Amphiphilic Poly[bis(trifluoroethoxy)phosphazene]−Poly(ethylene oxide) Block Copolymers: Synthesis and Micellar Characteristics

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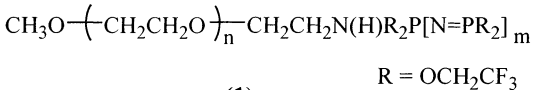
ABSTRACT: The micellar characteristics of the amphiphilic diblock copolymers based on a hydrophobic polyphosphazene (PN) and hydrophilic poly(ethylene oxide) (PEO) were investigated by using fluorescence techniques, dynamic light scattering, and transmission electron microscopy. The two diblock copolymers with different ratios of the length of block components form spherical micellar aggregates in an aqueous phase. The critical micelle concentrations of PN−PEO 1 and PN−PEO 2, determined by a fluorescence technique, were 12.4 and 5.2 mg/L, respectively. The mean diameters of the micelles of PN−PEO 1 and PN−PEO 2, measured by dynamic light scattering, were 100 and 120 nm. The steady-state fluorescence anisotropy values (*r*) of 1,6-diphenyl-1,3,5-hexatriene (DPH) were 0.103 for PN−PEO 1 and 0.108 for PN−PEO 2. The low anisotropy values for PEO−PN compared to those for other polymeric amphiphiles may be due to the intrinsic flexibility of the trifluoroethoxy-substituted polyphosphazene block, which would allow enhanced rotational diffusion of the probe.

Introduction

Amphiphilic block copolymers with hydrophilic and hydrophobic segments have been investigated extensively not only because of their unique self-organization characteristics but also for their wide range of potential applications such as drug delivery and separations technology.[1] The micellar characteristics of amphiphilic diblock copolymers depend on the nature of each block. The surface properties of self-organized micelles would be highly dependent on the structure of hydrophilic block. For example, poly(ethylene oxide) (PEO) block would provide a fairly biocompatible surface environment for micellar aggregates. On the other hand, the micellar core characteristics would be determined by the structure of hydrophobic blocks.[2−4]

Polyphosphazenes that contain an inorganic backbone of alternating nitrogen and phosphorus atoms with two different organic groups "R" on each phosphorus generate many different chemical and physical properties depending on the structure of the R group. In particular, polyphosphazenes bearing bioinert or biodegradable groups have attracted much attention as potential biomaterials.[5,6] Recently, we have developed the synthesis of novel poly(phosphazene−ethylene oxide) triblock copolymers by the cationic polymerization of a phosphoranimine via the use of $NH_2-PEG-NH_2$ as a macroinitiator.[7] This ambient-temperature polymerization provides an advanced method for the molecular weight control and gives narrow polydispersities.[8] The combination of polyphosphazenes with organic polymers provides a facile synthetic route to unique hybrid materials for bioapplications with a well-defined block copolymer structure. In addition, the diverse functionalities of poly(organophosphazenes) give an opportunity for the chemical and physical variations of phosphazene−organic block copolymers. In a previous work, it was reported that amphiphilic phosphazene−phosphazene diblock copolymers were able to form self-organized assemblies in an aqueous phase which can serve as nanosize vehicles for drug delivery.[9] In this work, we report the micellar characteristics of amphiphilic diblock copolymers (**1**) based on a hydrophilic poly(ethylene oxide) and a hydrophobic trifluoroethoxy-substituted polyphosphazene block, in which poly(ethylene oxide) would serve as an outer shell and the polyphosphazene block would determine the characteristics of the micellar core. Their micellar behavior was investigated by using fluorescence techniques, light scattering, and transmission electron microscopy (TEM).



(1)

Experimental Section

Materials. Monomethoxypoly(ethylene glycol) (CH_3O-PEO, $M_n = 5000$) and amine-terminated PEO ($CH_3O-PEG-CH_2-CH_2-NH_2$, $M_n = 5000$) were obtained from Shearwater Polymers Inc. and were purified by repeated precipitation from methylene chloride into *n*-hexane. Lithium bis(trimethylsilyl)-amide and allylamine were obtained from Aldrich and were used without further purification. Phosphorus pentachloride (Aldrich) was purified by sublimation under vacuum before use. 1,1,1-Trifluoroethanol was dried over CaH_2 and distilled before use. $Cl_3P{=}NSiMe_3$ and $Br(CF_3CH_2O)_2P{=}NSiMe_3$ were synthesized and purified by literature procedures.[7,8] Tetrahydrofuran and *n*-hexane (Aldrich) were distilled into the reaction flask from sodium benzophenone ketyl under an atmosphere of dry argon. Dichloromethane (Aldrich) was dried and distilled from CaH_2 and then from P_2O_5 into the reaction flask.

Equipment ^1H, ^{13}C, and ^{31}P spectra were recorded on a Bruker WM-360 NMR spectrometer operated at 360, 146, and 90.27 MHz, respectively. ^1H and ^{13}C NMR spectra were referenced to solvent signals while ^{31}P NMR chemical shifts are relative to 85% phosphoric acid as an external reference, with positive shift values downfield from the reference. Molecular weights were estimated using a Hewlett-Packard HP

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1090 gel permeation chromatograph equipped with an HP-1047A refractive index detector, American Polymer Standards AM gel 10 mm and AM gel 10 mm 10^4 Å column, and calibrated vs polystyrene standards (Polysciences). The samples were eluted at 40 °C with a 0.1 wt % solution of tetra-*n*-butylammonium nitrate (Aldrich) in THF (OmniSolv).

Synthesis of $CH_3-PEO-NH[(CF_3CH_2O)_2P{=}NSiMe_3]$ (2). A mixture of $CH_3O-PEO-NH_2$ (2.0 g, 0.4 mmol) and triethylamine (0.5 mL) in THF was cooled to −76 °C. To this solution, $(CF_3CH_2O)_2BrP{=}NSiMe_3$ (0.158 g, 0.4 mmol) was added dropwise over a 30 min period. The reaction mixture was stirred at −76 °C and then allowed to warm to room temperature. The salt (Et_3NHBr) was removed by filtration. All volatiles were removed under reduced pressure to produce a white solid, which was washed with *n*-hexane and dried under vacuum (1.67 g, yield 78%). For **2**: ^{1}H NMR ($CDCl_3$): δ −0.003 (d, J = 4 Hz, 9H), 1.40 (tri, 2H), 3.30 (s, 3H), 3.51 (s, 120H), 3.80 (tri, 2H), 4.23 (q, J = 16 Hz, 4H). ^{31}P NMR ($CDCl_3$): δ −0.76.

Synthesis of $CH_3-PEO-NH[(CF_3CH_2O)_2P{=}NPCl_3^+PCl_6^-]$ (3). To a stirred solution of PCl_5 (0.156 g, 0.8 mmol) in methylene chloride (15 mL) at −78 °C, **2** (0.4 mmol) in 25 mL of CH_2Cl_2 was added slowly. The reaction mixture was stirred at −76 °C for 1 h, and then the temperature was elevated to room temperature. After removal of the solvent, the product was washed with *n*-hexane to produce a light yellow solid (1.89 g, yield 87%). For **3**: ^{1}H NMR ($CDCl_3$): δ 1.29 (s, 2H), 3.30 (s, 3H), 3.50 (s, 120 H), 3.80 (tri, 2H), 5.47 (br, 4H). ^{31}P NMR ($CDCl_3$): δ −297 (s), −6.8 (d, J = 51 Hz), 4.3 (d, J = 54 Hz).

Synthesis of $CH_3-PEO-NH[(CF_3CH_2O)_2P-[(OCH_2CF_3)_2]_m$ (1). A methylene chloride solution (15 mL) of **3** (0.4 mmol) was added to a methylene chloride (15 mL) solution of $Cl_3P{=}NSiMe_3$ (10.2 g, 45 mol) at room temperature for 3 h. The reaction mixture was monitored by using ^{31}P NMR spectroscopy until complete conversion of $Cl_3P{=}NSiMe_3$ to polymer had occurred. After complete consumption of $Cl_3P{=}NSiMe_3$, all volatiles were removed under reduced pressure. The product was redissolved in THF and treated with an excess amount of $NaOCH_2CF_3$ (135 mmol) in THF. The reaction mixture was stirred at room temperature for 5 h. The product was precipitated into water (×2) and *n*-hexane (×2) to produce a light yellow solid (8.05 g, yield 62%). For **3**: ^{1}H NMR ($CDCl_3$): δ 1.41 (s, 2H), 3.35 (s, 3H), 3.51 (s, 120 H), 3.80 (tri, 2H), 4.23 (q, J = 16 Hz, 4H). ^{31}P NMR ($CDCl_3$): δ −7.3, 7.5 (d, J = 51 Hz).

Sample Preparation. To prepare micellar solutions, the PEO−PN block copolymer was dispersed in distilled water with gentle stirring for 3 h, followed by sonication for 30 min. For the measurement of fluorescence spectra of DPP in micellar solutions, samples were prepared following a literature procedure.[10] The concentrations of sample solutions were varied from 5×10^{-5} to 2.5 g/L. For the measurements of steady-state fluorescence anisotropy of DPH in micellar solutions, samples were prepared following a literature procedure.[9,11,12]

Fluorescence Measurements. All the fluorescence measurements were performed using an ISS K2 spectrofluorometer with a thermostat cell unit. Samples were excited using a 300 W xenon arc lamp (ILC Technology). The measurement of DPP emission spectra was performed using a reference method.[13] For the emission spectra, λ_{ex} = 333 nm. Steady-state fluorescence anisotropy values (r) of DPH were determined in the L-format geometry of detection.[12] The excitation wavelength was 360 nm, and the emission was measured at 430 nm.

Light Scattering Measurements. Dynamic light scattering measurements were performed using a Brookhaven BI-200SM goniometer, a BI-9000AT autocorrelator, and a He−Ne laser (632.8 nm) (Research Electro-Optics 35 mW). The sample solutions were purified by passing through a Millipore 0.45 μm filter. The hydrodynamic diameters (d) of micelles and the polydispersity factor of micelles, represented as μ_2/Γ^2, were calculated by using the Stokes−Einstein equation and the cumulant method, respectively.[14] CONTIN algorithms were used in the Laplace inversion of the autocorrelation function to obtain micelle size distribution.[15]

Table 1. Properties of Block Copolymers

	M_n (^{1}H NMR)	block ratio[a] (EO/PN)	wt % of PN	M_w/M_n[b]
PEO−PN 1	7500	1:0.1	20	1.01
PEO−PN 2	34000	1:1	85	1.94

[a] Measured by ^{1}H NMR. [b] Measured by GPC.

Transmission Electron Microscopy. Transmission electron microscopy (TEM) was performed using a Philips CM 200 unit, operated at an acceleration voltage of 80 kV. For the observation of size and distribution of micellar particles, a drop of sample solution (concentration = 1 g/L) was placed onto a 300 mesh copper grid coated with carbon. About 2 min after deposition, the grid was tapped with filter paper to remove surface water, followed by air-drying. Negative staining was performed by using a droplet of a 5 wt % uranyl acetate solution. The samples were air-dried before measurement.[16]

Results and Discussion

The synthesis of the PEO−polyphosphazene diblock copolymers **(1)** was carried out by using the method reported previously.[7] The block copolymers were synthesized via the controlled cationic polymerization of a phosphoranimine at ambient temperature using amine-terminated PEO, $CH_3O-PEG-CH_2CH_2-NH_2$, as a starting material. The macroinitiator, $CH_3-PEO-NH-P(OCH_2CF_3)_2{=}NSiMe_3$ **(2),** was synthesized by the reaction of $CH_3O-PEG-CH_2CH_2-NH_2$ with $BrP(OCH_2CF_3)_2{=}NSiMe_3$. After that reaction, 2 equiv of PCl_5 was added to obtain the cationic species $CH_3-PEO-NH-P(OCH_2CF_3)_2{=}N-PCl_3^+PCl_6^-$ **(3)**. Subsequent reaction of the initiator with $Cl_3P{=}NSiMe_3$ allowed the preparation of PEO−poly(dichlorophosphazene) block copolymers, which were then treated with $NaOCH_2CF_3$ to yield PEO−polyphosphazene block copolymers (**1**). The molar composition ratios of the repeating units of PEO to polyphosphazene block (n:m) were 1:0.1 for PEO−PN1 and 1:1 for PEO−PN2. The number-average molecular weights of PEO−phosphazene block copolymers were estimated by comparing the ^{1}H NMR peak integration ratio of the trifluoroethoxy protons at 4.23 ppm and the methylene protons of PEO at 3.51 ppm (Table 1). The PEO−PN block copolymers were soluble in THF, acetone, methanol, and DMF but were insoluble in chloroform, toluene, and *n*-hexane.

The PEO−PN block copolymers consist of the hydrophilic PEO and the hydrophobic trifluoroethoxy-substituted polyphosphazene blocks. The amphiphilic nature of these block copolymers provides an opportunity to form organized structures in an aqueous phase. The micellar characterization of the amphiphilic PEO−PN block copolymers was carried out by using fluorescence techniques, dynamic light scattering, and TEM. The critical micelle concentrations (cmc's) of the block copolymers in an aqueous phase were determined by using 1,3-(1,1′-dipyrenyl)propane (DPP) as a fluorescence probe.[13] The intramoecular excimer formation of DPP is highly dependent on local viscosity, and thus, the intensity ratio (I_E/I_M) of the excimer peak (473 nm) to the monomer peak (378 nm) of DPP was employed as a parameter to investigate the micellar characteristics of the hydrophobic core region of several polymeric amphiphiles. In Figure 1a, the excimer formation behavior of DPP is traced in an aqueous phase in the presence of PEO−PN 2. After increasing the block copolymer concentration, the excimer formation of DPP

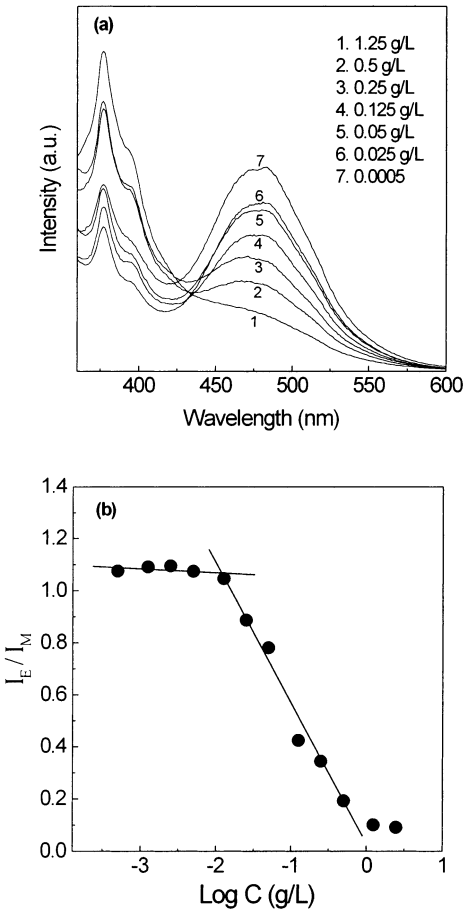

Figure 1. (a) Emission spectra of 1,3-(1,1′-dipyrenyl)propane (DPP) and (b) changes of intensity ratio (I_E/I_M) from emission spectra of DPP (3×10^{-7} M) with various concentrations of PEO−PN 2 in water.

is gradually hindered, reflecting the preferential partition of DPP into the hydrophobic micellar core region with limited mobility. Therefore, the change of the I_E/I_M after increasing the block copolymer concentration was utilized to determine the cmc of the PEO−PN block copolymers in an aqueous phase. The cmc's were determined from the onset concentration of a substantial decrease in the I_E/I_M ratio, which was represented by the intersection of two straight lines in Figure 1b. The cmc values of PEO−PN 1 and PEO−PN 2 were 12.4 and 5.2 mg/L, respectively. The longer phosphazene block induced a lower cmc value. These values are much lower than that of low molecular weight surfactants, e.g., 2.3 g/L for sodium dodecyl sulfate (SDS) in water, and were comparable with other polymeric amphiphiles.[17]

The spherical shape of the micellar aggregates and the broad size distribution were observed by using TEM (Figure 2).[16] The mean diameters (d) of the micelles of PEO−PN 1 and PEO−PN 2, measured by dynamic light scattering, were 100 and 120 nm, respectively (Table 2). A multicore type micellar structure might be suggested, considering the length of the block copolymer chain and the micelle size.

The microviscosity of the micellar core with the hydrophobic phosphazene block was estimated by measurement of the steady-state fluorescence anisotropy (r) originated from the depolarization of the fluorescence of 1,6-diphenyl-1,3,5-hexatriene (DPH) due to the rotational diffusion of DPH.[12] The anisotropy value decreases with decreasing microviscosity of the micellar

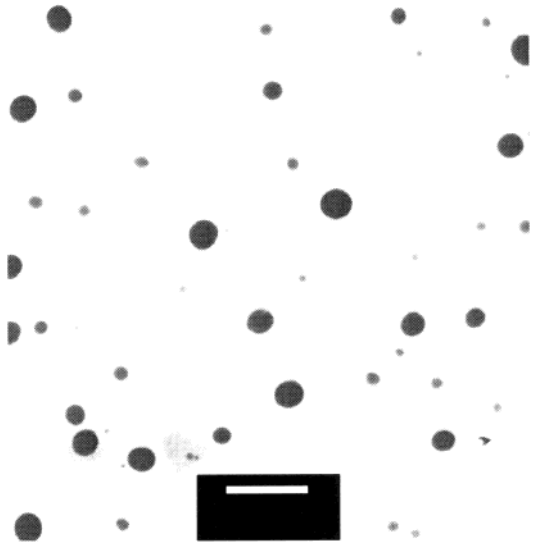

Figure 2. TEM image of micellar aggregates from PEO−PN 2.

Table 2. Micellar Characterization of PEO−PN Block Copolymers

	cmc[a] (mg/L)	d[b] (nm)	μ_2/Γ^2 [c]	r[d]
PEO−PN 1	12.4	100	0.144	0.103
PEO−PN 2	5.2	120	0.223	0.108

[a] Measured at 25 °C. [b] Measured by dynamic light scattering. [c] Polydispersity factor. [d] Steady-state fluorescence anisotropy.

core because the rotational diffusion of DPH is enhanced. The anisotropy values, r, measured for PEO−PN 1 and PEO−PN 2 are 0.103 and 0.108, respectively. The anisotropy values are not very dependent on the length of the hydrophobic blocks but more dependent on the chemical structure of the hydrophobic block.[9,11] Therefore, it is interesting to compare these values with those of other polymeric amphiphiles, i.e., poly(2-ethyl-2-oxazoline)−poly(L-lactide) (0.284), poly(2-ethyl-2-oxazoline)−poly(ϵ-caprolactone) (0.196),[11] poly(1-octadecene-*co*-maleic acid) (0.273).[18] The low anisotropy values for PEO−PN block copolymers compared to other polymeric amphiphiles may be due to the inherent flexibility of the trifluoroethoxy-substituted polyphosphazene block, which could allow enhanced rotational diffusion of the probe.

Conclusions

The micellar characteristics of amphiphilic diblock copolymers based on a hydrophobic polyphosphazene and hydrophilic PEO were examined by using fluorescence techniques, dynamic light scattering, and transmission electron microscope. The amphiphilic nature of the block copolymers provides an opportunity to self-organize in aqueous phase. The critical micelle concentrations of PN−PEO 1 and PN−PEO 2, determined by a fluorescence technique, were 12.4 and 5.2 mg/L, respectively. TEM and dynamic light scattering results indicate that the spherical micellar aggregates are formed with an average diameter 100−120 nm. The steady-state fluorescence anisotropy values (r) of 1,6-diphenyl-1,3,5-hexatriene (DPH) for the PEO−PN block copolymers (0.103−0.108) are lower than those for other organic polymeric amphiphiles. This result can be ascribed to the flexible nature of the phosphazene backbone which constitutes the hydrophobic micellar core.

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