Morphology of Poly(ethylene oxide)-*block*-Polycaprolatone Block Copolymer Micelles Controlled via the Preparation Method

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Summary: Biocompatible poly(ethylene oxide)-*b*-polycaprolatone (PEO-*b*-PCL) micelles have been successfully tested in many fields as nano-carries, especially in drug delivery. We demonstrate herein that using indirect dissolution methods to prepare PEO-*b*-PCL nano-objects in water, copolymers with volume fractions of PCL (ϕ_{PCL}) ranging from 0.35 to 0.58 self-assemble into spherical micelles when either DMF or THF is used as organic solvent to dissolve the diblocks before the micellization. Striking different, regular wormlike morphologies are however obtained using acetone as solvent for copolymers with $\phi_{PCL} = 0.50–0.58$. These results emphasize, therefore, that control over the shape of PEO-*b*-PCL micelles can be achieved not only by precise macromolecular engineering but also through clever processing strategies.

Keywords: block copolymers; micelles; morphology; poly(ethylene oxide)-*b*-polycaprolatone; self-assembly

Introduction

The ability of amphiphilic block copolymers to self-assemble into ordered structures when dissolved in a selective solvent (*i.e.*, a solvent thermodynamically good for one block and poor for the other) is well-known.[1–6] The size and thereby the aggregation number of those self-assembled nanoparticles are rather independent of the polymer concentration, but may change with the temperature, the copolymer composition (or volume fraction(ϕ)), the molar mass, the Flory-Huggins interaction parameter(χ), and, not surprisingly, the preparation method. As has been demonstrated in pioneer investigations by Lodge,[7] Bates,[2,3,6,8,9] Eisenberg,[10,11] Kataoka[12,13] and Borsali[14–16] during the last few years, tailor-made micellar nano-objects may be obtained through precise manipulation of such parameters. Indeed, numerous studies have proven that block copolymers can self-assemble into different morphologies such as spherical, rod-like or wormlike micelles, cylinders, vesicles, hollow spheres, branched tubules, etc.[2,3,6–9]

The methodology of micellar nanoparticles preparation, that is in fact the key parameter controlling the mechanism (kinetics and thermodynamics) of the self-assembling process, is neither fully described in the literature nor unique. Lately, the large variety of amphiphilic polymers (linear and cyclic blocks, stars, hyperbranched, etc.) synthesized through procedures often combining successive polymerization techniques and chemically distinct monomers[17] has demanded increasing efforts in terms of their manipulation, especially in solution. Frequently, the preparation of well-defined (monodisperse) self-assembled structures requires detailed optimization studies.

Three principal micelle preparation methods have been employed so far: **a**) direct dissolution, **b**) stimuli-induced self-organization and **c**) indirect dissolution or dialysis.[4–6,18] The choice of which method to use depends mostly on the copolymer

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solubility in the medium wherein micelles are to be formed. In an aqueous environment, for instance, direct dissolution is generally employed for marginally water-soluble AB and ABA block copolymers based on poly(ethylene oxide) and poly(propylene oxide) (''*Pluronics*''), and simply involves adding the desired amount of a copolymer to water or another aqueous medium such as buffer solutions. Likewise, stimuli-responsive macromolecules can be molecularly dissolved under certain experimental conditions, and lead to self-assembled structures upon changes in polymer-solvent interaction parameters in response to variations in the pH, temperature, ionic strength, etc. These two preparation methods (**a** and **b**) regularly show excellent reproducibility in terms of morphology and size of micellar nanoparticles.

Whenever the block copolymer solubility in water is too low, on the other hand, indirect methods of dissolution are needed. The dialysis method (**c**) consists in dissolving the block copolymer in a common organic solvent (*i.e.,* good for both blocks such as DMF, DMAc, THF, acetone) that is miscible with water. Subsequently, the copolymer/organic solvent/water mixture is dialyzed against water to remove the organic solvent, so that during this process the micellization is induced owing to amphiphilic nature of the polymeric chain. Albeit this procedure is experimentally simple, it involves a much higher number of controllable parameters (for instance, final polymer concentration, polymer concentration in the organic solution, organic solvent fraction, order and rate of solvent/solubilizate mixing – water onto organic solvent or organic solvent onto water –, temperature, equilibrium period prior to dialysis, etc), which ultimately affect the properties of self-assemblies, namely their size and morphology. As a consequence, intra-laboratory reproducibility is usually good, whereas inter-laboratory results are sometimes hardly comparable.

Poly(ethylene oxide)-*b*-polycaprolactone (PEO-*b*-PCL) copolymers have been increasingly and successfully tested as nanosized containers in many fields, especially in drug delivery.[18–23] In general, the preparation of PEO-*b*-PCL nanoparticles is carried out by the indirect dissolution (or dialysis) method (**c**),[4,5] where the polymer is firstly dissolved in a water-miscible organic solvent. Very often, DMF has been the choice to dissolve such biocompatible diblocks before micellization. However, increasing concerns, notably from biomedical industry, to such procedure have arisen due to potential carcinogenic effects of DMF. Meanwhile it is assumed that DMF is quantitatively eliminated after 48 h of extensive dialysis against water, comprehensive experimental proofs on this topic are not yet available, at the best of our knowledge. As a result, several research groups have contemplated alternative preparation methods using less hazardous and volatile solvents such as THF and acetone, in an attempt to decrease, yet not eliminating, these concerns.

Within this context, we have been studying preparation of PEO-*b*-PCL self-assemblies in aqueous medium by indirect dissolution method using THF,[24] acetone[24] and DMF (for the sake of comparison) as organic solvents. Herein we demonstrate that essentially the same nanoparticle size and morphology (spherical micelles) are obtained upon self-assembly of PEO-*b*-PCL with $0.34 < \phi_{PCL} < 0.58$ using either DMF or THF as organic solvent. In contrast, when acetone was used as solvent, self-organization of PEO-*b*-PCL samples $\phi_{PCL} = 0.50$–0.58 originated wormlike morphologies. In all the cases, solution morphologies were corroborated by combining Dynamic Light Scattering (DLS) experiments and (cryo-) Transmission Electron Microscopy (TEM and cryo-TEM) imagery. Cryo-TEM is a powerful microscopy technique capable of direct imaging molecular scale structures in thin films of vitrified aqueous solutions.

Experimental Part

Chemicals

Block copolymers samples used in this work were purchased from Polymer Source

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Table 1.
Characteristics PEO-*b*-PCL copolymers used to prepare micellar solutions.

PEO_x-*b*-PCL_y	M_n (g/mol)[a]	M_w/M_n	ϕ_{PCL}[b]
45-9	3 000	1.10	0.34
45-24	4 700	1.19	0.58
114-24	7 900	1.11	0.35
114-44	10 000	1.06	0.50

[a] Determined by combining GPC in THF (PEO standards) and ^1H NMR analysis.
[b] Volume fraction assuming that the polymer density is equal 1.0 g/mL.

Inc., and their molecular characteristics are summarized in Table 1. *N,N′*-dimethylformamide (DMF; J.T. Baker), tetrahydrofuran (THF; J.T. Baker) were distilled under reduced pressure prior to use. Acetone (Prolabo) was used as received. Distilled and deionized water was employed for all solution preparations.

Sample Preparation

Dilute PEO_x-*b*-PCL_y – here and throughout the text, subscripts *x* and *y* refer to the mean degree of polymerization (DP) of each block – aqueous solutions ($C_p = 0.5$–2.0 mg/mL) were prepared using the dialysis (or indirect dissolution) method. Typically, 3.5 mg of polymer were dissolved in 0.3 mL of organic solvent (DMF, THF or acetone) (good solvents for both blocks) in a closed vial. The solution was allowed to stir for at least 3h, and micellization was subsequently induced by slow (~0.3 mL/min) dropwise addition of 7.0 mL of water. The solution was then stirred overnight. When THF or acetone was used, the solution was purged gently with N_2 during ca. 12 h to speed up solvent evaporation. Micellar solutions prepared using DMF, conversely, were placed in 7.0-mL dialysis bags (Spectrum, MWCO = 25,000 g/mol), which were immersed in 2.0 L of water. The external medium was changed periodically during 48 h.

Dynamic Light Scattering (DLS) and (cryo-)Transmission Electron Microscopy

The experimental setups employed in this work for DLS, TEM and cryo-TEM experiments have been previously described in details elsewhere.[18,25,26]

Results and Discussion

The self-assembly of block copolymers has been the subject of comprehensive theoretical and experimental studies,[6,9] and it is nowadays well-established that the volume fraction (ϕ) of each constituting segments, along with the overall degree of polymerization (DP) and Flory-Huggins interaction parameter (χ), is a major driving force defining the final thermodynamic-stable morphology. The micellization behavior of poly(ethylene oxide)-*b*-polycaprolactone diblock copolymers with distinct molar masses and volume fractions (Table 1) was investigated in this work. The physicochemical parameters of the micellar nano-objects originated from their self-assembly using three different water-miscible organic solvents (DMF, THF and acetone) are summarized in Table 2.

Table 2.
Physicochemical parameters of PEO-*b*-PCL self-assemblies in water.

Diblock	DMF		THF		Acetone	
	$2R_H$ (nm)[c]	Type[d]	$2R_H$ (nm)[c]	Type[d]	$2R_H$ (nm)[c]	Type[d]
45-9[a]	16	S	16	S	16	S
45-24[a]	20	S	20	S	60/200/600	W
114-24[b]	40	S	80	S	120	S
114-44[b]	60	S	100	S	80/210/700	W

[a] $C_p = 1.0$ mg/mL.
[b] $C_p = 0.5$ mg/mL.
[c] Hydrodynamic diameter ($2R_H$) determined by DLS.
[d] Dominant morphology observed by TEM and/or cryo-TEM experiments; S: spherical; W: wormlike.

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It was observed that all PEO-*b*-PCL samples listed in Table 1 self-assemble into spherical (S) micelles when either DMF or THF is used as organic solvent to dissolve them before the micellization. Striking different, regular wormlike (W) morphologies are however obtained using acetone as solvent for copolymers with $\phi_{PCL} =$ 0.50–0.58, as described in the sequence.

Spherical Micelles Prepared from DMF or THF Solutions

Typical autocorrelation functions C(q,t) and distributions of the relaxation times A(t) at scattering angle of 90° as revealed by CONTIN analysis for selected samples (1.0 mg/mL PEO_{45}-*b*-PCL_{24} (A) and 0.5 mg/mL PEO_{114}-*b*-PCL_{44} (B)) prepared from THF are shown in Figure 1. In general, narrow distributions of relaxation times were observed for the dominant mode, which corresponds to the diffusive motion of the spherical micelles in solution, whose morphology was confirmed by TEM (see below). Their characteristic hydrodynamic diameter ($2R_H$) depended on the overall polymerization degree (DP) of both PEO and PCL segments (Table 2), irrespective of the organic solvent employed to prepare them. In general, $2R_H$-values increased with the length of the constituting blocks. These results are in very good agreement with other reports suggesting that the size of micelles is controlled by several factors, among which are the length of the corona- and core-forming blocks.[25,27,28] However, while the size of PEO_{45}-*b*-$PCL_{y\ (y=9\ \mathrm{or}\ 24)}$ micelles prepared from DMF or THF was basically the same, PEO_{114}-*b*-$PCL_{y\ (y=24\ \mathrm{or}\ 44)}$ originated slightly bigger micelles in THF (see data in Table 2).

The existence of a slow relaxation mode was regularly observed for all PEO-*b*-PCL micellar solutions, as illustrated in Figure 1 for two selected samples. The respective distribution amplitude decreased upon dilution, thus suggesting their dynamical behavior. Such large macromolecular aggregates (200–700 nm) are probably formed of small individual micelles, as indeed proposed earlier by Allen *et al.*[21,29] for PEO_{44}-*b*-PCL_{20} micellar solutions.

The spherical morphology of PEO-*b*-PCL micelles prepared using either DMF or THF during initial stages of their preparation was clearly confirmed by TEM experiments, which are shown in Figure 2 for (A) PEO_{45}-*b*-PCL_9 and (B) PEO_{45}-*b*-PCL_{24} solutions. These micrographs reveal rather monodisperse nanosized spherical micelles. Their mean diameters by TEM are slightly smaller than those determined by DLS measurements, in part because of the micelle dehydration

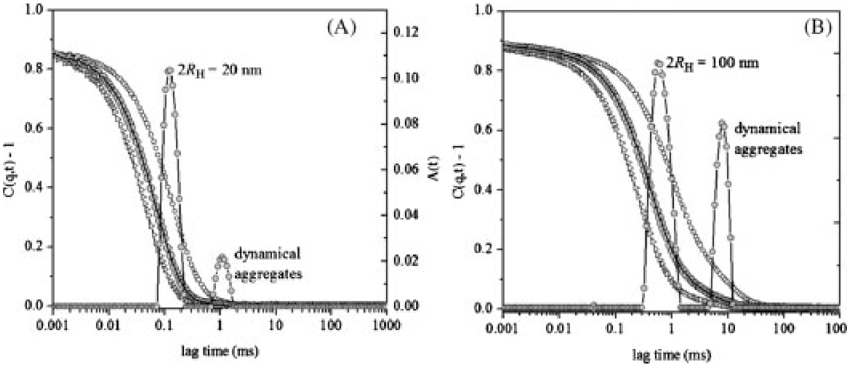

Figure 1.

Autocorrelation functions C(q,t) measured at 60°, 90° and 120° scattering angles and distributions of the relaxation times A(t) at 90° as revealed by CONTIN analysis for (A) 1.0 mg/mL PEO_{45}-*b*-PCL_{24} and (B) 0.5 mg/mL PEO_{114}-*b*-PCL_{44} micellar solutions prepared using THF as organic solvent.

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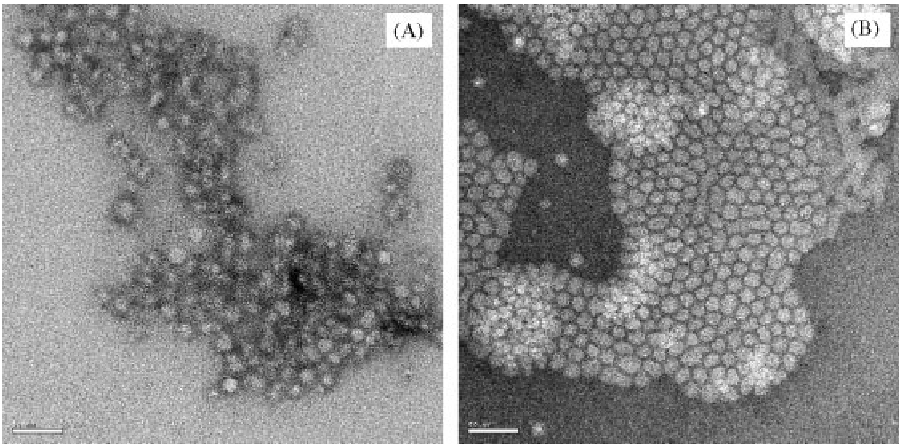

Figure 2.
TEM images of (A) PEO_{45}-*b*-PCL_9 and (B) PEO_{45}-*b*-PCL_{24} micelles prepared using THF as organic solvent.

caused by solvent evaporation under the high vacuum conditions employed during classical TEM imaging. However, discrepancies are also expected because DLS reports an intensity-average diameter, whereas TEM reports a number-average diameter.[25,26] The above comments also apply to micrographs (not shown) taken for the other samples listed in Table 2 and prepared using either DMF or THF.

Wormlike Micelles Prepared from Acetone Solutions

When PEO-*b*-PCL diblock copolymers with $\phi_{PCL} = 0.50–0.58$ were firstly dissolved in acetone, extended cylindrical (wormlike) micelles were formed upon self-assembling in aqueous solutions. For these solutions, DLS measurements revealed multiple (at least three) decay times. Such a behavior is illustrated in Figure 3, which shows the

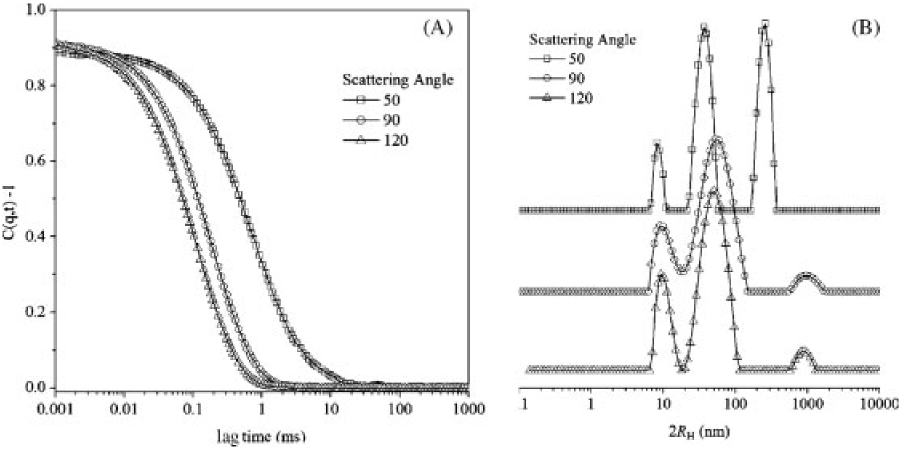

Figure 3.
(A) Autocorrelation functions C(q,t) measured at 50°, 90° and 120° scattering angles and (B) respective distributions of the relaxation times A(t) as revealed by CONTIN analysis for 1.0 mg/mL PEO_{45}-*b*-PCL_{24} micellar solutions prepared using acetone as organic solvent.

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autocorrelation functions C(q,t) and the distributions of the relaxation times A(t) at three scattering angles ($\theta = 50^\circ$, 90° and 120°) as revealed by CONTIN analysis for 1.0 mg/mL PEO_{45}-*b*-PCL_{24} solutions prepared from acetone. In this figure, it is possible to observe that the amplitude associated to the largest particles is much higher at low θ ($\theta = 50^\circ$, small q). Besides, the total scattered intensity recorded at $\theta = 50^\circ$, for instance, was higher than that measured at $\theta = 130^\circ$, thus suggesting heterogeneous distribution of sizes within the different the observation scales reached by varying the scattering angle (or q-values). Also, the relaxation frequencies (Γ) associated to each process eventually deviated from its linear q^2-dependence typical of diffusive scattering particles.

Indeed, direct imaging experiments carried on these micellar solutions have corroborated that self-assembling PEO-*b*-PCL block copolymers from acetone originates wormlike or extended cylindrical morphology instead of spherical. Figure 4 shows TEM and cryo-TEM micrographs taken exactly for the same solution as that one in Figure 3. Wormlike objects with nearly constant cross-sectional diameter and lengths of at least several microns are manifestly evident. Examination of large area micrographs shows long cylinders to be the dominant species in solutions, with only occasionally small individual spherical particles (Figure 4A). Such well-defined structures probably do exist in solution, since cryo-TEM allows for direct visualization of the aggregate structures in water. In addition, both electron microscopy techniques (cryo-TEM and TEM) gave similar results.

The formation of such elongated structures by PEO-*b*-PCL copolymers having $0.30 < \phi_{PCL} < 0.70$ has been previously reported by Bates[8] and Discher,[30] who used the so-called co-solvent/evaporation method. In those experiments, the copolymer was initially dissolved in methylene chloride[8] or chloroform.[30] After evaporation of the solvent, the resulting thin films were re-hydrated to give the desired aqueous micellar solutions.

Conclusions

The present results show that size and morphology of PEO-*b*-PCL self-assembled structures in aqueous media, when prepared by indirect dissolution (or dialysis) method, may be affected by the nature of the organic solvent chosen to dissolve the copolymer. For the series of PEO-*b*-PCL samples with $0.34 < \phi_{PCL} < 0.58$ herein investigated, the use of DMF or THF appear to have practically no influence on

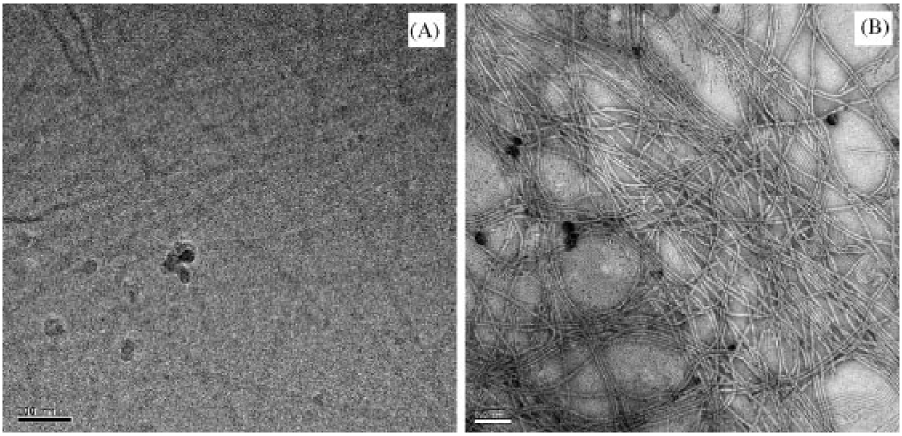

Figure 4.
(A) Cryo-TEM and (B) TEM images of PEO_{45}-*b*-PCL_{24} wormlike micelles prepared using acetone as organic solvent.

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the object's shape and little effect on the size, leading to well-defined spherical micelles. In contrast, the formation of micrometer-long wormlike micelles was favored in solutions prepared from acetone for $\phi_{\mathrm{PCL}} = 0.50$–$0.58$. Using this simple approach, one can thus control the shape of the PEO-*b*-PCL micelles using the same polymer precursor.

These observations emphasize once again that linear AB block copolymers afford access to completely different morphologies through clever processing strategies.

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