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Research paper

Porous biodegradable microparticles for delivery of pentamidine

T.K. Mandal*, L.A. Bostanian, R.A. Graves, S.R. Chapman, T.U. Idodo

Division of Pharmaceutics, College of Pharmacy, Xavier University of Louisiana, New Orleans, LA, USA

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Abstract

The primary objective of this study was to develop a method for the preparation of porous biodegradable controlled release formulation of poly(lactide/glycolide) (PLGA). The model drug used for this study was pentamidine. Scanning electron microscopy pictures showed that these microparticles are highly porous and spherical in shape. A comparison of particle size reveals a similar median particle size (54–68 μm) in all six batches. The particles are all smaller than 90 μm. Differential scanning calorimetry thermograms revealed that pentamidine was *mostly* present in the crystalline form in the microparticles and did not dissolve in PLGA. The efficiency of encapsulation of pentamidine was higher than 58% in all six batches. The amount of drug released from these microparticles was at least 12% within the first 60 min. At least 50% of the total drug was released within the first 4 h. Drug release from these microparticles continued for up to 12 h. This faster drug dissolution was due to the highly porous surface. This highly porous surface will allow large molecules to release at a much faster rate than the regular microcapsules/microspheres.

Keywords: Drug delivery; Controlled release; Pentamidine; Microspheres; Microparticles; Dissolution; Biodegradable; Poly(lactide/glycolide)

1. Introduction

Microspheres and microcapsules, collectively referred to as microparticles, have been widely used in pharmacy for drug delivery. These drug delivery systems contain a variety of polymers, including both biodegradable and non-biodegradable. A number of biodegradable polymers have been used, during the last two decades, to develop controlled release formulations of numerous therapeutic agents [1]. One of the advantages of these formulations is that no follow-up surgical removal is required once the drug supply is depleted.

In the controlled release technology, microencapsulation of drugs using poly(lactide/glycolide) (PLGA) has received the most attention because of the ease of fabrication. Once-a-month injectable microcapsules of leuprolide acetate using PLGA have been developed [2–4]. This product is now commercially available under the trade name Lupron-Depot®. Another similar product is ProLease® rhGH, which contains 24 mg recombinant human growth hormone–PLGA [5].

Over the last few years, several methods have been developed for the microencapsulation of a wide variety of drugs [6–15]. However, the double water-in-oil-in-water (w/o/w) emulsion method has been widely accepted for the encapsulation of a number of water soluble drugs [6,8,9,11,12,15]. The use of this double emulsion method to encapsulate pentamidine, having a water solubility of 1 in 10 at 25°C, in PLGA resulted in less than 16% efficiency of encapsulation [16]. The efficiency of encapsulation of pentamidine was increased up to 75% by a novel phase separation technique developed in our laboratory [7,17]. The method uses silicon oil as a dispersion medium. However, the efficiency of encapsulation of the active ingredient using this method was dependent on drug loading. The efficiency of encapsulation decreased significantly when the pentamidine loading was increased from 5 to 20%. Scanning electron micrographs of these microcapsules also showed that the surface of the particles was coated with free crystals at a higher drug loading (17). The microcapsules prepared using this method were mostly irregular in shape. The previously reported phase separation method, in general, produced relatively larger microcapsules. The efficacy of any controlled release microparticulate systems depends, primarily, on the physicochemical properties of the particles. Drug release from biodegradable polymer is generally slow because the release mechanism is mainly governed by the degradation of the polymer [1]. Typically, the drug release from PLGA microparticles (including microsphere and microcapsules) continued from 1 to 3

* Corresponding author. College of Pharmacy, Xavier University of Louisiana, 1 Drexel Dr., New Orleans, LA 70125, USA. Tel.: +1-504-483-7442; fax: +1-504-485-7930.

E-mail address: tmandal@xula.edu (T.K. Mandal).

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months with a 10–20% release within the first 24 h. Several investigators have tried to modify the surface morphology of the microparticles to achieve higher and faster drug release from the biodegradable polymer [18–22]. Changing the surface of the particles from smooth and non-porous to porous has increased the drug release significantly. Porous microparticles are useful for the delivery of large molecular weight protein and peptides. These are also useful for pulmonary drug delivery because of the smaller aerodynamic diameter, due to lower density, compared with the non-porous microparticles.

The primary objective of the present investigation was to develop a method for the preparation of a porous controlled release formulation of PLGA. The model drug used for this study was pentamidine. The secondary objectives were to find a suitable non-toxic oil and to study the effect of drug loading on the physicochemical characteristics.

2. Materials and methods

2.1. Materials

The copolymer poly(DL-lactic/glycolic acid), PLGA 50:50 (RG, 506; inherent viscosity, 0.8) was obtained from Boehringer Ingelheim (Germany). The surfactant L-α phosphatidylcholine was obtained from Avanti Polar-lipids, Inc. (Albaster, AL). Pentamidine and Span 85 were obtained from Sigma Chemical Co. (St. Louis, MO). Silicon oil, corn oil, mineral oil, dichloromethane, and petroleum ether (spectrophotometric grade) were also obtained from Sigma Chemical Co.

2.2. Experimental methods

Three different oils, silicon, corn, and mineral oil, have been evaluated for use as dispersion media in the preparation of these microparticles. A preliminary study was conducted to evaluate the miscibility of span 85 (1–10%) with 1–8 ml of dichloromethane and 1 ml of the specific oil. Based on the preliminary results, only corn and mineral oils were selected for these experiments. The effect of the drug/polymer ratio was studied at three levels, 1:8, 1:4, and 1:2.

2.3. Preparation of microparticles

In 2 ml of dichloromethane, 200 mg of PLGA (50:50) was dissolved. A specific amount of drug (as listed in Table 1) was added to the PLGA solution and vortexed for 15 s. A mixture of 10 ml oil (corn or mineral) and 50 ml dichloromethane containing 100 μl Span 85 was used as a dispersion medium. The polymer drug mixture was added dropwise to the dispersion medium while sonicating continuously at output 4 (50 W; ultrasonic probe, Sonic & Materials, Inc., CT, USA). The sonication continued for 5 min. The temperature of the bath was maintained at 8–10°C to control the evaporation rate of dichloromethane. Petroleum ether (5 ml) was added slowly in portions (0.5 ml every 10 min) to the mixture. Following sonication, the stirring was continued using a magnetic stirrer. Stirring was maintained until the dichloromethane had completely evaporated. The complete evaporation of dichloromethane was confirmed using residual organic solvent analysis by gas chromatography (GC; Hewlett Packard, Series II, CA) with a flame ionization detector [23]. Microparticles were then washed with cold petroleum ether, filtered, and dried using a Labconco CentriVap® Concentrator (Kansas City, MO) to obtain free flowing powder.

2.4. Particle size and morphology

The particle size and distribution were determined by a Coulter LS130 analyzer (Beckman Coulter, Inc., Fullerton, CA). This technique measures the size of particles dispersed in a medium by the scattering pattern of a laser light shown through the medium. The size calculations assume the presence of spherical particles. Therefore, percentage volume distributions assume the volumes of spheres. The samples were analyzed in a water medium and the Frauenhofer method was utilized to calculate the size distributions. For each sample, a background run of deionized water was performed. A sample of microcapsules (2 mg) was added to the deionized water in a micro-sample cell and counting was

Table 1
Description of pentamidine microparticles

Batch	Oil used	Amount of pentamidine (mg)	Drug/polymer	Median particle size (μm)[a]	Encapsulation[b] (%)	
					Mean (SD)	Results of SNK[c] test
A	Corn oil	25	1:8	68 (27–85)	65.1 (3.8)	A = B = C
B	Corn oil	50	1:4	60 (33–70)	65.8 (6.2)	
C	Corn oil	100	1:2	57 (15–81)	60.6 (7.9)	
D	Mineral oil	25	1:8	54 (28–70)	57.7 (2.8)	D < E = F
E	Mineral oil	50	1:4	54 (27–64)	74.2 (5.9)	
F	Mineral oil	100	1:2	59 (41–75)	73.6 (5.5)	

[a] Values in parentheses represent ranges.
[b] Efficiency of encapsulation = (core loading experimental)/(core loading theoretical) × 100.
[c] Student–Newman–Keul's multiple range test (α = 0.05).

performed for 120 s. After subtraction of the background, the particle size distribution calculation was performed. The morphology, and surface appearance of microparticles were examined by scanning electron microscopy (SEM; Amray AMR 1000A, Bedford, MA). Samples for SEM were mounted on metal stubs and coated with gold to a thickness of 200–500 Angstrom.

2.5. Thermal analysis

Differential scanning calorimetry (DSC; TA DSC 2920, New Castle, DE) of pentamidine, PLGA, empty microparticles, pentamidine-loaded microparticles (batches C and F) was performed in order to characterize their physical state after encapsulation. About 5 mg of a sample was weighed, crimped into an aluminum pan and analyzed at a scanning rate of 3°C/min. The glass transition temperature (T_g) was calculated using TA universal analysis software by extrapolating the linear portion of the thermograms above and below the glass transition point and determining the midpoint.

2.6. Determination of total content

For each formulation, a 20 mg sample was dissolved in 1 ml of dichloromethane. Methanol (4 ml) was added to the solution, followed by ultracentrifugation (35,000 revs./min at 15°C) to completely separate the precipitated copolymer. The efficiency of extraction and recovery of pentamidine were measured independently with five different samples. The efficiency of extraction was at least 98%. The amount of drug in each sample was determined using high performance liquid chromatography (HPLC).

2.7. HPLC analysis

The chromatographic system used in this study consisted of a Waters Model 600 programmable solvent delivery module, Waters Model 717plus auto sampler, and a Waters Model 996 photodiode array detector (Waters, Milford, MA). The chromatography was performed using a μbondpack C-18 column; the mobile phase consisted of 18% acetonitrile, 2% methanol, 0.2 M ammonium acetate and 0.5% triethylamine; and a flow rate of 1.5 ml/min. The identity of the eluting peaks was verified using a diode array detector. The concentration of pentamidine in each sample was determined by intrapolating the peak height to the pentamidine standard curve. Each experiment was performed in triplicate.

2.8. In vitro dissolution studies

For each formulation, a 40 mg sample was placed in a 10-ml tube and incubated in 5 ml of deionized water with constant shaking (20 revs./min) at 37°C. Samples (600 μl) were collected at scheduled times using a filter pipette and centrifuged for 10 min at 10,000 revs./min. The samples were analyzed for drug content using a HPLC method. Fresh double distilled water was added to the incubated sample (600 μl) to maintain sink conditions. Dissolution studies of each formulation were repeated three times.

2.9. Statistical analysis

The efficiency of encapsulation of pentamidine and the amount of drug released from the different formulations of microparticles during the in vitro study were compared using the SAS software package. A P value of <0.05 was considered as evidence of a significant difference.

3. Results and discussion

Six different batches of microparticles were prepared using two different oils, corn and mineral oil. Three of these batches (A, B, and C) were prepared to study the effect of a change in the drug/polymer ratio on the characteristics of the microparticles in the presence of corn oil. The other three batches (D, E, and F) were prepared to study the same effect in the presence of mineral oil. Figs. 1 and 2 show the SEM pictures of the microparticles prepared in the presence of corn (batch C) and mineral oil (batch F), respectively. Batch C is representative of all three batches prepared in the presence of corn oil. Only one SEM picture from each set of microparticles (corn and mineral oils) is included because all three similar batches (A, B, C and D, E, F, respectively) show similar surface morphologies. The SEM pictures show that these microparticles are porous and spherical in shape. The surface appearance of microcapsules/microspheres depends on the rate of polymer precipitation at the interface. The surface is generally smooth when the polymer precipitates slowly due to slow removal of the organic solvent [16]. In the present method, the use of ultrasonication resulted in an immediate precipitation, which was evident by the appearance of particles in the mixture. This relatively faster precipitation of the polymer may have resulted in the porous morphology of the microparticles. These microparticles

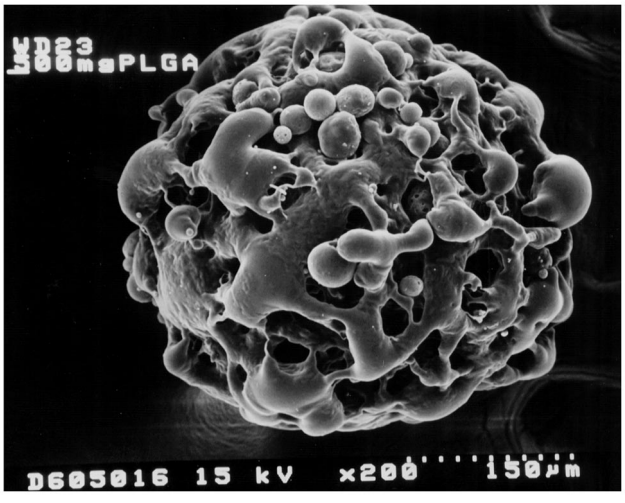

Fig. 1. Typical SEM photographs of microparticles prepared in presence of corn oil.

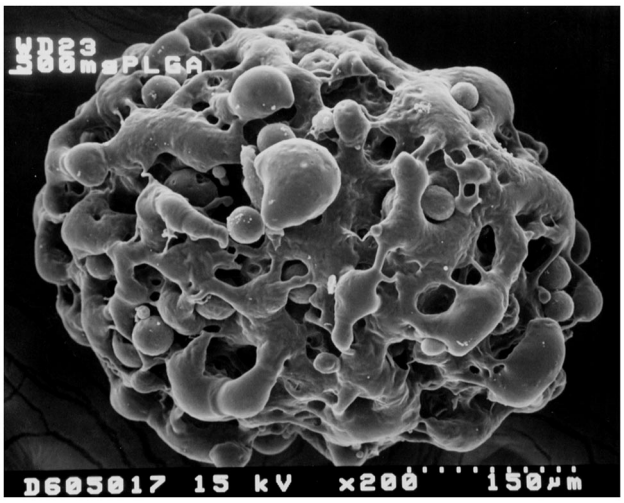

Fig. 2. Typical SEM photographs of microparticles prepared in presence of mineral oil.

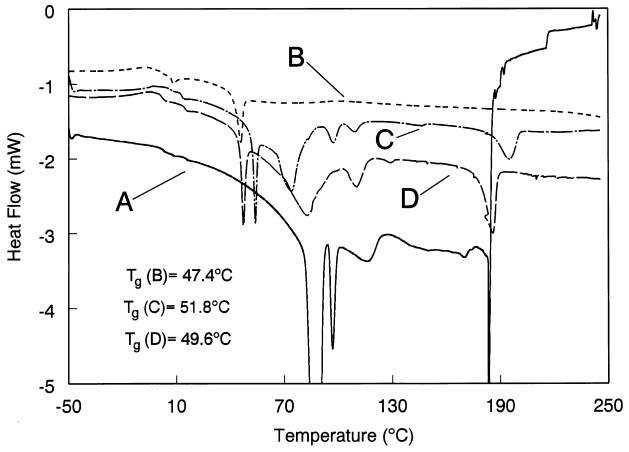

Fig. 3. DSC thermograms of: (A), pentamidine; (B), PLGA; (C), pentamidine-loaded microparticles prepared in presence of corn oil; and (D), pentamidine-loaded microparticles prepared in presence of mineral oil.

were analyzed for size distribution and the results are listed in Table 1. A comparison of particle sizes reveals a similar median particle size in all six batches; although in the presence of mineral oil, the particles are slightly smaller (median size ranges from 54 to 59 μm) than the particles prepared with corn oil (median size ranges from 57 to 68 μm). In the presence of corn oil, a change in the drug/polymer ratio from 1:8 to 1:2 did not have any significant effect on the particle size. Similar results were also observed when the microparticles were prepared using mineral oil. The particles were all smaller than 90 μm.

In cases where the drug formed a dispersion in the first stage of microencapsulation, we would expect that, at the end of the process, crystalline drug particles would be dispersed in the polymer matrix. In such a system, DSC would display two endotherms, each relating to the melting point of either drug or polymer. In cases where the drug is dissolved in the polymer solution, solvent removal causes the drug to either dissolve in the polymer or crystallize out and form a dispersion. In the latter case, DSC would again display two endotherms. In the former case, where the drug forms a solution inside the polymer, no separate event relating to the melting of the drug would occur. DSC thermograms of pentamidine, PLGA and the microparticles showed that there was no significant change in the glass transition temperature of PLGA in the microparticles (Fig. 3). The endothermic peaks, characteristic for melting of pentamidine, were also present in the microparticles. Therefore, it could be concluded that pentamidine was mostly present in the crystalline form in the microparticles and did not dissolve in PLGA. Only one thermogram from each set of microparticles (corn and mineral oils) is included because all three similar batches (A, B, C and D, E, F, respectively) show similar thermal behavior.

The efficiency of encapsulation of pentamidine was determined by measuring the total amount of pentamidine present in each 20 mg sample of the microparticles, i.e. core loading experimental, and comparing this value with the expected amount of pentamidine in each of the samples based on the drug loading during the preparation, i.e. core loading theoretical. The efficiency of encapsulation of pentamidine was higher than 58% in all six batches. In the presence of corn oil, the efficiency of encapsulation was between 60 and 66%. A change in the drug/polymer ratio from 1:8 to 1:2 did not produce any significant change ($P > 0.05$) in the efficiency of encapsulation (Table 1) when corn oil was used as the dispersion medium. However, in the presence of mineral oil, an increase in the drug/polymer ratio from 1:8 to 1:4 increased the efficiency of encapsulation from 58 to 74%. A further increase in the drug/polymer ratio up to 1:2 did not improve the efficiency of encapsulation. One of the reasons for this drug loss was due to the partial solubility of pentamidine, in presence of Span 80, in the oil phases. An increase in the efficiency of encapsulation due to an increase in the drug/polymer ratio may be

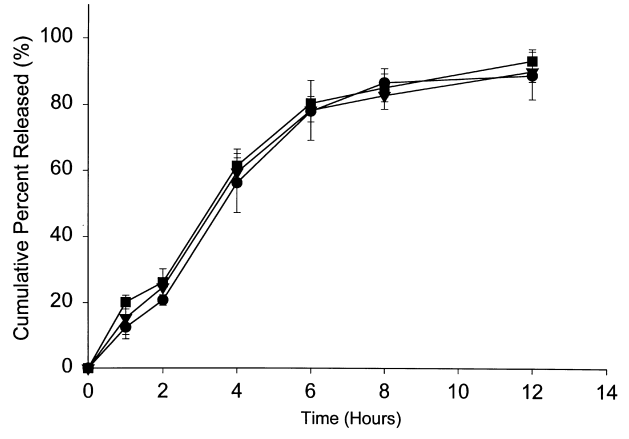

Fig. 4. Dissolution profiles of microparticles prepared in presence of corn oil: (●), batch A; (▼), batch B; (■), batch C.

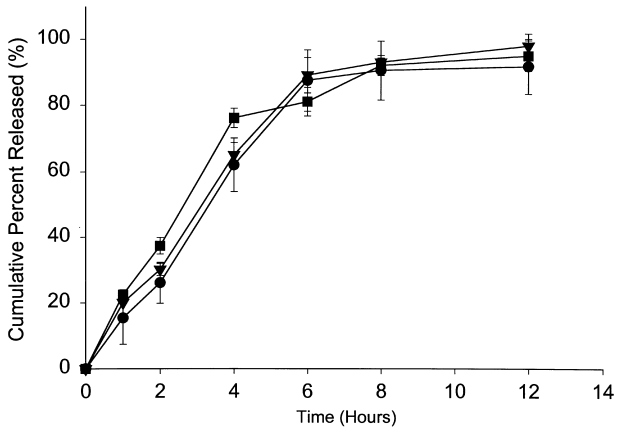

Fig. 5. Dissolution profiles of microparticles prepared in presence of mineral oil: (●), batch D; (▼), batch E; (■), batch F.

due to the partial saturation of the oil phase in the presence of mineral oil.

The dissolution of pentamidine was compared by calculating the cumulative percentage of the drug released at a specific sampling time. Figs. 4 and 5 show the dissolution profiles of the microparticles. The amount of drug released from these microparticles was at least 12% within the first 60 min. The microparticles prepared in the presence of mineral oil released a slightly higher amount of pentamidine during the first 60 min. However, these differences were not statistically significant ($P > 0.05$). At least 50% of the total drug was released within the first 4 h. The release rate was significantly high compared with the other PLGA microcapsules/microsphere formulations reported in the literature [24–29], including PLGA/pentamidine microcapsules [16]. This faster release was possibly due to the porous surface of the microparticles. PLGA microcapsules/microspheres release the encapsulated drug via diffusion through the polymer membrane during the first 1–2 weeks, followed by biodegradation rate of the polymer itself. Although polymer–drug interaction is one of the controlling factors in drug release, the size of the molecules is also important in controlling drug release. Large molecular weight compounds, like proteins/peptides, are released very slowly from these PLGA formulations. Hence, the present microparticles with the porous surface will allow large molecules to release at a much faster rate than the regular microcapsules/microspheres. Drug release from these microparticles continued for up to 12 h. In contrast, in a previous study, we reported that pentamidine release from PLGA microcapsules continued for at least 2–4 weeks [16]. One of the main reasons of this faster drug release was due to the porous surface.

4. Conclusions

The microparticles prepared using this technique were porous and spherical in shape. Irrespective of the oil (corn or mineral) used in the preparation, the microparticles were all smaller than 90 μm. A change in the drug/polymer ratio did not change the particle size. Pentamidine was mostly present in the microparticles in the crystalline form. The efficiency of encapsulation was higher than 58%. In the presence of corn oil, the efficiency of encapsulation was between 60 and 66%, whereas in the presence of mineral oil, the efficiency of encapsulation was between 58 and 74%. The rate of drug release from these microparticles was very high. This significantly high rate of drug release from PLGA microparticles was due to the porous surface morphology.

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