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Research Article

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Effect of D-/L-form ratio on properties and drug release behaviors of amorphous poly(D,L-lactide) microparticles

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ABSTRACT

In this study, the influence of the D-/L-form mole ratio of lactic acid on poly(D,L-lactide) (PDLLA) properties and its drug release behaviors were investigated and discussed. The PDLLAs were synthesized by ring-opening polymerization of DLLA monomers. The DLLA monomers with different D-/L-form ratios were prepared by polycondensation followed by a thermal decomposition processes for the D,L-/L-lactic acid mixtures. All the PDLLAs were complete amorphous for the D,L-/L-lactic acid mole ratios of 100/0-50/50. Indomethacin, a poorly-water soluble model drug was entrapped in the PDLLA microparticles. All drug-loaded PDLLA microparticles prepared by the oil-in-water emulsion solvent evaporation method were spherical in shape and had a smooth surface. In vitro drug release behaviors showed that the drug release from the PDLLA microparticles prepared with different D-/L-form ratios was similar. It is considered that the PDLLAs with a higher L-form ratio have the potential to be developed further as a lower-cost PDLLA for use in controlled release drug delivery applications.

Keywords: Lactic acid, Lactide, Polylactide, Microparticles, Drug delivery.

INTRODUCTION

Controlled release drug delivery systems made from biodegradable particles provides several benefits over traditional formulations [1]. Prior to release, the drug is protected from degradation or premature metabolism by the polymeric particle matrix. The release of the drug is sustained over days to months, thereby keeping the drug concentration in the plasma at an effective level for longer periods of time and reducing toxic side-effects. This decreases the frequency of drug dosing and increases patient compliance [2,3]. Biodegradable particles for drug delivery have been widely made from a variety of biodegradable polyesters.

Amorphous poly(D,L-lactide) (PDLLA) and poly(D,L-lactide-*co*-glycolide) (PDLLGA) are biodegradable and biocompatible polyesters that have been widely investigated for use as controlled release drug delivery systems [4-6]. The removal of these biodegradable polyester-based devices at the end of therapy is not required. The microparticles of the amorphous PDLLA and its copolymers have been widely investigated as controlled release drug delivery matrices due to their completely amorphous form [4]. Good drug distribution into the amorphous PDLLA matrices can be obtained. The semi-crystalline phases in poly(L-lactide) (PLLA) may induce drug aggregates into the PLLA matrices. A good distribution of the entrapped drug into the microparticle matrices could allow a consistent drug release rate.

Usually, PDLLA is prepared from the D,L-lactic acid monomer precursor. D,L-lactic acid is first changed to D,L-lactide monomer (DLLA) before its ring-opening polymerization to obtain the PDLLA. The D,L-lactic acid consists of a 50/50 D-/L-form mole ratio. However, the L-lactic acid is easier to find and cheaper than the D,L-lactic acid.

In the present work, we report the influence of the D-/L-form ratio of the DLLA monomer on the PDLLA properties. DLLA monomers with different D-/L-form ratios were prepared from mixtures of D,L-lactic acid and L-lactic acid. The characteristics of the drug-loaded PDLLA microparticles and drug release behaviors of a hydrophobic model drug were also determined.

EXPERIMENTAL SECTION

Materials

DLLA monomers were synthesized from mixtures of D,L-lactic acid (85% w/v, 50/50 D-/L-form ratio, Acros Organics) and L-lactic acid (88% w/v, 5/95 D-/L-form ratio, Purac) by direct polycondensation at 180 °C followed by thermal decomposition at 220 °C. Crude DLLA was purified by re-crystallization in ethyl acetate four times. The purified DLLA was dried under vacuum at 50 °C for 48 h before use in the polymerization. The DLLA monomers with D,L-/L-lactic acid mole ratios of 100/0, 90/10, 70/30 and 50/50 were prepared from D,L-lactic acid/L-lactic acid mixtures. 1-dodecanol (98%, Fluka) containing one-hydroxyl end group was purified by distillation under reduced pressure before use. Stannous octoate [$Sn(Oct)_2$, 95%, Sigma], indomethacin (99.95%, Sigma) and Tween80 (Acros Organics) were used without further purification. All reagents used were analytical grade.

Synthesis of poly(D,L-lactide)s

Poly(D,L-lactide)s (PDLLAs) with different D-/L-form ratios were synthesized by ring-opening polymerization of the DLLA monomers in bulk at 165 °C for 2.5 h under a nitrogen atmosphere. $Sn(Oct)_2$ was used as a catalyst at 0.01 mol% and 1-dodecanol was used as an initiator. The 1.44 mol% 1-dodecanol was used to synthesize the PDLLAs with a theoretical number-average molecular weight ($M_{n,\ theoretical}$) of 10,000 g/mol. The crude PDLLAs were purified by dissolving in chloroform before precipitating in cool *n*-hexane. They were then dried to a constant weight in a vacuum at 50 °C for 48 h.

Characterization of PDLLAs

The specific optical rotation of the PDLLA was determined in chloroform at a concentration of 1 g/dL at 25 °C with a Bellingham and Stanley Polarimeter ADP220 at a wavelength of 589 nm. The specific optical rotation of the polylactide was used to calculate the D and L enantiomer contents [7,8].

The number-average molecular weight (M_n) and molecular weight distribution (MWD) of the PDLLAs were determined by Gel Permeation Chromatography (GPC) with a Waters e2695 separations module equipped with PLgel 10 μm mixed B 2 columns operating at 40 °C and employing a refractive index (RI) detector. Tetrahydrofuran was used as the solvent at a flow rate of 1.0 mL/min.

The thermal transition properties of the PDLLAs were determined with a Perkin-Elmer Pyris Diamond differential scanning calorimeter (DSC) under a nitrogen flow. For DSC, samples of 5 – 10 mg in weight were heated at 10 ^oC/min over a temperature range of 0 to 200 °C (1^{st} heating scan) to observe their melting temperature (T_m). Then the samples were quenched to 0 °C according to the DSC instrument's own default cooling mode before heating from 0 to 200 °C (2^{nd} heating scan) to observe their glass transition temperature (T_g). The T_m was measured as the peak value of the endothermal phenomena in the DSC curve. The T_g was taken as the midpoint or half of the heat capacity increment associated with the glass-to-rubber transition.

Preparation of drug-loaded PDLLA microparticles

PDLLA microparticles entrapping the indomethacin model drug were prepared by the oil-in-water emulsion solvent evaporation method. The dichloromethane was used as an organic solvent. 90 mg of PDLLA and 10 mg of indomethacin were dissolved in 2.5 mL of dichloromethane (oil phase). The oil phase was slowly added-drop wise into 400 mL of a 2% w/v Tween80 solution in distilled water (water phase) under magnetic stirring. The organic solvents were evaporated in a fume hood for 6 h. The drug-loaded microparticles suspended in the water phase were obtained. The resulting microparticles were collected by centrifugation before freeze-drying.

Characterization of drug-loaded PDLLA microparticles

The morphology of the microparticles was observed by scanning electron microscopy (SEM, JEOL JSM-6460LV). The microparticles were sputter-coated with gold to enhance the surface conductivity before scanning. The average size of the microparticles was determined from several SEM images by counting a minimum of 100 particle diameters using the smile view software (version 1.02).

The drug loading of the microparticles was measured by UV-Vis spectrophotometry (Lamda 25, Perkin Elmer). For this purpose, the drug-loaded microparticles were dissolved in dichlorometane before analysis with a UV-Vis spectrophotometer at λ_{max} = 319 nm [9]. The amount of indomethacin model drug was calculated by comparing with

a standard equation of indomethacin solution in dichloromethane. The standard equation and R^2 were y = 0.0181x + 0.0158 and 0.9996, respectively.

The theoretical drug loading content ($DLC_{theoretical}$), actual drug loading content (DLC_{actual}) and drug loading efficiency (DLE) were calculated form Equations (1) – (3), respectively. The DLC_{actual} was an average value from three measurements.

$$DLC_{theoretical}\,(\%) = \frac{\text{Weight of feed drug}}{\text{Weights of feed drug and PDLLA}} \times 100 \quad (1)$$

$$DLC_{actual}\,(\%) = \frac{\text{Weight of entrapped drug in microparticles}}{\text{Weight of microparticles}} \times 100 \quad (2)$$

$$DLE\,(\%) = \frac{DLC_{actual}}{DLC_{theoretical}} \times 100 \quad (3)$$

where the weight of the entrapped drug in the microparticles was measured by dissolving the drug-loaded microparticles in dichloromethane before analysis with UV-Vis spectrophotometry at λ_{max} = 319 nm.

***In vitro* drug release tests**

An *in vitro* drug release test with the microparticles was performed as follows. About 10 mg of drug-loaded microparticles were placed in a pretreated dialysis bag before being incubated in a flask containing 200 mL of 0.02 M phosphate buffer saline (PBS, pH 7.4). The flasks were kept in an shaker incubator at 37 °C and 100 rpm for 48 h. At each desired time, some supernatant was withdrawn and replaced with an equal volume of fresh PBS medium. The release concentration of the indomethacin in the supernatant was determined by a UV-Vis spectrophotometer at λ_{max} = 319 nm [9].

The amount of indomethacin model drug was calculated by comparing with a standard equation of indomethacin solution in PBS. The standard equation and R^2 were y = 0.02x + 0.0047 and 0.9994, respectively. The cumulative release percentage of indomethacin (%drug release) was calculated based on the ratio of the drug release at in each time and the initial drug content in the microparticles. The drug release profiles were plotted between %drug release and release time. The *in vitro* drug release tests were performed in triplicate.

RESULTS AND DISCUSSION

Characterization of poly(D,L-lactide)s

The DLLA monomers with different D-/L-form ratios were prepared from mixtures of D,L-/L-lactic acid. The mole ratios of the D,L-/L-lactic acid mixtures were 100/0, 90/10, 70/30 and 50/50, which corresponded to the theoretical D-/L-form mole ratios of 50/50, 45.5/54.5, 36.5/63.5 and 27.5/72.5, respectively as reported in Table 1. The PDLLAs were synthesized by ring-opening polymerization of the DLLA. The yields of the PDLLAs obtained from the precipitation method were in the range of 78–85%. The actual D-/L-form ratios of the PDLLAs determined from the polarimetry are also summarized in Table 1. They were very close to the values of the theoretical D-/L-form ratios. The results suggest that the PDLLAs with different D-/L-form ratios can be prepared from the D,L-/L-lactic acid mixtures.

Table 1 also reports the number-average molecular weights (M_ns) and molecular weight distributions (MWDs) of the PDLLAs obtained from the GPC curves. All the GPC curves were unimodal. The M_ns and MWDs were in the ranges of 11,000-14,300 g/mol and 1.3-1.4, respectively. The M_ns obtained from the GPC were close to the values of the theoretical M_n (10,000 g/mol). The M_n of the PDLLA was controlled by the 1-dodecanol concentration. From the 1st heating scan DSC curves, the melting peaks of all the PDLLAs were not found. This suggests that the PDLLAs with the D-/L-form ratios in the range of this study were completely amorphous. The glass transition temperatures (T_gs) of the PDLLAs obtained from the 2nd heating scan DSC curves were similar and in the range of 49-51 °C as summarized in Table 1. The results indicate that the D-/L-form ratio did not affect the T_g of the PDLLA.

Characterization of PDLLA microparticles

The morphology of the drug-loaded PDLLA microparticles was investigated from the SEM images as shown in Fig. 1. The drug-loaded PDLLA microparticles with different D-/L-form ratios were spherical in shape and the surfaces

were smooth as shown in Fig. 2. The average particle sizes, determined from the several SEM images, are summarized in Table 2. They were in the range of 77-87 μm. The morphology results suggest that the D-/L-form ratio of the PDLLA did not significantly affect their microparticle morphology and size.

The theoretical drug loading content ($DLC_{theoretical}$) of all the PDLLA microparticles is 10 wt%. The actual drug loading content (DLC_{actual}) and the drug loading efficiency (DLE), as summarized in Table 2, were in the ranges of 4.93–5.91 wt% and 49.3–59.1%, respectively. Both DLC_{actual} and DLE did not change significantly with the D-/L-form ratio.

***In vitro* drug release**

The indomethacin release profiles are illustrated in Fig. 3. All drug-loaded PDLLA microparticles exhibited similar sustained release profiles. The release profile results suggest that the PDLLAs with different D-/L-form ratios showed potential for use in controlled release drug delivery applications. The plasma drug concentrations could be maintained at therapeutic levels for longer periods of time; therefore, the frequency of drug administration could be reduced. The D-/L-form ratio did not influence the drug release content.

Each drug release from the PDLLA microparticles consisted of an initial burst release of the drug near the particle surfaces within the first 3 h followed by slower drug release for the first 12 h. The predominant drug release mechanism in the release time of 3-12 h was proposed to be the drug diffusion process. The drug release was faster again after 12 h. The surface erosion process was proposed as the predominant drug release mechanism after 12 h. This was confirmed from the SEM images of the microparticle surfaces in Fig. 4. After 48 h of the drug release test, all the PDLLA microparticles were still spherical in shape, but the surface erosion was observed, as shown in Fig. 4. Their microparticle surfaces were rough. The drug release level remained constant after 24 h of release time. Thus, the drug release mechanisms of the PDLLA microparticles within 48 h of release time may include both drug diffusion and surface erosion mechanisms.

Table 1. Characteristics of PDLLAs

D,L-/L-lactic acid mole ratio	Theoretical D-/L-form mole ratio[a]	Actual D-/L-form mole ratio[b]	M_n (g/mol)[c]	MWD[c]	T_g (°C)[d]
100/0	50/50	49.0/51.0	14,200	1.3	50
90/10	45.5/54.5	45.9/54.1	11,000	1.4	49
70/30	36.5/63.5	36.2/63.8	14,100	1.3	51
50/50	27.5/72.5	26.4/73.6	14,300	1.3	50

[a] *Calculated based on 50/50 D-/L-form ratio and 5/95 D-/L-form ratio of D,L-lactic acid and L-lactic acid, respectively.*
[b] *Determined from polarimetry method.*
[c] *Determined from GPC curves.*
[d] *Measured from 2nd heating scan DSC curves.*

Table 2. Average particle size and drug loading of PDLLA microparticles

D,L-/L-lactic acid mole ratio	Average particle size (μm)[a]	DCL_{actual} (%)[b]	DLE (%)[c]
100/0	87 ± 15	5.91 ± 0.05	59.1
90/10	77 ± 18	4.93 ± 0.11	49.3
70/30	81 ± 17	5.46 ± 0.08	54.6
50/50	83 ± 22	5.53 ± 0.12	55.3

[a] *Determined from several SEM images.*
[b] *Calculated from Equation (2).*
[c] *Calculated from Equation (3).*

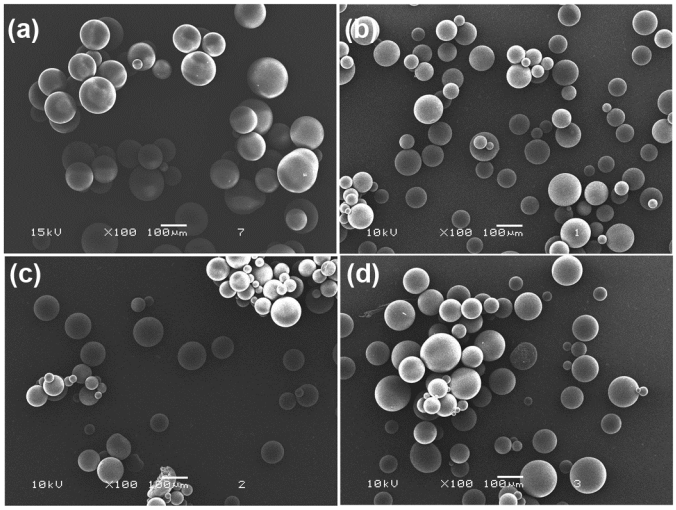

Fig. 1. SEM images of drug-loaded microparticles before drug release test prepared from PDLLAs with D,L-/L-lactic acid mole ratios of (a) 100/0, (b) 90/10, (c) 80/20 and (d) 50/50 (all scale bars = 100 μm)

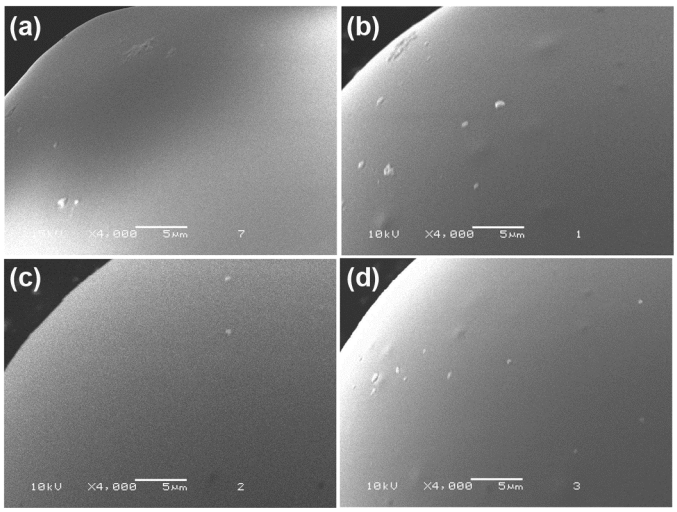

Fig. 2. Surfaces of drug-loaded microparticles before drug release test prepared from PDLLAs with D,L-/L-lactic acid mole ratios of (a) 100/0, (b) 90/10, (c) 80/20 and (d) 50/50 (all scale bars = 5 μm)

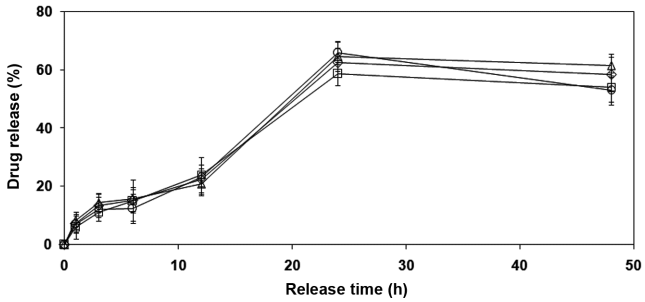

Fig. 3. *In vitro* drug release profiles of microparticles prepared from PDLLAs with D,L-/L-lactic acid mole ratios of (◊) 100/0, (□) 90/10, (Δ) 70/30 and (○) 50/50

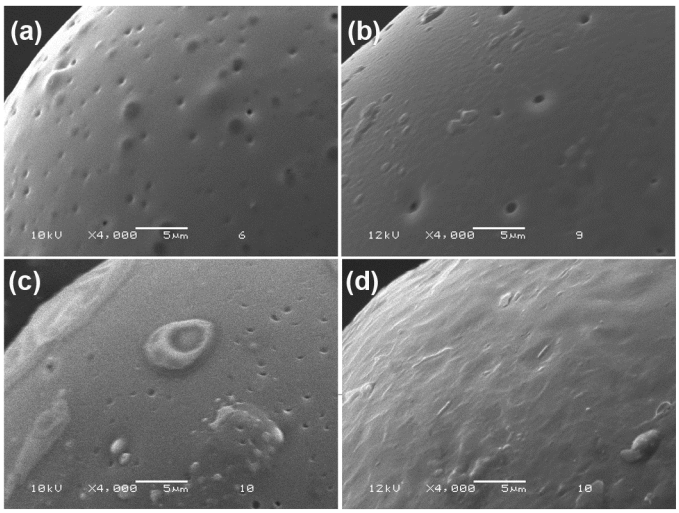

Fig. 4. Surfaces of drug-loaded microparticles after drug release test prepared from PDLLAs with D,L-/L-lactic acid mole ratios of (a) 100/0, (b) 90/10, (c) 80/20 and (d) 50/50 (all scale bars = 5 μm)

CONCLUSION

The biodegradable PDLLAs with different D-/L-form ratios were successfully synthesized via ring-opening polymerization of the DLLA monomers using 1-dodecanol and stannous octoate as the initiating system. The DLLA monomers with different D-/L-form ratios were prepared from the mixtures of D,L-/L-lactic acid. The PDLLAs with the D-/L-form mole ratios in the range of 49.0/51.0-26.4/73.6 were in an amorphous state. The drug-loaded PDLLA microparticles prepared by the oil-in-water emulsion solvent evaporation technique can be used as carriers of indomethacin, a poorly-water soluble model drug with a 49.3-59.1% loading efficiency. All the PDLLA microparticles were spherical in shape, had a smooth surface and showed similar in drug release profiles.

In conclusion, the results presented here show that the amorphous PDLLAs prepared in this work have the potential to be developed further as a drug delivery system. The amorphous PDLLAs containing a high L-form ratio could provide a viable lower-cost alternative to the commercial PDLLA containing the 50/50 D-/L-form ratio.

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