ATOMIC FORCE MICROSCOPY AND LIGHT SCATTERING STUDY OF ONION-TYPE MICELLES FORMED BY POLYSTYRENE-*block*-POLY(2-VINYLPYRIDINE) AND POLY(2-VINYLPYRIDINE)-*block*-POLY(ETHYLENE OXIDE) COPOLYMERS IN AQUEOUS SOLUTIONS

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The three-layer onion micelles formed in aqueous solution by hierarchical self-assembly of polystyrene-*block*-poly(2-vinylpyridine) micelles, PS-PVP, and poly(2-vinylpyridine)-*block*-poly(ethylene oxide) chains, PVP-PEO, were studied by a combination of light scattering (LS) and atomic force microscopy (AFM). Section analysis of AFM images of micelles deposited on mica in combination with LS data from micellar solutions provide distribution functions of sizes from which the number and mass distributions of molar masses of micelles can be evaluated. Both light scattering and AFM data reveal that the used preparation protocol yields onion micelles accompanied by an admixture of PVP-PEO micelles. It means that only a certain amount of PVP-PEO self-assembles with PS-PVP and forms onion micelles. The remaining PVP-PEO copolymer forms either small PVP-PEO micelles or participates in formation of large aggregates at longer times. The time-dependent measurements show that both onion-type and core-shell PVP-PEO micelles are fairly stable over a long time period and only a low fraction of large aggregate forms on the timescale of weeks and at longer times, the solution does not change any more.

Keywords: Atomic force microscopy; Light scattering; Polymer micelles; Polymeric nanoparticles; Self-assembly; Block copolymers; Stability in aqueous solutions.

Block copolymers belong to promising materials for application in nanotechnology because they form a variety of self-assembled nanostructures, both in melts[1a–1f] and in selective solvents[2,3a–3l]. We have been studying the behavior and structure of amphiphilic block copolymer micelles in aqueous solutions for almost two decades[4a–4d]. The copolymers containing long hydrophobic and long water-soluble blocks do not usually dissolve in aqueous

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media because water is too strong precipitant for the hydrophobic block. Nevertheless, aqueous solutions of micelles from high-molecular-weight amphiphilic copolymers can be prepared indirectly by adding water to micellar solutions in mild selective water-miscible organic solvents and by dialyzing against water or aqueous buffers. The copolymer micelles transferred in aqueous media by the above-described procedure are in a "kinetically frozen", i.e., in a nonequilibrium state and do not dissociate to unimers upon dilution.

A few years ago, we prepared and studied onion micelles[5a–5c] consisting of three layers of different polymers. The onion micelles were created by hierarchical self-assembly of two diblock copolymers, polystyrene-*block*-poly(2-vinylpyridine), PS-PVP, and poly(2-vinylpyridine)-*block*-poly(ethylene oxide), PVP-PEO. PS-PVP forms micelles with the glassy PS core and the shell of protonated PVP in acidic aqueous solutions (pH below 4.8). PVP-PEO, dissolves at low pH in the form of individual chains. When a mixture of PS-PVP micelles and PVP-PEO unimers in an acidic aqueous solution is titrated by an alkaline solution and pH exceeds 4.8, PVP blocks become water-insoluble. The PVP blocks from PS-PVP micelles and in PVP-PEO unimers collapse together and onion micelles with the inner PS core, middle neutral PVP layer and the outer water-soluble PEO shell are formed. The excess PVP-PEO unimers over the necessary amount for stabilization of onion micelles form small PVP-PEO micelles with PVP core.

Some time ago, we studied onion micelles by light scattering[5a,5b] as the principal method and by transmission electron microscopy as a supplementary method[5c]. In this paper, we (i) investigate the long-term stability of onion micelles and (ii) present detailed characterization of all types of nanoparticles present in the solution by a combination of atomic force microscopy (AFM) and light scattering (LS). We use AFM as the principal experimental technique with the aim to exploit its strength consisting in direct imaging of micellar assemblies. Although both AFM and LS provide information on size distributions of studied nanoparticles, experimental conditions, advantages and drawbacks of these two techniques differ significantly. A well-known drawback of LS is the strong dependence of scattering intensity on the size of scattering particles, which makes the observation of small scatters in mixtures with large ones often impossible. The other shortcoming of LS consists in the fact that while it yields direct and rather accurate integral information on all scattering particles (e.g., weight-average molar masses) information on the polydispersity is indirect and much less reliable.

The imaging of real particles is the main advantage of microscopy techniques, but the evaluation of size distributions of large assemblies of nanoobjects suffers from the fact that only a limited number of objects can be measured in a reasonable time. There are several other potential complications that have to be taken into consideration: The nanoobjects are often studied after their deposition on the surface and they may be deformed. The second danger consists in the fact that the deposition (even though it does not have to be direct adsorption) may be affected by adsorption and hence the dependence of amounts of adsorbed nanoparticles on their size and composition cannot be precluded. The third danger consists in potential re-arrangement of nanoparticles (aggregation, decomposition, etc.) during deposition and drying.

We addressed all the above complicating factors in our recent papers and we proved that the danger of size-dependent adsorption and reorganization of micelles with kinetically frozen cores deposited from very dilute solutions is negligible[6a–6d]. Further we have shown that micelles deposited on mica are pancake-deformed, but their size is proportional to that in the solution. Hence the combination of AFM with LS yields reasonable molar distribution of masses of dissolved nanoparticles.

Therefore we believe that the combination of AFM and LS yields reliable results and that the detailed analysis of AFM scans of systems containing several types of nanoparticles provides pieces of so far missing information and completes our knowledge of the behavior and stability of aqueous solutions containing onion micelles.

EXPERIMENTAL

Materials

Copolymer. Poly(2-vinylpyridine)-*block*-poly(ethylene oxide), PVP-PEO, was purchased from Polymer Source, Inc. (Dorval, Quebec, Canada). The molecular weight of the PVP block, of the PEO block, and the polydispersity index, provided by the manufacturer, were 14.2×10^3, 15.4×10^3, and 1.06, respectively.

Polystyrene-*block*-poly(2-vinylpyridine) (PS-PVP) was synthesized by living anionic polymerization at the University of Texas at Austin. The molecular weight of the PS-PVP was 70 000 and of the PS block 36 000, M_w/M_n was 1.14. Details on the synthesis and characterization are given in ref.[5b].

Preparation of micelles. PS-PVP micelles were prepared according to the following protocol: The sample was dissolved in a mixture of 1,4-dioxane (80 vol.%) and methanol and slowly titrated with methanol under very mild stirring until the methanol content was 50% by volume. Then the solution was carefully titrated with 0.01 M HCl aqueous solution until the water content was 50%. The final step was the dialysis of this solution (mass concentration

c = 3 g l^{-1}) against 0.01 M HCl. During the final dialysis, the HCl solution in the external bath was exchanged several times to assure the removal of all traces of organic solvents.

Onion micelles were prepared as follows: PVP-PEO was dissolved in the 0.01 M HCl and the solution of PS-PVP micelles in 0.01 M HCl was then added, so that both the concentrations of PS-PVP and PVP-PEO in the mixture were 1 g l^{-1}. In the last step, aliquot amount of 0.1 M NaOH was added for neutralization of the acid under vigorous stirring of the solution.

Methods

Light scattering. The light scattering setup (ALV, Langen, Germany) consisted of a 633 nm He-Ne laser, an ALV CGS/8F goniometer, an ALV High QE APD detector and an ALV 5000/EPP multibit, multitau autocorrelator. The solutions of the highest concentration were filtered through 0.45 μm Acrodisc filters, lower concentrations were prepared by mixing with adequate solvent filtrated through 0.20 μm Acrodisc filters. The measurements were carried out for different concentrations and different angles at 20 °C. Static light scattering (SLS) data were treated by the standard Zimm method using the equation

$$\frac{4\pi^2 n_0^2 (\mathrm{d}n/\mathrm{d}c)}{\lambda^4 N_A} \frac{c}{R^{cor}(q,c)} = \frac{1}{M_w P(q)} + 2A_2 c \qquad (1)$$

where n_0 is the refractive index of the solvent, dn/dc the refractive index increment of the polymer with respect to the solvent, λ the wavelength of the incident light, N_A the Avogadro constant, $R^{cor}(q,c)$ is the corrected Rayleigh ratio, which depends on the polymer concentration c and on the magnitude of the scattering vector, $q = (4\pi n_0/\lambda) \sin(\theta/2)$, where θ is scattering angle, M_w is the apparent weight-average molar mass of scattering polymeric particles, A_2 is the "light-scattering-weighted" second virial coefficient of the concentration expansion, and $P(q) = (1 + R_g^2 q^2/3 + \ldots)^{-1}$ is the particle scattering function (R_g is the radius of gyration). Refractive index increments of the copolymers with respect to the solvent, (dn/dc), were measured with Brice–Phoenix differential refractometer at the University of Texas at Austin[5b] and are as follows: for PS-PVP in water, 0.260 and for PVP-PEO, 0.194. Refractive index increment of onion micelles in water, dn/dc = 0.227, was calculated from values for PS-PVP and PVP-PEO as a mass-weighted average.

DLS data analysis was performed by fitting the measured normalized intensity autocorrelation function $g_2(t) = 1 + \beta|g_1(t)|^2$, where $g_1(t)$ is the electric field correlation function, t is the lag-time and β is a factor accounting for deviation from the ideal correlation. An inverse Laplace transform of $g_1(t)$ with the aid of a constrained regularization algorithm (CONTIN) provides the distribution of relaxation times, $\tau A(\tau)$.

$$g_1(t) = \int_{-\infty}^{\infty} \tau A(\tau) \exp(-t/\tau) \mathrm{d} \ln \tau \qquad (2)$$

Effective angle- and concentration-dependent hydrodynamic radii, $R_H(q,c)$, were obtained from the mean values of relaxation times, $\tau_m(q,c)$, of individual diffusive modes using the equation

$$R_H(q,c) = \frac{kT \tau_m(q,c) q^2}{6\pi\eta_0} \qquad (3)$$

where k is the Boltzmann constant, T is the temperature and η_0 is the solvent viscosity.

Atomic force microscopy (AFM). All measurements were performed in the tapping mode under ambient conditions using a commercial scanning probe microscope, Digital Instruments NanoScope dimensions 3, equipped with a Nanosensors silicon cantilever, typical spring constant 40 N m^{-1}. Details of the measurement and the principle of the evaluation of micellar polydispersity were given in our earlier papers. Polymeric micelles were deposited on a fresh (i.e., freshly peeled out) mica surface (flogopite, theoretical formula $KMg_3AlSi_3O_{10}(OH)_2$, Geological Collection of Charles University in Prague, Czech Republic) by a fast dip coating in a dilute micelle solution in 0.01 M HCl (core micelles) or pure water (small and onion micelles), $c \approx 10^{-2}$ g l^{-1}. After the evaporation of water, the samples for AFM were dried in vacuum oven at ambient temperature for ca. 5 h.

RESULTS AND DISCUSSION

Light Scattering Characterization

Core micelles (PS-PVP), small micelles (PVP-PEO) and onion micelles (PS-PVP/PVP-PEO) prepared as described in Experimental were characterized by SLS and DLS in aqueous solutions. Micelles of PS-PVP (core micelles) are fairly monodisperse. The PVP blocks are protonized in acidic media. In 0.01 M HCl and 0.1 M NaCl, they behave as expected according to our earlier studies[5a–5c]. The PVP blocks in the shell are fairly stretched due to the protonization of PVP segments, even though small ions screen partly the electrostatic forces. The weight-average molar mass, $(M_w)_{PS\text{-}PVP}$, radius of gyration and hydrodynamic radius extrapolated to the zero concentration and zero scattering angle are listed in Table I. The core micelles studied herein are larger than those in our previous study[5b] because a slightly modified preparation protocol was applied (the composition of the mild selective solvent from which PS-PVP was dialyzed into acidic water was

TABLE I
Characterization of micelles

Micelles	M_w, g mol^{-1} [a]	R_H, nm [b]	R_g, nm [a]	PDI [c]
PVP-PEO	2.53×10^6	29.4	23.0	1.91
PS-PVP	20.74×10^6	100.6	72.1	1.21
Onions	$40.64 \times 10^{6*}$			2.75
Small onions	$21.38 \times 10^{6**}$	51.8	41.9	
Large onions		83.0	146.0	

Determined by: [a] SLS, [b] DLS and [c] AFM. * The given value is $(M_w)_{mean}$ (see the Light Scattering part). ** The given value is $(M_w)^{ap} = \langle w \rangle (M_w)_{on}$ (see the Light Scattering part).

different). Small PVP-PEO micelles in pure water are much smaller than the core PS-PVP micelles and their scattering power is relatively low. Their molar mass, $(M_w)_{PVP\text{-}PEO}$, and size are listed in Table I. We have studied the time stability of individual micelles and it is worth-mentioning that size and polydispersity of PVP-PEO micelles do not almost change with aging.

The scattering response of aged onion micelles in water (mass ratio of PS-PVP and PVP-PEO copolymers 1:1; several weeks after preparation when changes in their size characteristics with time are very small and insignificant) is fairly complex. Static light scattering reveals that more types of micelles coexist in the solution. The scattering function $P(q)^{-1}$ increases significantly at small angles, as seen on the Zimm plot in Fig. 1, which resembles that for bimodal spherical systems[7]. The shape of angularly dependant curves can be explained assuming that a relatively small fraction of large particles is present in the solution and therefore the light scattering intensity increases at low scattering angles. The overall weight-average molar mass is $(M_w)_{mean} = 40.64 \times 10^6$ g mol^{-1} (Table I). When just the data for high angles are taken into account, the extrapolation yields $(M_w)^{ap} = \langle w \rangle (M_w)_{on} = 21.38 \times 10^6$ g mol^{-1} (Table I), where $(M_w)_{on}$ is molar mass of regular onion micelles. Nevertheless, the integral weight fraction $\langle w \rangle$ of non-aggregated onion micelles cannot be obtained by light scattering measurements only. The same conclusion on the coexistence of large and small scatters may be drawn from DLS measurement, which yields the angular-dependent appar-

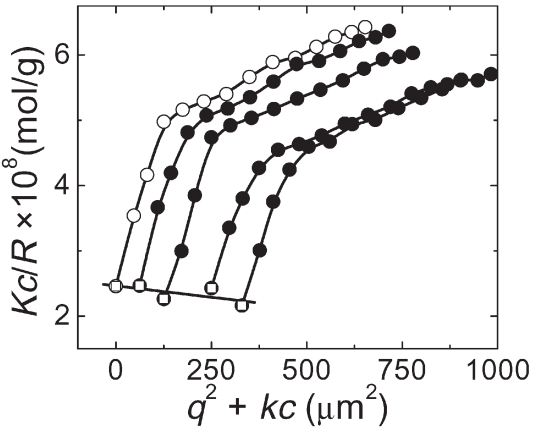

FIG. 1
Zimm plot of onion micelles in water for concentrations of polymer 0.125, 0.25, 0.5 and 0.66 g l^{-1}

ent hydrodynamic radius, R_H^{ap} (inset of Fig. 2). The value, R_H^{ex}, extrapolated to zero scattering angle from the wide-angle range is 51 nm, while the extrapolation from the small-angle range yields the value of 83 nm (Table I).

It was found earlier that a certain fraction of small PVP-PEO micelles is present in the solution together with onion micelles[5a–5c]. The fraction of small micelles was found to depend on the $c_{PVP\text{-}PEO}/c_{PS\text{-}PVP}$ ratio. In this paper we present data on the mixture with the ratio equal to one. The light scattering from small micelles is very weak and therefore hidden in the overall scattering intensity. Also the DLS distribution of relaxation times is monomodal (Fig. 2), despite the existence of three types of particles in the solution (small PVP-PEO micelles, regular onion micelles and micellar aggregates). The fraction of PVP-PEO adsorbed on core micelles during the formation of onions, δ, can be calculated indirectly from light scattering data according to the equation[5a]

$$\delta = \frac{c_{PVP\text{-}PEO}^{ads}}{c_{PS\text{-}PVP}} = \frac{(M_w)_{on}}{(M_w)_{PS\text{-}PVP}} - 1 . \qquad (4)$$

However, the light scattering measurement is not able to provide molar mass of regular onions, $(M_w)_{on}$. Instead, it gives $(M_w)_{mean}$. Assuming that the

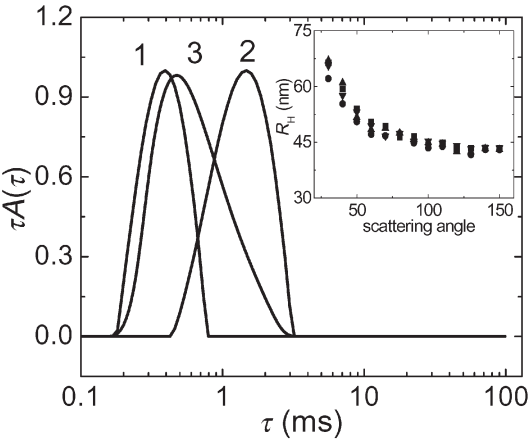

FIG. 2
Distribution of correlation times of small micelles in water (*1*), core micelles in 0.01 M HCl, 0.1 M NaCl (*2*) and onion micelles in water (*3*), measured at 90° and concentrations of polymers ca. 0.5 g l^{-1}. Inset: angle dependence of R_H of onion micelles for various concentrations of polymer (0.125, 0.25, 0.5 and 0.66 g l^{-1})

scattering power of onion micelles is considerably higher than that of small PVP-PEO micelles, we may write

$$\delta \approx \frac{(M_w)_{mean}}{(M_w)_{PS\text{-}PVP}} - 1 \qquad (5)$$

where δ is the mass fraction of PVP-PEO copolymer adsorbed on both regular onions and aggregates. Equation (5) yields the value δ = 0.96, which means that less than 4% of PVP-PEO remain in solution as small micelles. However, the presence of small micelles causes a decrease of $(M_w)_{mean}$ with respect to $(M_w)_{on}$ which cannot be evaluated, as well as the effect of potentially present micellar clusters. Therefore, the actual molar mass of true onion micelles and also δ may differ from the above estimates. It is very interesting to compare results for aged systems with those for onions shortly after preparation. The Zimm plot of fresh onion micelles is regular and there is no evidence of large clusters. Therefore we assume that it is dominated by scattering from regular high-molecular-weight onion micelles. The DLS-based distributions are bimodal indicating high fractions of both onion micelles and small PVP-PEO micelles. Consequently, it seems that for the fresh onion system prepared by the used dialyzation protocol, δ is lower than 0.96.

Atomic Force Microscopy

Samples for AFM were prepared by fast dip coating of freshly cleaved mica (i) in very dilute solutions of onion micelles (containing also small PVP-PEO micelles) and small micelles in water, or (ii) in a dilute solution of core micelles in 0.01 M HCl. As proved from AFM scans, micelles are evenly spread on the mica surface. Typical scans for all three types of micelles long time after solution preparation (more than one month) are shown in Fig. 3a–3c. It is evident that PVP-PEO micelles are much smaller than both PS-PVP core micelles and onion micelles. The core micelles deposited on mica are spherical and appear quite monodisperse. The mica-deposited onion micelles resemble core micelles covered by PVP-PEO. It is possible to distinguish both onion and small micelles. Based on our earlier observations[6a–6d], we assume that the deposition of micelles with the same corona composition on mica does not depend on the size of micelles. Therefore, the fraction of deposited micelles of a given size and type is proportional to their concentrations in the solution, i.e., to the number distribution of mo-

lar masses of micelles. Hence identical distributions of sizes and masses of adsorbed micelles and micelles in the solution can be expected. Critical evaluation of experimental data performed in this and in our recent papers supports this assumption.

The section analysis of AFM pictures yields sizes and shapes of deposited particles. We have performed the section analysis of several hundreds of onion, small and core micelles. Typical profiles of micelles on mica are shown in Fig. 3d. The shape is strongly nonspherical because of deformation of micelles due to attractive interactions with the surface, broadening effect of the AFM tip and also indentation of the tip into micelles. We constructed histograms of sizes and obtained distributions of horizontal radii, *R*, and heights, *z* (Figs 4a and 4b, respectively), of deposited micelles. Differences between radii corresponding to images of individual micellar types

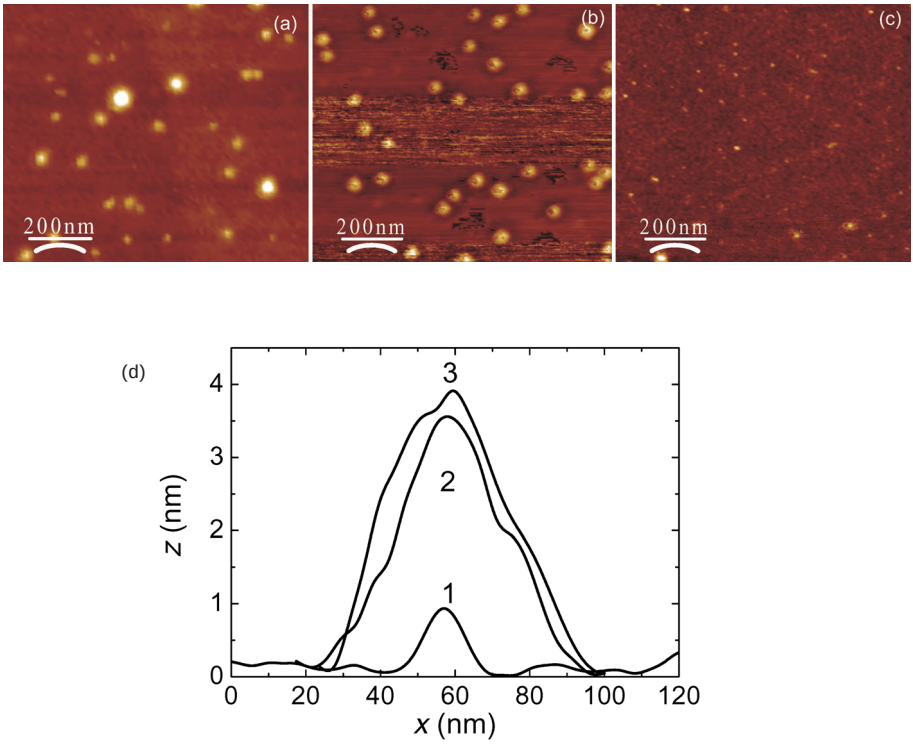

FIG. 3
Top view of AFM scans of onion micelles (a), core micelles (b) and small micelles (c); typical profiles (d) of small micelles (*1*), core micelles (*2*) and onion micelles (*3*)

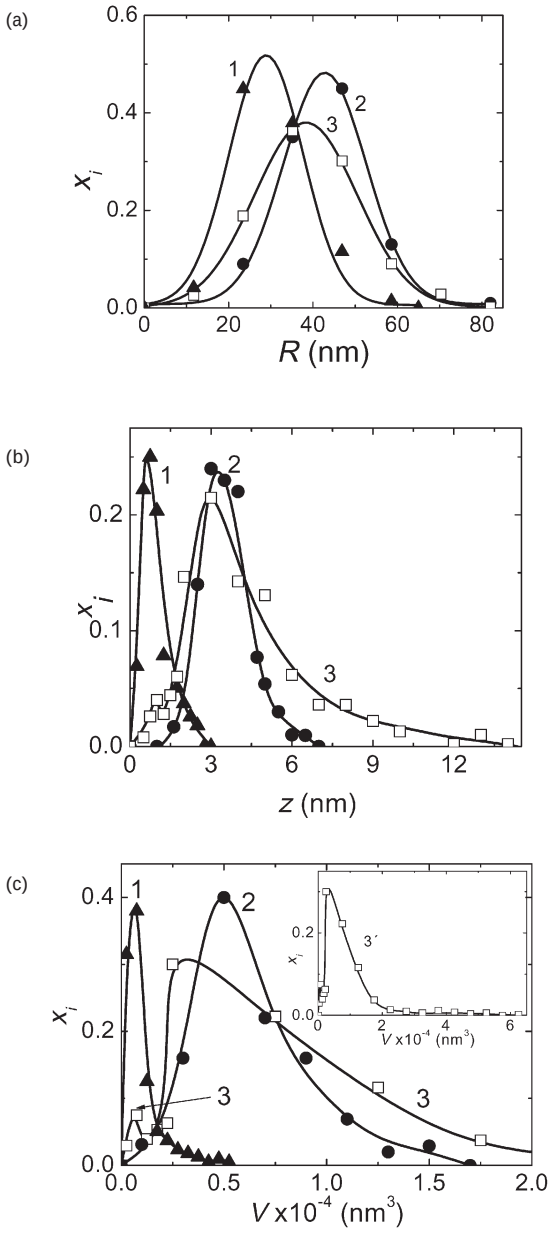

FIG. 4

Number-weighted distribution functions of radius (a), height (b) and volume (c) of small (*1*), core (*2*) and onion (*3*) micelles. Inset in Fig. 4c: whole curve for onion micelles

are similar as in the case of light scattering measurement of R_H. The PVP-PEO micelles are smaller than onion micelles and PS-PVP micelles appear to be slightly larger than onions, because the shell-forming PVP block are stretched in the solution due to protonization and as suggested by Minko et al., the PVP conformations are quenched at the mica surface and do not change during deposition and drying[8]. In minor part, due to the flexibility of the shell-forming blocks spread on the surface and, in major part, to the broadening effect of the AFM tip, the apparent polydispersity of micelles is partly suppressed with respect to *R*. Nevertheless, the distribution of vertical distances *z* provides very useful information on the size of kinetically frozen (and hence only little deformable) parts of micelles, i.e., on compact micellar cores and collapsed middle layers. The scans of deposited onion micelles show a small peak corresponding to the admixture of small PVP-PEO micelles. Further it is evident that the onion micelles are more polydisperse that both the core PS-PVP and small PVP-PEO micelles.

Onion micelles deposited on mica from a freshly prepared solution were studied for comparison (not shown). They are very similar to those in scan 3a; nevertheless, onions are accompanied by a high number of partly overlapped small micelles. Typical profiles of these micelles are almost the same as for regular onions or small micelles long time after preparation. The number fraction of small micelles yields approximately 75% for the freshly prepared sample. The section analysis of pure PVP-PEO micelles (prepared separately) and small micelles in the mixture with onions suggests that both deposited nanoparticles have identical sizes.

The long-term monitoring of stability of aqueous micellar solutions shows that the polydispersity of onion micelles changes with time (up to one month) and then it levels off. At early stages, a small fraction of large clusters forms and the number fraction of small PVP-PEO micelles decreases. The large clusters affect the light scattering data. However, the AFM analysis proves that their concentration is very small. Because the formation of large clusters stopped after one month and no other changes have been observed at longer times (up to one year), it is probable that large clusters form as a result of certain inhomogeneity of the PS-PVP copolymer. We can generally conclude that the onion micelles are fairly stable in neutral and alkaline solutions at ambient temperatures.

To be able to compare AFM and light scattering data, we use *R*- and *z*-histograms and reconstruct a new histogram for a combined size characteristic which is proportional to the molar mass of micelles. A suitable choice is the product $R^2z = V^{ef}$, which is proportional to the particle volume *V* of deposited particles and can be reasonably assumed to be proportional to

the molar mass M, i.e., $M = kV$. The constant k has to be determined for each sample by an independent method because the average structure and density of different types of deposited micelles may be different. We use the weight-average molar masses obtained by light scattering for conversion of the relative V^{ef} scale to the absolute M scale. The number distribution function $x(V^{ef}) = N(V^{ef})/N_{tot}$, where $N(V^{ef})$ is the number of micelles with V^{ef} and N_{tot} is the total number of scanned micelles, is depicted in Fig. 4c. The distribution function of molar masses may be obtained from the latter by transforming the horizontal axis for each type of particles separately, using light scattering data for determination of the proportionality constant k. For the weight distribution function, we have

$$w(V^{ef}) = \frac{V^{ef} x(V^{ef})}{\int_0^\infty V^{ef} x(V^{ef}) \mathrm{d}V^{ef}} . \qquad (6)$$

The number- and weight-average micellar molar masses and the polydispersity (PD) can be calculated

$$M_n = \frac{k\int_0^\infty V^{ef} x(V^{ef}) \mathrm{d}V^{ef}}{\int_0^\infty x(V^{ef}) \mathrm{d}V^{ef}} \qquad (7)$$

$$M_w = \frac{k\int_0^\infty (V^{ef})^2 x(V^{ef}) \mathrm{d}V^{ef}}{\int_0^\infty V^{ef} x(V^{ef}) \mathrm{d}V^{ef}} \qquad (8)$$

$$\mathrm{PD} = \frac{\int_0^\infty (V^{ef})^2 x(V^{ef}) \mathrm{d}V^{ef}}{\left(\int_0^\infty V^{ef} x(V^{ef}) \mathrm{d}V^{ef}\right)^2} . \qquad (9)$$

The polydispersity of individual types of micelles calculated from AFM data are given in Table I. In case of onion micelles, we can distinguish three types of different particles and calculate their amounts in the mixture (see Fig. 4c, curve *3*, and Inset). The first small peak can be assigned to small

PVP-PEO micelles ($\langle x \rangle$ = 12.4%, $\langle w \rangle$ = 1.2%). It is evident that onion micelles represent the majority of deposited particles, but it is not easy to set a border between non-aggregated onion micelles and micellar clusters. Nevertheless, the long tail in distribution can be ascribed to aggregates. Distribution functions $x(M)$ and $w(M)$ recalculated to the absolute M-scale are plotted in Fig. 5 for onion micelles. After recalculation, it is easier to find the border between individual small and large onions (regular micelles and clusters) from the shape of the weight distribution function, $w(M)$. We define aggregates as deposited nanoparticles with $M >$ ca. 70×10^6 g mol^{-1}; therefore the number fraction of aggregates (micellar clusters) is ca. 4%, while their weight fraction is much higher, ca. 23%.

The number fraction of small PVP-PEO micelles coexisting with onion micelles is about 12%, evaluated by AFM from all deposited micelles. To gain the weight fraction of the copolymer chains which form the small PVP-PEO micelles, the amount of all types of micelles in solution, their molar mass and the mass ratio of copolymers in sample have to be considered. If there is only one core particle (the original PS-PVP micelle) in each onion micelle, the fraction of PVP-PEO polymer which forms small micelles only (not regular and irregular onions) in solution can be calculated as follows:

$$f_{\text{PVP-PEO}} = \frac{x^{\text{mic}}_{\text{PVP-PEO}} M^{\text{mic}}_{\text{PVP-PEO}} c^{\text{mic}}_{\text{PS-PVP}}}{x^{\text{mic}}_{\text{onion}} M^{\text{mic}}_{\text{PS-PVP}} c^{\text{mic}}_{\text{PVP-PEO}}} \qquad (10)$$

where x is number fraction of micelles from AFM, M is molar mass of micelles from SLS, and the ratio of weight concentrations ($c_{\text{PS-PVP}}/c_{\text{PVP-PEO}}$)

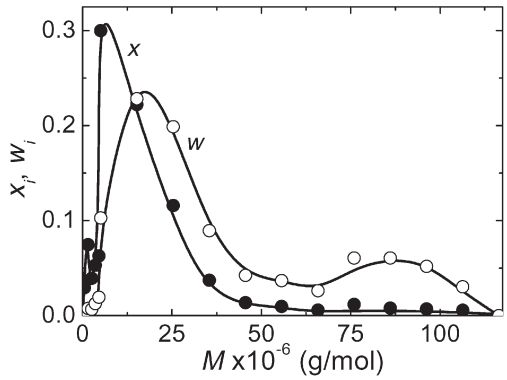

FIG. 5
Number- (x) and mass-weighted (w) distribution functions of molar mass for onion micelles

equals one in our case. Equation (*10*) yields $f_{PVP\text{-}PEO}$ = 1.7%, which means that the ratio $\delta = c^{ads}{}_{PVP\text{-}PEO}/c_{PS\text{-}PVP}$ is 0.983. It compares fairly well with the value 0.96 obtained by SLS, but as it was already mentioned in the light scattering section, the δ-value has to be corrected with respect to the mass ratio of small micelles. From AFM, the following data were obtained: $\langle w \rangle$ = 1.2% (PVP-PEO micelles), therefore $(M_w)_{mean} = 41.13 \times 10^6$ g mol^{-1} and δ = 0.982 and the weight-average molar mass of non-aggregated onion micelles ca. $(M_w)_{on} = 24 \times 10^6$ g mol^{-1}, i.e., one onion micelle carries on average ca. 100 PVP-PEO unimers. For comparison, the aggregation number of the small PVP-PEO micelles is 86 and that of core micelles is 296. This means that most PVP-PEO chains are engaged in onion clusters. Using the AFM distribution, we are able to find $(M_w)_{on}$ of non-aggregated micelles with ca. 10% precision. As already mentioned, the standard Zimm analysis based on wide scattering angles yields only the product $\langle w \rangle (M_w)_{on} = 21.38 \times 10^6$ g mol^{-1}. Hence, the agreement is fairly good.

As already mentioned, the time-dependent study shows that the three-layer onion PS-PVP/PVP-PEO micelles are stable in aqueous solutions. Some aggregation of micelles occurs on time scale of weeks. The number fraction of micellar aggregates is very low (only few percent). The aggregation is accompanied by an observable decrease in concentration of small PVP-PEO micelles. The large aggregates are soluble in neutral water and in alkaline buffers and do not precipitate. At later times, the solutions do not change any more. The study suggests that micellar aggregates contain only few aggregates of onion micelles together with a relatively large number of small PVP-PEO micelles. The observed aggregation may be caused by some heterogeneity in PS-PVP composition which results in a formation of low fraction of slightly less stable PS-PVP core micelles and later appreciably less stable onion micelles. While the protonization of PVP in acidic media generally stabilizes charged PS-PVP micelles and prevents aggregation, a low fraction of neutral onion micelles containing less soluble polymeric admixtures may serve as nuclei of secondary aggregation.

CONCLUSIONS

The kinetically frozen micelles formed by hierarchic comicellization of polystyrene-*block*-poly(2-vinylpyridine) and poly(2-vinylpyridine)-*block*-poly(ethylene oxide) during the alkalimetric titration of the acidic solution of PS-PVP micelles and PVP-PEO chains have been studied by a combination of atomic force microscopy and standard light scattering methods (SLS and DLS). For AFM study, the micelles were deposited on fresh flat mica

surface and the histogram of effective volumes, $V = R^2z$, based on section analysis of a high number of deposited micelles was constructed and converted to the distribution function of molar masses with help of light scattering data. The behavior of aqueous micellar solutions was monitored during a long time period after the formation of onion micelles.

The combination of AFM and light scattering proves that neutral and alkaline solutions contain a mixture of onion micelles, small PVP-PEO micelles (12%) and small fraction of large micellar aggregates (4%).

The study of the time-dependent behavior of aqueous solutions of onion micelles shows that some secondary aggregation together with a decrease of the number of small PVP-PEO micelles occurs at early stages on the time scale of weeks. Then the solutions do not change and remain stable (studied up to one year).

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