

Notes

Poly(4-(aminomethyl)styrene)-*b*-polystyrene: Synthesis and Unilamellar Vesicle Formation

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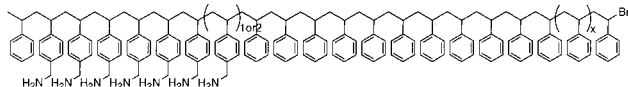
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Introduction

It has been demonstrated recently^{1,2} that phospholipid vesicles are inherently less robust than vesicles formed of thicker bilayered block copolymers and, therefore, that block copolymer vesicles may prove more useful for encapsulation technologies. Vesicles can be formed from block copolymers with hydrophobic blocks of poly(ethylene), polybutadiene, and polystyrene and various hydrophilic blocks (including poly(ethylene oxide)).^{1–6} Generally, the diameters reported^{1–6} for these vesicles were below 500 nm and long-term stability of single bilayered (unilamellar) block copolymer vesicles with diameters above 2 μm has not been reported to our knowledge.

We focus here on vesicle formation from new block copolymers of the composition poly(4-(aminomethyl)-styrene)-*b*-polystyrene [P4AMS_{*n*}-*b*-PS_{*m*}]: In contrast to



previous block copolymers that have been used for vesicle formation,^{1–6} both copolymer segments have similar backbone structures and the “hydrophilic” block (P4AMS) is more hydrophobic because of the associated styrene and is essentially “amphiphilic”. In addition, the amine

groups provide the option of performing further functionalization and may participate in hydrogen bonding. A block copolymer of somewhat similar structure, polystyrene-*b*-poly(4-vinylpyridine), was studied previously and used to form micelles.⁷

In the selection of the appropriate lengths for the hydrophobic and hydrophilic blocks, it must be recognized that chain stretching is an important factor in determining the ultimate structure formed (e.g. micelle vs vesicle).^{3–6} Chain stretching is important for PS-containing block copolymers, because it is necessary to dissolve the copolymer initially in a common solvent that swells the chains, and as water is exchanged for solvent, aggregates form and the environment gradually becomes less favorable for chain swelling. Therefore, we applied a predictive degree of stretching (*S*_c) correlation developed by Zhang and Eisenberg,³ for poly(acrylic acid)-*b*-PS initially dissolved in DMF, to choose block lengths that would yield vesicles. For the three P4AMS_{*n*}-*b*-PS_{*m*} block copolymers synthesized (7-*b*-46, 8-*b*-74, 8-*b*-130), vesicles formed when DMF and two other solvents, THF and dioxane, were used to form the initial block copolymer solutions. Surprisingly, the 8-*b*-130 species yielded very large vesicles, up to 10 μm in diameter, which appeared to be unilamellar utilizing optical and freeze-fracture transmission electron microscopy. Because the glass transition of PS is above room temperature, these structures were essentially trapped and have thus far remained stable for 1 year.

Experimental Section

Characterization during Synthesis. ¹H NMR (300 MHz) and ¹³C {¹H}NMR (75 MHz) spectra were recorded on a Varian Mercury 300 instrument, and chemical shifts were referenced to the residual proton signal of the solvent (CDCl₃) at 7.25 ppm or to the ¹³C signal of the solvent at 77.0 ppm. Number-averaged molecular weights (*M*_n), weight-averaged molecular weights (*M*_w), and molecular weight distributions (*M*_w/*M*_n) were determined using gel-permeation chromatography in THF at 30 °C and a flow rate of 1.00 mL min⁻¹. Three Polymer Standards Services columns (100 Å, 1000 Å, linear) were connected in series to a Thermoseparation Products P-1000 isocratic pump, autosampler, column oven, and Knauer refractive index detector. Calibration was performed using polystyrene samples (Polymer Standard Services; *M*_p = 300–1 000 000; *M*_w/*M*_n < 1.10).

(4-Vinylbenzyl)phthalimide (VBP) Preparation. Potassium phthalimide (13.1 g, 0.712 mol), 4-vinylbenzyl chloride (10.0 mL, 0.710 mol), and 4-*tert*-butylcatechol (0.971 g, 5.84 mmol) were stirred in 20 mL of DMF for 48 h at 70–78 °C. The solution was diluted using 100 mL of ethyl acetate and extracted using 3 × 50 mL of water to remove the excess potassium chloride and DMF. The organic layer was dried over anhydrous MgSO₄ and then gravity filtered. Volatile materials were removed by rotary evaporation, and the product was recrystallized from ethanol yielding 12.4 g (67%): IR (thin film) ν (cm⁻¹) 1768 and 1712 (ν (C=O(N))); ¹H NMR (300 MHz, CDCl₃) δ (ppm) 4.82 (s, 2H), 5.22 (d, *J* = 11.5, 1H), 5.70 (d, *J* = 17.7, 1H), 6.66 (dd *J*₁ = 17.4, *J*₂ = 11.1, 1H), 7.37 (m, 4H), 7.69 (m, 2H), 7.83 (m, 2H); ¹³C NMR (75 MHz, CDCl₃) δ (ppm) 168.1, 137.3, 136.5, 136.0, 134.2, 132.3, 129.1, 126.7, 123.5, 114.4, 41.7.

Poly((4-vinylbenzyl)phthalimide) Macroinitiator Preparation. The poly((4-vinylbenzyl)phthalimide) macroinitiator was

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Table 1. Conditions for Block Copolymer Formation and Characterization Data

	46 styrene/7 VBPH	74 styrene/8 VBPH	130 styrene/8 VBPH
amt of VBPH macroinitiator [no. of repeat units]	3.00 g (1.56 mmol) [$n = 7$]	1.03 g (0.476 mmol) [$n = 8$]	2.00 g (0.925 mmol) [$n = 8$]
M_n of macroinitiator	1.92×10^3	2.16×10^3	2.16×10^3
styrene [target no. of repeat units]	12.5 mL (0.109 mol) [$m = 70$]	5.00 mL (43.6 mmol) [$m = 92$]	10.0 mL (87.3 mmol) [$m = 94$]
CuBr	64.5 mg (0.450 mmol)	21.5 mg (0.150 mmol)	44.5 mg (0.310 mmol)
2,2'-bipyridine	0.151 g (0.967 mmol)	55.3 mg (0.354 mmol)	0.118 g (0.755 mmol)
<i>p</i> -xylene	15.0 mL	5.00 mL	5.00 mL
reaction time	12 h	16 h	16.5 h
yield of block copolymer	43%	45%	60%
M_n	6.71×10^3	9.88×10^3	1.59×10^4
M_w/M_n	1.28	1.13	1.12
yield of deprotected copolymer	83%	57%	57%

formed by mixing VBP (7.00 g, 26.6 mmol), (1-bromoethyl)benzene (0.180 mL, 1.32 mmol), CuBr (0.185 g, 1.30 mmol), 2,2'-bipyridyl (0.434 g, 2.78 mmol), and 20 mL of *p*-xylene under O₂-free conditions and heating at 110 °C for 6 h. The product was dissolved in THF and precipitated into methanol, and then volatile materials were removed under vacuum, yielding 63% of a white powder: IR (thin film) ν (cm⁻¹) 1770 and 1714 (ν (C=O(N))); ¹H NMR (300 MHz, CDCl₃) δ (ppm) 0.95–2.25 (br, 3H), 4.67 (br, 2H), 6.05–7.25 (br, 4H), 7.45–8.05 (br, 4H); GPC $M_n = 2.16 \times 10^3$, $M_w/M_n = 1.14$.

Poly(4-(4-vinylbenzyl)phthalimide)-*b*-polystyrene Preparation. The macroinitiator was reacted with the corresponding monomer under O₂-free conditions with copper bromide, 2,2'-bipyridyl, and *p*-xylene as a solvent at 110 °C. (The amounts and yields were as indicated in Table 1.) The block copolymer was then dissolved in THF, precipitated dropwise in methanol, and freeze-dried from benzene: IR (thin film) ν (cm⁻¹) 1772 and 1716 (ν (C=O(N))); ¹H NMR (300 MHz, CDCl₃) δ (ppm) 0.80–2.15 (br, 6H), 4.66 (br, 2H), 6.02–7.95 (br, 13H); GPC, see Table 1.

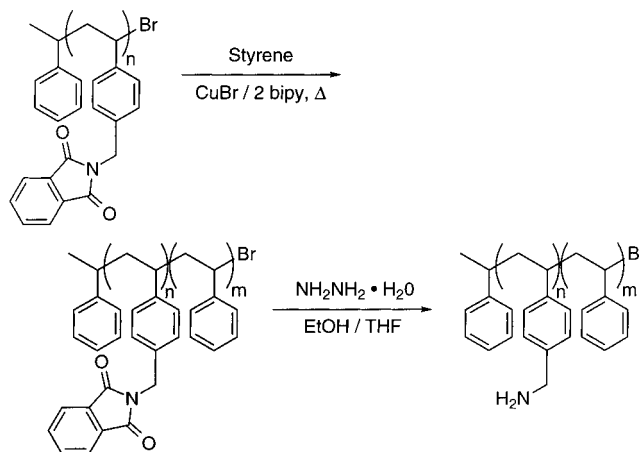
Poly(4-(aminomethyl)styrene)-*b*-polystyrene Preparation. The phthalimide group was removed from the block copolymer by heating ~2 g of the polymer at reflux for 2 h with 5.00 mL of hydrazine monohydrate, 8 mL of absolute ethanol, and 60 mL of THF. The mixture was cooled and gravity filtered. Volatile materials were removed under vacuum. The residue was dissolved in a minimal amount of THF and precipitated into 100 mL of petroleum ether. The precipitate was collected by filtration, and volatile materials were removed under vacuum. The yields are indicated in Table 1: IR (thin film) ν (cm⁻¹) no signals at 1770 or 1716; ¹H NMR (300 MHz, CDCl₃) δ (ppm) 1.15–2.20 (br, 6H), 3.78 (br, 2H), 6.25–7.35 (br, 9H).

Preparation of Block Copolymer Aggregates. The three block copolymers of P4AMS-*b*-PS (7-*b*-46, 8-*b*-74, 8-*b*-130) were dissolved in 2 mL of the solvents DMF, THF, and dioxane at a concentration of 2.5 mg/mL. Water was added dropwise to 2 mL of the copolymer–solvent solutions (while swirling by hand) using a syringe pump set at the following volume and rate settings: 500 μ L at 20 μ L/min; 500 μ L at 200 μ L/min; 1000 μ L at 2000 μ L/min. In all cases, the cloud point occurred during the slowest rate of water addition indicating aggregation of the copolymers. After addition of water, copolymer solutions were dialyzed for 4 days against water to remove residual solvent from the aggregates.

Results and Discussion

Block Copolymer Synthesis and Characterization.

A macroinitiator was prepared by the atom transfer radical polymerization (ATRP)⁸ of (4-vinylbenzyl)phthalimide (VBP). The ¹H NMR spectrum of the resulting oligomer showed a signal at 4.67 ppm due to the benzylic hydrogens next to the phthalimide group. The GPC data showed a unimodal distribution for a polymer of $M_n = 2.16 \times 10^3$ and $M_w/M_n = 1.14$. Chain extension of the polyVBP oligomer with styrene was performed next (Scheme 1). In the ¹H NMR spectra of the block copolymers, the signal at 4.67 ppm decreased in peak area relative to the signals

Scheme 1. Synthetic Scheme for Poly(4-(aminomethyl)styrene)-*b*-polystyrene

for the backbone protons and the side chain aromatic protons, indicating the addition of unfunctionalized styrene repeat units to the macromolecule. The compositions, as indicated in Table 1, were determined by the relative peak integrations in the spectra. GPC chromatograms of the block copolymers showed unimodal distributions with polydispersities of less than 1.30 and increases of molecular weights relative to the macroinitiator. The data were consistent with the formation of a PVBP-polystyrene block copolymer. In the final step, the phthalimide group was removed by heating the polymer in THF with hydrazine hydrate and ethanol (Scheme 1). In the ¹H NMR spectrum of the resulting copolymer, the signal for the benzylic hydrogens appeared at 3.73 ppm, consistent with the transformation of the phthalimide group to an amino group. The final block copolymer was soluble in polar organic solvents (CHCl₃, THF, DMF, dioxane). Differential scanning calorimetry performed on the lowest molecular weight P4AMS-*b*-PS_m, 7-*b*-46, yielded, after one thermal cycle, one glass transition temperature (T_g) of 99 °C and heat capacity change (ΔC_p) of 0.5 J/g °C. The T_g value and ΔC_p value are larger than expected by approximately 10 °C and 0.1 J/g °C for pure polystyrene of similar M_n ,⁹ possibly due to stronger interactions between the chains, attributed to the poly(4-(aminomethyl)styrene) block.

Aggregate Characterization. All samples were viewed with optical microscopy as well as freeze-fracture TEM to attain a fair assessment of aggregate sizes and morphology. For all solvents tested, the two P4AMS-*b*-PS copolymers with the shortest hydrophobic chains, 7-*b*-46 and 8-*b*-74, yielded only small aggregates (<300 nm), Figure 1, and the copolymer with the longest hydrophobic

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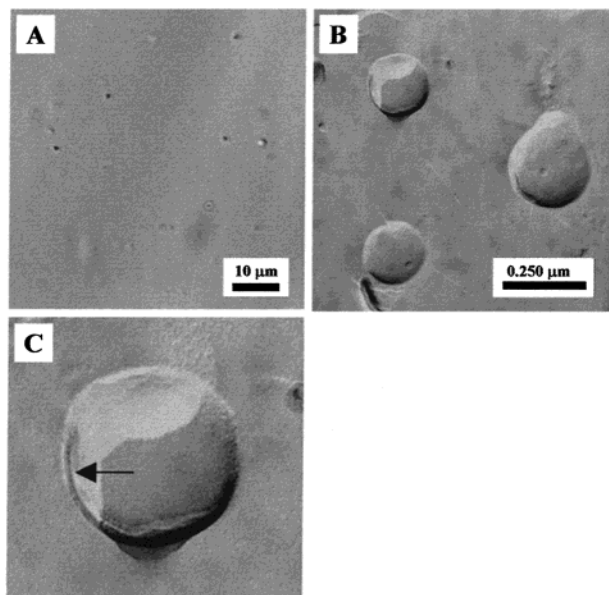


Figure 1. Vesicles formed of 8-*b*-75 (P4AMS-*b*-PS) block copolymer (original solvent dioxane): (A) optical microscopy image revealing only many small particles in rapid motion with image obtained using a 20× Hoffman Modulation Optics objective lens (Greenvalle, NY); (B) freeze-fracture TEM image of vesicles with replicas prepared in a RMC/JEOL RFD-9010 freeze-fracture system and imaged using a Phillips CM-12 TEM; (C) arrow pointing to vesicle shell that is revealed during the fracture process.

chain, 8-*b*-130, gave small aggregates as well as larger aggregates ($<10\ \mu\text{m}$), Figure 2. The morphology of these aggregates appeared unchanged for at least 1 year. Qualitatively, aggregate density appeared the same as phospholipid vesicles made at the same concentration. It is fortuitous that large aggregates formed since cross sections through the aggregates could be obtained using a 100× oil immersion lens (for a typical image, see Figure 2b). These images could then be compared to images of large unilamellar phospholipid vesicles of similar diameter (for a typical image, see Figure 2c) with a shell (lipid bilayer) thickness of 4.07 nm.¹⁰ In Figure 2c, the shell of the phospholipid vesicle is barely visible and the interior of the vesicle appears to be of the same material (water) as the exterior. In contrast, in Figure 2b the shell of the 8-*b*-130 aggregate is much more easily seen (note that quantitative measurements of shell thickness using optical microscopy are not reliable because the resolution is at best 200 nm and the images should be interpreted only qualitatively). Similar to the unilamellar phospholipid vesicle, the interior of the 8-*b*-130 aggregate appears to be of the same material as the exterior (water), we only ever observe one shell, and, qualitatively, there is no apparent thickness variation when comparing aggregates. We conclude from this comparison that the shells of the longer copolymer aggregates are significantly thicker than shells (bilayers) of phospholipid vesicles and are likely of single bilayer structure. Additional evidence that the aggregates formed here contained a single bilayer shell can be seen in freeze-fracture TEM images in which aggregates are occasionally fractured such that the shell appears to have been separated from the aqueous interior and exterior and a thin ring can be observed (Figure 1c). The smooth surfaces of the aggregates (with occasional adhered smaller vesicles) in the freeze-fracture replicas were also indicative of unilamellar vesicles.

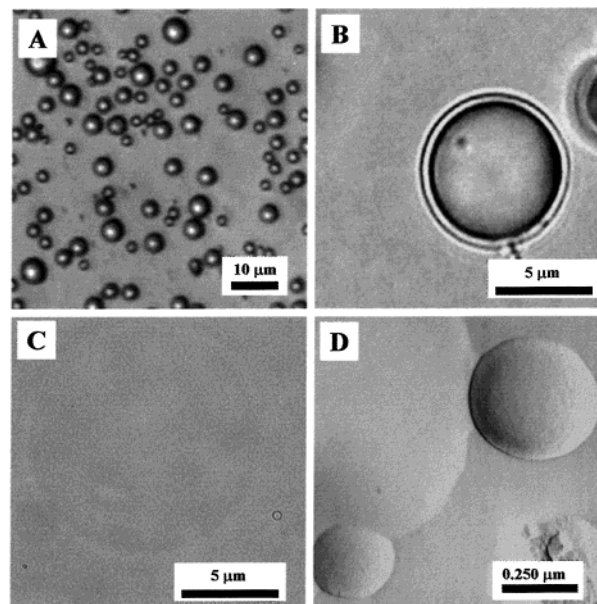


Figure 2. Vesicles formed of 8-*b*-130 (P4AMS-*b*-PS) block copolymer (original solvent THF): (A) Optical microscopy image revealed micrometer-scale vesicles. Note that these vesicles sank to the bottom cover glass due to their large size. If they were filled with THF (specific gravity = 0.884), they would have been expected to rise. Also note that the Hoffman Modulations optics used to obtain image A give vesicles a three-dimensional appearance. (B) Optical microscopy image of a single block copolymer vesicle. Images B and C were obtained using a 100× oil immersion lens. (C) For comparison to (B), we present an optical microscopy image of 1-stearoyl-2-oleoylphosphatidylcholine vesicle formed by electroformation technique.¹² Inside of the vesicle is 100 mM sucrose, and the exterior consists of 100 mM glucose. (D) Freeze-fracture TEM image of small block copolymer vesicles that would appear as small particles in optical microscopy images.

The tendency toward bilayer/vesicle formation, rather than micelle formation observed here, can be understood in terms of a chain stretching parameter (S_c),³ which is the ratio of the radius (for a micelle) or half-bilayer thickness (for a vesicle) to the end-to-end distance of the PS block in the unperturbed state. Data for a large number of poly(acrylic acid)-*b*-polystyrene copolymers that were initially dissolved in DMF and formed micelles upon water addition and dialysis were used by Zhang and Eisenberg³ to obtain the relationship that S_c is proportional to $N_{PS}^{-0.1}N_{PAA}^{-0.15}$ reflecting higher strain if either block length decreases with a slightly stronger correlation with the hydrophobic block length ($N_{PS}^{-0.1}$) than the hydrophilic block length ($N_{PAA}^{-0.15}$). Using the relationship, it was found that if S_c exceeded approximately 1.45, micelles were not formed; the entropic penalty for chain stretching was no longer balanced by the core–water interfacial tension reduction resulting from chain stretching. For example, for the PAA-*b*-PS block copolymer, 8-*b*-200, it was determined that S_c in micelles would be 1.62; therefore, vesicles ($S_c \sim 1$) not micelles were formed. From this value of 1.62 and the proportionality relationship given above (substituting N_{P4AMS} for N_{PAA}), we estimated S_c for 8-*b*-130, 8-*b*-74, and 7-*b*-46 (P4AMS-*b*-PS) as 1.69, 1.79, and 1.91, respectively, favoring vesicle formation. In addition, since P4AMS is more hydrophobic than PAA, we would expect DMF to swell P4AMS blocks more in comparison to PAA blocks, making micelle formation even less favorable. Micelle formation using THF and dioxane are ruled out, because they are even better solvents for PS chains than DMF. If micelles were to form, they would have even larger chain extension than we have calculated

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here, thus increasing both the aggregate numbers and strain when the solvents were removed.

The maximum size ($\sim 10\ \mu\text{m}$) of the 8-*b*-130 (P4AMS-*b*-PS) vesicles observed here is unusually large in comparison to vesicles formed from other copolymers.¹⁻⁶ Theoretically, the average number of monomers in a vesicle is proportional to the exponential of the bending modulus.¹¹ For phospholipid vesicles, the bending modulus is proportional to the square of the bilayer thickness.¹⁰ For block copolymers, the bending modulus does not increase nearly as rapidly in proportion to the bilayer thickness^{1,2} (otherwise, unimaginably large vesicles are predicted). However, the general trend of larger thickness leading to larger vesicles does explain our observations qualitatively. The bending moduli of the P4AMS-*b*-PS bilayers are probably larger than the bending moduli of PAA-*b*-PS bilayers with comparable thicknesses since the maximum vesicle diameters reported for PAA-*b*-PS bilayers were 500 nm. Possible explanations could be a difference in the depth of the hydrophilic/hydrophobic core interface (an important parameter in bending modulus determinations) or the formation of a hydrogen-bonding network that opposes bending between the hydrophilic block amino groups.

Conclusions

We have shown that poly(4-(aminomethyl)styrene)-*b*-polystyrene copolymers can be used to form vesicles. We

found that the copolymer with the longest PS chain (8-*b*-130) yielded surprisingly large vesicles, possibly indicating a large bending modulus. These copolymers may be good candidates for obtaining larger encapsulation volumes than normally could be obtained in other block copolymer vesicles and tougher, longer lived, encapsulation shells than are possible with phospholipid vesicles. The large size of these vesicles should allow us to obtain micromechanical measurements such as bending modulus and maximal strain, which we plan in the future. Additionally, further evaluation is required to determine if the proportionality relationship between block lengths and aggregate morphology utilized here is universally applicable to the poly(4-(aminomethyl)styrene)-*b*-polystyrene copolymers.

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