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Effects of solvent evaporation rate on the properties of protein-loaded PLLA and PDLA microspheres fabricated by emulsion-solvent evaporation process

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This paper investigated the effects of the rate of solvent removal by varying the ambient pressure at a fixed temperature on the morphology, particle size, encapsulation efficiency and release pattern of albumin-loaded PLLA and PDLA microspheres, prepared by the W/O/W emulsion-solvent evaporation process. For PLLA microspheres prepared either with a fast rate of solvent evaporation (FRSE) or a normal rate of solvent evaporation (NRSE) process, the difference in morphology was minor. In contrast, the different processes did affect the morphology of PDLA microspheres. Large (surface) pores were observed for PDLA microspheres fabricated with a FRSE process, while a smooth surface was seen in those with a NRSE process. With the FRSE process, both PLLA and PDLA microspheres showed smaller particle sizes and lower albumin encapsulation efficiencies than those prepared in the NRSE process. PLLA microspheres prepared with the NRSE process had higher drug encapsulation efficiencies than PDLA ones, but this was not the case for the FRSE process. An initial burst release of albumin was observed for both PLLA and PDLA microspheres prepared with the NRSE process, while a lesser burst release was seen for those prepared with the FRSE process. In subsequent stages of drug release, PLLA microspheres prepared with the two different processes showed differences, but this was not the case for PDLA ones.

Keywords: Poly(L-lactide), poly(D,L-lactide), solvent evaporation, microsphere, morphology.

Introduction

Poly(L-lactic acid) (PLLA), poly(D,L-lactic acid) (PDLA) or their derivatives such as poly(D,L-lactide-co-glycolide) (PLGA) have been widely applied as carriers for drug delivery. Since they are biodegradable and show no adverse tissue reactions (Conti *et al.* 1992, Gogolewski *et al.* 1993, Gupta *et al.* 1997) there is no need to retrieve the carrier after the drug is depleted.

The characteristics of drug-loaded PLLA, PDLA or PLGA microspheres are affected by process parameters such as the concentration of drug, the volume ratio of disperse-continuous phase, and the technique of solvent removal (Sato *et al.*

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1988, Arshady *et al.* 1991, Li *et al.* 1995, Jeyanthi *et al.* 1997, Atkins *et al.* 1998, Chung *et al.* 2001). In preparing PLLA or PDLA microspheres loaded with hydrophilic materials, such as peptides and proteins, W/O/W double emulsion techniques with dichloromethane solution (DCM) and polyvinyl alcohol solution (PVA) as a solvent and stabilizer of the suspension have been widely applied, respectively (Le Corre *et al.* 1994, Jeyanthi *et al.* 1997, Yang *et al.* 2000, Chung *et al.* 2001). After droplets are formed in the W/O/W suspension, the technique of solvent removal (e.g. temperature gradient or dilution of continuous phase) affects the characteristics of drug-loaded PLLA or other PLA-based microspheres, such as morphology, particle size and release profile (Li *et al.* 1995, Huang *et al.* 1997, Jeyanthi *et al.* 1997, Yang *et al.* 2000, Chung *et al.* 2001). Among those solvent removal techniques when preparing PLA related microspheres, temperature gradient or dilution of the continuous phase for solvent evaporation has been applied and investigated by some investigators (Li *et al.* 1995, Jeyanthi *et al.* 1997, Yang *et al.* 2000). Reducing the ambient pressure to facilitate solvent evaporation from the continuous phase or so-called 'fast rate of solvent evaporation' (FRSE) process also influenced the morphology and crystalline structure for hydrophobic drug-loaded PLLA or PDLA microspheres prepared by the O/W emulsion-solvent evaporation process (Chung *et al.* 2001). Whether the FRSE process would affect the characteristics of protein-loaded PLLA or PDLA microspheres, prepared by the W/O/W emulsion-solvent evaporation process, was interesting to investigate. The study was therefore conducted with albumin as a model protein.

Materials and methods

Materials

Albumin, PLLA (Lot 92H0795) and PDLA (Lot 117H0889) with average molecular weights of 93 and 106 kDa, respectively, were purchased from Sigma Company. PVA (degree of polymerization of 2000) were purchased from Nacclai Corp. (Nacclai Tesque, Kyoto, Japan). DCM, chloroform and other reagents (Alps Chemical Corp., Hsinchu, Taiwan) were LC grade and used as received.

Fabrication of PLLA and PDLA microspheres with FRSE or NRSE processes

The W/O/W/ double emulsion-solvent evaporation method was applied to fabricate PLLA and PDLA microspheres. Albumin was dissolved in 2 ml of deionized water that contained 0.05 wt% of PVA surfactant (Gander *et al.* 1995, Yang *et al.* 2000). The solution was poured into 0.5 g of PLLA or PDLA dissolved in 5 ml of DCM solution and emulsified by a probe ultra-sonicator (GE 50T, Cole-Palmer Co., USA) at 4°C to form an O/W emulsion. The O/W suspension was added to 50 ml of 0.05 wt% of PVA solution and emulsified by the probe ultra-sonicator to form a W/O/W emulsion suspension.

The emulsified suspension was then mechanically stirred, and the solvent was simultaneously evaporated at 25°C under different ambient pressures, which were 760 mmHg (or so-called 'normal rate of solvent evaporation' (NSRE)) process and 160 mmHg (or FRSE) process to form gel-type microspheres. After 2 and 6 h of solvent evaporation for FRSE and NRSE processes, respectively, solidified album-loaded PLLA or PDLA microspheres were formed. The hardened microspheres

were rinsed with distilled water several times to remove PVA and then dried in a vacuum chamber at 4 °C for 24 h to remove residual solvent.

Interfacial tension of DCM/PVA solution, particle sizes and surface morphology of microspheres

The interfacial tension between DCM containing 1–10% of PLLA or PDLLA and 0.5% of PVA solution was measured by a face-surface tensiometer (CBVP-A3, Kyowa Interface Science Co., Toyko, Japan) at 25 °C (Chung *et al.* 2001).

The particle size of albumin-loaded PLLA or PDLLA microspheres were determined by a laser particle analyser (Galai CIS counter, USA) after being suspended in 10 ml of 0.05 wt% of sodium dodecyl sulphate (SDS) solution.

The morphological difference of the surface of albumin-loaded PLLA or PDLLA microspheres prepared with the FRSE or NRSE process was examined by scanning electron microscopy (Hitachi S800, Tokyo, Japan).

Drug content and release studies of microspheres in vitro

The microspheres were dissolved in DCM, and the albumin content of microspheres was determined after extraction of albumin with pH = 7.4 isotonic phosphate buffer solution (PBS). In general, 1.5 mg of each batch of the PLLA or PDLLA microspheres was dissolved in DCM, and then 5 ml of PBS solution was added into the above solution for the extraction of albumin. The solution was mixed by a vortex mixer, clarified by standing for 15 min, and then centrifuged at 900g, the upper level of solution was analysed for albumin with the BCA method (Smith *et al.* 1985). The solution was incubated with BCA working reagents (1:20 in volume) for 1 h and then the absorbance of the solution was measured by a double beam of a UV/VIS spectrophotometer at a wavelength of 562 nm (Jasco UV/VIS 530, Kobe, Japan). The encapsulation percentage was determined by the ratio of actual content of drug/theoretical maximum loading of drug.

To study the release of albumin from microspheres, 10 mg of microspheres were suspended in 20 ml of isotonic phosphate buffer solution of pH = 7.4 and 0.1 wt% of sodium azide as dissolution medium, and the samples were shaken at 60 rpm at 25 °C; 0.5 ml of dissolution medium was periodically drawn out, and centrifuged to obtain albumin-containing solution (in upper level). The solution was then analysed by the BCA method. Same volumes of fresh medium were replaced. Data presented are mean $\pm$ SD.

Results and discussion

The rate of solvent removal during the hardening process of emulsified droplets when preparing PLLA and PLGA microspheres is an important factor to affect crystallinity and other characteristics of the microspheres (Sato *et al.* 1988, Arshady 1991, Li *et al.* 1995, Jeyanthi *et al.* 1997, Chung *et al.* 2001). For example, in preparing lidocaine or peptide-loaded PLLA or PLGA microspheres, differences in surface morphology and porosity of the core were observed when different solvent removal techniques, such as dilution of continuous phase, were applied (Jeyanthi *et al.* 1997, Yang *et al.* 2000, Chung *et al.* 2001).

A smooth surface of albumin-loaded PLLA microspheres was observed when the NRSE process was applied to remove solvent, although some of them has some quantities of pinholes scattered on their surface (figure 1(a)). A similar surface morphology of microspheres was also observed when the NRSE process was applied also with some pinholes scattered on the surface (figure 1(b)). These results are different from an earlier study on lidocaine loaded into PLLA microspheres in an O/W emulsion system (Chung *et al.* 2001). The difference may be due to different hydrophobicity of the substrates to be loaded (e.g. lidocaine and albumin, respectively) into different emulsion suspensions (O/W and W/O/W system, respectively). Figure 1(c) shows a smooth surface of albumin-loaded PDLA microspheres prepared with the NRSE process. In contrast, holes and/or pits were observed on the surface and inside albumin-loaded PDLA microspheres fabricated by the FRSE process (figure 1(d)). Similar results have been reported in preparing peptide-loaded PDLA microspheres with the FRSE process by other techniques (Jeyanthi *et al.* 1996, Yang *et al.* 2000). The different effects of the two processes on surface morphology for PLLA and PDLA microspheres might be due to crystalline and amorphous characteristics for L and D,L type PLA materials, respectively.

The FRSE and NRSE processes also affected the particle size profiles of albumin-loaded PLLA and PDLA microspheres. Both PLLA and PDLA microspheres prepared with the FRSE process showed smaller sizes than those prepared with the NRSE process (figure 2). It has been reported that the disperse phase viscosity, stirring rate and the concentration of PVA emulsifier during the emulsion process will affect the particle size of PDLA microspheres (Grandfils *et al.* 1992).

One possible explanation for the smaller size of PLLA or PDLA microspheres in the FRSE process might be interpreted by the Laplace equation, that could be expressed as $dP = 2\gamma/r$ when a liquid droplet is formed (Atkins 1998). dP is the pressure difference between outside and inside of the liquid film of a droplet, γ is the surface tension of the interface of the emulsion system, and r is the radius of a spherical droplet. Here, dP was assigned as the pressure differences between the external PVA solution and the internal PLLA or PDLA droplets during solvent evaporation. The γ was assigned as the interfacial tension between the solutions of different weight of PLLA or PDLA dissolved in DCM and the solution of 0.05 wt% of PVA at 25 °C. The γ values were -24.0 Nm^{-1} and -27.3 Nm^{-1} for 10% of PLLA/DCM and PDLA/DCM in PVA solution, respectively. It was interesting to find that the γ values of the above-mentioned systems were only slightly changed (e.g. less than 8%), when the weight ratios of PLLA and PDLA in DCM was varied more than 10 times. So, the γ values for those systems could be assumed to be constant during the solvent evaporation period. On the other hand, dP values during the solvent evaporation period for both materials were larger for the FRSE process than that for the NRSE process. According to the Laplace equation, it is reasonable to qualitatively estimate that the mean radius of microspheres yielded with FRSE process would be smaller than that for NRSE process for forming stabilized liquid droplets.

The encapsulation efficiency of albumin in PLLA and PDLA microspheres prepared with the NRSE process was 75.5 and 62.0%, respectively. However, there was only 47.2 and 45.2% of albumin encapsulated in PLLA and PDLA microspheres prepared with the FRSE process (figure 3), respectively. The

Figure 1. SEM photographs for albumin loaded PLLA microspheres prepared with (a) NRSE process, (b) FRSE process (the ambient pressure, 160 mm-Hg); and PDLLA microspheres prepared with (c) NRSE process, (d) FRSE process (the ambient pressure, 160 mm-Hg).

Figure 2. Particle sizes of PLLA and PDLLA microspheres prepared with NRSE and FRSE processes ($n = 3$, data presented is mean $\pm$ SD).

Figure 3. Albumin encapsulation efficiency of PLLA and PDLLA microspheres prepared with NRSE and FRSE process ($n = 3$, data presented is mean $\pm$ SD).

microspheres prepared with the NRSE process had higher encapsulation efficiency than those prepared with the FRSE process for both PLLA and PDLLA, similar to an earlier report (Chung *et al.* 2001). Interestingly, the encapsulation efficiency of albumin-loaded microspheres for this study varied with the rate of solvent evaporation while, in other techniques, it was only weakly related to solvent removal rates (Yang *et al.* 2000). Those differences might be due to applying

Figure 4. Albumin release from (a) PLLA and (b) PDLLA microspheres prepared with NRSE and FRSE processes ($n = 3$, data presented is mean $\pm$ SD).

different solvent removal techniques (e.g. reducing ambient pressure and dilution of continuous phase, respectively) that resulted in different conditions of solvent removal.

An initial burst release of albumin from PLLA and PDLLA microspheres prepared in both FRSE and NRSE processes were observed, although that for microspheres fabricated with NRSE was larger than FRSE ones within 5 h (figure 4(a)–(b)). In the subsequent stage, there was a slight difference in the rate of albumin releases for PLLA microspheres fabricated by those two processes, but not for PDLLA. To reduce burst release of the microspheres, double walled

microspheres could be applied (Huang *et al.* 1999, Chiou *et al.* 2001, Pekarek *et al.* 1994).

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