

Preparation of Surfactant-Resistant Polymersomes with Ultrathick Membranes through RAFT Dispersion Polymerization

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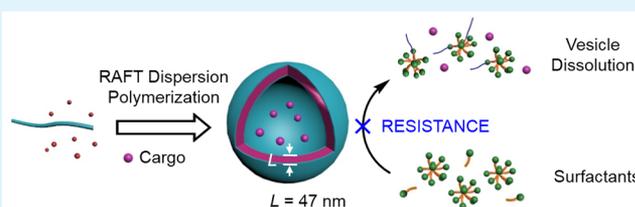
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S Supporting Information

ABSTRACT: Surfactant-resistant polymersomes have substantial potential to be used as delivery vehicles in industrial applications. Herein, we report the preparation of poly(ethylene oxide)-*block*-polystyrene copolymers with ultrahigh hydrophobic-block molecular weights through RAFT dispersion polymerization, which allows the polymerization-induced self-assembly into well-defined polymersomes with ultrathick membranes up to ~47 nm. These ultrathick membranes significantly enhance the resistance against surfactant solubilization of the vesicles, improving the vesicles' potential for use in industrial encapsulations. Vesicle-encapsulated actives are well retained in the presence of up to 40 wt % of various anionic and nonionic surfactants, with less than 7% active leakage being observed after 30 days.

KEYWORDS: polymersome, anionic and nonionic surfactants, surfactant resistance, RAFT dispersion polymerization, membrane thickness



Polymersomes (polymeric vesicles) have generated significant interest over the past few decades as a promising structural motif for the encapsulation and delivery of both hydrophobic and hydrophilic cargoes.^{1–4} Despite their enhanced toughness, higher membrane viscosity, and larger bending modulus as compared to liposomes,^{1,5,6} polymersomes remain leaky and susceptible to disassembly in the presence of surfactants, which are present in many industrial formulations where polymersomes are desired.^{7–9} The amphiphilic structure of surfactant molecules destabilizes polymersomes by solubilizing the diblock copolymer amphiphiles into micelles.¹⁰ Although cross-linkable functional handles incorporated into the membrane of polymersomes has improved the resistance to surfactants,^{11–13} this postassembly strategy requires the additional chemical modification of monomers or polymers that are expensive and time-consuming.

As an alternative to cross-linking, it is also possible to improve the resistance to surfactants by increasing the polymersome membrane thickness. As reported by Pata and co-workers, the dominant mechanism for surfactant-induced dissolution of polymersomes is surfactant transport through the bilayer, which can be suppressed by thicker membranes.¹⁰ A linear relationship was observed between membrane thickness and the critical surfactant concentration required to disassemble 50% of polymersomes. The thickest polymersome membranes

evaluated, however, were only 14.8 nm; these vesicles were still disrupted in the presence of only 1 wt % of nonionic surfactant, Triton X-100.¹⁰

Although it has been reported that thicker membranes improve the resistance of vesicles to surfactants, it remains unknown whether the linear thickness-resistance trend will continue upon further increasing the thickness of polymersomes. This lack of knowledge is due to the difficulty in synthesizing thicker polymersomes. In this communication, we report the synthesis and surfactant resistance of ~47 nm thick polymersomes assembled from poly(ethylene oxide)-*block*-polystyrene (PEO-*b*-PSt) (Figure 1A). These assemblies showed negligible dissolution and good retention of encapsulated cargo in the presence of up to 40 wt % anionic and nonionic surfactants. To the best of our knowledge, this is the first report of surfactant-resistant polymersomes that can survive in such a surfactant rich environment.

Because the polymersome membrane thickness is related to the molecular weight (MW) of the hydrophobic block,⁵ we first attempted to synthesize PEO-*b*-PSt amphiphilic diblock copolymers with ultrahigh PSt MWs in order to achieve

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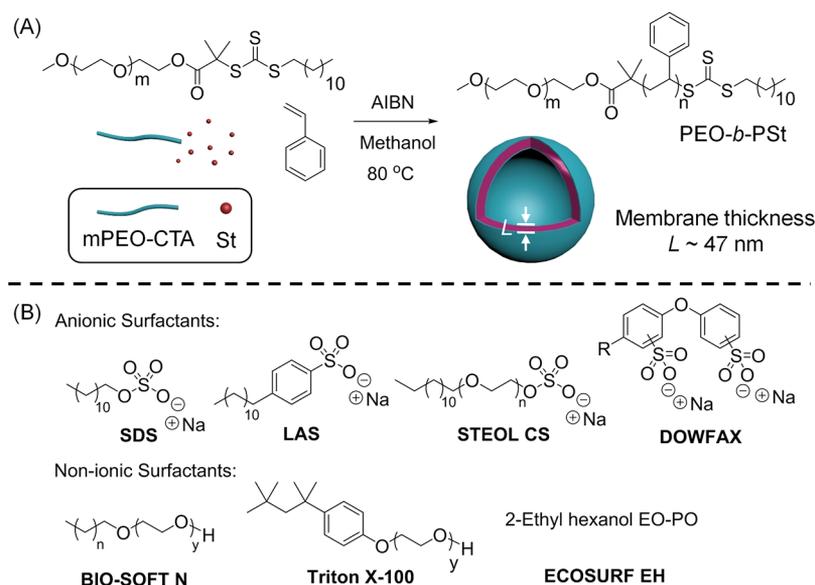


Figure 1. (A) Preparation of surfactant-resistant PEO-*b*-PSt polymersomes with ultrathick membranes through RAFT dispersion polymerization. (B) Chemical structures of anionic and nonionic surfactants tested in this study.

ultrathick polymersome membranes. The synthesis of these polymers in homogeneous systems, however, was difficult because of the low k_p value of styrene (St).¹⁴ Polymerization-induced self-assembly (PISA) was therefore selected instead to form PEO-*b*-PSt polymersomes during the polymerization, as PISA has been shown to be capable of synthesizing high MW polymers at an accelerated rate.^{15,16} In this technique, reversible addition–fragmentation chain transfer (RAFT) dispersion polymerization is used to prepare polymers with controlled MWs and narrow molecular weight distributions (MWDs).^{17–20}

The RAFT dispersion polymerization was conducted in methanol, which is known to be a good solvent for St and the methoxy PEO macromolecular chain transfer agent (mPEO-CTA) while being a poor solvent for the resulting PSt polymer (Figure 1A).¹⁹ As the polymerization of St proceeded, the initially clear methanol solution of St and mPEO-CTA gradually became turbid with time. The resulting morphology of the self-assembly was dictated by the block ratios,^{2,3} and hence directly related to the polymerization time (Table 1, Figures S1–S3). At early stages of polymerization where the

PSt block was short, the PEO-*b*-PSt diblock copolymer formed spherical and tubular micelles. A micelle-to-vesicle transition was observed upon further increasing the polymerization time to >16 h, where the degree of polymerization (DP) of PSt block exceeded 600. Continuing to increase the polymerization time resulted in vesicles with thicker membranes as observed via TEM. The thickest polymersome membranes were obtained after a 40 h polymerization time, leading to a copolymer composition of PEO₁₁₃-*b*-PSt₁₄₄₁ (Figure S4). The membrane thickness was (47 ± 7) nm (Figure S5), which agreed very well with the theoretical sum of the end-to-end distance of two PSt chains in the unperturbed state (bilayer structure, $L = 2 \times 0.067M^{1/2} = 52$ nm, where M is the molecular weight of the PSt block).²¹ The broad particle size distribution was attributed to the high monomer fraction of the RAFT dispersion polymerization (Figure S1).²² Although thicker polymersomes were targeted by even longer polymerization time, a different assembly morphology, large compound vesicles, was instead obtained, which is not the focus of this study.

The PEO₁₁₃-*b*-PSt₁₄₄₁ polymersome dispersion in methanol was purified using dialysis and ultrafiltration, resulting in a final aqueous dispersion of polymersomes. To evaluate the polymersome stability against surfactants, we first incubated the resulting suspension with different anionic and nonionic surfactants, including sodium dodecyl sulfate (SDS), sodium dodecylbenzenesulfonate (LAS), STEOL, DOWFAX, BIO-SOFT, Triton X-100, and ECOSURF (Figure 1B). After one month incubation in 5 wt % solutions of the various surfactants, all the polymersome dispersions remained opaque and showed an unappreciable change in turbidity at 500 nm, indicating negligible disassembly and dissolution of polymeric amphiphiles by the surfactant (Figure 2A and Figure S6).²³ The broad range of surfactant resistance was demonstrated by the fact that changes in surfactant structure do not significantly alter the stability of PEO-*b*-PSt vesicles. These surfactants selected contain various differences in structure including the incorporation of aromatic groups (aromatic LAS, compared with nonaromatic SDS and STEOL), the change in charge density (two anions for DOWFAX, compared with SDS, LAS, and STEOL with one anion per molecule), and the variation of

Table 1. Characterization of PEO-*b*-PSt Assemblies after RAFT Dispersion Polymerization^a

time (h)	composition ^b	M_n (kDa) ^c	PDI ^c	morphology ^d	L^e (nm)
2	PEO ₁₁₃ - <i>b</i> -PSt ₁₅₂	28	1.22		
6.5	PEO ₁₁₃ - <i>b</i> -PSt ₃₈₀	42	1.11	SM	
16	PEO ₁₁₃ - <i>b</i> -PSt ₆₆₃	69	1.10	SM, TM, V	
24	PEO ₁₁₃ - <i>b</i> -PSt ₁₀₅₈	111	1.08	V	42 ± 4
32	PEO ₁₁₃ - <i>b</i> -PSt ₁₁₄₁	120	1.13	V	44 ± 4
40	PEO ₁₁₃ - <i>b</i> -PSt ₁₄₄₁	147	1.09	V	47 ± 7
48	PEO ₁₁₃ - <i>b</i> -PSt ₁₆₉₆	191	1.06	V, LCV	

^aAll polymerizations were conducted in methanol at 80 °C with [St]:[mPEO-CTA]:[AIBN] = 10 000:1:0.1. ^bDetermined by NMR. ^cDetermined by GPC. ^dDetermined by TEM. SM = spherical micelle, TM = tubular micelle, V = vesicle, LCV = large compound vesicle. ^eMembrane thickness; measured by counting 100 polymersomes from 20 TEM images.

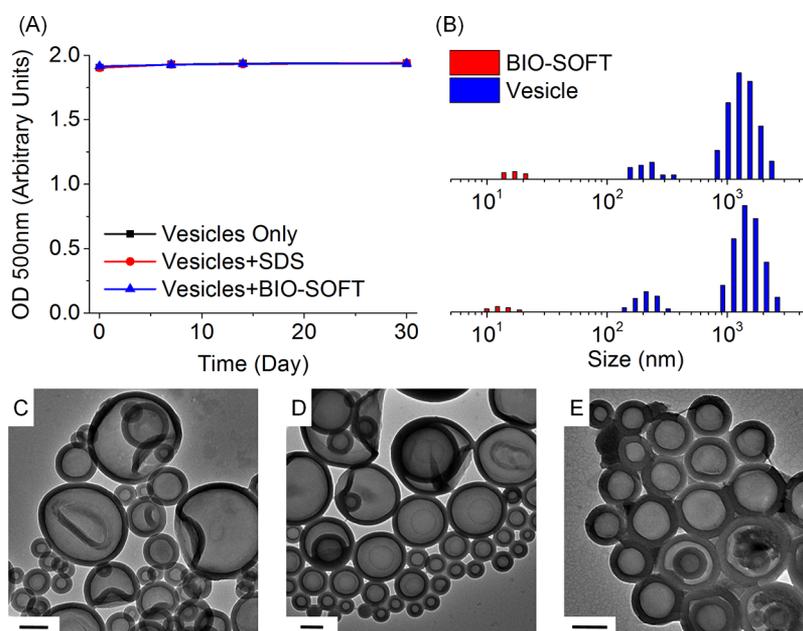


Figure 2. Surfactant resistance of PEO-*b*-PSt vesicles with ultrathick membranes. (A) Turbidity tests at 500 nm of vesicles in the presence of BIO-SOFT and SDS (5 wt %) over one month. (B) DLS results (size vs intensity) of vesicles incubated with BIO-SOFT (10 wt %) as prepared (top) and after 30 days (bottom). (C-E) TEM images of vesicles before surfactant treatment (C) and after treatment with BIO-SOFT (10 wt %) (D) or SDS (10 wt %) (E). Scale bar = 200 nm.

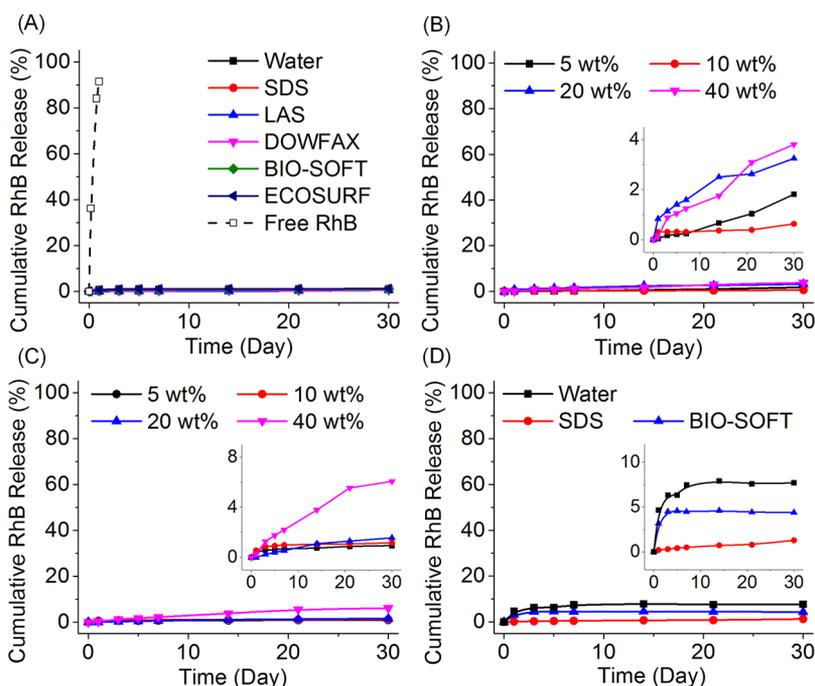


Figure 3. Cumulative release of encapsulated RhB from PEO-*b*-PSt polymersomes. (A) Release profile of RhB in the presence of different surfactants at 10 wt %; (B) Release profile of RhB in the presence of DOWFAX at different concentration; (C) Release profile of RhB in the presence of ECOSURF EH-6 at different concentration. (D) Release profile of RhB from shear force pretreated PEO-*b*-PSt polymersome in the presence of different surfactants at 10 wt %. A magnified view of the release profile could be found in the insets of Figure 3B-3D.

hydrophilic-to-hydrophobic ratios (three ECOSURF surfactants with different EO/PO ratios). The DLS intensity of polymersomes also did not change over this same time period (Figure 2B), confirming the absence of new particle populations and indicating insignificant formation of new mixed surfactant-polymer micelles that would form upon polymersome disassembly.¹⁰ The morphology and membrane thickness also showed no significant changes after surfactant

treatment, as verified by TEM and SEM (Figure 2C-2E and Figure S7). The superb resistance was further validated upon increase of the surfactant concentration up to 40 wt %, where the optical density at 500 nm stayed constant for one month (Figure S6). Although rupture of polymersomes in the presence of shear force such as sonication has been reported,²⁴ the ultrathick PEO-*b*-PSt vesicles remained stable after sonication even in the presence of SDS or BIO-SOFT (no turbidity

changes observed, Figure S6), further demonstrating the stability of the polymersomes.

In an attempt to provide more understanding on the mechanism of the surfactant resistance, we prepared PEO₂₀-*b*-PSt₂₅₀ polymersomes with thinner membranes (23 ± 4 nm from TEM) via solvent-switch method (Figure S9).²⁵ These polymersomes were partially solubilized by 40 wt % ECOSURF as verified by DLS and turbidity results (Figure S10), suggesting the critical role of membrane thickness in their stability in the presence of surfactant. On the other hand, the PEO₂₀-*b*-PSt₂₅₀ vesicles showed good stability against 10 wt % ECOSURF surfactant that outperformed most reported polymersomes with the hydrophobic blocks like polybutadiene, polymethacrylate, and polycaprolactone (Figure S10).^{10,12,13} Considering the difference in T_g of these hydrophobic blocks compared with PSt block (>100 °C),²⁶ we suggest the high T_g of hydrophobic PSt blocks may also contribute to the unprecedented surfactant stability of our PEO₁₁₃-*b*-PSt₁₄₄₁ polymersomes. The hydrophobic chains are “locked” to the collapsed state with limited mobility at room temperature,²⁷ which makes it difficult for surfactant to insert into the bilayer and solubilize the polymeric amphiphiles. The detailed resistance mechanism requires more experimental and simulation studies and will be included in our future work.

Although the turbidity tests provide important information on the lack of ability of surfactant molecules to solubilize polymersomes, it was unable to detect the disruption of vesicle membranes. To assess this, we utilized Rhodamine B (RhB, molecular size ~ 1.8 nm²⁸) as a model cargo to study the retention of encapsulated actives within the PEO-*b*-PSt polymersomes in the presence of surfactants. RhB loaded PEO-*b*-PSt polymersomes were prepared and purified in an analogous manner to blank polymersomes, instead starting with a methanol solution of RhB for the RAFT dispersion polymerization (Figure S8). No significant RhB leakage was observed when the vesicles were kept in water for as long as 1.5 years. Treatment with five representative surfactants at 10 wt % led to less than 1.5% release of RhB after one month of incubation (Figure 3A). Increasing the concentration of surfactants induced a greater release of encapsulated RhB, with the highest release (6.1%) occurring when the vesicles were treated with 40 wt % EXOSURF EH-6 for one month (Figure 3B, C). These results further validate the good retention of encapsulated actives from PEO-*b*-PSt vesicles at surfactant rich conditions. Exposing polymersomes to shear force before addition of surfactants resulted in slightly higher RhB release (less than 8% after one month of incubation), but the surfactant-added samples performed no worse than water control, indicating release is likely due to the shear-induced disruption of the membrane (Figure 3D).

In summary, surfactant-resistant PEO-*b*-PSt polymersomes were successfully prepared via RAFT dispersion polymerization in a single step. Ultrathick polymersome membranes were obtained after extended polymerization times, and these membranes had significantly improved stability against a wide variety of anionic and nonionic surfactants compared to conventional polymersomes. The release of actives from these polymersomes was less than 7% after one month incubation with up to 40 wt % surfactant. These thick polymersomes could provide a useful option for the design of stable delivery vehicles for industrial formulations.

■ ASSOCIATED CONTENT

§ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.6b05847.

Experiment details, TEM and SEM images of self-assemblies, the characterization of PEO-*b*-PSt diblock copolymers, turbidity test results, and the surfactant resistance of PEO-*b*-PSt polymersomes with thinner membranes (PDF)

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Notes

The authors declare no competing financial interest.

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