Polyelectrolytes and Oppositely Charged Surfactants in Organic Solvents: From Reversed Micelles to Soluble Polymer−Surfactant Complexes

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Received September 29, 1994; Revised Manuscript Received February 27, 1995[⊗]

ABSTRACT: This work studies aggregates formed by poly(*N*-ethyl-4-vinylpyridinium bromide) (PEVP) and oppositely charged sodium bis(2-ethylhexyl) sulfosuccinate (Aerosol OT) molecules in aliphatic hydrocarbon solvents (octane and hexane). It demonstrates that various polycation−surfactant structures are formed during PEVP solubilization in Aerosol OT solutions depending on the amount of water added to the system. At high degrees of hydration PVPE incorporates in the water pool of a reversed micelle formed by the surfactant where the polycation chain is substantially condensed. According to the light scattering and ultracentrifugation data, the incorporation of the polycation affects little the size of the reversed micelles. Under these conditions the water pools of PEVP-containing micelles may also incorporate other macromolecules (e.g., bovine serum albumin) simultaneously with the polycation. At the critical degree of hydration PEVP represents a swollen globule compressed by the micellar matrix. The density of the polycation in such globules equals about 0.5 g/cm^3 and about 50% of the globule volume is occupied by water molecules. Below the critical degree of hydration the polycation and the surfactant form a stoichiometric polymer−surfactant complex soluble in aliphatic hydrocarbons. The dimensions of such complexes are significantly higher than those of the initial empty reversed micelles, and they apparently represent "comblike" structures in which the Aerosol OT head groups are electrostatically bound with the charged units of PEVP, whereas the hydrophobic tails of the surfactant, "grafted" to the polycation, are exposed to the bulk solvent.

Introduction

Reactions of polyelectrolytes with oppositely charged ionic surfactants in aqueous solutions have attracted great attention during the last decades.[1−13] Polymer−surfactant complexes (PSC) form as a result of cooperative electrostatic binding of the polycation chains with the charged head groups of the surfactant during these reactions. Such complexes represent a specific class of polymer−surfactant compounds that have important applications in various areas.[5,11] Interactions of polyelectrolytes and surfactants in organic solvents have been studied using water−surfactant−organic solvent triple systems as models.[14−21] Various surfactant structures can be formed in these systems depending on the system composition, in particular, reversed micelles, which can physically entrap macromolecules. The remarkable fact that enzymes in such complexes remain active in organic media has been discovered[22−27] and then studied in detail (for a recent review, see ref 21). Although the major efforts in this area have been aimed at structure−function studies of solubilized biologically active macromolecules (enzymes, antibodies, DNA, etc.), several works studied the behavior of linear synthetic polyelectrolytes in such systems. In particular, reversed micelles of the anionic surfactant Aerosol OT (sodium bis(2-ethylhexyl) sulfosuccinate) in organic solvents have been used as matrix microreactors for controllable synthesis of conjugates of proteins with synthetic polyelectrolytes.[28,29] Compaction of the chains of various linear polyelectrolytes in the aqueous cavity of the reversed micelles and the formation of polyelectrolyte globules induced by the micellar matrix have been observed.[29,30] The complexes of the negatively charged random ionomer (sulfonated polystyrene) and the reversed micelles of Aerosol OT have been described recently.[31,32] Such complexes represent "beadlike" structures in which several reversed micelles are "strung" on the ionomer chain, each of them attached to the sulfonic groups of the ionomer which incorporate into the micelle surfactant layer. The noncharged polystyrene chains are dissolved in organic solvents and link these micelles. Finally, polyelectrolyte complexes with oppositely charged surfactants which are soluble in some organic solvents have been synthesized recently.[33,34] This work studies interactions of poly(*N*-ethyl-4-vinylpyridinium bromide) (PEVP) with the oppositely charged Aerosol OT molecules in aliphatic hydrocarbon solvents and reports new data on the structure formation and transitions in this system.

Experimental Section

Aerosol OT was purchased from Merck and used without additional purification. According to the data of IR spectroscopy,[35] it contained one water molecule per two surfactant molecules. Poly(4-vinylpyridine) was obtained by radical polymerization of 4-vinylpyridine, fractionated by a common technique,[36] and then quaternized by ethyl bromide or first by bromoacetic acid and then by ethyl bromide to obtain PEVP.[37,38] The degree of quaternization of PEVP was about 95% according to the IR spectroscopy data.[39] The polymer molecular weights were determined as described in ref 29. Two samples of PEVP were used in this work with weight-average molecular masses ($\bar{M}_w$) of 140 000 ($\bar{P}_w \approx 690$) and 200 000 ($\bar{P}_w \approx 980$), respectively. Twenty percent of the quaternized units in the sample with $\bar{M}_w$ = 140 000 contained *N*-carboxyethyl

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[⊗] Abstract published in *Advance ACS Abstracts,* April 1, 1995.

0024-9297/95/2228-3657$09.00/0

substituents.[29] Bovine serum albumin (BSA) was purchased from Sigma. It was modified by fluorescein isothiocyanate (FITC) using a standard technique, purified by gel filtration on Sephadex G-25, and lyophilized.

Light scattering measurements were carried out at 20 °C as follows. (A) The mean hydrodynamic radius of the scattering particles was determined using an Autosizer 2c (Malvern) small-angle laser photometer with a 20 mW He–Ne laser (633 nm). The autocorrelation functions of fluctuations of scattering intensity were determined using a correlator K 7032-05 (Malvern). The z-average coefficients of translation diffusion (D_z) were calculated by the cumulants method, and the R_h values were determined as radii of equivalent hydrodynamic spheres. (B) The light scattering study of the systems containing PSC was carried out with a He–Ne laser (KMX-6, Milton Roy) operated at a light intensity of 2 W and a wavelength of 630 nm, the scattering angle being equal to 6.5°. The autocorrelation function of fluctuations of the scattered light intensity was measured using a digital correlator (Langley Ford Instruments Model 1096), and the data were analyzed as described in Results and Discussion. Prior to light scattering, the samples were filtered 5–10 times through Millipore 0.22 μm filters.

The ultracentrifugation measurements were carried out at 20 °C on a Beckman E ultracentrifuge equipped with a photoelectric scanning device with a monochromator and a multiplexor using 12 nm section cells and a rotor An-G-Ti at 20 000, 30 000, and 44 000 rpm. Scanning was performed at 400 nm (picric acid) or 264–270 nm (PEVP). The reversed micelle systems were preincubated at 20 °C for 10 h prior to the measurements.

The fluorescence measurements were carried out on a Hitachi F-4000 spectrofluorimeter at 20 °C. The elemental analysis of PSC was performed using standard techniques in the analytical laboratory of the Institute of Elementoorganic Compounds, Russian Academy of Sciences.

Results and Discussion

Structure of Aerosol OT Reversed Micelles. It is well known that Aerosol OT forms reversed micelles in aliphatic hydrocarbons that can solubilize substantial amounts of water (up to several percent).[40–42] During solubilization of water, an aqueous pool is formed inside reversed micelles which is covered by a monolayer of Aerosol OT molecules. The hydrated micelles have a virtually spherical shape and are characterized by a very narrow size distribution.[43–45] Their size as well as the aggregation number of the surfactant in them increases with the elevation of the hydration degree (ω_0), i.e., the molar ratio [H_2O]/[Aerosol OT]. The molecular characteristics of reversed micelles of Aerosol OT formed in aliphatic hydrocarbons at various water contents are listed elsewhere.[46] At constant concentrations of the surfactant, the radius of the water pool ($r^{(e)}_{H_2O}$) linearly depends on ω_0 and is expressed as follows:

$$r^{(e)}_{H_2O} \approx 0.15\omega_0 + 0.4 \qquad (1)$$

Meanwhile, the thickness of the external surfactant layer formed by Aerosol OT molecules practically does not change with the variation of the water content and equals from 0.9 to 1.0 nm.

Solubilization of PEVP in Aerosol OT Reversed Micelles. PEVP was not soluble in aliphatic hydrocarbon solvents (e.g., octane or hexane) in the absence of the surfactant. On the contrary, PEVP solubilization took place in micellar solutions of Aerosol OT, which was registered visually and spectrophotometrically by measuring PEVP absorption at 270 nm. Various amounts of the concentrated aqueous solution of PEVP were added to the solutions of Aerosol OT in the organic solvent to introduce the polycation into the micellar system. The system was intensively shaken for 1–2 min, which was followed by formation of an optically transparent solution containing PEVP. At constant concentrations of Aerosol OT, the stability of micellar systems depended on the polycation concentration and ω_0. In the absence of the polycation, up to 60 molecules of water per molecule of surfactant ($\omega_0 = 60$) were introduced in the reversed micelles of 0.1 M Aerosol OT until opalescence was detected in the system. At relatively low PEVP concentrations (e.g., 10^{-3} basic mol/L), the system was stable at $\omega_0 \leq 45$.[47] At the same time, when the PEVP concentration increased by 10-fold, phase separation was observed already at $\omega_0 \geq 20$.

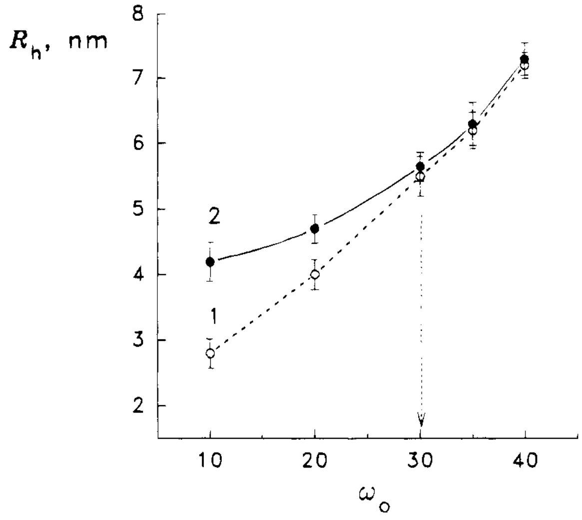

Figure 1. Dependence of the mean hydrodynamic radius (R_h) of (1) PEVP-free (○) and (2) PEVP-containing (●) particles of Aerosol OT in octane on the degree of hydration. Values are mean ± SD (n = 3–7). The vertical arrow shows the critical degree of hydration. [Aerosol OT] = 0.1 M; [PEVP] = 10^{-3} basic mol/L; PEVP $\bar{M}_w$ = 140 000.

Effect of PEVP on the Dimensions of Reversed Micelles. The effect of PEVP solubilization on the micelle dimensions was studied using the quasi-elastic light scattering technique (Figure 1). In these experiments the PEVP concentration was maintained low in order to obtain systems with high degrees of hydration. Two distinct areas were observed in the dependence of the mean hydrodynamic radius of the polycation-containing micelles on ω_0. At $\omega_0 \geq 30$ solubilization of PEVP did not affect practically the dimensions of the reversed micelles. With the decrease of ω_0 below 30, the mean hydrodynamic radius of the particles formed in the presence of PEVP was increasingly larger than that of the reversed micelles of Aerosol OT in the absence of the polycation. The degree of hydration equal to 30 will be termed herein the "critical degree of hydration" (ω_c).

Dimensions of the Particles at $\omega_0 < \omega_c$. The integral intensity of the light scattered by the particles of the disperse phase formed at $\omega_0 = 10$ and the mean radius significantly depended on the PEVP concentration (Figure 2). Our previous sedimentation study suggests that two different populations of micellar particles are formed under these conditions.[29] One type of these particles appears to be the empty micelles and another the polymer-containing particles. Therefore, the results of the light scattering experiments were analyzed under the assumption that two populations of the scattering particles differing in size were present in the system: the polymer-free reversed micelles and the PEVP-

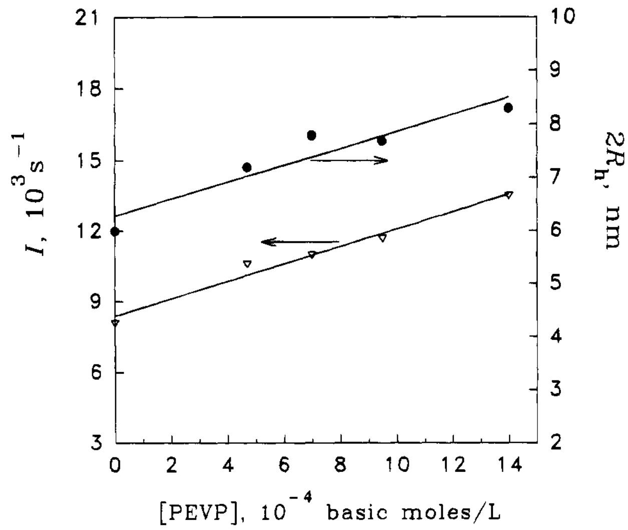

Figure 2. Dependencies of the integral intensity of scattered light, I (▽), and the mean hydrodynamic radius, $\bar{R}_h$ (●), of Aerosol OT particles on PEVP concentration in hexane. [Aerosol OT] = 0.1 M; ω_0 = 10; PEVP $\bar{M}_w$ = 200 000.

containing particles. In this case, the autocorrelation function of fluctuations of the intensity of scattered light ($G(t)$) can be expressed as follows:

$$G(t) = W_1G_1(t) + W_2G_2(t) \qquad (2)$$

where $G_1(t)$ and $G_2(t)$ are autocorrelation functions for the polymer-free reversed micelles and the PEVP-containing particles, respectively, W_1 and W_2 are parameters characterizing the impact of these particles on the integral intensity of the scattering. $G_1(t)$ was determined for the polymer-free reversed micelle systems having the same degree of hydration. $G_2(t)$ was expressed as a first approximation as an autocorrelation function of monodisperse spherical particles:

$$G_2(t) = e^{-D_z^{(f)}q^2t} \qquad (3)$$

Equations 2 and 3 described fairly well the experimental autocorrelation function. The coefficient of translation diffusion of the PEVP-containing particles ($D_z^{(f)}$) was determined by minimizing (by parameters $D_z^{(f)}$, W_1, and W_2) the sum of the squares of the deviations of the autocorrelation function and the function of eq 2. This approach permitted us to determine the hydrodynamic radius of the PEVP-containing particles, which at ω_0 = 10 was about 10 times larger than the size of the PEVP-free reversed micelles (e.g., in the case of PEVP with $\bar{M}_w$ = 200 000 it was about 30 nm).

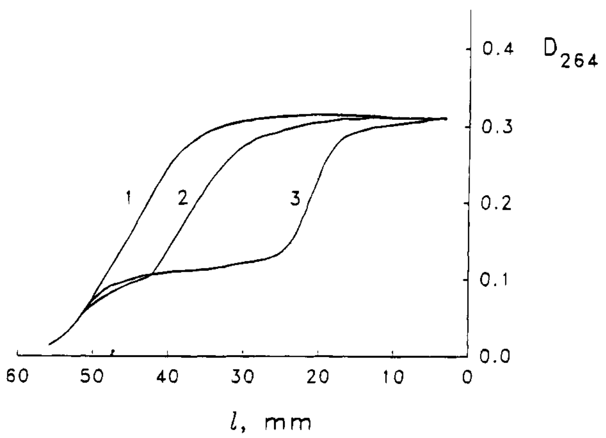

Figure 3. Typical sedimentation borders of (1) PEVP-free and (2, 3) PEVP-containing solutions of Aerosol OT in hexane. [Aerosol OT] = 0.1 M; ω_0 = 10; [PEVP] = 10^{-3} basic mol/L; PEVP $\bar{M}_w$ = 200 000.

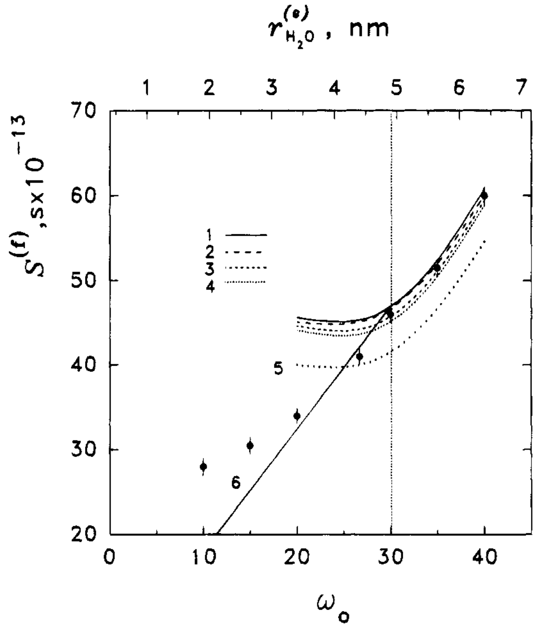

Figure 4. Experimental (filled symbols) and theoretical (curves 1–6) dependencies of the sedimentation coefficient of PEVP-containing particles on the degree of hydration in Aerosol OT solutions in octane. Curves 1–6 were obtained as described in Table 1 for the (1) fixed size, (2) increased volume 1, (3) increased volume 2, (4) increased volume 3, (5) increased volume 4, and (6) induced fit models. The vertical line shows the critical degree of hydration. The scale of radii of the inner cavity ($r^{(e)}_{H_2O}$) of reversed micelles formed in the PEVP-free Aerosol OT system is shown for comparison. The experiments were carried out under conditions described in the legend to Figure 1.

Sedimentation Study of Polycation-Containing Particles. The sedimentation study of reversed micelle systems containing PEVP was performed as previously described.[29] Picric acid, a water-soluble chromophore which is localized in the water cavity of reversed micelles, was added to the systems which were scanned at 400 nm. Under these conditions two populations of sedimenting particles were observed in the PEVP-containing systems: the polymer-free (slower sedimenting) and polymer-containing (faster sedimenting) ones. The former had sedimentation coefficients equal to those of the reversed micelles that formed in the absence of the polycation, their share accounting for more than 90% of the total amount of the sedimenting particles.[48] For a detailed study of the PEVP-containing particles, scanning was performed at the wavelength of polycation absorption at 270 nm where Aerosol OT micelles practically do not absorb the light. The typical sedimentation borders observed in this case are represented in Figure 3. The dependence of sedimentation coefficients (S) of the polycation-containing particles on the degree of hydration is shown in Figure 4.

This dependence was analyzed using the approach previously described for reversed micelles containing hydrophilic proteins.[46,49,50] As follows from these earlier studies, two distinct areas are observed on sedimentation dependencies of the protein-containing micelles. When the radius of the inner water cavity of the initial empty micelles is equal to or larger than the radius of the protein globule, sedimentation of the protein-containing micelles is described by the "fixed size"

Table 1. Models Used for Analysis of Sedimentation Coefficients ($S^{(f)}$) of the PVPE-Containing Particles

N	major features of the PEVP-containing reversed micelles	parameters characterizing the PEVP-containing reversed micelles[a]	expressions for the sedimentation coefficient of the filled RM[b]
1	*fixed size*: radius is equal to that of the empty reversed micelle; molecular mass is equal to the sum of the masses of PVPE and the empty micelle	$R_h^{(f)} = R_h^{(e)}$ $r_{H_2O}^{(f)} = r_{H_2O}^{(e)}$ $n_{AOT}^{(f)} = n_{AOT}^{(e)}$ $n_{H_2O}^{(f)} = n_{H_2O}^{(e)}$ $M^{(f)} = M^{(e)} + \bar{M}_w$	$S^{(f)} = S^{(e)}\left(1 + \frac{\bar{M}_w}{M^{(e)}}\frac{1}{1 - \nu^{(e)}\varrho}\right)$
2	*increased volume 1*: volume of the inner cavity of the reversed micelle increases by 10% of the PVPE volume	$R_h^{(f)} > R_h^{(e)}$ $r_{H_2O}^{(f)} > r_{H_2O}^{(e)}$ $n_{AOT}^{(f)} > n_{AOT}^{(e)}$ $n_{H_2O}^{(f)} = n_{H_2O}^{(e)}$ $M^{(f)} > M^{(e)} + \bar{M}_w$	$S^{(f)} = \frac{S^{(e)}}{\left(1 + \frac{4.78 \times 10^4 a}{(R_h{}^{(e)})^3}\right)^{1/3}}\left(1 + \frac{\frac{\bar{M}_w}{M^{(e)}} + \frac{\Delta m}{M^{(e)}} - \frac{4.78 \times 10^4 a}{(R_h{}^{(e)})^3}\nu^{(e)}\varrho}{1 - \nu^{(e)}\varrho}\right)$ $\Delta m = 444 n_{AOT}^{(e)}\left[\left(1 + \frac{4.78 \times 10^4 a}{(r_{H_2O}^{(e)})^3}\right)^{2/3} - 1\right]$ $a = 0.1$
3	*increased volume 2*: volume of the inner cavity of the reversed micelle increases by 20% of the PVPE volume	same as in 2	same as in 2; $a = 0.2$
4	*increased volume 3*: volume of the inner cavity of the reversed micelle increases by 30% of the PVPE volume	same as in 2	same as in 2; $a = 0.3$
5	*increased volume 4*: volume of the inner cavity of the reversed micelle increases by the PVPE volume	same as in 2	same as in 2; $a = 1.0$
6	*induced fit*: radius and aggregation number of Aerosol OT are equal to those of the empty reversed micelle at the critical degree of hydration	$R_h^{(f)} = R_h^{(c)}$ $r_{H_2O}^{(f)} = r_{H_2O}^{(c)}$ $n_{AOT}^{(f)} = n_{AOT}^{(c)}$ $n_{H_2O}^{(f)} = \omega_0 n_{AOT}^{(c)}$ $M^{(f)} < M^{(c)} + \bar{M}_w$	$S^{(f)} = S^{(c)}\left(1 + \frac{\bar{M}_w + \Delta m}{M^{(c)}}\frac{1}{1 - \nu^{(c)}\varrho}\right)$ $\Delta m = -18\, n_{AOT}^{(e)}(\omega_c - \omega_0)$

[a] Parameters n_{AOT}, n_{H_2O}, and M are the aggregation number, the number of water molecules per micelle, and the molecular mass of the micelle, respectively. The meaning of the superscript index is as follows: (f), PVPE-containing reversed micelle; (e) empty reversed micelle at a given hydration degree; (c), empty reversed micelle at $\omega_0 = \omega_c$. [b] These expressions were obtained using the Svedberg equation as previously described[46] under the assumption that micelles represent spheres covered by the monolayer of Aerosol OT. Sedimentation coefficients of empty micelles ($S^{(e)}$) in PEVP-free systems were determined experimentally. The volume of the PVPE molecule ($\bar{M}_w$ = 140 000) was estimated as the volume of the molecule of the amorphous polymer using the following expression: $V_{PEVP}/\mathrm{nm}^3 = 0.00143\bar{M}_w$.[29] Parameters ν and ϱ are the partial specific volume of the empty RM and the density of the surfactant, respectively, and Δm is the change of the molecular mass of the filled micelle due to redistribution of Aerosol OT or water.

model. According to this model (i) protein solubilization does not affect the micelle size and shape and (ii) the molecular mass of the filled micelle equals the sum of the masses of the empty micelle and the protein. When the size of the inner cavity of the empty micelle is smaller than the radius of the protein, sedimentation is consistent with the "induced fit" model, which presumes that the protein forms its own micelle in which it is tightly covered by the monolayer of hydrated Aerosol OT.

We compared the observed dependence with the theoretical curves obtained for various models which are listed in Table 1. In addition to the fixed size and induced fit models, four other models termed "increased volume" have been considered. According to these models (i) PVPE solubilization results in an increased volume of the water pool and (ii) the PEVP-containing reversed micelles remain spherical and the area occupied by the Aerosol OT molecule in the surfactant layer is constant; i.e., the aggregation number of the surfactant elevates due to the increase in the surface of the water pool. As follows from Figure 4, at $\omega_0 \geq 30$ sedimentation of the PEVP-containing particles is well described by the fixed size model and the models termed "increased volume 1,2,3". These models presume that the volume of the inner cavity of the reversed micelle is not altered or increases by 10–30% of the PEVP volume. Calculations performed for $\omega_0 = 30$ reveal that in these cases the increase in the hydrodynamic radius of the filled micelle does not exceed 0.45 nm. At higher degrees of hydration the change in the micelle radius must be even smaller according to these models. This result reveals that PEVP solubilization does not practically affect the dimensions of reversed micelles at $\omega_0 \geq 30$, which is consistent with the data of the light scattering experiment. No consistency between the experimental points and any of the theoretical curves (including the induced fit model) was observed at $\omega_0 < 30$. Contrary to the result obtained for $\omega_0 \geq 30$, the sedimentation coefficient of the PEVP-containing particles under these conditions significantly depended on the rotation speed, which usually indicates that particles are nonspherical (Table 2).

Table 2. Effect of Rotation Speed on the Sedimentation Coefficient of PEVP-Containing Particles Formed in 0.1 M AOT Solution in Octane

	sedimentation coefficient, 10^{-13} s	
rotation speed, rpm	$\omega_0 = 10$	$\omega_0 = 30$
20 000	75 ± 3	75 ± 3
30 000	60 ± 3	75 ± 3
44 000	42 ± 2	75 ± 3

Formation of the Polycation Globule at the Critical Degree of Hydration. Since PEVP is not soluble in bulk organic solvents, it is located in the water pool of reversed micelles. The above-described experiments suggest that at $\omega_0 = \omega_c$ the radius of the inner cavity of the PEVP-containing reversed micelle equals from 5.06 to 5.35 nm. At the same time the hydrodynamic radius of PEVP ($\bar{M}_w = 140\,000$) in 0.1 M NaCl in aqueous solution equals about 10.0 nm.[29] Therefore, under critical conditions the solubilized polycation is substantially condensed. This phenomenon has been studied recently using polyelectrolytes with various molecular masses as models.[29] The critical degrees of hydration were determined for these polyelectrolytes and it was demonstrated that the radius of the micelle inner cavity at ω_c, termed the "critical radius" ($r^{(c)}_{H_2O}$), is described by the equation

$$r^{(c)}_{H_2O}/\text{nm} \approx 0.09\bar{M}_w^{\ 1/3} \tag{4}$$

Globular macromolecules are characterized by proportionality of the linear dimensions to the cube root of the molecular mass. Therefore, it has been suggested that at critical degrees of hydration the polyion chain is compressed and represents a globule with the radius close to $r^{(c)}_{H_2O}$. The PEVP density in such a globule estimated from eq 4 equals about 0.5 g/cm^3, which is approximately 2 times lower than that of the amorphous polymer. This suggests that the globule is swollen and contains about 50 vol % of water.[29]

One of the possible reasons for polycation compaction is the compressing effect of the micellar matrix, which can be quantitatively estimated using the data on the polydispersity of reversed micelles. Let us consider the case when solubilization of PVPE does not result in its compaction and its radius in the micelle is equal to that of the PEVP chain in the homogeneous aqueous solution. In this case the radius of the inner cavity of the PEVP-containing micelle should be about 2 times larger than that of the initial empty micelle at $\omega_0 = 30$. The work (ΔW) of formation of such enlarged micelles can be expresed in first approximation as follows:[51]

$$\Delta W \approx \frac{kT}{2\sigma^2} \tag{5}$$

where T is temperature, k is the Boltzmann constant, and σ is the standard deviation characterizing the polydispersity of the micelles (=0.15–0.3 for Aerosol OT reversed micelles).[41,45] The unfavorable increase in the free energy due to micelle enlargement calculated using eq 5 per mole of the polycation is about 14–55 kJ/mol, which is comparable with the free energy of formation of the protein globule[52] and exceeds the energy of concentration of the polycation chain due to 2-fold compaction (≈10.4 kJ/mol).[53] This consideration does not take into account electrostatic repulsion of the PEVP positively charged units. It is probable that during formation of the globule condensation of the low molecular mass counterions (Br^-) with the PEVP units takes place. At the same time the net positive charge of condensed PEVP can be at least partially compensated by the negatively charged Aerosol OT molecules. The aggregation number of the surfactant in the reversed micelle at $\omega_0 = 30$ equals about 600, which is comparable with the polymerization degree of the polycation (≈690).

Water Pool in PEVP-Containing Reversed Micelles. It is important to know whether the water pool of PEVP-containing micelles can incorporate other water-soluble molecules simultaneously with the polycation. To answer this question the quenching of fluorescence of FITC-labeled BSA (BSA-FITC) with PEVP in Aerosol OT solutions has been studied. In these experiments equal volumes of solutions containing PEVP and BSA-FITC with the same degrees of hydration were mixed. The system was incubated for 40 min after mixing, and the quenching of fluorescence of the FITC tag was determined when the system reached equilibrium. The degree of quenching significantly depended on ω_0 (Figure 5): up to 55% of quenching took place at $\omega_0 \geq 30$, while at lower degrees of hydration it was decreased. This is consistent with the result of a previous experiment on PEVP covalent conjugation with BSA in reversed micelles of Aerosol OT.[29] The reaction did not proceed at $\omega_0 < 30$, while at higher ω_0 PEVP-BSA conjugates of various compositions were produced (Figure 5). The fluorescence and conjugation data suggest that at $\omega_0 \geq \omega_c$ the water pool of the PEVP-containing micelle contains enough space to accommodate the BSA molecule, whereas at $\omega_0 < \omega_c$ the polycation and BSA cannot be incorporated into the same structure simultaneously. The concept of a swollen globule explains why the interaction between PEVP and BSA takes place at $\omega_0 \approx 30$ when the polycation chain is apparently tightly surrounded by the surface layer. The volume of the BSA molecule equals about 115 nm^3, which is only 50% of the volume occupied by water in the PEVP-swollen globule ($r^{(c)}_{H_2O} \approx 5$ nm) at $\omega_0 = 30$. It is probable that an equal volume of water is expelled from the polycation-containing micelle into the polycation-free one to accommodate the protein molecule.

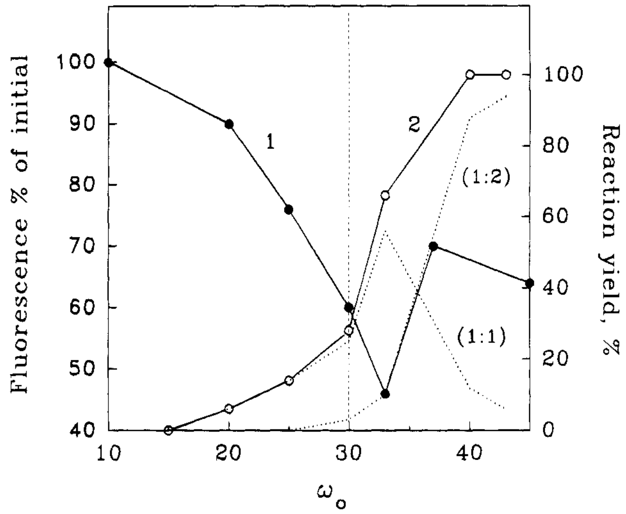

Figure 5. (1) Quenching of BSA-FITC fluorescence by PEVP (●) and (2) yield of the reaction of conjugation of PEVP with BSA (○) in Aerosol OT solutions in octane at various degrees of hydration. The yields of the PEVP:BSA conjugates of various compositions (1:1 and 1:2) are shown by the dashed lines. The vertical dashed line shows the critical degree of hydration. The quenching experiment was performed under the following conditions: [Aerosol OT] = 0.1 M; the water microphase contained 10 mM borate buffer, pH 9.2; [BSA-FITC] = 60 nM; [PEVP] = 5 × 10^{-4} basic mol/L; λ_{ex} = 498 nm; λ_{em} = 517 nm. To conjugate with BSA, the (4-vinyl-1-pyridino)-acetate units of PEVP were activated with *N*-cyclohexyl-*N*'-morpholinoethylcarbodiimide (data from ref 29). PEVP $\bar{M}_w$ = 140 000.

Formation of PSC in Organic Solvents and Water. The addition of the concentrated aqueous solution of PEVP (5 × 10^{-1} basic mol/L) to the Aerosol OT solution in hexane led to separation of the system into an aqueous and an organic phase when the resulting degree of hydration exceeded 20. The organic phase

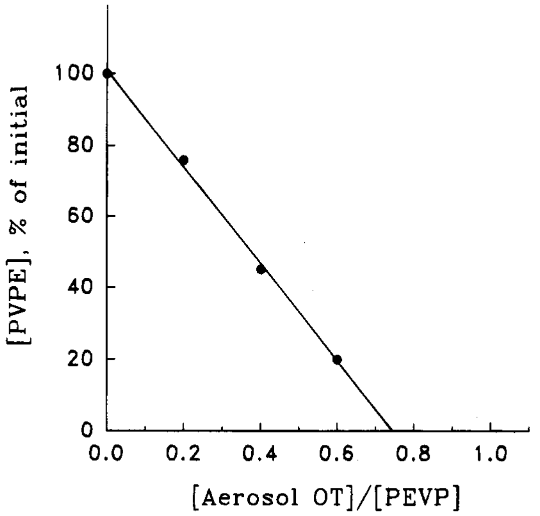

Figure 6. Dependence of the PEVP concentration in the supernatant on the concentration of Aerosol OT added during polycation precipitation from aqueous solution with the surfactant. PEVP $\bar{M}_w$ = 200 000.

Table 3. Elemental Analysis of PSC Obtained from Hexane and Aqueous Solutions[a]

solvent	composition	% C	% H	% N	% S	S/N
hexane	by weight	57.1	8.80	1.97	5.89	
	by moles	34.3	62.8	1	1.3	1.3
water	by weight	59.1	9.06	2.28	5.59	
	by moles	30.6	56.6	1	1.1	1.1

[a] PEVP $\bar{M}_w$ = 200 000.

formed under these conditions did not contain any PEVP. The aqueous phase contained a white precipitate in which both PEVP and Aerosol OT were revealed by the elemental analysis, the molar ratio S/N in it being equal to 1.3 (Table 3). This result was indicative of formation of PSC of Aerosol OT and PEVP with a composition close to equimolar.[54] This complex was also obtained in aqueous solutions of PEVP to which various amounts of Aerosol OT were added. This was followed by an increase in the turbidity of the system due to formation of insoluble PSC. The complex was separated by ultracentrifugation and the concentration of PEVP remaining in the supernatant was measured spectrophotometrically. As can be seen in Figure 6, the concentration of the polycation decreased linearly with the elevation of the concentration of the surfactant, which was indicative that the composition of PSC in the precipitate did not depend practically on the concentration of the reagents. The PSC composition of 0.75 was estimated by the crossing point of the dependence of Figure 6 with the concentration axis. According to the elemental analysis, the composition was 1.1, which is in reasonable agreement with the data of Figure 6 (Table 3).

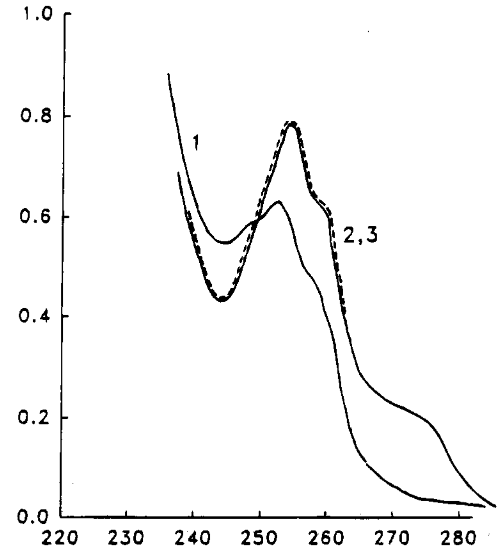

Figure 7. UV spectra of (1) PEVP in water, (2) PSC of PEVP and Aerosol OT in hexane, and (3) PEVP solubilized by the Aerosol OT reversed micelles in hexane. [Aerosol OT] = 0.1; ω_0 = 10; PEVP $\bar{M}_w$ = 200 000.

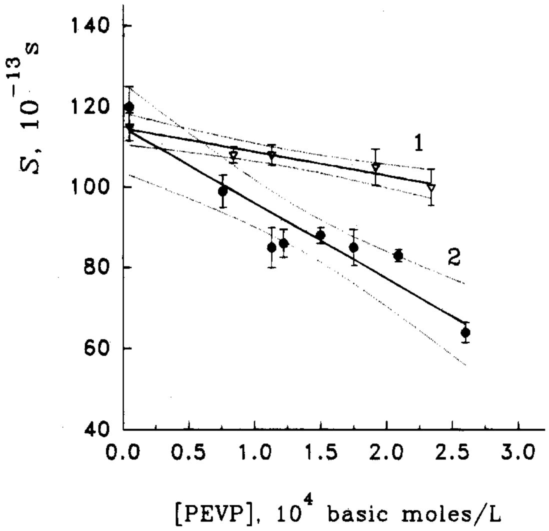

Figure 8. Concentration dependencies of sedimentation coefficients of (1) PSC of PEVP and Aerosol OT in hexane (▽) and (2) PEVP-containing particles formed in the Aerosol OT micellar solution, ω_0 = 10 (●). PEVP $\bar{M}_w$ = 200 000. Values are mean ± SD (n = 3). Linear regressions are shown by solid lines, and 95% confidence intervals by dotted lines.

Solubilization of PSC in Aliphatic Hydrocarbons. PSC obtained from aqueous solutions was dried and then dissolved in hexane (its solubility at 20 °C equaled about 0.7 g/L). As can be seen in Figure 7, the UV spectrum of PSC in hexane significantly differed from that of PEVP in aqueous solution: the hyperchromic effect and a shift of the absorption maximum from 251 to 255 nm were observed in the case of the complex. At the same time, this spectrum practically coincided with that observed in the case of PEVP solubilized in Aerosol OT reversed micelles at ω_0 = 10. Figure 8 represents concentration dependencies of sedimentation coefficients of PSC in hexane and PEVP-containing particles formed in Aerosol OT micellar solution. The value of the sedimentation coefficients ($S°$) obtained by extrapolation of these dependencies to infinite dilution are practically the same ($S°$ = 114.1 ± 2.8 for PSC and 115.8 ± 11.0 for the reversed micelle system). Finally, the mean hydrodynamic radius of PSC in hexane measured by quasi-elastic light scattering was about 25 nm, very close to the value (≈30 nm) obtained for PEVP-containing particles in Aerosol OT micellar solutions at ω_0 = 10. Taken together, the spectroscopy, ultracentrifugation, and light scattering data suggest that the PEVP-containing particles formed in Aerosol OT solutions at ω_0 = 10 represent PSC with the composition and characteristics similar to those of the complex obtained in water and then dissolved in hexane.

From Polycation-Containing Reversed Micelles to Soluble PSC. Our results demonstrate that various polycation–surfactant structures are formed during PEVP solubilization in Aerosol OT solutions in aliphatic hydrocarbons depending on the amount of water added to the system. At high degrees of hydration PVPE incorporates in the water pool of the reversed micelle where the polycation chain is substantially condensed. According to the light scattering and ultracentrifugation data, polycation incorporation does not affect significantly the size of the reversed micelle. Under these conditions the water pools of PEVP-containing micelles may also incorporate other macromolecules (e.g., BSA) simultaneously with the polycation. At the critical degree of hydration PEVP represents a swollen globule compressed by the micellar matrix. The density of the polycation in such a globule is about 0.5 g/cm^3, and about 50% of the globule volume is occupied by water molecules. Below the critical degree of hydration the polycation and the surfactant form a stoichiometric PSC, soluble in the aliphatic hydrocarbons. The dimensions of such a complex are significantly higher than those of the initial empty reversed micelles, and this complex apparently represents a "comblike" structure in which the Aerosol OT head groups are electrostatically bound with the charged units of PEVP, whereas the hydrophobic tails of the surfactant, "grafted" to the polycation, are exposed to the bulk solvent.

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(54) The composition of PSC is equal to the ratio of the molar concentration of the surfactant and the base molar concentration of the polycation.

MA946134C