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Paclitaxel-loaded poly(L-lactic acid) microspheres 3: blending low and high molecular weight polymers to control morphology and drug release

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Abstract

Microspheres were prepared from paclitaxel and binary polymer blends incorporating 1, 3, 40k and 100k g/mol PLLA. Thermal analysis was performed by DSC and in vitro paclitaxel release profiles were determined at 37 °C in phosphate buffer using an HPLC assay. In microspheres made with 3k/40k PLLA blends, the glass transition (T_g), crystallinity and melting temperature (T_m) all decreased with an increasing proportion of low molecular weight polymer in the blend. Similar trends were observed for 1k/100k blends. T_m values ranged from 175 to 110 °C and T_g values between 66 and 37 °C. However, for 1k/100k blends, melting point depression was linearly dependent on blend composition when plotted as $1/T_m = 0.000109 \times (\%1k \text{ in blend}) + 0.0223$, $R^2 = 0.97$. A similar plot with data from the 3k/40k system yielded a non-linear relationship. Furthermore, the decrease in T_g for both 1k/100k and 3k/40k blends followed the Fox equation, although experimental values were consistently 1–2 °C above predicted values. Paclitaxel release from microspheres made with a 1k/100k blend occurred in four distinct phases: a burst phase (day 0), a slower phase, a second burst (day 35) and a second slower phase (until day 70). The second burst coincided with visible degradation of the microspheres. Blends of low and high molecular weight PLLA display thermal properties indicating that 1k g/mol PLLA behaves as a diluent when blended with 100k g/mol PLLA, being excluded from the crystalline domains in the polymer matrix. In contrast, 3k g/mol PLLA is incorporated in both amorphous and crystalline regions of the polymer blend. Paclitaxel release profiles from 1k/100k PLLA microspheres demonstrate a multiphase profile due to the effects of both diffusion and degradation controlled release mechanisms.

Keywords: Polymer blending; PLLA; Drug release; Thermal properties; Paclitaxel; Microsphere

1. Introduction

Release profiles of drugs from microsphere systems are controlled by a number of parameters including polymer molecular weight (Burton et al., 2000). For paclitaxel-loaded PLLA in particular, we have characterized this effect from microspheres with polymer molecular weights ranging between 2k and 100k g/mol. The release characteristics of paclitaxel deviated from classical diffusion kinetics, because of the heterogeneity of paclitaxel dispersed within the matrix (Liggins and Burt, 2001). Based on these

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release profiles, a two-compartment kinetic model was proposed for microspheres with PLLA molecular weight up to 50k g/mol. At 100k g/mol, paclitaxel-loaded microspheres exhibited deposition of paclitaxel near the surface of the microspheres, resulting in an apparent zero-order release profile after an initial burst phase (Liggins and Burt, 2004). Low molecular weight (2k g/mol) PLLA microspheres were found to release drug rapidly over several days while 100k g/mol microspheres gave slower, incomplete release even after several weeks. Neither formulation provided prolonged release of the total dose over several weeks.

The strategy of blending multiple polymers in a formulation may be employed to alter drug release and improve efficacy. Burton et al. (2000) demonstrated improved efficacy and release characteristics of leuprolide by combining microspheres each made using different PLGA polymers, while others have proposed blending multiple polymers within a single matrix to modulate properties. Polyesters in particular have been blended with each other (Ravivarapu et al., 2000; Cao and Schoichet, 1999) or with more hydrophilic polymers such as polyethylene glycol (Nijenhuis et al., 1996; Jiang and Schwendenman, 2001) in order to achieve properties intermediate between those of the two polymers. To increase hydrophilicity, amorphous poly(DL-lactic acid) (PDLLA) matrices have been modified by the addition of a lower molecular weight PDLLA fraction (Bodmeier et al., 1989; Bain et al., 1999), resulting in enhanced drug release and polymer degradation rates relative to higher molecular weight PDLLA alone.

Despite the research interest in polymer blending, the effects of blending two molecular weights of a single semicrystalline polyester, such as PLLA, on drug release have not been well characterized for microsphere systems. We propose that the addition of very low molecular weight (1 or 3k g/mol) to high molecular weight (40 or 100k g/mol) PLLA can be used to modify the thermal and crystalline properties of the semicrystalline matrix, and therefore modulate and enhance drug release from the matrix. This approach should provide an improvement over the previously reported formulation of paclitaxel-loaded 100k g/mol PLLA microspheres (Demetrick et al., 1997; Liggins et al., 2000). This latter formulation was effective in preventing tumor growth following intraperitoneal injection after a tumor cell spill and was well tolerated at an intraperitoneal dose of 100 mg of 30% paclitaxel-loaded microspheres. However, paclitaxel release was incomplete meaning a higher total dose than was required for efficacy was administered.

2. Methods

Paclitaxel was obtained from Hauser (Boulder, CO). PLLA was obtained from Birmingham Polymers, Inc. (Birmingham, AB) and Polysciences, Inc. (Warrington, PA). L-Lactic acid was from Sigma-Aldrich. (St. Louis, MO). All solvents were HPLC grade (Fisher Scientific, Fairlawn, NJ) and all other reagents were of analytical grade (Sigma-Aldrich, St. Louis, MO). A polycondensation reaction using a method reported earlier (Liggins and Burt, 2001) was used to synthesize 1k g/mol PLLA from L-lactic acid. The molecular weights of all polymers were measured by GPC with universal calibration according to a previously reported method (Liggins and Burt, 2001).

Control, 10, 20 and 30% (w/w) paclitaxel-loaded microspheres were prepared using a previously described solvent evaporation method (Liggins and Burt, 2001). By altering the stirring speed and PVA concentration in the aqueous (internal) phase of the dispersion (Table 1), microspheres in two distinct size ranges were produced.

Particle size distributions of microspheres were determined using a Coulter LS-130 laser diffraction particle size analyzer. Immediately prior to analysis, microsphere samples were suspended in water with 0.01% polysorbate 80 and sonicated for 5 min to prevent aggregation.

The extent to which microspheres could be resuspended in water after drying was determined as follows. Fifty milligrams of microspheres were placed in a 1.5 ml Eppendorf tube and 1 ml of distilled water was added. The mixture was vortexed for 1 min and allowed to stand for 1 min to allow any aggregates to settle to the bottom. The supernatant was transferred to a glass vial and this process repeated twice more. The remaining aggregates in the Eppendorf tube were transferred to another glass vial. Both vials were centrifuged at 1500 rpm for 10 min and the supernatant removed. The vials were dried for 24 h and weighed to determine the masses of the resuspended and aggregated fractions.

Table 1
Total content of paclitaxel in 1–15, 10–35, 35–105 μm microspheres made with a blend of 60:40 1k:100k g/mol PLLA

PVA concentration (%, w/v)	Stir speed (rpm)	Microsphere size (μm)	Paclitaxel encapsulation efficiency (% of theoretical paclitaxel loading)	
			10% loading	30% loading
2.5	2100	1–15	128	79
2.5	900	10–35	115	109
1.0	900	35–105	112	109

Values are averages of three measurements from a single batch of microspheres. The precision in measurement was greater than 91% for all samples.

Microspheres were observed by scanning electron microscopy (SEM) at a magnification of 1000×. Samples were mounted onto carbon disks, coated with 100 Å gold–palladium and analyzed using an electron voltage of 10 kV with a S-2300 scanning electron microscope (Hitachi, Japan).

Thermal properties of microspheres were observed by differential scanning calorimetry (DSC). Samples weighing 3–5 mg were analyzed with a Dupont model 910S DSC (New Castle, DL) in unsealed pans (Perkin Elmer, Norwalk, CT). The degree of crystallinity (X_C) of PLLA was calculated using the equation:

$$X_C = \frac{\Delta H_f - \Delta H_c}{93.7\,J/g} \times 100\% \quad (1)$$

where ΔH_f and ΔH_c are the enthalpies of fusion and recrystallization, respectively, calculated from the area under the curve for both recrystallization and melting peaks and 93.7 J/g is the enthalpy of fusion for 100% crystalline polymer (Celli and Scandola, 1992). To control for thermal history, thermograms were obtained for samples stored at room temperature without any previous heating or cooling.

The total content of paclitaxel in microspheres was determined in a manner previously reported (Liggins and Burt, 2001). Five milligrams of microspheres were dissolved in 1 ml of dichloromethane and diluted with 20 ml of 60:40 acetonitrile:water. Two clear phases were allowed to separate with PLLA precipitating at the interface. The paclitaxel contents of each phase were determined by HPLC.

The procedure for measuring in vitro paclitaxel release from microspheres has been previously reported (Liggins and Burt, 2001). Briefly, 3 mg of microspheres were suspended in 50 ml of phosphate buffered saline with 0.4% albumin (PBS) and at given time intervals, paclitaxel was extracted from the PBS into dichloromethane. The organic extract was dried at 45 °C under nitrogen prior to reconstitution in 1 ml of 60:40 acetonitrile in water and analysis by reverse phase HPLC with UV detection at 232 nm.

3. Results

GPC analysis gave the molecular weights (M_{GPC}) of the PLLA polymers (labeled as 1, 2, 50k and 100k g/mol) as 1, 3, 40 and 100k g/mol, respectively. Henceforth, polymers are referred to by their M_{GPC} values.

Two series of blends were selected for study, one series being a combination of commercially obtained 40k and 3k g/mol PLLA and the other series being commercially obtained 100k g/mol PLLA and in-house synthesized 1k g/mol PLLA.

For 3k/40k g/mol PLLA blends, an aqueous phase composition of 10% PVA and a stirring rate of 900 rpm were used to prepare microspheres in a size range of 1–30 μm at all polymer blend ratios. Blend ratios were 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100 (3k:40k g/mol PLLA). Five batches of microspheres were made from each of the 3k and 40k g/mol PLLA alone and three batches were made from each blend composition. Scanning electron microscopy confirmed that the microspheres were spherical with smooth surfaces.

Representative DSC thermograms (scanned at 10 °C/min) of microspheres made with blends of 3k and 40k g/mol PLLA are shown in Fig. 1 and the effects of blend composition on thermal properties of the polymers are illustrated in Fig. 2. A single glass transition was observed for all blends, indicating that the amorphous components of the two polymers are miscible.

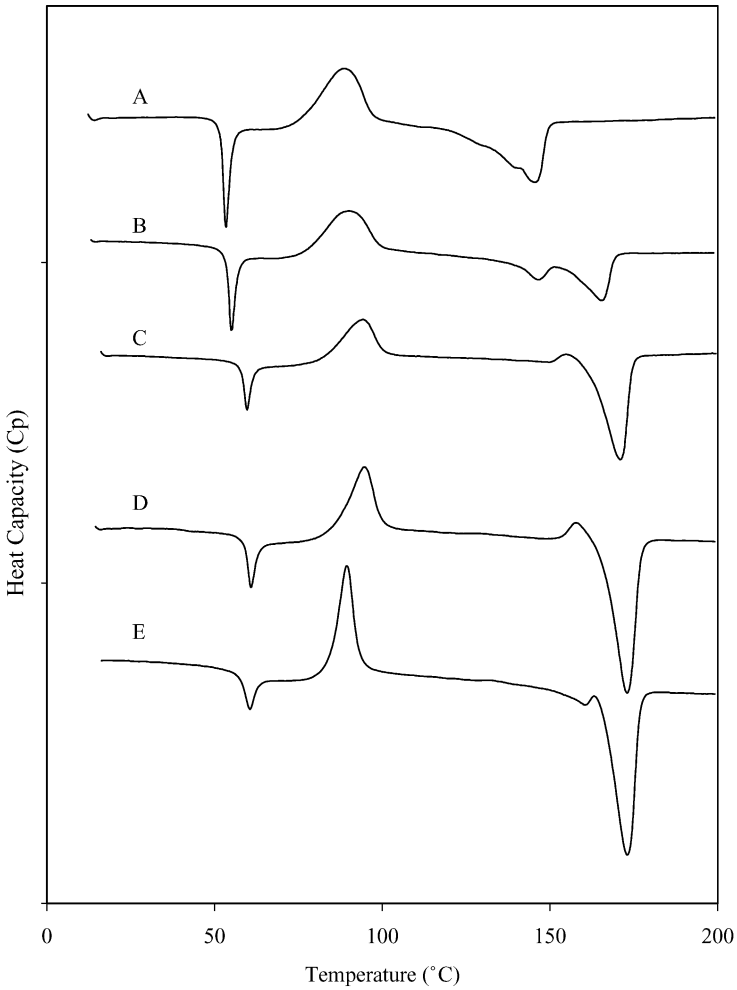


Fig. 1. Representative DSC thermograms of microspheres with size range of 1–30 μm prepared from blends of 3k and 40k g/mol PLLA with compositions of (A) 100:0, (B) 80:20, (C) 60:40, (D) 20:80 and (E) 0:100 3k:40k g/mol PLLA at 10 °C/min.

Microspheres prepared from all blend ratios showed recrystallization around 90 °C upon heating above the glass transition (Fig. 1). For PLLA blends, the degree of crystallinity decreased as the proportion of 3k g/mol PLLA increased. T_m was lower for 3k g/mol PLLA (145 °C) than 40k g/mol PLLA (173 °C) microspheres and T_m values for blends were between the two extremes (Fig. 2B). All microspheres, including 100% 3k and 40k g/mol PLLA, respectively, showed asymmetry in the melting transition due to the presence of a small shoulder peak (with slight exothermic deflection in the thermogram) prior to the main melting event. This phenomenon (a double melting endotherm) was most prominent in the 80:20 3k/40k blend (Fig. 1B).

PLLA with a molecular weight of 1k g/mol was blended in several ratios with 100k g/mol PLLA to make microspheres. A PVA concentration of 1% and stirring rate of 900 rpm were used for the preparation of these microspheres. Microspheres with a blend composition of up to 60% 1k g/mol PLLA were spherical and could be freely resuspended in water following the drying step in the manufacturing process. Microspheres with greater than 60% of the low molecular weight component were irregularly shaped and tended to aggregate at 90–100% 1k g/mol PLLA content.

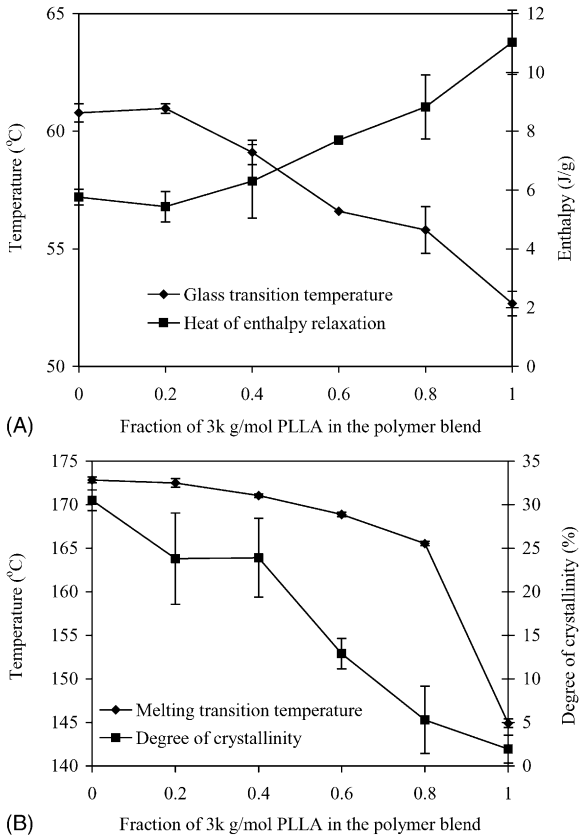


Fig. 2. The effects of polymer blend composition in microspheres made from 3k and 40k g/mol PLLA on (A) the glass transition and (B) the melting transition and degree of crystallinity ($n = 3$).

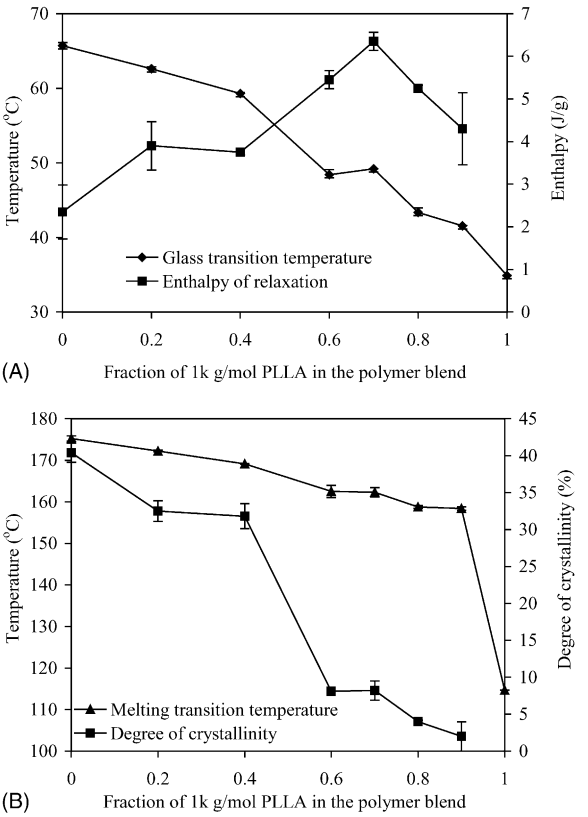


Fig. 3. The effects of polymer blend composition in microspheres made from 1k and 100k g/mol PLLA on (A) the glass transition and (B) the melting transition and degree of crystallinity ($n = 3$).

The thermal properties of the 1k/100k g/mol polymer blends are shown in Fig. 3. The trends were very similar to those for the 3k and 40k g/mol PLLA blends (Fig. 2). The glass transition and melting temperatures and the degree of polymer crystallinity were all lowered by the addition of 1k g/mol PLLA to the 100k g/mol PLLA. A double melting endotherm was observed for blends containing 70% or greater of the 1k g/mol polymer. The double endotherm was observed as a shoulder prior to T_m, between 100 and 130 °C.

The dependence of melting point depression on the blend composition for both 1k/100k and 3k/40k g/mol PLLA systems are shown in Fig. 4. The inverse T_m relationship (Eq. (2)) was previously described by Martinez-Salazar et al. (1996):

$$\frac{1}{T_{m2}} - \frac{1}{T^{\circ}_{m2}} = \frac{R}{\Delta h}\frac{1}{m_1}(1 - \phi_2) \quad (2)$$

where ϕ_2, Δh, T_{m2} and T°_{m2} are the weight fraction, heat of fusion of the polymer repeating unit, and depressed and equilibrium melting points of a semicrystalline component in a polymer blend, respectively, and m_1 the molar volume of the other component of the blend. In Fig. 4, blend composition data were adjusted assuming incomplete incorporation of the 1k g/mol polymer. Blend compositions were calculated based on the starting proportions of 1k and 100k g/mol polymers and the assumption that 34% of the 1k g/mol polymer was not incorporated. The is assumption is based on a 34% weight fraction of the 1k g/mol PLLA being water soluble, as previously determined (Liggins and Burt, 2001). Melting point data for 1k/100k g/mol

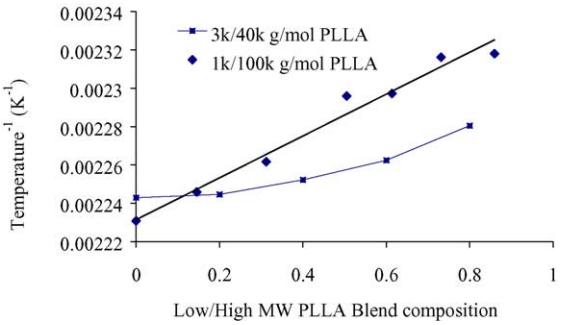


Fig. 4. Change in the observed melting temperature (T_m) with the addition of 1–100 and 3k–40k g/mol PLLA.

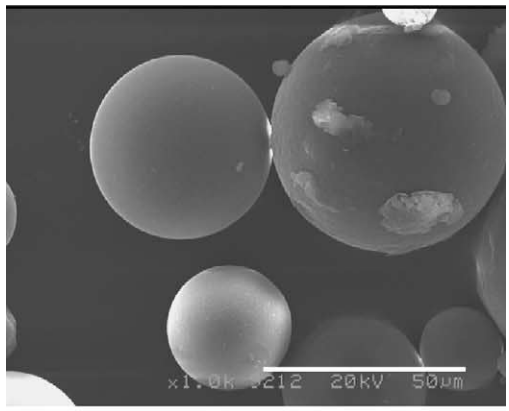
blends gave a linear plot ($R^2 = 0.97$) according to Eq. (2), whereas data for 3k/40k blends did not.

The blend ratio of 60% 1k g/mol PLLA to 40% 100k g/mol PLLA was selected for further study because it was the highest ratio of low/high molecular weight polymer which could form spherical microspheres and be resuspended in water. Microspheres made from this blend composition were called polyblend-60 or "PB60" microspheres.

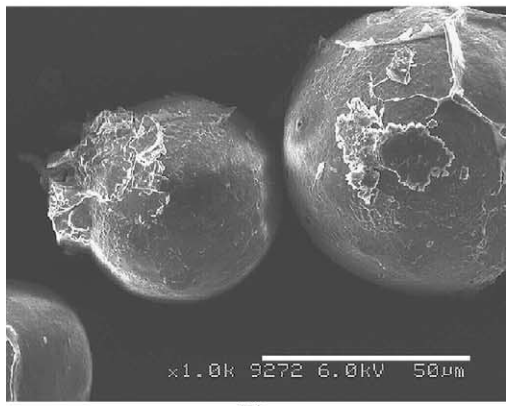
PB60 microspheres were made using the stirring speeds and PVA concentrations listed in Table 1 to produce size ranges of 1–15 and 35–105 μm. Particle size analysis yielded particle size distributions in which >90% (w/w) of the particles were within the defined ranges. Scanning electron micrographs showed that microspheres in all sizes and paclitaxel loadings (control, 10, 20 and 30%) were spherical with a smooth surface. Fig. 5A shows a representative micrograph for 20% paclitaxel-loaded 35–105 μm microspheres. All other sizes and loadings had a similar appearance.

PB60 microspheres exhibited generally good efficiency of incorporating paclitaxel (Table 1). Greater than 100% encapsulation efficiency was observed in the majority of formulations due to incomplete incorporation of the low molecular weight polymeric component. However, microspheres in the size range of 1–15 μm with theoretical loading of 30% had only 79% of the theoretical amount of paclitaxel in the microspheres.

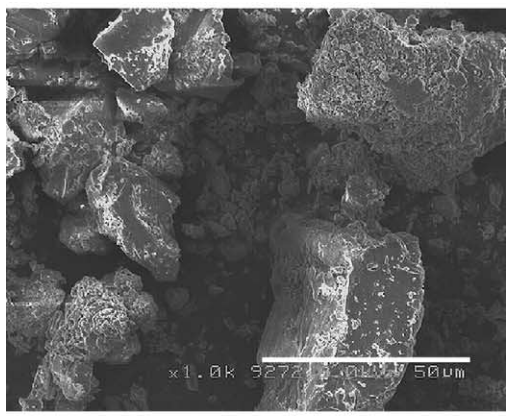
Representative thermograms of control and 20% paclitaxel-loaded PB60 microspheres in the 35–105 μm size range are shown in Fig. 6 and the transitions are summarized in Table 2 along with those in the 1–15 μm size range. Similar data were obtained



(A)



(B)



(C)

Fig. 5. The surface morphology of 20% paclitaxel-loaded microspheres in the size range of 35–105 μm made from a 60:40 blend of 1k and 100k g/mol PLLA after (A) day 0, (B) day 15 and (C) day 70 of an in vitro release study. (Magnification of micrographs is 1000×.)

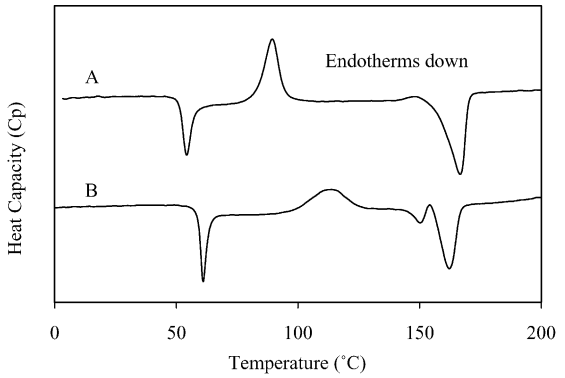


Fig. 6. DSC thermograms of (A) control or (B) 20% paclitaxel-loaded 35–105 μm microspheres made with a 60:40 blend of 1k and 100k g/mol PLLA containing.

for 10 and 30% paclitaxel-loaded PB60 microspheres. Control microspheres possessed a greater degree of crystallinity than did paclitaxel-loaded microspheres. A melting point depression of 2–4 °C was observed in the 20% loaded microspheres compared to control. This effect was drug loading dependent, with 30% paclitaxel-loaded 35–105 μm microspheres having a 6 °C depression in T_m. The addition of paclitaxel to PB60 microspheres was accompanied by a 7–10 °C increase in T_g (see Fig. 6 and Table 2). The elevation of T_g was observed in both size ranges and at all loadings. The recrystallization temperature (T_c) of the polymer was also higher in microspheres that contained paclitaxel. Control and 20% paclitaxel-loaded microspheres had values of T_c around 90 and 115 °C, respectively.

The addition of paclitaxel also resulted in a change in the shape of the melting endotherm. A small shoulder peak preceded the major polymer melting peak in paclitaxel-loaded microspheres when thermograms were obtained with a 10 °C/min heating rate (Fig. 6B). The peak temperature was 10 °C less than the major T_m for microspheres containing 20–30% paclitaxel and 15 °C lower for 10% loaded microspheres. The ΔH of the shoulder peak varied from approximately 5 to 30% of the total ΔH_f of the polymer as the paclitaxel loading level increased from 10 to 30%. However, at a scanning rate of 40 °C/min, the shoulder peak was not observed, a behavior typical of double melting endotherms.

In vitro paclitaxel release profiles for 10 and 30% loaded microspheres in the size ranges of 1–15 and 35–105 μm (formulations exhibiting the extremes in loading level and particle size) are shown in Fig. 7, expressed as a percentage of total loading. The features that were common to all the release profiles were an initial rapid release of 5–35% of total loading over approximately 3 days, a slower phase that lasted until around day 21 at which point the rate then increased, followed by continued slow release over the remaining 4–5 weeks of the study. By day 50 of the study, release rates had slowed to 0.1–0.3%/day. These values were similar to rates observed in the initial linear phase after day 21 (0.15–0.5%/day). Over the course of the study, only 30–60% of total paclitaxel was released. This partial release is similar to the amount released over 30 days for 100k g/mol PLLA microspheres (Liggins and Burt, 2004) however, the profile is much different. High molecular weight PLLA released paclitaxel with a larger initial burst and release slowed to almost no

Table 2
Thermal properties of control and 20% loaded paclitaxel 60:40 1k:100k g/mol PLLA microspheres with size ranges of 1–15, 10–35 and 35–105 μm

Theoretical loading	T_g[a] (°C) (ΔH_r[b], J/g)	T_C[a] (°C) (ΔH_c[b], J/g)	T_m[a] (°C) (ΔH_f[b], J/g)	X_C (%)
1–15 μm microspheres				
Control	55 (5.5)	83 (13)	166 (46)	18
20%	62 (7.4)	111 (18)	164 (41)	2
35–105 μm microspheres				
Control	53 (6.8)	92 (17)	167 (46)	8
20%	61 (7.6)	116 (22)	163 (41)	5

[a] Peak transition temperature data are averages of three measurements made from a single batch of microspheres for each size and loading. Peak temperature values varied by <2 °C for all transitions.
[b] Peak area data varied <1 J/g for ΔH_r and <5 J/g for ΔH_c and ΔH_f.

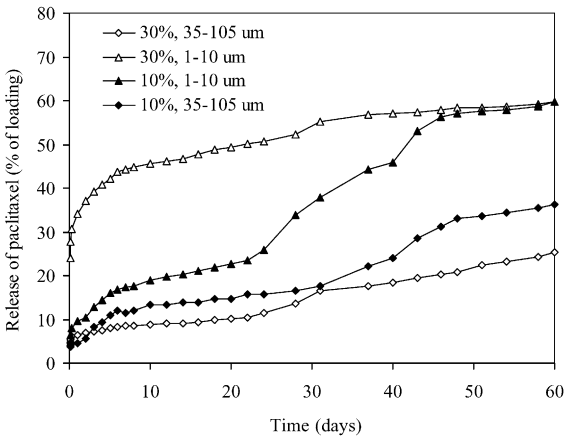


Fig. 7. In vitro release profiles of paclitaxel from (A) 10% and (B) 30% paclitaxel-loaded microspheres made with a 60:40 blend of 1k and 100k g/mol PLLA.

release after 21 days, whereas for PB60 microspheres, the burst was less pronounced and release continued for the duration of the study. Since no final plateau was reached, presumably release would have continued for some time after the 60 days studied.

The morphologies of PB60 microspheres at days 15 and 70 of an in vitro release study are shown in Fig. 6. By day 15 (Fig. 7B) the microspheres had begun to show signs of surface erosion. By day 70, PB60 microspheres had disintegrated leaving irregularly shaped particles with diameters of up to 75 μm (Fig. 7C).

4. Discussion

Two blend systems have been compared, one containing 3k and 40k g/mol PLLA and the other 1k and 100k g/mol PLLA. The properties of the two high molecular weight polymers (40k and 100k g/mol) used in this work were similar with respect to thermal properties and their ability to form microspheres. However, the 1k and 3k g/mol polymers were different from each other, with 1k g/mol PLLA having values of T_g and T_m, 18 and 30 °C lower, respectively, than values for 3k g/mol PLLA.

The composition of the 3k/40k g/mol blends affected the area of the endothermic peak that coincided with the T_g in the microspheres (Figs. 1and 2A). This peak was attributed to enthalpy relaxation at T_g, which has been reported for other PLLA polymers (Celli and Scandola, 1992). The magnitude of ΔH_r was greater for 3k g/mol than for 40k g/mol PLLA and was intermediate for the blends. Grandfils et al. (1996) also observed that enthalpy relaxation was a molecular weight dependent phenomenon for PDLLA. Enthalpy relaxation was observed in 3.5k g/mol PDLLA microspheres that was not observed in 65k g/mol PDLLA microspheres reflecting the so-called microstructure in the amorphous phase that can arise in polymers as they are precipitated from solution (Bodmeier et al., 1989), such as in solvent evaporation microsphere formation.

Recrystallization was observed when 3k/40k g/mol PLLA blend microspheres were heated above T_g. The T_c decreased as the proportion of 3k g/mol polymer increased in the blend. Both the T_c and T_g decreased approximately 7 °C as the proportion of 3k g/mol polymer increased from 0 to 100% so that the value of T_c was approximately 35 °C above the T_g for all blend compositions. Nijenhuis et al. (1996) also noted that for polymer blends of PLLA with PEG, the T_c of PLLA decreased with a decrease in the T_g so that T_c was always approximately 45 °C above the T_g. As the T_g is lowered, the mobility of the polymer chains is increased at any given temperature above T_g and recrystallization becomes possible at lower temperatures.

Melting of 3k and 40k g/mol PLLA blends occurred at temperatures that were dependent on the blend composition. Melting point depression data also confirmed that 3k and 40k g/mol PLLA were miscible (Figs. 2B and 4). However, the relationship between blend composition and melting point depression was non-linear when the data were plotted using Eq. (2) (Fig. 4). This non-linear relationship between melting point depression and blend composition means that Δh, the enthalpy of fusion per repeating unit, varies with blend composition. A change in Δh with an increase in the proportion of 3k g/mol PLLA is likely due to an increasing number of chain ends in the blend (Wünderlich, 1973). The presence of an increased number of chain ends with the addition of 3k g/mol PLLA resulted in more chain ends being included in crystallites, altering their surface free energy and thereby affecting their melting temperature. A non-linear relationship between melting point depression and blend composition for data plotted using Eq. (2) has also been noted for 8k and 83k g/mol PLLA blends (von Recum et al., 1995), although these were not in microspheres. Optical microscopy

of these blends showed that two different types of crystallites were present. The crystallites were termed "small" and "large" and were attributed to crystallites that were rich in 8k and 83k g/mol PLLA chains, respectively. It is therefore possible that the chains in the 3k/40k g/mol PLLA blends do not completely mix in the crystallites formed in microspheres. The formation of crystallites rich in 3k and 40k g/mol PLLA could be responsible for the double melting endotherm observed most clearly for the blend in Fig. 1B. Crystallites with more 3k than 40k g/mol PLLA would have a lower T_m than those containing proportionately more 40k g/mol PLLA.

The second polymer blend system studied was of 1k and 100k g/mol PLLA. In this blend system, thermal properties were similar in many respects to those of the 3k/40k g/mol PLLA blends. As with the 3k/40k g/mol polymer blends, a single T_g was observed for the 1k/100k g/mol PLLA blends, indicating polymer miscibility in the amorphous phase.

Similar to the 3k/40k blends, an increase in ΔH_r and a decrease in polymer crystallinity for 1k/100k blends were observed with an increase in the proportion of the low molecular weight component (Fig. 3A and B, respectively), that were similar to those observed for the 3k/40k g/mol polymer blends. However, in contrast to the 3k/40k g/mol polymer blends, the relationship between the melting point depression data and blend composition was linear when plotted using Eq. (2) (Fig. 4). Thus Δh, which is proportional to the slope of the linear melting point depression–molecular weight relationship, was not affected by the addition of 1k g/mol PLLA to the higher molecular weight 100k g/mol polymer. The short 1k g/mol chains of PLLA were too small to be incorporated into the polymer crystallites and did not affect Δh. We have identified an important dependence of blend properties on molecular weight for PLLA polymers. High molecular weight PLLA will incorporate 3k/g/mol PLLA into its crystallites, resulting in decreased perfection of these crystallites and the possibility for two types of crystallites to exist in the matrix. In contrast, 1k g/mol PLLA will not be incorporated into the 100k g/mol crystallites, being instead excluded into the amorphous phase. Thus while both blends exhibit melting point depression, the crystallites in the 1k/100k blends are structurally less affected by the low molecular weight component than are blends comprising the 3k g/mol polymer. Conversely, the amorphous phase of the 1k/100k blend will be more greatly affected on a per mass basis by the addition of the 1k g/mol polymer. This is consistent with the 30 °C drop in T_g for the 1k/100k blends in contrast to only a 8 °C drop in T_g in the 3k/40k blends.

Microspheres prepared with blends containing either 1k or 3k g/mol PLLA as the low molecular weight component also had different resuspension properties. Whereas the 3k/40k g/mol polymer blend microspheres were all freely resuspendable in water after drying, the proportion of 1k g/mol PLLA in the blend affected the resuspension index of the 1k/100k g/mol blend microspheres, despite the fact that T_g of both systems was above room temperature at which all microspheres were stored. The differences in resuspension properties coincided with changes in the surface morphology (Fig. 3) of the microspheres and with a sudden increase in polymer crystallinity in compositions having less than 60% low molecular weight component (Fig. 4B).

The 1k/100k g/mol polymer blend with 60% low molecular weight component (PB60) was chosen for further characterization since it represented the maximum amount of low molecular weight material that could be used to formulate spherical, resuspendable microspheres. A similar "critical" ratio was reported for a blend of 3k and 120k g/mol PDLLA used to make microspheres. A blend containing up to 75% of the 3k g/mol PDDLA could be used to manufacture intact microspheres (Bodmeier et al., 1989). The same limit was observed when blending 3.5k and 65k g/mol PDLLA (Grandfils et al., 1996). In using a semicrystalline polymer however, an even lower molecular weight component could be effectively used in the blend. By maximizing the amount of low molecular weight component and minimizing its molecular weight, it was expected that paclitaxel microspheres could be manufactured which would have maximally different properties compared to 100k g/mol PLLA microspheres, reported earlier (Liggins and Burt, 2004).

Paclitaxel loading did not affect the surface properties of PB60 microspheres, and all PB60 microspheres had smooth surfaces. The smooth surfaces were similar to those observed for microspheres made from PLLA with molecular weights of less than 10k g/mol (Liggins and Burt, 2001), whereas paclitaxel-loaded

100k g/mol microspheres had a dimpled appearance (Liggins and Burt, 2004), which was attributed to surface deposition of the drug. Providing the observed smooth surfaces indicate less extensive surface deposition of paclitaxel, and hence a slower rate of microsphere formation (Liggins and Burt, 2004) the work of Li et al. (1995) would support a more homogeneous distribution of drug in the matrix. This morphology is consistent with a less extensive burst phase observed for PB60 microspheres in contrast with the previously reported 100k g/mol PLLA microspheres, which gave up to 50% release in the burst phase.

In contrast to the effect of paclitaxel loading on the thermal properties of 100k g/mol PLLA microspheres (Liggins and Burt, 2004), the addition of paclitaxel to PB60 microspheres resulted in an increase in T_g indicating that paclitaxel is miscible with the amorphous phase and acts to stiffen the matrix, likely through interactions with the polymer. Increased ability for paclitaxel–polymer interactions may also contribute to less drug segregation that could be manifested as surface drug deposition. Enhanced interactions between paclitaxel and the polymer chains may occur through hydrogen bonding due to the 50–60-fold increase in the number of hydroxyl and carboxyl groups contributed by the chain ends of the 1k g/mol PLLA compared to 100k g/mol PLLA alone.

An increase in the T_c of PLLA was observed after the addition of paclitaxel to the microspheres (Fig. 6). This is consistent with paclitaxel being dispersed throughout the amorphous phase and acting as a diluent molecule in the polymer matrix, which is known to slow polymer recrystallization (Mandelkern, 1964). Interactions between paclitaxel and the 1k and 100k g/mol PLLA, such as hydrogen bonding with polymer chain ends, could also have contributed to the greater than 20 °C increase in T_c of the blend (Fig. 6). It is likely that paclitaxel interacts predominately with low molecular weight PLLA since only a 2–6 °C increase in T_c was observed for 100k g/mol PLLA microspheres (Liggins and Burt, 2004). Hydrogen bonding between paclitaxel molecules and polymer chains would be expected to reduce the rate of polymer chain diffusion through the amorphous regions of the polymer matrix as they migrate towards growing crystallites in the matrix.

As observed for 100k g/mol PLLA microspheres, addition of paclitaxel to PB60 microspheres resulted in a depression of T_m, providing further evidence for miscibility of paclitaxel in the PB60 matrix. A double melting endotherm was observed when paclitaxel was incorporated into the PB60 matrix that was not observed for control microspheres. It is possible that paclitaxel is in the amorphous phase, which resulted in a higher T_c and a broadening of the recrystallization exotherm, may also have altered the mechanism of crystallization so as to produce less perfect polymer crystallites with a lowered T_m (Mandelkern, 1964). This hypothesis was supported by observing only a single melting endotherm by DSC at a fast heating rate of 40 °C/min. Furthermore, no melting of crystalline paclitaxel was observed at 217 °C, as has been previously reported (Liggins et al., 1997). At faster heating rates, the imperfect crystallites melt but do not have time to recrystallize to form more perfect crystallites before the temperature rises above the equilibrium melting temperature of the polymer. This explanation is more likely than the presence of two different types of crystalline regions containing 1k and 100k g/mol PLLA, respectively. According to Fig. 3B, the degree of crystallinity for the 1k g/mol PLLA alone was almost zero. Thus, the 1k g/mol PLLA chains excluded from the crystallizing polymer in the PB60 blends are not believed to recrystallize on their own to form a second type of crystallite. In this behavior the 1k/100k g/mol blend system again differs from the 3k/40k g/mol blend system, which exhibits crystallinity in the low molecular weight component, providing for an alternative mechanism by which recrystallization may occur. Double melting endotherms were also observed in the non-drug loaded 3k/40k g/mol blends and in the 3k and 40k g/mol polymers alone. This phenomenon may also be due to the formation of less perfect crystallites, which melt, and may partly recrystallize, resulting in a slight exothermic event prior to the main melting peak. This was particularly evident in the 80:20 blend of 3k/40k g/mol polymers, for which less perfect crystallites are expected to form as a result of the incorporation of both molecular weight chains into the crystallites.

In vitro paclitaxel release from microspheres was modulated by the addition of 1k g/mol PLLA to the polymer matrix when compared to release profiles for paclitaxel-loaded microspheres made from 100k g/mol PLLA alone (Liggins and Burt, 2001). The initial rate of release of paclitaxel was greater from microspheres

manufactured from 100k g/mol PLLA alone than from PB60 microspheres, when comparing microspheres of similar sizes and loadings. The period of prolonged release was extended by several weeks, with an acceleration of release after the third week. In studies over a similar time range using 100k g/mol PLLA microspheres, no second burst was observed, thus blending low and high molecular weight PLLA affords more extensive or rapid release of paclitaxel than microspheres made from a single high molecular weight fraction.

In this report, we have demonstrated that as expected paclitaxel release from PLLA microspheres may be modulated by the addition of a low molecular weight component. However, the effects were not all as anticipated. The maximum burst effect was reduced, rather than accelerated, compared with high molecular weight microspheres. This may be explained by increased drug polymer interaction, which is supported by the calorimetric data, and by empirical evidence of less surface drug deposition, resulting in a smooth microsphere surface. In addition, the lower T_g for the blend may prolong the time for solidification during formation, resulting in a more homogeneous matrix as described by Li et al. (1995). Most importantly, two unique properties of the blend system were described. Firstly, the degree of interaction of paclitaxel and the low molecular weight polymer was different than that previously described for the high molecular weight polymer. Secondly, the thermal properties of two apparently similar blends were defined, demonstrating a dramatic difference in polymer crystallization behaviors for PLLA having 1k and 3k g/mol molecular weights. Whereas 3k g/mol PLLA can crystallize and become incorporated into crystallites with high molecular weight PLLA, 1k g/mol cannot. Thus, the lower molecular weight polymer results in more profound effects on the amorphous region. These effects together explain the release kinetics of paclitaxel from the microspheres, further extending the previously reported models of release of paclitaxel from semi-crystalline PLLA microspheres.

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