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# Paclitaxel loaded poly(L-lactic acid) (PLLA) microspheres II. The effect of processing parameters on microsphere morphology and drug release kinetics

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**Abstract**

The kinetics of solvent removal in microsphere preparation and their effect on the morphology and release characteristics of paclitaxel-loaded PLLA microspheres were determined. Microspheres were analyzed by SEM and DSC and in vitro paclitaxel release was monitored by HPLC. During manufacture, dichloromethane evaporated at a constant rate, which increased with dispersion stirring speed and decreased with increasing paclitaxel content. Paclitaxel-loaded microspheres had a dimpled surface, due to surface deposition of the drug, while controls were smooth. In the formation of larger microspheres, the deposition of drug in the surface slowed the solidification process resulting in drug-loading dependent thermal properties. Paclitaxel release did not follow diffusion kinetics, rather it was characterized by a large burst followed by a linear phase. We speculate that non-uniform (surface-rich) drug distribution in the microspheres may contribute to the deviation from the theoretical pattern of kinetics for diffusion from a sphere.


*Keywords:* Drug release kinetics; Solvent evaporation; Thermal properties; Paclitaxel; Microsphere

It has long been known that microsphere properties may be controlled by the processing conditions employed in their preparation (Tice and Gilley, 1985). Conditions such as stir speed and solvent ratio impact morphology and drug release behavior because they affect the rate of solvent efflux from the forming microspheres. Li et al. (1995a,b) and Jeyanthi et al. (1996) have reported correlations between solvent removal kinetics and drug release behavior and Berkland et al. (2004) have reported that precipitation behavior of drugs in microspheres due to differences in hydrophobicity can affect drug release. In this report, we demonstrate that for a given drug–polymer combination, drug loading and polymer molecular weight can exert an effect on microsphere morphology under certain processing conditions, while at others the effect is negligible. Solvent removal kinetics are correlated not only with surface morphology, drug loading and release profiles but also with thermal properties of microspheres, which to date have not been systematically compared with solvent removal kinetics.

Previously, we have characterized the effect of polymer molecular weight on the release of paclitaxel from low molecular weight PLLA microspheres and

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described a diffusion controlled "two compartment model" of drug release (Liggins and Burt, 2001). The model is based on diffusion of a fraction of the total amount of paclitaxel, the remaining portion being unavailable for diffusion. Paclitaxel loaded 100 K g/mol PLLA microspheres do not fit this model, nor standard diffusion kinetics. Morphological data are used to infer how drug deposition in microspheres during their formation resulted in this atypical drug release.

Paclitaxel was obtained from Hauser (Boulder, CO) and 100 K g/mol PLLA from Birmingham Polymers, Inc. (Birmingham, AB). Control, 10, and 30%w/w paclitaxel loaded microspheres were prepared using the solvent evaporation method (Liggins and Burt, 2001). Stirring rates of 900 and 2100 rpm and PVA concentrations of 1 and 2.5% in the aqueous (external) phase were used to manufacture microspheres in two size ranges. The progress of evaporation of the internal phase (dichloromethane) from the dispersion was measured gravimetrically at 5 min intervals. Particle size distributions were determined using a Coulter LS-130 particle size analyzer. Microspheres were observed by scanning electron microscope (Hitachi) with a 100 Å gold-palladium coating. Thermal properties of microspheres were measured by DSC with a Dupont model 910S DSC (New Castle, DL) with a scan rate of 10 °C/min. The degree of crystallinity ($X_C$) of PLLA was calculated using the equation of Celli and Scandola, 1992. The total content of paclitaxel and in vitro release from microspheres were assayed by HPLC in a manner previously reported (Liggins and Burt, 2001).

Microspheres in two size ranges (1–15 and 35–105 μm) were prepared, having mean radii of 12 and 75 μm, respectively. Thus the surface area to volume ratio for the smaller microspheres is approximately six times that of the larger microspheres.

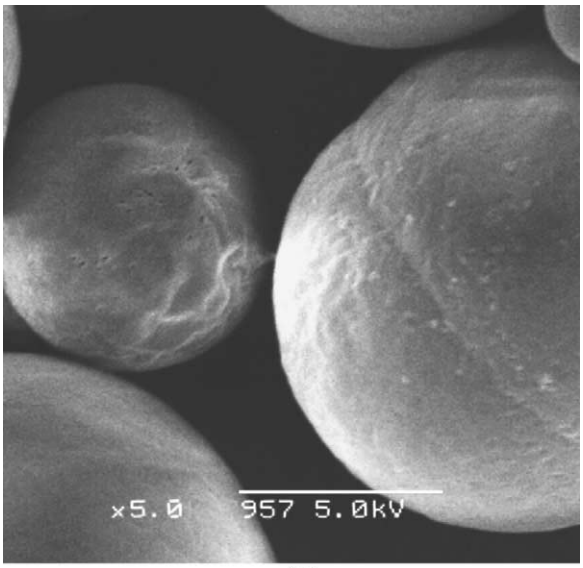
Weight loss of the dichloromethane dispersion during microspheres preparation was measured to calculate the time for weight loss equal to half of the solvent ($t_{50}$). Average values ($n = 3$) of $t_{50}$ for control, 10 and 30% loaded microspheres in two size ranges are listed in Table 1. After an initial 5 min lag phase (which accounted for the initial extraction of dichloromethane into the aqueous phase, described earlier by Li et al. (1995a) and Crotts and Park (1995), weight loss was constant until 90% of the dichloromethane was evaporated, after which the rate gradually decreased, accounting for >99% of the solvent mass. For large microspheres, $t_{50}$ was drug loading dependent. Evaporation in 30% loaded microspheres took almost twice as long as in unloaded controls. However, weight loss in small microspheres was independent of loading, with $t_{50}$ values equal to that for dichloromethane alone stirring in the aqueous phase.

While control microspheres exhibited smooth surfaces, paclitaxel loading gave microspheres a "dimpled" appearance (Fig. 1). Examination at higher magnifications confirmed that the dimples did not

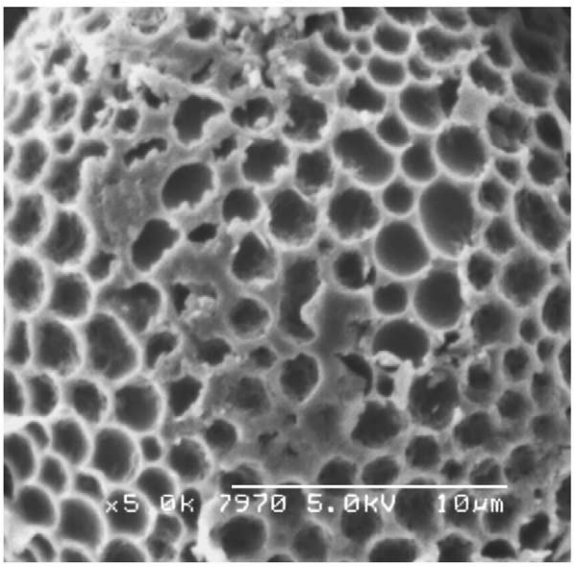
Table 1
Properties of small and large 100 K g/mol PLLA microspheres containing 0, 10, 30% paclitaxel. Values are averages of measurements from each of three batches ± standard deviation

| Paclitaxel loading | Rate of solvent evaporation ($t_{50}$) (min) | Paclitaxel released (%) in burst phase ($M_3$) | Release rate after burst phase (%/day) | $T_g$ (°C) | $T_m$ (°C) | $X_c$ (%) |
|---|---|---|---|---|---|---|
| 1–15 μm microspheres | | | | | | |
| Control | 25 ± 3 | na | na | 62 ± 2 | 174 ± 1 | 41 ± 4 |
| 10% | 25 ± 3 | 26 | 1.2[a] | 62 ± 3 | 173* | 41 ± 2 |
| 30% | 26 ± 3 | 50 | 2.4[b] | 64 ± 1 | 171* | 40 ± 5 |
| ANOVA $P$ value | 0.88 | na | na | 0.97 | <0.001 | 0.87 |
| 35–105 μm microspheres | | | | | | |
| Control | 34 ± 2 | na | na | 66 ± 1 | 176* | 36 ± 6 |
| 10% | 49 ± 2 | 5 | 1.2[a] | 63 ± 1 | 173 ± 1 | 38 ± 2 |
| 30% | 64 ± 2 | 9 | 2.1[a] | 60 ± 1 | 169 ± 1 | 44 ± 9 |
| ANOVA $P$ value | <0.001 | na | na | <0.001 | <0.001 | 0.10 |

[a] The confidence interval for the release rate is less than 0.2%/day.
[b] The confidence interval for the release rate is less than 0.4%/day.
* The standard deviation of the average melting temperatures was less than 1 °C.




(A)




(B)

Fig. 1. Surface morphology of (A) control and (B) 30% paclitaxel loaded 35–105 μm 100 K g/mol PLLA microspheres. (Magnification is 5000×).

extend as pores into the matrix, having a depth approximately equal to their diameters.

The solidification process occurs first on the surface of the organic droplets, forming a "skin" of precipitated material (Li et al., 1995b). Paclitaxel loading dependent solvent evaporation suggests that paclitaxel is a principal component in the initially formed surface layer. However, small microspheres exhibited a faster rate of solvent removal, which was independent of paclitaxel loading level, likely due to the six-fold higher specific surface area compared with large microspheres. Thus, drug loading does not impact microsphere morphology by slowing solidification at the high stirring rate used to produce smaller microspheres.

Thermal properties of the microspheres are summarized in Table 1. The microspheres exhibited enthalpy relaxation at $T_g$ and recrystallization upon heating to about 95 °C. Paclitaxel loading affected thermal properties, particularly in larger microspheres, for which drug loading also resulted in slower removal of dichloromethane during the solidification process. Microspheres in the 35–105 μm size range exhibited reduced enthalpy relaxation, extent of recrystallization, $T_g$, and $T_m$ as the drug loading increased. In 1–15 μm microspheres, only $T_m$ was significantly reduced by paclitaxel although to a lesser extent than was observed in larger microspheres. Paclitaxel was only an effective plasticizer of the amorphous regions in large microspheres, but not in the smaller size range. The absence of an effect of paclitaxel on the $T_g$ in small microspheres indicates that the paclitaxel may not be completely dissolved in the matrix, perhaps due to the more rapid solidification process or a greater specific surface area resulting in greater overall surface deposition of the drug. Phase separation of the drug may also contribute to the more extensive burst and faster linear release rates seen in small microspheres.

Paclitaxel loading efficiency was high, although 1–15 μm microspheres had lower efficiencies (87–88%) than larger microspheres (96–99%). In vitro release profiles of paclitaxel are shown in Fig. 2, expressed as percent of total loading. The burst phase was characterized by the total amount released in the first three days ($M_3$). Within both size ranges, $M_3$ increased two-fold as loading increased from 10 to 30%. Furthermore, for a given loading, $M_3$ increased five-fold as the external specific surface area increased six-fold. Following the initial phase of release a slower linear phase was observed between days 3 and 14 (Table 1) having $R^2 > 0.98$. In this phase of release, particle size had no effect on the release rate whereas a two-fold increase was observed as loading increased from 10 to 30%. After day 14 release rates decreased gradually and the profiles plateaued after

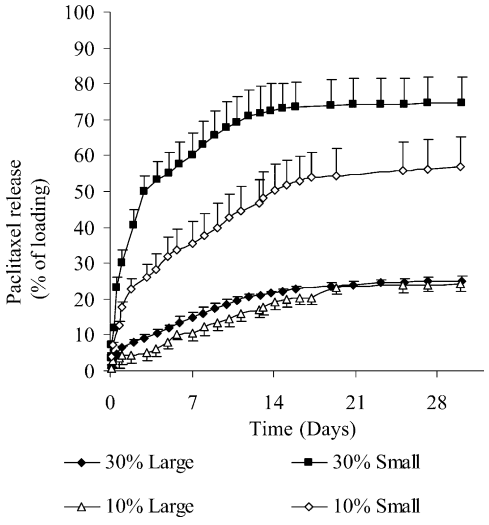



Fig. 2. In vitro release profiles of paclitaxel from 10 and 30% paclitaxel loaded 100 K g/mol PLLA 1–15 μm (small) and 35–105 μm (large) microspheres.

releasing 20–75% of encapsulated drug. Incomplete release of paclitaxel from the microspheres is consistent with our earlier findings for low molecular weight PLLA microspheres (Liggins and Burt, 2001) in which a fraction of the drug was entrapped in the semicrystalline matrix. However, our earlier "two compartment model" does not adequately describe release from 100 K g/mol PLLA microspheres. The disproportionate burst phase and linear phase after day 3 do not follow a Fickian release profile and the proportion of paclitaxel remaining after release was not dependent on polymer crystallinity, as described in our earlier results, but rather on particle size. The release profiles may be explained by surface drug deposition and drug loading dependent morphology arising from changing solvent evaporation rates.

Paclitaxel loaded PLLA microspheres with a distorted surface have been reported (Wang et al., 1997), while paclitaxel microspheres of PLGA (Wang et al., 1996) and PCL (Dordunoo et al., 1995) had smooth surfaces. PLLA microspheres containing paclitaxel do not exhibit the dimpled surface morphology when made from polymers having molecular weights in the range of 2–10 K g/mol (Liggins and Burt, 2001), nor do they exhibit such an extensive burst phase or apparently zero order phases of release. Thus, the interaction between specific drugs and polymers of specific molecular weights affect the extent of surface deposition of drug in microsphere formation, and the extent of deviation of release profiles from established kinetic models. We have demonstrated that although the impact of polymer and process condition selection is well known, individual drug–polymer combinations must be investigated using a variety of processing conditions in order to understand the exact effects on microsphere morphology and drug release. Thermal analysis coupled with solvent evaporation kinetic studies are useful methods elucidating microsphere morphology and explaining drug release kinetics.

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