Swollen Vesicles and Multiple Emulsions from Block Copolymers

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ABSTRACT: We engineer novel structures by "stuffing" the aliphatic regions of self-assembled aggregates with hydrophobic homopolymer. These "stuffed" vesicles and multiple emulsions are formed in a one-step process when we rehydrate stuffed films made of amphiphilic block copolymer and hydrophobic homopolymer. Without such homopolymer, this system forms micelles. With homopolymer, vesicles form; varying vesicle membrane thicknesses show that these structures incorporate different amounts of homopolymer. Multiple emulsions, containing more homopolymer than stuffed vesicles, are also fabricated using this single-amphiphile system. The system's incorporation of homopolymer to modify the properties and morphology of the resultant structures is a convenient strategy for preparing self-assembled macromolecular structures with controllable properties.

Introduction

In solution, amphiphilic molecules self-assemble into a rich array of intriguing and useful structures. Self-assembled structures in aqueous solution are useful for many encapsulation and delivery technologies. Such structures include vesicles, multiple emulsions, and micelles. A vesicle encloses an internal aqueous space in one or more bilayers of amphiphilic molecules; vesicles can encapsulate, protect, and release active agents.[1−4] A multiple emulsion contains internal aqueous droplets within a larger hydrophobic sphere; multiple emulsions can be used for encapsulation and extended release of hydrophobic and hydrophilic substances.[5−9] A micelle encloses a hydrophobic region via a spherical monolayer of amphiphiles with core-directed hydrophobic groups; micelles can solubilize hydrophobic substances in water and encapsulate nonaqueous contents for delivery in aqueous media. Self-assembled structures are often formed using small amphiphiles, such as lipids and surfactants,[10] but structures made using these materials have limited strength and stability. Ideal structures for encapsulation and release should have well-characterized and broadly adjustable stability, permeability, and mechanical strength. Structures formed from diblock copolymers, which have great varieties of possible molecular architectures, block chemistry, and molecular weights, have a broader range of possible properties than analogous structures made with lipids and small surfactants. Vesicles made from diblock copolymer are much tougher than their lipid counterparts.[11,12] The thickness and stability of the polymer membrane scale with the copolymer molecular weight.[13] This membrane's properties can be further modified by cross-polymerization.[14]

Beyond modifying vesicle membrane properties, the morphologies of self-assembled structures may also be adjusted using various methods. The morphology of self-assembled amphiphile aggregates in a given solution environment is governed by the geometric packing criteria that determine the interfacial curvature.[10] This curvature can be modified by adjusting the effective volume of the hydrophilic or the hydrophobic portion of the amphiphile. One strategy for forming polymer vesicles requires the synthesis of a specialty copolymer with a block ratio that favors vesicle structure.[15,16] Desired morphologies may also be achieved by manipulating the effective size of the amphiphile's hydrophilic region by adding salts to the solution environment.[17] Organic solvents have also been used to influence the formation of various types of aggregates.[18,19] Diblock copolymer systems offer an additional yet hitherto unexplored advantage: hydrophobic homopolymer can act as a building block for engineering structures with a broad range of properties by being incorporated into hydrophobic regions of self-assemblies, modifying membrane thickness and generating new morphologies.

In this paper, we explore this new route by fabricating structures that contain hydrophobic homopolymer and diblock copolymer. The homopolymer is incorporated, or "stuffed," within vesicle membrane bilayers; the membrane thickness depends on the amount of homopolymer in the membrane. Still more homopolymer may be incorporated to form multiple emulsions, containing several small drops of water within a larger sphere of hydrophobic polymer with interfaces stabilized by the diblock. This method for fabricating multiple emulsions requires only a one-step process and a single macromolecular surfactant. These stuffed vesicles and multiple emulsions represent a new class of self-assembled aggregate. Such structures have high potential for allowing control of structural properties and for encapsulating and releasing hydrophilic and hydropho-

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bic active agents in a controlled and adaptable fashion.

Experimental Section

Materials and Methods. We make and stabilize structures using the diblock copolymer poly(butyl acrylate)-*b*-poly(acrylic acid) (PBA−PAA), molecular weight 15 000 g/mol; this copolymer is synthesized by Madix radical polymerization.[20] The two blocks determine the amphiphilic character of the macromolecule: PAA is hydrophilic and PBA hydrophobic. For "stuffing", we use the homopolymer poly(butyl acrylate) (hPBA), chemically identical to the hydrophobic diblock region. Because the glass transition temperature, T_g, for PBA is −40 °C, these self-assembled structures are soft at room temperature. We prepare structures using two different techniques which are analogous to techniques commonly used for preparing phospholipid self-assemblies. The first technique is the dispersion of a powder in water; vortexing to mix the sample, we disperse a freeze-dried powder of the block copolymer without homopolymer in water or in an aqueous solution of tetrahydrofuran (THF) at 1 wt % to achieve a final polymer concentration of 0.5−1%. The second technique is rehydration of a dried film of polymer. To prepare a film for rehydration, stock solutions of PBA−PAA and hPBA are prepared in THF at concentrations 5 mg/mL and mixed at varying compositions of hPBA; we form thin films by pipetting 25 μL of each solution into a glass vial and evaporating the solvent. These films are then rehydrated with 250 μL of deionized water under bubbling nitrogen for 1 h.

Results and Discussion

The structures of the films to be rehydrated are evaluated by small-angle X-ray scattering (SAXS) and observation of film birefringence.[21] Films of 70−30 copolymers and films of 50−50 copolymers both display optical birefringence when they are observed under crossed polarizers. SAXS was performed at the University of Pennsylvania in the Laboratory for Research on the Structure of Matter. The SAXS spectrum of the 50−50 birefringent film has peaks at q ratios 1:2, which is indicative of a lamellar film morphology.[22] The SAXS spectrum of the 70−30 film has peaks at q ratios 1:$\sqrt{7}$, which is characteristic of cores of size 98 Å.[22] In combination with observations of film birefringence, this confirms that the 70−30 film contains inverted cylindrical structures of PAA cores in a hydrophobic PBA continuous phase.

The structure of these films is directly reflected in the structure of the assemblies formed upon rehydration. When a film of 50−50 diblock with no homopolymer is rehydrated and when a powder of symmetric 50−50 diblock is dispersed in water,[23] only micelles form. Micelles formed by film rehydration are imaged by transmission electron microscopy; a typical image is shown in Figure 1. When films of 70−30 diblock with no homopolymer are rehydrated, vesicles form. When a powder of 70−30 diblock is dispersed in a water−plasticizer solution (1 wt % THF), vesicles with diameters as large as tens of microns form.

We investigate the mechanical properties of these large vesicles using micropipet aspiration;[24] an example of such a vesicle being aspirated is shown in Figure 2. When a vesicle with initial surface area A is aspirated, the change in length of the vesicle projection in the micropipet, ΔL, is proportional, to first order, to the change in area of the vesicle, ΔA:

$$\Delta A \approx \pi D_p(1 - D_p/D_v)\Delta L$$

The diameters of the micropipet, D_p, and vesicle, D_v, determine the tension τ at any given applied aspiration pressure P:[24]

$$\tau = PD_p/[4(1 - D_p/D_v)]$$

The aspiration-induced membrane areal strain, $\Delta A/A$, increases linearly as a function of increasing membrane tension, as shown in Figure 3. The inverse slope of these data indicates an areal expansion modulus of about 1000 mN/m. Vesicles are aspirated until failure due to rupture; this occurs at a tension of about 40−60 mN/m, and their areal strain at rupture is about 5%. This areal expansion modulus is an order of magnitude greater than moduli of PEO−PEE and lipid vesicles,[11] and this rupture tension is about 2 times greater than that of PEO−PEE polymersomes[11] and about 5−10 times greater than those of typical lipid vesicles.[24] This strain at rupture is comparable to critical strains for

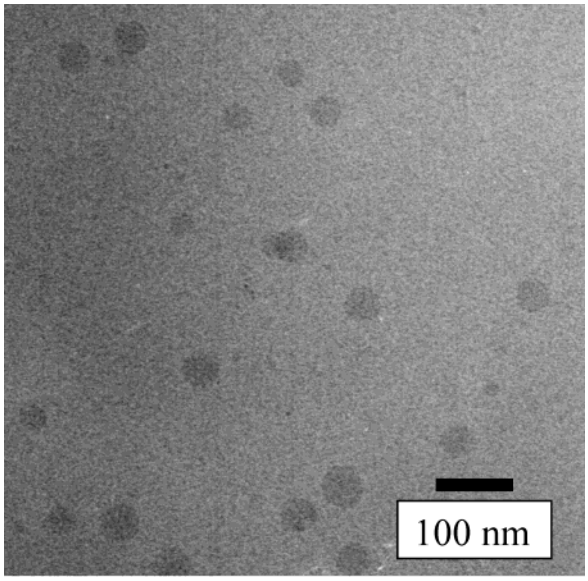

Figure 1. TEM imaging of a sample made by rehydrating 50−50 PBA−PAA from a film into dilute aqueous solution shows micelles.

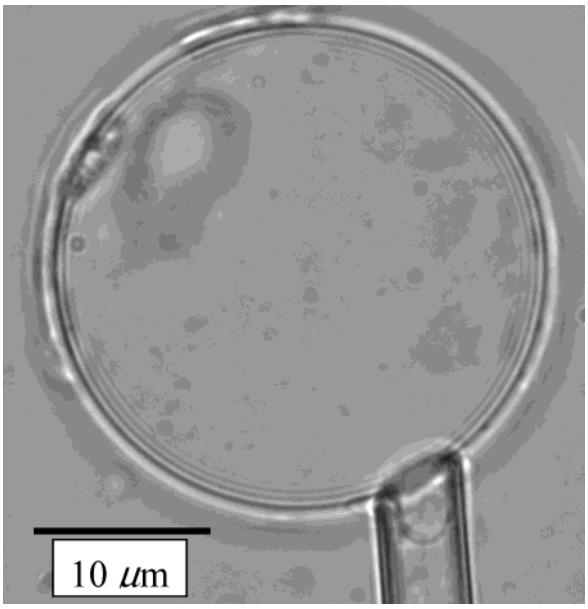

Figure 2. Vesicles made by dispersing a powder of 70−30 PBA−PAA in 1% aqueous solution of THF are aspirated into micropipets to measure membrane mechanical response.

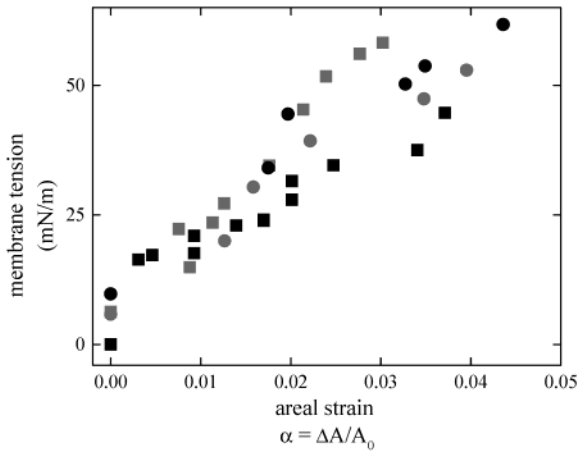

Figure 3. We use micropipet aspiration to probe the areal expansion of 70−30 PBA−PAA vesicle membranes as membrane tension increases. Distinct symbols represent different vesicles. The expansion modulus, $K_a = \Delta\tau/\Delta\alpha$, is approximately 1000 mN/m. Vesicles are aspirated until failure; vesicles rupture at a tension of about 40−60 mN/m, and their areal strain at rupture is about 5%.

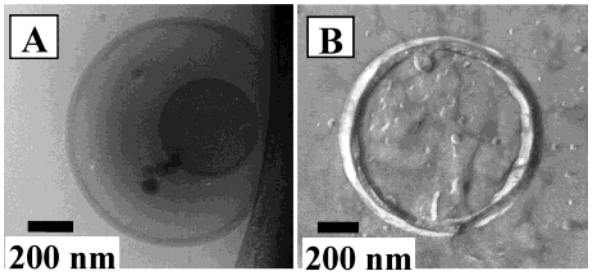

Figure 4. (A) Superposition of two vesicles made by dispersing 70−30 PBA−PAA powder in water show a membrane thickness of ∼50 nm when examined by cryogenic transmission electron microscopy. (B) Freeze−fracture electron microscopy of a 70−30 PBA−PAA vesicle made by film rehydration shows additional evidence that these structures have a hollow interior.

lipid vesicles but is much less than PEO−PEE vesicles' critical strain of 20%. Recent studies of polymer vesicle elasticity and stability indicate the onset of chain entanglement effects at molecular weights higher than 10 000 g/mol,[13] and the PBA−PAA diblock used here has a hydrophobic PBA block of 10 500 g/mol; the low critical strain of these PBA−PAA vesicles may result from chain entanglement of the hydrophobic regions of the diblock assemblies.

Vesicles made of 70−30 PBA−PAA are also probed with electron microscopy. Vesicles made by dispersing powder in water show a membrane thickness of ∼50 nm when imaged using cryogenic transmission electron microscopy (cryo-TEM), as Figure 4A shows; samples were prepared using a cryofixation technique. Freeze−fracture electron microscopy of vesicles made by film rehydration provides additional evidence that such structures are indeed hollow vesicles, as shown in Figure 4B.

Rehydration of films of 50−50 PBA−PAA with hPBA forms larger structures, "stuffed" vesicles and multiple emulsions, as well as smaller swollen micelles. These larger structures may be seen using optical microscopy, but we require electron microscopy to image swollen micelles; such electron micrographs are shown in Figure 5. Attempts to stuff the hydrophobic portion of the 70−30 PBA−PAA bilayer with hPBA are unsuccessful,

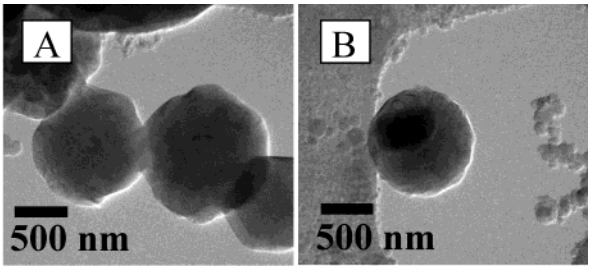

Figure 5. TEM images of samples made by rehydrating a polymer film composed of 50−50 PBA−PAA copolymer with 5% (A) and 20% (B) homopolymer PBA. These samples are characterized by polymer globules, which appear to be micelles swollen with hPBA.

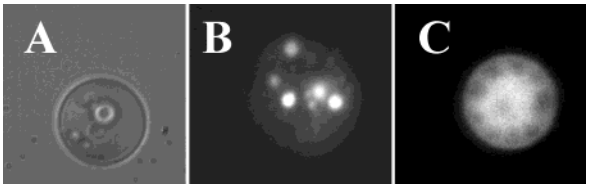

Figure 6. (A) A multiple emulsion drop is formed by rehydrating a film composed of 50−50 PBA−PAA and additional hPBA. (B) A multiple emulsion formed in water containing fluorescent dye shows internal droplets enclosing fluorescent water after quencher is added to the continuous aqueous phase. (C) A multiple emulsion formed from a film incorporating fluorescently tagged hPBA shows that the homopolymer is incorporated into the structure.

possibly as a result of macrophase separation of the homopolymer from the diblock.

In samples formed by rehydrating films of 50−50 PBA−PAA with hPBA, cryo-TEM shows a high number density of swollen micelles, suggesting that most of the homopolymer is emulsified into such micelles. Cumulant analysis of dynamic light scattering done at different scattering angles on hPBA-containing samples indicates sizes and polydispersity in agreement with those found by TEM. To confirm that most of the homopolymer is contained in swollen micelles, we make samples from films containing fluorescently tagged hPBA and use fluorimetry to measure sample fluorescence; the integrated fluorescence signal is proportional to the amount of homopolymer present. We then pass samples through a series of membrane filters, with smaller pores in each successive filter, and measure sample fluorescence after each filtering pass. The falloff in fluorescence with filtering indicates that most of the homopolymer is incorporated into structures between 100 and 800 nm in size. This agrees with the sizes indicated for swollen micelles by TEM images. Samples prepared with 5% and 20% hPBA show very similar decreases in intensity with decreasing filter pore size, indicating that micelle size does not vary significantly with the total homopolymer content of these samples. The preponderance of such swollen micelles demonstrates that these contain most of the hydrophobic homopolymer.

More intriguing than micelles, in the same samples we see microns-sized objects, vesicles and multiple emulsions; an image of a multiple emulsion taken using optical microscopy is shown in Figure 6A. To probe vesicle and multiple emulsion morphologies, we use fluorescence microscopy, rehydrating a film with aqueous solution of the fluorescent dye Dextran-Texas Red (10K, Molecular Probes Inc.). After structures form, encapsulating water and dye, the fluorescence signal from the bulk aqueous phase is then eliminated by

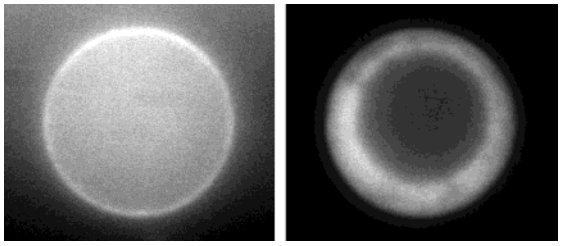

Figure 7. Fluorescent micrographs of vesicles formed by rehydrating films composed of 50−50 PBA−PAA with fluorescently tagged hPBA confirm that the homopolymer is incorporated into the membrane and that the amount incorporated can vary between vesicles.

addition of a quencher (anti-Texas Red, Molecular Probes Inc.). This allows us to visualize the encapsulated phase. For many structures the entire interior fluoresces, evidencing that these structures are vesicles, nonpermeable to the quencher. We also observe multiple emulsions in which discrete droplets fluoresce within the larger nonfluorescent structures, as seen in Figure 6B.

To probe the membranes and continuous structures of stuffed vesicles and multiple emulsions, we rehydrate films made with fluorescently tagged hPBA. Vesicles made in this way have fluorescent membranes, confirming that the hydrophobic homopolymer is incorporated into the membrane bilayer. Moreover, different amounts of hPBA are incorporated into different vesicles, varying the vesicle membrane thickness as the vesicles imaged in Figure 7 illustrate. Multiple emulsions have fluorescent continuous structures, but entrapped internal droplets do not fluoresce, as in Figure 6C. This demonstrates that multiple emulsions incorporate homopolymer in the hydrophobic regions of the continuous structures but not into discrete interior droplets. There is some indication that in some samples the amount of homopolymer in the film is reflected in the amount of homopolymer incorporated into microns-sized structures, so that rehydration of films containing more homopolymer results in thicker vesicle membranes and more multiple emulsions.

Our results present a novel type of macromolecular self-assembled structure with potentially tunable properties and demonstrate a novel one-step method for forming multiple emulsions with a single surfactant. Varying amounts of incorporated homopolymer observed in vesicles and multiple emulsions indicate that it should be possible to develop techniques for fabricating such structures that can control membrane thickness, tuning mechanical response, permeability, thermal stability, and other properties as may be required for particular technological applications.

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