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Synthesis and Micellar Behavior of Novel Amphiphilic Poly[bis(trifluoroethoxy)phosphazene]-*co*-poly[(dimethylamino)ethyl methacrylate] Block Copolymers

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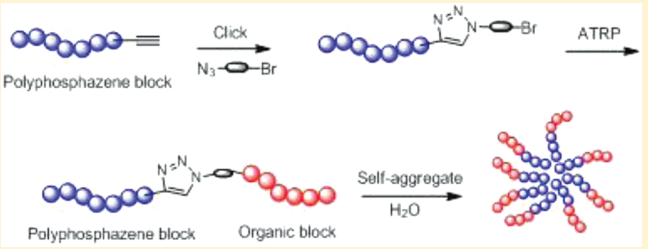
ABSTRACT: A number of amphiphilic diblock copolymers based on poly[bis(trifluoroethoxy)phosphazene] (TFE) as the hydrophobic block and poly[(dimethylamino)ethyl methacrylate] (PDMAEMA) as the hydrophilic block were developed. The TFE block was synthesized first by the controlled living cationic polymerization of a phosphoranimine, followed by replacement of all the chlorine atoms using sodium trifluoroethoxide. To allow for the growth of the PDMAEMA block, 3-azidopropyl-2-bromo-2-methylpropanoate, an atom transfer radical polymerization (ATRP) initiator, was grafted onto the end-cap of the TFE block via the "click" reaction followed by the ATRP of 2-(dimethylamino)ethyl methacrylate (DMAEMA). Once synthesized, micelles were formed by a standard method, and their characteristics were examined using fluorescence techniques, dynamic light scattering, and transmission electron microscopy. The critical micelle concentrations of the diblock copolymers as determined by fluorescence techniques using pyrene as a hydrophobic probe were between 3.47 and 9.55 mg/L, with the partition equilibrium constant of pyrene in these micelles ranging from 0.12×10^5 to 1.52×10^5. The diameters measured by dynamic light scattering were 100−142 nm at 25 °C with a narrow distribution, which were also confirmed by transmission electron microscopy.



INTRODUCTION

Amphiphilic diblock copolymers consisting of a hydrophobic and a hydrophilic segment have received considerable attention over the past few decades not only because of their unique self-aggregation properties in selective solvents to form micelles but also because of their potential applications in separation technologies, nanolithography, and drug/gene delivery.[1−6] In an aqueous environment the hydrophilic blocks will interact with the water and form the outer shell, allowing for the hydrophobic blocks to aggregate in the core to lower their interaction with the environment. By contrast, reverse micelles may be generated in an organic environment where the hydrophobic blocks form the shell and hydrophilic blocks form the core.[7] The cores of those micelles can then serve as microcontainers for various substances including drugs, dyes, and nanoparticles, while the outer shells determine the solubility and the interactions with the external environment.[8,9] Once formed, these micelles are thermodynamically stable with sizes that range from tens to a few hundreds of nanometers in diameter.[10] Therefore, it is important to develop new amphiphilic diblock copolymer structures with novel chemical and physical characteristics that can be easily tailored for specific applications.[11]

Polyphosphazenes are hybrid polymers consisting of an inorganic backbone of alternating phosphorus and nitrogen atoms with two side groups (usually organic) attached to each phosphorus.[12] These structures are synthesized via macromolecular substitutions, specifically by the replacement of chlorine atoms in poly(dichlorophosphazene) by various nucleophiles such as alkoxides,[13] aryloxides,[14] and primary or secondary amines.[15] The common synthetic route to poly(dichlorophosphazene) is through the thermal ring-opening polymerization of hexachlorocyclotriphosphazene at 250 °C in a sealed vacuum tube.[16] This route permits the facile synthesis of the poly(dichlorophosphazene), but it provides little control over molecular weight, polydispersity, and chain architecture.[17] These issues have been overcome by the recent development of an ambient-temperature living cationic via the PCl_5-induced polymerization of a phosphoranimine as reported by Allcock and Manners et al.[18] This polymerization allows for the production of a variety of polymeric phosphazene architectures with lower but controllable molecular weights and narrower polydispersities. This process has been used to synthesize a number of polyphosphazene-containing block copolymers.

Amphiphillic diblock copolyphosphazenes bearing two different side groups on two different polyphosphazene blocks, one hydrophobic and one hydrophilic, were first synthesized and their micelle properties tested in 1997 and 2001.[19,20] However, these initial diblock copolyphosphazenes had relative high critical micellar concentrations compared with other polymeric micelles.[8,9,11] To overcome these issues, unique hybrid block copolymers with a combination of polyphosphazene and organic polymer blocks were synthesized, and their ability to self-assemble into micelles in an aqueous environment was thoroughly studied. In previous work in our program, various organic blocks such as polystyrene,[21]

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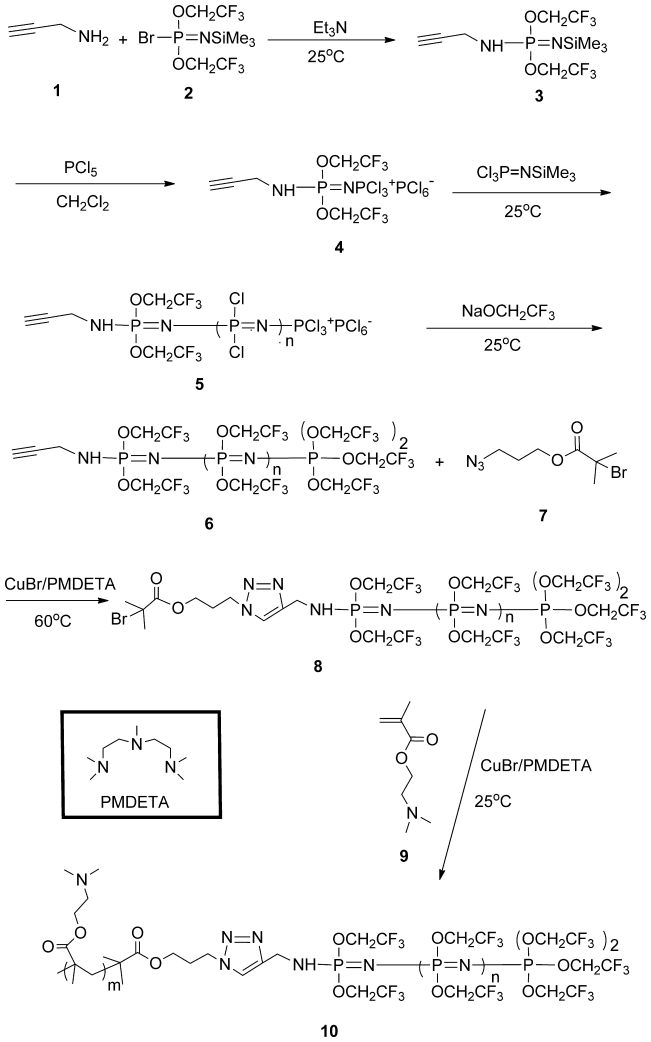
PPG,[22] and PEO[23] were linked to the polyphosphazene. These phosphazene−organic block copolymers showed advantageous micellar properties. However, they were synthesized following a "block-to" strategy, which involved the coupling two individual polymers via reaction sites at the polymer ends. Although this method was successful, it incurred disadvantages including low coupling efficiency, and the segment left unreacted after the coupling was hard to remove.[24]

In order to overcome the shortcomings of the "block-to" method, a new "block-from" approach has been widely utilized to synthesize diblock copolymers, especially after the development of atom transfer radical polymerization (ATRP).[25−28] In this approach, one block is synthesized first and the end-cap is modified to introduce a halogen-containing end group capable of acting as a macroinitiator to polymerize various monomers, including styrenes, acrylates, acrylamides, and acrylonitriles in the presence of Cu(I).[29] The resultant diblock copolymers usually have well-defined molecular weights and good chain uniformity.[25] In this present work, we report a new synthetic route to make polyphosphazene−organic amphiphilic diblock copolymers using the "block-from" method by combining the living cationic polymerization with ATRP (Scheme 1). The

Scheme 1. Synthetic Route of Diblock Copolymers



hydrophobic segment poly[bis(trifluoroethoxy)phosphazene] (TFE), which is produced via the living cationic polymerization, has been end-functionalized with an ATRP initiating site via an azide−alkyne "click" reaction. The subsequent macroinitiator was used to polymerize 2-(dimethylamino)ethyl methacrylate via ATRP to form the hydrophilic block. Once synthesized, the micellar behavior in an aqueous environment was investigated by fluorescence techniques, dynamic light scattering, and transmission electron microscopy.

EXPERIMENTAL SECTION

Materials. Lithium bis(trimethylsilyl)amide (Aldrich), propargylamine hydrochloride (TCI America), *N,N,N′,N″,N‴*-pentamethyldiethylenetriamine (PMDETA) (TCI America), CuBr (Aldrich), NaN_3 (Aldrich), 3-bromopropanol (Aldrich), and NaH (Aldrich) were used without further purification. Phosphorus pentachloride (Aldrich) was purified by sublimation under vacuum before use. 2,2,2-Trifluoroethanol was dried over calcium hydride and was distilled before use. Sulfuryl chloride (Aldrich) and phosphorus trichloride (Aldrich) were purified by distillation. Bromophosphoranime ($Br(CF_3CH_2O)_2P{=}NSiMe_3$) was synthesized and purified by literature procedures.[18,20] (Dimethylamino)ethyl methacrylate (DMAEMA) (Aldrich) was purified via an Al_2O_3 column to remove inhibitor. All the glassware was dried overnight at 120 °C before use. The synthesis reactions were carried out under an atmosphere of dry argon or nitrogen.

Equipment. ^1H and ^{31}P NMR spectra were recorded on a Bruker WM-360 NMR spectrometer operated at 360 and 145 MHz, respectively. ^1H NMR spectra were referenced to solvent signals while ^{31}P NMR chemical shifts were 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 1090 gel permeation chromatograph (GPC) equipped with an HP-1047A refractive index detector and American Polymer Standards AM gel 10 mm and AM gel 10 mm 104 Å columns and calibrated versus polystyrene standards (Polysciences). The samples were eluted at 40 °C with a 0.1 wt % solution of tetra-*n*-butylammonium nitrate (Aldrich) in tetrahydrofuran (THF).

Synthesis of Alkyne-Functionalized Trifluoroethoxyphosphoranimine (3). A mixture of propargylamine hydrochloride (0.10 g, 1.07 mmol) and triethylamine (0.11 g, 1.07 mmol) was suspended in THF (15 mL). The mixture was stirred under an inert atmosphere at 60 °C for 2 h while a white precipitate formed. The reaction mixture was then cooled to room temperature, and bromophosphoranime 2 (0.28 g, 0.71 mmol) and another 1 equiv of triethylamine (0.11 g, 1.07 mmol) were added to the mixture. The reaction mixture was then stirred at room temperature overnight. The white precipitate was filtered off, and all volatiles were removed under reduced pressure to give a colorless liquid **3**. Yield: 87%. ^1H NMR ($CDCl_3$): δ 4.23 (s, 4H), δ 3.45 (d, 2H), δ 2.26 (t, 1H), δ 0.13 (s, 9H). ^{31}P NMR ($CDCl_3$): δ −2.65.

Synthesis of Chlorophosphoranimine ($Cl_3P{=}NSiMe_3$). The synthesis of the chlorophosphoranimine monomer followed a previously reported procedures with some modifications.[18,20,30] Briefly, PCl_3 (46.25 g, 0.33 mol) was added dropwise to $LiN(SiMe_3)_2$ (56.93 g, 0.33 mol) in ether (500 mL) at 0 °C over 30 min. The mixture was allowed to remain at 0 °C and was stirred for another 1 h. SO_2Cl_2 (45.22 g, 0.33 mmol) was then added slowly over 30 min, and the reaction mixture was stirred at 0 °C for 2 h. After completion of the reaction, the salt was removed by filtration. The crude product was condensed to one-third of its volume and was purified by vacuum distillation at room temperature to yield a colorless liquid. Yield: 63%. ^1H NMR ($CDCl_3$): δ 0.16 (s, 9H). ^{31}P NMR ($CDCl_3$): δ −57.08.

Synthesis of Alkyne-Functionalized Poly[bis(trifluoroethoxy)phosphazene] (6). Compoud **3** (0.26 g, 0.71 mmol) was redissolved in CH_2Cl_2 (15 mL) along with PCl_5 (0.25 g, 1.19 mmol) to initiate polymerization, and the reaction mixture was stirred for 1 h. The chlorophosphoranime (4.00 g, 17.18 mmol) was then added rapidly, and the mixture was stirred for another 4 h under an inert atmosphere at room temperature. The solvent was removed under reduced pressure to yield a viscous liquid. The product was redissolved in THF (50 mL) and treated with an excess amount of CF_3CH_2ONa which was preprepared by treating CF_3CH_2OH (3.78 g, 37.80 mmol) with

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NaH (1.51 g, 37.80 mmol) in THF (20 mL). The reaction mixture was stirred at room temperature overnight, followed by concentration of the solution under reduced pressure and then precipitation of the polymer into water (500 mL × 3) and hexane (200 mL × 2) to isolate a white product **6**. Yield: 52%. ^{1}H NMR (acetone-d_6): δ 4.53 (180H). δ 3.60 (2H), δ 2.18 (1H). ^{31}P NMR (D_2O): δ −7.88

Synthesis of 3-Azidopropyl-2-bromo-2-methylpropanoate (7). Sodium azide (3.60 g, 55.40 mmol) was dissolved in water (80 mL), followed by the addition of 3-bromopropanol (3.84 g, 37.60 mmol) dropwise to the solution. The reaction mixture was stirred at 80 °C for 18 h. The solution was then extracted with ethyl acetate (100 mL × 3), and the organic layer was washed with saturated brine (100 mL × 3) and then dried over $MgSO_4$ overnight. The solvent was removed under reduced pressure to yield a colorless liquid. The liquid was then redissolved in CH_2Cl_2 (20 mL) and chilled to 0 °C before the addition of triethylamine (1.68 g, 16.61 mmol). In a second vial, 2-bromoisobutyl bromide was dissolved in CH_2Cl_2 (20 mL) before being added dropwise to the mixture at 0 °C over 1 h. The reaction mixture was then allowed to warm to room temperature and was stirred overnight. The precipitate was filtered off, and the solvent was removed under reduced pressure. The crude product was purified by passing through a silica column using CH_2Cl_2:hexane (2:1) as the mobile phase, and the sample was isolated as a light yellowish liquid **7**. Yield: 20.23%. ^{1}H NMR ($CDCl_3$): δ 4.23 (t, 2H), 3.41 (t, 2H), 1.93 (m, 2H), 1.89 (s, 6H).

Synthesis of Bromo-Functionalized Polyphosphazene Macroinitiator 8. Polymer **6** (1.13 g, 0.06 mmol) and 3-azidopropyl-2-bromo-2-methylpropanoate (**7**) (0.156 g, 0.63 mmol) were dissolved in THF (10 mL), along with PMDETA (0.04 g, 0.22 mmol). Nitrogen gas was bubbled through the solution for 20 min to remove any dissolved oxygen. A trace amount of CuBr (0.03 g, 0.22 mmol) was weighed in a vial, and the oxygen was removed by purging the system with nitrogen gas. Once the system was free from oxygen, the CuBr was added to the reaction solution rapidly, and the solution was stirred at 60 °C for 1 day under an inert atmosphere. The sample was forced through a silica plug using THF to remove the solids. The crude product was then precipitated from THF into water (500 mL × 3) and hexane (200 mL × 5) and was further purified by dialysis against acetone:methanol (2:1) for 2 days to remove any remaining compound **7**. The solvent was then removed under reduced pressure, and the resulting product was dried under vacuum overnight to yield a white powder (**8**). ^{1}H NMR (DMSO-d_6): δ 7.79 (1H), δ 4.45 (180H), δ 4.32 (2H), δ 4.20 (2H), δ 3.91 (2H), δ 2.41 (2H), δ 1.91 (6H). ^{31}P NMR (DMSO-d_6): δ −7.88.

Synthesis of Poly[bis(trifluoroethoxy)phosphazene]-*co*-poly[(dimethylamino)ethyl methacrylate] (10) (TFE-*b*-PDMAEMA). 2-(Dimethylamino)ethyl methacrylate (DMAEMA) was used as a monomer to grow a second block from the terminus of the polyphosphazene. Macroinitiator **8** (0.50 g, 0.03 mmol) was dissolved in THF (5 mL), followed by the addition of DMAEMA (1.97 g, 12.50 mmol) and PMDETA (0.017 g, 0.10 mmol) to the solution. Nitrogen gas was bubbled through the solution for 20 min to remove any dissolved oxygen. Copper(I) bromide (7.20 mg, 0.05 mmol) was weighed in a small vial and then purged with nitrogen to remove oxygen. It was then added to the solution, and the mixture was stirred for 6 h at room temperature (as an example for TFE-*b*-PDMAEMA-4). To terminate the polymerization, the catalysts were removed by passing the sample through a silica plug using THF as mobile phase, and the isolated crude product was dialyzed against acetone:methanol (2:1) for 2 days. All solvent was removed under reduced pressure to yield the diblock copolymer **10** as a yellowish powder. ^{1}H NMR (acetone-d_6): δ 4.55 ($-CH_2CF_3$), δ 4.09 ($-OCH_2-$), δ 2.62 ($-CH_2N$), δ 2.23 ($-N(CH_3)_2$), δ 1.88 ($-CH_2-$), δ 1.10 ($-CH_3$). ^{31}P NMR (acetone-d_6): δ −7.88.

Micelle Preparation. To prepare micellar solutions, nanopure water (20 mL) with a conductivity of 18.2 MΩ/cm was added dropwise to a mildly stirred solution of the diblock copolymer (200 mg) in THF (5 mL). Once the water was added, all the THF was removed under reduced pressure as monitored by ^{1}H NMR. The resulting solution was then diluted to obtain a micelle concentration in the range of 5 to 1 × 10^{-4} g/L. For fluorescence measurements, a pyrene solution in THF (1.2 × 10^{-3} M) was added to nanopure water to give a final pyrene concentration of 12 × 10^{-7} M. Following dilution, the THF was removed under reduced pressure, and its removal was confirmed by ^{1}H NMR spectroscopy. The pyrene solution was then mixed with the diblock copolymer solutions to obtain copolymer concentrations ranging from 2.5 to 5 × 10^{-5} g/L, while the pyrene concentration of the samples was maintained at 6 × 10^{-7} M. All the samples were sonicated for 10 min and were allowed to stand for 1 day before fluorescence measurements.

Fluorescence Measurements. Excitation spectra of pyrene were measured using a Photon Technology International (PTI) fluorescence spectrometer using an 814 photomultiplier detection system. For the excitation spectra, the emission wavelength was set at 391 nm. All the samples were measured in a 1 × 1 cm quartz cuvette at room temperature.

Light Scattering Measurements. The sizes and size distributions of the diblock copolymer micelles were evaluated by dynamic light scattering using a particle size analyzer (Zetasizer Nano S, Malvern Instruments Ltd.) at room temperature (25 °C) with a scattering angle of 90°. Samples were filtered through a 0.45 μm syringe filter before measurement of particle size for each sample. The hydrodynamic radius (R_h) of the micelles was calculated by using the Stokes−Einstein equation $R_h = k_BT/6\pi\eta D$, where k_B is the Boltzmann constant, T is the absolute temperature, η is the solvent viscosity, and D is the diffusion coefficient.[31] The polydispersity factor of micelles, represented as μ_2/Γ^2, where μ_2 is the second cumulant of the decay function and Γ is the average characteristic line width, was calculated by the cumulant method.[32]

Tranmission Electron Microscopy. Transmission electron microscopy (TEM) was performed using a KEOL 2010 unit, operated at an acceleration voltage of 200 kV. For observation of the size and distribution of the micellar particles, a drop of sample solution (concentration = 1 g/L) was placed onto a 400-mesh copper grid coated with carbon. About 1 min after deposition, the grid was tapped with a filter paper to remove surface water, followed by air-drying. Negative staining was performed by using a droplet of a 2.5 wt % uranyl acetate solution.[33] The samples were air-dried at room temperature overnight.

RESULTS AND DISCUSSION

Synthesis of Block Copolymers. Conventional free radical polymerization lacks control over the polymer structure due to the slow initiation, fast propagation, and subsequent transfer or termination and makes it unsuitable for synthesizing well-defined block copolymer structures.[34] The development of atom transfer radical polymerization (ATRP) is one of the most robust controlled/living radical polymerization techniques with a diversity of monomers, desirable molecular weight control, and narrow polydispersity. This comes about because the low radical concentrations present during the polymerization reduce the contribution of inter- and intramolecularly terminated chains.[26] In this work, ATRP was chosen to form the organic block linked to a polyphosphazene backbone, and it is the first report to apply ATRP to synthesize polyphosphazene-containing diblock copolymers. In order to introduce the ATRP initiation site to the end of polyphosphazene backbone, azide−alkyne "click" chemistry was applied due to its excellent reaction efficiency, high functional group tolerance, and solvent compatibility.[35,36] In this work, a small molecule linker (**7**), an ATRP initiation site, was "click" coupled onto the polyphosphazene block and used for subsequently growing the organic block at the end of the polymer chain ("block-from"). This avoids the preparation of two distinct polymers with azide or alkyne functionality on the two segments and then coupling them together ("block-to"). This "block-from" method showed a higher reaction efficiency than the "block-to" method due to the better diffusion of small molecules to the

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reaction site at the polymer end-cap in solution as compared to the reaction of two polymer chains.[24,26]

A series of phosphazene−organic block copolymers was prepared by the synthetic procedures illustrated in Scheme 1. The phosphoranimine readily underwent bromine replacement reactions in the presence of amines to produce an alkyne-functionalized initiator **3**. To produce the cationic species **4**, the initiator **3** was treated with 2 equiv of PCl_5 at room temperature in CH_2Cl_2. Once formed, the chloro monomer $Cl_3P{=}NSiMe_3$ was added to propagate the living cationic polymerization to give poly(dichlorophosphazene) with a predetermined chain length.[18,19,21] The resultant poly(dichlorophosphzene) was then treated with an excess amount of $NaOCH_2CF_3$ in THF to yield the hydrophobic poly[bis(trifluoroethoxy)phosphazene] block with a pendent triple bond attached to the end of the chain. The entire process was carried out in an inert anhydrous atmosphere to prevent uncontrollable cross-linking.[12,13]

Compound **7** with an azide and bromine at its opposing ends was prepared as both the linker between the phosphazene and organic blocks and the initiator for growing the organic block. Initial attempts with $CuSO_4{\cdot}5H_2O$/sodium ascorbate as the catalytic system for the "click" reaction in THF at room temperature were unsuccessful probably due to the poor solubility of the catalyst in THF and the donor coordinating nature of the polyphosphazene backbone. Thus, a stronger catalyst CuBr/PMDETA complex in THF was chosen at an elevated temperature of 60 °C for 1 day to yield the ATRP macroinitiator **8**.[37] Unlike the $CuSO_4{\cdot}5H_2O$/sodium ascorbate catalyst system, which is tolerant to limited amounts of oxygen in solution by *in situ* reduction of $CuSO_4$,[36] the CuBr/PMDETA catalyzed "click" reaction in this work required the careful removal of all the oxygen dissolved in the solution by exchanging it with nitrogen to prevent the oxidation of Cu^+. In order to remove all of the uncoupled initiator **7** from the polyphosphazene polymer, the sample was purified extensively by precipitation from THF into hexane 5 times, followed by dialysis against acetone:methol (2:1) for 2 days. DMAEMA was selected as the second monomer due to its high reactivity, and CuBr/PMDETA was used as the catalyst in this polymerization. ATRP is usually carried out by bulk polymerization by directly dissolving the initiators in the pure monomers without utilizing solvents.[25,29,38] However, in this work, THF was used as a solvent at a sacrifice of reaction efficiency due to the poor solubility of the macroinitiator **8** in DMAEMA.

The existence of both the trifluoroethoxyphosphazene block and PDMAEMA signals in the ^{1}H NMR (Figure 1) confirmed

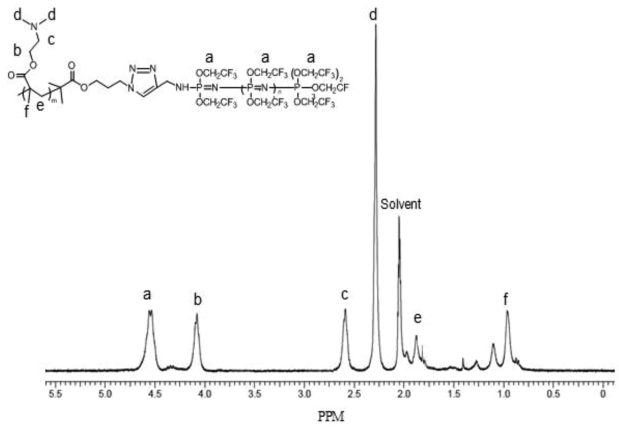

Figure 1. ^{1}H NMR of TFE-*b*-PDMAEMA-3 block copolymer at ambient temperature referenced to acetone-d_6.

the structure, together with the ^{31}P NMR (Figure 2) of the diblock copolymer. The molecular weight increased from 27

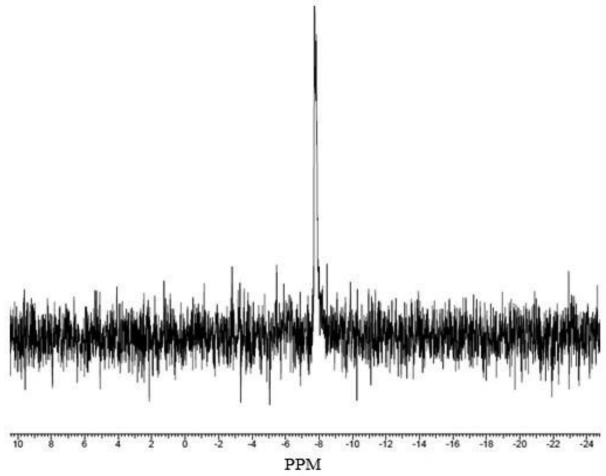

Figure 2. ^{31}P NMR of TFE-*b*-PDMAEMA-3 block copolymer at ambient temperature referenced to acetone-d_6.

900 to 42 300 (varying by ATRP reaction time) as determined by gel permeation chromatograph (GPC). All these items of evidence support the diblock copolymer structure. The length of both the hydrophobic and hydrophilic blocks can be tuned by controlling the ratio of initiator to monomer and the reaction time. In this work, the length of the hydrophobic block remained constant, and the length of the hydrophilic segment was controlled by the varying reaction times at the same monomer/initiator ratio. Table 1 shows the structural

Table 1. Characterization of TFE-*b*-PDMAEMA Block Copolymers

block copolymers	ATRP time (h)	block ratio (n:m)[a]	TFE (wt %)	M_n (g/mol) (GPC)[b]	M_n (g/mol) (NMR)[c]	PDI[d]
TFE-*b*-PDMAEMA-1	12	1:1.23	44	48 270	19 800	1.27
TFE-*b*-PDMAEMA-2	10	1:0.95	61	47 290	17 800	1.17
TFE-*b*-PDMAEMA-3	8	1:0.71	66	44 300	16 100	1.25
TFE-*b*-PDMAEMA-4	6	1:0.63	71	42 300	15 500	1.08

[a]The ratio of the hydrophobic block to the hydrophilic block was calculated from the integration obtained from ^{1}H NMR. [b]The number-average molecular weight (M_n) was calculated from ^{1}H NMR. [c]M_n was measured by gel-permeation chromatography (GPC). [d]Polydispersity index (PDI) = M_w/M_n.

characterization of a series of TFE-*b*-PDMAEMA diblock copolymers. The molecular weights calculated by ^{1}H NMR spectroscopy were estimated by comparing peak integration ratios of the end group on propargylamine (−CH_2−, 3.60 ppm), the trifluoroethoxy groups on the polyphosphazene (−CH_2−, 4.55 ppm), and the DMAEMA block (−CH_2−, 4.09 ppm). The significant difference of M_n between the GPC measurement and the ^{1}H NMR calculation as shown in Table 1 was attributed to (i) error caused by integration from the ^{1}H NMR peaks if the peak intensities of the end groups were too low and (ii) the difference of hydrodynamic radius between TFE-*b*-PDMAEMA and polystyrene standards which were used to calibrate the GPC. The final block copolymers were soluble in THF, acetone, DMSO, and DMF but were insoluble in hexane and toluene.

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Self-Association of Block Copolymers in the Aqueous Phase. The TFE-*b*-PDMAEMA block copolymer consists of the hydrophilic PDMAEMA and hydrophobic trfluoroethoxy-substituted polyphosphazene segments (TFE), which imparts the ability to form organized micellar structures in an aqueous environment. Generally, micelles can be formed in an aqueous environment by one of these three ways: (i) direct addition of block copolymers into stirred water; (ii) dissolution of the block copolymer in an organic solvent, followed by dialysis against water; or (iii) dissolution of the block copolymer in an organic solvent, followed by the addition of water dropwise to the solution using a mild stirring.[39,40] The last two methods are preferred, as the gradual exchange of the organic solvent with water can give more uniform micelle structures.[22] However, the organic solvent has to be removed completely from the aqueous medium to prevent it from influencing the micelle characterization. In this work, the third route was selected, and ^{1}H NMR was utilized to monitor for THF residues in the micelle solutions. The micellar behavior of the amphiphilic block copolymers was monitored by a fluorescence technique, dynamic light scattering, and TEM. The critical micelle concentrations (cmc's) of the diblock copolymers in an aqueous phase were determined by a fluorescence technique using pyrene as a probe. Previous studies have shown that pyrene has distinct fluorescence spectra depending on the environment utilized, aqueous or organic.[41,42] Figure 3 shows

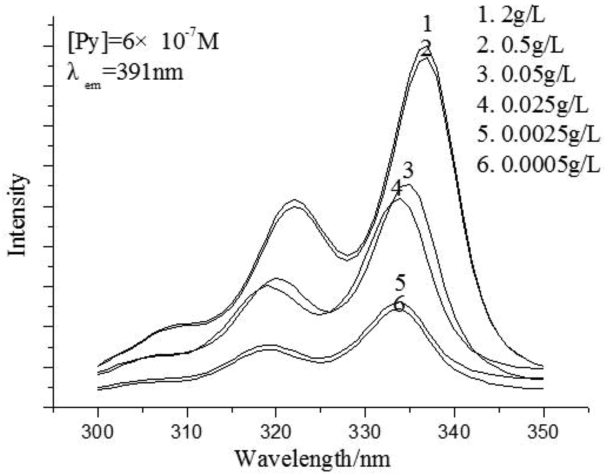

Figure 3. Excitation spectra of pyrene as a function of TFE-*b*-PDMAEMA-3 concentration in water.

the excitation fluorescence spectra of polymer and pyrene sample (TFE-*b*-PDMAEMA-3 as an example), in which the concentration of the pyrene was kept constant while varying the concentrations of the polymer. In the spectra, the symmetry-forbidden (0,0) band shifted from 332 to 337 nm, and the intensity gradually increased as the pyrene transferred from the aqueous environment to the hydrophobic micelle cores. Meanwhile, the pyrene fluorescence spectrum obtained in pure water is identical to the one obtained from a pyrene solution with low concentrations of block copolymers in Figure 3, and the block copolymers themselves give no fluorescence signals in this region. The ratios of the peak intensities at 337 and 332 nm were utilized to determine the cmc value.[41] Figure 4 shows the intensity ratios (I_{337}/I_{332}) of the pyrene excitation spectra versus the logarithm of concentrations of TFE-*b*-PDMAEMA-3 (log *C*). At low concentrations of the diblock copolymer, the change in the intensity ratio (I_{337}/I_{332}) was

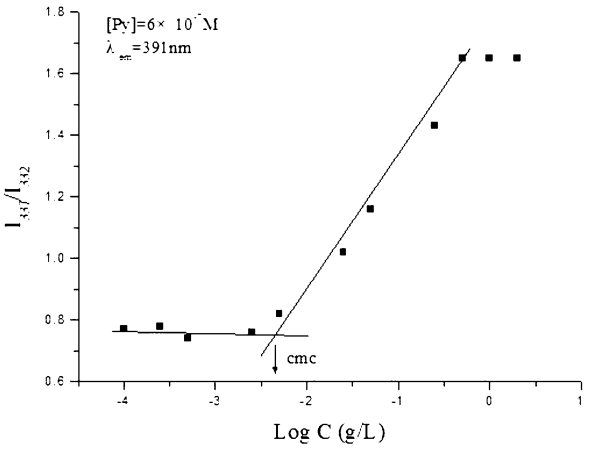

Figure 4. Plot of I_{337}/I_{332} (from pyrene excitation spectra) vs log *C* for TFE-*b*-PDMAEMA-3.

negligible since the concentrations of the block copolymer were insufficient to self-aggregate and form micelles. But at a threshold concentration of the diblock copolymer, the intensity ratios began to show a substantial increase with an increase in the concentration of the diblock copolymer. This reflects the shift of the pyrene probe from an aqueous environment to a hydrophobic one. This threshold indicated the minimum concentration of the diblock copolymer needed for the formation of micelles, the inner cores of which were able to act as hydrophobic containers to incorporate the pyrene. Using these data, the cmc values can be determined from the turning point of the curve as shown in Figure 4. The cmc values of the block copolymers were in the range 3.47–9.55 mg/L depending on the block composition (Table 2). The

Table 2. Properties of TFE-*b*-PDMAEMA Micelles

block copolymers	cmc (mg/L)	diam (nm)	μ_2/Γ^2	K_v ($\times 10^{-5}$)
TFE-*b*-PDMAEMA-1	9.55	100	0.29	0.12
TFE-*b*-PDMAEMA-2	6.31	130	0.29	0.40
TFE-*b*-PDMAEMA-3	5.46	134	0.16	0.48
TFE-*b*-PDMAEMA-4	3.47	142	0.20	1.52

results showed that the cmc values decreased with an increase in the proportion of the hydrophobic segment, which is in agreement with other studies on micelles.[11,42] These values are much lower than those of low molecular weight surfactants (e.g., 2.3 g/L for sodium dodecyl sulfate) and diblock copolyphosphazenes (e.g., 80 mg/L for methoxyethoxyethoxy and phenyl containing species)[20,41] but comparable to those of other polymeric amphiphiles.[1,5,39]

Dynamic light scattering (DLS) was carried out to determine the diameters of the micelles. Figure 5 shows an example of the size distribution of TFE-*b*-PDMAEMA-3 at 25 °C. The diameters of the micelles for the four polymers investigated were in the range of 100–142 nm with a narrow size distribution in an aqueous phase as summarized in Table 2. The diameters increased slightly with the increase in the proportion of the hydrophobic block. The polydispersity factors (μ_2/Γ^2) of the micelles are fairly low (0.16–0.29), which suggests a narrow size distribution.[31,32]

The size and shape of the micelles were also examined by TEM. In order to amplify the contrast of the micelles and background, negative staining was performed by using uranyl acetate solution to make the background dark gray.[33] Figure 6 shows the micelles formed from a solution with a polymer

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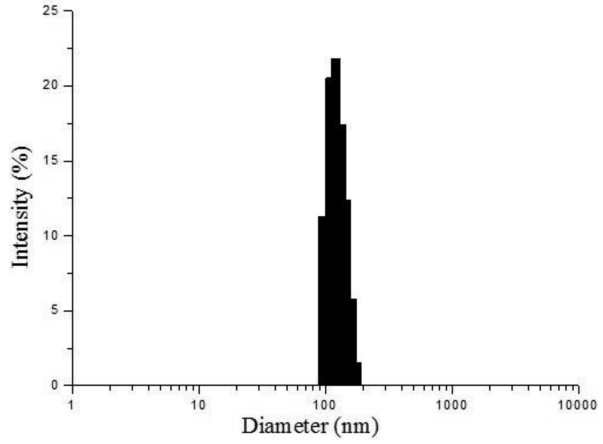

Figure 5. Diameters of TFE-*b*-PDMAEMA-3 from dynamic light scattering.

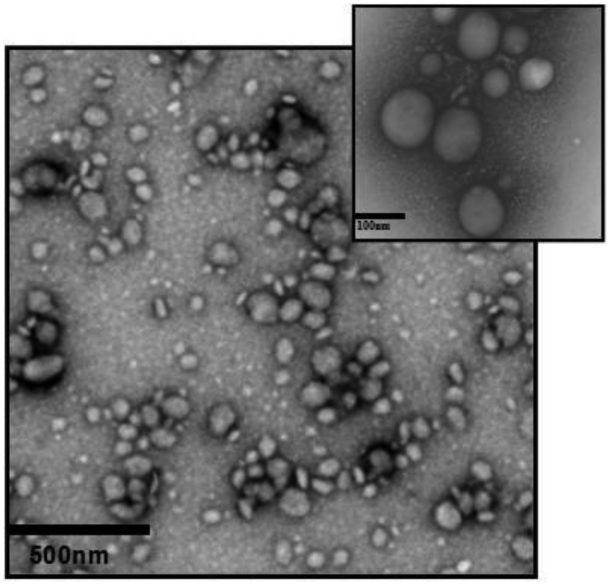

Figure 6. TEM image of TFE-*b*-PDMAEMA-3.

(TFE-*b*-PDMAEMA-3) concentration of 1 g/L. Although the average size of the micelles was about 100 nm, micelles with a size range of several hundreds of nanometers were often observed. This resulted from intermicellar aggregates that form a multicore structure through the association of individual micelles.[44,45] Most of the micelles had a spherical shape, and the average diameter from TEM was in agreement with the mean diameter measured from dynamic light scattering.

Partitioning of Pyrene in Micellar Solutions. The hydrophobicity of the micelle core was estimated by measuring the equilibrium constant K_v for the partitioning of pyrene between the micelle core and the aqueous media. A higher K_v indicates a higher hydrophobicity of the microdomain (micelle cores) constructed by the hydrophobic segments.[22,23] In this work, the equilibrium constant K_v was calculated following the approach reported by Wilhelm.[41] A simple equilibrium between pyrene in the bulk aqueous environment and pyrene incorporated into the micelle was assumed. The ratio of the pyrene concentration inside the micelle to that of pyrene dissolved in the bulk water phase ($[Py]_m/[Py]_w$) can be correlated to the ratio of the volumes of each phase as expressed in eq 1. The K_v here is the partition equilibrium constant of pyrene between the micelle core phase and water phase.

$$[Py]_m/[Py]_w = K_v V_m/V_w \quad (1)$$

Equation 1 can be rewritten as

$$[Py]_m/[Py]_w = K_v x(c - \mathrm{cmc})/1000\rho \quad (2)$$

where x is the weight fraction of the hydrophobic polyphosphazene block, c is the concentration of the block copolymer, and ρ is the density of the core of the micelles, which is assumed to be the bulk density of the poly[bis-(trifluoroethoxy)phosphazene] (1.10 g/mL). In the intermediate range of polymer concentration with a substantial increase of intensity ratio (I_{337}/I_{332}), $[Py]_m/[Py]_w$ can be written as

$$[Py]_m/[Py]_w = (F - F_{min})/(F_{max} - F) \quad (3)$$

where F_{max} and F_{min} correspond to the average magnitude of I_{337}/I_{332} in the flat region of the high and low concentration ranges, respectively, in Figure 4, and F is the intensity ratio (I_{337}/I_{332}) in the intermediate concentration range of the block copolymers. Combining eqs 2 and 3, K_v values for pyrene can be determined as the slope by using a plot of $(F - F_{min})/(F_{max} - F)$ versus block copolymer concentrations as shown in Figure 7.

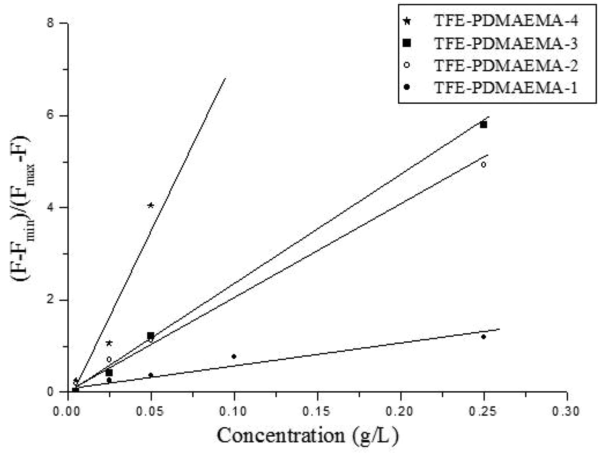

Figure 7. Plots of $(F - F_{min})/(F_{max} - F)$ vs concentration of block copolymers.

The K_v values, as summarized in Table 2, were in the range of 0.12×10^5 to 1.52×10^5 for the TFE-*b*-PDMAEMA system, which are much larger than those of amphiphillic diblock copolyphosphazenes (e.g., 7×10^3 for a methoxyethoxyethoxy and phenoxy containing copolyphosphazene).[20] The data also showed that, as the proportion of the hydrophobic blocks in the amphiphilic block copolymers increased, the K_v value also increased, suggesting an increase in the hydrophobic characteristic of the micelle cores.

CONCLUSION

A series of amphiphilic TFE-*b*-PDMAEMA diblock copolymers has been synthesized via the controlled living cationic polymerization of $Cl_3N{=}PSiMe_3$, azide−alkyne "click" chemistry, and atom transfer radical polymerization of DMAEMA. The length of each block was well controlled, and the polydispersity index was relatively low. The block copolymers self-aggregated into

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organized micelle structures in an aqueous environment. The micelles which were formed were characterized by the use of fluorescence techniques, dynamic light scattering, and transmission electron microscopy. The critical micelle concentrations of the block copolymers were determined from fluorescence spectra using pyrene as a probe. The cmc values depended on the proportion of the hydrophobic blocks in the copolymer and were in the range of 3.47−9.55 mg/L. TEM and dynamic light scattering results indicated that the spherical micelle aggregates were formed with an average diameter of 100−142 nm. The hydrophobicity of the micellar core was estimated by measurement of the partition equilibrium constant of pyrene in the micelle solution, and the values were in the range of 0.12×10^5 to 1.52×10^5. The combination of "click" reaction chemistry, and ATRP has opened a new facile route ("block-from") for synthesizing well-defined hybrid phosphazene−organic block copolymer structures with high synthetic tunability. The properties of the micelles can be tailored by changing either the phosphazene block or the organic block following the synthetic procedures described above. This can be achieved by varying the nucleophiles during the substitution of poly(dichlorophosphazene) or by varying the organic monomers during ATRP. For instance, to synthesize block copolymers containing biodegradable substituents, similar synthetic procedures can be followed except the side group nucleophiles used could be changed to amino acid esters instead of the trifluoroethoxy group to confer biodegradability to the micelles.[15]

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Notes

The authors declare no competing financial interest.

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