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Synthesis and characterization of temperature and pH-responsive pentablock copolymers

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Abstract

A family of amphiphilic ABCBA pentablock copolymers based on commercially available Pluronic® F127 block copolymers and various amine containing methacrylate monomers was synthesized via Cu(I) mediated controlled radical polymerization. The block architecture and chemical composition of the pentablock copolymers were engineered to exhibit both temperature and pH responsive self-assembly by exploiting the lower critical solution temperature of the poly(ethylene oxide)/poly(propylene oxide) blocks and the polycationic property of the poly(amine methacrylate) blocks, respectively. In aqueous solutions, the pentablock copolymers formed temperature and pH-responsive micelles. Concentrated aqueous solutions of the copolymer formed a pH-responsive, thermoreversible gel phase. The controlled radical synthesis route yielded well-defined copolymers with narrow molecular weight distributions with the benefit of mild reaction conditions. Small angle X-ray scattering, laser light scattering, cryogenic transmission electron microscopy and dynamic mechanical analysis have been used to characterize the self-assembled structures of the micellar solution and gel phases of the aqueous copolymer system. These copolymers have potential applications in controlled drug delivery and non-viral gene therapy due to their tunable phase behavior and biocompatibility.

Keywords: pH Sensitive; Thermoreversible; Gel

1. Introduction

Amphiphilic block copolymers that self-assemble into micelles and gels in response to environmental stimuli are an important class of materials. These stimuli-responsive water-soluble copolymers have many biological applications, such as drug delivery and gene therapy. Amphiphilic block copolymers and amphiphilic polyelectrolytes often exhibit stimuli responsive behavior due to reversible self-assembly in solution [1,2]. Recent advances in controlled radical polymerization techniques have led to the facile synthesis of well-defined block copolymers with a wide range of functional monomers. Atom transfer radical polymerization (ATRP) [3–5], reversible addition fragmentation chain transfer (RAFT) polymerization [6] and nitroxide-mediated radical polymerization [7] techniques have been utilized to develop well-defined functional polymers which exhibit stimuli responsive behavior in solution.

This paper describes the ATRP synthesis of a family of novel gel forming pentablock copolymers with different amine containing methacrylate blocks. A difunctional ATRP macroinitiator was prepared from commercially available Pluronic® F127 triblock copolymer. Pluronic® F127 is a amphiphilic triblock copolymer of poly(ethylene-oxide)-*block*-poly(propyleneoxide)-*block*-poly(ethyleneoxide) (PEO-*b*-PPO-*b*-PEO). The micelle and gel formation of Pluronic® copolymers in solution has been extensively investigated [8–12]. Due to the block architecture and lower critical solution temperature (LCST) of PEO and PPO,

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aqueous solutions of Pluronics® exhibit temperature-dependent micellization. Concentrated solutions of the copolymer form a thermoreversible gel phase as a result of micellar packing [13]. The family of pentablock copolymers synthesized in this work, initiated by a Pluronic® F127 macroinitiator, contain blocks of either poly(2-diethylaminoethyl methacrylate) (PDEAEM), poly(2-dimethylaminoethyl methacrylate) (PDMAEM), poly(2-diisopropylaminoethyl methacrylate) (PDiPAEM) or poly(*tert*-butylaminoethyl methacrylate) (P*t*BAEM) as the outer blocks. These amine methacrylate end blocks are hydrophilic when the amine groups are charged at low pH, and exhibit various degrees of hydrophobic character above critical pH values where they become uncharged. The pentablock copolymers exhibit reversible temperature and pH-dependent micelle and gel formation in solution. The different hydrophobic character of the pendant amino groups of the monomers result in a range of pH responsive solution properties. Additionally, the ability to control the molecular weight of the pentablock copolymers offers further opportunities to tune the materials' responsive behavior.

Recently, we reported the synthesis of a pentablock copolymer composed of Pluronic® F127 and PDEAEM [14]. The pentablock copolymer was synthesized via oxyanionic polymerization of DEAEM initiated by a difunctional carbanion of the Pluronic® F127 copolymer. The oxyanionic synthesis scheme required rigorous purification of solvents and exclusion of oxygen and water. Additionally, due to the rapid rate of propagation in oxyanionic synthesis, uniform chain growth from macroinitiators was difficult to control and resulted in low initiation efficiency and multimodal molecular weight distributions. ATRP synthesis of the pentablock copolymers requires relatively mild conditions, less rigorous monomer purification and is applicable to a wider range of functional monomers. Careful selection of reaction conditions resulted in a controlled polymerization, and molecular weight can be effectively controlled with the initiator-to-monomer ratio. Triblock copolymers that incorporate tertiary amine methacrylate monomers have recently been synthesized via the difunctional macroinitiator ATRP approach, in aqueous [15] and organic media [5]. These materials were found to exhibit reversible lypotropic and pH dependent properties. Herein, we describe the synthesis of well-defined pentablock copolymers based on commercially available Pluronic® block copolymers and amine methacrylate monomers via ATRP in both hydrocarbon and alcoholic media. The temperature and pH dependent-micellization of these materials were monitored with small angle X-ray scattering (SAXS) and quasi-elastic and multi-angle light scattering. Pentablock copolymer micelles were directly visualized with cryogenic transmission electron microscopy (CryoTEM). The temperature dependent sol–gel behavior was characterized by dynamic mechanical analysis (DMA) to investigate the self-assembly process of these polymers in solution.

2. Experimental section

2.1. Reagents

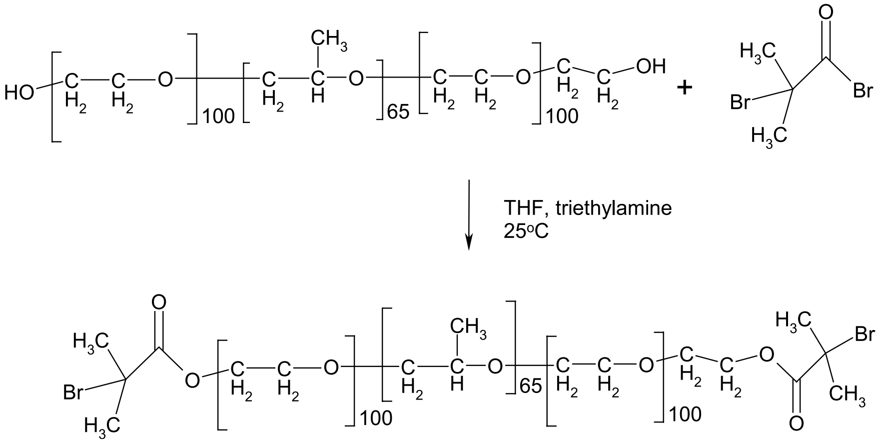
2-(*N*,*N* Diisopropylaminoethyl methacrylate) (DiPAEM), *tert*-butylaminoethyl methacrylate (*t*BAEM) and the inhibitor removal column were purchased from Scientific Polymer Products Inc., Ontario New York. 1-propylamine, 2-pyridinecarbaldehyde, *N*,*N*-(diethyl amino)ethyl methacrylate (DEAEM), *N*,*N*-(dimethylamino)ethyl methacrylate (DMAEM), 2-bromoisoburyryl bromide, copper(I) bromide (CuBr 99.99%), Pluronic® F127 ($M_n = 12600$, 70% PEO) and copper powder (99) for organic synthesis were purchased from Sigma-Aldrich, St. Louis, MO. Triethylamine, tetrahydrofuran (THF), toluene and all other chemicals were purchased from Fisher Scientific and used with no further purification. *N*-Propyl-pyridynyl methanimine (NPPM) was prepared by reacting 1-propylamine with 2-pyridinecarbaldehyde [16].

2.2. Preparation of Pluronic® ATRP macroinitiator

The difunctional 2-bromo propionate Pluronic® F127 was synthesized as illustrated in Scheme 1. Pluronic® F127 (10 g, 7.94×10^{-4} mol) and triethylamine (3 molar excess) were dissolved in THF at 25 °C. 2-Bromoisobutyryl bromide (6 molar excess) was added and the solution was stirred overnight. The reaction mixture was filtered to remove the precipitated hydrobromide salt. The cleared solution was stirred with activated charcoal for 2 h and then dried with $MgSO_4$. The mixture was filtered to remove the charcoal and $MgSO_4$, and the excess solvent was removed with a rotary evaporator before the product was precipitated in −72 °C *n*-hexane and dried under vacuum. ^1H NMR of the functionalized Pluronic® F127 was used to verify the quantitative modification of the end groups as shown in Fig. 1 The integral area of the sharp singlet at $\delta = 1.97$, was assigned to the two methyl groups α to the terminal bromine atoms. The product is an off white powder (9.1 g, 91% yield); GPC (THF) $M_w = 13.64$ kDa, $M_w/M_n = 1.133$; ^1H NMR ($CDCl_3$) δ 3.63 (s, 800H), δ 3.56–3.48 (m, 108H), δ 3.40–3.35, (m, 58H), δ 1.92 (s, 12H), δ 1.13–1.11, (m, 168H).

2.3. Synthesis of pentablock copolymer

Successful ATRP, resulting in uniform initiation and growth, requires a dynamic equilibrium between active, propagating radicals and dormant alkyl halides. The dormant species must be favored in the equilibrium to maintain a low concentration of active radicals and suppress normal radical termination reactions. A transition metal catalyst/ligand complex mediates the equilibrium. Copper catalyst was employed in this study due to proven versatility in mediating ATRP of methyl methacrylates [17]. Due to the low solubility of the copper catalyst in most solvents, a



Scheme 1. Synthesis of difunctional ATRP macroinitiator based on Pluronic® F127.

complexing ligand is employed to increases the solubility and enhances the activity of the catalyst. The *N*-propyl-pyridynyl methanimine (N-PPM) ligand was utilized in this investigation. NPPM has been shown to successfully mediate ATRP of PDEAEM and PDMAEM [15]. The N-PPM ligand was initially developed to overcome the difficulties of polymerizing methacrylate monomers in nonpolar solvents such as toluene [16]. Additionally, N-PPM has been shown to mediate well-controlled ATRP reactions in aqueous media [18]. The effect of varying the relative molar amounts of ligand and catalyst, as well as the solvent on the initiation efficiency, monomer conversion and polydispersity of the product were studied.

A typical synthesis, as depicted in Scheme 2, was carried out as follows. Monomers were passed over an inhibitor removal column immediately prior to use. The macroinitiator was dissolved either in toluene or in a (1:1) water:isopropanol solution, and was added to an argon-flushed round bottom flask with copper(I)bromide (99.99%), ligand and monomer, and degassed via 3 freeze–pump–thaw cycles. A small quantity of copper(0) powder (<1 mg) was added to the reactions to enhance the stability of the Cu(I)/Cu(II) equilibrium [17]. In some cases, the reaction kinetics were monitored by periodically sampling the reaction with a degassed syringe. The reaction was stopped by precipitation of the copolymer into cold n-hexane. The crude product was dissolved in a 1:1 THF:methylene chloride mixture, passed over a short column of basic alumina and precipitated in n-hexane, yielding a light green or light brown solid. The final product was collected and dried under vacuum overnight. ^{1}H NMR (CDCl$_3$) for PDMAEM$_{50}$-F127-PDMAEM$_{50}$ copolymer

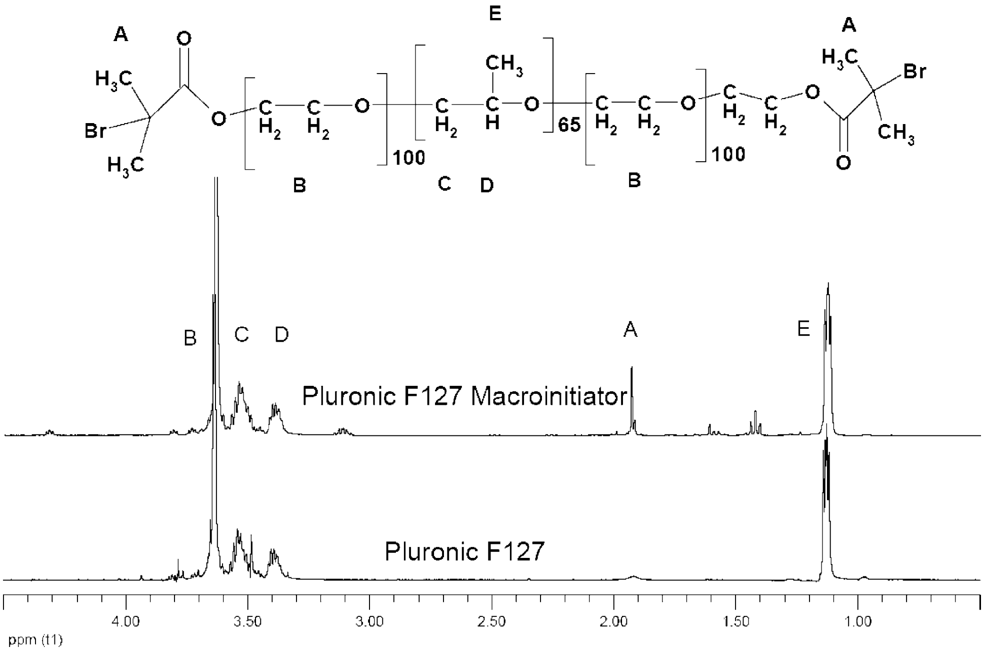
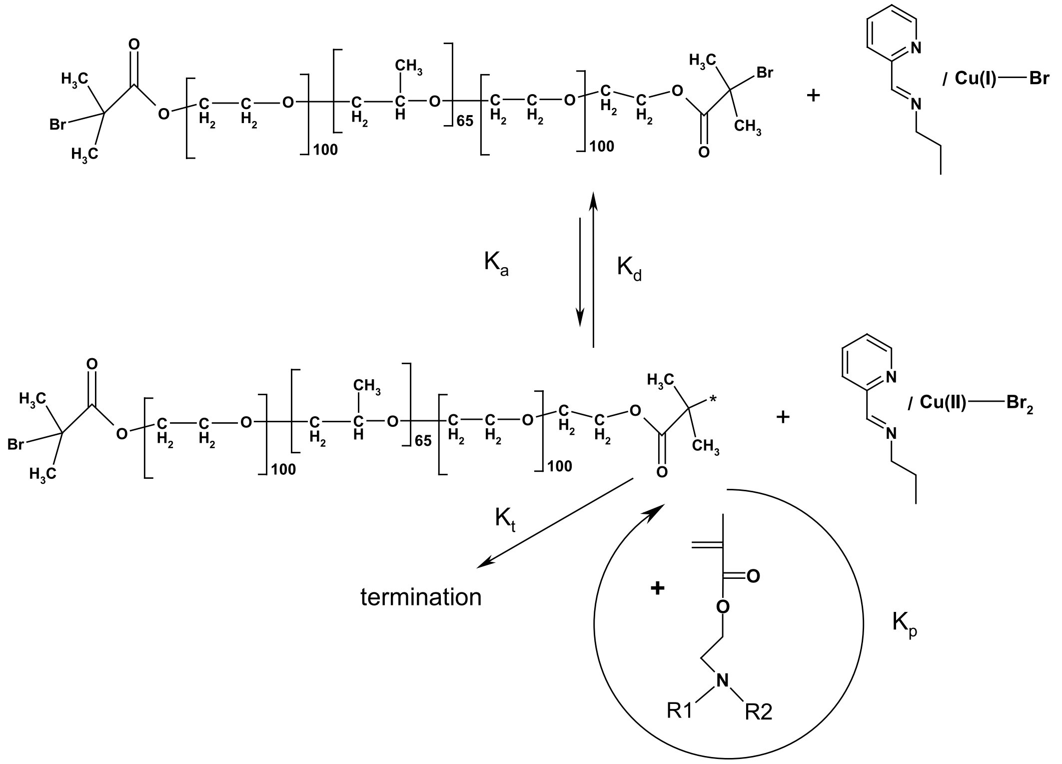


Fig. 1. Schematic of Pluronic® F127 ATRP macroinitiator and ^{1}H NMR of Pluronic® F127 and Pluronic® F127 ATRP macroinitiator.



Scheme 2. Synthesis of pentablock copolymers. DEAEM R1 = R2 = –CH_2–CH_3; DMAEM R1 = R2 = –CH_3, DiPAEM R1 = R2 = –$CH(CH_3)_2$, *t*BAEM R1 = H, R2 = –$C(CH_3)_3$.

(3.1 g, 92% yield): δ 4.02 (s, 200H), δ 3.62 (s, 800H), δ 3.52 (m, 108H), δ 3.37 (m, 62H), δ 2.5 (s, 204H), δ 2.25 (s 604H), δ 2.03–1.794 (m, 165H), δ 1.12–1.09 (m, 184H), δ 1.03 (m, 99H), δ 0.86 (s, 158H). ^{1}H NMR ($CDCl_3$) for P*t*BAEM$_{50}$-F127-P*t*BAEM$_{50}$ copolymer (2.9 g, 89% yield): δ 4.02 (s, 216H), δ 3.61 (s, 800H), δ 3.52 (m, 124H), δ 3.37 (m, 69H), δ 2.78 (s, 210H), δ 1.92–1.79 (m 228H), δ 1.09 (m, 1100H), δ 1.04 (s, 105H), δ 0.88 (m, 150H). ^{1}H NMR ($CDCl_3$) for PDEAEM$_{50}$-F127-PDEAEM$_{50}$ copolymer (3.3 g, 98% yield): δ 3.954 (s, 187H), δ 3.6 (s, 800H), δ 3.51 (m, 123H), δ 3.36 (m, 64H), δ 2.66 (m, 195H), δ 2.54 (m 399H), δ 1.86–1.76 (m, 131H), δ 1.11–1.08 (m, 186H), δ 1.04–0.976 (m, 690H), δ 0.844 (s, 177H). ^{1}H NMR ($CDCl_3$) for PDiPAEM$_{50}$-F127-PDiPAEM$_{50}$ copolymer (3.5 g, 91% yield): δ 3.83 (s, 200H), δ 3.62 (s, 800H), δ 3.53 (m, 121H), δ 3.38 (m, 66H), δ 2.98 (s, 202H), δ 2.62 (s, 200H), δ 1.90–1.79 (m, 161H), δ 1.14–1.11 (m, 187H), δ 0.99 (s, 1254H), δ 0.87 (s, 212H).

2.4. Characterization

All ^{1}H NMR spectra were collected using a Varian VXR400 (400 MHz) spectrometer, and chemical shifts are given in ppm. Chloroform-*d* (98%, Fisher) and D_2O (99%) were used as solvents. The pH of the ^{1}H NMR in D_2O was adjusted by addition of DCl (30% in D_2O) and NaOD (5 M in D_2O). The copolymer molecular weight and polydispersity index (PDI) were determined using gel permeation chromatography (GPC). The GPC system consisted of a Waters 510 pump, Waters 717 autosampler, a Wyatt Optilab DSP refractometer, and a Wyatt Dawn EOS light scattering detector. Poly(methyl methacrylate) (PMMA) standards from Polymer Laboratories were used for column calibration. The mobile phase was THF with 1% v/v triethylamine at a flow rate of 1 ml/min on four columns (Polymer Laboratories, PLgel 100, 500, 1×10^4, 1×10^5 Å at 40 °C).

A Corning 313 pH/temperature meter was used to conduct potentiometric titrations. All titrations were performed at room temperature under constant stirring with 100 ml of 1.0 wt% pentablock copolymer solution. The polymer was dissolved in dionized water and the pH is adjusted to pH 2.0 with 1.0 M HCl, and subsequently titrated with 25 mM NaOH while pH was monitored. A one-minute lag time was allowed for the reaction to reach equilibrium between additions of base. The meter was periodically calibrated against NIST traceable buffer solutions. Assuming that all dissociated protons effectively protonate amine groups, the degree of protonation was calculated as the ratio of the net molarity of H^+ ions (C_H–C_{OH}) and amine groups (C_N) in solution, $\alpha = (C_H - C_{OH})/C_N$ [19].

A Wyatt DAWN EOS Multi Angle Light Scattering (MALS) instrument equipped with a Wyatt QELS detector was used to for static and dynamic light scattering experiments. The absolute values of the scattered intensities were calibrated against filtered toluene and the instrument detectors were normalized with bovine serum albumin (Sigma Aldrich) for aqueous solutions and polystyrene (30 kDa) for THF solutions. The experiments were conducted in micro-batch configuration with the samples being delivered to the flow cell of the DAWN EOS via a syringe pump at 0.5 ml/min. Samples were prepared by removing aliquots of solution from a potentiometric titration of a pentablock copolymer solution as described previously. The samples were diluted to 0.5 mg/ml. The QELS measurements were carried out at 27±0.2 °C and were clarified with 0.22 μm filters immediately prior to analysis. Data collection and analysis for the light scattering measurements were carried out with ASTRA® and QELSBatch® software (Wyatt Technology Co.).

Small angle X-ray scattering (SAXS) samples were prepared by dissolving the copolymer samples in the aqueous solutions at the desired ionic and pH conditions. The solutions were allowed to completely dissolve for several days at 4 °C. The samples were then transferred to 1.3 mm OD quartz capillary tubes with 0.1 mm nominal wall thicknesses and sealed to prevent evaporation. SAXS measurements were carried out on the instrument at the 12-ID beam line at the Advanced Photon Source [20]. A 15 cm × 15 cm CCD detector was used to measure the intensity of scattering and the direct beam intensity was measured using a photodiode. A Peltier heating device controlled the temperature of the sample holder. Five data sets were collected at each temperature with exposure times of 0.5 s at incident beam energies of 12 keV ($\lambda = 1.035$ Å). The distance between the detector and the sample was 2 m. The scattering data were appropriately corrected and azimuthally averaged to obtain $I(Q)$; $Q = 4\pi \sin\theta/\lambda$, where 2θ is the scattering angle and λ is the wavelength of X-rays. The one-dimensional data for the five runs were averaged to obtain high precision scattering data for each condition. The scattered intensity was then corrected by subtracting the scattering from a quartz capillary filled with deionized water and normalized to an absolute scale with a polyethylene standard.

Transmission electron microscopy (TEM) allows real-space imaging of objects in the nanometer size regime. Sample solutions at room temperature, were wetted onto the sample grid and blotted prior to rapid cryogenic vitrification. This technique enables direct imaging of micelle nanostructures. A 3 wt% solution of pentablock copolymer dissolved in 0.1 M pH 7.4 phosphate buffer solution was imaged at the cryo-TEM housed in the Materials Research Science and Engineering Center (MRSEC) at the University of Minnesota. Sample preparation and instrumentation details are presented elsewhere [21]. Image analysis was done with MetaMorph® 5.0 software from Universal Imaging Corporation.

A Perkin–Elmer 7e Dynamic Mechanical Analyzer (DMA) was used to monitor the mechanical properties of pentablock gels as a function of temperature [22]. A cup-and-plate geometry (5 mm diameter) was utilized and the sample was tested in compression at 1 Hz. Gel forming solutions of Pluronic® F127 copolymer were prepared by mixing deionized water with the copolymer and allowing the solution to homogenize at 4 °C for several days. Pentablock copolymer solutions were prepared similarly and the pH was adjusted with the 0.1 M HCl. Cooled polymer solutions were poured into the cup assembly and were allowed to equilibrate at 50 °C. The sample was then mounted in the analyzer. The applied static and dynamic forces were 9 and 10 mN, respectively. The temperature of the sample was decreased at 2 °C/min. The temperature control chamber was humidified to keep the sample from drying over the course of the experiment. Temperature calibration of the instrument was conducted with deionized water with the cup and plate sample holder used for this study.

3. Results and discussion

3.1. Synthesis and macromolecular characterization

The goal of the work was to develop a relatively easy method of synthesizing a pH and temperature responsive block copolymer. The thermoreversible micellization and gelation of the Pluronic® F127 copolymers and the pH responsive micelle formation of block copolymers incorporating the tertiary amine methacrylate motif served as inspiration to develop the pentablock copolymers described in this report [23].

The preparation of the difunctional macroinitiator based on Pluronic® F127 was accomplished in a single step with quantitative yields via the reaction shown in Scheme 1. The ^{1}H NMR spectra in Fig. 1 were used to confirm the conversion of hydroxyl end groups to bromides capable of initiating the ATRP reaction. The sharp singlet at 1.9 ppm in the spectra of the macroinitiator, assigned to the methyl groups in the alpha position relative to the bromide, was integrated relative to the peaks arising from the F127 backbone protons to confirm conversion to difunctional ATRP initiating end groups.

Scheme 2 illustrates the ATRP, initiated by the Pluronic® F127 macroinitiator, of the amine methacrylate blocks, resulting in pentablock copolymers with polydispersities ranging from 1.22 to 1.44. The results of these polymerizations are summarized in Table 1. Because the pentablock copolymer synthesized via ATRP has a relatively low polydispersity and the reaction typically proceeds to high conversions, the average polymer molecular weight can be controlled by controlling the initial initiator to monomer ratio.

The ^{1}H NMR in $CDCl_3$ of four pentablock copolymers (A, B, C and D in Table 1) synthesized are shown in Fig. 2. Monomer conversion was measured with ^{1}H NMR by utilizing the F127 macroinitiator as an internal reference.

Table 1
Summary of the conversion and molecular weight data for the Pluronic® F127- based pentablock copolymers synthesized by ATRP. The concentration of copper(I) bromide catalyst [Cu(I)] and ligand [Ligand] are given relative to molar equivalents of the concentration of initiator

ID	Monomer	Target DP	Solvent	[Cu(l)]	[Ligand]	Polymer concentration (g/ml)	Temperature (°C)	Conversion (NMR) (%)	$\langle M_n \rangle \times 10^{-4}$ (g/mol) (GPC)	$\langle M_w \rangle \times 10^{-4}$ (g/mol) (GPC)	PDI
A	DMAEM	100	Toluene	0.5	1	0.5	70	1	3.18	4.00	1.26
B	tBAEM	100	Toluene	1	2	0.5	70	9	2.63	3.42	1.30
C	DEAEM	100	Toluene	0.5	1	0.5	70	1	2.89	3.72	1.29
D	DiPAEM	100	Toluene	1	2	0.5	70	1	2.66	3.35	1.26
E	DMAEM	40	Toluene	0.5	1	0.5	70	1	2.43	3.02	1.24
F	DEAEM	50	Toluene	0.5	1	0.5	70	9	2.61	3.53	1.35
G	DiPAEM	50	Toluene	1	2	0.5	70	9	2.56	3.26	1.22
H	DEAEM	100	IPA:water	1	2	0.3	50	9	2.13	2.95	1.38
I	DEAEM	100	IPA:water	0.5	1	0.3	50	8	2.09	2.94	1.40
J	DEAEM	100	IPA:water	0.25	0.5	0.3	50	8	2.38	3.24	1.36
K	DEAEM	100	IPA:water	0.125	0.25	0.3	50	9	2.37	3.43	1.45

Fig. 3 shows GPC chromatograph traces of the macroinitiator and a PDEAEM$_{40}$-F127-PDEAEM$_{40}$ pentablock copolymer. The molecular weight distribution of the Pluronic® F127 includes a triblock PEO-*b*-PPO-*b*-PEO and a lower molecular weight PEO-*b*-PPO diblock copolymer fraction that is a result of the commercial synthetic process. As shown in Table 1 the PDI of the pentablock copolymers, synthesized in toluene, was typically below 1.3. The molecular weight of the pentablock copolymer obtained by GPC in THF compared very well with the molecular weights calculated from monomer conversion via ^{1}H NMR, indicating good initiating efficiencies.

Synthesis of the pentablock copolymer was carried out in several solvents in an effort to minimize the PDI of the final product. The solubility of the catalyst/ligand complex is a critical factor determining the relative rates of reaction in ATRP. Solvents were selected based on accounts from the literature of ATRP of methylacrylates with the N-PPM ligand [16,18]. Toluene, isopropyl alcohol (IPA) and water were identified as potential solvents for the reaction, spanning a wide range of polarities. Fig. 4 contains first

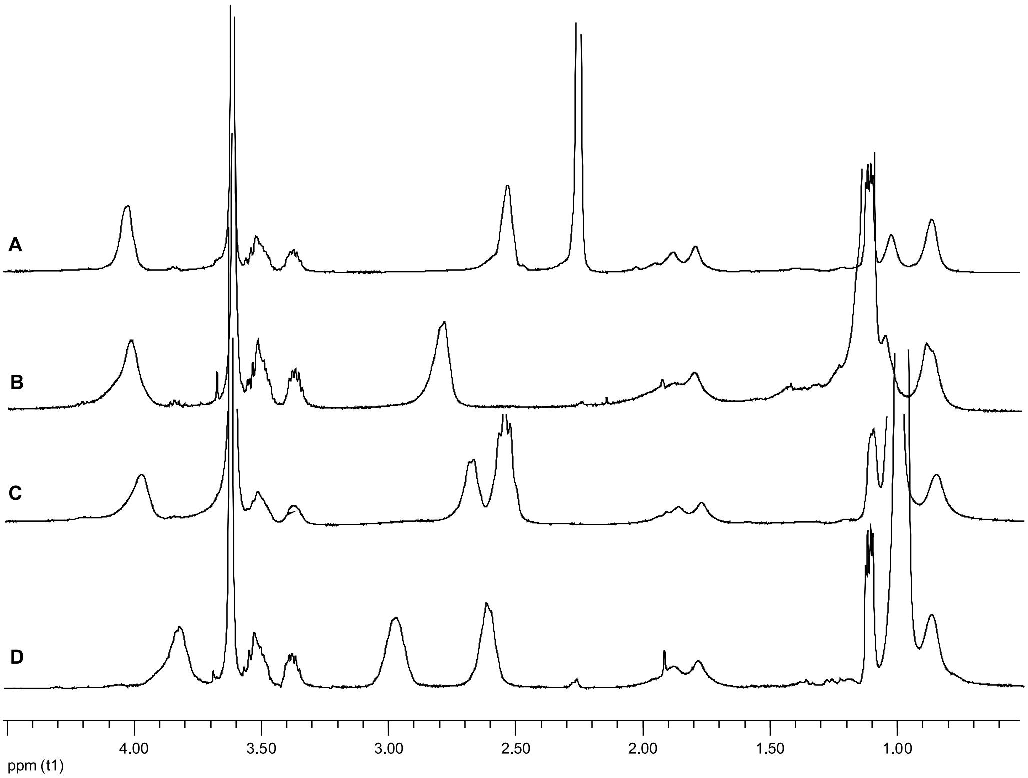


Fig. 2. ^{1}H NMR of pentablock copolymers: (A) PDMAEM$_{50}$-F127-PDMAEM$_{50}$, (B) P*t*BAEM$_{50}$-F127-P*t*BAEM$_{50}$, (C) PDEAEM$_{50}$-F127-PDEAEM$_{50}$, (D) PDiPAEM$_{50}$-F127-PDiPAEM$_{50}$.

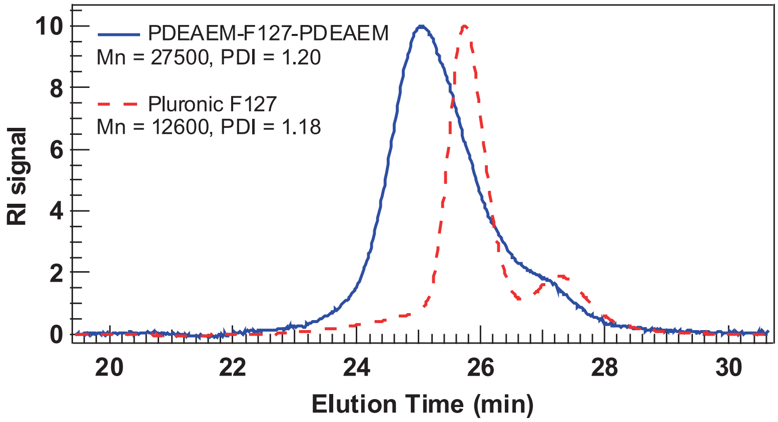


Fig. 3. GPC chromatograph of Pluronic® F127 macroinitiator (dashed) and a pentablock copolymer (solid).

order kinetics plots of the ATRP of PDEAEM in toluene, and in 1:1 IPA:water. A linear fit was found to describe the monomer consumption kinetics for the pentablock ATRP in toluene up to very high conversion. The linear fit indicates that the concentration of propagating radical species is constant and that radical termination reactions are not significant on the time scale of the reaction. ATRP is usually carried out with molar equivalents of catalyst and ligand to initiator. Because of potential biomedical applications for this material, we investigated the possibility of minimizing the catalyst concentration necessary to produce a well-defined pentablock copolymer product. PDEAEM-F127-PDEAEM copolymers were synthesized in toluene with various concentrations of ATRP ligand and catalyst. As shown in Fig. 5, the reaction resulted in near complete conversion of monomer at acceptable PDI over a range of catalyst concentrations. Catalyst concentrations of 0.25–0.50 molar equivalents, relative to initiator concentrations, consistently resulted in the lowest PDI copolymer product. ATRP carried out with catalyst concentrations below 0.125 molar equivalent resulted in very low conversion and initiation efficiency (data not shown). Increasing the concentration of the catalyst and ligand above [1.0]: [24] molar equivalents did not result in any improvement in the PDI of the pentablock copolymer.

ATRP in polar solvents, such as water and lower alcohols have been shown to proceed more rapidly than in less polar solvents such as toluene [17]. ATRP of the PDEAEM pentablock copolymers in water resulted in rapid monomer conversion reaching 100% conversion in less than an hour as judged by ^{1}H NMR. However, the product was only fractionally soluble in THF and chloroform, possibly indicating that a crosslinking side reaction had taken place. ATRP of the PDEAEM pentablock copolymers in isopropyl alcohol resulted copolymer with PDIs greater than 1.5 and multimodal molecular weight distributions due to significant fractions of unreacted macroinitiator. We attributed these results to slow initiation of ATRP from the F127 macroinitiator in this solvent. However ATRP of the pentablock copolymer was successfully achieved in a

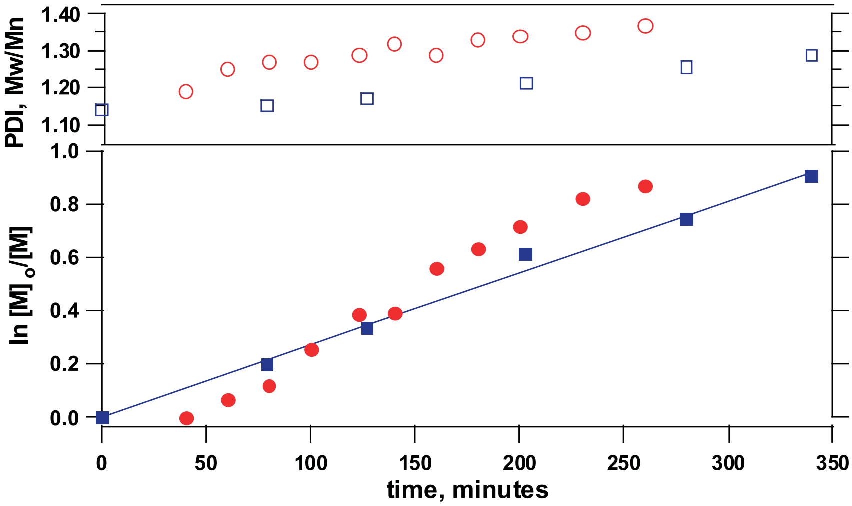


Fig. 4. Kinetics of the polymerization of PDEAEM in IPA/water at 50 °C, [N-PPM]:[CuBr]:[F127]:[DEAEM] = 2:1:1:100, ●, ○ ($\ln[M]_0/[M]$ and PDI); and in toluene at 70 °C, [N-PPM]:[CuBr]:[F127]:[DEAEM] = 1:0.5:1:100, ■, □ ($\ln[M]_0/[M]$ and PDI); line represents the best fit through the data. $[M]_{\mathrm{o}}$ = initial monomer concentration, [M] = monomer concentration during the reaction.

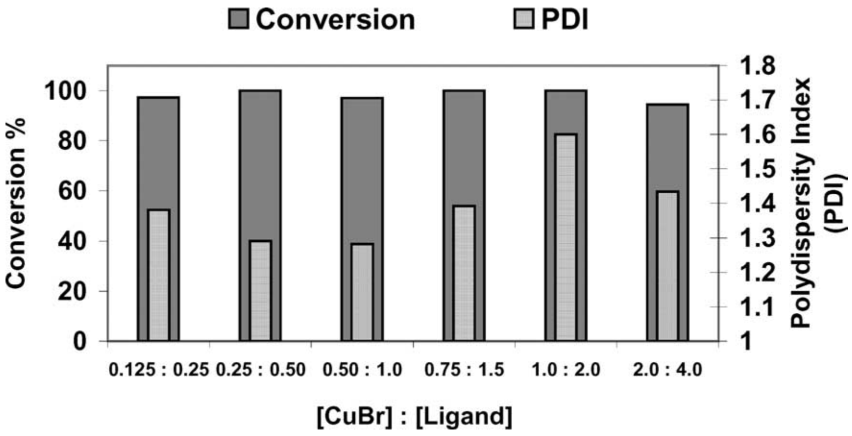


Fig. 5. Monomer conversion and polydispersity of pentablock copolymer PDEAEM$_{50}$-F127-PDEAEM$_{50}$ with varying molar amounts of N-PPM and CuBr in toluene at 70 °C.

1:1 IPA:water solution, resulting in monodisperse molecular weight distributions. Table 1 contains a summary of the ATRP of the PDEAEM-F127-PDEAEM copolymers in IPA:water solutions at various catalyst concentrations. These reactions consistently reached lower conversion and typically had slightly higher PDIs than the analogous reactions in toluene. Fig. 4 contains a first order kinetics plot of the PDEAEM-F127-PDEAEM pentablock copolymer synthesized in IPA:water. We observed a significant ‘initiation period’ for the ATRP reaction in the IPA:water solution. In contrast to the reaction kinetics in toluene, the kinetics plot for this reaction is linear at low conversions but exhibits curvature at higher conversion, indicating a less controlled ATRP reaction. The more rapid rate of ATRP in the IPA:water solution did not allow for a significant reduction in catalyst concentration necessary to yield pentablock copolymers with PDIs below 1.3.

The effective removal of catalyst residues from polymers synthesized via ATRP is especially important for materials in biological applications [5,25]. Cytotoxicity studies were carried out to investigate the effectiveness of the simply flash chromatography and repeated precipitation technique employed to remove the ATRP catalyst from these pentablock copolymers. Cytotoxicity studies of these pentablock copolymers dissolved in media and exposed to SKOV-3 cells confirmed that the cells were viable in the presence of the polymer and that these polymers can be used for biomedical applications [26].

3.2. Characterization of solution behavior

Several techniques were used for characterization of the temperature and pH dependent self-assembly of the pentablock copolymers. The critical micellization concentration (CMC), and the critical micellization temperature (CMT), the concentration and temperature at which micelles start forming, are important parameters in the characterization of self associating block copolymers. In the case of Pluronic® copolymers, the CMC and the CMT values decrease with increasing molecular weight of the hydrophobic PPO block [10]. Additionally, ionic copolymers that exhibit pH-sensitive behavior are commonly characterized with critical pH values that correspond to significant conformational changes in solution [19,27]. The pendent tertiary amine groups of the methacrylate blocks in the pentablock copolymers are weakly basic. These blocks are electrostatically charged and hydrophilic at low pH and are relatively hydrophobic at high pH. Potentiometric titrations were conducted to determine the critical pH values at which the pendent amines became uncharged. The pH of 10 mg/ml pentablock solutions, as a function of degree of amine protonation (α) are shown in Fig. 6. The buffering region of the curves indicates the pH range at which the pendent amine groups are partially ionized. $\alpha > 1$ and $\alpha < 0$ correspond to excess acid and excess base in solution, respectively. Plotted this way, it is easy to observe the pH range where the amine blocks become uncharged. At low

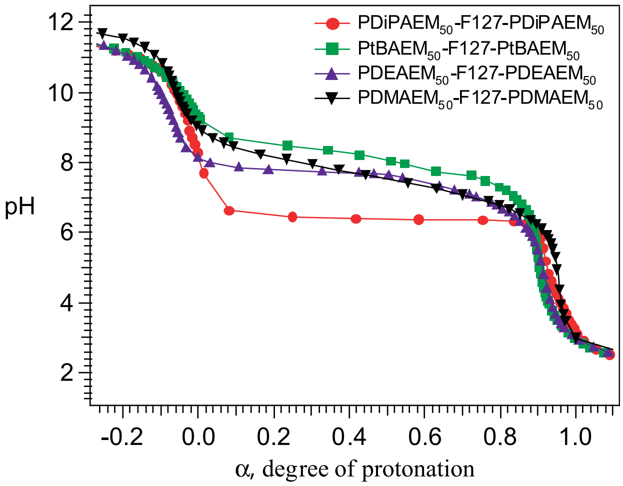


Fig. 6. Titration curves for four pentablock copolymers. The pH at the inflection point of the curves was taken as the pK_a value of the copolymer: pK_a(PDiPAEM$_{50}$-F127-PDiPAEM$_{50}$) = 6.37; pK_a(PDEAEM$_{50}$-F127-PDEAEM$_{50}$) = 7.63; pK_a(PDMAEM$_{50}$-F127-PDMAEM$_{50}$) = 7.52; pK_a(P*t*BAEM$_{50}$-F127-P*t*BAEM$_{50}$) = 8.06.

pH, electrostatic interactions between the charged groups along the copolymer chain likely results in extended chain conformations, while uncharged amine methacrylate blocks likely exist in a more compact conformation at higher pH. The hydrophilic character of the charged pendent amine groups decreases in the order *t*BAEM > DMAEM > DEAEM > DiPAEM. The PDiPAEM pentablock precipitated above pH 8.3 due to the hydrophobic isopropyl groups. The PDEAEM pentablock copolymer solution precipitated above pH 11 and the P*t*BAEM and PDMAEM pentablock copolymer solutions did not exhibit pH-induced precipitation. This indicates that the P*t*BAEM and PDMAEM blocks remain hydrophilic even when completely deprotonated.

The pH-responsiveness of the pentablock copolymers was investigated with ^{1}H NMR in D_2O. Fig. 7 shows the ^{1}H NMR spectrum obtained for the PDEAEM$_{25}$-F127-PDEAEM$_{25}$ copolymer (sample F in Table 1) in D_2O/DCl at pH values of 2.9, 7.4 and 8.3. The copolymer solutions were prepared at 2 mg/ml, and measurements were taken at room temperature. The intensity of the peaks associated with protons adjacent to the amine of the PDEAEM blocks (A, D and E in Fig. 7) were observed to attenuate with increasing pH relative to the signal associated with the protons in the PEO backbone (B). This reflects the dehydration and reduced mobility of the PDEAEM blocks as they become deprotonated [23]. As the PDEAEM blocks transition from hydrophilic polyelectrolytes to uncharged hydrophobic blocks, upon increasing pH, the copolymers undergo an association process. To further examine the isothermal pH-dependent micellization of the pentablock copolymers, quasielastic light scattering (QELS) and multi angle light scattering (MALS) were utilized to determine the critical micellization onset pH and size of the micellar aggregates formed. Fig. 8 shows the dependence of the hydrodynamic radius (R_h) of pentablock copolymer micelles as a function of pH. In Fig. 9, MALS was used to measure the degree of multimerization of the copolymers as they associated into micelles in response to changes in pH. The hydrodynamic radii of Pluronic® F127 micelles showed little dependence on pH as expected. For the pentablock copolymers at low pH, charged unimers or small micelles with $R_\text{h} \sim 10$ nm were observed. Within the resolution of these QELS measurements, the low pH pentablock copolymer micelles and the Pluronic® micelles appeared to have the same hydrodynamic radii. The pentablock micelles exhibited an abrupt increase in size above a critical pH value. The amine methacrylate blocks became hydrophobic at high pH, resulting in attractive hydrophobic interactions between micelles. The aggregation of several micelles may explain the large increase in micelle size and aggregation number at high pH. The pH-induced onset of micellar association of the PDEAEM pentablock copolymers occurred when only 20% of the cationic amines had been neutralized. The PDMAEM pentablock micelles did not begin to aggregate until more than half of the pendant groups had become deprotonated. This presumably reflects the greater hydrophobic character

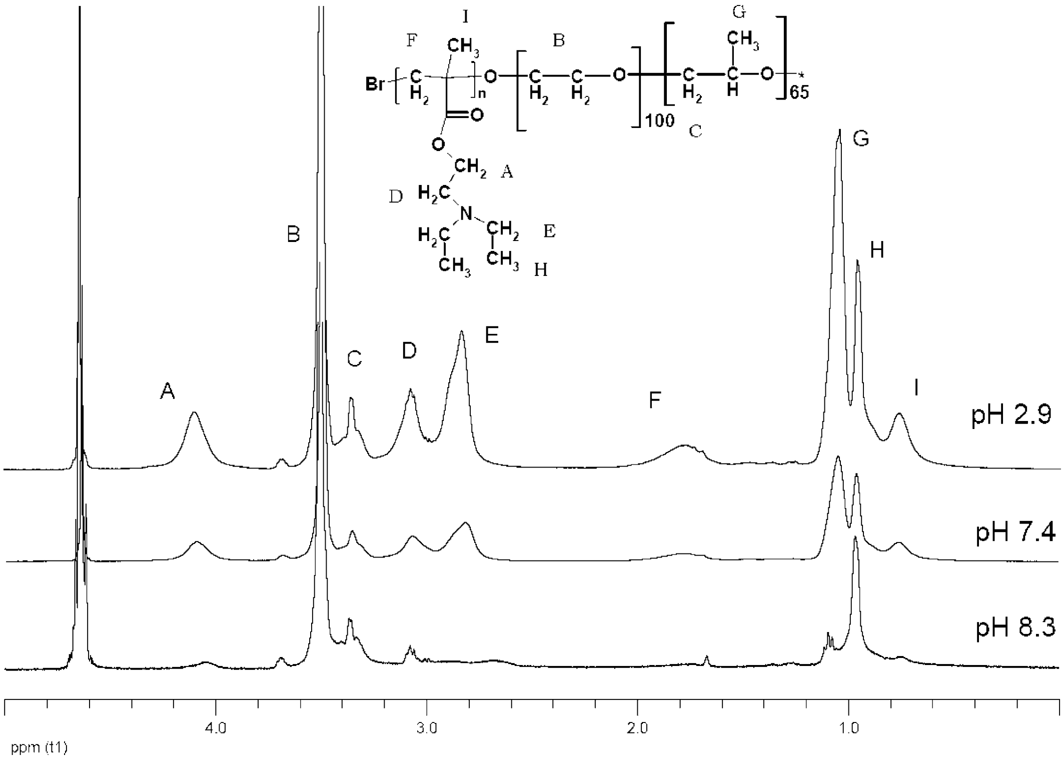


Fig. 7. ^{1}H NMR spectra of obtained for PDEAEM$_{25}$-F127-PDEAEM$_{25}$ pentablock copolymers at pH values of 2.9, 7.4 and 8.3 in D_2O.

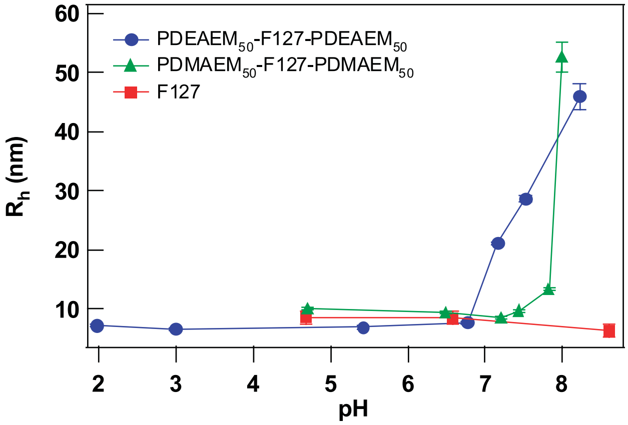


Fig. 8. Micelle hydrodynamic radius, R_h, as determined by QELS measurements, for 5.0×10^{-4} g/ml pentablock and Pluronic® F127 copolymers as a function of pH.

of the unprotonated PDEAEM compared to PDMAEM. Lowering the sample pH resulted in a decrease in aggregation number and hydrodynamic radius, demonstrating the reversibility of this aggregation process.

Cryo-TEM was used to directly visualize micelles formed from the pentablock copolymers. Fig. 10 is a cryo-TEM micrograph of micelles of pentablock copolymers (PDEAEM$_{20}$-F127-PDEAEM$_{20}$). The polymer solution, at 27 °C and 30 mg/ml in pH 7.4 phosphate buffer, was rapidly vitrified in liquid ethane to preserve the microstructure originally present in the ambient solution. Micelles with dark, electron dense cores surrounded by light coronas were observed. The pentablock copolymer micelles were significantly larger and more polydisperse than Pluronic® F127 micelles visualized with cryo-TEM. Lam and coworkers observed Pluronic® F127 micelles with a mean diameter of approximately 6 nm in a 5% solution [28]. There was a considerable size distribution of the pentablock copolymer micelles, and the micelles did not appear to exhibit any long-range order. The micellar dimensions and size distribution were quantified with Metamorph® image analysis software. Fig. 11 shows the radial size distribution of the micelles and the micelle cores. The average radii of the micelles and micelle cores were 39.4 and 15.2 nm, respectively. The dimensions of the micelles visualized with cryo-TEM were in good qualitative agreement with the QELS measurements of the dilute solution of PDEAEM pentablock micelles at pH 7.2.

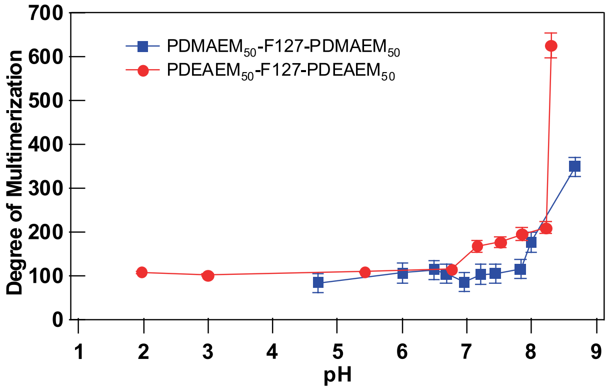


Fig. 9. Degree of multimerization of pentablock copolymer micelles as a function of solution pH, determined by MALS.

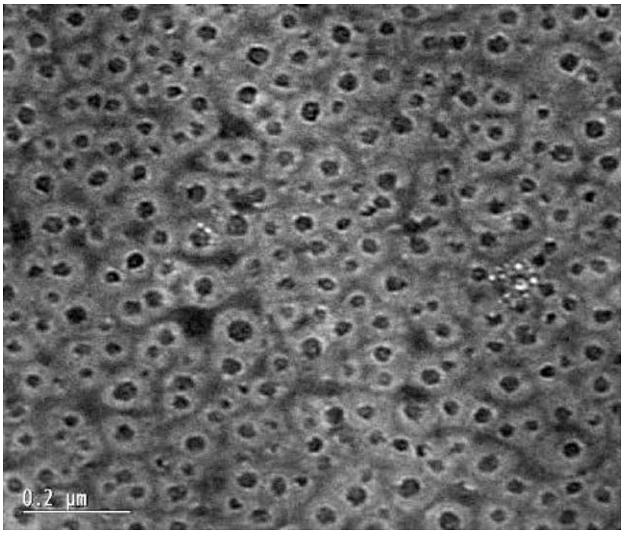


Fig. 10. Cryo-TEM micrograph of a 3 wt% PDEAEM$_{20}$-F127-PDEAEM$_{20}$ pentablock copolymer solution in 0.1 M PBS at a pH of 7.4.

Small angle X-ray scattering (SAXS) was used to monitor temperature dependent self-association of the pentablock copolymers in solution. SAXS spectra collected at several temperatures from 50 mg/ml solutions of PDEAEM$_{50}$-F127-PDEAEM$_{50}$ at pH 7.4 and 4.4 are shown in Figs. 12 and 13, respectively. The formation of nanometer scale copolymer micelles with increasing temperature was observed as an increase in the scattering intensity in the low Q region. With an increase in temperature, the increase in degree of association of copolymers into micelles further contributed to the intensity of scattering at low Q for both samples.

No correlation peaks emerged in the low angle scattering for the pH 7.4 pentablock solutions but the continuous increase in scattering intensity could be attributed to the formation of a disordered micellar solution phase. Scattering from the pentablock copolymer solutions at pH 4.4 exhibited a more pronounced temperature dependence. Below 20 °C, the scattering from the pH 4.4 solution exhibited a broad peak centered on $Q=0.065$ which may correspond to interactions between charged unimers in solution. Above 20 °C, a correlation peak was observed at $Q=0.0175$ in the scattering pattern from the pH 4.4 pentablock copolymer solution. This suggests the presence of spherical micelles with a correlation length of about 35.7 nm.

The intensity, at $Q=0.02\ \text{Å}^{-1}$, for the pentablock copolymers at pH 4.4 and 7.4 and Pluronic® F127 are shown as a function of temperature in Fig. 14. The CMT of the Pluronic® F127 and the pH 4.4 pentablock solutions

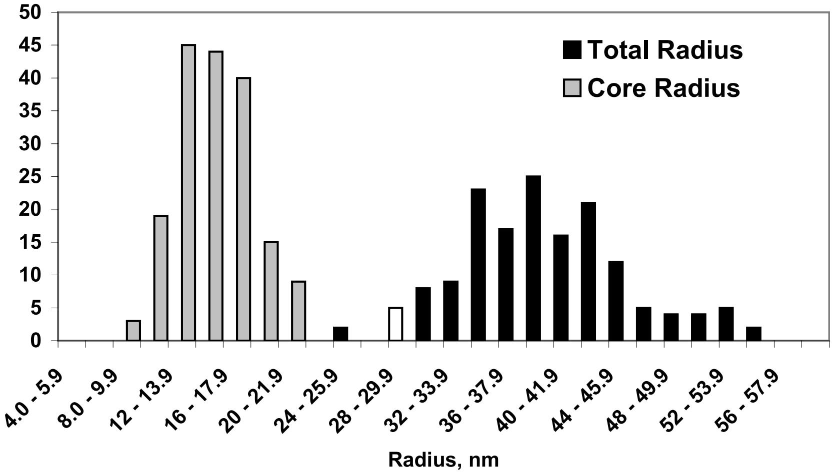


Fig. 11. Radial size distribution of spherical micelles and the spherical micelle cores obtained by cryo-TEM measurements of 3 wt% PDEAEM$_{20}$-F127-PDEAEM$_{20}$ pentablock copolymer in pH 7.4 PBS.

both occurred near 20 °C. This suggests that micellization of the pentablock in the charged state, at low pH, is due to the LCST of the PPO block. The intensity of scattering at low Q from the pH 7.4 pentablock copolymer solutions increased upon warming and reached a plateau at 25 °C. The uncharged pentablock copolymers at pH 7.4 appeared to self-associate in a manner distinct from the micellization of the pentablock copolymer at pH 4.4. Future small angle neutron scattering studies will examine the detailed structures of the micelles along with pH, and temperature dependent phase behavior of the pentablock copolymers.

The sol–gel transition temperatures and the viscoelastic properties of pentablock copolymer gels were measured with dynamic mechanical analysis (DMA). The sol-gel transition temperature of F127, as determined by this technique, agreed well with literature reports [29,30]. The elastic modulus of the gel underwent dramatic transitions at the sol–gel temperature. The transition temperature was found to be independent of frequency in the range of 1–50 Hz. The storage moduli of gels (20% by weight) formed by PDEAEM$_{25}$-F127-PDEAEM$_{25}$ pentablock copolymer (F in Table 1) at several pHs and Pluronic® F127 gels are shown in Fig. 15. The pentablock copolymers exhibited a thermoreversible sol–gel transition similar to Pluronic® F127. The solutions tested formed transparent, rigid gel phases at elevated temperatures and were free flowing liquids at lower temperatures. In the case of Pluronic® F127 solutions, micelles form in aqueous solutions when the PPO blocks aggregate and dehydrate to form compact cores surrounded by hydrated PEO chains that extend into the solvent forming a corona. Upon warming, PEO blocks become less hydrophilic, leading to the coronal interdigitation and packing into a physical network [31,32]. The transition from liquid to gel phases is completely reversible. Solutions of PDEAEM$_{25}$-F127-PDEAEM$_{25}$ pentablock copolymer (20% by weight) at pH 3.0, 5.5 and 7.7 were found to form thermoreversible gels with transition temperatures around 20 °C. At pH 8.3 the pentablock

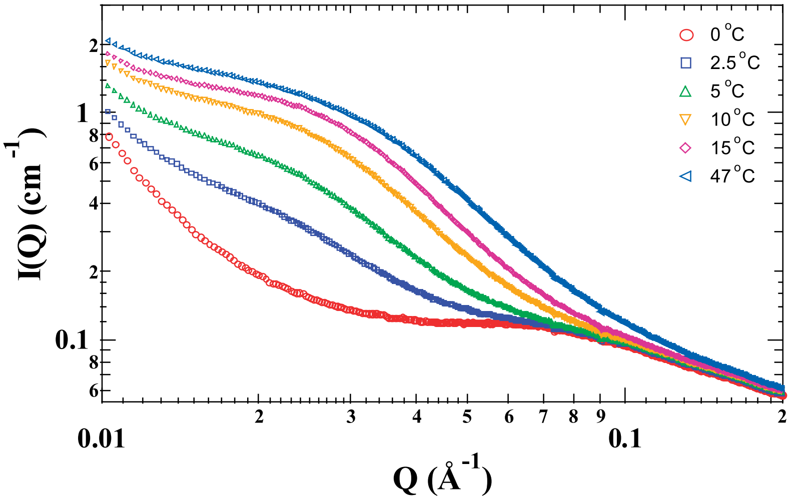


Fig. 12. Effect of temperature on a pentablock copolymer solution of PDEAEM$_{50}$-F127-PDEAEM$_{50}$, 0.05 g/ml, pH 7.4, monitored by SAXS.

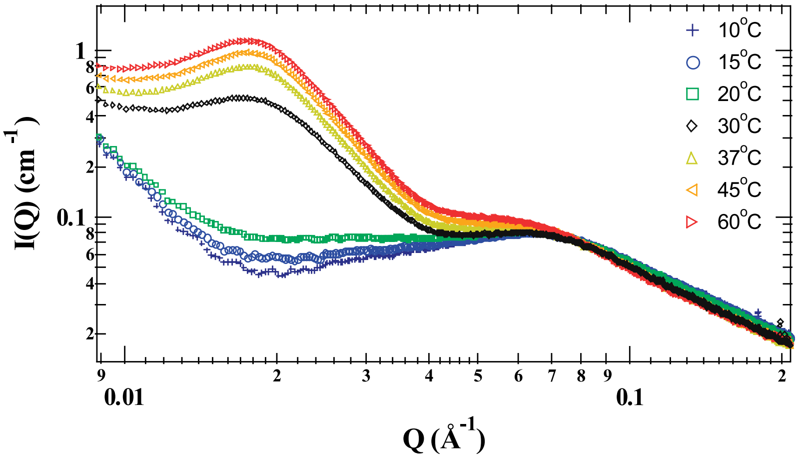


Fig. 13. Effect of temperature on a pentablock copolymer solution PDEAEM$_{50}$-F127-PDEAEM$_{50}$, 0.05 g/ml, pH 4.4, monitored by SAXS.

solution remained in the gel phase down to 7 °C. This is presumably due to the presence of deprotonated and hydrophobic PDEAEM blocks forming non-temperature sensitive intermicellar network. The pentablock copolymer gels formed a less rigid gel phase compared to F127 gels. The presence of the PDEAEM blocks in the pentablock copolymer likely disrupts the regular micellar lattice structure, resulting in a softer gel phase.

In keeping with the trends observed in Pluronic® F127 systems, the sol–gel transition temperatures of the pentablock copolymer solutions decreased with increasing concentration (data not shown) [12]. Pentablock copolymers with PDMAEM blocks were found to form thermoreversible gels with similar properties to the PDEAEM containing pentablock copolymer. The copolymers containing PDEAEM blocks were found to form more rigid gels with lower transition temperatures. The more hydrophobic ethyl groups of the PDEAEM blocks likely result in stronger hydrophobic interactions between micelles than the methyl groups of the PDMAEM blocks resulting in a more rigid macroscopic gel phase. The PDiPAEM pentablock copolymer was not easily dissolved at concentrations high enough to form gel phases, presumably do to the increased hydrophobicity of the PDiPAEM blocks. Pentablock copolymers incorporating the P*t*BAEM blocks did not form gels. We hypothesize that the presence of the bulky pendent *t*-butyl group inhibits micellar packing and coronal interdigitation.

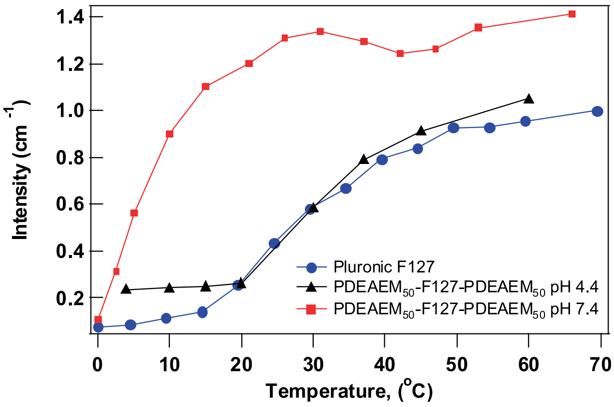


Fig. 14. Effect of temperature on scattering intensity at $Q = 0.02$ Å^{-1} in Pluronic® F127 and pentablock copolymer solutions.

The pentablock copolymers have potential applications in injectable depot drug delivery formulations. The thermoreversible sol-gel transition and the pH-dependent association properties impart environmentally sensitive solution properties that may be utilized to improve current injectable depot formulations [33].

4. Conclusions

An ATRP method for the synthesis of a family of novel pentablock copolymers with well-defined molecular weights under mild conditions has been developed. These pentablock copolymers were found to exhibit pH-sensitive micellization and LCST behavior and thermoreversible sol-gel transitions, in dilute and concentrated aqueous solutions, respectively. The reversible gelation at around physiological temperatures and pH-dependent micellization make these pentablock copolymers potential candidates for use in injectable drug delivery devices that exhibit pH-regulated release and for injectable gene delivery applications [26]. Further investigations of the nanostructures of the micellar solutions and sol–gel transitions with detailed modeling analysis of small angle neutron scattering data and cryo-TEM will further elucidate the rich phase behavior of these materials.

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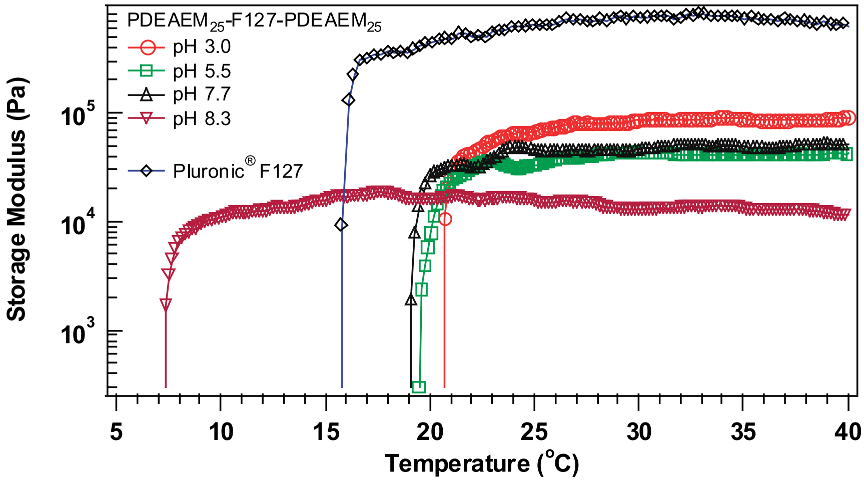

Fig. 15. The storage modulus, as measured by DMA, of self-assembled gels formed by Pluronic® F127 and the pentablock copolymer.

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