Pharmaceutical Research, Vol. 4, No. 6, 1987

Research Article

The Preparation and Evaluation of Drug-Containing Poly(*dl*-lactide) Microspheres Formed by the Solvent Evaporation Method

Roland Bodmeier[1,2] and James W. McGinity[1]

Received March 9, 1987; accepted July 14, 1987

Several compounds such as caffeine, diazepam, hydrocortisone, progesterone, quinidine, quinidine hydrochloride, quinidine sulfate, and theophylline were evaluated for incorporation into poly(*dl*-lactide) (PLA) microspheres using the solvent evaporation technique. The process is generally limited to the entrapment of water-insoluble drugs. Adjustment of the pH of the aqueous phase to minimize drug solubility resulted in increased drug contents within the microspheres in the case of ionizable drugs. The release profile of quinidine from the microspheres was characterized by three different release phases, a lag time with no drug release, a burst effect of rapid drug release within a short period of time, and a slow release phase, respectively. The structure of the microsphere surface layer, which was a function of the pH of the aqueous phase at preparation, strongly influenced the rate and amount of drug released. Thermal analysis of quinidine-loaded microspheres revealed three thermal events, corresponding to the glass transition temperature of the polymer and to the recrystallization and melting of quinidine.

KEY WORDS: microspheres; poly(*dl*-lactide); quinidine; solvent evaporation method; biodegradable drug delivery systems.

INTRODUCTION

The biocompatibility and biodegradability of polyesters such as polylactic and polyglycolic acid have been extensively studied. Since the mid 1970s, considerable interest has evolved in the use of these polymers as drug carrier materials. The early applications of poly(*dl*-lactide) (PLA) and other polyesters as biodegradable drug carriers included a wide range of polymer/drug composites of different sizes and shapes. Various techniques such as film casting (1,2), molding (3,4), air suspension (5), and extrusion (6) were used in the preparation of these systems. However, most of these devices required surgery at the site of administration. This drawback has led to the development of biodegradable microparticulate drug delivery systems. Phase separation techniques such as nonsolvent addition and the solvent evaporation method have been successfully applied to the preparation of biodegradable microcapsules or microspheres.

In the solvent evaporation process, the active ingredient is dissolved or dispersed in a solution of the polymer in a suitable water-immiscible and volatile organic solvent. This solution or dispersion is emulsified in an aqueous medium to form microdroplets. The organic solvent then diffuses into the aqueous phase and evaporates at the water/air interface. The microdroplets solidify and solid, free-flowing microspheres are obtained after complete organic solvent evaporation, filtration, and drying. Many variables can influence the preparation and properties of the microspheres (7–11). The solvent evaporation method works best for water-insoluble drugs. Water-soluble drugs will favorably partition into the aqueous medium, resulting in low core loadings. Nonaqueous phase separation techniques using nonsolvent addition have been used for the preparation of PLA microcapsules containing water-soluble compounds (12,13).

The objective of the present study was to evaluate the suitability of the solvent evaporation process for the entrapment of active compounds with different physicochemical properties and to investigate the structure, thermal properties, and *in vitro* release characteristics of the resulting microspheres.

MATERIALS AND METHODS

Poly(*dl*-lactide) (Southern Research Institute, Birmingham, Ala.) had an inherent viscosity of 1.2 dl/g in chloroform at a concentration of 5 mg/ml. The following chemicals were obtained from commercial suppliers and used without further purification: caffeine (MCB Manufacturing Chemist, Inc., Gibbstown, N.J.), diazepam (Hoffman-La Roche, Inc., Nutley, N.J.), hydrocortisone, progesterone, quinidine, quinidine hydrochloride, quinidine sulfate, theophylline (Sigma Chemical Co., St. Louis, Mo.), methanol, methylene chloride, and salicylic acid (Fisher Scientific Co., Fair Lawn, N.J.).

[1] College of Pharmacy, The University of Texas at Austin, Austin, Texas 78712-1074.

[2] To whom correspondence should be addressed.

0724-8741/87/1200-0465$05.00/0

Microspheres were prepared by the solvent evaporation process as previously described (14). The drugs were dissolved or, in the case of insoluble drugs, dispersed in a solution of PLA in methylene chloride. The organic solution was poured into the agitated aqueous phase. The resulting emulsion was stirred continuously at room temperature and under ambient pressure until the solvent evaporation was completed. The microspheres were collected by filtration, washed with deionized water, and dried in a desiccator. The dried spheres were screened through stainless-steel sieves into the desired size fractions and stored at room temperature.

The drug content of the microspheres was determined after extracting the microspheres with methanol or chloroform by ultraviolet spectroscopy at the wavelength of maximum absorbance. The theoretical drug content within the microspheres was 30%, unless otherwise mentioned.

Scanning electron microscopy (SEM) was used to characterize the microsphere structure. The dried microspheres were coated for 70 sec under an argon atmosphere with gold–palladium (Pelco Model 3 sputter coater). The surface morphology of the microspheres was examined with a scanning electron microscope (Jeol JSM 35C).

Thermograms of the drugs, polymers, and microspheres were obtained by using a computer-interfaced Perkin–Elmer differential scanning calorimeter, Model DSC 2. An enclosed, air-cooled refrigeration unit (Perkin–Elmer Intracooler II) was attached to the DSC cell for controlled cooling to subambient temperatures. The temperature calibration was accomplished with the melting transition of indium. The samples (4–7 mg, 8–70 μm), which were stored in a desiccator prior to the analysis, were sealed in aluminum pans. The scanning rate throughout the investigation was 20°C/min for heating and 320°C/min for cooling. All tests were run in a nitrogen atmosphere.

In vitro release profiles of the drugs from the microspheres were obtained by the rotating bottle method at 37°C. Triplicate samples of microspheres (20–30 mg, 8–70 μm) were suspended in test tubes containing 50 ml of prewarmed medium (phosphate buffer, pH 7). The flasks were sealed and rotated at 26 rpm. Agitation was stopped and samples of the medium (1 ml) were withdrawn at predetermined time intervals after the microspheres had settled. The samples were assayed spectrophotometrically either directly or after appropriate dilution with the release medium. The sample volumes were immediately replaced with fresh medium kept at 37°C to maintain the original volume. The maximum concentration of the drug in the release medium was below 1% of the saturation concentration.

RESULTS AND DISCUSSION

Several drugs with different solubility characteristics were chosen as model compounds. The drugs are listed in Table I together with the resulting drug content within the microspheres. Quinidine, caffeine, progesterone, salicylic acid, and diazepam were completely soluble in the polymer solution. Quinidine sulfate dissolved in methylene chloride only under moderate heat. Microspheres could thus be prepared from a homogeneous quinidine sulfate–polymer solution or from a quinidine sulfate suspension prepared without heating the organic phase. Theophylline and hydrocortisone were insoluble and they were emulsified as suspensions.

Table I. Drug Entrapment in the Microspheres as a Function of Drug and of Different pH Values of the Aqueous Phase

Drug	Drug content (wt%)[a]		
	pH 2	pH 7	pH 12
Quinidine	—	5.09	23.76
Quinidine sulfate	—	—	23.93
Quinidine hydrochloride	—	—	24.36
Salicylic acid	—	—	—
Diazepam	14.51	21.10	20.96
Hydrocortisone	12.22	12.41	13.06
Progesterone	20.62	20.79	20.46
Theophylline	—	—	—
Caffeine	—	—	—

[a] Thirty percent theoretical drug content.

The successful entrapment of drug within the microspheres was highly dependent on the solubility of the drug in the aqueous phase (15). Partitioning of drug was expected to take place between the organic and the aqueous phase. Water-soluble drugs such as theophylline, caffeine, and salicylic acid could not be entrapped within the spheres because of complete partitioning into the aqueous phase. Conversely, drugs with low water solubility were successfully retained within the microspheres. Diffusion and loss of ionizable drugs such as quinidine and diazepam into the aqueous phase could be minimized by adjusting the pH to values of low drug ionization. Only minor differences in drug content were observed between samples prepared from a quinidine sulfate solution or a suspension. Progesterone precipitated in the aqueous phase after approximately 35 min. Methylene chloride diffused into the aqueous phase and then evaporated at the air/water interface. Once the level of organic solvent dropped below a critical concentration in the aqueous phase, progesterone crystals appeared in the aqueous phase. The progesterone crystals could not be washed off the microspheres during the regular washing step. However, an additional wash with a 75 wt% aqueous ethanol solution rapidly removed the crystals without extracting progesterone from the microspheres. The other

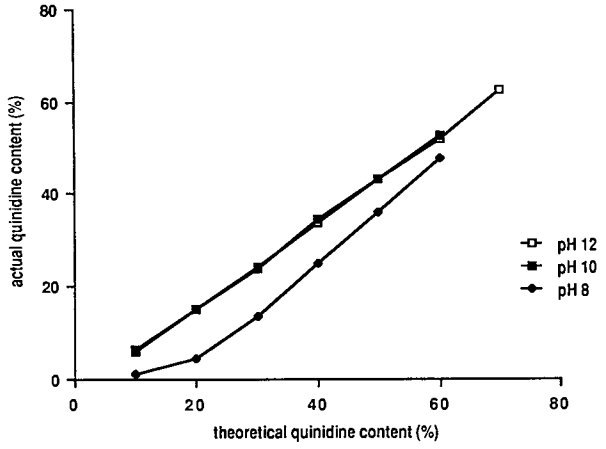

Fig. 1. Effect of quinidine payload on the actual quinidine content in the microspheres.

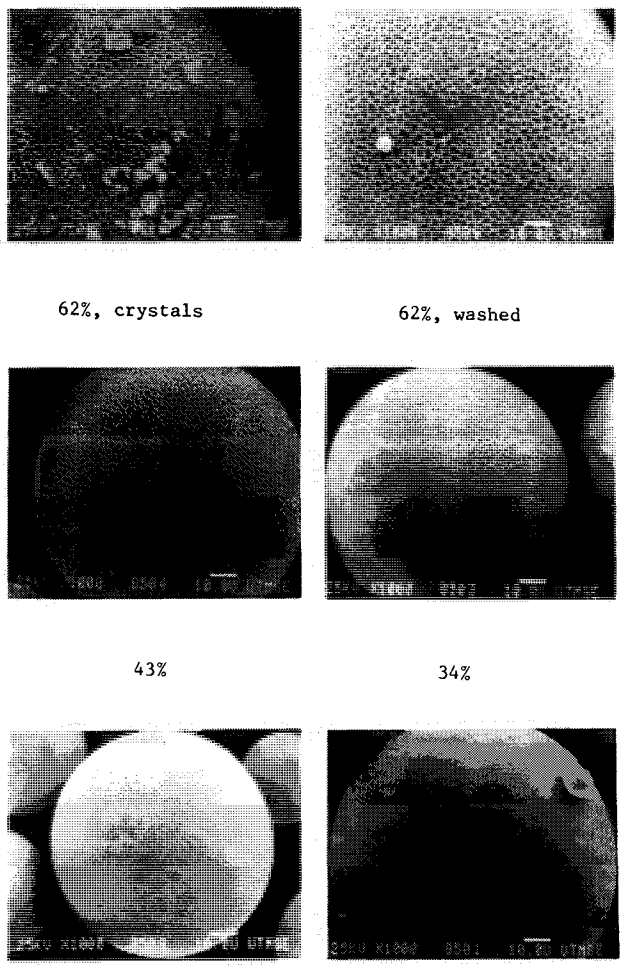

Fig. 2. Scanning electron micrographs of quinidine-loaded microspheres prepared at pH 12.

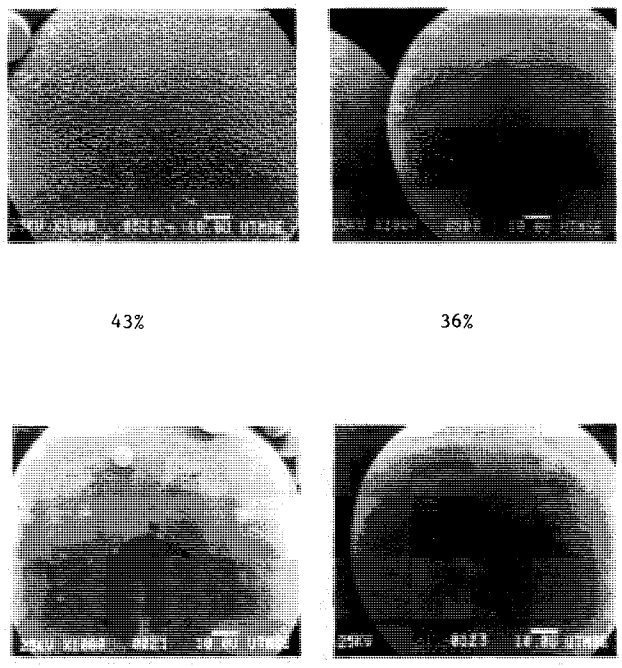

Fig. 3. Scanning electron micrographs of quinidine-loaded microspheres prepared at pH 8.

drugs did not precipitate in the aqueous phase at the drug concentrations tested.

The effect of drug loading on the quinidine content in the resulting microspheres was studied at three pH levels, pH 8, 10, and 12 (Fig. 1). With an increase in the quinidine content in the organic phase at preparation, the drug content in the microspheres was increased, also on the basis of the percentage drug content of the theoretical value. This was indicated in the increasing positive slope of the curves. The effect was more pronounced at pH values of higher drug solubility (pH 8). No differences in drug content were observed between microspheres prepared at pH 10 and those prepared at pH 12, corresponding to the same solubility of quinidine in the two buffer solutions.

Distinct differences in the surface structure of the microspheres at different drug contents and pH values can be seen in Figs. 2 and 3. The surface changed from a virtually smooth texture at low drug loadings to a honeycomb-like structure containing small holes at high loadings at pH 12 (Fig. 2). The number and size of pores increased accordingly. Structural changes in the microsphere surface prepared at different pH values were reported earlier. The surface layer or skin peeled off at high pH values, revealing the subsurface or porous substructure (15). Actual degradation of the polymer via hydrolytic cleavage of the ester links probably contributed significantly to the changes in surface structure under these extreme pH conditions. A partition method which circumvented the surface degradation observed at high pH values was developed to load the drug into the microspheres at pH values of high drug solubility (16). Free drug crystals appeared on the surface at a theoretical drug loading of 70%. Precipitation of quinidine occurred after evaporation of methylene chloride from the aqueous phase, once its saturation solubility in the aqueous medium was exceeded. The drug crystals could be removed by washing the microspheres in an acidified medium to give crystal free surfaces. SEM pictures of microspheres prepared at pH 8 reveal the same substructure under the skin (Fig. 3). The top polymer layer was not removed at this pH value.

The effect of drug loading on the release pattern is shown in Fig. 4. Three different release phases were distinguishable. A lag time with no drug release was followed by an apparent burst effect of rapid drug release within a short period of time, followed again by a slow release phase. The lag time was caused by the time required for water to penetrate into the polymer matrix. The long lag time confirmed that no free drug crystals on the surface were available for rapid dissolution. The *in vitro* drug release from the drug-containing PLA microspheres could be explained by two diffusional processes. In the first process, the aqueous dissolution medium will diffuse into the polymer matrix and dissolve the drug, which will then diffuse directly through channels or pores formed within the matrix to the external

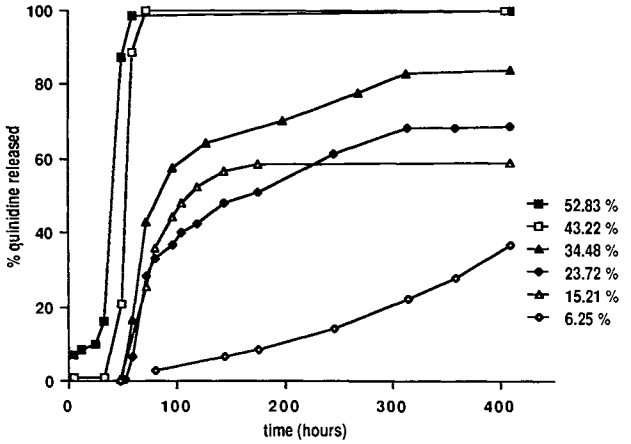

Fig. 4. Effect of quinidine loading in the microspheres on drug release.

medium. The initial burst was attributed to the rapid leaching of the drug through pores and channels, which represented the second phase of release. In the second process, the active compound dissolves in the polymer and passes by diffusion through the polymer matrix to the external fluid. The second process could be equated to the third phase of slow release. Both diffusional processes will contribute to the overall release of drug, the extent of each depending on parameters such as drug loading, matrix porosity, and drug solubility in the polymer.

Microspheres of equivalent size released the drug at a faster rate with increasing payloads. The duration of drug delivery was inversely proportional to the drug loading level and the total percentage of drug released increased with increasing payloads. Leaching and pore diffusion as expressed in the burst effect became more dominant at higher drug concentrations. In highly loaded microspheres, the drug will diffuse into the release medium, leaving empty pores or interconnected channels. When all drug within the pores was dissolved, drug release continued from the remaining less accessible drug, which was either finely dispersed or dissolved in the PLA matrix. Drug release was small after the initial burst, suggesting that drug diffusion in the polymeric matrix was very slow. The burst effect was eliminated at

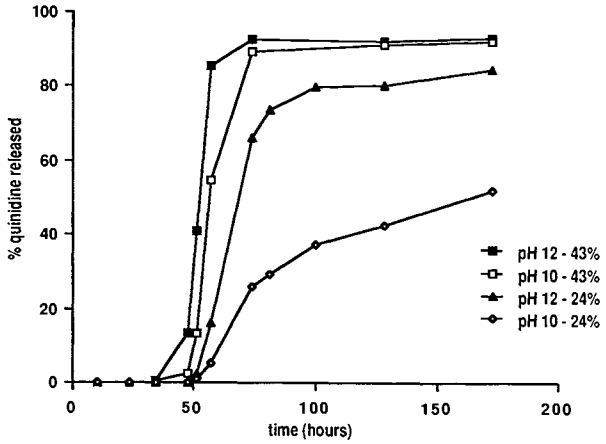

Fig. 5. Effect of the pH of the aqueous phase during microsphere preparation on drug release.

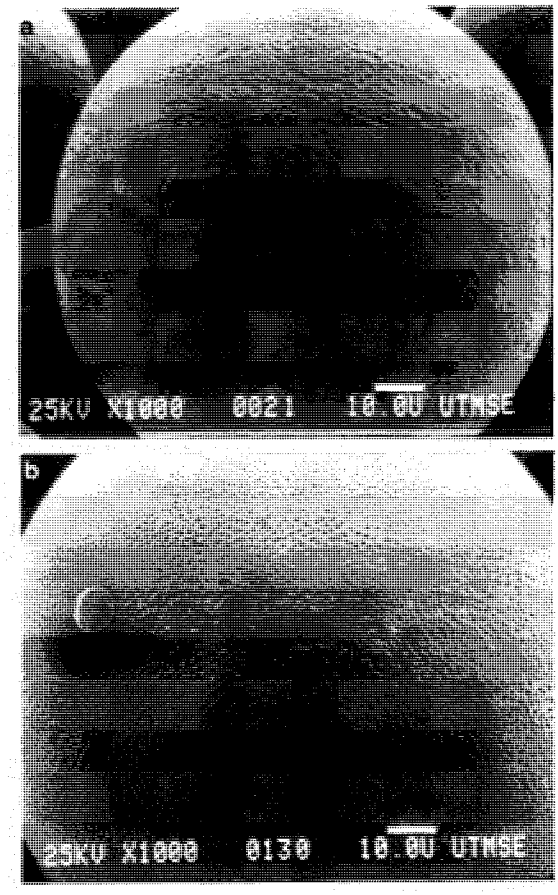

Fig. 6. Scanning electron micrographs of quinidine-loaded microspheres prepared at (a) pH 10 and (b) pH 12.

lower drug levels (6.25%). A payload below the concentration where leaching occurs is desirable to obtain very slow release rates. The release might become erosion-controlled *in vivo* if the polymer exhibits a very low permeability for the drug (17). The distribution of drug between the polymer and the pores during microsphere formation will influence the amount of drug available for rapid pore diffusion. No structural changes in the microsphere surface were observed during the course of the dissolution study by SEM.

Figure 5 shows the effect of surface structure of microspheres containing quinidine prepared at different pH levels on drug release. Dramatic differences were seen in the release rates from microspheres prepared at pH 10 and pH 12 containing quinidine. The drug release was slower from microspheres containing 24 and 43% quinidine prepared in an aqueous phase of pH 10, the difference in release rates being more pronounced at the lower drug loading. These differences could be explained by observing the surface structure of the microspheres (Fig. 6). As mentioned earlier (15), skinning occurred at high pH values in the aqueous phase, revealing a pore containing surface at pH 12. These pores provided sites for accelerated drug transport into the release medium by increasing the accessible surface for water penetration. The presence of an intact surface layer or the absence of pores at pH 10 explained the retardation in drug release. The lag time was approximately the same, while the burst was less dramatic at pH 10.

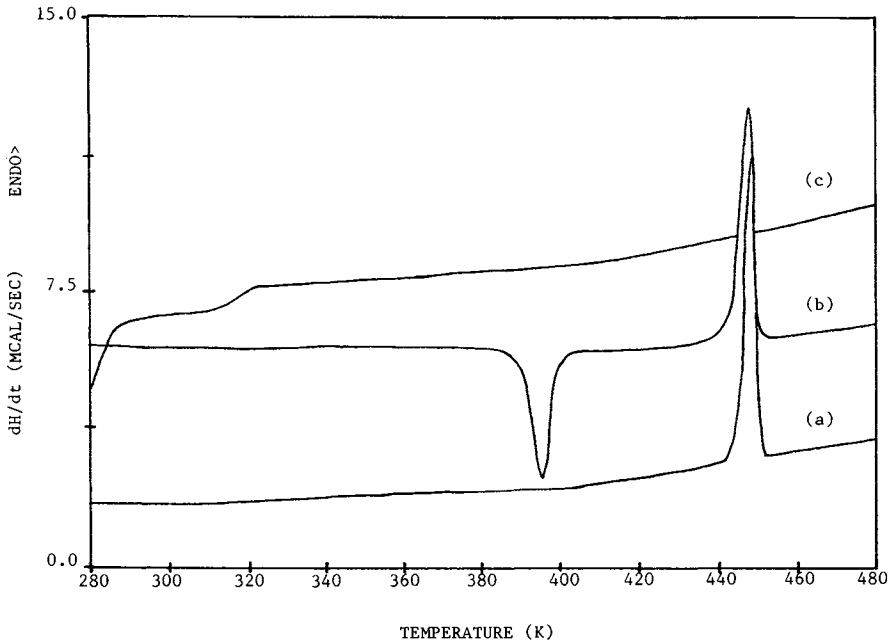

Fig. 7. DSC thermograms of quinidine (a) first run and (b) second run and (c) a melt-quenched sample of *dl*-PLA.

DSC analysis of the polymers, drugs, and drug-loaded microspheres was performed in order to characterize the physical state of the polymer and drug before and after microsphere preparation. A sharp endotherm was observed for quinidine at 171°C, corresponding to its melting phase transition, and the T_g of PLA could be clearly detected at 55°C after melt-quenching the sample (Fig. 7).

Three thermal events, which were reasonably sharp and well separated, were detectable in thermograms of drug-loaded microspheres (Fig. 8). These events corresponded to the T_g of the polymer, to the recrystallization of quinidine, and to the melting of quinidine. The melting point of quinidine was depressed and the melting range broadened with increasing PLA concentrations in the microspheres, while the recrystallization exothermic peaks were relatively insensitive to variations in drug content. The thermogram of a

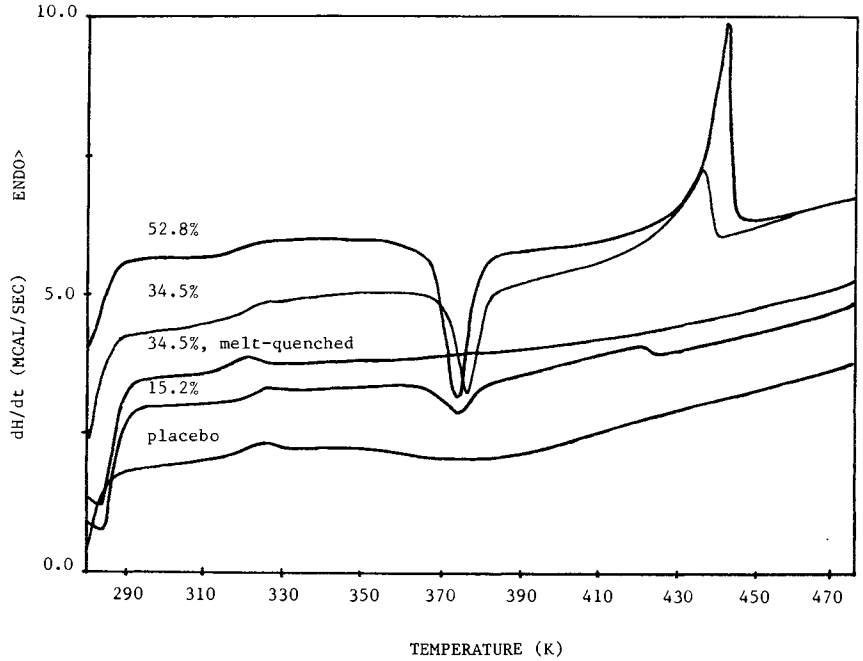

Fig. 8. DSC thermograms of *dl*-PLA microspheres containing different amounts of quinidine.

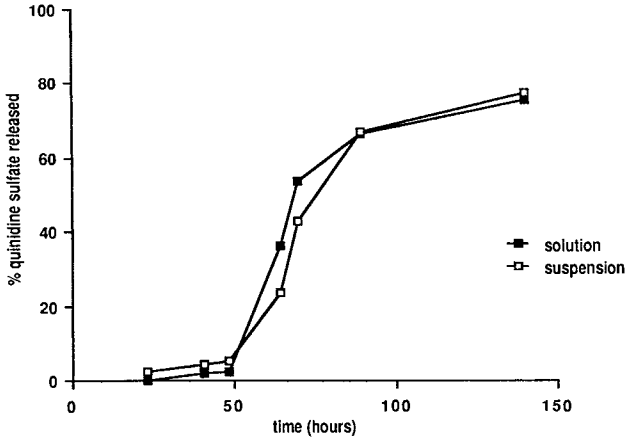

Fig. 9. Effect of the physical state of quinidine sulfate in the organic phase at preparation on drug release.

melt-quenched quinidine sample also exhibited a recrystallization peak (Fig. 7). Placebo microspheres containing no drug showed only one thermal event corresponding to the T_g of the PLA. This would indicate that the drug in the microspheres was at least partially in a noncrystalline state and that the crystalline phase developed during the test run. A dispersion of precipitated drug particles in the polymeric matrix was formed during microsphere preparation. The drug molecules had insufficient time to nucleate to form crystalline domains.

The recrystallization and melting peaks of the drug disappeared completely after melt-quenching microspheres at 320°C/min (Fig. 8). This suggested the formation of a glass solution. Heating the microspheres above the melting point of quinidine resulted in an extremely viscous melt. Supersaturation of the drug in the polymer glass was likely to occur due to the difficult growth of crystals in the viscous melt (18). Additionally, the crystallite size of the drug may also be very fine if the drug greatly exceeds its solubility in the polymer.

Quinidine sulfate-loaded microspheres could be prepared either from a homogeneous drug–polymer solution or from a drug suspension prepared without heating the organic phase. The emulsification of a drug solution results in a more homogeneous drug distribution within the microspheres, especially at low drug contents. However, microspheres prepared from a drug suspension tend to be more stable. Quinidine sulfate was released faster during the initial period or drug burst from microspheres prepared from the clear solution (Fig. 9). The actual particle size of quinidine sulfate within the polymer matrix as well as its physical form will greatly influence the drug release. The physical state of the drug in the microspheres prepared from the suspension should be the same as the form being added to the polymer solution in addition to the drug fraction which dissolved in the polymer solution. If the drug dissolved completely in the PLA solution, either it could precipitate within the microspheres during polymer precipitation in a crystalline or amorphous form or it could be present in a molecular dispersed state within the PLA matrix. DSC studies revealed a noncrystalline form of the drug within the microspheres, as represented by the recrystallization peak, with the amount being higher in the microspheres prepared from the quinidine sulfate solution (Fig. 10). The recrystallization peak was smaller for microspheres prepared from the suspension. This was expected since more quinidine sulfate was in the crystalline state when added as a suspension. Recrystallization could stem only from the drug fraction that dissolved in the polymer solution. This would explain the initial faster release from the microspheres which were prepared from the solution and thus contained the higher fraction of noncrystalline drug. Additionally, the drug might precipitate into smaller particles within the polymer matrix from the solution, also enhancing drug dissolution.

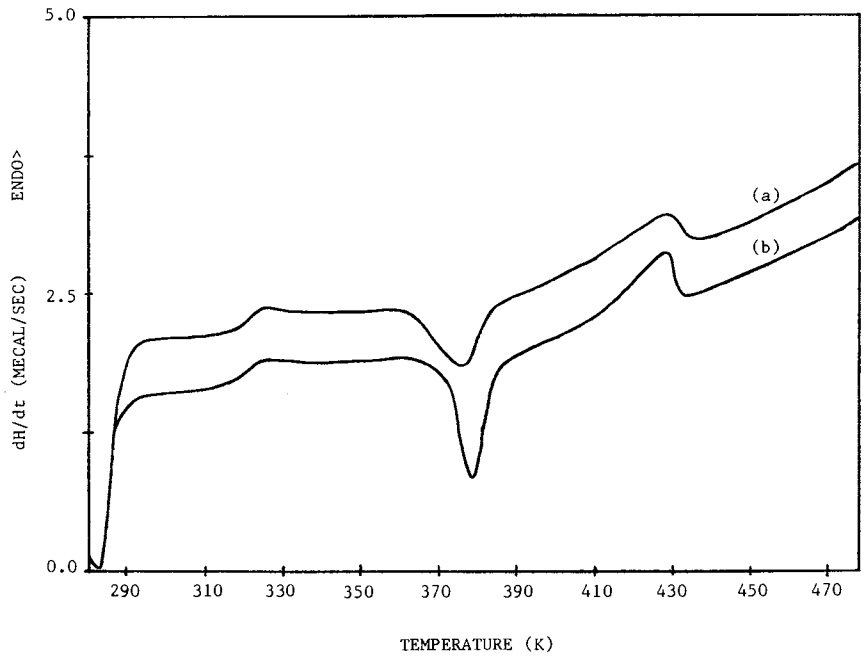

Fig. 10. DSC thermograms of *dl*-PLA microspheres containing 24% quinidine sulfate prepared from (a) a drug suspension and (b) a drug solution.

ACKNOWLEDGMENT

This work was supported in part by a grant from the FMC Corporation, Philadelphia, Pennsylvania 19103.

REFERENCES

1. T. M. Jackanicz, H. A. Nash, D. L. Wise, and J. B. Gregory. *Contraception* 8(3):227–234 (1973).
2. J. H. R. Woodland and S. Yolles. *J. Med. Chem.* 16(8):897–901 (1973).
3. A. D. Schwope, D. L. Wise, and J. F. Howes. *Life Sci.* 17:1877–1886 (1975).
4. A. D. Schwope, D. L. Wise, and J. F. Howes. *Natl. Inst. Drug Abuse Res. Monogr. Ser.* 4:13–18 (1976).
5. E. S. Nuwayser, D. L. Williams, J. H. Kerrigan, W. A. Nucefora, and J. C. Armstrong. In G. I. Zatuchni, A. Goldsmith, J. D. Shelton, and J. J. Sciarra (eds.), *Long-Acting Contraceptive Delivery Systems,* PARFR Series on Fertility Regulations, Harper & Row, Philadelphia, 1984, pp. 64–76.
6. C. A. Sharon and D. L. Wise. In R. E. Willette and G. Barnett (eds.), *Naltrexone,* NIDA Research Monograph 28, U.S. Government Printing Office, Washington D.C., 1980, pp. 194–213.
7. N. Wakiyama, K. Juni, and M. Nakano. *Chem. Pharm. Bull.* 30(7):2621–2628 (1982).
8. K. Suzuki and J. C. Price. *J. Pharm. Sci.* 74(1):21–24 (1985).
9. J. P. Benoit, F. Courteille, and C. Thies. *Int. J. Pharm.* 29:95–102 (1986).
10. M. Cavalier, J. P. Benoit, and C. Thies. *J. Pharm. Pharmacol.* 38:249–253 (1986).
11. T. R. Tice and R. M. Gilley. *J. Contr. Release* 2:343–352 (1985).
12. P. C. Gopalratnam, N. S. Mason, and R. E. Sparks. *Appl. Biochem. Biotechnol.* 10:213–220 (1984).
13. L. M. Sanders, J. S. Kent, G. I. McRae, B. H. Vickery, T. R. Tice, and D. H. Lewis. *J. Pharm. Sci.* 73(9):1294–1297 (1984).
14. R. Bodmeier and J. W. McGinity. *Proceedings of the 4th International Conference on Pharmaceutical Technology,* Paris, France, 1986, Vol. II, pp. 168–172.
15. R. Bodmeier and J. W. McGinity. *J. Microencapsulat.* (in press).
16. R. Bodmeier and J. W. McGinity. *J. Microencapsulat.* (in press).
17. L. M. Sanders, G. I. McRae, K. M. Vitale, and B. A. Kell. *J. Contr. Release* 2:187–195 (1985).
18. W. L. Chiou and S. Riegelman. *J. Pharm. Sci.* 60(9):1281–1302 (1971).