A Novel One-Step Drug-Loading Procedure for Water-Soluble Amphiphilic Nanocarriers

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Purpose. The lack of water-solubility hampers the use of many potent pharmaceuticals. Polymeric micelles are self-assembled nanocarriers with versatile properties that can be engineered to solubilize, target, and release hydrophobic drugs in a controlled-release fashion. Unfortunately, their large-scale use is limited by the incorporation methods available, especially when sterile dosage forms are sought.
Methods. In this manuscript, we describe a straightforward, economical, and innovative drug-loading procedure that consists in dissolving both the drug and an amphiphilic diblock copolymer in a water/*tert*-butanol mixture that is subsequently freeze-dried.
Results. We demonstrate that monodisperse 20–60 nm-sized drug-loaded polymeric micelles are produced directly and spontaneously upon rehydration of the freeze-dried cake. To establish the proof-of-principle, two hydrophobic taxane derivatives were solubilized in the micelles, and their partition coefficient was determined.
Conclusions. This approach is efficient yet astonishingly simple and may be of great interest for scientists working in nanotechnology and pharmaceutical sciences.

KEY WORDS: diblock copolymers; polymeric micelles; poorly water-soluble drugs; self-assembly.

INTRODUCTION

Exhaustive research in the field of nanoscience along with ground-breaking advances in polymer chemistry have allowed the design of a variety of well-defined structures that find practical applications in many areas like electronics (1) and analytical chemistry (2). Such knowledge is now being exploited by pharmaceutical scientists to design nanocarriers capable of sequestering different compounds such as therapeutic polyions (e.g., DNA) and hydrophobic drugs within their core and target them to their site of action (3–8). Among the nanometric particles developed over the past two decades, polymeric micelles (PM) are generally recognized as versatile and promising water-soluble carriers (9,10). PM present a core-shell architecture that results from the self-assembly of amphiphilic block copolymers in a selective solvent above a threshold concentration referred to as the critical association concentration (CAC) (11). Their structure is such that the core provides an isolated cargo space where hydrophobic drugs can partition. This is of great significance given that the lack of water solubility hampers the use of many potent pharmaceutical agents.

The unique features of PM account for their qualities as efficient drug delivery systems. For instance, their nanometric size range (10–100 nm) and flexible highly hydrated corona minimize nonselective scavenging and rapid clearance by the mononuclear phagocyte system. These drug carriers can extravasate and accumulate passively in regions presenting leaky vasculatures such as tumors; inflamed and infarcted tissues (12). Very recently, micelles have also been shown to distribute to defined cytoplasmic organelles (13), and increasing efforts are now directed at targeting subcellular components.

A major limitation to the development of treatments based on PM ensues from the restricted applicability and efficacy of the drug-loading procedures currently available. For highly hydrophilic low-molecular-weight polymers, drug incorporation can be performed by simply dissolving the polymer and the drug in water. Polymer chains rapidly self-assemble to yield micelles where the drug can diffuse. Unfortunately, this procedure often results in low levels of incorporated drug and applies only to polymers that readily dissolve in water (10). Highly hydrophobic drugs and most amphiphilic polymers used in PM formulation need first to be dissolved in an organic solvent. Incorporation is thereafter carried out either by dialysis or oil-in-water emulsification (14,15). The dialysis method involves the dissolution of the polymer and drug in a water-miscible solvent. Micelle formation and drug incorporation occur as water slowly replaces the good solvent. However, the process requires that large volumes of water be used. The dialysis step can span over more than 2 days and is often associated with drug loss. As for the oil-in-water emulsion case, the polymer partitions at the solvent/water interface and the drug is incorporated as the solvent evaporates. This procedure generally requires the use of chlorinated solvents, and thus may not be ideal from an ecological viewpoint. One last method consists in dissolving the two components in an organic solvent that is removed under reduced pressure to yield a polymer/drug solid dispersion. Drug-loaded micelles are formed by addition of hot water on the preheated dried cake (16). This process is proscribed for thermo-sensitive drugs. All the incorporation methods described above further require sterilization and freeze-drying steps to yield long shelf-life injectable formulations.

Hence, there clearly is a need to further improve the drug-loading procedures. In that respect, we patented a simple, flexible, and innovative one-step loading procedure that consists in dissolving both drug and polymer in a water/*tert*-butanol (TBA) mixture and freeze-drying the solutions. Drug-loaded PM are spontaneously obtained by rehydrating the freeze-dried cake in an injectable vehicle (Fig. 1). This low-cost, straightforward procedure works with several water/TBA ratios and is clearly more amendable to scale-up. The main objectives of this paper are to disclose for the first time the foundation of this novel approach and to better understand the dynamics of micellization in the water/TBA system. The study was conducted with two different poly(*N*-vinyl-2-pyrrolidone)-*block*-poly(D,L-lactide) (PVP-*b*-PDLLA) polymers. The water/TBA copolymer solutions were characterized by different methods, and the proof-of-principle was es-

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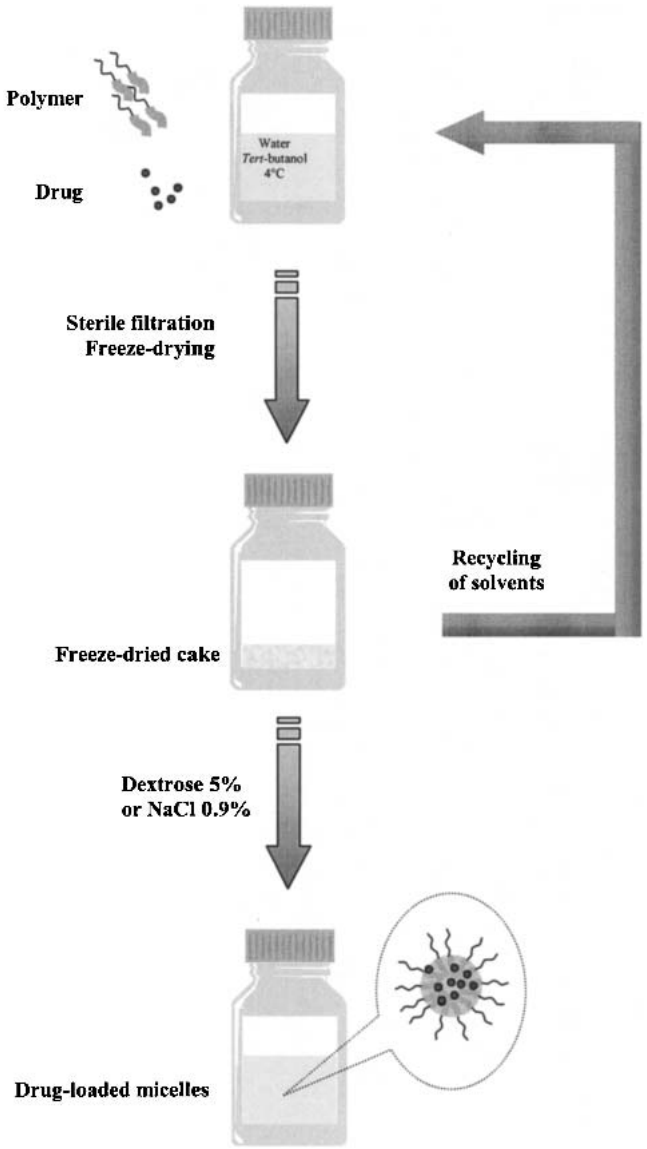

Fig. 1. Scheme of the freeze-drying procedure.

tablished with two widely used hydrophobic anticancer drugs, namely paclitaxel (PTX) and docetaxel (DCTX).

MATERIALS AND METHODS

Copolymers

The PVP-*b*-PDLLA diblock copolymers were prepared by ring-opening polymerization of D,L-lactide using a PVP-OH macroinitiator (Table I) as described by Benhamed *et al.* (17) and Luo *et al.* (18). The polymers were characterized by size-exclusion chromatography (SEC) using refractive index and light-scattering detectors and by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy (18) (Table I). The copolymer CACs were estimated in water at 25°C by a steady-state fluorescence method on a Series 2 Aminco Bowman fluorimeter (Spectronic Instruments Inc., Rochester, NY, USA) using pyrene as a probe, as described elsewhere (19).

Characterization of the Water/TBA Copolymer Solutions

The solutions were prepared by first dissolving the polymer in water and adding TBA to different proportions (final polymer concentration 2 mg/ml). In the case of the 100% TBA solution, the polymer was directly dissolved in the organic solvent. The clear solutions were gently stirred overnight at 4°C. All solvents and water were filtered before use (0.22 μm), and PM were prepared in a laminar flow hood to minimize dust contamination.

Dynamic Light-Scattering Study

Dynamic light-scattering (DLS) measurements (hydrodynamic mean diameter and size distribution) were performed at 25°C at a fixed scattering angle of 90° using a Malvern Autosizer 4800 equipped with a uniphase argon-ion laser (Malvern Instruments, Worcestershire, UK). All size measurements were at least conducted in triplicate. Size data were only recorded for samples with high enough scatter counts (above the detection limit). Refractive indexes and viscosity data for the water/TBA mixtures were determined at 25°C using a differential Rudolph J157 automatic refractometer (Rudolph Research Analytical, Flanders, NJ, USA) and an Ubbelohde viscometer, respectively.

^{1}H-NMR Spectroscopy

^{1}H-NMR spectra were recorded on a Bruker ARX400 spectrometer (Milton, ON, Canada) in deuterated water/TBA solutions (Sigma-Aldrich Co., St Louis, MO, USA). The self-association of the hydrophobic blocks was monitored by calculating the PDLLA signal suppression using the following equations:

$$\%\text{PDLLA content} = \frac{I_{5.1\,ppm}}{\left[\frac{(I_{1.2\text{–}4.0ppm} - 3\,I_{5.1\,ppm})}{9} + 1\right]} \quad (1)$$

where I is the intensity of the NMR signals. The peak at 5.1 ppm corresponds to the –CH– group of the PDLLA block backbone and was normalized to 1. Signals comprised between 1.2 ppm and 4.0 ppm are associated to the VP repeating units and the pendant methyl group of the PDLLA block.

Table I. Characterization of Polymers

Polymer	Number-average molecular weight[a] (M_n)	M_w/M_n	PDLLA content (mol%)	Critical association concentration (mg/L)
PVP-*b*-PDLLA$_{38\%}$	4400	1.1	38	2.6
PVP-*b*-PDLLA$_{27\%}$	4900	2.1	27	4.3
PVP-OH$_{\text{PVP-}b\text{-PDLLA38\%}}$	2600	1.6		
PVP-OH$_{\text{PVP-}b\text{-PDLLA27\%}}$	2900	2.0		

[a] Determined by ^{1}H-NMR spectroscopy.

The term ($I_{1.2–4.0\ ppm} - 3\ I_{5.1ppm}$) corresponds to the net intensity of the PVP signals. The relative proportion of PVP is obtained when the latter is divided by 9 (the total number of protons per VP repeating unit).

$$\%\text{PDLLA suppression} = \frac{\left(\begin{array}{c}\text{mol\%PDLLA}_{\text{CDC13}} \\ -\ \text{mol\%PDLLA}_{\text{TBA/water}}\end{array}\right) \times 100}{\text{mol\%PDLLA}_{\text{CDC13}}} \quad (2)$$

The reference value (mol%$\text{PDLLA}_{\text{CDCl3}}$) was the PDLLA content as determined by ^{1}H-NMR in deuterated chloroform. Chloroform is a good solvent for both polymer blocks.

Multiangle Static Light Scattering

Polymer solutions in water/TBA mixtures were prepared as described above. Solutions were gently stirred overnight, rapidly frozen at –50°C, and freeze-dried for 48 h. The freeze-dried cakes were rehydrated and subjected to multiangle static light scattering (MASLS) at 25°C using a Malvern Autosizer 4800 (Malven instruments, Worcestershire, UK). Measurements were conducted at angles ranging from 50° to 110° on 10 diluted solutions (final polymer concentrations ranging between 1 and 5 mg/ml). All solutions were passed through 0.22-μm filters prior to analysis. The refractive index increments with concentration (dn/dc) were determined using a differential Rudolph J157 automatic refractometer (Rudolph Research Analytical, Flanders, NJ, USA).

The micelle weight-average molecular weight (M_{Wmic}) was computed using Equation 3

$$\frac{Kc}{R_0} = \frac{1}{M_{Wmic}P(\theta)} + 2A_2c + 3A_3c^2 \quad (3)$$

where c stands for the copolymer solution concentration, R_0 the Rayleigh ratio of the solution, and A_2 and A_3 the second and third virial coefficients. K is in turn defined as:

$$K = \frac{4\pi^2 n_0^2 \left(\frac{dn}{dc}\right)^2}{\lambda^4 N} \quad (4)$$

where n_0 is the refractive index of the solvent, λ the laser wavelength *in vacuo*, and N the Avogadro's number. Radius of gyration (R_g) and M_{Wmic} were computed using Zimm analysis. The aggregation number (N_{agg}) was determined by dividing Mw_{mic} by the copolymer weight-average molecular weight.

Atomic Force Microscopy

Atomic force microscopy (AFM) images were recorded at room temperature using a Nanoscope III Dimension 3100 atomic force microscope (Digital Instruments, Santa Barbara, CA, USA). Imaging was performed in tapping mode with a silicon tip (tapping mode etched Si probes – RTESP7) operating at a 250–300 KHz resonance frequency and a 42 N/m constant force. A PVP-*b*-PDLLA solution was prepared in 70/30 water/TBA as described above. The solution was gently stirred for 3 h, rapidly frozen at –50°C, and freeze-dried for 48 h. The AFM sample was prepared by letting a drop of the rehydrated micelles (0.02 mg/ml) dry on a freshly cleaved mica surface.

Head Space Gas Chromatography

Aliquots of freeze-dried micelles prepared in 70/30 water/TBA were dissolved in *N,N*-dimethylformamide in 10-ml clear glass, aluminum-sealed vials fitted with PTFE/silicone septa. Samples were conditioned in a Varian Genesis Headspace Autosampler, and residual TBA in micelles was assayed using a Varian 3800 GC system equipped with a CP5865 capillary column (30 m × 0.32 mm × 1 μm film thickness; composition: 95% dimethylpolysiloxane/5% phenyl groups) and a flame ionization detector (FID) (Varian S.A., Les Ulis, France).

Determination of the Partition Coefficient (K_v) of PTX and DCTX Between Micelles and Water

PTX and DCTX (Shanghai Fudan taxusal New Technology Co., Shanghai, China)-loaded PM were prepared by dissolving the drug in TBA prior to mixing with the aqueous polymer solution (water/TBA 70/30). Drug-loaded PM were prepared with drug present in large excess and the polymer concentration increasing from zero to 85 mg/mL. The solutions were stirred overnight and then centrifuged at 16,100*g* for 6 h. The drug concentration was assayed in the supernatant using a Waters high-performance liquid chromatography (HPLC) system equipped with a 1525 Binary pump, a 2487 Dual Wavelength Absorbance Detector, and a Breeze Chromatography Software (Waters, Milford, MA, USA). The mobile phase consisted of acetonitrile, methanol, and water (48:11:41 v/v). The column was a Waters Nova-Pack C18 60 Å 4 μm (3.9 × 300 mm). The flow rate, detection wavelength, and injection volume were set at 1.0 ml/min, 232 nm and 55 μl, respectively. K_v was obtained by plotting the ratio of the drug solubility in the presence of PM (S_{tot}) over that in pure water (S_w) against micelle concentration according to Eq. 5 (20):

$$S_{tot}/S_w = 1 + K_v \,.\, C_{mic} \,.\, v_m \quad (5)$$

where v_m is given by:

$$v_m = (M_{Wmic} \,.\, \phi_{PDLLA})/d_{PDLLA} \quad (6)$$

C_{mic} is the micellar concentration (defined as the polymer concentration minus the CAC), v_m the micellar partial molar volume, M_{Wmic} the molecular weight of micelles (1,500,000 as ascertained by MASLS), and ϕ_{PDLLA} the PDLLA weight fraction (0.284). The density of the core, d_{PDLLA}, was assumed to be that of a PDLLA segment of similar molecular weight. The absolute PDLLA density (1.30 g/cm^3) was determined using an ultrapycnometer 1000 version 2.12 (Quantachrome, Boynton Beach, FL, USA) on a PDLLA sample having a M_n of 1080. The water solubilities (S_w) of PTX and DCTX were 0.4 and 1.9 μg/ml, respectively.

Drug-Loaded Micelle Stability

PTX and DCTX (Shanghai Fudan Taxusal New Technology Co., Shanghai, China)-loaded PM (5, 7.5, and 10% w/w) were prepared by dissolving the drug in TBA prior to mixing with the aqueous polymer solution (water/TBA 70/30). The clear solutions were gently stirred for 3 h at 4°C and freeze-dried. The lyophile was rehydrated with 5% dextrose, subjected to DLS analysis and to visual examination for any sign of precipitation over 48 h.

The stability of the solid-state formulations at 48 h, 2 weeks, and 1 month was evaluated by differential scanning calorimetry (DSC) (DSC Q1000, TA Instruments, New Castle, DE, USA) as described elsewhere (18) and by X-ray diffraction [XRD; Siemens Kristalloflex 760 (cathode: Cu K_α. α_1 = 1,540562 Å. α_s = 1,544390 Å) equipped with a Wire Grid Bruker AXS detector (Madison, WI, USA)].

RESULTS AND DISCUSSION

Remarkable features of our one-step drug-loading procedure include the formation of a clear polymer/drug solution that can be filter-sterilized prior to freeze-drying (Fig. 1). Lyophilization of the solution yields a dry powder with increased shelf-life. Narrowly distributed drug-loaded micelles are directly obtained from the reconstitution of the cake in aqueous media. Unlike other drug incorporation methods, the procedure involves none of the harsh heating or time-consuming emulsification and dialysis steps. Last but not least, the freeze-dried solvents can be recycled, which further reinforces the feasibility of a scale-up.

The choice of TBA as the cosolvent was based on both its physicochemical properties and its qualities as a pharmaceutical adjuvant. TBA is miscible with water in any proportion at room temperature, as opposed to its linear butyl analogs. It is argued that its spherical shape permits water molecules to gather around it to form a structured hydration shell presenting minimal distortions in the hydrogen bond angles (21). TBA possesses many of the attributes required of an ideal freeze-drying medium. Its melting point around room temperature (24°C) and its high vapor pressure (26.8 mmHg at 20°C) induce its rapid sublimation and accelerate the freeze-drying process (22). Alternatively, TBA modifies the crystallization pattern of water to give fine needle-shaped ice crystals that readily sublime. This structure further enhances the freeze-drying efficiency and produces more porous cakes. TBA belongs to the class 3 category of solvents and is thus generally considered as safe. The water/TBA system is used in the manufacturing of a marketed injectable pharmaceutical product (Caverject) and is used to dissolve lipids in the preparation of vesicles by the freeze-drying procedure (23). The water/TBA cosolvent system has been extensively studied to stabilize labile molecules during lyophilization and to solubilize hydrophobic drugs (24–28). Such abilities are of particular interest given that the use of potent drugs is often impaired by their poor water solubility or stability. Our approach consisted in using the water/TBA mixture to dissolve hydrophobic drugs prior to the freeze-drying step and allow their incorporation into PM.

PM with a core-shell architecture are obtained from the self-assembly of amphiphilic block copolymers in water. The concentration at which micellization occurs depends on factors such as the polymer composition, the hydrophobic/hydrophilic balance, and the nature of the polymer-solvent interactions. Two PVP-*b*-PDLLA polymers with either 27 or 38 mol% PDLLA content, that is, PVP-*b*-$PDLLA_{27\%}$ and PVP-*b*-$PDLLA_{38\%}$, were investigated. They self-associated in water at concentrations of 4.3 and 2.6 mg/l (Table I) to give PM with mean hydrodynamic diameters of 47 and 30 nm, respectively. As predicted, an increase in the hydrophobic block length resulted in a decrease of the CAC (29).

The TBA effect on the dynamics of micellization was monitored by DLS (Fig. 2A). For both copolymers, addition of the first aliquots of TBA scarcely affected size. Subsequent increases in the TBA concentration eventually led to increases in micelle size and even micelle dissociation. In the case of PVP-*b*-$PDLLA_{27\%}$, the scattered light intensity decreased sharply above 50% TBA and no micelles were detected. The affinity between the copolymers and solvent serves to rationalize such behaviors. Polymer/solvent interactions are favored when their solubility parameters are similar. The solubility parameters of water and TBA are 47.9 and 21.7 $(MPa)^{1/2}$, respectively (30). Those of PVP and PDLLA were approximated to be 29.6 and 23.3 $(MPa)^{1/2}$ using the group additivity method proposed by Hoftyzer and Van Krevelen (31). It is readily seen that if water is a better solvent for PVP, TBA is likely to help solubilize both the PVP and PDLLA segments. In that respect, addition of TBA increases the quality of the solvent for the PDLLA blocks. Stronger PDLLA-solvent interactions are associated with an increase in the solvent content of the core, elongation of the hydrophobic polymer chains, and increase in core dimension. If no micelles could be detected above 50% TBA for PVP-*b*-$PDLLA_{27\%}$, suggesting good solvation of the polymer chains, the PVP-*b*-$PDLLA_{38\%}$ micellar structure persisted over the whole TBA concentration range to yield increasingly swollen aggregates. It is speculated that the greater hydrophobic balance of PVP-*b*-$PDLLA_{38\%}$ accounts for its phase separation at a lower critical water concentration (32,33). In that case, TBA is not good enough of a solvent to completely solubilize the more hydrophobic PVP-*b*-$PDLLA_{38\%}$ chains and induce micelle dissociation.

^{1}H-NMR analyses were performed at various deuterated water/TBA ratios as another means of studying the influence

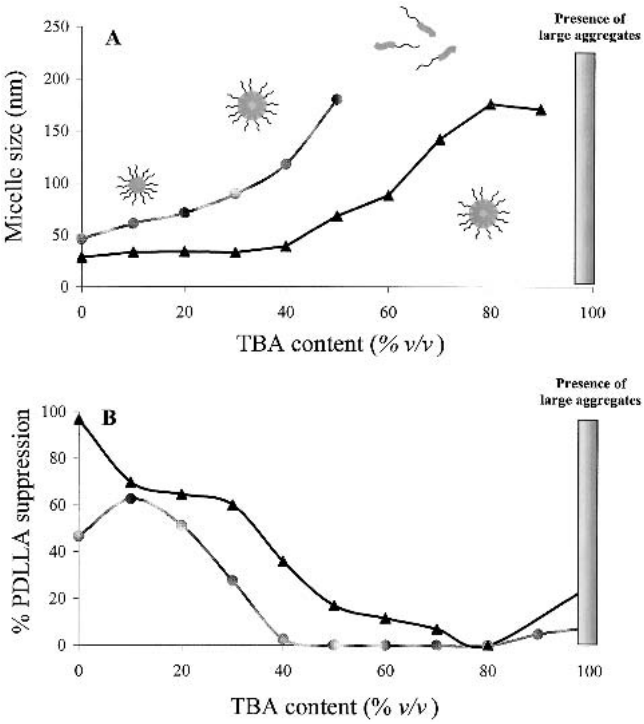

Fig. 2. (A) PM swelling and (B) %PDLLA signal suppression with increases in TBA content for PVP-*b*-$PDLLA_{27\%}$ (●) and PVP-*b*-$PDLLA_{38\%}$ (▲) solutions.

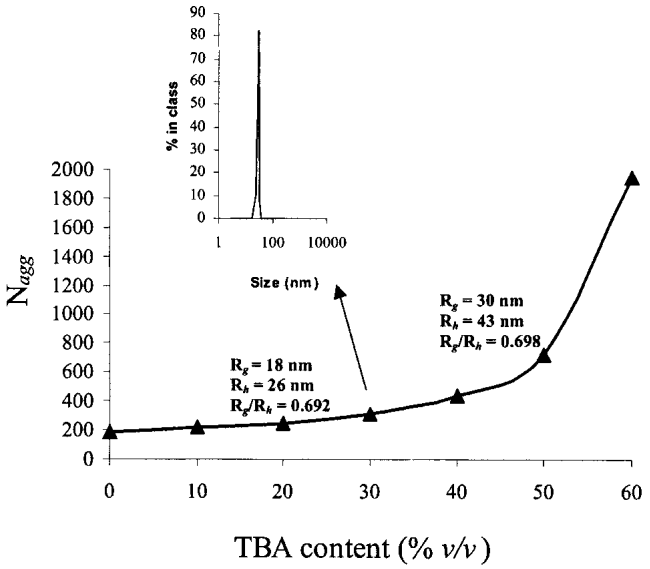

Fig. 3. N_{agg} of PVP-*b*-PDLLA$_{38\%}$ micelles vs. TBA content after rehydration in water. Gyration (R_g) and hydrodynamic (R_h) radii are indicated as examples for the 20% and 50% water/TBA ratios. PM prepared in 70% TBA yielded an opalescent solution in water that prevented their analysis by MASLS. Inset: Size distribution of PVP-*b*-PDLLA$_{38\%}$ micelles prepared in a 70/30 water/TBA mixture.

of TBA on micellization (Fig. 2B). The suppression of signals associated with the core blocks of amphiphilic copolymers reflects their segregation in the micelle core and is attributed to lower chain mobility and decreased solvation. In the 0–50% TBA concentration range, micelle formation was evidenced by a decreasing suppression of the peaks assigned to the micelle core (i.e., PDLLA protons). The PDLLA signal intensity generally increased as the TBA content was raised. Increases in the TBA content indeed favored PDLLA-solvent interactions and accounted for increased mobility and reduced signal suppression. No PDLLA signal suppression was observed between 50–80% TBA for PVP-*b*-PDLLA$_{27\%}$, suggesting that the copolymer chains were perfectly solvated and found as unimers.

The two ^{1}H-NMR and DLS techniques correlated and clearly showed the presence of PVP-*b*-PDLLA$_{27\%}$ unimers above 50% TBA. As for the more hydrophobic PVP-*b*-PDLLA$_{38\%}$ copolymer, PDLLA suppression was minimized at 80% TBA. This concentration compared well to TBA content at which the most swollen micelles were observed by DLS. Surprisingly, in pure TBA, solutions of both polymers were optically transparent but large aggregates were detected by DLS and approximately 5–25% PDLLA signal suppression was measured by ^{1}H-NMR. This unexpected behavior cannot be rationalized yet, but the absence of water seems to impair the complete dispersion of PVP-*b*-PDLLA in TBA.

Freeze-drying of the water/TBA mixtures yielded porous polymer cakes that were readily redispersed in water. The freeze-dried powder contained 20 ppm residual TBA, as assayed by head space gas chromatography. As TBA is a class 3 solvent, this quantity falls far below the authorized limits (34). DLS analyses on rehydrated samples showed that unimodal size distributions were obtained for the 70/30 and 80/20 water/TBA initial ratios. Under these conditions, the diameters of PVP-*b*-PDLLA$_{27\%}$ micelles were of 80–140 nm whereas those of PVP-*b*-PDLLA$_{38\%}$ micelles were of 20–60 nm. This latter polymer was selected for the remaining experiments because its micelles were narrowly distributed and smaller over a larger TBA concentration range. The inset in Fig. 3 specifically illustrates the size distribution of PVP-*b*-PDLLA$_{38\%}$ micelles prepared at a 70/30 water/TBA initial ratio. AFM imaging of PVP-*b*-PDLLA$_{38\%}$ copolymer micelles in turn revealed spherical particles with sizes ranging from 40 to 50 nm, as observed by section analysis (Fig. 4). The image also illustrates the narrowness of the size distribution.

Multiangle static light-scattering measurements were conducted on rehydrated PVP-*b*-PDLLA$_{38\%}$ micelles. Both the micelle molecular weight (M_{Wmic}) and the number of polymer chains per micelle (N_{agg}) were determined. Increases in N_{agg} and size were observed for micelles originating from polymer solutions with increasing proportions of TBA (Fig. 3). N_{agg} values were of the order of 200–400 chains/micelle for TBA contents lower than 50%. As TBA increased from 50–60%, N_{agg} sharply raised from 700 to 2000. As aforementioned, the presence of TBA leads to stronger PDLLA-solvent interactions and swollen micelles. Freeze-drying of such swollen micelles yielded lyophiles with exposed PDLLA segments that interacted hydrophobically with one another upon rehydration to induce chain entanglements and micelle reorganization. The greater the TBA fraction, the more exposed the PDLLA blocks in the freeze-dried cake, and the larger N_{agg}.

MASLS results further allowed the calculation of the radius of gyration (R_g) over the hydrodynamic radius (R_h) ratio, a value that is indicative of the aggregate shape. For

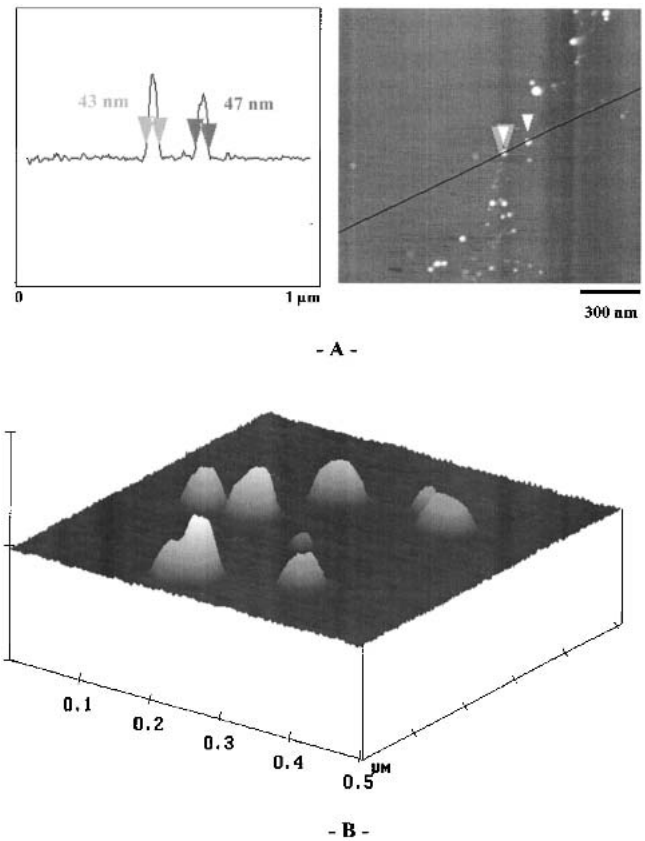

Fig. 4. AFM imaging of PVP-*b*-PDLLA$_{38\%}$ micelles; (A) section analysis and (B) perspective.

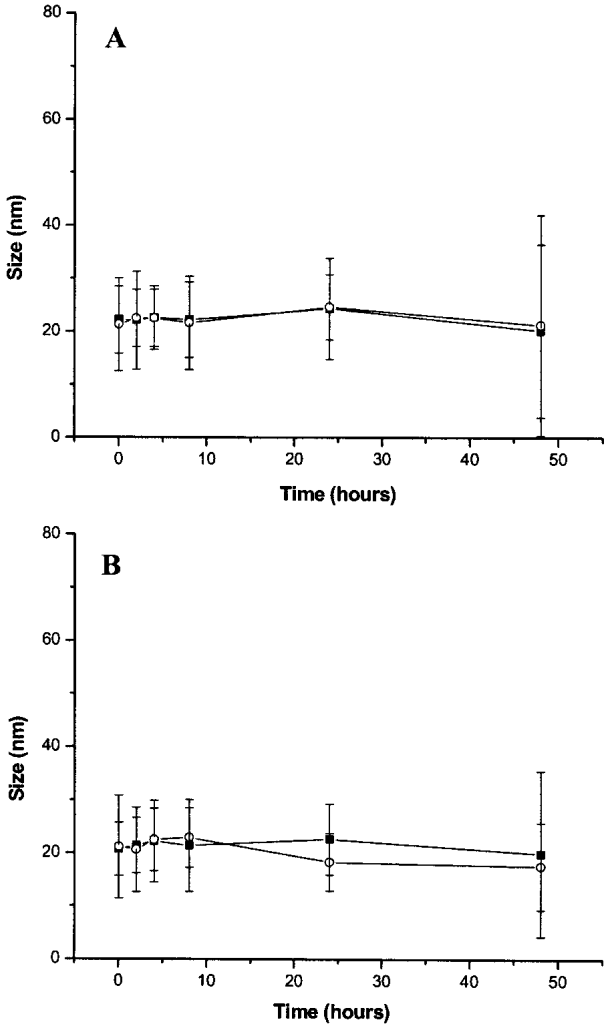

Fig. 5. (A) DCTX- and (B) PTX -loaded micelle stability at room temperature as a function of time, for the 5% (■) and 7.5% (w/w) (○) initial feed ratios.

instance, it is established that the theoretical R_g/R_h value of a hard sphere is 0.778 (35). The values obtained here were slightly lower (~0.700) and were constant with increases in the initial TBA content. This discrepancy is reasonable considering that we are dealing with core-shell structures rather than homogeneous spheres. Harada *et al.* (36) obtained similar values for spherical polyion complex micelles and argued that the radius of gyration most probably is underestimated considering that the density of micelles decreases from the center to the outer boundary.

As monodisperse PM with minimal residual TBA content could readily be obtained, two water-insoluble compounds (i.e., PCTX and DCTX) were incorporated in the swollen micelles by dissolving the drug in TBA. The mixtures (final 70/30 water/TBA) were filter-sterilized, freeze-dried, and rehydrated in 5% dextrose. At a 10% (w/w) initial feed ratio, drug precipitation occurred after 24 h for both drugs. Drug-loaded PM prepared with 5 and 7.5% (w/w) initial feed ratios exhibited sizes comparable to that of unloaded micelles during the whole observation period, with the standard deviation increasing substantially after 2 days (Fig. 5). The formation of such drug-loaded micelles allowed taxane solubilities as high as 20 mg/ml to be reached, without any signs of precipitation of the drug over 48 h. DSC (18) and X-ray measurements both correlated to show the absence of crystallization of the drug in the solid-state dosage forms after up to 1 month (data not shown).

Drug-loading efficiencies were estimated by subtracting the solubility of the drug in water from that of the drug in presence of PVP-*b*-PDLLA. Values superior to 99.5% and 99.9% were calculated for DCTX and PTX, respectively. Alternatively, partition coefficients were determined to assess the solubilization capacity of PVP-*b*-$PDLLA_{38\%}$ and its affinity for the drugs. The partition coefficients (K_v) of PTX and DCTX between PVP-*b*-$PDLLA_{38\%}$ and water were evaluated as per Eq. 5. All parameters of the equation could be calculated or determined experimentally. The S_{tot}/S_w ratio was plotted against the micellar concentration and the K_v values extracted from the slope of the curves (Fig. 6). The calculated K_v were 5.07×10^4 and 2.46×10^4 for PTX and DCTX, respectively, which demonstrates that partitioning of the drugs into the PDLLA phase was highly favored. High affinity of hydrophobic molecules for various biodegradable block copolymer micelles has been reported by several groups. For example, the K_v values for pyrene and dihydrosterone in PM of poly(2-ethyl-2-oxazoline)-*b*-poly(ε-caprolactone) and poly(ethylene oxide)-*b*-poly(ε-caprolactone) are 5.4×10^5 (37) and 1.9×10^4 (38), respectively. The process that we propose here is thus highly suitable for incorporating hydrophobic drugs. Before freeze-drying, the water/solvent mixture conferred a liquid-state character to the micelle core and allowed drug molecules to interact with the PDLLA segments. The swollen state was maintained for a sufficient time-period and made it possible to incorporate large amounts of drug.

In conclusion, the incorporation method reported in this paper represents a novel and valuable tool to prepare nanocarriers. Other experiments exploiting the drug-loading procedure are being carried out in our laboratory and illustrate the versatility of the method. For instance, other water/cosolvent systems, such as water/dioxane mixtures, were also shown to lead to the formation of diblock copolymer micelles. Moreover, various other drugs were incorporated by this technique. In essence, the drug only needs to be soluble in the water/cosolvent mixture and to present a good affinity for the swollen micelle core for the procedure to be successful.

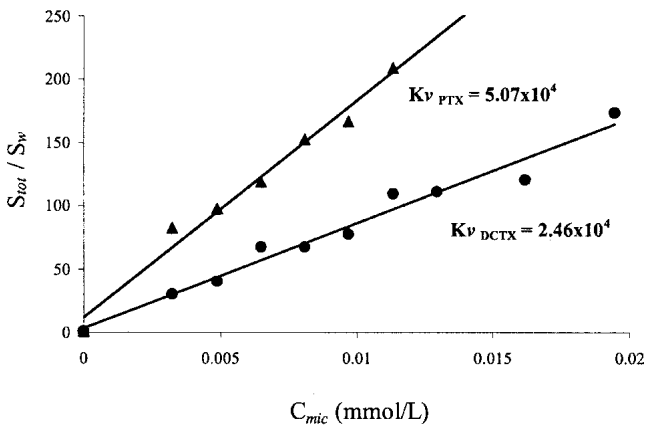

Fig. 6. Plots of S_{tot}/S_w against C_{mic} for PTX (▲) and DCTX (●). The curve slopes are proportional to K_v.

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