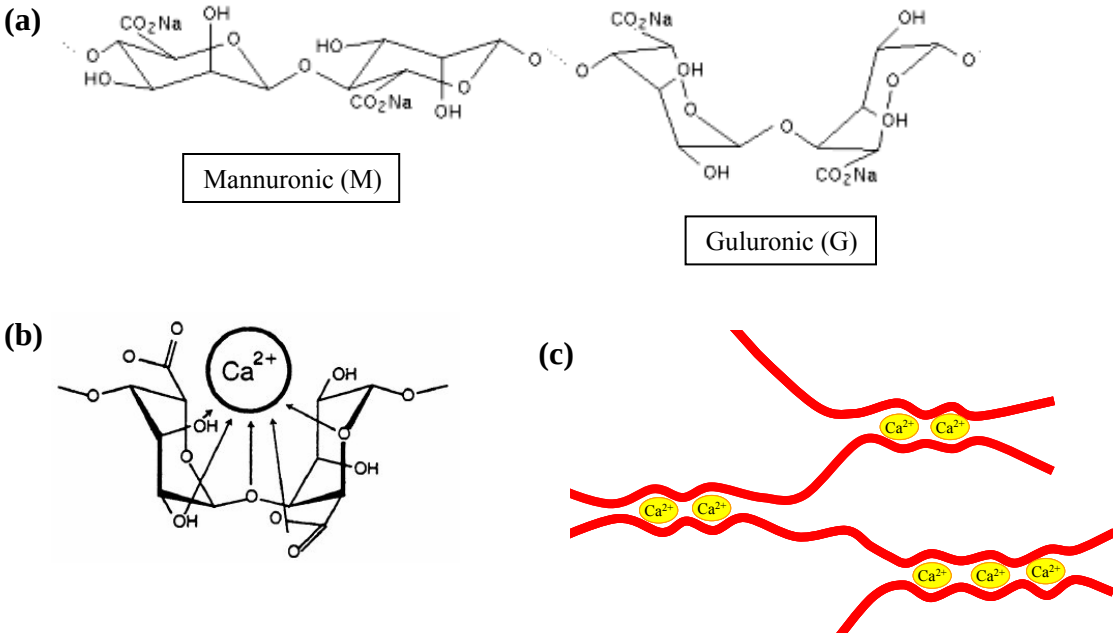

Figure 2.2. (a) Structure of β-D-mannuronic (M) and (b) α-L-guluronic (G) residues in a sodium alginate polymer. (b) The ionic coordination of G residues with divalent Ca^{2+} is indicated. (c) Structure of alginate-Ca^{2+} gels as per the "egg-box" model, which involves co-operative binding of the ions to adjacent chains.[11]

2.2. SURFACTANT STRUCTURES

Surfactants are water-soluble surface-active agents comprised of a hydrophobic portion, usually a long alkyl chain, attached to hydrophilic functional groups. When added to water, surfactants can form a variety of structures depending on their concentration and molecular geometry. These include micelles and vesicles, with the latter discussed in more detail below.

Vesicles

Vesicles are self-assembled capsules formed in water by surfactants or lipids.[6] The molecules that form vesicles are amphiphilic, with a hydrophilic head and hydrophobic tail(s). The shell of the vesicle is a bilayer (*ca*. 2-5 nm in thickness) of these amphiphilic molecules, with the hydrophilic heads on both sides of the bilayer and thereby exposed to water, while the hydrophobic tails inside the bilayer are shielded from water. A vesicle can be considered to form by the folding of amphiphilic bilayers. Vesicles with only a single bilayer (or lamella) are called unilamellar vesicles (ULVs), while vesicles with several concentric bilayers are called multilamellar vesicles (MLVs) and these are also referred to as "onions".

PCDA Vesicles

In this thesis, we are particularly interested in vesicles formed by diacetylenic surfactants, such as 10,12-pentacosadiynoic acid (PCDA). This surfactant has a single tail with a diacetylenic moiety in the middle (Figure 2.3a). When PCDA is added to water and mixed by sonication, it forms unilamellar vesicles (Figure 2.3b). The tails forming

the bilayer of PCDA vesicles can be polymerized by UV light at 254 nm.[7] The polymerization involves a 1,4-addition reaction and results in the bilayer being converted into a conjugated polymer with an alternating ene-yne sequence (Figure 2.3c). Polymerized PCDA vesicle solutions accordingly exhibit an intense blue color. When the vesicles are exposed to environmental perturbations, such as temperature, pH, or solvent, the solution changes color from blue to red, which is believed to be due to strain on the conjugated bilayers. This property has led to the use of PCDA-based vesicles and Langmuir-Blodgett films for sensing applications.[8]

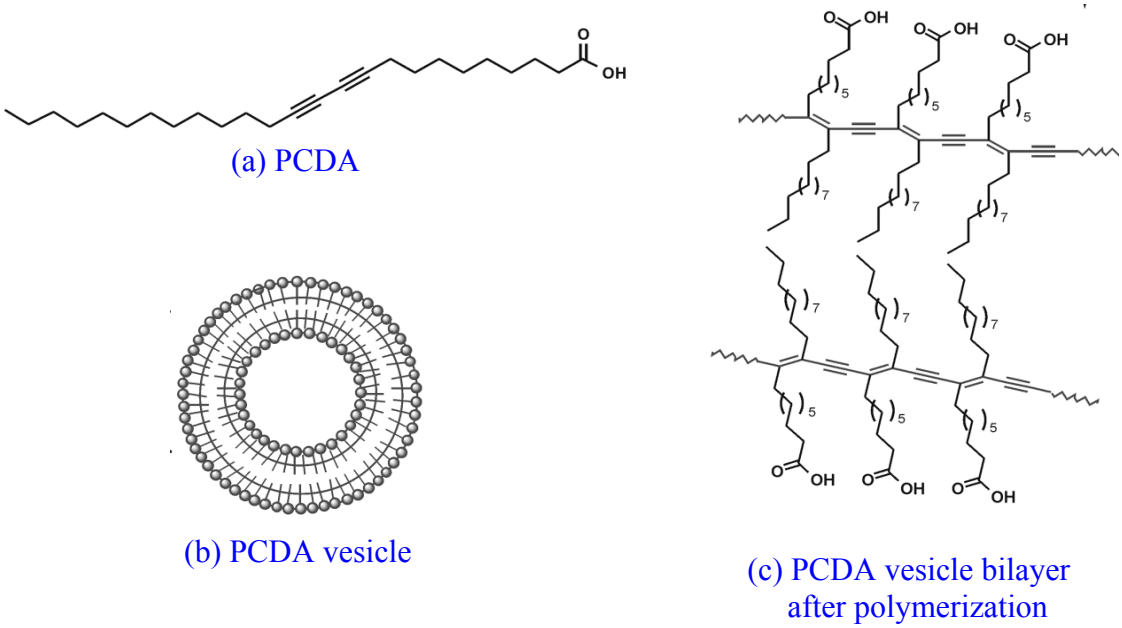

Figure 2.3. (a) Structure of the diacetylenic surfactant, PCDA. (b) Schematic of a unilamellar PCDA vesicle formed by dispersing PCDA in water. (c) Structure of the polymerized PCDA bilayer obtained by irradiating PCDA vesicle solutions with 254 nm UV light. Note that the tails in the bilayer become linked to each other in this process.

2.3. NANOPARTICLES

Nanoparticles are solid structures within the size range of 1-1000 nm, and can be based on organic polymers, metals or ceramics. For the purposes of this thesis, we will focus on clay (laponite) nanoparticles and superparamagnetic iron oxide nanoparticles, which are each discussed below.

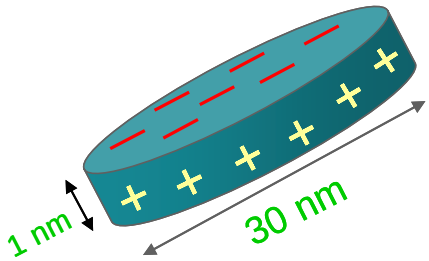

Figure 2.4. Schematic of a disk-like laponite nanoparticle bearing a strong negative charge on its faces.

Laponite

Laponite is a type of synthetic clay nanoparticle in the form of discs of diameter 30 nm and thickness 1 nm. The faces of the particles are negatively charged while the edges bear positive charge (Figure 2.4). Due to the negative charge on their faces, they have an affinity for cationic species – the cations are basically exchanged for the native sodium counterions. Accordingly, these nanoparticles have the ability to sense and separate cationic solutes from solution.[9]

Iron Oxide

Superparamagnetic iron oxide (SPIO) particles are composed of very small crystallites (1–10 nm), with both ferric iron (Fe^{3+}) and ferrous iron (Fe^{2+}) present. Superparamagnetism means that under a magnetic field, the entire particle aligns its

magnetic moment with the applied field. The effective magnetic moment of a material containing SPIO particles is thus very high. SPIO particles are usually coated with a protective layer of a hydrophilic material such as the polysaccharide, dextran so as to be stable in aqueous media. These particles find a variety of applications, for example as contrast agents for magnetic resonance imaging (MRI).[10-12]

2.4. POLYMER CAPSULES AND BEADS

Capsules and beads ranging in size from a few microns to several millimeters can be prepared both from biopolymers as well as synthetic polymers. A structure is referred to as a capsule if it has a thin shell enclosing a liquid-filled core. On the other hand, a bead is a more homogeneous material where there is no clear-cut distinction between a shell and a core.

Capsule Formation

The method we will use to form capsules involves *polyelectrolyte complexation*. This process requires two polymers of opposite charge, or one polymer and a surfactant of opposite charge. For example, one could use chitosan as the cationic polymer and sodium alginate as the anionic polymer. To form the capsules, the chitosan solution is added drop-wise to a bath containing a solution of alginate (or vice versa). As illustrated in Figure 2.5, contact between the oppositely charged polymers at the drop interface leads to electrostatic complexation, thus forming a polymer shell around the drop. In this way, capsules of given size (equal to the size of the generating drop) can be created by a simple, mild process (room temperature, no organic solvents). Smaller capsules can be

created by a modification to the above process, where instead of drop-wise addition, the chitosan is sprayed into the alginate as a fine mist – this yields microcapsules having a size around 10 to 100 microns. Alternately, microcapsules can also be used by creating smaller drops using different nozzle designs.

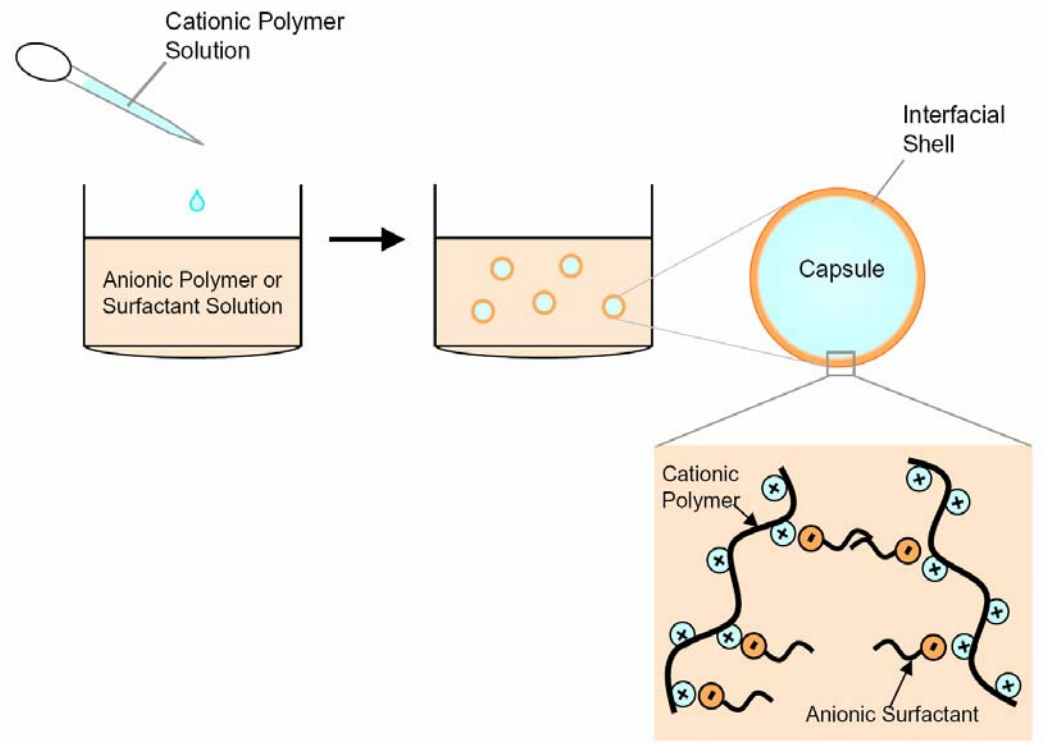

Figure 2.5. Creation of chitosan capsules by electrostatic complexation. A drop of chitosan is added to a solution of anionic biopolymer or surfactant. The resulting capsules have a shell consisting of chitosan complexed with the anionic moiety.

In terms of the materials composing the capsule shell, a limited number of choices exist for the cationic polymer. In addition to chitosan, the only other common cationic that finds frequent use is poly-L-lysine (PLL). On the other hand, a variety of choices exist for the anionic polymer – in addition to alginate, one could use gelatin (at high pH), gellan gum, xanthan gum etc. Also, as mentioned, one could form capsules using one polymer and an oppositely charged surfactant. In Chapter 3, we will form chitosan

capsules using the anionic surfactant, sodium dodecyl benzene sulfonate (SDBS). Some of the other variables that influence capsule structure and properties are discussed in Chapter 3 as well.

Bead Formation

Polymer beads are created by a method similar to the one above. As an example, beads of alginate are created by drop-wise addition of sodium alginate to a bath containing calcium salt (Figure 2.6). Ca^{2+} ions diffuse into the drop and crosslink the alginate chains, converting the liquid drop into a gel bead. Unlike capsules, beads are more homogeneous and do not have a distinct shell. However, depending on the Ca^{2+} concentration in the bath, the bead can still be somewhat inhomogeneous, with the crosslink density near the center of the bead being less than that near the periphery.

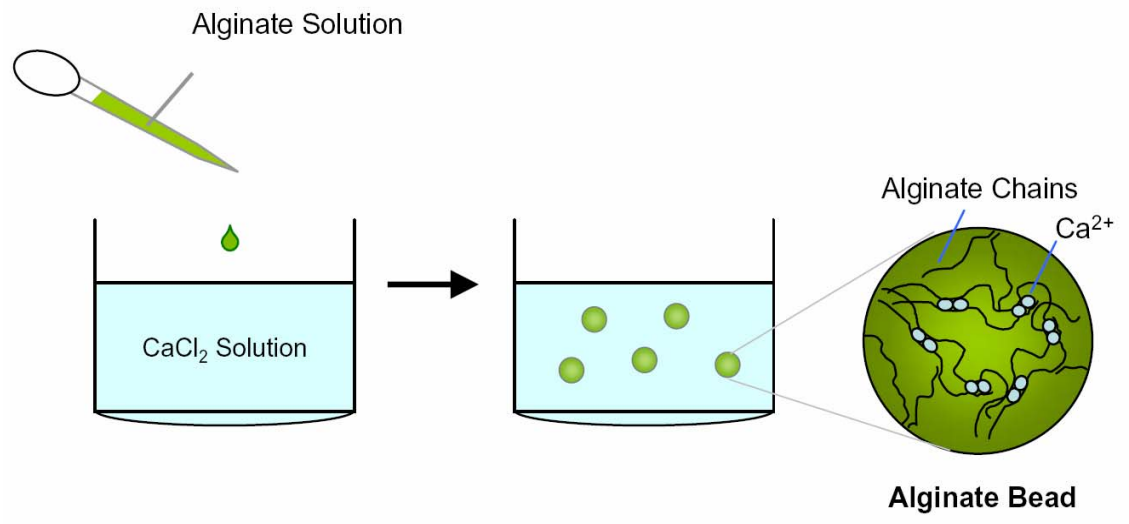

Figure 2.6. Creation of alginate beads by ionic crosslinking. A drop of alginate is added to a solution of $CaCl_2$. The ions diffused into the drop and crosslink the alginate chains to form a solid bead.

A notable point regarding the above methods is that they facilitate encapsulation or entrapment within the capsule/bead. Any chemicals or colloidal structures in the generating drop are generally preserved in the interior of capsules/beads, and examples include biological cells, vesicles, and nanoparticles. Note that encapsulation and capsule/bead formation are integrated within a single step. We will exploit this aspect to create hybrid capsules and beads having new functionalities.

2.5. UV-VIS SPECTROSCOPY

UV-Vis absorption spectroscopy is an analytical technique used to study molecules that adsorb radiation in the ultraviolet (200 to 400 nm) and visible (400 to 800 nm) regions of the electromagnetic spectrum. When a molecule absorbs radiation in the UV-Vis range, the absorbed energy generally moves electrons into higher energy levels. The molecule does not absorb energy continuously throughout the spectral range because the absorbed energy is quantized; that is, the molecule will absorb only at wavelengths that provide the exact amount of energy necessary to promote electrons to higher energy levels.[13] Each compound will thus have a unique UV-Vis spectrum. UV-Vis can thus serve as an analytical technique, especially for compounds that have an aromatic group.

A typical UV-Vis experiment is done with a solution of low solute concentration (10^{-5} to 10^{-2} M), which is then placed in a cuvette into the sample cell of a UV-Vis spectrometer. Light is broken down into its component wavelengths in the spectrometer and passed through the sample. The absorption intensity is measured for each wavelength and a UV-Vis spectrum (plot of absorbance vs. wavelength) is produced for the sample.

UV-Vis spectroscopy can be used as a *quantitative* analytical method to determine the concentration of the solute. This is done using the Beer-Lambert law:[33]

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

where A is the measured absorbance at a particular wavelength, c is the concentration of the solute in mol/L, ℓ is the path length of the sample, and ε is the molar extinction coefficient or molar absorptivity at that wavelength.

Chapter 3: CAPSULES: FUNDAMENTAL STUDIES & NEW CONCEPTS

3.1. INTRODUCTION

In Chapter 3, we study the microstructure and properties of biopolymer capsules/beads loaded with different materials like vesicles and nanoparticles. Following a brief study of some fundamental aspects pertaining to capsules, we will explore three new concepts, as mentioned in Chapter 1: (a) PCDA vesicle-loaded capsules having pH-responsive properties; (b) beads loaded with clay nanoparticles (laponite) and thus having ion-sensing capabilities; and (c) artificial earthworms, which are chains of magnetic particle-filled capsules that show motility under a magnetic field.

With regard to pH sensing, the simplest and most well known method is using litmus paper, which changes color based on the pH of a test solution. A variety of pH-responsive indicator dyes, polymer gels, and polymer blends also exist, and some of them have been integrated onto optical fibers or other devices to create electronic pH sensors. The concept we will explore in this study is to integrate pH-sensitive *vesicles* into capsules. The vesicles are formed from the diacetylenic surfactant, PCDA, which has been discussed in Chapter 2. Polymerized PCDA vesicles are known to undergo colorimetric transitions upon exposure to different pH, temperatures, and solvents.[14-17] Here, we will show that PCDA vesicles remain stable within the lumen of capsules, with the net result that the capsules now have the same colorimetric properties as the vesicles.

With regard to cation sensing and separation, a number of approaches have been investigated, especially for removal of metal cations from water. Alginate gels, beads, and fibers are actually known for their ability to sequester metal ions (such as Cu^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+} and Cr^{3+}) from solution. Likewise, clay particles are also known to bind cations strongly from solution.[18-22] Note that cation binding in both cases actually involves a process of ion exchange, where the cations of interest get bound to the alginate or clay while sodium counterions are released into the solution. Here, we investigate alginate beads containing a type of synthetic clay particles (laponite). We will show that the hybrid beads outperform conventional alginate beads quite significantly with regard to their cation sensing and separation capabilities.

With regard to artificial earthworms, the general goal is to create soft structures that are motile under an external field. For example, polymer gel strips have been set in motion either by vibrations, by an electric field, or by a magnetic field.[23-24] Polymer gels undergoing volume phase transitions in response to temperature or solvent quality have also been exploited in actuators and valves.[25-26] The new concept we explore here is to link a series of chitosan-gelatin capsules into a chain using glutaraldehyde. Each capsule in the chain has magnetic particles in its interior. The idea of putting superparamagnetic iron oxide (SPIO) particles inside capsules has been around since 1978 when albumin microcapsules containing magnetic particles as well as chemotherapeutic agents were used to deliver site-specific chemotherapy to tumors.[27] However, the connection of magnetic capsules into a chain has not been demonstrated thus far to our knowledge. The chains we create show motion guided motility under an external magnetic field.

3.2. EXPERIMENTAL SECTION

Capsule Materials: Polymers, Surfactants, Salts

Chitosan, gelatin, and sodium alginate were all obtained from Sigma-Aldrich. The chitosan was of medium molecular weight (190–310K), with a Brookfield viscosity of 286 cps and a degree of deacetylation *ca*. 80%. Chitosan is soluble only under acidic conditions, i.e., at a pH < 6.5, and we used 1% acetic acid to control the pH in chitosan solutions. The gelatin was of grade 300 bloom, while the sodium alginate had a Brookfield viscosity between 20-40 cps. The anionic surfactant, sodium dodecyl benzene sulfonate (hard type) was obtained from TCI America.

Nanoparticles, Dyes, and Other Chemicals

Laponite RD clay was obtained from Southern Clay Products. The particles are discs having an average diameter of 30 nm and a thickness of 1 nm. Dispersions of laponite in deionized water were prepared by vortex mixing. The samples had a basic pH of 9.8. The magnetic capsules (γ-Fe_2O_3) were purchased from Alfa Aesar. Their size was specified to be 32 ± 18 nm, and their average surface area was 42 m^2/g. The crosslinking agent, glutaraldehyde (grade I, 50%) was obtained from Sigma Aldrich, as were the cationic dye, methylene blue and the anionic dye 5(6)-carboxyfluorescein.

PCDA Vesicles and Their Polymerization

10, 12-Pentacosadiynoic acid (PCDA) was obtained from Sigma Aldrich. PCDA vesicles were created by dispersing the compound in deionized water, followed by sonication using a tip sonicator at 62°C for 60 min. The resulting solution was stored

overnight at 4°C. To polymerize the vesicles, the solution was irradiated at room temperature for 5 min using UV radiation at 254 nm from a low-pressure Oriel Hg pen light, with a light intensity of roughly 10^{-4} W/cm^2. Upon polymerization, the solution turned deep blue, and this was stored in a refrigerator until needed.

Dynamic Light Scattering

A Photocor-FC instrument with a 5 mW laser light source at 633 nm was used to analyze the size of vesicles. Studies were done at 25°C with the scattering angle being 90°. A logarithmic correlator was used to obtain the autocorrelation function, which was analyzed by the method of cumulants to yield a diffusion coefficient for the vesicles analyzed. The apparent hydrodynamic radius of the vesicles was obtained from the diffusion coefficient through the Stokes-Einstein relationship.

UV-Vis Spectroscopy

A Varian Cary 50 UV-Vis spectrophotometer was used to monitor the concentration of dyes in solution as well as the color transition of PCDA vesicles in response to change in pH.

Optical Microscopy

The Zeiss Axiovert 135 TV inverted microscope equipped with Motic Image Plus imaging system has been used for high-quality transmission microscopy. Capsules were imaged with a 2.5X objective.

3.3. Results and Discussion

3.3.1. Fundamental Studies with Capsules

In our initial studies, we focused on variables that influence the size, stability, and mechanical integrity of biopolymer capsules. These studies were done with chitosan-SDBS capsules, with the biopolymer chitosan being the cationic moiety and the anionic surfactant, SDBS being the anionic moiety. Capsules were prepared by adding drops of chitosan to a bath of SDBS. Variables include the concentration of chitosan, the concentration of SDBS, and the incubation time, i.e., the time that the drop stays in the SDBS solution. Other key variables are the molecular weight of the chitosan and the presence of hydrophobes on the chitosan backbone, but those were not explored here.

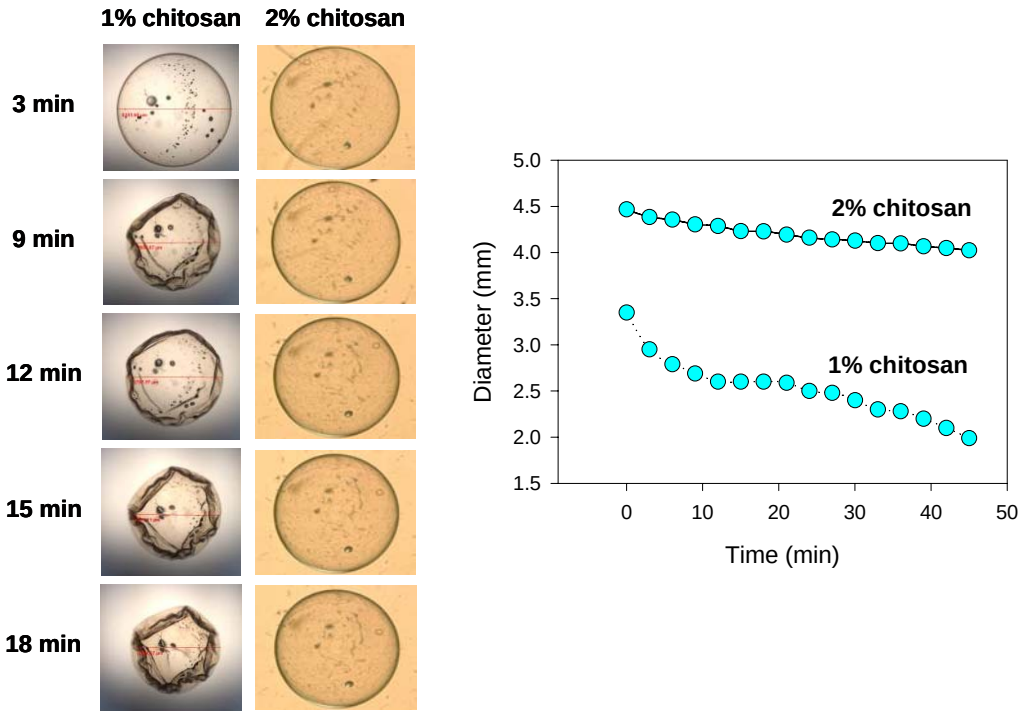

Figure 3.1. Typical images of chitosan-SDBS capsules at various incubation times. The capsule diameter extracted from these images is shown as a function of time on the plot.

First, the chitosan concentration was explored as a variable. Capsules were made with 0.5, 1, 1.5 and 2 wt% chitosan solutions, with the SDBS concentration in the receiving bath fixed at 5 wt%. In each case, capsules corresponding to various incubation times were examined by optical microscopy for their size and shape. We observe from Figure 3.1 that with increasing incubation time, the capsules shrink in overall diameter while their shell thickness increases. The shrinking with time is especially apparent at low chitosan concentrations. For 1 wt% chitosan, the capsule has a uniform spherical shape for incubation times around 3 min, but at higher times, the capsule shrivels, i.e., the shell of the capsule ceases to have a uniform structure. Note that if the incubation time is too low (< 3 min), the capsule does not have any mechanical integrity and cannot be picked up from solution – this is because the capsule shell is too thin.

When the chitosan concentration is increased to 2%, the capsules show a uniform spherical shape and shell structure even for much longer incubation times. There is only a slight decrease in capsule diameter and a modest increase in shell thickness over this period. Note also that the 2% chitosan capsules are larger than the 1% ones – this is because the drops were generated by a transfer pipette and the higher viscosity of the 2% chitosan gave rise to bigger drops. Indeed, there is a limitation with using too high a chitosan concentration (above 2 wt%) since the solution becomes very viscous and difficult to handle.

We then examined the effect of SDBS concentration over the range of 1 to 5 wt%, with the chitosan concentration fixed at 2 wt%. For low SDBS concentrations (less than

about 3 wt%), capsules are not formed – instead, addition of chitosan leads to a liquid-liquid phase separation. At higher SDBS concentrations from 3 to 4 wt%, capsules are formed, but they are not sturdy – they collapse when perturbed slightly by a spatula or by stirring. For 5 wt% SDBS, capsules with good mechanical integrity are formed. Taken together, our results suggest that an optimal composition for forming chitosan-SDBS capsules is by combining 1.5–2 wt% chitosan and 5 wt% SDBS, with an incubation time of about 3–4 min.

3.3.2. pH-Sensitive Capsules Loaded with PCDA Vesicles

The next set of studies are on capsules loaded with PCDA vesicles. For these studies, we used the chitosan-SDBS capsule formulation described above. The following procedure was adopted to prepare vesicle loaded capsules. First, polymerized PCDA vesicles were prepared at a concentration of 1 mM (~ 0.3 wt%), as described in the Experimental Section. Note that polymerization confers a deep blue color to PCDA vesicles, as seen by the photographs in Figure 3.2. Before polymerization, the sample has a light blue tinge, which is simply an indication of light scattering from vesicles (it is seen for all vesicles, not just PCDA). The deep blue color after polymerization, on the other hand, is due to the conjugated nature of polydiacetylenes. One further point is that the size of the vesicles, as characterized by DLS, remained about the same before and after polymerization (~ 90 nm diameter in both cases).

Once the PCDA vesicles were polymerized, we added 1.5 wt% chitosan to the sample. The solution became slightly viscous, but otherwise appeared to be stable and

homogeneous. This chitosan-PCDA solution was then added dropwise into a solution of 5 wt% SDBS. After an incubation time of about 3–4 min, the capsules were removed and placed in a solution of phosphate buffer at pH 5.

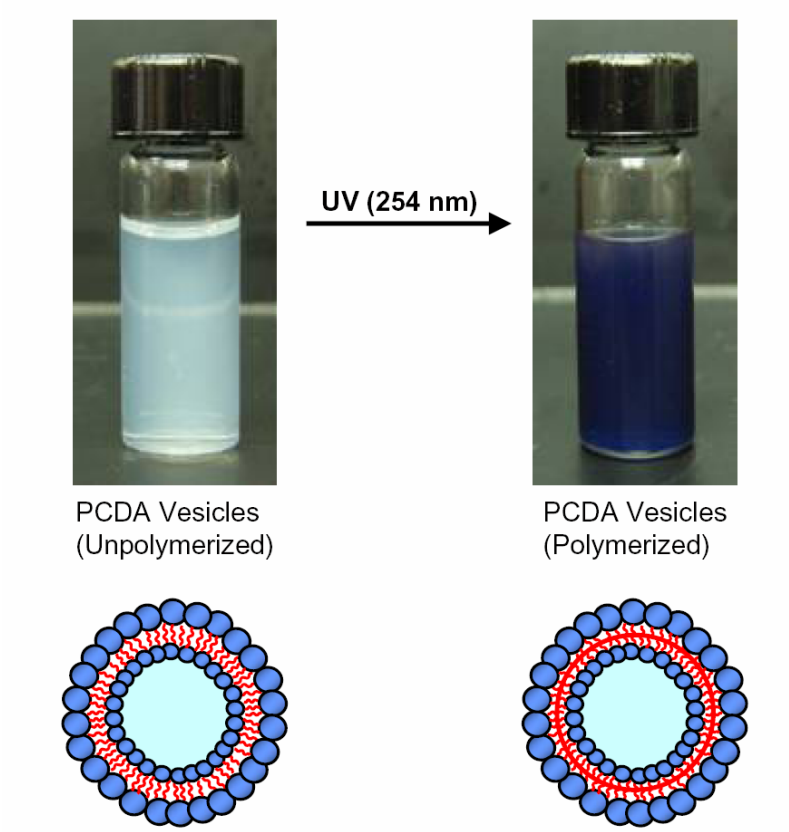

Figure 3.2. Photographs of a PCDA vesicle solution before and after UV polymerization of the vesicle bilayers. Upon polymerization, the solution turns a deep blue color.

For studies as a function of pH, it is useful to compare the results for PCDA vesicle-bearing capsules with those for PCDA vesicles. Towards this purpose, we added NaOH to each sample and monitored the results as a function of pH by visual observation and UV-Vis spectroscopy. As mentioned earlier, polymerized PCDA vesicles show an

irreversible colorimetric transition from blue to red with increasing pH. This can be seen clearly from the UV-Vis spectra (Figure 3.3) as well as the photographs in Figures 3.4. Figure 3.3 shows that, with increasing pH, there is a decrease in absorbance at 635 nm (blue) while that at 549 nm (red) gradually increases. The photographs in Figure 3.4 show the solution to be purple around pH 10 and a deep red at pH 11. For a pH of 12 or above, PCDA vesicles precipitate out of solution. The molecular basis for the colorimetric transition is that the higher pH causes a disordering of the polydiacetylene bilayers in PCDA vesicles (because hydrogen-bonds between the side chains are disrupted). Note that the transition is irreversible, presumably because the disordered state of the bilayers is thermodynamically more stable than the ordered state.

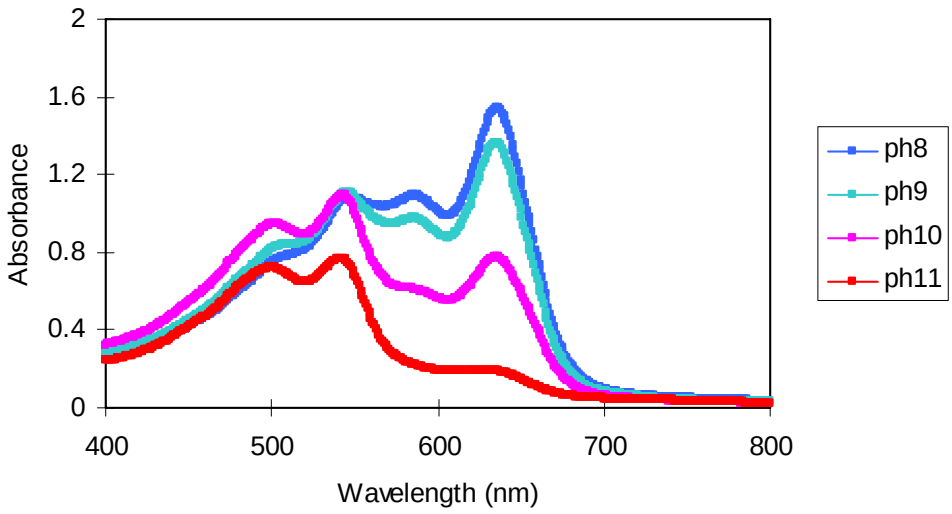

Figure 3.3. UV-Vis spectra of a PCDA vesicle solution at various solution pH. The data show a shift from blue to red peaks with increasing pH.

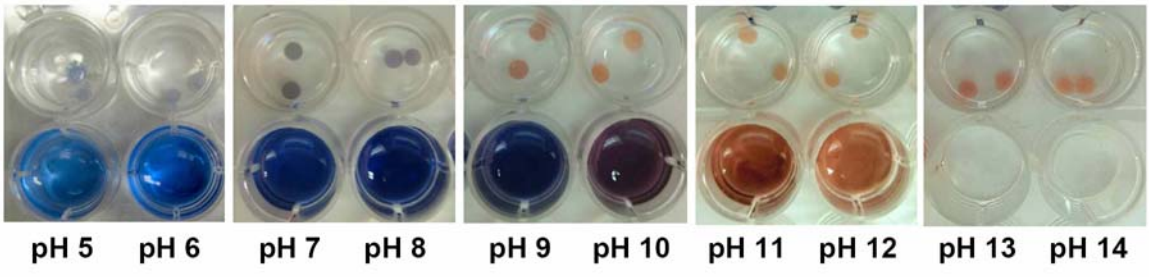

Figure 3.4. Colorimetric transitions as a function of pH in PCDA vesicle solutions (top) and PCDA vesicle-bearing capsules (bottom).

Figure 3.4 shows the effect of solution pH on chitosan capsules loaded with PCDA vesicles. The capsules have a blue color around pH 6, a purple color around pH 7, and a red color around pH 9. Thus a colorimetric transition with pH does occur for the capsules, i.e., the capsules are pH-responsive. Interestingly, the transitions are slightly shifted relative to those observed for the vesicle solutions: for example, the capsules are red at pH 9 whereas the solution turns red only at pH 11. The origin for these differences is yet to be deciphered, although it is probably related to interactions between chitosan and the PCDA vesicles.

Regarding the timescale of the colorimetric transition with the capsules, we noticed that, as soon as the pH of the solution was changed, a moving front corresponding to the new color developed inside the capsule. The entire capsule reached a uniform color within the span of a few minutes. The color change is thus quite rapid and is very reproducible. Another point worth mentioning is that the capsules can be responsive to pH even at very high pH values (> 12). Although PCDA vesicles precipitate out of solution at such high pH, it does not cause a problem when they are

localized within capsules. One can thus envision vesicle-loaded capsules as a miniaturized pH sensor. While our studies were done with millimeter-sized capsules, we expect the same results to hold for smaller capsules. Individual microcapsules bearing PCDA vesicles could be used as pH sensors in microfluidic devices.

3.3.3. ION-SENSITIVE CAPSULES LOADED WITH CLAY NANOPARTICLES

Next, we study alginate beads loaded with laponite (clay) nanoparticles. The affinity of these hybrid beads for cationic species in solution is the focus of our study. To make the hybrid beads, we combined a 1% alginate solution with 1% laponite and added the resulting mixture drop-wise to a 5 wt% solution of calcium chloride ($CaCl_2$). After incubation for about 5 min, the beads were removed and stored in deionized water. Also, as a control, we prepared alginate beads that did not contain laponite. Equal weights of the two sets of beads were then added to separate vials containing 1 mM solutions of methylene blue (MB), a cationic dye. The dye concentration in solution was monitored as a function of time by UV-Vis spectrometry.

The results are shown in Figure 3.5. We note that after a period of 24 h, the solution containing alginate-laponite beads is practically colorless, indicating that most of the dye has been absorbed by the beads. The hybrid beads are clearly more efficient at absorbing dye compared to the alginate beads. The differences between the two are quantified using UV-Vis spectrometry. While the dye concentration in the presence of alginate reaches a plateau in about an hour, the concentration continues to decrease in the presence of alginate-laponite. The concentration of dye after 24 h is 50% lower in the

case of alginate-laponite compared to alginate. It is clear that the superior separation is due to the adsorptive capability of laponite particles due to the strong negative charge on the particle faces. Note, however, that such separation could not have been achieved by simply adding laponite particles to the water, since the particles have a tendency to aggregate into a gel and/or precipitate in aqueous solution in the presence of cationic species. The laponite-bearing beads, in contrast, remain quite stable during the process.

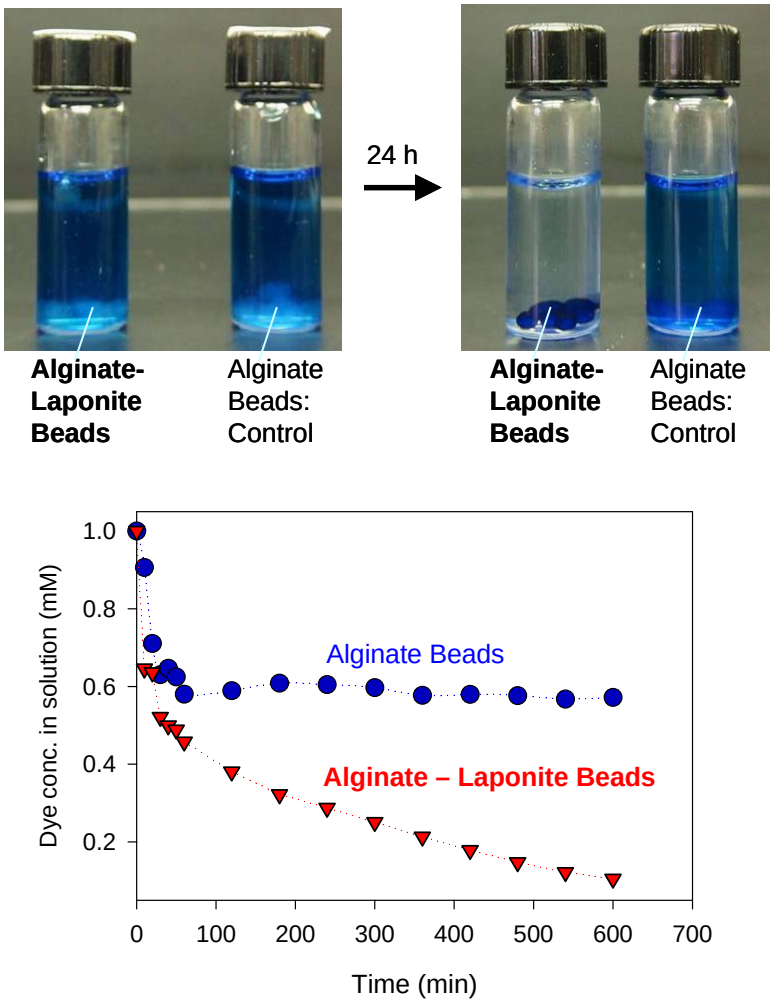

Figure 3.5. Binding of a cationic dye to alginate beads and alginate-laponite beads. Initially (photos on the left), the dye solutions are incubated with the same quantity of beads. After 24 h (photos on the right) the alginate-laponite beads are able to absorb much more of the dye from solution than the alginate beads. The visual results correlate with the UV-Vis data shown in the plot below.

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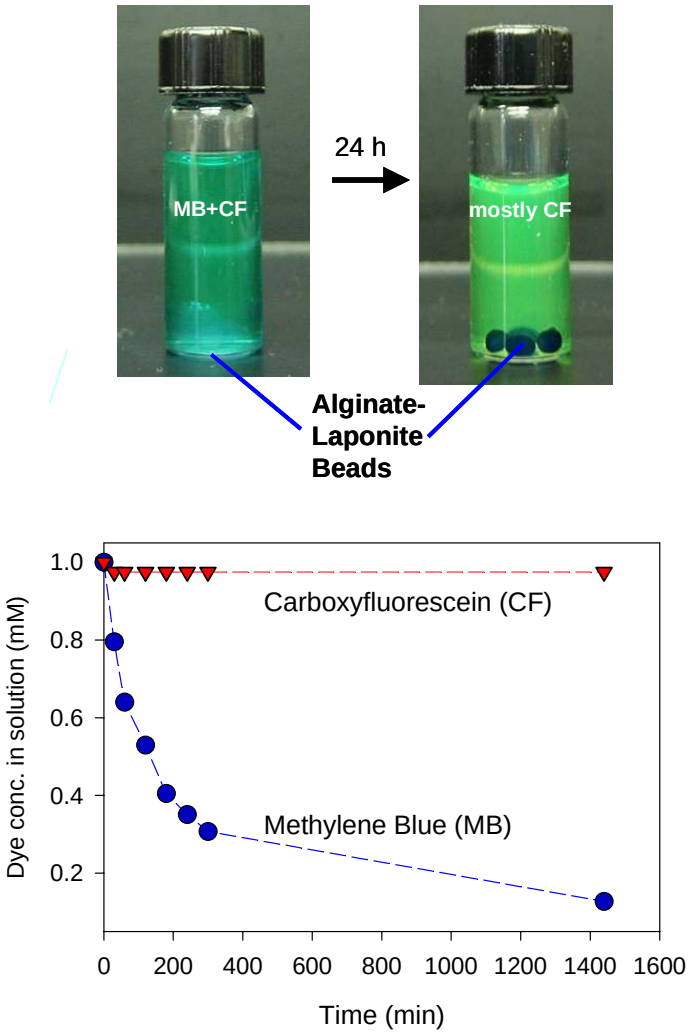

Figure 3.6. Ability of alginate-laponite beads to separate a cationic dye from a mixture of cationic and anionic dyes. Initially (left photo), the solution is blue-green due to the presence of MB and CF, whereas it turns yellow after 24 h (right photo), indicating that the MB has been selectively removed by the beads. The visual results again correlate with the UV-Vis data shown in the plot below.

We then conducted another experiment to test the suitability of alginate-laponite beads for cation separation. In this case, a blue cationic dye (MB) was mixed with a yellow anionic dye (carboxyfluorescein, CF), each at a concentration of 1 mM. This mixture was then combined with alginate-laponite beads and the results are shown in Figure 3.6. We find that the beads are able to selectively remove the MB from the

MB+CF mixture. The solution thus turns from a blue-green to a yellow color as time progresses. The UV-Vis results show that the CF concentration remains roughly constant whereas the MB concentration drops by two orders of magnitude over a period of 24 h. The results highlight the advantages of alginate-laponite beads in removing cationic species from water.

3.3.3. Artificial Earthworms Based On Magnetic Capsules

The last section of our work deals with the creation of "artificial earthworms". The building blocks for these are magnetic capsules, wherein we introduce magnetic ferrite nanoparticles into conventional capsules. Magnetic chitosan-SDBS capsules have been prepared as part of earlier work in our laboratory. For the present study, the capsule recipe was slightly modified: first, we made a mixture of 1 wt% chitosan and 0.5 wt% gelatin along with 0.25 wt% ferrite particles. This mixture was then added drop-wise to a 5 wt% SDBS solution, and after an incubation time of about 4 min the capsules were removed and placed into deionized water. The next crucial step was to link these individual spherical capsules to form a chain. For this, we added the above capsules to a vial containing 1 mM glutaraldehyde. Due to the higher density of the ferrite particles, the capsules settled to the bottom of the vial. At this point, the capsules were manually collected against the vial wall to form a chain. The glutaraldehyde serves to bond the capsules to each other, thus forming a chain. After about 14 h of incubation, the chain of capsules was removed for further study.

The key to chain formation is thus glutaraldehyde ($CHO-CH_2-CH_2-CH_2-CHO$), a dialdehyde that is known for its ability to form covalent bonds with amine groups. Glutaraldehyde thus acts as an efficient crosslinker for amine-containing polymers and proteins (it is used as a tissue fixative for this reason). Since amines are present both on chitosan and gelatin, glutaraldehyde can crosslink both polymers. Our studies, however, showed that capsule chains could not be formed using glutaraldehyde if we used chitosan alone or gelatin alone.[28-29] However, chitosan-gelatin capsules could be conveniently linked into chains by this method. It is known in the literature that chitosan-gelatin mixtures have improved mechanical properties over either of the parent polymers alone. Such a synergistic interaction between the two probably facilitiates formation of capsule chains. We should point out that the individual capsules also become more crosslinked during the chain formation process.

Each chain ("artificial earthworm") is thus composed of a series of semi-hard spheres, with the overall structure having reasonably good mechanical integrity and stability. Their only drawback is that the chains are slightly brittle, tending to break up into smaller units when subjected to mechanical stress. Chains can be stored in DI water or they can be made to float in a density-matched liquid, such as water containing sugar. The latter is an important property that may be useful in building biomimetic structures having the ability to swim or otherwise survive in water.

We have briefly studied the magnetic field-induced motility of our artificial earthworms using a simple, permanent magnet. These studies have been done both in

deionized water as well as the density-matched sugar solution (Figure 3.7). In both liquids, the earthworms usually align themselves perpendicular to the magnet. Further studies on these unique structures are under way in our laboratory. Future experiments will also examine new designs involving chains with alternating magnetic and non-magnetic capsules, or chains with blocks of each kind of capsule. Also, the motility of the chains in viscous and viscoelastic fluids will also be of interest.

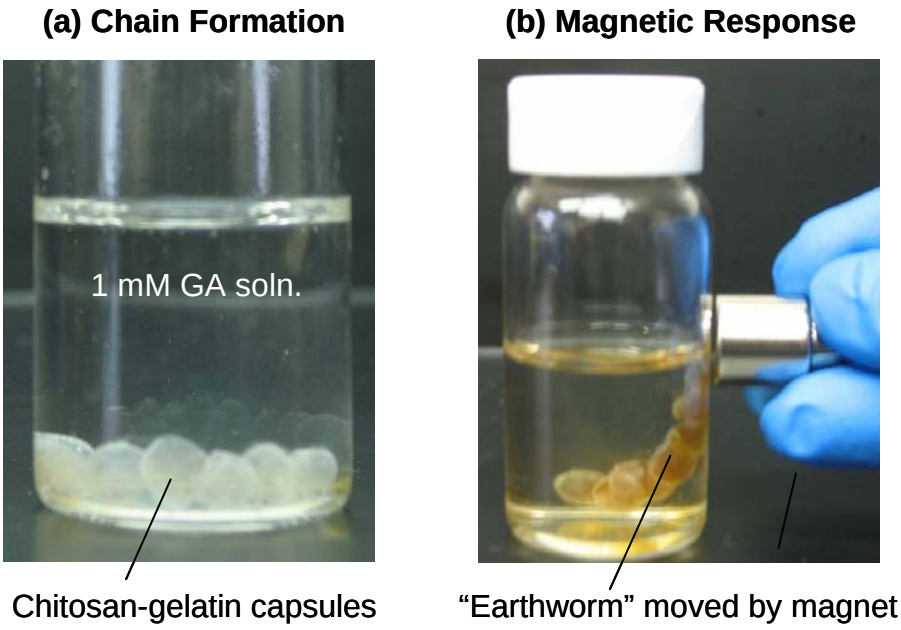

Figure 3.7. Magnetic earthworms in different geometry and solution (a) initially the capsules are colorless in the glutaraldehyde solution (b) after crosslinking for 12 hours they turn yellowish and the capsule chain can be moved by a magnet. Note that in (b), the chain has been placed in a sugar solution of matching density.

3.4. CONCLUSIONS

We have studied hybrid polymer capsules or beads, each encapsulating a different type of colloidal structure in their interior. The presence of the colloids imparts unique

properties to the capsules/beads. First, we studied capsules containing polymerized PCDA vesicles. We demonstrated the ability of the capsules to respond to pH changes by exhibiting chromatic transitions. Secondly, we demonstrated that alginate beads containing clay (laponite) nanoparticles can be used for the removal of cationic species from solution. Lastly, we have developed a unique *artificial earthworm* that can move in response to a magnetic field. The earthworm design was based on linking magnetic chitosan-gelatin capsules into a chain via glutaraldehyde. The biomimetic potential of this structure is immense: movement patterns can be varied based on the geometry and constiution of the earthworm, as well as medium in which it is placed.

Chapter 4: CONCLUSIONS & RECOMMENDATIONS

4.1. CONCLUSIONS

The common theme underlying this thesis is the development of new "*smart*" materials that have the ability to respond to physical or chemical stimuli. We have shown that such responsive capabilities can be imparted to polymer capsules and beads. The key is to encapsulate various colloidal or nano-structures within the interior of the capsule or bead. The fact that the capsule interior remains fluid permits such encapsulation. The properties of the encapsulated structures can thus be imparted to the capsule.

We studied three different concepts for multi-functional capsules. In the first case, capsules containing pH responsive PCDA vesicles were made. These capsules responded to a change in external pH of the solution by exhibiting a color change. The chromatic response of the capsules was in some ways better than that of PCDA vesicles due to increased stability to pH. Thus, the capsules could serve as pH sensors for pH values ranging from pH 5 up to pH 14. In the second study, we devised a new type of ion-sensing material by combining the unique properties of laponite clay nanoparticles with alginate beads. These hybrid beads were highly effective in selectively absorbing cationic species from an aqueous solution. In the final study, we developed novel *artificial earthworms* by linking chitosan-gelatin capsules into a chain with glutaraldehyde. We were able to study the magnetic field-induced movement of these chains in deionized water and in a density-matched sugar solution.

4.2 FUTURE DIRECTIONS

For these future studies, different types of perturbations like solvent change, temperature change can be induced to trigger by a chromatic change in the PCDA-chitosan microcapsules. *In vitro* and *in vivo* studies of vesicle loaded gels and capsules can be done in the future. Other possibilities for ion exchange resin studies include application of these composite systems to real- life waste water treatment experiments where the functionality of these beads can be tested. We can also carry out various studies on these beads to evaluate the absorption characteristics of divalent and monovalent cationic species. Further more one can study the recovery of these dyes from the alginate bead structure. Finally we will evaluate the targeting ability of magnetic *earthworm* capsules in vivo using MRI. Capsule surface may be modified in several manners to increase targeting capability. We also plan to study these earthworms based on hm-chitosan as opposed to chitosan there by possibly increasing the stability of the capsule chain.

References

[1] Uhrich, K.E., et al., Polymeric systems for controlled drug release. Chem. Rev. 1999, 99, 3181.

[2] Alberts, B., Molecular Biology of the Cell. 1994: Garland Publishing, New York.

[3] A.G Ward and A. Courts, The Science and Technology of Gelatin. 1977: New York: Academic Press

[4] Smidsrod, O.; Whitting.Sg; Haug, A. "Molecular Basis for Some Physical Properties of Polyuronides.", J. Phys. Chem., 1972, 26, 2563.

[5] Braccini, I.; Perez, S. "Molecular basis of Ca2+-induced gelation in alginates and pectins: The egg-box model revisited.", Biomacromolecules, 2001, 2, 1089-1096.

[6] Murthy, A. K.; Kaler, E. W.; Zasadzinski, J. A. N. "Spontaneous Vesicles from Aqueous-Solutions of Aerosol Ot and Choline Chloride Compounds.", J. Phys. Chem., 1991, 145, 598-600.

[7] Huo, Q.; Russell, K. C.; Leblanc, R. M. "Chromatic studies of a polymerizable diacetylene hydrogen bonding self-assembly: A "self-folding" process to explain the chromatic changes of polydiacetylenes.", Langmuir, 1999, 15, 3972-3980.

[8] Potisatityuenyong, A.; Tumcharern, G.; Dubas, S. T.; Sukwattanasinitt, M. "Layer-by-layer assembly of intact polydiacetylene vesicles with retained chromic properties.", Langmuir, 2006, 304, 45-51.

[9] Van, O.H., An Introduction to Clay Colloid Chemistry. 1963: Interscience, New York.

[10] Jayaraman, K.; Okamoto, K.; Son, S. J.; Luckett, C.; Gopalani, A. H.; Lee, S. B.; English, D. S. "Observing capillarity in hydrophobic silica nanotubes.", J. Am. Chem. Soc., 2005, 127, 17385-17392.

[11] Perez, J. M.; O'Loughin, T.; Simeone, F. J.; Weissleder, R.; Josephson, L. "DNA-based magnetic nanoparticle assembly acts as a magnetic relaxation nanoswitch allowing screening of DNA-cleaving agents.", J. Am. Chem. Soc., 2002, 124, 2856-2857.

[12] Vemury, S.; Pratsinis, S. E.; Kibbey, L. "Electrically controlled flame synthesis of nanophase TiO2, SiO2, and SnO2 powders.", Powder Technol. ,1997, 12, 1031-1042.

[13] C. Wagner, W. Riggs, L. Davis, and J. Moulder, in Handbook of x-ray photoelectron spectroscopy, edited by G.E. Muilenberg (Perkin Elmer Corporation, Eden Prairie, Minnesota, 1979).

[14] Peterson, J. I.; Goldstein, S. R.; Fitzgerald, R. V.; Buckhold, D. K. "Fiber Optic Ph Probe for Physiological Use.", Anal. Chem., 1980, 52, 864-869.

[15] Grant, S. A.; Bettencourt, K.; Krulevitch, P.; Hamilton, J.; Glass, R. "In vitro and in vivo measurements of fiber optic and electrochemical sensors to monitor brain tissue pH.", Sensors and Actuators B. Chemical, 2001, 72, 174-179.

[16] Grant, S. A.; Glass, R. S. "Sol-gel-based biosensor for use in stroke treatment.", Sensors and Actuators B. Chemical, 1999, 46, 1207-1211.

[17] Gerlach, G.; Guenther, M.; Suchaneck, G.; Sorber, J.; Arndt, K. F.; Richter, A. "Application of sensitive hydrogels in chemical and pH sensors.", Sensors and Actuators B. Chemical ,2004, 210, 403-410.

[18] Williams, C. J.; Aderhold, D.; Edyvean, R. G. J. "Comparison between biosorbents for the removal of metal ions from aqueous solutions.", Water Res., 1998, 32, 216-224.

[19] Dhakal, R. P.; Ghimire, K. N.; Inoue, K.; Yano, M.; Makino, K. "Acidic polysaccharide gels for selective adsorption of lead(II) ion.", Sep. Purif. Technol., 2005, 42, 219-225.

[20] Reddad, Z.; Gerente, C.; Andres, Y.; Le Cloirec, P. "Lead removal by a natural polysaccharide in membrane reactors.", Water Sci. Technol., 2004, 49, 163-170.

[21] Pandey, A. K.; Pandey, S. D.; Misra, V.; Devi, S. "Role of humic acid entrapped calcium alginate beads in removal of heavy metals.", J. Hazard. Mater, 2003, 98, 177-181.

[22] Ming, J.H. and J.G. Hering, "Arsenate sorption by Fe(III) doped alginate gels", Water Res. , 1998,32,1544–1552.

[23] Okuzaki, H.; Funasaka, K. "Electromechanical properties of a humido-sensitive conducting polymer film.", J. Biomater. Sci., 2000, 33, 8307-8311.

[24] Okuzaki, H.; Hattori, T.; Morikage, H.; Yamada, Y. "Electrically induced contraction of zone-drawn polypyrrole films.", J. Biomater. Sci., 2005, 153, 105-108.

[25] Liu, R. H.; Yu, Q.; Beebe, D. J. "Fabrication and characterization of hydrogel-based microvalves.", J Microelectromech. Syst, 2002, 11, 45-53.

[26] Kobayashi, K.; Suzuki, H. “A sampling mechanism employing the phase transition of a gel and its application to a micro analysis system imitating a mosquito.”, Sensors and Actuators B. Chemical, 2001, 80, 1-8.

[27] Widder, K. J.; Senyei, A. E. “Magnetic Microspheres - a Vehicle for Selective Targeting of Drugs.”, Sensors and Actuators B. Chemical, 1983, 20, 377-395.

[28] Vanessa L Gonsalves, Mauro C.M. Laranjeria, Valfredo T. Favere,” Effect of crosslinking agents on chitosan microspheres in controlled release of Diclofenac Sodium”, Polimeros: Ciencia e Tech.,2005.15: p. 6-12

[29] Yan Huang, Stella Onyeri, Mbonda Siewe, Aliakbar Moshfeghian and Sundararajan V. Madihally, “In vitro characterization of chitosan–gelatin scaffolds for tissue engineering”, Biomaterials, 2005, 26,7616-7627