Novel Polymeric Micelles Based on the Amphiphilic Diblock Copolymer Poly(*N*-vinyl-2-pyrrolidone)-*block*-poly(D,L-lactide)

Amina Benahmed,[1] Maxime Ranger,[1] and Jean-Christophe Leroux[1,2]

Received September 1, 2000; accepted December 12, 2000

Purpose. The purpose of this work was to synthesize a new amphiphilic diblock copolymer of poly(*N*-vinyl-2-pyrrolidone and poly(D,L-lactide) (PVP-*b*-PDLLA) capable of self-assembling into polymeric micelles with multiple binding sites and high entrapment efficiency.

Methods. The copolymer was synthesized by ring-opening polymerization of D,L-lactide initiated by potassium PVP hydroxylate. It was characterized by gel permeation chromatography, ^{1}H- and ^{13}C-NMR spectroscopy. The ability of the copolymer to self-assemble was demonstrated by dynamic and static light scattering, spectrofluorimetry and ^{1}H-NMR. The hydrophobic model drug indomethacin was incorporated into the polymeric micelles by a dialysis procedure.

Results. A series of amphiphilic diblock copolymers based on PVP-*b*-PDLLA were successfully synthesized. The critical association concentrations in water were low, always below 15 mg/L. Micellar size was generally bimodal with a predominant population between 40 and 100 nm. PVP-*b*-PDLLA micelles were successfully loaded with the poorly water-soluble drug indomethacin and demonstrated an entrapment efficiency higher than that observed with control poly(ethylene glycol)-*b*-PDLLA micelles. It was hypothesized that specific interactions with the hydrophilic outer shell could contribute to the increase in drug loading.

Conclusion. PVP-*b*-PDLLA micelles appear to exhibit multiple binding sites and thus represent a promising strategy for the delivery of a variety of drugs.

KEY WORDS: poly(*N*-vinyl-2-pyrrolidone) (PVP); poly(D,L-lactide) (PDLLA); colloids; diblock copolymer; polymeric micelles; drug carrier, indomethacin.

INTRODUCTION

In recent years, water-soluble supramolecular assemblies such as polymeric micelles and polyion complex micelles have emerged as promising new colloidal carriers for the delivery of hydrophobic drugs (1) and polyions (*e.g.* antisense oligonucleotides) (2,3), respectively. Polymeric micelles are generally prepared from amphiphilic diblock or multiblock copolymers (4). Their hydrophobic inner core serves as a microenvironment for the incorporation of poorly water-soluble drugs, while the hydrophilic outer shell is responsible for micelle stability, protecting the system against opsonization (2) and uptake by the mononuclear phagocyte system (5). Compared to low-molecular weight surfactant micelles, polymeric micelles are generally more stable because of their lower critical association concentration (CAC), and show slower dissociation which allows the longer retention of loaded drugs and higher drug accumulation at the target site (5,6).

Until now, in most studies dealing with the preparation of polymeric micelles, poly(ethylene glycol) (PEG) has been used for formation of the hydrophilic shell (7,8). Thus, the uniqueness associated with different copolymer systems largely originates from the choice of the hydrophobic block (8). Indeed, several biodegradable (*e.g.* poly(D,L-lactide, PDLLA) as well as non-biodegradable (*e.g.* poly(propylene oxide) biocompatible polymers have been employed as core-forming blocks (8). Although PEG remains the "gold standard" in the stabilization of colloidal drug carriers because of its remarkable properties in water, De Jaeghere et al. (9) have demonstrated that, under certain circumstances, PEG may promote the aggregation of PDLLA nanoparticles after freeze-drying.

Poly(*N*-vinyl-2-pyrrolidone) (PVP) is a well-known water-soluble, biocompatible and relatively amphiphilic polymer. The highly polar amide confers hydrophilic and polar-attracting properties to the polymer, while the apolar methylene group in the backbone and the methine group in the ring contribute to its hydrophobic properties (10). The amide group of PVP easily forms hydrogen bonds and, as a result, PVP has a high binding affinity for water and several other small and large molecules (11,12). The interaction between PVP and water is so strong that high PVP concentrations can prevent water from freezing. This unique behavior has led to the use of PVP as a cryoprotectant for a wide variety of cells (13) and as a lyoprotectant for proteins (14). It has been shown that interactions between PVP and DNA protect DNA from degradation by extracellular nucleases (12) and that PVP can serve to stabilize proteins (15). Like PEG, PVP can increase the circulation time of peptides/proteins (16) and colloids *in vivo* (17). Moreover, PVP is remarkable for its capacity to interact with a wide variety of hydrophilic and hydrophobic pharmaceutical agents. For instance, it can enhance the solubility of several poorly water-soluble nonsteroidal anti-inflammatory drugs (18,19). Drug solubilization in water is due to hydrophobic interactions with PVP which lead to the formation of a soluble complex.

The objective of the present study was to synthesize a new biocompatible and biodegradable diblock copolymer based on PVP and PDLLA. To the best of our knowledge, only a random graft copolymer, poly(*N*-vinyl-2-pyrrolidone)-*graft*-poly(L-lactide), has been previously described in the literature (20). Since PVP is in itself a lyoprotectant, it should self-assemble in micelles with excellent storage properties (after freeze-drying), and should be able to incorporate a variety of drugs in the inner core and the outer shell with a high loading capacity (Fig. 1A). Several diblock copolymers were synthesized, characterized and assessed for micelle formation. The incorporation of a model hydrophobic drug, *i.e.* indomethacin, into PVP-*b*-PDDLA micelles was compared to control PEG-*b*-PDLLA micelles prepared under identical conditions.

MATERIALS AND METHODS

Chemicals

Solvents and reagents were purchased from Moquin Scientifique (Terrebonne, QC, Canada) and Aldrich (Oakville,

[1] Faculty of Pharmacy, University of Montreal, C.P. 6128, Succ. Centre-ville, Montréal (Qc) H3C 3J7, Canada.

[2] To whom correspondence should be addressed. (e-mail: leroujea@pharm.umontreal.ca)

0724-8741/01/0300-0323$19.50/0

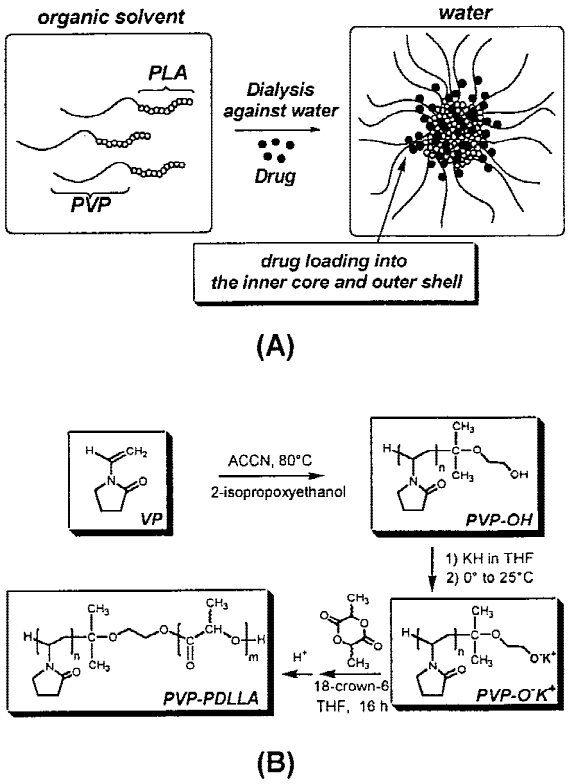

Fig. 1. Schematic illustration of the incorporation of a pharmaceutical agent into the inner core and outer shell of polymeric micelles (A) and synthesis of PVP-OH and PVP-*b*-PDLLA (B).

ON, Canada). *N*-vinyl-2-pyrrolidone (VP), PEG monomethyl ether (MeO-PEG, M_n = 5000), 2-isopropoxyethanol, potassium hydride (KH) 35% (*w/w*) dispersion in mineral oil, 18-crown-6 and pyrene were used without further purification. D,L-lactide was recrystallized 3 times from anhydrous ethyl acetate at 60°C and then dried over P_2O_5 at room temperature for 24 h under reduced pressure. Tetrahydrofuran (THF) was refluxed and distilled over sodium under an inert atmosphere. 1,1′-Azobis(cyclohexane-carbonitrile) (ACCN) was purified by precipitation into water from an ethanol solution and dried under vacuum for 4 days. Sepharose 2B was obtained from Sigma (St. Louis, MO) and equilibrated with water. The dialysis bags used for micelle preparation and drug incorporation were Spectra/Por membranes (Por 1, molecular weight cut-off: 6000–8000, Spectrum, Rancho Dominguez, CA). All reactions were carried out in round-bottom flasks fitted with a rubber septum under an anhydrous and inert atmosphere.

Synthesis of Polymers

Hydroxy-Terminated PVP (PVP-OH)

PVP-OH was prepared by radical polymerization using 2-isopropoxyethanol as a chain transfer agent (21). VP (5 mL, 47 mmol) and ACCN (0.11 g, 0.45 mmol) were solubilized in volumes of 2-isopropoxyethanol ranging from 60 to 300 mL. These solutions were degassed with argon. Polymerization was carried out at 80°C with stirring under a dry argon atmosphere for 24 h. After evaporation of 2-isopropoxyethanol, the polymer was precipitated in diethyl ether. The white powder obtained was purified by 3 successive solubilizations in CH_2Cl_2 and reprecipitations in diethyl ether. The polymer was finally dried in vacuum.

PVP-b-PDLLA and PEG-b-PDLLA

Diblock copolymers PVP-*b*-PDLLA and PEG-*b*-PDLLA were obtained by anionic ring-opening polymerization (22,23). KH (0.567 g, 14 mmol) was placed in a round-bottom flask under inert atmosphere. Anhydrous THF was added *via* a double-tipped needle, the resulting dispersion was briefly stirred, and then THF was removed. Thirty mL of THF was added to the flask and the dispersion was cooled to 0°C. PVP-OH or MeO-PEG (1.34 mmol, based on the molecular weight of the repeating unit), previously dried in vacuum at 60°C over P_2O_5, was solubilized in 30 mL of THF at 60°C. This solution was added to the stirred dispersion through a double-tipped needle, and the resulting solution was maintained at 0°C for 1 h. After warming to room temperature, stirring was maintained for 4 h. Then, the dispersion was transferred to another flask, 18-crown-6 (0.085 g, 0.32 mmol) was added at room temperature, and stirred for 30 min. D,L-lactide polymerization was initiated by its quick introduction (1.5 g, 10 mmol) in 20 mL of THF. After 16 h, polymerization was stopped by adding 1 mL of acetic acid and 5 mL of water. The polymer solution was dialyzed against water for 24 h at 4°C to form micelles. After dialysis, the solution was centrifuged for 30 min at 40,790 g and 20°C to remove PDLLA homopolymers. Free PVP-OH (MeO-PEG) was removed by passage over Sepharose 2B. The micellar solution was lyophilized for 2 days and stored at -20°C until use to avoid degradation of PDLLA segments. All the copolymers were synthesized with a yield of approximately 60%.

Nuclear Magnetic Resonance (NMR) Spectroscopy

^{1}H- and ^{13}C-NMR spectra of the polymers were recorded on Varian 300 and Bruker AMX 600 spectrometers in $CDCl_3$ at 25°C. ^{1}H-NMR spectra of PVP-*b*-PDLLA were also obtained in D_2O.

Determination of Copolymer Molecular Weights

Molecular weights were determined by gel permeation chromatography (GPC) (Waters Model 600, Milford, MA) employing the Millennium software program. HR1, HR3 and HR4 Styragel columns (Waters, 4.6 × 300 mm) and a differential refractometer detector (Waters 2410) were used. The mobile phase was $CHCl_3$ (30°C and 1mL/min). The calibration curve was produced with polystyrene standards (Aldrich).

Critical Association Concentration (CAC)

CAC was measured by the steady-state pyrene fluorescence method (24). It has been shown previously that with increasing concentrations of amphiphilic polymers in aqueous pyrene solution, there is a shift of the (0,0) band from 333 to 338.5 nm in the excitation spectra of pyrene. This change, as measured by the I_{338}/I_{333} intensity ratio, accompanies the transfer of pyrene molecules from a water environment to the hydrophobic micellar cores, and can be used to estimate the

apparent CAC (24). Several polymeric solutions in water containing 10^{-7} M of pyrene were prepared and stirred overnight in the dark at 4°C. Steady-state fluorescent spectra were measured (λ_{em} = 390 nm) after 5 min under stirring at 20°C using a Series 2 Aminco Bowman fluorimeter (Spectronic Instruments Inc., Rochester, NY). Experiments were run in duplicate.

Mean Diameter and Molecular Weight of Polymeric Micelles

Hydrodynamic mean diameter and size distribution were determined at a 90° angle by dynamic laser scattering (DLS) using differential size distribution processor intensity analysis (N4Plus, Coulter Electronics, Haieleah, FL). Micelle size was measured at 20°C in water and phosphate-buffered saline (PBS) at a concentration of 5 g/L and after filtration through a 0.22-μm pore size filter. Size measurements were done in triplicate. The weight-average molecular weight (M_w) of the polymeric micelles was evaluated by static light scattering in a Dawn DSP Laser Photometer (Wyatt Technology Corp., Santa Barbara, CA). The laser wavelength was 633 nm. A magic optical glass was used to normalize the signal of each detector. The specific refractive increment (*dn/dc)* was evaluated with a Water Millipore Model 590 pump coupled to a Model 903 interferometric refractometer (Wyatt Technology Corp.). The eluent was either PBS or deionized water. The eluent flow rate was kept at 0.2 mL/min to avoid dilution of the samples. The concentration of each solution exceeded the CAC. PVP-*b*-PDLLA presented a *dn/dc* of 0.124 ± 0.003 in water and 0.140 ± 0.002 in PBS. PEG-*b*-PDLLA showed a *dn/dc* of −0.052 ± 0.005 in water.

Preparation of Indomethacin-Loaded Polymeric Micelles

The drug and the copolymer (10 mg/mL) were dissolved in *N,N*-dimethylformamide (DMF) or ethanol and dialyzed for 24 h, in the dark, against water. The solutions were filtered through a 0.22-μm pore size filter and freeze-dried. The drug loading was determined by spectrophotometry in DMF at 320 nm, using a Hewlett Packard 8452A diode array spectrophotometer (Boise, ID).

RESULTS AND DISCUSSION

PVP-OH was synthesized by radical polymerization using 2-isopropoxyethanol and ACCN, as chain transfer agent and initiator respectively (Fig. 1B) (21). The chain transfer agent can react with the initiator-derived radical or the propagating radical and reinitiate the polymerization (25). This allows the grafting of functional groups (-OH) at one extremity of the polymeric chain (21). Its chemical structure was confirmed by NMR spectroscopy. ^{1}H-NMR δ (ppm): 1.15 (m, C<u>H</u>$_3$), 3.50-4.00 (broad signal, C<u>H</u> PVP); ^{13}C-NMR δ (ppm): 175.74 (<u>C</u>=O PVP), 63.05 (<u>C</u>H$_2$OH). M_ws of the PVP-OH obtained were between 4600 and 8600 with low polydispersities (<2) (Table I). The molecular weight of PVP-OH was controlled by varying the solvent/VP ratio, a higher ratio resulting in decreased molecular weight.

Stannous octanoate is the most commonly used catalyst for the synthesis of PEG-*b*-poly(lactide) diblock copolymers by ring–opening polymerization of lactide initiated from MeO-PEG (26). Poly(*N*-isopropylacrylamide)-*b*-poly(D,L-lactide) (PNIPAm-*b*-PDLLA) can be obtained under the same conditions from hydroxy-terminated NIPAm (27). Our attempts to prepare PVP-*b*-PDLLA from PVP-OH with stannous octanoate were unsuccessful, favoring the formation of the PDLLA homopolymer rather than the PVP-*b*-PDLLA copolymer. Accordingly, to enhance the reactivity of PVP-OH, the diblock copolymers were prepared by anionic polymerization of lactide using potassium PVP hydroxylate as the initiator (Fig. 1B) (22,23). Ether crown was employed to increase the rate of polymerization by amplifying the nucleophilic character of the generated potassium alkoxide. Several diblock PVP-*b*-PDLLA copolymers with variable compositions were synthesized with this method. The residual PDLLA homopolymer was removed by centrifugation, while unreacted PVP-OH was eliminated by size exclusion chromatography. The molecular weights of the resulting copolymers after purification are shown in Table II. All PVP-*b*-PDLLA copolymers presented M_ns between 3700 and 12100 with polydispersity between 1.2 and 1.9. PVP/PDLLA mass ratios, as estimated by GPC, varied from 33:67 to 75:25 (Table 2). In the case of PEG-*b*-PDLLA (<u>6</u>), the evaluated weight estimated by GPC was of the same order of magnitude as that obtained from NMR, confirming the validity of our molecular weight analysis. The GPC analysis did not reveal the presence of monomers or oligomers after purification of the copolymers by dialysis and gel filtration. The chemical structure of the copolymer was characterized by NMR spectroscopy: ^{1}H-NMR δ (ppm): 5.20 (m, C<u>H</u> PDLLA), 3.50-4.00 (broad signal,

Table I. Weight- (M_w) and Number-Average (M_n) Molecular Weights of PVP-OH

PVP-OH[a]	Solvent/VP (*v/v*)	M_w	M_n	M_w/M_n
1	12	8600	4800	1.8
2	30	5800	4100	1.4
3	30	7200	4000	1.8
4	40	6000	3300	1.8
5	60	4600	2500	1.8

[a] By ^{13}C NMR, it was found that more than 80% PVP chains had a terminal hydroxy group (semi-telechelic polymer).

Table II. Weight- (M_w) and Number-Average (M_n) Molecular Weights of Diblock PVP-b-PDLLA copolymers

PVP-OH	Diblock copolymer	PVP:PLA (% *w/w*)[a]	M_w	M_n	M_w/M_n
1	1a	60:40	15200	8000	1.9
2	2a	75:25	8500	5500	1.5
3	3a	57:43	9000	7000	1.3
3	3b	33:67	14500	12100	1.2
4	4a	60:40	7200	5500	1.3
4	4b	65:35	8000	5100	1.5
5	5a	68:32	5800	3700	1.5
6[c]	6a[c]	41:59	18000	12000	1.4
		37:63[b]	—	7900[b]	—

[a] Determined by GPC, using the M_n of PVP-OH and the corresponding diblock copolymers PVP-b-PDLLA.

[b] Determined by ^{1}H-NMR, using the terminal methoxy group of the PEG chain.

[c] <u>6</u>: MeO-PEG-OH and <u>6a</u>: PEG-b-PDLLA.

CH PVP); ^{13}C-NMR δ (ppm): 175.78 (C=O PVP), 169.77 (C=O PDLLA), 69.82 (CH-CH_3 PDLLA), 17.20 (CH-CH_3 PDLLA).

Figure 2 shows the ^{13}C-NMR spectra of PVP-OH (A) and PVP-*b*-PDLLA (B). The signal (**e**) at 63 ppm was associated with the primary carbon linked to the terminal hydroxy group and disappeared after coupling to D,L-lactide. On the other hand, the signals (**j**), (**k**) and (**l**) appeared in the ^{13}C spectrum (B) of PVP-*b*-PDLLA. The presence of only 2 peaks, related to the carbonyl groups of PVP and PDLLA, and the suppression of signal (**e**) confirmed that a diblock structure was obtained.

The formation of PVP-*b*-PDLLA (PEG-*b*-PDLLA) micelles was investigated by a steady-state fluorescence method, using pyrene as a probe (24). The pyrene intensity ratio I_{338}/I_{333} was determined as a function of the diblock copolymer and PVP-OH concentration (Fig. 3). In the case of the diblock copolymer, the major change in the slope of the fluorescence intensity ratio indicated the onset of micellization. The CACs of all block copolymers were always below 15 mg/L (Table III) and reached values as low as 2 mg/L (samples ***4a*** and ***6a***). In the case of PVP-OH only (without PDLLA segments), the ratio was enhanced above 100 mg/L. As micellization is *a priori* excluded with pure PVP-OH, the relative increase in fluorescence intensity can be explained by the binding of pyrene to the hydrophobic domains of PVP-OH.

Mean micelle sizes and standard deviations obtained by DLS are reported in Table III. The copolymers gave micelles with a bimodal size distribution, except for PEG-*b*-PDLLA ***6a*** where the size was unimodal in water and bimodal in PBS. Eisenberg and coworkers (28) also observed a bimodal size distribution with polycaprolactone-*block*-poly(ethylene oxide) micelles. They suggested that the larger population reflected the aggregation of small individual micelles, governed by a secondary order of aggregation. However, these aggregates depended largely on the preparation of the solution, *i.e.* the more diluted solution favored the first population (smaller sizes). The micelle size of the small particles was generally between 40 and 100 nm, whereas that of the larger aggregates reached values up to 500 nm. The micelle sizes obtained by static light scattering correlated with the first population determined by DLS (data not shown). The aggregation number of polymeric micelles was usually between 100 and 300 (when no apparent secondary aggregation was observed) according to an average micelle M_w of about 1–2 × 10^6.

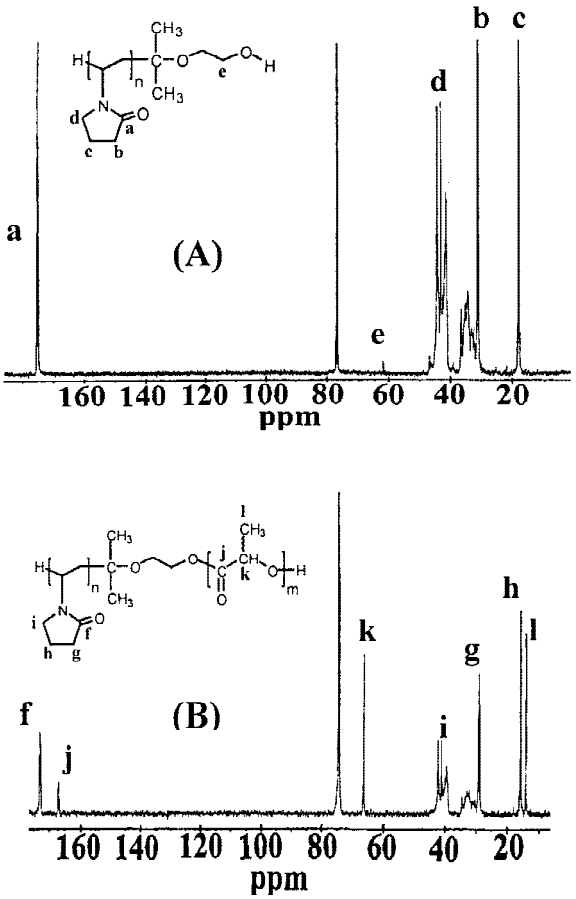

Fig. 2. ^{13}C-NMR spectra of (A) PVP-OH *1* and (B) PVP-*b*-PDLLA *1a* in $CDCl_3$.

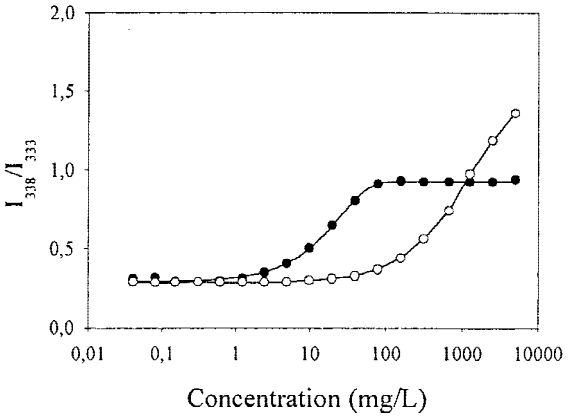

Fig. 3. Plots of the I_{338}/I_{333} ratio as a function of PVP-OH *5* (open circles) and PVP-*b*-PDLLA *5a* (closed circles) concentrations.

To further characterize micelle formation, the ^{1}H-NMR spectra of the copolymers taken in D_2O and $CDCl_3$ were compared (Fig. 4). In $CDCl_3$, where micelle formation is not expected, all peaks proper to the hydrophilic (PVP) and hydrophobic parts (PDLLA) were detected (Fig. 4A). In D_2O, however, the formation of micelles modified the ^{1}H-NMR spectrum, resulting in a loss of resolution of PDLLA signals (Fig. 4B). NMR analysis in water showed mainly the signals of PVP chains while those of the hydrophobic core were suppressed, suggesting the formation of a highly viscous internal core (26).

Indomethacin is a very poorly water-soluble, nonsteroidal anti-inflammatory drug. Its entrapment efficiencies in PVP-*b*-PDLLA (**3a** and **5a**) and control PEG-*b*-PDLLA (**6a**) micelles were compared (Fig. 5). At low initial drug concentrations, no significant difference was observed between the 3 different diblock copolymers. At an initial drug concentration of 40%, PVP-*b*-PDLLA ***3a*** presented a drug loading level about 30% higher than PEG-*b*-PDLLA ***6a***. Moreover, in spite of the much higher molecular weight of the PDLLA segment of ***6a***, copolymer ***5a*** demonstrated comparable entrapment efficiencies. It is believed that at low levels of drug loading, indomethacin is first incorporated in the core, whereas at higher levels, it also interacts with the PVP outer shell. The binding of indomethacin to PVP probably occurs

Table III. Characterization of Polymeric Micelles: Micelle Size and CAC

# Batch	Diblock copolymer	CAC (mg/L)	Micelle size in water* (nm)		Micelle size in PBS* (nm)	
1a	PVP-PDLLA	10	85%	62 ± 29	63%	41 ± 13
	(60:40)		15%	419 ± 133	34%	290 ± 60
2a	PVP-PDLLA	4	50%	29 ± 6	44%	36 ± 12
	(75:25)		50%	199 ± 54	56%	124 ± 51
3a	PVP-PDLLA	2	60%	106 ± 22	18%	38 ± 12
	(57:43)		30%	328 ± 111	82%	154 ± 69
3b	PVP-PDLLA	3	37%	100 ± 15	4%	40 ± 13
	(33:67)		63%	319 ± 46	96%	243 ± 138
4a	PVP-PDLLA	2	72%	48 ± 26	52%	37 ± 11
	(60:40)		28%	158 ± 51	48%	89 ± 21
4b	PVP-PDLLA	3	64%	39 ± 23	14%	26 ± 8
	(65:35)		36%	248 ± 88	86%	119 ± 37
5a	PVP-PDLLA	4	55%	36 ± 10	38%	36 ± 11
	(68:32)		45%	109 ± 46	62%	106 ± 36
6a	PEG-PDLLA	2	100%	122 ± 53	61%	188 ± 70
	(41:59)				39%	502 ± 126

via intermolecular hydrogen bonding (29). At maximal loading, PVP-*b*-PDLLA micelle size increased by approximately 30% (data not shown). The influence of the organic solvent on loading efficiency was also evaluated. The entrapment efficiency of indomethacin PVP-*b*-PDLLA was similar in DMF and ethanol (data not shown). This is an interesting finding, since ethanol might be preferred over DMF for safety reasons. In the case of PEG-*b*-PDLLA, no comparison could be made because this polymer was found to be insoluble in ethanol.

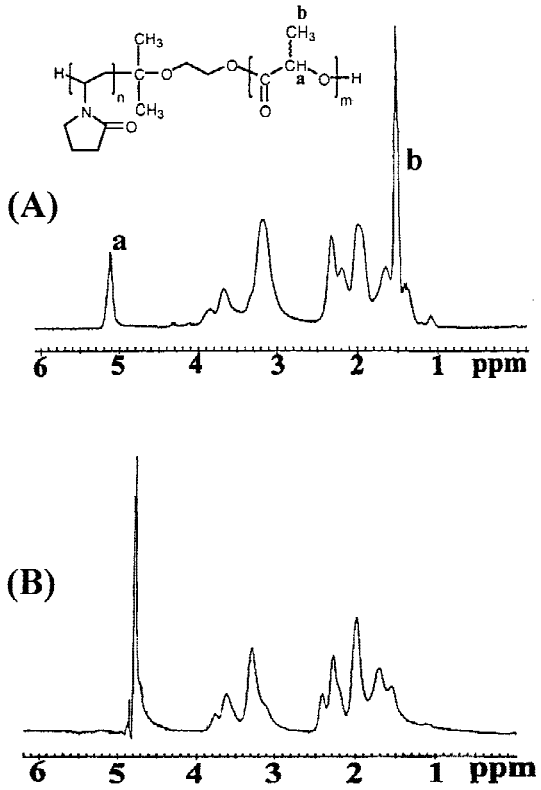

Fig. 4. ^{1}H-NMR spectra of PVP-*b*-PDLLA *5a* (A) in $CDCl_3$ and (B) in D_2O.

CONCLUSION

A series of amphiphilic diblock copolymers based on PVP-*b*-PDLLA were successfully synthesized, using anionic and ring-opening polymerization of D,L-lactide from a PVP-OH macroinitiator. These copolymers self-assemble in micelles at a low CAC. Specific interactions can take place with the hydrophobic core as well as the hydrophilic outer shell. These interactions could explain the higher drug entrapment efficiencies observed for PVP-*b*-PDLLA comparatively to PEG-*b*-PDLLA micelles. Moreover, as reported in the literature, PVP polymers present several other interesting properties such as being good cryo- and lyoprotectants. Finally, owing to the diversity of interactions they can show, PVP-*b*-PDLLA micelles could probably be loaded with several drugs simultaneously and exhibit selective release patterns, as described previously by Jansen *et al.* (30) for unimolecular dendritic micelles.

ACKNOWLEDGMENTS

This research was supported financially by the Natural Sciences and Engineering Research Council of Canada

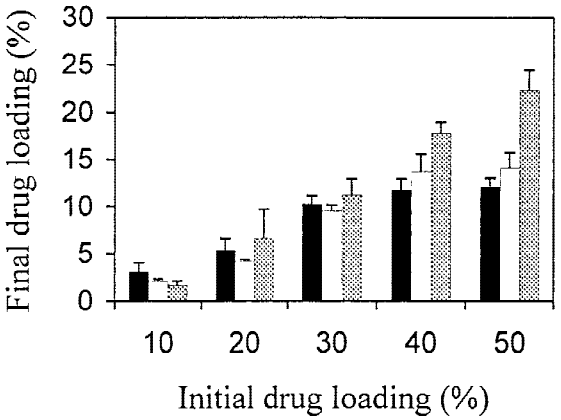

Fig. 5. Incorporation of indomethacin into PEG-*b*-PDLLA (*6a*, black column) and PVP-*b*-PDLLA (*5a*, white column; *3a*, gray column) micelles. Mean ± sd (n = 3).

(NSERC) and Labopharm Inc. (Laval, Canada). Technical support was provided by CERSIM (Laval University, Canada). Dr. Vincent Lenaerts is acknowledged for his critical reading of this manuscript.

REFERENCES

1. M. Yokoyama, T. Okano, Y. Sakurai, S. Fukushima, K. Okamoto, and K. Kataoka. Selective delivery of adriamycin to a solid tumor using a polymeric micelle carrier system. *J. Drug Target.* **7**:171–186 (1999).
2. S. V. Vinogradov, T. K. Bronich, and A. V. Kabanov. Self-assembly of polyamine-poly(ethylene glycol) copolymers with phosphorothioate oligonucleotides. *Bioconjugate Chem.* **9**:805–812 (1998).
3. K. Kataoka, H. Togawa, A. Harada, K. Yasugi, T. Matsumoto, and S. Katayose. Spontaneous formation of polyion complex micelles with narrow distribution from antisense oligonucleotide and cationic block copolymer in physiological saline. *Macromolecules* **29**:8556–8557 (1996).
4. M. Jones and J. Leroux. Polymeric micelles—A new generation of colloidal drug carriers. *Eur. J. Pharm. Biopharm.* **48**:101–111 (1999).
5. G. S. Kwon, S. Suwa, M. Yokoyama, T. Okano, Y. Sakurai, and K. Kataoka. Enhanced tumor accumulation and prolonged circulation times of micelles-forming poly(ethylene oxide-aspartate) block copolymer-adriamycin conjugate. *J. Control. Release* **29**:17–23 (1994).
6. K. Kataoka, G. S. Kwon, M. Yokoyama, T. Okano, and Y. Sakurai. Block copolymer micelles as vehicles for drug delivery. *J. Control. Release* **24**:119–132 (1993).
7. K. Yasugi, Y. Nagasaki, M. Kato, and K. Kataoka. Preparation and characterization of polymer micelles from poly(ethylene glycol)-poly(D,L-lactide) block copolymers as potential drug carrier. *J. Control. Release* **62**:89–100 (1999).
8. C. Allen, D. Maysinger, and A. Eisenberg. Nano-engineering block copolymer aggregates for drug delivery. *Colloids and Surfaces B: Biointerfaces* **16**:3–28 (1999).
9. F. De Jaeghere, E. Alleman, J.-C. Leroux, W. Stevels, J. Feijen, E. Doelker, and R. Gurny. Formulation and lyoprotection of poly(lactic acid-co-ethylene oxide) nanoparticles: Influence on physical stability and in vitro cell uptake. *Pharm. Res.* **16**:859–866 (1999).
10. W. G. Rothschild. Binding of hydrogen donors by peptide groups of lactams. Identity of interaction sites. *J. Am. Chem. Soc.* **94**: 8676–8683 (1972).
11. M. Otagiri, T. Imai, H. Koinuma, and U. Matsumoto. Spectroscopic study of the interaction of coumarin anticoagulant drugs with polyvinylpyrrolidone. *J. Pharm. Biomed. Anal.* **7**:929–935 (1989).
12. R. J. Mumper, J. G. Duguid, K. Anwer, M. K. Barron, H. Nitta, and A. P. Rolland. Polyvinyl derivatives as novel interactive polymers for controlled gene delivery to muscle. *Pharm. Res.* **13**:701–709 (1996).
13. G. F. Doebbler. Cryoprotective compounds. *Cryobiology* **3**:2–11 (1966).
14. M. Townsend and P. P. DeLuca. Use of lyoprotectants in the freeze-drying of a model protein, Ribonuclease A. *J. Parent. Sci. Technol.* **37**:190–199 (1988).
15. W. R. Gombotz, S. C. Pankey, R. Phan, R. Drager, K. Donaldson, K. P. Antonsen, A. S. Hoffman, and H. V. Raff. The stabilization of a human IgM monoclonal antibody with poly(vinylpyrrolidone). *Pharm. Res.* **11**:624–632 (1994).
16. H. Kamada, Y. Tsutsumi, S. Tsunoda, T. Kihira, Y. Kaneda, Y. Yamamoto, S. Nakagawa, Y. Horisawa, and T. Mayumi. Molecular design of conjugated tumor necrosis factor-a: Synthesis and characteristics of polyvinyl pyrrolidone modified tumor necrosis factor-a. *Biochem. Biophys. Res. Comm.* **257**:448–453 (1999).
17. V. P. Torchilin. Polymer-coated long-circulating microparticulate pharmaceuticals. *J. Microencapsulation* **15**:1–19 (1998).
18. G. P. Bettinetti, P. Mura, A. Liguori, G. Bramanti, and F. Giordano. Solubilization and interaction of naproxen with polyvinylpyrrolidone in aqueous solution and the solid state. *Farmaco* **43**: 331–343 (1988).
19. M. C. Tros de llarduya, C. Martin, M. M. Goni, and M. C. Martinez-Oharriz. Solubilisation and interaction of sulindac with polyvinylpyrrolidone K30 in the solid state and aqueous solution. *Drug Dev. Ind. Pharm.* **24**:295–300 (1998).
20. J. L. Eguiburu, M. J. Fernandez-Berridi, and J. M. Roman. Graft copolymers for biomedical applications prepared by free radical polymerization of poly(L-lactide) macromonomers with vinyl and acrylic monomers. *Polymer* **37**:3615–3622 (1996).
21. E. Ranucci, G. Spagnoli, F. Bignotti, P. Ferruti, O. Schiavon, P. Caliciceti, and F. M. Veronese. Synthesis and molecular weight characterization of end-functionalized N-vinyl-2-pyrrolidone oligomers. *Macromol. Chem. Phys.* **196**:763–774 (1995).
22. Z. Jedlinski, P. Kurcok, W. Walach, H. Janeczek, and I. Radecka. Synthesis of ethylene glycol-L-lactide block copolymers. *Makromol. Chem.* **194**:1681–1689 (1993).
23. Y. Wang, S. Chen, and J. Huang. Synthesis and characterization of a novel macroinitiator of poly(ethylene oxide) with a 4-hydoxy-2,2,6,6-tetramethylpiperidinyloxy end group: Initiation of the polymerization of styrene by a "living" radical mechanism. *Macromolecules* **32**:2480–2483 (1999).
24. I. Astafieva, X. F. Zhong, and A. Eisenberg. Critical micellization phenomena in block polyelectrolyte solutions. *Macromolecules* **26**:7339–7352 (1993).
25. Y.G. Takei, T. Aoki, K. Sanui, N. Ogata, T. Okano, and Y. Sakurai. Temperature-responsive bioconjugates. 1. Synthesis of temperature-responsive oligomers with reactive end groups and their coupling to biomolecules. *Bioconjugate Chem.* **4**:42–46 (1993).
26. S. A. Hagan, G. A. Coombes, M. C. Garnett, S. E. Dunn, M. C. Davies, L. Illum, S. S. Davis, S. E. Harding, S. Purkiss, and P. R. Gellert. Polylactide-poly(ethylene glycol) copolymers as drug delivery systems. 1. Characterization of water dispersible micelle-forming systems. *Langmuir* **12**:2153–2161 (1996).
27. F. Kohori, K. Sakai, T. Aoyagi, M. Yokoyama, Y. Sakurai, and T. Okano. Preparation and characterization of thermally reponsive block copolymer micelles comprising poly(N-isopropylacrylamide-b-DL-lactide). *J. Control. Release* **55**:87–98 (1998).
28. C. Allen, Y. Yu, D. Maysinger, and A. Eisenberg. Polycaprolactone-b-poly(ethylene oxide) block copolymer micelles as a novel drug delivery vehicle for neurotrophic agents FK506 and L-685,818. *Bioconjugate Chem.* **9**:564–572 (1998).
29. L. S. Taylor and G. Zografi. Spectroscopic characterization of interactions between PVP and indomethacin in amorphous molecular dispersions. *Pharm. Res.* **14**:1691–1698 (1997).
30. J. F. G. A. Jansen, E. M. M. de Brabander-van den Berg, and E. W. Meijer. Encapsulation of guest molecules into a dendritic box. *Science* **266**:1226–1229 (1994).