Solid-State Solubility Influences Encapsulation and Release of Hydrophobic Drugs from PLGA/PLA Nanoparticles

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Received 16 November 2003; revised 8 February 2004; accepted 25 February 2004

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.20094

ABSTRACT: Biodegradable nanoparticles formulated from poly(D,L-lactide-*co*-glycolide) (PLGA) and polylactide (PLA) polymers are being extensively investigated for various drug delivery applications. In this study, we hypothesize that the solid-state solubility of hydrophobic drugs in polymers could influence their encapsulation and release from nanoparticles. Dexamethasone and flutamide were used as model hydrophobic drugs. A simple, semiquantitative method based on drug–polymer phase separation was developed to determine the solid-state drug–polymer solubility. Nanoparticles using PLGA/PLA polymers were formulated using an emulsion–solvent evaporation technique, and were characterized for size, drug loading, and *in vitro* release. X-ray powder diffraction (XRD) and differential scanning calorimetry (DSC) were used to determine the physical state of the encapsulated drug. Results demonstrated that the solid-state drug–polymer solubility depends on the polymer composition, molecular weight, and end-functional groups (ester or carboxyl) in polymer chains. Higher solid-state drug–polymer solubility resulted in higher drug encapsulation in nanoparticles, but followed an inverse correlation with the percent cumulative drug released. The XRD and DSC analyses demonstrated that the drug encapsulated in nanoparticles was present in the form of a molecular dispersion (dissolved state) in the polymer, whereas in microparticles, the drug was present in both molecular dispersion and crystalline forms. In conclusion, the solid-state drug–polymer solubility affects the nanoparticle characteristics, and thus could be used as an important preformulation parameter. J Pharm Sci 93:1804–1814, 2004

Keywords: sustained release; biodegradable polymers; dexamethasone; X-ray powder diffraction; differential scanning calorimetry; particle size; preformulation parameter

INTRODUCTION

Biodegradable nanoparticles are currently being investigated for the purpose of sustained and localized administration of different therapeutic agents such as antiproliferative agents,[1–3] local anesthetics,[4] and macromolecules such as DNA and proteins.[5] Although nanoparticles can be formulated from a wide variety of synthetic and natural polymers,[6] biodegradable polymers such as poly(D,L-lactide-*co*-glycolide) (PLGA) and polylactides (PLA) are especially suitable for sustained drug delivery applications due to their biodegradable nature and biocompatibility with cells and tissue.[7]

Drug loading and release rates are important parameters in nanoparticle-mediated drug delivery to optimize the therapeutic efficacy of the encapsulated drug.[8] In general, hydrophobic

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Journal of Pharmaceutical Sciences, Vol. 93, 1804–1814 (2004)

drugs are encapsulated into nanoparticles using an oil-in-water emulsion–solvent evaporation technique. In this method, typically drugs and polymers are dissolved in an organic solvent or a cosolvent system, which is then emulsified into an aqueous phase containing emulsifier(s) to form oil-in-water emulsion. The organic solvent(s) from the emulsion is removed by either diffusion or by evaporation to form drug-loaded nanoparticles. Although some of the parameters such as the effect of stirring and polymer concentration on particle size, particle size distribution, yield, and drug encapsulation have been studied,[9,10] most often an empirical approach is used in selecting PLGA/PLA polymers in the formulation of nanoparticles.

Although many studies have investigated the formulations of PLGA/PLA nanoparticles for sustained release of different hydrophobic drugs,[3,11–13] there is no simple technique that can be used for the easy selection of different formulation parameters (polymer composition and/or molecular weight, polymer–drug ratio) to design nanoparticles of desired properties in terms of drug loading and release. We hypothesize that the solid-state solubility of the drug in polymer could be an important determinant that could influence the drug loading in nanoparticles as well as the release characteristics of the encapsulated drug. To test the above hypothesis, we determined the solid-state drug–polymer solubility as a function of polymer characteristics such as lactide mol %, molecular weight, and end group chemistry using dexamethasone and flutamide as model hydrophobic drugs. Nanoparticles were formulated using different polymers to determine the relationship between the solid-state solubility, and drug loading in nanoparticles and its release under *in vitro* conditions. Although the solubility of drugs in polymers can be calculated based on the solubility parameters or from theoretical models,[14] such information may not always be readily available for a particular drug–polymer combination or for new therapeutic agents and polymers. Therefore, we developed a simple, semiquantitative technique to determine the solid-state drug–polymer solubility. Formulations with dexamethasone were characterized for different parameters, and some of the studies were repeated using flutamide as another model hydrophobic drug to establish the generality of the proposed hypothesis.

MATERIALS AND METHODS

Materials

Polymers (polylactide and polylactide-*co*-glycolide) of different composition and molecular weight were obtained from Birmingham Polymers, Birmingham, AL. Dexamethasone, flutamide, lithium fluoride, polyvinyl alcohol (average molecular weight 30,000–70,000) were purchased from Sigma Chemicals (St. Louis, MO). Other chemicals and solvents were of analytical grade.

Semiquantitative Solid-State Solubility of Drugs in the Polymer

To determine the solid-state drug–polymer solubility, different quantities of drug (stock solution of drug in methanol, 25 mg/mL) were added to polymer solutions of different composition and molecular weight (30 mg/mL in chloroform) (Table 1), solutions were vortexed, and 100 μL of each drug–polymer solution was spread on a glass

Table 1. Phase Separation of Dexamethasone and Flutamide from PLGA/PLA Polymers of Different Composition and Molecular Weight

Variation	Molecular Weight (Da)	Lactide mol %	End-Group Chemistry	Phase Separation Concentration of Dexamethasone (% w/w)	Phase Separation Concentration of Flutamide (% w/w)
Effect of lactide/ glycolide ratio	53,100	50	Ester	10	15
	103,800	75	Ester	15	15
	87,800	100	Ester	15	20
Effect of molecular weight	12,000	50	Ester	15	20
	53,100	50	Ester	10	15
	143,000	50	Ester	10	15
Effect of end function group	12,000	50	Ester	15	20
	10,000	50	Acid	10	15

microscope slide. The solvent was allowed to evaporate undisturbed overnight at room temperature, and the dried films were observed visually for drug precipitation. Phase separation of drugs from polymers was visible from the opacity of the films, whereas when there was no phase separation, the polymer films remained transparent.

Quantitative Solubility of Drugs in the Polymer

Solid-state solubility of dexamethasone in polymers was also determined quantitatively by a previously reported differential scanning calorimetry (DSC) method.[15] In brief, polymer–drug solutions were prepared as described in the previous section and the solutions (~100 μL, equivalent to 3–4 mg of polymer–drug mixture) were then transferred to aluminum pans (Shimadzu, Columbia, MD). Organic solvent was allowed to evaporate, and the pans were then crimped and weighed. Samples were then heated at the rate of 10°C/min in Shimadzu DSC-50 equipment calibrated with an indium standard. The heats of melting associated with the melting endotherm were calculated using the instrumental software and plotted as a function of dexamethasone concentration. The intercept on Y-axis represented the solubility of the drug in the polymer at its melting temperature.

Solubilization of Dexamethasone in PVA Solution

Solubilization of dexamethasone in PVA solution was determined by UV spectrophotometry. A saturated solution of dexamethasone in different concentrations (0.5, 1.0, and 2.5% w/v) of aqueous PVA was prepared, and the UV absorbance of the supernatant was measured at 240 nm.

Nano- and Microparticle Formulation

An emulsion–solvent evaporation technique was used to formulate dexamethasone-loaded nanoparticles.[16] Tritiated-labeled dexamethasone was used in the nanoparticle formulations. In brief, dexamethasone solution in methanol (6 mg in 240 μL) was mixed with 1 mL of 30 mg/mL polymer solution (polymers of different molecular weights and lactide–glycolide ratios were used; see Table 2) in chloroform, which was then added to 6 mL of 2.5% w/v aqueous polyvinyl alcohol solution (PVA, 30–70 kDa, Sigma) and sonicated for 5 min. The emulsion was stirred at room temperature for 18 h and then in a desiccator under vacuum for 1 h to evaporate organic solvents. Nanoparticles were recovered by ultracentrifugation (35,000 rpm for 30 min at 4°C, Optima™ LE-80K, Beckman, Palo Alta, CA), washed two times with distilled water to remove unentrapped drug and PVA, and then lyophilized (−80°C and <10 μm mercury pressure, LYPHLOCK® 12, Labconco, Kansas City, MO) for 48 h to obtain a dry powder. All the washings were carefully collected and analyzed for dexamethasone levels to determine the amount of drug that was not encapsulated in nanoparticles. In addition, the last washing was analyzed separately to ensure that the free drug (unencapsulated) is washed off from nanoparticles. Drug loading in nanoparticles was determined from the amount of dexamethasone that is not entrapped and subtracting this from the total amount of dexamethasone added in the formulation. Direct extraction of drug from nanoparticles using chloroform as an extracting solvent was also attempted to determine the drug-loading in nanoparticles; however, this method resulted in about 20 to 30% recovery of the encapsulated drug. This could be because of

Table 2. Effect of Polymer Characteristics on Physical Characteristics of Dexamethasone-Loaded Nanoparticles

Lactide Mole %	Molecular Weight (Da)	End-Group Chemistry	Mean Hydrodynamic Diameter (nm)	Polydispersity Index	Zeta Potential (mV)	Drug Loading (% w/w)[a]	Crystalline Drug (%)[b]
100	87,800	Ester	260	0.255	−23.9 ± 3.5	8.7 ± 0.5	nd[c]
75	103,800	Ester	260	0.115	−22.8 ± 4.1	5.8 ± 1.1	nd
50	143,000	Ester	270	0.228	−19.6 ± 1.5	6.0 ± 0.4	nd
50	12,000	Ester	740	0.394	−28.1 ± 3.2	9.3 ± 2.5	10.0 ± 1.2
50	10,000	Acid	240	0.225	−22.1 ± 3.8	6.3 ± 1.7	nd

[a] $n = 3$.
[b] % of total drug content.
[c] nd: none detected.

a fraction of PVA (used as an emulsifier) that remains associated with the nanoparticle surface (could not be washed off)[17] and forms a layer around the nanoparticles, rendering them insoluble in an organic solvent. The drug-loading data obtained with the extraction method do not correlate with the drug release from nanoparticles under *in vitro* conditions because the cumulative percent drug released from nanoparticles is greater than the drug loading in nanoparticles as determined by direct extraction method. However, with microparticles, the amount of PVA associated with surface is insignificant (as surface-associated PVA increases with decrease in particle size[18]), and does not interfere in the dissolution of microparticles in an organic solvent. Therefore, the direct extraction method may be adequate to determine the drug loading in PLGA/PLA microparticles but not in nanoparticles. For measuring radioactivity, about 100 μL each of the washing solutions were mixed with 4 mL of scintillation cocktail (Scinti Verse®, Fisher Scientific, Hanover Park, IL) and the radioactivity was measured using a liquid scintillation counter (Tri-Carb® 2500 TR/AB, Packard Instrument Company, Meriden, CT). Similar procedure was used to formulate flutamide-loaded nanoparticles. Flutamide concentration in the washings was analyzed by UV spectrophotometry (λ_{max} 300 nm).

Microparticles were prepared by using the same protocol used for nanoparticles except that vortexing (5 min, Highest energy setting, Fisher Vortex Genie 2™, Fisher Scientific, Pittsburgh, PA) was used to formulate the emulsion instead of sonication. Microparticles were recovered and washed by centrifugation at 3200 rpm (Sorvall RTH-750, DuPont, Newton, CT) and processed as described for nanoparticles.

Particle Size Analysis and Zeta Potential

Particle size and size distribution were determined by photon correlation spectroscopy (PCS) using quasielastic light-scattering equipment. A dilute suspension of nanoparticles (100 μg/mL) was prepared in double-distilled water and sonicated on an ice bath for 30 s. Nanoparticles after lyophilization form a fluffy mass that can be easily dispersed in an aqueous media using sonication without affecting their integrity.[16] The sample was subjected to particle size analysis in the ZetaPlus™ particle size analyzer (Brookhaven Instrument Corp, Holtsville, NY). The particle size of microparticles was measured by scanning electron microscopy. A sample of microparticles was placed on a double-stick tape over aluminum stubs to get a uniform layer of particles. The sample was gold coated (~300 Å) using a sputter gold coater (Emscope SC500, Ashford, England) at 40-mA current, and 50-millitorr pressure for 200 s. Gold-coated particles were then viewed using a scanning electron microscope (JEOL 840A, Peabody, MA). To measure the zeta potential of nanoparticles, a suspension of nanoparticles was prepared as above in distilled water. The zeta potential was measured immediately using the ZetaPlus™ zeta potential analyzer.

X-ray Powder Diffractometry (XRD)

Preparation of Standard Mixtures and Test Samples

Physical mixtures containing various proportions of dexamethasone (1%, 5%, and 10% w/w) and blank nano- or microparticles were prepared for different formulations. Lithium fluoride (10% w/w) was added as an internal standard because it has many of the ideal properties described for internal standards for XRD.[19] To obtain homogenous mixing of dexamethasone and internal standard with nano- and microparticles, dexamethasone and lithium fluoride were added to blank particle dispersion in distilled water and lyophilized. For dexamethasone-loaded nano- and microparticles, lithium fluoride (10% w/w) was added to the nanoparticle dispersion in distilled water and lyophilized.

Instrumentation

Nano- and microparticle samples were filled into a cavity-mount quartz holder aluminum sample holder, and were exposed to CuKα radiation (40 kV and 30 mA) on a Rigaku D-Max/B Horizontal Q/2Q X-Ray Diffractometer (The Woodlands, TX). The scans were run from 5 to 40° 2θ at 0.01° 2θ/s. A high-intensity peak of dexamethasone at 14.35° 2θ and a 38.7° 2θ peak of lithium fluoride were chosen for quantitative analyses. Peak intensities were compared rather than integrated intensities because the peaks of the nanoparticle samples were small, and it was difficult to obtain a good fit after background subtraction using the integrated peak intensities. Also, it is assumed that the shape of the diffraction peak is not affected by variations in disorder or particle size.[19] Appropriate background subtraction was performed in each case.

Dexamethasone Content in Nano- and Microparticles

The peak intensities were plotted as a function of dexamethasone concentration in the physical mixtures of dexamethasone with blank nanoparticles, and a linear fit obtained therein was used to calculate the content of crystalline dexamethasone in nanoparticle formulations.

DSC for Detecting Amorphous Dexamethasone

To determine the presence of amorphous dexamethasone in nanoparticles, the samples were subjected to DSC and investigated for the presence of glass transition. Amorphous dexamethasone was initially prepared by melt-quench method.[20] Dexamethasone was heated in the DSC, at 10°C/min, up to 200°C. The melt was held at this temperature for 5 min, and cooled back to −30°C using liquid nitrogen. When the sample was reheated again, at 10°C/min, loss in melting endotherm and a glass transition at 18.9°C were observed.

In Vitro Release of Dexamethasone

In vitro release of the tritium-labeled dexamethasone from nanoparticles was carried out under sink conditions (drug concentration in the medium was kept five times lower than the saturation solubility of dexamethasone in buffer) using a side-by-side double diffusion apparatus.[21] Nanoparticle suspension (1 mg/2.5 mL) in phosphate-buffered saline (PBS, pH 7.4, 0.15 M) was placed in the donor chamber, and 2.5 mL of PBS was placed in the receiver chamber. The chambers were separated by a 0.1 μm Millipore® membrane (Type VV, Bedford, MA). Drug is freely diffusible across the membrane but not nanoparticles. The apparatus was then placed in an orbital shaker (Environ® orbital shaker, Lab Line, Melrose Park, IL) maintained at 37°C and 100 rpm. At different time intervals, the entire volume of the receiver chamber was removed and replaced with fresh PBS. The amount of drug released was measured by counting the radioactivity in the samples recovered from the receiver chamber. About 100 μL each of the release samples were mixed with 4 mL of scintillation cocktail and the radioactivity was measured using a liquid scintillation counter (Tri-Carb® 2500 TR/AB). To calculate the drug released between the two data points, the total drug in 5 mL (2.5 mL in the donor and 2.5 mL in the receiver chamber) was calculated from the concentration. From the above total drug amount in 5 mL, the drug left in the donor chamber (amount in 2.5 mL) from the previous time point was subtracted. The above calculations are necessary to account for the drug left in the donor chamber because the buffer from the receiver chamber only was collected and replaced with fresh buffer at each time point.

RESULTS

Semiquantitative Solid-State Solubility of Drugs in the Polymer

The semiquantitative solid-state solubility of dexamethasone in PLGA and PLA films of different composition and molecular weight is given in Table 1. Representative slides from a phase separation study are shown in Figure 1. It was found that the solid-state solubility of the drug in the polymer increased with an increase in the lactide content in the polymer and with a decrease in the molecular weight. The presence of free acid end groups in the polymer resulted in a decrease in the drug's solubility in the polymer. The semiquantitative solid-state solubility of flutamide as a function of polymer properties is shown in Table 1. The solubility profile of flutamide was similar to that of dexamethasone, with higher solubility in more hydrophobic or low molecular weight polymers.

Quantitative Solid-State Solubility of Dexamethasone in the Polymer

To confirm the above semiquantitative solubility studies, the solubility of dexamethasone in selected polymers were quantified using DSC. Solubility values were obtained from the intercept of the heat of fusion versus the concentration plot (Fig. 2). Similar to the solubility data obtained with semiquantitative method, dexamethasone demonstrated higher solubility (67 mg/g versus 32 mg/g) in the low molecular weight (12,000 Da) polymer than in the high molecular weight (143,000 Da) polymer.

Particle Size and Zeta Potential

Particle size and the zeta potential of different dexamethasone nanoparticle formulations are shown in Table 2. In general, nanoparticles had

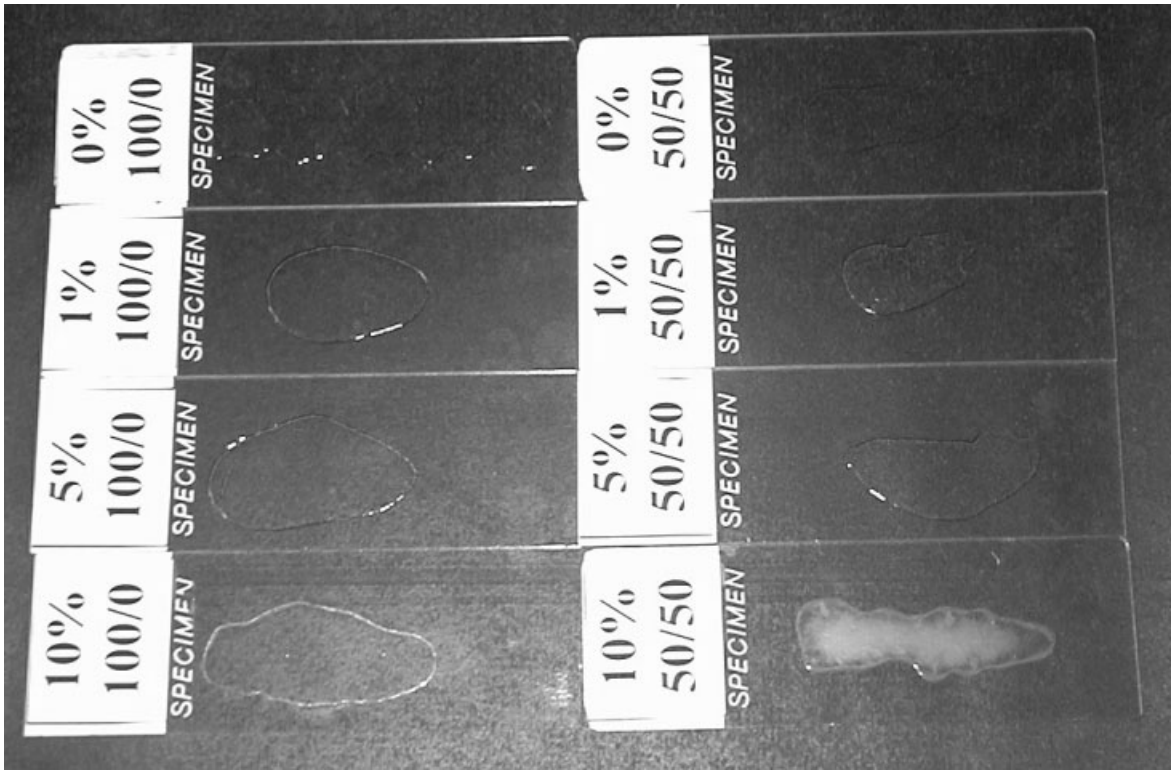

Figure 1. Semiquantitative evaluation of dexamethasone's solid-state solubility in polymer matrix. Increasing amounts of drug was dissolved in polymer solution and the mix was cast into films. Films were evaluated for phase separation. Picture shows representative slides showing phase separation occuring at 10% w/w drug concentration for high molecular weight PLGA (right) (143,000 Da, 50/50 lactide-to-glycolide ratio). Polylactide (87,800 Da100/0) (left) did not show phase separation at 10% w/w.

a mean hydrodynamic diameter between 240 and 270 nm. Nanoparticles formulated using low molecular weight (12,000 Da) polymer had a relatively larger mean hydrodynamic diameter (740 nm). The zeta potentials of all the formulations were negative, with zeta potential varying from −19 to −28 mV for the different formulations. Based on the SEM characterization, microparticles had a mean diameter of 4.8 ± 2.1 μm. Nanoparticles loaded with flutamide demonstrated similar particles size and zeta potential values as dexamethasone-loaded nanoparticles (Table 3).

Drug Loading in Nano- and Microparticles

Dexamethasone loading in different nanoparticle formulations (Table 2) was found to correlate with the semiquantitative solid-state polymer–drug solubility profiles shown in Table 1. Higher drug loading was obtained for polymer of high lactide content (100%) compared to that with polymer containing a fraction of glycolide and for lower molecular weight PLGA (12,000 Da) than higher molecular weight PLGA. Similarly, nanoparticles prepared with a polymer containing ester end groups had higher drug loading compared to those with free acid end groups. Flutamide loading in nanoparticles also demonstrated a similar dependence on the drug's solid-state solubility in the polymer. Nanoparticles formulated from hydrophobic PLA (100:0 lactide-to-glycolide ratio) demonstrated higher loading of flutamide than those formulated from a relatively hydrophilic PLGA (50:50 lactide-to-glycolide ratio) ($11.0 \pm 0.6\%$ versus $7.1 \pm 0.3\%$, Table 3). Dexamethasone loading in microparticles was 7.5% w/w.

Solid-State Nature of the Drug in Nano- and Microparticles

XRD was used to determine the crystalline content of the drug, while DSC was used to

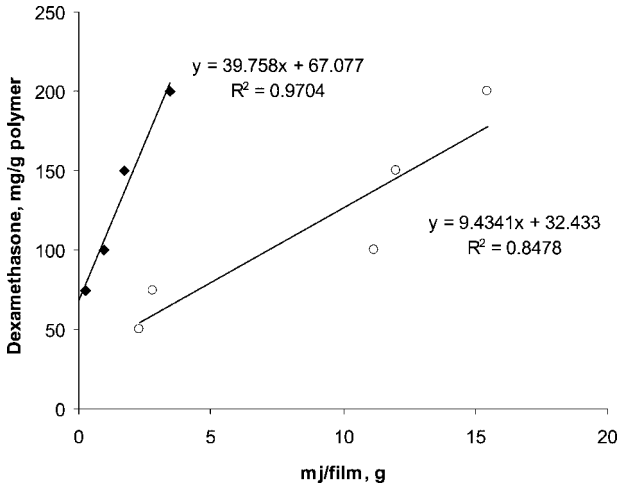

Figure 2. Dexamethasone concentration of the polymer–drug film samples as a function of the observed heat (mj = millijoules). Intercept on the Y-axis represents the drug's solid-state solubility in the polymer matrix. Filled squares: 12,000 Da polymer; Open circles: 143,000 Da polymer.

determine the presence of amorphous drug. No crystalline dexamethasone was detected in any of the nanoparticle formulations except those formulated using the low molecular weight (12,000 Da) polymer (Fig. 3). About 1% w/w of crystalline dexamethasone (~10% of the total drug content) was detected in these nanoparticle formulations (Table 2). However, about $53 \pm 5\%$ of the total drug content was in the crystalline form in the larger size microparticles (Fig. 4).

DSC studies showed no glass transition corresponding to that of amorphous dexamethasone in any of the nanoparticle formulations (Fig. 5), suggesting the absence of amorphous dexamethasone.

In Vitro Drug Release from Nanoparticles

In vitro release of the dexamethasone from nanoparticles was found to have an inverse relationship with the solid-state drug–polymer solubility. Higher cumulative percent drug release was obtained from nanoparticle formulations prepared with the polymers exhibiting lower solid-state drug solubility. Thus, nanoparticles formulated from 100% lactide content, which demonstrated higher solid-state solubility for the drug, released lower percent of the encapsulated drug than those prepared from polymers containing glycolide (Fig. 6A). However, the drug release from nanoparticles formulated using 50/50 and 75/25 lactide/glycolide ratio was almost similar despite the difference in their lactide content. One possibility is that the change in the lactide content of the polymer from 75 to 50% does not significantly affect the properties of nanoparticles. This is also evident from the similar drug loading in nanoparticles formulated using these polymers (Table 2). Nanoparticles formulated using low molecular weight polymer showed lower percent cumulative release (Fig. 6B). Also, lower cumulative percent drug release was obtained from nanoparticles prepared using polymers containing ester-end groups than from nanoparticles prepared using polymers containing acid end groups (Fig. 6C).

DISCUSSION

In the solvent evaporation technique used to formulate particulate systems, the organic phase consisting of polymer and drug dissolved in an organic solvent such as chloroform or methylene chloride is emulsified in a continuous phase containing an emulsifier such as PVA to stabilize the emulsion formed. The emulsion is then stirred to evaporate the organic solvent, which results in the polymer droplets containing the drug hardening into particles.[22] Depending on the energy input used to form the emulsion, nano- or microparticles are formed.[18] As the solvent evaporates from the emulsion, the drug distributes between the polymeric droplets and the surrounding emulsifier phase. If the surfactant utilized to

Table 3. Effect of Polymer Characteristics on Physical Characteristics of Flutamide-Loaded Nanoparticles

Lactide Mole %	Molecular Weight (Da)	End-Group Chemistry	Mean Hydrodynamic Diameter (nm)	Polydispersity Index	Zeta Potential (mV)	Drug Loading (% w/w)[a]
100	87,800	Ester	294	0.16	-17.2 ± 1.8	11.0 ± 0.6
50	143,000	Ester	289	0.19	-19.8 ± 0.8	7.1 ± 0.3

[a] $n = 2$.

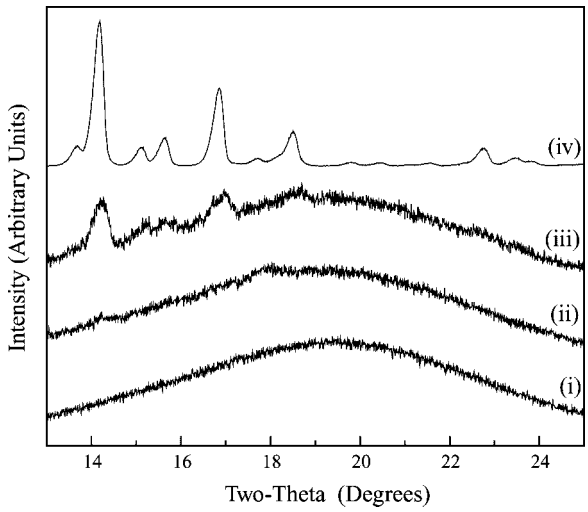

Figure 3. XRD patterns of (i) blank nanoparticles, (ii) dexamethasone-loaded nanoparticles (12,000 Da molecular weight polymer), (iii) 5% w/w physical mixture of dexamethasone with blank nanoparticles, and (iv) dexamethasone.

stabilize the emulsion is present at a concentration greater than its critical micellar concentration, then surfactant micelles, which can solubilize the drug, are also present in the continuous phase of the emulsion.[14] The organic solvent used to dissolve the polymer can diffuse into the continuous phase because of its miscibility in water, which can temporarily increase the drug solubility in the continuous phase. However, the drug diffused into the continuous phase could precipitate once the organic solvent is evaporated completely, and then deposit onto the nanoparticle surface.

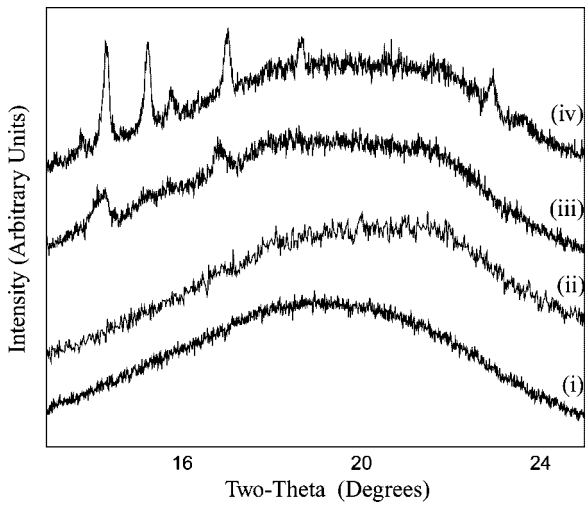

Figure 4. XRD patterns of (i) dexamethasone-loaded nanoparticles (50/50 lactide-to-glycolide, 143,000 Da), (ii) blank microparticles, (iii) dexamethasone-loaded microparticles (50/50 lactide-to-glycolide, 143,000 Da), and (iv) 5% w/w physical mixture of dexamethasone with blank microparticles.

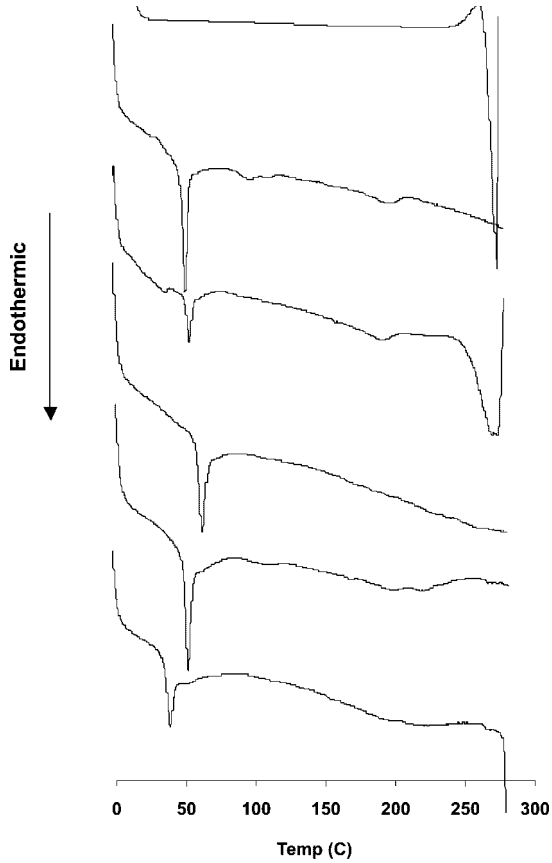

Figure 5. The DSC curves (from top to bottom) of (i) dexamethasone, (ii) blank nanoparticles, (iii) physical mixture of dexamethasone and blank nanoparticles, (iv) dexamethasone-loaded nanoparticles (100/0 lactide), (v) dexamethasone-loaded nanparticles (50/50 lactide-to-glycolide, 143,000 Da), and (vi) dexamethasone-loaded nanparticles (50/50 lactide-to-glycolide, 12,000 Da).

Based on the above model, drug loading in particulate systems prepared by the emulsion solvent evaporation technique would depend on (1) the solubility of the drug in the polymeric phase, (2) solubility of the drug in the continuous phase, and (3) drug that might separate as a solid in the continuous phase and deposit onto the nanoparticle surface. Because the drug solubility in PVA is not significantly different from than that in water, the continuous phase affecting the drug loading in nanoparticles due to its micellar solubilization can be ruled out. Second, because nanoparticles

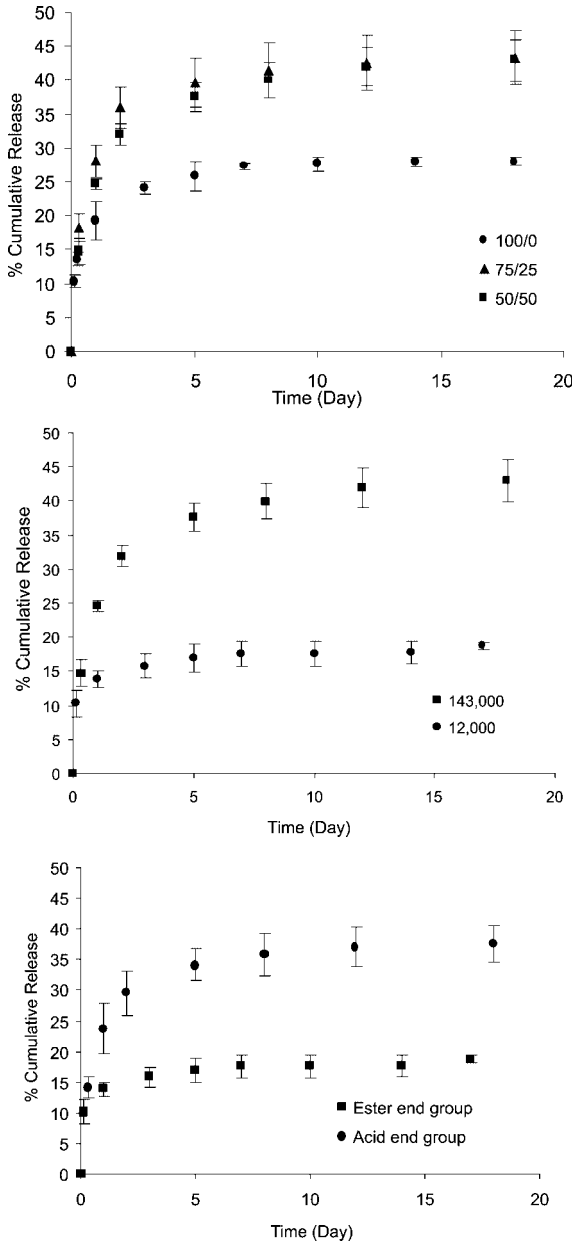

Figure 6. Effect of (A) lactide-to-glycolide ratio, (B) molecular weight, and (C) end-group chemistry on the *in vitro* release of dexamethasone from nanoparticles.

are washed thoroughly after they are formed; the drug loading data eliminate the drug adsorbed onto the nanoparticle surface. In other words, drug loading in the particles would be governed by the partitioning of the drug between the polymer phase and the continuous phase and its subsequent separation from the continuous phase and deposition on the nanoparticle surface.[14]

Thus, for a given continuous phase volume and concentration, drug loading in nanoparticles should be governed mainly by the drug's distribution in the polymeric phase. This distribution in the polymeric phase would be influenced by drug's solid-state solubility and by the ability of the polymeric matrix to entrap drug in dispersed state. Hence, in this study, we determined the solid-state solubility of dexamethasone in polymer as a function of polymer characteristics such as lactide mol %, molecular weight, and end-group chemistry to determine the correlation between solid-state drug–polymer solubility and drug loading in nanoparticles and also the nature of the drug in nanoparticles.

Relatively higher solid-state drug–polymer solubility obtained for polymer with 100% lactide content compared to polymers that contained glycolide or for polymer with an ester end group than a polymer with a carboxyl group can be explained based on increasing hydrophobicity of the polymer with increasing lactide content or with ester end groups, resulting in better solid-state solubility of the hydrophobic drug in the hydrophobic polymer matrix (Fig. 1, Table 1). The increase in solid-state solubility with decrease in molecular weight of the polymer can be explained based on the fact that the free energy of mixing of the drug with the polymer decreases with the molecular weight of the polymer due to the increased entropic contribution.[23] Results from the semiquantitative solubility studies were also verified for some of polymers using quantitative DSC studies (Fig. 2).

Drug loading in nanoparticles closely matched with the respective solid-state drug–polymer solubility profiles obtained above. In general, polymers that showed higher solid-state solubility resulted in higher drug loading than those that showed lower solid-state solubility, suggesting that solid-state polymer–drug solubility is a major predictor of drug loading in nanoparticles. A similar trend was obtained for another hydrophobic drug, flutamide, which also demonstrated higher drug loading in nanoparticles formulated using a polymer in which it had higher solid-state solubility. Also, compared to dexamethasone, flutamide demonstrated higher solubility in all the polymers studied, and accordingly, the drug had higher loading in nanoparticles compared to dexamethasone. The higher solid-state drug–polymer solubility for flutamide compared to that for dexamethasone could be because of more lipophilic nature of flutamide compared to dexamethasone, as indicated by the differences in their octanol/water partition coefficient (Log *P* for

Dexamethasone = 2.06 ± 0.57 and for Flutamide = 4.05 ± 0.33). The above relation between the drug's solid-state solubility in polymer and drug loading in nanoparticles also suggested that most of the drug could be present in the dissolved state rather than in the dispersed state in the polymer matrix.

To determine the physical state of the drug in nanoparticles, XRD and DSC were used. XRD studies suggested that there was no crystalline drug present in most of the nanoparticles formulations. Absence of crystalline drug in PLGA nanoparticles has been reported for amphotericin B.[24] An exception to the above general observation was the nanoparticle formulation prepared from a lower molecular weight polymer (~12,000 Da PLGA), which had about 10% of the total drug in crystalline form. A possible explanation for the presence of crystalline drug in this nanoparticle formulation could be due to their larger size compared to other nanoparticle formulations favoring entrapment of drug crystals (Table 2). The particle size effect on entrapment of crystalline drug is also evident from our studies where microparticles demonstrated over 50% dexamethasone in crystalline form (Fig. 4). Alternatively, the presence of crystalline drug in larger size nano- and microparticles could have been because of higher drug loading (Table 2). Polakovic et al. have also demonstrated the presence of crystalline drug in PLA nanospheres that have higher drug (lidocaine) loading.[4] The presence of crystalline drug in PLGA/PLA microparticles has been reported previously by others.[25] The DSC studies indicated the absence of amorphous dexamethasone in nanoparticles. Thus, the XRD and DSC studies indicate that dexamethasone is present mainly in the dissolved state (molecular dispersion) in nanoparticles with the exception of the formulation prepared with 12,000 Da PLGA polymer and microparticles where the drug was present in both crystalline and dissolved states (Figs. 3–5).

As a part of the above studies, the influence of polymer characteristics on the *in vitro* release of dexamethasone was also examined. Nanoparticles formulated from polymers that demonstrated higher solid-state drug–polymer solubility showed relatively lower drug release compared to those prepared with polymers that demonstrated lower solid-state solubility. Thus, nanoparticles with the lower drug loading demonstrated relatively higher percent cumulative drug release (Fig. 6). The release of the drug would be governed by its partitioning between the polymeric phase and the aqueous release medium. Thus, the higher the solubility of the drug in the polymer, the lower the partitioning of the drug from the polymer to the external aqueous phase, resulting in lower rates of drug release. The release of miconazole, a hydrophobic drug from different hydrophobic matrices was found to correlate with the difference in solubility parameters of the matrix and the drug.[26] Drug release was found to be faster when the difference between the solubility parameters of the matrix and of the drug was higher, suggesting that drug release increases with the decrease in drug–polymer solubility. It has been know that the drug release from PLGA/PLA nano- and microparticles is biphasic, with an initial release phase followed by a lag period and a second release phase, which is characterized by the bulk degradation of polymer matrix.[27] In this article, we have determined the effect of solid-state polymer solubility on the initial phase of drug release from different formulations of nanoparticles, during which time period nanoparticles are not completely degraded. It would be interesting to study the effect of solid-state polymer drug solubility on the lag phase and the second phase of drug release from nanoparticles.

CONCLUSIONS

Our studies suggest that the solid-state solubility of the drug in PLGA/PLA polymers influences the drug encapsulation and release from nanoparticles. Increased drug loading and lower release were observed for nanoparticle formulated using polymers that demonstrated higher solid-state solubility of the drug. Furthermore, it was demonstrated that only the dissolved state of the drug in the polymer matrix was detected in nanoparticles, and that the particle size influences the form of the drug that is present in the formulation. Thus, the solid-state solubility of the drug in the polymer could be used as an important preformulation parameter for the selection of the polymer to be used in the formulation of nanoparticles.

ACKNOWLEDGMENTS

Grant support from the National Institutes of Health (HL 57234) and the Nebraska Research Initiative was appreciated. J.P. was supported by a predoctoral fellowship from the American Heart Association. Administrative assistance from Ms. Elaine Payne is greatly appreciated.

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