Methoxy Poly(ethylene glycol)-*b*-Poly(L-lactic acid) Copolymer Nanoparticles as Delivery Vehicles for Paclitaxel

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ABSTRACT: Methoxy poly(ethylene glycol)-*b*-poly(L-lactic acid) (MPELLA) was prepared by the melt polycondensation of methoxy poly(ethylene glycol) and L-lactic acid. The structure and properties of MPELLA were characterized by IR, ^{1}H-NMR, differential scanning calorimetry, and wide-angle X-ray diffraction. To estimate its feasibility as a vehicle for paclitaxel, MPELLA nanoparticles were prepared by a self-emulsification/solvent evaporation method. The paclitaxel-loaded nanoparticles (PMTs) showed a spherical morphology with an inner core and an outer shell. The size, size distribution, and loading capacity of PMTs were also measured. The release kinetics of paclitaxel from PMTs *in vitro* was studied. The results show that paclitaxel can be effectively incorporated into MPELLA nanoparticles, which provide a delivery system for paclitaxel and other hydrophobic or toxic compounds. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 98: 2116–2122, 2005

Key words: biodegradable; block copolymers; nanoparticles

INTRODUCTION

There has been considerable interest in developing biodegradable nanoparticles as effective drug delivery vehicles over the past 2 decades.[1] Various polymers have been used in drug delivery research because they can effectively deliver drugs to a targeted site; as a result, the therapeutic benefit increases, and some side effects are minimized.[2] So far, polylactide (PLA) and its derivatives have been extensively studied in this regard because of their excellent biocompatibility and biodegradability, and some have already been commercialized as microspheres for controlled drug delivery.

Plasma protein adsorption and phagocytosis of nanoparticles are subjects that have been widely studied at present. When nanoparticles are administered intravenously, they are easily recognized by the body's immune systems and then cleared from the circulation systems. Hence, for avoiding the interaction of blood proteins with nanoparticles, it is necessary to design long-circulating nanoparticles through surface modification. The surface modification of biodegradable and long-circulating polymeric nanoparticles has been achieved mainly by two methods: (1) surface coating with hydrophilic polymers and surfactants and (2) the development of biodegradable copolymers with hydrophilic segments such as poly(ethylene glycol) (PEG).

Nanoparticles formed from amphiphilic block copolymers have been exploited as carriers for hydrophobic drugs.[3,4] In an aqueous environment, the hydrophobic blocks of the copolymer form the core of the nanoparticles, whereas the hydrophilic blocks form the corona or outer shell. The hydrophobic nanoparticle core serves as a microenvironment for the incorporation of lipophilic drugs, whereas the corona shell serves as a stabilizing interface between the hydrophobic core and the external medium.

The unique application of PEG–PLA block copolymers (PELAs) in the field of medicine involves their roles as carriers for drug delivery.[5–10] This is based on the nanoparticle-forming propensity of PELAs in an aqueous medium through multimolecular association. The polymer nanoparticles are characterized by a core–shell architecture, in which a segregated core of PLA blocks is surrounded by a dense palisade of PEG blocks. Diverse hydrophobic drugs can be loaded with high efficacy into the core of nanoparticles. Because of the reduced interaction with biological components, the nanoparticles loading the hydrophobic drug have a long half-life in the blood compartment. PELAs are generally synthesized by two methods. First, PELAs are prepared through the ring-opening polymerization of PEG and lactide with stannous compounds as a catalyst.[11] Second, PELAs are prepared through a

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TABLE I
Compositions and Molecular Weights of MPELLAs

Polymer	MPELLA1	MPELLA2	MPELLA3	MPELLA4
LLA/mPEG (w/w) ratio used in the synthesis	1 : 1	2 : 1	3 : 1	4 : 1
$M_{n(\text{MPELLA})}$[a]	3515	4247	7127	10026
$M_{n(\text{MPELLA})}$[b]	3792	4421	7481	10477
Polydispersity[b]	1.13	1.04	1.08	1.40
$M_{n(\text{PLLA segments in MPELLA})}$	1515	2247	5127	8026
$M_{n(\text{mPEG segments in MPELLA})}$	2000	2000	2000	2000
$\text{Content}_{(\text{mPEG segments in MPELLA})\ (\%)}$	56.9	47.1	28.1	19.9

[a] Measured by NMR.
[b] Measured by GPC.

successive ring-opening polymerization of ethylene oxide and lactide with an anionic initiator.[12,13]

There have been some reports on paclitaxel-loaded PELA nanoparticles.[14–17] Paclitaxel is a promising antitumor agent with poor water solubility. It is effective for various cancers, especially ovarian and breast cancer. The intravenous administration of a current formulation in a nonaqueous vehicle containing Cremophor EL may cause allergic reactions and precipitation upon aqueous dilution. Moreover, the extensive clinical use of this drug is somewhat delayed by the lack of appropriate delivery vehicles. Because of this, there is a need for the development of an alternative formulation of paclitaxel that has good aqueous solubility and is, at the same time, free of any side effects. Various approaches employed so far include cosolvents, emulsions, micelles, liposomes, microspheres, nanoparticles, cyclodextrins, pastes, and implants.[18] This article mainly reports the characterization of methoxy poly(ethylene glycol)-*b*-poly(L-lactic acid) (MPELLA) self-prepared by melt polycondensation in our laboratory and the properties of the paclitaxel-loaded MPELLA nanoparticles (PMTs) prepared by a self-emulsification/solvent evaporation method.

EXPERIMENTAL

Materials

A commercial L-lactic acid (LLA) aqueous solution (88%) was obtained from Purac Co. (Arkelsedijk, the Netherlands). Commercial methoxy poly(ethylene glycol) [mPEG; number-average molecular weight (M_n) = 2000] and pyrene were purchased from Aldrich (St. Louis, MO). Stannous chloride ($SnCl_2 \cdot 2H_2O$) was supplied by the Tianjin First Chemical Reagent Factory (Tianjin, China). Paclitaxel was provided by Hengrui Co. (Shanghai, China). All other reagents were analytical-grade and were used without further purification. Hyperpure water was prepared with a Milli-Q Plus apparatus (Waters, Millipore, Milford).

Synthesis of MPELLA

MPELLA was synthesized by the melt polycondensation of mPEG and LLA with a mixture of stannous chloride and *p*-toluene sulfonic acid (1 : 1 molar ratio) as a catalyst. The weighed amounts of LLA and mPEG were mixed in a glass round-bottom flask connected with a vacuum joint. The flask was placed in an oil bath, and the water in the system was evaporated at 120°C *in vacuo* for 2 h. After it was cooled, the multiplex catalyst was added under nitrogen protection, and then polymerization was carried out in a high vacuum and at 160–200°C. When the copolymerization was completed, the product was cooled at the ambient temperature and then dissolved in $CHCl_3$. The solution was precipitated in an excess amount of ice-cold diethyl ether to eliminate the low-molecular-weight impurities. The purified copolymers were dried in a vacuum oven at 40°C for approximately 24 h. In this article, four MPELLA samples with different lengths of poly(L-lactic acid) (PLLA) segments were prepared by changes in the ratios of LLA to mPEG, as shown in Table I. The molecular weights measured by NMR and gel permeation chromatography (GPC) are shown in Table I.

Fluorescence measurements

To determine the critical association concentration (CAC) of MPELLA, fluorescence measurements were carried out with pyrene as reported in refs. 10 and 11. The fluorescence emission spectra of pyrene were measured on an F-3010 spectrofluorometer (Hitachi, Tokyo, Japan).

Preparation of PMTs

PMTs were prepared by a self-emulsification/solvent evaporation method.[19] Briefly, MPELLA (100 mg) was dissolved in 3 mL of dichloromethane (DCM); this was followed by the addition of paclitaxel with various weight ratios to the polymer. Hyperpure water (10 mL) was dropped slowly with stirring with a magnetic stirring bar to the copolymer–drug solution, and this solution formed discrete droplets in the aqueous continuous phase. To induce micelle formation, DCM had to first diffuse into the aqueous phase and then evaporate at the water/air interface. Agitation of the

system was continued until DCM partitioned into the aqueous phase and was removed by evaporation. This process resulted in a hardened inner core of nanoparticles that contained paclitaxel. Then, the dispersion was centrifuged with an LD5-2A centrifuge (Beijing Medical Centrifuge Factory, Beijing, China) to eliminate the unloaded paclitaxel and the aggregated particles. The supernatant was frozen and lyophilized by a freeze dryer system (LGJ-10, Four-Ring Science Instrument Plant, Beijing, China) to obtain PMT powder.

Characterization

Fourier transform infrared (FTIR) spectroscopy (FT3000, Bio-Rad, Hercules, CA) was used to confirm the structure of MPELLA. Polymer samples were pressed into KBr pellets (1 : 100 copolymer/KBr ratio) and analyzed with IR data manager software.

GPC measurements of the molecular weights and molecular weight distributions of MPELLA were conducted with an Alliance high-pressure liquid chromatography (HPLC) system (Waters, United States). HPLC-grade tetrahydrofuran (THF) was used as both the solvent and eluent. Samples dissolved in THF were injected into the GPC circuit, and elution was performed at a temperature of 30°C and at a flow rate of 1.0 mL/min. Molecular weight calibration was done with a series of standard polystyrene with various molecular weights. The composition and M_n of each copolymer in a $CDCl_3$ solution were determined by 400-MHz ^{1}H-NMR (Unity-Plus 400, Varian, Dallas, TX) with tetramethylsilane as the internal standard.

Wide-angle X-ray diffraction (WAXD) patterns were recorded with graphite-filtered Cu Kα radiation produced with a Rigaku Denki model D/MAX-RA diffractometer (Rigaku, Tokyo, Japan).

Differential scanning calorimetry (DSC) measurements were carried out with a PerkinElmer DSC 7 (Shimadzu, Kyoto, Japan). All measurements were carried out at a heating rate of 10°C/min from −50 to 200°C under a liquid nitrogen purge.

The sizes of the PMTs were determined with a BI 9000 AL photon correlation spectroscope (Brookhaven, Upton, NY) with an Ar-ion laser (λ = 514.5 nm, measurement angle = 90°, temperature = 25°C).

After MPELLA nanoparticles or PMTs were dispersed in diethyl ether, morphological evaluations of the MPELLA nanoparticles and PMTs were performed with transmission electron microscopy (TEM; EM400ST, Philips, Eindhoven, the Netherlands).

Measurement of the drug-loaded content

PMTs were dissolved in acetonitrile (5 mL; a common solvent for both MPELLA and paclitaxel) and injected into a high-pressure liquid chromatograph (Waters) with a Hypersil ODS-2 (250 mm × 4.6 mm × 5 μ) C18 column. The mobile phase, composed of acetonitrile and water (75 : 25 v/v), was performed at a temperature of 30°C and at a flow rate of 1.0 mL/min. The paclitaxel peak was detected at 273 nm. The drug-loaded content (% w/w) was defined as the weight ratio of paclitaxel in PMTs to preweighted PMTs. Before this analysis, the standard curve of paclitaxel was calibrated by HPLC.

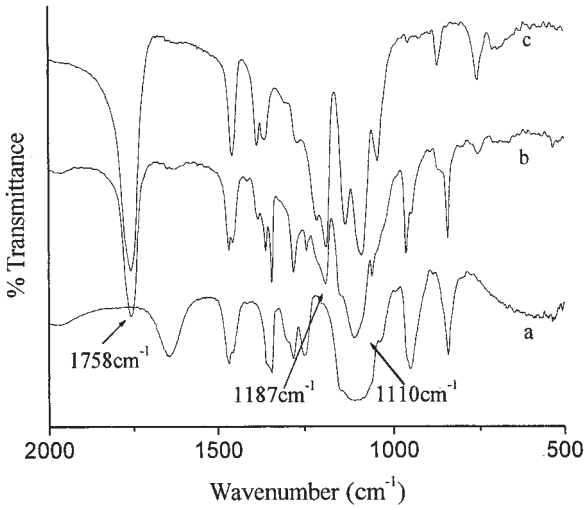

Figure 1 FTIR spectra of the polymers: (a) mPEG, (b) MPELLA1, and (c) PLLA.

Releasing kinetics of paclitaxel from PMTs

PMTs were well dispersed in a phosphate buffer solution (PBS, pH 7.4, 2 mL) and then placed in a dialysis bag, which was immersed in a large vial containing warmed PBS (28 mL, pH 7.4, 37°C) with stirring with a magnetic stirring bar. At the appropriate time intervals, a 20-mL aliquot of PBS outside the dialysis bag in the large vial was removed to measure the amount of paclitaxel released from PMTs. After the removal of each 20-mL aliquot, a 20-mL aliquot of fresh PBS was supplemented. The amount of paclitaxel in the release medium was determined by HPLC. The accumulated release was calculated as follows:

$$E_r = \frac{V_e \sum_{1}^{n-1} C_i + V_0 C_n}{m_{\text{drug}}}$$

where E_r is the accumulated release (%), V_e is the sampling volume (20 mL), V_0 is the initial volume (30 mL), C_i and C_n are the paclitaxel concentrations (μg/mL), i and n are the sampling times, and m_{drug} is the mass of paclitaxel in PMTs (μg).

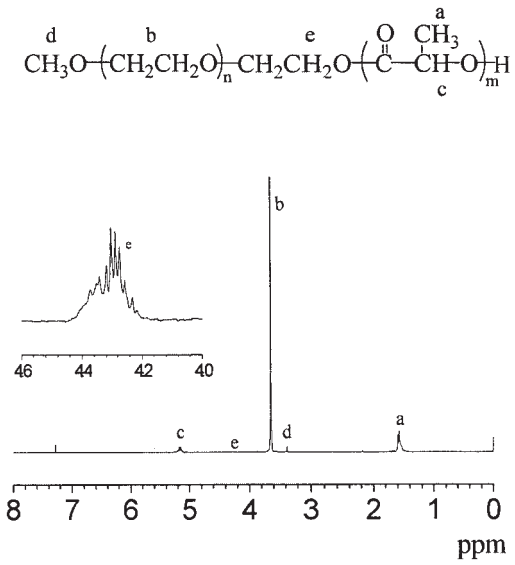

Figure 2 ^{1}H-NMR spectrum of MPELLA1.

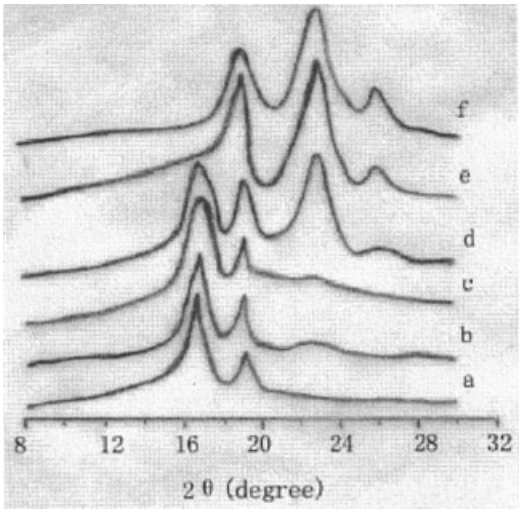

Figure 3 WAXD patterns of the polymers: (a) PLLA, (b) MPELLA4, (c) MPELLA3, (d) MPELLA2, (e) MPELLA1, and (f) mPEG.

RESULTS AND DISCUSSION

Physicochemical characterization of MPELLA

In this study, MPELLA was synthesized by the melt polycondensation of mPEG and LLA with a mixture of stannous chloride and *p*-toluene sulfonic acid (1 : 1 molar ratio) as the catalyst. Figure 1 shows FTIR spectra of mPEG, MPELLA1, and PLLA. A strong carbonyl band appears at 1758 cm^{-1} and a C—O stretching band appears at 1187 cm^{-1} in Figure 1(b); they are similar to those of PLLA. Absorption at 1110 cm^{-1} in Figure 1(a,b) is due to C—O—C stretching of mPEG. These IR spectroscopy results suggest that there are mPEG and PLLA segments in MPELLA.

The structure, compositions, and molecular weights of MPELLA were determined by ^{1}H-NMR spectroscopy. Figure 2 shows a typical ^{1}H-NMR spectrum of MPELLA1. The insert in Figure 2 is a magnified image of peak e, which is the signal of the methylene proton of the mPEG chain bonding with PLLA. The appearance of peak e proves the covalent bond between mPEG and PLLA. Because of only one hydroxyl group in the mPEG chain, it can be concluded that MPELLA is an amphiphilic diblock copolymer, as shown in the top of Figure 2.

The compositions and M_n values of MPELLA were estimated from the ^{1}H-NMR spectrum (Fig. 2) on the basis of the peak intensity ratio of the methylene protons of mPEG (OCH_2CH_2, δ = 3.65 ppm) and the methine proton of PLLA [$COCH(CH_3)$, δ = 5.18 ppm]. The measured molecular weights of MPELLA samples are summarized in Table I. The GPC results show that low-molecular-weight homopolymers are absence in MPELLA.

The thermal properties of MPELLA, that is, the fusion temperatures (T_m's), were determined by DSC, and the results are shown in Table II. There are two fusion temperatures when the concentration of PLLA in the copolymer is about 50–80%, but only one T_m was found for MPELLA samples with lower or higher PLLA contents. T_m of the mPEG segments obviously decreases and that of PLLA segments increases with an increase in the weight fraction of PLLA segments. That is because the increase in the PLLA content in the copolymer is beneficial to the regularity and crystallinity of PLLA segments but restricts those of mPEG segments.

The results of DSC are further validated by WAXD. Figure 3 shows typical WAXD patterns of MPELLA block copolymers. mPEG has three characteristic crystalline peaks at 2θ values of 19, 23, and 27°, and PLLA has two characteristic crystalline peaks at 2θ values of 17 and 19°. MPELLA2 and MPELLA3 show the characteristic crystalline peaks of PLLA at 2θ = 17° and the characteristic crystalline peaks of mPEG at 2θ = 19° and 2θ = 23°; this indicates that they are semicrystal-

TABLE II
T_m Values of the Polymer Samples

Polymer	mPEG	MPELLA1	MPELLA2	MPELLA3	MPELLA4	PLLA
$T_{m(PEG)}$ (°C)	59	57.2	55	50	—	—
$T_{m(PLLA)}$ (°C)	—	—	135	140	146	148

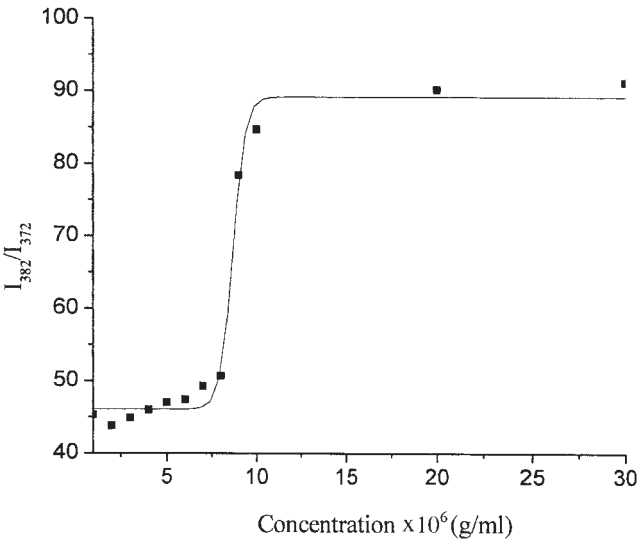

Figure 4 I_{382}/I_{372} versus the concentration of MPELLA1 (pyrene concentration = 2.0×10^{-6} mol/L).

line polymers and there is phase separation of mPEG crystallized domains and PLLA crystallized domains. For MPELLA, the crystalline peak of mPEG at $2\theta = 19°$ and $2\theta = 23°$ decreases, whereas the characteristic peak of PLLA at $2\theta = 17°$ increases, with increasing PLLA moiety.

Fluorescence spectroscopy measurements

Pyrene was chosen as a fluorescent probe because of its photochemical properties suitable for an effective probe, such as the remarkably long life span of pyrene monomers, the efficient formation of excimers, and the emission intensity increasing along with the environment polarity decreasing, especially with an increasing ratio of the emission intensity at 382 nm to that at 372 nm (I_{382}/I_{372}).[20,21] The fluorescence emission spectra of pyrene at various concentrations of MPELLA were obtained at an excitation wavelength of 339 nm and at a constant pyrene concentration of 2.0×10^{-6} mol/L. The fluorescence intensity increases with increasing MPELLA concentration. This is because pyrene molecules have a strongly hydrophobic character and very low solubility in water. Pyrene preferentially is solubilized into the hydrophobic cores of MPELLA nanoparticles, and this leads to the environmental polarity of pyrene decreasing and the emission intensity increasing. Therefore, the fluorescence intensity is affected by the change in the copolymer concentration.

Figure 4 plots the relationship between I_{382}/I_{372} and the concentration of MPELLA1. There is a critical concentration, below which I_{382}/I_{372} is low and mildly increases and above which I_{382}/I_{372} increases sharply and then tends to be constant. This critical concentration can be seen as the CAC of MPELLA. According to Figure 4, the CAC value of MPELLA1 was estimated to be 2.57×10^{-6} mol/L. In accordance with this method, the CAC values were estimated to be 2.14×10^{-6}, 0.9×10^{-6}, and 0.69×10^{-6} mol/L for MPELLA2, MPELLA3, and MPELLA4, respectively. The CAC values decrease with increasing hydrophobic chain length.

Physicochemical characterization of PMTs

Figure 5 presents TEM images of the MPELLA1 nanoparticles before and after the loading of paclitaxel. These images confirm that the unloaded nanoparticles and PMTs have a spherical morphology with a core/shell structure and discrete particles in aqueous media.

The MPELLA composition exerts a strong influence on PMTs.[22] When the concentration of mPEG in MPELLA is higher than 30%, the drug-loaded nanoparticles are stably dispersed in aqueous media. Otherwise, the dispersions are unstable. The highest drug-loaded concentration is higher than 15%. As shown in Table III, the particle size of PMTs increases with an increasing drug-loaded concentration. These results indicate that a highly lipophilic model molecule such as paclitaxel can be effectively incorporated into MPELLA nanoparticles, and a delivery system for paclitaxel is provided. This delivery system may be used for highly hydrophobic or toxic compounds.

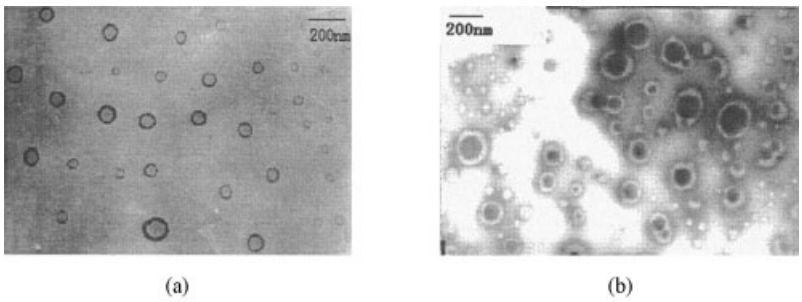

Figure 5 TEM images of (a) MPELLA1 nanoparticles and (b) PMT_2 (5% paclitaxel loading).

TABLE III
Physicochemical Characterization of PMT

PMT	Copolymer	Drug-loaded content (%)	Mean particle size (nm)
PMT_1	MPELLA1	1.97	66
PMT_2	MPELLA1	4.85	79.4
PMT_3	MPELLA1	9.43	100
PMT_4	MPELLA1	12.8	783
PMT_5	MPELLA2	4.91	85.4

Paclitaxel release of PMTs *in vitro*

Paclitaxel is a lipophilic drug. Therefore, paclitaxel release from PMTs *in vitro* is influenced by the character of the inner core of the nanoparticles. The release kinetic profiles of two kinds of PMTs over a 4-day period are plotted in Figure 6. As shown in Figure 6, the initial burst release is not observed, and the release of paclitaxel is slow. The release rate of paclitaxel from PMT_2 is much faster than that from PMT_5. This is due to the differences in both the affinity of the hydrophobic core of the copolymer to the drug and the degradation behavior of the copolymer resulting from the molecular weight of the PLLA moiety of MPELLA. Because MPELLA2 has a longer hydrophobic PLLA segment, the affinity of MPELLA2 to hydrophobic paclitaxel must be greater than that of MPELLA1, and the degradation of MPELLA2 should be faster than that of MPELLA1.[19] However, it seems that the effect of the molecular weight is not a large, dominating factor because PLLA degrades quite slowly and more slowly within an *in vitro* medium without any enzymes. Therefore, the effect of affinity is much greater than that of degradation on a release profile in these PMTs.

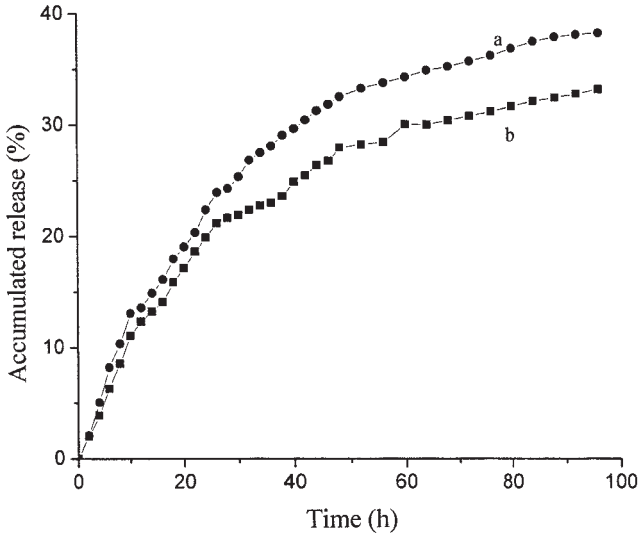

Figure 6 Release profiles of paclitaxel from PMTs with various PLLA chain lengths in MPELLA: (a) PMT_2 and (b) PMT_5. The release medium was PBS (pH 7.4) at 37°C.

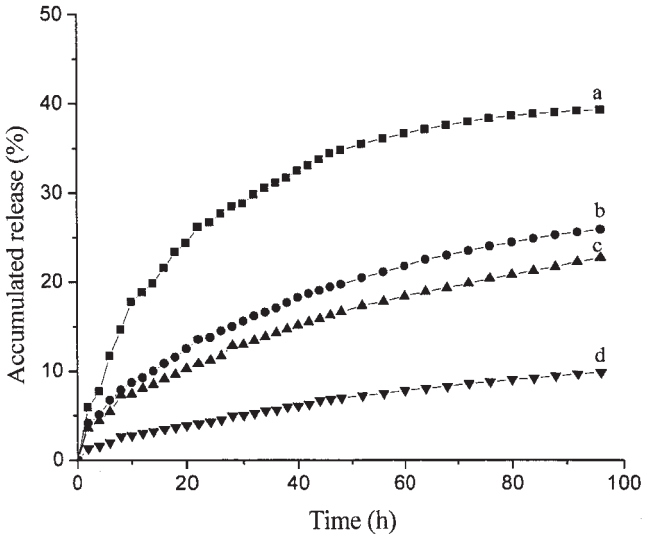

Figure 7 *In vitro* release profiles of paclitaxel from PMTs with various drug-loaded contents: (a) PMT_1, (b) PMT_2, (c) PMT_3, and (d) PMT_4.

Figure 7 presents the release profiles of paclitaxel from PMTs with different drug-loaded contents. As shown in Figure 7, the relative rate of drug release is fastest with the lowest drug-loaded contents, and it is slowest with the highest drug-loaded contents. For example, PMT_1 reached an accumulated release of 5% in 3–4 h, whereas PMT_4 reached that after 20 h. A similar phenomenon was discovered by Allen et al.[23] when they studied dihydrotesterone-loaded poly-(ε-caprolactone)-*b*-poly(ethylene glycol) copolymer nanoparticles. This phenomenon is explained by the formation of a homogeneous matrix with the drug randomly distributed throughout the polymer particles at low loadings, whereas the formation of a heterogeneous matrix is assumed at high loadings. The drug in PMT_1 directly diffuses into the receiving liquid. However, the drug crystal in PMT_4 is first dissolved in water and then diffuses into the receiving liquid. Therefore, the release rate of paclitaxel from PMTs increases with decreasing drug-loaded content.

CONCLUSIONS

MPELLA was prepared by the melt polycondensation of mPEG and LLA. MPELLA nanoparticles were prepared by the self-emulsification/solvent evaporation method and shown to be efficient carriers of paclitaxel. As the hydrophobic components of the copolymers increased, the critical micelle concentration decreased. PMTs showed a spherical morphology with an inner core and an outer shell. No initial burst release was observed in the release patterns of all the samples. The

drug-release rate and the accumulation release decreased along with increases in the molecular weight of the hydrophobic chain in the copolymer and the drug-loaded concentration. From these results, we can suggest that the MPELLA diblock copolymer nanosphere system is a biocompatible drug vehicle candidate for hydrophobic drugs in injectable delivery systems.

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