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Paclitaxel loaded poly(L-lactic acid) microspheres: properties of microspheres made with low molecular weight polymers

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Abstract

Microspheres were prepared from poly(L-lactic acid) polymers having molecular weights between 500 and 50k g/mol. The polymers were synthesized using two initiator molecules, L-lactic acid oligomer (PLLA-LA) or stearyl alcohol (PLLA-SA). For both PLLA-LA and PLLA-SA polymers, glass (Tg) and melting (Tm) transition temperatures and enthalpy of melting all increased as the polymer molecular weight increased. PLLA-SA showed the greatest change in Tg (-13 to 54°C) as molecular weight increased from 500 to 10k g/mol, compared to 25 to 55°C for PLLA-LA polymers. Changes in Tm and enthalpy of melting with increasing molecular weight were similar for both PLLA-LA and PLLA-SA. Paclitaxel release from 30% paclitaxel loaded microspheres in the size range of 50–90 μm was affected by these changes in polymer properties as molecular weight increased. As the molecular weight increased from 2k to 50k g/mol the amount of drug released from microspheres over 14 days decreased from 76 to 11% of the initial drug load. The release profiles were consistent with a diffusion controlled mechanism provided a two-compartment model was employed. According to this model, the total amount of 'available' drug (compartment 1) was released by diffusion in 14 days while the remainder (compartment 2) was confined within the polymeric matrix and could not diffuse out at a measurable rate. Following the in vitro release study, microsphere made from 2k–10k g/mol polymers showed significant signs of disintegration whereas 50k g/mol polymer microspheres remained intact.

1. Introduction

Biodegradable microspheres prepared using polymers such as poly(L-Lactic acid) (PLLA) have been used extensively to deliver drugs to the body over prolonged periods of time (Mumper & Jay, 1992; Jalil & Nixon, 1990). The release rate profiles of drugs from microspheres are a function of the physicochemical properties of both the drug and the polymer. For a given drug-polymer combination, control of the release profile may also be achieved by varying properties such as drug loading (Kishida et al., 1990) and polymer molecular weight (Omelczuk & McGinity, 1992). Studies using poly(DL-lactic acid) (PDLLA) in the molecular weight range of 4k–500k g/mol

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(Omelczuk & McGinity, 1992) showed that drug release from microspheres was inversely proportional to the polymer molecular weight. However, polymers with molecular weights below 4k g/mol have received less attention, likely due to unfavorable thermal and mechanical properties at extremely low molecular weights. For example, PDLLA with a molecular weight of 1400 g/mol has a Tg below 20°C (Asano et al., 1991) and would therefore be expected to exhibit plastic flow at room temperatures.

We have reported that paclitaxel loaded microspheres prepared using high molecular weight (100k g/mol) PLLA were effective in the prevention of tumor growth in the peritoneal cavity when administered intra-peritoneally, after a simulated tumor cell spill (Demetrick et al., 1998; Liggins et al., 2000). However, release of paclitaxel from the microspheres was incomplete and there was no microscopic evidence of degradation of the microspheres after 6 weeks in vivo. The use of low molecular weight PLLA to prepare paclitaxel loaded microspheres should produce delivery systems with more rapid drug release and erosion rates.

In this work we have investigated PLLA polymers with molecular weights ranging from 500 to 50k g/mol. Because PLLA is a semicrystalline polymer, with a Tg above ambient temperatures (Engelberg & Kohn, 1991), we hypothesized that very low molecular weight PLLA may be used to produce intact microspheres. The effects of PLLA molecular weight on microsphere crystallinity, thermal properties, resuspendability and paclitaxel release rates were evaluated.

2. Methods

2.1. Materials

Paclitaxel was obtained from Hauser (Boulder, CO). PLLA, molecular weight 50k g/mol, and polystyrene molecular weight standards ranging from 300 g/mol to 100k g/mol were obtained from Polysciences (Warrington, PA). All solvents were HPLC grade (Fisher Scientific, Fairlawn, NJ) and all other reagents were of analytical grade (Sigma-Aldrich, St. Louis, MO).

PLLA was synthesized by ring-opening polymerization (Schindler et al., 1982) from L-lactide (Polysciences, Warrington, PA) with an initiator (L-lactic acid (LLA) oligomer or stearyl alcohol) and 0.5% stannous 2-ethyl hexanoate to catalyze the reaction. The reaction was carried out at 150°C for 4 h in evacuated glass ampoules. To control the molecular weight of the polymer, the masses of reactants were added stoichiometrically according to the equation:

$$\left(\frac{\mathrm{Mn}^*}{\mathrm{Mol.\ Weight}_{\mathrm{Initiator}}}\right) - 1 = \frac{\mathrm{Mass}_{\mathrm{Lactide}}}{\mathrm{Mass}_{\mathrm{Initiator}}} \qquad (1)$$

where Mn* is the molecular weight predicted for each synthesis. Polymers initiated with stearyl alcohol and L-lactic acid oligomer were called 'PLLA-SA' and 'PLLA-LA', respectively.

The L-lactic acid oligomer was prepared by polycondensation of L-lactic acid at 170°C for 7 h, with nitrogen bubbled through the reaction mixture. Prior to its use as a polymerization initiator, the oligomer water content was determined by Karl Fischer titration and its molecular weight was measured by titration of the carboxylic acid end-groups with potassium hydroxide in benzyl alcohol. The titration end point was observed colorimetrically with phenolphthalein.

The molecular weight (M_{GPC}) of polymers was measured by gel permeation chromatography (GPC) using a Hewlett Packard 10^3 Å PLgel column (5 μm × 7.5 × 300 mm) and a Shimadzu GPC system (Tokyo, Japan) with refractive index detection and a mobile phase of chloroform flowing at 1 ml/min. M_{GPC} was calculated from a universal calibration curve constructed using the Mark-Houwink constants for PLLA, $K = 5.45 \times 10^{-4}$ and $a = 0.73$ (Schindler & Harper, 1979).

Control and 30% w/w paclitaxel loaded microspheres were prepared using the oil in water solvent evaporation method (Jalil & Nixon, 1990). PLLA and paclitaxel, total weight 0.5 g, were dissolved in 10 ml dichloromethane. The organic phase was added to 100 ml of an aqueous solution of 2.5% 13–20k g/mol PVA in a beaker and stirred at 24 ± 2°C and 600 rpm with an overhead stirrer. After 2 h the aqueous suspension containing microspheres was passed through 90 and 50 μm sieves and particles in this size range were

retained. Microspheres were air dried for 12–16 h at ambient temperature to form a solid cake. The dried materials were stored at ambient temperature in sealed glass containers.

The extent to which microspheres could be resuspended in water after drying was determined and expressed as a 'resuspension index' (R.I.):

$$\text{R.I.} = \frac{(\text{Mass Resuspended})}{(\text{Mass Resuspended} + \text{Mass Aggregated})} \times 100\% \quad (2)$$

The masses of resuspended and aggregated materials were measured as follows. Approximately 50 mg (accurately weighed) of microspheres were placed in a 1.5 ml Eppendorf tube and 1 ml of distilled water 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 supematant 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.

Microspheres were observed by scanning electron microscopy (SEM). 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).

X-ray powder diffraction (XRPD) patterns for microspheres were obtained using a Geigerflex X-ray diffractometer (Rigaku, Japan). The X-ray source was CuKα radiation (45 kV, 40 mA). The range of 5–40°2θ was scanned at a rate of 5°2θ/min in increments of 0.03°2θ.

Thermal properties of microspheres were observed by differential scanning calorimetry (DSC). Samples weighing 3–5 mg were analyzed with a Dupont model 9108 DSC (New Castle, DL) in unsealed pans (Perkin Elmer, Norwalk, CT) with a heating rate of 10°C/min. The degree of crystallinity (X_c) of PLLA was expressed in terms of the enthalpy of fusion (J/g) of polymer sample analyzed by DSC and may be converted to a percentage using the equation:

$$X_c = \frac{\Delta H_f - \Delta H_c}{93.7\ \text{J/g}} \times 100\% \quad (3)$$

where ΔH_f and ΔH_c are the enthalpies of fusion and recrystallisation, respectively, calculated from the area under the curve for both recrystallisation and melting peaks and 93.7 J/g is the enthalpy of fusion for 100% crystalline polymer (Celli & Scandola, 1992), the enthalpy value being calculated from data collected for PLLA in the range of 5–90 k g/mol molecular weight.

The total content of paclitaxel in microspheres was determined in the following manner. Approximately 5 mg of microspheres were accurately weighed and dissolved in 1 ml of dichloromethane. To the solution were added 20 ml of 60:40 acetonitrile:water, and two clear phases allowed to separate, with PLLA precipitating at the interface. The paclitaxel content of the organic (top) and aqueous (bottom) phase were determined by HPLC. The HPLC sample was prepared by diluting 100 µl of each phase with 900 µl of acetonitrile. Mean values of total content of paclitaxel are calculated from four replicates for each formulation.

The procedure for measuring in vitro paclitaxel release from microspheres was based on the method given by Burt et al. (1995) but with some modifications. Into 50 ml glass, screw capped tubes were placed 50 ml of pH = 7.4 10 mM phosphate buffered saline with 0.4% albumin (PBS) and 3 mg paclitaxel loaded microspheres. The tubes were tumbled end-over-end at 30 rpm and 37°C in a thermostatically controlled oven. At given time intervals, the tubes were centrifuged at 1500 rpm for 10 min and 5 ml of the supernatant saved for analysis. The remainder of the supernatant was removed and the microsphere pellets were resuspended in fresh PBS (50 ml). The buffer was replaced at each sampling interval in order to maintain sink conditions, which were taken to be a paclitaxel concentration in the PBS not exceeding 15% of its solubility. The amount of paclitaxel in 5 ml of the supernatant was determined by extraction of paclitaxel into 1 ml dichloromethane followed by evaporation to dryness at 45°C under a stream of nitrogen, reconstitution in 1 ml of 60:40 acetonitrile in water and analysis by HPLC. The chromatographic condi-

tions used to quantify paclitaxel were a C_{18} reverse phase column, a mobile phase of 58:37:5 acetonitrile:water:methanol flowing at 1 ml/min and UV detection at 232 nm.

3. Results

Prior to its use as an initiator, the LLA oligomer was characterised as having a water content of 3% w/w and a molecular weight (M_n) of 337 g/mol, equivalent to an average of 4.7 repeating units per chain.

Fig. 1 relates the predicted values of Mn* of PLLA polymers based on Eq. (1) to corresponding values of M_{GPC} for both PLLA-SA and PLLA-LA. The molecular weight of PLLA-SA was controlled by the stoichiometric ratio of stearyl alcohol to L-lactide addition over the entire predicted molecular weight range. However, as the intercept of the regression curve indicates, the experimentally determined or observed molecular weight was overestimated by Eq. (1) by approximately 1.2k g/mol for all molecular weights.

For PLLA-LA polymers (Fig. 1) the relationship between predicted and observed molecular weight was linear up to 2k g/mol and the predicted and observed molecular weight values were similar. However, above 2k g/mol, observed molecular weights were much less than those predicted. At a predicted Mn* of 10k g/mol, M_{GPC} was 3.5k g/mol, which represented the maximum molecular weight achieved by this method of synthesis. The M_{GPC} value for the commercially obtained PLLA (50k g/mol) was found to be 41k g/mol.

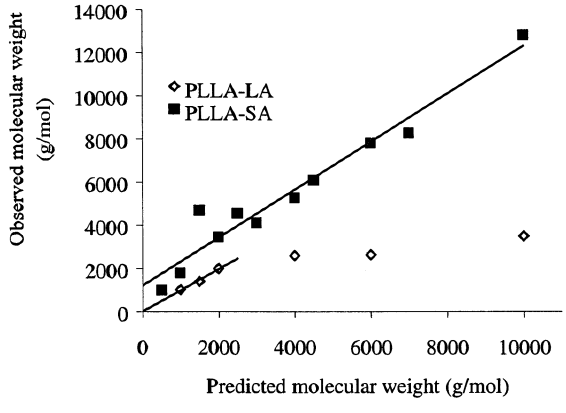


Fig. 1. The relationship between predicted molecular weights (Mn*) and observed values of M_{GPC} for PLLA-SA and PLLA-LA.

Table 1
Total content of paclitaxel in 50–90 μm PLLA microspheres with theoretical loading of 30%[a]

Microsphere composition (molecular weight (g/mol) of PLLA)	Total content of paclitaxel (% w/w)	Efficiency of loading (%)
2k	36	120
4k	34	112
10k	30	99
50k	29	96

[a] Values are averages of three measurements made from each batch of microspheres.
The realtive standard deviation of the measurements is less than 6%

To examine the water solubility of low molecular weight PLLA, samples of the 1k g/mol PLLA-LA were incubated in distilled water at room temperature for several hours to leach out water soluble components. The leaching process was continued over 12 h until the material reached a constant dry weight. The final weight was 67.7 ± 4.8% of the original, indicating that approximately one third of the original material was water soluble. It is possible that more water soluble material was present but was not leached out. The physical appearance of the 1k g/mol PLLA-LA changed from a soft, translucent tacky material to a brittle white solid, after leaching out the low molecular weight components. It was anticipated that the water soluble components would be at least partly leached out of the material during the microsphere manufacturing process and therefore not all of the polymer would be incorporated into the microsphere matrix.

Values of total paclitaxel content in microspheres with a theoretical loading of 30% paclitaxel prepared using 2k g/mol PLLA-LA, 4k and 10k g/mol PLLA-SA and 50k g/mol PLLA are listed in Table 1. A higher than expected content was observed in microspheres made with polymers with molecular weights below 50k g/mol. To

encapsulate greater than 100% of the expected total content of paclitaxel, the extent of incorporation of the polymer must have been less than that of paclitaxel. Assuming firstly, that as little as 67.7% of the polymer may be incorporated due to the loss of water soluble components and secondly, a paclitaxel content equal to the initial paclitaxel loading of 30%, a maximal theoretical loading efficiency of 129% would be expected based on the following relationship:

$$\text{Loading efficiency} = \frac{\text{Paclitaxel content}}{\text{Polymer content} + \text{Paclitaxel content}} \times \frac{1}{\text{Initial Paclitaxel Loading}} \quad (4)$$

Generally, the loading efficiencies decreased from 120 to approximately 100% as the molecular weight of the polymers increased (Table 1).

Representative XRPD patterns of stearyl alcohol and microspheres prepared using PLLA-SA and PLLA-LA are shown in Fig. 2A–C. Two peaks at 21.3 and 24.4°2θ were observed in the pattern of stearyl alcohol (Fig. 2B), which were absent in the patterns for microspheres. All PLLA-SA and PLLA-LA microspheres had similar XRPD patterns, with the most intense peak located between 16.2 and 16.5°2θ and a less intense diffraction peak at 18.8°2θ. These peaks were also evident in a XRPD pattern for high molecular weight PLLA microspheres containing paclitaxel (Fig. 2D). Based on a comparison with previously reported XRPD patterns for paclitaxel (Liggins et al., 1998), no peaks could be attributed to crystalline drug in the microsphere matrix. In XRPD patterns for all microspheres, all peaks were attributed to crystallites comprising mainly the L-lactic acid repeat unit. No contribution to crystallinity by stearyl alcohol was observed by XRPD, indicating its contribution was less than 3%, the sensitivity of the instrument.

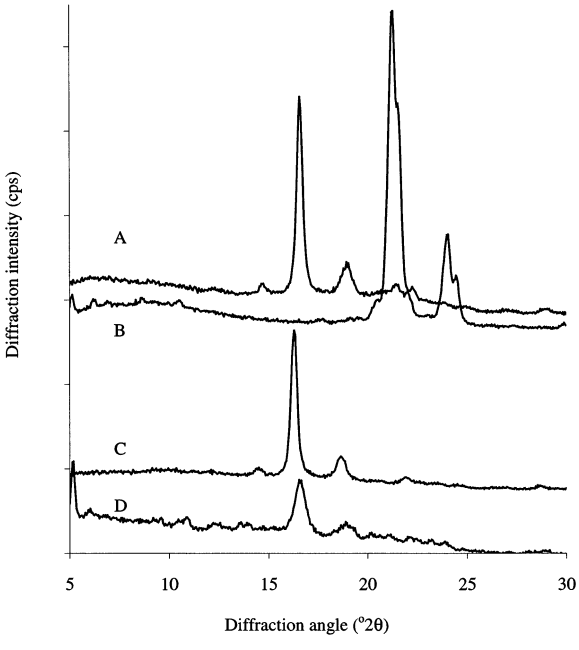


Fig. 2. X-ray powder diffraction patterns of microspheres prepared using (A) 1k PLLA-SA, (B) stearyl alcohol, (C) 1k g/mol PLLA-LA, and (D) 100k g/mol PLLA with 30% paclitaxel.

Fig. 3 shows representative DSC thermograms of microspheres prepared from PLLA-SA polymers. For these microspheres, trends of increasing Tg and Tm with molecular weight were observed. The glass transition increased from −13°C–54°C as the molecular weight of PLLA-SA increased (Fig. 3, inset A). The Tg for 10k g/mol PLLA-SA (Fig. 3D) was taken as the peak temperature of enthalpy relaxation and the Tg of the other PLLA-SA polymers (Fig. 3A–C) were taken as the mid-point in the change in heat capacity (Cp). The endothermic peaks observed around 30°C (Fig. 3A and B) were attributed to the melting of polymer crystallites rich in hydrocarbon chains from stearyl alcohol, while the peaks above 100°C (Fig. 3A-D) were attributed to the melting of polymer crystallites rich in L-lactic acid chains. A recrystallisation exotherm was observed around 80°C for 10k g/mol PLLA-SA.

DSC thermograms of 1k g/mol PLLA-LA as synthesised and the water insoluble fraction of the 1k g/mol PLLA-LA (after incubation in water) are shown in Fig. 4A and B. Thermograms for microspheres made from PLLA-LA polymers are shown in Fig. 4C to E. As synthesised, 1k g/mol PLLA-LA did not show a clear glass transition and during the melting transition, discontinuities were observed in the thermogram which may indicate degradation of the material (Fig. 4A). To

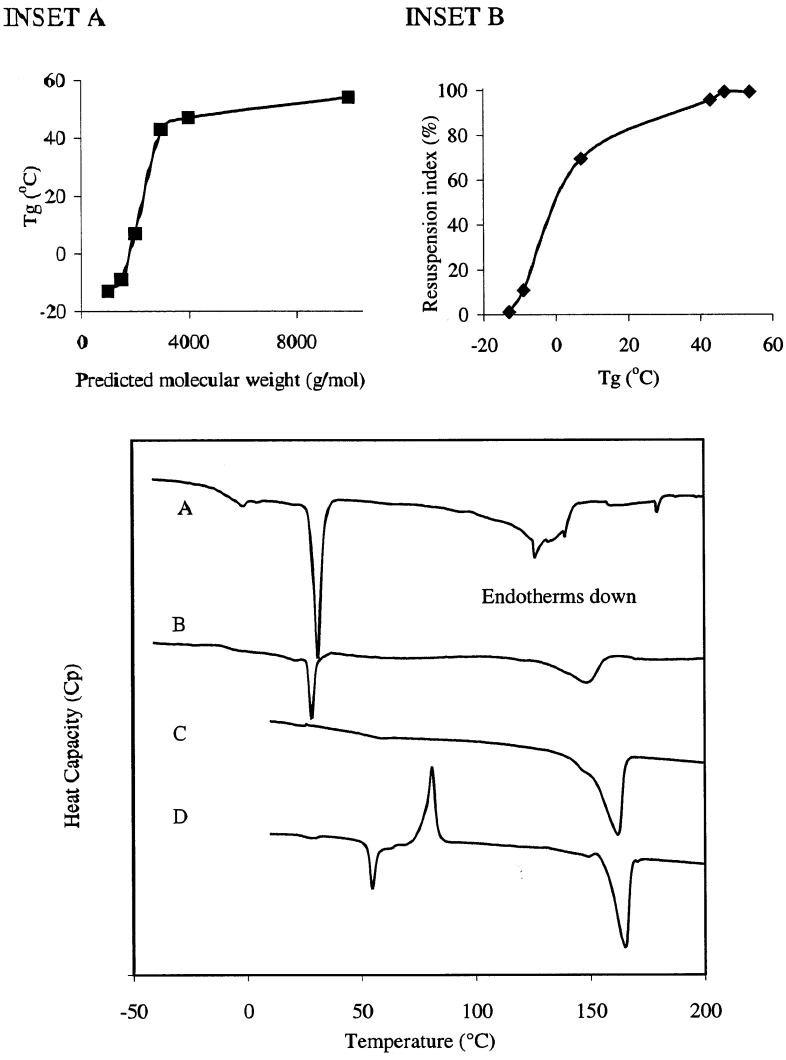


Fig. 3. DSC thermograms of microspheres prepared using (A) 1k, (C) 1.5k, (D) 4k and (E) 10k g/mol PLLA-SA. Inset A: the effect of molecular weight on the Tg. Inset B: the effect of the Tg on the resuspension index of microspheres.

confirm that the discontinuities observed were not a result of solvent evolution (residual water or dichloromethane), thermogravimetric analysis was performed by heating a sample of 1k g/mol PLLA-LA at 10°C/min to 200°C using a Pyris/ Perkin Elmer TGA system. No weight loss was observed until 146°C, at which point the sample became discoloured, consistent with degradation.

Enthalpy relaxation accompanied the glass transition for PLLA-LA in microspheres, partial recrystallisation of the amorphous phase in microspheres was observed around 80°C and melting occurred between 115–150°C (Fig. 4C–E).

The DSC data were obtained from a single heating scan of the microspheres and thus reflect the thermal history imparted by the microsphere preparation process. The history was assumed to be identical for each sample because an identical process was used to prepare microspheres of all polymer molecular weights. The first scan data were collected in order to measure the thermal properties of the microspheres whereas a second

scan would reflect a thermal history imparted by cooling from the molten state. In order to ascertain the effects of thermal history, microspheres were analysed by DSC including a first scan, followed by cooling and re-heating. For DSC samples that were cooled and reheated, no evidence of enthalpy relaxation at the glass transition was observed in the second scan. Furthermore, only the 10k and 50k g/mol polymers samples recrystallised upon heating above the Tg and the melting transition for all samples was characterised by a 1–4°C reduction in peak temperature.

For both PLLA-SA and PLLA-LA polymers, Fig. 5A and B show the relationship of Tg and Tm to molecular weight, according to Eq. (5) and Eq. (6), respectively:

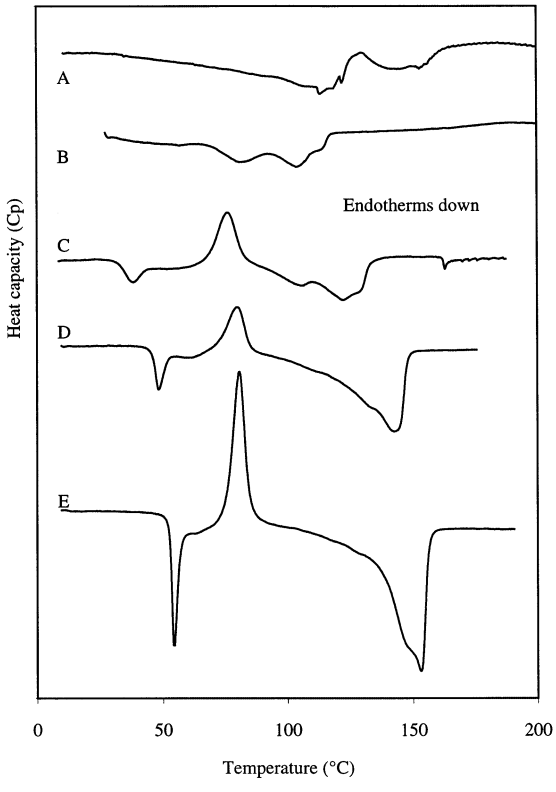
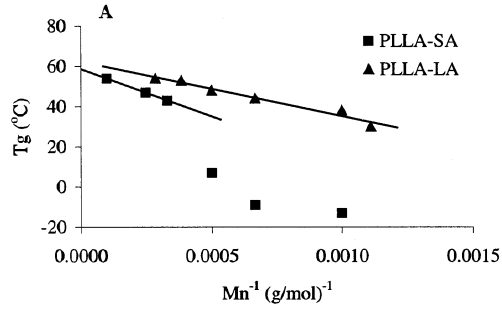
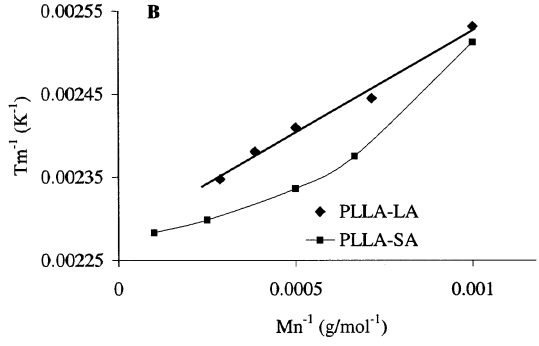


Fig. 4. DSC thermograms (A) 1k g/mol PLLA-LA as synthesised and (B) 1k g/mol PLLA-LA after incubation in water to remove water soluble material, and of microspheres manufactured from PLLA-LA polymers with molecular weights of (C) 1k, (D) 2k and (E) 3.5k g/mol.



Regression analysis parameters (y=mx + b). R^2

PLLA-SA: y = -27000 x + 62, R^2=0.97 (data plotted using Mn*)
PLLA-LA: y = -47000 x + 59, R^2=1.00 (data plotted using Mn)



Regression analysis parameters:

PLLA-LA: y = 0.246 x + 0.0023, R2 = 0.99 (data plotted using Mn)
Data are plotted using Mn* for PLLA-SA

Fig. 5. The effect of polymer molecular weight on (A) Tg and (B) Tm of PLLA-SA and PLLA-LA microspheres, plotted using Eq. (6) and Eq. (7), respectively.

$$\mathrm{Tg} = \mathrm{Tg}^{\infty} - \frac{k}{\mathrm{M_n}} \tag{5}$$

where Tg and Tg^{∞} are the glass transition temperatures of the polymer with molecular weight Mn and infinity, respectively (Eisenberg, 1993). The constant k is a value directly proportional to the free volume per chain end (Kelley & Bueche, 1961) and,

$$\frac{1}{\mathrm{Tm}} - \frac{1}{\mathrm{Tm}^{\circ}} = \frac{2R}{\Delta h} \times \frac{1}{\mathrm{M_n}} \tag{6}$$

where Tm is the observed melting point, Tm° is the equilibrium melting point, Δh is the enthalpy of fusion per repeat unit and R is the gas constant (Mandelkern, 1993).

Tg varied in a linear fashion with 1/Mn* for PLLA-SA with a molecular weight greater than 3k g/mol, deviating from the relationship described in Eq. (5) at lower molecular weights. In contrast, for PLLA-LA polymers, Tg varied in a linear fashion with l/Mn for all molecular weights. For PLLA-LA polymers, the 1/Tm versus 1/Mn relationship was linear (Fig. Fig. 5B) yielding a value of Δh equal to 68 J/g^{-1}. However, the relationship was non-linear for PLLA-SA (Fig. Fig. 5B). Thus for PLLA-SA microspheres, Δh was dependent on molecular weight.

As polymer molecular weight increased, crystallinity also increased. Fig. 6 shows the increase in crystallinity, expressed as the enthalpy of melting as polymer molecular weight increased. Generally, PLLA-LA polymer microspheres increased with molecular weight more rapidly and at a lower molecular weight than did PLLA-SA polymer microspheres. The data are plotted using a log scale on the molecular weight axis to allow closer examination of the data in the low molecular weight range. In general, crystallinity increased with molecular weight. However, the 2k g/mol PLLA-LA was more crystalline than the 4k g/mol PLLA-SA.

The resuspension index of microspheres manufactured from PLLA-SA polymers increased with the Tg of PLLA-SA (Fig. 3, inset B). Greater than 95% w/w of microspheres prepared from polymers with Tg's above storage and ambient temperature (25°C) could be readily resuspended in water.

The resuspension index of microspheres manufactured from PLLA-LA polymers could not be quantified because the material adhered to the walls of the tubes and pipettes used in the assay, owing to the increased hydrophilicity of PLLA-LA compared to PLLA-SA. In the absence of a resuspension index for these microspheres, samples were vortexed in water in an Eppendorf tube and the suspension observed visually and by optical microscopy for the presence of aggregates. PLLA-LA microspheres with molecular weights equal to, or greater than, 1.5k g/mol could be readily resuspended in water. Microspheres manufactured from 50k g/mol PLLA were all completely resuspended. Paclitaxel loading had no effect on the resuspension of microspheres.

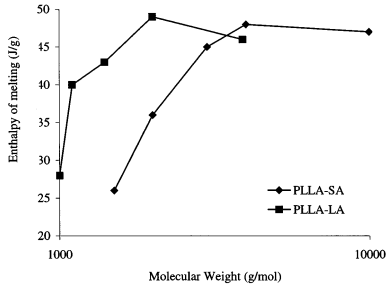


Fig. 6. The effect of polymer molecular weight on the enthaipy of melting of PLLA-LA and PLLA-SA incorporated into microspheres.

The surface morphology of microspheres was observed by scanning electron microscopy and representative micrographs are shown in Fig. 7. Below 2k g/mol, both PLLA-SA and PLLA-LA polymers did not form spherical microspheres. The 1k g/mol PLLA-SA resulted in a single aggregated mass (Fig. 7A) while 1k g/mol PLLA-LA gave irregularly shaped particles (Fig. 7B). Microspheres made from polymers with molecular weights of at least 2k g/mol were all spherical with a smooth surface (Fig. 6C). Microscopic observations were consistent with the evaluation of resuspendability for both PLLA-SA and PLLA-LA polymer microspheres.

In vitro release profiles of paclitaxel from microspheres prepared PLLA polymers with molecular weights between 2k and 50k g/mol are shown in Fig. 8. Paclitaxel release was greatest during the first 2 days. A slower phase was observed between days 2–12. By day 13 of the study, the amount of paclitaxel released from 5 mg of microspheres in all samples decreased below the detectable limit of the assay despite the fact that not all of the paclitaxel was released. Based on a solubility value for paclitaxel in the release medium of 3 μg/ml at 37°C (Winternitz & Zhang, 1997), it was evident that concentrations near saturation were encountered in the first 3 days of the study. If sink conditions had been maintained, a greater amount of paclitaxel would be expected to be released over this time period. However, this would not be expected to alter the cumulative amount released in the course of the entire study. Using ANOVA and Tukey tests, microspheres prepared from 2k g/mol PLLA-LA, 4k and 10k g/mol PLLA-SA

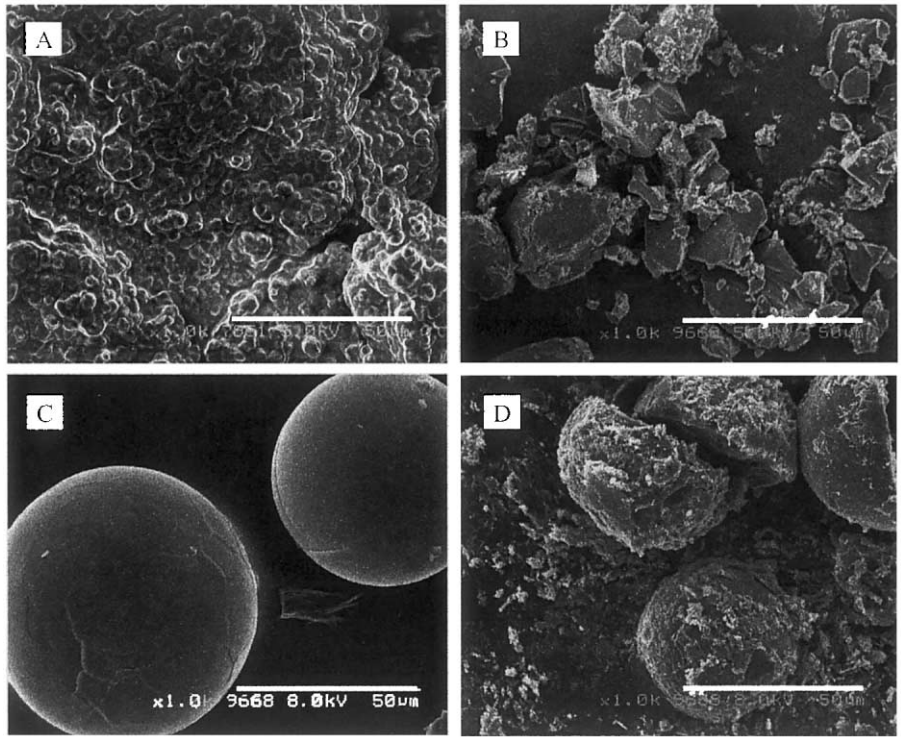


Fig. 7. The surface morphologies of microspheres prepared using (A) 1k g/mol PLLA-SA, (B) 1k g/mol PLLA-LA, (C) 2k g/mol PLLA-LA and (D) 2k g/mol PLLA-LA with 30% paclitaxel, after 9 days in an in vitro drug release study. (Magnification of all micrographs is 1000 ×).

were found to release paclitaxel to a significantly greater extent after 14 days compared to microspheres made from 50k g/mol PLLA ($P < 0.05$).

Based on the total content data from Table 1, between 11 and 76% of the total paclitaxel was released over the 14 days of the release study. These data are shown in Table 2. After the release study was completed, the remaining material was assayed to quantify the amount of residual paclitaxel in the polymer matrix. Based on the total paclitaxel content (refer to Table 1) values of total amount of paclitaxel recovered for each formulation, called percent mass balance, were 78, 98, 112 and 102% for 2k, 4k, 10k and 50k g/mol PLLA polymers, respectively.

Because of the incomplete release of paclitaxel from the microspheres and the negligible release rate observed after day 12 of the release study, it was hypothesised that release kinetics could be described using what we will refer to as a 'two-compartment model'. Compartment 1 was hypothesised to contain paclitaxel that could freely diffuse from the microspheres, while compartment 2 contained paclitaxel immobilised by the semi-crystalline polymer matrix. To test this hypothesis, the fraction of paclitaxel remaining after the release study was related to polymer crystallinity and molecular weight. These values are summarised in Table 2. Generally, as polymer molecular weight and crystallinity increased, the fraction of paclitaxel remaining increased. For the 2k and 4k molecular weight polymers, the effect of crystallinity was more pronounced whereas for 10k and 50k g/mol polymers the crystallinity was unchanged and the fraction remaining increased with molecular weight.

Theoretical curves for release profiles were fit to the data using the equations approximating diffusion controlled release from a sphere, as described by Baker (1987):

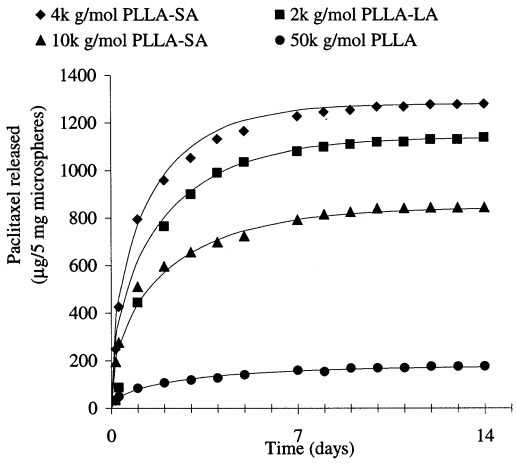


Fig. 8. In vitro release profiles of paclitaxel from 30% paclitaxel loaded microspheres made from PLLA polymers with molecular weights ranging from 2k to 50k glmol. Best-fit curves for diffusion controlled release (Baker, 1987) are shown with the data.

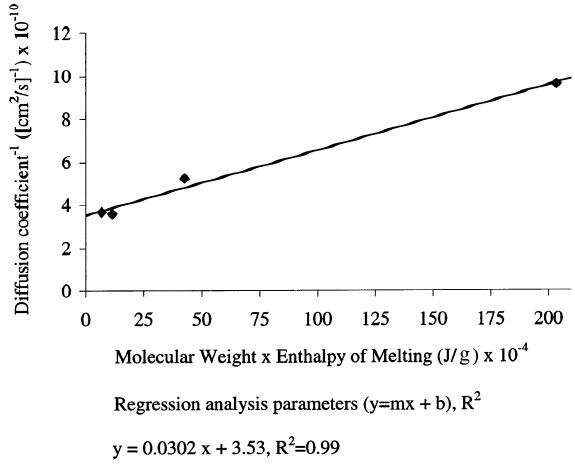


Fig. 9. Empirical relationship of the diffusion coefficient of paclitaxel through microspheres to polymer molecular weight and crystallinity, expressed as enthalpy of fusion.

$$\frac{M_t}{M_0} = 6\left(\frac{Dt}{r^2\pi}\right)^{1/2} - \frac{3Dt}{r^2} \quad \text{for } 0 \le M_t/M_0 \le 0.4 \tag{7}$$

$$\frac{M_t}{M_0} = 1 - \frac{6}{\pi^2} e^{\left(\frac{-\pi^2 Dt}{r^2}\right)} \quad \text{for } 0.6 \le M_t/M_0 \le 1 \tag{8}$$

where M_t and M_0 are the total amount released and total amount of drug loaded into the matrix, respectively, r is the microsphere radius (an average of 70 μm) and t is time. The theoretical curves were in agreement with the data (Fig. 8) and yielded diffusion coefficients (D) in the range of 1.04 to 2.78×10^{-11} cm^2/s for all microspheres (Table 2). Because the diffusion coefficient of a drug through a polymer is expected to increase with decreasing molecular weight and polymer crystallinity an empirical relationship to these parameters was sought. Fig. 9 illustrates the empirical inverse-linear relationship of diffusion coefficient to the product of molecular weight and polymer crystallinity. These data show that when both variables are considered, values of D change as expected. However, when the same data (Table 2) are examined separately, values of D are not well correlated to either molecular weight or enthalpy alone.

The microspheres were observed in the release medium on a daily basis and it was observed that by the end of the first week, the 2k and 4k g/mol PLLA microspheres were no longer freely suspended in the medium within the tumbling tube,

Table 2
Parameters affecting paclitaxel release from 30% loaded 50–90 μm microspheres and the fraction of paclitaxel remaining in microspheres after a 14 day in vitro release study

Molecular weight of PLLA in 30% paclitaxel loaded microspheres (g/mol)	Enthalpy of melting (J/g)	Remaining fraction of paclitaxel after in vitro release (%)	Diffusion coefficient of paclitaxel in microspheres ($\times 10^{-11}$ cm^2/s)
2k	34	36	2.72
4k	29	23	2.78
10k	42	43	1.91
50k	40	87	1.04

rather the microspheres had begun to aggregate and adhere to the bottom of the tube.

Degradation of the microspheres made from PLLA with molecular weights between 2k and 50k g/mol was observed by SEM, following removal of samples from the release medium on various days during the in vitro release experiment. Initially, the microspheres were smooth and spherical. By day 5, the microspheres showed loss of their spherical shape and by day 9, small particles (< 10 μm in diameter) along with larger debris were visible. A representative micrograph for 2k g/mol microspheres after 9 days of in vitro degradation is shown in Fig. 6D. Similar results were observed for all microspheres made from PLLA with molecular weight below 10k g/mol while microspheres made with 50k g/mol PLLA remained intact for over 14 days.

4. Discussion

PLLA polymers were synthesised in the molecular weight range of 500 to 10k g/mol by ring opening polymerisation using a tin catalyst and either stearyl alcohol (PLLA-SA) or L-lactic acid oligomer (PLLA-LA) as initiators. A reaction time for the synthesis of PLLA-SA and PLLA-LA was selected based on reaction times for similar syntheses reported to produce high rates of conversion while minimizing the potential for side-reactions such as ester-interchange between chains (Schindler et al., 1982; Gilding & Reed, 1979).

Stearyl alcohol has several characteristics that make it a good initiator. It possesses only a single functional group, is non-volatile and stable at the reaction temperature, and has a monodisperse molecular weight. The L-lactic acid oligomer is a promising initiator for PLLA polymers because it does not introduce any structural heterogeneity into the polymer chain. However the oligomer used in this work also has a number of drawbacks. It possesses a carboxylic acid group in addition to its hydroxyl group and thus chain termination reactions may occur via condensation reactions. As well, Karl Fischer analysis of the oligomer showed that greater than 0.5 mole of water was present per mole of oligomer molecules. Schindler et al. (1982) noted that hydroxyl groups from water may lower the polymer molecular weight. However this process may not be predictable since some water would be vaporised and less likely to interfere with polymerisation.

In reporting molecular weight data, two expressions of molecular weight are used. For PLLA-SA, the Mn* value predicted by the synthetic parameters is used, while for PLLA-LA the peak value obtained by GPC, M_{GPC} is reported. For PLLA-SA, M_{GPC} had a high correlation with Mn* (Fig. 1) indicating that the synthetic method was able to control and predict molecular weight. Mn* was therefore selected for further reference as the peak values from GPC data are less well defined, being derived from molecular weight standards of a different material, polystyrene, and from empirical constants. However, the data in Fig. 1 show that the synthetic method could not be used to predict Mn for PLLA-LA polymers above 2000 g/mol. In this case, M_{GPC} was considered to be a more accurate value than Mn*. Despite the limitations of M_{GPC} which arise from the method of its determination, it is believed to be representative of a true molecular weight for low molecular weight PLLA-LA polymers. This is because the synthetic method used traditionally produces polymers with narrow molecular weight distributions, meaning all expressions of molecular weight will have a value that is close to M_n. In other experiments using this synthetic method the authors have obtained molecular weight distributions between 1.04 and 1.10 (data not shown).

Regression analysis of the data in Fig. 1 demonstrates the good correlation between Mn* values predicted by Eq. (1) and M_{GPC} values for PLLA-SA polymers in the molecular weight range of 500–10k g/mol. Thus, the molecular weight of PLLA-SA initiated using stearyl alcohol can be controlled by the ratio of monomer to stearyl alcohol used in synthesis (Eq. (1)). However, the intercept of the regression equation shows that Mn* values were 1.2k g/mol less than M_{GPC}. Differences in M_{GPC} and Mn* most likely reflect changes in composition of the polymer chains. Low molecular weight PLLA-SA polymers may be considered to be 'AB' block copolymers with a

block of $–CH_2–$ repeating units contributed by stearyl alcohol, attached to a block of $–OCH(CH_3)CO–$ repeating units contributed by lactide. With a total molecular weight of 500 g/mol, each chain would contain approximately 55% w/w $–CH_2–$ repeat units. However, as the molecular weight increases, the contribution of hydrocarbon by weight decreases rapidly so that a 2k g/mol polymer chain would contain only 14% w/w $–CH_2–$ groups. For this reason, stearyl alcohol is best suited to the synthesis of polymers with higher molecular weight, i.e. Mn greater than 2k g/mol. The values of K and a used to calculate M_{GPC}, are derived for homopolymers of PLLA and will not accurately reflect the molecular weight-viscosity relationship for very low molecular weight PLLA with a structure approaching that of a block copolymer. Thus these polymers were referred to by their Mn* values rather than M_{GPC}.

Values of Mn* and M_{GPC} were identical for PLLA-LA polymers in the range of 500 to 2k g/mol (Fig. 1). For PLLA-LA it was expected that Mn* would not deviate from M_{GPC} since it does not possess a copolymer nature at low molecular weights, making it a better initiator than stearyl alcohol for PLLA at molecular weights up to 2k g/mol. The deviation of M_{GPC} from Mn* above 2k g/mol may have occurred because the properties of the oligomer (water content, presence of an acid group and polydispersity of molecular weight) make it a poor initiator with respect to molecular weight control for Mn* greater than 2k g/mol.

Despite the differences observed in thermal properties when comparing PLLA-SA and PLLA-LA polymers, the XRPD patterns of microspheres made from the two polymers were identical with respect to peak positions (Fig. 2A and C, respectively), indicating similar crystal structure in both polymers. The XRPD pattern of stearyl alcohol had peaks that were distinct from all those observed for PLLA microspheres (Fig. 2B). These peaks were not observed even in the lowest molecular weight PLLA-SA, indicating that crystallinity in the microspheres due to stearyl alcohol, while observed by DSC, was not great enough to be detected by the less sensitive X-ray diffraction method.

Glass transitions observed in microspheres exhibited concurrent enthalpy relaxation in DSC thermograms (Fig. 3). Enthalpy relaxation is due to short range order that arises in the glassy phase of a polymer matrix after physical aging. In microspheres, short range order may arise within the matrix, without aging, during the microsphere formation process (Bodmeier et al., 1989). The enthalpy relaxation phenomenon observed for PLLA microspheres is similar to that observed by Bodmeier et al. since no enthalpy relaxation was observed for samples analysed by DSC by cooling and re-heating to eliminate the thermal history imparted by the microsphere formation process.

Polymer molecular weight had a profound effect on thermal properties of PLLA-SA microspheres over the range of 1–10k g/mol (Figs. 3–5). The relationship between polymer molecular weight and Tg, described by Eq. (5) was linear only at molecular weights above 3k g/mol for PLLA-SA. Thus 3k g/mol represents a critical molecular weight threshold, below which the thermal properties change not only as a function of molecular weight but also of the polymer chain composition. The discontinuity in the Tg-molecular weight relationship and the broad range observed for the Tg of PLLA-SA over the 1–10k g/mol molecular weight range reflect the rapidly changing composition of the polymer. The presence of a C_{18} hydrocarbon group on the end of each chain due to stearyl alcohol results in a change in the proportion of stearyl alcohol to L-lactic acid as molecular weight increases. This would cause the thermal properties to vary in the same manner as those of copolymers with varying monomer ratios (Grijpma et al., 1990).

The melting behaviour of PLLA-SA was also affected by changes in molecular weight. A plot of 1/Tm versus 1/Mn* (Eq. (6)) should be linear, with the slope of the line being dependent on the enthaipy of fusion for the polymer repeat unit, Δh. Fig. 5B shows a deviation from linearity as the molecular weight of PLLA-SA decreased. A change in the slope indicates that Δh was dependent on molecular weight. This could be due to changes in the polymer composition due to changes in the stearyl alcohol:L-lactic acid ratio or due to the increase in the concentration of

polymer chain ends, which can affect the structure of crystallites Wünderlich, 1973.

In contrast to PLLA-SA, the effect of polymer molecular weight on Tg was linear for PLLA-LA at molecular weights down to 1k g/mol (Fig. 5A). These data were consistent with the values of Tg for PLLA polymers with different molecular weights taken from literature sources (Jamshidi et al., 1988; Celli & Scandola, 1992). The linear relationship for PLLA-LA reflects its homopolymer composition over the entire molecular weight range, in contrast to the PLLA-SA polymers. As well, the range of Tg values did not vary over a broad range at low molecular weights as observed for PLLA-SA polymers. The melting temperature of PLLA-LA decreased as the molecular weight decreased. The Tm-molecular weight relationship, plotted using Eq. (6), showed a linear relationship between 1/Tm and 1/MW. These data indicate that Δh is independent of molecular weight in PLLA-LA, reflective of its homogeneous chain structure.

Based on the requirements of an intact and spherical microsphere morphology and microsphere resuspendability, 2k g/mol PLLA-LA was identified as the lowest molecular weight polymer that could be used to manufacture paclitaxel loaded microspheres. For microspheres made from the lowest molecular weight polymer, PLLA-LA was selected over PLLA-SA because of its simpler structure, having no stearyl alcohol component, and because the minimum molecular weight required to achieve resuspension of microspheres from the dry state was higher than for PLLA-SA.

Paclitaxel loaded microspheres prepared using PLLA polymers with molecular weights of 2k–10k g/mol had smooth surfaces as did control microspheres. This is in contrast to the dimpled morphology of 50k g/mol PLLA paclitaxel loaded microspheres which were similar to the morphology of other paclitaxel loaded PLLA microspheres previously reported earlier (Liggins et al., 2000). The difference may be explained by the differences in hydrophilicity of the polymers. Jalil & Nixon (1990) and Chang et al. (1986) have suggested that polymers with different molecular weights precipitate from a given solvent at different rates based on differences in their hydrophobicity. Chang et al. (1986) reported that poly(ε-caprolactone) microspheres were dimpled in appearance, but addition of a more hydrophilic cellulose polymer gave smooth microspheres. Wang et al. have also reported that paclitaxel loaded PLLA microspheres are dimpled in appearance whereas smooth microspheres are seen when paclitaxel is incorporated into poly(lactide-co-glycolide), a more hydrophilic polymer (Wang et al., 1996, 1997)

The total content of paclitaxel in microspheres was influenced by the molecular weight of PLLA-LA. At lower molecular weights, greater than 100% of the expected amount of paclitaxel was incorporated into the polymer. This occurred due to less efficient incorporation of the polymer than of the paclitaxel into the matrix. Less efficient incorporation of polymer than paclitaxel may be expected given the relative hydrophilicity of each component.

Weight loss data indicated that approximately one third of the 1k g/mol PLLA-LA was water soluble. The 1k g/mol PLLA is more hydrophilic than paclitaxel and therefore there is less complete retention of PLLA in the organic phase during microsphere formation compared to paclitaxel. Mumper & Jay (1992) encountered a similar loss of low molecular weight PLLA in the manufacture of microspheres. As expected, the effect of incomplete polymer incorporation on paclitaxel loading efficiency decreased with increasing polymer molecular weight (see Table 1) and a corresponding decrease in hydrophilicity. The effect of incomplete incorporation of a low molecular weight polymer into microspheres has been characterised semi-quantitatively by Grandfils et al. (1996) by measuring relative GPC peak areas for a polymer blend of low and high molecular weight PDLLA incorporated into microspheres. Based on GPC analysis of the water soluble fraction of low molecular weight PDLLA, the highest molecular weight that dissolved in water was found to be approximately 650 g/mol. In order to eliminate this phenomenon, isopropyl alcohol has been incorporated into the organic phase to more rapidly precipitate the low molecular weight components present (Wichert & Rhodewald, 1990).

In vitro paclitaxel release profiles from microspheres prepared using PLLA polymers with molecular weights ranging from 2–50k g/mol (Fig. 8) show that the rate and extent of release are dependent on polymer molecular weight. This finding is consistent with the work of Heya et al. (1991) and Omelczuk & McGinity (1992) who showed that a decrease in polymer molecular weight increased the rate and extent of drug release from PDLLA matrices. In these studies using semi-crystalline polymers, the effect of molecular weight was considered in conjunction with polymer crystallinity (Fig. 9). For low molecular weight PLLA microspheres, the rate, characterised by D values, increased with a decrease in enthalpy. At higher molecular weights, D was dependent on molecular weight. The empirical relationship in Fig. 9 effectively weights the data, with the effects of crystallinity and molecular weight being greatest at low and high molecular weights, respectively.

The observation of incomplete release and slowing of release rate of paclitaxel after 12 days in vitro led to the development of what we refer to as a 'two-compartment model' that described the release kinetics. It was assumed that release from the two-compartments was independent and that compartment 1 was diffusion controlled while compartment 2 did not give any release as this compartment did not allow paclitaxel diffusion at a measurable rate. It was also assumed that the first compartment was depleted over the course of the 14 day release study and that in the second compartment paclitaxel was essentially immobilised by the crystalline domains in the matrix such that diffusion was almost negligible over the time course of these studies. This nature of compartment 2 was consistent with the increase in extent of release of paclitaxel observed from microspheres as the crystallinity decreased and molecular weight increased (Table 2).

Ike et al. (1992) reported release profiles for cisplatin from PDLLA microspheres that showed between 40–90% release of the drug over a 10 day period. In this case the extent of release was dependent on drug loading as the polymer was largely amorphous and of constant molecular weight. This would suggest that the relative size of each compartment is also dependent on pore formation within the matrix as drug is released. It would therefore be expected that paclitaxel loading may also affect the two-compartment model describing release from PLLA microspheres. Furthermore, the release profiles reported by Ike et al. (1992) showed that a second phase of release began after 20 days and continued until the total drug load was depleted. It is anticipated that a second phase of release would also be observed for paclitaxel loaded PLLA microspheres, as the polymer crystallites comprising the second compartment are degraded releasing the remaining drug. Further studies are therefore being undertaken by the authors to study the effects of both drug loading and polymer degradation on release kinetics using the two-compartment model.

Other kinetic models have been used to describe drug release from microspheres. Leelarasamee et al. (1986) reported that hydrocortisone release from PDLLA microspheres followed Higuchi's equation for a homogeneous sphere. Another mathematical model was proposed by Sparks et al. (1979) which incorporates boundary layer and solution saturation phenomena into the kinetic equation. In both cases, the models were dependent on pore formation within the matrix. Presumably, the two-compartment model would be reduced to the Higuchi model if a pore density existed within a polymer matrix sufficient to affect the polymer domains of compartment 2.

In this work, we have demonstrated that very low molecular weight semi-crystalline polymers such as PLLA have the potential to be used in microsphere formulations to deliver drugs over several days to weeks. These low molecular weight PLLA polymers have been characterised as having thermal properties that vary greatly with changes in molecular weight. Paclitaxel release profiles were consistent with a two-compartment model with diffusion controlled release for all molecular weights. We suggest this two-compartment release model may be useful in interpreting release from a polymeric matrix in which incomplete release is observed.

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