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Microfluidic Fabrication of Pluronic Vesicles with Controlled Permeability

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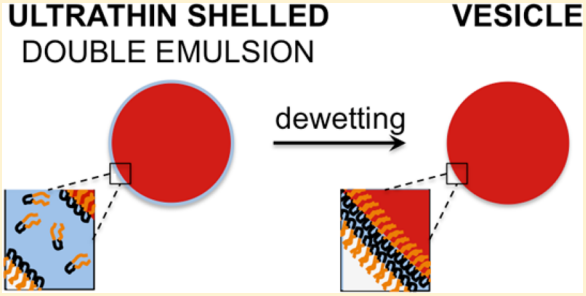
Supporting Information

ABSTRACT: Block copolymers with a low hydrophilic-to-lipophilic balance form membranes that are highly permeable to hydrophilic molecules. Polymersomes with this type of membrane enable the controllable release of molecules without membrane rupture. However, these polymersomes are difficult to assemble because of their low hydrophobicity. Here, we report a microfluidic approach to the production of these polymersomes using double-emulsion drops with ultrathin shells as templates. The small thickness of the middle oil phase enables the attraction of the hydrophobic blocks of the polymers adsorbed at each of the oil/water interfaces of the double emulsions; this results in the dewetting of the oil from the surface of the innermost water drops of the double emulsions and the ultimate formation of the polymersome. This approach to polymersome fabrication enables control of the vesicle size and results in the efficient encapsulation of hydrophilic ingredients that can be released through the polymer membrane without membrane rupture. We apply our approach to the fabrication of Pluronic L121 vesicles and characterize the permeability of their membranes. Furthermore, we show that membrane permeability can be tuned by blending different Pluronic polymers. Our work thus describes a route to producing Pluronic vesicles that are useful for the controlled release of hydrophilic ingredients.



INTRODUCTION

Polymer vesicles, or polymersomes, are aqueous droplets stabilized by an amphiphilic block copolymer membrane.[1−4] These are promising delivery vehicles for many different types of cargo such as drugs and cosmetics;[5−7] these applications depend critically on the mechanical stability and permeability of the polymer membrane, parameters that can be tuned by varying the molecular weight and chemical nature of the block copolymers.[3,8] For example, increasing the molecular weight of the polymer yields polymersomes with thicker membranes; these are more stable and less permeable than those with thinner membranes.[2] Although highly stable polymersomes are desirable for encapsulation,[9] their cargo can be released only by introducing pores into the membrane[10] or rupturing it completely[11] because their permeability is extremely low; this burst release is one of the major limitations in the development of vehicles for controlled release.[12] To overcome this limitation, the chemical nature of the block copolymer must be adjusted. Unfortunately, the ratio of hydrophilic to hydrophobic blocks required for vesicle formation is quite narrow and cannot be varied.[3,13] Polymersome permeability, therefore, must be controlled by adjusting the hydrophilic-to-lipophilic balance (HLB) of the block copolymer that forms the polymersome membrane. If the HLB is low, then the core of the polymer membrane is only slightly hydrophobic, and this results in polymersomes with enhanced permeability compared to that of those with highly hydrophobic membrane cores. Block copolymers of polyoxyethylene-polyoxypropylene-polyoxyethylene (PEO-PPO-PEO), commonly known under the trademark Pluronic, have low HLB values and are thus ideal for fabricating such highly permeable membranes. In addition, they are biocompatible.[14] However, because these membranes are only slightly hydrophobic, they are difficult to assemble. Conventionally, small Pluronic vesicles, with diameters of tens of nanometers, are assembled by hydration, and their sizes are subsequently homogenized by extrusion.[15−17] However, this method yields encapsulation efficiencies lower than 35% and vesicles that lack the stability required for use as delivery vehicles. Their stability increases if they are assembled in the presence of silklike proteins[18] or core cross-linked polymer micelles.[19] Alternatively, large Pluronic vesicles can be prepared by hydration[20] or electroformation.[21] These have a higher

Received: April 13, 2016
Revised: May 9, 2016
Published: May 18, 2016

DOI: 10.1021/acs.langmuir.6b01399
Langmuir 2016, 32, 5350−5355

Table 1. Properties of the Three Pluronic Polymers Used in This Study and Compositions C1−4[a] Used to Fabricate the Different Pluronic Membranes

commercial name	chemical name	chemical formula	Mw (g/mol)	f	HLB	C1	C2	C3	C4
Pluronic L121	Poloxamer	$PEO_5PPO_{67}PEO_5$	4400	0.12	1-7	100	59	46	58
Pluronic L101	Poloxamer 331	$PEO_4PPO_{58}PEO_4$	3800	0.12	1-7	0	41	54	0
Pluronic L61	Poloxamer 181	$PEO_2PPO_{30}PEO_2$	2000	0.12	3	0	0	0	42

[a]Expressed in mole %.

loading capacity for hydrophilic ingredients than do small vesicles; however, this method still results in a low encapsulation efficiency. Microfluidic technologies overcome many of these limitations. Monodisperse water-in-oil-in-water (W/O/W) double-emulsion drops with relatively thick shells can be used as templates to form Pluronic vesicles through the occasional spreading of the oil phase of the double emulsions at the air/water interface.[22] Unfortunately, the yield of this technique is relatively low, and in addition to vesicles, aggregates of vesicles are formed. Thus, despite the potential of Pluronic vesicles for encapsulation and controlled release, their widespread use remains severely restricted because of the limitations in their fabrication.

In this article, we report a microfluidic approach to the production of monodisperse Pluronic vesicles using water-in-oil-in-water (W/O/W) double-emulsion drops with ultrathin shells as templates to produce stable vesicles with controllable permeability and high encapsulation efficiency. Importantly, these oil shells are ultrathin;[23] this enhances the stability of the double-emulsion drops and facilitates the dewetting process that transforms the emulsion drops into vesicles. The dewetting transition is triggered by the attraction between the two polymer monolayers adsorbed at the surfaces of the thin oil shell[24−26] and requires that random fluctuations bring them into contact;[27] this is difficult to achieve in typical double-emulsion templates because their shells are significantly thicker.[22] With our technique, we efficiently encapsulate a model hydrophilic cargo, a small fluorescent compound, in the cores of the vesicles and characterize the permeability of these membranes. Moreover, we show that membrane permeability can be tuned by blending polymer Pluronic L121 with different amounts of other Pluronic polymers such as Pluronic L61; this blending strategy provides a straightforward means for controlling material release. Our work thus provides an approach to Pluronic vesicle fabrication that enables control of the vesicle size and results in efficient encapsulation of hydrophilic ingredients; this further enhances the potential of membranes with low HLB values for the controlled release of hydrophilic cargo.

EXPERIMENTAL SECTION

Materials. We fabricate the polymersome membranes with Pluronic L121, Pluronic L101, and Pluronic L61 (BASF), at the compositions detailed in Table 1. All of these Pluronic polymers are liquid at room temperature, as indicated by the first digit of their names: L. The last digit of their names, which is 1 in all cases, indicates the mass fraction of the PEO block, which is 10% for the three Pluronic polymers considered in this study. The digits between the letter and the last digit indicate the length of their PPO central block; multiplying this number by 300 gives the approximate molecular weight of the PPO block.[28] Therefore, from the molecular weight of the polymer and the information contained in their commercial names, we calculate the chemical formula of each polymer, which is shown in the third column of Table 1. The densities of the PEO and PPO blocks are 1.13 and 1.036 g/cm^3, respectively.[21] Therefore, we calculate a volume ratio of the hydrophilic to hydrophobic block, f, of 0.12 for the three Pluronic polymers used in this study; this value points to their potential as vesicle-forming polymers.

We use mixtures of chloroform and hexane (Sigma) as a solvent for the Pluronic polymers. For the inner and outer aqueous phases, we dissolve poly(ethylene glycol) (PEG, Mw = 6 kDa, Sigma) and poly(vinyl alcohol) (PVA, Mw = 13−23 kDa, Sigma) in Milli-Q water (Millipore, resistivity of 18.2 M Ω /cm) to 10 wt % each; these solutions are then filtered through 5 μm filters (Acrodisc) before their injection into the microfluidic device. We purchase the phosphate-buffered saline (PBS) solution that we use as the collection phase from Sigma. We add sulforhodamine B (Sigma) to the PEG solution to determine the permeability of the membrane from fluorescence intensity measurements.

Osmolarity. We measure the osmolarity of the inner, outer, and collection phases using a Micro-Osmometer (Advanced Instruments, Inc.); these are 100, 100, and 270 mOsm/L, respectively.

Fabrication of the Glass Capillary Device. A square capillary (Atlantic International Technology, Inc.) of inner dimension 1.05 mm and length 40 mm is fixed onto a microscope slide using 5 min Epoxy (Devcon). We then simultaneously heat and pull a cylindrical capillary of inner and outer diameters 0.58 and 1.00 mm, respectively (World Precision Instruments, Inc.), using a micropipette puller (P-97, Sutter Instrument, Inc.). The resultant diameter of the tip after pulling is 20 μm. We use sand paper to carefully sand the tips of the cylindrical capillaries to final diameters of approximately 80 and 150 μm for injection and collection, respectively. The injection capillary is treated with *n*-octadecyl-trimethoxysilane (Aldrich) to render its surface hydrophobic, and the collection capillary, with 2-[methoxy (polyethyleneoxy)propyl]trimethoxysilane (Gelest, Inc.) to render its surface hydrophilic. For this treatment, the tips of the capillaries are immersed in the silane solutions for 20 min. They are then dried using compressed air. We insert the two tapered capillaries in the opposite ends of the square capillary and align them on the microscope, maintaining a separation distance between them of approximately 80 μm. After alignment, we fix them in place with epoxy glue. Next, we stretch another cylindrical capillary using a flame and insert it into the injection cylindrical capillary. Finally, we place dispensing needles (type 304, McMaster-Carr) at the junctions between capillaries and their ends and fix them to the slide with epoxy glue. These needles are used to inject the different liquid phases.

Operation of the Microfluidic Device. We pour the inner and outer aqueous phases into 10 mL plastic syringes (Becton) and the middle oil phase into a 5 mL glass syringe (Hamilton). We connect the needles of the syringes to the needles of the device with polyethylene tubing of inner diameter 0.86 mm (Scientific Commodities, Inc.). We inject the liquid phases at constant flow rates using pumps (Harvard Apparatus). We work in the discontinuous dripping regime; this regime produces single-emulsion drops and double-emulsion drops with ultrathin shells alternately. The single-emulsion drops are separated from the double-emulsion drops with ultrathin shells upon collection as a result of their different densities. Double-emulsion drops rapidly sink, whereas single-emulsion drops remain at the top of the collection vial; this collection vial contains a large volumetric excess of PBS.

Imaging. We record the microfluidic production of double-emulsion drops using a 5× dry objective on an inverted microscope (Leica) equipped with a high-speed camera (Phantom V9). We monitor the formation of the vesicles from the double-emulsion drops and their changes in size using bright field microscopy. Simultaneously,

DOI: 10.1021/acs.langmuir.6b01399
Langmuir 2016, 32, 5350−5355

we monitor the evolution of the fluorescence intensity in the core of the vesicles using confocal fluorescence microscopy. This simultaneous acquisition of bright field and confocal fluorescence micrographs is performed on a confocal fluorescence microscope (Leica) using a 10× dry objective with a numerical aperture of 0.3. The excitation source is a HeNe (543 nm) laser. The fluorescence emission is collected by the PMT through a bandpass filter between 560 and 670 nm. All of our experiments are performed at room temperature.

Image Analysis. We use a custom MATLAB code to measure the size and average fluorescence intensity of the vesicles. The code identifies circles within the confocal slices based upon the fluorescence intensity and then combines the circles to recreate spheres. The radius of the sphere is taken from the equatorial plane.

RESULTS

We use a glass capillary microfluidic device with coflow geometry to fabricate double-emulsion drops with ultrathin shells.[23] The device consists of two tapered cylindrical capillaries inserted into the opposite ends of a square capillary whose inner dimension fits the outer diameter of the cylindrical capillaries; this configuration aligns the axes of the cylindrical capillaries. The injection capillary, on the left, contains an additional smaller cylindrical capillary, as illustrated in Figure 1A, to inject the innermost aqueous phase; this consists of an aqueous solution of 10 wt % poly(ethylene glycol) (PEG, 6 kDa) and also contains 4 μg/mL sulforhodamine B (0.56 kDa) as model hydrophilic cargo. To avoid wetting of the innermost aqueous phase onto the capillary wall, we coat the injection capillary with *n*-octadecyl trimethoxysilane to make its surface hydrophobic. We use the injection capillary to inject the middle oil phase, which is a solution of 20 wt % Pluronic L121 in a mixture of 36 vol % chloroform and 64 vol % hexane. Both the innermost and middle phases flow at 0.5 mL/h, forming a train of large water emulsion drops, spaced and surrounded by oil. The interstices between the outer wall of the cylindrical injection capillary and the inner wall of the square capillary are used to inject the outer aqueous phase, which is a 10 wt % poly(vinyl alcohol) (PVA, 13−23 kDa) aqueous solution. This phase flows at a typical flow rate of 4 mL/h, forcing the water drops to break up at the capillary tip, and yields double-emulsion drops with ultrathin shells. The oil that separates the large water emulsion drops also breaks up at the capillary tip, thereby yielding single-emulsion drops.[23] Double- and single-emulsion drops flow through the collection capillary, on the right, toward the device outlet and are then collected in a vial, which contains a large volumetric excess of sodium phosphate buffered saline solution (PBS, pH 7.4). Double-emulsion drops sink rapidly because of the presence of PEG within their cores; this enables us to separate them from the single-emulsion drops, which remain on the surface of the buffered solution as a result of their lower density. The double emulsions are monodisperse in size and efficiently encapsulate the model hydrophilic cargo as shown in the confocal fluorescence microscope image of Figure 1B. The typical coefficient of variation, CV, in size is lower than 5% and in fluorescence intensity is lower than 10%, as shown in Figure 1C,D, respectively.

We use these double-emulsion drops as vesicle templates. Importantly, to facilitate the formation of vesicles through a dewetting transition, as illustrated in Figure 2A, the oil shell around the innermost water drop of the double emulsions must be ultrathin.[23,27] Also crucial for this dewetting transition is the chemical nature of the middle oil phase. We use a mixture of a highly volatile good solvent and a less volatile poor solvent for the Pluronic, which are chloroform and hexane, respectively. As the good solvent evaporates, the quality of the solvent is reduced and the polymer that remains dissolved in the hexane starts to precipitate; this ultimately induces the attraction of the two polymer monolayers adsorbed at the interfaces of the oil shell,[24−26] when a random fluctuation bring them closer.[27] This

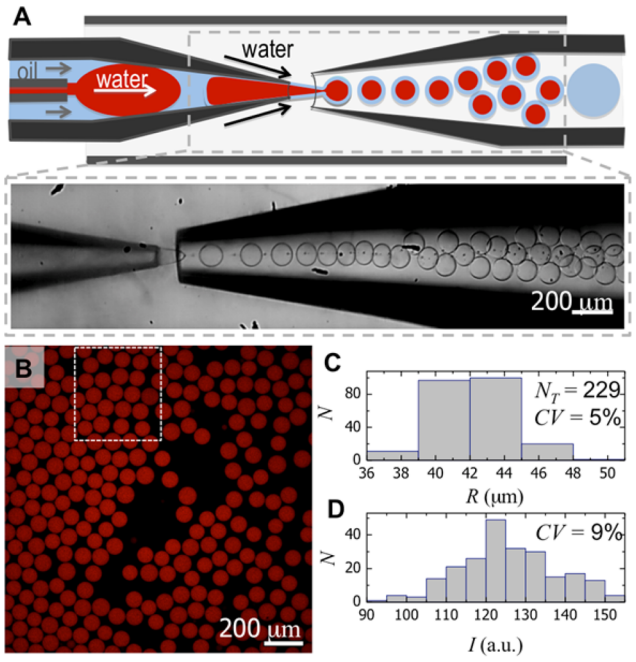


Figure 1. (A) Schematic illustration (top) and optical microscope image (bottom) of the microfluidic device used to produce double-emulsion drops with ultrathin shells. (B) Confocal fluorescence microscope image of the equatorial plane of double-emulsion drops encapsulating sulforhodamine B. (C, D) Distribution of the radius and fluorescence intensity, respectively, of the double-emulsion drops.

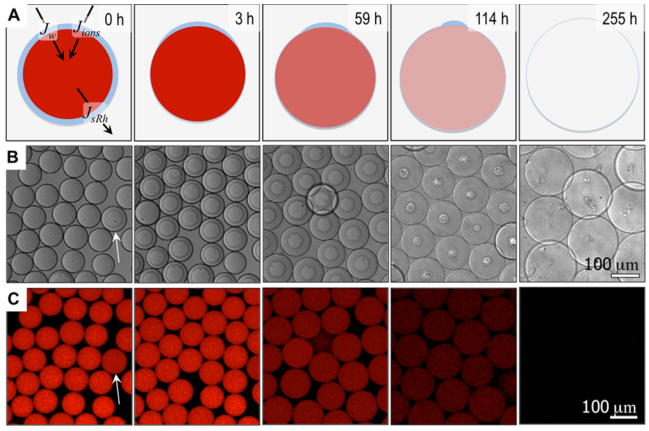


Figure 2. (A) Schematic illustration of the time evolution of the size and fluorescence intensity of the Pluronic vesicles due to an inflow of water and ions and an outflow of sulforhodamine B. (B) Time series of bright field images showing incomplete dewetting of the middle-phase solvent, further evaporation of the middle phase, and vesicle swelling. (C) Time series of confocal fluorescence images showing a decrease in the fluorescence intensity of the cores of the vesicles during vesicle swelling. The white arrow indicates a dewetting event that results in a decrease in fluorescence intensity.

DOI: 10.1021/acs.langmuir.6b01399
Langmuir 2016, 32, 5350−5355

transition occurs over the duration of several minutes, as exemplified by the white arrow in the first optical microscope image of Figure 2B. The oil then accumulates at the top part of the drops and continues to evaporate over the course of several hours as shown in the second and third optical microscope images of Figure 2B. The resultant Pluronic vesicles thus retain a small oil pocket at the top. The oil evaporates further over the course of several days, through diffusion into the outer aqueous phase, thereby leaving an aggregate of polymer in the membrane region of the oil pocket, as shown in the fourth optical microscope image of Figure 2B. This polymer reservoir disappears upon swelling of the vesicle, as shown in the last optical microscope image of Figure 2B.

We thus expect the membrane formed by this approach to be a bilayer; however, because of the triblock nature of Pluronic polymers, it is possible that they rearrange into a monolayer upon dewetting of the middle-phase solvent. Assuming that the Pluronic membrane is a bilayer, we can estimate the thickness of the Pluronic membrane, t, on the basis of the scaling with polymer molecular weight, Mw, known for other amphiphilic polymers: $t \propto Mw^{0.55}$;[29] this yields an estimate for the thickness of the membrane of Pluronic L121 of approximately 11 nm. We expect this thickness to be independent of the initial polymer concentration as excess polymer accumulates at the top of the vesicle upon dewetting of the middle-phase solvent and to disappear upon vesicle swelling. However, the polymer concentration needs to be sufficient to yield stable templates and not too high because this causes vesicle destabilization. This behavior is in stark contrast to that of capsules with polymer membranes, whose thickness depends on the polymer concentration and the thickness of the double-emulsion template; this is due to the different nature of the polymers used for vesicle and capsule formation. Polymers that form capsules are not amphiphilic and thus self-arrange in a structure different from a bilayer upon evaporation of the middle-phase solvent of the double-emulsion template. Polymersome stability depends on the polymer molecular weight and chemical nature of the block copolymers. Therefore, because the Mw of polymers is larger than that of lipids, polymersomes are in general more stable than liposomes. However, because of the weak hydrophobic nature of the midblock of Pluronic polymers, Pluronic polymersomes are less stable than polymersomes formed by other polymers with more hydrophobic midblocks. Nevertheless, Pluronic L121 polymersomes formed using our approach are stable for more than 10 days, unlike those formed by hydration.[15]

We observe that the size of the double-emulsion drops increases during the duration of vesicle formation, as shown in Figure 2(B); this is due to the inflow of water into the cores of the vesicles. Because the osmolarity of the innermost water drops of the double emulsions equilibrates rapidly to that of the outer solution, the observed water inflow indicates that permeating solutes, driven by a gradient in chemical potential, are crossing the membrane to penetrate the vesicle cores; this creates an osmotic imbalance, which is restored by an instantaneous inflow of water. The rate of the water inflow, obtained as the variation of the vesicle radius over time, shown in Figure 3A, provides a measurement of the permeability of the Pluronic membrane to the permeating solutes initially dissolved in the outer solution. Because the outer water phase is a sodium phosphate buffered solution, it is likely that the ions in this solution penetrate the Pluronic membrane. Within this picture, the vesicle radius should increase in time as

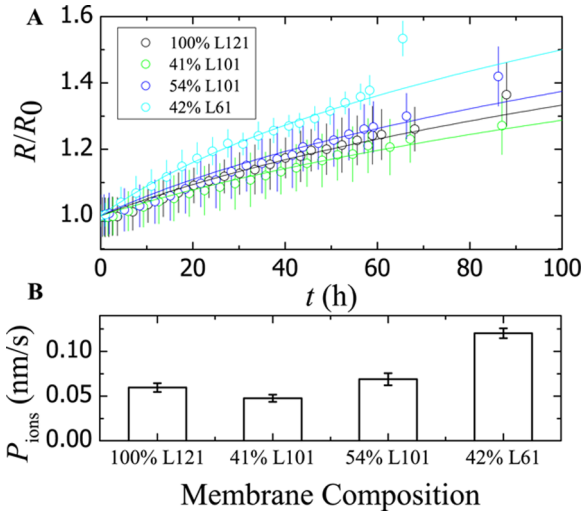


Figure 3. (A) Time evolution of the size of the vesicle relative to the initial size of the double emulsions for different membrane compositions. The curves indicate the best fit to eq 1. (B) Permeability to ions of the buffered saline solution as a function of membrane composition. All of the compositions include Pluronic L121.

$$\frac{R}{R_0} = \left(1 + \frac{P_{ions} t}{R_0}\right)^{1/4} \quad (1)$$

where R_0 is the initial radius of the vesicles and P_{ions} quantifies the permeability of the vesicle membrane to the ions in the outer solution, as further detailed in the Supporting Information.[30,31] Therefore, by fitting our experimental data to eq 1, we calculate the permeability of the Pluronic L121 membrane to the ions, which is approximately 0.06 nm/s.

We also observe that the fluorescence intensity of the vesicles decreases over time as shown in Figure 2C. Vesicle swelling should result in dilution of the model hydrophilic cargo. From dilution, we expect the total fluorescence intensity of the vesicles, I_T, defined as the average intensity of a vesicle multiplied by its volume, to remain constant over time. However, we observe an exponential decrease in the total fluorescence intensity over time as shown in Figure 4A; this indicates that the cargo penetrates the membrane and is successfully released into the outer aqueous phase. This outer phase acts as a sink of material. Therefore, we do not need to flush the outer solution in the course of the measurement. Theoretically, we expect the total intensity of the innermost water core of the vesicles to vary as

$$\frac{I_T}{I_T(0)} = \exp\left(-\frac{P_{sRh} t}{2R_0}\right) \quad (2)$$

where P_{sRh} is the membrane permeability to sulforhodamine B, as detailed in the Supporting Information. By fitting our experimental data to eq 2, we calculate the membrane permeability of the Pluronic L121 membrane, which is approximately 0.36 nm/s. This value corresponds to a characteristic release time, $\tau = 2R_0/P_{sRh}$, of approximately 60 h. Therefore, our Pluronic vesicles may be used as delivery vehicles for the release of hydrophilic molecules with a typical molecular weight of 500 Da, which corresponds to a typical size of approximately 1 nm, over the course of several days. This permeability value is close to that measured for phospholipid vesicles with α-hemolysin pores.[27]

DOI: 10.1021/acs.langmuir.6b01399
Langmuir 2016, 32, 5350−5355

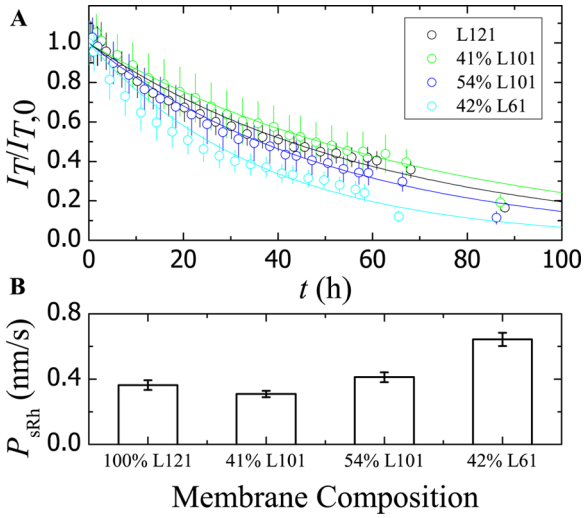


Figure 4. (A) Time evolution of the average fluorescence intensity of the vesicle relative to the initial average fluorescence intensity of the double emulsions for different membrane compositions. The curves indicate the best fit to eq 2. (B) Permeability to sulforhodamine B as a function of membrane composition. All of the compositions include Pluronic L121.

To control the duration of this release, we modify the permeability of the vesicle membrane by blending Pluronic L121 with another Pluronic polymer. We use Pluronic L61 as a blender because it is insoluble in water and it has HLB and f values similar to those of Pluronic L121 but its molecular weight is lower than that of Pluronic L121. This decrease in molecular weight results in a decrease in membrane thickness. On the basis of this scaling,[29] we estimate that changing the polymer from Pluronic L121 to Pluronic L61 produces a decrease in membrane thickness from 11 to 7 nm. We hypothesize that this decrease in the thickness of the vesicle membrane may increase membrane permeability, thereby accelerating material release. To test this hypothesis, we monitor the radius and total fluorescence intensity over time for vesicles with membranes composed of a mixture of 58 mol % Pluronic L121 and 42 mol % Pluronic L61. We observe that the variation of the vesicle size and fluorescence intensity over time of this membrane composition is similar to that of the pure Pluronic L121 membrane, as shown in Figures 3A and 4A. Therefore, we again use eqs 1 and 2 to determine the permeability of the membrane to both ions and sulforhodamine B molecules, as shown in Figures 3B and 4B, respectively. Our data show that the permeability to ions doubles upon introduction of 42 mol % Pluronic L61 into the Pluronic L121 membrane. We also show that the permeability to sulforhodamine B molecules slightly increases for the Pluronic L61 blend, which results in a characteristic release time about half of that of the pure Pluronic L121 membranes. Therefore, the use of mixtures of Pluronic L121 and Pluronic L61 at different molar fractions may provide a good strategy for finely tuning membrane permeability. In contrast to the effect of Pluronic L61, the incorporation of Pluronic L101 into Pluronic L121 has little effect on membrane permeability, as shown in Figures 3 and 4, for the compositions detailed in Table 1 because the molecular weights of these two polymers are similar.

CONCLUSIONS

We report a microfluidic technique for the production of monodisperse Pluronic vesicles. We use W/O/W double-emulsion drops with ultrathin shells as templates to form vesicles through dewetting of the oil from the surface of the innermost water drop of the double emulsions. This transition is facilitated by the small thickness of the oil shell, which enhances the stability of the template and enables the attraction of the two monolayers initially adsorbed at the interfaces of the thin oil shells. Using this technique, we efficiently encapsulate a model hydrophilic cargo, a fluorescent compound, in the cores of the vesicles to study the release of material through different Pluronic membranes. We show that membranes composed of pure Pluronic L121 are ideal for the slow release of small molecules. Moreover, we show that the release can be accelerated by blending Pluronic L121 with Pluronic L61, a polymer with lower molecular weight that yields thinner membranes. Moreover, our blending strategy can be applied to produce Pluronic vesicles using a wide range of polymer compositions; this may impart additional functionalities to the vesicles such as responsiveness to pH and temperature. Our work thus describes a route to produce Pluronic vesicles useful for the slow and controlled release of hydrophilic cargo.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.6b01399.

Further details on the formalism used to obtain permeability values from the variations of vesicle radius and fluorescence intensity over time (PDF)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the National Science Foundation (DMR-1310266), the Harvard Materials Research Science and Engineering Center (DMR-1420570), and a Marie Curie International Outgoing Fellowship within the EU Seventh Framework Programme for Research and Technological Development (2007−2013). D.F.d.N. was supported by a scholarship from CAPES, CNPq, and Ciencias Sem Fronteiras.

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DOI: 10.1021/acs.langmuir.6b01399
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DOI: 10.1021/acs.langmuir.6b01399
Langmuir 2016, 32, 5350−5355