About the methods of preparation of poly(ethyleneoxide)-b-poly(ε-caprolactone) nanoparticles in water
Analysis by dynamic light scattering

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Abstract

Self-assembly of nonionic amphiphilic poly(ethylene oxide)-b-poly(ε-caprolactone) (PEO-b-PCL) and poly(ethylene oxide)-b-poly(γ-methyl-ε-caprolactone) (PEO-b-PMCL) has been studied in water by dynamic light scattering (DLS). The aqueous solutions have been prepared by three methods, i.e., dialysis of solutions in a common organic solvent against water, rapid addition of water to organic solutions, and rapid addition of organic solutions into water. Several common organic solvents have been used: tetrahydrofuran (THF), dimethylformamide (DMF), dimethylacetamide (DMAc) and dimethylsulfoxide (DMSO). The dialysis method is not reproducible and leads to very large and polydispersed particles (~1μm). In contrast, nanoparticles are formed by the two other methods with a low polydispersity and a size in the 30-10 nm range, depending on the organic solvent used. The particle size increases when the concentration in the organic solvent is decreased. The effect of temperature and length of the hydrophobic block has also been studied.

Keywords: Dynamic light scattering; Poly(ethylene oxide)-block-poly(ε-caprolactone); Preparation method; Amphiphilic copolymer; Organic co-solvent

1. Introduction

A series of applications such as detergency, painting, surface conditioning [1], drug delivery [2] requires the availability of water-soluble amphiphilic block copolymers. These copolymers aggregate into micelles with a hydrophobic core in which hydrophobic molecules (e.g., drugs) can be dissolved, and a hydrophilic corona which imparts stability to the colloidal dispersion.

When the attention is paid to drug delivery devices, several requirements have to be fulfilled [3]. The critical micellar concentration (CMC) must be low enough for the particles to resist dilution caused by intraveneous injections. The degradation rate must be low for increasing the drug delivery period of time. Particles with a bimodal size distribution are not appropriate because of a too large difference in circulation time, biodistribution [4], and mechanism of entry into cells [5]. For instance, smaller particles can escape uptake by the reticuloendothelial system, whereas the drug loading capacity increases with the size of the micelles [3a,6]. The choice of the core forming block has a direct influence on the interactions with the hydrophobic drug, thus on the drug loading and release kinetics.

The method used for the preparation of aqueous so lutions of amphiphilic copolymers has an impact on the size, size distribution and stability of the (nano)particles formed [2f,3a,7]. Few examples of direct solubilization of nonionic copolymers in water have been reported, mainly poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) copolymers, known as "pluronics", and amphiphilic copolymers with a low hydrophobic content [8]. Whenever the water solubility is too low, indirect methods of dissolution have to be used, which require the temporary use of a common organic solvent miscible with water. Eisenberg et al. proposed two methods for the preparation of "crew-cut" micelles: (i) dropwise addition of water to the organic solution until aggregation occurs, (ii) preparation of a micellar solution in an appropriate organic solvent/water mixture, followed by addition of a large amount of water in order to freeze in the micelles, and by dialysis against pure water [9]. De Jaeghere et al., who used the latter method, designated it as a "salting-out" method [10]. Precipitation or emulsification by addition of the copolymer solution in an organic solvent, into water (under stirring or not) is widely used [2f,11]. Dialysis of an organic solution against water or water/organic solvent mixtures of increasing water content, is an alternative [12]. This work deals with the preparation of particles of nonionic amphiphilic diblock copolymers that contain a hydrophilic poly(ethylene oxide) (PEO) block and a hydrophobic poly(ε-caprolactone) (PCL) or poly(γ-methyl-ε-caprolactone) (PMCL) one. The nonionic hydrosoluble and biocompatible PEO is known to inhibit the adsorption of biological components. The semi-crystalline PCL is biodegradable and resorbable in a suitable period of time [11b,13]. It has already been used as carrier for a variety of drugs [6a,14]. To our best knowledge, the amorphous PMCL has never been used in biomedical applications. The main purpose is to prepare particles of different sizes with narrow particle size

distribution and good stability. Several preparation methods have been considered, including dialysis, addition of the copolymer solution in an organic solvent into water, and the reverse, i.e., addition of water into the organic solution. Four water miscible organic solvents have been tested, i.e., tetrahydrofuran (THF), dimethylformamide (DMF), dimethylacetamide (DMAc) and dimethylsulfoxide (DMSO). Several experimental parameters have been varied, such as the molecular weight of the hydrophobic block and the initial concentration of the copolymer in the organic solvent.

2. Experimental part

2.1. Copolymer synthesis

The diblock copolymers were synthesized by ring-opening polymerization of ε-caprolactone initiated by α-methoxy,ω-hydroxy poly(ethylene oxide) as reported elsewhere [15]. Briefly, the poly(ethylene oxide) (Mn = 5000) macroinitiator was prepared by anionic polymerization of ethylene oxide initiated by a triethylene glycol monomethyl ether in the presence of KOH. It was recrystallized three times from ethyl acetate, dried by azeotropic distillation of toluene and finally at 45 °C in vacuo overnight. ε-Caprolactone was supplied by Aldrich, and γ-methyl-ε-caprolactone was prepared by reaction of 1.4 eq. of *m*-chloroperbenzoic acid with 1 eq. of 4-methyl-cyclohexanone. The lactones were dried over calcium hydride and distilled under reduced pressure. 1.1 eq. of triethyl aluminum (Fluka) was added to 1 eq. of α-methoxy,ω-hydroxy poly(ethylene oxide) in order to convert the hydroxyl end-group into the diethyl aluminum alkoxide initiator. The lactones were polymerized in dichloromethane in the presence of 1 eq. of pyridine, at room temperature. The polymerization was stopped by the addition of 1M HCl. The copolymer was purified by precipitation in heptane and was dialysed against water before lyophilisation.
PEO and the diblocks were analyzed by size exclusion chromatography (SEC) and proton nuclear magnetic resonance (^{1}HNMR). SEC was performed in THF with a Hewlett Packard 1090 liquid chromatograph equipped with a Hewlett Packard 1037A refractometer index detector, and calibrated with PEO standards. ^{1}HNMR spectra were recorded at 400 MHz with a Brucker AM 400 spectrometer. Molecular weight of the second block was calculated from the ^{1}HNMR spectrum and the signals for PEO and PCL at 3.6 and 4.06 ppm, respectively. The molecular characteristics for all the copolymers used in this study are listed in Table 1.

Table 1
Composition and molecular weight distribution of PEO-b-PCL and PEO-b-PMCL block copolymers

Sample PEO$_x$-b-P(M)Cl$_y$	Mn PEO[a]	Mn PCL[b] (or PMCL)	N_A[c]	N_B[c]	EO/CL (mol%)	EO/CL (wt.%)	Polydispersity
PEO$_{114}$-b-PCL$_9$	5000	1000	114	9	93	83/17	1.16
PEO$_{114}$-b-PCL$_{16}$	5000	1850	114	16	88	73/27	1.16
PEO$_{114}$-b-PCL$_{19}$	5000	2200	114	19	86	69/31	1.14
PEO$_{114}$-b-PCL$_{34}$	5000	3900	114	34	77	56/44	1.17
PEO$_{114}$-b-PMCL$_3$	5000	350	114	3	97	93/7	1.07
PEO$_{114}$-b-PMCL$_{12}$	5000	1500	114	12	90	77/23	1.12
PEO$_{114}$-b-PMCL$_{27}$	5000	3450	114	27	81	59/41	1.15

[a] *Determined by SEC with PEO standards.*
[b] *Calculated from Mn$_{PEO}$ and composition determined by ^{1}HNMR analysis.*
[c] *N_A and N_B are the polymerization degree for the PEO and the PCL blocks, respectively.*

2.2. Preparation of micellar solutions

A stock solution of each copolymer was prepared with a well-defined concentration (e.g., 0.5 wt.%) in the appropriate organic solvent (p.a. grade, used as received). Three methods were used to form the copolymer particles in water. (i) The organic solution (5 mL) was dialyzed against distilled water (1L) for 8h, through cellulose dialysis membranes (Spectra por, Cut-off 6-8000, Polylab). Water was replaced every hour; (ii) bidistilled water (20 mL) was added to the organic solution (5 mL) under vigorous stirring, which was maintained for half an hour. The micellar solution was then dialyzed against water (cfr supra); (iii) 5 mL of the organic solution were added to 20 mL of water under vigorous stirring, followed by dialysis against water.

2.3. Dynamic light scattering (DLS)

DLS measurements were performed with a Brookhaven Instruments Corp. DLS apparatus, which consists of a BI-200 goniometer, a BI-2030 digital correlator, and an Ar ion laser (Lexel) with a wavelength of 488 nm. The scattering angle used for the measurements was 90°. A refractive index matching bath of filtered decalin

surrounded the scattering cell, and the temperature was controlled usually at 25 °C. All the glass vessels were cleaned with detergent and rinsed abundantly with bidistilled water and dried at 60 °C. All the solutions were filtered through a 0.45 (im nylon filter (Gelman).

The experimental correlation function *G2 (t)* was measured. For single-exponential decay, *G2 (t)* can be expressed by Eq. (1):

$$G_2(t) = B[1 + b\exp(-2\Gamma t)] \tag{1}$$

where *B* is the baseline, β is an optical constant that depends on the instrument, and .Γ is the decay rate for the process, *t* being the time. This rate is given by

$$\Gamma = Dq^2 \tag{2}$$

where *D* is the translation diffusion coefficient, and *q* is the absolute value of the scattering vector, which can be calculated by Eq. (3).

$$q = [4\pi n \sin(\theta/2)]/\lambda \tag{3}$$

Here *n* is the refractive index of the solvent, θ is the diffusion angle, and λ is the wavelength of the incident light. The diffusion coefficient extrapolated to zero concentration *(D_0)* for spherical particles is related to the hydrodynamic radius, R_h, by the Stokes—Einstein equation:

$$D_0 = \frac{k_B T}{6\pi\eta R_h} \tag{4}$$

where k_B is the Boltzmann constant, *T* is the absolute temperature, and η is the viscosity of the solvent. When particles of different size are formed in solution, the experimental correlation function depends on all the individual decay processes. In this case, the data analysis was performed with the CONTIN routine, a constrained regularization method program for the inverse Laplace transformation of the dynamic light scattering data. The CONTIN program gives access to the distribution of the relaxation times. The *Z*-averaged distribution of R_h was then calculated. Each value corresponds to the average of five measurements.

The polydispersity of the particles was then calculated by the $\mu_2\Gamma^{-2}$ ratio, in which μ_2 was determined by analysis of the first-order correlation function by the method of the cumulants.

$$G_1(t) = \exp[-\Gamma t + (\mu_2/2)t^2 - (\mu_3/3!)t^3 + \cdots] \tag{5}$$

3. Results and discussion

With the purpose to prepare diblock copolymer nanoparticles of well defined characteristic features, the efficiency of three methods of preparation has been compared. Each of them requires the temporary use of a water-miscible organic solvent. Four solvents have been tested, i.e., DMSO, THF, DMF and DMAc. Self-association of the PEO_{114}-b-PCL_{16} copolymer has been systematically analyzed by changing the copolymer concentration in the organic solvent and the final content of the nanoparticles in water. Nanoparticles have been prepared by the best of the three methods, and their characteristics have been studied in relation to temperature and to the copolymer molecular weight and composition.

3.1. Dialysis method

0.5 wt.% solutions of the PEO_{114}-b-PCL_{16} diblock in each of the aforementioned organic solvents have been dialyzed against water. A cloudy solution is formed whatever the organic solvent used, and the final micellar solution is diluted 120 times before analysis by DLS. Large particles were observed with high standard deviation, i.e., 1095 ± 85 nm in DMSO; 540 ± 100nm in THF; 2720 ± 140nm in DMF and 1070 ± 133 nm in DMAc. Fig. 1A illustrates the formation of micrometric particles in case of dialysis against DMSO. Moreover, reproducibility is a problem, making the experimental size uncertain. Filtration of the micellar solutions before DLS measurement has been disregarded because an important part of the copolymer is lost. Therefore preparation of nanoparticles by direct dialysis of organic solutions against water is not reliable enough for being considered further.

3.2. Addition of water to copolymer solution in an organic solvent

Size and polydispersity index for the particles formed by this method from organic solutions at three concentrations (1, 0.5 and 0.1% w/w) are collected in Table 2a. The average particle size may change importantly with the organic solvent used. Much larger particles are formed from DMSO and THF solutions, and without clear dependence on the concentration of the organic solution. In contrast, the particle diameter is rather low in case of DMF and DMAc, and it increases upon decreasing concentration of the organic phase, particularly when DMAc is the solvent. As a rule, the particle size decreases in the following order
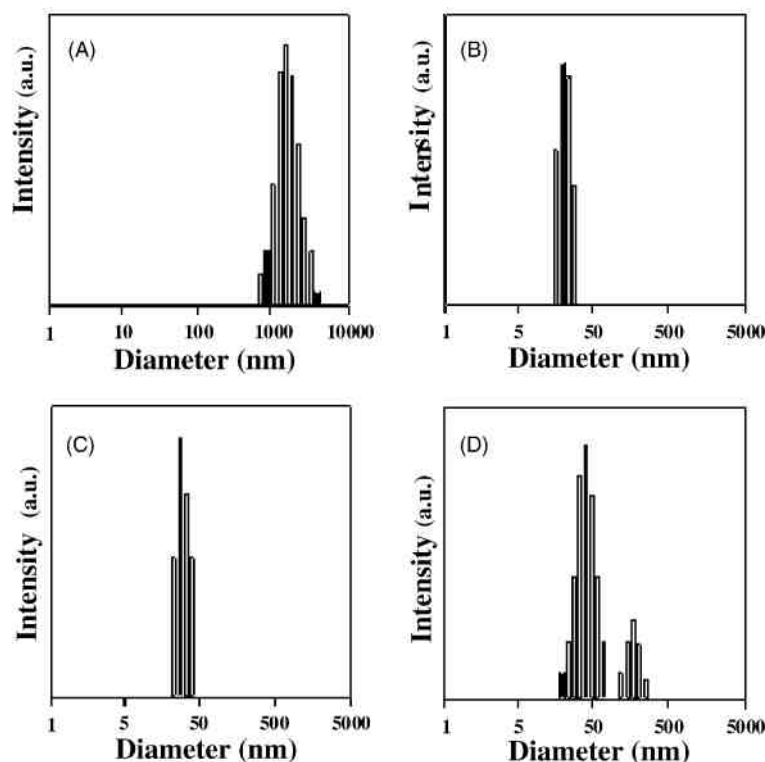THF > DMSO > DMAc ≈ DMF.

Fig. 1. CONTIN size distribution for PEO_{114}-b-PCL_{16} particles formed in water and born from DMF (A, B, C) and DMSO (D), by (A) the dialysis method; (B) the addition of the organic solution (0.5 wt.%) to water; (C, D) the addition of water to the organic solution (0.5 wt.%).

For each organic solvent used, the polydispersity index tends to increase slightly when the concentration of the organic solution is decreased. As illustrated by DMF solution (0.5 wt.%) in Fig. 1C, the CONTIN distribution analysis shows a relatively narrow particle size distribution. However, some CONTIN analysis, e.g., in case of DMSO (Fig. 1D; Table 2a), shows occasionally a bimodal distribution.
The effect of dilution on the particle size has also been investigated in order to check the stability of these colloidal solutions. Table 3a reports how size and size distribution change with dilution for particles prepared from 1 wt.% organic solutions. In all the cases, no significant change is reported.

3.3. Addition of the organic phase into water

Comparison of Table 2a and b shows that the addition order of the liquid phases has an effect on the particles formed.

A substantial increase in diameter is observed when the concentration of the copolymer in the organic phase is decreased, whatever the organic solvent. At the lower concentration, the size is the same (ca. 63 nm) at least for three of the four organic solvents. At high concentration (1 wt.%) the particle diameter lies in the 30–55 nm range depending on the solvent, i.e., decreasing from DMSO to THF to DMAc and finally to DMF.
Again, the polydispersity index increases when the copolymer concentration in the organic solvent is decreased. Fig. 1B shows the CONTIN analysis for the particles born from the 0.5 wt.% copolymer solution in DMF. The size distribution is again relatively narrow.
Table 3b confirms that once the particles are "frozen in" by precipitation, they are insensitive to dilution.

Table 2a
Addition of water to the organic solution: effect of the copolymer concentration in the organic solvent on the particles size

Initial concentration (%)	DMSO Diameter (nm)	μ_2/Γ^2 [a]	THF Diameter (nm)	μ_2/Γ^2	DMF Diameter (nm)	μ_2/Γ^2	DMAc Diameter (nm)	μ_2/Γ^2
1.0	71.5±0.6	0.18	102.0 ± 1.5	0.16	34.5±0.6	0.17	30.0±1	0.18
0.5	80.5±0.6	0.24	81.0±2	0.19	35.5±2.9	0.18	39.0±3.5	0.22
0.1	70.5±2.9	0.22	90.5 ± 0.6	0.20	38.0±1.0	0.20	58.5±2.5	0.23

[a] *Polydispersity factor.*

Table 2b
Addition of the organic solution to water: effect of the copolymer concentration on the particles size

Initial concentration (%)	DMSO Diameter (nm)	μ_2/Γ^2 [a]	THF Diameter (nm)	μ_2/Γ^2	DMF Diameter (nm)	μ_2/Γ^2	DMAc Diameter (nm)	μ_2/Γ^2
1.0	55.5±0.6	0.19	44.5±0.6	0.16	31.0±0.1	0.18	37.5 ± 1.5	0.12
0.5	58±0.1	0.23	60.5±1.5	0.18	32.5±0.6	0.23	39.5 ± 3	0.26
0.1	63.3±3.2	0.24	62.0±1.0	0.18	65.0 ± 10.3	0.27	81.5 ± 1.5	0.29

[a] *Polydispersity factor.*

Table 3
Effect of dilution on the size of particles

Dilution	DMSO Diameter (nm)	μ_2/Γ^2 [a]	THF Diameter (nm)	μ_2/Γ^2	DMF Diameter (nm)	μ_2/Γ^2	DMAc Diameter (nm)	μ_2/Γ^2
(a) Addition of water to 1 wt.% organic solution								
no	71.7±0.6	0.17	102 ±1.5	0.16	34.7±0.6	0.17	30±1	0.18
2*	68.0±0.1	0.19	104±2.5	0.16	36.3±0.6	0.22	32±0.1	0.17
4*	65.7±1.1	0.18	102±2.1	0.16	34.5±2.1	0.23	30±2.6	0.20
(b) Addition of 1 wt.% organic solution to water								
no	55.3±0.6	0.19	44.3±0.6	0.16	31.0±0.1	0.18	42.3±1.5	0.12
2*	54.0±0.1	0.18	46.3±1.5	0.14	35.0±1.7	0.23	43.0±1.7	0.14
4*	54.6±2.5	0.18	44.6±1.1	0.21	36.3±1.5	0.23	41.8±1.0	0.16

[a] *Polydispersity factor.*

3.4. Temperature effect

The stability of the particles dispersed in water has also been tested against temperature in the 25–75 °C range. For this purpose, particles of the PEO_{114}-b-PCL_{16} copolymer have been prepared by addition of the copolymer organic solution (1 wt.%) in water. The particle size has been measured by DLS at temperatures increasing from 25 to 75 °C and finally at 25 °C again (for the sake of clarity, the latter data have been plotted in Fig. 2, as open symbols at 27 °C instead of 25 °C).

In case of copolymer solutions in DMF and THF, the particle diameter is independent of temperature in the range under consideration. The size variation remains in the limits of uncertainties when DMAc is the organic solvent. Only the size of the particles prepared from a DMSO solution, decreases above 40 °C (from 56 to 48 nm) and then levels off. For the particles born from DMSO solutions, the size measured at 25 °C after heating at 75 °C is the same as that one previously noted at 75 °C.
The polydispersity index for all the samples tested lies between 0.18 and 0.22, without showing a regular trend with temperature.
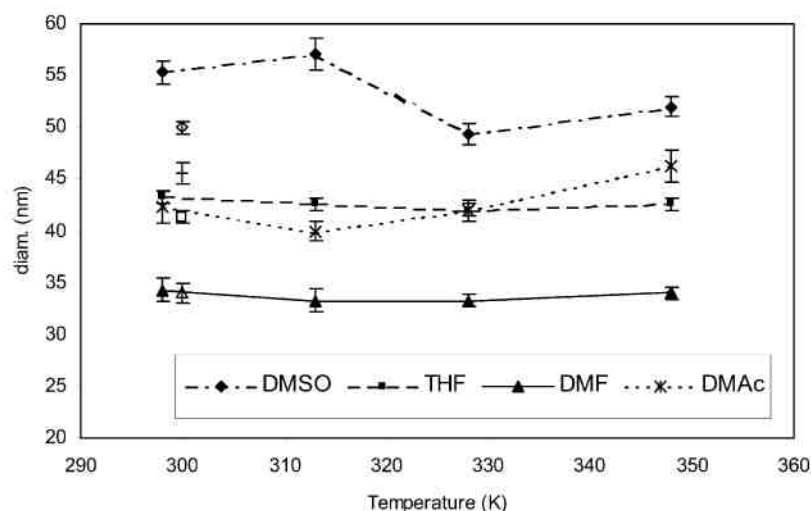
Fig. 2. Effect of temperature on the size of PEO_{114}-b-PCL_{1850} particles prepared by the addition of the organic solution (1 wt.%) to water.

3.5. Effect of the hydrophobic block

Until now, only the PEO_{114}-b-$PCL16$ copolymer has been analyzed. Copolymers containing the same PEO block and a polyester block (including the semi-crystalline poly(ε-caprolactone) and the amorphous poly(γ-methyl-ε-caprolactone) [16] of increasing molecular weight have been compared. Particles have been prepared by addition of the organic solution in water.
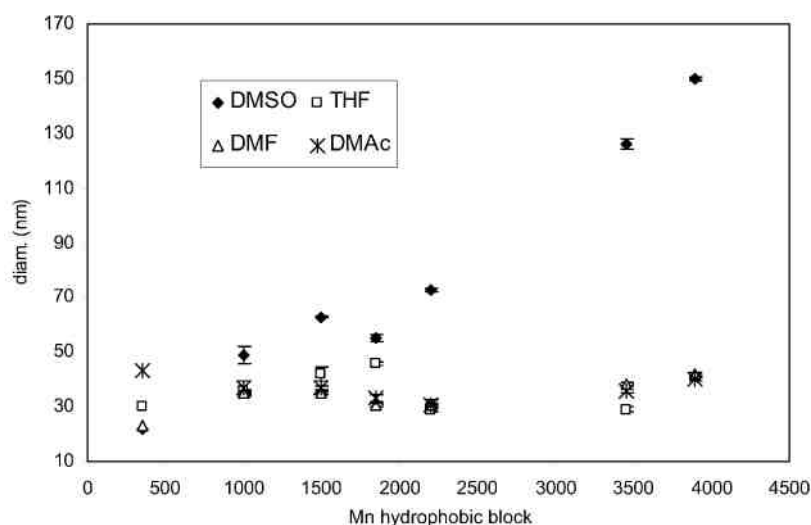
Fig. 3. Effect of PCL (PMCL) molecular weight on the size of PEO_{114}-b-$PCLy$ particles prepared by the addition of the organic solution (1 wt.%) to water.

According to Fig. 3, particles prepared from DMF, DMAc and THF, have a size which is basically independent of the hydrophobic block molecular weight. In sharp contrast, the diameter of the particles born from DMSO shows a linear dependence on the length of the hydrophobic polyester block. A scaling law with a 0.8 power has been extracted from Fig. 4.

These experimental data confirm that PCL and PMCL are interchangeable. Indeed, the two series of copolymers PEO_{114}-b-PCL and PEO_{114}-b-PMCL obey the same law whatever the organic solvent.
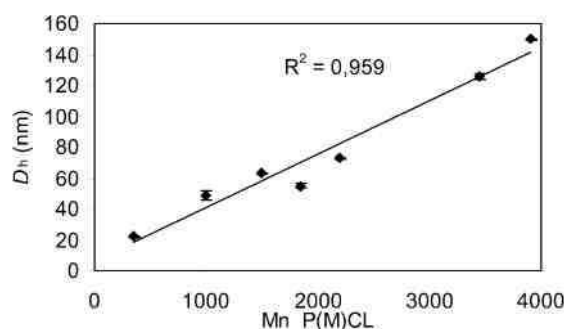
Fig. 4. Effect of PCL (PMCL) molecular weight on the size of PEO$_{114}$-b-PCL$_y$ particles prepared by the addition of DMSO solution (1 wt.%) to water.

3.6. Discussion

Direct dialysis of the copolymer solutions in a water-miscible organic solvent against water leads to very large particles with a micrometric size. These colloidal solutions could not be filtered without loss of copolymer, and the final sizes are not reproducible. Lee and coworkers used this method for preparing PEO-b-PGA (PGA stands for polyg lycolide) and PEO-b-PCL nanoparticles [12,17]. Solutions were sonicated and centrifugated before size measurement, and nanoparticles in the 100–150 nm range were observed. Kataoka and coworkers prepared PEG-b-PLA particles with a diameter of ca. 30 nm from a DMAc solution [18]. They considered that dialysis proceeds by a slow exchange between the organic phase and water through the membrane. In this study, however, the organic solution becomes cloudy within a few minutes, whatever the organic solvent, which indicates that the exchange is fast. In this respect, the membrane porosity must play an important role, and any reliable comparison requires to keep this porosity constant. At least with the membrane available to us, dialysis is not well suited to the preparation of PEO-b-PCL nanoparticles, because part of the copolymer that forms large size aggregates should be eliminated.

The two other methods that consist of the rapid addition of the organic solution in water and vice versa, allow well-defined particles to be formed, within a size range which, however, depends on the organic solvent. In case of DMF and DMAc, the order of addition does not matter. When the copolymer concentration is 1 wt.%, the particle size lies in the 30–35 nm range. Whenever DMSO and THF are the organic solvent, addition of the organic solution in water results in smaller particles (55 and 45 nm, respectively) than when the addition order is reversed (71 and 102 nm, respectively) as shown in Table 2a and b for 1 wt.% copolymer solutions. As a rule, whatever the addition mode, the particle size and the polydispersity index increase upon decreasing copolymer concentration in the organic phase. All the particles dispersed in water are stable against small dilution. Only the particles formed from DMSO have a size which depends on temperature and molecular weight of the hydrophobic block (at constant PEO block).

Table 4
Main properties of the solvents and polymers used in this study (from ref. [19]).

	e	ηa (N s/m^2)	δ (J/cm^3)$^{1/2}$	$^\delta$Hoyb (J/cm^3)$^{1/2}$	$^\delta$VanKreve lenb (J/cm^3)$^{1/2}$
DMSO	46.68	2.0	26.5–26.7		
DMAc	37.78	0.92	22.1–22.8		
THF	7.58	0.36	19.5		
DMF	36.71	0.80	24.9		
H$_2$O	80.37	0.89	47.9–48.1		
PEO			20.2	21.46	21.58
PCL			/	20.38	20.94

^{a} Measured at 25 ℃.
^{b} Calculated according to ref. [19].

The effect of each experimental parameter on the particle formation is discussed hereafter.

Although Kim and coworkers changed the organic solvent in the preparation of particles by the dialysis method [12b], they did not discuss the solvent effect. Eisenberg and coworkers [7b,9] showed that size and morphology of crew-cut micelles changed with the organic solvent, under conditions of very slow addition of water, such that the equilibrium may be reached before the particles are frozen in by an excess of water. Observations were

rationalized on the basis of the polymer–solvent interactions (i.e., the Hildebrand solubility parameters), and the dielectric constant of solvent in case of polar polymers. In this work, the precipitation is fast and the control of the particles formation is basically kinetic. Indeed, no clear dependence emerges between the data reported in Tables 2 and 3, and the Hildebrand solubility parameter for each solvent and for the constitutive blocks (calculated for PEO and PCL) [19] (Table 4). Dielectric constant and intrinsic viscosity for each solvent are also reported in Table 4. A rapid precipitation under stirring is expected to form small nanoparticles with a narrow size distribution. Consistently larger particles are born from DMSO as possible results of a higher viscosity, thus a slower water-organic phase mixing and a lower precipitation rate. In case of THF, a lower miscibility with water (in relation to a low dielectric constant compared to water and the other organic solvents) might slow down the mixing rate and be responsible for larger particles. In parallel, the CONTIN analysis shows a larger size distribution in case of DMSO and THF compared to the two other solvents in which the fast precipitation would be favorable to the formation of better defined nanoparticles.

According to Zhang and Eisenberg [20] the thermodynamic control operates at water content lower than 6.5 wt.%. Above this content, results depend on kinetic parameters and thus on the preparation method itself. In this study, the excess of water is very high (80 wt.%), which means that both the addition rate of one phase into the other one and the stirring rate must be controlled. Whenever these rates are maintained constant, size data are reproducible for the same experiment (deviation ≤ 2 nm), and difference between repeated experiments does not exceed 5 nm, which is consistent with the unavoidable small changes in both the stirring and addition rates. The effect of the experimental parameters discussed hereafter has been studied under constant rates of addition and stirring.

Davis and coworkers prepared PEO-b-PLA nanoparticles by fast precipitation [3b]. Whenever PEO was the minor component (weight fraction lower than 0.14), the particle size was independent of the copolymer concentration in the organic phase in contrast to neat PLA. Gautier et al. confirmed this observation even at higher PEO content (weight fraction from 0.25 up to 0.50) [7a]. However, when PEO dominates (weight fraction = 0.62), the particle size increases upon decreasing the copolymer concentration in the organic phase.

The same behavior is noted, whatever the addition order, for the PEO_{114}-b-PCL_{16} diblock, in which PEO is the major block, except when DMSO and THF are the organic solvents. As a rule, copolymers with a short PCL or PLA block are not prone to fast precipitation upon water addition, which is favorable to the formation of larger aggregates. From DMSO and THF, the precipitation would be slow enough for the concentration effect to be erased. The addition order, however, has an effect. Indeed, the intrinsically low precipitation rate is expected to be increased when the organic solution is added to a large amount of water compared to the reverse addition, i.e., water to the organic phase. As a result, smaller particles are formed upon addition of the organic solution in water.

Molecular weight of the hydrophobic block (at constant PEO block) has no effect on the size of the particles prepared by addition of the organic solution in water, except when DMSO is the organic solvent. This general observation is in line with a non thermodynamic control. Indeed, the size of block copolymer micelles usually depends on the degree of polymerization of each block according to a scaling law [21]. Davis and coworkers reported that the size of PEO-b-PLA particles, prepared by precipitation from acetone, changed with the PLA molecular weight [3b]. In contrast, Wu and coworkers did not observe large variation in size for particles of PEO-b-PCL copolymers of different hydrophilic/hydrophobic balance, prepared from THF [11a]. So, the conclusions are not clear and more likely depend on the preparation method. In this study, the particles born from DMSO obey a scaling law as shown in Fig. 4, consistent with a strong separation regime [22]. This unique characteristic of the particles formed from DMSO may be reasonably accounted for by the immobilization of residual solvent in the micellar core due to high viscosity (as long as the weight fraction of the core is high). This explanation is also consistent with the irreversible decrease in size on increasing temperature, in contrast to the size of particles born from the other solvents, which is temperature independent (Fig. 2). A loss of residual DMSO at higher temperature is therefore suggested. Further experiments are needed to confirm this hypothesis. Furthermore, the melting of the PCL core (below 75 $^{\circ}$C) [23] has no effect on the particle size (no discontinuity in the temperature dependence).

Finally, the chemical structure of PCL and PMCL is similar enough for these polymers to precipitate the same way under the same experimental conditions, as supported by the data in Fig. 3.

4. Conclusions

Nanoparticles of amphiphilic diblocks of PEO and either PCL or PMCL as the hydrophobic block, have been prepared by precipitation from water-miscible organic solvents. Compared to fast precipitation under stirring, dialysis of the organic solution against water does not allow for an acceptable size control (i.e., formation of monodisperse and relatively narrow size distribution).

Formation of nanoparticles by precipitation is under kinetic control and depends on the organic solvent used. Small nanoparticles (ca. 35 nm) with a narrow size distribution are born from DMF and DMAc. They are stable

against dilution and temperature (at least up to 75 °C). When DMSO is the organic solvent, the observations change more likely because residual solvent is immobilized in the micellar core, consistent with a size which increases with PCL (PMCL) molecular weight (at constant PEO block) and decreases upon increasing temperature. These dependencies are no longer observed for particles born from DMF, DMAc, and THF. No difference is observed when PMCL is substituted for PCL, all the other conditions being the same.

Acknowledgements

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