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Interfacial Self-assembly of Sugar-based Amphiphiles: Solid- and Liquid-core Capsules

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Interfacial Self-assembly of Sugar-based Amphiphiles: Solid- and Liquid-core Capsules

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Abstract

Sugar-swollen reverse micelles and alternating polymer vesicles were prepared to examine interfacial self-assembly of both small and macromolecular sugar-based amphiphiles. Anhydrous, glassy sugar-sucrose laurate mixtures spontaneously dissolve in hydrocarbon oil at moderate temperatures and form sugar-swollen reverse micelles. The size of the micelles and the microemulsion viscosity depend on the mass ratio of sugar to surfactant. As sugar loading increases, the micelles increase in size and bulk viscosity decreases. Formation of sugar-oil complex fluids is related to the dynamics of the sugar in the supercooled liquid state, investigated by modulated differential scanning calorimetry.

Alternating polymers of N-alkylmaleimides and vinyl gluconamide spontaneously form ultra small (10 to 20 nm) and medium (50 to 300 nm) sized vesicles when dissolved in water at room temperature. These materials have molecular weights approximately one hundred times higher than alternating oligomers of alkylmaleate and vinyl ether monomers, and demonstrate conclusively that alternating copolymers can form vesicles. The size and shape of the vesicles are characterized thoroughly by cryogenic-transmission electron microscopy (cryo-TEM), dynamic light scattering (DLS), and small angle neutron scattering (SANS). The copolymer vesicles exhibit alkyl chain length dependent release characteristics and bilayer thickness (1.7, 2.0, and 2.6 nm for alkyl chains of 10, 12, and 14 carbons, respectively).

These nonionic alternating polymers contain no evident acidic or basic groups yet exhibit pH reversible self-assembly. The polymers form vesicles spontaneously in
neutral, deionized water, precipitate under acidic conditions, and re-dissolve into
optically clear, blue-tinted vesicular solutions upon neutralization of the turbid mixture.
Cryo-TEM and DLS confirm that the size and structure of the vesicles remain the same
after pH cycling. In the absence of ionic, acidic, and basic functionalities, the pH
reversible self-assembly of this alternating polymer is likely driven by coordinated
hydrogen bonding of protons within electron rich pockets of alternating vinyl
gluconamide groups. This is consistent with measured titration curves and shifts in the
infrared O-H stretch (3380 cm$^{-1}$) of the gluconamide hydroxyl groups following
acidification and neutralizing of the polymer in D$_2$O.
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<tr>
<td>$N_s$</td>
<td>Surfactant packing parameter</td>
</tr>
<tr>
<td>$H$</td>
<td>Mean curvature of surfactant film</td>
</tr>
<tr>
<td>$R$</td>
<td>Mass ratio of sugar to surfactant</td>
</tr>
<tr>
<td>$T_g$</td>
<td>Glass transition temperature</td>
</tr>
<tr>
<td>CMC</td>
<td>Critical micelle concentration</td>
</tr>
<tr>
<td>O/W</td>
<td>Oil in water microemulsion</td>
</tr>
<tr>
<td>W/O</td>
<td>Water in oil microemulsion</td>
</tr>
<tr>
<td>PIT</td>
<td>Phase inversion temperature</td>
</tr>
<tr>
<td>HLB</td>
<td>Hydrophilic-lipophilic balance</td>
</tr>
<tr>
<td>L595</td>
<td>Sucrose laurate, HLB 5</td>
</tr>
<tr>
<td>L1695</td>
<td>Sucrose laurate, HLB 16</td>
</tr>
<tr>
<td>MI</td>
<td>Maleimide</td>
</tr>
<tr>
<td>VG</td>
<td>Vinyl gluconamide</td>
</tr>
<tr>
<td>C10</td>
<td>$N$-$n$-decylmaleimide-co-vinyl gluconamide</td>
</tr>
<tr>
<td>C12</td>
<td>$N$-$n$-dodecylmaleimide-co-vinyl gluconamide</td>
</tr>
<tr>
<td>C14</td>
<td>$N$-$n$-tetradecylmaleimide-co-vinyl gluconamide</td>
</tr>
<tr>
<td>C10 25%M+</td>
<td>$N$-$n$-decylmaleimide-co-(75 mol% vinyl gluconamide, 25 mol% cationic vinyl ether)</td>
</tr>
<tr>
<td>DLS</td>
<td>Dynamic light scattering</td>
</tr>
<tr>
<td>Cryo-TEM</td>
<td>Cryogenic transmission electron microscopy</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>SANS</td>
<td>Small angle neutron scattering</td>
</tr>
<tr>
<td>SAXS</td>
<td>Small angle x-ray scattering</td>
</tr>
<tr>
<td>MDSC</td>
<td>Modulated differential scanning calorimetry</td>
</tr>
<tr>
<td>XPCS</td>
<td>X-ray photon correlation spectroscopy</td>
</tr>
<tr>
<td>ATR-FTIR</td>
<td>Attenuated total reflectance Fourier transform infrared spectroscopy</td>
</tr>
</tbody>
</table>
Chapter 1 Introduction

1.1 Research Significance

This dissertation is a compiling of three investigations that focus on interfacial self-assembly of sugar-based amphiphiles for solid- and liquid-core capsules and technologies. Sugar-swollen reverse micelles are discussed to highlight recent discoveries in the self-assembly of sugar-based surfactants and formation of sugar-oil complex fluids. Next, we discuss alternating copolymers that spontaneously form vesicles when dissolved in water at room temperature. These investigations have either been published or are in preparation for being published in peer-reviewed scientific journals.

Dating back to their initial founding as a science in the nineteenth century, colloids have been integral in the discovery of many important scientific phenomena, including Brownian motion and principles of diffusion,¹ and are under continual investigation by scientists and researchers for many interesting and exciting applications. Colloids exist in multiple combinations of the three phases of matter (solids, liquids, and gases) and have found important roles in food, pharmaceutical, and agriculture industries, to name a few. These systems may be composed of sols, macromolecules, or aggregates of small molecules and may take on a variety of structural morphologies both at the macro and molecular level.

Amphiphiles are molecules that have both water-loving and water-hating parts. Systems based on these materials have exceptionally interesting properties and features that are advantageous for applications where mixing two or more immiscible compounds is important. These molecules have the ability to self-assemble into many different
structures depending on concentration, temperature, and ionic strength of the dispersing medium. The domain sizes of self-assembled structures are important features which affect how the material or fluid may be implemented and range from a few to hundreds of nanometers, resulting in large internal surface area to volume ratios. Surface area is important for chemical reactions, especially for reactions between immiscible species where diffusion and molecular interaction is severely hindered by the thermodynamic instability of the two opposing surfaces. Thin films of amphiphiles bridge this hindrance by reducing the interfacial free energy of the two phases to a thermodynamic minimum.

In this dissertation, we focus on colloidal systems with thin films, either monolayers or bilayers, of self-assembled sugar-based amphiphiles. The interest in sugar-based complex fluids is motivated by the current environmental and industrial push for systems and processes employing renewable resources, such as sugars. Sugar-oil complex glasses and alternating polymer vesicles are relatively new discoveries and much remains to be understood about their properties and behavior. This dissertation attempts to provide further understanding of these interesting and promising materials through three experimental investigations.

1.2 Dissertation Outline

A literature survey of the fundamentals of amphiphilic self-assembly, important properties of surfactants, phase behavior and morphology, microemulsions, and amphiphilic macromolecules is presented in Chapter 2. This chapter covers recent reports of the self-assembly behavior of sugar-in-oil microemulsion glasses as well as the first known report of alternating polymers that spontaneously form vesicles in aqueous solution.
Chapter 3 details the experimental work done to investigate solid-core capsules obtained through microemulsification of sugar in oil, along with synthesis of amphiphilic alternating polymers that spontaneously form vesicle type liquid-core capsules and exhibit pH reversible self-assembly. Chemicals used, synthesis procedures, material characterization, structure elucidation, and other experimental techniques are all described in this chapter.

Results and thorough discussion of sugar-swollen reverse micelles are presented in Chapter 4, while Chapters 5 and 6 discuss characterization of the alternating polymer vesicle systems and the observed pH reversible self-assembly, respectively. Chapter 7 summarizes the general conclusions for each topic and provides an outlook on future investigations.
Chapter 2 Background

2.1 Amphiphiles

2.1.1 Surfactants and Self-assembly

Surfactants are amphiphilic molecules consisting of a polar head group coupled to a hydrophobic tail. Because of this polar contrast, these molecules rarely form ideal solutions in aqueous or hydrocarbon media, instead preferring to coalesce into aggregates or assemble in monolayers at the liquid-air interface. The polar head groups may be ionic or nonionic, and the hydrophobic tails may be saturated or partially unsaturated. In very dilute aqueous solution, these molecules are found at the liquid-air interface with their hydrophobic tails oriented toward the gaseous phase and very few individual molecules will be found in free solution. However, as more surfactant is added, the liquid-air interface becomes saturated and, in attempt to reduce the free energy of the system, the free molecules in solution self-assemble into aggregates, which may take on a variety of structures.

The simplest known aggregate structure is a micelle, shown in Fig. 2.1. The hydrocarbon tails sequester themselves inside the core of the structure while the polar head groups orient toward the bulk polar phase. The critical micelle concentration, CMC, is defined as the point where addition of more surfactant to a solution results only in the formation of new micelles. Dependent on temperature and unique for each surfactant, the CMC is the result of two competing forces.\(^1\) Migration of the hydrophobic chains into the aggregate core drives self-assembly while repulsion between head groups as they are
drawn close together opposes it. From this balance of forces, spherical micelles may only
grow to certain size. The packing of the tails in the oil-like core must remain
energetically favorable for the system to be stable. The radius of the micelle is then
limited by the number of carbon atoms in the alkyl tails and the addition of more
surfactant molecules does not increase the size of existent micelles; instead, new micelles
are formed.

Using NMR techniques, the hydrophobic core of micelles was found to have liquid-
like properties. The surfactant tails have mobility similar to the molecular mobility of
liquid hydrocarbons containing the same number of carbon atoms, shown in Fig. 2.2 in a
plot of characteristic time of rotation versus hydrocarbon chain length.²

As mentioned previously, a variety of structures may be formed when surfactants
aggregate in polar or nonpolar solvent. The energetically preferred structure depends on
many factors, including temperature, surfactant concentration, ionic strength of the
solvent, pH, and the chemical structure of the surfactant molecules. For ionic surfactants,
electrolyte concentration is decisive for aggregate structure and head group interactions,
whereas temperature plays that role for nonionic surfactants.³ All the aforementioned
properties may be conveniently quantified except the latter; numerical designation of
chemical structure requires a closer look. Amphiphilic molecules consist of a polar head
group coupled to one or more hydrophobic tails. Considering the micelle structure, the
size and shape of micelles are governed by the cross sectional areas of the head group and
the hydrophobic tail, which dictate molecular packing at the micelle surface and in the
core, respectively. The packing parameter $N_v$, defined in Equation 2.1, relates those
molecular parameters to the microstructure where $v$ is the volume, $l$ is the length of the
hydrophobic tail and $a_0$ is the effective area of the polar head group. Hence, $N_s$ is effectively the ratio of the hydrophobic to hydrophilic cross-sectional areas and the surfactant aggregates take on different structures as $N_s$ increases, summarized in Fig. 2.3. Conventional structures, having a small surfactant parameter, are designated as oil-in-water while inverse structures, having surfactant parameters above 1, are designated as water-in-oil.

In addition to the packing parameter, another tool for characterizing aggregate structure is the mean interfacial curvature of a surfactant film. The mean curvature $H$ is calculated by Equation 2.2 using the radii of two perpendicular planes, $R_1$ and $R_2$. Shown in Fig. 2.4, the radii may be both positive in the case of a sphere, cylinder, or bilayer, while for saddle-shaped surfaces, observed in cubic and bicontinuous structures, the radii are of opposite signs resulting in mean curvatures near zero. By convention then, the spontaneous curvature of a film is positive if it wraps around the hydrophobic part and is negative if it instead wraps around the polar part of the amphiphilic molecules. The mean curvature is related to the packing parameter by Equation 2.3 where $K$ is the Gaussian curvature parameter, defined in Equation 2.4.

$$N_s = \frac{v}{a_0 l}$$ (2.1)

$$H = \frac{1}{2} \left( \frac{1}{R_1} + \frac{1}{R_2} \right)$$ (2.2)

$$\frac{v}{l a_0} = 1 + Hl + \frac{Kl^2}{3}$$ (2.3)
Self-assembly occurs spontaneously as a result of the solvophobic effect, where a molecule or part of a molecule has very low solubility and prefers to aggregate rather than exist discretely in free solution a single molecule.\textsuperscript{1,5} This phenomena is attuned to the "like dissolves like" behavior commonly observed among organic materials. In an aqueous solution, the hydrocarbon tail of a surfactant molecule has very weak interactions with a liquid of high cohesive energy, thus preferring to cluster together and induce structure formation. In a bulk oil phase, it is the polar head groups that find themselves having weak interactions and, therefore, migrate toward the center of a cluster, pointing the hydrocarbon tails into the oil and forming an inverted structure.

2.1.2 Microemulsions

Emulsions constitute some of the most often used methods for mixing water and oil in food, agriculture, cosmetic, and pharmaceutical industries. These systems have the ability to mix insoluble materials with each other for numerous encapsulation and entrapment technologies. Emulsions are grouped into two classes based on thermodynamic stability, although both share similar structural features. Large droplets of one fluid in another are called macro emulsions and are thermodynamically unstable. Microemulsions are thermodynamically stable, isotropic dispersions of two immiscible phases separated by a monolayer of surfactant. While the name suggests otherwise, the domain sizes of microemulsions are actually sub-micron, often only a few nanometers in the case of oil swollen micelles in a continuous aqueous phase. Because the structure of

\[ K = \frac{1}{R_1 R_2} \] (2.4)
a microemulsion is dependent on the behavior of the self-assembled surfactants at the oil-water interface, microemulsion phase diagrams and structures have much resemblance to surfactant phase behavior in binary systems, as discussed in Section 2.1.1.

In the simplest case, microemulsions are spherical droplets of O/W (micelles) or W/O (reverse micelles) where the radius of the core is constrained by the curvature of the surfactant film surrounding the droplet and how much of the secondary phase is enclosed. For highly curved interfaces in an O/W system, the droplets have small radii and the volume fraction of oil not sequestered by the micelles exists in a second continuous phase. In the case of smaller mean curvatures, the micelles have larger radii and, therefore, greater emulsification capacities.

When the mean curvature is approximately zero, the surfactant film has no preferred curvature and the two immiscible phases are continually woven around each other in a sponge-like manner, termed bicontinuous. Both the polar and non-polar phases have equivalent roles in the complex fluid. Besides micelles, reverse micelles, and bicontinuous structures, other phases may also be present in microemulsions, such as hexagonal, lamellar, and cubic, but discussion of these is beyond the scope of this section.

Temperature plays a crucial role in determining the structure of nonionic microemulsions as it directly affects the curvature of the surfactant film. The phase inversion temperature, PIT, is defined as the temperature where a system transitions from a water continuous phase at low temperature to an oil continuous phase at higher temperature.\(^1,3\) At \(T = \text{PIT}\), the water and oil of the microemulsion exist in near equal parts, regardless of whether or not the system contains higher overall fractions water or oil. That is, the system may be wholly one phase bicontinuous or a three-phase mixture
of water, oil, and microemulsion. This phenomena is illustrated quite well in the fish cut of a phase diagram, shown in Fig. 2.5. At 5 wt% C12E5, the system is two phase at 40 °C, then transitions to three phase just below 45 °C, and finally becomes two phase again just above 50 °C. As surfactant loading increases, the temperatures where these transitions occur converge to a single point around 47 °C, which is designated as the PIT. Moreover, since the microemulsion takes on bicontinuous structure at equal loadings of oil and water at this temperature, the PIT can also be identified as the temperature where the spontaneous curvature equals zero.6,7

Double-chain surfactants have increased hydrophobic volume and exhibit remarkably different phase behavior than their single-chained analogs. Since the volume of the hydrophobic region of the molecule is doubled, the packing parameter is increased and the spontaneous curvature of interfacial films may be near zero or negative rather than positive. In ternary systems consisting of multi-tailed surfactants lamellar, bicontinuous, and inverted structures are more energetically favorable than conventional structures for a wide range of surfactant concentrations.1

2.1.3 Sugar-based Surfactants and Microemulsions

The polar head groups of sugar-based surfactants are often derived from naturally occurring compounds, including glucose, fructose, sucrose, and glycerol. Glucose is among the most commonly used sugars for synthesizing nonionic amphiphiles, being the source for alkyl polyglucosides. These surfactants have wide ranging applications and significant industrial production rates.8 Sorbitan, a derivative of glucose, also has been used extensively as an industrial material for nonionic surfactants.
Sucrose esters were first developed over 50 years ago and have been used since for a variety of different studies that have focused on their production and use in applications.\textsuperscript{9-15} Sucrose contains eight hydroxyl groups available for esterification, allowing a wide range of compounds with different amphiphilic properties based on the degree of substitution. Moreover, these surfactants self-assemble into various structures depending on the degree of substitution, the length of the alkyl chains, and surfactant concentration. Monoesters are very hydrophilic and readily form micelles in aqueous solution.\textsuperscript{16} Hydrophobicity is significantly increased in di- and tri-esters due to shielding of the polar head group. Octaesters are only soluble in oil and are commonly used as fat substitutes in food products.\textsuperscript{17}

Reverse micelle water-in-oil (W/O) microemulsions have been demonstrated using sucrose monoester surfactants.\textsuperscript{12-14} Reverse structures are not immediately apparent possibilities due to the large sucrose head group which yields a packing parameter much less than one for the mono-substituted ester. Addition of co-surfactant helps drive the spontaneous curvature of the surfactant film toward inverse structures and both initial and recent reports of sugar ester microemulsions indicate the addition of short to medium chained alcohols is necessary for generating reverse micelle W/O systems.\textsuperscript{12-15,18} However, systems of four chemically different species are unfavorable for practical applications because additional compounds increase cost and may participate in side reactions that could alter the physical properties of the microemulsion through compositional changes.

Nonionic sugar-based surfactants have been used recently to prepare sugar-in-oil microemulsions from concentrated aqueous sugar solutions.\textsuperscript{19-21} Mixtures of octyl and
dodecyl glucosides form bicontinuous microemulsions with 70-80 wt% solutions of equimolar sucrose and trehalose in oil. These complex fluids are optically clear and exhibit solid-like properties at room temperature despite containing over 50 vol% liquid oil, shown in Fig. 2.6. When the oil is a UV curable monomer, photo-initiated polymerization yields a sugar-polymer composite with identical structure and domain sizes of the original surfactant template. The solid sugar network resists thermodynamic rearrangement of the surfactant template during polymerization of the oil phase, which is commonly observed in reactions with surfactant templates. Washing away the rigid sugar matrix produces a flexible porous polymer membrane, shown in Fig. 2.7A and B. SEM images of the photopolymerized membrane after dissolution of the sugar, shown in Fig. 2.7C for varying oil loading in the original complex glasses, confirms the structure of the polymer material.

Monoesters of sucrose laurate and stearate form microemulsions with anhydrous mixtures of equimolar sucrose and trehalose in edible oil. Glasses are prepared by controlled drying of the aqueous sugar-surfactant solution and are readily dispersed in oil at elevated temperatures to yield optically clear, bicontinuous microemulsions containing equal amounts of sugar and oil, shown in Figure 2.8. DSC measurements, shown in Figure 2.9 as reversible heat capacity versus temperature, show glass transition temperatures, $T_g$, of these complex fluids vary with the amount of oil present (oil loading represented by $\alpha$) and are below the temperature required to form the microemulsion. However, no first order phase transitions (melting point) are observed in the heat flow versus temperature plot. This evidence suggests the sugar is in supercooled liquid state.
during microemulsification and the molecular mobility of this state facilitates rearrangement and self-assembly of the microstructure.
Figure 2.1 Spontaneous self-assembly of surfactants into micelles in aqueous solution.

The hydrocarbon tails gather within the core of the aggregate while the polar head groups orient toward the bulk phase.\textsuperscript{22}
Figure 2.2 Characteristic time of rotation, $\tau$, versus hydrocarbon chain length of various surfactants. The neat hydrocarbon oil is shown by the dotted line, indicating similar molecular mobility between the hydrophobic core of a micelle and the pure hydrocarbon.$^2$
Figure 2.3 Surfactant parameters and preferred self-assembled structures. Conventional structures, i.e. micelles, are termed oil-in-water while inverted structures are termed water-in-oil.3
Figure 2.4 Radii of curvature, $R_1$ and $R_2$, for a simple surface\textsuperscript{23} (top) and a saddle-shaped surface\textsuperscript{1} (bottom). Per convention, both radii of the simple surface are positive while in the saddle-shaped case one is positive and the other is negative.
Figure 2.5 The fish cut of a phase diagram showing phase behavior for temperature versus nonionic surfactant loading in a sample with equal amounts of oil (O) and water (W). μE and L_α designate microemulsion and lamellar phases, respectively. The phase inversion temperature (PIT) occurs at the mirror pane of the fish shape.¹
Figure 2.6 Optically clear and brittle microemulsion glass containing equal masses of sugar and liquid isobutylacrylate oil.\textsuperscript{19}
Figure 2.7 Images of sugar-oil microemulsion glass before polymerization (A) and after
the sugar has been washed away post-polymerization, yielding a flexible polymer
membrane (B). SEM images of the bicontinuous network for varying oil loading, $\alpha$.
(C).
Figure 2.8 Spontaneous dissolution of glassy sugar-surfactant powder into a bicontinuous microemulsion at 92 °C. The complex fluid cools to a hard solid at room temperature.²⁴
Figure 2.9 DSC scans of sugar glass for varying oil loading, $\alpha$.\textsuperscript{24}
2.2 Macromolecular Amphiphiles

2.2.1 Surface Active Polymers

Amphiphilic block copolymers are a continually developing field of colloid science due the vast array of starting materials available for polymerization and the interesting collection of structures that are possible, analogous to well known surfactant phase behavior discussed in Section 2.1. Amphiphilic macromolecules are readily found in nature such as lipopolysaccharides, which have hydrophobic side chains tethered to a hydrophilic backbone, and glycoproteins, which have hydrophilic side chains tethered to hydrophobic backbones. These materials serve important physiochemical roles within organisms and have inspired researchers to investigate the amphiphilic features and phase behavior of synthetic polymers.

Like surfactants, self-assembly of block polymers is driven by the solvophobic effect. Considering an aqueous system, polymer chains of poly(ethylene oxide) (PEO) or poly(acrylic acid) (PAA) are very hydrophilic and dissolve readily whereas hydrophobic polymers such as poly(butadiene) (PB), poly(styrene) (PS), or poly(propylene oxide) (PPO) are essentially insoluble. However, when blocks of these polymers are joined end-to-end through covalent bonds, the resulting macromolecule has amphiphilic properties and exhibits self-assembly and aggregation phenomena. Indeed, structures across the entire phase diagram have been observed with such materials as Pluronic P105 tri-block polymer surfactants, shown in Fig. 2.10. Block copolymers have also demonstrated microemulsion capabilities within wholly polymer systems.
Referring to the discussion in Section 2.1.1, the packing parameter isn't a useful tool in predicting structure for these polymeric materials because the dynamics involved with very long molecules are quite different from their short chain surfactant analogs. For surfactants, chains of 10-16 carbon atoms may be assumed to be fully extended when part of an aggregate, at least for the purpose of predicting the structure by the packing parameter. However, chain bending, coiling, and self-aggregation of high molecular weight chains make predicting the structure much more difficult and experimental investigation is usually the only reliable method to determine the phase behavior of amphiphilic polymers.

### 2.2.2 Polymersomes

Due to their ease of preparation and encapsulation capabilities, vesicles are among the more interesting and applicable structures for amphiphilic polymers and have been under investigation for just over a decade. In the first reports of these materials, di-block copolymers of polyethyleneoxide-co-polyethylethylene (PEO-co-PEE) were synthesized and found to form vesicles with thick, tough bilayer walls.\(^{25}\) In polymer vesicles, dubbed polymersomes, the molecules are oriented in parallel with the hydrophilic blocks extending into the polar phase and the hydrophobic blocks pointing to the inside of the bilayer, as diagramed in Fig. 2.11. This occurs because amphiphilicity is aligned vertically along the polymer backbone and it is energetically favorable for the insoluble polymer blocks to sequester themselves from the polar environment.

Due to the orientation of the polymer blocks, the thick bilayer is robust compared to lipid and surfactant vesicles and is a key feature which gives these polymersomes advantages in various applications.\(^{28}\) Polymersomes may be on the order of microns or
less than a hundred nanometers in diameter, and may be unilamellar, multilamellar, or a mixture of both. Since the first report of polymersomes described above, many new block copolymers have been developed for making self-assembled structures including synthetic peptides and materials sensitive to changing pH and temperature.

2.2.3 Alternating Polymer Vesicles

Alternating polymers are a new class of self-assembling material. As discussed in Sections 2.2.1 and 2.2.2, amphiphilicity of block polymers is aligned in parallel to the polymer backbone with the hydrophobic block in tandem with the hydrophilic block. One arrangement for alternating polymers to exhibit amphiphilicity is to have polarity perpendicular to the backbone. That is, an alternating sequence of hydrophilic and hydrophobic monomers along the polymer backbone creates amphiphilicity that mimics a series of aligned surfactant molecules covalently linked together at the junction of the polar and non-polar groups.

A recent report describes such polymer systems using sugar-based vinyl gluconamide linked to alkyl maleates through alternating free radical polymerization. These polymers spontaneously form vesicles when dissolved in water at moderate temperatures. Cryo-TEM images, shown in Fig. 2.13, confirm vesicular structure and reveal that the vesicles have a bimodal distribution and very thin, flexible bilayer shells. Vesicles dissolved in water by mechanical stirring range from 50 nm to over 300 nm in diameter (Fig. 2.13A). High shear energy, via horn sonication, reduces them to much smaller sizes, many having diameters as small as 10 nm (Fig. 2.13C). DLS measurements of the sonicated vesicles confirm bimodal distributions of ultra-small (10-20 nm) and medium-
sized (50-300 nm) vesicles, shown in Fig. 2.14 in comparison with distributions obtained from cryo-TEM images.

Small angle neutron scattering (SANS), which probes lengths scales beyond the lower limits of TEM resolution, was used to determine the thickness of the vesicle bilayers. The scattering spectra are shown in Fig. 2.15 for polymer vesicles in D$_2$O. An analytical model using core-shell form factors and bimodal, polydisperse distributions was developed and fitted to the data, shown as solid lines in the figure. For each of the polymers investigated, the bilayer thickness was calculated to be ~1.5 nm, which is comparable to lipid and surfactant bilayer thickness and strongly supports the presumption that the polymer chains are oriented horizontally with the hydrophilic groups extended into the polar phase and the alkyl tails pointing toward the interior of the bilayer.

Alternating polymer vesicles with bilayer thicknesses of ~1.5 nm exhibit rapid release of encapsulated rhodamine B dye, shown in Fig. 2.16 for polymers containing butyl, hexyl, and octyl maleate groups. Such release characteristics are comparable to observed release rates from lipid vesicles$^{35-37}$ but much faster than release from polymersomes made from block copolymers.$^{38,39}$
Figure 2.10 Phase diagram of concentration vs. temperature for Pluronic P105 triblock polymer surfactant in water. L₁, I, E, D, L₂, and P designate micellar, cubic, hexagonal, lamellar, isotropic polymer-rich, and paste-like phases, respectively.
Figure 2.11 Schematic of vesicle bilayer organization for various block copolymers (b-d) in comparison to phosphatidylcholine, a common vesicle forming lipid (a). The polymer chains orient themselves so the hydrophilic chains (blue) extend into the polar phase and the hydrophobic chains (gray) are contained within the interior of the bilayer.\textsuperscript{28}
Figure 2.12 Cryo-TEM images of block polymer vesicles (PDMS$_{10}$-block-PEO$_{12}$) in dilute solution showing large unilamellar vesicles in coexistence with small multilamellar vesicles (left) and in concentrated solution showing the evolution of lamellar structures.\textsuperscript{29}
Figure 2.13 Cryo-TEM images of vinyl gluconamide-co-alkyl maleate alternating polymer vesicles before (A) and after (B) sonication. Images C and D exemplify the ultra-small vesicles.\textsuperscript{34}
Figure 2.14 Size distributions of calculated from cryo-TEM and DLS measurements for alkylmaleate-co-vinyl gluconamide alternating polymer vesicles.\textsuperscript{34}
Figure 2.15 Small angle neutron scattering data for alkylmaleate-co-vinyl gluconamide alternating polymer vesicles, 1 wt% in D$_2$O. Solid lines are model calculations using two Shultz distributions of polydisperse spheres and a core-shell form factor.$^{34}$
Figure 2.16 Release profiles of rhodamine dye from vesicles with varying alkyl chain length.\textsuperscript{34}
Chapter 3 Experimental

3.1 Introduction

This chapter details the experimental work up of the solid- and liquid-core capsules examined in this dissertation. First, the preparation and characterization of sugar-swollen reverse micelles in decane are described, followed by a detailed account of synthesis, characterization, and application of vesicle-forming alternating polymers. These polymers also exhibit responses to changing pH and relevant experimental techniques for probing this behavior are presented.

3.2 Sugar-swollen Reverse Micelles

3.2.1 Introduction

This section describes reverse micelle microemulsions of sugar in oil using food grade sucrose laurate surfactant. Sample preparation and experimental techniques used to characterize the phase behavior, size distributions, and dynamics of the sugar cores are discussed therein.

3.2.1 Materials

L-595 sucrose laurate ester, HLB 5, was received from Mitsubishi Kagaku Corp (Japan). All other chemicals and reagents were purchased from Fisher Scientific or Sigma-Aldrich and used as received. Deionized water with specific resistance of 18.2 MΩ cm was used to prepare all samples.
3.2.2 Phase Diagram Determination

Precursor sugar-surfactant glasses were prepared by mixing aqueous sugar solution with surfactant powder at 80 °C followed by pre-drying under an Ar gas stream and careful vacuum drying through controlled foaming of the hot viscous solution. The mixtures were dried to >99.5% dryness, typically taking 1 to 2 days at 30-40 °C. Absolute dryness requires elevated temperatures and longer drying periods, increasing the risk of sugar crystallization. Once dry, the sugar-surfactant glasses were crushed into powder and mixed with decane at 70 °C for 5 minutes by magnetic stirring. Samples prepared using 50/50 mixtures of glucose/fructose were stirred in decane at 60 °C. Temperatures of 80-85 °C were needed for samples containing <40 wt% oil to overcome mixing difficulties of the highly viscous mixtures. Samples were cooled and equilibrated at room temperature for at least 24 h, after which phase behavior was recorded.

3.2.3 Dynamic Light Scattering

Size distributions of the reverse micelles were measured by dynamic light scattering (DLS) on an ALV/LSE-5003 CGS-3 instrument at 90° angle using a HeNe laser. Intensity autocorrelations were acquired by ALV Correlator Ver. 3.0A and analyzed by CONTIN for number weighted distributions.

3.2.4 Modulated Differential Scanning Calorimetry

Modulated differential scanning calorimetry measurements were recorded using a TA Instruments Q100 calorimeter with modulation amplitude, period, and heating rate of ±1 °C, 60 s, and 2 °C min⁻¹, respectively. Glass transition temperatures were determined by the mid-point of the step-wise increase in the reversible heat capacity.
3.3 Alternating Polymer Vesicles

3.3.1 Introduction

Alternating polymers were synthesized through the electron donor-acceptor mechanism of maleimide-vinyl ether free radical polymerization. These materials spontaneously form vesicles when dissolved in water and this section describes the experimental procedures used to synthesize and characterize the polymers and also the techniques used to investigate and confirm vesicle structure and possible applications.

3.3.2 Materials

All chemicals and solvents were purchased from either Sigma-Aldrich or Acros Organics/Fisher Scientific and used without further purification. Reactions were performed under argon atmosphere in oven-dried glassware. Acetylene gas (99.6%) was obtained from Wright Brothers, Inc. Deionized water (Milli-Q, 18.2 MΩ cm) was used to prepare all samples and deuterium oxide was purchased from Cambridge Isotope Laboratories, Inc.

3.3.3 Synthesis

2-(Vinyloxy)ethylamine (I) was synthesized by addition of ethanolamine to acetylene gas. A three-neck round bottom flask was fitted with a jacketed short path distillation unit and charged with ethanolamine (40 g) and sodium hydroxide (2 g). The system was purged with argon for 1 hour and heated to 140 °C, upon which sodium hydroxide dissolved and the solution became clear. Acetylene gas was bubbled through the liquid with rapid stirring and the crude product was collected via simultaneous distillation over 19 hours and distilled twice under reduced pressure to yield (I) (10.7 g, 19%). $^1$H NMR
(D$_2$O, 250 MHz) δ 2.79 (t, $J$=5.3 Hz, 2H), 3.72 (t, $J$=5.3 Hz, 2H), 4.06 (d, $J$=6.6 Hz, 1H), 4.27 (d, $J$=14.4 Hz, 1H), 6.45 (dd, $J$=14.4, 6.6 Hz, 1H); HRMS (ESI, +) Calcd for C$_4$H$_{10}$NO (M+1): 88.0757. Found: 88.0757.

2,3,4,5,6-Pentahydroxy-N-[2-(vinyloxy)ethyl]hexanamide (vinyl gluconamide, VG) (2) was synthesized from δ-gluconolactone and (1) as previously described. Briefly, (1) (8 mL, 83.4 mmol) was added to a suspension of δ-gluconolactone (13.5 g, 75.8 mmol) in methanol (50 mL) and refluxed for one hour. Upon cooling, a white solid precipitated, was collected by suction filtration, and recrystallized from methanol to give (2) (10.5 g, 51%).

$N$-$n$-alkylmaleimides (4a-c) were synthesized by ring opening addition to maleic anhydride followed by ring closing amide condensation. In a 50 mL three-neck round bottom flask fitted with a condenser, maleic anhydride (4.59 g, 46.9 mmol) was suspended in 10 mL anhydrous dichloromethane. A suspension of $n$-tetradecylamine (10.0 g, 46.9 mmol) in 20 mL anhydrous dichloromethane was added incrementally and the reaction mixture was refluxed for 30 min where it partially solidified. The mixture was chilled and $N$-$n$-tetradecylmaleamic acid (3c) was collected by suction filtration, washed with cold dichloromethane, and dried (13.3 g, 91%). $^1$H NMR (CDCl$_3$, 250 MHz) δ 0.90 (t, $J$=6.6, 3H) 1.28 (m, 22H) 1.60 (app quintet, $J$=6.8, 2H) 3.38 (q, $J$=6.8, 2H) 6.28 (d, $J$=12.9, 1H) 6.36 (d, $J$=12.9, 1H) 7.10 (br s, 1H); HRMS (ESI, +) Calcd for (M+1): 312.2539. Found: 312.2531.

The maleamic acid product (10 g, 32 mmol) was added to a 100 mL round bottom flask along with sodium acetate (1.8 g, 22 mmol) and 20 mL acetic anhydride. The mixture was heated to 100°C for 2 h. After cooling, 50 mL water was added and the
contents were transferred to a separation funnel where the product was extracted with diethyl ether, 2 x 30 mL, washed with 2% KOH, 2 x 30 mL, and water, 1 x 30 mL. The organic phase was concentrated by rotary evaporation and dried overnight. The solid residue was dissolved in 20 mL dichloromethane, dried with MgSO₄, cold filtered, and again concentrated by rotary evaporation and vacuum drying. The crude product was recrystallized from ethanol to yield \( N-n \)-tetradecylmaleimide (4c) (7.18 g, 76%). \(^1\)H NMR (CDCl₃, 250 MHz) \( \delta \) 0.90 (t, \( J=6.6 \), 3H) 1.28 (m, 22H) 1.59 (m, 2H) 3.52 (t, \( J=7.3 \), 2H) 6.7 (s, 2H); HRMS (ESI, +) Calcd for (M+1): 294.2428. Found: 294.2457.

\( N-n \)-dodecylmaleimide and \( N-n \)-decylmaleimide were obtained in an analogous fashion to \( N-n \)-tetradecylmaleimide except the latter was recrystallized from petroleum ether. \( N-n \)-dodecylmaleimide (4b) (46%) \(^1\)H NMR (CDCl₃, 250 MHz) \( \delta \) 0.90 (t, \( J=6.6 \), 3H) 1.28 (m, 18H) 1.58 (m, 2H) 3.52 (t, \( J=7.3 \), 2H) 6.70 (s, 2H); HRMS (ESI, +) Calcd for (M+1): 266.2115. Found: 266.2113. \( N-n \)-decylmaleimide (4a) (63%) \(^1\)H NMR (CDCl₃, 250 MHz) \( \delta \) 0.90 (t, \( J=6.6 \), 3H) 1.28 (m, 14H) 1.59 (m, 2H) 3.52 (t, \( J=7.3 \), 2H) 6.70 (s, 2H); HRMS (ESI, +) Calcd for (M+1): 238.1802. Found: 238.1806.

### 3.3.4 Free Radical Polymerization

All polymerization reactions were performed without additional photoinitiators. MI and VG monomers were dissolved in methanol in equimolar amounts, purged with argon, and immersed in a water bath at 60 °C. Polymerization was induced by UV irradiation (365 nm, output 900 μW/cm²) at a distance of 1 cm for 20 hours. The polymer product was dried under vacuum, dissolved in a small amount of methanol, precipitated with diethyl ether, and dried. The partially purified copolymer was dissolved in deionized water (Milli-Q, 18.2 MΩ-cm) by magnetic stirring at room temperature and centrifuged
at 2400 g for 15 min to remove dust, debris, and insoluble material. The clear supernatant was carefully removed and fully dried to yield the copolymer product. Losses from centrifugation were between 0.8 and 3.6 wt% and overall yields of the copolymers were about 40%.

The conversion of MI groups was verified by measuring absorption at 300 nm before and after polymerization using a Cary 50 Bio UV-Vis spectrophotometer (Varian Inc., USA). Values are reported as a percentage of initial absorption. Molecular weights were determined by gel permeation chromatography (GPC) using a Viskotek HPLC system with three single-pore columns (PolyAnalytik, Canada) and DMF with 0.2 M LiBr at 55 °C as eluant. Samples were injected at 0.6 mL/min and analyzed by triple detection (light scattering, differential refractive index, and viscometry) to obtain absolute molecular weights distributions. Samples were analyzed twice to confirm reproducibility.

3.3.5 Vesicle Preparation

Aqueous vesicle solutions were prepared by bulk hydration of copolymer powder in deionized water. Solutions were stirred overnight although they dissolved completely within 1 hour to yield clear, blue-tinted solutions.

3.3.6 Characterization

SANS spectra were acquired at Argonne National Laboratory’s Intense Pulsed Neutron Source using a small angle neutron diffractometer with wavelengths between 1 and 14 Å ($q$ range 0.0035 to 0.6 Å$^{-1}$) and 2 mm quartz cells. Samples were prepared in D$_2$O (1wt% polymer) to minimize incoherent scattering and enhance the scattering contrast between the polymer and solvent. Background correction and data reduction
were performed using the onsite instrument software. DLS measurements were performed on an ALV/LSE-5003 CGS-3 instrument at 90° angle using a HeNe laser. Intensity correlation was acquired by ALV Correlator Ver. 3.0A and number weighted distributions were calculated through CONTIN analysis for vesicles using bilayer thickness information obtained from SANS.

Cryo-TEM images were obtained by preparing samples in the controlled environment vitrification system (CEVS) at 24 °C and at water saturation to avoid evaporation. A 7.0 μL drop of each solution was placed on a TEM copper grid covered with a perforated carbon film (Pelco International, USA) and blotted with a filter paper to form a thin liquid film of the sample (100–200 nm thick). Vitrification was achieved by plunging the thinned sample into liquid ethane at its freezing temperature (-183 °C). The vitrified specimen was transferred to liquid nitrogen (-196 °C) for storage until examination. Specimens were studied in a Philips CM120 transmission electron microscope (Philips, The Netherlands) operating at an accelerating voltage of 120 kV with an Oxford CT3500 (Oxford Instruments, UK) cryo-holder, operated below -175 °C. Digital images were recorded on a cooled Gatan MultiScan 791 CCD camera (Gatan, UK) using the DigitalMicrograph 3.1 software (Gatan, UK). Imaging was done in the low-dose mode to minimize beam exposure and electron-beam radiation damage.

3.3.7 Controlled Release

The release behavior of the vesicle systems was examined by encapsulation and release of the fluorescent dye rhodamine B (λ<sub>ex</sub> 554 nm, λ<sub>em</sub> 575 nm). The dye was encapsulated by hydrating the copolymer with an aqueous solution of rhodamine B (5.2×10<sup>-6</sup> g/mL) and stirred overnight. After preparation, 2 mL was transferred to 25,000
MWCO regenerated cellulose dialysis tubing (Spectrum Laboratories Inc., USA) and immersed in 80 mL of fresh deionized water. The bulk solution was assayed for rhodamine B content at periodic intervals using a Cary Eclipse fluorescent spectrophotometer (Varian Inc., USA). The control experiment was done in an identical manner but without copolymer encapsulation. Release of rhodamine B from the vesicles is reported as a percentage of the total amount released from the control. Error bars represent one standard deviation of triplicate independent experiments.

### 3.3.8 Transfection and Cytotoxicity

For transfection of mammalian cells, C10 copolymer was cationically modified by substituting 25 mol% of VG with N-ethyl-N,N-dimethyl-2-(vinyloxy)ethylammonium chloride\(^{34}\) (cat-VE) during polymerization. This modification introduced positive charge to promote complex formation with the negatively charged phosphate groups in the backbone of DNA.

Solutions of this cationic copolymer (C10 25%M+) were prepared at 0.2 wt% in sterile, DNase, RNase free water (Fisher Scientific) by stirring at room temperature. GFP mammalian expression vector gWiz-GFP (Genlantis, USA) was amplified by *Escherichia coli* and purified using a GenoPure Plasmid Maxi Kit (Roche Applied Science, USA). The transfection reagent was prepared by diluting 50 µL of vesicle solution with 50 µL serum free Opti-MEM media (Invitrogen, USA) and incubated for 5 min at room temperature. The solutions were then mixed with 1.5 µg pDNA in 100 µL serum free media and incubated for 20 min at room temperature to form polyplexes. One day before transfection, NIH 3T3 fibroblasts were seeded at 1 × 10\(^5\) cells/well in 12 well plates and incubated at 37 °C and 5% CO\(_2\) in media supplemented with 10% newborn calf serum.
(NCS) to achieve 80-90% confluency. On the day of transfection, cells were washed with PBS and supplemented with 200 µL serum free media and 200 µL of the polyplexes. The cells were incubated at 37 °C and 5% CO₂ for 7 h, after which the media was replaced with media containing 10% NCS and incubated for 24 h. GFP expression was observed by fluorescent microscopy ($\lambda_{\text{ex}}$ 470-480 nm, $\lambda_{\text{em}}$ 510 nm) and quantified by flow cytometry 24 h after transfection, acquiring 20,000 events for each experiment. Lipofectamine 2000 (Invitrogen, USA) was used as a positive control following the manufacturer's instructions with an identical amount of pDNA. Untreated cells and cells treated with "naked" pDNA served as negative controls. Cells receiving naked pDNA were treated analogously to cells treated with the polyplexes. All experiments were performed in triplicate.

Cytotoxicity was evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Briefly, 24 h after transfection 100 µL 0.5% MTT in PBS was added to each well and incubated for 3 h. The medium was removed and the purple formazan crystals were dissolved in 3 mL DMSO. Solutions were centrifuged at 2400 g for 5 min and absorption was measured at 545 nm. Cell survival is reported as a mean percentage of the control group and error bars represent one standard deviation of triplicate independent experiments.

### 3.4 pH Reversible Vesicle Self-assembly

#### 3.4.1 Introduction

Nonionic alternating polymer vesicles exhibit pH reversible self-assembly during cyclic titration with dilute acid and base. This section describes the methods used to
investigate this behavior, confirm reversibility of vesicle formation, and provide insight into the underlying mechanism.

### 3.4.2 Materials

*-$n$*-Alkylmaleimides (MI) and vinyl gluconamide (VG) were prepared as previously described. Deionized water (Milli-Q, 18.2 MΩ cm) was used to prepare all samples and stock solutions and deuterium oxide was purchased from Cambridge Isotope Laboratories, Inc. All other chemicals and solvents were purchased from either Sigma-Aldrich or Fisher Scientific and used as received.

### 3.4.3 Polymerization

Monomers were polymerized by UV initiated free radical polymerization and purified as previously described with slight modification. Briefly, $N$-$n$-alkylmaleimides and VG were dissolved in methanol at 60 °C and irradiated with UV light (365 nm, 900 μW cm$^{-2}$) at a distance of 1 cm for 20 h. No additional initiator is necessary for this monomer pair because maleimides absorb light around 300 nm and generate free radicals, thereby self-propagating the polymerization through an electron acceptor/donor complex with vinyl ethers. After irradiation, the reaction mixture was dried under vacuum, redissolved in a small amount of methanol, and precipitated in 30 mL diethyl ether. The polymer product was washed twice more with diethyl ether, collected, and dried to yield off-white powders with overall yields of 45-50%. C10, C12, and C14 copolymer nomenclature refer to materials synthesized with $N$-$n$-decylmaleimide, $N$-$n$-dodecylmaleimide, and $N$-$n$-tetradecylmaleimide, respectively.
3.4.4 Vesicle Preparation and Titration

Aqueous vesicles were prepared by stirring polymer powder in deionized water for 1-2 hours. Full dissolution of the powder at neutral to slightly basic conditions yields optically clear, blue tinted solutions. Solutions were titrated with 0.01 M HCl and 0.01 M NaOH to examine pH reversible self-assembly of the polymer and pH values were recorded from ColorpHast pH indicator strips (EMD Chemicals, Inc.).

Quantitative titration experiments were performed under continuous flow of argon gas to prevent CO$_2$ absorption. Sample solutions were prepared from fresh deionized water that was partially degassed by boiling for 1-2 h under reduced pressure. Approximately 15 mL of solution, containing either VG or polymer vesicles, was titrated with 0.01 M HCl or 0.01 M NaOH by dropwise addition from a buret. MI monomers are insoluble in water and could not be titrated in this manner. pH measurements were recorded using a 290Aplus pH meter (Thermo Orion).

3.4.5 Characterization

Cryo-TEM images were acquired by methods described in Section 3.3.6. Dynamic light scattering (DLS) measurements were performed on an ALV/LSE-5003 CGS-3 instrument at 90° angle using a HeNe laser. Intensity autocorrelation was acquired by ALV Correlator Ver. 3.0A and number weighted distributions were calculated through CONTIN analysis for vesicles using bilayer thickness information obtained from prior small angle neutron scattering measurements and analyses.$^{46}$
3.4.6 ATR-FTIR

Coordinated hydrogen bonding of protons and polymer were probed by measuring the O-H vibrational signal at ~3400 cm$^{-1}$ on a Nicolet Nexus 870 FTIR spectrophotometer (Thermo Electron Corp.) operating at 4 cm$^{-1}$ resolution and 128 scans using a ZnSe horizontal ATR crystal. To isolate O-H stretching of the polymer, samples and stock solutions were prepared fresh in D$_2$O and samples were analyzed within one to two hours after preparation to minimize the effects of proton-deuterium exchange. The ATR crystal was fully wetted with 500 µL of sample solution and aliquots of acid or base were added using a micro-pipetter and mixed by pipetting action. Background subtraction was performed for each measurement to remove the absorbance of residual water and account for any effect the added acid or base may have on the signal.
Chapter 4 Sugar-swollen Reverse Micelles

4.1 Introduction

Microemulsions, glasses, and supercooled liquids have interesting properties and wide ranging applications to pharmaceutical, cosmetic, flavor, fragrance, and agricultural industries. Convergence of microemulsion and glassy systems, recently demonstrated by Gao et al.\textsuperscript{19,20} and Dave et al.,\textsuperscript{21} may provide avenues to new materials and complex fluids that capture the advantages of each. In these reports, water was replaced by concentrated sugar solutions which resulted in bicontinuous sugar-in-oil microemulsions. After complete removal of water by desiccation, these complex fluids remain one phase and possess solid-like properties despite containing more than 50 vol\% liquid oil. The rigid, glassy sugar matrix permits polymerization of the oil phase without reaction induced rearrangement of the microstructure, which is difficult to achieve in aqueous systems.\textsuperscript{51} The procedure results in a nano-structured, porous, polymer material with nearly identical domain sizes as the original microemulsion. Moreover, it was later reported that anhydrous, glassy sugar-surfactant powders also spontaneously dissolve in oil at elevated temperatures and form bicontinuous structures that remain one phase when cooled to ambient conditions,\textsuperscript{24} demonstrating a new method of arresting self-assembled, nano-structured materials at room temperature.\textsuperscript{52}

Reported recently, anhydrous sugar-surfactant mixtures containing equimolar amounts of sucrose and trehalose spontaneously dissolve in oil at elevated temperatures and form bicontinuous microemulsions.\textsuperscript{24} Examination of these complex glasses by differential scanning calorimetry indicates no first-order phase transitions (melting point)
occur between the glass transition temperature and the temperature used to prepare the complex fluids. This result suggests the anhydrous sugar is a supercooled liquid and there is sufficient molecular mobility for rearrangement of the components and self-assembly of the microstructure.\textsuperscript{53-55}

The above reports focused on bicontinuous structures and suggested sugar-in-oil microemulsion glasses do not form beyond \(~60\) wt\% loading of oil (surfactant-free basis). However, these results do not preclude the possibility of other structures commonly observed in self-assembled systems. Here we examine anhydrous sugar-swollen reverse micelles in hydrocarbon oil using food grade sucrose laurate surfactant. Dynamic light scattering (DLS) measurements confirm micelle size while molecular dynamics are investigated by modulated differential scanning calorimetry (MDSC). Altogether, these measurements provide further insight to sugar-oil complex glasses and how their properties and features may be advantageous for important applications.

\section*{4.2 Results and Discussion}

\subsection*{4.2.1 Phase Behavior}

In an earlier report, sugar-in-oil microemulsions were prepared using a mixture of oleate and caprylate mono-substituted sucrose esters.\textsuperscript{24} The mixture of different tail lengths provides the spontaneous curvature needed for bicontinuous structures. Much greater curvature is required for reverse micelles so we adopted di- and tri-substituted sucrose esters to drive the curvature toward inverted structures by increasing the number of hydrophobes in the surfactant film.
Dissolution of the glassy, anhydrous sugar-surfactant powder in decane at moderate temperatures is rapid and spontaneous. Fig. 4.1 shows the phase diagram for a ternary system of sucrose, sucrose laurate, and decane observed at 25 °C, the behavior of which is comparable to aqueous systems. Isotropic, one phase microemulsions are located within the dotted line and have an assortment of viscosities and appearances. Samples with 75-90 wt% oil loading near the phase boundary (triangles) are optically clear, non-birefringent, exhibit low viscosity, and have a bluish tint characteristic of microemulsions. As sugar content is reduced at constant oil loading, the bluish tint gradually fades until samples become colorless (circles). Further decrease of sugar loading results in dramatically increased viscosity at room temperature (squares), which suggests the spherical micelles have now become elongated, rod-like micelles or even transitioned to an interconnected network. As seen in the subset image in Fig. 4.1, samples with low sugar loading or very high surfactant loading (squares) exhibit gel-like resistances to flow. Low viscosity systems (triangles and circles) only exist above ~60 wt% oil loading.

Sugar-swollen reverse micelles were prepared by dissolving glassy sugar-surfactant powders in oil at 70 °C (80-85 °C for samples containing <40 wt% oil) and allowed to equilibrate at room temperature before recording their phase behavior. The one phase region at 70-80 °C is identical to the area enclosed by the dotted line in Fig. 4.1; that is, samples that are one phase at elevated temperatures do not phase separate when cooled to room temperature, though viscosity increases significantly for samples with low sugar loading. In fact, the samples shown in Fig. 4.1 remain stable down to -20 °C, although slight changes in viscosity are observed. This phase stability is evidence of the structure becoming arrested during cooling of the sugar. Glucose, fructose, trehalose, and
mixtures of sugars may also be microemulsified with similar results, as represented in Fig. 4.2 for an equimolar mixture of glucose and fructose. Phase boundary lines are drawn as lobes to show that binary systems of surfactant and oil are not stable at room temperature.

Reported earlier, bicontinuous sugar-in-oil systems require equimolar mixtures of sucrose and trehalose to suppress crystallization. Here, we observe a secondary sugar is not necessary for the time scale used to prepare microemulsions of sucrose. These complex fluids form readily in a few minutes and are stable at room temperature for at least eight months. However, below the melting temperature the crystalline state is the only true thermodynamically stable state. Continually undergoing time and temperature dependent structural relaxation toward free energy minima, glasses and supercooled liquids are considered thermodynamically unstable and metastable, respectively. Phase separation is observed when samples of higher sugar loading are held at elevated temperatures because crystallization disrupts the stability of the microstructure.

4.2.2 Micelle Size and Structure

DLS measurements were performed on low viscosity samples (triangles and circles in Fig. 4.1) that were diluted to 1 wt% in decane at room temperature. Number weighted size distributions are shown in Figs. 4.3 through 4.5 and indicate the micelles are 5-15 nm in diameter and monodisperse. The size distributions are comparable to those of water swollen reverse micelles. Fig. 4.3 shows calculated distributions for two systems of different $R$, where $R$ is defined as the mass ratio of sugar to surfactant:

$$R = \frac{\text{sucrose}}{\text{sucrose ester}}$$  \hfill (4.1)
Reverse micelles prepared at $R = 0.25$ (bottom) and 0.5 (top) have average diameters of 9 and 12 nm, respectively. Increasing particle size is consistent with mass balance and surface area considerations. Both systems were prepared at constant 90 wt% decane so the system of higher $R$ actually contains less surfactant and more sugar than the system of lower $R$, making it more likely for the former to include the extra sugar in larger micelles rather than in a larger number of smaller micelles. The volume ratio calculated from the average diameters is ~2, which agrees well with the ratio of the two $R$ values. Seen in the subset images in Fig. 4.3, the bluish tinted and colorless appearances of the samples further suggests that increasing $R$ corresponds to increasing length scale.

Fig. 4.5 shows the calculated size distributions for sugar-swollen reverse micelles prepared at equal $R$ but at 25 wt% (top) and 10 wt% (bottom) overall loading of solids in decane. The average diameter of the micelles remains fixed at ~9 nm under these conditions, indicating inter-particle interactions are not a key variable in the self-assembly of the microstructure. The total interfacial surface area, and thus the mass of sucrose at the interface, is approximately doubled without significantly altering the viscosity, which may be advantageous for reactions occurring at the interfaces of micelles. Comparison of Figs. 4.3 and 4.5 suggests particle size in this region of the phase diagram is dependent only on $R$ and not on the overall concentration of solids in oil.

### 4.2.3 Sugar-core Dynamics

MDSC measurements of the anhydrous, glassy sugar-surfactant powders and subsequent microemulsions are shown in Fig. 4.6A and B, respectively, as a plot of reversible heat capacity versus temperature. The glass transition temperature $T_g$ was determined by the mid-point method and is indicated by arrows for each curve. For
increasing sugar loading, or increasing \( R \), the observed \( T_g \) increases for both powder and microemulsion samples. As \( R \) increases, sugar-sugar hydrogen bonding has a more significant contribution to the observed \( T_g \) than sugar-surfactant and surfactant-surfactant hydrogen bonding. This effect is most evident in the microemulsion samples in Fig. 4.6B, where the surfactant is partially solubilized in oil at the interface and whose hydrophobes have liquid-like mobility. In this molecular arrangement, \( T_g \) is depressed considerably when only small amounts of sugar are present within the micelle core (Fig. 4.6B, \( R = 0.1 \)).

For \( R = 0.5 \), two glass transitions are observed in the glassy powder curve shown in Fig. 4.6A. The first may be counted as the primary transition of the glassy matrix while the second, being very near the \( T_g \) of sucrose,\(^{55}\) may be a result of micro-heterogeneities arising from non-ideal mixing of the sugar and surfactant. The sugar-surfactant glassy powders likely have some type of microstructure based on the nature of their preparation and glasses are inherently unstable by classical thermodynamics. Some phase separation or crystallization may occur during drying, resulting in compositional gradients within the mixture. Once the powders are dissolved in oil, the second glass transition vanishes (Fig. 4.6B). This disappearance is evidence that the molecular dynamics of the supercooled liquid state are sufficient for relaxation of micro-heterogeneities present in the precursor glass. The final sugar-in-oil microemulsions have a single \( T_g \) and are isotropic.

Sugar-swollen reverse micelles were prepared by spontaneous dissolution of anhydrous glassy mixtures of sugar and surfactant in decane. However, this is not the only method available for making sugar-oil complex fluids. Direct microemulsification of sugar is possible via the supercooled liquid state, as shown in Fig. 4.7. An aqueous
solution of equimolar glucose and fructose was dried by methods described in Section 3.2.2 (a small amount of L-1695 was added to promote foaming during the vacuum drying step) and the glassy sugar product was added directly to a 25 wt% solution of L-595 in decane at 70 °C (arrow in Fig. 4.7A). With mechanical stirring, the sugar became dispersed into the non-polar phase and eventually microemulsified, yielding an optically clear, isotropic fluid (Fig. 4.7F). The $T_g$ of both glucose and fructose are below 70 °C so the coupled molecular mobility and solvophobicity of the supercooled liquid sugar is what drives microemulsification. Glassy sucrose exhibits similar behavior at ~90 °C but crystallization competes significantly with microemulsification at such temperatures.

4.3 Conclusions

Sugar-swollen reverse micelles in hydrocarbon oil are readily prepared from anhydrous, glassy sucrose-sucrose laurate mixtures. These ternary systems are stable at room temperature and display a wide range of physical properties depending on the mass ratio of sugar to surfactant. Analysis of MDSC data reveal the sugar is a supercooled liquid at the temperatures required to make the microemulsions, indicated molecular mobility of the sugar molecules is crucial to self-assembly of the microstructure.

Reverse micelle microemulsions have been well studied for use as micro-reactors for aqueous and polar organic media based reactions. Microemulsification of sugar may provide avenues for more carbohydrate-based reactions and processes to become industrially viable due to increased surface area to volume ratio of the glassy sugar. In our lab, a new method of synthesizing sucrose ester surfactants using K$_2$CO$_3$-doped sugar-swollen reverse micelles dispersed in a continuous methyl laurate phase is being investigated currently.
Figure 4.1 Ternary phase diagram for sucrose, sucrose laurate (L-595), and decane at 25 °C. The region enclosed by the dotted line indicates the one phase window and the subset picture shows representative samples: (Δ) blue tinted, low viscosity; (○) colorless, low viscosity; (□) colorless, gel-like viscosity. Note the sample on the far right is turned on its side to illustrate the gel-like resistance to flow. Exceptionally high viscosity precluded examination of samples with lower than 25 wt% oil.
**Figure 4.2** Ternary phase diagram for equimolar glucose/fructose (Sugar) and sucrose laurate (L-595) in decane. Symbols are analogous to those in Fig. 4.1.
Figure 4.3 Calculated particle size distributions from DLS measurements for samples with $R = 0.5$ and 0.25, where $R$ is the mass ratio of sugar to sugar ester surfactant. Both systems were initially prepared at 10 wt% solids in decane and diluted to approximately 1 wt% at room temperature prior to measurement. At $R$ values near the phase boundary (dotted line in Fig. 4.1) the microemulsions have a bluish tint whereas they become colorless as $R$ decreases.
Figure 4.4 Size distributions for equimolar glucose/fructose microemulsions in decane for varying $R$. 
Figure 4.5 Calculated particle size distributions from DLS measurements for samples with $R = 0.25$, prepared at different concentrations in decane. Samples were diluted to approximately 1 wt% at room temperature prior to taking measurements.
Figure 4.6 Modulated DSC scans, plotted as reversible heat capacity versus temperature, for sucrose-sucrose laurate precursor glasses (A) and sugar-in-oil microemulsions (B). Indicated by arrows, the glass transition temperature is identified by a step-wise increase in the heat capacity.
Figure 4.7 Spontaneous microemulsification of equimolar glucose/fructose glass (arrow) into 25 wt% L-595 solution in decane at 70 °C. Images correspond to times of 0 (A), 1 (B), 2 (C), 5 (D), 10 (E), and 30 min (F), respectively.
Chapter 5 Alternating Polymer Vesicles

5.1 Introduction

Liquid-core capsules have drawn significant attention from biomedical, pharmaceutical, cosmetic, flavoring, and agriculture industries for encapsulation and release applications. Fabrication methods have been well studied and common techniques for obtaining such capsules include emulsion polymerization, intrabilayer polymerization of vesicles, self-assembly of colloidal particles and amphiphilic macromolecules, and cross-linking of polymers absorbed at oil-water interfaces. Lipid and surfactant based vesicles, whose flexible shells can be advantageous compared to the often rigid capsules obtained through interfacial polymerization, have also been widely investigated throughout scientific literature. However, polymer chemistry offers an extensive library of materials compared to lipids and with the initial report of block copolymer vesicles by Discher et al., polymeric vesicles, dubbed polymersomes, have elicited broad interest ranging from fundamental research of bilayer physics to practical controlled delivery applications using a diverse collection of hydrophilic and hydrophobic polymer blocks.

Last year, a new class of vesicle forming polymers was reported wherein alternating sequences of hydrophilic and hydrophobic monomers yield copolymers with laterally derived amphiphilicity that self-assemble into vesicles. In particular, alternating copolymers of alkylmaleates and vinyl gluconamide were shown to form vesicles upon dissolution in water and ultra small sizes of 10 to 20 nm in diameter could be obtained through horn sonication. However, the molecular weights of these alternating
copolymers, or rather, co-oligomers, were on the order of a few kDa, suggesting only three to nine repeat units. The influence of the charged initiator, tethered on one or both ends of the oligomers, could also be significant. Thus, it was uncertain whether alternating copolymers of significant molecular weight can form vesicles.

This question is addressed here by studying the vesicle forming properties of alternating copolymers made from alkylmaleimides and vinyl ethers. Systems of this monomer pair have been reported to polymerize rapidly and alternately when the initial monomer ratio is equimolar\cite{47,76} or when the vinyl ether is in excess.\cite{48,49} Assuming comparable termination rates, we expected higher molecular weight alternating polymers could be obtained using monomers with these functional groups. Moreover, because maleimides absorb UV light to self-induce free radical polymerization,\cite{47-49} no additional initiator is necessary and eliminates potential end-group contributions to the capacity of these alternating polymers to form vesicles.

Monomers of \(N\)-\(n\)-alkylmaleimides (MI) and sugar derived vinyl gluconamide (VG) were synthesized, polymerized to high molecular weight, and the resulting copolymers spontaneously formed vesicles when dissolved in water. The size, shape, and bilayer thickness of the vesicles were investigated by cryo-TEM, DLS, and SANS while potential applications of these vesicles were examined by studying the controlled release of encapsulated dye and the transfection of mammalian cells with a green fluorescent protein (GFP) vector.
5.2 Results and Discussion

5.2.1 Polymerization

The copolymers recently prepared from the alkylmaleate-VG system had low molecular weights, bringing into question if vesicle formation is limited to oligomers with alternating side chain functionality.\(^{34}\) Listed in Table 5.1, the molecular weights of the MI-VG copolymers are approximately 100× higher than their maleate counterparts, as expected from their rapid polymerization kinetics and presumably comparable termination rates. UV-Vis measurements confirmed that the reactions went to high conversion of MI groups in 20 hours. The polydispersity index, \(M_w/M_n\), however, was found to be unusually low, less than 1.1 in all cases, for a free radical polymerization mechanism.\(^{77}\) To remove unreacted monomers and contaminants, the copolymer product was precipitated from methanol and dissolved in water followed by centrifugation. This narrow distribution may be partly due to these purification steps, which resulted in low overall yields of 35-40%. Nonetheless, the polydispersity is unusually low following just two liquid-fractionations and prompted us to verify that the polymerization is non-living - polymerization of the monomers ceases when the UV light source is removed.

5.2.2 Vesicle Size and Structure

Alkylmaleimide-VG copolymer vesicles form readily at room temperature with magnetic stirring whereas vesicles from the previously reported alkylmaleate-VG system required elevated temperatures and inclusion of a small amount of cationic monomer to promote solubility.\(^{34}\) We suspect that the single hydrocarbon tail of the alkylmaleimides, in comparison to the two hydrocarbon tails of alkylmaleates, results in greater flexibility
of the polymer chain, enabling water to access the hydroxyl groups of VG more readily and promote hydration and self-assembly of the bilayer at room temperature.

The size distributions of the vesicle systems were characterized by DLS and cryo-TEM. Shown in Figs. 5.1 – 5.3, DLS measurements yield a bimodal distribution of ultra small (10-20 nm) and medium (50-300 nm) sized vesicles for the three polymer systems. Cryo-TEM images are in good agreement with the DLS results and two representative images for each sample are shown to illustrate the bimodal size distributions. Numerous vesicles with diameters between 50-100 nm are observed in Fig. 5.1 and ultra-small vesicles are clearly observed in Fig. 5.2. Overall, the size distributions of the spontaneously formed vesicles are rather insensitive to the chain length of the alkyl hydrophobe present. Although limited by imaging resolution, it is evident that the bilayer thicknesses of the vesicles are less than 5 nm. More precise determination of the bilayer thickness comes from analysis of the SANS spectra of the vesicles.

SANS data for the three copolymer vesicle solutions in $D_2O$ are plotted in Fig. 5.4. All samples exhibit $I \propto q^{-2}$ behavior, which is characteristic of thin sheets and vesicular structures.\textsuperscript{65,78-80} The thickness of the vesicle bilayer may be calculated from the high $q$ turnover of the $I \propto q^{-2}$ region using a Kratky-Porod plot and the Guinier approximation,\textsuperscript{80-82} although it's been suggested that this simplified method may only be useful in calculating relative changes in thickness rather than absolute values.\textsuperscript{83} To obtain the vesicle bilayer thickness, we developed a model based on Vrij's equations for scattering from mixtures of hard spheres\textsuperscript{84} using core-shell form factors and two Schulz distributions adopted from DLS measurements. Model parameters were constrained by the known concentration of polymer and the relative fraction of medium-sized vesicles to
ultra small vesicles as determined from DLS. The scattering length density of the bilayer was assumed to be homogenous over the entire thickness.

The average diameters, standard deviations, and bilayer thicknesses calculated from the SANS model are summarized in Table 2 and the corresponding model SANS spectra are shown as the solid lines in Fig. 2. Departures from $I \propto q^{-2}$ at both low and high-$q$ due to the finite size of the vesicles are captured accurately by the model. The average vesicle diameters determined from SANS and DLS are in agreement to within their expected accuracy. Also, the bilayer thicknesses of 1.7, 2.0, and 2.6 nm for C10, C12 and C14 copolymer vesicles are consistent with the increasing alkyl-chain length and in good agreement with those of unilamellar surfactant vesicles. These measured thicknesses also confirm that the copolymer chains are oriented such that all hydrophobic tails extend toward each other and the polyhydroxy side chains of VG extend into the aqueous phase.

### 5.2.3 Release Kinetics

The rate of release of rhodamine B dye from copolymer vesicles was found to be dependent on the length of the alkyl group on the maleimide, indicating decreasing permeability with increasing bilayer thickness, shown in Fig. 5.5. Release of the dye reached completion after 50 h, which is comparable to the release kinetics of liposomes and alkylmaleate-VG copolymer vesicles but much faster than release from block copolymer vesicles which typically have thicker walls.
5.2.4 Transfection and Cytotoxicity

Synthetic gene delivery vectors have been studied quite extensively and many novel systems and materials have been developed including liposomes, dendrimers, and cationic polymers of polyethyleneimine (PEI), poly-ε-lysine (PLL), and polyethylene glycol (PEG). \textsuperscript{85-89} Sugar and polyhydroxy functional groups have been demonstrated to reduce cytotoxicity in polymeric gene delivery vehicles\textsuperscript{90} and to be suitable head groups for vesicle forming materials.\textsuperscript{74} We found that VG has low toxicity in mammalian cells and we hypothesized that vesicles containing VG would also have low toxicity. Fig. 5.6 shows C10 25%M+ is moderately nontoxic (>80% survival) and slightly less toxic than commercially available Lipofectamine 2000. Optical and fluorescent microscopy images of cells expressing GFP by C10 25%M+ mediated transfection are also shown in Fig. 5.7. However, transfection efficacy was low for polyplexes of C10 25%M+ and quantification of GFP positive cells by flow cytometry revealed that Lipofectamine was approximately 10 times more efficacious.

Many studies have indicated the efficacy of a synthetic transfection agent is related to the ratio of available nitrogens in the polymer to phosphates in the DNA backbone, denoted as N/P.\textsuperscript{85,86,91,92} N/P for C10 25%M+ as used here was approximately 10 while values of 20-50 have been reported as optimum conditions for synthetic vectors. It may be reasonable to conclude that cationically modified C10 copolymer mediated transfection was not efficacious because N/P was too low. However, simply increasing the amount of copolymer used in transfection did not improve GFP expression. Also, increasing the loading of cationic monomer during polymerization failed to yield a
vesicle forming material, presumably because the increased number of charged species disrupted the free radical polymerization mechanism.

The low efficacy may also be attributed to instability of the polyplexes in media. Aggregates of solid material were clearly visible during transfection and may have entrapped a majority of the plasmid, preventing delivery into the cells. Investigations are underway to study how the incorporation of PEG and other functional groups affect the stability of these complexes and promote targeting to various cell types. Following the flexible chemistry of VG synthesis shown in Scheme 5.1, the lactone may be replaced by other groups susceptible to nucleophilic attack by primary amines, providing access to a library of vinyl ether monomers that may be incorporated into alternating copolymers. Such materials may also be well suited for catalytic reactions where the proximity of specific functional groups is important.

5.3 Conclusions

Free radical polymerization of N-\(n\)-alkylmaleimides and vinyl gluconamide yields alternating copolymers that spontaneously form vesicles when dissolved in water at room temperature. These vesicles can be as small as 10-20 nm in diameter without sonication or other high shear procedures. Cryo-TEM and DLS data show a bimodal distribution of very small and medium-sized vesicles and analysis of SANS data confirmed vesicular structure and alkyl side chain dependent bilayer thicknesses ranging from 1.7 to 2.6 nm. Inclusion of cationic vinyl ethers yields alternating copolymers that complex with plasmid DNA and although these materials exhibit poor transfection efficacy, they have low toxicity in mammalian fibroblasts.
Table 5.1 Maleimide conversion, overall yield, and molecular weight of the alkylmaleimide-co-vinyl gluconamide polymers

<table>
<thead>
<tr>
<th>Copolymer</th>
<th>Maleimide</th>
<th>Conversion</th>
<th>Yield</th>
<th>$M_W$, kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>C10</td>
<td>$N$-$n$-decylmaleimide</td>
<td>92%</td>
<td>35%</td>
<td>127</td>
</tr>
<tr>
<td>C12</td>
<td>$N$-$n$-dodecylmaleimide</td>
<td>91%</td>
<td>38%</td>
<td>353</td>
</tr>
<tr>
<td>C14</td>
<td>$N$-$n$-tetradecylmaleimide</td>
<td>88%</td>
<td>37%</td>
<td>290</td>
</tr>
</tbody>
</table>

Table 5.2 Results from SANS model calculations\(^a\)

<table>
<thead>
<tr>
<th>Copolymer</th>
<th>$\langle D_1 \rangle$ (nm)</th>
<th>$\langle D_2 \rangle$ (nm)</th>
<th>$t$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C10</td>
<td>7.0 ± 1.1</td>
<td>62 ± 23</td>
<td>1.7</td>
</tr>
<tr>
<td>C12</td>
<td>6.7 ± 0.7</td>
<td>75 ± 27</td>
<td>2.0</td>
</tr>
<tr>
<td>C14</td>
<td>7.2 ± 0.6</td>
<td>84 ± 31</td>
<td>2.6</td>
</tr>
</tbody>
</table>

\(^a\)Average shell diameters, $\langle D_1 \rangle$ and $\langle D_2 \rangle$, and standard deviations of the bimodal distributions and bilayer thickness $t$.

Scheme 5.1 Synthesis of sugar derived vinyl gluconamide

![Scheme 5.1 Synthesis of sugar derived vinyl gluconamide](image1)

Scheme 5.2 Synthesis of hydrophobic $N$-$n$-alkylmaleimides

![Scheme 5.2 Synthesis of hydrophobic $N$-$n$-alkylmaleimides](image2)
Scheme 5.3 Copolymerization of the maleimide-vinyl ether system and hypothetical structure of the vesicle wall
**Figure 5.1** Cryo-TEM images and DLS measurements of C10 vesicles
Figure 5.2 Cryo-TEM images and DLS measurements of C12 vesicles
Figure 5.3 Cryo-TEM images and DLS measurements of C14 vesicles
Figure 5.4 Symbols: small angle neutron scattering data for the copolymer vesicles in D$_2$O, exhibiting $I \propto q^{-2}$ behavior characteristic of bilayer structures. Lines: model calculations using a bimodal distribution of polydisperse spheres from DLS measurements and a core-shell form factor. Data sets vertically scaled for clarity.
**Figure 5.5** Release of rhodamine B dye from vesicles contained within dialysis tubing.

Fractional release was calculated as a percentage of the total amount released from the control. Error bars represent one standard deviation of triplicate independent experiments.
Figure 5.6 Survival of NIH 3T3 fibroblasts after C10 25%M+ copolymer mediated transfection compared with cells treated with naked pDNA, cells transfected using Lipofectamine 2000, and a non-transfected control group.
Figure 5.7 NIH 3T3 cells transfected by C10 25%M+ copolymer complexed with pDNA containing a GFP reporter gene under optical (top) and fluorescent microscopy (bottom). Cells positive for GFP expression are seen as bright green.
Chapter 6 pH Reversible Vesicle Self-assembly

6.1 Introduction

pH reversible self-assembly is typically associated with surfactants or polymers containing acidic and basic functional groups, such as amines and carboxylic acids, that play an integral role in establishing amphiphilicity. Poly(2-vinylpyridine)-block-poly(ethylene oxide) for example, forms vesicles in neutral and alkaline solutions. Upon acidification, protonation of the vinylpyridine block renders the entire polymer hydrophilic and the vesicles disappear.\(^{30}\) Conversely, carboxylic groups, protonated under acidic conditions, become hydrophobic and induce self-assembly when coupled to hydrophilic polymer blocks. When neutralized with base, de-protonation renders these carboxyl block co-polymers fully water soluble and the microstructures disband.\(^{93}\) Polymers containing both amines and carboxylic groups,\(^{94}\) bioinspired polymers, polypeptides, and zwitterionic materials have also been reported to form pH responsive self-assembled structures.\(^{31,32,95,96}\) Such systems have wide ranging applications for controlled release of active agents in agricultural, pharmaceutical, cosmetic, and flavor industries. Protonation and de-protonation of acidic and basic groups are effective modulators of amphiphilicity and self-assembly. Here we report a new class of alternating polymers that contain no evident ionic, acidic, or basic groups, but whose self-assembly is nonetheless reversibly tunable by small changes in pH.

Alternating polymers are a new class of vesicle forming material and recent reports present exciting opportunities for their use in interesting applications.\(^{34,46}\) Nonionic polymers of alternating hydrophilic and hydrophobic groups spontaneously form vesicles...
as small as 10-20 nm in diameter in aqueous solution. Due to the physical orientation of the polymer chains, these vesicles have thin flexible bilayers, yielding polymersomes with release characteristics comparable to liposomes.

Here, we report the pH reversible self-assembly of nonionic alternating polymer vesicles composed of $N$-alkylmaleimides and vinyl gluconamide. These polymers form vesicles under near neutral conditions but precipitate rapidly upon addition of dilute acid. The polymer re-assembles into vesicles upon neutralization of the acid, as confirmed by cryo-TEM imaging. Precipitation and re-assembly is repeatable many times over and DLS measurements show that the size distributions of re-assembled vesicles are comparable to the size distributions before precipitation. We hypothesize that this pH reversible self-assembly of vesicles is the result of coordinated hydrogen bonding between protons and the non-bonded electron pairs that occupy the interstitial sites between VG residues in the polymer backbone. This is supported by titration experiments and ATR-FTIR spectroscopy.

6.2 Results and Discussion

6.2.1 Vesicle Self-assembly

Complementary techniques of DLS, SANS, and cryo-TEM have confirmed earlier that C10, C12, and C14 alternating polymers spontaneously form vesicles in aqueous solution. These systems are optically clear and exhibit a bluish tint characteristic of structured fluids and microemulsions, represented by C14 in Fig. 6.1A. Titration of the polymer with dilute acid causes precipitation, resulting in a turbid solution with visible white aggregates (Fig. 6.1B). Precipitation occurs catastrophically, however, overall a
small pH range around 3.5 rather than gradually as acidity is increased. The precipitated polymer re-dissolves upon neutralization with dilute base to yield solutions with optical clarity and bluish tint (Fig. 6.1C). Precipitation and re-dissolution of the polymer can be cycled repeatedly, shown in Fig. 6.1D and E, and solution stability is not affected by excess base (Fig. 6.1E).

Imide and amide bonds are susceptible to acid hydrolysis at elevated temperatures and extreme pH but such reactions would destroy the amphiphilicity of the polymer and re-dissolution after neutralization would not be possible. Since the pH reversible dissolution can be cycled repeatedly at ambient conditions, we conclude that there are no reactions occurring that chemically alter the polymer.

At pH ~6, the precipitated polymer re-dissolves to yield an optically clear solution with bluish tint. Cryo-TEM imaging confirms that the polymer has re-assembled into vesicles. Fig. 6.2 compares images of the original vesicles (A) with those reconstituted from precipitated solutions (B). Evident from the images, the re-assembled vesicles have a wide range of sizes and very thin bilayers, consistent with our previous report on vesicles prepared under low shear. DLS measurements confirm that the vesicles have reversible size distributions before precipitation and after re-assembly as shown in Fig. 6.3 for C12 (A and B) and C14 (C and D) polymer vesicles.

To probe the transition between self-assembled vesicles and precipitates, size distributions of C10 vesicles were measured by DLS for decreasing pH. The range for each mode of the distribution remains relatively constant with decreasing pH, as shown in Fig. 6.4. The small vesicles are consistently 10-20 nm in diameter and the medium-sized vesicles have diameters of 50-400 nm. Precipitation occurs catastrophically at pH ~3.5
and precluded DLS measurement at lower pH. Following precipitation, neutralization of the turbid mixture returns the vesicles to the original size distribution as indicated by the long arrow. Although the range of sizes remains steady with decreasing pH, the number of vesicles within the two modes exhibits exponential behavior as the solution approaches critical pH, shown in Fig. 6.5 for C10 vesicles (data adopted from Fig. 6.4). Initially, the vesicles are not affected by changes in pH, having relatively constant size distributions from neutral conditions to pH ~4.5. As solution acidity increases further, the number of small- and medium-sized vesicles decrease and increase asymptotically, respectively. This result suggests the acidic solution may trigger changes to the curvature of the self-assembled polymer chains. Further increase in acidity results in precipitation at pH ~3.5.

6.2.2 Titration

Synthesis of the monomers used in the polymerization reaction begins with primary amines, which are readily protonated in acidic conditions. pH reversible self-assembly of the polymer may be due to cyclic protonation/de-protonation of residual amines incorporated into the polymer. However, titration of aqueous monomer and polymer vesicle solutions confirm that such impurities are not present.

Forward titration curves of 0.5 wt% VG and 0.5 wt% C14 vesicles with 0.01 M HCl (circles and triangles, respectively) and back-titration of the polymer with 0.01 M NaOH (squares) are shown in Fig. 6.6. Titration of VG reveals the pH at the equivalence point is around 6, which is equivalent to that of water containing a small amount of dissolved CO₂ as carbonic acid. The lack of an equivalence point at elevated pH, as typically observed for weak bases, confirms there are no residual amines in the monomer. Furthermore, titration of the polymer, both forward and backward, shows the pH at the
equivalence point is also near 6 and confirms the absence of typical acid/base functional
groups in the polymer.

Precipitation of the polymer at pH ~3.5 and re-assembly near neutral conditions are
indicated in Fig. 6.6 by arrows. There are no observable inflection points in the titration
curves associated with these significant physical events, which is contrary to other
systems that exhibit pH dependent self-assembly related to the pK$_a$ of constituent
functional groups.$^{30}$ However, the slopes of the monomer and polymer curves at the
equivalence point differ. The more gradual slope of the polymer titration curve is
associated with affinity for protons, commonly observed in polyelectrolytes whose pK$_a$
depends on the degree of protonation.$^{97-99}$ Although the polymer vesicles do not exhibit
characteristics typically observed for weak acids or bases, in terms of buffering or
altering the pH at the equivalence point, they do exhibit affinity for protons which is
likely due to coordinated hydrogen bonding.

6.2.3 Hydrogen Bonding

Titration of the polymer reveals the pH reversible self-assembly is not due to
protonation of acidic or basic groups. To reiterate, the slope of the polymer vesicle
titration curve at the equivalence point is smaller than the slope of the monomer curve,
indicating the self-assembled polymer has a higher affinity for protons than VG in free
solution. Since the polymer contains no residual acid or base functional groups, we
hypothesize that protonation occurs through coordinated hydrogen bonding of proton
within the electron rich pockets formed cooperatively by the multiple hydroxyl groups of
adjacent VG residues. This effect is not observed when VG is in free aqueous solution
because the molecules, isolated by large pools of water, have significant translational and
rotational freedom that precludes formation of these electron-rich pockets. In the self-assembled polymer vesicle, however, the sequence and order of VG groups are comparatively fixed. Thus, the proximity of hydroxyl and amide functional groups creates pockets of non-bonded electron pairs that may coordinate with protons. These pockets have selective affinity for protons, and vesicle stability is not affected by alkaline conditions (Fig. 6.1E). This hypothesis is illustrated in Fig. 6.7.

Measurements of O-H stretching frequencies by ATR-FTIR spectroscopy of the vesicles in D₂O support this hypothesis. Upon addition of HCl (D₂O solution) the O-H stretching band shifts to shorter wave numbers as shown in Fig. 6.8. When the solution of C14 vesicles is neutralized with NaOH (D₂O solution) and the polymer re-assembles, the O-H stretching band returns close to its original wave number (open symbol). Nonetheless, it remains unclear if ATR-FTIR, or presumably any other alternative method, is detecting changes in hydrogen bonding caused by addition of protons or by alteration of the polymer conformation and bilayer structure.

Protons sequestered within the electron-rich pockets bring along counter anions and waters of hydration that increases the effective size of the hydrophilic groups. This alters the preferred conformation of the polymer chain, disrupts the bilayers, and ultimately leads to precipitation. Neutralization of the precipitated polymer solution to pH ~6 restores the favorable balance between the effective size of the hydrophilic and hydrophobic groups necessary for bilayer/vesicle formation. Although another system of nonionic glycolipid vesicles have been reported to elicit morphological responses to changing pH, these glycolipids do not precipitate in acidic conditions. Rather, vesicles of these surfactants form clusters that disaggregate reversibly near
neutral/alkaline conditions. The lack of precipitation suggests the mechanisms of pH reversible behavior of the glycolipid vesicles and the polymer vesicles reported here differ.

The accumulation of salt during repeated acid/base cycling does not affect the stability of the vesicles up to ~0.5 M concentration of NaCl. Since only millimolar concentrations of acid and base are necessary to traverse the small pH hysteresis between vesicle precipitation (pH~3.5) and vesicle reassembly (pH~6.0), this process can be repeated hundreds of times rapidly and with little shear. Efforts are ongoing to develop even more salt-resistant reversible vesicle systems for applications such as the cleanup of water soluble compounds that are selectively captured within the precipitated polymer.

6.3 Conclusions

Alternating polymer vesicles of N-n-alkylmaleimides and vinyl gluconamide exhibit pH reversible self-assembly in aqueous solution. Although containing no typical acidic, basic, or ionic functionalities these systems are nonetheless sensitive to small changes in pH and oscillate between clear, blue tinted solutions and turbid, precipitated mixtures during cyclic titration with acid and base. The polymers, which precipitate at pH ~3.5, re-assemble into vesicles at near neutral conditions as confirmed by cryo-TEM and DLS measurements. This phenomenon likely arises from the selective coordination of protons to the multiple hydroxyl side groups of regularly spaced gluconamide groups.
Figure 6.1 Solutions of C14 alternating polymer vesicles are optically clear and blue tinted (A) and upon addition of 0.01 M HCl they become turbid with visible white precipitates (B). Neutralizing the acid with 0.01 M NaOH restores the solution back to its original clarity and bluish tint (C). This behavior may be cycled repeatedly (D, E) and solution stability is not affected by basic conditions (E).
Figure 6.2 Cyro-TEM images of C12 alternating polymer vesicles before precipitation (A) and after re-dissolution (B), i.e. Fig. 6.1A and C, respectively.
Figure 6.3 Size distributions for C12 (A and B) and C14 (C and D) vesicles by DLS measurements. (A) and (C) show distributions for the original vesicles and (B) and (D) show distributions for re-assembled vesicles after acid-induced precipitation.
Figure 6.4 Size distributions for C10 vesicles for decreasing pH. The polymer precipitates catastrophically at pH ~3.5, which precludes measurement by DLS, and re-
assembles into vesicles at near neutral conditions with the same size distribution, as depicted by the arrows.
Figure 6.5 For decreasing pH, the number of small vesicles begins to drop off rapidly as the solution nears precipitation (filled symbols and arrow). Conversely, the number of larger vesicles begins to increase near the onset of precipitation (open symbols and arrow). Data are adopted from Fig. 6.4 and lines are drawn to guide the eye.
Figure 6.6 Titration of monomer (VG) and polymer vesicle (C14) solutions. VG (circles) behaves analogously to water, exhibiting no weak acid or base characteristics. Precipitation of the polymer occurs at pH ~3.5 during the forward titration of adding acid (triangles). Neutralization with base (squares) restores the polymer to solution via re-assembly into vesicles at pH ~6.
Figure 6.7 Schematic representation of putative bilayer disruption caused by coordinated hydrogen bonding between protons and vinyl gluconamide groups. Under neutral conditions, the conformation of the alternating polymers yields a comparatively flat monolayer favoring the formation of vesicular structures. Under acidic conditions, protons are sequestered within the electron-rich pockets of regularly spaced gluconamide groups. Accompanying counter-ions and waters of hydration swells and changes the conformation of the polymer chains disrupting the bilayer structure.
Figure 6.8 O-H stretching vibrations for decreasing pH, as measured by ATR-FTIR spectroscopy of C14 polymer vesicles in D₂O. As pH decreases, the O-H stretching band shifts to lower wavenumbers, indicating changes in hydrogen bonding. Neutralization of the precipitated solution returns the band to near its original value (open symbol).
Chapter 7 Conclusions and Proposed Work

This dissertation has discussed interfacial self-assembly of small molecule and macromolecule amphiphiles for applications in solid- and liquid-core capsules. The experimental investigations focused on sugar-swollen reverse micelles and alternating polymer vesicles. This chapter summarizes the findings and provides an outlook for further investigations and research.

7.1 Sugar-swollen Reverse Micelles

Sugar-swollen reverse micelles were prepared from food grade sucrose laurate surfactant, sugar, and hydrocarbon oil. These microemulsions exhibit phase behavior related to the mass ratio of sugar to surfactant and overall concentration of solids in oil. DLS measurements show that micelles are 5-15 nm in diameter and monodisperse. MDSC measurements of glass transition temperatures provided insight into the supercooled liquid state of sugar and how molecular mobility is important for self-assembly in sugar-oil complex fluids. Sucrose was used principally for this investigation but other sugars, such as trehalose, glucose, and fructose, may also be used.

Reverse micelle microemulsions have been well studied for use as micro-reactors for aqueous and polar organic media based reactions. Since sugars do not readily dissolve in most common solvents, the scope of sugar-based processes available for industrial scale-up is limited. Microemulsification of sugar may provide avenues for more carbohydrate-based reactions and processes to become industrially viable due to increased surface area to volume ratio of the glassy sugar. In our lab, a new method of synthesizing sucrose ester surfactants using K₂CO₃-doped sugar-swollen reverse micelles
dispersed in a continuous methyl laurate phase is being investigated currently. Anhydrous sucrose-surfactant glasses may be prepared with up to 10 wt% K$_2$CO$_3$ (relative to sugar) and microemulsified in methyl laurate at 55 °C. Also, anhydrous sucrose glass loaded with 25 wt% glycerol is readily emulsified by sucrose laurate solutions in methyl laurate. Such systems may provide a new synthesis route for sucrose esters by transesterification of sucrose with methyl laurate or triglycerides, offering an alternative biodiesel fuel that retains the abundant glycerol byproduct.

Direct microemulsification of sugar via the supercooled liquid state, discussed in Section 4.2.3 and Fig. 4.7, may hold exciting potential for continuous reaction systems. Here, we have described microemulsification via a glassy sugar-surfactant mixture but it may be very beneficial to develop a method to replenish the sugar consumed in a reaction scheme without the assistance of costly surfactant, as demonstrated in Fig. 4.7 with equimolar glucose/fructose glass. Recent observations have indicated direct microemulsification of glassy sucrose is also possible using mixtures of L-595 and L-1695 (sucrose monolaurate) in decane at 90 °C. The mixture of surfactants is presumably necessary to increase the solvation capabilities of the reverse micelle core. However, at such high temperatures, rates of crystallization and microemulsification compete fiercely.

### 7.2 Alternating Polymer Vesicles

Alternating polymers of N-$n$-alkylmaleimides and vinyl gluconamide were synthesized and found to spontaneously form vesicles when dissolved in water. The vesicles have bimodal size distributions of 10-20 nm and 50-300 nm diameters, as confirmed by cryo-TEM images and dynamic light scattering. Analysis of small angle neutron scattering data confirms vesicular structure and alkyl side chain dependent
bilayer thicknesses ranging from 1.7 to 2.6 nm. The controllable thickness permits modulation of release characteristics, which are more similar to release from liposomes than vesicles constructed of block copolymers. Functionalization of the vesicle surface with cationic groups facilitates complexing with DNA and transfection of mammalian cells.

These vesicles, despite containing no acidic or basic functionalities, exhibit pH reversible self-assembly. Between pH ~6.5 and ~3.5 the polymer cycles between self-assembled vesicles and insoluble precipitate, respectively. Cryo-TEM images confirm the polymer re-assembles into vesicles at near neutral pH and DLS measurements confirm reversibility of the size distributions. The pH reversible self-assembly is attributed to coordinated hydrogen bonding within the pockets of neighboring VG groups in the polymer backbone.

Looking forward, the chemistry used to synthesize these polymers has exciting potential due to the number of chemical functionalities that are compatible with free radical polymerization mechanisms, providing endless possibilities for vesicle surface customization. Crosslinking of the polymer chains may create more robust capsules and provide further control and modulation of release characteristics. Such systems would require multiple, compatible polymerization mechanisms to ensure crosslinking does not occur before the polymer assembles into vesicles.
Bibliography


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