การเตรียมอนุภาคไมโครบรรจุยาของพอลิดี,แอล-แล็กไทด์ผสมกรดสเตียริกและพฤติกรรมการปลดปล่อยยา

Preparation of Drug-loaded Poly(D,L-lactide)/Stearic acid Blend Microparticles and Their Drug Release Behaviors

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บทคัดย่อ

อนุภาคไมโครพอลิดี,แอลแล็กไทด์ผสมกรดสเตียริกเตรียมได้โดยวิธีการระเหยตัวทำละลายของอิมัลชั่นแบบน้ำมันในน้ำสำหรับนำส่งยาแบบควบคุมการปลดปล่อยยาอินโดเมธาซินซึ่งเป็นยาตัวอย่างที่ละลายน้ำได้น้อย โดยศึกษาอิทธิพลอัตราส่วนผสมพอลิดี,แอลแล็กไทด์และกรดสเตียริกต่อลักษณะเฉพาะของอนุภาคไมโครและพฤติกรรมการปลดปล่อยยา อนุภาคไมโครถูกวิเคราะห์ด้วยเทคนิคอิเลคตรอนแบบส่องกราด ดิฟเฟอเรนเชียลสแกนนิ่งแคลอรีเมทรี และยูวี-วิสิเบิลสเปกโทรสโคปี อนุภาคไมโครผสมบรรจุยาที่มีอัตราส่วนผสมพอลิดี,แอลแล็กไทด์/กรดสเตียริกในช่วง 100/0—92.5/7.5 โดยน้ำหนัก มีรูปร่างใกล้เคียงทรงกลมและผิวเรียบ พบว่าอนุภาคไมโครผสมมีขนาดอนุภาค (84—114 ไมครอน) และร้อยละประสิทธิภาพการบรรจุยา (24.85—33.30 เปอร์เซ็นต์) ใกล้เคียงกัน จากการทดสอบการปลดปล่อยยาแบบอินวิโทร แสดงให้เห็นว่าปริมาณการปลดปล่อยยาลดลงเมื่ออัตราส่วนกรดสเตียริกเพิ่มขึ้น จากผลการทดลองที่ได้ชี้ว่ามีความเป็นไปได้ในการใช้อนุภาคไมโครพอลิดี,แอลแล็กไทด์ผสมกรดสเตียริกสำหรับเป็นระบบนำส่งยาแบบควบคุมการปลดปล่อย

คำสำคัญ: พอลิเมอร์แตกสลายทางชีวภาพได้ พอลิเมอร์ผสม อนุภาคไมโคร ระบบนำส่งยา

Abstract

The poly (D,L-lactide) (PDLL)/stearic acid blend microparticles were prepared by an oil-in-water emulsion solvent evaporation method for controlled-release drug delivery of indomethacin, a poorly water-soluble model drug. The effect of PDLL/stearic acid blend ratio on microparticle characteristics

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and drug release behaviors were investigated. The microparticles were characterized using a combination of scanning electron microscopy (SEM), differential scanning calorimetry (DSC) and UV-vis spectrophotometry. The drug-loaded blend microparticles with a PDLL/stearic acid blend ratio in the range 100/0—92.5/7.5 (w/w) showed a spherical shape with a smooth surface. Blend microparticles with a similar size (84—114 µm) and drug loading efficiency (24.85—33.30%) were obtained. An *in vitro* drug release test showed that the content of drug release decreased as the stearic acid ratio increased. The results indicated that it was possible to use PDLL/stearic acid blend microparticles as a controlled-release drug delivery system.

Keyword: biodegradable polymers, polymer blends, microparticles, drug delivery systems

Introduction

Controlled-release drug delivery is a term referring to drug delivery in response to time and used to identify drug delivery systems that is designed to deliver the precise amount of a drug at a pre-programmed rate in order to achieve the drug level necessary for the treatment after administration.[1] Poly(D,L-lactide) (PDLL) has been widely reported as a matrix for controlled-release drug delivery systems in film and particle forms due to its biodegradability and biocompatibility. The removal of the PDLL matrices at the completion of therapy is not required. Drug release mechanisms from a PDLL matrix consists of drug diffusion out and matrix erosion processes.

The PDLL is an amorphous polymer. This induces uniform drug distribution in the PDLL matrix. PDLL microparticles have been widely investigated for use as controlled-release drug delivery systems.[2-5] The oil-in-water emulsion solvent evaporation method has been usually chosen to produce the PDLL microparticles containing poorly water-soluble model drugs.[2,5] Drug release from polymeric matrix depended upon the polymer molecular weight, initial drug content, particle size and blend polymers.[6-8]

Lipids are low cost biocompatible substances that have been widely also used as a matrix for drug delivery.[9-11] Solid lipid microparticles attain high drug loading efficiency for poorly water-soluble drugs due to their hydrophobic nature. Stearic acid is a solid lipid. Stearic acid microparticles combine the advantages of liposome and polymer microparticles, while avoiding some of their disadvantages such as toxicity, biodegradability problems and raw material costs.[12] However, the PDLL/stearic acid blend microparticles for use as controlled-release drug delivery systems have not been reported.

In this work, the PDLL/stearic acid blend miroparticles containing a poorly water-soluble model drug were prepared by the oil-in-water emulsion solvent evaporation method. The influence of PDLL/stearic acid blend ratio on morphology, particle size, thermal transition properties, drug loading and drug release behavior of the blend microparticles was investigated.

Materials and Method

Materials

Poly (D,L-lactide) (PDLL) with an average-viscosity molecular weight of 50,000 g/mol was synthesized by ring-opening polymerization of a D,L-lactide monomer in bulk at 140°C for 24 h under a nitrogen atmosphere. 1-Dodecanol (98%, Fluka, Switzerland) and stannous octoate (95% Sigma, USA) were used as the initiating system. The 1-dodecanol, stannous octoate, stearic acid (95%, Sigma-Aldrich, USA), indomethacin model drug (99%, Sigma, USA) and Tween80 (Labchem, Australia) were used without further purification. Dichloromethane (Carlo Erba, USA) in analytical grade was used.

Preparation of drug-loaded PDLL/stearic acid blend microparticles

The PDLL/stearic acid blend microparticles containing the indomethacin, a poorly water-soluble model drug were fabricated by the oil-in-water emulsion solvent evaporation method. Briefly, 0.1 g of PDLL/stearic acid mixture and 0.01 g of indomethacin were completely dissolved in 2.5 mL of dichloromethane before pouring into 400 mL of 2 wt% Tween80 aqueous solution under magnetic stirring (800 rpm) to form an oil-in-water emulsion. The dichloromethane was evaporated in a fume hood for 3 h. The blend microparticles were filtered and rinsed with distilled water. The blend microparticles were then freeze-dried overnight and stored in a desiccator before characterization and *in vitro* drug release testing.

Characterisation of drug-loaded PDLL /stearic acid blend microparticles

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

Thermal transition properties of the microparticles were determined by means of differential scanning calorimetry (DSC) using a Perkin-Elmer DSC Pyris Diamond. For DSC analysis, 5–10 mg of sample was heated at 10°C/min under a nitrogen flow in range 0-200°C.

The drug loading content (DLC) of the indomethacin in the blend microparticles was determined by dissolving the blend microparticles in dichloromethane. The drug concentration in clear solution was analyzed by UV-vis spectrophotometry from the absorbance at λ_{max} = 319 nm using a Perkin-Elmer Lambda 25 and compared to a standard curve of indomethacin. Theoretical DLC ($DLC_{theoretical}$), actual DLC (DLC_{actual}) and drug loading efficiency (DLE) were calculated from equations (1)–(3), respectively. The DLC_{actual} is an average value from three determinations.

$$DLC_{theoretical}(\%) = \frac{\text{Weight of drug}}{\text{Weight of drug in PDLL and stearic acid}} \times 100 \quad (1)$$

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

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

In vitro drug release test

An *in vitro* drug release test was performed in a 0.1 M phosphate buffer solution (pH 7.4) at 37 $^{\circ}$C under shaking. The blend microparticles (~10 mg) were placed in a dialysis tube before immersing in 200 mL of buffer. At predetermined time intervals, 10 mL of release medium was withdrawn. Then 10 mL of fresh buffer was added to the original for maintain the total volume. The drug release was determined by UV-vis spectrophotometry at λ_{max} = 319 nm and compared to a standard curve of the drug solution. Cumulative drug release was calculated in terms of the ratio of the cumulative mass of the released drug at a given time against the initial drug loading in the blend microparticles. *In vitro* drug release tests were performed in triplicate (n = 3).

Results

Figure 1 shows SEM micrographs of the drug-loaded blend microparticles. It can be seen that the resulting blend microparticles with PDLL/stearic acid blend ratios of 100/0, 97.5/2.5, 95/5 and 92.5/7.5 (w/w) were nearly spherical in shape. While the 90/10 (w/w) PDLL/stearic acid blend microparticles were irregular in shape with a deflated surface. The average particle sizes determined from several SEM images were similar in range 84–114 µm.

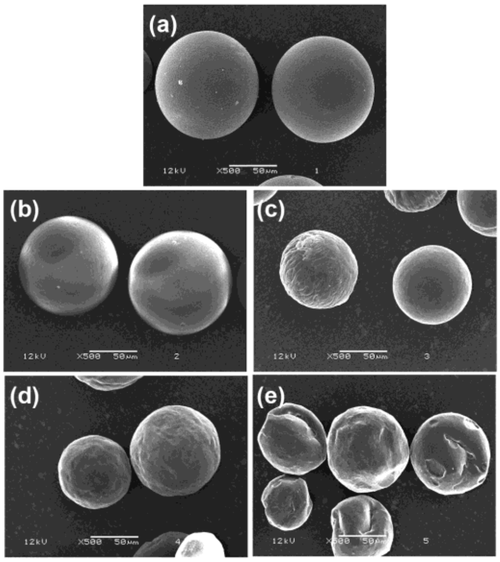

Figure 1 SEM micrographs of drug-loaded blend microparticles with PDLL/stearic acid blend ratios of (a) 100/0, (b) 97.5/2.5, (c) 95/5, (d) 92.5/7.5 and (e) 90/10 (w/w), all bars = 50 µm

The thermal transition properties of the blend microparticles were studied by DSC, as shown in Figure 2. The DSC data are summarized in Table 1. The PDLL microparticles showed only a single glass transition temperature (T_g) at 50°C. The T_g of the blend microparticles decreased as the stearic acid ratio increased. The blend microparticles also exhibited a single melting temperature (T_m) of stearic acid crystallinity in the range 55–57°C. The heat of melting (ΔH_m) for the blend microparticles, which was directly related to stearic acid crystallinity, increased with the stearic acid ratio.

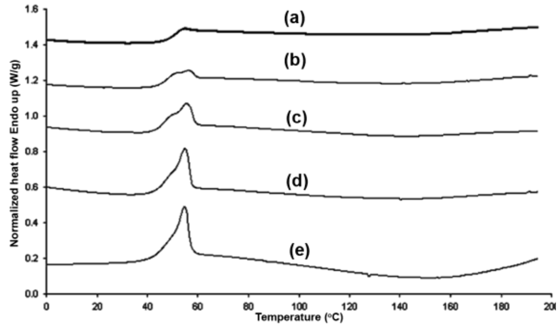

Figure 2 DSC thermograms of drug-loaded blend microparticles prepared with PDLL /stearic acid blend ratios of (a) 100/0, (b) 97.5/2.5, (c) 95/5, (d) 92.5/7.5 and (e) 90/10 (w/w)

Table 1 DSC results of the drug-loaded blend microparticles

PDLL/stearic acid (w/w)	T_g (°C)	T_m (°C)	ΔH_m (J/g)
100/0	50	-	-
97.5/2.5	46	57	0.6
95/5	44	56	2.4
92.5/7.5	43	55	4.2
90/10	40	55	6.0

Table 2 reported the actual drug loading content (DLC_{actual}) and drug loading efficiency (DLE) of the blend microparticles. The theoretical drug loading content ($DLC_{theoretical}$) of the all blend microparticles was 9.09%. All blend microparticles showed similar DLC_{actual} and DLE values in the ranges 2.26–3.03% and 24.85–33.30%,respectively.

The *in vitro* drug release from the blend microparticles was measured at 37°C for 48 h in a phosphate buffer pH 7.4. Figure 3 shows the influence of the PDLL/stearic acid blend ratio on the drug release behavior of the blend microparticles. The drug release is plotted between cumulative drug release and time. The PDLL microparticles exhibited complete drug release within the first 6 h. The blend microparticles showed slower drug release than the PDLL microparticles. The total cumulative drug release of the blend microparticles decreased when the stearic acid ratio was increased. The total cumulative drug release at 48 h decreased from 77% to 48% when the stearic acid ratio was increased from 2.5% to 10%.

Table 2 Drug loading of the drug-loaded blend microparticles

PDLL/stearic acid (w/w)	DLC_{actual} (%)	DLE (%)
100/0	2.30 ± 0.33	25.31 ± 3.64
97.5/2.5	2.26 ± 0.08	24.85 ± 0.83
95/5	2.72 ± 0.01	29.96 ± 0.10
92.5/7.5	3.03 ± 0.29	33.30 ± 3.15
90/10	3.03 ± 0.05	33.29 ± 0.59

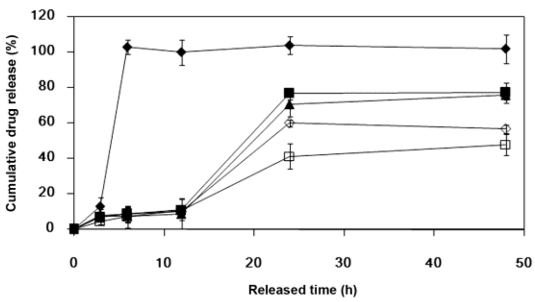

Figure 3 *In vitro* drug release from the blend microparticles prepared with PDLL/stearic acid blend ratios of (◆)100/0, (■) 97.5/2.5, (▲) 95/5, (◇) 92.5/7.5 and (□) 90/10 (w/w)

Discussions

The morphology of the blend microparticles was investigated from their SEM micrographs as shown in Figure 1. The PDLL and all blend microparticles with fine dispersibility were observed. The results indicate that the preparation conditions, including polymer concentration (4% w/w), volumes of oil (2.5 mL) and water phases (400 mL), stirring speed (800 rpm) and evaporation time (3 h) for the oil-in-water emulsion solvent evaporation method used in this study were appropriate for fabricating the drug—loaded blend microparticles with a good dispersibility.

The drug-loaded blend microparticles with PDLL/stearic acid blend ratios of 100/0, 97.5/2.5, 95/5 and 92.5/7.5 (w/w) were nearly spherical in shape with a smooth surface as shown in Figures 1(a)—1(d). This suggests that the PDLL/stearic acid blend ratio in this range did not affect the morphology of the microparticles. However, the 90/10 (w/w) PDLL/stearic acid blend microparticles were irregular in shape with a deflated surface as shown in Figure 1(e). This may be due to the self-aggregate of steraric acid molecules to induce irregular microparticles. The average particle sizes of the blend microparticles determined from their SEM micrographs were similar. The PDLL/stearic acid blend ratio did not affect the average particle size.

Thermal transition properties have been used to characterize the blend particles.[13] Each blend component showed its thermal transition properties, such as glass transition temperature (T_g) and melting temperature (T_m). The PDLL has a T_g at 40—50°C. The stearic acid has a T_m at 55°C. The DSC thermograms of the drug-loaded blend microparticles are compared in Figure 2. The DSC results are summarized in Table 1. The The PDLL microparticles showed a single glass transition temperature (T_g) at 50°C without a melting peak. The PDLL is amorphous. The T_g of the blend microparticles steadily decreased as the stearic acid ratio increased which suggested that the PDLL and stearic acid were partial miscible. The blend microparticles also exhibited a single melting temperature (T_m) in the range 55—57°C due to stearic acid crystallinity. The heat of melting (ΔH_m) for the blend microparticles, which was directly related to stearic acid crystallinity, increased with the stearic acid ratio, which supports the blend microparticles with various stearic acid ratios can be prepared.

The drug loading results in Table 2 showed that the DLE values of the blend microparticles slightly increased with the stearic acid ratio. This may be explained by the interactions of the stearic acid indomethacin model drug that were stronger than the PDLL-indomethacin interactions during the microparticle formation due to hydrophobic characteristic of stearic acid. Polymer blending has been widely used to adjust the drug release behaviors by varying the blended ratio.[14,15] Different properties of polymer components such as crystallinity and hydrophobicity affected on drug release behaviors. As shown in Figure 3, the drug release content decreased when the stearic acid ratio was increased. This may be due to the hydrophobicity of the stearic acid component induced slower release of poorly water-soluble model drug.

Conclusions

The present work demonstrated that drug-loaded blend microparticles with various PDLL/stearic acid blend ratios could be successfully prepared by the oil-in-water emulsion solvent evaporation method. The microparticles with PDLL/stearic acid blend ratios in the range of 100/0—92.5/7.5 (w/w) were spherical in shape and had smooth surfaces, but the 90/10 (w/w) blend microparticles were not. Particle size and drug loading efficiency of the PDLL and all the blend microparticles were similar. The thermal transition properties of the blend microparticles strongly depended upon the blend ratio. The drug release from the blend microparticles can be tailored by adjusting the PDLL/stearic acid blend ratio. These blend microparticles are considered to be promising drug carriers for controlled release of poorly water-soluble drugs.

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References

1. O'Hagan DT, Singh M, Gupta RK. Poly(lactide-*co*-glycolide) microparticles for the development of single-dose controlled-release vaccines. Adv Drug Del Rev 1998;32:225-46.
2. Chung T-W, Huang Y-Y, Liu Y-Z. Effects of the rate of solvent evaporation on the characteristics of drug loaded PLLA and PDLLA microspheres. Int J Pharm 2001;212:161-9.
3. Cui F, Cun D, Tao A, Yang M, Shi K, Zhao M, et al. Preparation and characterization of melittin-loaded poly(DL-lactic acid) or poly(DL-lactic acid-co-glycolic acid) microspheres made by the double emulsion method. J Controlled Release 2005;107:310-9.
4. Liu M, Zhou Z, Wang X, Xu J, Yang K, Cui Q, et al. Formation of poly(L,D-lactide) spheres with controlled size by direct dialysis. Polymer 2007;48:5767-79.
5. Baimark Y, Srisa-ard M. Preparation of drug-loaded microspheres of linear and star-shaped poly(D,L-lactide)s and their drug release behaviors. J Appl Polym Sci 2012;124:3871-8.
6. Jain RA. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. Biomaterials 2000;21:2475-90.
7. Freiberg S, Zhu XX. Polymer microspheres for controlled drug release. Int J Pharm 2004;282:1-18.
8. Dong Y, Feng S-S. Nanoparticles of poly (D,L-lactide)/methoxy poly (ethylene glycol) - poly(D,L-lactide) blends for controlled release of paclitaxel. J Biomed Mater Res 2006;78A:12-9.
9. Muller RH, Mader K, Gohla S. Solide lipid nanoparticles (SLN) for controlled drug

delivery – a review of the state of the art. Euro J Pharm Biopharm 2000;50:161-77.
10. Jaspart S, Bertholet P, Piel G, Dogne J-M, Delattre L, Evrard B. Solid lipid microparticles as a sustained release system for pulmonary drug delivery. Euro J Pharm Biopharm 2007;65:47-56.
11. Perge L, Robitzer M, Guillemot C, Devoisselle J-M, Quignard F, Legrand P. New solid lipid microparticles for controlled ibuprofen release: formulation and characterization study. Int J Pharm 2012;422:59-67.
12. Dalpiaz A, Mezzena M, Scatturin A, Scalia S. Solid lipid microparticles for the stability enhamcement of the polar drug cyclopentyladenosine. Int J Pharm 2008;355:81-6.
13. Baimark Y, Srisuwan Y. Thermal characterization of biodegradable methoxy poly (ethylene glycol) – b – poly (D, L-lactide) /methoxy poly (ethylene glycol)-b-poly(caprolactone) blend nanoparticles. J Therm Anal Calorim 2013;112:795-803.
14. Mi F-L, Lin Y-M, Wu Y-B, Shyu S-S, Tsai Y-H. Chitin/PLGA blend microspheres as a biodegradable drug-delivery system: phase-separation, degradation and release behavior. Biomaterials 2002;23:3257-67.
15. Kang Y, Yin G, Ouyang P, Huang Z, Yao Y, Liao X, Chen A, Pu X. Preparation of PLLA/PLGA microparticles using solution enhanced dispersion by supercritical fluids (SEDS). J Colloid Interf Sci 2008;322:87-94.