Effect of Block Size and Sequence on the Micellization of ABC Triblock Methacrylic Polyampholytes

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ABSTRACT: Fluorescence spectroscopy and static and dynamic light scattering were employed to probe the effect of block size and sequence on the micellization properties of a series of water-soluble, low-polydispersity, low-molecular-weight, block and random methacrylic polyampholytes: $B_8M_{12}A_{16}$, $B_{12}M_{12}A_{12}$, $B_{16}M_{12}A_8$, $B_{12}A_{12}M_{12}$, and $(B\text{-}co\text{-}M\text{-}co\text{-}A)_{12}$, where B is 2-(dimethylamino)ethyl methacrylate (DMAEMA), M is methyl methacrylate (MMA), and A is methacrylic acid (MAA). The values of the pyrene fluorescence emission intensity ratio I_1/I_3, indicative of micelle formation, are reported for both acidic and basic solution pH values, over a wide range of copolymer concentrations (0.0001–0.5% w/w) and at three different temperatures (10, 25, and 50 °C). The critical micellization concentration (CMC) values ordered as CMC($B_{16}M_{12}A_8$) > CMC($B_{12}M_{12}A_{12}$) > CMC($B_8M_{12}A_{16}$) at low solution pH. Under basic conditions, the CMC for $B_{16}M_{12}A_8$ was slightly lower than that for $B_{12}M_{12}A_{12}$. Micelles were not formed by the random copolymer $(B\text{-}co\text{-}M\text{-}co\text{-}A)_{12}$ at either pH value, nor by the triblock $B_{12}A_{12}M_{12}$ at basic pH. A small increase in the CMC with increasing temperature was observed for the BMA copolymers at basic pH. Dynamic light scattering data indicated that, for the micelles formed by the BMA polyampholytes, both DMAEMA and MAA blocks were in the corona regardless of which block was charged, while the middle hydrophobic MMA block constituted the micellar core. The constraints imposed by such a conformation of the polyampholyte blocks resulted in relatively low micelle aggregation numbers of 10–20 and a micelle size about half the contour length of the polymer. The $B_{12}A_{12}M_{12}$ copolymers formed larger micelles than the BMA polyampholytes; with the MMA block in the center, the micelle radius is determined by the length of the fully-stretched polymer.

Introduction

A class of polymers with potentially interesting applications in a range of areas is the family of block polyampholytes, copolymers in which at least two of the blocks are of opposite charge. To date, however, polymers of this type have received little attention. Stille's group in 1972 was the first to synthesize block polyampholytes; they used anionic polymerization to form block copolymers of 2-vinylpyridine with methacrylic acid (MAA) or acrylic acid.[1,2] Varoqui et al.[3] synthesized a diblock polyampholyte based on styrenesulfonic acid and 2-vinylpyridine, whereas Miyaki and co-workers[4–9] synthesized cross-linked pentablock polyampholytes, the second block comprising quaternary ammonium residues, the fourth block styrenesulfonate, and the other three blocks being neutral cross-linkable isoprene blocks. More recently, Bekturov et al.[10,11] 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 an otherwise diblock polyampholyte leads to an ABC triblock polymer structure, which can be expected to exhibit a richer solution behavior than the corresponding diblock polyampholytes. The literature on ABC triblock polymers is modest and primarily concerns studies of morphology and film properties.[12] We recently reported[13] the synthesis of low-molecular-weight random, diblock, and ABC triblock polyampholytes, containing methacrylic acid (MAA), methyl methacrylate (MMA), and (dimethylamino)ethyl methacrylate (DMAEMA), employing the group transfer polymerization (GTP) method.[14] Unlike the random terpolymers which are water soluble over the entire pH range, the block polymers show a strong tendency to precipitate near the isoelectric point.[13]

The hydrophobic block provides a driving force for the micellization of the triblock polyampholytes, thus offering the opportunity for utilizing these polymers in solubilization applications.[15] The ability to vary the polymer solubility as well as micellar stability while still in the same solvent (i.e., water) by manipulating the solution pH will be an advantage in this case. Polyampholyte-mediated protein separation methods such as ion-exchange displacement chromatography,[16,17] precipitation,[18,19] and aqueous two-phase partitioning[20,21] are other potential applications for ABC polyampholytes.

The results of Patrickios et al.[13] suggest that the structure of a polyampholyte affects its solution properties significantly. Preliminary light scattering measurements on one triblock polyampholyte indicated the formation of micelles, attributed to the presence of the MMA hydrophobic block in the copolymer. In this paper, we provide a more systematic and detailed investigation of the micellization behavior of the ABC triblock methacrylic polyampholytes synthesized by Patrickios et al.[13] Fluorescence spectroscopy and light

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scattering have been used to probe the effect of block size and sequence on the formation of micelles and the micelle size under both acidic and basic solution conditions. Our results are discussed in terms of the electrostatic interactions between the charged blocks as modulated by the solution pH and of the conformation adopted by the polyampholyte molecule in the micelle.

Materials and Methods

Materials. The synthesis of the polyampholytes used in the present study is described elsewhere;[13] their chemical structure, theoretical molecular weight, isoelectric point, and solubility are listed in Table 1. The polydispersity values of the polyampholytes studied here are tabulated in ref 13. The M_w/M_n values were in the range 1.15–1.25, indicating that the polyampholytes were of low polydispersity. The notation used for the monomers comprising the polyampholytes is B for 2-(dimethylamino)ethyl methacrylate (DMAEMA), A for methacrylic acid (MAA), and M for methyl methacrylate (MMA). Benzo[*def*]phenanthrene (pyrene) (CA No. 129–00–0) was purchased from Sigma Chemical Co., St. Louis, MO, and was recrystallized three times from ethanol. Sodium hydroxide (NaOH), hydrochloric acid (HCl), and potassium chloride (KCl) were all purchased from Mallinckrodt, Paris, KY, and used as received.

Sample Preparation. The solid polyampholyte was initially dissolved in HCl. After diluting with deionized water to approach the desired polyampholyte w/w concentration, the pH was adjusted by adding 0.5 N HCl or 0.5 N NaOH; the copolymer concentration was calculated accordingly. The solution pH was measured using a Fisher Accumet pH-meter (Model 825MP). One microliter of pyrene/acetone solution was added to 3 mL of copolymer solution for the fluorescence measurements.

Spectroscopy. Fluorescence spectra were recorded on a SPEX FluoroMax spectrofluorometer (SPEX Industries, Inc., Edison, NJ) using a 0.3 nm band-pass in the "s&r" mode to account for possible variations in lamp intensity. Wavelengths of excitation (λ_{ex}) were chosen according to the maximum intensity obtained in the excitation spectra; depending on the solution, $\lambda_{ex} \approx 335$ nm and $\lambda_{em} \approx 372$ nm. Sample PM counts were always smaller than 10^5 counts/s to ensure a linear response of the detector.[22,23] Step increments and integration times were set at 0.5 nm and 0.5 s, respectively. All samples were aerated, magnetically stirred, temperature controlled using a thermostated cuvette holder connected to a circulating water bath, and examined at right-angle geometry. Each spectrum was obtained by averaging three scans, corrected for scatter using an equivalent blank solution. In all cases the values of the ratio I_1/I_3 of the intensities of the first and third vibronic peaks in the pyrene fluorescence emission spectrum were averaged over four different experiments. The reproducibility of these results was better than 3% and the standard deviation on the I_1/I_3 values was smaller than 0.03. The pyrene concentration was 3×10^{-7} M in all samples; no excimer formation was observed, even in the micellar solutions. The measured values of I_1/I_3 depend on the wavelength of the excitation light,[24,25] and they are biased toward the hydrophobic state (low I_1/I_3 values) when the excitation wavelength is 339 nm and toward the hydrophilic state (high I_1/I_3 values) at 333 nm. At the excitation wavelength of 335 nm used in the present study, the I_1/I_3 ratio is about equally weighted for the two states.[26]

Light Scattering. Dynamic and static light scattering measurements were performed using a Brookhaven Model BI-200SM instrument (Brookhaven Instrument Corp., Holtsville, NY).[22] The light source was a Lexel 8 W argon ion laser operating at $\lambda = 514$ nm (Lexel Laser, Inc., Fremont, CA). A BI-9000AT digital correlator (Brookhaven Instrument Corp., Holtsville, NY) was used for signal analysis. The dynamic light scattering was carried out at a scattering angle of 90°, while for the static light scattering (Zimm plot) the angle ranged from 30 to 145° in 15° intervals. The scattering cells were first rinsed with a polymer solution which was filtered through a 0.22 mm filter to remove dust and then filled with the rest of the solution. Temperature control was achieved using a Neslab RTE-110 refrigerated bath/circulator.

Table 1. Chemical Structure, Theoretical Molecular Weight, Isoelectric Point, and Solubility of the Polyampholytes Used in This Study (Extracted from Ref 13)

polyampholyte	theor M_w	isoelectric point (pI)	pH insolubility range
$B_8M_{12}A_{16}$	3812	5.4	4.2–6.0
$B_{12}M_{12}A_{12}$	4116	6.6	5.5–7.9
$B_{16}M_{12}A_8$	4400	8.0	6.6–8.3
$B_{12}A_{12}M_{12}$	4116	6.6	5.4–8.0
(B-co-M-co-A)$_{12}$	*4116*	*6.6*	*none*

Results and Discussion

Polyampholyte Micelle Formation. The fluorescence emission of pyrene is sensitive to the polarity of its environment.[27,28] As a result, the I_1/I_3 intensity ratio of the pyrene emission spectrum is widely employed for the determination of the critical micellization concentration (CMC) and temperature (CMT) of surfactants and amphiphilic block copolymers in aqueous solutions.[22,23,27,29,30] When micelles are formed, pyrene partitions preferentially toward the hydrophobic domain afforded by the micelle core and experiences a nonpolar environment; consequently, the I_1/I_3 ratio decreases from a value of ~2 in water to a value of ~1.5 in a micelle.[23] The micellization behavior of the polyampholytes listed in Table 1 was studied using pyrene as the probe over a wide range of polymer concentrations (0.0001–0.5% w/w) at three different temperatures (10, 25, and 50 °C).

The pyrene I_1/I_3 emission intensity ratio values are shown in Figure 1 as a function of polyampholyte concentration for a number of polyampholytes; data at 25 °C for two pH values are presented (note that the pH is not exactly the same for each polymer studied here; however, our measurements suggest that small pH changes do not affect the micellization behavior as long as the pH used is ~1–2 units away from the isoelectric point, pI, of the polyampholyte). At low polyampholyte concentrations (generally smaller than 0.001%) the I_1/I_3 ratio was the same as that observed in water (I_1/I_3 in water ranges from 1.8 to 2.0, depending on the temperature[23,30]), indicating that the presence of the copolymer did not result in well-defined hydrophobic domains, i.e, micelles. The formation of micelles at higher polyampholyte concentrations was evident from the decrease in the I_1/I_3 ratio to a value of approximately 1.5, as observed in solutions of $B_{12}A_{12}M_{12}$ at pH = 4 and $B_8M_{12}A_{16}$, $B_{12}M_{12}A_{12}$, and $B_{16}M_{12}A_8$ for both pH values studied here (see Figure 1a–d).

This decrease in I_1/I_3 was gradual for $B_{16}M_{12}A_8$, spanning two concentration decades, less gradual for $B_{12}M_{12}A_{12}$, and steep for $B_8M_{12}A_{16}$. Similar experiments, conducted with aqueous solutions of Pluronic poly(ethylene oxide)-*block*-poly(propylene oxide)-*block*-poly(ethylene oxide) copolymers (not reported here), resulted in a micellization transition spanning approximately one concentration decade. The CMC of the Pluronic copolymers, determined from dye solubilization[31] and surface tension[32] studies, was shown to correspond to the large change in slope of the sigmoidal I_1/I_3 vs log-concentration curve at the higher concentration. The determination of CMC for poly(ethylene oxide)–poly(styrene) block copolymer solutions from I_1/I_3 measurements has been discussed by Wilhelm et al.[25]

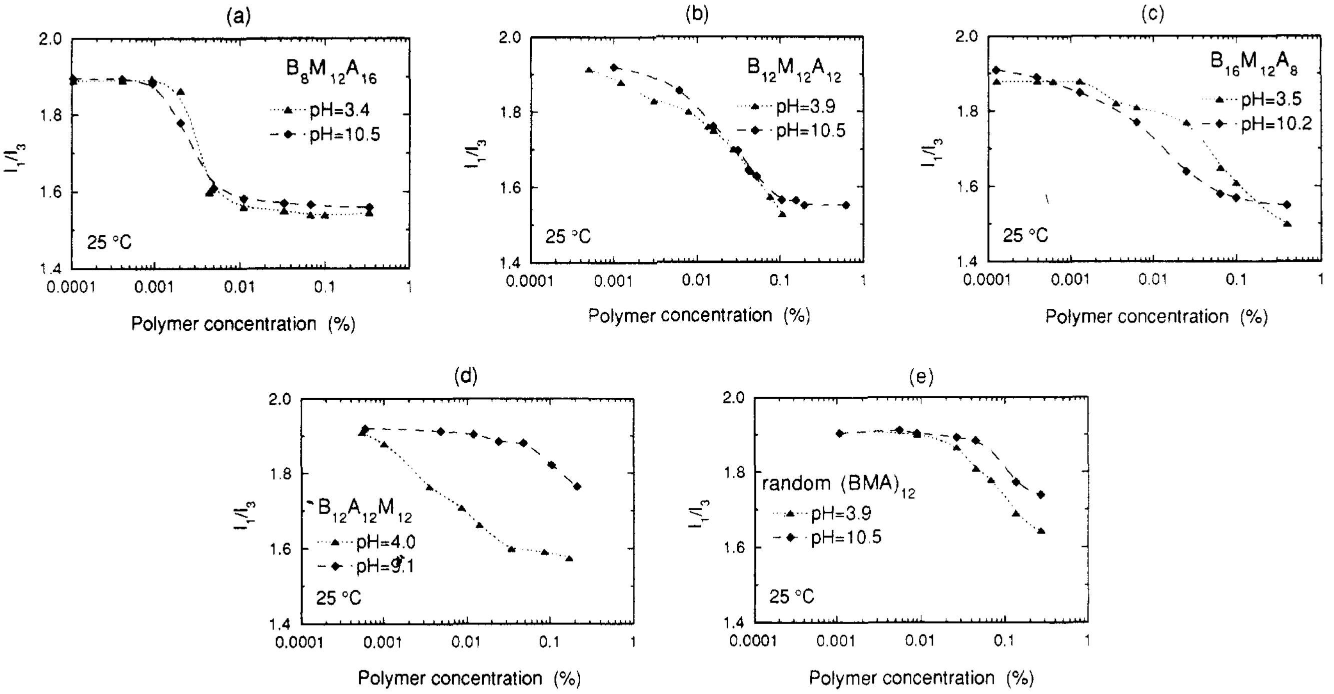

Figure 1. Pyrene I_1/I_3 emission intensity ratio as a function of polymer concentration for a number of polyampholytes: (a) $B_8M_{12}A_{16}$, (b) $B_{12}M_{12}A_{12}$, (c) $B_{16}M_{12}A_8$, (d) $B_{12}A_{12}M_{12}$, and (e) (B-*co*-M-*co*-A)$_{12}$. Data at 25 °C for two pH values are presented.

Table 2. Polyampholyte Concentration (% w/w) Range over Which the Pyrene I_1/I_3 Ratio Exhibits a Decrease Indicative of the Unimer-to-Micelle Transition (25 °C)

polyampholyte	low pH (≈3.7)	high pH (≈10.3)
$B_8M_{12}A_{16}$	0.003–0.006	0.002–0.008
$B_{12}M_{12}A_{12}$	0.006–0.08	0.009–0.08
$B_{16}M_{12}A_8$	0.01–0.2	0.003–0.06
$B_{12}A_{12}M_{12}$	0.002–0.02	does not form micelles
(B-*co*-M-*co*-A)$_{12}$	does not form micelles	does not form micelles

Maltesh et al.[33] attributed the first (low-concentration) break of the sigmoidal I_1/I_3 vs log-concentration curve observed in aqueous solutions of a maleic anhydride α-olefin comb copolymer to intrapolymer association, and the high-concentration change in slope to the formation of micelles by interpolymer association of the hydrophobic groups on the polymer; surface tension measurements supported this interpretation.[33] On the basis of these results, it is reasonable to assume that the CMC for the block polyampholytes is given by the high-concentration break in the sigmoidal curves.

The gradual decrease in the I_1/I_3 ratio with increasing polyampholyte concentration seen in Figure 1 can be attributed to molecular weight polydispersity, the presence of hydrophobic impurities, association of pyrene with individual polymer chains (unimers),[24] or premicellar aggregates; all these will result in the creation of hydrophobic domains at polyampholyte concentrations lower than the ones corresponding to the primary unimer-to-micelle transition (CMC). Fluorescence is a very sensitive technique and can detect the presence of some pyrene in hydrophobic domains while most of the pyrene is still in the aqueous solution, because the quantum yield of pyrene in a hydrophobic environment is higher than that in water.[27] Mono- and diblock impurities[34] are most likely the cause of the gradual decrease in I_1/I_3 in the experiments reported here. Note that the I_1/I_3 ratio for the random (B-*co*-M-*co*-A)$_{12}$ polyampholyte (which is not expected to form micelles[13]) started decreasing for copolymer concentrations of approximately 0.03% (Figure 1e), indicating either the influence of impurities or the presence of some hydrophobic domains through loose intra- or interpolymer association.

Pyrene has been used as a fluorescence probe in other polyelectrolyte solutions. Zana and co-workers[26,35–37] studied the microdomains formed in aqueous solutions of poly(maleic acid-*co*-alkyl vinyl ether) ("polysoap") using pyrene, interpreting the results in terms of "binding" of pyrene to the copolymer[26] with a polysoap concentration-dependent binding constant. The sigmoidal decrease in the I_1/I_3 ratio, spanning four polymer concentration decades, was attributed to intramolecular hydrophobic domains that were present even at vanishingly low polysoap concentrations because a single polysoap molecule was composed of many amphiphilic segments. A polymeric intramolecular micellar system, PA-18K2 (potassium salt of a 1:1 copolymer of maleic anhydride and 1-octadecene),[38] and a polymerized micelle, poly(sodium undecanoate),[39] were investigated using pyrene and its derivatives. Pyrene was solubilized in the interior of PA-18K2 over the entire pH range, whereas poly(sodium undecanoate) expanded and exposed pyrene to water at pH > 10, and poly(methacrylic acid) acted as a host for pyrene only in the low-pH range.[38,39]

The effect of pH on the I_1/I_3 ratio in the case of the block polyampholytes studied here is discussed below in conjunction with dynamic light scattering data.

Critical Micellization Concentration (CMC). The CMC of the polyampholytes can be estimated from the high-concentration break in the slope of the I_1/I_3 curve, as discussed above. The polyampholyte concentration range over which the I_1/I_3 ratio exhibits a decrease indicative of the unimer-to-micelle transition is listed in Table 2 for solutions at 25 °C. The high concentration value in the micellization transition range corresponds to the CMC. The micellization transition range is wider for $B_{16}M_{12}A_8$ and narrower for $B_8M_{12}A_{16}$ for both pH values. Note that the polyampholyte concentration at which the I_1/I_3 ratio starts decreasing does not vary much between the different polymers (see discussion above on the origin of this decrease); the broadness of the transition reflects differences in the CMC, the high-concentration limit of the I_1/I_3 transition.

The effect of block size on the micellization of the BMA (DMAEMA–MMA–MAA) polyampholytes is apparent from the results shown in Figure 1. At acidic pH (≈3.4–3.9) the CMC values were ordered as CMC($B_{16}M_{12}A_8$) > CMC($B_{12}M_{12}A_{12}$) > CMC($B_8M_{12}A_{16}$). At pH values 1–2 units lower than the p*K* of the methacrylic acid (5.35) the MAA segments do not dissociate appreciably and, therefore, are not charged, or are only slightly charged, whereas the DMAEMA segments are highly protonated and bear a strong positive charge. The micellization process is a delicate balance of the "attraction" of the hydrophobic surfactant tails for each other, favoring micelle formation and growth, and of the hydrophilic (and often charged) surfactant headgroups repelling each other to limit micelle formation.[40] The ranking of the CMC values according to composition is a direct reflection of the increasing electrostatic repulsions between the positively charged DMAEMA segments as this block is increased in size, coupled with the simultaneous decrease in the size of the uncharged MAA block, making the association of polyampholyte molecules into micelles more difficult.

The effect of block size on the micellization of the BMA polyampholytes at basic pH is more difficult to interpret than that at acidic pH. It was anticipated that with increasing size of the uncharged basic group, and decreasing size of the charged acidic block, the ranking of the CMC would be the reverse of that observed under acidic conditions. This is true for the two larger polymers, where the CMC for $B_{16}M_{12}A_8$ is slightly lower than that for $B_{12}M_{12}A_{12}$, but it is surprising that $B_8M_{12}A_{16}$ has a significantly lower CMC than $B_{12}M_{12}A_{12}$ at this pH. Also surprising is that each of these last two polymers has what appears to be almost identical micellization profiles under very different charging conditions.

An increase in the solution temperature resulted in a general decrease in the I_1/I_3 ratio, as shown in Figure 2, reflecting a decrease in the overall polarity of the solution.[23] For the BMA polyampholytes at acidic pH, this shift of I_1/I_3 to lower values occurred across the entire polyampholyte concentration range, but there was no change in the micellization transition or in the CMC (data not shown). A small increase in CMC with increasing temperature has been observed for these polymers at basic pH, though, as shown in Figure 2. This implies a small negative or exothermic enthalpy of micelle formation.[41] The opposite, i.e, an endothermic enthalpy of micellization, has been observed in the micellization of ionic and nonionic surfactants and nonionic block copolymers in water, while the micellization of block copolymers in nonaqueous media is usually exothermic (see refs 31 and 42 and references cited therein).

The precise location of the different blocks, either in the core of the micelle or in the corona region, cannot be elucidated from the fluorescence measurements. The MMA block (M) in the middle of the BMA molecule is hydrophobic and is expected to be in the micelle core. At low pH, the uncharged MAA block may also be in the core region of the micelle, located in the center, as shown in Figure 3a. Under these conditions, the micelle size would be determined by the full contour length of the polymer molecule. Since the uncharged MAA block is still fairly polar, however, the polyampholyte molecule could adopt a configuration favoring both DMAEMA and MAA in the corona, with MMA in the micelle center, in which case the micelle radius will be approximately half the polymer contour length, as in Figure 3b. For high pH values, similar structures can be proposed, with the DMAEMA block now being the one that can be either in the corona or in the core region of the micelle. The possible configurations are illustrated schematically in Figure 3c,d.

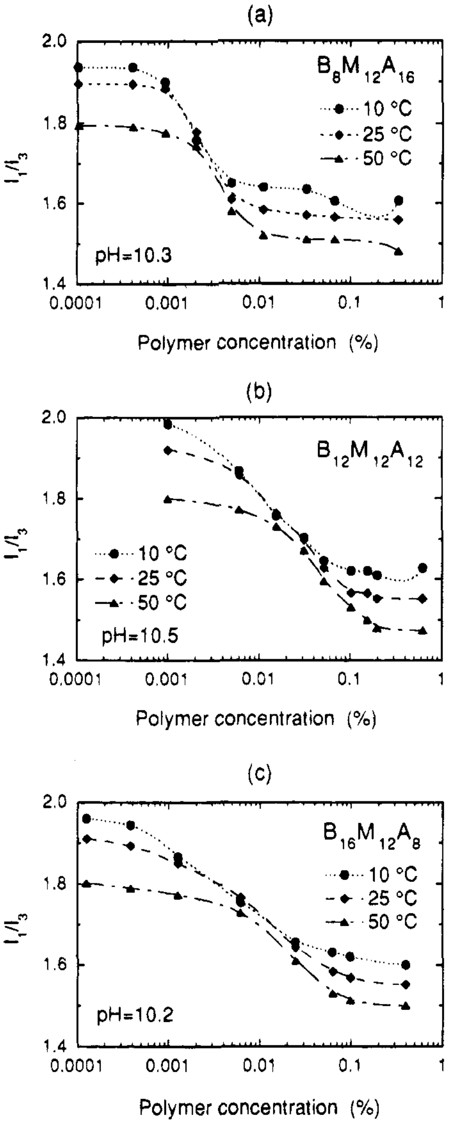

Figure 2. Effect of temperature on the micellization of BMA triblock polyampholytes: (a) $B_8M_{12}A_{16}$, (b) $B_{12}M_{12}A_{12}$, and (c) $B_{16}M_{12}A_8$. Data are presented at basic pH for three temperatures (10, 25, 50 °C).

The polyampholytes $B_{12}M_{12}A_{12}$ and $B_{12}A_{12}M_{12}$ can be expected to behave differently in solution owing to their different block sequences. These polymers have the same pI and, under the conditions of our experiment at low pH, carry the same charge at one end of the molecule. However, $B_{12}A_{12}M_{12}$ has the hydrophobic MMA block at the end and, at acidic pH, should behave as a diblock with the charged DMAEMA as one block and the neutral MAA and MMA as the other. The CMC of $B_{12}A_{12}M_{12}$ at pH ≈ 4 is lower than that of $B_{12}M_{12}A_{12}$, suggesting an "easier" micellization (see Table 2 and Figure 1b,d). This can be a result of better packing of $B_{12}A_{12}M_{12}$ in the micelle core (if both BAM and BMA behave like diblocks, Figure 3a,e) or of the differences

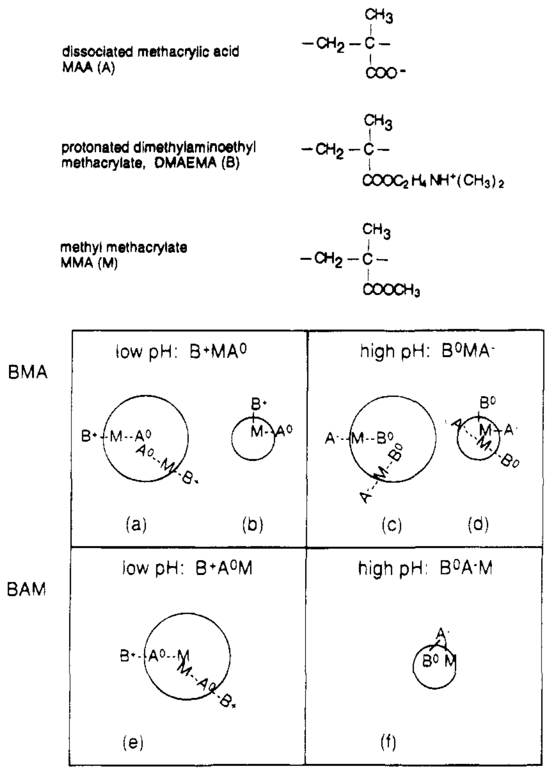

Figure 3. Chemical structure of the DMAEMA, MMA, and MAA monomers and a schematic of the different possible configurations the polyampholytes may assume in the micelles, depending on the block charge and sequence. As discussed in the text, configurations a, c, and f were not attained by the polyampholytes studied here.

in micellization behavior of triblocks and diblocks (if BMA behaves as a triblock, Figure 3b); for block copolymers of the same molecular weight and composition, diblocks form micelles more readily than do triblocks.[42] Light scattering data are presented in the following section in an attempt to resolve this issue.

The effect of block sequence on the micellization of the polyampholytes is clearly demonstrated when the I_1/I_3 ratios of $B_{12}A_{12}M_{12}$ and $B_{12}M_{12}A_{12}$ in basic solution are compared. At this high pH (1−2 units greater than the DMAEMA pK of 8.0) the MAA block is fully dissociated (negatively charged) while the DMAEMA has zero charge. As a result, $B_{12}A_{12}M_{12}$ has the charged block in the middle of the molecule. This makes the packing of the polyampholyte molecules in the micelle (see Figure 2f) difficult or almost impossible,[43] as evidenced by the high I_1/I_3 values obtained in $B_{12}A_{12}M_{12}$ solutions of polymer concentrations of approximately 0.25% (see Figure 1d), which are indicative of the absence of significant hydrophobic domains. In fact, the I_1/I_3 ratio afforded by $B_{12}A_{12}M_{12}$ solutions is similar to that of the random polyampholyte (B-*co*-M-*co*-A)$_{12}$ (Figure 1e) which does not form micelles.[13] In contrast, $B_{12}M_{12}A_{12}$ forms micelles at pH = 10.5 with a CMC similar to that at pH = 3.9 (see Figure 1b).

Hydrodynamic Diameter of the Polyampholyte Micelles. Dynamic and static light scattering experiments were performed to obtain information on the hydrodynamic size and "molecular" weight, respectively, of the block polyampholytes, and to shed some light on the structural characteristics of the micelles that are formed under certain solution conditions.

The hydrodynamic diameters of the aggregates of $B_{12}M_{12}A_{12}$ formed in aqueous solutions in the absence of salt are presented in Figure 4a at different pH values; the polyampholyte concentration used was 0.6% w/w. At pH values lower than ≈3 the hydrodynamic diameter detected from dynamic light scattering was 3−4 nm, suggesting either that only unimers (individual polymer coils) are present in solution or that very small, compact micelles are formed. The I_1/I_3 ratios for $B_{12}M_{12}A_{12}$ solutions over a range of pH values shown in Figure 4b show the presence of hydrophobic domains even at the lowest pHs, suggesting that it is small micelles that are formed here, or that "collapsed" MAA blocks[45] provide a hydrophobic environment leading to low I_1/I_3 ratios in the presence of unimers. We show below that static light scattering experiments point to the formation of very small aggregates of about five polymer molecules under these solution conditions for this polymer. The electrostatic repulsion between the positively charged DMAEMA blocks is the main reason for such solution behavior. An increase in the solution pH toward the pI value of the polyampholyte resulted in a gradual decrease in the net charge per polymer molecule, reducing electrostatic repulsions and facilitating the formation of micelles with a hydrodynamic diameter of ~10 nm. Large aggregates[44] were formed at pHs around the isoelectric point of the polyampholyte, reflecting the macroscopic precipitation of the polymer from solution at a low polymer charge density (weak electrostatic repulsion). Hydrodynamic radii consistent with the presence of micelles were observed again at pH values higher than the pI. However, no unimers or small micelles were detected even at extreme pH values (pH = 12); this can be partly attributed to the interactions between the relatively hydrophobic DMAEMA blocks. It is possible that the presence of Na$^+$ ions in solution (from adjusting the pH) could diminish the repulsion between the negatively charged MAA blocks to a certain degree, but this effect is not considered to be significant.

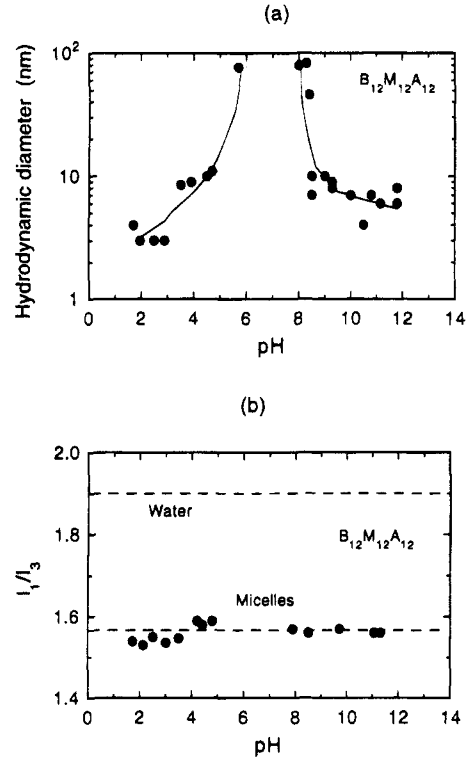

Figure 4. Effect of pH (a) on the hydrodynamic diameter of the aggregates formed in aqueous solutions of $B_{12}M_{12}A_{12}$ and (b) on the I_1/I_3 ratio afforded by the aggregates formed in aqueous solutions of $B_{12}M_{12}A_{12}$. The polymer concentration was 0.6% w/w.

The dependence of the hydrodynamic diameter of polymer $B_{12}M_{12}A_{12}$ on pH shown in Figure 4a is in agreement with the solubility curves presented by Patrickios et al.,[13] and in fact, this figure can be considered as a phase diagram for $B_{12}M_{12}A_{12}$ in aqueous solutions. A similar behavior was observed by Webber and co-workers[45-47] with the polystyrene-*block*-poly(methacrylic acid) and polystyrene-*block*-poly(*tert*-butyl methacrylate) copolymers which exhibited a strong dependence of aggregation state on solvent quality. The unimer-to-micelle transition of polystyrene-*block*-poly(methacrylic acid) copolymers was studied in 1,4-dioxane/water mixtures,[46] 1,4-dioxane/methanol mixtures,[47] and buffered water.[45] In aqueous media and at low pHs the poly(methacrylic acid) block collapsed onto the polystyrene core due to low solubility, whereas at high pHs the MAA block was stretched out as a polyelectrolyte because of deprotonation of the acid groups. The hydrodynamic radius of polystyrene-*block*-poly(methacrylic acid) micelles increased linearly with increasing pH to pH $\approx$ 7 and then remained steady on a further increase in pH, to pH $\approx$ 10, indicating that the MAA block did not deprotonate further.[45]

In the presence of 0.5 M KCl, the hydrodynamic diameter detected in a 0.6% w/w polyampholyte solution remained approximately constant at ~12 nm, indicating that micelles formed over the whole pH range examined. Furthermore, no phase separation in the vicinity of the polyampholyte pI similar to that seen in Figure 4a was observed. The effect of increasing salt concentration at a given pH is to decrease the dissociation of the acidic residues or decrease the protonation of the basic residues, whichever group is charged at that pH. This weakening of the acidic or basic character of the polymer blocks by the salt can be attributed to the decrease in intramolecular attractive electrostatic interactions and has been predicted theoretically and observed experimentally in the titration of biological polyampholytes (proteins) and synthetic polyampholytes (see Patrickios et al.[13] for references). The salting-in effect, well-known for protein solutions, is evident from the increase in the polyampholyte solubility around the isoelectric point with increasing salt concentration, a result of the screening of interpolymer electrostatic attractions between oppositely charged blocks on different polymers.[13] A dynamic light scattering study on polyampholyte $B_{12}M_{12}A_{12}$ at pH 5 showed that the hydrodynamic diameter remained ~11 nm for all of the combinations of KCl and polymer concentrations employed (the KCl concentration was varied from 0.0 to 1.0 M, and the polymer concentration from 0.1 to 5%).[13]

Temperature does not have a significant effect on the hydrodynamic diameter of the micelles formed in aqueous solutions of $B_8M_{12}A_{16}$ in the presence of 0.5 M KCl over the temperature range investigated. This is consistent with the fluorescence measurements which showed little (at pH $\approx$ 10.3) or no (at pH $\approx$ 3.7) effect of temperature on the CMC of the polyampholyte solutions.

Hydrodynamic diameters of micelles formed by $B_8M_{12}A_{16}$, $B_{12}M_{12}A_{12}$, and $B_{16}M_{12}A_8$ are reported in Figure 5 over the 2–12 pH range. The hydrodynamic size of $B_{12}M_{12}A_{12}$ micelles is generally smaller than that of $B_8M_{12}A_{16}$, which, in turn, is smaller than that of $B_{16}M_{12}A_8$ micelles. These findings can be reconciled if both DMAEMA and MAA blocks are located in the micelle corona, as in Figure 3b,d. In this case micelles of both $B_{16}M_{12}A_8$ and $B_8M_{12}A_{16}$ will have blocks consisting of 16 segments in the corona and would thus be larger than the $B_{12}M_{12}A_{12}$ micelles having blocks of 12 segments. Furthermore, DMAEMA, being more bulky than MAA, has a greater Kuhn length and may accommodate a higher number of waters of hydration than MAA and, therefore, can be expected to have a more extended structure with less coiling in the corona than MAA, resulting in a larger hydrodynamic size for the $B_{16}M_{12}A_8$ micelles than for the others.

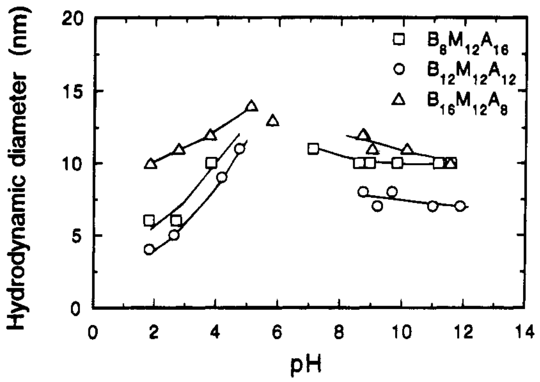

Figure 5. Hydrodynamic diameters of micelles formed by the triblock polyampholytes $B_8M_{12}A_{16}$, $B_{12}M_{12}A_{12}$, and $B_{16}M_{12}A_8$, in aqueous solutions over the 2–12 pH range. The polymer concentration was 0.6% w/w.

It appears from the dynamic light scattering data that, in the micelles formed from the BMA polyampholytes, both DMAEMA and MAA are in the corona (Figure 3b,d) regardless of the solution pH (and the positive or negative charge of the polyampholyte), while the middle hydrophobic block MMA constitutes the micellar core. The hydrodynamic diameter of 11 nm for the BMA micelles suggests that the chains are "stretched" 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 per residue).[13] A similar configuration in the micelle is attained by the Pluronic poly(ethylene oxide)-*block*-poly(propylene oxide)-*block*-poly(ethylene oxide) copolymers. In this case the micelle size is bounded by the length of the polymer chain.[48,49] In contrast, the hydrodynamic diameter of the micelles formed by $B_{12}A_{12}M_{12}$ is approximately 20 nm (more specifically, the diameter values measured were 18, 18, 33, and 25 nm at pH = 3.25, 5.56, 8.23, and 9.62, respectively, at 0.25% polymer concentration), suggesting that this polyampholyte forms aggregates in a manner different from that of the BMA polymers. The configuration of BAM molecules in the micelle is probably as shown in Figure 3e.

Micelle Aggregation Number. Static light scattering experiments were conducted for the $B_8M_{12}A_{16}$, $B_{12}M_{12}A_{12}$, and $B_{16}M_{12}A_8$ triblock polyampholytes at 25 °C. Zimm plots[41] were constructed by measuring the scattered light intensity at several angles for each of several solution concentrations at which micelles were known to exist and for a pure solvent at each angle. The micelle weight was obtained by extrapolating to zero concentration and zero angle. The pH values used were the same for all concentrations of a certain polymer, and similar to those of the fluorescence experiments. The "molecular" or "micellar" weight, second virial coefficient, and aggregation number (N_{agg}) of the micelles formed by the BMA polyampholytes are listed in Table 3 for two solution pH values (N_{agg} was calculated by dividing the micellar weight by the theoretical molecular weight of the copolymers). The average number of

Table 3. Micellar Weight, Second Virial Coefficient (10^4 cm^3 mol g^{-2}), and Aggregation Number of the Micelles Formed by the Polyampholytes at the pH Values at Which the Fluorescence Experiments Were Conducted

	low pH (≈3.1)			high pH (≈10.3)		
polyampholyte	micellar wt	A_2	N_{agg}	micellar wt	A_2	N_{agg}
$B_8M_{12}A_{16}$	37 200	−5.0	10	35 000	8.1	9
$B_{12}M_{12}A_{12}$	22 600	−1.9	5	41 100	12.1	10
$B_{16}M_{12}A_8$	86 000	−2.8	42	68 500	−2.8	16

polymer molecules associating in a micelle was relatively low for all triblock polyampholytes. N_{agg} was ≈10 for $B_8M_{12}A_{16}$ and $B_{12}M_{12}A_{12}$ and 20−40 for $B_{16}M_{12}A_8$. At pH ≈ 3, the aggregation number increases in the sequence $B_{12}M_{12}A_{12} < B_8M_{12}A_{16} < B_{16}M_{12}A_8$, the same trend as observed in the hydrodynamic diameters derived from dynamic light scattering experiments (see Figure 5). The effect of pH on micelle molecular weight and aggregation number also parallels the effect on the hydrodynamic diameters observed in the dynamic light scattering study.

Information on the interactions between the micelles can be obtained from static light scattering measurements through the second virial coefficient (A_2), as determined from the Zimm plot. A_2 was negative for all polyampholytes at pH ≈ 3, consistent with attractive interactions between micelles, while it was positive for $B_{12}M_{12}A_{12}$ and $B_8M_{12}A_{16}$ at pH ≈ 10, indicating repulsive micellar interactions. The larger negative A_2 values observed for $B_8M_{12}A_{16}$ at pH ≈ 3 and for $B_{16}M_{12}A_8$ at pH ≈ 10 can be attributed to the relatively smaller differences between the solution pH and the isoelectric points of these polyampholytes (the pI of $B_8M_{12}A_{16}$ is 5.4 and the pI of $B_{16}M_{12}A_8$ 8.0) than for the other polymers; this increases the tendency of the polymers to phase separate (precipitate).

Size exclusion chromatography (SEC) in water has also been performed to determine the sizes of polyampholyte micelles.[50] The value of the pH (8.5) and the ionic strength (1 M KCl) used in SEC were such that the polyampholytes were negatively charged and the electrostatic interactions suppressed, thus minimizing adsorption of the polyampholyte on the SEC column. The peak retention times were converted to molecular weights using both poly(ethylene oxide) and protein calibration curves. The aggregation number of the copolymer micelles was estimated by dividing the molecular weight of the triblock SEC peak by the molecular weight of the random polymer (B-*co*-M-*co*-A)$_{12}$ which does not form micelles. The aggregation numbers calculated from the two different calibration curves agreed well and ranged between 10 and 40. The larger micelles were formed by polymer $B_{12}A_{12}M_{12}$ that bears the hydrophobic MMA block at the end of the molecule (N_{agg} ≈ 40). It was anticipated that micelles formed by triblocks bearing the hydrophobic block in the middle of the molecule (BMA polyampholytes) would have a size approximately half of that of the aggregates of $B_{12}A_{12}M_{12}$ (the aggregation numbers of $B_{12}M_{12}A_{12}$, $B_{16}M_{12}A_8$, and $B_8M_{12}A_{16}$ micelles were estimated to ≈11, 17, and 25, respectively).[50] This has been confirmed by our dynamic light scattering data, which showed the hydrodynamic diameter of the $B_{12}A_{12}M_{12}$ micelles to be 20 nm, while that of the BMA copolymers was approximately 10 nm.

The values for N_{agg} obtained from our static light scattering results are comparable to the aggregation numbers obtained from SEC. The discrepancies could be attributed to the different pH and salt environments in the two studies. We were not able to obtain static light scattering information for $B_{12}A_{12}M_{12}$ because of the presence of impurities of ~100 nm. However, our dynamic light scattering data (in which we were able to resolve the bimodal distribution and determine the peak corresponding to micelles) suggest that $B_{12}A_{12}M_{12}$ molecules indeed aggregate in micelles larger than those of the BMA polyampholytes.

Conclusions

The micellization properties of some recently-synthesized, water-soluble, low-polydispersity, low-molecular-weight block methacrylic polyampholytes have been investigated using fluorescence spectroscopy and dynamic and static light scattering techniques. It is inferred that the structure of the micelles is such that the hydrophobic MMA block is always in the center of the micelle, irrespective of the net charge born by the other blocks in the polymer. Thus, if the MMA block is between the acid and basic blocks, the micelle *diameter* is restricted to be about the copolymer contour length, while if the MMA block is at the end of the molecule, the micelle *radius* is determined by the polymer contour length. These results are consistent with estimates of the aggregation numbers obtained from static light scattering experiments. Micelles were not formed by the random (B-*co*-M-*co*-A)$_{12}$ (at both pH values) and the triblock $B_{12}A_{12}M_{12}$ at basic pH, reflecting the lack of well-defined hydrophilic−hydrophobic nature in the case of the random copolymer and packing constraints imposed by having the charged block in the middle of the molecule in the case of $B_{12}A_{12}M_{12}$ at basic pH. A small increase in CMC with increasing temperature has been observed for the BMA polymers at basic pH, while no effect of temperature was detected at acidic pH.

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