In Vitro Extended-Release Properties of Drug-Loaded Poly(DL-Lactic Acid) Nanoparticles Produced by a Salting-Out Procedure

Eric Allémann,[1] Jean-Christophe Leroux,[1] Robert Gurny,[1,2] and Eric Doelker[1]

Received March 17, 1993; accepted May 14, 1993

Savoxepine-loaded poly(DL-lactic acid) (PLA) nanoparticles were prepared using an emulsion technique involving a salting-out process which avoids surfactants and chlorinated solvents. After their formation, the nanoparticles were purified by cross-flow microfiltration and subsequently freeze-dried. The drug loading and the drug entrapment efficacy were improved by using savoxepine base rather than the methanesulfonate salt and by modifying the pH of the aqueous phase. A drug entrapment efficacy as high as 95% was obtained with a 9% drug loading. The overall yield of the procedure can rise up to 93%. *In vitro* release studies have demonstrated that by varying the mean size of the nanoparticles and their drug loading, the release of the drug from the nanoparticles can be modulated to last from several hours to more than 30 days, thus allowing the preparation of an injectable extended-release dosage form.

KEY WORDS: nanoparticle; polymeric aqueous dispersion; poly(DL-lactic acid); salting-out; injectable dosage form; *in vitro* release.

INTRODUCTION

Aqueous suspensions of polymeric nanoparticles (latexes) have been used in various pharmaceutical applications such as film coating (1,2), drug targeting (3), and extended release (4). These aqueous dispersions can be divided into two main categories, according to their method of preparation. They can be produced either by polymerization reactions (5) or by dispersion of preformed polymers (pseudolatexes) (6–8). The presence of monomer, surfactant, and sometimes initiator residues may render the former techniques unsuitable for pharmaceutical preparations such as injectable dosage forms (9). Most of the latter techniques rely on the emulsification of water-immiscible organic solutions of preformed polymers in aqueous phases containing surfactants, followed by the removal of the solvents under reduced pressure. Residual surfactants and chlorinated solvents are an important drawback of these methods for pharmaceutical applications.

The aim of this work was to produce drug-loaded poly(DL-lactic acid) nanoparticles for intramuscular injection to provide an extended release over a period of 1 month of a neuroleptic drug (savoxepine). The advantage of using poly(DL-lactic acid) as polymer lies in its biocompatibility and biodegradability. Poly(lactic acid) nanoparticles can be produced essentially by three techniques: the emulsion-evaporation technique (4) based on a patent to Vanderhoff *et al.* (10), the precipitation procedure proposed by Fessi *et al.* (11), and the salting-out procedure (12–14). The latter technique, which has been used in this study, has proved promising with regard to scale-up possibilities and purification of preparations intended for parenteral administration (15,16).

In order to improve the nanoparticle drug loading and the drug entrapment efficacy, several process parameters were varied: savoxepine base or methanesulfonate was used as drug, an acidic electrolyte (magnesium chloride), or a basic one (magnesium acetate), was tested as salting-out agent, and finally, the influence of nanoparticle size on drug entrapment efficacy was studied.

In this study, the release profiles were determined by a "sample and separate" technique (17,18). In this technique, the carrier is diluted into a sink, which is sampled at intervals. The continuous phase of the sample is then separated from the dispersed phase, usually by filtration or centrifugation, and the released drug is assayed. Here centrifugation was chosen to avoid drug adsorption during filtration. Since the release of the drug from the poly(lactic acid) nanoparticles was expected to last several days or weeks, a 2-hr ultracentrifugation of the samples was not considered to be long enough to influence the release profile. However, in the case of a release expected to last a day or less, the time needed to centrifuge should be taken into account in the interpretation of the release profiles.

In this study, the influence of process parameters such as the nanoparticle size, the drug loading, and the rate of dilution on the release characteristics were analyzed. Storage and sterilization were also considered as possible sources of modifications in the *in vitro* release patterns.

MATERIALS AND METHODS

Materials

Based on previous work (15), nanoparticles were produced using acetone as the organic water-miscible solvent, because of its favorable physicochemical properties (19) and its pharmaceutical acceptability with regard to toxicity (20). The particles were loaded with savoxepine, a new neuroleptic drug: 3-(cyclopentylmethyl)-2,3,4,5-tetrahydro-1-H-dibenzo[2,3:6,7]oxepino-[4,5-*d*]azepine-7-carbonitrile, either as the methanesulfonate salt or as the base (Ciba-Geigy, Basel, Switzerland). Poly(DL-lactic acid) (PLA) (Medisorb Technologies International L.P., Cincinnati, Ohio) was used as polymer (intrinsic viscosity, 0.72 dL/g). Magnesium chloride hexahydrate (Fluka, Buchs, Switzerland) or magnesium acetate tetrahydrate (Merck, Darmstadt, Germany) were used as salting-out agents. Poly(vinyl alcohol) (PVAL) with a molecular mass of 26,000 was selected as stabilizing colloid (Mowiol 4-88 Hoechst, Frankfurt/M, Germany). Higher molecular weight PVALs are difficult to eliminate during the cross-flow filtration procedure (16).

Nanoparticle Preparation

The preparation method consisted of adding a water-

---

[1] School of Pharmacy, University of Geneva, CH-1211 Geneva 4, Switzerland.

[2] To whom correspondence should be addressed.

0724-8741/93/1200-1732$07.00/0

Table I. PLA Nanoparticle Batches Loaded with Savoxepine Methanesulfonate

Batch no.	Salting-out agent	Phase ratio	Initial drug content (m/m %)	Drug loading (m/m %)	Entrapment efficacy (%)[a]	Weight yield (%)[b]	Size (nm)	Residual PVAL conc. (%)
1	$MgCl_2$	70/100	1.0	0.20	20.7	71	358	3.47
2	$MgCl_2$	70/100	1.6	0.25	16.3	93	437	3.98
3[c]	$MgCl_2$	70/100	1.6	0.19	12.3	92	421	3.09
4	$Mg(Ac)_2$	70/100	1.6	1.00	64.2	16	427	2.63
5	$Mg(Ac)_2$	40/100	1.6	1.12	77.3	89	254	9.45

[a] Drug/polymer ratio in the nanoparticles divided by initial drug/polymer ratio.

[b] Batch yield after preparation, purification, and freeze-drying of the nanoparticles.

[c] Dilution was carried out in 30 min for batch 3 and in 10 min for all other batches.

soluble polymer (PVAL) to a saturated electrolyte (magnesium chloride or magnesium acetate) solution, so as to obtain a viscous gel (aqueous phase). PLA and savoxepine were dissolved separately in acetone (organic phase). Although this organic solvent is miscible in all proportion with pure water, a liquid–liquid two-phase system formed when a portion of the gel was added to the organic solution. Such a system was obtained when the salt dissolved in the aqueous phase was capable of causing the salting-out of the organic solvent. Upon further addition of gel to the organic phase under mechanical stirring, an oil-in-water emulsion formed at room temperature. Pure water was then added to the resulting emulsion in a sufficient amount for the acetone to diffuse into the aqueous phase, thus inducing the formation of nanoparticles suspended in the resulting organic-aqueous medium (14).

Typically, the aqueous phase contained 60% (m/m) of magnesium chloride hexahydrate or 35% (m/m) of magnesium acetate tetrahydrate and a variable amount of PVAL, depending on the size desired for the nanoparticles. The aqueous phase was emulsified with an organic phase containing 17.7% (m/m) of PLA and an amount of drug ranging from 1 to 20% based on the weight of the polymer. Generally, 100 g of aqueous phase was added to 70 or 40 g of organic phase, which corresponds to phase ratios of 70:100 and 40:100, respectively. After emulsification under mechanical stirring, pure water (100 mL) was added to induce the diffusion of acetone in water and the formation of the nanoparticles.

The raw nanoparticulate suspension was then purified by cross-flow microfiltration using a Sartocon Mini device (Sartorius, Göttingen, Germany) mounted with a polyolefin cartridge filter with a 100-nm pore size (16). Purification was stopped after the collection of 10 L of filtrate containing the PVAL, the electrolyte, and the unbound drug. The purified nanoparticles were then freeze-dried in 24 hr at 0.08 mbar with a Lyolab BII (Secfroid, Aclens, Switzerland).

Determination of Drug Loading

Nanoparticles were dissolved in 50.0 mL of chloroform and the solution was assayed spectrophotometrically for savoxepine in the wavelength range from 284 to 294 nm (Hewlett Packard, diode array spectrometer 8452 A, Waldbronn, Germany).

Assay for Residual Poly(Vinyl Alcohol) in the Nanoparticles

PVAL in solution forms stable complexes with iodine in the presence of boric acid (21). Two hundred milligrams of nanoparticles was mixed in 25 mL of chloroform to dissolve the PLA and the drug and sonicated for 15 min. The solution was then filtered on a cellulose filter and the residual PVAL retained on the filter was washed with 25 mL of chloroform. The filter was dried at atmospheric pressure and then introduced into a flask, and pure water was added to 100 g. The water was heated to 94°C to dissolve the residual PVAL. After cooling, an aliquot of the PVAL solution was assayed according to a previously described method (16). Namely, 15 mL of a 4% boric acid solution and 3 mL of an iodine solution (1.27% iodine and 2.5% potassium iodide in water) were added to 5.0 mL of the PVAL solution. The volume was then

Table II. PLA Nanoparticle Batches Loaded with Savoxepine Base

Batch no.	Salting-out agent	Phase ratio	Initial drug content (m/m %)	Drug loading (m/m %)	Entrapment efficacy (%)	Weight yield (%)	Size (nm)	Residual PVAL conc. (%)
6	$MgCl_2$	70/100	2.00	0.32	16.8	50	228	5.00
7	$MgCl_2$	70/100	6.00	0.45	7.9	82	350	4.74
8	$Mg(Ac)_2$	40/100	1.10	0.91	89.5	89	247	7.52
9	$Mg(Ac)_2$	40/100	5.00	3.98	87.7	91	245	9.20
10	$Mg(Ac)_2$	40/100	9.10	7.14	86.2	92	274	8.97
11	$Mg(Ac)_2$	40/100	20.0	16.7	94.6	93	246	11.77
12[a]	$Mg(Ac)_2$	40/100	9.10	6.82	82.9	51	265	9.50

[a] Dilution was carried out in 30 min for batch 12 and in 10 min for all other batches.

Table III. Savoxepine Base-Loaded PLA Nanoparticles Produced with Aqueous Phases Containing Variable Amounts of PVAL

Batch	PVAL conc. %	Initial drug content (m/m %)	Drug loading (m/m %)	Entrapment efficacy (%)	Weight yield (%)	Size (nm)	Residual PVAL conc. (%)
10	12	9.10	7.14	86.2	92	274	8.97
13	10	9.10	7.76	90.3	80	303	5.57
14	8	9.10	8.44	94.8	92	671	2.22
15	8	9.10	8.48	94.9	62	641	1.88
16	8	5.00	4.70	95.4	80	735	1.53

adjusted to 50.0 mL with water. The absorbance was measured in the integrated wavelength range from 620 to 690 nm. Values given in the tables are means of three assays.

Particle Size Determination

Nanoparticles were measured by photon correlation spectroscopy (PCS) using a Zeta-sizer III (Malvern, Worcestershire, England). Values given are means of three measurements.

In Vitro Release Kinetics

The release properties of the nanoparticles were studied in a 0.15 *M* phosphate buffer, pH 7.4, containing 40 mmol/L of sodium lauryl sulfate to reach sink conditions (22,23). A sufficient amount of nanoparticles (corresponding to 3 mg of savoxepine) was suspended in the buffer. The suspension was agitated at 100 rpm in a horizontal shaker maintained at 37°C (GFL, Burgwedel, Germany). At selected intervals of time up to 28 days, 2.5 mL of the suspension was sampled and centrifuged at 55,000*g* for 2 hr. The absorbance of the supernatant was measured spectrophotometrically in the wavelength range from 278 to 290 nm.

RESULTS AND DISCUSSION

Drug Loading

The first drug loading trials were carried out with savoxepine methanesulfonate (pK_a, 8.3). Due to its low solubility in acetone (310 mg/100 mL), the highest initial concentration of drug in the acetone solution was 0.283% (m/m), which corresponds to an initial drug concentration of 1.6% based on the polymer weight (Table I). Batches 1 and 2 exhibited very low drug loadings and entrapment efficacies. The entrapment efficacy is defined as the drug/polymer ratio in the final product divided by the initial drug/polymer ratio. For batches 1 and 2, the dilution of the emulsion with 100 mL of water was achieved in 10 min. The entrapment efficacy of the drug was not improved by increasing the dilution time to 30 min (batch 3). This demonstrates that a reduction of the diffusion rate of acetone in water does not improve the entrapment efficacy. The entrapment efficacy was, however, significantly improved by replacing the magnesium chloride by magnesium acetate (batch 4) as the salting-out agent. With this modification, the pH of the aqueous phase rose from 6 to 8, thus preventing the solubilization of the drug in the aqueous phase. However, by using magnesium acetate, a lower phase ratio was needed to allow a complete phase inversion of the emulsion and thus to achieve high batch yields (Table I, batch 5). The low batch yield of batch 4 can be explained by the incomplete phase inversion when using a 70/100 phase ratio. The batch yield is calculated after preparation, purification and freeze-drying.

Since savoxepine methanesulfonate is only slight soluble in acetone, savoxepine base was tested to obtain higher drug loadings. Table II (batches 6 and 7) shows that, by using an acidic aqueous phase, the entrapment efficacies remained very low as was observed previously with batches 1 and 2. Upon replacement of magnesium chloride by magnesium acetate, entrapment efficacies were higher than 83%, with

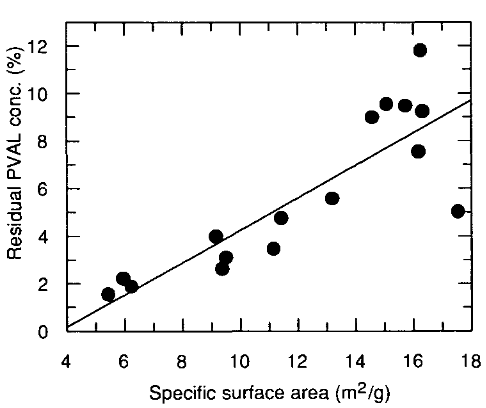

Fig. 1. Residual PVAL concentration in 16 batches of savoxepine-loaded PLA nanoparticles, expressed as a function of the specific surface of the particles.

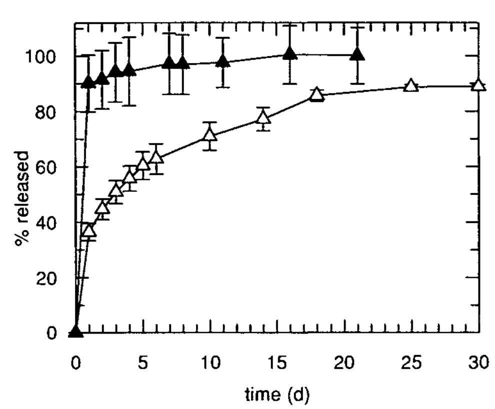

Fig. 2. Influence of drug loading on the release rate for particles smaller than 300 nm: (△) 7.1% (batch 10); (▲) 16.7% (batch 11). Mean ± SD; $n = 4$.

Table IV. Drug Release Characteristics of Savoxepine Base-Loaded PLA Nanoparticles as a Function of Drug Loading and Mean Nanoparticle Size

Batch no.	Drug loading (m/m %)	Size (nm)	$t_{25\%}$ (days)	$t_{50\%}$ (days)	$t_{75\%}$ (days)
10	7.14	274	<1	2.5	12.4
10 (sterilized)	7.14	301	<1	1.4	4.9
11	16.7	246	<1	<1	<1
12 (slow dilution)	6.82	265	<1	<1	7.3
13	7.76	303	<1	2.7	13.9
14	8.44	671	3.2	18.1	n.a.
15	8.48	641	3.0	17.9	n.a.
15 (9 months)	8.48	641	1.6	13.1	n.a.
16	4.70	735	2.3	20.3	n.a.

batch yields exceeding 90%. Drug loadings reached 16.7% (m/m) were obtained with this technique. Batch 12 was prepared in the same conditions as batch 10, except for the dilution time, which was prolonged to 30 min. As it was observed previously with batch 5, this modification improved neither the drug loading nor the entrapment efficacy. Based on a previous study (15), the size of the drug-loaded nanoparticles was varied by changing the concentration of PVAL in the aqueous phase (Table III). The batches with larger mean particle sizes had also higher entrapment efficacies (up to 95%) with good batch yields.

Residual Poly(Vinyl Alcohol) in the Nanoparticles

Residual PVAL concentrations are presented in Tables I, II, and III. Since the concentration of residual PVAL appeared to be related to the mean size of the nanoparticles, specific surface areas of the particles were calculated to determine if PVAL residues were related to them. Considering spherical particles (15) showing a monodispersed distribution, the specific surface area S_w of the nanoparticles can be calculated by Eq. (1):

$$S_w = \frac{S}{v \cdot \rho} = \frac{6}{d \cdot \rho} \qquad (1)$$

with S being the surface, v the volume, ρ the true density, and d the diameter of the spheres. Since the density of the nanoparticles has an average value of 1.5 g/cm^3 (measured with a comparative air pycnometer Beckman, Model 930), the specific surface areas can be calculated. For example, particles with a mean diameter of 228 nm (batch 1) have a specific surface area of 17.5 m^2/g and large particles of 735 nm (batch 15) have a specific surface area of 7.5 m^2/g. Figure 1 shows that the residual amount of PVAL in the 15 batches tested is roughly linearly related to the S_w of the particles. These results confirm that the hydrocolloid forms a stable layer at the surface of the particles, which cannot be eliminated during the purification procedure by cross-flow microfiltration. This hydrophilic layer has the advantage of allowing an almost-instantaneous redispersion of the freeze-dried particles in water. The consequences of the PVAL layer on the biodistribution of the particles will be discussed in a subsequent paper.

In Vitro Release Kinetics

Among the experimental methods available for determining the release profiles from drug-loaded nanoparticles *in vitro*, the most widely used are membrane diffusion techniques and "sample and separate" techniques (17). Due to the necessary addition of a surfactant to reach sink conditions with the drug used in this study, preliminary testing revealed the impossibility of using a membrane diffusion technique (dialysis), due to the accumulation of the drug in surfactant micelles in the donor compartment and which led to a retarded equilibrium between the two compartments. Consequently, a "sample and separate" technique was used in this study.

Batches 1 to 9 had an insufficient drug loading to be considered for further investigations. Batches 10 to 16 were tested for their *in vitro* release properties. Figure 2 and Table IV show the significant influence of drug loading on the release rate of savoxepine from PLA nanoparticles. As already discussed, particles prepared by a prolonged dilution procedure of the emulsion (batch 12) did not demonstrate a higher entrapment efficacy, and they did not present enhanced extended-release properties (Fig. 3), compared to the particles produced with a rapid dilution (batch 10). For particles having a mean size below 275 nm, Table IV shows that half of the

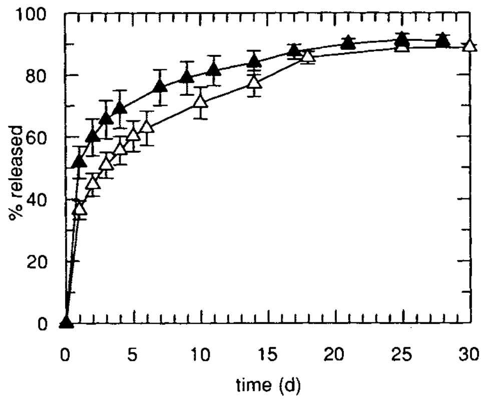

Fig. 3. Influence of the dilution rate of the emulsion on the release rate of savoxepine from PLA nanoparticles: (△) Rapid dilution of 10 min (batch 10); (▲) slow dilution of 30 min (batch 12). Mean ± SD; $n = 4$.

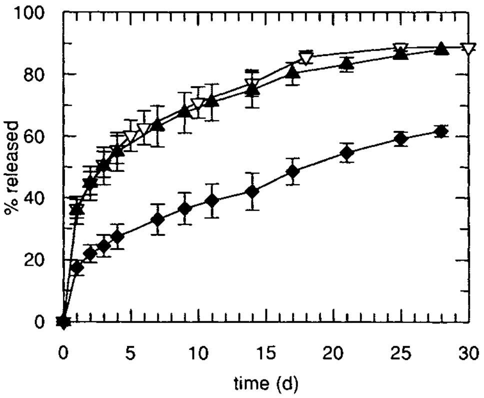

Fig. 4. Influence of the nanoparticle size on the release rate of savoxepine from PLA nanoparticles: (▽) 274 nm (batch 10); (▲) 303 nm (batch 13); (◆) 671 nm (batch 14). Mean ± SD; $n = 4$.

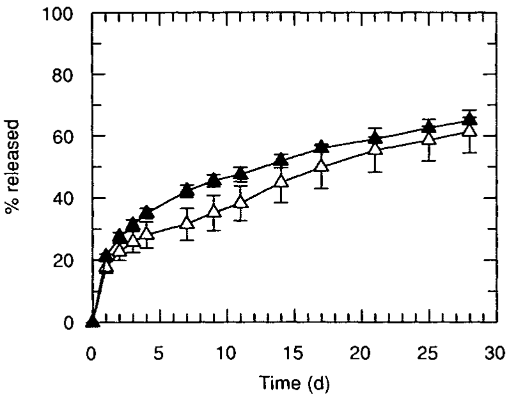

Fig. 6. Influence of aging on the release rate of savoxepine from PLA nanoparticles (batch 15); (△) 1 week; (▲) 9 months. Mean ± SD; $n = 4$.

entrapped dose of drug was released in all cases in less than 2.5 days ($t_{50\%}$). Since the aim of this study was to produce a nanoparticulate dosage form releasing its drug content over a period of 1 month, batches with larger particle sizes were tested *in vitro* (Fig. 4). A significant increase in the time needed to release 25% ($t_{25\%}$) and 50% ($t_{50\%}$) of the dose was observed with batch 14, having a mean size of 671 nm. The important "burst" release observed with smaller particles was reduced to 17.3% of the drug released after 24 hr. A last attempt to slow down the release was achieved by reducing the drug loading in "large" nanoparticles (Table IV, batch 16). Compared to batches 14 and 15, batch 16, with a slightly larger particle size, exhibited an improved $t_{50\%}$. However, the burst release was not reduced as shown by the $t_{25\%}$ value.

The *in vitro* release patterns obtained in this study demonstrate that an extended release of drug over a period exceeding 1 month is possible with these polymeric particles carrying drug entrapped in them. As a comparison, drugs entrapped in or adsorbed to poly(alkylcyanoacrylate) nanoparticles are usually released within only a few hours (24). On the other hand, PLA nanocapsules containing indomethacin release their lipophilic drug content in less than 24 hr (25). It should be mentioned that *in vitro* release studies with poly(alkylcyanoacrylate) have been conducted under different experimental conditions.

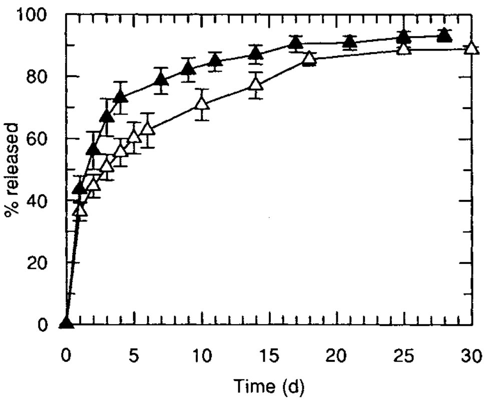

Fig. 5. Influence of γ-sterilization (2.5 Mrad) on the release rate of savoxepine from PLA nanoparticles (batch 10). (△) Not sterile; (▲) sterilized. Mean ± SD; $n = 4$.

For the production of an injectable dosage form, the sterility of the final product must be considered. Since no information is available in the literature on γ-sterilization of PLA nanoparticles, the effect of this technique on the release rate of savoxepine was also evaluated in this study. Figure 5 shows that a 2.5 Mrad dose had an important effect on the release rate, the $t_{50\%}$ and $t_{75\%}$ being reduced from 2.5 to 1.4 and from 12.4 to 4.9 days, respectively. Such an increase in the release rate has been reported by Lewis (26) in the case of microcapsules consisting of lactide/glycolide polymers. This is apparently due to a dramatic decrease in the number-average molecular weight at sterilization doses above 1.0 Mrad.

The stability of the nanoparticles after storage was tested on batch 15. An *in vitro* release trial was performed immediately after preparation and after 9 months during which the freeze-dried particles were kept at −24°C. Aging of the particles resulted in a faster release of the drug (Fig. 6). The basic nature of the drug could be responsible for the relatively high micro pH in particles, inducing a progressive hydrolysis of the polymer.

CONCLUSIONS

It has been demonstrated in the present study that a salting-out procedure can be used for entrapping efficiently a lipophilic drug in PLA nanoparticles of sizes ranging from 230 to 730 nm. Drug loading can be adjusted in a range up to 16.7%, for example, depending on the release rate desired for the dosage form. For example, particles with an 8.5% drug loading and a mean size of 640 nm release their content over more than 1 month. This holds promise for the preparation of biocompatible injectable extended-release dosage forms based on nanoparticulate suspensions.

In a subsequent paper, the *in vivo* distribution, kinetics of release, and excretion in rats will be described and correlated with the present data.

ACKNOWLEDGMENTS

Eric Allémann acknowledges a grant and the support of this work by Ciba-Geigy, Switzerland.

Jean-Christophe Leroux acknowledges a grant from the Medical Research Council of Canada.

REFERENCES

1. C. Bindschaedler, R. Gurny, and E. Doelker. Osmotically controlled drug delivery systems produced from organic solutions and aqueous dispersions of cellulose acetate. *J. Contr. Rel.* 4:203–212 (1986).
2. F. Gumowski, E. Doelker, and R. Gurny. The use of a new redispersible aqueous enteric coating material. *Pharm. Technol.* 11(2):26–32 (1987).
3. J. W. Vanderhoff and M. S. El-Aasser. Theory of colloids. In H. A. Liebermann, M. M. Rieger, and G. S. Banker (eds.), *Pharmaceutical Dosage Forms: Disperse Systems,* Marcel Dekker, New York, 1988, pp. 93–149.
4. R. Gurny, N. A. Peppas, D. D. Harrington, and G. S. Banker. Development of biodegradable and injectable latices for controlled release of potent drugs. *Drug Dev. Ind. Pharm.* 7:1–25 (1981).
5. C. Vauthier-Holtzscherer, S. Benabbou, G. Spenlehauer, M. Veillard, and P. Couvreur. Methodology for the preparation of ultra-dispersed polymer systems. *S.T.P. Pharma* 1:109–116 (1991).
6. R. Gurny. Latex systems. In D. D. Breimer and P. Speiser (eds.), *Topics in Pharmaceutical Science 1983,* Elsevier, Amsterdam, 1983, pp. 277–288.
7. R. Gurny, T. Boye, and H. Ibrahim. Ocular theory with nanoparticulates systems for controlled drug delivery. *J. Contr. Rel.* 2:353–361 (1985).
8. H. Ibrahim, C. Bindschaedler, E. Doelker, P. Buri, and R. Gurny. Concept and development of ophtalmic pseudo-latexes triggered by pH. *Int. J. Pharm.* 77:211–219 (1991).
9. R. C. Oppenheim. Solid colloidal drug delivery systems: nanoparticles. *Int. J. Pharm.* 8:217–234 (1981).
10. J. W. Vanderhoff, M. S. El-Aasser, and J. Ugelstad. Polymer emulsification process. U.S. Patent 4,177,177 (1979).
11. H. Fessi, J. P. Devissaguet, F. Puisieux, and C. Thies. Procédé de préparation de systèmes colloïdaux dispersibles d'une substance, sous forme de nanoparticules. French Patent 2,608,988 (1986).
12. C. Bindschaedler, R. Gurny, and E. Doelker. Process for preparing a powder of water-insoluble polymer which can be redispersed in a liquid phase, the resulting powder and utilization thereof. U.S. Patent 4,968,350 (1990).
13. C. Bindschaedler, R. Gurny, and E. Doelker. Process for preparing a powder of water-insoluble polymer which can be redispersed in a liquid phase, the resulting powder and utilization thereof. European Patent 363549B1 (1993).
14. H. Ibrahim, C. Bindschaedler, E. Doelker, P. Buri, and R. Gurny. Aqueous nanodispersions prepared by a salting-out process. *Int. J. Pharm.* 87:239–246 (1992).
15. E. Allémann, R. Gurny, and E. Doelker. Preparation of aqueous polymeric nanodispersions by a reversible salting-out process, influence of process parameters on particle size. *Int. J. Pharm.* 87:247–253 (1992).
16. E. Allémann, E. Doelker, and R. Gurny. Drug loaded poly(lactic acid) nanoparticles produced by a reversible salting-out process: Purification of an injectable dosage form. *Eur. J. Pharm. Biopharm.* 39:13–18 (1993).
17. C. Washington. Drug release from microdisperse systems: A critical review. *Int. J. Pharm.* 58:1–12 (1990).
18. M. Y. Levy and S. Benita. Drug release from submicrozed o/w emulsion: A new in vitro kinetic evaluation model. *Int. J. Pharm.* 66:29–37 (1990).
19. C. E. Matkovitch. Salting-out of acetone from water-basis of a new solvent extraction system. *Anal. Chem.* 45(11):1915–1921 (1973).
20. J. Rabiant. La limitation des solvants résiduels. Aspect réglementaire. *S.T.P. Pharma* 1:278–283 (1991).
21. J. H. Finley. Spectrophotometric determination of polyvinylalcohol in paper coatings. *Anal. Chem.* 33:1925–1927 (1961).
22. B. Gander, R. Gurny, and E. Doelker. Einfluss der Zerfallseigenschaften von Tabletten auf die Freigabe und Absorption einer quasi unlöslischen Modellsubstanz. *Acta Pharm. Technol.* 31(2):77–84 (1985).
23. B. Gander, K. Ventouras, R. Gurny, and E. Doelker. In vitro dissolution medium with supramicellar surfactant concentration and its relevance for in vivo absorption. *Int. J. Pharm.* 27:117–124 (1985).
24. L. Illum, M. A. Khan, E. Mak, and S. S. Davis. Evaluation of carrier capacity and release characteristics for poly(butyl 2-cyanoacrylate) nanoparticles. *Int. J. Pharm.* 30:17–28 (1986).
25. N. Ammoury, H. Fessi, J. P. Devissaguet, F. Puisieux, and S. Benita. In vitro release kinetic pattern of indomethacin from poly(D,L-lactide) nanocapsules. *J. Pharm. Sci.* 79:763–767 (1990).
26. D. H. Lewis. Controlled release of bioactive agents from lactide/glycolide polymers. In M. Chasin and R. Langer (eds.), *Biodegradable Polymers as Drug Delivery Systems,* Marcel Dekker, New York, 1990, pp. 1–41.