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International Journal of Pharmaceutics 196 (2000) 115–125

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Gentamicin encapsulation in PLA/PLGA microspheres in view of treating *Brucella* infections

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Received 26 June 1999; received in revised form 11 November 1999; accepted 1 December 1999

Abstract

In view of treating intracellular *Brucella* infections, microspheres made of poly(lactide) (PLA) and poly(lactide-co-glycolide) (PLGA) were developed as delivery system for the cationic and highly hydrophilic antibiotic gentamicin sulphate. Drug microencapsulation by spray drying yielded microspheres with regular morphology, an average particle size of approximately 3 μm and encapsulation efficiencies of up to 45%. Different copolymers of similar molecular weights gave varying encapsulation efficiencies and particle size distributions. The encapsulation efficiency generally increased with polymer hydrophilicity, except for the hydrophilic copolymer PLGA50:50H carrying carboxylic end groups. Encapsulation also depended on the pH value of the aqueous drug solution to be encapsulated. Moreover, increasing nominal gentamicin sulphate loading yielded lower efficiencies. For comparison, some formulations were also prepared by a $(W_1/O)W_2$-solvent evaporation method, which yielded lower encapsulation efficiencies, in the order of 13%. Finally, drug bioactivity was found to remain intact after microencapsulation, MS storage and MS incubation in aqueous medium. The results suggest that PLA/PLGA microspheres prepared by spray drying may be an appropriate delivery system for gentamicin sulphate to be used in the treatment of intracellular *Brucella* infections.

Keywords: Gentamicin sulphate; Poly(lactide); Poly(lactide-co-glycolide); Microspheres; Brucellosis; Intracelullar infections

1. Introduction

Brucellosis is a typical intracellular bacterial infection that causes major economic losses and also has important socio-economic implications. Apart from being a major animal health problem, brucellosis also constitutes a serious risk to human health, mainly in rural areas. Infections in animals mainly lead to abortions and reproductive disorders in males and females, resulting in infertility. The intracellular localisation of *Brucella* species, particularly in the cells of the mononuclear phagocytic system (MPS), renders treatment difficult, since most antibiotics known

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0378-5173/00/$ - see front matter
PII: S0378-5173(99)00448-2

to be efficient in vitro do not actively pass through cellular membranes (Hall, 1990). Hence, suitable delivery systems, such as liposomes, nanoparticles or biodegradable microparticles, should be developed to reach these intracellular sites (Bakker-Woudenberg, 1995). Parentally administered microparticles represent foreign particulate material to the body. Thus, they are captured by the cells of the mononuclear phagocytic system, such as blood monocytes and macrophages of the liver, spleen, and bone marrow (Bradfield, 1984; Kersten and Gander, 1996; Anderson and Shive, 1997; Zhang et al., 1998). Although phagocytic uptake could be a disadvantage for the treatment of some types of infection, it would provide great benefit against infections, which target the mononuclear phagocytic system, such as brucellosis. Liposomes containing gentamicin have already been developed and shown to be quite efficient for targeting (Dees et al., 1985; Hernández-Caselles et al., 1989; Vitas et al., 1996). However, liposomes suffer from low encapsulation efficiency, stability problems, and their therapeutic efficacy is not always optimal. Conversely, to our knowledge, microparticles have not yet been used as delivery system for antibiotic brucellosis treatment.

Biodegradable microspheres are useful for prolonged drug release and targeting drugs to specific infection sites. The need for improving actual treatments of microbial infections, such as brucellosis, encouraged us to study the physical targeting of antibiotics by these delivery systems. According to the physico-chemical properties of gentamicin sulphate (cationic, polar, H-donator), biodegradable poly(lactide) (PLA)/poly(lactide-co-glycolide) (PLGA) microspheres should entrap efficiently this drug and release it in a sustained and controlled manner (Gander et al., 1996). Nonetheless, size, entrapment efficiency, release profiles and pharmacological effects also depend greatly on the microencapsulation method (Pavanetto et al., 1993), polymer characteristics and biodegradation behaviour, hence copolymer composition and molecular weight (Thomasin et al., 1996).

In this study, five types of PLA/PLGA of similar molecular weight but different hydrophobicity were used to encapsulate gentamicin sulphate (GS) at different nominal loadings. The microspheres (MS) were characterised for drug entrapment efficiency, particle size distribution and drug antibacterial activity in view of its potential use in *Brucella* infections.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide) (end-group capped PLA, 14.6 kDa and end-group uncapped PLA-H, 14.6 kDa) and poly(D,L-lactide-co-glycolides) (PLGAs) of differing copolymer ratio and hydrophilicity were used, i.e. end-group capped PLGA 75:25, 17kDa and PLGA 50:50, 13.7 kDa; and end-group uncapped PLGA 50:50H, 13.7 kDa. The corresponding commercial products Resomer® R202, R202H, RG752, RG502 and RG 502H were purchased from Boehringer Ingelheim (Ingelheim, Germany); the letter H in the product names end-group uncapped polymers. Gentamicin sulphate (GS) was from Selectchemie (Zürich, Switzerland) and conformed to pharmacopoeial specifications. Poly(vinyl alcohol) (PVA, MW 15 000) was from Fluka (Buchs, Switzerland), Synperonic® F68 from ICI (Middlesborough, UK) and polyoxyethylene-sorbitan monolaurate (Tween 20) was from Sigma (St. Louis). American bacteriological agar was purchased from Pronadisa, Hispanlab S.A. (Alcobendas, Madrid, Spain), Trypcase-soy broth was from bioMérieux (Marcy l'Etoile, France) and Mueller Hinton broth and Mueller Hinton agar were from Difco Laboratories (Detroit, MI). Lyophilised *Staphylococcus aureus* ATCC® 25923 discs were from Difco Laboratories (Detroit, MI). All other chemicals and solvents used were of analytical grade.

2.2. Preparation of microspheres (MS) by spray drying

Typically, 20–100 mg GS were dissolved in 1 ml phosphate buffer (67 mM, pH 6.0 or 7.4). This solution was dispersed in 20 g of a 5% (w/w) polymer solution in ethyl formate by ultrasonica-

tion under cooling. The resulting W/O emulsion was spray-dried (Büchi 190, Flawil, Switzerland) at a flow rate of 3 ml min^{-1}, while being sonicated using a pulsatile regimen (Vibra Cell, Sonic and Materials, Danbury, USA) to maintain emulsion homogeneity. The inlet/outlet temperatures were at 52–53 and 44–45°C, respectively, and the spraying air flow was varied between 450 and 600 NL h^{-1}. The MS were collected on a 0.8 μm regenerated cellulose acetate membrane filter, washed with 0.1% (w/w) Synperonic F68 solution, rinsed with distilled water and dried under 10 mbar vacuum for 24 h. Residual water was eliminated by dispersing the dried MS in n-hexane, followed by additional vacuum drying for 24 h. The final product was stored under dry conditions at 4°C. Placebo MS were produced accordingly but without the presence of the gentamicin solution. Some formulations were prepared by simply dispersing GS powder of defined particle sizes in a 5% (w/w) polymer solution in ethyl formate, by the use of ultrasonication. In some cases, surfactants were added to improve the dispersion stability. Defined powder granulometries were achieved through treating the commercial GS powder by ball mill grinding (Fritsch Pulverisette, Fritsch, Idar-Oberstein, Germany), followed by ultrasonic wet-sieving through a 20 μm mesh (A 75 NS, Alpine Augsburg, Germany), or through micronisation in a counterjet air-mill (TF-Forschung, A. Bauermann Verfahrenstechnik, Solingen, Germany) (Thomasin et al., 1997).

2.3. Preparation of microspheres by $(W_1/O)W_2$ solvent evaporation

Typically, 8 or 40 mg of GS were dissolved in 0.4 ml phosphate buffer (67 mM, pH 6.0), and 0.4 g of PLGA 50:50 was dissolved in 8 ml of a mixture of DCM and acetone (85:15, v/v). The two solutions were mixed by ultrasonication for 30 s under cooling (output 4, 40% duty cycle) to form a W_1/O emulsion. This so-called inner emulsion was slowly added to 100 ml of a 1% (w/w) aqueous PVA solution (W_2), which was homogenised with a high-speed mixer (Polytron®) for 8 min, increasing progressively the rotor rate from 5500 to 8500 rpm. The resulting $(W_1/O)W_2$ emulsion was stirred in a double-jacketed vessel at a rate of 300 rpm. Evaporation of the organic solvent was facilitated under vacuum and by increasing the temperature from ambient to 37°C. The microspheres were collected and washed three times with water by re-suspending and centrifuging the particles at 12 000 rpm during 15 min (SS-34 rotor, Sorvall, Wilmington, USA). The MS were collected on a 0.8 μm regenerated cellulose acetate filter and dried for 24 h under 10 mbar vacuum. Residual water was eliminated by re-dispersing the dried MS in n-hexane and drying again under vacuum, as specified above. Finally the MS were stored under dry conditions at 4°C.

2.4. Size and morphology of the microspheres

MS size was measured by laser light scattering (Mastersizer X, Malvern, UK). MS were carefully mixed in a mortar with two to three drops of polysorbate 20, followed by the addition of water. The dispersion was poured into a small presentation unit (Malvern) and sonicated for 1–2 min. The values were expressed as number average size distributions.

The morphology of the MS was examined by scanning electron microscopy (SEM). The spray-dried MS were mounted on double-faced adhesive tape on metal stubs, coated with gold to a thickness of 8 nm and further analysed in a Zeiss DSM 940A scanning electron microscope.

2.5. GS content of the MS

Measurement of GS content in the MS was performed by three different methods to determine the optimal extraction procedure. Using the here called filtration method (method A), encapsulated GS was determined by dissolving 40 mg of MS in 3 ml of dichloromethane and collecting the undissolved GS on 0.2 μm cellulose acetate filters. The dried filters were transferred into test tubes. GS was eluted with 2 ml of water by vortexing and assayed photometrically (320–350 nm) after derivation with *o*-phthalaldehyde (Hartke et al., 1991). In the partition method (method B), 40 mg of MS were dissolved first in 2 ml of either chloroform or dichloromethane or ethyl formate.

Then, 2 ml of water was added, and the mixture was vortexed and centrifuged. In this procedure, GS partitioned into the aqueous phase, which was collected and assayed. In the hydrolysis method (method C), 40 mg of MS were dispersed in 3 ml of 0.1 N or 1 N NaOH solution and kept at room temperature for 24 h to hydrolyse the polymer. Then, the samples were centrifuged, and the GS contents determined in the supernatant, using the above described assay method. Finally, the actual loading (μg GS/mg MS) of the microparticles and the encapsulation efficiency (%) were determined. The latter is defined here as the percentage of determined loading relative to the nominal (theoretical) loading.

2.6. Antimicrobial activity of encapsulated GS

Approximately 10 mg (weighed accurately) of spray-dried MS loaded with gentamicin sulphate were suspended by short sonication in 4 ml of aqueous solution (phosphate buffer 67 mM, pH 7.4, 0.05% Tween 20 and 0.02% sodium azide) contained in borosilicate vials (Chromacol®, Welwyn Garden City, UK). The MS were incubated at 37°C under shaking for 24 h. Supernatant samples of 500 μl were collected by centrifugation and stored at 4°C until checked for antimicrobial activity. Biological activity of encapsulated GS was measured by a bacterial growth inhibition assay using an agar diffusion method with *Staphylococcus aureus* ATCC® 25923 as test organism. Two days prior to the bioassay, one disc of lyophilised *S. aureus* was suspended in saline solution and kept overnight at 37°C. Afterwards, the suspended bacteria were seeded onto Trypcase-soy agar plates and further incubated for 24 h. Two to three isolated colonies were transferred into Mueller Hinton broth and incubated for 3 h at 37°C under shaking. The suspension was diluted in Mueller Hinton broth to an optimal OD (590 nm) for adequate bacteria concentration and used for the preparation of a bacteria in top Mueller Hinton agar layer. Petri dishes containing solid agar were prepared several hours in advance, and 45 ml of warm Mueller Hinton agar mixed with 5 ml of the bacterial suspension was poured onto the solid agar base and left to dry at room temperature. Triplicates of 50 μl of supernatant from individual incubated MS samples were added to 6 mm wells cut into the top of agar layer. Plates were incubated for 18 h at 37°C, and the diameters of the growth inhibition zones of each well were measured to 0.1 mm accuracy with a Calibrating Viewer (Kallestad Laboratories Inc., Chaska, MN, USA). Gentamicin sulphate concentrations were determined by reference to a standard curve made by diluting a freshly prepared GS solution in the incubation buffer and compared to photometrical determinations. Incubation buffer was used as control.

3. Results

3.1. Evaluation of the methods of microencapsulation and of determining drug content in the microspheres

In preliminary experiments, GS was microencapsulated by the methods of spray drying and $(W_1/O)/W_2$-solvent evaporation to select the more suitable technique for this drug. Two PLGA 50:50 formulations with nominal loadings of 2 and 10% GS were prepared. Both methods produced comparable MS size distributions (Table 1). Conversely, encapsulation efficiency was significantly higher with spray drying than with solvent evaporation. Thus, spray drying was used for all further experiments.

Comparison of the three methods for extracting GS from the MS revealed that the filtration and partitioning methods gave comparable results (Fig. 1); GS extraction by particle hydrolysis, however, did not provide reliable data, as the assay for GS was sensitive to pH. Thus, all further drug loading data were determined by the filtration method.

3.2. Morphology and size range of microspheres produced by spray drying

Microencapsulation of GS into PLA/PLGA by spray drying yielded small-sized particles, mainly a result of the type of equipment and the low polymer solution viscosity used. SEM-micropho-

Table 1
Size distribution and GS-encapsulation efficiency of PLGA 50:50 microspheres prepared by spray drying and solvent evaporation[a]

GS nominal loading (%, w/w)	MS size		GS actual loading (μg GS/mg MS)	Encapsulation efficiency (%, w/w)
	Number averaged (μm)	90% Undersize (μm)		
Solvent evaporation				
2	n.d.[b]	n.d.[b]	2.7 ± 0.2	13.3 ± 1.0
10	3.4	6.0	6.5 ± 0.3	6.5 ± 0.3
Spray drying				
2	3.5	6.1	8.9 ± 0.1	44.5 ± 0.2
10	3.7	7.0	23.6 ± 1.0	23.6 ± 1.0

[a] For encapsulation, GS was dissolved in a pH 6.0 buffer solution.
[b] n.d., not determined.

tographs revealed homogeneous and spherically shaped particles with smooth surfaces for all unloaded MS and for GS-loaded PLGA 50:50 MS (Fig. 2). Occasionally, small indentations were observed on the latter particles. Conversely, the surface of GS-loaded PLGA 50:50H MS was porous, and a substantial fraction of coalesced particles appeared, which depended on nominal drug loading.

MS size was affected by the polymer type, drug content, and the physical form of the drug during processing (aqueous solution or solid particles) (Fig. 3), but not by the spraying-air flow (450–600 NL h^{-1}) or polymer concentration. In general, PLGA 75:25, PLA-H and PLA yielded smaller particles than PLGA 50:50H and PLGA 50:50. In addition, higher drug loadings increased the particle size slightly. Interestingly, microencapsulation of GS powder yielded particles in the sub-micron size range, whereas encapsulation of GS solution produced MS in the low micrometer range.

3.3. Gentamicin sulphate encapsulation efficiency

GS encapsulation efficiency depended on the polymer type, nominal drug loading and the physical form of the drug (solid or in solution) during processing. It was highest (45%) with PLGA 50:50 at 2% nominal loading and using a pH 6.0 buffer for GS-encapsulation (Fig. 4). With the exception of PLGA 50:50H, carrying carboxyl end-groups, the more hydrophilic polymers entrapped GS more efficiently. At a nominal loading of 10%, the encapsulation efficiency dropped generally by approximately 10–20%, except for the PLA-H where the nominal loading had no significant effect. When GS was encapsulated as powder, the encapsulation efficiency was only 2.3%. Apparently, the solid drug particles were too large for efficient encapsulation into the small microspheres, although the process was optimised by previous milling and micronisation of GS and the use of emulsifying agents in the drug–polymer dispersion.

The effect of nominal loading (from 2 to 10%) on encapsulation efficiency was examined in more

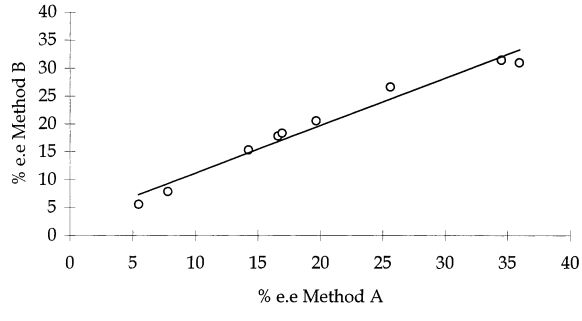

Fig. 1. Comparison between encapsulation efficiency values determined after extraction of GS from PLGA 50:50 or PLGA 50:50H MS by the filtration (A) and partition methods (B). Correlation parameters were $y = 0.8544x + 2.657$; $R^2 = 0.9717$.

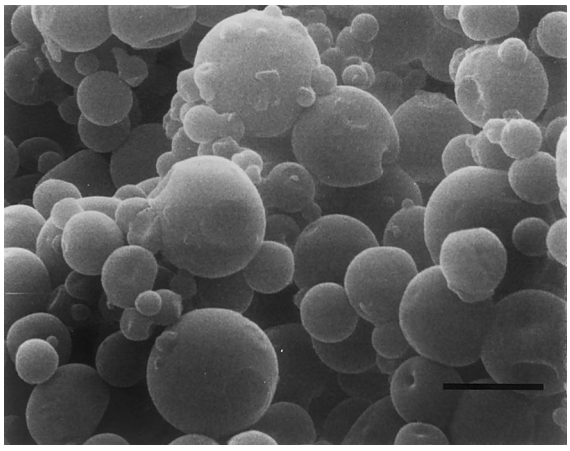

Fig. 2. SEM-microphotograph of PLGA 50:50 microspheres loaded with 2% (nominal) gentamicin sulphate (bar = 3 μm).

detail with PLGA 50:50 (Fig. 5). Increasing the nominal loading from 2 to 6% decreased the encapsulation efficiency from 40 to 15%, while further increase in nominal loading up to 10% did not further affect drug entrapment.

A further parameter studied was the pH of the GS-solution to be encapsulated (Table 2). The H-type polymers showed an improved encapsulation efficiency when the pH value of the buffer in drug solution was 7.4 rather than 6.0. However, this pH effect was inversed and less pronounced with the end-group capped PLGA 50:50.

3.4. Antimicrobial activity of encapsulated GS

Effects of microsphere encapsulation and storage on the biological activity of the encapsulated drug were studied by an antibacterial assay using aqueous incubation medium after incubating the MS for 24 h at 37°C. The assay measured growth inhibition of *S. aureus* ATCC® 25923 on Muller Hinton agar. Inhibition diameter versus the log of GS concentration (2.5–30 μg/ml) of standard solutions closely correlated ($R^2 = 0.9808$). The inhibition zones of MS incubation media corresponded to a GS concentration of 17.4 ± 0.3 and 11.5 ± 0.2 μg/ml for A and B, respectively (Fig. 6). From the amount of MS and the actual drug loading, GS concentrations of 17.5 ± 0.4 and 9.3 ± 0.2 μg/ml were expected. Thus, GS fully retained its biological activity upon encapsulation and subsequent storage (18–21 months under dry conditions at 4°C).

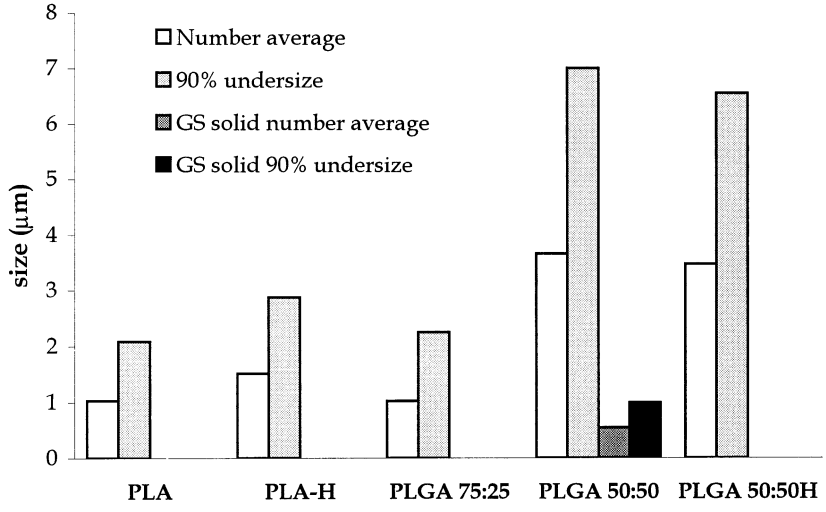

Fig. 3. Number averaged particle size and 90% undersize values of microspheres prepared by spray drying different polymer types. Gentamicin sulphate was generally encapsulated as aqueous solution, although the drug powder was use in one particular PLGA 50:50 formulation. The nominal loading was 2%.

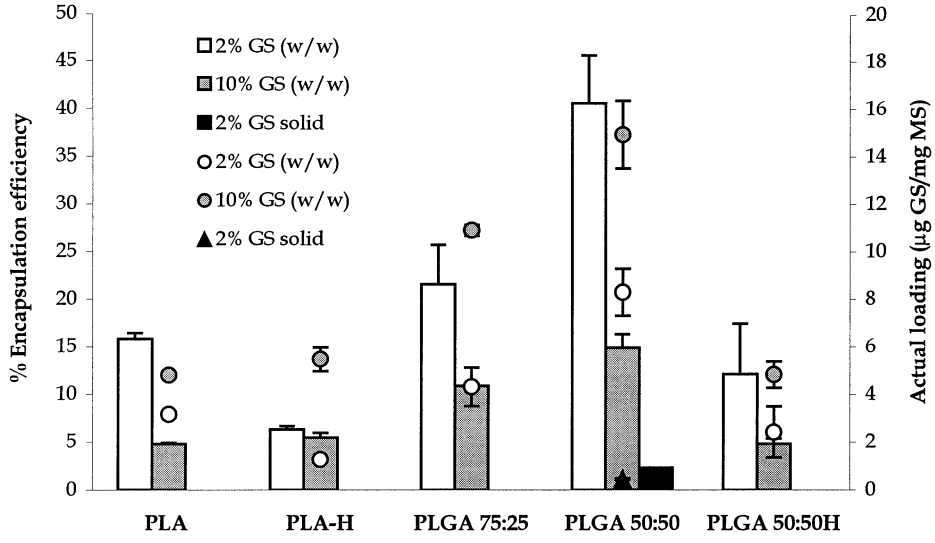

Fig. 4. Effect of polymer type, nominal loading (%, w/w) and physical state of the GS, i.e. in a pH 6.0 buffer or as powder, on encapsulation efficiency (bars) and actual loading (symbols).

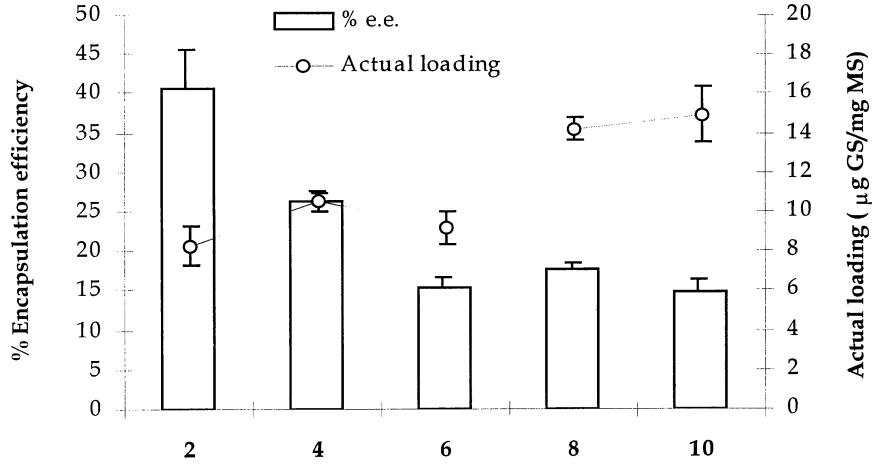

Fig. 5. Effect of nominal GS loading (%) on encapsulation efficiency (bars) and actual loading (line) in PLGA 50:50 microspheres.

4. Discussion

Treatment of intracellular bacterial infections such as brucellosis requires prolonged chemotherapy with a combination of agents with at least one drug having high intracellular penetration capacity for infected cells. Relapsing infections due to low therapeutic efficacy and poor patient compliance during the long regimens represent important problems (Solera et al., 1997). The intended therapeutic use of the described GS loaded microspheres is the treatment of *Brucella* infected cells of the mononuclear phagocytic system (MPS). Thanks to the appropriate size for phagocytosis and prolonged release properties, microspheres might be useful to control the acute infectious stage, prevent complications and relapse, as well as to overcome practical disadvan-

Table 2
Encapsulation efficiency, actual loading and MS size observed with different polymer types and pH values of the GS-solution to be encapsulated

Polymer type	PH	GS actual loading (μg GS/mg MS)	Encapsulation efficiency (%, w/w)	MS size	
				Number average (μm)	90% Undersize (μm)
PLA-H	6.0	1.3	6.4	1.5	2.9
	7.4	3.7	18.6	3.5	6.0
PLGA 50:50	6.0	8.3 ± 1.0	40.6 ± 5.1	3.7 ± 0.2	7.0 ± 1.0
	7.4	7.0 ± 0.7	30.6 ± 2.4	3.5 ± 0.1	6.3 ± 0.9
PLGA 50:50H	6.0	2.4 ± 1.7	12.1 ± 5.3	3.5 ± 0.0	6.6 ± 0.2
	7.4	6.6 ± 0.7	32.8 ± 3.7	3.8 ± 0.1	7.6 ± 0.4

tages of long-term therapies. In addition, drugs delivered by microspheres that target specifically infected cells of the MPS should reduce drug toxicity associated with undesired biodistribution of the free form of the drug. In light of this, we aimed here at producing microspheres having the following characteristics: (i) sizes below 5–10 μm, (ii) satisfactory drug entrapment efficiency, (iii) preserved bioactivity of the encapsulated compound, and (iv) controlled drug release.

Particles of sizes below 5–10 μm are known to be potentially suitable for uptake by macrophages (Illum et al., 1982; Tabata and Ikada, 1991) and, hence, for targeting *Brucella* infected cells. PLA/PLGA-microspheres of this typical size range were obtained here by spray drying and $(W_1/O)W_2$-solvent evaporation. By spray drying, PLGA 50:50 and PLGA 50:50H yielded slightly larger particles than PLGA 75:25, PLA-H and PLA. This may be ascribed to differences in the physico-chemical properties of the polymers. It is known that PLGA 50:50 forms crystalline domains which may cause gelification of the polymer in solution (Bendix, 1990). If polymer gelification occurs during spraying, larger droplets should be expected due to increased viscosity. The larger particle sizes obtained in the presence of the aqueous drug solution may also be related to polymer gelification. In general, higher nominal loadings generated slightly broader particle size distributions, in agreement with data in the literature (Thomasin et al., 1997). From SEM-micrographs, increasing nominal gentamicin loadings increased surface porosity and microsphere coalescence of the more hydrophilic PLGA 50:50H MS, while the surface smoothness of PLGA 50:50 MS was not affected. This effect might be related to preferential water uptake of this polymer type, causing insufficient drying during preparation. The residual water can then plasticise the polymer

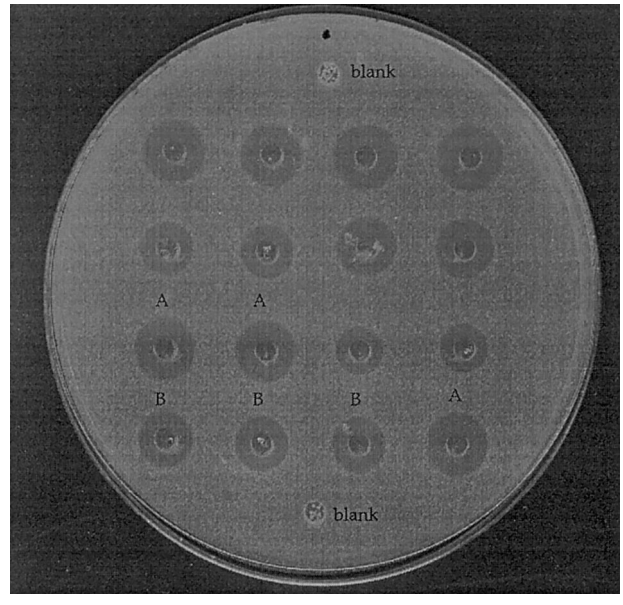

Fig. 6. Antibacterial assay with media obtained from MS incubation for 24 h at 37°C. The wells contained incubation buffer alone (blanks), two samples of MS incubation medium in triplicates (A and B), and gentamicin sulphate standard solutions (2.5–30 μg ml^{-1}).

matrix, as suggested by others (Thomasin et al., 1996).

The encapsulation of gentamicin sulphate into the polyesters used was generally modest. Nonetheless, spray drying yielded more efficient GS encapsulation than conventional $W_1/O/W_2$-solvent evaporation. This confirms the suitability of spray drying for highly water-soluble drugs (Gander et al., 1995a). The entrapment efficiency of GS also depended on the polymer type and increased generally with increasing hydrophilicity. The enhanced microencapsulation in more hydrophilic polymers may be ascribed to enhanced molecular interactions between the drug and the polymer (Gander et al., 1995b, 1996; Thomasin et al., 1996). Nagata et al. (1994) observed that the terminal free carboxyl group of the polymer was necessary for efficient GS entrapment in PLGA microspheres. For the highly water soluble leuprorelin, NMR measurements recently showed that a rigid structure is formed in the MS due to ionic interaction between amino groups of the drug and the terminal carboxylic anions of the polymer (Okada, 1997). This was assumed to be responsible for improved drug entrapment and lowered release rates. Mauduit et al. (1993) and Vert et al. (1994) suggested strong acid–base interactions for gentamicin base and gentamicin sulphate with PLA blends by ^{13}C-NMR and FTIR spectroscopy, polymer degradation and drug release studies. The modest drug encapsulation by PLGA 50:50H in our experiments was unexpected, as a strong interaction between the cationic drug and PLGA 50:50H was assumed; the latter should indeed have a more pronounced anionic character than the regular end-group capped PLA/PLGAs. Our results suggest mechanisms other than ionic interactions between GS and the end-group uncapped PLGA type. Thus, we studied the effect of pH of the buffer solution on gentamicin entrapment in the H-type polymers. With these polymers, a significant higher encapsulation efficiency was obtained at pH 7.4 as compared to pH 6.0, which, however, was not observed for PLGA 50:50. We speculate that the H-type polymers, carrying carboxyl end-groups, exhibit at higher pH values a more negatively charged interface to the aqueous phase (Gabor et al., 1999; Govender et al., 1999), thus favouring interaction with the cationic drug. On the other hand, such an effect should not be expected with the end-group capped polymer types. Here, it became evident that encapsulation of gentamicin sulphate into PLA/PLGAs by spray drying must be influenced by a combination of physico-chemical interactions (H-bonding, acid–base, polar, hydrophobic and ionic interactions) between polymer, drug and solvents. Therefore, elucidation of these interactions would require further studies.

The physical form of the drug (powder or aqueous solution) also affected substantially the encapsulation efficiency. The very poor encapsulation of GS powder agrees with observations made with bovine serum albumin (BSA) powder (Thomasin et al., 1997). We assume two properties to be responsible — (i) the relatively unfavourable drug particle-to-microsphere size ratio and (ii) a presumably weaker interaction between polymer and solid drug powder as compared to the aqueous drug solution. This view is consistent with the observations of Mauduit et al. (1993) and Vert et al. (1994), finding that the interaction between gentamicin and carboxylic end-groups depended on the chemical state of the drug. Hence, those interactions will not take place unless GS is dissolved in an aqueous medium, where the salt dissociates and interacts with the polymer carboxyl end-groups. With aqueous drug solutions, encapsulation efficiency depended on nominal loading and was generally higher at low (2%) as compared to high (10%) nominal loadings. This is a common phenomenon with water soluble drugs (Rafati et al., 1997) which tend to generate a more porous microsphere structure at higher drug content, as we also observed by SEM.

The relatively modest GS encapsulation efficiency achieved here might be considered as an obstacle for antibiotic therapy. However, considering the expected ability of the microspheres to target the MPS infected cells, where *Brucella* is localised, the gentamicin dose might still be sufficient to provide adequate doses in the intracellular sites.

Drug stability upon microencapsulation remains a problem for many compounds, especially

peptides and proteins. The exposure to organic solvents, interfaces, and stringent physical conditions of temperature and cavitational or shear forces can lead to structural changes which can have an effect on drug integrity (Gander et al., 1995a). It is known that gentamicin sulphate is very stable in aqueous buffers over a wide pH and temperature range (Rosenkrantz et al., 1980). Nevertheless, we conducted a bioassay, which demonstrated that the antimicrobial activity of the encapsulated gentamicin sulphate was fully preserved. On the other hand, the antibacterial activity of gentamicin decreases with a lowering of pH (Rubenis et al., 1964). Thus, lower efficiency of GS formulations under certain low pH in intracellular compartments could also occur. However, the antibiotic intracellular accumulation by means of microsphere drug delivery systems, might minimise this effect.

In conclusion, the present work suggests that the highly hydrophilic and cationic gentamicin sulphate can be entrapped with acceptable efficiency into PLA/PLGA MS by spray drying. Thereby, drug bioactivity was maintained. Nevertheless, formulation parameters such as polymer type and concentration, physical state of the drug and nominal loading all influenced substantially encapsulation efficiency and microsphere morphology and size distribution. Gentamicin sulphate containing MS may constitute an appropriate delivery system for this aminoglycoside antibioticum, since the appropriate particle size should be suitable for monocyte–macrophage uptake and may therefore be useful in the treatment of intracellular *Brucella* infections.

Acknowledgements

This work was supported by 'Proyectos de Investigación Universidad de Navarra' (PIUNA-96 240501) from Spain. Fellowship support for S.P. from 'Empresa-Universidad' Foundation and 'Asociación Amigos de la Universidad' is gratefully acknowledged. The authors would also like to thank Professor R. Jordana, Zoology Department, University of Navarra, Pamplona, Spain, for the SEM-microphotographs, taken by a DSM 940A-Zeiss digital scanning electron microscope obtained thanks to the grant CICYT IN92-0225.

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