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Development of amphiphilic diblock copolymers as micellar carriers of taxol

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Abstract

Diblock copolymers of poly(DL-lactide)-*block*-methoxy polyethylene glycol were synthesized from monomers of DL-lactide (DLLA) and methoxy polyethylene glycol (MePEG) by a ring opening bulk polymerization in the presence of stannous octoate. The copolymer molecular weight and composition were controlled by changing the monomer weight ratio. The copolymers dissolved in water to form polymeric micelles with a hydrophobic poly(DL-lactide) (PDLLA) core and a water soluble MePEG shell. The critical micelle concentrations (CMC), measured by fluorescence techniques, depended on copolymer molecular weight and ranged from millimolar to micromolar. The PDLLA core of the micelle was in a highly viscous state since the ^{1}H-NMR peaks of PDLLA in the copolymers presented in $CDCl_3$ and disappeared in D_2O due to the restriction of PDLLA chain mobility. Up to 25% taxol could be loaded into matrices of PDLLA-MePEG (MePEG molecular weight: 2000; DL-lactide/MePEG compositions: 40:60 or 50:50) using the solution casting method. Dissolution of the taxol/copolymer matrices in water, 0.9% NaCl, or 5% dextrose solutions resulted in complete solubilization of taxol within the copolymer micelles. Evidence of strong association or binding of taxol to the PDLLA block of the copolymer even below the polymer CMC is presented.

Keywords: Diblock copolymer; Micellar solubilization; Polylactide; Polyethylene glycol; Taxol

1. Introduction

Taxol has been used successfully in the treatment of cancers such as advanced ovarian and breast cancer (Spencer and Faulds, 1994). Due to its very low water solubility, taxol is formulated in a 50:50 mixture of Cremophore EL (polyethoxylated castor oil) and dehydrated alcohol. However, serious hypersensitivity reactions have been associated with its current formulation (Onetto et al., 1993; Spencer and Faulds, 1994). Therefore a

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great deal of effort has been directed towards developing aqueous based formulations for taxol. Soluble semi-synthetic taxol derivatives have been synthesized (Greenwald and Shorr, 1994) and carriers such as surfactants (Alkan-Onyuksel et al., 1994) and liposomes (Sharma and Straubinger, 1994) have been used to solubilize taxol.

Amphiphilic diblock copolymer micelles are effective vehicles for the solubilization of hydrophobic drugs (Kataoka et al., 1993). The drugs can be covalently coupled to block copolymers to form micellar structures or can be physically incorporated within the hydrophobic cores of polymeric micelles (Kwon et al., 1994a). The diblock copolymer, poly(aspartic acid)-*block*-polyethylene glycol, has been evaluated as a micellar carrier of the anti-cancer drug adriamycin for intravenous administration (Kataoka et al., 1993). A high drug payload, long circulation times in the blood, and good anti-tumor activity were observed with this system (Kwon et al., 1994a,b, 1995; Yokoyama et al., 1994).

Gref et al. (1994) synthesized diblock copolymers of methoxy polyethylene glycol with either poly(DL-lactide) (PDLLA), poly(lactide-co-glycolide) or polycaprolactone and prepared nanospheres of these copolymers. The PEG coating on the nanosphere surface resulted in a significant increase in blood circulation time due to a dramatically decreased nanosphere uptake by the reticuloendothelial system (Gref et al., 1994; Bazile et al., 1995). High entrapment efficiencies for lidocaine and prednisolone in the nanospheres were achieved (Gref et al., 1994).

In this work we have investigated the use of poly(DL-lactide)-*block*-methoxy polyethylene glycol (PDLLA-MePEG) diblock copolymers as micellar carriers for the solubilization of taxol. The effects of the molecular weight of the MePEG block and the weight ratio of PDLLA/MePEG on the ability of the diblock copolymer micelles to solubilize taxol were investigated. The physicochemical characteristics of the taxol/copolymer matrices and micellar dispersions were determined.

2. Experimental

2.1. Materials

DL-lactide was purchased from Aldrich. Stannous octoate, 1,6-diphenyl-1,3,5-hexatriene (DPH), and methoxy polyethylene glycol (MePEG) with molecular weights of 750, 2000, and 5000, were obtained from Sigma. Taxol was obtained from Hauser Chemicals, Boulder, CO. Polystyrene standards of narrow molecular weight distributions and various molecular weights were purchased from Polysciences (Warrington, PA). Acetonitrile (ACN), chloroform, methylene chloride, ethanol, methanol, ethyl acetate, and tetrahydrofuran (THF) were HPLC grade (Fisher Scientific).

2.2. Synthesis of PDLLA-MePEG

A ring opening polymerization procedure was employed to synthesize the copolymer of poly(DL-lactide-co-methoxy polyethylene glycol) (termed PDLLA-MePEG) (Reed and Gilding, 1981; Zhu et al., 1990). Appropriate quantities of the DL-lactide, MePEG, and 0.5 wt% stannous octoate were added to a 10 ml glass ampoule. The ampoule was evacuated under vacuum for 20 min and heat sealed. The sealed ampoule was immersed in a mineral oil bath at 150°C to start the polymerization. Immediately after the melting, the ingredients in the ampoule were vortex mixed and re-immersed in the oil bath. The polymerization was stopped after 3.5 h by pulling the ampoule out from the oil bath.

2.3. Gel permeation chromatography (GPC)

The molecular weights of the PDLLA-MePEG copolymers were determined at ambient temperature by GPC using a Shimadzu LC-10AD HPLC pump and a Shimadzu RID-6A refractive index detector (Kyoto, Japan) coupled to a 10^3 Å Hewlett Packard Plgel column. The mobile phase was chloroform with a flow rate of 1 ml/min. The injection volume of the sample was 20 μl at a polymer concentration of 0.2% (w/v). The molecular weights of the polymers were determined relative to polystyrene standards.

2.4. Preparation of micellar taxol

Taxol and the copolymer samples were dissolved in acetonitrile followed by evaporation of the solvent under a stream of nitrogen at 60°C for about 2 h to obtain a solid taxol/PDLLA-MePEG matrix. Residual acetonitrile remaining in the taxol/copolymer matrix was determined by gas chromatographic (5830A GC and 18850A GC terminal) analysis using flame ionization detection and the following conditions: column Carbopack at 0.2% Carbowax 20 M, 2 m × 2 mm I.D.; N_2 gas carrier (16 ml/min); injection volume 5 μl. Dissolution of the solid taxol/copolymer matrix was carried out by preheating the matrix in a warm water bath (about 60°C) to obtain a transparent gel-like sample. This was followed by the addition of water at about 60°C and stirring by a vortex mixer or a glass rod to obtain a clear micellar solution. Samples of solid taxol/copolymer matrix were also prepared by mixing solid taxol into the copolymer melted at about 60°C.

2.5. Differential scanning calorimetry (DSC)

DSC was carried out using a TA Instruments 2000 controller and DuPont 910S DSC (Newcastle, Delaware). The heating rate was 10°C/min and the copolymer and taxol/copolymer matrix samples were weighed (3–5 mg) into crimped open aluminum sample pans.

2.6. X-ray diffraction

X-ray diffraction of polymers and the matrices of polymer/taxol were performed with a Rigaku Geigerflex X-ray Diffractometer System (Rigaku Corporation, Tokyo, Japan). The X-ray source was Kα radiation from a copper target with a nickel filter. Samples were scanned from 5 to 40° 2θ at a scanning speed of 1°/min and a step size of 0.05° 2θ. The X-ray tube was operated at a potential of 40 kV and a current of 20 mA.

2.7. Determination of critical micelle concentration

Critical micelle concentrations (CMC) of the copolymers were determined by fluorescence intensity and anisotropy techniques (Zhang et al., 1996). To measure the fluorescence intensity and anisotropy, a DPH stock solution of 10 mM in tetrahydrofuran (THF) was prepared and a given volume of DPH solution was added to aqueous PDLLA-MePEG solutions of various concentrations (0.0001–0.5%). These samples were equilibrated overnight in a dark chamber. A Shimadzu RF 540 spectrofluorophotometer with a polarization accessory was used to measure the intensity of DPH fluorescence. The wavelengths of excitation and emission were 355 nm and 428 nm, respectively. The temperature was controlled with a water bath circulator (SLM Instruments, Inc.). The fluorescence anisotropy (r) was calculated using the equation: $r = (I_{vv} - I_{vh})/(I_{vv} + 2I_{vh})$, where I_{vv} and I_{vh} are the emission intensities detected via a polarizer oriented parallel and vertical, respectively, to vertically polarized monochromatic excitation light.

2.8. ^{1}H-nuclear magnetic resonance (NMR)

^{1}H-NMR spectra of PDLLA-MePEG and taxol loaded PDLLA-MePEG were obtained in $CDCl_3$ or D_2O using a NMR instrument (Bruker, AC-200E) at 200 MHz. The concentration of the polymer in $CDCl_3$ or D_2O was 5–10%.

2.9. Binding of taxol to the copolymer

A micellar solution of 10% taxol loaded PDLLA-MePEG 2000-50:50 was added to a large volume of distilled water with stirring at 37°C. The final copolymer concentration was made to 1.13 or 45 μM. An aliquot (0.4 ml) was taken at different time intervals up to 48 h and passed through an ultrafiltration unit using centrifugation (Ultra Free®-MC, 5000 NMWL Filter Unit, Millipore) to obtain an aqueous solution of free taxol. The centrifugation was done using an Eppendorf centrifuge (20 min, 10 000 × g). The taxol concentration was determined by reverse-phase HPLC. HPLC analysis was performed using a 110A pump and C-8 ultrasphere column (Beckman), and a SPD-6A UV detector set at 232

Table 1
Molecular weights, composition, critical micelle concentration, and taxol loading capacity of PDLLA-MePEG copolymers

Copolymer	MW of PDLLA-MePEG		Polymerization degree		Weight ratio[a]	CMC at 25°C (μM)[b]		Taxol loading (%)
	Measured[c]	Calculated[d]	PDLLA[e]	MePEG[f]	PDLLA/MePEG	Intensity	Anisotropy	
750-50:50	2046	1500	5.2	17.1	0.98	-	-	-
750-30:70	-	1071	2.2	17.1	-	2800	2300	-
2000-50:50	5860	4000	13.9	45.5	1.11	40	5	25%
2000-40:60	5240	3333	9.3	45.5	0.737	90	23	25%
2000-30:70	4620	2857	5.95	45.5	0.468	210	27	-
5000-30:70	119	7143	14.9	113.6	0.412	42	22	10%

[a]Determined by ^{1}H-NMR.
[b]Calculated polymer molecular weight was used.
[c]Measured by GPC, relative to polystyrene standard.
[d]Calculated by $MW_{PDLLA-MePEG} = (1 + W_{DLLA}/W_{MePEG}) \times MW_{MePEG}$.
[e]Calculated by $P_{PDLLA} = W_{DLLA}/W_{MePEG} \times MW_{MePEG}/144$.
[f]Calculated by $P_{MePEG} = MW_{MePEG}/44$.

nm, a SIL-9A autoinjector and a C-R3A integrator (Shimadzu). The injection volume was 20 μl and the flow rate was 1 ml/min. The mobile phase was 58% acetonitrile, 5% methanol, and 47% distilled water.

2.10. Taxol solubilization by copolymer

Given amounts of aqueous PDLLA-MePEG solutions were added to capped glass tubes containing excess amounts of taxol suspensions in distilled water. The tubes were incubated at 37°C with gentle rotational mixing for different time periods to establish an equilibration time. In subsequent studies, tubes were incubated overnight (approximately 16 h). The suspensions were centrifuged at 10000 × g for 10 min at room temperature. The concentrations of taxol in the supernatants were analyzed using HPLC.

3. Results and discussion

3.1. Polymer synthesis, composition, molecular weight and dissolution in water

Three series of PDLLA-MePEG copolymers were synthesized using MePEG with molecular weights of 750, 2000, or 5000. Each series were prepared with PDLLA/MePEG weight ratios of 50:50, 40:60, 30:70, 20:80, and 10:90. The polymer nomenclature is designated PDLLA-MePEG $X-Y/Z$, where X is the molecular weight of MePEG and Y and Z are the weight percentage of monomers DLLA and MePEG, respectively. The polymerization went almost to completion under these experimental conditions as shown by the ^{1}H-NMR spectrum (see Fig. 5), where peaks representing DL-lactide and its oligomers were small. Other studies have suggested that the ring opening polymerization should give high yields under similar polymerization conditions (Eenink, 1987; Zhang et al., 1994). The copolymers PDLLA-MePEG 750 series were viscous liquids or waxy semi-solids at room temperature, while copolymers PDLLA-MePEG 2000 and 5000 series were all whitish solids.

The chemical compositions of the copolymers determined by NMR corresponded closely to the percentage of the monomers used in the polymerization reaction (Table 1). The hydroxy group is believed to be responsible for initiating the ring opening polymerization (Schindler et al., 1982; Kricheldorf et al., 1995). Stannous octoate dramatically increases the polymerization rate and may also form complexes with the hydroxy group

to initiate the polymerization (Eenink, 1987; Zhang et al., 1994; Kricheldorf et al., 1995). In the polymerization, the lactide ring opens and inserts into the initiation site resulting in a growing polylactide chain starting from the hydroxy group. In the case of MePEG, the hydroxy group at one of the MePEG ends is acting as the initiation site to allow the growth of the PDLLA chain. Therefore a diblock copolymer of PDLLA and MePEG is obtained.

The molecular weights of PDLLA-MePEG copolymers measured by GPC increased with an increase in the molecular weight of MePEG and decreasing amounts of MePEG (Table 1). It has been shown that, assuming conditions of insignificant side reactions, the polymerization degree of PDLLA (P_{PDLLA}) can be calculated by the theoretical equation $P_{PDLLA} = [M_{DLLA}]/[M_{OH}]$, where $[M_{DLLA}]$ and $[M_{OH}]$ are the molar concentrations of DL-lactide and hydroxy groups, respectively (Schindler et al., 1982; Li and Kissel, 1993; Kricheldorf et al., 1995). The calculated molecular weights of PDLLA-MePEG (Table 1) are believed to be closer to the true molecular weights of the copolymers than the measured molecular weights since the latter were based on polystyrene standards directly, due to the lack of Mark-Houwink parameters for the copolymers. It has been shown in our laboratory that the calculated molecular weight of poly(L-lactide) using the same synthesis conditions but with stearyl alcohol as an initiator corresponds well to the molecular weight measured by GPC, in the range of molecular weights up to 10 000 (data not included).

Depending on the composition, PDLLA-MePEG copolymers could be dissolved in water and other aqueous solutions such as 0.9% NaCl and 5% dextrose. For the synthesized copolymers, those with molecular weights of MePEG of 750, 2000, and 5000 were water soluble at weight ratios of PDLLA/MePEG of 50:50–10:90, 50:50–10:90, and 30:70–10:90, respectively. The water solubility of the copolymer is a function of the molecular weight of the PDLLA block. The higher the PDLLA molecular weight, the lower the solubility. A reduced PDLLA molecular weight could be obtained from low molecular weight MePEG and using less DL-lactide monomer in the synthesis (Table 1). The copolymers readily dissolved in common organic solvents such as chloroform, methylene chloride, acetone, acetonitrile, ethyl acetate, THF, ethanol and methanol.

3.2. Characterization of the copolymer matrices

The residual acetonitrile remaining in the taxol/copolymer matrix after evaporation of the solvent at 60°C for 2 h was 158 ± 75 ppm. We found that the residual acetonitrile level in the matrix could be reduced to below detectable level (< 10 ppm) by drying for 20 h in a vacuum oven at 60°C. To ensure there was no degradation of taxol in the matrix after prolonged drying at 60°C, we determined the content of taxol in the matrix by HPLC after 96 h storage at 60°C and found that there was no evidence of degradation of taxol. DSC thermograms revealed endothermic peaks for taxol, MePEG 2000, PDLLA-MePEG, and taxol/PDLLA-MePEG matrices (Fig. 1, Table 2). All scans were run up to 250°C; however, with the exception of the thermogram for pure taxol, no thermal changes were detected beyond 75°C. The peak at 53°C for MePEG 2000 with an enthalpy of 154 J/g represented its melting transi-

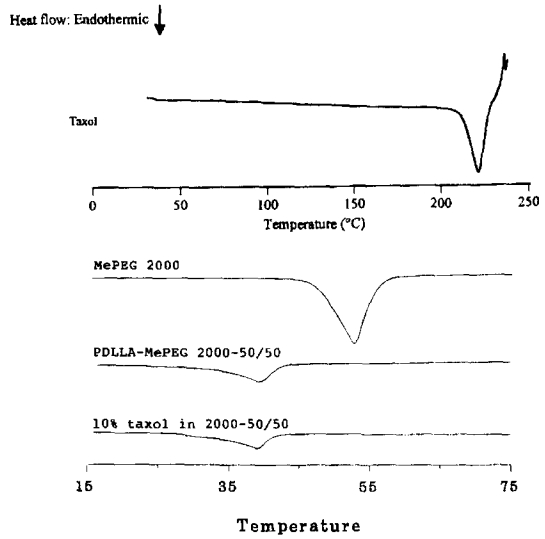

Fig. 1. DSC thermograms of taxol, MePEG, PDLLA-MePEG copolymer, and taxol loaded PDLLA-MePEG copolymer. The heating rate was 10°C/min. See Table 2 for melting temperature and enthalpy.

Table 2
Thermal analysis and X-ray diffraction of copolymer

Material	Peak melting temperature (°C)	ΔH (J/g) of melting transition	X-ray diffraction d spacing (Å)
Taxol	221	59.3	15.36, 9.71, 6.99
MePEG 2000	53	153.9	4.49, 3.78
PDLLA-MePEG 2000-50:50	39	55.9	4.70, 3.82
PDLLA-MePEG 2000-40:60	45	78.7	—
PDLLA-MePEG 2000-30:70	46	71.1	—
PDLLA-MePEG 5000-30:70	54	97.4	—
10% Taxol in 2000-50:50, ACN cast	41	49.5	4.60, 3.77
10% Taxol in 2000-50:50, melt mix	52	45.8	16.35, 10.10, 4.60, 3.92

tion. The endothermic peaks for PDLLA-MePEG copolymers between 39°C to 54°C were probably due to the melting of the MePEG region in the copolymer indicative of the formation of separated MePEG and PDLLA phases in the solid copolymer matrix. The reduced melting temperatures and reduced enthalpies of the MePEG region in the copolymers compared with MePEG alone indicated a lower degree of MePEG crystallinity in the copolymer. As the content of PDLLA in the copolymer decreased, the melting temperature of the copolymer approached that of MePEG alone (Table 2). It is possible that the PDLLA block interferes with the crystallization of the MePEG block resulting in an imperfect crystal (therefore a decreased melting temperature). A lower PDLLA content should result in less interference with MePEG block crystallization. Taxol did not affect the crystallization of MePEG in the copolymer, indicating that taxol was probably in the hydrophobic PDLLA region.

The X-ray diffraction patterns of taxol, MePEG and copolymer matrices are shown in Fig. 2 and the d-spacings corresponding to the two or three most intense peaks are given in Table 2. Taxol showed three intense peaks at 2θ of 5.75°, 9.10° and 12.65° and numerous small peaks between 15°–25° (Fig. 2a). MePEG gave two peaks at 18.85° and 23.25° 2θ (Fig. 2b) and these peaks were also present in the solid PDLLA-MePEG copolymer (Fig. 2c), providing further evidence of phase separation in the solid block copolymer. When 10% taxol was co-dissolved with PDLLA-MePEG 2000-50:50 in acetonitrile and allowed to dry by evaporation of the acetonitrile, the resulting mixture gave no taxol peaks (Fig. 2d). Similarly, no taxol peaks were observed when taxol was mixed with either MePEG 2000 or PDLLA (intrinsic viscosity 0.6 dl/g in $CHCl_3$ at 30°C Birmingham Polymers, AL) using the same method (data not shown). This indicated that taxol was either molecularly dispersed in the polymers or distributed in the polymer in an amorphous state. When the matrix was made by mixing taxol crystals into PDLLA-MePEG melt at a temperature of about 60°C (melt mixing), taxol crystal peaks were observed (Fig. 2e). Using optical microscopy, taxol crystals were clearly seen in the matrix made by melt mixing. DSC thermograms of the matrix from melt mixing did not reveal a melting peak for taxol. This was probably due to the dissolution of taxol into the polymer when the temperature was increased during thermal analysis. It has been observed previously under hot stage microscopy that taxol crystals in a melt mixed taxol/PCL matrix disappeared when temperature was increased up to 200°C (Winternitz et al., 1996).

3.3. Solubilization of taxol

In the development of solubilized taxol using the copolymers as carriers, our goal was to obtain a taxol loaded polymer matrix which could be

dissolved readily in various fluids such as water, 0.9% NaCl, and 5% dextrose. We found that the method of matrix manufacture was critical to the reconstitution and formation of a clear aqueous taxol/copolymer solution. Physical mixing of solid taxol in the copolymer melt did not produce a one-phase system following reconstitution in water. The solution casting method was attempted, where both taxol and the copolymer were dissolved in a solvent followed by evaporation of the solvent at 60°C under a stream of nitrogen. Different solvents such as chloroform, methylene chloride, ethyl acetate, acetone, methanol, ethanol, THF and acetonitrile were evaluated. None of the solvents except acetonitrile resulted in a taxol/copolymer matrix which produced a clear solution following reconstitution in aqueous vehicles. Different methods of dissolution of the taxol/copolymer matrices included heating, sonication, vortex mixing and mechanical mixing and methods were evaluated for ease of dissolution of the taxol/polymer matrix in aqueous solutions. It was found that the matrices could be most readily dissolved by preheating both the matrix and water to 60°C followed by mechanical and vortex mixing.

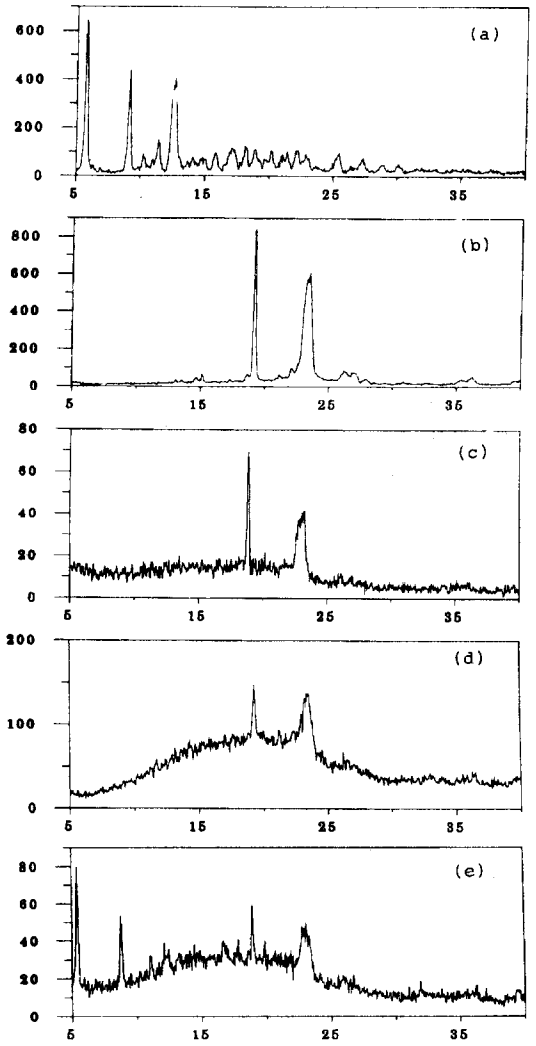

Fig. 2. Powder X-ray diffraction patterns for taxol and copolymer matrices. (a) Taxol; (b) MePEG 2000; (c) PDLLA-MePEG 2000-50:50; (d) 10% taxol loaded PDLLA-MePEG 2000-50:50, solution casting method; (e) 10% taxol loaded PDLLA-MePEG 2000-50:50, melt mixing method.

The effect of the different solvents is not well understood but may be related to a particular morphology of the phase separated taxol/PDLLA and MePEG in the copolymer matrices. One possibility is that the differing solubilities of taxol or polymer in the solvents may induce different morphologies. A higher solubility may inhibit phase separation as the solvent evaporates. It is also possible that residual acetonitrile in the matrix may have played a role in the solubilization process. The effects of the different solvents on the physicochemical properties of the taxol/copolymer matrix and taxol solubilization require further study.

Factors which promoted a greater extent of taxol solubilization in the copolymer micelles were a higher PDLLA content and higher molecular weight of the copolymer. Low molecular weight copolymer 750-50:50 formed a precipitate following dissolution in water, whereas 25%, 25% and 10% taxol were loaded into the higher molecular weight copolymers 2000-50:50, 2000-40:60, 5000-30:70, respectively, without precipitation after the dissolution step. The copolymer matrices could be dissolved in water with a polymer concentration up to 20%. The high taxol solubilization capacity was in contrast to small molecular weight surfactant pluronics F127, P103, and L101, where precipitates formed even at loading of 0.5% taxol.

The physical stability of the solid matrices and the solubilized solutions were evaluated. It was

found that solid taxol/copolymer matrices of 2000-50:50, 2000-40:60 and 5000-30:70 could be stored at 4°C for at least 2 months without changing the reconstitution and dissolution behavior. Using microscopic methods we found no evidence of taxol recrystallization in the matrices after 2 months storage at 4°C. The appearance of solubilized solutions of 10% taxol in 2000-50:50, 2000-40:60 and 5000-30:70 copolymer micellar solutions was unchanged at 4°C for 2 months, 2 months, and 2 days, respectively. Taxol solubilized (10%) in the 2000-40:60 copolymer remained in solution for about 1 day at room temperature.

Attempts were made to analyze the particle size of the copolymer micelles using a Submicron Particle Sizer (Nicomp Model 270, Pacific Scientific, CA). The particle sizes were less than the detection limit (about 50 nm) of the instrument indicating that the size was probably smaller than 50 nm. The general size range is in agreement with literature values of micellar sizes (Kwon et al., 1993; Gref et al., 1994).

3.4. Formation of micelles

The formation of micelles of the copolymers in an aqueous environments was supported by the detection of critical micelle concentrations (CMC). The CMCs were determined by fluorescence intensity and fluorescence anisotropy techniques using DPH as a probe. These techniques are based on significant changes of fluorescence intensity and anisotropy at the CMC. The fluorescence intensity of DPH increased slowly at lower polymer concentrations and then increased rapidly due to the formation of micelles (Fig. 3A). Since there was no distinctive abrupt change in the intensity, the concentration where the most abrupt change occurred (the largest tangential change) was taken as the CMC. Fluorescence anisotropy increased with increasing polymer concentration initially and then leveled off (Fig. 3B). The CMC was taken as the lowest polymer concentration at which there was no further change in anisotropy since a distinctive abrupt anisotropy change can be seen.

In Fig. 3B, anisotropies of the copolymers increased with polymer concentration at the initial

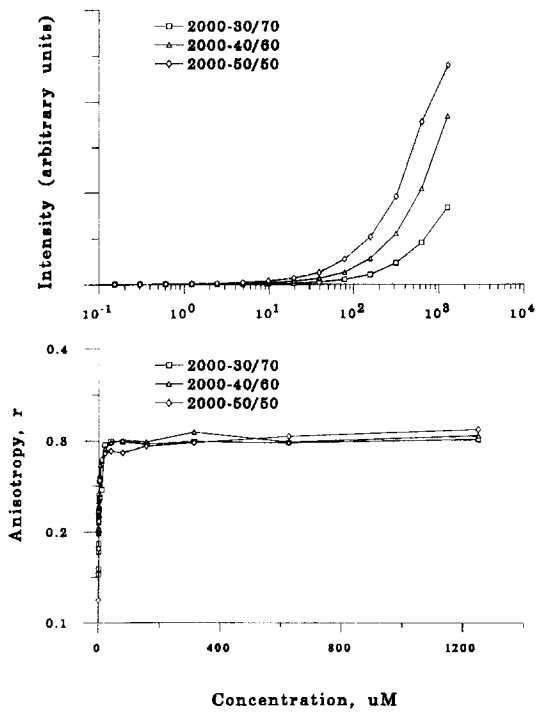

Fig. 3. Determination of critical micelle concentrations (CMC) of PDLLA-MePEGs by fluorescence intensity (A) and fluorescence anisotropy (B) methods (25°C). The abscissa units are concentration (μM) for both (A) and (B).

stage. This was in contrast to low molecular weight surfactants (i.e. Triton X-100, SDS, and sodium cholate), where anisotropy decreased initially and then leveled off (Zhang et al., 1996). The hydrophobicity of the hydrophobic segment of amphiphilic copolymers is significantly greater than that of small molecule surfactants. Indeed, the copolymer can exhibit core-shell structure in water even with a single molecular chain (Evans and Wennerstrom, 1994). The DPH molecule may bind to the hydrophobic segment of the copolymer below the CMC, therefore giving a higher vibrational freedom to DPH compared with its self-associated state and also its micelle associated state. Anisotropy values are directly related to the rotational freedom of DPH, the higher the local viscosity of the DPH associated region, the lower the anisotropy. Therefore the decreasing order of anisotropy values are self-associated DPH, micelle associated DPH, and single polymer chain associ-

ated DPH. Below the CMC, the single chain associated DPH dominates the anisotropy since it gives a much higher fluorescence intensity over self-associated DPH. This results in a lower anisotropy value below the CMC. With increasing polymer concentration, DPH associates with more polymer chains and finally micelles, resulting in the anisotropy increasing and leveling off.

The CMC values obtained from the fluorescence intensity and fluorescence anisotropy measurements for PDLLA-MePEG 750-30:70, 2000-50:50, 2000-40:60, 2000-30:70, and 5000-30:70 are given in Table 1. The CMC values for the 750-30:70 matrix determined by the fluorescence intensity and anisotropy methods are in good agreement but there are 2- to 8-fold differences in the CMC values measured by the two methods for the other copolymers. We believe that the differences are due to our difficulty in interpreting the CMC values from Fig. 3A given that the intensity changes are not abrupt at the CMC and occur over a large copolymer concentration range.

At the same MePEG molecular weight, higher molecular weights of PDLLA resulted in lower CMCs (Table 1, 2000-50:50, -40:60, and -30:70). This is because higher molecular weight PDLLA is more hydrophobic, which promoted micelle formation at lower polymer concentrations. On the other hand, at the same PDLLA molecular weight, an increased molecular weight of MePEG resulted in increased CMC value (Table 2, 5000-30:70, 2000-50:50) due to the increased hydrophilicity of the copolymer.

The CMCs of PDLLA-MePEG 2000-40:60 in water, 0.9% saline, and 5% dextrose were the same in each solvent and were 23 μM measured by fluorescence anisotropy and 94 μM measured by fluorescence intensity. The insensitivity of the CMCs to NaCl and dextrose was probably due to the non-ionic nature of the polymer. CMCs were not significantly changed by solubilization of 10% taxol in the micelles. The reason for the absence of any measurable change in CMC upon taxol solubilization is not clear but may be due to the strong hydrophobic association of taxol with PDLLA chains.

There was a long equilibration time for PDLLA-MePEG single polymer chains forming micellar structures as indicated by the time required for DPH molecules to become fully associated or solubilized within PDLLA-MePEG micelles (about 2.5 h) (Fig. 4). Solubilization of DPH within low molecular weight surfactant micelles such as sodium dodecyl sulfate (SDS), Triton X-100, and sodium cholate took only a few minutes (Chattopadhyay and London, 1984). The slow equilibration of the single polymer chains forming micelles may due to the strong association among PDLLA chains in the micelles. In fact, the PDLLA core of the copolymer micelle was probably in a highly viscous state as indicated by the NMR data. The NMR peaks of both PDLLA and MePEG were detected in $CDCl_3$, which is a good solvent for both PDLLA and MePEG (Fig. 5A). This indicated that the movement of PDLLA and MePEG was not restricted in $CDCl_3$. In D_2O, the MePEG peak was still present while the PDLLA peaks disappeared (Fig. 5B). The expansion of the PDLLA peak positions in Fig. 5B revealed scattered small peaks resulting from oligomers of DLLA. This indicated that the movement of PDLLA was restricted due to the high viscosity of the PDLLA core.

3.5. The interaction between taxol and polymer

Equilibrium solubility values for taxol/copolymer solutions were attained within 10–15 h (Fig.

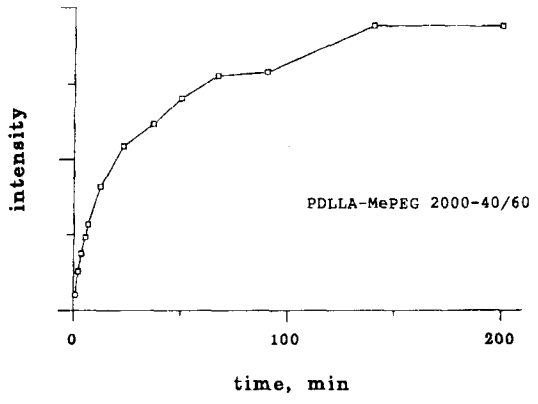

Fig. 4. Time course of DPH solubilization within PDLLA-MePEG 2000-40:60 micelles in water at 25°C.

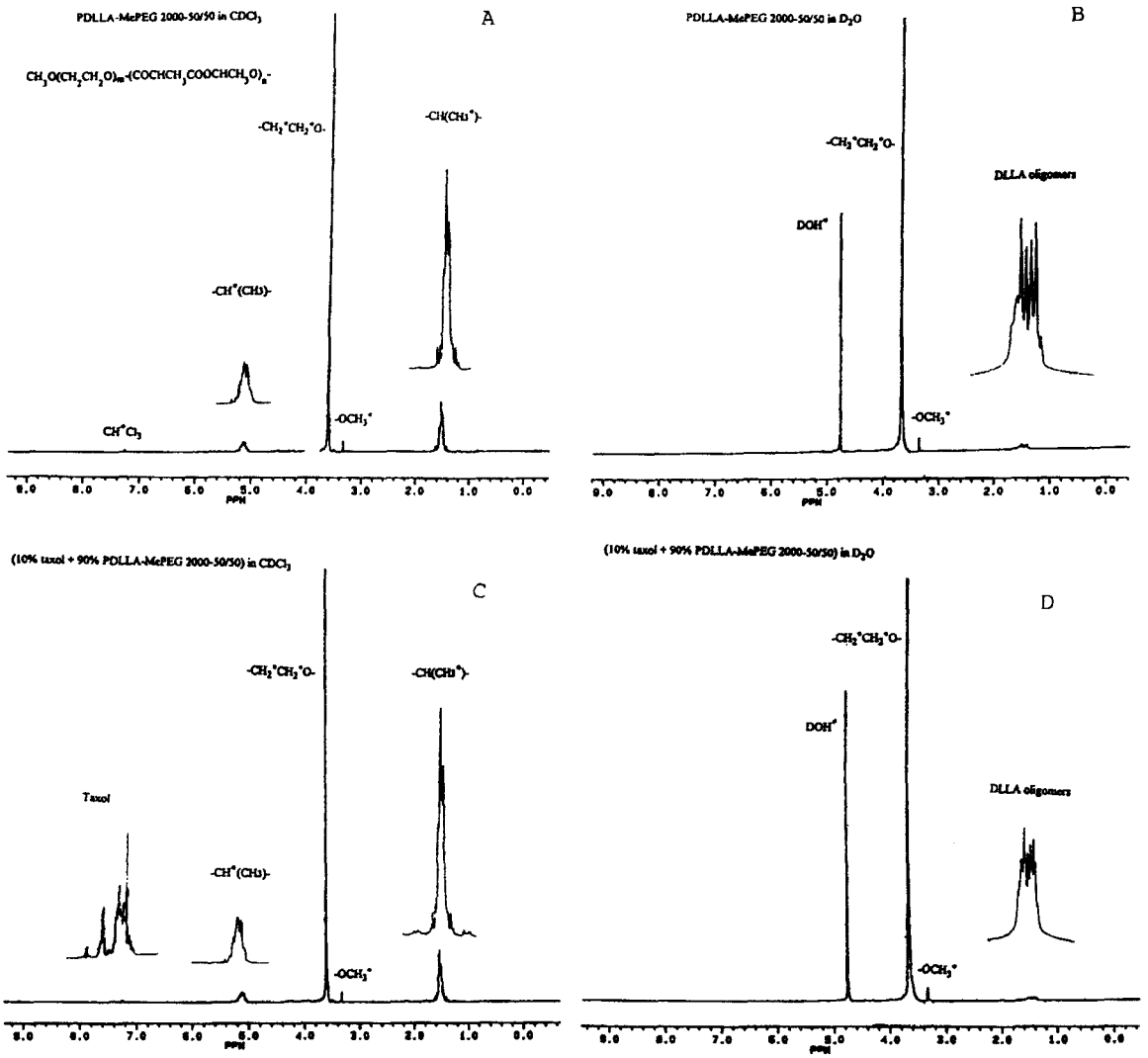

Fig. 5. ^{1}H-NMR spectra of PDLLA-MePEG and taxol/PDLLA-MePEG. (A) PDLLA-MePEG 2000-50:50 in $CDCl_3$; (B) PDLLA-MePEG 2000-50:50 in D_2O; (C) 10% taxol loaded PDLLA-MePEG 2000-50:50 in $CDCl_3$; (D) 10% taxol loaded PDLLA-MePEG 2000-50:50 in D_2O.

6A). The solubility of taxol increased with the increasing copolymer concentration ranging from below to above the CMCs (Fig. 6). The large error bars on some data points in Fig. 6 are probably due to problems with very small amounts of taxol crystals being sampled along with the supernatants following centrifugation. The solubilization of taxol below the CMC was due to its association with the hydrophobic PDLLA block of the single copolymer chain or self-associated copolymer chains. Above the CMC, taxol was solubilized in the PDLLA core of the copolymer micelle. The ability to solubilize taxol decreased in the order, PDLLA-MePEG 2000-50:50, 2000-40:60, and 5000-30:70 corresponding to the decrease in PDLLA content. The increased solubility of taxol even below the copolymer CMC indicated that taxol interacts strongly with the hydrophobic PDLLA segment.

An attempt was made to measure the free taxol concentration in a 10% taxol loaded PDLLA-MePEG 2000-50:50 aqueous solution (taxol 0.5 μg/ml, copolymer 1.125 μM below the CMC) up to 48 h, using the centrifugal ultrafiltration technique. No significant amount of free taxol was detected (data not shown) indicating the strong hydrophobic binding of taxol to the single polymer chain. When the concentrations of total taxol and copolymer 2000-50:50 were 20 μg/ml and 45 μM (above the CMC), respectively, the concentration of free taxol increased to a peak value of 2 μg/ml in 1 h followed by a decrease and stabilization at 0.5 μg/ml by 5 h (data not shown).

The NMR spectra of taxol/PDLLA-MePEG matrices dissolved or solubilized in $CDCl_3$ or D_2O are given in Fig. 5. While there were NMR peaks from both taxol and PDLLA of dissolved taxol/PDLLA-MePEG matrices in $CDCl_3$ (Fig. 5C), these peaks disappeared in D_2O where the matrices formed micelles (Fig. 5D). The peak disappearance was due to the restricted mobility of PDLLA and taxol. This suggested that taxol was entrapped in the PDLLA core of copolymer micelles. The solubilization of taxol within the PDLLA-MePEG micelles did not alter the chemical nature of taxol based on the following evidence. Taxol solubilized in the micelles could be completely recovered following methylene chloride extraction. The HPLC chromatograms for free taxol and the extracted taxol was the same (data not shown). The fluorescence spectra of free taxol and taxol in micelles were also identical (excitation wavelength 237 nm, emission wavelength range 280–440 nm).

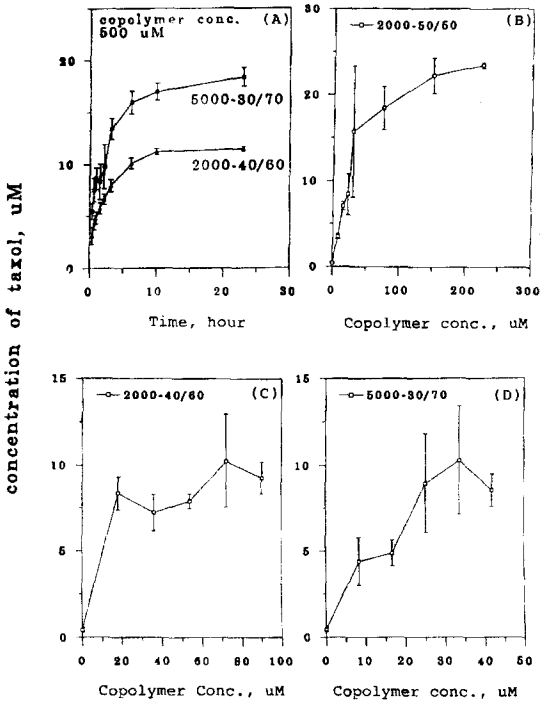

Fig. 6. Effect of time (A) and copolymer concentration (B–D) on the solubilization of taxol in different PDLLA-MePEG copolymer aqueous solutions at 25°C.

In conclusion, diblock copolymers of poly(DL-lactide)-*block*-methoxy polyethylene glycol formed micelles with a hydrophobic PDLLA core of high microviscosity and a water soluble MePEG shell, in an aqueous environment. Taxol could be loaded into the hydrophobic core up to 25% to achieve solubilization. Taxol bound strongly to the PDLLA segment of the copolymers resulting in its slow release from the copolymers.

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