Process Variable Implications for Residual Solvent Removal and Polymer Morphology in the Formation of Gentamycin-Loaded Poly (L-lactide) Microparticles

Rick F. Falk[1] and Theodore W. Randolph[1,2]

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Purpose. The purpose was to determine the influence of process parameters in the precipitation with a compressed antisolvent (PCA) process on the morphology and residual dichloromethane (DCM) levels in gentamycin-loaded PLA microparticles.

Methods. The three variables studied were the rate of CO_2 co-flowed during the polymer and drug co-precipitation, the post-precipitation pure CO_2 flush rate, and the post-precipitation CO_2 flush volume. Residual DCM levels were determined from headspace gas chromatography — mass spectroscopy (GC-MS) with single ion monitoring. X-ray diffraction (XRD) and differential scanning calorimetry (DSC) were used to estimate the crystallinity within microparticles. DCM was extracted from drug-loaded microparticles by both supercritical CO_2 extraction and vacuum drying for up to two days to determine a lower limit for solvent removal.

Results. Increasing either the post-precipitation CO_2 flow rate or flush volume resulted in lower residual DCM levels in the microparticle. The CO_2 co-flow rate showed an opposite trend. Increasing its value resulted in a higher DCM value after precipitation. XRD and DSC analysis on these samples suggest that those produced at lower CO_2 co-flow rates have a higher degree of crystallinity, which increases the diffusivity of DCM through the polymer matrix. Finally, samples subjected to extended (48 hr) CO_2 extraction resulted in DCM levels on the order of one to three ppm.

Conclusions. Specific PCA process conditions during microparticle formation have a strong influence on the residual solvent levels within the microparticles. Polymer morphology affects the diffusivity of solvent through the polymer matrix, which in turn determines the solvent removal rates.

KEY WORDS: controlled drug delivery; hydrophobic ion pairing (HIP); gentamicin; supercritical carbon dioxide; precipitation with a compressed antisolvent (PCA); residual solvent.

INTRODUCTION

Poly (L-lactide) (PLA) has many desirable attributes as a releasing matrix for controlled drug delivery and surgical applications. *In vivo*, PLA hydrolyzes to lactic acid over the course of months to over a year, making it possible to release pharmaceutical agents for this span of time as well. A disadvantage to using PLA is its solubility limitations. The only common solvents that solubilize PLA are chloro- and fluorocarbons, with dichloromethane (DCM) and trichloromethane (TCM) being the most common. As a result, any device fabrication process which calls for a PLA solution in any of its steps will most likely use one of these solvents. The U.S.P. limits for these solvents in a pharmaceutical device are 50 and 500 ppm for TCM and DCM, respectively (1). Therefore, removal of this solvent after production is an important issue.

Drug-loaded microparticles are an attractive form for a PLA delivery system which can be used for inhalation therapy (2), or injected subcutaneously (3). Several techniques exist for the production of such microparticles, most of which involve some sort of emulsion evaporation step (4–8). These techniques usually require a post-production drying step, wherein residual solvent is removed over the course of several days. Precipitation with a compressed antisolvent (PCA) is an alternate process for microparticle production (9–14). This process involves spraying a solution of drug and polymer into a compressed fluid which acts as a non-solvent for the drug and polymer solutes, resulting in precipitation of microparticles containing the drug. Supercritical carbon dioxide, commonly used as the antisolvent, has favorable extraction properties with respect to lipophilic compounds such as DCM (15,16). Using PCA, it is possible to combine the microparticle precipitation and solvent removal steps into one unit operation. Randolph *et al.* reported elemental chlorine levels below the limits of detection in pure PLA particles produced using the PCA process (17). Bleich *et al.* produced PLA microparticles containing a DCM-soluble drug by such a process with low residual DCM levels, but measured a large initial drug burst, with most of the drug being released within a few hours, indicating that most of the drug is on the surface of the microparticle (12). Others have also shown that co-precipitation of DCM-soluble drugs with PLA results in microparticles containing a non-uniform drug dispersion (10) or low encapsulation of the drug within the polymer due to drug solubility in the CO_2 antisolvent phase (9,10).

The microparticles produced in this work contain a water-soluble drug which has been solublized in DCM using a technique termed hydrophobic ion-pairing (18–20). This serves to raise the solubility of the drug in DCM to a useful level for PCA, but not so high as to encounter the problem of enhanced solubility in the CO_2 antisolvent phase (9,10). The objective of this work is to determine how PCA process variables affect residual DCM levels in PLA microparticles containing a model drug, gentamycin. We have previously reported on the release characteristics of ion-paired gentamycin from PLA microparticles formed using the PCA process (10). Polymer morphological changes resulting from addition of ion-paired drug and PCA processing were also investigated for their role in solvent extraction efficiency.

MATERIALS AND METHODS

Materials

Gentamycin sulfate, 1,2 dichloroethane, decalin, and sodium dioctyl sulfosuccinate (AOT) were obtained from Sigma Chemical Co, (St. Louis, MO). Poly (L-lactide) (PLA) was purchased from Boehringer Ingelheim (Montvale, NJ) sold under the name Resomer® L 206 (intrinsic viscosity = 10 g/ml). Medical grade carbon dioxide was obtained from U.S. Welding (Denver, CO). Dichloromethane (DCM), 2-propanol,

[1] Department of Chemical Engineering, University of Colorado, Boulder, Colorado 80309.

[2] To whom correspondence should be addressed. (e-mail: randolph@pressure.colorado.edu)

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mercaptoethanol, sodium borate, *o*-phthalaldehyde (OPA), and methanol were purchased from Fisher Scientific (Pittsburgh, PA). All reagents were used as received.

Preparation of Drug-Loaded Microparticles

Gentamycin-loaded PLA microparticles were produced using precipitation with a compressed antisolvent (PCA) described in detail elsewhere (10). Briefly, a solution of the drug and PLA in DCM is sprayed through an atomizing nozzle into supercritical CO_2, resulting in precipitation of PLA microparticles containing uniformly dispersed gentamycin. Pure CO_2 is co-flowed with the DCM phase during precipitation as DCM-rich CO_2 is vented from the system. After precipitation is complete, pure CO_2 is flushed through the system to remove DCM and prevent condensation during depressurization. The CO_2 co-flow rate during microparticle precipitation, along with the post-precipitation flush rate and flush volume, are three process variables which were adjusted to help elucidate the mechanism by which DCM is removed from the polymer. All microparticle samples in this study were prepared at 85 bars and at 35°C with a DCM solution flow rate of 1 ml/min. To achieve uniform drug distribution, the PCA process requires the dissolution of the drug and the polymer in the same solvent prior to precipitation. Gentamycin sulfate is an ionic, water-soluble molecule, with negligible solubility in DCM. Water as a solvent is incompatible with both PCA and the dissolution of PLA. Accordingly, gentamycin was solubilized in DCM using hydrophobic ion-pairing by replacing the polar sulfate counter ions with more hydrophobic dioctyl sulfosuccinate (AOT) counter ions (10,18–20). A solution of ion-paired gentamycin and PLA in DCM was then sprayed into supercritical CO_2 as described previously [10]. DCM containing solutions of 9 mg/ml PLA, 1 mg/ml gentamycin, and a corresponding AOT concentration of 5 mg/ml was used for all preparations in this work. Gentamycin loading factors were measured by hydrolyzing a known amount of microparticles in 1 N sodium hydroxide for one hour, followed by a hundred-fold dilution with water. Gentamycin concentration in this solution was then measured using the OPA derivatization technique of Sampath *et al.* (21), as modified by Goosen *et al.* (22). A sodium borate buffer of 0.1 M was also used instead of the 0.04 M buffer used by Goosen *et al.* to help maintain the proper derivatization pH.

Microparticle Morphology Analysis

Microparticle size and shape were characterized by scanning electron microscopy (SEM). Particles were gold-coated for five minutes on a Polaron SEM coating system and examined using an ISI-SX-30 SEM (International Scientific Instruments).

Residual Dichloromethane Analysis

Residual DCM in the microparticles was measured by headspace gas chromatography-mass spectroscopy (GC-MS) using single ion monitoring. A Hewlett-Packard 5890 gas chromatograph and a Hewlett-Packard 5972 mass spectrometer were used with an HP5MS column (30 m L × 0.25 mm I.D.) Approximately 20 mg of gentamycin-loaded PLA was added to 200 μl of decalin with 1,2 dichloroethane as an internal standard at a concentration of 0.2 μg/mL. This mixture was incubated at 60°C for ten minutes in a two-dram vial with a teflon-lined septum to volatilize DCM and 1,2 dichloroethane. 5 ml of the headspace was used for the GC injection at a flowrate of 1 ml/min using helium as the carrier gas. The column temperature was ramped from 40°C to 250°C at 30°C/min. The response signals of the characteristic ions in the vial headspace of both DCM (ions 62, 63, and 64) and 1,2 dichloroethane (ions 84, 86, and 88) were measured and the ratio of the response of DCM to the response of 1, 2 dichloroethane was taken. Residual DCM levels were determined from a linear calibration curve constructed from the ratios of DCM standards to 1,2 dichloroethane internal standards. This calibration had a correlation coefficient r^2 of 0.99.

Thermal Analysis

Differential scanning calorimetry (DSC) was used to estimate polymer phase transitions. A Perkin-Elmer DSC-7 was used with a heating rate of 10°C/min. 10 mg samples were heated in aluminum pans from 25°C to 90°C and back down to 25°C to standardize their thermal history following the procedure of Hiljanen-Vainio *et al.* (23). The standardization temperature of 90°C was chosen so as to remain well below the crystallization temperature of 105°C. Samples were then heated to 225°C at 10°C/min to measure thermal melt and glass transition temperatures. Instrument calibration was performed using indium and zinc standards.

X-Ray Diffraction

X-ray diffraction (XRD) was performed on a Scintag powder XRD (Sunnyvale, CA) model PAD V at 25 mA and 40 kV. 5 mg samples on a glass slide were scanned from 5 to 40° at a scanning rate of 2°/min. A step of 0.02° was used with a count time of 0.6 seconds.

Extended DCM Extraction

Extended solvent extraction experiments using supercritical CO_2 were performed at a pressure and temperature of 85 bar and 35°C, respectively. 100 mg samples of PLA microparticles containing ion-paired gentamycin were extracted in a 5 ml stainless steel pressure vessel for either 24 or 48 hours. Extended vacuum extraction experiments were performed at a vacuum level of 430 mm Hg. 100 mg samples were extracted in small open glass vials placed in a 1000 mL vacuum flask for 24 or 48 hours.

RESULTS AND DISCUSSION

Microparticle samples of ion-paired gentamycin in PLA at a loading of 6.7% (w/w) were produced under a variety of process conditions as described in *Materials and Methods*. Figure 1 is an SEM photomicrograph of ion-paired gentamycin-loaded PLA microparticles. Particles produced under the various conditions were indistinguishable by SEM analysis. Even at magnification of 10,000×, no evidence of particle porosity was seen (data not shown). Three variables, CO_2 co-flow rate, CO_2 flush rate, and CO_2 flush volume were examined in a 2 × 3 factorial experiment with values of either high or low. The co-flow rate was either 15 or 30 ml/min, the flush rate was either 10 or 20 ml/min, and the flush volume was either 200 or 400 ml. Table I summarizes the residual DCM in each

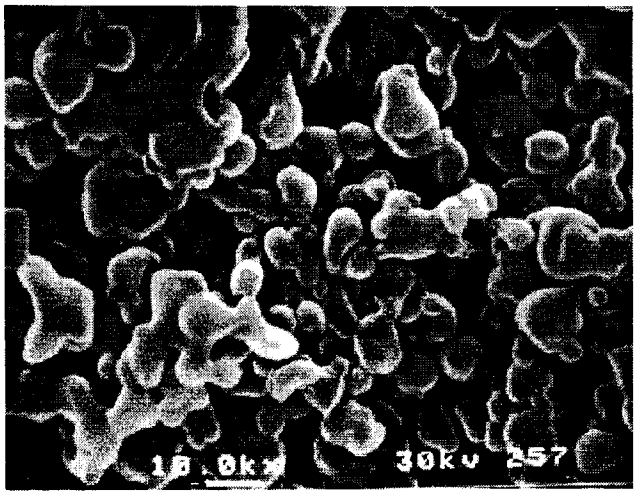

Fig. 1. SEM photomicrograph of ion-paired gentamycin-loaded PLA microparticles (gentamycin loading factor of 6.7% w/w). The larger white bar at the bottom of the photomicrograph is a 1 micron length scale.

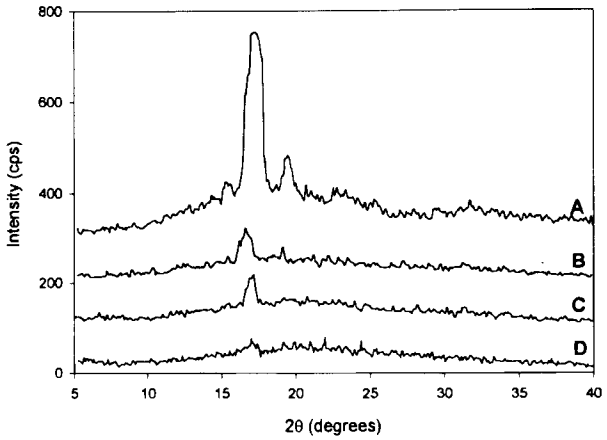

Fig. 2. X-ray diffraction patterns of (A) pure as-received PLA, (B) pure PLA microparticles after PCA processing, (C) gentamycin-loaded PLA microparticles after PCA processing at 15 ml/min CO_2 co-flow rate, and (D) gentamycin-loaded PLA microparticles after PCA processing at 30 ml/min CO_2 co-flow rate. Both (C) and (D) were produced with post precipitation CO_2 flush rates of 20 ml/min for 200 ml total.

of these samples after processing. The two post-precipitation variables, CO_2 flush rate and volume, show predictable trends with higher flow rates and larger flush volumes resulting in lower residual DCM levels. The effect of CO_2 co-flow rate, however, is less intuitive. The rate of CO_2 co-flow will determine the steady-state composition of the antisolvent phase. A higher CO_2 co-flow rate at a constant drug/polymer solution flow rate will decrease the DCM concentration in the antisolvent phase during microparticle precipitation. Microparticles produced at a polymer/drug solution flow rate of 1 ml/min and a CO_2 flow of 15 ml/min resulted in a steady state DCM concentration in the antisolvent phase of 8% (w/w) while those produced with a CO_2 flow rate of 30 ml/min (at a polymer/drug solution flow rate of 1 ml/min) resulted in a steady state DCM level of 4% (w/w) in the antisolvent phase. Samples prepared at the lower CO_2 co-flow rate of 15 ml/min resulted in higher DCM levels after minimal post-precipitation CO_2 contact, but that remaining solvent was more easily extracted than from those samples prepared at a co-flow rate of 30 ml/min after equivalent post-precipitation CO_2 contact. These data suggest that the rate at which make-up CO_2 is added during microparticle precipitation has an effect on the morphology of the polymer, and ultimately, the diffusion rate of DCM through PLA.

X-ray diffraction (XRD) patterns were determined for microparticle samples containing ion-paired gentamycin produced using PCA. Those patterns, along with ones for control samples of pure PLA before and after PCA processing are shown in Figure 2. The two samples containing gentamycin were produced at a CO_2 co-flow rate of 15 or 30 ml/min with a 20 ml/min pure CO_2 flush and a 200 ml pure CO_2 flush volume (sample ID gent 15 and gent 30). These data show that most of the crystallinity initially present in the polymer is lost during PCA processing. Also, gentamycin-containing microparticles produced at the low CO_2 co-flow rate exhibit similar levels of crystallinity as pure PLA microparticles, while the

Table I. Residual Dichloromethane Levels of Gentamycin-Loaded Microparticles as a Function of CO_2 Co-Flow Rate, Post-Precipitation CO_2 Flush Rate, and Post-Precipitation CO_2 Flush Volume

CO_2 co-flow rate (ml/min)	Post-PCA CO_2 flush rate (ml/min)	Post-PCA CO_2 flush volume (ml)	Residual DCM (ppm)
30	10	200	91 (10.6)[a]
30	10	400	71 (24.8)[a]
30	20	200	157 (20)[a]
30	20	400	139 (39)[a]
15	10	200	346 (74)[a]
15	10	400	252 (54)[a]
15	20	200	55 (9.4)[a]
15	20	400	56 (37)[a]

[a] Two standard deviations based on three measurements.

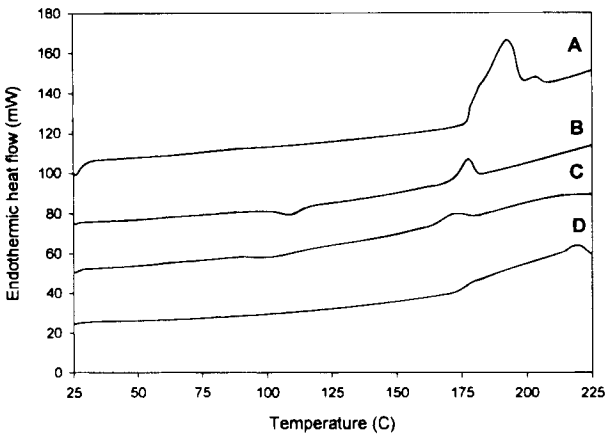

Fig. 3. DSC thermograms of (A) pure as-received PLA, (B) pure PLA microparticles after PCA processing, (C) gentamycin-loaded PLA microparticles after PCA processing at 15 ml/min CO_2 co-flow rate, and (D) gentamycin-loaded PLA microparticles after PCA processing at 30 ml/min CO_2 co-flow rate. Both (C) and (D) were produced with post precipitation CO_2 flush rates of 20 ml/min for 200 ml total.

Table II. Residual Dichloromethane Levels of Gentamycin-Loaded Microparticles After Extended Solvent Extraction Conditions

Sample I.D.[a]	Type of Extraction	Length of Time (hours)	Residual DCM (ppm)
gent15	CO_2	24	3.44 (1.42)[b]
gent15	CO_2	48	6.28 (—)[c]
gent15	Vacuum	24	2.16 (1.10)[b]
gent15	Vacuum	48	1.29 (0.52)[b]
gent30	CO_2	24	1.86 (—)[c]
gent30	CO_2	48	1.11 (0.98)[b]
gent30	Vacuum	24	1.52 (0.52)[b]
gent30	Vacuum	48	2.01 (1.34)[b]
gent15	Normal PCA processing		55 (9.4)[b]
gent30	Normal PCA processing		157 (20)[b]
pure PLA microparticles	Normal PCA processing		46 (—)[c]

[a] Gent15 and gent30 indicate gentamycin-loaded microparticles produced at 15 and 30 ml/min CO_2 co-flow rates, respectively. Both samples were produced at post-precipitation CO_2 flush rates of 20 ml/min for 200 ml total.
[b] Two standard deviations based on three measurements.
[c] One measurement.

drug-containing samples produced at the higher co-flow rate show no XRD peaks.

Figure 3 shows DSC thermograms for samples gent15, gent30, pure PLA microparticles (post-PCA), and as-received PLA. All four samples show melt temperatures of 170 to 180°C. As-received PLA shows the largest heat of melting, followed by pure PLA microparticles, then gent15, and finally gent30 having the smallest heat of melting. The degree of sample crystallinity deduced from this heat of melting data follows the same trend. These results, like the XRD data, suggest that PCA processing reduces the crystallinity of the resulting microparticles. One explanation for this is that as the CO_2 co-flow rate is decreased, the steady-state concentration of DCM in the CO_2 phases increases. CO_2 has been shown to have a plasticizing effect on polymers (9,12). Adding DCM to the CO_2 phase can intensify this effect. So, as the steady-state DCM concentration in the CO_2 phase is increased (by lowering the CO_2 co-flow rate), the extent to which this mixture will plasticize the microparticles increases. This can increase the mobility of the polymer in the microparticle and allow the polymer chains to rearrange into a more crystalline state, with a concomitant increase in the diffusivity of DCM through the polymer. A second explanation is that at higher steady state DCM concentrations, solvent evaporates more slowly from the spray, allowing crystallization to proceed to a greater degree than at lower steady state DCM concentrations. PLA with a higher degree of crystallinity will have a greater amount of grain boundaries separating the crystalline from the amorphous domains. An increase in grain boundaries can enhance the diffusion rate of DCM from a more crystalline polymer.

Two techniques were used to further reduce the residual DCM levels of gentamycin-loaded microparticles: supercritical CO_2 extraction, and vacuum drying. The same drug-loaded microparticles used for the XRD experiments described above were used for the extraction experiments. For the CO_2 extraction experiments, samples of microparticles were contacted with supercritical CO_2 at 85 bar and 35°C for 24 and 48 hours under static conditions. The volume of the extraction chamber was sufficiently large to maintain sink conditions. Vacuum drying consisted of pulling vacuum at 430 mm Hg on samples for 24 and 48 hours. For comparison, PLA microparticles containing no gentamycin were produced with no extended extraction time and the residual DCM was measured. This level and the results of the extraction experiments are summarized in Table 2. In all cases, the residual DCM levels are decreased to near zero. It would be possible to avoid the prolonged extraction steps described here with additional post-PCA CO_2 contact and truly combine the production and extraction steps into one. These levels show that the gentamycin:AOT ion pair complexes within the microparticle do not permanently bind DCM in what could be visualized as a 'hydration' layer of solvent around the hydrophobic surfactant tails. Rather, DCM extraction is mass transfer limited in nature.

CONCLUSIONS

PCA process variables during and after microparticle precipitation can influence the amount of carrier solvent remaining in the polymer. Specifically, increasing the post-precipitation CO_2 flush rate and volume resulted in lower residual DCM levels in gentamycin-loaded PLA microparticles. Increasing CO_2 co-flow rate during precipitation resulted in higher residual DCM. This parameter also changes the morphology of the resulting polymer microparticle. Both XRD and DSC show an increase in crystallinity in drug-load microparticles produced at lower CO_2 co-flow rates as well as a general reduction in crystallinity after PCA processing. Also, the ease with which DCM can be removed from the polymer after precipitation is a function of polymer crystallinity. The gent15 sample had both higher crystallinity and higher initial DCM levels than the gent30 sample, but under similar solvent removal conditions, ended up with lower DCM levels. Higher DCM diffusion rates through crystalline versus amorphous PLA might explain this. Finally, experiments have shown that any of these samples can be cleaned of DCM to essentially the same level (1–3 ppm) given enough extraction time.

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