

***p*H- and temperature-dependent phase behavior of a PEO-PPO-PEO-based pentablock copolymer in aqueous media**

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We investigated the structural features of micelles formed by the self-association of the pentablock copolymer poly[*N,N*-(diethyl amino)ethyl methacrylate]-block-poly(ethylene oxide)-block-poly(propylene oxide)-block-poly(ethyleneoxide)-block-poly[*N,N*-(diethylamino)ethyl methacrylate] (PDEAEM-PEO-PPO-PEO-PDEAEM) in aqueous solutions by using small-angle neutron scattering SANS. The pentablock copolymer solutions exhibit micellar and gel phases in response to changes in both the temperature and *p*H by virtue of (1) the lower critical solution temperature of the PPO blocks and (2) the polyelectrolyte character of the pendant PDEAEM blocks. Two modeling schemes were employed to describe the SANS data of semidilute copolymer solutions at higher temperature as they contain interacting charged micelles at *p*H < 7.5 and interacting neutral micelles at higher *p*H. We have elucidated the structures of the micelles in terms of size, shape, polydispersity, association number, number density, and surface charge. At low *p*H the charged spherical micelles are less packed with the copolymers presumably due to the electrostatic repulsion between the charged pendant groups. On the other hand, at higher *p*H the hydrophobic character of the neutral pendant groups enable them to sequester within the micelle core along with the PPO, thus increasing the number density and the core size of the spherical micelles. At higher copolymer concentration reversible thermoresponsive sol-gel transitions were observed at all *p*H conditions and the rheological behavior of the gels nicely correlates with different organization of micelles with different shapes.

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I. INTRODUCTION

Amphiphilic block copolymers with temperature and *p*H-dependent properties offer as versatile materials in a number of applications, ranging from drug delivery to personal care formulations. With the advent of living radical polymerization techniques such as atom transfer radical polymerization (ATRP) and reversible addition-fragmentation transfer polymerization (RAFT), a number of stimuli responsive block copolymers have been developed using the polymerization of water-soluble, functional monomers [1,2]. These systems self-assemble to form micelles with nanometer dimensions in response to changes in solution conditions. Recently, a number of block copolymers has been synthesized that exhibited both temperature [3] and *p*H responsive [4–8] behaviors. The pentablock copolymer considered in this study was designed for the use in a stimuli-responsive drug delivery formulation. Cell culture cytotoxicity studies of the pentablock copolymer gels indicated its good overall biocompatibility [9]. We have explored the use of the pentablock copolymer for the solubilization and release of low molecular weight hydrophobic drugs and therapeutic DNA complexes [10,11]. The copolymer was synthesized by ATRP with a Br-terminated Pluronic© F127 PEO-PPO-PEO triblock copolymer macroinitiator as previously described [2]. The resulting poly[*N,N*-(diethyl amino)ethyl methacrylate]-block-

poly(ethylene oxide)-block-poly(propylene oxide)-block-poly(ethyleneoxide) - block - poly [*N,N* - (diethylamino)ethyl methacrylate] copolymer has been observed to form micelles and gels as a function of temperature and *p*H [12].

The PEO-PPO-PEO triblock copolymers have been extensively studied and their rich phase behavior in aqueous media as a function of polymer concentration, temperature, the type and concentration of anion additives is well documented [13–19]. In these systems the micellar phenomenon is dominantly governed by the lower critical solution temperature (LCST) behavior of the central PPO block [13,19] that becomes hydrophobic either at high temperatures or in the presence of anions [18] and both. The pentablock copolymer also exhibits a similar thermoreversible micellar phenomenon due to the LCST of the central PPO block, resulting in micelles with fairly dehydrated PPO cores surrounded by hydrated PEO-PDEAEM corona at low *p*H [12]. The pendant tertiary amine groups of the PDEAEM blocks are charged and hydrophilic at low *p*H, resulting in a micellar corona of ionized electrolyte chains. At *p*H above 7.5 the PDEAEM blocks become increasingly deprotonated and hydrophobic [11]. Under these conditions the hydrophobic PDEAEM segments have to reorganize and sequester within the micellar core in order to decrease the overall free energy of the system. Coarse grained molecular dynamics simulations [20] provide clues to the difference in morphologies of the micelles formed by the pentablock at different *p*H and temperature. Experimental studies on the surface properties of this system at the air-water interface showed the *p*H and

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temperature-dependent micelle-to-hydrogel transition [21]. However, experimental data on the structure of the micelles in bulk solutions are not yet available. In this paper we present detailed structural analysis of the micelles formed by the pentablock copolymer in aqueous solutions as a function of pH , temperature, and concentration.

Small angle x-ray and neutron scattering techniques are versatile in elucidating detailed information on the phase behavior and the morphological transitions of the self-assembled micelles and gels. In addition, the unique scattering contrasts and the absolute scattering cross section obtainable with small-angle neutron scattering (SANS) enable quantitative determination of the structure and morphology of micelles and vesicles as well as their organization in ordered arrays in response to changes in temperature and electrolyte concentration. SANS and 1H NMR were used to probe the geometry, size, and charge of the pentablock micelles in the aqueous media as a function of pH and temperature. Rheology was used to investigate the viscoelastic properties of the hydrogels of the pentablock copolymer at high concentrations (>12 wt %) in the aqueous medium.

II. EXPERIMENTAL SECTION

A. Materials

The PDEAEM₂₅-PEO₁₀₀-PPO₆₅-PEO₁₀₀-PDEAEM₂₅ copolymer investigated was synthesized as previously reported [11]. The molecular weight of the copolymer, characterized as previously reported was, $M_n=21900$ and $M_w/M_n=1.27$ as determined by 1H NMR and gel permeation chromatography (GPC)

B. NMR

Solutions of the pentablock copolymer were prepared by dissolving the copolymer in D₂O (99.9% deuterium, Aldrich) by 4 wt % at $pH=3$. The quoted pH values are the readings from the pH meter, not the pD . The pH of the solution was adjusted by the addition of DCl (30% in D₂O) and NaOD (5 M in D₂O). Aliquots were periodically removed as the solution was carefully titrated to pH 11. Samples were diluted to 2 wt % with D₂O. All 1H NMR spectra were collected using a Varian VXR400 (400 MHz) spectrometer, and chemical shifts are given in ppm.

C. Small-angle neutron scattering

SANS experiments were carried out using the time-of-flight small-angle neutron diffractometer (SAND) at the Intense Pulsed Neutron Source (IPNS) at Argonne National Laboratory. This instrument provided data in the Q ($4\pi \sin \theta/\lambda$ where 2θ is the scattering angle and λ is the wavelength of the neutrons) range of $0.004-0.5 \text{ \AA}^{-1}$ in a single measurement by using a $40 \times 40 \text{ cm}^2$ position-sensitive 3He gas detector and neutrons with wavelengths in the range of $1-14 \text{ \AA}$ ($\Delta\lambda=0.05$ at each λ). Pentablock copolymer solutions were sealed in Suprasil cells with a 2 mm path length for the SANS measurements. Temperature was maintained to within $\pm 1 \text{ }^\circ\text{C}$ during measurements by using a thermostated water bath. The scattering data were corrected

for empty cell and solvent scattering, detector sensitivity, and sample transmission, and were calibrated to the absolute scale in units of cm^{-1} with scattering standards.

D. Small-angle scattering analysis

The organization of the block copolymer molecules in micelles is strongly influenced by the extent of ionization of the pendant groups. Hence the micelles formed at different pH conditions are expected to have different organization of the various moieties of the block copolymer, requiring different modeling schemes to elucidate their structural properties. Here we describe the two different models used to elucidate the micellar structures at different solvent conditions.

The scattering intensity of a system of monodisperse particles can be expressed into contributions of form factor and structure factor in a decoupled form as

$$I(q) = N_s(\Delta b)^2 F(q)S(q) + I_{\text{inc}}, \quad (1)$$

where N_s is the number density of the scattering particles, Δb is the excess scattering length of a single particle with respect to its surrounding medium, $F(q)$ is the single particle form factor, $S(q)$ is the structure factor due to interparticle interactions, and I_{inc} is the incoherent background scattering.

At low temperatures most of the copolymer molecules exist as non-associated individual particles (unimers) dispersed in solution and their conformation can be approximated by a flexible random coil (Gaussian chain) whose scattering profile is given by the Debye function

$$F_c(q, R_g) = \frac{2[\exp(-z) - 1 + z]}{z^2}, \quad (2)$$

where $z=q^2 R_g^2$ with R_g being the radius of gyration of the polymer chain. We also used the Gaussian coil approximation to account for the unimer contribution to the scattering profile at conditions when micelles coexist with unimers.

At elevated temperatures, the PPO segments become increasingly hydrophobic and self-assemble to form the micelle core. The PEO segments are hydrophilic and remain in the hydrated micelle corona. The charges carried by the PDEAEM segments will vary with pH of the solution due to the variation in the extent of ionization. When they are charged at $pH < 7.7$ (see Fig. 1) they are fairly hydrophilic and hence they are expected to organize at the corona region along with the PEO segments. The charged spherical micelles will experience electrostatic repulsion in solution and this can be accounted for by using a structure factor of macroions [22,23], along with the form factor for the spherical micelles. Recent reviews provide analytical expressions for the form factors of a number of geometrical shapes along with structure factors that can be used to model the scattering data from block copolymer micelles. [24–26]. The cryo-TEM images of the pentablock copolymer in solution indicated the presence of spherical micelles [11], hence the use of a form factor for a spherical geometry is appropriate for modeling the scattering data.

Micelles in solution generally have polydispersity in their size. Furthermore, the solution also contains unimers in equilibrium with the micelles. Hence Eq. (1) needs to be inte-

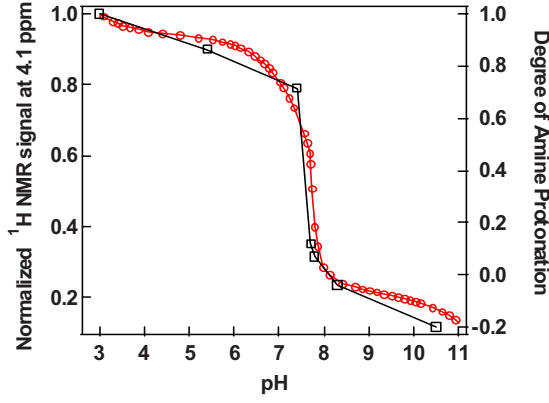


FIG. 1. (Color online) The normalized ^1H NMR signal at 4.1 ppm, corresponds to the degree of PDEAEM block hydration (\square , left axis), and the degree of amine protonation (solid line, right axis) determined by potentiometric titration.

grated over the population distribution of micelles with different size. For our study, we consider only the influence of polydispersity on the particle scattering form factor $F(q)$ while using a structure factor $S(q)$ for an average particle size for simplicity. The scattering intensity for such a system is

$$I(q) = I_{\text{inc}} + S(q) \int \Omega(R) (\Delta b)^2 F(q, R) dR, \quad (3)$$

where $\Omega(R)$ is the number distribution function of micelles with radius R . For our current data modeling we chose a Gaussian distribution to describe the polydispersity of the overall micelle size and allowed all individual regions of the micelle to scale proportionally.

Micelles with uncharged PDEAEM segments at pH 8.1. At pH 8.1, the PDEAEM segments are uncharged. Even though the PDEAEM segments are at the termini of the pentablock copolymer, their uncharged nature render them hydrophobic, and thus they will assemble together with the PPO segments to form the micelle core, while the micelle corona is composed of hydrated PEO segments whose two termini loop back into the core (our NMR data discussed later supports this hypothesis). In this case, the micelle can be modeled nicely by the Pedersen core-chain form factor [25] corresponding to a densely packed spherical core whose surface is attached with flexible, hydrated Gaussian chains of PEO. We have used this model previously to describe the size and shape of Pluronic® F88 micelles [18] in D_2O and it can be written as

$$(\Delta b)^2 F(q) = n^2 (\Delta b_s)^2 F_s + 2n (\Delta b_c)^2 F_c + 2n(2n-1) (\Delta b_c)^2 F_{cc} + 4n^2 \Delta b_s \Delta b_c F_{sc}, \quad (4)$$

where n is the micelle association number, Δb_s is the total excess scattering length of the hydrophobic segments per molecule in the core (one PPO segment plus 2 PDEAEM segments: -0.135 \AA), and Δb_c is the total excess scattering length of one hydrophilic PEO segment in the corona (-0.042 \AA). We also use this model to fit the scattering data from the F127 micelles.

The self-correlation term $F_c(q, R_g)$ for an attached Gaussian chain in the corona has the same form as Eq. (2) with R_g now being the radius of gyration of the PEO segment. The self-correlation term $F_s(q, R_0)$ for the spherical dense core with a radius R_0 , the interference cross-term F_{sc} between the spherical core and an attached Gaussian chain, and the interference term F_{cc} between Gaussian chains are given by

$$F_s(q, R_0) = \frac{9[\sin(qR_0) - qR_0 \cos(qR_0)]^2}{(qR_0)^6}, \quad (5)$$

$$F_{sc} = \frac{3[\sin(qR_0) - qR_0 \cos(qR_0)]}{(qR_0)^3} \times \frac{[1 - \exp(-q^2 R_g^2)] \sin[q(R_0 + dR_g)]}{(qR_g)^2 q(R_0 + dR_g)}, \quad (6)$$

$$F_{cc} = \frac{[1 - \exp(-q^2 R_g^2)]^2}{(qR_g)^4} \left\{ \frac{\sin[q(R_0 + dR_g)]}{q(R_0 + dR_g)} \right\}^2, \quad (7)$$

where $R_0 + dR_g$ is the effective distance between the center of the core and the center of an attached Gaussian chain, with d being a constant (dR_g is the distance of the center of the attached Gaussian chain to the core surface).

The structure factor $S(q)$ in Eq. (3) for spherical micelles is formulated by assuming a hard sphere interaction potential of the Percus-Yevick approximation and spatial correlation fluctuations of the classical Ornstein-Zernike approximation. The structure factor $S(q)$ is analytically expressed for the average micelle size as

$$S(q) = \frac{1}{1 + 24\phi G(x, \phi)/x}, \quad (8)$$

where $G(x, \phi)$ is a trigonometric function of $x = 2qR_{HS}$ and the volume fraction ϕ of the hard spheres with a radius R_{HS} :

$$G(x, \phi) = [(1 + 2\phi)^2 / (1 - \phi)^4] [(\sin x - x \cos x) / x^2] - [6\phi(1 + \phi/2)^2 / (1 - \phi)^4] \times \{ [2x \sin x + (2 - x^2) \cos x - 2] / x^3 \} + [(\phi/2)(1 + 2\phi)^2 / (1 - \phi)^4] [4(x^3 - 6x) \sin x - (x^4 - 12x^2 + 24) \cos x + 24] / x^5. \quad (9)$$

It should be noted that the hard sphere radius should not be taken the same as the micelle physical size because of the presence of excluded volume which makes micelles rarely in direct physical contact with each other. Instead, the hard sphere radius should be treated as a fitting parameter and the fitted value is a representation of the hydrodynamic radius which is larger than the physical radius. Standard least-square fitting procedures were used to fit the SANS data to the integral form factor for the spherical micelles of pentablock copolymers as in Eq. (3) and the structure factor in Eq. (8). The fitted parameters were the average core radius R_0 , the average micelle association number n , the radius of gyration R_g of the PEO segments in the corona, the displacement dR_g of the PEO segment gyration center from the core surface, the hard sphere radius R_{HS} , the micelle number density N_s , and the polydispersity parameter which has been

chosen to be the Gaussian standard deviation σ . Because polydispersity in micelle size is caused by the variation of the association number and hence the association number in Eq. (4) should also be polydisperse and thereby calculated from the micelle size polydispersity accordingly. The total excess scattering length of the hydrophobic segments per molecule in the core (one PPO segment plus 2 PDEAEM segments: -0.135 \AA) and the total excess scattering length of one hydrophilic PEO segment in the corona (-0.042 \AA) were held constant in the fitting process. In this case the scattering contribution from unimer molecules was neglected owing to the significantly higher scattering intensity from the larger micelles.

Micelles with charged PDEAEM segments at pH 3 and pH 7.4. The PDEAEM segments are highly charged at a low pH of 3 and become partially charged at pH 7.4 (Fig. 1) and they will exist as fairly stretched due to the repulsion between like charges. The charged PDEAEM segments are hydrophilic and will form a separate outermost corona region due to the electrostatic repulsion between the PDEAEM segments. The stretching renders this outer corona very thick, to a size comparable to the full length of the PDEAEM segment, or larger in a staggered configuration wherein they are not evenly aligned relative to each other. The charged PDEAEM segments will attract free counterions in solution; hence the apparent number of charges will be much smaller than the actual number of charges on the PDEAEM segments. Furthermore, due to the location of the charged segments in the micelle and repulsion between them, micelles will be more difficult to form than in the case of uncharged PDEAEM segments, and hence we expect the micelle association number to be smaller.

Based on the above arguments the micelle geometry can be described to consist of a hydrophobic PPO core, an inner hydrated PEO shell, and an outer most thick shell composed of stretched PDEAEM segments with charges that are largely shielded by counter ions. Instead of using the above Perdersen core-chain model [25] for $(\Delta b)^2 F(q)$ in Eq. (3), we now use a more appropriate form factor for a spherical core with two shells of different scattering length densities

$$\begin{aligned}
 (\Delta b)^2 F(q) = 9n^2 & \left[\frac{2R_2^3 \Delta b_2 J_1(qR_2)}{R_2^3 - R_1^3} \frac{1}{qR_2} \right. \\
 & + \left(\frac{2R_1^3 \Delta b_1}{R_1^3 - R_0^3} - \frac{2R_1^3 \Delta b_2}{R_2^3 - R_1^3} \right) \frac{J_1(qR_1)}{qR_1} \\
 & \left. + \left(\Delta b_0 - \frac{2R_0^3 \Delta b_1}{R_1^3 - R_0^3} \right) \frac{J_1(qR_0)}{qR_0} \right]^2, \quad (10)
 \end{aligned}$$

where n is the micelle association number, R_0 , R_1 , and R_2 are the radii of core, inner, and outer coronas, Δb_0 , Δb_1 , and Δb_2 are the total excess scattering lengths of single PPO, PEO, and PDEAEM segments in the core, inner, and outer coronas, respectively, and $J_1(x) = (\sin x - x \cos x)/x^2$ is the first-order spherical Bessel function.

For describing the interaction between charged pentablock copolymer micelles, the Hayter-Penfold macroion structure factor [22,23] has been used:

$$S = \frac{1}{(1 - 24\phi a)}, \quad (11)$$

where ϕ is the micelle volume fraction and a is a function of ϕ , macroion radius R , ionic strength I and the effective number of surface charges Z . The expression for this structure factor is lengthy and the readers are referred to Hayter and Penfold [23] and Hansen and Hayter [22].

The fitting parameters are R_0 , $T_1 (=R_1 - R_0)$, $T_2 (=R_2 - R_1)$, n , N_s , Z , and the polydispersity parameter σ . The macroion radius was set as the micelle outer radius since it is the physical limit where micelles tend to feel the strongest repulsion between each other. The known parameters in the fitting are temperature and water dielectric constant, total excess scattering length for a single PPO segment ($\Delta b_0 = -0.0376 \text{ \AA}$), single PEO segment ($\Delta b_1 = -0.0422 \text{ \AA}$), and single PDEAEM segment ($\Delta b_2 = -0.0489 \text{ \AA}$). The calculated ionic strength I for the added salt did not give a satisfactory fit and was thus treated as a fitting parameter. This treatment is consistent with the fact that many of the free ions in solution move to the vicinity of the charged PDEAEM segments to shield the charged moieties and thus reduce the effective free ions in the medium. The low association number due to the repulsion of charged PDEAEM segments leads to much lower scattering intensity of the micelles than the uncharged micelles at high pH. Hence scattering contribution from the unimers must be taken into account while fitting the scattering data. The scattering profile of unimers at very low temperatures (micelles do not form at low temperatures) can be well approximated by that of a Gaussian coil. The fitted unimer dimension was then used as a fixed parameter in the fitting process for micelle scattering profiles at high temperatures to account for the contribution from the unimers by treating the scale of unimer scattering as an additional parameter.

E. Rheometry

A Haake Rheostress RS80 controlled-stress rheometer (Thermo Haake, Karlsruhe, Germany) was employed for the rheological measurements. A stainless steel C40/4 cone-and-plate geometry (diameter 40 mm and angle 4°) with a shear stress and frequency of 1 Pa and 1 Hz was used to obtain the dynamic viscosity data of the copolymer solution. For each sample, a stress sweep test was performed first to determine that the strain amplitude response at the applied stress of 1 Pa was in the linear viscoelastic regime. Temperature sweeps were conducted at $5^\circ \text{C}/\text{min}$ between 10 and 75°C . A thin layer of Dow Corning Fluid (oil) was used to cover the cone edge of the measuring geometry for preventing evaporation of water from the copolymer solution over the course of the measurement.

III. RESULTS AND DISCUSSION

A. ^1H NMR of pentablock copolymer

The pK_a of the PDEAEM blocks were determined by potentiometric titration of a 2 wt % pentablock copolymer solution at 25°C as shown in Fig. 1. The inflection at pH 7.5

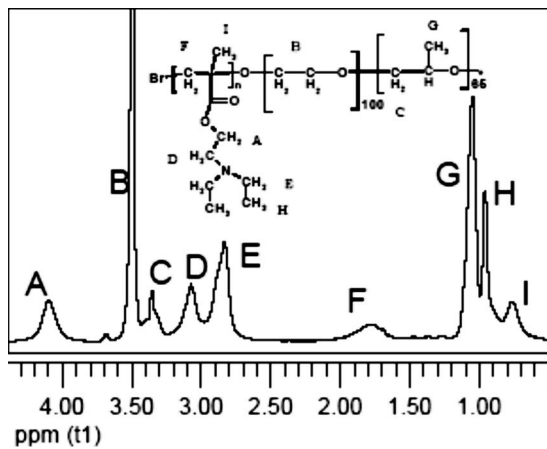


FIG. 2. ¹H NMR in D₂O of pentablock copolymer, pH=3.0 and 25 °C.

is taken as the pK_a . The titration data is plotted as the degree of amine protonation, or the fraction of DEAEM amine groups that are ionized, based on the acid-base stoichiometry of the copolymer solution. The uncharged PDEAEM is hydrophobic and hence is expected to bury within the micelle core to reduce the overall free energy of the system in aqueous solution. The ¹H NMR of the pentablock copolymer solutions as a function of pH was performed to examine the extent of hydration of the PDEAEM blocks. A ¹H NMR spectrum of the pentablock copolymer in D₂O at pH=3.0 is shown in Fig. 2. The spectra were recorded at 25 °C for the 2 wt % pentablock copolymer aliquots taken during the course of the titration. The ¹H NMR peaks associated with the pendant amine groups of the PDEAEM, labeled as A, D, E, and H in Fig. 2, are progressively attenuated with increasing pH due to the decrease in hydration and mobility of these groups as the tertiary amines become deprotonated. The extent of hydration as a function of pH was characterized by using the integral of the signal at $\delta=4.1$. At pH=3 the PDEAEM blocks are completely protonated and hydrophilic. As seen in Fig. 1 as the pH of the solution increases the degree of hydration decreases and around pH=7.5 a sharp decrease in the degree of hydration is indicated due to the large extent of deprotonation of the pendant amines. We speculate that at higher pH when the end blocks become uncharged they bury within the hydrophobic core of the micelles. These results are consistent with the SANS results discussed below as well as the previous dynamic laser light scattering experiments [12].

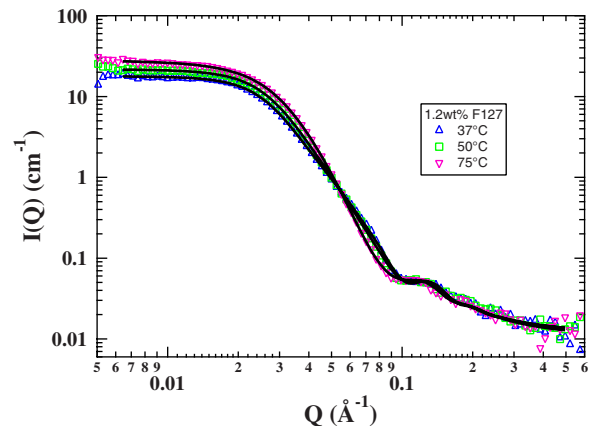


FIG. 3. (Color online) SANS data of 1.2 wt % F127 in D₂O solution as a function of temperature. The data for the micelles were fitted by using Pedersen’s core-shell model.

B. SANS of pentablock copolymer micelles

SANS provided direct information on the micelle size and morphology of pentablock copolymer solutions at various conditions. Solutions of 2 wt % copolymer at pH 3, 7.4, and 8.1 were measured at several temperatures to investigate the temperature-dependent self-assembly of the copolymers in solution. Based on ¹H NMR and previous light scattering studies [12] a significant transition of micellar morphology was anticipated to occur around the pK_a of the PDEAEM blocks. As a reference, solutions of Pluronic® F127 micelles were also examined. These samples were similarly prepared with D₂O and an equivalent weight percent of PPO expected in the 2 wt % pentablock that correspond to 1.2 wt % of Pluronic triblock copolymer. As expected, the F127 solutions at pH 3, 7, and 10 exhibited identical scattering curves (data not shown) and hence pH does not affect the micellar properties of the triblock Pluronic solutions. On the other hand, the pentablock copolymer solutions exhibited strong pH dependence in their scattering data.

C. Micelles of F127

The SANS data of the Pluronic® F127 solutions and fits using the Pedersen core-chain model [25] with the Percus-Yevick hard sphere potential are shown in Fig. 3 and the micellar parameters from the fits in Table I. The size of the unimer in solution was determined by fitting the SANS data of the F127 solution at room temperature using Eq. (2) that yielded an R_g of 22.4 Å. In the transition region between 25

TABLE I. Fitted micelle parameters for 1.2 wt % F127 aqueous solutions.

T (°C)	R_0 (Å)	R_g (Å)	d	σ	n	R_{HS} (Å)	N_s ($1/\mu\text{m}^3$)	Micelle c (mg/ml)	χ^2
25	N/A	N/A							
37	43.5 ± 0.5	36 ± 0.5	0.68 ± 0.02	0.08 ± 0.01	39 ± 1	111 ± 1	12760 ± 500	10.4	2.33
50	44.5 ± 0.5	33 ± 0.5	0.68 ± 0.02	0.1 ± 0.01	42.5 ± 1	109.5 ± 1	12000 ± 500	10.7	1.70
75	46.5 ± 0.5	29 ± 1	0.6 ± 0.03	0.09 ± 0.01	47.5 ± 1	101 ± 2	10780 ± 700	10.7	0.90

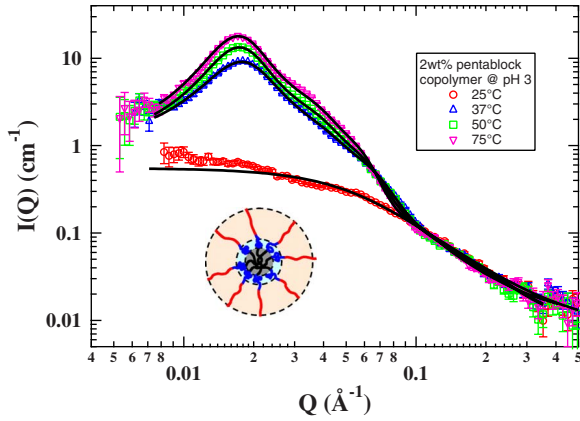


FIG. 4. (Color online) SANS data of pentablock copolymers in D₂O solution at pH 3 as a function of temperature. The data were fitted by using a form factor for a spherical core and two shells, one constituted by PEO and the exterior by the charged PDEAEM groups and the macroion structure factor. The inset is the schematic of the micelle structure with the PPO core and two shells.

and 37 °C, the equilibrium of the copolymer shifts from predominantly unimers in solution to micelles. The overall micelle radius, association number, and volume fraction in solution are 110 Å, 39, and 7% at 37 °C that change to 110 Å, 43, and 6.5% at 50 °C, and 100 Å, 48, and 4.5% at 75 °C. The micelle core size slightly increases from 43.5 Å at 37 °C to 46.5 Å at 75 °C, while the R_g of the PEO Gaussian chains in the corona slightly decreases from 36 Å at 37 °C to 29 Å at 75 °C. All these observations can be interpreted as a consequence of progressive dehydration of PPO and PEO segments at increasing temperatures which leads to higher packing of the PPO segments in the core as well as partial insertion of the PEO segments into the core. It is also clear that the transition from unimers to micelles mainly happens at a temperature between 25 and 37 °C, and that the drop in volume fraction of micelles above 37 °C is due to the reduced hydration of the micelles at elevated temperatures. The increase in association number compensated the collapse of PPO and PEO segments, leading to basically no change in the micelle size other than a significant reduction in the number density of micelles in the solution. The size of the Pluronic® F127 micelles was in good agreement with the previous SANS studies of F127 micelles [17].

Figure 4 shows the SANS data of the charged pentablock copolymer micelles at pH 3 together with the fits. The scattering data at 25 °C do not show much contribution from micelles and its profile can be fitted with a flexible Gaussian chain with $R_g=29$ Å. It should be noted that at low pH when the end groups are charged they likely are rigid and deviate

from a Gaussian chain. This is clearly seen in the low Q data for the unimer in Fig. 4. The data at higher temperatures show significant scattering, implying the formation of micelles. The increase in the SANS intensity with temperature indicates that the size of the pentablock copolymer micelles and their volume fraction strongly depend on the temperature of the solution. This behavior is similar to that of the Pluronic® F127 solutions where the micelle formation is driven by the water becoming a poor solvent to the PPO segment at elevated temperatures. However, the scattering data for the pentablock micelles exhibit a strong correlation peak at $Q=0.016$ Å⁻¹ that indicates the presence of micelles separated by the repulsive electrostatic interaction due to the presence of polyelectrolyte PDEAEM blocks in the micelle corona. As described earlier, the micelles can be modeled as a compact hydrophobic core surrounded by double corona layers. A form factor of concentric spherical shell of three regions and the Hayter-Penfold macroion potential structure factor [22,23], that accounts for the electrostatic interactions between charged colloidal particles, when used together, yielded better fits with lower χ^2 to the SANS data. During the fitting process the contribution from the unimers was accounted for by using the scattering data from a Gaussian random coil with an $R_g=29$ Å measured at 25 °C.

The fitted parameters are presented in Table II. The fitted ionic strength is so small that a trial fit with an ionic strength of zero had no effect on the fitted values of other parameters. The fitted apparent number of surface charges was also quite small. These two observations support our expectation that most of the free ions in solution move to the vicinity of the charged PDEAEM segments to shield these charged moieties to make the apparent number of surface charges small and also leave very few free ions in solution. The fitted association number is around 15 that is much smaller than that for uncharged micelles at pH 8.1 as discussed later and the association number of ~40 for the 1.2 wt % Pluronic 127 micelles at 37 °C. Thus the structure of the pentablock micelles formed at low pH is governed by both the hydrophobic PPO and the partially charged PDEAEM groups.

At the physiological pH of 7.4, the PDEAEM segments are partially charged, and the SANS data from the pentablock copolymer solutions were nearly identical to those at pH 3.0. We believe that the shielding effect of free ions in solution leads to similar micelle properties to the fully charged situation at pH 3. Hence we adopted the same fitting scheme used above for the fully charged case. Figure 5 shows the fitted solid lines plotted together with the scattering data at different temperatures. The scattering profile at 25 °C again can be fitted well with a Gaussian chain conformation with the same $R_g=29$ Å as in the case of pH 3. It is

TABLE II. Fitted micelle parameters for the Pentablock copolymer solutions at pH 3.

T (°C)	R_0 (Å)	T_1 (Å)	T_2 (Å)	σ	n	N_s (1/ μm^3)	Z	Micelle c (mg/ml)	χ^2
37	25 ± 1	26 ± 1.5	64 ± 1	0.16 ± 0.01	13.5 ± 2	10300 ± 3200	35 ± 4	5	3.41
50	29.5 ± 0.5	22 ± 1	71 ± 1	0.17 ± 0.01	13 ± 0.5	15950 ± 200	32 ± 1	7.5	6.14
75	33 ± 0.5	16 ± 1	79 ± 1	0.17 ± 0.01	16 ± 1	15300 ± 1000	33 ± 2	9	9.62

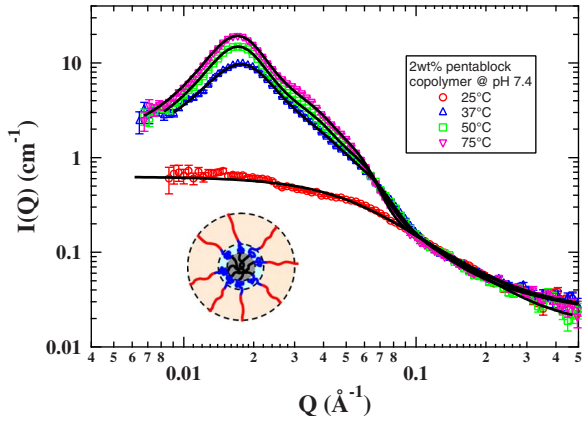


FIG. 5. (Color online) SANS data of pentablock copolymers in D₂O solution at pH 7.4 as a function of temperature. The data were fitted by using a form factor for a spherical core and two shells, one constituted by PEO and the exterior by the charged PDEAEM groups and macroion structure factor. The inset is the schematic of the micelle structure with a core and two shells.

interesting to note that the low Q scattering data for the unimer at pH 7.4 deviates only slightly from the form factor for a Gaussian coil. The identical unimer size verifies the expectation that micelles at both pH 3 and 7.4 should have similar properties. This is further confirmed by the fitted similar parameters at higher temperatures shown in Table III. At both pH 3.0 and 7.4, the micelle radius, association number and micelle volume fraction of the pentablock copolymer solutions increased with increasing temperature. Indeed the increase was substantial between 25 and 37 °C.

At $pH > 8.1$, the PDEAEM in the pentablock copolymer is essentially deprotonated and the SANS data are better described by setting the effective surface charge to zero. Fits to the data were carried out using Pedersen core-chain form factor [25] corresponding to a densely packed spherical core discussed above. Figure 6 shows the fits in solid lines plotted along with the scattering data of the pentablock copolymer micelles in 0.05 M NaCl at pH 8.1 and different temperatures. The fitted parameters are presented in Table IV. The deprotonation of the outer PDEAEM blocks at pH 8.1 results in significantly higher scattering intensity in the low- Q region at a given temperature when compared to solutions at low pH. This is due to the combined effects of a decrease in the interparticle electrostatic interaction and the growth of micelles. This change in the scattering pattern was reflected in the calculated micellar parameters such as larger size and association number of the micelles at a given temperature. Increasing the pH from 3.0 to 8.1 resulted in around a five-fold increase in the association number and slightly less than

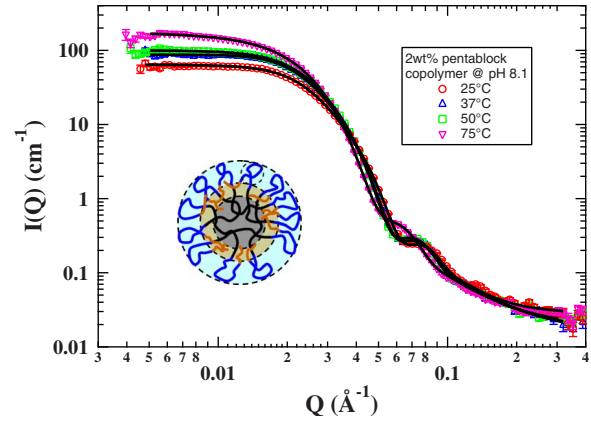


FIG. 6. (Color online) SANS data of pentablock copolymers in D₂O solution at pH 8.1 as a function of temperature. The data for the uncharged micelles were fitted by using Pedersen’s core-shell model. The inset shows the schematic of the micelle structure where the uncharged PDEAEM groups bury and increase the size of the hydrophobic core of the micelle and the PEO forming the shell.

a doubling of the average micelle volume. This is consistent to the expectation that in the absence of electrostatic repulsion between the uncharged deprotonated PDEAEM segments at pH 8.1 the pentablock copolymer can readily form well packed micelles.

D. Pentablock copolymer hydrogel

Aqueous solutions of the pentablock copolymer exhibit a reversible thermoresponsive sol-gel transition at concentrations greater than 12 wt %. Shear rheometry and SANS were used to investigate the viscoelastic properties and the structure of the hydrogel of 14 wt % pentablock copolymer solutions. The strong attractive inter-micellar interactions at 14 wt % precluded the quantitative modeling of the SANS data.

The viscosity of the pentablock copolymer solution was monitored with constant stress, oscillatory shear rheometry at several temperatures between 10 and 75 °C. In Fig. 7, the measured viscosity of solutions at pH 3.0, 7.7, and 8.3 are shown. The viscosity of the pentablock copolymer solution at pH=3 increases more than 3 orders of magnitude at the sol-gel transition temperature around 20 °C. This is qualitatively consistent with the SANS data shown in Fig. 8 for the same solution (under quiescent condition) at 10 and 50 °C. At pH=3.0 and 10 °C the copolymer solution exhibited very low levels of coherent scattering, which is indicative of the presence of only unimers in solution. At pH=3.0 and 50 °C, broad peaks in the SANS data exhibit features similar to

TABLE III. Fitted micelle parameters for the Pentablock copolymer solutions at pH 7.4.

T (°C)	R_0 (Å)	T_1 (Å)	T_2 (Å)	σ	n	N_s (1/ μm^3)	Z	Micelle c (mg/ml)	χ^2
37	24 ± 1	24 ± 1	63 ± 1	0.19 ± 0.02	11.5 ± 2	13840 ± 4500	28 ± 5	5.8	2.51
50	30 ± 1	23 ± 1	73 ± 1	0.15 ± 0.01	14.5 ± 0.5	15320 ± 120	32 ± 1	8	6.01
75	32.5 ± 0.5	17 ± 1	79 ± 1	0.17 ± 0.01	16.5 ± 0.5	15180 ± 200	32 ± 1	9	9.85

TABLE IV. Fitted micelle parameters for the pentablock copolymer solutions at pH 8.1.

T (°C)	R_0 (Å)	R_g (Å)	d	σ	n	R_{HS} (Å)	N_s ($1/\mu\text{m}^3$)	Micelle c (mg/ml)	χ^2
25	66 ± 0.5	38 ± 1	0.53 ± 0.02	0.16 ± 0.01	44.5 ± 0.5	124 ± 1	8170 ± 230	13	3.03
37	69 ± 0.5	27 ± 1	0.63 ± 0.02	0.15 ± 0.01	62 ± 1	136 ± 1	5450 ± 150	12	1.18
50	70 ± 0.5	23 ± 1	0.62 ± 0.02	0.15 ± 0.01	68 ± 1	129 ± 1	4800 ± 180	12	1.26
75	76 ± 0.5	18 ± 1	0.71 ± 0.02	0.17 ± 0.01	107 ± 3	125 ± 2	2560 ± 170	10	1.24

hexagonal packed macroassembly of wormlike micelles. It is well known that such wormlike micelles will exhibit high viscosity as shown in Fig. 7 for the 14 wt % sample at higher temperatures.

At pH=7.7, which is near the pK_a of the copolymer, the solution formed a lower viscosity gel phase compared to that of the pH=3.0 solution. The broad peak at low Q in the SANS data of the pH=7.7 solution suggests the presence of large, poorly ordered micelles at 10 °C. At pH=7.7 the micelles become uncharged and the increasingly hydrophobic PDEAEM blocks microphase separate from solution as observed in the ^1H NMR results. This could be due to the aggregation of several micelles into clusters, resulting in high scattering intensity at low Q and the broad correlation peak. At 50 °C, where a gel phase occurs, a more distinct peak was observed and the peak shifts from 0.018 to 0.023 Å⁻¹. The strong scattering at low Q also suggests the presence of larger aggregates of micelles. We surmise that the large aggregation of micelles with different sizes may decrease the overall ordering in the micellar gel, resulting in a softer gel phase.

At pH=8.3 the PDEAEM blocks of the pentablock copolymer becomes completely uncharged. At 10 °C the solution became notably more viscous than the solutions at pH 3.0 and 7.7 at that temperature. SANS data indicate the presence of interacting cylindrical micelles, inferred by the $I(Q) \sim Q^{-1}$ region at low Q and a strong correlation peak at $Q \sim 0.025$ Å⁻¹. The pH=8.3 solution exhibited a modest increase in viscosity between 20 and 35 °C, which corresponds to an increase in the SANS intensity. The overall shape of the SANS data remained relatively constant between 10 and 50 °C with a small increase in the intensity at

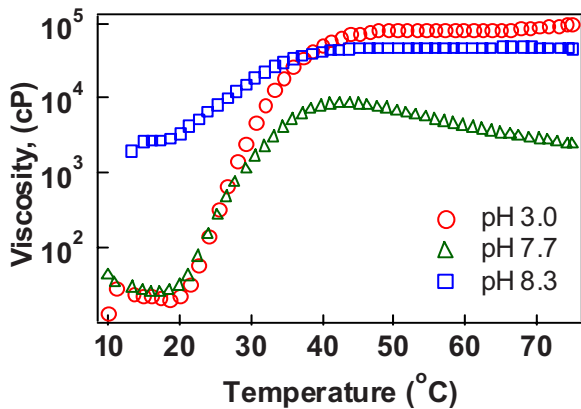


FIG. 7. (Color online) Shear rheometry temperature sweep data of 14 wt % pentablock copolymer solutions at different pH.

low Q . This suggests that the increase in viscosity is due to an increase in the size and the number density of the wormlike micelles.

IV. CONCLUSIONS

SANS and NMR together showed that the pentablock copolymers form multi responsive micelles and their self-assembly is governed by both the LCST of PPO block and the magnitude of the charge on the PDEAEM blocks that depends on the pH. Rheology was used to examine the viscoelastic properties of the gels formed at different tempera-

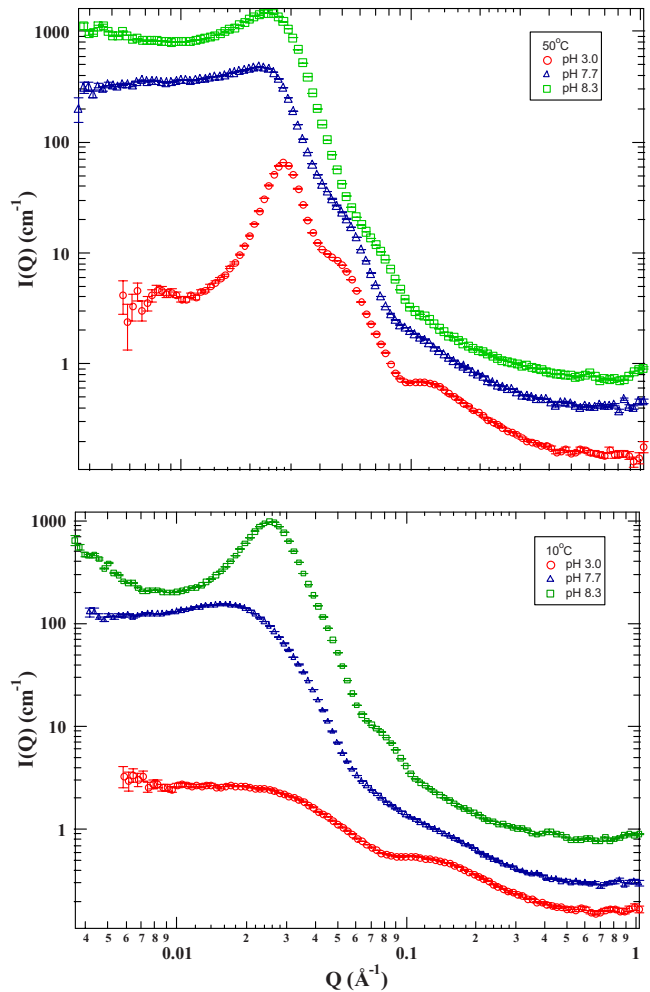


FIG. 8. (Color online) SANS data of 14 wt % pentablock copolymer in D₂O solution at different pH and temperatures.

ture and solution pH. By using the core-chain form factor [25] and the macroion structure factor [22,23] for the quantitative analysis of the SANS data we derived detailed information on the micellar dimensions, morphology and surface charge. Micelles formed below the pK_a of the PDEAEM blocks interact as charged colloidal particles and exhibit sharp correlation peaks due to the repulsive columbic interactions. Furthermore, at low pH the micelle association number is significantly low due to the electrostatic repulsion between the charged PDEAEM segments. The deprotonation of the PDEAEM blocks at pH higher than 7.5 results in higher association number, as well as larger size of the micelles. At higher pH and temperature the progressive dehydration of

the PPO blocks causes the formation of wormlike micelles and this is consistent with the higher viscosity of the solutions.

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