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Note

In vitro evaluation of gentamicin released from microparticles

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Abstract

In this study, the preparation, characterization and drug release behaviour of gentamicin (GM)-loaded poly(D,L-lactide-co-glycolide) microspheres are described. The microspheres were produced using a double emulsion solvent evaporation technique. All the microspheres preparation resulted in spherical shape and the mean diameter was 3 μm (for empty microspheres) and between 5 and 9 μm for microparticles loaded with GM. The encapsulation efficiency (EE) ranged from 3.4 to 90% depending on the formulation. Increasing the volume of the external aqueous phase, increased the EE. Encapsulation also depended on the pH value of the internal aqueous phase, the highest value was achieved when maintained the internal aqueous phase at pH 6, where GM was more soluble. Moreover, increasing nominal GM loading yielded lower encapsulation efficiencies. The release profiles of GM from microparticles resulted in biphasic patterns. After an initial burst, a continuous drug release was observed for up to 4 weeks. Finally, the formulations with higher loading released the drug faster. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Brucellosis; Poly(lactide-co-glycolide); Microencapsulation; Gentamicin

The need for improving actual treatments of microbial infections by intracellular pathogens, such as *Brucella*, encouraged us to study the physical targeting of antibiotics by drug delivery systems (Gamazo et al., 1999). Antibiotics are effective against *Brucella* in vitro, however, during infection the bacterium is localized intracellularly making the treatment difficult (Hall, 1990). The therapeutics requires the association of more than one antimicrobial for weeks (Ariza, 1999) and that often lead to poor patient compliance, contributing to low therapy efficiency (Solera et al., 1997). Particulate carriers such as biodegradable microspheres, may target the antibiotics to the intracellular sites where the bacterium is found, and as being in a sustained manner, would permit to reduce the number of doses and decrease drug toxicity (Gamazo et al., 1999).

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In a previous work, Prior et al. (2000) found that gentamicin (GM)-loaded microparticles prepared by spray-drying and using the uncapped poly(D,L-lactide-co-glycolide) (PLGA, RG 502H) polymer showed a low burst and a continuous release profile. This property makes this formulation suitable as a possible therapy in brucellosis. Nevertheless, using spray-drying, a strong particle aggregation was observed making difficult its administration in mice. Therefore, the objective of this work was to prepare GM loaded microparticles by a double solvent evaporation method, in order to avoid particle aggregation, and to study GM in vitro release.

PLGA (50:50) with free carboxyl end groups was purchased from Boehringer Ingelheim (Ingelheim, Germany; RG 502H, inherent viscosity of 0.2 dl/g). Polyvinyl alcohol (PVA) 115 000 MW was supplied by BDH (Poole, England) and dichloromethane was provided by Prolabo (Fontenay, France). Freeze–dried GM sulphate was obtained from Sigma Chemical Co. (Madrid, Spain).

Microparticles were obtained by the double emulsion solvent evaporation technique (Blanco-Príeto et al., 1996). Briefly, different amounts of GM dissolved in 0.2 ml of a PVA 0.5% aqueous solution (W_1) and 0.2 g of PLGA dissolved in 5 ml of dichloromethane (O) were mixed by ultrasonication for 30 s under cooling to form a W_1/O emulsion. This inner emulsion was added to 10 or 30 ml of an aqueous PVA 1% solution (W2), and homogenized using an Ultraturax®. The resulting (W_1/O/W_2) solution was stirred for at least 3 h under room temperature to allow solvent evaporation and microspheres formation. After preparation, the microspheres were isolated by centrifugation $7000 \times g$ for 10 min, washed with distilled water and freeze–dried.

Ten batches of microspheres were prepared which characteristics are shown in Table 1 and Table 2. For batches E–J, the inner water phase was maintained at pH 6, where GM is more soluble. Microparticles diameter was measured by laser diffractometry, using a Matersizer S (Malvern Instruments, Worcestershire, UK).

Encapsulated GM was determined by dissolving the loaded microspheres in 1 ml of dichloromethane and the drug was extracted with 2 ml of phosphate buffer pH 6. The GM content in the PLGA microspheres was determined fluorometrically using a Cytofluor 2300/2350

Table 1
Microspheres characteristics

Batch	W_2 (ml)	Size (μm)	mg GM (W_1)	EE (%)	μg GM/mg polymer
A	30	6.0	5	8.7	2.12
B	30	6.6	10	9.2	4.36
C	10	5.4	5	7.1	1.72
D	10	5.7	10	3.4	1.61

W_1: PVA 1%.

Table 2
Microspheres characteristics

Batch	W_2 (ml)	Size (μm)	mg GM (W_1)	EE (%)	μg GM/mg polymer
E	10	5.6	5	66	12
F	10	5.8	10	24	16
G	10	6.0	50	11.5	23
H	30	7.1	5	90	22
I	30	7.4	10	54	26
J	30	9.0	50	27	54

W_1: PVA 1% pH 6.

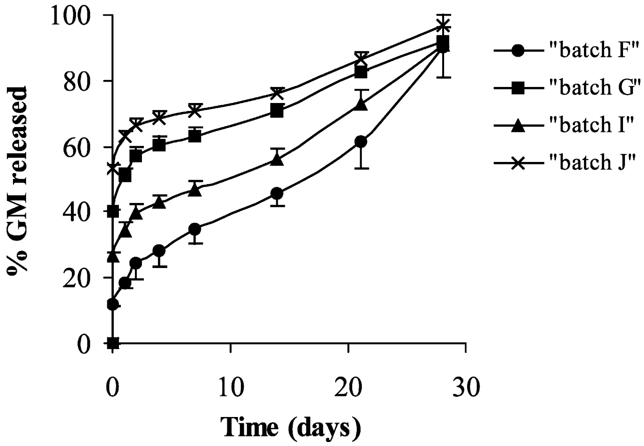

Fig. 1. Cumulative in vitro release profiles of GM from PLGA microparticles, in PBS.

(Millipore). Fluorescence was measured after derivation with *o*-phthaldehyde with excitation and emission wavelengths of 360 and 460 nm, respectively (Benson and Hare, 1975).

In vitro drug release profiles were obtained by incubating the microspheres in 1.5 ml of phosphate buffer saline (PBS) pH 7.4 under continuous shaking and at 37 °C. At regular intervals, the vials were centrifuged at $986 \times g$ for 10 min and GM was quantified in the supernatant, as described above.

All the microspheres resulted in spherical shape and the mean diameter was 3 μm (for empty microspheres) and between 5 and 9 μm for microparticles loaded with GM (Tables 1 and 2). The resuspension characteristics after lyophilization were good and the particles showed no aggregation. The effect of the volume of W_2 and the amount of GM introduced in W_1 in the encapsulation efficiency (EE) is shown in Table 1 and Table 2. Increasing the volume of W_2 from 10 to 30 ml of PVA 1% also increased the EE. Jeffery et al. (1993) also observed an increase in the EE of ovalbumin into PLGA microspheres when the volume of the external aqueous phase was risen. This effect could be attributed to the increase of the particle size which enable more drug to be incorporated into the microspheres. On the other hand, increasing the amount of GM in the initial emulsion to a value of 50 mg reduced the EE as described also by Prior et al. (2000). Finally, the highest EE was achieved when maintained W_1 at pH 6, where GM was more soluble (Table 2). For formulation J, an encapsulation rate of 54 μg of GM/mg of polymer was obtained when 50 mg of GM were dissolved in the inner aqueous phase of the emulsion.

The in vitro release of GM from microparticles is shown in Fig. 1, indicating that microparticles with a higher loading (batches G and J) released GM faster. This results agree with the observations by Sah et al. (1994). For all the formulations, after an initial burst, a continuous GM release was observed for up to 4 weeks (Fig. 1).

In this work size, loading, resuspension characteristics after lyophilization and GM released of PLGA microspheres prepared by a $(W_1/O/W_2)$ solvent evaporation method were investigated. It was found that maintaining the inner aqueous phase at pH 6 improved GM encapsulation and the microparticles showed no aggregation. Furthermore, a continuous GM release was observed for up to 4 weeks. The obtained microspheres could be useful as a prolonged drug delivery system for brucellosis treatment. Accordingly, the next step of this work will be to study the therapeutic effect of this particles in vivo.

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