Preparation and Characterization of Self-Assembled Nanoparticles Formed by Poly(ethylene oxide)-*block*-poly(ε-caprolactone) Copolymers with Long Poly(ε-caprolactone) Blocks in Aqueous Solutions

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Aqueous solutions of self-assembled nanoparticles formed by biocompatible diblock copolymers of poly(ε-caprolactone)-*block*-poly(ethylene oxide) (PCL-PEO) with the same molar mass of the PEO block (5000 g mol^{-1}) and three different molar masses of the PCL block (5000, 13 000, and 32 000 g mol^{-1}) have been prepared by a fast mixing the copolymer solution in a mild selective solvent, tetrahydrofuran (THF)/water, with an excess of water, that is, by quenching the reversible micellization equilibrium, and a subsequent removal of THF by dialysis of the water-rich solution against water. The prepared nanoparticles have been characterized by static and dynamic light scattering and atomic force microscopy imaging. It was found that stable monodisperse nanoparticles are formed only if the initial mixed solvent contained 90 vol % THF. The results show that the prepared nanoparticles are spherical vesicles with relatively thick hydrophobic walls, that is, spherical core/shell nanoparticles with the hollow core filled with the solvent.

Introduction

The potential application of self-assembled nanoparticles formed by different amphiphilic polymers as vessels for controlled drug release and/or targeted drug delivery in pharmacology has been a subject of numerous studies.[1] Most investigations deal with nanoparticles of AB diblock and ABA- or BAB-type triblock copolymers, where A is a hydrophobic, core-forming block, and B is a hydrophilic, shell-forming block. In many systems, the hydrophilic block is poly(ethylene oxide), PEO, because particles coated by this polymer are tolerated by living organisms and exhibit very low adsorption affinity to proteins.[2] The core-forming blocks are usually polymers that undergo hydrolysis in aqueous solutions and decompose, that is, polyamides, like poly(γ-benzyl-L-glutamate)[3] and poly(β-benzyl-L-aspartate),[4] or polyesters, like poly(DL-lactide)[5] and poly(ε-caprolactone) (PCL).[6] Nanoparticles formed by such block copolymers in aqueous solutions are thus both biocompatible and biodegradable, and their cores are able to solubilize various hydrophobic compounds.

Nanoparticles of PCL and PEO diblock or triblock copolymers have been studied as carrier vessels for the immunosuppressive Cyclosporine,[6] the calcium antagonist Nimodipine,[7] and other drugs. Most studies of PCL-PEO and PCL-PEO-PCL micelles, as drug delivery vessels, concentrate on their preparation and characterization[8,9] and on kinetic studies of the release of solubilized compounds[6,7] to the bulk solution or their decomposition both in vitro[10,11] and in vivo.[12]

As water is an extremely strong precipitant for hydrophobic blocks of the above-mentioned copolymers, their direct dissolution in water is either difficult or impossible. Vangeyte et al.[8] prepared nanoparticles of PCL-PEO diblock copolymers with relatively short PCL blocks (M_n not exceeding 3900 g mol^{-1}) by direct dissolution of the copolymer in water at 80 °C, but this procedure cannot be used for the preparation of nanoparticles from amphiphilic copolymers with a long hydrophobic block.

The most common method of preparation of aqueous solutions of nanoparticles formed by amphiphilic block copolymers is the dissolution of the copolymer in a mixture of a good solvent for the hydrophobic block with water, in which the reversible multimolecular micelles with swollen cores form spontaneously. The organic solvent content is subsequently decreased by the injection of the solution in an excess of water, by distillation, or by dialysis of the solution against water. The thermodynamic quality of the solvent for the hydrophobic blocks deteriorates with decreasing content of the organic solvent, and the association number increases.[13] A further decrease in the content of the organic solvent causes the collapse of hydrophobic cores, and the nanoparticles kinetically "freeze". Their association number does not change any more. Nevertheless, it depends on the

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copolymer concentration, its composition and architecture, rate of the organic solvent removal, temperature, and other factors. The fact that the association number and size of resulting nanoparticles can be influenced by the modification of the preparation protocol is very promising for potential applications in medicine. It allows for tuning the properties of the delivery vessel and minimizes the number of syntheses of samples differing in length and chemical composition. Vangeyte et al.[9] demonstrated that the choice of the organic solvent and the method of the copolymer transfer from the mild selective solvent into the aqueous solution affect the self-assembly of the PCL-PEO block copolymer.

The small-angle X-ray and neutron scattering measurements revealed that PCL-PEO nanoparticles prepared by direct dissolution of the copolymer in water at elevated temperature are spherical micelles of about 20 nm in diameter.[8] However, the nanoparticles prepared using an organic cosolvent were found substantially larger. Their hydrodynamic radius measured by dynamic light scattering ranges up to 50 nm, and with respect to the lengths of both core- and shell-forming blocks the formed particles are too large to possess the micellar structure.[9]

In this paper, we report on the preparation and characterization of nanoparticles formed by PCL-PEO copolymers in aqueous solutions. Unlike in refs 8 and 9, we use the PCL-PEO copolymers whose PCL blocks have the same length or are longer than the PEO block, which results in very poor solubility in water. The used preparation protocol is based on the injection of PCL-PEO dissolved in tetrahydrofurane (THF)-rich aqueous mixtures into an excess of water. For the characterization of prepared nanoparticles, we employ static and dynamic light scattering and atomic force microscopy.

Experimental Section

Materials. *Copolymer Samples.* Three PCL-PEO samples, PCL-(5)-PEO, PCL(13)-PEO, and PCL(32)-PEO, all with the declared polydispersity index less than 1.4, were purchased from Aldrich. The numbers in brackets indicate number-averaged molar masses, M_n, of the PCL block in kg/mol; M_n of the PEO blocks was 5 kg/mol for all three samples.

Preparation of Nanoparticles. The aqueous solutions of PCL-PEO nanoparticles were prepared according to the following protocol: 10 mg of each copolymer was added to 2 mL of THF or THF/water mixtures differing in composition and left shaken overnight. Full dissolution of all three copolymers was achieved in THF/water mixtures containing more than 60 vol % of THF. The THF-rich solutions were then added drop-by-drop to 4 mL of water under vigorous stirring. Finally, the solutions were dialyzed extensively against water several times to remove THF completely.

Solvents. THF, luminescence spectroscopy grade, from Fluka, and deionized water were used as solvents.

Techniques. *Light Scattering Measurements.* 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 for measurements were filtered through 0.45 μm Acrodisc filters. The measurements were carried out for different concentrations (0.4–1.8 g/L) and different angles at 20 °C.

Static light scattering (SLS) measurements were treated by the Berry method using the equation:

$$\sqrt{\frac{Kc}{R(q,c)}} = \sqrt{\frac{1}{M_w}}\left(1 + \frac{1}{6}R_g^2q^2\right)(1 + A_2M_wc) \qquad (1)$$

where M_w, R_g, and A_2, respectively, are the weight-averaged molar mass, z-averaged radius of gyration, and the "light-scattering-averaged" osmotic second virial coefficient of the polymer in the solution, and $R(q,c)$ is the corrected Rayleigh ratio that depends on

the polymer concentration c and on the magnitude of the scattering vector $q = (4\pi n_0/\lambda)\sin(\vartheta/2)$, where ϑ is the scattering angle, n_0 is the refractive index of the solvent, and λ is the wavelength of the incident light. The contrast factor K is given by the relationship $K = 4\pi^2n_0^2(dn/dc)^2/(\lambda^4N_A)$, where (dn/dc) is the refractive index increment of the polymer with respect to the solvent, and N_A is the Avogadro constant.

Refractive index increments of PCL-PEO samples were calculated as the weighted average of literature data for PCL and PEO on the basis of copolymer compositions.[14,15] As PCL is water-insoluble and the pertinent value in water is thus not available, the literature dn/dc value for PCL in THF[15] was recalculated to that in water, assuming that, in the first approximation, the difference between increments in two solvents equals the difference of refractive indices of the solvents.[16] The same approximation was employed for the calculation of increments in mixed THF/water solvents, using the literature data on the refractive index dependence on the composition of THF/water mixtures.[17] We compared the values of (dn/dc) for 90% THF, in which the copolymers are directly soluble, obtained by the measurement with a Brice–Phoenix differential refractometer with those calculated by this procedure, and the results agreed very well for all three samples. Therefore, the used approximation should neither invalidate nor significantly affect the data.

Dynamic light scattering measurements were evaluated by fitting of the measured normalized time autocorrelation function of the scattered light intensity, $g_{(2)}(t)$, related to the electric field autocorrelation function, $g_1(t)$, by the Siegert relation, $g_{(2)}(t) = 1 + \beta|g_{(1)}(t)|^2$. The data were fitted with the aid of the constrained regularization algorithm (CONTIN), which provides the distribution of relaxation times τ, $A(\tau)$, as the inverse Laplace transform of $g_{(1)}(t)$ function:

$$g_{(1)}(t) = \int_0^\infty A(\tau)\exp(-t/\tau)\,d\tau \qquad (2)$$

To obtain the averaged hydrodynamic radius of the particles, R_H (z-average for R_H^{-1}), the $g_{(1)}(t)$ functions for various copolymer concentrations and scattering angles were fitted to the second-order cumulant expansion:

$$g_{(1)}(t) = \exp(-D_{app}q^2t + \mu_2t^2/2) \qquad (3)$$

where D_{app} is the apparent diffusion coefficient, and μ_2 (the second cumulant) is the second moment of the distribution function of relaxation times. The D_{app} values obtained from the fits were further extrapolated to zero q and c to yield the z-averaged diffusion coefficient of the particles, $\langle D\rangle_z$, using the relationship:[18]

$$D_{app}(q,c) = \langle D\rangle_z(1 + k_Dc + CR_g^2q^2) \qquad (4)$$

where k_D is the hydrodynamic virial coefficient, and C is a coefficient determined by the slowest internal motion and polydispersity of the scattering particles. The hydrodynamic radius was calculated from $\langle D\rangle_z$, by means of the Stokes–Einstein formula:

$$R_H = \frac{k_BT}{6\pi\eta_0\langle D\rangle_z} \qquad (5)$$

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

Atomic Force Microscopy (AFM). Atomic force microscopy measurements were performed in the tapping mode under ambient conditions using a commercial scanning probe microscope, Digital

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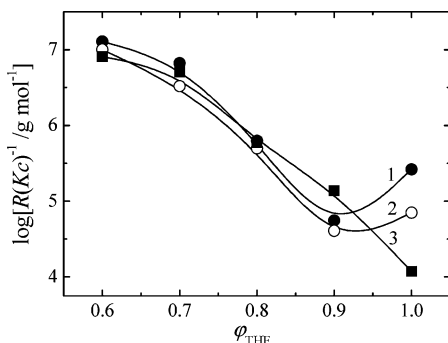
Figure 1. Logarithm of the apparent molar mass (R/Kc at the scattering angle $\vartheta = 90°$) of PCL(5)-PEO (curve 1), PCL(13)-PEO (curve 2), and PCL(32)-PEO (curve 3) aggregates in THF/water mixtures, as a function of THF volume fraction, φ_{THF}. The mass concentration of the copolymers in the solutions was $c = 1$ g L^{-1}.

Instruments NanoScope dimensions 3, equipped with a Nanosensors silicon cantilever, typical spring constant 40 N m^{-1}. Nanoparticles were deposited on a fresh (i.e., freshly peeled out) mica surface (flogopite, theoretical formula KMg$_3$AlSi$_3$O$_{10}$(OH)$_2$, Geological Collection of Charles University in Prague, Czech Republic) by a fast dip coating in a dilute PCL-PEO solution in water (c of ca. 10^{-2} g L^{-1}). After the evaporation of water, the samples for AFM were dried in a vacuum oven at ambient temperature for ca. 5 h.

Results and Discussion

Characterization of PCL-PEO Nanoparticles by Light Scattering. To find the optimum composition of the mixed solvent for the preparation of well-defined PCL-PEO nanoparticles in aqueous media, we varied the composition of THF/water mixtures, from which the dissolved copolymers were transferred into aqueous solutions, and characterized the copolymer associates formed in these solvents.

Not surprisingly, the solubility of the PCL-PEO copolymers in THF/water mixtures decreases with increasing length of the water-insoluble PCL block. The minimum THF content in THF/water mixtures for the complete dissolution of all three samples (1 mL of the solvent per 5 mg of the copolymer) was 60 vol %.

Figure 1 shows the dependence of the scattering intensity at the scattering angle $\vartheta = 90°$, of the PCL-PEO solutions in THF/water mixtures on the volume fraction of THF. The changes are large, and the scattering intensity was expressed as R/Kc (i.e., the apparent molar mass). The refractive index increments of the copolymers for individual mixed solvents were determined as described in the Experimental Section. The measured quantity, which is roughly proportional to the molar mass of the PCL-PEO aggregates formed in THF/water solutions, decreases with increasing volume fraction of THF, φ_{THF}, until $\varphi_{THF} = 0.9$ where it passes a minimum for PCL(5)-PEO and PCL(13)-PEO. In pure THF, which is a poor solvent for PEO, the copolymers aggregate due to limited solubility of PEO blocks, and R/Kc thus increases with the increasing weight fraction of PEO in the copolymer.

The injection of PCL-PEO solutions in THF-rich/water mixtures in an excess of water leads to the formation of stable nanoparticles only in the case of the copolymer solutions in 80% and 90% THF. The DLS relaxation time distributions of final aqueous solutions (i.e., after removal of THF by dialysis) are plotted in Figure 2, which shows that the best results (unimodal, relatively narrow distributions for all three samples) were achieved if the initial solvent contained 90% THF, in which the PCL-(5)-PEO and PCL(13)-PEO copolymers formed aggregates with the lowest apparent molar mass. With 80% THF as the initial solvent, the relaxation time distributions for final aqueous
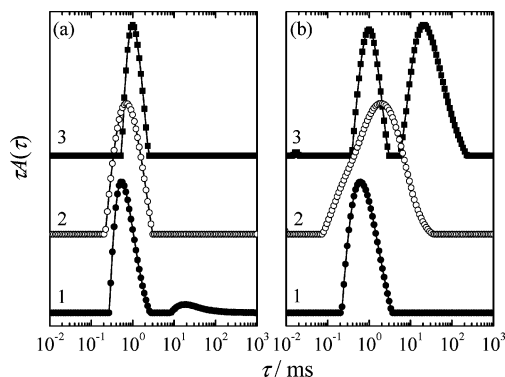

Figure 2. DLS relaxation time distributions (measured at the scattering angle $\vartheta = 90°$) of PCL(5)-PEO (curve 1), PCL(13)-PEO (curve 2), and PCL(32)-PEO (curve 3) aqueous solutions prepared from copolymer solutions in (a) 90% THF and (b) 80% THF (the final copolymer concentration, $c = 0.4$ g L^{-1}).
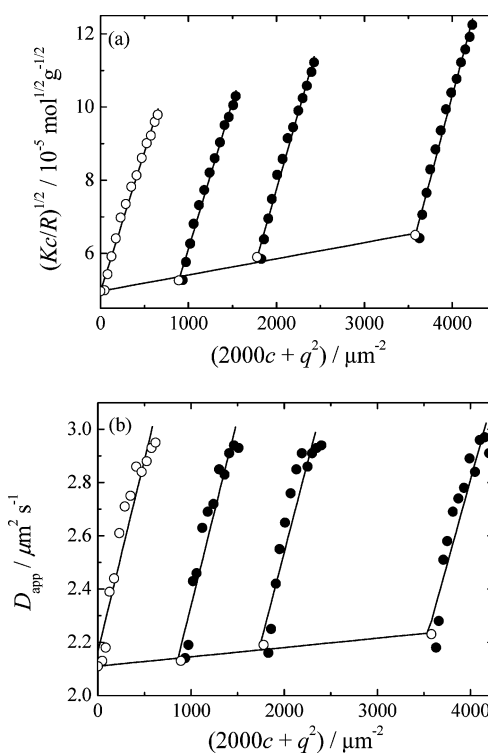

Figure 3. (a) The Berry plot and (b) the dynamic Zimm plot of light scattering data for PCL(32)-PEO aqueous solutions.

solutions are broader and in the case of PCL(32)-PEO nano-particles even bimodal (curve 3 in Figure 2b). The admixture of the large aggregates in the PCL(32)-PEO aqueous solution can be explained as a consequence of the copolymer polydispersity: The system is close to the solubility limit, and the small fraction PCL(32)-PEO chains with longer PCL blocks (i.e., less soluble chains in aqueous solutions) tend to precipitate and form large aggregates. (One has to keep in mind that the shown distibutions are intensity-weighted and the aggregates scatter much stronger than the small nanoparticles. The mass fraction of the aggregates is thus very small.)

For other THF/water solvent compositions, highly turbid unstable suspensions of PCL-PEO nanoparticles precipitating on the time scale of days were formed after their transfer into aqueous media. Therefore, only the aqueous solutions prepared from the PCL-PEO solutions in 90 vol % THF were used for further study.

Table 1. Molecular Characteristics of PCL-PEO Nanoparticles in Aqueous Solutions

copolymer	$M_w \times 10^{-6}$, g mol^{-1}	R_g, nm	R_H, nm	R_g/R_H	Z	ρ, g cm^{-3}
PCL(5)-PEO	22.7 ± 0.2	40 ± 1	42.2 ± 0.7	0.95 ± 0.04	2270 ± 5	0.12 ± 0.01
PCL(13)-PEO	77.6 ± 1.5	57 ± 1	60.7 ± 0.3	0.94 ± 0.02	4310 ± 60	0.13 ± 0.01
PCL(32)-PEO	406 ± 16	96 ± 2	99 ± 3	0.97 ± 0.05	$10\,900 \pm 1800$	0.16 ± 0.02

Figure 3a and b shows the Berry plot (SLS data) and the dynamic Zimm plot (i.e., D_{app} vs $kc + q^2$ from DLS data), respectively, for PCL(32)-PEO nanoparticles in water transferred from the solution in 90 vol % THF. The results of measurements for PCL(5)-PEO and PCL(13)-PEO solutions are qualitatively similar and are not shown. The concentration dependences of the scattered intensity and distributions of relaxation times do not indicate any dissociation of nanoparticles at low concentrations and confirm the assumption that the nanoparticles are kinetically frozen.

The molecular characteristics of PCL-PEO nanoparticles obtained by light scattering for the aqueous solutions obtained by quenching the solutions in 90 vol % THF are summarized in Table 1, together with other parameters, that is, R_g/R_H ratio, aggregation number, Z, calculated as $Z = M_w/(M_n)_u$, where $(M_n)_u$ is the number-averaged molar mass of the unimers, and the average density, ρ, is given by the relationship $\rho = 3M_w/(4\pi N_A R_H^3)$.

It is interesting and slightly surprising that the association number of micelles follows the scaling relation fairly well despite the fact that the prepared PCL-PEO micelles are kinetically frozen nonequilibrium nanoparticles. In this case, their molar mass should depend mainly on the preparation protocol. According to our opinion, it is due to the fact that the used protocol is based on quenching, that is, on a fast transfer from the fully reversible equilibrium state (for all three samples) to conditions that are very bad for the PCL core. In Figure 4, log Z is plotted as a function of the logarithm of the number of PCL units. The fit of the data yields the relation $Z \approx N_{PCL}^\mu$, where $\mu = 0.85$ is the scaling exponent.

Although this value fits between exponents predicted by scaling theory[19] (i) for the model of the hairy micelle with the core negligibly small in comparison with the size of the shell, $\mu = 4/5$, and (ii) for the model of the crew cut micelle whose size is approximately the same as the size of its core, $\mu = 1$, one must bear in mind that PCL-PEO micelles in aqueous solutions should exhibit different scaling relations. Micelles of amphiphilic copolymers such as PCL-PEO in aqueous solutions are characterized by large Flory–Huggins interaction parameters χ_{AB} and χ_{AS}, where indices A, B, and S refer to the core-forming block monomer, the shell-forming block monomer, and the

solvent, respectively. These values correspond to a strong segregation of (i) the core from the shell and (ii) the solvent from the core. For micelles under such conditions, the scaling theory[19] predicts $Z \approx N_A^2$, irrespective of the ratio between N_A and N_B. The validity of this scaling relation was confirmed experimentally for polystyrene-*block*-poly(4-vinylpyridine) micelles in toluene[20] and for polystyrene-*block*-poly(methacrylic acid) micelles in dioxane(80 vol %)/water mixture,[21] where the experimental scaling exponents μ were found to be 1.93 and 1.89, respectively.

Not only the observed scaling relation but also the sizes and molar masses of PCL-PEO nanoparticles are slightly surprising. The nanoparticles are clearly too large and their aggregation numbers are too high for simple core/shell micelles. For comparison, Winnik et al. found that diblock copolymers of polystyrene-*block*-poly(ethylene oxide) with molar masses from 8.5 to 50.8 kg/mol formed the core/shell micelles with hydrodynamic radii ranging from 10 to 23 nm and aggregation numbers from 350 to 1850.[22] However, hydrodynamic radii of about 50 nm and aggregation numbers of about 5000, that is, values comparable to those obtained in this study, were reported by Eisenberg et al. for vesicles formed by asymmetrical polystyrene-*block*-poly(acrylic acid) with molar masses 32 and 4 kg/mol for polystyrene and poly(acrylic acid) blocks, respectively.[23] This comparison suggests that the prepared PCL-PEO nanoparticles could have the vesicle-like structure.

Block copolymer vesicles, also called polymersomes,[23–26] have a concentric spherical structure similar to that of liposomes (phospholipid vesicles); that is, they contain a cavity filled with the solvent, surrounded by a spherical layer formed by insoluble blocks, which is separated from the solvent inside by an inner layer and from outside by an outer layer, both formed by soluble blocks of the same type. Besides the high association numbers, two following observations support the assumption of the vesicular structure of PCL-PEO nanoparticles:

(i) The measured R_g/R_H ratios are close to the theoretical value for the hollow sphere, $(R_g/R_H)_{hs} = 1$. The slightly lower values obtained by light scattering could be caused by the polydispersity and finite wall thickness of the formed vesicles.

(ii) The average densities of the nanoparticles are relatively low, indicating that a major part of the nanoparticle volume should be filled with the solvent. As the solvent-swollen shells of the nanoparticles are formed by relatively short PEO chains, such low-density values suggest the presence of the solvent also in a cavity in the nanoparticle core.

There is also a supporting argument for the formation of vesicles in our system raising from the observations of Luo and Eisenberg,[24] who showed that block copolymer vesicles are
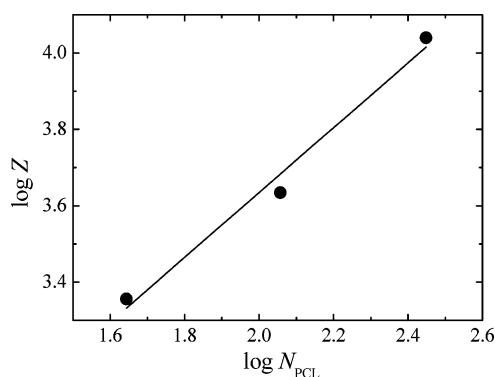

Figure 4. Logarithm of the aggregation number, Z, of PCL-PEO nanoparticles as a function of the logarithm of the number of PCL units, N_{PCL}.

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stabilized by segregation of chains with short hydrophilic blocks to the inside of the vesicles and of the long hydrophilic chains to the outside. This mechanism of vesicle stabilization is especially relevant in our case with respect to the relatively high polydispersity (M_w/M_n is ca. 1.4) of the PCL-PEO copolymers.

However, the copolymer vesicles prepared and described, for example, by Eisenberg et al.[23] exhibit broad size distributions, while the wall thickness distributions are relatively narrow. On the contrary, the radii of the studied PCL-PEO nanoparticles are controlled by copolymer molar masses, which suggests that their cavities are fairly small.

Because all structural characteristics obtained by light scattering are indirect, the question of the shape and inner structure of nanoparticles cannot by solved by LS only. Hence, we performed an additional study of PCL-PEO nanoparticles by atomic force microscopy, as described in the following section.

Atomic Force Microscopy Imaging of PCL-PEO Nanoparticles. For the atomic force microscopy study, the PCL-PEO nanoparticles were deposited from aqueous solutions on a freshly peeled-off mica surface. When comparing AFM data with results of techniques used for studying the solutions, one has to be careful. The thermodynamic conditions and forces acting on polymeric nanoparticles at the surface are very different from those in the solution before deposition. Hence, the nanoparticles may reorganize, for example, aggregate or decompose, during the deposition on the mica surface. On the basis of the experience gained in our earlier studies,[27−29] we do believe that we can (i) decide if the comparison is relevant or not (i.e., if an important reorganization occurred or not), in the case that the AFM study is relevant, (ii) prevent or, at least, substantially minimize the effect of complicating factors (by tuning the conditions during the deposition), and finally (iii) differentiate between the core/shell micelles and vesicles.

Figure 5a and b, respectively, shows the top view of 1 μm × 1 μm scans of PCL(5)-PEO and PCL(32)-PEO nanoparticles. (Scans of PCL(13)-PEO nanoparticles are similar to those of PCL(32)-PEO and are not shown.) The images show clearly that many particles are "ring-shaped", with a hollow space in the center. The horizontal profiles of the "ring-shaped" particles obtained by the section analysis of both scans are shown in Figure 6.

In our earlier study,[27] we found that, if major complications are prevented, sizes of block copolymer micelles evaluated from AFM scans are proportional to those measured in the solution by light scattering, although the deposited micelles are pancake-deformed due to their interaction with the surface and their heights correspond approximately to the core diameter. The pancake deformation is observed also in the case of PCL-PEO nanoparticles. The section analysis of the sizes and shapes of PCL-(5)-PEO and PCL(32)-PEO nanoparticles provides roughly the same horizontal diameter for both particles, D, about 220 nm, as well as the same diameter of the hollow center. The diameter, estimated in the half of the particle height, is ca. 40 nm. The particles formed by different samples differ significantly in the height, which is ca. 6 nm for PCL(5)-PEO and 12 nm for PCL-(32)-PEO. The presence of the hollow space in the center of the deposited nanoparticles supports the assumption that the nanoparticles are vesicles, because the presence of a "crater" can be
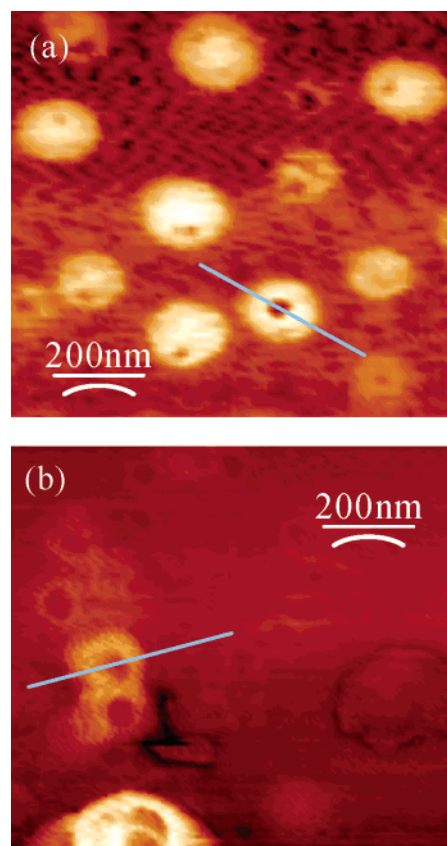

Figure 5. AFM scans (top view, 1 μm × 1 μm) of (a) PCL(5)-PEO and (b) PCL(32)-PEO nanoparticles deposited on the mica surface.
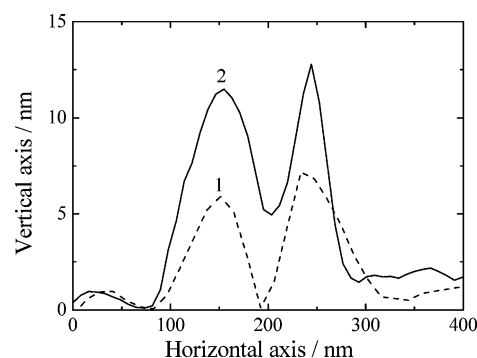

Figure 6. Section analysis of AFM scans of typical PCL(5)-PEO and PCL(32)-PEO nanoparticles deposited on the mica surface, curves 1 and 2, respectively.

explained as a result of the collapse of the vesicle wall after the evaporation of the solvent.

Conclusions

In this paper, we report on the preparation of aqueous solutions of nanoparticles formed by poly(ϵ-caprolactone)-*block*-poly-(ethylene oxide) copolymer and their characterization by static and dynamic light scattering and atomic force microscopy. We studied three copolymer samples, with the same length of the PEO block (5 kg mol^{-1}) and with different lengths of the PCL blocks (5, 13, and 32 kg mol^{-1}). We employed tetrahydrofuran/water mixtures as initial mild selective solvents, from which the reversible and spontaneously formed nanoparticles were transferred (quenched) by fast injection into an excess of water. The preparation of purely aqueous solutions was accomplished by extensive dialysis of water-rich solutions against water for the complete removal of THF. The results of the study are as follows:

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(28) Humpolíčková, J.; Procházka, K.; Hof, M.; Tuzar, Z.; Špírková, M. *Langmuir* **2003**, *19*, 4111.
(29) Štěpánek, M.; Matějíček, P.; Humpolíčková, J.; Procházka, K. *Langmuir* **2005**, *21*, 10783.

(i) The best results in terms of stability and polydispersity of the prepared nanoparticles were achieved when the initial THF/water mixture contained 90 vol % of THF.

(ii) The aggregation numbers, Z, and hydrodynamic radii, R_H, of the nanoparticles in aqueous solution prepared by quenching from solutions in 90% THF are 2270 and 42 nm, 4310 and 61 nm, and 10 900 and 99 nm for PCL(5)-PEO, PCL(13)-PEO, and PCL(32)-PEO, respectively. The values of Z scale with the number of PCL units, N_{PCL}, as $Z \approx N_{PCL}^{0.85}$. High values of both Z and R_H with respect to the molar mass of the copolymers suggest that the nanoparticles are rather vesicles than micelles.

(iii) AFM scans of PCL-PEO nanoparticles deposited on the mica surface show that the deposited nanoparticles are "ring-shaped". The presence of hollow centers of nanoparticles deposited on mica supports the assumption that the nanoparticles are vesicles (polymersomes) with the cavity inside the PCL core, filled with PEO blocks and the solvent.