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VEHICLES FOR DRUG DELIVERY

DRUG RELEASING CHARACTERISTICS OF PDLLA-MPEG DI- AND MPEG-PDLLA-MPEG TRIBLOCK COPOLYMER MICELLES

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Key Words: Copolymer Micelle, Biodegradation, Drug Delivery Systems, Poly(lactic acid), Poly(ethylene glycol)

ABSTRACT

Poly(DL-lactic acid)-methoxy poly(ethylene glycol) (PDLLA-MPEG) di- and MPEG-PDLLA-MPEG triblock copolymer systems with varying MPEG block length were synthesized and their fundamental properties were characterized. Micellar structures were formed in aqueous milieu by amphiphilic characteristics of block copolymers. The critical micelle concentration and size distribution of the copolymer micelles were determined. Drug loading and releasing experiments were performed in the phosphate buffer solution of pH 7.4 at 37°C. It was observed that the loading content in the block copolymer micelles increased with increasing MPEG block segment length, resulting in the difference in initial burst strength. There was not a big difference in the releasing rate for the same type of block copolymer systems even with different MPEG segment length, but triblock systems illustrated slightly lower releasing rates than the diblock systems.

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INTRODUCTION

Polymeric drug delivery systems have long been studied to maintain the remedial effectiveness by releasing the drug with controlled rate. Among many polymeric systems, either biodegradable or not, nano-scaled polymeric micelles are a strong candidate for the injectable or inhalable applications [1].

Poly(lactic acid) (PLA), one of the well known biodegradable hydrophobic polymers, has been applied for lots of drug delivery systems, as it possesses good bio-adaptability, and does not have a severe reverse effect on blood or tissues, nor does it possess toxicity itself. Poly(ethylene glycol) (PEG), a typical hydrophilic polymer, has also been applied to many biomedical systems to increase hydrostability of blood contacting materials.

Due to such unique properties, several types of PLA-PEG block copolymer systems were studied, focused on their synthesis, characterization, and applications. Zhu *et al.* [2-4] synthesized PLA-PEO copolymers from D,L-lactide and ethylene oxide by solution polymerization. A number of catalysts were used in the synthesis and their effects were observed. Synthesis of PLA-PEG copolymers from PEG and D,L-lactide was also reported using catalyst of stannous octoate by bulk polymerization. Younes and Cohn [5, 6] investigated morphological behavior and physical and mechanical properties of PLA-PEG copolymers covering a wide range of compositions and segmental lengths. Cerrai and Tricoli [7] reported the synthesis and properties of PLA-PEG block copolymers through a non-catalyzed route from L-lactide and PEG. Vert *et al.* [8, 9] characterized PLA-PEO-PLA triblock copolymers with short and long PLA chains synthesized from L-lactide in the presence of bi-functional OH-terminated PEG using Zn metal or CaH_2 catalyst. Jedlinski *et al.* [10] synthesized PLA-PEO-PLA triblock copolymers by anionic polymerization of L-lactide in the presence of sodium poly(ethylene glycol)ate in tetrahydrofuran at room temperature. They investigated morphology and mechanical properties according to their compositions and thermal treatment. Langer *et al.* [11] addressed the injectable applications of PLGA-PEG block copolymer nanospheres and investigated their biocompatibility for site-specific drug delivery system. Piskin *et al.* [12] synthesized PDLLA-PEG multi-block copolymers from the condensation reaction of PDLLA and PEG. They investigated drug releasing characteristics of copolymers in the form of micelles. Bazile *et al.* [13] reported the characterization of macrophage avoidance behavior of nanoparticles prepared from the methoxy poly(ethylene glycol)(MPEG)-poly(d,l-lactic acid)(PDLLA) block copolymers and blends.

In the present contribution, PDLLA-MPEG di- and MPEG-PDLLA-MPEG triblock copolymers were synthesized from DL-lactic acid and MPEG, and their properties were characterized. Each block copolymer system was prepared in the micelle form and its releasing characteristics was studied.

EXPERIMENTAL

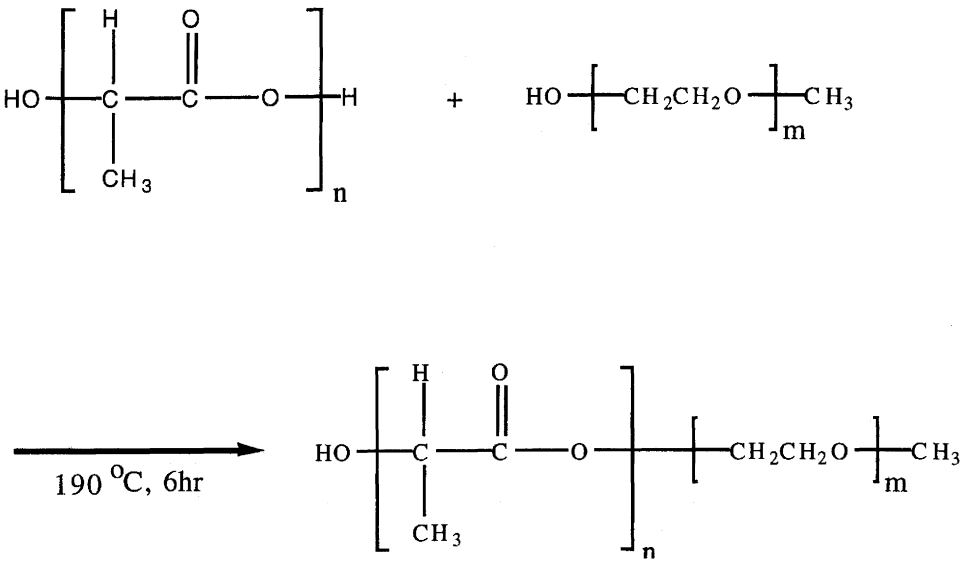
Raw Materials

DL-lactic acid (85% solution in water), the monomer for the synthesis of poly(DL-lactic acid) (PDLLA), was purchased from Aldrich Chemical Company. Poly(ethylene glycol) methyl ethers (MPEG) with varying molecular weights of 750, 2000, and 5000 g/gmol, respectively, were purchased from Aldrich Chemical Company. Before use, those were stored in the vacuum oven at 30°C for at least 100 hours. Toluene was purchased from Duksan Phamaceutical Company as the solvent for the solution polymerization of MPEG-PDLLA-MPEG triblock copolymer systems. Humidity was eliminated by distillation with calcium anhydride at 120°C. Hexamethylene diisocyanate (HDI, Duksan Pharmaceutical Company) and stannous octoate (Sigma Chemical Company) were used as the coupling agent and catalyst, respectively, for the synthesis of MPEG-PDLLA-MPEG triblock copolymers.

Synthesis of PDLLA-MPEG and MPEG-PDLLA-MPEG Block Copolymer Systems

PDLLA was synthesized from DL-lactic acid without catalyst by bulk condensation polymerization technique in the 250 ml three-neck flask at 200°C for 24 hours. Nitrogen gas was continuously purged until the reaction was completed.

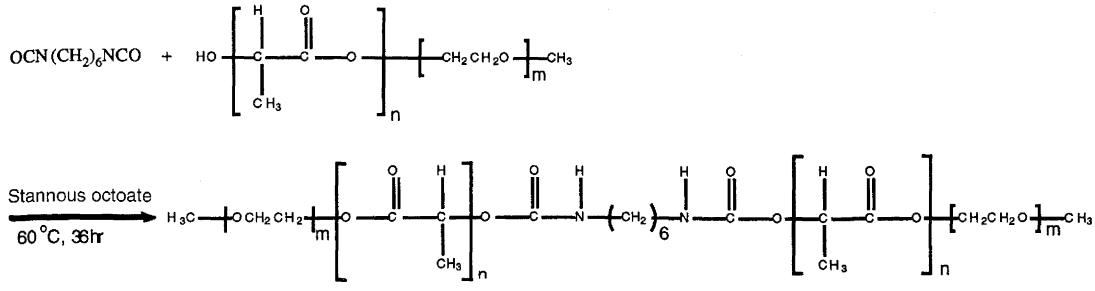
PDLLA-MPEG diblock copolymers were synthesized by esterification reaction of PDLLA with MPEG of varying molecular weights of 750, 2000, and 5000 g/gmol, respectively. Two types of homopolymers equivalently weighed were dissolved in acetone to the concentration of 1 g/ml. After the polymer solution was placed in the 250 ml three-neck flask, most of acetone solvent was evaporated at 100°C for 2 hours. The polymerization reaction was conducted as in Scheme 1 at 190°C for 6 hours after gradually raising the temperature from 100°C. The reaction was conducted in the presence of nitrogen gas with mechanical agitation.



Scheme 1.

The synthesized diblock copolymers were purified by separating the two unreacted homopolymers of PDLLA and MPEG as follows. The diblock copolymers were dispersed by mild agitation in the distilled water to the concentration where the micelle structures were formed. PDLLA homopolymers were precipitated, and then were separated from polymer solution by centrifugation and filtration. After filtration of homo PDLLA, the PDLLA-MPEG copolymer micelles in the distilled water were precipitated by changing the ionic strength of water solution with the addition of sodium chloride. The precipitated block copolymers were also separated by filtration technique from the residual polymer solution including homo MPEG and lactic acid oligomers.

MPEG-PDLLA-MPEG triblock copolymers were synthesized from diblock copolymers by solution polymerization method with HDI of which the diisocyanate groups reacted with the hydroxyl end groups in the PDLLA-MPEG diblock copolymers to produce urethane linkage as shown in Scheme 1. 5 g of diblock copolymers were dissolved in 100 ml of toluene with the addition of 55.59 mg of HDI and 5.356 mg of stannous octate catalyst. The reaction was conducted as shown in Scheme 2 at 60°C for 36 hours in the presence of nitrogen gas. The reaction products were precipitated with diethyl ether, and then separated from bulk solution by filtration. The residual solvents were removed by vacuum evaporation technique.



Scheme 2.

Drug Loading and Releasing Experiments

1 g of each block copolymer was dissolved with 0.2 g of indomethacin in 10 ml of methylene chloride. The homogeneous polymer solution was dispersed in 50 ml of distilled water at the concentrations above the critical micelle concentration (CMC). Since the minute amount of drug agglomerates unloaded to the polymer micelles were precipitated after centrifugation, those were separated by filtration. 40 ml of drug-loaded micelle solution was concentrated to 20 ml using freeze dryer at -40°C. The concentrated micelle solution was placed in the dialysis membrane with cut off molecular weight of 1000 g/gmol, and then the drug releasing experiments were performed in the phosphate buffer solution of pH 7.4 at 37°C. To maintain the constant temperature and the uniform concentration, the releasing experiments were conducted in the temperature controllable shaker (model KMC-1205SW1, Vision Scientific). The content of the drug released from the polymer micelles was periodically measured using UV/Vis spectrophotometer (model 3210, Hitachi) until the released drug concentration was invariant. Before this measurement the UV calibration curve which related the drug content with solution concentration was prepared by calculating the area of indomethacin characteristic band appeared at 320 nm.

Characterization

The molecular weights of PDLLA, PDLLA-MPEG, and MPEG-PDLLA-MPEG homo and block copolymer systems synthesized were determined by either end group analysis or using gel permeation chromatography (GPC) (model 410, Waters). The chemical structure was characterized using Fourier Transform infrared spectroscopy (FTIR) (model 1000, Matton) and ^{1}H-nuclear magnetic resonance spectroscopy (^{1}H-NMR) (500 MHz, Varian Unity).

The thermal properties were analyzed using differential scanning calorimetry (DSC) (model 2910, Du Pont) with the scanning rate of 10°C/min in the temperature range from -50 to 200°C in the presence of the nitrogen gas. The CMC values and size distribution of block copolymer micelles were determined using UV/Vis spectrophotomer (model 3210, Hitachi) and He-Ne laser light scattering system (model BI-9000AT, Brookheaven), respectively.

RESULTS AND DISCUSSION

Characterization of PDLLA, PDLLA-MPEG, and MPEG-PDLLA-MPEG Homo and Block Copolymer Systems

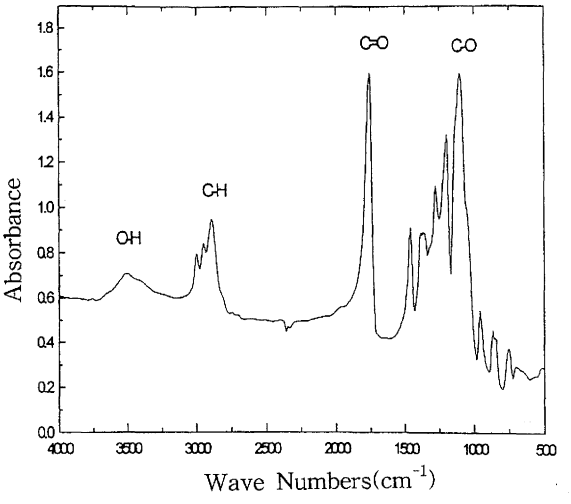
The number average molecular weight of homo PDLLA was determined to be 2,000 g/gmol using the end group analysis as given by Equation 1 and its result was very similar to that using GPC measurement.

$$\text{Molecular weight} = 1/[(\text{meq COOH} + \text{meq OH})/2 \times 1{,}000 \times \text{sample weight}] \quad (1)$$

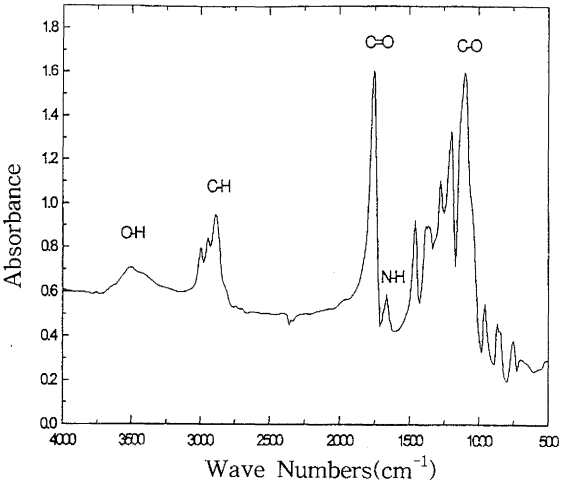
Figures 1 (a) and (b) show the IR spectra of the PDLLA-MPEG diblock and MPEG-PDLLA-MPEG triblock copolymers, respectively. In Figures 1 (a) and (b) the C=O stretching band at 1750 cm^{-1} and CH stretching band at 2880 cm^{-1} illustrate the presence of PDLLA and MPEG block segments, respectively. In Figure 1 (b) the additional N-H stretching IR band at 1650 cm^{-1} represents the presence of urethane group which was produced when the diisocyanate group in HDI was reacted with hydroxyl end groups in PDLLA-MPEG diblock copolymers during the synthesis of triblock copolymers.

Figures 2 (a) and (b) show the ^{1}H-NMR spectra of the PDLLA-MPEG diblock and MPEG-PDLLA-MPEG triblock copolymers, respectively. In Figures 2 (a) and (b), the characteristic peaks at 5.22, 3.65, and 1.55 ppm correspond to the protons of -CH-, -CH_2-, and -CH_3 in the PDLLA and MPEG block segments, respectively. The additional peak at 3.15 ppm in Figure 2 (b) was caused by the existence of methylene protons (-CH_2-) in the HDI which was incorporated in the triblock copolymer synthesis from two PDLLA-MPEG diblock molecules.

Figure 3 shows the DSC thermogram of PDLLA-MPEG diblock copolymers. Glass transition temperature was observed at around -10°C.



(a)

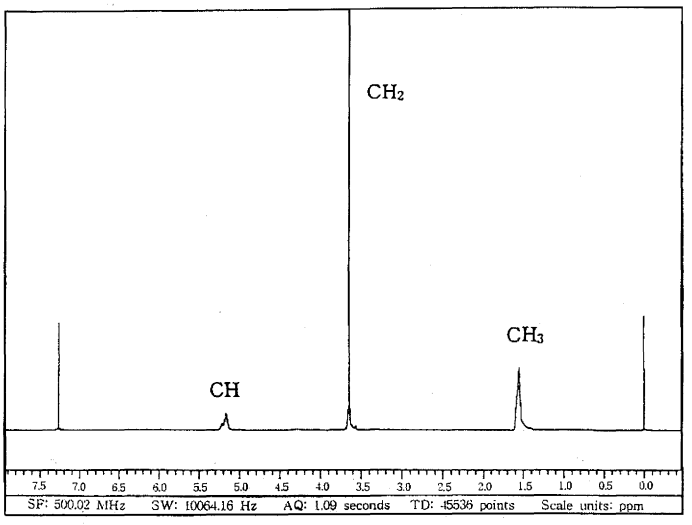


(b)

Figure 1. IR spectra of (a) PDLLA-MPEG diblock and (b) MPEG-PDLLA-MPEG triblock copolymers.

Characterization of Block Copolymer Micelles

In order to determine the critical micelle concentration (CMC), the UV absorbance at 490 nm was measured for varying polymer solution concentration. As shown in Figure 4, the CMC value of 0.0001 g/ml was determined at the concentration where the UV absorbance was abruptly increased. Figure 5 shows the



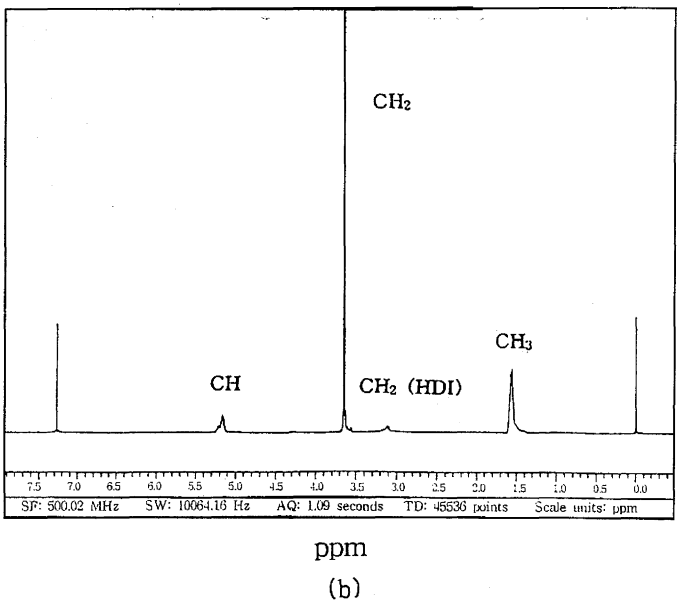


Figure 2. ^{1}H-NMR spectra of (a) PDLLA-MPEG diblock and (b) MPEG-PDLLA-MPEG triblock copolymers.

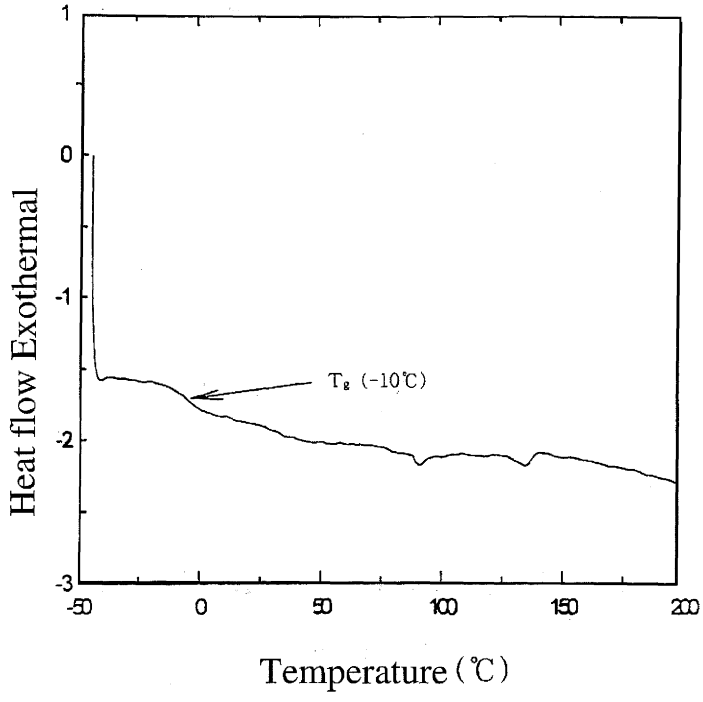


Figure 3. DSC thermograms of PDLLA(2000)-MPEG(2000) block copolymer.

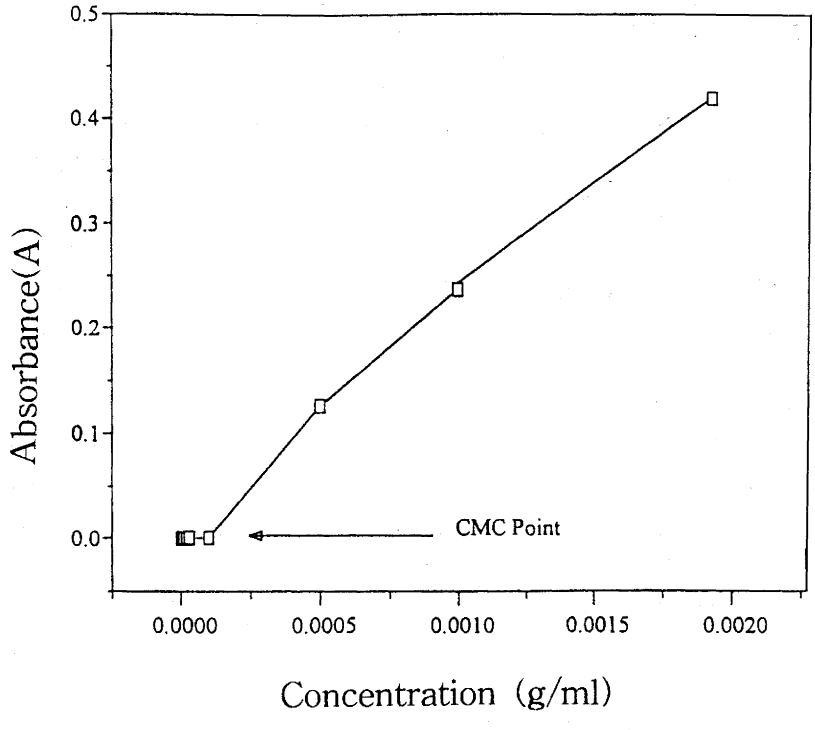


Figure 4. UV absorbance as a function of concentration of PLA(2000)-MPEG(2000) block copolymer.

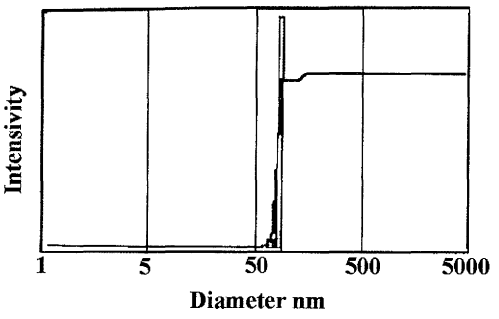


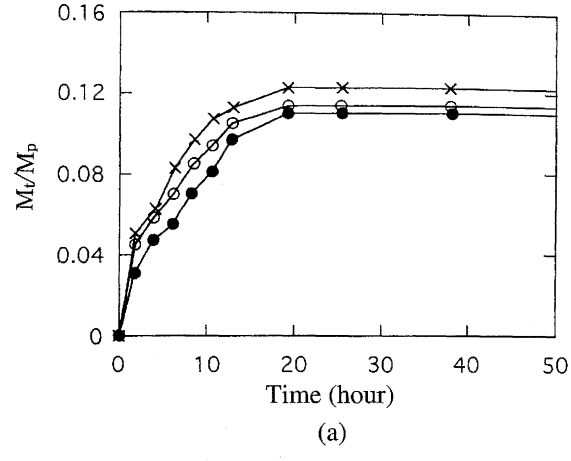
Figure 5. Size distribution of PDLLA(2000)-MPEG(2000) block copolymer micelles.

size distribution of copolymer micelles measured by laser light scattering apparatus at the solution concentrations of 0.01 g/ml. The resulting micelle diameter was in the range from 100 to 120 nm with relatively narrow distribution. This size range was reported to be appropriate for the application of the intravenously administered carriers [11].

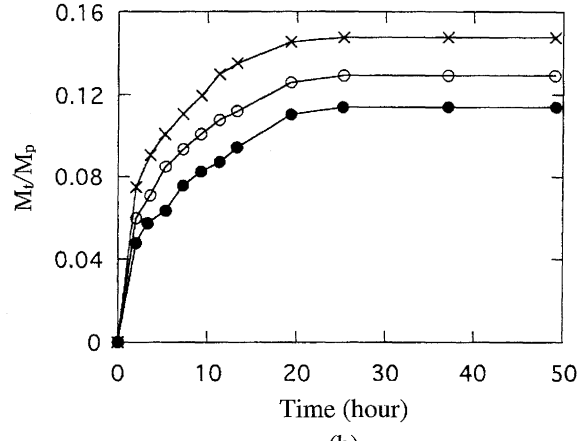
Drug Loading and Releasing Characteristics

In Figures 6 and 7, the drug releasing behavior of PDLLA-MPEG di and MPEG-PDLLA-MPEG triblock copolymer micelles are shown. M_t/M_p indicates the mass of drug released from the unit mass of PDLLA cores and M_t/M_{eqm} the mass fraction of the drug released from the total drug initially loaded in polymer micelles.

From the equilibrium values of M_t/M_p in Figures 6 (a) and (b), the drug loading content in the polymer micelles was estimated. Drug loading content (final drug releasing contents) was observed to be increased with increasing molecular weights of MPEG for both di and triblock copolymer systems. It was caused by the difference in the drug loading contents in the MPEG segments which occurred mainly in the freeze drying processes. As the freeze drying process was operated at -40°C which was much lower than the glass transition temperature of block copolymers of -10°C, certain content of drug was possibly captured in the less mobile MPEG block segments closely linked to hydrophobic PDLLA core surfaces, and this inclination might become stronger for micelles with longer MPEG segments. This presumption is supported by the burst release phenomenon observed in the initial drug releasing process. The first data points in Figure 6s (a) and (b) represent the content of drug released burstly from the MPEG block chains especially located close to the PDLLA cores



(a)



(b)

Figure 6. Time dependent drug content released from the unit mass of (a) PDLLA-MPEG diblock and (b) MPEG-PDLLA-MPEG triblock copolymer micelles, respectively, with varying MPEG molecular weights of 750 (●), 2000 (○), and 5000 (x) g/gmol.

which were originally frozen at the dry-freezing experiment, but then became mobile at the releasing experiments conducted at 37°C. Thus, this burst content was increased with increasing molecular weight of MPEG for the same types of copolymer systems. The effect of MPEG block length on the drug loading amount (burst amount) was greater for the triblock copolymer systems than the diblock systems, because the triblock copolymer systems had more MPEG block segments than diblock systems. The releasing data in the absence of burst points

illustrate that the equilibrium releasing contents (loading contents) were in the same range from 0.067 to 0.079 g/g, indicating that the drug loading content in the PDLLA cores were not in big difference with different MPEG block sizes and types.

Figures 7 (a) and (b) show the time dependence of fractional drug releasing rate, $d(M_t/M_{eqm})/dt$ for di- and triblock copolymer systems, respectively. The releasing rate was not significantly affected by MPEG block length for both di-

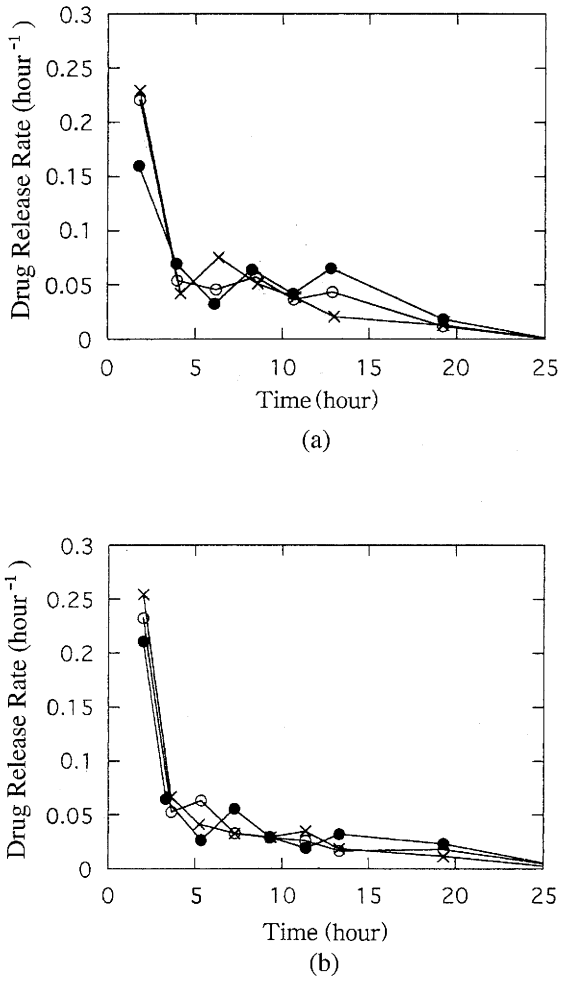


Figure 7. Time dependence of fractional mass of drug released from (a) PDLLA-MPEG diblock and (b) MPEG-PDLLA-MPEG triblock copolymer micelles, respectively, with varying MPEG molecular weights of 750 (●), 2000 (○), and 5000 g/gmol (x).

and triblock copolymer systems. The duration time to release all drug was somewhat longer for triblock copolymer systems than for diblock systems, when the component block sizes were the same. When the first data point indicating the burst effect was subtracted form all releasing data, the releasing kinetics seemed to follow zero-order rate, up to about 20 hours.

CONCLUSION

PDLLA-MPEG di- and MPEG-PDLLA-MPEG triblock copolymers with varying MPEG block sizes were synthesized, and their structures and properties were characterized using FTIR, ^{1}H-NMR, and DSC. The CMC value of 0.0001 g/ml was determined using UV/Vis spectrophotometer. The micelle diameter in the range of 100 to 120 nm was measured using the light scattering system. The loading content in the block copolymer micelles increased with increasing MPEG block segment length, attributed to the freeze drying process operated well below the glass transition temperature of copolymer systems. The difference in the drug loading content led to the difference in burst strength in the initial releasing process, and its effect was more significant for triblock systems than diblock systems. Otherwise there is little difference in the releasing behavior for different MPEG block segment lengths. From this drug releasing behavior it is anticipated that the overall releasing kinetics can be controlled by the modification of block size of each polymer component in copolymer systems as well as their preparation methods.

ACKNOWLEDGEMENTS

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