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Cryo-TEM of Polyelectrolyte–Micelle Complexes

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Cryo-TEM was carried out on samples containing polyelectrolyte–micelle complexes, formed by combining poly(diallyldimethylammonium chloride) (PDADMAC), a strong cationic polyelectrolye, with oppositely charged mixed micelles of sodium dodecyl sulfate (SDS) and nonionic Triton X-100 (TX100), in 0.40 *M* NaCl. Complexation appears to involve the formation of micelle-rich regions, presumably within the domains of polymer chains, without any particular organization or restructuring of the micelles. At polymer concentrations (C_P) ≤ 0.4 g/L, the observation of such clusters with dimensions as low as 50 nm suggests that intrapolymer complexes may exist at low C_P. With increasing polymer concentration, aggregates grow in size, with dimensions that indicate the involvement of many polymer chains. Upon increase in the ratio of SDS:TX100, the system coacervates, and the micelle/polymer-rich phase forms a continuous matrix. © 1997 Academic Press

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INTRODUCTION

Polyelectrolytes form complexes with oppositely charged mixed micelles (1–5). Since the interaction between these two macroions is primarily coulombic, its magnitude depends on the micelle surface charge density, σ, the polymer linear charge density, ξ, and the ionic strength, *I*. As predicted by theoretical treatments for the binding of polyelectrolytes to oppositely charged surfaces (6–10), there is no detectable interaction when the quantity $\sigma\xi/\kappa$ is smaller than some critical value (here κ is the Debye-Hückel parameter, proportional to $1/\sqrt{I}$). Above this critical value, polyion–micelle association typically leads to phase separation, often in the form of coacervation (11). For most systems studied to date, there is a region between the onset of interaction and subsequent bulk phase separation in which soluble, equilibrium complexes are formed. Under such conditions, complexes may be studied by a wide range of techniques, including static and dynamic light scattering, electrophoretic light scattering, turbidimetry, NMR, microcalorimetry, and fluorescence spectroscopy.

One system that forms soluble complexes over a wide range of experimental conditions is composed of the strong (i.e., pH-independent) polycation, polydimethyldiallylammonium chloride (PDADMAC); and anionic/nonionic mixed micelles formed from sodium dodecyl sulfate (SDS) and Triton X-100 (TX100) (12). In this system, σ can be conveniently varied by changing the surfactant mole fraction of SDS (Y). When Y is increased at constant ionic strength, a well-defined point of incipient complexation "Y_c" may be identified, which is subsequently followed by a point of phase separation "Y_p". The condition $Y_c < Y < Y_p$ is the regime of soluble complexes, which may be studied by numerous spectroscopic and hydrodynamic methods, including NMR (13), fluorescence spectroscopy, and others. Dubin and coworkers have characterized PDADMAC–SDS/TX100-soluble complexes using dynamic and static light scattering (14, 15), turbidimetry (16), electrophoretic light scattering (11), dialysis equilibrium (17), viscometry (17), and microcalorimetry (18) to investigate the effects of polymer $\bar{M}_W$ (19), ionic strength (15), and polymer concentration (20) on complex structure.

The aforementioned studies indicate that SDS/TX100 mixed micelles interact with high molecular weight ($\bar{M}_W$) PDADMAC ($>10^5$) at low polymer concentration (<1 g/L) to form intrapolymer complexes of very high molecular weight ($>10^7$) but not particularly large dimensions. Thus the complexes are quite dense. Binding of micelles to the polycation leads to charge neutralization, and consequently to intermacromolecular association and the formation of higher order complexes. While this general picture could be deduced from available data, a fundamental question has remained unanswered: whether micelle structure is preserved upon binding. The literature is ambivalent on this matter. In general, it has been concluded that SDS binds to nonionic polymers such as poly(ethylene glycol) in aggregates that are smaller than the free micelles (21–26), but for charged polymers, the results are less congruent. Abuin and Scaiano (27) found $N/N_0 \cong 0.1$ (where N and N_0 are the aggregation numbers for complexed and uncomplexed micelles, respec-

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tively) for sodium poly(styrenesulfonate)–dodecyltrimethylammonium bromide (DTAB); for the same system Almgren *et al.* (28) obtained $N/N_0 \cong 0.5$. On the other hand, Chu and Thomas obtained $N > N_0$ for sodium poly(methacrylate)–DTAB (29). Thalberg *et al.* found N for DTAB to be relatively insensitive to sodium hyaluronate (30); and, similarly, Hansson and Almgren found little effect of sodium polyacrylate on the aggregation number of DTAB (31).

With regard to the PDADMAC–TX100/SDS system, there has been only indirect evidence available about the structure of polymer-bound micelles. Dye solubilization studies showed no effect of complex formation on the apparent CMC, or on the magnitude of solubilization (32). While it has been proposed that the Gibbs energy of polymer–micelle complexation could be deduced from the reduction in the CMC upon complexation, the failure to observe a change in CMC in the presence of interacting polymer was also reported by Brackman and Engberts (33) for poly(propylene oxide)-*n*-octylthioglucoside (OTG), and by Winnik for hydroxymethylcellulose-OTG (34).

A priori, one might anticipate various reasons for modification of micelle structure upon polymer binding. Charged micelles at low ionic strength tend to exhibit large surface curvature which reduces the surface density of ionic head groups; conversely, micelles tend to grow with increasing ionic strength. The binding of polycations to the micelle surface should lower the electrostatic potential and reduce the electrostatic energy of regimes of low curvature, thus stabilizing larger micelles. One may also presuppose the existence of micellar distributions with respect to both composition, i.e., the microscopic mole fraction of SDS in a single micelle, y, and microscopic aggregation number (n). Polymers should preferentially bind micelles with $y > Y$. Little evidence exists to support any of these speculations, and the description of the structure of the complexes achieved to date is supported by experiment only indirectly.

The uncertainties described above have led us to conduct exploratory cryo-TEM measurement on PDADMAC–SDS/TX100 complexes. Cryo-TEM is now a well-established technique for direct observation of microstructures in dilute systems, relatively free from artifacts which are normally found when using staining methods. We chose here to work at an ionic strength of 0.4 *M* NaCl, at about 10 m*M* surfactant, and in the range of 1 g/L polymer, since these were suitable for cryo-TEM, and because soluble complexes are readily formed under these conditions (17).

EXPERIMENTAL

Materials. A high molecular weight polycation, PDADMAC, reported molecular weight, $\bar{M}_W = 2.5 \times 10^5$, and a low molecular weight PDADMAC, $\bar{M}_W = 5.0 \times 10^4$, from Calgon Corp., were dialyzed and lyophilized. SDS was ''Purissima'' grade (Fluka). Triton X-100 (*p*-polyethoxylated isooctylphenol), with average degree of ethoxylation 9.5 (approx. $\bar{M}_W$ 646 g M^{-1}) and hydrogenated to reduce UV absorption, was purchased from Sigma and used as supplied. Sodium chloride was analytical grade. Highly pure (MilliQ-plus) water was used to prepare the solutions.

The polymer-free samples were prepared in 0.4 *M* NaCl and used as stock solutions to prepare the samples with PDADMAC. The concentration of TX100 was kept at 10 m*M* and the SDS concentration was varied over a range of SDS mole fractions (Y), expressed as $Y = [\mathrm{SDS}]/([\mathrm{SDS}] + [\mathrm{TX100}])$. The samples with either 0.1% or 0.2% (w/v) polymer were prepared by carefully weighing PDADMAC into a volumetric flask and dissolving it at 25°C by mechanical stirring for 12 h in a mixed-micellar stock solution of known composition and ionic strength.

Cryo-transmission electron microscopy (cryo-TEM). Samples for cryo-TEM were prepared using a modified controlled environment vitrification system (CEVS). The procedure has been described earlier (35) and permits cryofixation of the specimen at controlled temperature and humidity (36). Bulk samples of the polymer and polymer-free SDS/TX100 mixed micellar solutions in electrolyte were equilibrated at 25°C in an atmosphere with humidity (98–99% rh) after which approximately 3 μL of the sample were withdrawn and deposited on a grid with a holey polymer film. After carefully spreading the drop, excess liquid was blotted away with a filter paper to produce a thin sample film spanning the holes. The grid was then plunged into liquid ethane maintained close to its freezing point. The vitrified specimen was examined in a Zeiss EM 902 electron microscope, operated in the filtered brightfield image mode at DE = 0 eV using an underfocus of 1–3 μm. This was done by first focusing the image and then defocusing the final adjustments by turning the knob about 1–3 μm from the focus position before photographing the image. Consequently, objects smaller than 5 nm would appear as 5-nm images. The accelerating voltage was 80 kV and the stage temperature was kept below 108 K. The imaging in the microscope was carried out with considerable underfocus due to the low contrast between solvent and microstructure. All of the micrographs presented are representative images based on repeated samples.

RESULTS AND DISCUSSION

The results presented in Figs. 1–3 are micrographs taken from bulk samples with a composition $Y = 0.26$ in 0.4 *M* NaCl at 25°C, with and without high molecular weight PDADMAC (concentration 0.2%, w/v). Fig. 1 is for a polymer-free composition of SDS/TX100 mixed micelles, while Fig. 2 and Fig. 3 are for PDADMAC-containing solutions at the same composition $Y = 0.26$. The micrograph presented in Fig. 3 is for the polymer-containing sample in Fig. 2, after fivefold isoionic dilution (i.e., fixed ionic strength, $I =$ 0.4 *M*). From the micrograph of the polymer-free solution,

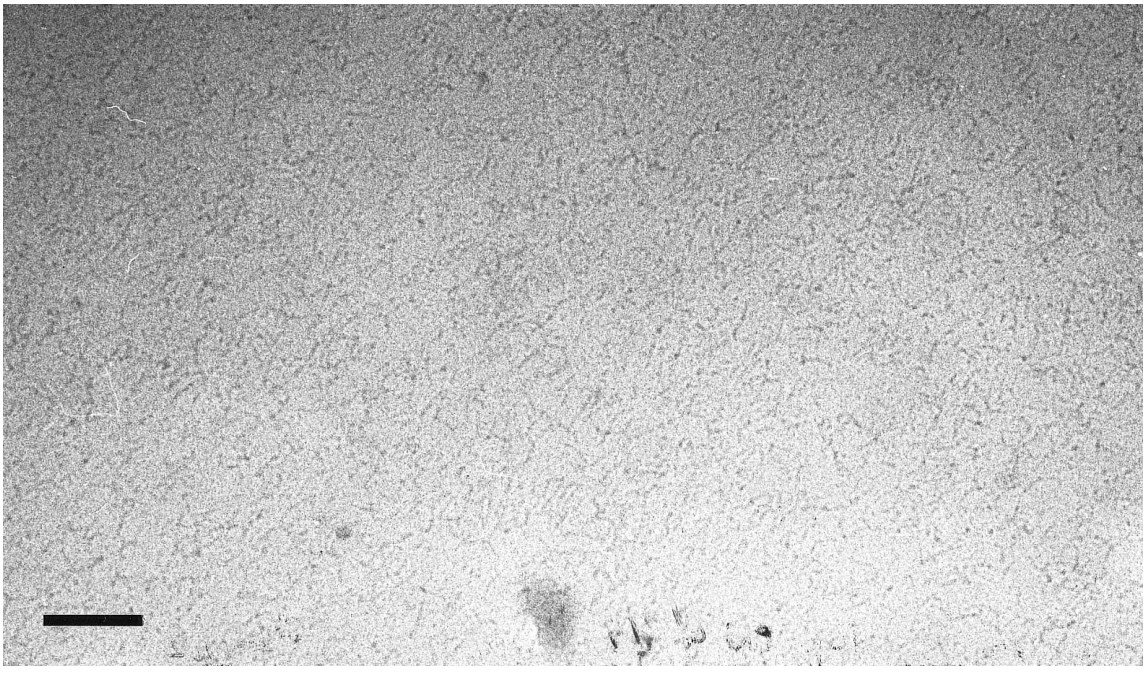

FIG. 1. Mixed micellar structures of SDS/TX100 observed by cryo-TEM in 0.4 *M* NaCl at 25°C. The mole fraction of SDS is $Y = 0.26$, [TX100] = 10 m*M*. Bar = 100 nm.

we see that the mixed micelles are nonspherical, i.e., cylindrical in shape. Previous measurements of the size of these micelles by dynamic light scattering (17), static light scattering (17), and size-exclusion chromatography (37) gave apparent mean radii of $R_S = 90$ Å, $R_g = 210$ Å, and $R_{sec} = 70$ Å, respectively. If the micelle were considered as a rigid prolate ellipsoid, with a minor radius somewhere in the range of 10–35 Å nm, then the measured Stokes radius of 90 Å corresponds to a major radius of 250–400 Å (38). The limited contrast of Fig. 1 and the high surfactant concentra-

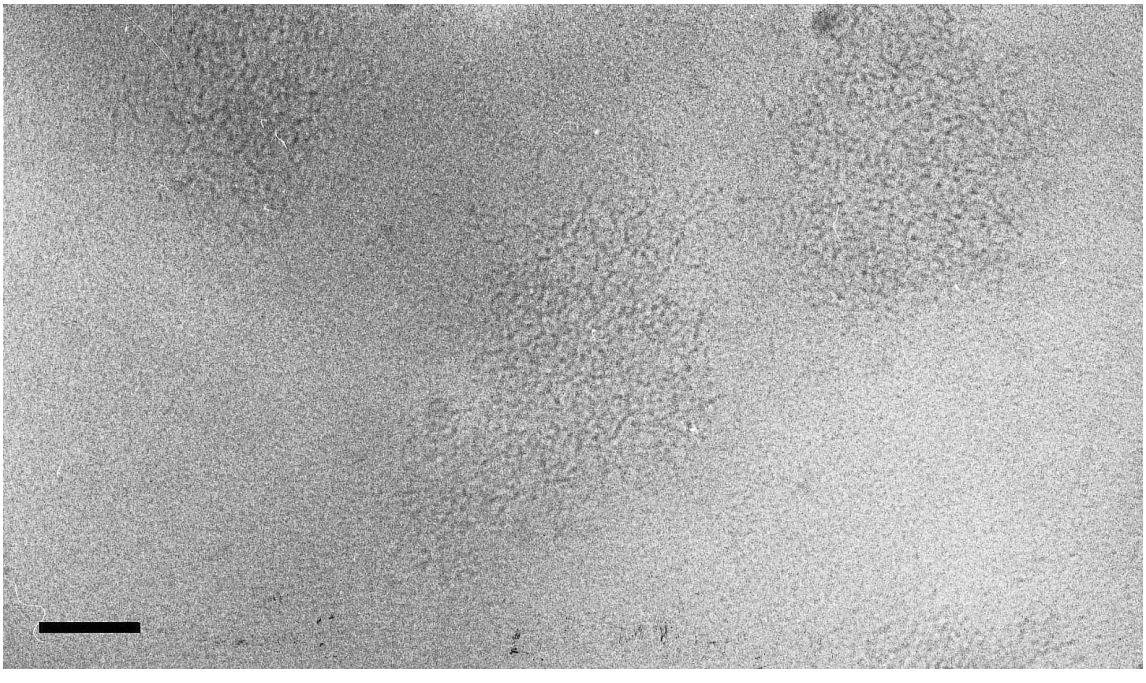

FIG. 2. Dense microstructures of polyelectrolyte–micelle complexes found after high molecular weight PDADMAC is added to SDS/TX100 with the same composition as in Fig. 1. $C_p = 2$ g L^{-1}, $T = 25$°C. Bar = 100 nm.

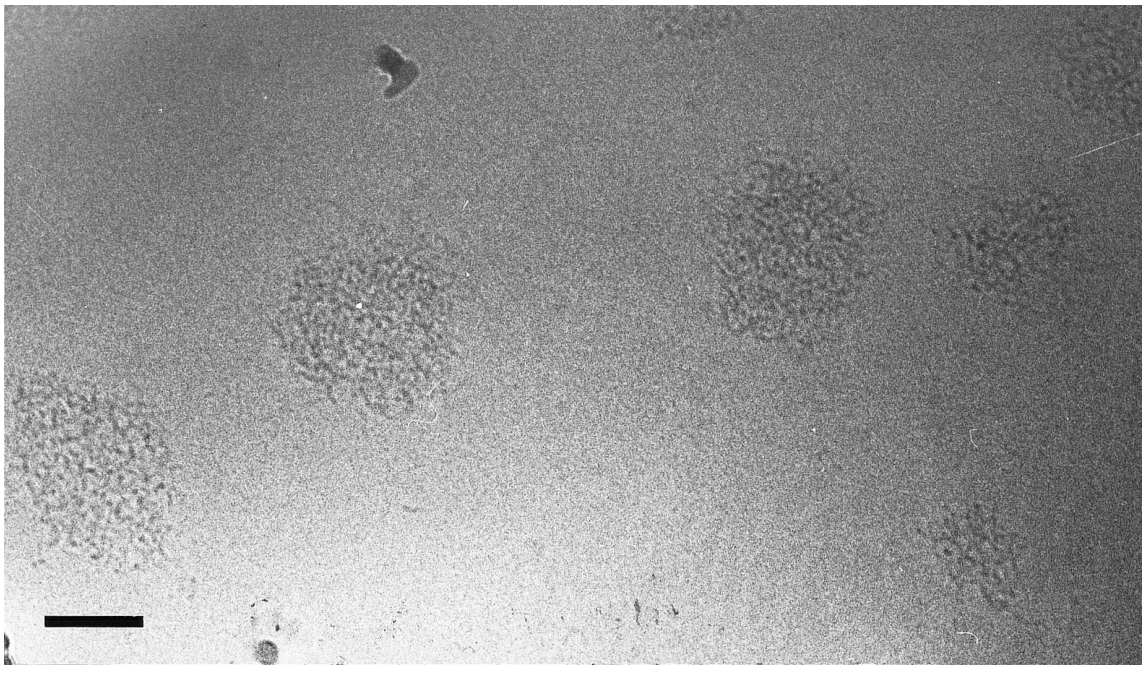

FIG. 3. Microstructures found when the sample used in Fig. 2 was diluted five times at fixed ionic strength (0.4 *M*). The smaller clusters (about 50 m*M*) may be intrapolymer complexes. Bar = 100 nm.

tion (10 m*M* TX100) lead to overlapping images, making precise evaluation of size difficult; nevertheless, one may discern particles of length about 30–60 nm, which may be compared with the diameters of 14–80 nm obtained in the various methods described above.

Figure 2 is obtained from a solution prepared by adding 2 g/L high molecular weight PDADMAC to the surfactant solution of Fig. 1. One observes several dense concentrated micellar domains, about 300–500 nm in diameter. The large size of the complex and its rather dense microstructure probably implies that the mixed micelles are not bound to single polyions but rather numerous polymer chains (multipolymer complex). Li *et al.* (12) recently observed a maximum in both the turbidity and R_S with polymer concentration at $C_P \cong 2.5$ g/L for $Y = 0.3$, in 31 m*M* TX100. While the difference in surfactant concentration limits the validity of comparison, it is clear that the aggregation state of the complex is C_P-dependent. Within the limits of resolution and contrast, no particular change in micelle size can be observed between bound and free micelles (Fig. 2 vs. Fig. 1).

Isoionic dilution of soluble PDADMAC/SDS–TX100 complexes at $Y = 0.3$, in 0.40 *M* NaCl, from ca. 2 to 0.2 g/L in PDADMAC, has been found to decrease R_S by almost a factor of 3, from 85 to 30 nm (12). Fivefold isoionic dilution of the sample used for Fig. 2 similarly produces smaller clusters, shown in Fig. 3, with diameters ranging from 70 to 150 nm. If we consider that dynamic and static light scattering give, respectively, diameters of 40 nm ($2 \times R_S$) and 90 nm ($2 \times R_g$) for soluble complexes, in the limit of low C_P ($\leqslant 0.01$ g/L) (17), then it is reasonable to conclude that the smallest clusters identifiable in Fig. 3 correspond to intrapolymer complexes.

The same experiment as above was repeated at $Y = 0.22$ slightly below Y_c (in 0.40 *M* NaCl, $Y_c = 0.23$ independent of molecular weight, C_P, or C_S of the polymer (17)), at $I = 0.4$ *M*, and $C_p = 2$ g/L. The results were identical to those in Fig. 1, confirming the absence of complex formation below Y_c. Increasing the SDS concentration (i.e., to $Y = 0.3$, 0.34, etc.) at fixed $I = 0.4$ and C_p (2 gL^{-1}) showed an increase in the size of the soluble complex to about 400 nm, although the contrast was poor. Despite the low contrast of the specimens, it was possible to observe, as in Fig. 4, the formation of a continuous interconnected network of micelle-rich domains, surrounding particle-free ''holes,'' at $Y = 0.37$, corresponding to the point of macroscopic coacervation. To confirm that this observation is not the result of specimen preparation, we obtained the same results for samples prepared on different days, and also with repetitions of freshly prepared samples. However, since the diameter of the structures is not much smaller than the thickness of the vitrified film, it is possible that the confinement of the network within the thin film affects the structure. In other words, the image may not exactly represent a section of the original 3D structure, and the arrangement of the micelle-free domains with respect to each other may be distorted. Nevertheless, the appearance of an interconnected biphasic state upon coacervation merits further study.

To test the effect of molecular weight on the formation

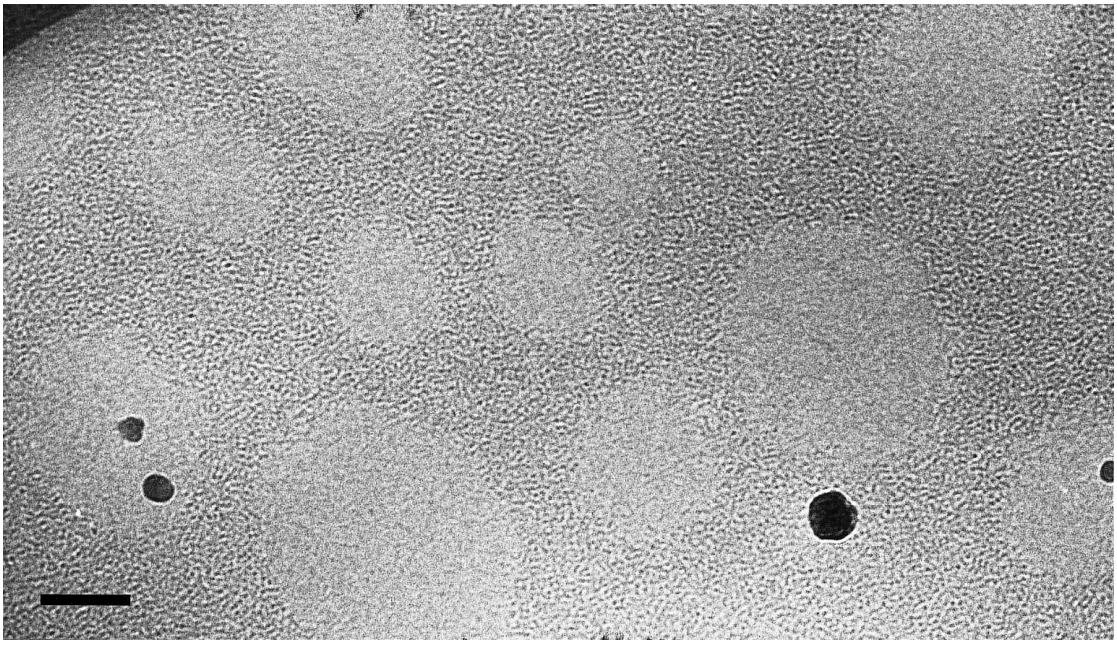

FIG. 4. Continuous microstructures observed at $Y = 0.37$, $I = 0.4$, $C_p = 2$ g/L. These conditions correspond to coacervate formation. Bar = 100 nm.

of soluble complexes two samples with lower molecular weight polymer were prepared; one below Y_c ($Y = 0.22$, $I = 0.6$ *M*, $C_p = 1$ g L^{-1}) and the other above Y_c ($Y = 0.35$, $I = 0.4$ *M*, $C_p = 1$ g L^{-1}). Both compositions failed to give evidence of complex formation although some interaction is expected at the second composition. The size of the lower molecular weight polymer is in the same order as that of the mixed micelles and if only few mixed micelles are associated with each polymer chain it probably is difficult to observe them by cryo-TEM. Indeed, Li *et al.* (20) observed a transition in the behavior of the complexes when the molecular weight of the polymer fell below ca. 1×10^5, which they ascribed to the difficulty of forming multimicellar complexes ($R_g \cong 15$ nm was observed independent of molecular weight, for MW $< 1 \times 10^5$) when the dimensions of the polyion failed to exceed those of the micelle.

Some limited measurements were made to explore the effect of ionic strength. Micrographs were obtained in 0.75 *M* NaCl, with 1 g/L high-molecular weight polymer, at $Y = 0.40$ (about 10% larger than Y_c at this ionic strength). Large clusters of somewhat aligned threadlike micelles were observed (picture not shown). While the increase in micelle size with ionic strength is expected, the limited nature of these results precludes detailed discussion.

CONCLUSIONS

Complex formation between polycations and anionic/nonionic mixed micelles has been observed by cryo-TEM. Microscopic observations are largely consistent with the conclusions of earlier light scattering studies on such systems. Cryo-TEM reveals no particular ordering or organization of the micelles in these complexes, but rather suggests that complex formation involves a concentration of micelles within the domain of the polymer chain. Depending on polymer concentration, these domains may consist of one or many polymer chains.

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