Dynamic Light Scattering vs ^{1}H NMR Investigation of pH-Responsive Diblock Copolymers in Water

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ABSTRACT: The solution properties and the micellization behavior of double hydrophilic diblock copolymers depend on the degree of ionization of the pH-tunable block. This is investigated by dynamic light scattering and ^{1}H NMR spectroscopy in aqueous solutions of diblock copolymers comprising a neutral hydrophilic poly(hexa(ethylene glycol) methacrylate), PHEGMA, block and an ionizable poly(2-(diethylamino)ethyl methacrylate), PDEAEMA, block. At low pH the copolymer is in its unimer state due to the hydrophilicity of the protonated tertiary amine units, while an increase of the solution pH results in the deprotonation of the amine residues, which become hydrophobic, and leads to the formation of micelles consisting of a PDEAEMA core and a PHEGMA corona. The critical degree of ionization for chain aggregation and micelle formation is determined. It is found that the polymer exists as unimers in the aqueous solution for as low as 30% protonated amine groups, whereas for 20% degree of ionization a slight increase in polymer aggregation is observed. When the fraction of ionized amines decreases further to 10%, hydrated micelles are initially formed, followed by the formation of equilibrium micellar structures only upon the complete deprotonation of the PDEAEMA block which thus becomes fully hydrophobic. This unimer to aggregate to micelle transition is observed in the quantitative analysis of the NMR data at similar degrees of ionization to those obtained by DLS, signifying that NMR spectroscopy can be used to follow the micellization process in block copolymer systems.

Introduction

Responsive materials are of fundamental importance in many scientific areas and have been proposed for use in a variety of applications such as in drug delivery,[1] in biotechnology,[2] and in the development of sensors.[3] Responsive or "smart" polymers[4] are polymers which are able to undergo significant changes in their physicochemical properties in response to applied stimuli. "Smart" polymers are investigated in terms of their response to variations in temperature,[5−8] pH,[9−14] or ionic strength[15,16] or to the application of UV or visible light[17−19] radiation or of a magnetic field.[20,21]

Self-assembly[22] offers one of the most general strategies for generating ordered nanostructures such as micelles.[23] Micelles are formed in dilute block copolymer solutions in solvents which are selective for one of the copolymer blocks. The micellar structures consist of a dense core and a protective corona. The core is formed by the insoluble blocks, while the solvent-soluble blocks form the corona, a shell that extends in solution and confers stabilization to the micelle structure.

In recent years, great attention has been focused in the area of aqueous block copolymer micellar systems[7,9,10,24,25] due to their great potential as environmental friendly and biocompatible substitutes to micelles formed in organic media. The former micelles are formed by block copolymers comprising one hydrophilic, water-soluble part and a second hydrophobic part that dislikes water. By changing the copolymer molecular characteristics (size and/or architecture of the two parts) various micellar shapes have been observed in water, which range from spherical micelles[9−11] to vesicles,[26−28] tubules,[27,29] and complex super-aggregates.[27] The use of such aqueous micellar systems in a number of applications such as targeted drug delivery and catalysis has been also discussed.[30,31]

Two main types of block copolymers are used for micelle formation in aqueous media: amphiphilic[27] and double-hydrophilic[32,33] block copolymers. Amphiphilic block copolymers typically compose of a permanently hydrophobic, water-insoluble block that associates in aqueous solution and a hydrophilic block that prevents the aggregates from precipitation.[34−36] However, the direct dissolution of amphiphilic block copolymers in water has been proven problematic, in particular for systems comprising hydrophobic segments of high T_g which require the temporary use of an organic cosolvent, such as methanol, dimethylformamide, or tetrahydrofuran for the preparation of stable and well-defined micelles. The organic solvent plays the role of a plasticizer for the hydrophobic core and increases the unimer/micelle exchange rate, which results in the formation of equilibrium micelles. However, after the removal of the organic solvent by dialysis, the micelles formed in such copolymer systems comprise glassy cores and are thus kinetically frozen.[9,11]

One way to avoid the use of cosolvents in micelle formation is to employ the so-called double-hydrophilic block copolymers.[33] These copolymers comprise two different hydrophilic blocks, one of which undergoes physical or chemical transfor-

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Table 1. Copolymer Molecular Characteristics

copolymer[a]	M_n[b]	M_w/M_n[b]	theoretical composition (mol %)	composition by ^{1}H NMR (mol %)	effective pK_α
PHEGMA$_{50}$-*b*-PDEAEMA$_{50}$	13500	1.14	50−50	49−51	6.9

[a] The numbers denote degrees of polymerization. [b] Determined by SEC using PMMA standards.

mations in aqueous solution and becomes hydrophobic, while the other remains soluble in water. Thus, micelles are formed in these systems by a simple change in the solution conditions, i.e., pH, temperature, and/or salt concentration.[8,10,15,32,37−40] The main advantage of these copolymers is that micellization is an almost fully reversible process.

Increasing attention is paid nowadays to the micellization induced by changing the pH of the aqueous solution of a double-hydrophilic diblock copolymer. Such copolymers comprise a pH-sensitive block which undergoes a transition from hydrophilic to hydrophobic as the solution pH is changed and forms the micelle core, while the second block is hydrophilic and forms the corona of the micelle. For instance, poly(ethylene oxide)-*block*-poly(2-vinylpyridine), P2VP-*b*-PEO, diblock copolymers form unimers at low pH values when P2VP is protonated and soluble in aqueous solution and micelles at pH values higher than 5.0 when P2VP becomes deprotonated and insoluble in water.[9,41] Similar pH-sensitive poly(ethylene oxide)-*block*-poly-(2-(diethylamino)ethyl methacrylate), PEO-*b*-PDEAEMA, diblock copolymers were synthesized by oxyanionic polymerization.[7] ^{1}H NMR spectroscopy and dynamic light scattering studies have shown the molecular dissolution of the copolymer chains at low pH, when the PDEAEMA block is fully protonated and behaves as a cationic polyelectrolyte, while at high pH values, when the DEAEMA segments become deprotonated and, thus, hydrophobic, micelles are formed. The formation of both conventional and reverse micelles has been reported in aqueous media for copolymers with one pH-responsive and one salt-concentration-sensitive block,[42] whereas the associating behavior becomes more complicated when both copolymer blocks are ionizable.[24] pH-sensitive diblock copolymers can, in principle, be used as pH sensors as a result of their transformation from nonassociated chains to micelles at a particular pH range. They could also serve as model delivery systems, in which the solute is encapsulated within the micelle cores under certain pH values and is released as the micelles break apart when reaching the target pH.

In such applications the precise determination of the polymer solution properties as a function of the acidity or basicity of the solution is critical. A specific parameter that very accurately determines the physics underlying the micellization process, and thus, the polymer behavior in pH-sensitive diblock copolymers is the degree of ionization α of the ionizable block rather than the pH. This is because the pH is a property characteristic for the solution surrounding the polymer chains while the degree of protonation is responsible for the changes in the polymer structure upon addition of acid or base. Moreover, α can change from 0 to 1 in the buffering region (close to the effective pK_α of the polyelectrolyte) where the pH remains fairly constant; i.e., α allows the accurate control of the ionization of the electrolyte. However, very few studies have investigated the effect of the degree of ionization of the polymer on its solution properties. Gast et al. have used potentiometric titrations, fluorescence spectroscopy, light scattering, and small-angle neutron scattering measurements to characterize the formation of micelles in pH-sensitive block copolymer solutions as a function of the degree of ionization of the ionizable core-forming block.[10,43]

In the present work the aqueous solution properties of a symmetric poly(hexa(ethylene glycol) methacrylate)-*block*-poly-(2-(diethylamino)ethyl methacrylate), PHEGMA-*b*-PDEAEMA, diblock copolymer are investigated as a function of the degree of ionization α of the PDEAEMA block by dynamic light scattering (DLS) and proton nuclear magnetic resonance spectroscopy (^{1}H NMR). Previous studies[41] have shown that at pH 2 the copolymer is in its unimer state due to the hydrophilicity of the protonated DEAEMA units, while an increase of the solution pH to 10 results in the deprotonation of the amine groups, which become hydrophobic and lead to the formation of micelles. Herein, this unimer-to-micelle formation is followed as a function of the degree of ionization α of the PDEAEMA block. The critical degrees of ionization for chain aggregation and the sequential micelle formation are determined. It was found that for up to 70% deprotonated amine groups both blocks remain well solvated in the aqueous medium, and the polymer chains exist as unimers in solution. When the degree of ionization becomes as low as 20%, aggregation occurs followed by the formation of hydrated micelles at 10% ionized amines. The final equilibrium micellar structures are only obtained upon complete deprotonation of the PDEAEMA block which thus becomes fully dehydrated, suggesting that even a small number of charged units on the core forming block has a significant effect on the micelle structure. A good agreement in the determination of the critical degree of ionization is obtained between the DLS and ^{1}H NMR results.

Experimental Section

Materials. 2-(Diethylamino)ethyl methacrylate), PDEAEMA, monomer is commercially available and was purchased from Aldrich, whereas the hexa(ethylene glycol) methacrylate, HEGMA, monomer that was kindly donated by Inspec U.K. The PHEGMA-*b*-PDEAEMA amphiphilic block copolymer (Figure 6) was synthesized at Sussex University using group transfer polymerization chemistry, following the procedures described earlier.[44] The molecular weight, polydispersity index, and composition of the copolymer were determined by size exclusion chromatography in THF and ^{1}H NMR spectroscopy in $CDCl_3$, respectively, and are shown in Table 1.

Sample Preparation. Polymer solutions of different values of the effective degree of ionization α were prepared in water. We define the effective α as the ratio C_H/C_m, where C_H is the concentration of added acid and C_m is the polymer concentration in terms of monomeric DEAEMA units. When defined in this way, α approximates the degree of protonation of the polymer or the fraction of charged tertiary amine units of the diblock copolymers and takes values between zero and one, if one assumes that all of the protons from the added acid protonate the polymer. Polymer solutions were prepared by dissolving the appropriate amount of copolymer in Milli-Q water prefiltered through a 0.2 μm syringe filter and previously adjusted to $C_H/C_m = 2$ using 0.1 M HCl. The samples were allowed to stir overnight to ensure complete polymer dissolution. Next, the degree of ionization of the PDEAEMA block was adjusted to the required value using 0.1 M NaOH, and the solution was stirred for another 24 h before the measurement. For the DLS measurements the polymer solutions were filtered carefully through a 0.45 μm pore size filter to eliminate any dust and were left to equilibrate for about 1 h before being measured.

Dynamic Light Scattering (DLS). The autocorrelation function of the polarized light scattering intensity $G_{VV}(q,t) = \langle I(q,t)I(q,0)\rangle/$

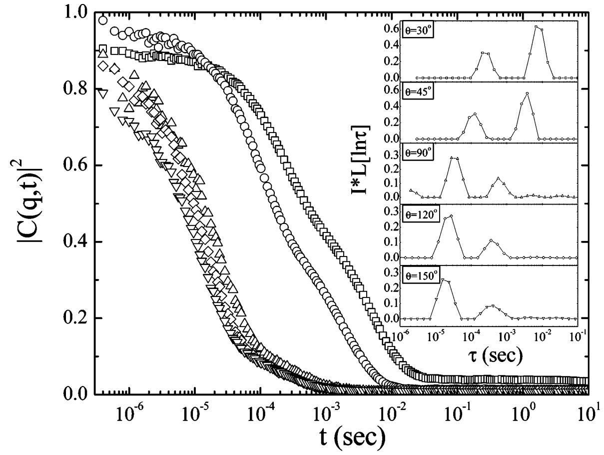

Figure 1. Intensity autocorrelation functions of a 0.1 wt % solution of PHEGMA$_{50}$-*b*-PDEAEMA$_{50}$ at degree of ionization $\alpha = 0.3$ and scattering angles 30° (□), 45° (○), 90° (△), 120° (◇), and 150° (▽). The distributions of relaxation times multiplied by the total scattering intensity (normalized to that of toluene) are shown in the insets for the respective scattering angles.

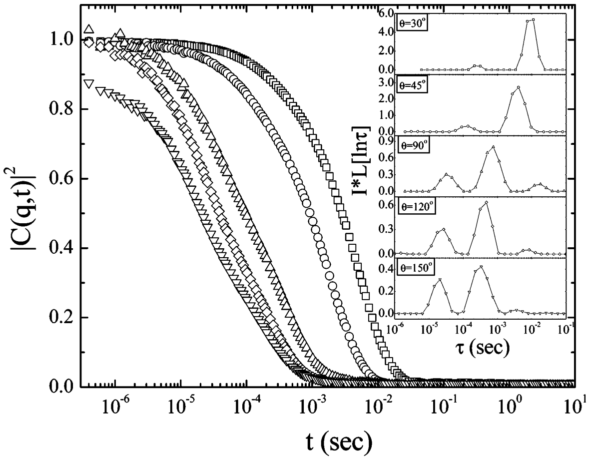

Figure 2. Intensity autocorrelation functions of a 0.1 wt % solution of PHEGMA$_{50}$-*b*-PDEAEMA$_{50}$ at degree of ionization $\alpha = 0.2$ and scattering angles 30° (□), 45° (○), 90° (△), 120° (◇), and 150° (▽). The distributions of relaxation times multiplied by the total scattering intensity (normalized to that of toluene) are shown in the insets for the respective scattering angles.

$\langle I(q,0)\rangle^2$ was measured at different scattering angles, θ, using an ALV spectrophotometer and an ALV-5000 full digital correlator over the time range $10^{-7}-10^{3}$ s; $I(q,0)$ is the mean scattering intensity. Generally both the incident and the scattered beam were polarized perpendicular to the scattering plane (VV geometry). An Adlas diode-pumped Nd:YAG laser was used as the light source with wavelength $\lambda = 532$ nm and single mode intensity 100 mW. The magnitude of the scattering wavevector is $q = (4\pi n/\lambda)\sin(\theta/2)$, where n is the refractive index of the medium.

Under homodyne conditions, $G_{VV}(q,t)$ is related to the desired scattered field autocorrelation function $C(q,t)$ by $C(q,t) = \{[G_{VV}(q,t) - 1]/f^*\}^{1/2}$, where f^* is an experimental factor calculated by means of a standard. The experimental correlation functions $C(q,t)$ are analyzed by performing the inverse Laplace transform (ILT) using the routine CONTIN assuming a superposition of exponentials for the distribution of relaxation times $L(\ln \tau)$, i.e., $C(q,t) = \int_{-\infty}^{\infty} L(\ln \tau) \exp[-t/\tau]\, d(\ln \tau)$. The rate Γ of each process is calculated as the inverse of its relaxation time $1/\tau$, whereas their dynamic intensities are calculated from the integrals under the peaks of $L(\ln \tau)$ multiplied by $I(q,0)$. In the case of a diffusive process, its diffusion coefficient D is obtained from the slope of Γ vs q^2 by $\Gamma = Dq^2$. The latter is related to the hydrodynamic radius R_h of the diffusing moiety by the Stokes−Einstein equation $R_h = k_BT/(6\pi\eta D)$, where η is the viscosity of the solvent, k_B is the Boltzmann constant, and T is the temperature of the sample. (It is assumed at these low concentrations that D corresponds to its limit for concentration $c \rightarrow 0$.) All measurements were performed at 20 °C.

^{1}H Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR). The aqueous solution properties of the pH-sensitive diblock copolymers were also investigated by ^{1}H NMR spectroscopy using a Bruker AMX-500 spectrometer. Samples were prepared as described above at a 2 wt % polymer concentration in D_2O 99.9%, whereas the degree of ionization was accurately adjusted to the required value using either a 0.1 wt % DCl or a 0.1 wt % NaOD solution in D_2O. All samples were allowed to stir for 1 day in order to equilibrate before being measured.

Results and Discussion

Dynamic Light Scattering Measurements. The aqueous solution properties and the micellization process for a PHEGMA$_{50}$-*b*-PDEAEMA$_{50}$ diblock copolymer in dilute solution as a function of the degree of protonation of the PDEAEMA block were studied by DLS.

Figure 1 shows the intensity autocorrelation functions of a 0.1 wt % PHEGMA$_{50}$-*b*-PDEAEMA$_{50}$ diblock copolymer solution for degree of protonation $\alpha = 0.3$ at different scattering angles. The distributions of relaxation times are shown in the inset. The observed behavior is identical to that obtained for all α between 1 and 0.3 (not shown). Two relaxation processes both with low intensities are observed. The fast process with diffusivity $D_1 = \lim_{q\rightarrow 0}(\Gamma_1/q^2) = 5.9 \times 10^{-7}$ cm^2/s, which corresponds to a hydrodynamic radius $R_{h,1} = 3.6 \pm 0.2$ nm, is attributed to single polymer chains, while a second slower process with diffusivity $D_2 = \lim_{q\rightarrow 0}(\Gamma_2/q^2) = 3.0 \times 10^{-8}$ cm^2/s, which corresponds to $R_{h,2} = 71 \pm 2$ nm, is also observed. The latter process possesses a q-dependent intensity, which, when fitted with the Guinier expression $I(q) = I(0) \exp(-q^2R_g^2/3)$, results in a radius of gyration $R_g \approx 89 \pm 4$ nm and signifies some kind of polymer aggregation suggested in earlier studies for low-pH copolymer solutions.[41] It is worth noting that both processes exhibit low intensities; this is expected for the unimer chains due to their small size, whereas for the polymer aggregates, it suggests that they are either very few in number or highly hydrated and, thus, have a low scattering contrast. The situation is somehow modified for $\alpha = 0.2$ regarding the intensities due to the aggregates. As can be seen in Figure 2, two processes are again identified with diffusivities $D_1 = 5.6 \times 10^{-7}$ cm^2/s and $D_2 = 3.2 \times 10^{-8}$ cm^2/s, corresponding to hydrodynamic radii $R_{h,1} = 3.9 \pm 0.2$ nm and $R_{h,2} = 66 \pm 2$ nm, respectively, which are attributed to the diffusion of single polymer chains and polymer aggregates as discussed above. However, the intensities of the slow process are higher than those for $\alpha = 0.3$. This is because the fraction of deprotonated DEAEMA units increases with decreasing α and so do the hydrophobic interactions of the polymer chains, leading to an enhanced polymer aggregation. It should be noted that it is the number of polymer aggregates and/or degree of dehydration which increases, while their hydrodynamic size does not change significantly from that obtained at higher values of α. When the effective degree of protonation decreases further to $\alpha = 0.1$, the light scattering data are very different (see Figure 3). The scattering intensities associated with both processes are now significantly high (insets of Figure 3), whereas the two processes identified in the intensity autocorrelation functions possess diffusivities $D_1 = 1.9 \times 10^{-7}$ cm^2/s and $D_2 = 2.8 \times 10^{-8}$ cm^2/s, which correspond to hydrodynamic radii $R_{h,1} = 11.3 \pm 0.5$ nm and $R_{h,2} = 76 \pm 2$ nm. The fast process is now due to the formation of micelles consisting of a core of PDEAEMA and a corona of PHEGMA chains, while the slower process is

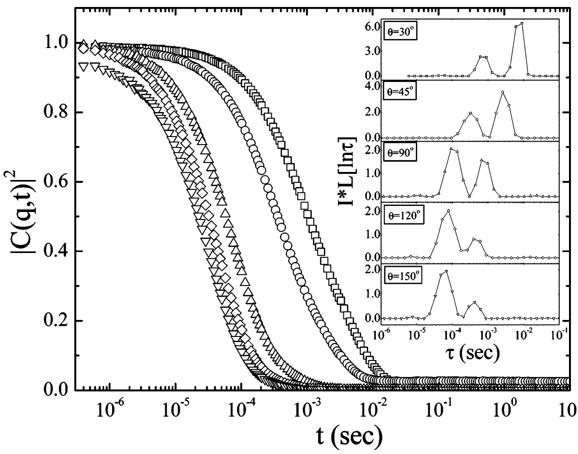

Figure 3. Intensity autocorrelation functions of a 0.1 wt % solution of PHEGMA$_{50}$-*b*-PDEAEMA$_{50}$ at degree of ionization α = 0.1 and scattering angles 30° (□), 45° (○), 90° (△), 120° (◇), and 150° (▽). The distributions of relaxation times multiplied by the total scattering intensity (normalized to that of toluene) are shown in the insets for the respective scattering angles.

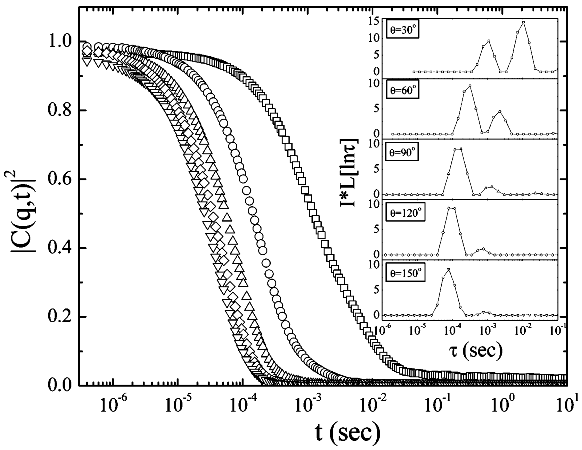

Figure 4. Intensity autocorrelation functions of a 0.1 wt % solution of PHEGMA$_{50}$-*b*-PDEAEMA$_{50}$ at degree of ionization α = 0 and scattering angles 30° (□), 60° (○), 90° (△), 120° (◇), and 150° (▽). The distributions of relaxation times multiplied by the total scattering intensity (normalized to that of toluene) are shown in the insets for the respective scattering angles.

attributed to the diffusion of a few polymer aggregates. Both the size and the intensity of the micelles are lower than those obtained in previous studies for the copolymer solution at high pH.[41]

At α = 0.0 (see Figure 4) a sharp increase in the scattering intensity is observed (insets of Figure 4), and two diffusive processes are obtained in the distributions of relaxation times with $D_1 = 1.4 \times 10^{-7}$ cm^2/s and $D_2 = 1.9 \times 10^{-8}$ cm^2/s and corresponding hydrodynamic radii $R_{h,1} = 15.1 \pm 0.5$ nm and $R_{h,2} = 113 \pm 4$ nm, respectively; the fast process is now due to the diffusion of equilibrium polymer micelles with size similar to that found in previous studies for high pH copolymer solutions, while the second slower process is attributed to the diffusion of a few micellar aggregates.[41] The sharp increase in the scattering intensity is consistent with the increase in the hydrophobicity of the PDEAEMA block, which results in the formation of micelles with fully dehydrated cores of increased contrast and/or in the formation of a large number of micelles. Moreover, because of the small size of these micelles, their scattering intensities are independent of the wavevector *q* while the slower process exhibits a strongly *q*-dependent intensity, which, when fitted with the Guinier expression, results in a radius of gyration $R_g \approx 125 \pm 4$ nm.

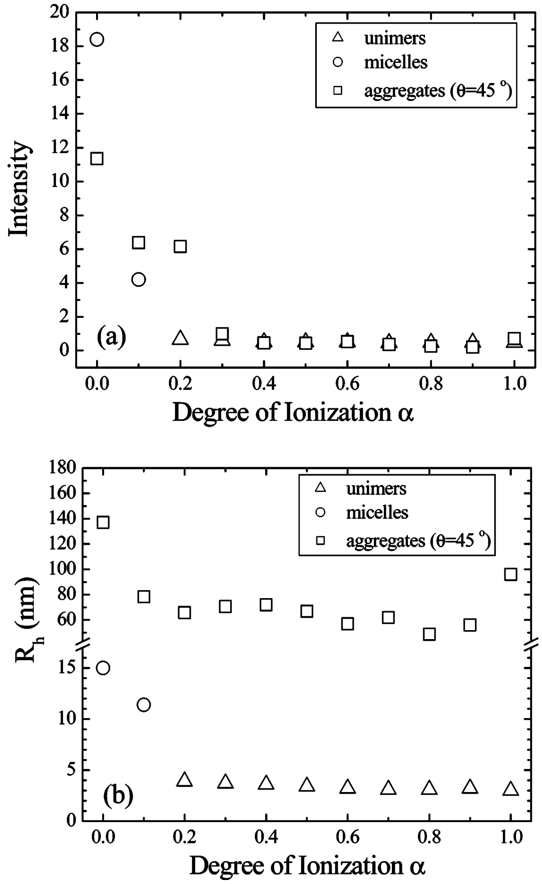

Figure 5. Scattering intensities normalized to that of toluene (a) and hydrodynamic radii (b) of a 0.1 wt % PHEGMA$_{50}$-*b*-PDEAEMA$_{50}$ diblock copolymer solution as a function of the degree of ionization α of the PDEAEMA block.

Figure 5 shows the scattering intensities and the hydrodynamic radii of the diffusing species vs the degree of protonation of the DEAEMA units for a 0.1 wt % aqueous solution of the PHEGMA$_{50}$-*b*-PDEAEMA$_{50}$ diblock copolymer. For $0.3 \leq \alpha < 1.0$ two processes of low intensity are consistently obtained. The fast process is attributed to the diffusion of single copolymer chains with hydrodynamic radius $R_{h,1} \sim 4$ nm and a *q*-independent intensity $I \sim 0.5$, while the slower process is attributed to the diffusion of polymer aggregates with an $R_{h,2} \sim 70$ nm and a very low *q*-dependent intensity ($I(\theta = 45°) \sim 0.5$), which, when fitted with the Guinier expression, results in a radius of gyration $R_g \approx 80 \pm 4$ nm. The scattering intensity of the aggregates remains low, suggesting that they are either very few in number or highly hydrated and, thus, have low contrast. At α = 0.2 both the intensity and the size of the unimers remain constant while the scattering intensity due to the aggregates increases at constant size. This increase in the intensity of the aggregates suggests an increase in either their number or their contrast, which is attributed to the increased fraction of deprotonated DEAEMA units leading to more hydrophobic interactions between the polymer chains and, thus, to polymer aggregation. For α = 0.1, a strong increase of the scattering intensity is observed, accompanied by a large increase of the hydrodynamic size of the fast process to $R_h = 11$ nm. This latter process is attributed to the diffusion of polymer micelles, which are formed at α = 0.1 when only 10% of the amines remain charged and hydrophilic. Finally, for α = 0.0 a sharp increase of the scattering intensity accompanied by an

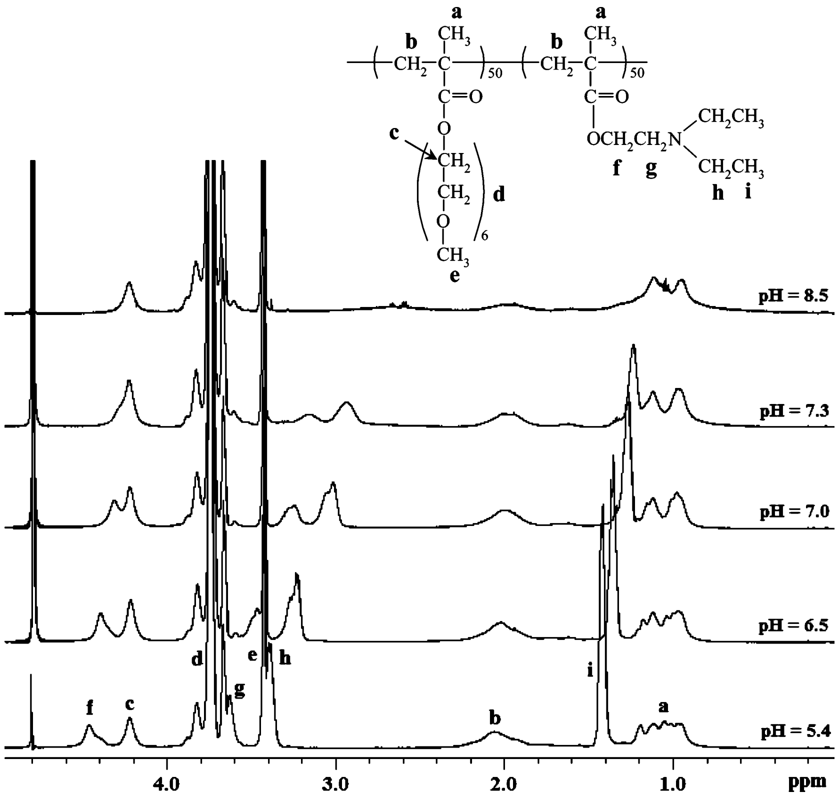

Figure 6. ^{1}H NMR spectra of a 2 wt % PHEGMA$_{50}$-*b*-PDEAEMA$_{50}$ copolymer solution in D_2O at pH values 5.4, 6.5, 7.0, 7.3, and 8.5.

increase in the micelle size ($R_h = 15$ nm) are observed, which reach their equilibrium values obtained in a previous work at high pH (>7) copolymer solutions. An increase of the scattering intensity and the hydrodynamic radius of the slow process are also observed at $\alpha = 0.0$ attributed to the formation of a few micellar aggregates. These micellar aggregates are attributed to a noneffective steric stabilization conferred by the short hydrophilic PHEGMA blocks that form the micelle corona.

^{1}H NMR Experiments. Proton nuclear magnetic resonance spectroscopy was also used to investigate the effect of the degree of ionization of the PDEAEMA block on the molecular properties of the PHEGMA$_{50}$-*b*-PDEAEMA$_{50}$ diblock copolymer and its solution behavior in water.

This study was stimulated by an earlier NMR investigation of a PEO$_{45}$-*b*-PDEAEMA$_{34}$ diblock copolymer solution as a function of pH, which allowed the determination of the core forming block that becomes insoluble upon micellization, while the second block remained well solvated in the micelle corona conferring the stability to the copolymer micelles.[7] The latter was observed in the ^{1}H NMR spectrum of the copolymer as a disappearance of the peaks attributed to the protons of the insoluble core-forming block upon micellization, while the peaks of the protons of the block in the corona of the micelles remained visible both before and after micellization. In the present study, spectra of the PHEGMA$_{50}$-*b*-PDEAEMA$_{50}$ copolymer are initially recorded in the pH range from 5.4 to 8.5, where deprotonation of the PDEAEMA block occurs and micellization takes place as confirmed earlier by DLS.[37] This allowed a detailed investigation of the copolymer solution properties in the critical pH region in which changes in the copolymer molecular structure occur and structure formation is induced. The ^{1}H NMR spectra of the PHEGMA$_{50}$-*b*-PDEAEMA$_{50}$ diblock copolymer from pH 5.4 to 8.5 are shown in Figure 6. The signals observed at 1.4, 3.3−3.4, 3.6−3.7, and 4.4−4.5 ppm are due to the protons of the DEAEMA units, while signals at 3.4−3.5, 3.7−3.8, and 4.2 correspond to the protons of the HEGMA units. At pH 5.4 all signals attributed to the protons of the PHEGMA and PDEAEMA blocks are visible. However, as the pH increases from 6.5, to 7.0, and then 7.3, the intensities of the signals due to the protons of the DEAEMA units decrease. Finally, at pH 8.5 the PDEAEMA block becomes completely deprotonated, and the signals due to the protons of the DEAEMA units disappear, indicating the formation of micelles with fully dehydrated cores. Moreover, besides the decrease in their intensity the peaks attributed to the PDEAEMA block seem to shift to lower ppm as the pH is increased. These are related to the decrease of the effective degree of ionization of the DEAEMA units as the pH increases.

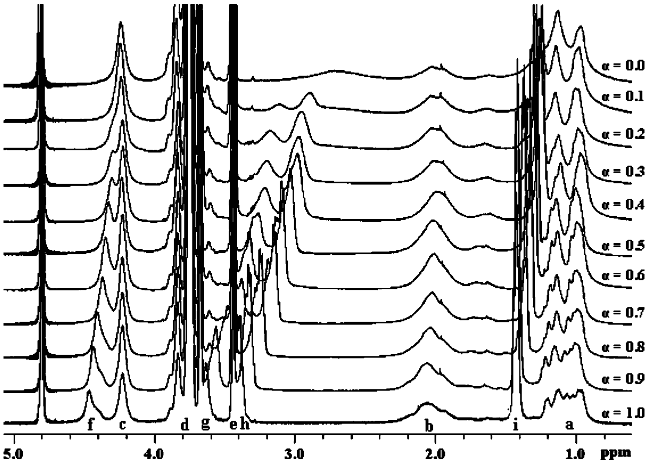

Figure 7. ^{1}H NMR spectra of a 2 wt % PHEGMA$_{50}$-*b*-PDEAEMA$_{50}$ copolymer in D_2O as a function of the degree of ionization α of the PDEAEMA block of the copolymer from $\alpha = 1.0$ to $\alpha = 0.0$.

Figure 7 shows the ^{1}H NMR spectra for 2 wt % PHEGMA$_{50}$-*b*-PDEAEMA$_{50}$ diblock copolymer solutions as the degree of ionization α is varied from 0.0 to 1.0, with a step of 0.1, which corresponds to a 10% change in the degree of ionization. The

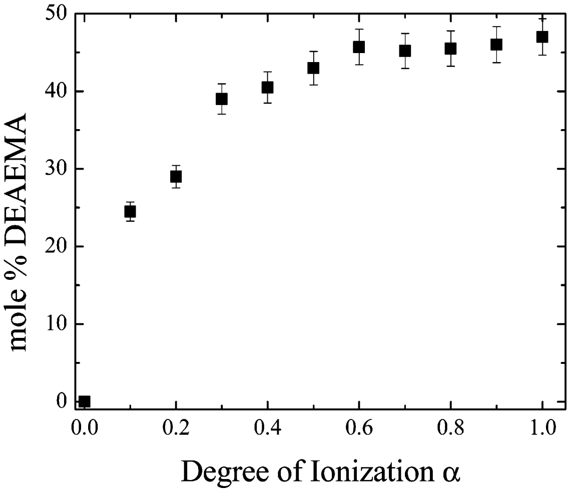

Figure 8. Mole percentage of the soluble DEAEMA units as a function of the degree of ionization α of the PDEAEMA block for the PHEGMA$_{50}$-*b*-PDEAEMA$_{50}$ copolymer.

gradual shift of the peaks at 1.4, 3.3−3.4, 3.6, and 4.4−4.5 ppm corresponding to the hydrogen atoms of the DEAEMA side groups to lower ppm is clearly observed as α is lowered, and the DEAEMA units become deprotonated. This is attributed to the more effective shielding of the DEAEMA hydrogen atoms from the electron pair of the deprotonated nitrogen at low α values, causing them to shift upfield. Moreover, it is consistent with the exchange of the protons between the charged and noncharged amine groups, thus leading to an observed mean electron shielding of the DEAEMA hydrogen atoms, which appears as a gradual change in the position of the peaks attributed to the PDEAEMA block. The latter seems at first to contradict results obtained earlier for partially quaternized amine-based polymers where distinct hydrogen signals at constant ppm values were obtained for the neutral and charged monomer units.[45] However, in those systems no exchange mechanism could take place, and thus, the difference in the electron density around the nitrogen atom resulted in the appearance of discrete hydrogen signals the relative integrals of which depict the degree of quaternization. The disappearance of the PDEAEMA peaks at $\alpha = 0.0$ is observed as well in Figure 7, which confirms the formation of micelles with the deprotonated PDEAEMA blocks being immobilized in the micelle core, while the well-solvated PHEGMA blocks comprise the micelle corona. The above effect has been reported previously for systems that can self-assemble when changing the solution temperature. In these studies, structure formation due to intermolecular interactions resulted in changes of the shielding effects on the hydrogen atoms and a decrease of freedom of movement which was observed as a gradual shift and broadening of the position of the proton signals.[8,46] In a similar work, ^{1}H NMR has been used to probe the intramolecular ordering as a function of chain length of synthetic molecules. The ordering resulted in upfield shifts of the aromatic hydrogen atoms of the molecules due to their more effective shielding by the electrons of the aromatic ring.[47] However, the present work is the first example where such a shift in the NMR signals is observed upon changing the solution pH and thus the degree of ionization α of ionizable monomer units along a polymer molecule.

Figure 8 illustrates the quantitative ^{1}H NMR data, where the mole fraction of DEAEMA units that are present in solution (calculated from the peak integrals of the NMR spectra) are plotted as a function of α. A gradual decrease in the number of DEAEMA units that remain in solution is observed from $\alpha = 1.0$ to $\alpha = 0.3$, while for $\alpha = 0.2$ a rather sharp decrease of the soluble DEAEMA moles is obtained. This decrease becomes even more abrupt below $\alpha = 0.1$, which is in good agreement with the formation of micelles at such low degrees of protonation, as observed above by DLS.

The consistency between the DLS and ^{1}H NMR results as a function of the degree of protonation α of the PHEGMA$_{50}$-*b*-PDEAEMA$_{50}$ diblock copolymer, suggesting clearly that the changes in the solution properties of the polymer as α is varied between 1.0 and 0.0 are closely related to the changes in the polymer molecular structure for the same values of α. Thus, for $0.3 \leq \alpha \leq 1.0$ the number of soluble DEAEMA units obtained by NMR decrease only gradually, and the polymer exists mainly as unimers in solution as observed by DLS. However, for $\alpha = 0.2$ the intensity of the polymer aggregates increases, as observed by DLS, accompanied by a simultaneous decrease in the number of soluble DEAEMA residues, found by NMR. This result suggests an increase in the hydrophobicity of the PDEAEMA block at this value of α, thus leading to increased polymer aggregation before the formation of the equilibrium micelles at $\alpha = 0.0$, which is also observed as a disappearance of the peaks corresponding to the protons of the PDEAEMA block in the NMR spectrum and, thus, a sharp drop to zero of the soluble DEAEMA units.

It is interesting to note that both the DLS and ^{1}H NMR results suggest that the polymer remains well solvated for as low as 30% protonated DEAEMA units, while micelles are only formed when 10% of the amine units are ionized. The latter seems to contradict earlier findings by Gast et al.[10] for a symmetric PDMAEMA-*b*-PDEAEMA diblock copolymer where micellization was observed for higher degrees of ionization $\alpha = 0.31$. This may be explained by the lower molecular weight of the polymer investigated in the present study, which thus remains in solution for a broader range of α and requires a larger fraction of deprotonated hydrophobic amine groups per polymer chain for micelles to form. Finally, equilibrium micelles are only formed when all DEAEMA groups become deprotonated, and no charges are present along the polymer chain. The latter is in good agreement with the results presented by Gast et al. for a similar PEO-*b*-PDEAEMA diblock copolymer where a decrease in the R_h of the micelles with increasing α was found and was attributed to a decrease in the aggregation number caused by electrostatic repulsions between the core-forming PDEAEMA blocks.[43] The triggering of the micellization process of the PHEGMA$_{50}$-*b*-PDEAEMA$_{50}$ diblock copolymer investigated in this study in such a narrow range of α could be advantageous for the development of very sensitive sensing elements, where even very small changes in the acidity/basicity of the solution can be observed or in biomedical applications where, for example, a very small change in the pH could trigger the release of a drug that has been previously loaded in the micellar structures.

Conclusions

DLS and ^{1}H NMR spectroscopy have been used to investigate the solution properties of a symmetric pH-sensitive PHEGMA-*b*-PDEAEMA diblock copolymer in aqueous media as a function of the degree of ionization α of the pH-sensitive PDEAEMA block. The polymer remains well solvated in its unimer state for degrees of ionization as low as 0.3, while chain aggregation, observed by DLS as an increase in the scattering intensity and by NMR as an abrupt reduction of the soluble DEAEMA monomer units, is induced for only 20% ionized amines. This aggregation results in the formation of precursor micelles at degree of ionization 0.1, which obtain their equilibrium nonhydrated structure only upon complete deprotonation of the PDEAEMA block when all groups within the micelle core become uncharged. The equilibrium micellar size was found around 15 nm with the PDEAEMA blocks forming the micelle cores and the PHEGMA ones comprising the micelle coronas. Both DLS and NMR results are in good agreement over the whole range of α and reveal a detailed picture of the copolymer solution properties and the micellization process upon subtle changes on the molecular level.

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