Diblock, ABC Triblock, and Random Methacrylic Polyampholytes: Synthesis by Group Transfer Polymerization and Solution Behavior

C. S. Patrickios,[†] W. R. Hertler,[*,‡] N. L. Abbott,[§] and T. A. Hatton[*,†]

Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, Du Pont Central Research and Development, Experimental Station, Wilmington, Delaware 19880-0328, and Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138

Received June 14, 1993; Revised Manuscript Received November 16, 1993[⊗]

ABSTRACT: Water-soluble, low-polydispersity, low-molecular-weight random, AB diblock, and ABC triblock polymers of methacrylic acid (MAA), 2-(dimethylamino)ethyl methacrylate (DMAEMA), methyl methacrylate (MMA) and 2-phenylethyl methacrylate (PEMA) were synthesized by group transfer polymerization (GTP). The isoelectric points, titration behavior, and solubility curves of these polyampholytes are reported. Light scattering studies revealed that the triblocks form micelles as a result of the presence of the MMA hydrophobic block. A fluorescence study demonstrated that the triblock copolymers associate more strongly with pyrene than do the corresponding random terpolymers.

Introduction

Random and block copolymers can exhibit contrasting behaviors both in the bulk and in solution. For example, in the bulk, the modulus of a random copolymer undergoes a sharp decrease at a single temperature defined by the copolymer composition. In contrast, the modulus of the corresponding block copolymer undergoes one decrease at the glass transition temperature of each component block and, between the decreases, it assumes a plateau value, defined by the copolymer composition.[1] In solution, the fluorescence quenching of a block copolymer of 9-vinylphenanthrene and methacrylic acid in water, a selective solvent, is more efficient than that of the corresponding random copolymer because of the larger size of the hydrophobic phenanthrene microdomain, which results in more efficient binding of the quencher and longer range energy migration.[2] At interfaces, Monte Carlo simulations show that random copolymers orient along the interface, while diblocks tend to assume a configuration perpendicular to the interface.[3,4]

We are interested in polyampholyte-mediated protein separation methods such as ion-exchange displacement chromatography,[5–7] precipitation,[8,9] and aqueous two-phase partitioning.[10,11] Utilization of block instead of random polyampholytes in these applications may have a dramatic effect on the performance of these separation processes, as the properties of the block copolymers are expected to be different from those of the random. For instance, in displacement chromatography, we expect to have enhanced performance because the block architecture will strengthen the separation driving force which is the electrostatic interaction between the polyampholyte and the chromatography column. The localization of the similar charges within a block will generate an electric field which will be stronger than that for the random copolymer. Another implication of the strengthening of the electrostatic interactions by block copolymer architecture is that, at the isoelectric point of the polymer, the interpolyampholyte attractions will be stronger, leading to a more extensive precipitation. The ability to precipitate the polymer is very crucial in industrial applications because it will facilitate recycling. Random acrylic polyampholytes do precipitate when they are of relatively high molecular weight, typically above 15 000, but they do not when they are of much lower molecular weight.[12]

Reports on the synthesis and characterization of block polyampholytes are very few and not systematic. On the other hand, there are more than 200 reports for random polyampholytes,[13] which were synthesized for the first time in the 1950s.[14–20] The dual-charge nature of these polymers and their resulting isoelectric points make them attractive nonbiological models for proteins. The polyampholyte solubility and titration curves presented in this study are very similar to those of proteins. Recent studies of random polyampholytes have focused on viscosity,[21,22] swelling of gels,[23,24] solubility changes,[25,22] scaling theory,[26] Monte Carlo simulation,[27,28] and adsorption and surface forces.[29] Stille's group in 1972 was the first to synthesize block polyampholytes, via anionic polymerization.[30,31] These copolymers, based on 2-vinylpyridine and methacrylic acid (MAA) or acylic acid, were evaluated for their salt-rejecting properties in dynamic reverse osmosis membranes. Following this study, Varoqui et al.[32] synthesized a diblock polyampholyte based on styrenesulfonic acid and 2-vinylpyridine for study of the intramolecular complexation of the anionic poly(styrenesulfonate) block with the cationic poly(2-vinylpyridinium) block. Miyaki and co-workers[33–38] synthesized charge-mosaic membranes, which are cross-linked pentablock polyampholytes, the second block comprising quaternary ammonium residues, the fourth block being sytrenesulfonate, and the other three blocks being neutral cross-linkable isoprene blocks. The charge-mosaic membranes could find application in desalination. Most recently, Bekturov and co-workers[39,40] studied the precipitation and polymer complexation of two diblock polyampholytes of high molecular weight (600 000) comprising methacrylic acid and 1-methyl-4-pyridinium chloride residues.

Inclusion of an uncharged, hydrophobic block in a block polyampholyte leads to an ABC triblock polymer structure. Water-soluble methacrylic ABC triblock polymers appear to be a new class of polymers, and they are expected to exhibit a richer solution behavior than the corresponding

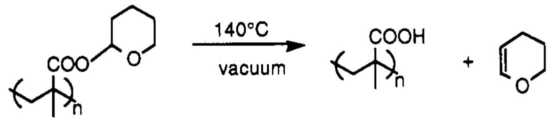
---

[*] Authors to whom correspondence should be addressed.
[†] Massachusetts Institute of Technology.
[‡] Du Pont.
[§] Harvard University.
[⊗] Abstract published in *Advance ACS Abstracts,* January 1, 1994.

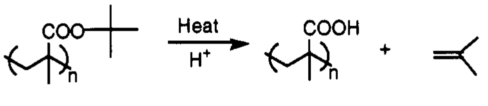
0024-9297/94/2227-0930$04.50/0

Scheme 1

Scheme 2



Scheme 3



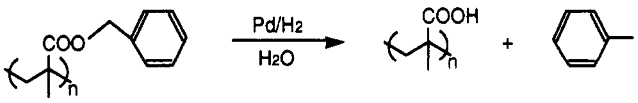
diblocks. The literature on ABC triblock polymers is modest and primarly concerns studies of morphology and film properties.[41] Recently, Sdranis and Kosmas[42] provided a theoretical consideration of solution properties of nonionic ABC triblock polymers, while Wu and Slater[43] calculated the static structure factor and shape of ABC triblock polyampholytes (one of the blocks being nonhydrophobic and neutral) reptating in a gel in the presence of an electric field.

The most straightforward way to prepare acrylic triblock polyampholytes is to use a living polymerization method with sequential addition of monomers. Because group transfer polymerization (GTP)[44] is convenient and amenable to a wide variety of functional and nonfunctional methacrylates,[45,46] we chose to use this process to prepare block polymers containing DMAEMA and MAA. Müller and co-workers[47] have reported the preparation by GTP of diblock polymers comprising DMAEMA and MMA or decyl methacrylate. Since carboxylic acids cause termination of GTP, it is necessary to use an ester of MAA, which, after polymerization by GTP, can easily be converted to the free carboxylic acid. Four protected monomers which have been used in GTP to prepare precursors to MAA-containing polymers are *tert*-butyl methacrylate,[48-51] trimethylsilyl methacrylate (TMSMA),[52,53] tetrahydropyranyl methacrylate (THPMA),[54-56] and benzyl methacrylate.[51,57]

For our synthesis of polyampholytes by GTP, we elected to use TMSMA and THPMA. TMSMA is a very attractive precursor to poly(MAA) because of its commercial availability and the ease of methanolysis or hydrolysis of poly(TMSMA) with, or without, mild acid catalysis (Scheme 1). However, the reactivity of the trimethylsilyl ester toward nucleophilic GTP catalysts results in significant slowing of polymer growth. Thus, it is generally desirable to (i) add supplementary amounts of catalyst during the polymerization of TMSMA, (ii) leave TMSMA until the last block in block copolymerizations, and (iii) use alternative monomers for the synthesis of polymers of molecular weight higher than about 10 000. For polymers of higher molecular weight THPMA is a better choice. This monomer, however, is not commercially available. Although several procedures have been reported for the synthesis of THPMA,[58,59] careful purification is required to remove traces of methacrylic acid which interfere with GTP.[54,58] Poly(THPMA) is smoothly converted to poly(MAA) by heating at 140 °C under vacuum for several hours as illustrated in Scheme 2. Schemes 3 and 4 show the deprotection of the *tert*-butyl and the benzyl esters, respectively.

This report describes the synthesis by GTP of low-molecular-weight methacrylic polyampholytes, most of which are triblock copolymers and two of which are random terpolymers. The polymer solution characterization includes molecular weight determination by GPC, structural characterization by light scattering, water-solubility determination as a function of pH and salt concentration, isoelectric point determination, hydrogen ion titration, and a fluorescence study.

Scheme 4



Materials and Methods

Solvent. Tetrahydrofuran was distilled from sodium and benzophenone immediately prior to use.

Initiator. 1-Methoxy-1-(trimethylsiloxy)-2-methyl-1-propene was prepared by the method of Ainsworth,[60] distilled in a spinning-band column, and stored under nitrogen.

Monomers. Commercially available monomers were purified by passage over a column of basic alumina under an argon atmosphere to remove inhibitors and protonic impurities, except for trimethylsilyl methacrylate, which was used as received. Tetrahydropyranyl methacrylate was prepared by the reaction of MAA with 3,4-dihydro-2*H*-pyran (Aldrich Chemical Co.) using cross-linked poly(4-vinylpyridine hydrochloride) (Fluka Chemie AG) as catalyst by the method of Hertler.[58] Two distillations over calcium hydride provided tetrahydropyranyl methacrylate of sufficient purity for GTP.

Catalyst. Tetrabutylammonium biacetate[61] was prepared in a drybox by addition of 1 equiv of acetic acid to a solution of tetrabutylammonium acetate (Fluka Chemie AG) in THF. The resulting precipitated tetrabutylammonium biacetate was collected by filtration and dissolved in freshly distilled propylene carbonate to give a 0.1 M stock solution of cataylst for GTP. The use of propylene carbonate, rather than THF, as solvent avoids the use of 6 molar equiv of water required to solubilize tetrabutylammonium biacetate in THF.[61]

Polymerizations. Polymerization reactions were performed in a 250-mL three-necked flask fitted with an addition funnel, two rubber septa, and a magnetic stirrer. A thermocouple was inserted through one septum. The second septum was used for injection by syringe of solvent, catalyst, and initiator. All glassware and syringes were stored overnight at 120 °C in an oven, and the glassware was assembled while hot, heated at 100 °C with a heat gun, and allowed to cool to room temperature under a flow of argon. The syringes and syringe needles were cooled in nitrogen-blanketed bell jars. All transfers of liquid were performed with syringes. First, 40 mL of THF was transferred to the reactor, and the amount of monomer corresponding to the first block, typically about 15 mL, was transferred to the addition funnel. Catalyst solution, typically 1 mL, corresponding to 1 mol % of initiator, was injected. Then the initiator, typically 2 mL, was injected, followed immediately by dropwise addition of the monomer at a feed rate of 1 mL/min. The polymerization exotherm was monitored by a digital thermometer. When the temperature fell to near room temperature, addition of the next monomer was begun. The concentration of polymer after the addition of all of the monomers was typically 50% w/w. Since the polymerization of MMA, DMAEMA, and THPMA is much faster than that of TMSMA, TMSMA was the last block for all but one of the reactions. During the polymerization of the TMSMA, after all of the TMSMA had been added, an additional 3 or 4 1-mL aliquots of catalyst solution were periodically injected to obtain satisfactory polymerization rates (as evidenced by increasing or steady temperature). In the case of random polymerizations, the three monomers were mixed and loaded into the addition funnel. At the end of the polymerizations the living chain ends were quenched by addition of 5 mL of methanol. The complete consumption of monomers was confirmed by ^{1}H NMR, which indicated absence of the peaks characteristic of the olefinic hydrogens.

Removal of the Protecting Groups. The tetrahydropyranyl functionality was removed by heating the neat polymer in a vacuum oven at 140 °C for 48 h. The extent of deprotection was

Table 1. Methacrylic Polyampholytes (MW = 4000)

polymer	comp of blocks mole ratio			pH insol range
1	MMA 1	MMA 1	DMAEMA 1	
2	DMAEMA 1	MMA 1	MMA 1	5.5–7.9
3[a]	MMA 1	MMA 1	DMAEMA 1	
4[a]	MMA 1	MMA 1	DMAEMA 1	
5	DMAEMA 1	MMA 2	MMA 1	5.6–7.7
6	DMAEMA 1	PEMA 1	MAA 1	<9
7	PEMA-*b*-DMAEMA 1 12	MMA 12	MAA 12	5.2–7.9
8	DMAEMA 12	*b*-MMA-PEMA-*b*-MMA 6 1 6	MAA 12	5.2–7.5
9	DMAEMA 1.3	MMA 1	MMA 0.65	6.6–8.3
10	DMAEMA 0.65	MMA 1	MAA 1.3	4.2–6.0
11[b]	DMAEMA 1	MAA 1		5.6–7.7
12		DMAEMA-*co*-MMA-*co*-MAA 1 1 1		none
13		DMAEMA-*co*-MMA-*co*-MAA 1.3 1 0.65		none

[a] MW = 15 000, prepared using THPMA. [b] MW = 2400.

followed by the decrease in weight resulting from loss of dihydropyran. The weight loss was just as expected from stoichiometry. The trimethylsilyl functionality was removed by refluxing the polymerization reaction mixture at 60 °C after the addition of a 5-fold molar excess of methanol, a 5-fold molar excess of water, and 0.5 mol % of dichloroacetic acid, all of the percentages referring to TMSMA. The completion of the reaction was confirmed by titration.

Molecular Weight Determination. Molecular weights and molecular weight distributions were determined by gel permeation chromatography using a series of four Waters Ultrastyragel columns (10 000, 1000, 500, and 100 Å) on a Hewlett-Packard 1090 HPLC system connected to a Hewlett-Packard 1037A refractometer. The mobile phase was THF at a flow rate of 1 mL/min. Four narrow-molecular-weight poly(MMA) standards (M_p = 2700, 9800, 17 500, and 33 500) were used for calibration. The logarithm of M_p was determined to vary linearly with retention time, with a correlation coefficient of 1.000.

Isoelectric Point Determinations. The isoelectric points were determined by two methods. First, neutral polymer powder was equilibrated with deionized water, and the pH of the resulting 10% w/w suspension was measured. This is called the isoionic pH and, for the spectrum of the compositions of our polyampholytes and the high polymer concentration in the suspension, it is practically the same as the isoelectric pH.[62] In the second method, the midpoint of the pH range of precipitation during the titration of 1% w/w solutions of polymers in 0.1 M KCl was used as an indication of the isoelectric point.

Hydrogen Ion Titrations. A model 825 MP Fisher Accumet pH meter with a miniature glass electrode and a microreference electrode with a glass barrel was used for the measurement of the pH. Titration of 5 mL of 1% w/w solutions of polymer in 0.02, 0.1, and 0.5 M KCl were performed from pH 2 to 12 at room temperature (23 ± 1 °C).

Solubility Determinations. Different amounts of acid or base, as calculated from the experimental titration curves, were added to basic (typically pH 8) and acidic (typically pH 5) 10% w/w copolymer stock solutions to adjust the pH at different values within the range 5–8. The samples were vortexed, centrifuged for 1 h at 4000 rpm, and allowed to equilibrate for at least 1 day. The polymer concentrations in the supernatant phase were determined at 25 °C using an American Optical Abbe refractometer. Different amounts of solid potassium chloride were added for ionic strength adjustment. The polymer concentrations were determined by subtracting the salt contribution to the refractive index and dividing by the polymer refractive index increment, which was determined to be 0.181 mL/g at 25 °C and constant up to 15% w/w polymer concentration.

Light Scattering. Static and dynamic light scattering were performed on a Brookhaven Instrument Corp. instrument with an argon laser light source at 488 nm. A 2030 autocorrelator was used for the analysis of the dynamic scattering data. Prior to measurements, samples were filtered five times through 0.2- or 0.5-μm Millipore filters to remove dust. Preliminary dynamic light scattering experiments were performed using a Microscope Laser Light Scattering apparatus equipped with a light source at 633 nm.

Fluorescence. A 10 mg/L suspension of pyrene in water was formed by a 100-fold dilution in water of a 1 g/kg solution of pyrene in ethanol. A small amount of the freshly prepared suspension was transferred to the polymer solutions, which were buffered at pH 4.5 with 0.01M sodium acetate. The volume ratio of the polymer solution to the pyrene suspension was 100, so that the polymer concentration was practically unchanged, the final pyrene concentration in the polymer solution was 0.1 mg/L, which is close to the solubility of pyrene in water, and the ethanol concentration was 100 ppm. A Spex Fluoromax fluorimeter was used for the measurement of the steady-state fluorescence spectra of the pyrene-containing polyampholyte solutions at an excitation wavelength of 333 nm.

Results and Discussion

Polymer Synthesis. Table 1 lists the acrylic polyampholytes synthesized along with their molecular weight, sequence, composition, and pH range of precipitation during titration of 1% w/w in 0.1 M KCl. Ten ABC triblocks, one diblock, and two random terpolymers were prepared. The polymers are of relatively low molecular weight, the highest molecular weight examples being the two triblock polymers, 3 and 4, which are 15 000. Our efforts to make random polymers with molecular weights 15 000 and 30 000 were unsuccessful, probably due to the presence of TMSMA in the monomer mixture. The slowing down effect of TMSMA in the above polymerization reactions may have been most dramatic because of the small amount of initiator required for synthesis of the higher molecular weight polymers. The acid-to-base molar ratio in the polymers is a very important quantity because it determines the isoelectric point of the polymers and,

Table 2. GPC Molecular Weights of Block Polymers

polymer	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$	$\bar{M}_n$ theory
3	22200	17500	1.27	15000
4	19300	14500	1.33	15000
6A[a]	1000	800	1.25	1550
6B[b]	3600	3200	1.14	3440
9A[a]	1600	1200	1.37	2520
9B[b]	4900	4000	1.22	3730
10A[a]	730	580	1.28	1260
10B[b]	2500	2100	1.17	2460

[a] First block. [b] First two blocks.

therefore, the charge and pH-dependence of solubility. Most of the block polyampholytes carry acidic and basic monomers in equimolar amounts and, therefore, they have the same pH range for insolubility 5.5–8. The differences among these polymers lie in molecular weight, block sequence, percentage of MMA, and presence of 2-phenylethyl methacrylate (PEMA). The molar percentage of MMA in the triblocks, except in copolymer **5**, is kept constant at 33%.

The PEMA labeling of polymers **7** and **8** results in an increase in the UV absorbance (250–280 nm) by a factor of 2 and renders the polymers more easily detectable. The ability in GTP to introduce the label as a polymerizable monomer is more convenient than the alternative of chemical label attachment after polymerization. Similar labeling was performed by Webber and co-workers,[63–65] who copolymerized short sequences of vinyl-2-naphthalene within their block copolymers based on polystyrene and poly(*tert*-butyl methacrylate). Polymer **6**, which contains a block of PEMA residues and is expected to exhibit an even higher UV absorbance, is unfortunately insoluble, probably due to the high hydrophobicity of the PEMA block.

Molecular Weights. The weight-average and number-average molecular weights, the polydispersity index, and the theoretical molecular weight of some representative polymers and polymer blocks are listed in Table 2. The low polydispersities, lower than 1.4 for all of the polymers, are typical for GTP. The samples indexed A and B represent the first block and the first two blocks of the triblock polymers, respectively. The third block of these polymers was TMSMA, the presence of which prevented GPC analysis, as the polymer is retained on the column. GPC for triblock copolymers **3** and **4** was performed because they contained not TMSMA, but THPMA, which is not retained on the column. The higher polydispersity of the first block of polymers **6**, **9**, and **10**, which was poly(DMAEMA), as compared to that of the first two blocks, as well as the difference between theoretical and experimental molecular weights of the first blocks, is due to the interaction of poly(DMAEMA) with the column, which results in broadening and shift of the peak to larger retention times (lower molecular weights). Poly(amine) adsorption on GPC columns has been observed by other researchers and has been attributed to the presence of residual acidic functionalities on the column.[34] Figure 1 shows the GPC traces of samples **3**, **6A**, **6B**, **9A**, **9B**, **10A**, and **10B**. After the second block is added, the distribution is still unimodal and narrow, which is indicative of the molecular weight homogeneity of our polymers, which has already been manifested by the low polydispersity indices. The tails of the curves observed toward larger retention times are typical for polymers prepared by GTP.

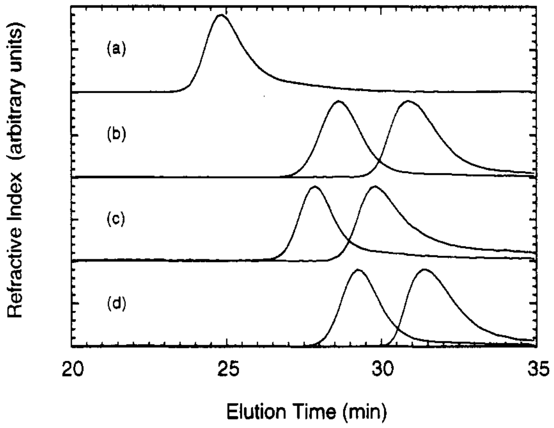


Figure 1. Molecular weight distributions of the block copolymers by GPC: polymer **3** before thermolysis (a); first and first two blocks of polymer **6** (b), polymer **9** (c), and polymer **10** (d).

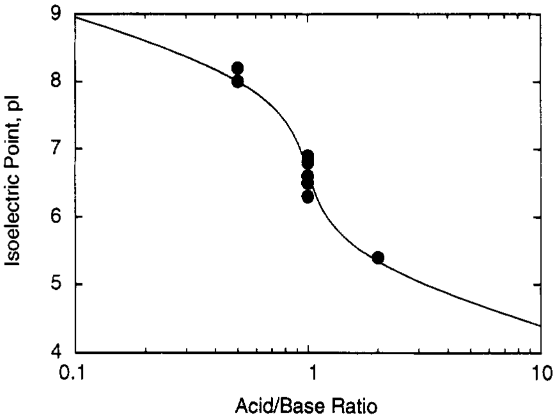


Figure 2. Experimental isoelectric points of the polyampholytes. The solid line represents a theoretical prediction.

Table 3. Isoelectric Points

polymer	$(\mathrm{p}I)_{\text{isoionic pH}}$	$(\mathrm{p}I)_{\text{precip}}$
2	6.6	6.7
5	6.5	6.6
7	6.3	6.5
8	6.8	6.4
9	8.0	7.5
10	5.4	5.1
11	6.9	6.7
12	6.6	
13	8.2	

Isoelectric Points. The isoelectric points of the polymers as approximated by the isoionic pHs as well as the midpoints of the pH range of precipitation are listed in Table 3. The values obtained from the two methods are in good agreement. The values of the isoelectric points from the isoionic pHs are considered more reliable because they were measured in the absence of salt, which may interact with the polyampholyte. It has been reported that anions may bind to proteins[66] and that cations may interact with ampholytic latices,[67] leading to a shift in the isoelectric point or the pH region of precipitation. The determination of the isoelectric pH from the isoionic pH is particularly useful for the random copolymers which are completely soluble and for which the precipitation method is not applicable. No acid, base, or salt was added during the polymer preparation procedures with the exception of the catalytic amounts of dichloroacetic acid needed in the deprotection step. This implies that the polymers are essentially free of any ions other than H^+ or OH^- and, therefore, are in an isoionic state. Figure 2 illustrates the isoionic points of the polymers along with a theoretical prediction, based on the requirement that the net charge of the polymer is zero and that the dissociation constants are not composition dependent. According to this equation the isoelectric point depends on the acid to base molar ratio, *R*, and the dissociation

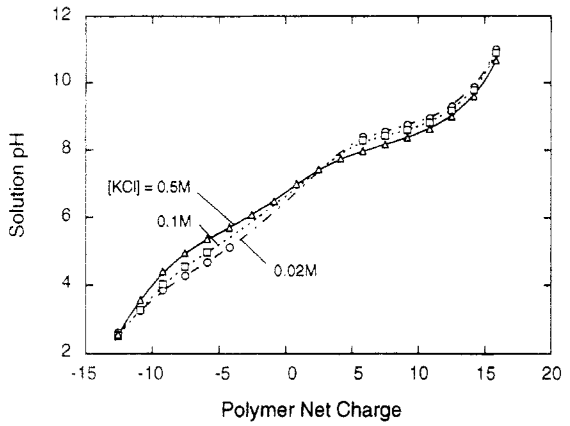


Figure 3. Titration of triblock copolymer **2** at different KCl concentrations.

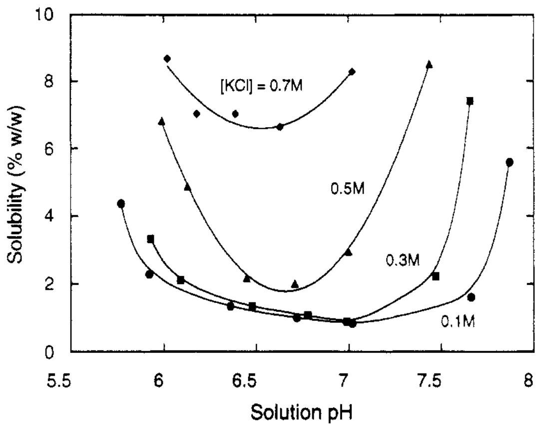


Figure 4. Solubility of triblock copolymer **2** as a function of pH at different KCl concentrations.

constants of the negative and positive charges, $\mathrm{p}K_a$ and $\mathrm{p}K_b$.

$$\mathrm{p}I = \mathrm{p}K_b + \log\left\{\frac{1}{2}\left[\frac{1-R}{R} + \left(\left(\frac{1-R}{R}\right)^2 + \frac{4}{R}\,10^{\mathrm{p}K_a-\mathrm{p}K_b}\right)^{1/2}\right]\right\}$$

The values of the dissociation constants, taken from a previous study[68] on polyampholytes comprising the same monomers, are $\mathrm{p}K_a = \mathrm{p}K_{\mathrm{MAA}} = 5.35$ and $\mathrm{p}K_b = \mathrm{p}K_{\mathrm{DMAEMA}} = 8.00$.

It should be noted that the equations that lead to this expression were first presented by Ehrlich and Doty[18] and Mazur et al.[20] but no analytical solution was derived. This equation can be very useful for estimating protein isoelectric points from the amino acid composition (the ratio of acidic to basic amino acids). For proteins with low contents of histidine ($\mathrm{p}K = 6.2$) and arginine ($\mathrm{p}K = 12$) the appropriate dissociation constants should be $\mathrm{p}K_a = \mathrm{p}K_{\text{aspartic acid}} = 4.5$ and $\mathrm{p}K_b = \mathrm{p}K_{\text{lysine}} = 10.04$.[69]

Hydrogen Ion Titrations. The titration curves of 1% w/w solutions of polymer **2** at 0.02, 0.1, and 0.5 M KCl are shown in Figure 3. The curves are generated by interpolation between the experimental points and are not based on any model. It should be pointed out that there are no experimental points around the isoelectric point in the titration curves at the two lower salt concentrations because the polymer precipitates. The portion of the curve at high pH corresponds to the titration of the basic groups and that at low pH to the titration of the acidic groups. The interpolated curves intersect at a pH near the isoelectric point. In the calculations for the construction of the curves, the pH of zero net charge was fixed at 6.6 for all three salt concentrations. The effect of increasing salt concentration at constant pH is to decrease the charge of the group being titrated in that pH region (decrease in the dissociation of the acidic residues or decrease in the protonation of the basic residues). This weakening of the acidic or basic character of the polymer groups can be attributed to the decrease in intrapolymer attractive electrostatic interactions by the salt and has been predicted theoretically[20,68,70,71] and observed experimentally in the titration of biological polyampholytes (proteins)[62] and synthetic polyampholytes.[68]

Solubility Curves. Figure 4 shows the pH dependence of the solubiltiy of polymer **2** at different salt concentrations, 0.1, 0.3, 0.5, and 0.7 M KCl. The solubility around the isoelectric point, which is 6.6, is much lower than that 1 pH unit away. By increasing the salt concentration, the solubility around the isoelectric point increases. At 0.9 M KCl the polymer is completely (at least 10% w/w) soluble, even at the isoelectric point. The polymer net charge is zero at the isoelectric point and, therefore, the electrostatic repulsion, which keeps the polymer in solution, is at a minimum. In contrast, the electrostatic attraction between the positive and negative charges, which are equal in number, is maximized. The increase in salt concentration leads to the screening of the attractions around the isoelectric point and results in increased solubility. The above behavior was observed for the first time with proteins,[62,72,73] in which a solubility minimum was observed at their isoelectric point and in which addition of salts led to an increase in protein solubility, called the salting-in effect. Tanford used the linearized Poisson–Boltzmann equation to develop expressions for the charge and salt concentration dependence of the solubility of globular proteins. In these expressions the protein solubility increases exponentially with the square of the net charge.[62]

Comparison of triblock copolymer **2** with its random counterpart copolymer **12** indicates that factors other than the net charge can influence the solubility. Polymer **12** is soluble, at least 15% w/w, over the entire pH range, even at the isoelectric point and in the absence of salt. This can be attributed either to the smaller effective size of the random copolymer (no micelles) or to the random distribution of the positive and the negative charges on the polymer, which leads to a dipole moment lower than that necessary for enhancing interpolymer association at the isoelectric point. Supporting the latter interpretation is the precipitation of diblock copolymer **11** at the isoelectric point despite the absence of micelles, the lower molecular weight (2400), and lower hydrophobicity (no MMA residues). It should be mentioned, however, that the diblock polyampholyte salts-in very easily.

It was expected that the solubility of the triblock polymer **2** should be essentially zero because in a parallel study[8,9] the same polymer could be precipitated from solutions of polymer concentration as low as 0.004%. The minimum solubility shown in Figure 4 is 1% and it is much higher than the expected value of 0.004%. This discrepancy is due to the presence of impurities which are taken as polyampholyte by the nonselective refractive index technique. We estimate that our triblock copolymers contain 5–10% impurities of homopolymer (terminated first block) and diblock. This is consistent with the long tails in the GPC plots (Figure 1) as well as with the findings of Müller and co-workers.[47] Since the total average polymer concentration of the samples was 10%, 10% of which was impurities, it can be calculated that the concentration of impurities was 1%.

Light Scattering. A light scattering study was conducted to probe the micellization behavior of the block polymers. We compared triblock polymer **2** and the corresponding random terpolymer, polymer **12**, which have

Table 4. Light Scattering Data for DMAEMA:MMA:MAA (1:1:1) Polyampholytes at pH = 5

	quasielastic light scattering		static light scattering (Zimm plot)			
copolymer	d_h (Å)	aggregation no.	$\bar{M}_w$	A_2[a]	d_g (Å)	aggregation no.
12 (random)	40		5500			
2 (triblock)	110	21[b]	125000	+20.4	188	23[b]

[a] mL·mol·kg^{-1}·kg^{-1}. [b] See text for details of calculation.

the same molecular weight (4000) and composition, DMAEMA:MMA:MAA = 1:1:1. Solutions of the two polymers at pH 5 were studied by both static and dynamic light scattering. The information obtained by these techniques is summarized in Table 4. The molecular weight of the random terpolymer, as determined by static light scattering, is 5500 ± 500, in reasonable agreement with the expected value of 4000. The molecular weight of the triblock polymer, as determined by the same method, was found to be 125 000 ± 5000, which implies that aggregation occurs. To estimate the aggregation number, the effective molecular weight of the triblock polymer was divided by that of the random terpolymer, and the result is 23. Cubing the ratio of the corresponding hydrodynamic radii, as determined by dynamic light scattering gives a similar result, 21. If the same segment density for the two polymers is assumed, this agreement implies that the aggregates are roughly spherical in shape. The second virial coefficient of the triblock polymer, as determined by static light scattering, is positive, indicating a repulsion between the positively charged micelles.

From these results we envision the block copolymer micelles to comprise 20–25 polymer chains, with the middle hydrophobic block constituting the micellar core and the ionic blocks constituting the corona. The hydrodynamic diameter of 11 nm of the micelles suggests that the chains are in an extended configuration in the micelles because the contour length of the chains (based on the theoretical molecular weight) is approximately 9 nm (36 residues × 0.25 nm/residue).

A subsequent QELS study on polymer 2 at pH 5 showed that the hydrodynamic size remains 11 nm for all of the combinations of salt and polymer concentrations employed. The salt concentration was varied from 0.0 to 1.0 M KCl, and the polymer concentration was varied from 0.1 to 5%. Additional QELS studies showed that, while all the triblocks form micelles, the diblock copolymer does not.

The dependence of the hydrodynamic diameter of polymer 2 on pH appears in Figure 5. The qualitative trends in this figure are in agreement with those of the solubility curves of Figure 4. Around the isoelectric point, big aggregates of size 100 nm form because of the weak electrostatic repulsion. At intermediate pH, smaller micellar aggregates of size 11 nm form. At extreme pH, the strong electrostatic repulsions destroy the micelles and the polymer molecules occur in solution as separate chains. A similar behavior was observed by Webber and colleagues[63–65] with the polystyrene–poly(*tert*-butyl methacrylate) and polystyrene–poly(methacrylic acid) block copolymers, which exhibited a strong dependence of aggregation state on solvent quality.

Fluorescence. The intensity ratio of peak 3 (382 nm) to peak 1 (371 nm) of pyrene emission appears in Figure 6 as a function of polymer concentration for block copolymer 2 and random copolymer 12. While the behavior of the two copolymers is identical at very low (<10^{-3}%) and at very high (>1%) polymer concentrations, it differs at intermediate concentrations.

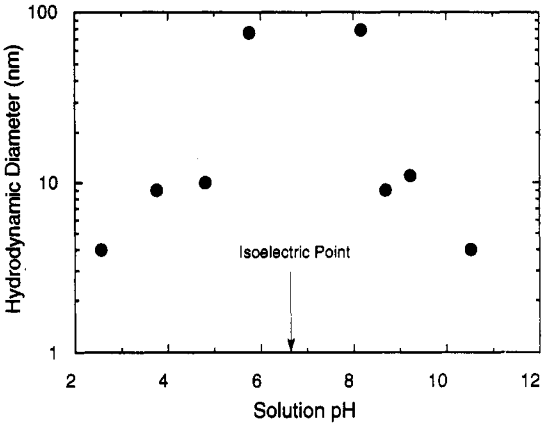


Figure 5. Hydrodynamic diameter of triblock copolymer 2 at different pHs with no added salt.

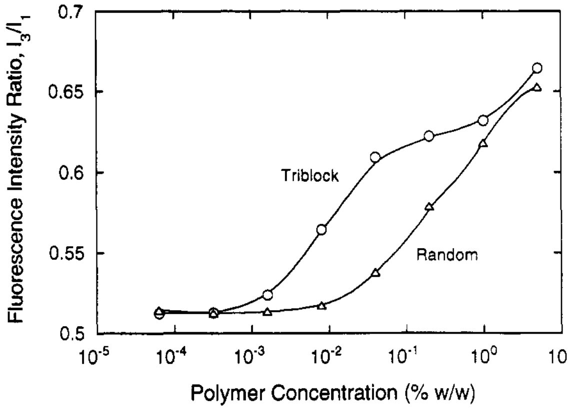


Figure 6. Comparison of the intensity ratio of pyrene fluorescence of triblock polymer 2 and random copolymer 12.

In the low-concentration regime polymer–pyrene interactions are absent as the pyrene intensity ratio remains constant and equal to the control (pyrene solution without polymer) value of 0.52.

In the intermediate-concentration region the pyrene intensity ratios for the two polymers increase with increasing polymer concentration, reaching a value of 0.63 at 1% polymer concentration. This increase indicates that pyrene is transferred from an aqueous to a more hydrophobic environment as a result of the increasing polymer concentration. For block copolymer 2, the onset of the increase occurs at a concentration of 0.001%, while for random copolymer 12 the same change occurs at a concentration an order of magnitude higher. This can be attributed to the longer hydrophobic domains of the block copolymer which are offered for more efficient binding and, therefore, higher affinity for the hydrophobic fluorescent probe. For the same amount of pyrene to associate hydrophobically with the random copolymer, higher polymer concentrations are necesary. Another explanation for the different behavior of the two copolymers can be attributed to the micellization of the block copolymer. This can be supported by the observation that, in the case of the block polymer, the increase in the intensity ratio is sharp in the concentration range 10^{-3}–4×10^{-2}, potentially due to micellization, and becomes shallow in the concentration range 4×10^{-2}–1%, possibly due to completion of micellization at 4×10^{-2}%.

In the high-concentration region, covering polymer concentrations higher than 1%, the intensity ratios of the two polymers become again the same and increase with increasing polymer concentration. The equality of their intensity ratios is in agreement with the fact that the two polymers have identical chemical compositions. The increase in intensity with polymer concentration may be due to a higher order of polymer–pyrene association or it may be simply due to the lower scattering of peak 3 at 382 nm as compared to that of peak 1 at 371 nm by the concentrated polymer solution. The scattering is inversely proportional to the fourth power of the wavelength (assuming independence of the refractive index on the wavelength),[74] and such a calculation can satisfactorily account for this increase. Supporting the latter interpretation is the trend in the absolute intensities of peaks 1 and 3, which increase monotonically up to 0.2% polymer concentration and then decrease at higher concentrations.

The onset of the increase in the intensity ratio of pyrene in solutions of block copolymers has been claimed to occur at the critical micelle concentration (cmc)[75,76] and was utilized for its determination. We might be tempted to conclude that the cmc of the triblock copolymer is at 10^{-3}%. However, the increase in the intensity ratio of the random copolymer, which should not form micelles, suggests that such a behavior can also be explained by pyrene–unimer association as opposed to pyrene–micelle association.

Conclusions

Low-molecular-weight random, diblock, and ABC triblock polyampholytes containing MAA, MMA, and DMAEMA were synthesized by GTP. Unlike the random terpolymers which are soluble over the entire pH range, the block polymers show a strong tendency to precipitate near the isoelectric point. The presence of the hydrophobic block leads to the micellization of the triblocks. This micellization offers the opportunity for utilization of these polymers in solubilization applications, which is further reinforced by the ability to vary polymer solubility as well as micellar stability by pH manipulations. Although it did not result in the determination of the cmc of the block copolymer, a pyrene fluorescence study showed that the hydrophobic probe binds to the triblock polymer **2** more strongly than to its random counterpart, polymer **12**. Our results suggest that the structure of the polyampholytes affects significantly their properties. We expect to be able to use GTP to prepare novel polyampholytes of various other structures such as stars,[77] ladders,[78,79] grafts,[80] combs,[81] and gels.

Acknowledgment. We thank S. W. Glatfelter and M. K. Heyman (Du Pont Central Research and Development) for their assistance in polymer synthesis. We also thank Professor T. Tanaka and M. Orkisz of the Physics Department at MIT for their help in performing preliminary QELS of the polyampholytes with their Microscope Laser Light Scattering apparatus. This work was supported by the National Science Foundation through the Biotechnology Process Engineering Center at MIT.

References and Notes

(1) McGrath, J. E. In *Block Copolymers: Science and Technology*; Meier, J. D., Ed.; MMI Press Symposium Series; Harwood: New York, 1983; Vol. 3; p 3.
(2) Morishima, Y. *Prog. Polym. Sci.* **1990**, *15*, 949.
(3) Balazs, A. C.; Gempe, M.; Lantman, C. W. *Macromolecules* **1991**, *24*, 168.
(4) Balazs, A. C.; Siemasko, C. P.; Lantman, C. W. *J. Chem. Phys.* **1991**, *94*, 1653.
(5) Patrickios, C. S.; Lansbury, P. T., Jr.; Hatton, T. A. Paper presented at the 203rd National Meeting of the American Chemical Society, San Francisco, 1992.
(6) Patrickios, C. S.; Gadam, S. D.; Cramer, S. M.; Hertler, W. R.; Hatton, T. A. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1993**, *34* (1), 1073.
(7) Patrickios, C. S.; Gadam, S. D.; Cramer, S. M.; Hertler, W. R.; Hatton, T. A. In *Polyelectrolytes*; Schmitz, K. S., Ed.; ACS Symposium Series, in press.
(8) Patrickios, C. S.; Jang, C. J.; Hertler, W. R.; Hatton, T. A. *Polym. Prep. (Am. Chem. Soc., Div. Polym. Chem.)* **1993**, *34* (1), 954.
(9) Patrickios, C. S.; Jang, C. J.; Hertler, W. R.; Hatton, T. A. In *Polyelectrolytes*, Schmitz, K. S., Ed., ACS Symposium Series, in press.
(10) Hughes, P.; Lowe, C. R. *Enzyme Microb. Technol.* **1988**, *10*, 115.
(11) Patrickios, C. S.; Abbott, N. L.; Foss, R. P.; Hatton, T. A. In *New Developments In Bioseparation*; Ataai, M. M., Sikdar, S. K., Eds.; AIChE Symposium Series; AIChE: New York, 1992; Vol. 88; p 80.
(12) Righetti, P. G. *Isoelectric Focusing: Theory, Methodology and Applications*; Elsevier: Amsterdam, 1983; p 50.
(13) Bekturov, E. A.; Kudaibergenov, S. E.; Rafikov, S. R. *Macrmol. Sci. Rev., Macromol. Chem. Phys.* **1990**, *C30* (2), 233.
(14) Alfrey, T.; Morawetz, H.; Fitzgerald, E. B.; Fuoss, R. M. *J. Am. Chem. Soc.* **1950**, *72*, 1864.
(15) Alfrey, T.; Fuoss, R. M.; Morawetz, H.; Pinner, S. H. *J. Am. Chem. Soc.* **1952**, *74*, 438.
(16) Alfrey, T.; Morawetz, H. *J. Am. Chem. Soc.* **1952**, *74*, 436.
(17) Alfrey, T.; Pinner, S. H. *J. Polym. Sci.* **1957**, *23*, 533.
(18) Ehrlich, G.; Doty, P. *J. Am. Chem. Soc.* **1954**, *76*, 3764.
(19) Katchalsky, A.; Miller, I. R. *J. Polym. Sci.* **1954**, *14*, 57.
(20) Mazur, J.; Silberberg, A.; Katchalsky, A. *J. Polym. Sci.* **1959**, *35*, 43.
(21) McCormick, C. L.; Salazar, L. C. *Macromolecules* **1992**, *25*, 1896.
(22) Corpart, J.-M.; Candau, F. *Macromolecules* **1993**, *26*, 1333.
(23) Annaka, M.; Tanaka, T. *Nature* **1992**, *355*, 430.
(24) Baker, J. P.; Stephens, D. R.; Blanch, H. W.; Prausnitz, J. M. *Macromolecules* **1992**, *25*, 1955.
(25) Ebersole, R. C.; Foss, R. P.; Ward, M. D. *Bio/Technology* **1991**, *9*, 450.
(26) Higgs, P. G.; Joanny, J.-F. *J. Chem. Phys.* **1991**, *94*, 1543.
(27) Higgs, P. G.; Orland, H. *J. Chem. Phys.* **1991**, *95*, 4506.
(28) Kantor, Y.; Li, H.; Kardar, M. *Phys. Rev. Lett.* **1992**, *69*, 61.
(29) Kamiyama, Y.; Israelachvili, J. *Macromolecules* **1992**, *25*, 5081.
(30) Kamachi, M.; Kurihara, M.; Stille, J. K. *Macromolecules* **1972**, *5*, 161.
(31) Kurihara, M.; Kamachi, M.; Stille, J. K. *J. Polym. Sci., Polym. Chem. Ed.* **1973**, *11*, 587.
(32) Varoqui, R.; Tran, Q.; Pefferkorn, E. *Macromolecules* **1979**, *12*, 831.
(33) Fujimoto, T.; Ohkoshi, K.; Miyaki, Y.; Nagasawa, M. *J. Membr. Sci.* **1984**, *20*, 313.
(34) Miyaki, Y.; Iwata, M.; Fujita, Y.; Tanisugi, H.; Isono, Y.; Fujimoto, T. *Macromolecules* **1984**, *17*, 1907.
(35) Miyaki, Y.; Nagamatsu, H.; Iwata, M.; Ohkoshi, K.; Se, K.; Fujimoto, T. *Macromolecules* **1984**, *17*, 2231.
(36) Takahashi, S.; Matsumura, K.; Toda, M.; Fujimoto, T.; Hasekawa, H.; Miyaki, Y. *Polym. J.* **1986**, *18*, 41.
(37) Fujimoto, T.; Miyaki, Y.; Nagasawa, M. *Science* **1984**, *224*, 74.
(38) Itou, H.; Toda, M.; Ohkoshi, K.; Iwata, M.; Fujimoto, T.; Miyaki, Y.; Kataoka, T. *Ind. Eng. Chem. Res.* **1988**, *27*, 983.
(39) Bekturov, E. A.; Frolova, V. A.; Fudaibergenov, S. E.; Schulz, R. C.; Zöller, J. *Makromol. Chem.* **1990**, *191*, 457.
(40) Bekturov, E. A.; Kudaibergenov, S. E.; Khamzamulina, R. E.; Frolova, V. A.; Nurgalieva, D. E.; Schulz, R. C.; Zöller, J. *Makromol. Chem., Rapid Commun.* **1992**, *13*, 225.
(41) Spontak, R. J.; Zielinski, J. M. *Macromolecules* **1992**, *25*, 663 and references cited therein.
(42) Sdranis, Y. S.; Kosmas, M. K. *Macromolecules* **1991**, *24*, 1341.
(43) Wu, S. Y.; Slater, G. W. *Macromolecules* **1993**, *26*, 1905.
(44) Webster, O. W.; Anderson, B. C. In *New Methods for Polymer Synthesis*; Mijs, W. J., Ed.; Plenum: New York, 1992; p 1.
(45) Sogah, D. Y.; Hertler, W. R.; Webster, O. W.; Cohen, G. M. *Macromolecules* **1987**, *20*, 1473.
(46) Webster, O. W.; Hertler, W. R.; Sogah, D. Y.; Farnham, W. B.; RajanBabu, T. V. *J. Am. Chem. Soc.* **1983**, *105*, 5706.
(47) Müller, M. A.; Augenstein, M.; Dumont, E.; Pennewiss, H. *New Polym. Mater.* **1991**, *2*, 315.
(48) Doherty, M. A.; Müller, A. H. E. *Makromol. Chem.* **1989**, *190*, 527.
(49) Choi, W.-J.; Kim, Y.-B.; Kwon, S.-K.; Lim, K.-T.; Choi, S.-K. *J. Polym. Sci., Polym. Chem. Ed.* **1992**, *30*, 2143.
(50) Wei, Y.; Wnek, G. N. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1987**, *28* (1), 252.
(51) Mykytiuk, J.; Armes, S. P.; Billingham, N. C. *Polym. Bull. (Berlin)* **1992**, *29*, 139.
(52) Dicker, I. B.; Hertler, W. R.; Ma, S. H. U.S. Patent 5,219,945, 1993.
(53) Ma, S. H.; Matrick, H.; Shor, A. C.; Spinelli, H. J. U.S. Patent 5,085,698, 1992.

(54) Taylor, G. N.; Stillwagon, L. E.; Houlihan, F. M.; Wolf, T. M.; Sogah, D. Y.; Hertler, W. R. *Chem. Mater.* **1991**, *3*, 1031.
(55) Taylor, G. N.; Stillwagon, L. E.; Houlihan, F. M.; Wolf, T. M.; Sogah, D. Y.; Hertler, W. R. *J. Vac. Sci. Technol.* **1991**, *B9*, 3348.
(56) Raymond, F. A.; Hertler, W. R. *J. Imag. Sci. Technol.* **1992**, *36*, 243.
(57) Rannard, S. P.; Billingham, N. C.; Armes, S. P.; Mykytiuk, J. *Eur. Polym. J.* **1993**, *29*, 407.
(58) Hertler, W. R. U.S. Patent 5,072,029, 1991.
(59) Kearns, J. E.; McLean, C. D.; Solomon, D. H. *J. Macromol. Sci., Chem.* **1974**, *A8*, 673.
(60) Ainsworth, C.; Chen, F.; Kuo, Y.-N. *J. Organomet. Chem.* **1972**, *46*, 59.
(61) Dicker, I. B.; Cohen, G. M.; Farnham, W. B.; Hertler, W. R.; Laganis, E. D.; Sogah, D. Y. *Macromolecules* **1990**, *23*, 4034.
(62) Tanford, C. *Physical Chemistry of Macromolecules*; Wiley: New York, 1961; pp 240, 461, 465, 466, 472, 555, 561.
(63) Prochazska, K.; Kiserow, D.; Ramireddy, C.; Tuzar, Z.; Munk, P.; Webber, S. E. *Macromolecules* **1992**, *25*, 454.
(64) Kiserow, D.; Prochazska, K.; Ramireddy, C.; Tuzar, Z.; Munk, P.; Webber, S. E. *Macromolecules* **1992**, *25*, 461.
(65) Kiserow, D.; Chan, J.; Ramireddy, C.; Munk, P.; Webber, S. E. *Macromolecules* **1992**, *25*, 5338.
(66) Velick, S. F. *J. Phys. Colloid Chem.* **1949**, *53*, 135.
(67) Healy, T. W.; Homola, A.; James, R. O.; Hunter, R. J. *Faraday Discuss. Chem. Soc.* **1978**, *65*, 156.
(68) Merle, Y. *J. Phys. Chem.* **1987**, *91*, 3092.
(69) Creighton, T. E. *Proteins: Structures and Molecular Properties*; Freeman: New York, 1984; p 7.
(70) Rice, S. A.; Harris, F. E. *J. Chem. Phys.* **1956**, *24*, 326.
(71) Harris, F. E.; Rice, S. A. *J. Chem. Phys.* **1956**, *24*, 336.
(72) Green, A. A. *J. Biol. Chem.* **1931**, *93*, 517.
(73) Melander, W.; Horváth, C. *Arch. Biochem. Biophys.* **1977**, *183*, 200.
(74) Hiemenz, P. C. *Principles of Colloid and Surface Chemistry*; Marcel Dekker: New York, 1986; p 238.
(75) Zhao, C.-L.; Winnik, M. A.; Riess, G.; Croucher, M. D. *Langmuir* **1990**, *6*, 514.
(76) Wilhelm, M.; Zhao, C.-L.; Wang, Y.; Xu, R.; Winnik, M. A.; Mura, J.-L.; Riess, G.; Groucher, M. D. *Macromolecules* **1991**, *24*, 1033.
(77) Spinelli, H. J. Int. Patent Appl. WO 8,600,626, 1986.
(78) Sogah, D. Y. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1988**, *29* (2), 3.
(79) Sogah, D. Y. U.S. Patent 4,906,713, 1990.
(80) Hertler, W. R.; Sogah, D. Y.; Boettcher, F. P. *Macromolecules* **1990**, *23*, 1264.
(81) Fleischmann, G.; Eck, H.; Schuster, J. Eur. Patent Appl. EP 355826, 1990.