Notes

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

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Introduction

It has been demonstrated recently 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 oxide).1

It has been demonstrated recently that phospholipid vesicles have been used for vesicle synthesis.1,2 Poly(ethylene oxide) can also be used to form the initial block copolymer solutions. Suprisingly, vesicles formed when DMF and other solvents, THF and dioxane, were used to form the initial block copolymer solutions. Suprisingly, 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 (Mn), weight-averaged molecular weights (Mw), and molecular weight distributions (Mw/Mn) 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 standards (Polymer Standard Services; Mn = 300–10,000,000; Mw/Mn < 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-butylcatalech (0.971 g, 5.84 mmol) were stirred in 20 mL of DMF for 48 h at 70 °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 (2H, 2H), 5.22 (d, J = 11.5, 1H), 5.70 (d, J = 17.1, 1H), 6.66 (dd, J1 = 174, J2 = 11, 1H), 7.37 (m, 4H), 7.69 (m, 2H), 7.83 (m, 2H); 13C NMR (75MHz, CDCl3) δ (ppm) 168.1, 137.3, 136.5, 136.0, 134.2, 132.3, 129.1, 126.7, 123.5, 114.4, 47.7.

Poly(4-(vinylbenzyl)phthalimide) Macriniitator Preparation. The poly(4-(vinylbenzyl)phthalimide) macriniitator was prepared by the reaction of a stoichiometric amount of the poly(4-(vinylbenzyl)phthalimide) macriniitator with anhydrous potassium phthalimide...
formed by mixing VBP (7.00 g, 26.6 mmol), (1-bromomethyl)benzene (0.180 g, 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 solid.

<table>
<thead>
<tr>
<th>1H NMR (300 MHz, CDCl3)</th>
<th>IR (thin film)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.15 ppm</td>
<td>1770 or 1716</td>
</tr>
</tbody>
</table>

The copolymer was then dissolved in THF, precipitated dropwise in 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 Mₙ = 2.16 × 10³, Mₘ/Mₙ = 1.14.

**Poly(4-(vinylbenzyl)phthalimide)-b-polystyrene Preparation.** The macroinitiator was reacted with the corresponding monomer and copolymer 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 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 by heating the polymer in THF with hydrazine hydrate and ethanol (Scheme 1). 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): 1770 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 block copolymer was dissolved in THF with hydrazine hydrate and ethanol (Scheme 1). 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): 1770 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.

**Results and Discussion**

**Block Copolymer Synthesis and Characterization.** A macroinitiator was prepared by the atom transfer radical polymerization (ATRP)² of (4-vinylbenzyl)phthalimide (VBP). The 1H NMR spectrum of the resulting oligomer showed a signal at 4.67 ppm due to the benzyl hydrogen and next to the phthalimide group. The GPC data showed a unimodal distribution for a polymer of Mₙ = 2.16 × 10³ and Mₘ/Mₙ = 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 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-b-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 benzyl hydrogen signals 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-P5m, 7-b-46, yielded, after one thermal cycle, one glass transition temperature (Tg) of 99 °C and heat capacity change (ΔCp) of 0.5 J/g·°C. The Tg value and ΔCp value are larger than expected by approximately 10 °C and 0.1 J/g·°C for pure polystyrene of similar Mn, 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-P5m 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 chain, 8-b-130, yielded quite large aggregates (~2 μm), Figure 2.


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**Table 1. Conditions for Block Copolymer Formation and Characterization Data**

<table>
<thead>
<tr>
<th>amo of VBPH macroinitiator</th>
<th>46 styrene/7 VBP</th>
<th>74 styrene/8 VBP</th>
<th>130 styrene/8 VBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>[no. of repeat units]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mₙ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[target no. of repeat units]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CuBr</td>
<td>12.5 mL (0.109 mol)</td>
<td>5.00 mL (43.6 mmol)</td>
<td>10.0 mL (87.3 mmol)</td>
</tr>
<tr>
<td>2,2'-bipyridine</td>
<td>0.151 g (0.967 mmol)</td>
<td>55.3 mg (0.354 mmol)</td>
<td>0.118 g (0.755 mmol)</td>
</tr>
<tr>
<td>p-xylene</td>
<td>15.0 mL</td>
<td>5.00 mL</td>
<td>5.00 mL</td>
</tr>
<tr>
<td>reaction time</td>
<td>12 h</td>
<td>16 h</td>
<td>16.5 h</td>
</tr>
<tr>
<td>yield of block copolymer</td>
<td>43%</td>
<td>45%</td>
<td>60%</td>
</tr>
<tr>
<td>Mₙ</td>
<td>6.71 × 10³</td>
<td>9.88 × 10³</td>
<td>1.59 × 10⁴</td>
</tr>
<tr>
<td>yield of deprotected copolymer</td>
<td>83%</td>
<td>57%</td>
<td>57%</td>
</tr>
</tbody>
</table>

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

[Scheme image]
were also indicative of unilamellar vesicles.

adhered smaller vesicles) in the freeze-fracture replicas and exterior and a thin ring can be observed (Figure 1c). It appears to have been separated from the aqueous interior

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). It appears to have been separated from the aqueous interior

aggregates formed here contained a single bilayer shell

single bilayer structure. Additional evidence that the shells (bilayers) of phospholipid vesicles and are likely of

longer copolymer aggregates are significantly thicker than

we conclude from this comparison that the shells of the vesicle appears to be of the same material (water) and, qualitatively, there is no obvious thickness variation when comparing aggregates.

vesicle, the interior of the 8-b-130 aggregate is much more easily seen (note that in optical microscopy images.

1.79, and 1.91, respectively, favoring vesicle formation. In addition, since P4AMS is more hydrophobic than PAA, we would have expected to rise. Also note that the Hoffman Modulations optics used to obtain 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 vesicles 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 a small particle 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 ($S_c$), 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 pol(yacrylic 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 $N_{PS}^{-1.15}N_{PAA}^{-0.15}$ reflecting higher strain if either block length decreases with a slightly stronger correlation with the hydrophobic block length ($N_{PS}$) than the hydrophilic block length ($N_{PAA}$). 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 ($S_c$ - 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

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

The maximum size (~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.\(^1\)\(^1\) For phospholipid vesicles, the bending modulus is proportional to the square of the bilayer thickness.\(^1\)\(^0\) 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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