Drug-Releasing Behavior of MPEG/PLA Block Copolymer Micelles and Solid Particles Controlled by Component Block Length

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ABSTRACT: Three series of poly(ethylene glycol)methyl ether (MPEG)/poly(lactic acid) (PLA) block copolymer systems, composed of MPEG in the molecular weights of 2000, 5000, and 750 g/gmol, respectively, and PLA in different molecular weights were synthesized. The chemical structure and physical properties were investigated using FTIR, NMR, DSC, TGA, and GPC. Different crystallization and melting transition behavior were observed according to the variation of the component block length. Either nano-sized micelles or solid particles were formed in water, depending on the component block length and its ratio. The mean diameter of spherical drug carriers was controlled by the preparation conditions and was characterized using DLS and SEM. Indomethacin was loaded into nano-sized spherical polymer carriers. The releasing rates were significantly affected by not only the sizes but also by the physical state of the drug carriers in the media, resulting from the difference in the component block length and the molecular entanglement of the PLA cores. © 2002 John Wiley & Sons, Inc. J Appl Polym Sci 83: 435–445, 2002

Key words: micelles; biodegradable; diffusion; drug delivery system

INTRODUCTION

Poly(glycolic acid) (Dexon®), poly(glicolide-*co*-lactide) (Vicryl®), polydioxanone, and polyglyconate (Maxon®) are in a major class of biodegradable polymers with a variety of medical applications such as for surgical suture, drug-delivery carriers, fractured bone adhesives, and artificial skin.[1–3] In application for drug-delivery systems, these polymeric materials act as drug carriers by controlling the release rate of the drug initially loaded. Poly(lactic acid) (PLA) has been of importance for this type of drug-delivery system because of its excellent biocompatability and biodegradability. As the hydrophobic and brittle nature of PLA, however, limits its application for intravenous administration, it has been usually copolymerized with poly(ethylene glycol) (PEG), a typical hydrophilic polymer, which has been approved by the Food and Drug Administration (FDA) for the use of inner-body application.[4] Low molecular weight PEG is readily excreted through the kidneys. Because of the applicable strength of PEG/PLA copolymers, there have been a number of fundamental studies on their synthesis and properties.[4–12]

The amphiphilic nature of PEG/PLA block copolymer systems has led to different phase equilibria in water. It was reported that PEG/PLA

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block copolymers existed in a soluble, micellar, gel, or insoluble state, depending on the component block length and their ratio.[13,14] According to the literature about the molecular weight and block length ratio dependence of the sol–gel transition behavior of PEG/PLA copolymers, PEG/PLA copolymers are generally soluble in water when the molecular weight of the PLA block is less than about 2000 g/gmol. Also, these were reported insoluble when the molecular weight is higher than about 2000 g/gmol and the block length ratio of PLA to PEG is higher than a certain value. Micellar structures can be formed in the soluble state, when the copolymer concentration is above the so-called critical micelle concentration (CMC). Even the physical states of copolymers in water are different, either being micelles or solid particles; they can be called nano-sized spheres. Nano-sized micelles can be stabilized by a general hydrophobic–hydrophilic balance, and nano-sized solid particles, by buoyant suspension in water. The aqueous solution including micelles is transparent, but nanoparticles are translucent.

In this contribution, three series of block copolymers composed of different block lengths of PLA and poly(ethylene glycol)methyl ether (MPEG) were synthesized, leading to either micellar or water-insoluble characteristics. The micelle-forming series, MPEG2000, was composed of MPEG with a molecular weight of 2000 g/gmol and PLA with a relatively low molecular weight. Two water-insoluble series, MPEG5000 and MPEG750, were composed of MPEG with molecular weights of 5000 and 750 g/gmol, respectively, with PLA having relatively high molecular weights. The copolymer systems synthesized were prepared into micelles or (water-insoluble) nanoparticles according to their amphiphilic strength in order to examine and compare their drug-loading and -releasing characteristics. These polymeric nanospheres have injectable drug carrier applications.[15]

EXPERIMENTAL

Materials

All chemicals used in the synthesis of the block copolymers were purified before use. The solvents used in the present experiments, acetone, hexane, ethyl acetate, and toluene, were vacuum-distilled with calcium hydride. MPEG with molecular weights of 750, 2000, and 5000 g/gmol (Aldrich Chemical Co., Milwaukee, WI) was recrystallized in hexane after being dissolved in acetone at 70°C. (3*S*)-*cis*-3,6-Dimethyl-1,4-dioxane-2,5-dione-(L-lactide) (Aldrich Chemical Co.) was first dissolved in ethyl acetate at 80°C. After filtration of other impurities, pure L-lactide was precipitated below room temperature. The solvents incorporated in the precipitated L-lactide were removed in a vacuum oven at 50°C for 3 days. Stannous 2-ethyl hexanoate (Aldrich Chemical Co.) was used as the catalyst, and indomethacin, as the drug to be loaded into the polymeric nanospheres. Methylene chloride was used as the cosolvent for dissolving both the drug and block copolymers. Sodium phosphate dibasic $12H_2O$ and sodium phosphate monobasic $2H_2O$ were used for the preparation of the drug-releasing medium.

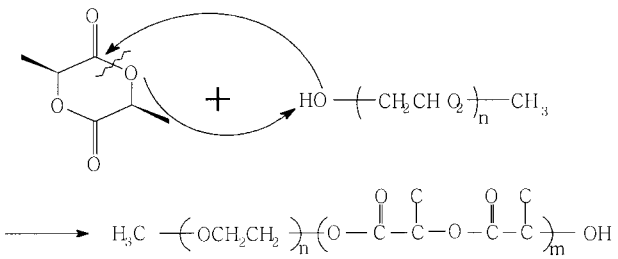


Figure 1 Ring-opening mechanism for the synthesis of MPEG/PLA block copolymers.

Synthesis of Block Copolymers

MPEG/PLA block copolymers were synthesized using the solution polymerization technique with the ring-opening mechanism as shown in Figure 1. A predetermined amount of L-lactide and MPEG was dissolved in toluene with 0.5 wt % of the catalyst. An acyl–oxygen bond in the L-lactide ring was opened to react with the hydroxyl end group in the growing chains. This reaction was conducted at 130°C for about 50 h. Solid products of the diblock copolymers were obtained by addition of diethyl ether to the polymer solution. The lactide monomer and homo-PLA in the products were separated by dissolution in diethyl ether. Unreacted homo-MPEG in the products was separated by dissolution in distilled water. Final copolymer products were obtained after placement in a vacuum oven for 50 h to remove other residual solvents.

Characterization

Fourier transform infrared spectroscopy (FTIR, Unicam, Mattson 1000) was used to investigate

the presence of the ester carbonyl group in the synthesized copolymer products. ^{1}H- and ^{13}C-Fourier transform nuclear magnetic resonance spectroscopy (FT-NMR, Varian, Unity Inova 500) was used to characterize the chemical structure of the synthesized polymer molecules.

The molecular weight and polydispersity indices of the copolymer products were measured using ^{1}H-NMR spectroscopy and gel permeation chromatography (GPC, Waters, Millennium 2.01). A polymer solution in the concentration of 0.8 g/mL was prepared in HPLC-grade tetrahydrofuran (THF) for the GPC measurement. The elution rate of the mobile THF phase was 1 mL/min.

The thermal properties were characterized using differential scanning calorimetry (DSC, Perkin–Elmer, DSC7) and a thermogravimetric analyzer (TGA, Perkin–Elmer, TGA7). In the DSC measurement, 6–9 mg of polymer samples were heated from −70 to 250°C with a scanning rate of 10°C/min in the presence of nitrogen gas. The second heating scan was conducted after quenching the first heated sample with a cooling rate of 200°C/min. The glass and melting transition and crystallization temperatures were determined. The decomposition temperatures of the synthesized copolymers were determined from the TGA measurements. Ten milligrams of the polymer sample was heated from 25 to 400°C with a scanning rate of 10°C/min in the presence of nitrogen gas.

Preparation and Characterization of Nanospheres

Twenty milligrams of the copolymers were dissolved with 2 mg of indomethacin in 1.6 mL of methylene chloride. The mixture was poured into a 50-mL beaker containing 12 mL of distilled water and then magnetically stirred for about 7 min. The water-insoluble series, the MPEG5000 and MPEG750 series, were sonicated using an ultrasonic sonifier (Branson) for 0.5, 1, 1.5, or 2 min with 40 W output power. The drug-loaded polymer particles were obtained by removing water and solvent using a freeze dryer.

A dynamic light-scattering instrument (DLS, Brookhaven, BI-200SM) with a Ne–He laser was used to measure the size distribution of polymeric spheres in distilled water. Each MPEG series was magnetically dispersed in distilled water and then filtrated using 0.45-μm pore-sized filter paper to remove oversized materials. The light intensities scattered from the polymeric spheres were measured at the angle of 90°.

The mean size of the polymeric spheres obtained by the DLS measurement was confirmed using a scanning electron microscope (SEM, Hitachi, S-2400). The polymer-dispersed water solution was put onto a glass slide with the dimension of 5 × 7 mm using a microsyringe. After this slide was fixed on the circular holder, the particles were coated with platinum in a vacuum and then the microphotographs were obtained.

Drug-releasing Experiments

The drug-loaded block copolymer nanocarriers were placed in the cellulose dialysis membranes with a cutoff molecular weight of 1000 g/gmol. The membranes containing nanospheres were put into the phosphate buffer solution of pH 7.4. The concentration of the drug in the buffer solution was kept uniform by locating the test tubes in a water-bath shaker maintained at a constant temperature of 37°C. One milliliter of the buffer solution was sampled periodically to measure the drug concentration. Each sample was dried in a vacuum to remove water. The drugs were separated from the phosphate solid by dissolution in 5 mL of chloroform. The drug concentration was measured using ultraviolet spectroscopy (UV, Hitachi, U-3210). The peak intensity of the experimental sample was compared with that of the calibrated one obtained from the standard sample.

RESULTS AND DISCUSSION

Characterization of MPEG/PLA Block Copolymers

Table I shows the molecular weights and polydispersity indices of the three block copolymer series synthesized. The molecular weights of the PLA blocks in the MPEG2000 series were from about 420 to 1860 g/gmol, and those of the MPEG750 and MPEG5000 series, from 2750 to 5650 g/gmol and from 7400 to 13,000 g/gmol, respectively. The molecular weight distributions of the MPEG750 and MPEG2000 series were very narrow, as their polydispersity indices were measured very close to 1. The polydispersity indices of the MPEG5000 series were in the range of 1.24–1.48, which was much higher than those of the other two lower molecular weight series. The block-length ratio of hydrophobic PLA to hydrophilic MPEG was reported as an important parameter in determining the phase equilibria in water.[13,14] In simplicity, when the hydrophilic segments are rather longer

Table I Characteristics of MPEG–PLA Diblock Copolymers Synthesized

Copolymer Series	$\bar{M}_n$	Polydispersity Index	Solubility in Water
MPEG2000 series	MPEG(2000)/PLA(420)	1.06	Soluble (micelle)
	MPEG(2000)/PLA(890)	1.08	Soluble (micelle)
	MPEG(2000)/PLA(1510)	1.09	Soluble (micelle)
	MPEG(2000)/PLA(1860)	1.09	Soluble (micelle)
MPEG750 series	MPEG(750)/PLA(2750)	1.03	Insoluble
	MPEG(750)/PLA(3450)	1.05	Insoluble
	MPEG(750)/PLA(5650)	1.04	Insoluble
MPEG5000 series	MPEG(5000)/PLA(7400)	1.24	Insoluble
	MPEG(5000)/PLA(9200)	1.48	Insoluble
	MPEG(5000)/PLA(13000)	1.28	Insoluble

than are the hydrophobic segments, the equilibrium phase is reported to be in solution or a micellar state. In the present polymeric systems with the block length represented in Table I, the MPEG2000 series was observed to be micellar, but the other two series, insoluble in water.

The presence of ester groups originating from the reaction of the hydroxyl group of MPEG with the acyl–oxygen of L-lactide was examined by the IR spectra shown in Figure 2. The IR band observed at 1760 cm^{-1} stemmed from the carbonyl group with the production of the diester group. The IR bands observed from 2775 to 3103 cm^{-1} and from 1427 to 1573 cm^{-1} originated from the methylene groups in the MPEG segments. The area ratio of the band at 1760 cm^{-1}, indicated by (2), to that from 1427 to 1573 cm^{-1}, indicated by (1), increased with an increasing PLA block length.

Figure 3 shows the NMR spectra of the synthesized copolymers. The chemical structure of the synthesized polymers was examined by analyzing peak splitting and integration in the proton and ^{13}C-NMR spectra. The presence of CH and CH_3 groups in PLA was observed at 5.17 (1) and 1.59 ppm (3), respectively, and the CH_2 group in MPEG at 3.65 ppm (2) in the ^{1}H-NMR spectra. The presence of C═O, CH, and CH_3 groups in the PLA block was at 170.43 (1), 69.84 (3), and 17.37 ppm (4) in the ^{13}C-NMR spectra, respectively, and the CH_2 group in the MPEG block, at 71.42 ppm (2).

Figure 4(a–c) shows the second heated DSC thermograms of the three copolymer series,

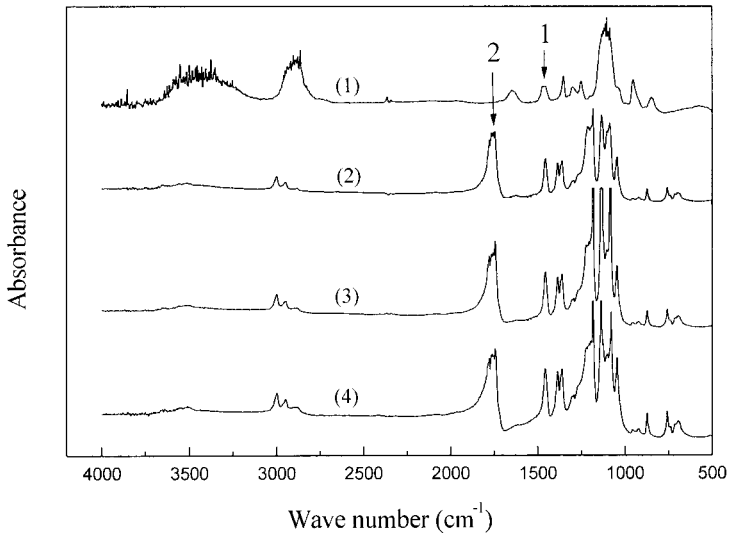


Figure 2 FTIR spectra of MPEG750 series with PLA in varying molecular weights of (1) 0, (2) 2750, (3) 3450, and (4) 5650 g/gmol.

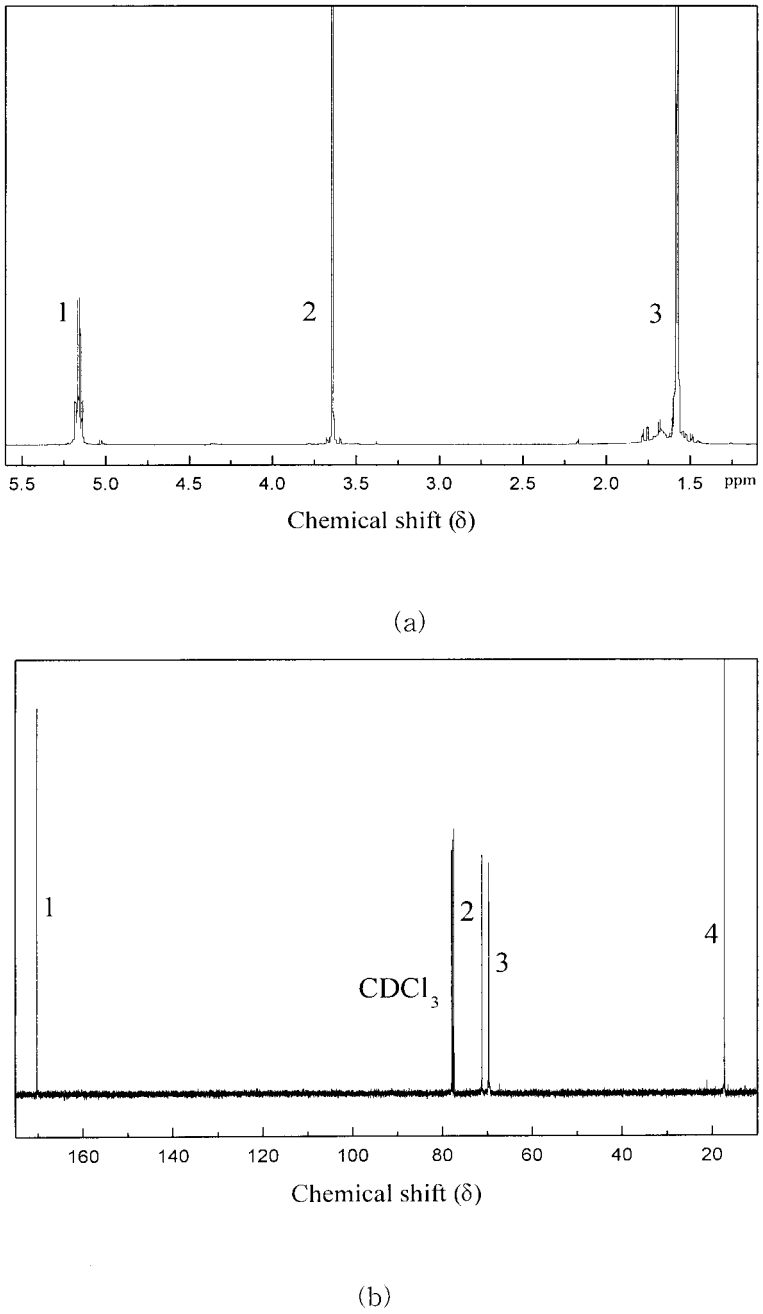


Figure 3 (a) ^{1}H-NMR and (b) ^{13}C-NMR spectra of MPEG(5000)/PLA(7400) diblock copolymers.

MPEG2000, MPEG750, and MPEG5000, respectively, after being quenched from the melt. For the MPEG2000 series shown in Figure 4(a), only the melting transition of the PEG crystalline phase was clearly observed. Because of the relatively short PLA block length, melting or crystallization of the PLA block was barely observed up to the PLA molecular weight of 1740 g/gmol. A slight crystallization peak of the PLA block was observed at around 70°C for the PLA molecular weight of 1860 g/gmol. Figure 4(b) shows the DSC thermograms of the MPEG750 series. In this case, single crystallization followed by a melting transition was observed. As the MPEG segment with this very low molecular weight was in an almost amorphous state, its presence had influence on the overall thermal behavior by shifting the crystallization and melting transition temperatures of PLA. An increase in the block-length ratio of PLA to MPEG led to decrease of the crys-

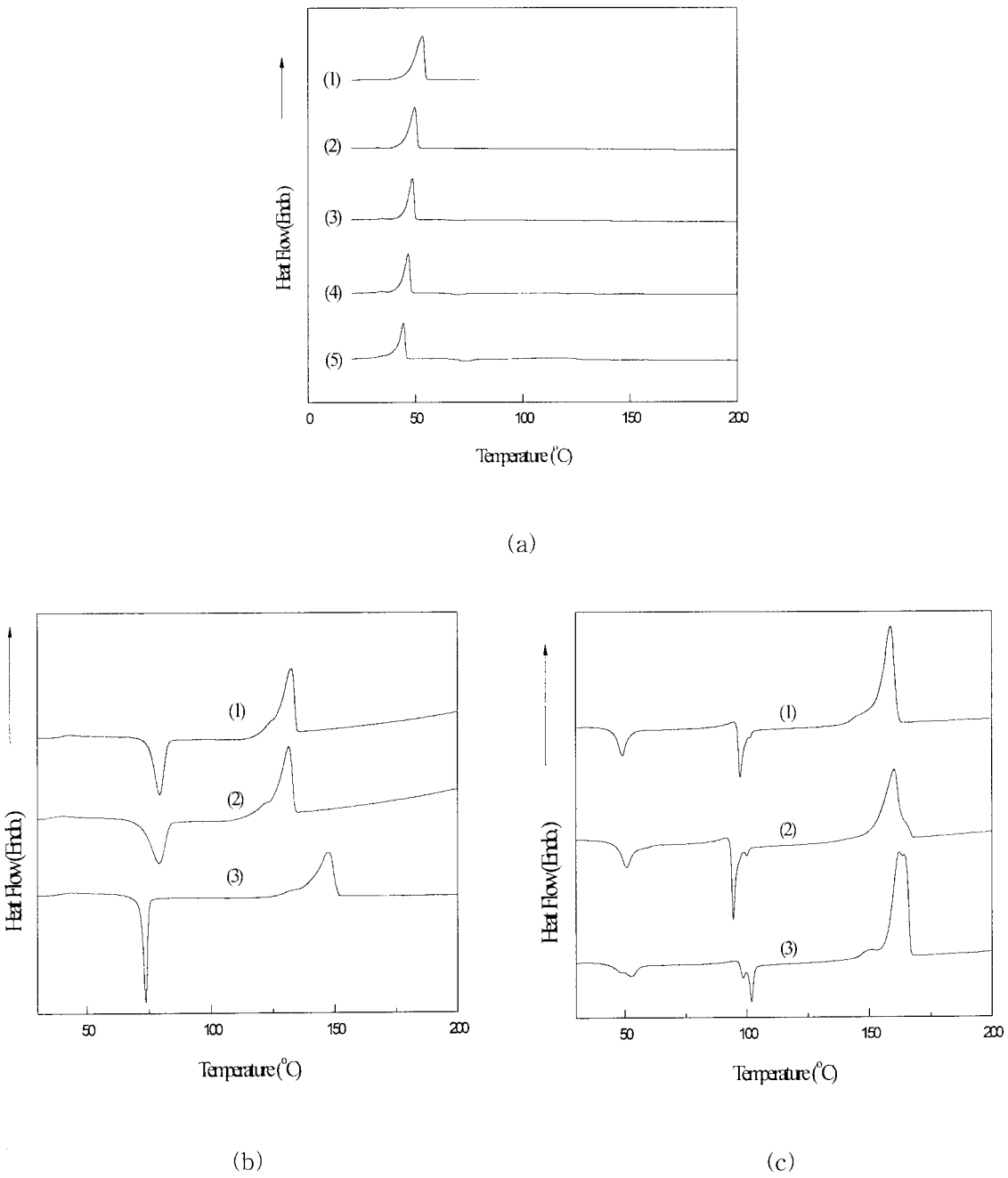
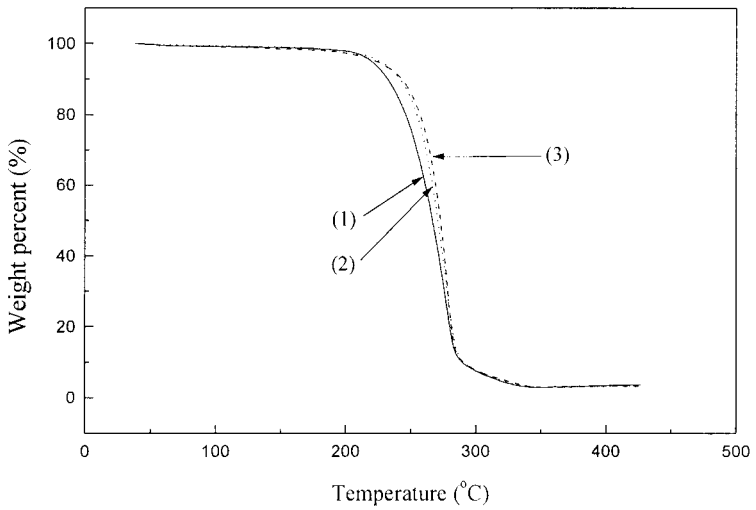


Figure 4 DSC thermograms of (a) MPEG2000 series with PLA in varying molecular weights of (1) 0, (2) 420, (3) 890, (4) 1510, and (5) 1860 g/gmol. (b) MPEG750 series with PLA in varying molecular weights of (1) 2750, (2) 3450, and (3) 5650 g/gmol. (c) MPEG5000 series with PLA in varying molecular weights of (1) 7400, (2) 9200, and (3) 13,000 g/gmol.

tallization but also to an increase of the melting transition temperatures. As it was reported that PLA blocks crystallize more readily than do MPEG blocks due to the higher inherent crystallization tendency of the LA repeating units,[7] the short segment of MPEG retarded the crystallization of PLA and this trend was more significant with an increasing block length ratio of MPEG to PLA. For the MPEG5000 series in Figure 4(c), however, two crystallization peaks, followed by a melting peak, were observed. The crystalline phase was not fully developed at room temperature due to the quenching effect. The complex molecular rearrangements from this single amor-



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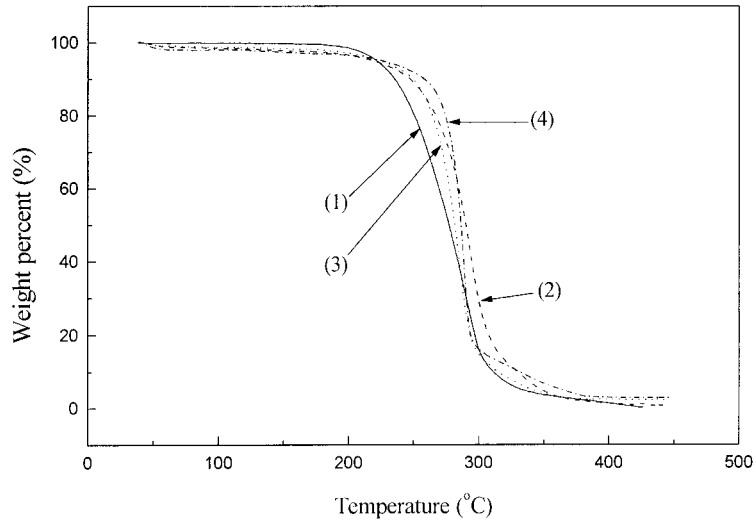
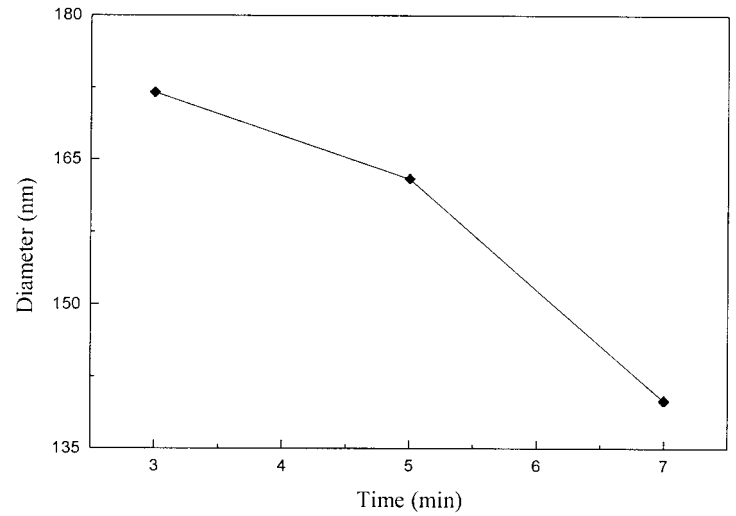


(b)

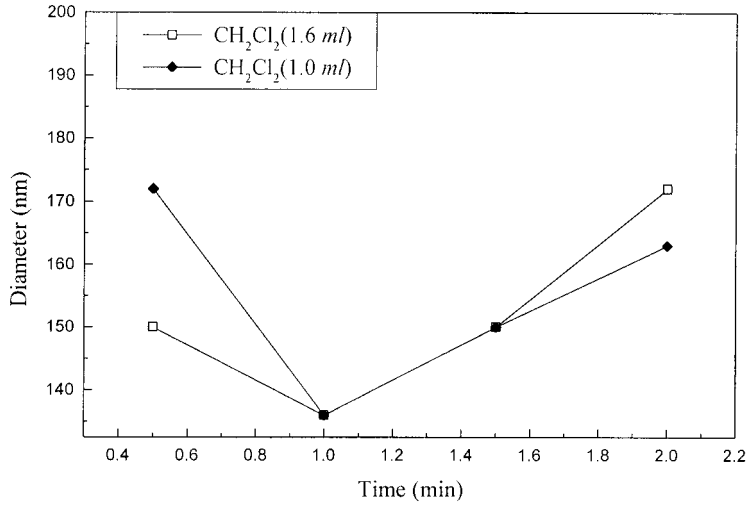
Figure 5 TGA thermograms of (a) MPEG750 series with PLA in varying molecular weights of (1) 7250, (2) 3450, and (3) 5650 g/gmol and (b) MPEG5000 series with PLA in varying molecular weights of (1) 5000, (2) 7400, (3) 9200, and (4) 13,000 g/gmol.

phous phase led to additional crystallization, as illustrated by the two exothermic peaks. The melting peaks observed from 150 to 170°C were slightly shifted to a lower temperature with a decreasing PLA block length. As the melting transition of homo-MPEG around 50°C was much lower than that of homo-PLA around 160°C, the decrease in the PLA block length resulted in a reduction of the overall melting temperature.

Figure 5 shows TGA thermograms of the block copolymers. The MPEG750 series was thermally stable to about 200°C, and the MPEG5000 series, to about 250°C. One of the main reasons affecting this thermal behavior was the total molecular weight of the block copolymer system. The composition effect on the thermal stability was not significant in the present range of the PLA block lengths, as MPEG block sizes were very different. No significantly different trend was observed for the MPEG2000 series.



(a)



(b)

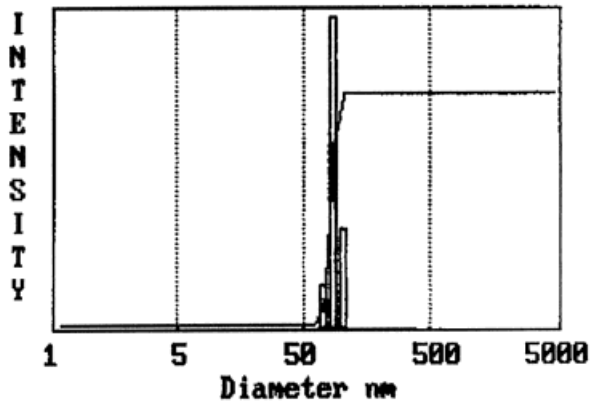
Figure 6 (a) Stirring and (b) sonification time effects on the mean diameter of MPEG(5000)/PLA(9200) diblock copolymer particles in distilled water.

Preparation and Characterization of Nanospheres

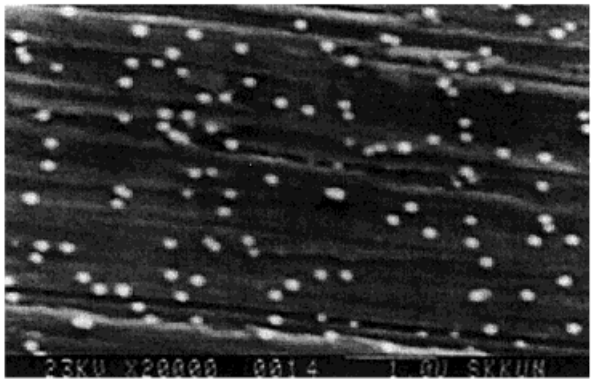
PEG/PLA block copolymers were prepared into nano-sized spherical forms, whether those were micellar or in a water-insoluble state, by the o/w emulsion technique described in Experimental section. When the PLA segments became longer, the block copolymers became more hydrophobic and they can be present as solid particles rather than as micelles in water. Being different from micelles, water-insoluble nanoparticles exist as either precipitates or suspensions. As the physical states of the nanoparticles were significantly affected by the preparation conditions, especially the dispersing condition without any use of a surfactant, their effect on the nanoparticle size was of importance. Figure 6(a) shows the stirring-time effect on the size of the nanoparticles prepared. The mean particle diameters decreased with increasing stirring time. However, a longer stirring than 7 min resulted in a poor size distribution due

to the evaporation of methylene chloride. After the stirring process, the particle size was also controlled by ultrasonification. As shown in Figure 6(b), the particle size was reduced with an increasing sonification time up to 1 min, but then increased. The increase in the particle size after 1 min was attributed to the floculation of the particles due to the heat generated from a sonifier tip. As these effects of the MPEG750 series were very similar to those of the MPEG5000 series, the stirring and sonification time was chosen as 7 and 1 min, respectively, for all the present copolymer systems.

Figure 7(a) shows the size distribution and the mean diameter of the nanospheres prepared from the MPEG 750 series measured using DLS. The prepared nanospheres showed a very narrow size distribution. The particle size measured using the DLS experiment was complemented by the microphographs obtained from SEM. In Figure 7(b), the particles were illustrated in white circles, and their diameters were in a similar range of those measured using DLS. The nanospheres prepared from other MPEG series also had narrow size distributions, but had somewhat different mean diameters. The mean diameters of the micelles produced from the MPEG2000 series were around 180 nm. The mean diameters of the solid particles from the MPEG5000 series were around 140 nm, and those from the MPEG750 series, around 115 nm.



(a)



(b)

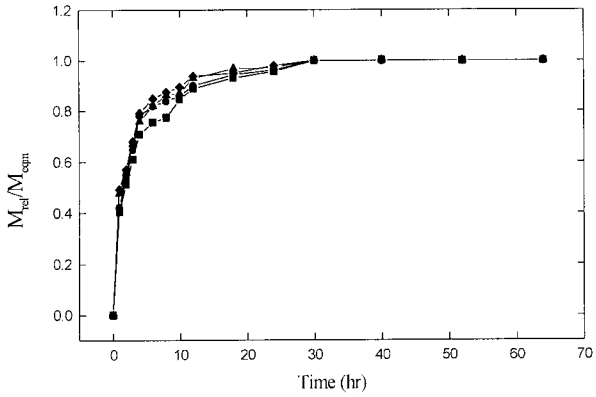
Figure 7 (a) Particle-size distribution and (b) SEM photographs of MPEG(750)/PLA(2750) diblock copolymer particles.

Drug-releasing Characteristics

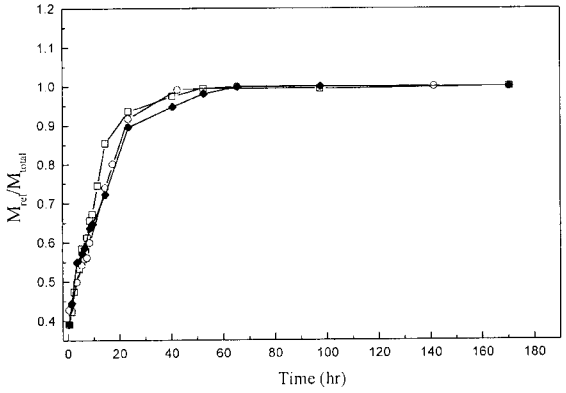
Figure 8 shows the drug-releasing characteristics of three block copolymer series. The releasing rates from micelles produced from the MPEG2000 series were higher than those from the water-insoluble nanoparticles produced from the MPEG750 and MPEG5000 series, even though the mean micelle diameter was higher than were the mean particle diameters. This was because of the less dense PLA cores for the micellar structure than for the solid nanoparticle structure. For the solid nanoparticle systems, the releasing rate of the MPEG750 series was much higher than that of the MPEG5000 series. This was caused by two differences—particle size and molecular entanglement in the PLA core. As the nanoparticles prepared from the MPEG750 series were smaller than those from the MPEG5000 series, it took a shorter time to release all of the drug. Also, the molecular entanglement in the PLA core for the MPEG750 series was less than that for the MPEG5000 series, because the higher molecular weight PLA in the MPEG5000 series had a higher tendency of entanglement. More entanglement might lead to a longer path and a higher resistance for drugs to reach the free surface of the particles. The time to release all the drugs from the particles, whether it was for the MPEG750 series or for the MPEG5000 series, was still affected by the PLA block length, even if its effect was not significant in this PLA molecular weight range. The loading amount of the drug was about 8–9 wt %.

For the MPEG750 series, the releasing kinetics was very close to following zero order and was not significantly affected by the PLA block length. The releasing rates of the MPEG5000 series, however, were more fluctuated than were those of the MPEG750 series for different PLA segmental lengths. It might be caused by a higher molecular weight and a broader distribution for the MPEG5000 series than those for the MPEG750 series.

Figure 9 shows the IR bands for the pure drug and block copolymers before and after the release experiments. In Figure 9(a), the characteristic bands at 1693 and 1589 cm^{-1} originated from the carbonyl group in COOH and from the amide group of N—C═O of indomethacin. A comparison of Figure 9(b) and (c) implies that all the drugs were released from the carriers when equilibrium was reached, as there were no differences in the IR band positions between them and no IR characteristic bands from indomethacin were observed.



(a)



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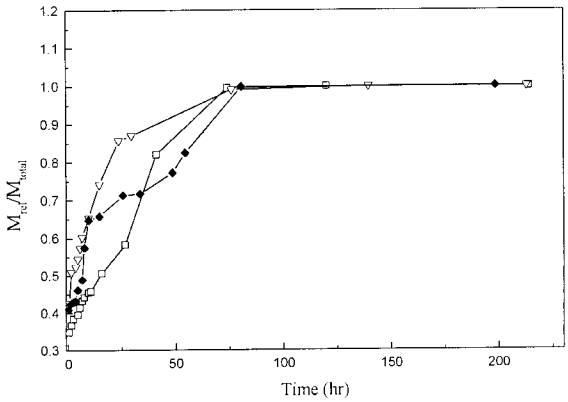

(c)

CONCLUSIONS

MPEG/PLA block copolymers were synthesized by a ring-opening reaction of L-lactide with MPEG at the reaction temperature of 130°C for 50 h using the solution polymerization technique. Synthesis of the three block copolymer systems of the MPEG2000, MPEG5000, and MPEG750 series was investigated using GPC, FTIR, and NMR spectroscopy. The thermal behavior of quenched polymer samples characterized by DSC showed no crystallization for the MPEG2000 series, two crystallization exotherms for the MPEG5000 series, and single crystallization exotherm for the MPEG750 series. The optimal condition for the preparation of stable nanoparticles was determined as the stirring time of 7 min and the sonification time of 1 min. From DLS and SEM measurements, the mean diameter of micelles prepared from the MPEG2000 series was about 180 nm, and those of water-insoluble particles from the MPEG5000 and MPEG750 series, about 140 nm and 115 nm, respectively. The releasing rates of micelle-forming nanospheres were much higher than those of water-insoluble nanoparticles, even though the mean diameter of the micelles was higher than that of the particles. For water-insoluble particles, the time to release all the drugs was longer for the MPEG5000 series than for the MPEG750 series. These results were caused by differences in not only particle size but also in the density and molecular entanglement of the PLA cores.

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Figure 8 Drug-releasing kinetics of (a) MPEG2000 series with PLA in varying molecular weights of (■) 420, (●) 890, (▲) 1510, and (♦) 1860 g/gmol, respectively; (b) MPEG750 series with PLA in varying molecular weights of (□) 2750, (▽) 3450, and (♦) 5650 g/gmol; and (c) MPEG5000 series with PLA in the varying molecular weights of (□) 7400, (▽) 9200, and (♦) 13,000, g/gmol.

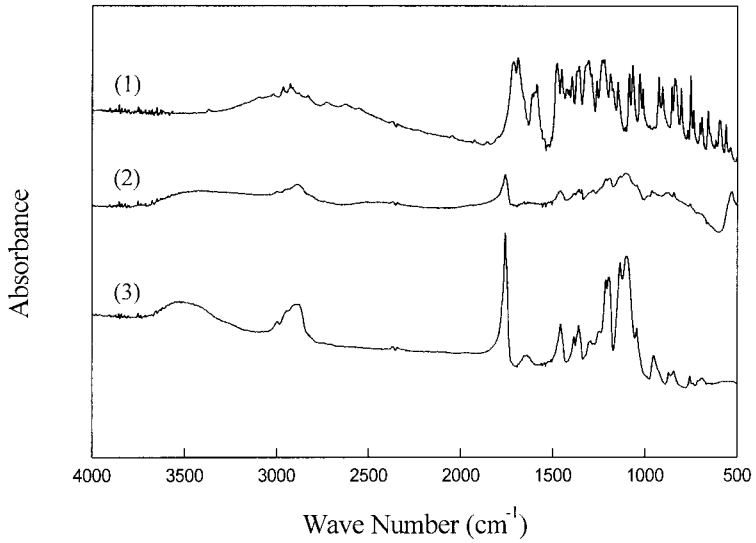

Figure 9 FTIR spectra of (1) pure drug, (2) MPEG/PLA copolymers after release experiments, and (3) MPEG/PLA copolymers before release experiments.

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