Aqueous RAFT Synthesis of Micelle-Forming Amphiphilic Block Copolymers Containing *N*-Acryloylvaline. Dual Mode, Temperature/pH Responsiveness, and "Locking" of Micelle Structure through Interpolyelectrolyte Complexation†

Brad S. Lokitz,‡ Adam W. York,‡ Jonathan E. Stempka,‡ Neil D. Treat,‡ Yuting Li,‡ William L. Jarrett,‡ and Charles L. McCormick*,‡,§

Department of Polymer Science and Department of Chemistry and Biochemistry, University of Southern Mississippi, Hattiesburg, Mississippi 39406

Received April 19, 2007; Revised Manuscript Received June 25, 2007

ABSTRACT: Temperature- and pH-responsive, micelle-forming, amphiphilic block copolymers were prepared from *N*,*N*-dimethylacrylamide (DMA), *N*-isopropylacrylamide (NIPAM), and *N*-acryloylvaline (AVAL) utilizing aqueous reversible addition−fragmentation chain transfer (RAFT) polymerization. A series of block copolymers were synthesized by employing DMA as a macro-chain transfer agent to mediate the statistical copolymerization of NIPAM with AVAL. Structural organization and solution behavior were investigated utilizing dynamic light scattering, two-dimensional NMR spectroscopy, and transmission electron microscopy. It has been demonstrated that the critical micellization temperature for the block polymers can be tuned to range from ≈10 to 36 °C by adjusting the solution pH. Micelles with apparent hydrodynamic diameters from 45 to 86 nm are formed between pH 2 and 5. Above pH 5, a sufficient number of the AVAL units are deprotonated which prevents micellization. The extent of pH and temperature changes on the apparent hydrodynamic diameters have been illustrated via 3-D plots. Significantly, micelles assembled within a specified range of pH and temperature can be "locked" by interpolyelectrolyte complexation of anionic AVAL segments with those of a cationic polymer, in this case a RAFT-generated poly([ar-vinylbenzyl]trimethylammonium chloride) (PVBTAC). When the temperature is lowered to room temperature, the polymeric micelles remain "locked" in their multimeric structures which remain dispersed in water. Addition of 0.3 M NaCl to the aqueous solution results in dissociation of the complexes into the respective water-soluble components.

Introduction

Well-defined amphiphilic block copolymers have attracted a great deal of interest in targeted delivery and controlled release due to their ability to reversibly self-assemble into micelles,[1−8] vesicles,[9−13] and mesoglubles.[14−16] Association/dissociation is most often triggered by external stimuli including pH, temperature, or added electrolyte. It has long been established that temperature-responsive polymers such as poly(*N*-isopropylacrylamide) (PNIPAM) undergo sharp coil-to-globule transitions in water that lead to phase separation at the lower critical solution temperature (LCST).[17−19] Tuning of the LCST can be achieved through copolymerization of the thermally responsive monomer with comonomers of varying degrees of hydrophobicity.[20−27] Additionally, copolymerization with amphoteric monomers can lead to a triggered response through a change in pH at a given temperature.[20,21] For example, both acrylic acid and methacrylic acid have been copolymerized with NIPAM using conventional free radical polymerization to produce random copolymers with both pH- and temperature-responsive properties.[21−24] Recently, Stayton and co-workers[25] used reversible addition−fragmentation chain transfer (RAFT) polymerization to synthesize a random copolymer of NIPAM and propylacrylic acid that showed a sharp phase transition in response to temperature and pH. Block copolymers that are capable of self-assembling into ordered structures, such as multimeric micelles, have also been investigated. Determan et al.[26,27] used ATRP to synthesize a pentablock copolymer containing a pH-responsive block of diethylaminoethyl methacrylate and a temperature-responsive block of propylene oxide. Block copolymers that respond to two stimuli provide for increased versatility and control when tuning the self-assembly properties of macromolecules for pharmaceutical and diagnostic applications.

Multimeric micelles often experience dilution upon mixing with physiological fluids, thus allowing the concentration to fall below the critical micelle concentration (cmc) and leading to the dissociation of the micelles into unimers. To circumvent this limitation, several research groups are currently exploring avenues for increasing the robustness of the micelles via chemical cross-linking of the "shell" or "core" components of the assemblies. Some of the first stabilized micelles, known as shell cross-linked (SCL) micelles, were reported by the groups of Wooley[28−30] and Armes.[31−34] Building on current cross-linking strategies, our group reported the formation of SCL micelles[35] and reversible SCL micelles[36] through diamine coupling of the active monomer *N*-acryloxysuccinimide.

Traditional cross-linking technologies, however, are often limited by a number of factors including poor reagent solubility and low reaction efficiency, especially in water where diffusional factors and structural organization of the amphiphilic block copolymers dominate behavior. Additionally, reagents are often toxic and must be removed prior to application.[2,30,37,38] Recently, an alternative strategy based on simple interpolyelectrolyte complex formation among multimeric micelle units has been

† Paper no. 129 in a series titled "Water Soluble Polymers".
‡ Department of Polymer Science.
§ Department of Chemistry and Biochemistry.

10.1021/ma070921v CCC: $37.00
Published on Web 08/01/2007

used to "cross-link" or "stabilize" these assemblies. For example, Armes and co-workers[39] reported SCL micelles composed of a cationic ABC triblock copolymer and an anionic (co)polymer. Our group has recently reported such interpolyelectrolyte complexation to stabilize triblock copolymer micelles containing anionic, amino acid-based monomers[40] and thermally assembled vesicles.[41] Although reports of interpolyelectrolyte-stabilized micelles are relatively few, the behavior of interpolyelectrolyte complexes (IPECs) and block ionomer complexes (BICs) in water has been the subject of extensive research.[42−45]

N-Acryloyl derivatives of amino acids which can be synthesized in a facile manner are of interest to our group due to their chirality, ability to self-assemble into higher order structures, and potential for interpolyelectrolyte stabilization.[46−48] Rapid developments of controlled radical polymerization techniques which allow preparation of well-defined functional polymers and block copolymers have recently been reviewed.[49−51] Of significance is the fact that the RAFT technique allows direct synthesis of well-defined water-soluble, stimuli-responsive polymers of various architectures, often directly in water.[52,53] For example, our group has reported the aqueous RAFT polymerization and copolymerization of hydrophilic (meth)-acrylamido monomers including anionic,[52−55] cationic,[52,53,56] zwitterionic,[52,53,57,58] and neutral derivatives.[52,53,59−63]

Herein, we report a facile method for the synthesis of self-ordering block copolymers that have dual modes of responsiveness. Block copolymers containing a hydrophilic *N*,*N*-dimethylacrylamide (DMA) block and doubly (temperature and pH) responsive statistical blocks of *N*-isopropylacrylamide (NIPAM) and *N*-acryloylvaline (AVAL) have been prepared via aqueous RAFT polymerization. These copolymers undergo reversible pH- and temperature-induced unimer-to-micelle transition. The self-assembled micelles can be reversibly "cross-linked" above the critical micelle temperature (CMT) via interpolyelectrolyte complexation of the poly(AVAL) segments in the core of the micelle with the cationic homopolymer poly(ar-vinylbenzyl)-trimethylammonium chloride (VBTAC). Finally, we demonstrate the reversibility of this process by addition of sufficient electrolyte [NaCl] which results in dissociation of the complexes into the respective water-soluble components.

Experimental Section

Materials. All reagents were purchased from Aldrich at the highest purity available and used as received unless otherwise stated. 2-Ethylsulfanylthiocarbonylsulfonyl-2-methylpropionic acid (EMP) was previously prepared.[62] A monofunctional macroCTA of *N*,*N*-dimethylacrylamide (M_n= 11 400 g/mol, M_n/M_w = 1.09) and poly-([ar-vinylbenzyl]trimethylammonium chloride), PVBTAC (M_n= 26 000 g/mol, M_n/M_w = 1.21), were previously synthesized.[40] 4,4′-Azobis(4-cyanopentanoic acid) (V-501) and 2,2′-azobis[2-(2-imidiazolin-2-yl)propane] dihydrochloride (VA-044) were donated by Wako Chemicals and were recrystallized twice from methanol prior to use. NIPAM was recrystallized twice from hexanes prior to use.

Synthesis of *N*-Acryloylvaline (AVAL) The synthesis of the valine-based acrylamido monomer, *N*-acryloylvaline (AVAL), was performed as follows: L-valine (0.40 mol, 46.8 g) and NaOH (0.80 mol, 32.0 g) were dissolved in deionized (DI) water (180 mL) and stirred using a mechanical stirrer. Once the solids had completely dissolved, the solution was cooled to 4 °C using an ice bath. Acryloyl chloride (0.40 mol, 36.2 g) was added dropwise over 2 h, maintaining the temperature of the reaction at 4 °C. Upon complete addition of acryloyl chloride, HCl was added dropwise to neutralize the monomer and induce precipitation. The afforded crystals were then collected by filtration and recrystallized from benzene. Melting point 119−120 °C. ^{1}H NMR: CH_2CHCO 5.71 (d), CH_2CHCO 6.22 (m), HNCH(COOH)CH$(CH_3)_2$ 4.15 (d), HNCH(COOH)C$H(CH_3)_2$ 2.01 (m), HNCH(COOH)CH(C$H_3)_2$ 0.98 (d).

General Procedure for the RAFT Polymerization of AVAL. Polymerizations were conducted at 30 and 70 °C, employing 2,2′-azobis[2-(2-imidiazolin-2-yl)propane] dihydrochloride (VA-044) or 4,4′-azobis(4-cyanopentanoic acid) (V-501) as the primary radical source and 2-ethylsulfanylthiocarbonylsulfonyl-2-methylpropionic acid EMP as the RAFT CTA. Polymerizations were performed directly in water (pH 6.5) with an initial monomer concentration ($[M]_0$) of 1.00 M in individual, septa-sealed vials, which were purged with nitrogen at 5 °C for 30 min prior to reaction. The initial monomer to CTA ratio ($[M]_0/[CTA]_0$) was 210, while the initial CTA to initiator ratio ($[CTA]_0/[I]_0$) was held constant at 5:1. For example, to determine the kinetics for the RAFT polymerization of AVAL mediated by EMP at 70 °C, 5.00 g (0.029 mol) of AVAL, 31.4 mg (1.40×10^{-4} mol) of EMP, and 7.85 mg (2.80×10^{-5} mol) of V-501 were added to 29 mL of deionized (DI) water. The polymerization kinetics and the absolute molecular weights were determined from aliquots (0.5 mL) taken at predetermined time intervals and quenched via rapid cooling and exposure to oxygen.

Block Copolymer Synthesis. A macroCTA of DMA was used for preparing diblock copolymers of DMA-*b*-(NIPAM-*s*-AVAL). The polymerizations were conducted directly in water (pH 6.5) with an initial monomer concentration of 1.00 M at 30 °C with VA-044 as the initiator. The $[PDMA]_0$:$[VA\text{-}044]_0$ ratio was maintained at 5:1, and the target M_n for the second block was 25 000 g/mol. The feed ratio for AVAL was varied between 10 and 40 mol %. For example, DMA_{115}-*b*-($NIPAM_{202}$-*s*-$AVAL_{27}$) was prepared by adding 1.09 g (9.6×10^{-3} mol) of NIPAM, 0.171 g (1.0×10^{-3} mol) of AVAL, 0.516 g (4.5×10^{-5} mol) of $PDMA_{115}$ macroCTA, and 2.93 mg (9.1×10^{-6} mol) of VA-044 to 10 mL of DI water and allowing the polymerization to proceed for 360 min (87% conversion; M_n = 38 800 g/mol; PDI = 1.19). Polymerizations were conducted under a nitrogen atmosphere in round-bottomed flasks equipped with magnetic stir bars and sealed with rubber septa. The products were purified by dialysis against deionized water and isolated by lyophilization.

Aqueous Size Exclusion Chromatography. Homopolymers of AVAL were analyzed directly by aqueous size exclusion chromatography (ASEC) using an aqueous eluent of 20% acetonitrile/80% 0.5 M Na_2SO_4. DMA-*b*-(NIPAM-*s*-AVAL) were analyzed by ASEC using an aqueous eluent of 0.1 M $NaNO_3$. A flow rate of 0.5 mL/min at 25 °C, TOSOH Biosciences TSK-GEL columns [G3000 PWXL (<50 000 g mol^{-1}, 200 Å), and G4000 PWXL (2000−300 000 g mol^{-1}, 500 Å)], a Polymer Labs LC 1200 UV/vis (λ = 275 nm), Wyatt Optilab DSP interferometric refractometer (λ = 690 nm), and Wyatt DAWN EOS multiangle laser light scattering detectors (λ = 690 nm) were used. Wyatt DNDC for Windows was used for d*n*/d*c* determinations.

^{1}H NMR Spectroscopy. ^{1}H NMR spectra were recorded with a temperature-controlled Varian UNITY INOVA spectrometer operating at a frequency of 499.8 MHz for protons and using a 5 mm three-channel HCN probe. Samples were prepared as 10% w/w solutions in D_2O (HOD internal standard). Copolymer compositions were determined by a comparison of resonances associated with the comonomers (DMA dimethyl group ~2.8, ppm, NIPAM dimethyl ~1.1 ppm, AVAL dimethyl groups ~0.8 ppm). Nuclear Overhauser enhancement spectroscopy (NOESY) data were acquired using a standard NOESY sequence with WET suppression.[64] Acquisition parameters were as follows: the recycle delay was 2 s, the 90° pulse width was 6.85 μs, the sweep width in both dimensions was 16 ppm, and the acquisition time was 150 ms. The number of t1 increments was 400 with 16 scans acquired per increment. Phase-sensitive phase cycling (States) was used, and Gaussian filters were applied to data in both F1 and F2. A mixing time of 500 ms was used.

Preparation of Micelles. Copolymers were dissolved directly in HPLC grade water containing 0.01 M NaCl (1 mg/mL, 10 mL), and the pH was adjusted using 0.1 N HCl or NaOH. Micellization

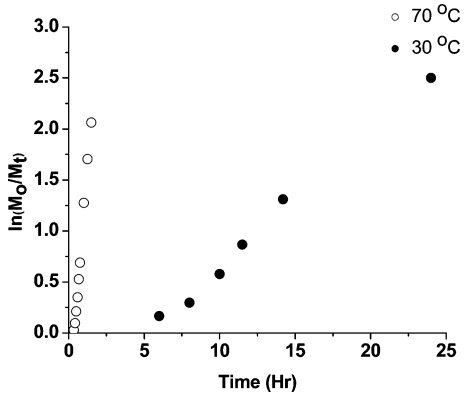
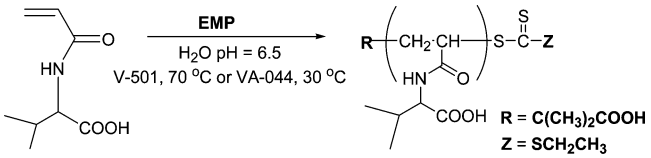


Figure 1. Pseudo-first-order kinetic plot for the 2-ethylsulfanylthiocarbonylsulfonyl-2-methylpropionic acid-mediated homopolymerizations of *N*-acryloylvaline at 70 °C (○) and 30 °C (●).

Scheme 1. Synthetic Pathway for the Aqueous Reversible Addition−Fragmentation Chain Transfer Polymerization of *N*-Acryloylvaline with 2-Ethylsulfanylthiocarbonylsulfonyl-2-methylpropionic Acid and 4,4′-Azobis(4-cyanopentanoic acid) or 4,4′-Azobis[2-(imidazolin-2-yl)propane] Dihydrochloride as the Free Radical Initiator



of the copolymers was achieved by adjusting both the solution pH and temperature.

Interpolyelectrolyte Cross-Linked Micelles. Core cross-linked micelles were prepared from an aqueous stock solution of the copolymer(1 mg/mL, 3 mL). The pH of the solution was adjusted to 4.2 ± 0.2 equilibrated at 50 °C for 30 min. PVBTAC, a cationic polymer (M_n= 26 000 g/mol, M_n/M_w = 1.21), was then added at a 1:1 mole ratio based on the deprotonated carboxylic acid groups of AVAL. The micelle solution was then stirred at 50 °C for 15 min.

Dynamic Light Scattering. Dynamic light scattering studies of the copolymers at concentrations of 1.00 g/L in an aqueous 0.01 M NaCl solution were conducted using a Malvern Instruments Zetasizer Nano ZS instrument equipped with a 4 mW He−Ne laser (λ = 633 nm) at an angle of 173°, an avalanche photodiode detector with high quantum efficiency, and an ALV/LSE-5003 multiple τ digital correlator electronics system. The CONTIN analysis method was used.

Transmission Electron Microscopy. Transmission electron microscopy (TEM) measurements were conducted using a JEOL JEM-2100 electron microscope at an acceleration voltage of 200 kV. The specimens were prepared by placing a 10 μL drop of the 0.005% w/w micelle solution on a Formvar-coated copper grid followed by water evaporation.

Results and Discussion

Aqueous RAFT Polymerization of AVAL. *N*-Acryloylvaline, a structural isomer of the pH-responsive monomer 3-acrylamido-3-methybutanoate (AMBA), was selected for this study on the basis of its facile synthesis from readily available amino acid sources and its amphiphilic nature, which allows for polymerization, purification, and characterization directly in aqueous solution. In order to incorporate the monomer into pH- and temperature-responsive block copolymers, the RAFT polymerization of AVAL was conducted directly in water utilizing the diazo initiators V-501 (70 °C) or VA-044 (30 °C) and 2-ethylsulfanylthiocarbonylsulfonyl-2-methylpropionic acid (EMP) as the chain transfer agent (CTA) (Scheme 1).

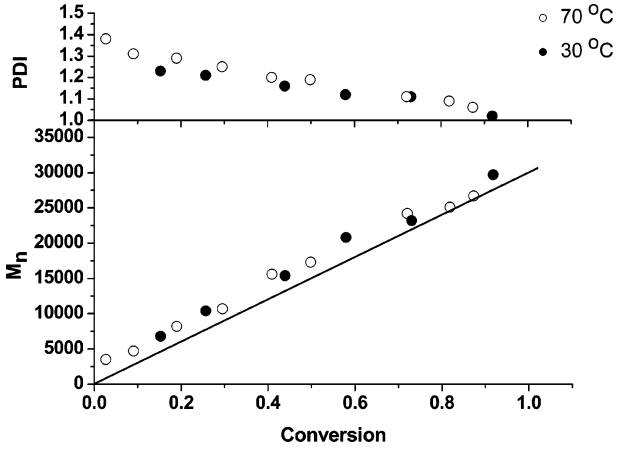


Figure 2. Number-average molecular weight (M_n) and polydispersity (PDI) vs conversion for the aqueous homopolymerizations of *N*-acryloylvaline mediated by 2-ethylsulfanylthiocarbonylsulfonyl-2-methylpropionic acid at 70 °C (○) and 30 °C (●).

Scheme 2. Synthetic Route for Preparation of AB Diblock Copolymers with a *N*,*N*-Dimethylacrylamide A Block and a *N*-Acryloylvaline and *N*-Isopropylacrylamide Statistical B Block via Aqueous Reversible Addition−Fragmentation Chain Transfer Polymerization

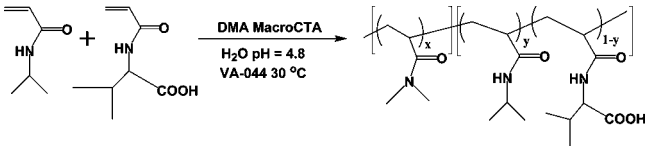


Figure 1 shows the pseudo-first-order kinetic plots for the EMP mediated hompolymerizations of AVAL at 30 and 70 °C with an initial monomer to CTA ratio ($[M]_0$:$[CTA]_0$)of 210:1. As expected, the apparent rate of polymerization at 70 °C is significantly higher than at 30 °C. This is attributed to a larger number of initiator radicals and a faster fragmentation rate of the intermediate radical species, thus yielding higher values of $k_p[M^\bullet]$ and a faster rate of propagation at 70 °C. It should be noted that an induction period is observed at 30 °C.[65]

Evident from Figure 2 are the linear increases in molecular weight vs conversion, excellent agreement between theoretical (solid line) and experimental molecular weights, and low polydispersities for the aqueous RAFT polymerization of AVAL at 30 and 70 °C. These initial experiments indicate that the RAFT-mediated polymerization of AVAL at both 30 and 70 °C proceeds in a controlled fashion. Of particular interest is the ability to control the polymerization of AVAL 30 °C, which allows for copolymerization with a temperature-responsive monomer, such as NIPAM, directly in water.

pH- and Temperature-Responsive Block Copolymers. Having established conditions for the controlled polymerization of AVAL, pH- and temperature-responsive diblock copolymers were synthesized as detailed in the Experimental Section and shown in Scheme 2. The initial hydrophilic, neutral block synthesized from DMA utilizing EMP as the chain transfer agent had an M_n of 11 400 g/mol and a PDI of 1.07. Subsequent block copolymerization with NIPAM and AVAL yielded the pH- and temperature-responsive diblock copolymers utilized for micellization studies.

The structural data for the copolymers (Table 1) were determined utilizing aqueous size exclusion chromatography with multiangle laser light scattering (ASEC-MALLS) detection and ^{1}H NMR.

^{1}H NMR spectra for the diblock copolymers are shown in Figure 3. The compositions of the statistical block containing

Table 1. Degree of Polymerization (DP) for *N,N*-Dimethylacrylamide (DMA), *N*-Isopropylacrylamide (NIPAM), and *N*-Acryloylvaline (AVAL), NIPAM/AVAL Mole Percents, Molecular Weight (M_n), and Polydispersity (PDI) for DMA_{115}-*b*-NIPAM-*s*-AVAL Block Copolymers 1−5

entry	DP DMA[a]	DP NIPAM[b]	DP AVAL[b]	NIPAM/AVAL (mol %)[b]	M_n[b]	PDI[a]
1	115	252	0	100/0	39 900	1.18
2	115	202	27	88/12	38 800	1.19
3	115	182	47	79/21	39 900	1.15
4	115	161	67	71/29	41 000	1.13
5	115	131	80	62/38	39 900	1.16

[a] As determined by aqueous size exclusion chromatography (ASEC). [b] Determined by proton nuclear magnetic resonance (^{1}H NMR) spectroscopy in D_2O.

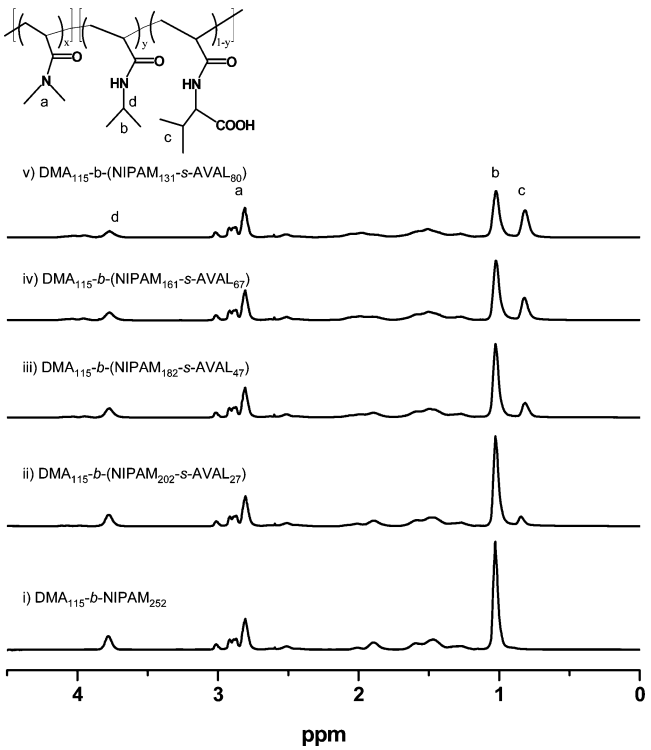


Figure 3. Proton nuclear magnetic resonance (^{1}H NMR) spectra for block copolymers of *N,N*-dimethylacrylamide, *N*-isopropylacrylamide, and *N*-acryloylvaline: DMA_{115}-*b*-$NIPAM_{252}$ (i), DMA_{115}-*b*-($NIPAM_{202}$-*s*-$AVAL_{27}$) (ii), DMA_{115}-*b*-($NIPAM_{182}$-*s*-$AVAL_{47}$) (iii), DMA_{115}-*b*-($NIPAM_{161}$-*s*-$AVAL_{67}$) (iv), DMA_{115}-*b*-($NIPAM_{131}$-*s*-$AVAL_{80}$) (v).

NIPAM and 10−40 mol % (monomer feed ratio) AVAL statistical blocks were determined by comparing resonances of the DMA dimethyl group (∼2.8 ppm) to those associated with NIPAM dimethyl at (1.1 ppm) and AVAL dimethyl groups (0.8 ppm). From Table 1 it is evident that the actual copolymer composition of the statistical blocks is in relatively good agreement with the targeted (feed ratio) compositions, thus providing an appropriate series for studies of the self-assembly behavior and responsiveness to pH and temperature.

Temperature-Induced Micellization. Dynamic light scattering (DLS) was utilized to study the pH- and temperature-induced assembly (micellization) of the block copolymers shown in Table 1. It was anticipated that the statistical NIPAM/AVAL block composition could be utilized to "tune" the critical micelle temperature (CMT) at polymer concentrations above the critical micelle concentration (CMC) since the NIPAM block undergoes phase separation above its LCST[47,48] and AVAL units are pH responsive ($pK_a \sim 4.2$). The unimer-to-micelle transition was followed using dynamic light scattering. The CMT for the control, a diblock copolymer of DMA_{115}-*b*-$NIPAM_{252}$, was

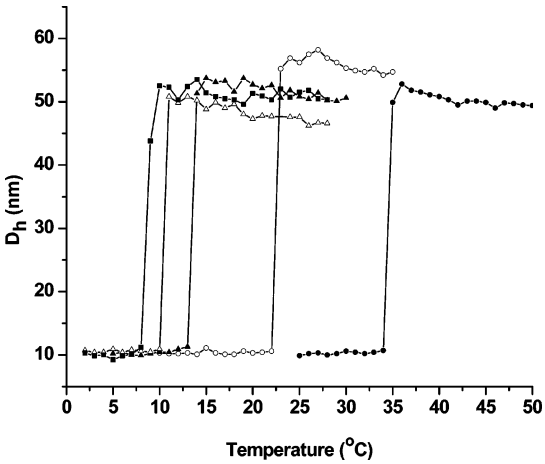


Figure 4. Apparent hydrodynamic diameters for DMA_{115}-*b*-$NIPAM_{252}$ (●), DMA_{115}-*b*-($NIPAM_{202}$-*s*-$AVAL_{27}$) (○), DMA_{115}-*b*-($NIPAM_{182}$-*s*-$AVAL_{47}$) (▲), DMA_{115}-*b*-($NIPAM_{161}$-*s*-$AVAL_{67}$) (△), and DMA_{115}-*b*-($NIPAM_{131}$-*s*-$AVAL_{80}$) (■) measured by dynamic light scattering (polymer concentration = 1.0 g/L) at pH 2.

determined to be 35−36 °C. By copolymerizing 10−40 mol % AVAL into the NIPAM block and adjusting the pH of the solution to 2, the CMT of the block copolymers is shifted to significantly lower temperatures. Figure 4 shows the temperature-induced changes in the apparent hydrodynamic diameters for aqueous solutions of the respective diblock copolymers at pH 2. The CMTs for the diblock copolymers at pH 2 range from 23 °C for DMA_{115}-*b*-($NIPAM_{202}$-*s*-$AVAL_{27}$)(10 mol % AVAL) to 9 °C for DMA_{115}-*b*-($NIPAM_{131}$-*s*-$AVAL_{80}$)(40 mol % AVAL).

Similar trends were observed at pH 4 where roughly 50% of the AVAL units are deprotonated. The CMTs range from 32 °C for DMA_{115}-*b*-($NIPAM_{202}$-*s*-$AVAL_{27}$)(10 mol % AVAL) to 28 °C for DMA_{115}-*b*-($NIPAM_{161}$-*s*-$AVAL_{67}$)(30 mol %). It should be noted that the range is much smaller at pH 4 than at pH 2 and that DMA_{115}-*b*-($NIPAM_{161}$-*s*-$AVAL_{67}$)(30 mol %) actually has the lowest CMT of 28 °C. At pH 8, essentially all of the AVAL units are deprotonated and impart enough hydrophilicty to the statistical block to suppress the hydrophobic interactions of PNIPAM above its LCST, thus preventing micellization. The CMTs for the diblock copolymers at pH values 2, 4, and 8, and the apparent D_h[66] values below and above the CMT are summarized in Table 2.

Combined pH and Temperature Effects on Micelle Size. Three-dimensional plots (Figure 5) were constructed to demonstrate the combined effects of pH and temperature on the CMT and micelle size. In general, increasing the solution pH leads to an increase in the copolymer CMT value up to a critical pH at which micellization does not occur. A large increase in the apparent hydrodynamic diameter of the assemblies is observed between pH 4 and 5. This is attributed to the deprotonation of the AVAL units which increases electrostatic repulsions and chain stretching. Above pH 5, a sufficient number of the AVAL units are deprotonated and impart enough hydrophilicity to prevent association.

Interpolyelectrolyte Cross-Linking. The thermally assembled entities were ionically cross-linked by allowing the micelle solution (pH 4.2 ± 0.2) to equilibrate at 50 °C for 30 min before adding a predetermined amount (1:1 mole ratio anionic:cationic repeat units) of a 0.1% (w/w) solution of the RAFT-polymerized, poly(ar-vinylbenzyl)trimethylammonium chloride (PVBTAC) (M_n= 26 000 g/mol, M_n/M_w = 1.21). DLS indicates that the micelles remain intact upon lowering the

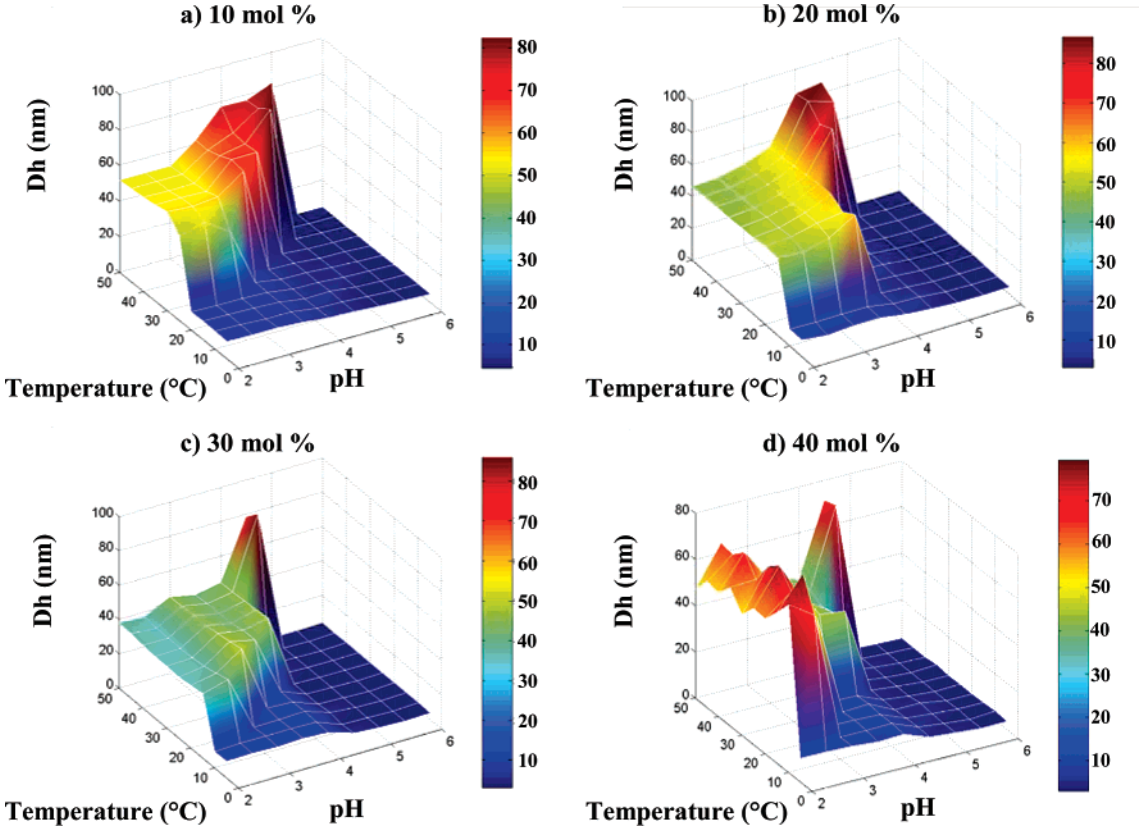


Figure 5. Apparent hydrodynamic diameters for the block copolymers (DMA-*b*-(NIPAM-*s*-AVAL)) (polymer concentration = 1.0 g/L) as a function of pH and temperature: (a) 10 mol % AVAL; (b) 20 mol % AVAL; (c) 30 mol % AVAL; (d) 40 mol % AVAL. (Actual data points occur at intersection of grid lines.)

Table 2. Critical Micelle Temperature (CMT) Values at pH 2, 4, and 8 and Apparent Hydrodynamic Diameter (D_h) (nm) at T < CMT and D_h (nm) at T > CMT for DMA_{115}-*b*-$NIPAM_{252}$ (Entry 1), DMA_{115}-*b*-($NIPAM_{202}$-*s*-$AVAL_{27}$) (Entry 2), DMA_{115}-*b*-($NIPAM_{182}$-*s*-$AVAL_{47}$) (Entry 3), DMA_{15}-*b*-($NIPAM_{161}$-*s*-$AVAL_{67}$) (Entry 4), and DMA_{115}-*b*-($NIPAM_{131}$-*s*-$AVAL_{80}$) (Entry 5) Measured by Dynamic Light Scattering (Polymer Concentration = 1.0 g/L)

	CMT (°C)			D_h (nm) (T < CMT)			D_h (nm) (T > CMT)		
entry	pH 2	pH 4	pH 8	pH 2	pH 4	pH 8	pH 2	pH 4	pH 8
1	35	36	36	10.2	10.3	10.1	49.4	49.5	49.1
2	23	32	NT[a]	10.2	6.7	7.7	52.2	67.7	7.7
3	14	30	NT	10.0	6.8	7.3	49.0	82.3	7.3
4	11	28	NT	10.4	6.3	7.0	44.9	85.8	7.0
5	9	29	NT	10.0	6.7	7.1	48.9	73.2	7.1

[a] NT = no observed CMT.

solution to 25 °C, indicating the formation of interpolyelectrolyte cross-linked micelles. To further demonstrate interpolyelectrolyte complexation and to show spatial correlations between protons in the micelles, 2D ^{1}H NMR nuclear Overhauser effect spectroscopy (NOESY) was utilized. NOESY is a two-dimensional NMR technique that probes internuclear distances through the nuclear Overhauser effect (NOE).[67] NOE scales at r^{-6}, where r is the internuclear distance between two protons. Thus, only protons that are spatially near each other will exhibit cross-peaks in the spectrum. The results of 2D ^{1}H NMR NOESY experiments are typically displayed in a contour plot, where the 1-dimensional ^{1}H NMR spectra are plotted on the horizontal and vertical axes. Cross-peaks between two unlike protons will appear off-diagonal but symmetrical with respect to the diagonal.[68−70] Shown in Figure 6 is the 2D ^{1}H NMR NOESY contour plot for the interpolyelectrolyte complexed micelles of DMA_{115}-*b*-($NIPAM_{161}$-*s*-$AVAL_{67}$) with PVBTAC (pH 4.2, 25 °C, C_p = 10 g/L) in D_2O. From Figure 6, several intermolecular and intramolecular cross-peaks between DMA_{115}-*b*-($NIPAM_{161}$-*s*-$AVAL_{67}$) and PVBTAC (solid red circles) can be seen. In this case, we are primarily concerned with the intermolecular cross-peaks between the $NIPAM_{161}$-*s*-$AVAL_{67}$ core blocks and the cationic cross-linker PVBTAC (dashed blue circles). Intermolecular correlations of the aromatic protons (e, f) from PVBTAC with the methyl and methine protons (b, d) of NIPAM and the methyl protons (c) of AVAL are highlighted with dashed blue circles and provide evidence of the close spatial proximity between the core forming blocks and the cross-linker due to electrostatic interactions in the micelle core. It should also be noted that intermolecular interactions between the vinyl protons (e, f) of PVBTAC and the methyl protons (a) in the DMA corona are observed and can be attributed to the interfacial proximity of the core and corona.[70]

The self-assembled morphology of the block copolymers can be observed by transmission electron microscopy (TEM). Figure 7 shows the TEM images for DMA_{115}-*b*-($NIPAM_{161}$-*s*-$AVAL_{67}$) (30 mol %) at pH 2 and 25 °C and at pH 4 and 25 °C after interpolyelectrolyte complexation with the cationic polymer PVBTAC. The images show the relatively spherical shape of the micelles.

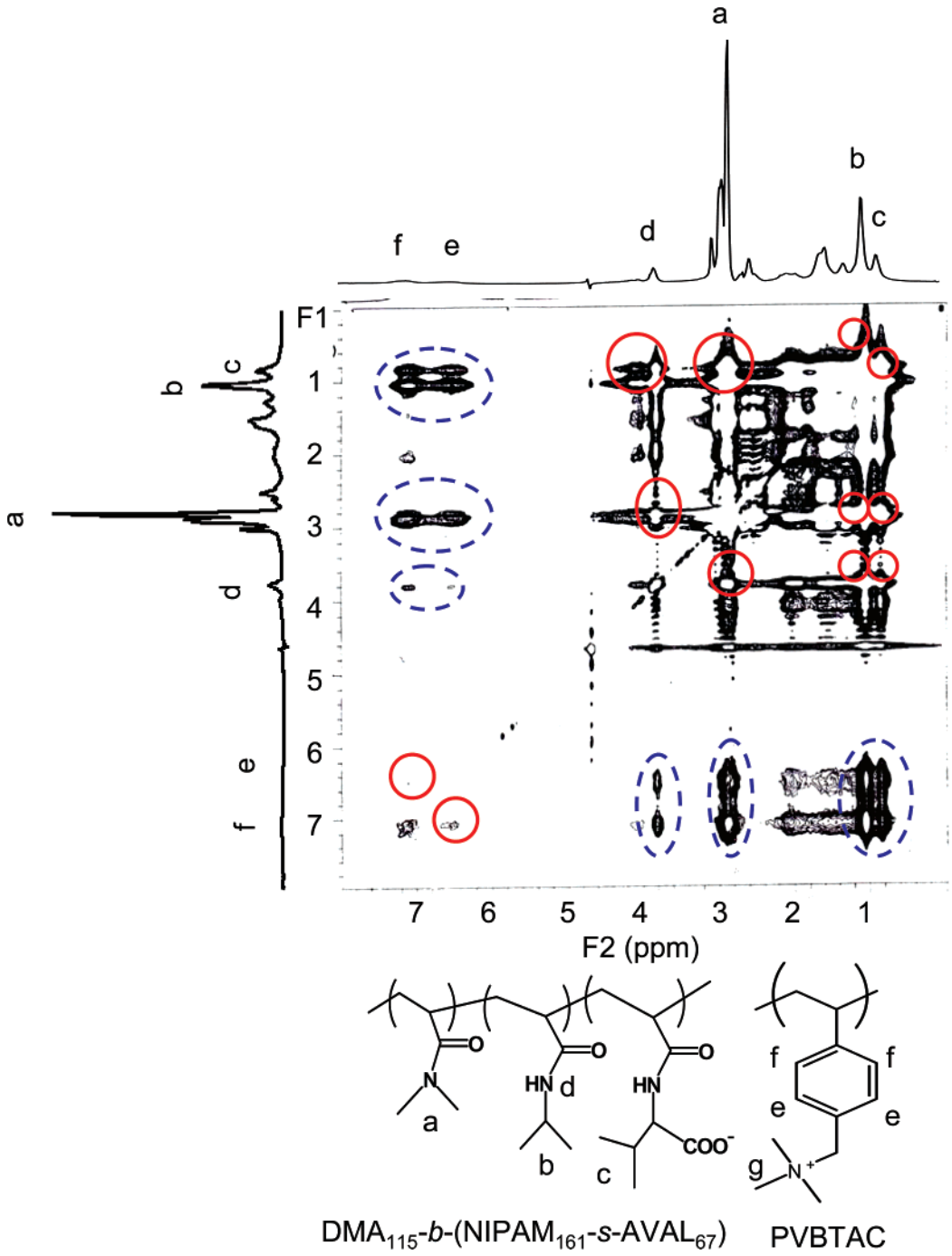


Figure 6. 2D ^{1}H NMR NOESY contour plot of DMA_{115}-*b*-($NIPAM_{161}$-*s*-$AVAL_{67}$) micelles cross-linked with the cationic polymer arvinylbenzyltrimethylammonium chloride (pH = 4.2, 25 °C, C_p = 10 g/L) in D_2O. Solid red circles indicate intramolecular cross-peaks, and dashed blue circles indicate intermolecular cross-peaks.

Of significance to potential applications is that the addition of a simple electrolyte to the interpolyelectrolyte complexes effectively screens the Coulombic interactions and leads to the dissociation of the micelles into the respective water-soluble components in aqueous solution. The reversibility of these interpolyelectrolyte complexes is illustrated in Figure 8. At 25 °C, DMA_{115}-*b*-($NIPAM_{161}$-*s*-$AVAL_{67}$) polymers exist as unimers with an apparent D_h of 7.0 nm (Figure 8, peak A). Raising the temperature to 50 °C induces the formation of micelles with average apparent D_h values of 96.9 nm (Figure 8, peak B). Interpolyecletrolyte complexed micelles are formed through the addition of an equimolar (anionic:cationic) amount of PVBTAC to the micelle solution at 50 °C (Figure 8, peak C). This is accompanied by a decrease in the apparent D_h to 52.4 nm due to charge neutralization. "Locking" of the self-assembled structures is evidenced by the existence of micelles below the LCST of the NIPAM segments which are "swollen" to apparent D_h values of 57 nm after lowering the solution temperature to 25 °C (Figure 8, peak D). The addition of 0.3 M NaCl is then shown to induce dissociation of the complexed micelles into their unimeric components with an average apparent D_h of 7.7 nm (Figure 8, peak E). The thermodynamic stability of the complexed micelles and the kinetics of the reversible unimer-to-micelle process is obviously related to the strength and extent of complex formation as well as the resulting morphology. Studies are underway in our laboratories to further elucidate the behavior of these nanoassemblies and will be the subject of subsequent reports.

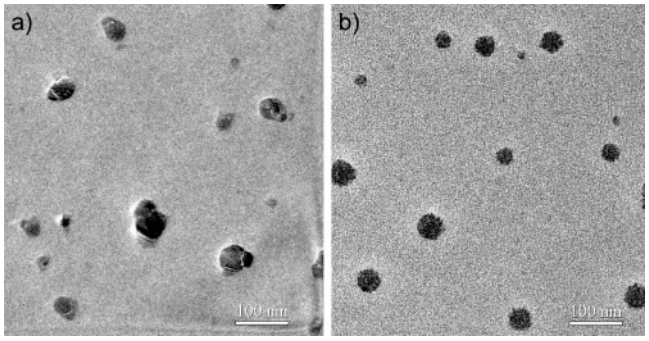


Figure 7. Transmission electron microscopy images of polymeric micelles of (DMA_{115}-*b*-($NIPAM_{161}$-*s*-$AVAL_{67}$)) at (a) pH 2 and 25 °C and (b) at pH 4 and 25 °C after addition of the cationic polymer ar-vinylbenzyltrimethylammonium chloride.

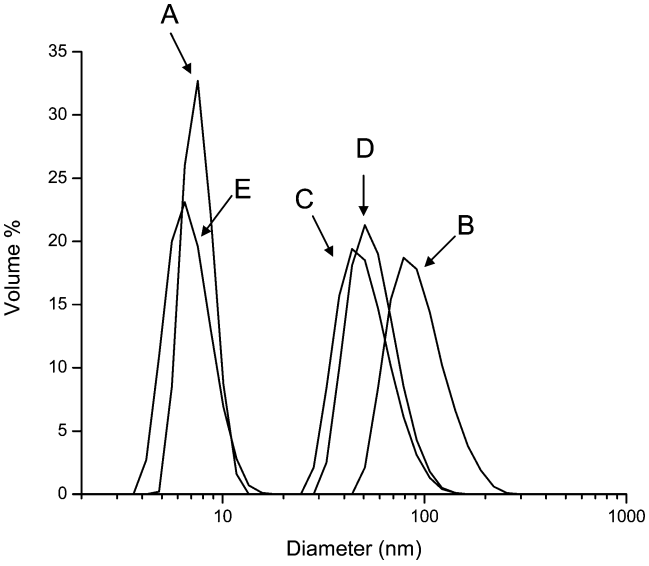


Figure 8. Size distribution (measured by dynamic light scattering) of the DMA_{115}-*b*-($NIPAM_{161}$-*s*-$AVAL_{67}$) copolymer under specific conditions: (A) unimers at pH 4.5 and 25 °C; (B) micelles at pH 4.5 and 50 °C; (C) interpolyelectrolyte-complexed micelles after the addition of ar-vinylbenzyltrimethylammonium chloride at pH 4.5 and 50 °C; (D) interpolyelectrolyte-complexed micelles after addition of the cationic polymer at pH 4.5 and 25 °C; (E) unimeric complex components after addition of 0.3 M NaCl at pH 4.5 and 25 °C.

Conclusions

In this work, we have shown the controlled polymerization of AVAL directly in water utilizing RAFT. Excellent control over the molecular weight and molecular weight distribution can be achieved at both 30 and 70 °C. The amino acid-based monomer AVAL was then copolymerized with NIPAM to form pH- and temperature-responsive block copolymers. The hydrophilic DMA block length was kept constant while 10−40 mol % of AVAL was copolymerized with NIPAM in order to synthesize a series of pH-triggered, temperature-responsive block copolymers and to investigate their self-assembly behavior. It was shown that the critical micellization temperature for the block copolymers can be tuned by adjusting the solution pH. Micelles with apparent hydrodynamic diameters ranging from ≈45 to 86 nm with CMT values between 9 and 36 °C were formed at pH values between 2 and 5. Above pH 5, a sufficient number of the AVAL units are deprotonated and impart enough hydrophilicity to the block compolymers to prevent micellization. The effects of pH and temperature on the apparent hydrodynamic diameter were measured by dynamic light scattering and illustrated through 3-D plots. Additionally, it was shown that reversible polyelectrolyte complexed micelles can be formed through the addition of the cationic polymer PVBTAC. These interpolyelectrolyte "locked" micelles are stable in aqueous solution at room temperature (below CMT), but complexation can be reversed by addition of sufficient electrolyte. The facility by which these nanocomplexes are formed, the ability to tune the CMT by adjusting the solution pH, and the reversibility of these interpolyelectrolyte complexed micelle assemblies suggest that such systems may have significant future applications in targeted delivery and controlled release of active agents.

Acknowledgment. The Department of Energy (DE-FC26-01BC15317), Genzyme, MRSEC program of the National Science Foundations (NSF) (DR-0213883), and the Robert M. Hearin Foundation are gratefully acknowledged for financial support. The authors also acknowledge the NSF Division of Materials Research Major Research Instrumentation Awards 0079450 and 0421406 for the purchase of the Varian Unity Inova 500 MHz NMR spectrometer and JEOL JEM-2100 electron microscope and Wako Chemicals for donating V-501 and VA-044.

References and Notes

(1) Wooley, K. L. *J. Polym. Sci., Part A: Polym. Chem.* **2000**, *38*, 1397−1407.
(2) Thurmand, K. B.; Kowalewski, T.; Wooley, K. L. *J. Am. Chem. Soc.* **1997**, *119*, 6656−6665.
(3) Huang, H.; Remsen, E. E.; Kowalewski, T.; Wooley, K. L. *J. Am. Chem. Soc.* **1999**, *121*, 3805−3806.
(4) Ma, Q.; Remsen, E. E.; Kowalewski, T.; Wooley, K. L. *J. Am. Chem. Soc.* **2001**, *123*, 4627−4628.
(5) Sanji, T.; Nakastuka, Y.; Ohnishi, S.; Sakurai, H. *Macromolecules* **2000**, *33*, 8524−8536.
(6) Underhill, R. S.; Liu, G. *Chem. Mater.* **2000**, *12*, 2082−2091.
(7) Butuen, V.; Wang, X. S.; de Paz Banez, M. V.; Robinson, K. L.; Billingham, N. C.; Armes, S. P. *Macromolecules* **2000**, *33*, 1−3.
(8) Fujii, S.; Cai, Y.; Weaver, J. V. M.; Armes, S. P. *J. Am. Chem. Soc.* **2005**, *127*, 7304−7305.
(9) Zhang, L.; Eisenberg, A. *Science* **1995**, *268*, 1728−1731.
(10) Zhang, L.; Eisenberg, A. *Macromolecules* **1996**, *29*, 8805−8815.
(11) Luo, L.; Eisenberg, A. *Angew. Chem., Int. Ed.* **2002**, *41*, 1001−1004.
(12) Ghoroghchian, P. P.; Li, G.; Levine, D. H.; Davis, K. P.; Bates, F. S.; Discher, D. E.; Hammer, D. A.; Therien, M. J. *Macromolecules* **2006**, *39*, 1673−1675.
(13) He, Y.; Li, Z.; Simmone, P.; Lodge, T. P. *J. Am. Chem. Soc.* **2006**, *128*, 2745−2750.
(14) Kujawa, P.; Tanaka, F.; Winnik, F. M. *Macromolecules* **2006**, *39*, 3048−3055.
(15) Kujawa, P.; Aseyev, V.; Tenhu, H.; Winnik, F. M. *Macromolecules* **2006**, *39*, 7686−7693.
(16) Qiu, X.; Winnik, F. M. *Macromolecules* **2007**, *40*, 872−878.
(17) Heskins, M.; Guillet, J. E. *J. Macromol. Sci., Chem.* **1968**, *A2*, 1441−1455.
(18) Schild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163−249.
(19) Cho, E. C.; Lee, J.; Cho, K. *Macromolecules* **2003**, *36*, 9929−9934.
(20) Feil, H.; Bae, Y. H.; Feijen, J.; Kim, S. W. *Macromolecules* **1992**, *25*, 5528−5530.
(21) Chen, G.; Hoffman, A. S. *Nature (London)* **1995**, *373*, 49−52.
(22) Brazel, C. S.; Peppas, N. A. *Macromolecules* **1995**, *28*, 8016−8020.
(23) Zhou, S.; Chu, B. *J. Phys. Chem. B* **1998**, *102*, 1364−1371.
(24) Bulmus, V.; Ding, Z.; Long, C. J.; Stayton, P. S.; Hoffman, A. S. *Bioconjugate Chem.* **2000**, *11*, 78−83.
(25) Yin, X.; Hoffman, A. S.; Stayton, P. S. *Biomacromolecules* **2006**, *7*, 1381−1385.
(26) Determan, M. D.; Cox, J. P.; Seifert, S.; Thiyagarajan, P.; Mallapragada, S. K. *Polymer* **2005**, *46*, 6933−6946.
(27) Determan, M. D.; Liang, G.; Thiyagarajan, P.; Mallapragada, S. K. *Langmuir* **2006**, *22*, 1469−1473.
(28) Thurmand, K. B.; Kowalewski, T.; Wooley, K. L. *J. Am. Chem. Soc.* **1996**, *118*, 7239−7240.
(29) Huang, H.; Kowalewski, T.; Remsen, E. E.; Geertzmann, R.; Wooley, K. L. *J. Am. Chem. Soc.* **1997**, *119*, 11653−11659.
(30) Zhang, Q.; Remsen, E. E.; Wooley, K. L. *J. Am. Chem. Soc.* **2000**, *122*, 3642−3651.
(31) Butuen, V.; Billingham, N. C.; Armes, S. P. *J. Am. Chem. Soc.* **1998**, *120*, 12135−12136.

(32) Butuen, V.; Lowe, A. B.; Billingham, N. C.; Armes, S. P. *J. Am. Chem. Soc.* **1999**, *121*, 4288−4299.
(33) Pilon, L. N.; Armes, S. P.; Findlay, P.; Rannard, S. P. *Langmuir* **2005**, *21*, 3808−3813.
(34) Liu, S.; Weaver, J. V. M.; Save, M.; Armes, S. P. *Langmuir* **2002**, *18*, 8350−8357.
(35) Li, Y.; Lokitz, B. S.; McCormick, C. L. *Macromolecules* **2006**, *39*, 81−89.
(36) Li, Y.; Lokitz, B. S.; Armes, S. P.; McCormick, C. L. *Macromolecules* **2006**, *39*, 2726−2728.
(37) Liu, S.; Armes, S. P. *J. Am. Chem. Soc.* **2001**, *123*, 9910−9911.
(38) Stewart, S.; Liu, G. *J. Chem. Mater.* **1999**, *11*, 1048−1054.
(39) Weaver, J. V. M.; Tang, Y. Q.; Liu, S.; Iddon, P. D.; Grigg, R.; Armes, S. P.; Billingham, N. C.; Hunter, R.; Rannard, S. P. *Angew. Chem., Int. Ed.* **2004**, *43*, 1389−1392.
(40) Lokitz, B. S.; Convertine, A. J.; Li, Y.; McCormick, C. L. *Macromolecules* **2006**, *39*, 8594−8602.
(41) Li, Y.; Lokitz, B. S.; McCormick, C. L. *Angew. Chem., Int. Ed.* **2006**, *45*, 5792−5795.
(42) Kabanov, A. V.; Bronich, T. K.; Kabanov, V. A.; Yu, K.; Eisenberg, A. *Macromolecules* **1996**, *29*, 6797−6802.
(43) Solomatin, S. V.; Bronich, T. K.; Bargar, T. W.; Eisenberg, A.; Kabanov, V. A.; Kabanov, A. V. *Langmuir* **2003**, *19*, 8069−8076.
(44) Harada, A.; Kataoka, K. *Macromolecules* **2003**, *36*, 4995−5001.
(45) Gohy, J. F.; Varshney, S. K.; Jerome, R. *Macromolecules* **2001**, *34*, 2745−2747.
(46) Sanda, F.; Abe, T.; Endo, T. *J. Polym. Sci., Part A: Polym. Chem.* **1997**, *35*, 2619−2629.
(47) Sanada, F.; Abe, T.; Endo, T. *Macromol. Chem. Phys.* **1999**, *200*, 2651−2661.
(48) Mori, H.; Sutoh, K.; Endo, T. *Macromolecules* **2005**, *38*, 9055−9065.
(49) Hawker, C. J.; Bosman, A. W.; Harth, E. *Chem. Rev.* **2001**, *101*, 3661−3688.
(50) Matyjaszewski, K.; Xia, J. *Chem. Rev.* **2001**, *101*, 2921−2990.
(51) Moad, G.; Rizzardo, E.; Thang, S. H. *Aust. J. Chem.* **2005**, *58*, 379−410.
(52) Lowe, A. B.; McCormick, C. L. *Prog. Polym. Sci.* **2007**, *32*, 283−351.
(53) McCormick, C. L.; Lowe, A. B. *Acc. Chem. Res.* **2004**, *37*, 312−325.
(54) Sumerlin, B. S.; Donovan, M. S.; Mitsukami, Y.; Lowe, A. B.; McCormick, C. L. *Macromolecules* **2001**, *34*, 6561−6564.
(55) Sumerlin, B. S.; Lowe, A. B.; Thomas, D. B.; McCormick, C. L. *Macromolecules* **2003**, *36*, 5982−5987.
(56) Vasilieva, Y. A.; Thomas, D. B.; Scales, C. W.; McCormick, C. L. *Macromolecules* **2004**, *37*, 2728−2737.
(57) Donovan, M. S.; Sumerlin, B. S.; Lowe, A. B.; McCormick, C. L. *Macromolecules* **2002**, *35*, 8663−8666.
(58) Donovan, M. S.; Lowe, A. B.; Sanford, T. A.; McCormick, C. L. *J. Polym. Sci., Part A: Polym. Chem.* **2003**, *41*, 1262−1281.
(59) Thomas, D. B.; Convertine, A. J.; Myrick, L. J.; Scales, C. W.; Smith, A. E.; Lowe, A. B.; Vasilieva, Y. A.; Ayres, N.; McCormick, C. L. *Macromolecules* **2004**, *37*, 8941−8950.
(60) Convertine, A. J.; Ayres, N.; Scales, C. W.; Lowe, A. B.; McCormick, C. L. *Biomacromolecules* **2004**, *5*, 1177−1180.
(61) Mitsukami, Y.; Donovan, M. S.; Lowe, A. B.; McCormick, C. L. *Macromolecules* **2001**, *34*, 2248−2256.
(62) Convertine, A. J.; Lokitz, B. S.; Vasilieva, Y. A.; Myrick, L. J.; Scales, C. W.; Lowe, A. B.; McCormick, C. L. *Macromolecules* **2006**, *5*, 1724−1730.
(63) Thomas, D. B.; Sumerlin, B. S.; Lowe, A. B.; McCormick, C. L. *Macromolecules* **2003**, *36*, 1436−1439.
(64) Smallcombe, S. H.; Patt, S. L.; Keifer, P. A. *J. Magn. Reson., Ser. A* **1995**, 295−303.
(65) For discussion of the induction period, see the following reference: Barner-Kowollik, C.; Buback, M.; Charleux, B.; Coote, M. L.; Drache, M.; Fukuda, T.; Goto, A.; Klumperman, B.; Lowe, A. B.; McCleary, J. B.; Moad, G.; Monteiro, M. J.; Sanderson, R. D.; Tonge, M. P.; Vana, P. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 5809−5831.
(66) The unimer sizes reach minimum values at pH 4 (pK_a of AVAL) which may be attributed to the presence of a sufficient number of deprotonated carboxylic acid groups that prevent unimer association. A slight increase in size of the unimers is observed at pH 8, in which case the AVAL units are completely charged resulting in a more extended conformation.
(67) Neuhaus, D.; Williamson, M. *The Nuclear Overhauser Effect in Structural and Conformational Analysis*; VCH Publishers: New York, 1989.
(68) Atkins, P. W. *Physical Chemistry*, 7th ed.; Oxford University Press: Oxford, 2002.
(69) Mo, H. P.; Pochapsky, T. C. *Prog. Nucl. Magn. Reson. Spectrosc.* **1997**, *30*, 1−38.
(70) Voets, I. K.; Keizer, A.; Stuart, M. A. C.; Waard, P. *Macromolecules* **2006**, *39*, 5952−5955.

MA070921V