Dynamic and Static Light Scattering Studies on Self-Aggregation Behavior of Biodegradable Amphiphilic Poly(ethylene oxide)−Poly[(*R*)-3-hydroxybutyrate]−Poly(ethylene oxide) Triblock Copolymers in Aqueous Solution

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The self-aggregation behavior of two amphiphilic poly(ethylene oxide)−poly[(*R*)-3-hydroxybutyrate]−poly-(ethylene oxide) (PEO−PHB−PEO) triblock copolymer samples with nearly identical PHB block lengths but different PEO block lengths, PEO−PHB−PEO(2000−810−2000) and PEO−PHB−PEO(5000−780−5000), was studied with dynamic and static light scattering (DLS and SLS), in combination with fluorescence spectroscopy and transmission electron microscopy (TEM). The formation of polymeric micelles by the two PEO−PHB−PEO triblock copolymers was confirmed with fluorescence technique and TEM. DLS analysis showed that the hydrodynamic radius (R_h) of the monodistributed polymeric micelles increased with an increase in PEO block length. The relative thermostability of the triblock copolymer micelles was studied by SLS and DLS at different temperatures. The aggregation number and the ratio of the radius of gyration over hydrodynamic radius were found to be independent of temperature, probably due to the strong hydrophobicity of the PHB block. The combination of DLS and SLS studies indicated that the polymeric micelles were composed of a densely packed core of hydrophobic PHB blocks and a corona shell formed by hydrophilic PEO blocks. The aggregation numbers were found to be ∼53 for PEO−PHB−PEO(2000−810−2000) micelles and ∼37 for PEO−PHB−PEO(5000−780−5000) micelles. The morphology of PEO−PHB−PEO spherical micelles determined by DLS and SLS measurements was further confirmed by TEM.

Introduction

Amphiphilic block copolymers can self-assemble into highly ordered supramolecular architectures in aqueous solution with different morphologies, such as spherical,[1] vesicular,[2] cylindrical,[3] and planar[4] geometries. Potential applications for these polymers include nanoreactors,[5] drug delivery,[6] and templates for synthesis of mesoporous materials and nanoparticles.[7] Among the amphiphilic block copolymers employed for drug delivery, triblock copolymers consisting of poly(ethylene oxide) (PEO) and poly(α-hydroxyalkanoic acid)s such as poly(lactide) (PLA)[8] and poly(lactic-*co*-glycolide) (PLGA)[9] have been very attractive because of their biocompatibility and biodegradability. In their core−shell structured micelles, the hydrophobic polyester core serves as a loading reservoir for lipophilic agents and the hydrophilic shell acts as a steric stabilizer for the hydrophobic core. In the end, the hydrophobic polyester core is degraded and excreted from living systems.

Optically active poly[(*R*)-3-hydroxybutyrate] (PHB), as a poly(β-hydroxyalkanoic acid), is produced by many microorganisms as an intracellular carbon and energy storage material.[10] Consisting entirely of *R* units, natural PHB exhibits a relatively higher crystallinity and stronger hydrophobicity but lower glass transition temperature (∼5 °C) compared to synthetic poly(α-hydroxyalkanoic acid).[11] Furthermore, natural PHB has a lower

in vivo degradation rate.[12] Therefore, biodegradable and biocompatible PHB has attracted much attention lately with its potential environmental, pharmaceutical, and biomedical applications.[13] Recently, we reported the synthesis and characterization of PEO−PHB−PEO triblock copolymers and their inclusion complexation with cyclodextrins.[14,15] Their micellization phenomena were also studied by use of the pyrene fluorescence absorption technique. Unlike the temperature-sensitive micellization of triblock copolymers consisting of PEO and poly(α-hydroxyalkanoic acid), the micelle formation of triblock copolymers consisting of PEO and poly(β-hydroxyalkanoic acid) was temperature-insensitive, inferred from their unchanged critical micelle concentration (cmc) in water at various temperatures.[16]

Light scattering was well used to determine the diffusion coefficient (*D*), the hydrodynamic radius (R_h), the root-mean-square *z*-average radius of gyration ($\langle R_g^2 \rangle_z^{1/2}$) (normally written as R_g), the weight-average molecular weight ($M_{w,agg}$), the second virial coefficient (A_2), and the aggregation phenomena of polymers in solution.[17] It is probably the most convenient technique for studying the actual shapes of polymers and for investigating the polymer−solvent interactions and intra- and interpolymer interactions in dilute solution.[17a] Thus, compared to the fluorescence absorption technique, more information on PEO−PHB−PEO triblock copolymer micelles, such as the actual shape and the aggregation number of the micelle and the interaction between polymer and solvent, can be obtained from laser light scattering studies. Herein, two PEO−PHB−PEO triblock copolymers with fixed PHB block length ($M_n =$

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Amphiphilic Triblock Copolymers in Solution

J. Phys. Chem. B, Vol. 110, No. 12, 2006 **5921**

TABLE 1: Molecular Characteristics of PEO–PHB–PEO Triblock Copolymers

| copolymer[a] | M_n[b] | M_w[b] | M_w/M_n[b] | block length (M_n) | |
				PEO[b]	PHB[c]
PEO–PHB–PEO(2000–810–2000)	5050	5260	1.04	1820	810
PEO–PHB–PEO(5000–780–5000)	10390	11200	1.08	4740	780

[a] The numbers in parentheses show the indicative block length of each block in g mol^{-1}. [b] Determined by GPC. [c] Calculated from ^{1}H NMR results.

800) but varied PEO block length were synthesized, and their self-aggregation in water at different temperatures was studied by use of dynamic and static laser light scattering for the first time. We have found that the micelle size and the aggregation number can be well controlled by changing the copolymer composition and that the micelles are quite stable at high temperature.

Experimental Section

Materials. Natural-source poly[(R)-3-hydroxybutyrate] (PHB) was supplied by Aldrich, and purified by being dissolved in chloroform followed by filtration and subsequent precipitation in hexane before use. The M_n and M_w of the purified PHB were 8.7×10^4 and 2.3×10^5, respectively. Methoxy poly(ethylene oxide) monopropionic acid (M-PEO-A) with a molecular weight of ca. 5000 was purchased from Shearwater Polymers, Inc. Methoxy poly(ethylene oxide) (M-PEO) with a molecular weight of ca. 2000 was supplied by Sigma. Bis(2-methoxyethyl) ether (diglyme, 99%), ethylene glycol (99%), dibutyltin dilaurate (95%), 1,3-N,N′-dicyclohexylcarbodiimide (DCC, 99%), 4-(dimethylamino)pyridine (DMAP, 99%), succinic anhydride (97%), triethylamine (99%), pyrene, and phosphotungstic acid were obtained from Aldrich. Diglyme was dried with a molecular sieve, and methylene chloride was distilled from CaH$_2$ before use.

Synthesis of PEO–PHB–PEO Triblock Copolymers. Telechelic hydroxylated PHB (PHB-diol) prepolymers were prepared from natural-source PHB and diethylene glycol, and M-PEO-monocarboxylic acid (M-PEO-A) prepolymer with M_n 1820 was prepared from M-PEO and succinic anhydride, as reported previously.[18,19]

The synthesis and characterization of PEO–PHB–PEO triblock copolymers were reported previously.[14] Typically, 1.82 g (1.00 mmol) of M-PEO-A (M_n 1820, M_w/M_n 1.03), 0.30 g (0.40 mmol) of PHB-diol (M_n 750, M_w/M_n 1.06) and 0.04 g (0.33 mmol) of DMAP were dried in a 250 mL two-neck flask at 60 °C under high vacuum overnight. Then, 30 mL of anhydrous methylene chloride was added to the flask, and any trace water in the system was removed through azeotropic distillation with 10 mL of methylene chloride being left in the flask. When the flask was cooled to room temperature, 1.60 mL of DCC in anhydrous methylene chloride (1 M) was added, and the reaction mixture was stirred overnight at room temperature under dried nitrogen. The precipitated dicyclohexylurea was filtered off and the filtrate was precipitated in diethyl ether. The desired PEO–PHB–PEO triblock copolymer was collected by filtration and fractionated in methanol and diethyl ether mixture to remove remaining M-PEO-A. Yield: 0.93 g, 46%. Two triblock copolymers were prepared, and their molecular characteristics are given in Table 1.

Molecular Characterization. Gel permeation chromatography (GPC) analysis was carried out with a Shimadzu SCL-10A and LC-8A system equipped with two Phenogel 5μm, 50 and 1000 Å columns (size 300 × 4.6 mm) in series and a Shimadzu RID-10A refractive index detector. Tetrahydrofuran (THF) was used as eluent at a flow rate of 0.30 mL/min at 40 °C.

Monodispersed poly(ethylene glycol) standards were used to obtain a calibration curve. The ^{1}H NMR spectra were recorded on a Bruker AV-400 NMR spectrometer at 400 MHz at room temperature. The ^{1}H NMR measurements were carried out with an acquisition time of 3.2 s, a pulse repetition time of 2.0 s, a 30° pulse width, 5208 Hz spectral width, and 32K data points. Chemical shift was referred to the solvent peaks ($\delta = 7.3$ ppm for CHCl$_3$).

Fluorescence Spectroscopy. A series of PEO–PHB–PEO triblock copolymer aqueous solutions with concentrations ranging from 0.0025 to 3 mg/mL for PEO–PHB–PEO(2000–810–2000) and from 0.0025 to 50 mg/mL for PEO–PHB–PEO-(5000–780–5000) were prepared by diluting its stock solution with deionized water, respectively. An equal amount of pyrene–methanol solution was transferred to a number of cylindrical vials, and methanol was then evaporated under nitrogen flow before an equal volume of prepared PEO–PHB–PEO solutions was added to give a final pyrene concentration of 6.0×10^{-7} M. The fluorescence excitation spectra of pyrene in PEO–PHB–PEO solution with varied concentration were finally recorded between 290 and 360 nm with an emission wavelength of 373 nm by using a Shimadzu RF-5301 PC spectrofluorophotometer at 23 °C. The ratio of the intensities at 337 and 334 nm (I_{337}/I_{334}) were then analyzed to the determine critical micelle concentration (cmc).

Refractive Index Increment Measurements. The refractive indices of PEO–PHB–PEO triblock copolymers aqueous solutions at concentrations of 0.1–0.5 mg/mL for PEO–PHB–PEO(2000–810–2000) and 1.0–1.8 mg/mL for PEO–PHB–PEO(5000–780–5000) were measured by using a Brookhaven differential refracttometer at a wavelength of 620 nm to determine the refractive index increment (dn/dc). Prior to measurement, the instrument was calibrated with potassium chloride (KCl) aqueous solution. To study the temperature effect on the apparent molecular weight and the average aggregation number of triblock copolymer micelles, dn/dc values at different temperatures from 15 to 45° were determined.

Dynamic and Static Light Scattering. Dynamic and static light scattering (DLS and SLS) studies were performed with a Brookhaven light scattering instrument that applies vertically polarized laser light of wavelength 632.8 nm. The spectrometer was calibrated by using polystyrene standard solution of 97 ± 3.2 nm size. Light scattering studies were carried out in the concentrations ranges of 1–3 mg/mL for PEO–PHB–PEO-(2000–810–2000) and 6–10 mg/mL for PEO–PHB–PEO-(5000–780–5000). Prior to both DLS and SLS measurements, all the solvents were filtered through 0.2 μm Millipore membrane filters (Whatman) to remove dust particles; the solutions were centrifuged at 8000 rpm for 30 min and then filtered with 0.22 μm Millipore filters directly into the light scattering cell.

In DLS measurements, the intensity correlation function with a maximum number of 256 channels was measured by using a BI-9000AT digital autocorrelator. Then, the diffusion coefficient (D) of the triblock copolymer micelle was calculated from

5922 *J. Phys. Chem. B, Vol. 110, No. 12, 2006*

Li et al.

$$\Gamma = Dq^2 \qquad (1)$$

Γ is the relaxation rate ($\Gamma = 1/\tau$, τ is the relaxation time) and q is the scattering vector determined by the scattering angle θ [$q = 4\pi n/\lambda \sin\theta/2$, where n is the refractive index of the liquid medium and λ is the wavelength of the laser]. The hydrodynamic radius (R_h) was then evaluated from D according to the following equation:

$$R_h = \frac{k_B T}{6\pi\eta_s D} \qquad (2)$$

where k_B, T, and η_s are the Boltzmann constant, the absolute temperature, and the viscosity of the solvent, respectively.

The SLS measurements were carried out at different scattering angles (30°−130°) to determine the apparent molecular weight ($M_{w,agg}$), the radius of gyration (R_g), and the second virial coefficient (A_2) of PEO−PHB−PEO triblock copolymers. By measuring the optical constant (K) and the excess Rayleigh ratio (ΔR_θ) in an infinite dilution, $M_{w,agg}$, R_g, and A_2 can be determined by the extrapolation of $Kc/\Delta R_\theta$ to zero angle and zero concentration according to the following relation:

$$\frac{Kc}{\Delta R_\theta} = \frac{1}{M_w}\left[1 + \frac{16\pi n^2}{3\lambda^2}R_g^2 \sin^2\left(\frac{\theta}{2}\right)\right] + 2A_2 c \qquad (3)$$

where K is the optical constant, which depends on the refractive index increment (dn/dc) of the polymer solution ($K = 4\pi^2 n^2\cdot(dn/dc)^2/N_A\lambda^4$, where N_A is Avogadro's number, n is the refractive index of the liquid medium, and λ is the wavelength of the laser), and ΔR_θ is the excess Rayleigh ratio [$\Delta R_\theta = R_\theta$(solution) $- R_\theta$(solvent)], respectively.

Transmission Electron Microscopy (TEM). TEM micrographs were obtained using a Philips CM300 FEGTEM operating at an accelerating voltage of 300 kV. A 180 μL PEO−PHB−PEO triblock copolymer micelle solution was mixed with 20 μL of phosphotungstic acid aqueous solution (1%) through a vortex. A drop of the mixed solution was then cast onto a 200-mesh carbon-coated copper grid. After 30 s, the excess solution was removed by tapping the grid using a Kimwipe delicate wipe. The samples were finally dried at room temperature prior to measurement.

Results and Discussion

Two PEO−PHB−PEO triblock copolymers with fixed PHB block length but varied PEO block length were synthesized, and they were characterized by using GPC and ^{1}H NMR. Table 1 presents their molecular weights, molecular weight distributions, and compositions (block length). Relatively narrow molecular weight distributions were observed for both PEO−PHB−PEO triblock copolymers, and their chemical structure is shown in Chart 1.

With an amphiphilic characteristic, both PEO−PHB−PEO-(2000−810−2000) and PEO−PHB−PEO(5000−780−5000) are soluble in water and can self-assemble into polymeric micelles. Because pyrene preferentially partitions into the hydrophobic core of polymeric micelles with a concurrent change in its photophysical properties, the formation of polymeric micelles was confirmed by a fluorescence technique using pyrene as a probe as reported previously.[16] In a fluorescence spectroscopic study, the critical micelle concentration (cmc) defined as the onset of micellization was found to be 1.3 × 10^{-4} g/mL for PEO−PHB−PEO(2000−810−2000) and 7.0 × 10^{-4} g/mL for PEO−PHB−PEO(5000−780−5000), respectively, which indicates that the cmc for PEO−PHB−PEO

**CHART 1: Chemical Structure of PEO−PHB−PEO Triblock Copolymer**
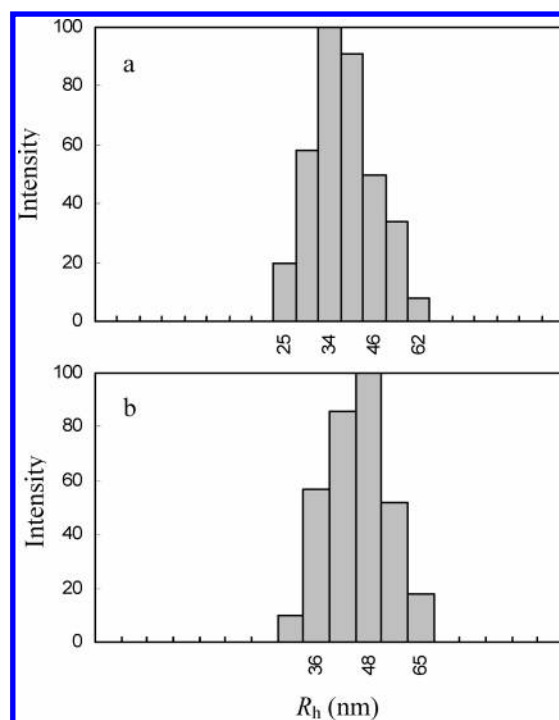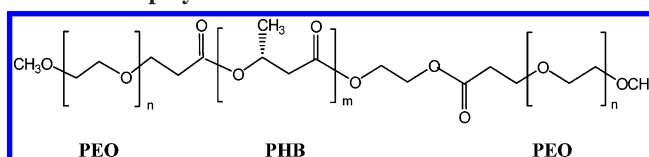
Figure 1. Hydrodynamic radius (R_h) distributions of PEO−PHB−PEO(2000−810−2000) at 2 mg/mL concentration (a) and PEO−PHB−PEO(5000−780−5000) at 8 mg/mL concentration (b) in water measured at 90°.

triblock copolymer with fixed hydrophobic PHB block length increases with an increase in hydrophilic PEO block length, as pointed out in our previous report.[16] The triblock copolymer with longer PEO block will need a higher concentration to form micelles in aqueous solution since the whole copolymer chain is more hydrophilic due to the longer hydrophilic PEO block. Next, the dynamic and static light scattering measurements (DLS and SLS) of PEO−PHB−PEO(2000−810−2000) and PEO−PHB−PEO(5000−780−5000) were carried out at concentrations well above their cmc's.

In DLS, the distributions of relaxation times were obtained by measuring the intensity time correlation functions and analyzed by means of nonnegatively constrained least-squares fit (NNLS) software provided by Brookhaven Co. Figure 1 shows the hydrodynamic radius (R_h) distributions of PEO−PHB−PEO(2000−810−2000) and PEO−PHB−PEO(5000−780−5000) in aqueous solution measured at 90° with a concentration of 2 mg/mL for PEO−PHB−PEO(2000−810−2000) and 8 mg/mL for PEO−PHB−PEO(5000−780−5000). The observation of a monomodal distribution peak with relatively narrow size distribution indicates that only one size of aggregation was found in the solution, which is identified as the polymeric micelle. With increasing PEO block length, the average R_h of PEO−PHB−PEO polymeric micelle increased from ~33 to ~44 nm.

Figure 2 shows the dependence of relaxation rate (Γ) on the square of the scattering vector (q) at 25 °C for PEO−PHB−PEO(5000−780−5000) with a concentration of 8 mg/mL. As

Amphiphilic Triblock Copolymers in Solution
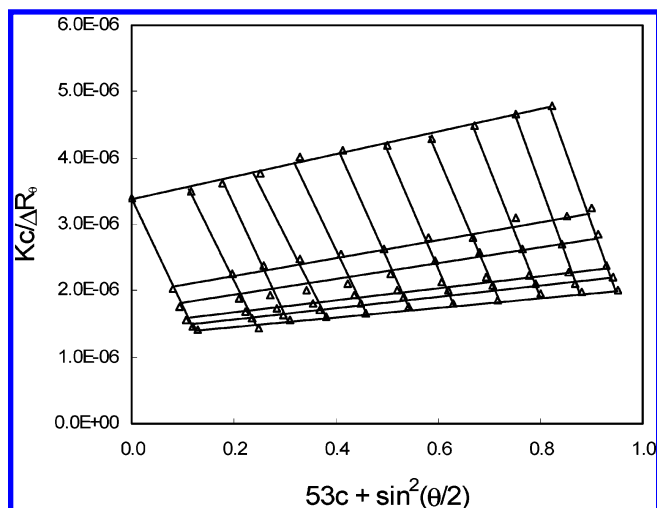
J. Phys. Chem. B, Vol. 110, No. 12, 2006 **5923**
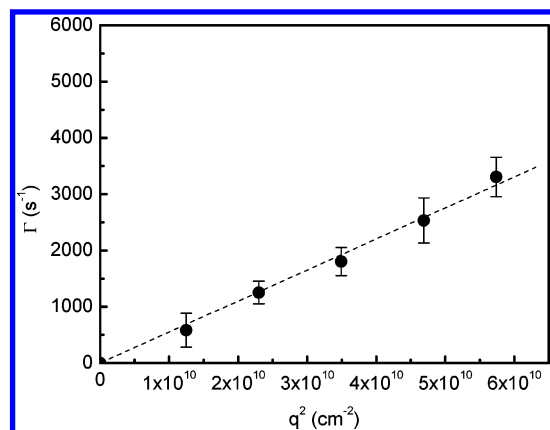
Figure 2. Dependence of relaxation rate on scattering vector for PEO–PHB–PEO(5000–780–5000) polymeric micelle in water at a concentration of 8 mg/mL.
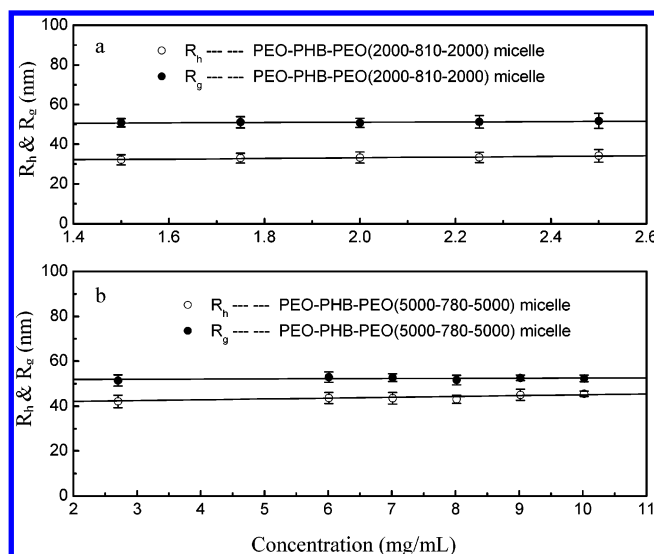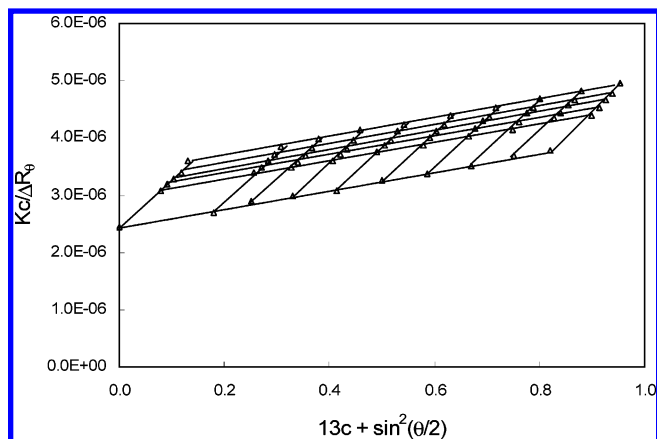

Figure 3. Dependence of hydrodynamic radii (R_h) and radii of gyration (R_g) of PEO–PHB–PEO(2000–810–2000) (a) and PEO–PHB–PEO-(5000–780–5000) (b) in water against its concentration at 25 °C.

Figure 4. Zimm plot of PEO–PHB–PEO(2000–810–2000) in water at 25 °C, where c changes from 1.5 to 2.5 mg/mL. The lines are drawn to visualize the data points.

Figure 5. Zimm plot of PEO–PHB–PEO(5000–780–5000) in water at 25 °C, where c changes from 6 to 10 mg/mL. The lines are drawn to visualize the data points.

shown in Figure 2, the relaxation rate of polymeric micelle is linearly dependent on the square of the scattering vector (q^2) passing through the intercept (0,0), indicating that the relaxation mode shows a translational diffusion behavior. The diffusion coefficient (D) was then determined from the slopes of Γ vs q^2 plots according to eq 1 to calculate the R_h value of PEO–PHB–PEO(5000–780–5000) polymeric micelles through the Stokes–Einstein equation (eq 2). Translational diffusion behavior was also observed with PEO–PHB–PEO(2000–810–2000), and its diffusion coefficient and R_h were determined accordingly.

Figure 3 shows the concentration dependence of R_g and R_h for PEO–PHB–PEO(2000–810–2000) and PEO–PHB–PEO-(5000–780–5000) in aqueous solution at 25 °C. The R_g values were obtained from the intensity measurement in SLS at each concentration. As shown in Figure 3, within the concentration range, no significant variations of R_g and R_h values are observed for both PEO–PHB–PEO(2000–810–2000) and PEO–PHB–PEO(5000–780–5000), indicating that the solutions were measured at the dilute concentration regime without interference from the neighboring micelle and the polymeric micelle is so stable that its aggregation behavior is not dependent on polymer concentration. The values of R_g and R_h at infinite dilute aqueous solution are determined through extrapolating the concentration to zero and are found to be 51.0 and 32.3 nm for PEO–PHB–PEO(2000–810–2000) and 51.8 and 42.1 nm for PEO–PHB–

PEO(5000–780–5000), respectively. Although the R_h value of PEO–PHB–PEO(2000–810–2000) is comparatively lower than that of PEO–PHB–PEO(5000–780–5000), the values of R_g are similar within the experimental error for both triblock copolymers, which will be interpreted later.

Figures 4 and 5 show the angular (θ) and the concentration (c) dependence of $Kc/\Delta R_\theta$ (Zimm plot) for PEO–PHB–PEO-(2000–810–2000) and PEO–PHB–PEO(5000–780–5000) aqueous solution measured at 25 °C. The values of apparent molecular weight ($M_{w,agg}$) were estimated by extrapolation of c and θ to zero, and the radius of gyration (R_g) and the second virial coefficient (A_2) were estimated from the slope of the angular and concentration dependence of the Zimm plots, respectively. The results are presented in Table 2. The much higher $M_{w,agg}$ for both PEO–PHB–PEO(2000–810–2000) and PEO–PHB–PEO(5000–780–5000) compared to the corresponding M_w of single triblock copolymer chain determined by GPC confirms the presence of polymeric micelles in water. The values of R_g obtained from Zimm plots (Figures 4 and 5) are in good agreement with the results determined by the intensity measurement within the experimental error, and the large R_g value also substantiates the presence of polymeric micelles in solution. Interestingly, while a positive value of A_2 is obtained for PEO–PHB–PEO(5000–780–5000), the value of A_2 for PEO–PHB–PEO(2000–810–2000) is found to be negative (shown in Table 2). This is because, with shorter PEO block

TABLE 2: Dynamic and Static Light Scattering Measurements of PEO−PHB−PEO Triblock Copolymers in Water at 25 °C

	PEO−PHB−PEO(2000−810−2000)	PEO−PHB−PEO(5000−780−5000)
R_h (nm)	32.5 ± 2.2	42.6 ± 2.8
R_g (nm)	50.0 ± 3.8	52.0 ± 2.5
R_g/R_h	1.54	1.23
$M_{w,agg}$ (g mol^{-1})	(2.78 ± 0.38) × 10^5	(4.09 ± 0.25) × 10^5
A_2 (cm^3 mol g^{-2})	(−3.2 ± 0.4) × 10^{-4}	(2.7 ± 0.5) × 10^{-5}
N_{agg}	53 ± 7	37 ± 2

TABLE 3: Refractive Index Increments and Static Light Scattering Measurements of PEO−PHB−PEO Triblock Copolymers in Water at Various Temperatures

temp (°C)	PEO−PHB−PEO(2000−810−2000)			PEO−PHB−PEO(5000−780−5000)		
	dn/dc (mL/g)	$M_{w,agg} \times 10^{-5}$ (g mol^{-1})	N_{agg}	dn/dc (mL/g)	$M_{w,agg} \times 10^{-5}$ (g mol^{-1})	N_{agg}
15	0.1254 ± 0.003	2.74 ± 0.25	52 ± 5	0.1306 ± 0.003	4.10 ± 0.45	37 ± 4
25	0.1244 ± 0.002	2.78 ± 0.38	53 ± 7	0.1299 ± 0.001	4.09 ± 0.25	37 ± 2
35	0.1237 ± 0.003	2.78 ± 0.25	53 ± 5	0.1288 ± 0.001	4.05 ± 0.24	36 ± 2
45	0.1221 ± 0.002	2.66 ± 0.22	51 ± 4	0.1238 ± 0.001	4.13 ± 0.36	37 ± 3

length, PEO−PHB−PEO(2000−810−2000) exhibits more hydrophobic character which substantiates the unfavorable interaction between polymer and solvent leading to a negative A_2 value. The average aggregation number (N_{agg}) for the PEO−PHB−PEO triblock copolymer micelle was calculated from its apparent molecular weight ($M_{w,agg}$) according to the following equation and listed in Table 2.

$$N_{agg} = \frac{M_{w,agg}}{M_w} \qquad (4)$$

where M_w is the weight-average molecular weight for a single PEO−PHB−PEO triblock copolymer chain determined by GPC. The N_{agg} decreases from ∼53 for PEO−PHB−PEO(2000−810−2000) to ∼37 for PEO−PHB−PEO(5000−780−5000) with increasing PEO block length. This is also due to the more hydrophobic character of PEO−PHB−PEO(2000−810−2000) than that of PEO−PHB−PEO(5000−780−5000) as discussed in A_2 measurements. The decrease in N_{agg} for amphiphilic block copolymer with increasing hydrophilic block length has been reported previously.[20,21] Because of the larger N_{agg} of PEO−PHB−PEO(2000−810−2000) polymeric micelle compared to that of PEO−PHB−PEO(5000−780−5000), the values of R_g relating to mass distribution for both PEO−PHB−PEO(2000−810−2000) and PEO−PHB−PEO(5000−780−5000) are relatively the same even though the R_h values are significantly smaller in PEO−PHB−PEO(2000−810−2000), as shown in Table 2.

It is well established that the ratio of R_g/R_h is a characteristic parameter indicating the conformation of polymer chain. The values of R_g/R_h for hard-sphere micelle, random coil, and rodlike structure are reported to be 0.78, 1.78, and ≥2, respectively.[22] As shown in Table 2, the values of R_g/R_h for PEO−PHB−PEO(2000−810−2000) and PEO−PHB−PEO(5000−780−5000) are 1.54 and 1.23, respectively. Therefore, the large values of R_g/R_h in our study suggest that the PEO−PHB−PEO triblock copolymers do not form uniform spherical micelles. In contrast, due to the strong hydrophobicity and the small composition of the PHB block, PEO−PHB−PEO triblock copolymers self-assemble in aqueous solution into spherical micelles with a densely packed core of hydrophobic PHB blocks and the hydrophilic PEO blocks stretch out into the aqueous medium to form shell-like star polymers with many arms.[23] Recently, we found that cubic silsesquioxane−poly(ethylene oxide) aggregated to form core−shell structured micelles in water with a large ratio of R_g/R_h of 1.46.[17f]

The radius of PHB core (R_c) of polymeric micelles was calculated according to the following equation and found to be 2.6 nm for PEO−PHB−PEO(5000−780−5000) micelle and 2.2 nm for PEO−PHB−PEO(2000−810−2000) micelle, respectively.

$$R_c = \left(\frac{3N_{agg}M_{w,PHB}}{4\pi N_A \rho_{PHB}}\right)^{1/3} \qquad (5)$$

where $M_{w,PHB}$ is the molecular weight of the PHB block and ρ_{PHB} is the density of PHB with a value of 1.18 g/cm^3.[24] Thus, the shell thickness ($R_h - R_c$) is found to be ∼30 nm for PEO−PHB−PEO(2000−810−2000) micelle and ∼40 nm for PEO−PHB−PEO(5000−780−5000) micelle, respectively. The end-to-end distance of PEO block in PEO−PHB−PEO(2000−810−2000) and PEO−PHB−PEO(5000−780−5000) triblock copolymers was calculated according to the literature assuming a helical configuration of PEO[25] and found to be ∼14 and ∼35 nm, respectively. Comparison of the thickness of polymeric micelle shell with these calculated end-to-end distances of PEO block showed that the PEO blocks are stretched in PEO−PHB−PEO(2000−810−2000) micelles, while the PEO blocks in PEO−PHB−PEO(5000−780−5000) micelles are in folding state.

To study the thermostability of PEO−PHB−PEO micelle, DLS and SLS measurements were carried out for both PEO−PHB−PEO(2000−810−2000) and PEO−PHB−PEO(5000−780−5000) aqueous solution at various temperatures ranging from 15 to 50 °C. The refractive index increments (dn/dc) of PEO−PHB−PEO(2000−810−2000) and PEO−PHB−PEO(5000−780−5000) at various temperatures were also measured to determine the $M_{w,agg}$ and N_{agg} of polymeric micelle, and the results are presented in Table 3. As shown in Table 3, both $M_{w,agg}$ and N_{agg} are constant at all temperatures, indicating that the polymeric micelle is stable within the measuring temperature range. This is quite different from amphiphilic triblock copolymers containing PLGA or poly(propylene oxide) (PPO) as the middle hydrophobic block, which are usually thermosensitive, and an increased N_{agg} has been observed with increasing temperature.[27] Generally, amphiphilic block copolymers will become more hydrophobic at higher temperature, intermicellar aggregation will occur, and a larger N_{agg} is expected. However, the strong hydrophobicity of the PHB block in PEO−PHB−PEO triblock copolymers is believed to lead to the formation of a polymeric micelle with densely packed a PHB hydrophobic core which is not further influenced by increasing temperature. Thus, the obtained polymeric micelles are quite stable with temperature.

The dependence of R_h and R_g values of the PEO−PHB−PEO triblock copolymers on temperature is shown in Figure 6.

Amphiphilic Triblock Copolymers in Solution

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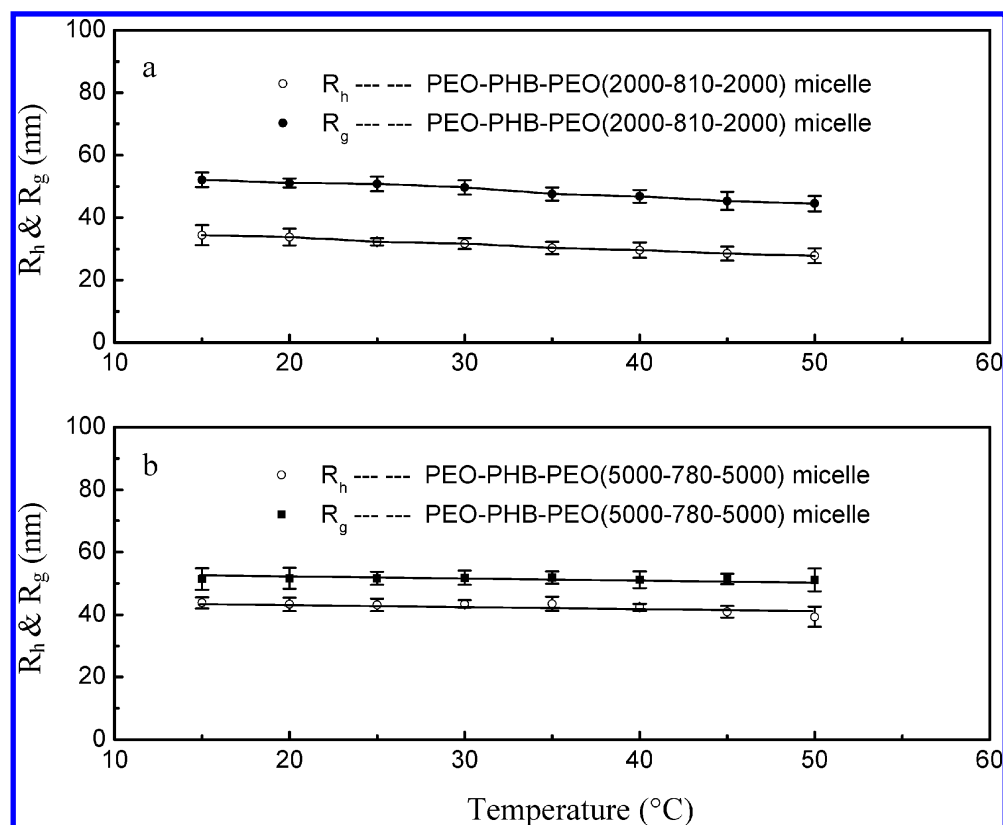
Figure 6. Dependence of hydrodynamic radii (R_h) and radii of gyration (R_g) of PEO−PHB−PEO(2000−810−2000) (a) and PEO−PHB−PEO-(5000−780−5000) (b) in water against temperature with concentrations of 2 mg/mL for PEO−PHB−PEO(2000−810−2000) and 8 mg/mL for PEO−PHB−PEO(5000−780−5000).

Both R_g and R_h values of PEO−PHB−PEO(2000−810−2000) and PEO−PHB−PEO(5000−780−5000) slightly decrease as temperature increases from 15 to 50 °C. As both $M_{w,agg}$ and N_{agg} are constant in the measuring temperature range as observed above, the decrease in R_h relating to the excluded volume with increasing temperature is ascribed to the dehydration of PEO blocks at higher temperature. Because the composition of the PEO block is significantly higher than that of the PHB block in PEO−PHB−PEO triblock copolymers, the R_g defined as the density distribution of the micelles is mainly contributed by the corona segments of PEO blocks. Thus, the dehydration of PEO blocks at higher temperature also leads to a decrease in R_g value. The weaker dependence of R_g and R_h values of PEO−PHB−PEO(5000−780−5000) on temperature compared with that of PEO−PHB−PEO(2000−810−2000) is observed in Figure 6. The reason is that the decrease in R_h and R_g due to the gradual dehydration of PEO blocks in PEO−PHB−PEO(5000−780−5000) micelles is compensated by the conformational change of longer PEO blocks in the corona to become extended with increasing temperature. As R_g and R_h values decrease in parallel with increasing temperature as shown in Figure 6, the ratio of R_g/R_h is constant indicating that the actual shape of PEO−PHB−PEO triblock copolymer micelles is unchanged within the measured temperature range. The temperature insensitivity of PEO−PHB−PEO micelle indicated by DLS and SLS studies is in good agreement with that obtained from the pyrene fluorescence absorption technique.[16]

To confirm the morphologies of PEO−PHB−PEO micelles obtained from light scattering measurements, the direct observation of polymeric micelles was carried out by use of transmission electron microscopy (TEM). Figure 7 shows the TEM images of PEO−PHB−PEO(2000−810−2000) and PEO−PHB−PEO-(5000−780−5000) micelle. As shown in Figure 7, spherical
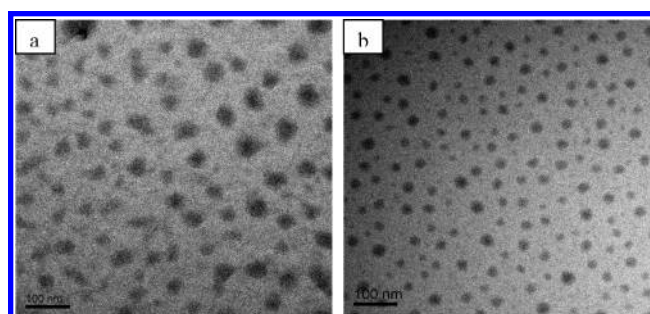
Figure 7. TEM images of PEO−PHB−PEO(2000−810−2000) (a) and PEO−PHB−PEO(5000−780−5000) (b) polymeric micelles.

micelles were observed with an averaged particle radius of ∼20 nm for PEO−PHB−PEO(2000−810−2000) micelle and ∼13 nm for PEO−PHB−PEO(5000−780−5000) micelle, respectively, which are smaller than those measured by DLS (R_h = 33 nm for PEO−PHB−PEO(2000−810−2000) and R_h = 43 nm for PEO−PHB−PEO(5000−780−5000)). This is because TEM micrographs show the micelle with corona shrinkage after evaporation of water during sample preparation, while DLS gives the size of swollen nanoparticles in aqueous solution. In contrast to the higher R_h for PEO−PHB−PEO(5000−780−5000) micelle compared to that of PEO−PHB−PEO(2000−810−2000) micelle determined from DLS, the micelle size for PEO−PHB−PEO(5000−780−5000) micelle observed by TEM is smaller than that of PEO−PHB−PEO(2000−810−2000) micelle, which is ascribed to the higher N_{agg} of PEO−PHB−PEO(2000−810−2000) micelle with more hydrophobic character.

To further elucidate the smaller PEO−PHB−PEO(5000−780−5000) micelle size observed by TEM, the average particle density (ρ_p) of PEO−PHB−PEO micelle was calculated from

$M_{\text{w,agg}}$ and R_{h} according to the following equation and found to be 3.4×10^{-3} g/cm³ for PEO−PHB−PEO(2000−810−2000) micelle and 2.0×10^{-3} g/cm³ for PEO−PHB−PEO(5000−780−5000) micelle, respectively.

$$\rho_{\text{p}} = \frac{3M_{\text{w,agg}}}{4\pi N_A R_{\text{h}}{}^3} \quad (6)$$

The smaller value of ρ_{p} for PEO−PHB−PEO(5000−780−5000) micelle indicates that more water is included in its hydrodynamic volume compared to that in the hydrodynamic volume of PEO−PHB−PEO(2000−810−2000) micelle. Thus, after evaporation of water, the size of PEO−PHB−PEO(5000−780−5000) micelle in the dry state could be smaller than that of PEO−PHB−PEO(2000−810−2000) micelle.

Conclusions

PEO−PHB−PEO triblock copolymers can self-assemble spontaneously into water to form polymeric micelles, which was confirmed by a fluorescence technique. With increasing PEO block length, the cmc value determined by the fluorescence technique increased from 1.3×10^{-4} g/mL for PEO−PHB−PEO(2000−810−2000) to 7.0×10^{-4} g/mL for PEO−PHB−PEO(5000−780−5000).

DLS analysis showed the R_{h} of polymeric micelle increases with increasing PEO block length. The combination of SLS and DLS measurements indicated that the spherical micelles formed from PEO−PHB−PEO triblock copolymers are composed of a densely packed core of hydrophobic PHB blocks and hydrophilic PEO blocks stretching out into the aqueous medium to form the corona. With increasing PEO block length, the N_{agg} of polymeric micelle changed from ∼53 for PEO−PHB−PEO-(2000−810−2000) micelle to ∼37 for PEO−PHB−PEO(5000−780−5000) micelle. The stronger hydrophobic character of PEO−PHB−PEO(2000−810−2000) is responsible for its negative A_2 and higher N_{agg}. Both R_{g} and R_{h} values of PEO−PHB−PEO micelles decreased slightly with increasing temperature, whereas the ratio of $R_{\text{g}}/R_{\text{h}}$, $M_{\text{w,agg}}$, and N_{agg} of the polymeric micelle are constant, indicating that the obtained polymeric micelles are quite stable with temperature and their actual shape is unchanged within the measured temperature range.

The spherical structure visualized in TEM images of polymeric micelle confirmed the morphology of spherical micelles obtained by a combination of DLS and SLS measurements. Attributed to the higher N_{agg} of PEO−PHB−PEO(2000−810−2000) micelle with higher particle density, a larger size with PEO−PHB−PEO(2000−810−2000) micelle was observed by TEM even though its R_{h} is smaller than that with PEO−PHB−PEO(5000−780−5000) micelle in aqueous solution as determined in DLS measurements.

Due to the stronger hydrophobicity of PHB block, the PEO−PHB−PEO micelle is temperature-insensitive at body temperature. The size and the aggregation number of polymeric micelles can be controlled by adjusting polymer composition. Thus, the biodegradable amphiphilic PEO−PHB−PEO triblock copolymers may find promising biomedical applications as stable drug carriers.

Supporting Information Available: Full description of fluorescence spectroscopic study. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Savić, R.; Luo, L. B.; Eisenberg, A.; Maysinger, D. *Science* **2003**, *300*, 615. (b) Allen, C.; Han, J.; Yu, Y. S.; Maysinger, D.; Eisenberg, A. *J. Controlled Release* **2000**, *63*, 275. (c) Soo, P. L.; Luo, L. B.; Maysinger, D.; Eisenberg, A. *Langmuir* **2002**, *18*, 9996. (d) Lazzari, M.; López-Quintela, M. A. *Adv. Mater.* **2003**, *15*, 1583.

(2) (a) Discher, D. E.; Eisenberg, A. *Science* **2002**, *297*, 967. (b) Battaglia, G.; Ryan, A. J. *J. Am. Chem. Soc.* **2005**, *127*, 1012. (c) Discher, B. M.; Won, Y.; Ege, D. S.; Lee, J. C.-M.; Bates, F. S.; Discher, D. E.; Hammer, D. A. *Science* **1999**, *284*, 1143. (d) Chécot, F.; Lecommandoux, S.; Gnanou, Y.; Klok, H. A. *Angew. Chem., Int., Ed.* **2002**, *41*, 1339. (e) Zhou, Y. F.; Yan, D. Y. *Angew. Chem., Int. Ed.* **2004**, *43*, 4896.

(3) (a) Wang, X. S.; Winnik, M. A.; Manners, I. *Macromolecules* **2005**, *38*, 1928. (b) Won, Y. Y.; Davis, H. T.; Bates, F. S. *Science* **1999**, *283*, 960.

(4) (a) Gomez, E. D.; Rappl, T. J.; Agarwal, V.; Bose, A.; Schmutz, M.; Marques, C. M.; Balsara, N. P. *Macromolecules* **2005**, *38*, 3567. (b) Jenekhe, S. A.; Chen, X. L. *Science* **1998**, *279*, 1903.

(5) Riess, G. *Prog. Polym. Sci.* **2003**, *28*, 1107.

(6) (a) Kim, S. Y.; Lee, Y. M. *Biomaterials* **2001**, *22*, 1697. (b) Kim, S. Y.; Ha, J. C.; Lee, Y. M. *J. Controlled Release* **2000**, *65*, 345. (c) Rosler, A. V.; Guido, W. M.; Klok, H. A. *Adv. Drug Delivery Rev.* **2001**, *53*, 95. (d) Kataoka, K.; Harada, A.; Nagasaki, Y. *Adv. Drug Delivery Rev.* **2001**, *47*, 113. (e) Ahmed, F.; Discher, D. E. *J. Controlled Release* **2004**, *96*, 37.

(7) (a) Hartgerink, J. D.; Beniash, E.; Stupp, S. I. *Science* **2001**, *294*, 1684. (b) Neiser, M. W.; Muth, S.; Kolb, U.; Harris, J. R.; Okuda, J.; Schmidt, M. *Angew. Chem., Int. Ed.* **2004**, *43*, 3192. (c) Tang, C.; Qi, K.; Wooley, K. L.; Matyjaszewski, K.; Kowalewski, T. *Angew. Chem., Int. Ed.* **2004**, *43*, 2783. (d) Bao, X. Y.; Zhao, X. S.; Li, X.; Chia, P. A.; Li, J.; *J. Phys. Chem. B* **2004**, *108*, 4684.

(8) (a) Jeong, B.; Bae, Y. H.; Lee, D. S.; Kim, S. W. *Nature* **1997**, *388*, 860. (b) Yasugi, K.; Nagasaki, Y.; Kato, M.; Kataoka, K. *J. Controlled Release* **1999**, *62*, 89.

(9) (a) Gref, R.; Minamitake, Y.; Peracchia, M. T.; Trubetskoy, V.; Torchilin, V.; Langer, R. *Science* **1994**, *263*, 1600. (b) Gref, R.; Luck, M.; Quellec, P.; Marchand, M.; Dellacherie, E.; Harnisch, S.; Blunk, T.; Muller, R. H. *Colloids Surf., B* **2000**, *18*, 301.

(10) Doi, Y. *Microbial Polyesters*; VCH Publishers: New York, 1990.

(11) Abe, H.; Doi, Y. *Biomacromolecules* **2002**, *3*, 133.

(12) Gogolewski, S.; Jovanovic, M.; Perren, S. M.; Dillon, J. G.; Hughes, M. K. *J. Biomed. Mater. Res.* **1993**, *27*, 1135.

(13) (a) Anderson, A. J.; Dawes, E. A. *Microbiol. Rev.* **1990**, *54*, 450. (b) Müller, H. M.; Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 477. (c) Lenz, R. W.; Marchessault, R. H. *Biomacromolecules* **2005**, *6*, 1.

(14) Li, J.; Li, X.; Ni, X. P.; Leong, K. W. *Macromolecules* **2003**, *36*, 2661.

(15) Li, X.; Li, J.; Leong, K. W. *Macromolecules* **2003**, *36*, 1209.

(16) Li, J.; Ni, X. P.; Li, X.; Tan, N. K.; Lim, C. T.; Ramakrishna, S.; Leong, K. W. *Langmuir* **2005**, *21*, 8681.

(17) (a) Huglin, M. B. *Light Scattering from Polymer Solutions*; Academic Press: London, 1972; p 90. (b) Zhou, Z.; Yang, Y. W.; Booth, C.; Chu, B. *Macromolecules* **1996**, *29*, 8357. (c) Tan, J. F.; Ravi, P.; Too, H. P.; Hatton, T. A.; Tam, K. C. *Biomacromolecules* **2005**, *6*, 498. (d) Altinok, H.; Yu, G. E.; Nixon, S.; Gorry, P. A.; Attwood, D.; Booth, C. *Langmuir* **1997**, *13*, 5837. (e) Zhao, Y.; Liang, H. J.; Wang, S. G.; Wu, C. *J. Phys. Chem. B* **2001**, *105*, 848. (f) Mya, K. Y.; Li, X.; Chen, L.; Ni, X. P.; Li, J.; He, C. B. *J. Phys. Chem. B* **2005**, *109*, 9455.

(18) Thomas, D. H.; Neuenschwander, P.; Suter, U. W. *Macromol. Chem. Phys.* **1996**, *197*, 1659.

(19) Bae, Y. H.; Huh, K. M.; Kim, Y.; Park, K. *J. Controlled Release* **2000**, *64*, 3.

(20) (a) Kelarakis, A.; Havredaki, V.; Rekatas, C. J.; Mai, S. M.; Attwood, D.; Booth, C.; Ryan, A. J.; Hamley, I. W.; Martini, L. G. A. *Macromol. Chem. Phys.* **2001**, *202*, 1345. (b) Booth, C.; Attwood, D. *Macromol. Rapid Commun.* **2000**, *21*, 501.

(21) Liu, S.; Weaver, J. V. M.; Tang, Y.; Billingham, N. C.; Armes, S. P. *Macromolecules* **2002**, *35*, 6121.

(22) (a) Burchard, W. In *Light Scattering: Principles and Development*; Brown, W., Ed.; Clarendon Press: Oxford, 1996; Chapter 13. (b) Wu, P.; Mohammad, S.; Chen, H.; Qiang, D.; Wu, C. *Macromolecules* **1996**, *29*, 277.

(23) Burchard, W.; Schmidt, M.; Stockmayer, W. H. *Macromolecules* **1980**, *13*, 1265.

(24) Sudesh, K.; Abe, H.; Doi, Y. *Prog. Polym. Sci.* **2000**, *25*, 1503.

(25) Johnson, J. A.; Saboungi, M. L.; Price, D. L.; Ansell, S. *J. Chem. Phys.* **1998**, *109*, 7005.

(26) (a) Mao, G.; Sukumaran, S.; Beaucage, G.; Saboungi, M. L.; Thiyagarajan, P. *Macromolecules* **2001**, 34, 552. (b) Jeong, B.; Bae, Y. H.; Kim, S. W. *Colloids Surf., B* **1999**, *16*, 185.