Arch Pharm Res Vol 26, No 2, 173-181, 2003

Archives of Pharmacal Research
http://apr.psk.or.kr

A Polymeric Micellar Carrier for the Solubilization of Biphenyl Dimethyl Dicarboxylate

Sang-Cheol Chi[1], Dae-Il Yeom[1], Sung-Chul Kim[1,2], and Eun-Seok Park[1]

[1]Sungkyunkwan University, 300 Chonchon-Dong, Changan-Gu, Suwon, Kyonggi-Do 440-746, Korea and [2]Samyang Pharmaceuticals R&D, Samyang Corporation, 63-2 Hwaam-Dong, Yusung-Gu, Taejeon 305-717, Korea

(Received October 17, 2002)

A polymeric micelle drug delivery system was developed to enhance the solubility of poorly-water soluble drug, biphenyl dimethyl dicarboxylate, DDB. The block copolymers consisting of poly(D,L-lactide) (PLA) as the hydrophobic segment and methoxy poly(ethylene glycol) (mPEG) as the hydrophilic segment were synthesized and characterized by NMR, DSC and MALDI-TOF mass spectroscopy. The size of the polymeric micelles measured by dynamic light scattering showed a narrow monodisperse size distribution with the average diameter less than 50 nm. The MW of mPEG-PLA, 3000 (MW of mPEG, 2 K; MW of PLA, 1 K), and the presence of hydrophilic and hydrophobic segments on the polymeric micelles were confirmed by MALDI-TOF mass spectroscopy and NMR, respectively. Polymeric micelle solutions of DDB were prepared by three different methods, i.e. the matrix method, emulsion method and dialysis method. In the matrix method, DDB solubility was reached to 13.29 mg/mL. The mPEG-PLA 2K-1K micelle system was compared with the poloxamer 407 micelle system for their critical micelle concentration, micelle size, solubilizing capacity, stability in dilution and physical state. DDB loaded-polymeric micelles prepared by the matrix method showed a significantly increased aqueous solubility (>5000 fold over intrinsic solubility) and were found to be superior to the poloxamer 407 micelles as a drug carrier.

Key words: Polymeric micelles, Biphenyl dimethyl dicarboxylate, DDB, Polylactide, Methoxy poly(ethylene glycol), mPEG-PLA, Block copolymer, Solubilization, Poloxamer 407

INTRODUCTION

Biphenyl dimethyl dicarboxylate (dimethyl-4, 4'-dimethoxy-5, 6, 5', 6'-dimethylene dioxy biphenyl-2, 2' dicarboxylate, DDB) (Fig. 1) is a hepatoprotective agent used to improve the liver functions of the patients with hepatitis or the patients receiving cancer chemotherapy. DDB is freely soluble in chloroform, dimethylformamide and acetone, and slightly soluble in methanol and ethanol. It is, however, practically insoluble in water, with its solubility being 2.5-4.0 μg/mL over a pH range from 1.2 to 6.5 (Hyun and Chun, 1994). When given as a suspension or tablet, oral bioavailability of DDB is only 20-30%. Studies have been conducted to enhance the dissolution and solubility of DDB. For instance, solid dispersions of DDB with soluble carriers such as polyethylene glycol 6000, polyvinyl pyrrolidone and urea have been prepared by the melting and solvent method (Gu *et al.*, 1989). Also, a water-soluble DDB derivative, (Bis (2-(methylamino)ethyl)-4,4-dimethoxy-5,5',6,6'-dimethylene-dioxy-biphenyl-2,2'-dicarboxylate), has been synthesized to increase the solubility of DDB (Moon *et al.*, 1997).

The use of colloids in the pharmaceutical sciences has grown rapidly over the last several decades. Recently, to overcome the problems in solubilizing poorly soluble drugs, much interest has focused on the use of polymeric micelles formed in an aqueous system. While many researches have been conducted on the polymeric micellization in organic media (Pispas and Hadjichristidis, 1996; Quintana *et al.*, 1995), fewer studies have been devoted to the polymeric micellar formation in aqueous systems. Major characteristics of these systems are high thermodynamic stability, solubilization of hydrophobic drugs, prolonged storage stability, and low interaction with biological components.

Block copolymers composed of hydrophilic and hydro-

Correspondence to: Eun-Seok Park, College of Pharmacy, Sungkyunkwan University, 300 Chonchon-Dong, Changan-Gu, Suwon, Kyonggi-Do 440-746, Korea
Tel. +82-31-290-7715, Fax. +82-31-290-7729
E-mail espark@skku.ac.kr

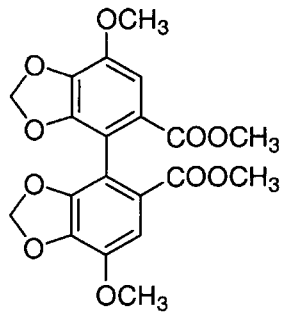

Fig. 1. Molecular structure of biphenyl dimethyl dicarboxylate (DDB)

phobic segments have the potential to form a micellar structure in an aqueous medium (Yokoyama, 1992). AB type block copolymers composed of the hydrophilic and hydrophobic segments can form a micellar structure due to their amphiphilic properties. There are two spherical concentric regions of the polymeric micelles, a densely packed core consisting of the hydrophobic segments and a shell consisting of a dense brush of the hydrophilic segments. Polymeric micelles are an effective vehicle for the solubilization of hydrophobic drugs (Kataoka *et al.*, 1993). The drug can be covalently coupled to the block copolymers or physically incorporated into the hydrophobic core of micelles (Kwon *et al.*, 1994a). The diblock copolymer, poly(ethylene oxide)-block-poly(aspartic acid), has been evaluated as a micellar carrier of an anticancer drug, adriamycin, for intravenous administration (Kwon *et al.*, 1994b). A high drug payload, long circulation times in blood and good antitumor activity were observed with this system.

Indomethacin- and cisplatin-incorporated poly(ethylene glycol)-block-poly(β-benzyl-L-aspartic acid) micelle systems have been investigated (La *et al.*, 1996; Yokoyama *et al.*, 1996). Zhang *et al.* (1996) synthesized diblock copolymer of methoxy poly(ethylene glycol)-block-poly(D,L-lactide) as a micellar carrier for the solubilization of taxol.

In this study, we have investigated the use of poly(D,L-lactide)-block-methoxy poly(ethylene glycol) diblock copolymers as micellar carriers for the solubilization of DDB. The triblock copolymer poloxamer 407 was also investigated and compared with the mPEG-PLA diblock copolymers as a drug carrier.

MATERIALS AND METHODS

Materials

D,L-lactide and methoxy PEG (poly(ethylene glycol) methyl ether, Ca. 2000) were obtained from Aldrich Chemical Co. (Milwaukee, U.S.A.). D,L-lactide was purified by recrystallization with ethyl acetate and sublimation before use. Stannous octoate and stannous 2-ethylhexanoate were obtained from Sigma Chemical Co. (St. Louis, U.S.A.). Biphenyl dimethyl dicarboxylate (DDB) was kindly supplied by Dae-Wha Pharm. Co. (Seoul, Korea). Acetonitrile (HPLC-grade) was obtained from Mallinckrodt Baker, Inc. (Paris, U.S.A.). Chloroform, dimethyl acetamide and diethyl ether were of analytical grade (Kanto Chemical Co., Tokyo, Japan). Poloxamer 407 was from BASF Co. (Ludwigshafen, Germany). All other chemicals were from Sigma Chemical Co. (St. Louis, U.S.A.). Purified deionized water was prepared by the Milli-Q Plus system (Millipore, Milford, U.S.A.).

Synthesis of mPEG-PLA diblock copolymers

The mPEG-PLA diblock copolymers were synthesized by the ring opening polymerization of D,L-PLA in the presence of methoxy PEG using stannous 2-ethylhexanoate as a catalyst at 110°C (Reed and Gilding, 1981; Zhu *et al.*, 1990). Copolymerization in the melt state was accomplished at various ratios of the monomer to the initiator ([M]/[I], where [M] and [I] represent the D,L-lactide unit and mPEG

Table I. Compositions and physical properties of mPEG 2K and mPEG-PLA diblock copolymers

Sample	Feed molar ratio[a] (lactide/mPEG)	Mola composition[b] (lactide/mPEG)	Weight % composition of mPEG[c]	Number average molecular weight ($\times 10^3$)		Polydispersity (Mw/Mn)	Physical properties[d]		
				Calc.	Est.[c]		Tg (°C)	Tm (°C)	H (J/g)
mPEG 2K	-	-	100.00	2.00	2.04	1.01	-	54.00	153.90
mPEG-PLA 2K-0.5K	3.87	3.79	79.79	2.50	2.56	1.05	-61.30	42.22	120.50
mPEG-PLA 2K-0.7K	5.09	4.99	74.65	2.70	2.73	1.04	-55.93	40.42	108.12
mPEG-PLA 2K-1K	6.70	6.57	68.84	3.00	2.97	1.03	-51.69	40.40	104.69
mPEG-PLA 2K-1.2K	8.01	7.85	64.71	3.20	3.15	1.05	-46.94	38.63	86.87
mPEG-PLA 2K-1.4K	9.66	8.88	60.17	3.40	3.39	1.05	-46.88	35.32	44.38
mPEG-PLA 2K-1.8K	12.37	12.12	53.95	3.80	3.78	1.05	-33.60	36.23	54.34

[a] Calculated on the basis of the Mn of mPEG(2000) measured by Aldrich Chemical Co.
[b] Estimated as the difference between the experimental total Mn of Copolymer and mPEG homopolymer in MALDI-TOF experiments
[c] Measured by MALDI-TOF analysis
[d] Measured by DSC analysis

homopolymer, respectively) (Table I). A preweighed amount of mPEG was added to a 30 mL reaction vessel. The reaction vessel was evacuated under vacuum for 2 h at 120°C. The reaction vessel was cooled and was added with preweighed amounts of D,L-lactide and 0.1% (w/w) stannous octoate. The vessel was then evacuated under vacuum for 30 min, sealed, and placed in a silicon oil bath at 130°C to start the polymerization. After 24 h, the reaction product was cooled to ambient temperature. The obtained viscous material was dissolved with chloroform and then precipitated with cold diethyl ether to remove unreacted D,L-lactide monomer. The resulting product was dried in a vacuum oven at 30°C for 2 days.

MALDI-TOF mass spectroscopy

The molecular weights of mPEG-PLA copolymers were determined by the matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectroscopy (VoyagerTM^{RP}, PerSeptive Biosystem, U.S.A.). The copolymer solution (1 mg/mL in H_2O) and HABA (2-(4-hydroxy-phenylazo benzoic acid) matrix solution (1 mg/mL in 50% acetonitrile) were mixed to give a final copolymer concentration of 0.05% (w/v). A portion (1 μL) of the aliquot was added to the sample probe of the mass spectrometer and was allowed to dry under reduced pressure.

Preparation of DDB loaded polymer matrix

To compare the capacity for the formation of a clear aqueous DDB/copolymer solution, three different methods, i.e., the matrix method, emulsion method and dialysis method, were used to prepare DDB loaded polymer matrix.

Matrix method : DDB and the copolymer were dissolved in acetonitrile. The solvent was evaporated under a gentle stream of nitrogen at 60°C for about 3 h to obtain solid DDB/mPEG-PLA matrix. Dissolution test was conducted for the DDB/copolymer matrix after preheating in a heating block to obtain a transparent gel-like sample. This was followed by the addition of water at about 60°C and stirring on vortex mixer to obtain a clear micellar solution.

Oil in water emulsion method : DDB was dissolved in appropriate volumes of chloroform. The chloroform solution was added dropwise to a polymeric micelle aqueous solution under vigorous stirring at room temperature. The reaction mixture was stirred in an air open system to remove chloroform by evaporation.

Dialysis method : Appropriate amounts of DDB and mPEG-PLA copolymer were added to DMAC (dimethyl acetamide) and were dissolved completely. Formed micelles were separated by dialysis (Spectrapor, membrane m.w. cutoff 12,000-14,000 g/mol) against distilled water over 8 h. The water was exchanged every 2 h for 8 h.

To remove unloaded free drug, micellar solutions were filtered using 0.45 μm nylon syringe filter (Target®, National Scientific Co., U.S.A.).

Differential scanning calorimetry (DSC)

Thermal analysis of mPEG-PLA copolymer and DDB/copolymer matrix was carried out on a differential scanning calorimeter (Pyris 1, Perkin Elmer, U.S.A.). Approximately 2.5-5 mg of the copolymer and DDB/copolymer matrix samples were weighed into the aluminum sample pans. All scans were run from -150°C to 100°C with the heating rate of 10°C/min after quenching from the melt.

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

^{1}H-NMR spectra of mPEG-PLA copolymer and DDB loaded mPEG-PLA were obtained in $CDCl_3$ or D_2O using a NMR instrument (Unity Inova 500 MHz, Varian, U.S.A.). The concentration of the polymer in $CDCl_3$ or D_2O was 1-2%.

Transmission electron microscopy (TEM)

One drop of mPEG-PLA micellar solution (1%, w/v) was placed on a carbon-coated grid (200 mesh, Formvar/Carbon, Electron Microscope Science, U.S.A.). The grid was held horizontally for 20 s to allow the molecular aggregates to settle and then excess fluid was removed from the grid surface with filter paper (Whatman, No. 1). The grid was returned to the horizontal position and a drop of 1% phosphotungstic acid was added to provide negative stain. The grid was then left to stand for 20 s before removing excess stain as above. Specimen was air-dried before examination using a JEOL 1220 transmission electron microscope (Japan).

HPLC conditions

The concentration of DDB was determined by high performance liquid chromatographic (HPLC) method using the following system and conditions. The HPLC system consists of an isocratic pump (Hitachi, Model L-7110), a manual injector (Rheodyne, Model 7125), an UV/Vis detector (Hitachi, Model L-7400) and an integrator (Hitachi, Model D-7500). A Cosmosil $5C_{18}$ AR column (4.6 mm × 150 mm, 5 μm particle size, Nacalai Tesque, Japan) was used. The mobile phase consisting of acetonitrile:water (50:50, v/v) was pumped at a flow rate of 1.0 mL/min. The UV/Vis detector was set at 278 nm and the injection volume was 50 μL. All operations were carried out at room temperature.

Determination of critical micelle concentration

The critical micelle concentrations (CMC) of the copolymers were determined by a fluorescence probe technique (Kwon *et al.*, 1993). To measure the fluorescence intensity, excess amounts of pyrene were added to the aqueous copolymer solutions at various concentrations (10, 5, 1, 0.5, 0.1, 0.05, 0.01, 0.005, 0.001 mg/mL) and these

samples were equilibrated overnight in a shaker. A Hitachi F-4010 fluorescence spectrophotometer (Japan) was used to measure the intensity of pyrene fluorescence. The wavelengths and bandwidths for excitation were 339 and 3.0 nm and 390 and 1.5 nm for emission. The experiments were performed at 20°C.

Determination of micelle radii

The hydrodynamic radii of the mPEG-PLA micelles were measured dynamic light scattering method using a He-Ne laser (Lexel Laser Inc., Model 127, New York, U.S.A.) with BI 200 SH Goniometer, BI-8000 AT digital correlator (Brookhaven Instrument Co., New York, U.S.A.) and circulator (Neslab, Model RTE-100, U.S.A.).

Physical stability of polymeric micelles

Physical stability tests were performed for the polymeric micelles in solution. DDB loaded polymeric micelle solutions prepared from mPEG-PLA 2K-1K and poloxamer 407 (DDB concentration; about 200 μg/mL) were stored in dark place at room temperature for 2 weeks. To observe changes in DDB concentration, micelle solutions were analyzed by HPLC.

RESULTS AND DISCUSSION

Synthesis and characterization of mPEG-PLA diblock copolymers

A series of mPEG-PLA copolymers was synthesized using mPEG with molecular weight of 2000 at varying mPEG/PLA weight ratios. The copolymerization went almost to completion under the experimental conditions as shown by the NMR spectrum, where peaks representing D,L-lactide and its oligomers were small. Other studies have suggested that the ring opening polymerization should give high yields under similar polymerization conditions (Zhang *et al.*, 1996; Zhu *et al.*, 1990). Copolymers were all whitish solids and were freely soluble in acetonitrile, chloroform, ethanol, methylene chloride, acetone and dimethyl acetamide. These copolymers showed high water solubility (>200 mg/mL).

The chemical composition of the copolymers determined by ^{1}H-NMR corresponded closely to the amount of the monomers used in the polymerization reaction. In polymerization, the lactide ring opens and inserts into the initiation site resulting in a growing polylactide chain starting from the hydroxy group. In case of mPEG, the hydroxy group at one of the mPEG ends is acting as the initiation site to allow the growth of the PLA chain (Zhang *et al.*, 1996). Therefore a diblock copolymer of mPEG and PLA is obtained.

The molecular weight of mPEG-PLA copolymers was measured by MALDI-TOF mass spectroscopy. The mass spectra shown in Fig. 2 reveal only a single peak. The polydispersity indices of mPEG-PLA copolymers were all under 1.05.

The thermal behavior of the copolymers was investigated by DSC analysis (Fig. 3). At glass transition temperature, Tg, a small endothermic reaction was observed as the specific heat of the copolymer increased. Tg was defined as the midpoint of the step change in energy. Beyond Tg, the plot appeared as a shifted baseline until an exothermic reaction became evident, i.e., the copolymer released heat during crystallization. The plot returned to the baseline at the end of the exothermic reaction. On further heating, another endothermic reaction was observed as the copolymer melted. The glass transition temperatures of the copolymers were increased with increasing the molecular weight, whereas the melting point and enthalpies were decreased with increasing the molecular weight (Table I). Reduced melting temperatures and enthalpies of the mPEG region of the copolymer compared with mPEG alone indicated a lower degree of mPEG crystallinity in the copolymer. As the content of PLA in the copolymer was decreased, the melting temperature approached that of mPEG alone. It is possible that the PLA block interferes with the crystallization of the mPEG block resulting in imperfect crystals. Therefore, copolymers showed a decreased melting temperature.

To ensure that mPEG-PLA copolymer was compatible with DDB, physical mixtures of the mPEG-PLA copolymer and DDB were scanned by DSC. All the scans were run up to 200°C. Obtained DSC thermograms (Fig. 4) revealed the endothermic peaks for mPEG-PLA copolymers and DDB. Therefore, the mPEG-PLA copolymers appeared compatible with DDB.

Identification of micelle formation

Micelle formation was identified by ^{1}H-NMR. The NMR spectra of DDB-loaded mPEG-PLA matrices dissolved or solubilized in $CDCl_3$ or D_2O are given in Fig. 5. While there were NMR peaks from both DDB and PLA of dissolved DDB loaded mPEG-PLA matrices in $CDCl_3$, these peaks disappeared in D_2O because the matrices formed micelles. The peak disappearance was due to the restricted mobility of PLA and DDB. This suggested that DDB was incorporated in the PLA core of the micelle. The solubilization of DDB within micelles did not alter the chemical nature of DDB as evidenced by the identical HPLC chromatograms obtained for free and micellar DDB (UV wavelength 278 nm) (Chromatograms were not shown).

DDB solubilization

DDB loaded polymeric micelles were prepared from mPEG-PLA copolymers and poloxamer 407. Prepared polymeric micelle solutions showed enhanced aqueous

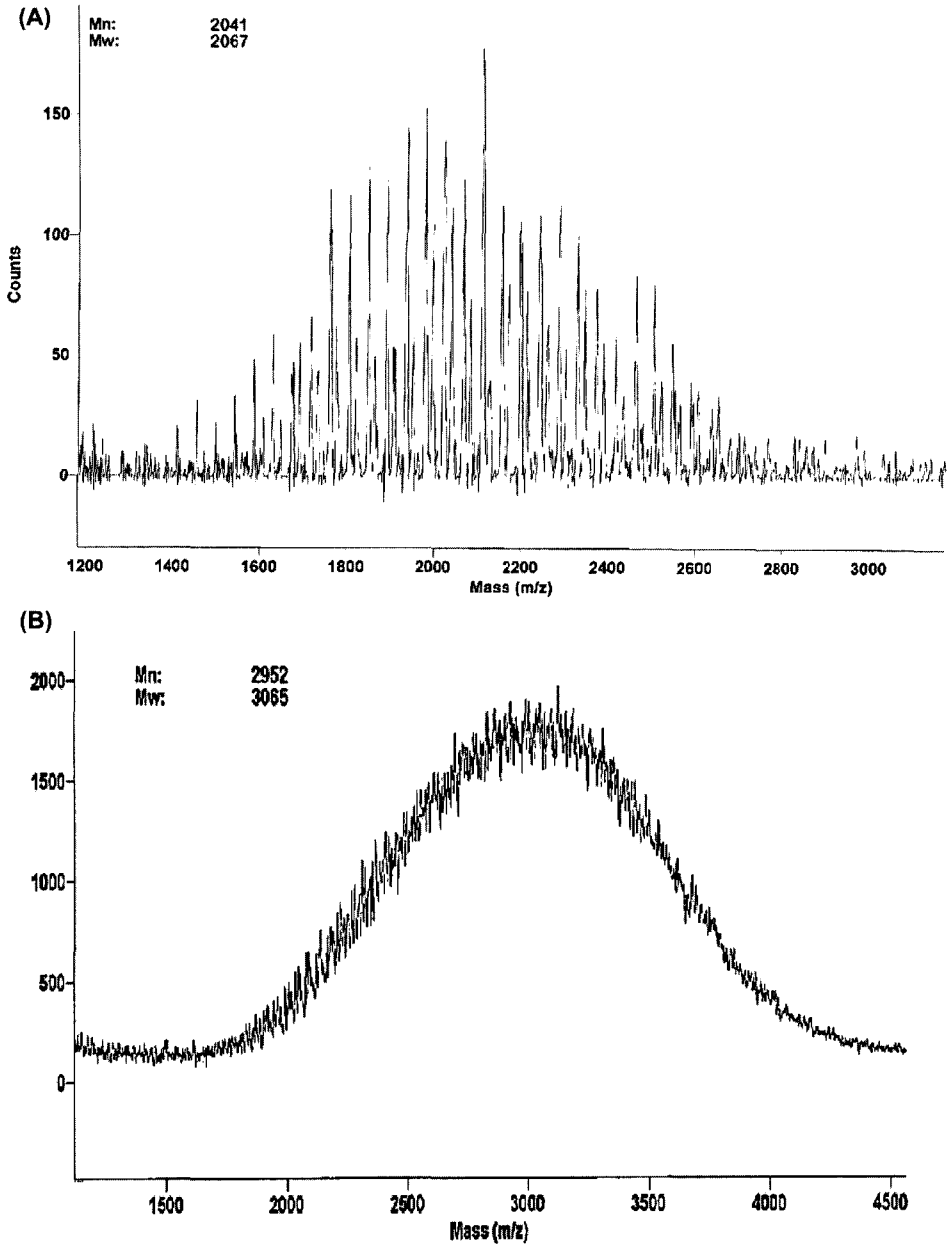

Fig. 2. MALDI-TOF Mass spectra of (A) mPEG 2K and (B) mPEG-PLA 2K-1K.

solubility of DDB. Among the mPEG-PLA copolymers of various molecular weights, mPEG-PLA 2K-1K (MW of mPEG, 2K; MW of PLA, 1K) showed the highest increase in DDB solubility.

Solubility profiles of DDB achieved by three different preparation methods are given in Fig. 6. The micellar DDB solution of mPEG-PLA 2K-1K copolymer showed the highest solubility when prepared by the matrix method, whereas the micellar DDB solution of poloxamer 407 showed the highest solubility when prepared by the dialysis method. The polymeric micelles prepared with mPEG-PLA 2K-1K resulted in >5000 fold increases in DDB solubility. In case of mPEG-PLA 2K-1K, the loading efficiency of DDB in polymeric micelles was all greater than 95% at 1-15% copolymer ratio, indicating mPEG-PLA 2K-1K has a relatively high loading capacity for DDB.

Characterization of polymeric micelles

The CMC of copolymers were determined by the fluorescence probe method based on significant changes in fluorescence intensity. The fluorescence intensity of pyrene was increased slowly at lower copolymer concentrations and then increased rapidly due to the formation of micelles. It has been reported that there is a change in the vibrational structure of pyrene monomer emission upon micellization of poly(ethylene oxide)-block-poly(β-benzyl-L-aspartate)

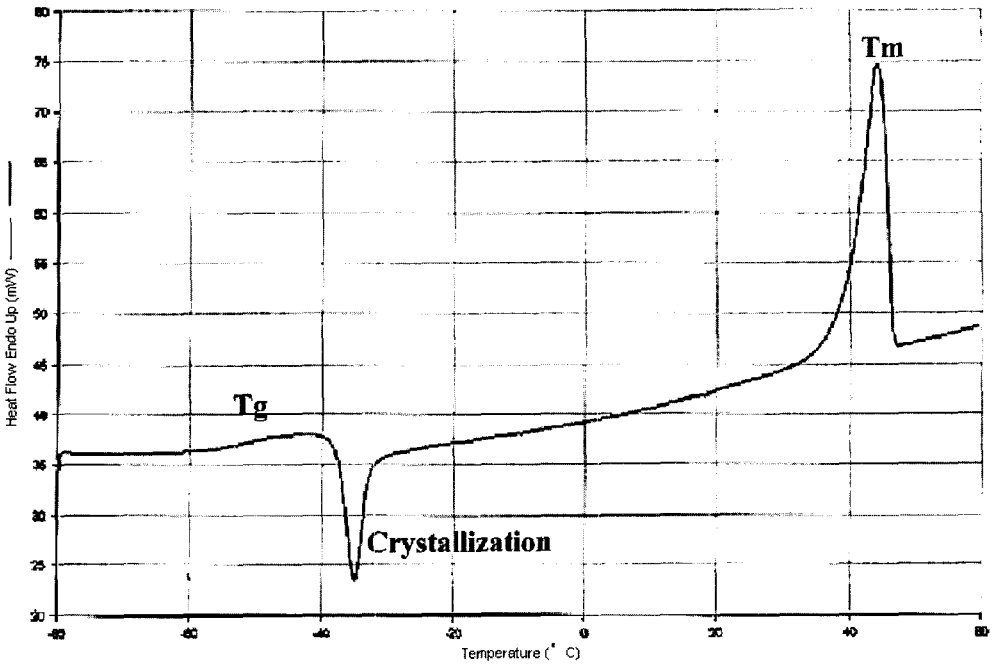

Fig. 3. DSC thermogram of mPEG-PLA 2K-1K.

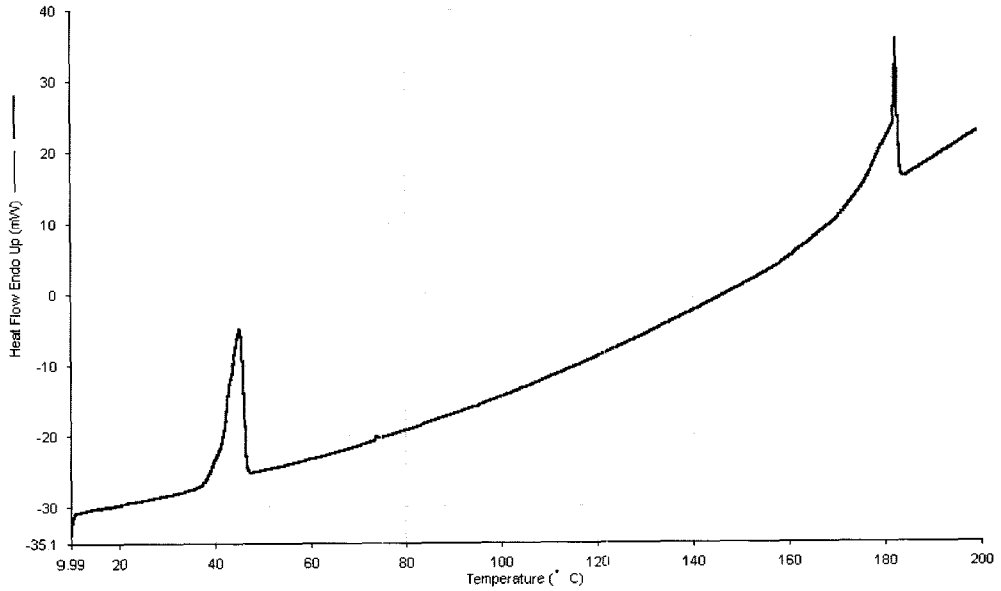

Fig. 4. DSC thermogram of mPEG-PLA 2K-1K copolymer-DDB (1:1) physical mixture.

(Kwon *et al.*, 1993). The vibrational structure of pyrene monomer emission is known to be dependent on the local polarity. The preferential partitioning of pyrene into the hydrophobic domains may be used to determine the micropolarity of molecular assemblies.

The CMC of mPEG-PLA 2K-1K was about three times higher than that of poloxamer 407, indicating that the mPEG-PLA 2K-1K system has a more stable micelle structure than the poloxamer 407 system (Table II). Polymeric micelles prepared from mPEG-PLA 2K-1K are expected to retain the micellar structure even when diluted at body temperature. Therefore, the mPEG-PLA 2K-1K system appears to be a useful drug vehicle.

Both mPEG-PLA and poloxamer 407 formed micelles of very small size. Dynamic light scattering measurements revealed that mPEG-PLA micelles have the hydrodynamic diameter of about 10-20 nm (Fig. 7). These values were similar to those reported for other micelle systems having a core-shell structure (Scholz *et al.*, 1995). Alexandridis and Hatton (1995) reported that the radius of the micelle

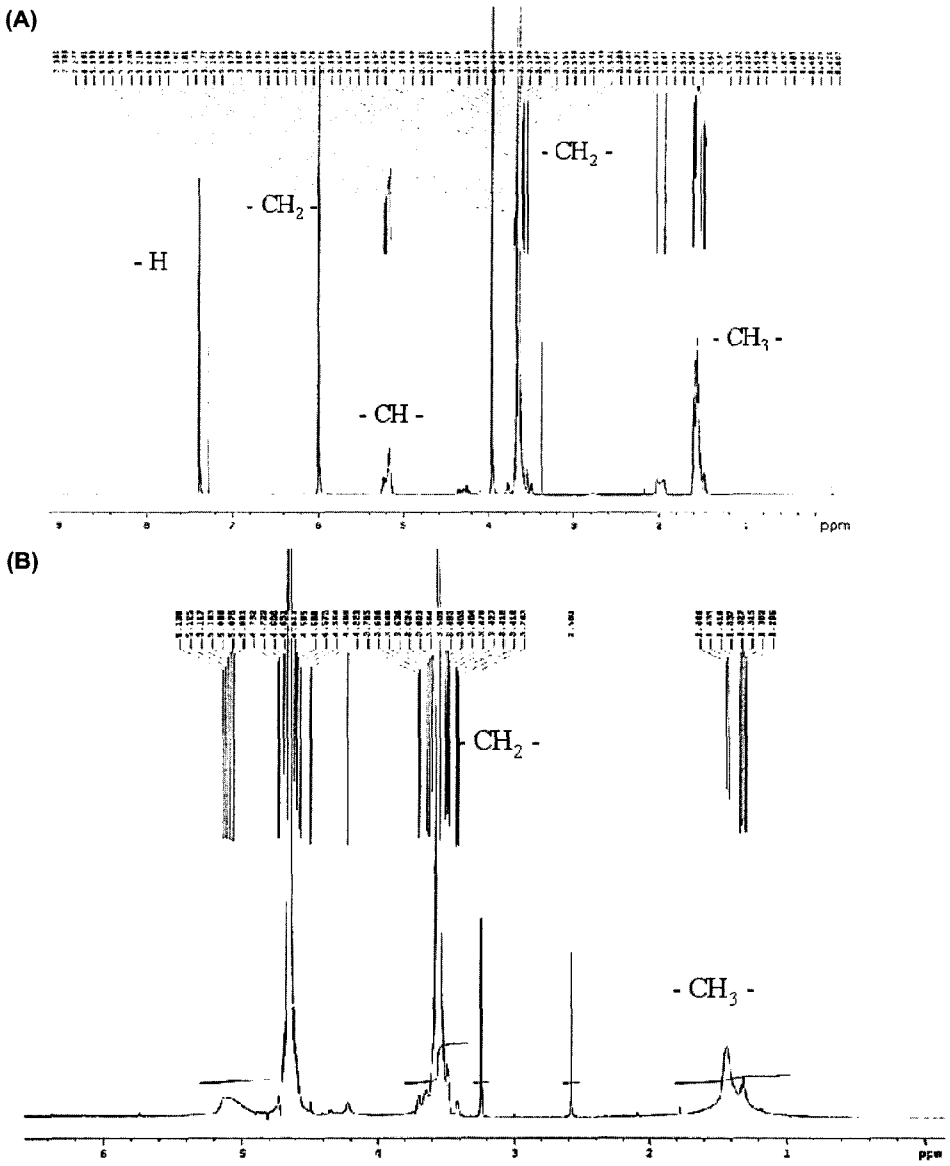

Fig. 5. ^{1}H-NMR spectra of DDB loaded mPEG-PLA 2K-1K in (A) $CDCl_3$ and (B) D_2O.

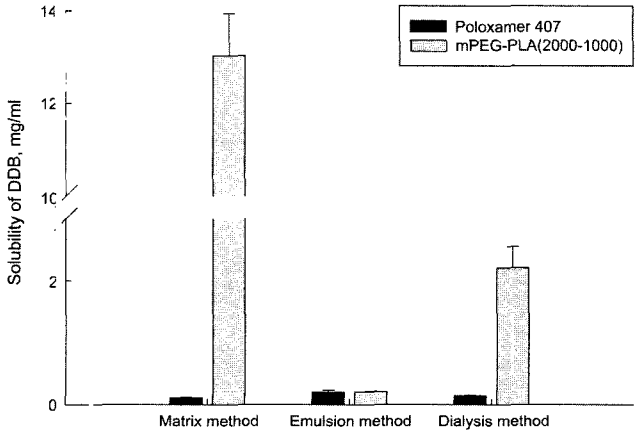

Fig. 6. The maximum solubility of DDB in polymeric micelle solutions prepared from mPEG-PLA 2K-1K and poloxamer 407.

Table II. Comparison of polymeric micelle solutions prepared from mPEG-PLA 2K-1K and poloxamer 407

	mPEG-PLA 2K-1K	Poloxamer 407
Type	Diblock copolymer	Triblock copolymer*
M.W.	3000	12600*
Tm (°C)	40	56
Tg (°C)	-52	-
CMC (mg/L, 20°C)	401.20	1125.81
Radii of micelle (nm)	13	10.2*
HLB	>13	18-23*
Micelle stability	+++	+
Maximum DDB solubility (mg/mL)	13.29	0.20

*Alexandridis and Hatton (1995)

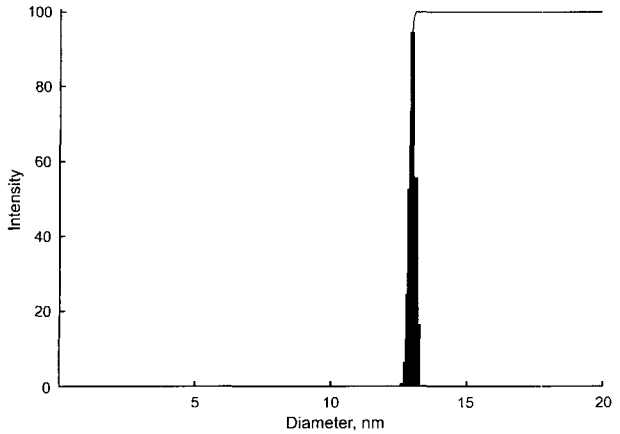

Fig. 7. Size distribution of micelles prepared from mPEG-PLA copolymer.

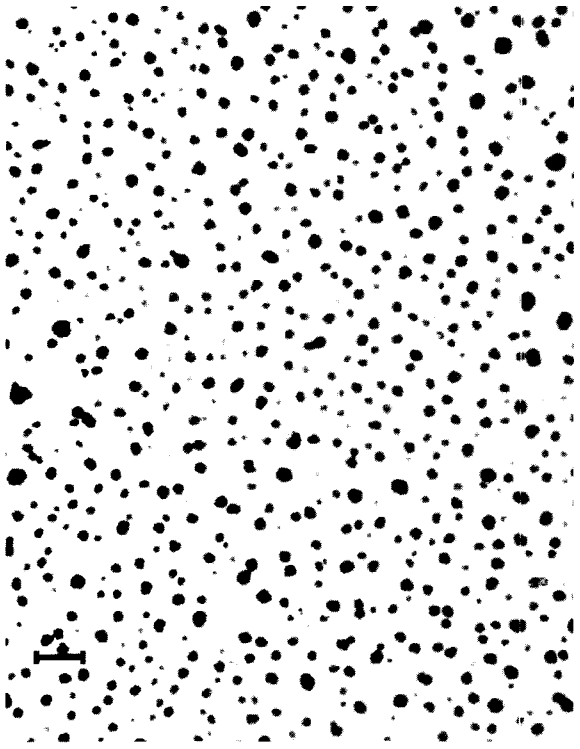

Fig. 8. *Transmission electron micrograph of a 1% (w/v) mPEG-PLA 2K-1K dispersion (magnification 30k) (scale bar = 200 nm).*

solution of poloxamer 407 obtained from light and neutron scattering studies was 10.2 nm.

TEM can resolve nanoscopic colloids such as polymeric micelles. Obtained TEM pictures revealed spherical mPEG-PLA micelles, confirming earlier dynamic light scattering measurements on the polymeric micelles (Fig. 8). The mean diameter of mPEG-PLA 2K-1K micelles has been measured directly from 200 randomly selected polymeric micelles in TEM photographs. The mean diameter of DDB loaded mPEG-PLA 2K-1K micelles was 15 ± 3.5 nm. The size distribution of mPEG-PLA 2K-1K micelles is very narrow,

Table III. The physical stability of micelle solutions prepared from mPEG-PLA 2K-1K and poloxamer 407 (n = 3)

		Concentration of DDB (μg/mL)
Poloxamer 407	Before test	190.94 ± 6.85*
	2 weeks later	30.55 ± 1.78
	% remaining	16.00
mPEG-PLA 2K-1K	Before test	212.08 ± 8.34
	2 weeks later	187.82 ± 1.64
	% remaining	88.56

*Mean ± S.D.

characteristic of polymeric micelles. Both the polymeric micelles prepared from mPEG-PLA 2K-1K and poloxamer 407 have the potential to decrease RES (reticuloendothelial system) uptake because their diameter ranges (ca. less than 100 nm) are considered small enough to evade non-specific capture by RES. If they are administered intravenously, their size may be advantageous for extravasation, which should be an essential process for the carrier to reach the target located outside of the capillaries.

The physical stability of DDB loaded polymeric micelle solutions prepared from mPEG-PLA 2K-1K and poloxamer 407 are shown in Table III. mPEG-PLA 2K-1K formed more stable micelles than poloxamer 407. DDB loaded micellar solutions prepared from both mPEG-PLA and poloxamer 407 precipitated under the storage conditions tested. When the same amount of DDB was loaded, however, the mPEG-PLA 2K-1K micellar solution was physically more stable than the poloxamer 407 micellar solution.

The physical characteristics of mPEG-PLA 2K-1K polymeric micelles and poloxamer 407 micelles were summarized in Table II. Based on our results, the micelle solution prepared from mPEG-PLA 2K-1K was superior to that prepared from poloxamer 407. This observation is consistent with that of Yokoyama (1992) in that among the block copolymers, AB type block copolymers are the most appropriate one in the micelle stability, aggregation number and size due to the simple structure of their molecules. Xu *et al.* (1992) reported a micellization of poly(ethylene oxide)-polystyrene (PEO-PS) diblock copolymers and PEO-PS-PEO triblock copolymers in water. The aggregation number was increased as either the molecular weight or the PS content was increased. Diblock copolymer micelles had a higher aggregation number than those of triblock copolymer micelles. Nagarajan and Ganesh (1996) reported that for the identical molecular weights and block compositions, the diblock (PEO-PPO) copolymer micelles had a much larger core radius, shell thickness, aggregation number, and volume fraction of the hydrocarbon solubilized in the core as compared with the symmetric triblock (PEO-PPO-PEO) copolymer micelles.

Zhang *et al.* (1996) showed the NMR spectra of taxol/

mPEG-PLA matrices dissolved or solubilized in $CDCl_3$ or D_2O. While there were NMR peaks from both taxol and PLA of dissolved taxol/mPEG-PLA matrices in $CDCl_3$, these peaks disappeared in D_2O where the matrices formed micelles. The peak disappearance was due to the restricted mobility of PLA and taxol. Nivaggioli *et al.* (1995) showed the NMR spectra of benzene/poloxamers dissolved or solubilized in D_2O. In NMR spectra, the solubilized benzene peak was not disappeared, suggesting that the hydrophobic core of the polymeric micelles prepared from mPEG-PLA is more tightly packed than that from poloxamer. Therefore, mPEG-PLA can form a more stable micelle structure than poloxamers.

In summary, the mPEG-PLA system used in this study has distinct advantages over the poloxamer system regard to the formation of multi-molecular micelles with a core-shell structure, biodegradability and biocompatibility. On the other hand, the poloxamer system may be used to increase the solubility of DDB, although the formation of multi-molecular micelles with a core-shell structure is unlikely.

REFERENCES

Alexandridis, P. and Hatton, T. A., Poly(ethylene oxide)-poly (propylene oxide)-poly(ethylene oxide) block copolymer surfactants in aqueous solutions and at interfaces: thermodynamics, structure, dynamics, and modeling. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 96, 1-46 (1995).

Gu, S. J., Gao, W. W., Qiao, P. X., Wang, A. G., and Qiang, Z. Y., The dissolution rate and physical dispersion state of preparation of biphenyl dimethyl dicarboxylate. *Yao Hsueh Hsueh Pao,* 24(11), 859-864 (1989).

Hyun, J. and Chun, I. K., Dissolution characteristics of biphenyl dimethyl dicarboxylate from solid dispersions and permeation through rabbit duodenal mucosa. *J. Kor. Pharm. Sci.*, 24(2), 57-65 (1994).

Kataoka, K., Kwon, G. S., Yokoyama, M., Okano, T., and Sakurai, Y., Block copolymer micelles as vehicles for drug delivery. *J. Control Rel.*, 24, 119-132 (1993).

Kwon G. S., Naito, M., Kataoka, K., and Yokoyama, M., Block copolymer micelles as vehicles for hydrophobic drugs. *Colloids and Surfaces B; Biointerfaces*, 2, 429-434 (1994).

Kwon G., Naito, M., Yokoyama, M., Okano, T., Sakurai, Y., and Kataoka, K., Micelles based on AB block copolymers of poly (ethylene oxide)-poly(-benzyl-L-aspartate). *Langmuir*, 9(4), 945-949 (1993).

Kwon G. S., Suwa, S., Yokoyama, M., Okano, T., Sakurai, Y., and Kataoka, K., Enhanced tumor accumulation and prolonged circulation times of micelle-forming poly(ethylene oxide-aspartate) block copolymer-adriamycin conjugate. *J. Control Rel.*, 29, 17-23 (1994).

La, S. B., Okano, T., and Kataoka, K., Precipitation and characterization of the micelle-forming polymeric drug indomethacin-incorporated poly(ethylene oxide)-poly(-benzyl-L-aspatate) block copolymer micelles. *J. Pharm. Sci.*, 85(1), 85-90 (1996).

Moon, J. O., Jung, K. W., Kim, S. H., Kim, N. D., Lee, S. K., Yang, H. S., and Lee, C. H., Effect of water soluble DDB derivative on the carbon tetrachloride induced hepatotoxicity in rats. *J. Kor. Pharm. Sci.*, 27(3), 173-205 (1997).

Nagarajan, R. and Ganesh, K., Comparison of solubilization of hydrocarbons in (PEO-PPO) diblock versus (PEO-PPO-PEO) triblock copolymer micelles. *J. Colloid Interf. Sci.*, 184, 489-499 (1996).

Nivaggioli, T., Tsao, B., Alexandridis, P., and Hatton, T. A., Microviscosity in Pluronic and tetronic poly(ethylene oxide)-poly(propylene oxide) block copolymer micelles. *Langmuir*, 11(1), 119-126 (1995).

Pispas, S. and Hadjichristidis, N., Micellization of model graft copolymers of the H and type in dilute solution. *Macromolecules*, 29, 7378-7385 (1996).

Quintana, J. R., Salazar, R. A., and Katime, I., Micelle formation and polyisobutylene solubilization by polystyrene-block-poly (ethylene-co-butylene)-block-polystyrene block copolymers. *Macromol. Chem. Phys.*, 196, 1625-1634 (1995).

Reed, A. M. and Gilding, D. K., Biodegradable polymers for use in surgery-poly(glycolic acid)/poly(lactic acid) homo and copolymers: 2. *In vitro* degradation. *Polymer*, 22, 494-498 (1981).

Scholz, C., Iijima, M., Nagasaki, Y., and Kataoka, K., A novel reactive polymeric micelle with aldehyde group on its surface. *Macromolecules*, 28, 7295-7297 (1995).

Xu, R. and Winnik, M. A., Micellization of polystyrene-poly (ethylene oxide) block copolymers in water. 5. A test of the star and mean-field models. *Macromolecules*, 25, 644-652 (1992).

Yokoyama, M., Block copolymers as drug carriers. *Critical Reviews in Therapeutic Drug Carrier System*, 9(3,4), 213-248 (1992).

Yokoyama, M., Okano, T., Sakurai, Y., Suwa, S., and Kataoka, K., Introduction of cisplatin into polymeric micelle. *J. Control. Rel.*, 39, 351-356 (1996).

Zhang, X., Jackson, J. K., and Burt. H. M., Development of amphiphilic diblock copolymers as micellar carrier of taxol. *Int. J. Pharm.*, 132, 195-206 (1996).

Zhu, K. J., Xiangzhou, L., and Shilin, Y., Preparation, characterization, and properties of polylactide(PLA)-poly(ethylene glycol)(PEG) copolymers : A potential drug carrier. *J. App. Polymer Sci.*, 39, 1-9 (1990).