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Key parameters affecting the initial release (burst) and encapsulation efficiency of peptide-containing poly(lactide-*co*-glycolide) microparticles

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Abstract

The objective of this study was to identify key variables affecting the initial release (burst) and the encapsulation of leuprolide acetate-containing poly(lactide-*co*-glycolide) (PLGA) microparticles, which were prepared by the cosolvent evaporation method. Adjusting parameters, which affected the PLGA precipitation kinetics, provided efficient ways to increase the encapsulation efficiency and to control the initial release. Addition of 0.05 M NaCl to the external aqueous phase increased the encapsulation efficiency and the initial release; in contrast, NaCl at high concentration (0.5 M) delayed polymer precipitation and resulted in non-porous microparticles with a low initial release. The presence of ethanol in the external phase led to porous microparticles with an increased initial release but a decreased encapsulation efficiency. The initial release also decreased with decreasing volume of the external phase and homogenization speed, as well as with covering the preparation apparatus; however, these variations had no significant effect on the encapsulation efficiency. Scale-up of the laboratory size by a factor of 5 and 25 showed insignificant influence on the encapsulation efficiency, particle size, and drug release.

Keywords: Burst; Encapsulation efficiency; Initial release; Microencapsulation; Poly(lactide-*co*-glycolide); Scale-up; Solvent evaporation method

1. Introduction

Microparticles based on the biodegradable polymer PLGA have been extensively investigated as controlled drug delivery system because of their excellent biocompatibility and biodegradability (Jain, 2000). In recent years, a continued interest in PLGA microparticles has been triggered by their application for the controlled release of macromolecular drugs (Okada et al., 1994; Herrmann and Bodmeier, 1995a; Woo et al., 2001; Kim and Park, 2001).

The drug release from PLGA microparticles can usually be divided into an initial release (burst) phase followed by a slower continuous release phase. The initial release, which plays an important role in the therapeutic efficacy and toxicity of microparticles, is normally defined as the amount of drug released during the first 24 h. Depending on the drug, a lower or higher initial release is required in order to initiate a pharmacological effect; an undesirable high initial release may exhaust the encapsulated drug from microparticles too rapidly and even cause toxicity problems. Thus, the proper control of the initial release phase is one of the key issues in the design of PLGA microparticles. The effect of formulation variables such as molecular weight of the PLGA (Ravivarapu et al., 2000a), drug loading (Ravivarapu et al., 2000b), and formation of a hydrophobic ion pair of the peptide drug (Choi and Park, 2000) on the initial release have been investigated.

The initial release is commonly attributed to the release of drug located close to the surface of microparticles (Wang et al., 2002; Batycky et al., 1997; Cohen et al., 1991). It is related to the microstructure (porosity) of the microparticles. A high porosity correlates with a large surface area and rapid penetration of the release medium and consequently a high initial release (Herrmann and Bodmeier, 1995b).

A popular method for the preparation of microparticles is the solvent evaporation method (Bodmeier and Chen, 1989). The drug is dissolved, dispersed or emulsified into an organic polymer solution. After emulsification of the polymer phase into an external (mostly aqueous) phase, the solvent diffuses into the external phase and evaporates; simultaneously, the external

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phase (nonsolvent) penetrates into the surface of the polymer droplets. The precipitation kinetics of the polymer droplets determines the microstructure of the solidified microparticles. In general, a rapid polymer precipitation causes the formation of porous microparticles because of a hardening of the droplets with still significant amount of solvent present, while a slower precipitation results in more concentrated polymer droplets and denser microparticles (Schlicher et al., 1997; Graham et al., 1999). Although having the same final composition, different microstructures of the particles with different release profiles can be obtained.

From a mechanistic point of view, many similarities exist between the formation of filtration membranes by phase inversion and microparticles by the solvent evaporation method. The polymer precipitation in ternary systems of polymer, solvent and nonsolvent in the formation of phase inversion membrane has been investigated in detail (Strathmann and Kock, 1977; Kimmerle and Strathmann, 1990). The resulting membrane structure was mainly dependent upon the velocity of the solvent/nonsolvent exchange. A fast solvent/nonsolvent exchange led to the formation of membranes with a thin surface skin and a highly porous finger-like inner structure; in contrast, a slow exchange resulted in a thicker surface skin and a denser spongy inner structure. The PLGA precipitation kinetics in an in situ PLGA implant system was examined by McHugh et al. (Graham et al., 1999; Brodbeck et al., 1999). Parameters leading to a faster PLGA precipitation (e.g., PVP or water addition to the PLGA solution or a decreasing polymer concentration) resulted in more porous implants and a high initial release. In contrast, a slower precipitation resulted in denser sponge-like implant with a low initial release.

Another key evaluation parameter for microparticles is the encapsulation efficiency, which is influenced many variables including the amount, physical state, and solubility of the drug (Freytag et al., 2000; Dunne et al., 2003), the properties of the polymer (Ravivarapu et al., 2000a; Boury et al., 1997) and additives in the polymer solution or the external aqueous phase (Freytag et al., 2000; Herrmann and Bodmeier, 1998).

In the present study, peptide-loaded PLGA microparticles were prepared by a solvent evaporation (cosolvent) method. The objectives were to identify key variables affecting the initial release and the encapsulation of the microparticles and to scale-up the lab size standard formulation by a scale-up factor up to 25.

2. Materials and methods

2.1. Materials

The following chemicals were used as received: poly(D,L-lactide-*co*-glycolide) (PLGA, 50:50) polymers, Resomer® RG 503H (Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany), leuprolide acetate (leuprolide, Lipotec S.A. Barcelona, Spain), polyvinyl alcohol (PVA, Mowiol 40-88, Clariant GmbH, Frankfurt am Main, Germany), methylene chloride, methanol, sodium hydroxide, sodium chloride, sodium azide (Merck KGaA, Darmstadt, Germany), polyethylene sorbitan monooleate (Tween 80) and mannitol (Sigma–Aldrich Chemie GmbH, Steinheim, Germany), ethanol and acetonitrile (Rotisolv® HPLC Gradient Grade, Carl Roth GmbH & Co., Karlsruhe, Germany).

2.2. Preparation of the microparticles by the cosolvent method

Three hundred and fifty milligrams PLGA and the 88 mg leuprolide acetate were dissolved in a solvent mixture of 2.5 g methylene chloride and 0.5 g methanol. This solution was emulsified into 800 ml 0.25% (w/w) PVA aqueous solution (external phase) using a homogenizer (Ultra-Turrax T 25, Janke & Kunkel, IKA-Labortechnik, Staufen, Germany) at 8000 rpm. The emulsion/suspension was stirred at 400 rpm for 2 h with a magnetic stirrer (Variomag® Electronicrührer, Multipoint HP 6, H&P Labortechnik GmbH, Oberschleissheim, Germany) to extract and evaporate the methylene chloride. The solidified microparticles were recovered by filtration and vacuum-dried for 1 day at room temperature or freeze-dried.

The linear scale-up of the formulation by a factor of 25 (all formulation parameters were multiplied by 25) was achieved by using a larger homogenizer (Ultra-Turrax T 50, Janke & Kunkel, IKA-Labortechnik, Staufen, Germany) at 4500 rpm. The stirring time was adjusted to 5.5 h with a propeller stirrer at 400 rpm. The microparticles were freeze-dried to avoid agglomeration of the microparticles.

2.3. Freeze-drying of an aqueous microparticle suspension

Microparticles were suspended in 16% (w/w) mannitol solution. The suspension was frozen at −40 °C for 2 h and freeze-dried (primary drying: chamber pressure 0.01 mbar over 24 h with a shelf temperature of −15 °C, second drying: chamber pressure 0.01 mbar with a shelf temperature of 20 °C for 12 h) (Gamma 2-20, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany).

2.4. Determination of drug loading/encapsulation efficiency of the microparticles

Leuprolide acetate-containing PLGA microparticles (∼13 mg) were dissolved in 10 ml 0.1N NaOH aqueous solution during 12 h of shaking on a horizontal shaker (HS 501 Digital, Janke & Kunkel, IKA-Labortechnik, Staufen, Germany). The drug concentration was measured by UV (UV–vis scanning spectrophotometer 2101 PC, Shimadzu, Kyoto, Japan) at 279 nm to obtain the drug loading ($n = 2$). The percent encapsulation efficiency is calculated as (actual drug loading/theoretical drug loading) × 100%.

2.5. In vitro drug release

In vitro release was determined by suspending the microparticles (∼10 mg) in 6 ml phosphate buffer (1/30 M, pH 7.0, 0.01% (w/w) Tween 80 and 0.01% (w/w) sodium azide). The suspensions were incubated in glass test tubes at 37 °C in an incubation

shaker (GFL 3033, Gesellschaft für Labortechnik GmbH & Co., KG, Burgwedel, Germany). The samples were centrifuged at predetermined time points. Five millilitres supernatant was collected and replaced. The leuprolide concentration was determined by UV at 279 nm ($n = 2$).

2.6. Reverse phase high performance liquid chromatography (RP-HPLC) assay for the determination of the encapsulation efficiency and in vitro drug release

Five milligrams microparticles were suspended in a mixture of 8 ml phosphate buffer (1/30 M, pH 7.0) and 3 ml methylene chloride. The suspension was shaken 24 h at 120 rpm at ambient temperature on a horizontal shaker. After centrifugation, the aqueous supernatant was collected. The concentration of leuprolide in the supernatant (encapsulation efficiency) or in the release medium (in vitro release) was analyzed by RP-HPLC (SCL-10A VP, Shimadazu, Japan), C18 Eurospher-100 column (150 mm × 4 mm, Knauer GmbH, Germany) (mobile phase: phosphate buffer (1/30 M, pH 7), acetonitrile 70:30 v/v; flow rate: 1.2 ml/min; UV detection at 280 nm). No statistical difference was observed between results obtained by UV or HPLC analysis for the encapsulation efficiency and the initial release (data not shown).

2.7. Particle size distribution

Microparticles were suspended in 0.1% Tween 80 aqueous solution and the size distribution was determined by laser diffractometry (LD) (LS 230, Beckman Coulter GmbH, Krefeld, Germany). Unless otherwise mentioned, the particle size of the microparticles was in the range of 5–40 μm for all investigated batches.

2.8. Characterization of the microparticle morphology

Scanning electron microscopy was used to image the surface and interior morphology of the microparticles. Samples were coated under an argon atmosphere with gold to a thickness of 8 nm (SCD 040, Bal-Tec GmbH, Witten, Germany), and were then observed with a scanning electron microscope (S-4000, Hitachi High-Technologies Europe GmbH, Krefeld, Germany).

3. Results and discussion

The model peptide leuprolide acetate was encapsulated in PLGA microparticles by a solvent evaporation (cosolvent) method. Drug and PLGA were dissolved into a solvent mixture of methanol and methylene chloride, which was then emulsified into an external aqueous phase. Methanol is a water-miscible solvent for leuprolide, but a nonsolvent for PLGA, while methylene chloride is a water-immiscible nonsolvent for leuprolide but a solvent for PLGA. The diffusion rate of methylene chloride into the external aqueous phase is crucial to the PLGA precipitation and thus the morphology of and drug release from the resulting microparticles. The influence of various parameters on the initial release and encapsulation efficiency was investigated.

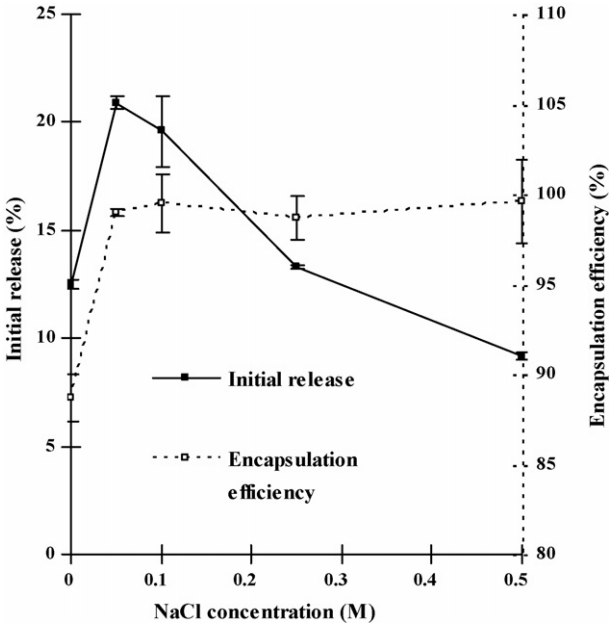

Fig. 1. Influence of NaCl addition to the external phase on the encapsulation efficiency and initial release of leuprolide acetate-loaded microparticles (drug loading 20%, external phase 800 ml containing 0.25% PVA).

3.1. Addition of NaCl to the external phase

The addition of 0.05 M NaCl to the external phase increased the encapsulation efficiency from 88.7 to 99.0%. An increase in NaCl concentration to 0.5 M showed no further improvement (Fig. 1). The increase in encapsulation efficiency could be attributed to the increased osmotic pressure of the external phase by addition of salts, which resulted in denser microparticles and a reduced the drug loss from the formulation. This result is in agreement with the literature (Herrmann and Bodmeier, 1995b, 1998).

The addition of NaCl showed a concentration-dependent effect on the initial release with an increase at low NaCl and a decrease at higher NaCl concentrations. A low salt concentration (0.05 M NaCl) in the external phase increased the initial release from 12.5 to 20.1%; however, a further increase in NaCl concentration to 0.25 and 0.5 M resulted in a decreased initial release to 13.3 and 9.2%, respectively (Fig. 1). The presence of 0.05 M NaCl in the external phase increased the encapsulation efficiency and actual drug loading (from 17.8 to 19.8%). The higher drug loading might be responsible for the higher the initial release. In general, the release increases with increasing loading in the case of water-soluble drugs (Ravivarapu et al., 2000b; Bodmeier and McGinity, 1987; Bodmer et al., 1992). To verify this assumption, microparticles with different leuprolide acetate loading were prepared. As expected, the initial release increased with increased actual drug loading (Fig. 2). In particular, the initial release jumped from 11.4 to 20.3% when the actual drug loading increased over a fairly narrow range from 17.7 to 20.0%. Thus, the increase in the initial release caused by the addition of the low concentration of NaCl (0.05 M) to the external phase could be attributed to the increased actual drug loading.

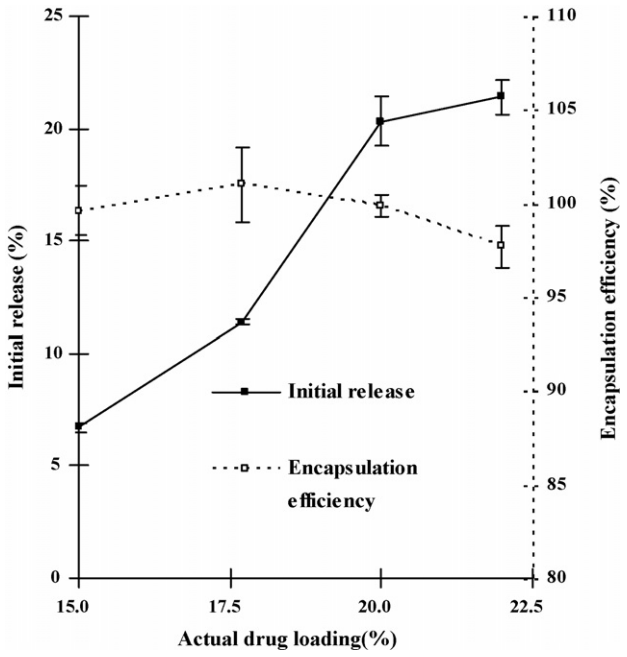

Fig. 2. Influence of the actual drug loading on the encapsulation efficiency and initial release of leuprolide acetate-loaded microparticles (external phase 800 ml containing 0.1 M NaCl and 0.25% PVA).

The decrease in the initial release at higher NaCl concentrations could be explained with a denser structure of the resulting microparticles. The presence of NaCl probably reduced the solubility of methylene chloride in the external aqueous phase. This then delayed the polymer precipitation and led to the formation of less porous microparticles. The porosity of the microparticles decreased with increasing NaCl concentration (Fig. 3). Microparticles prepared without NaCl addition showed a porous surface and inner structure, while microparticles prepared with 0.5 M NaCl in the external phase led to the formation of particles with a smooth surface and a dense inner structure. The decrease in porosity reduced the drug accessibility to the release medium and thus correlated with a lower initial release (Fig. 1).

3.2. Addition of ethanol to the external phase

NaCl at high concentration in the external phase reduced the affinity between the PLGA solvent methylene chloride and the nonsolvent water and led to a slower polymer precipitation and formation of nonporous microparticles with increased encapsulation efficiency and reduced initial release. To further confirm this result and investigate the precipitation kinetics, ethanol (miscible with both water and methylene chloride) was added to the external aqueous phase to increase the affinity between the

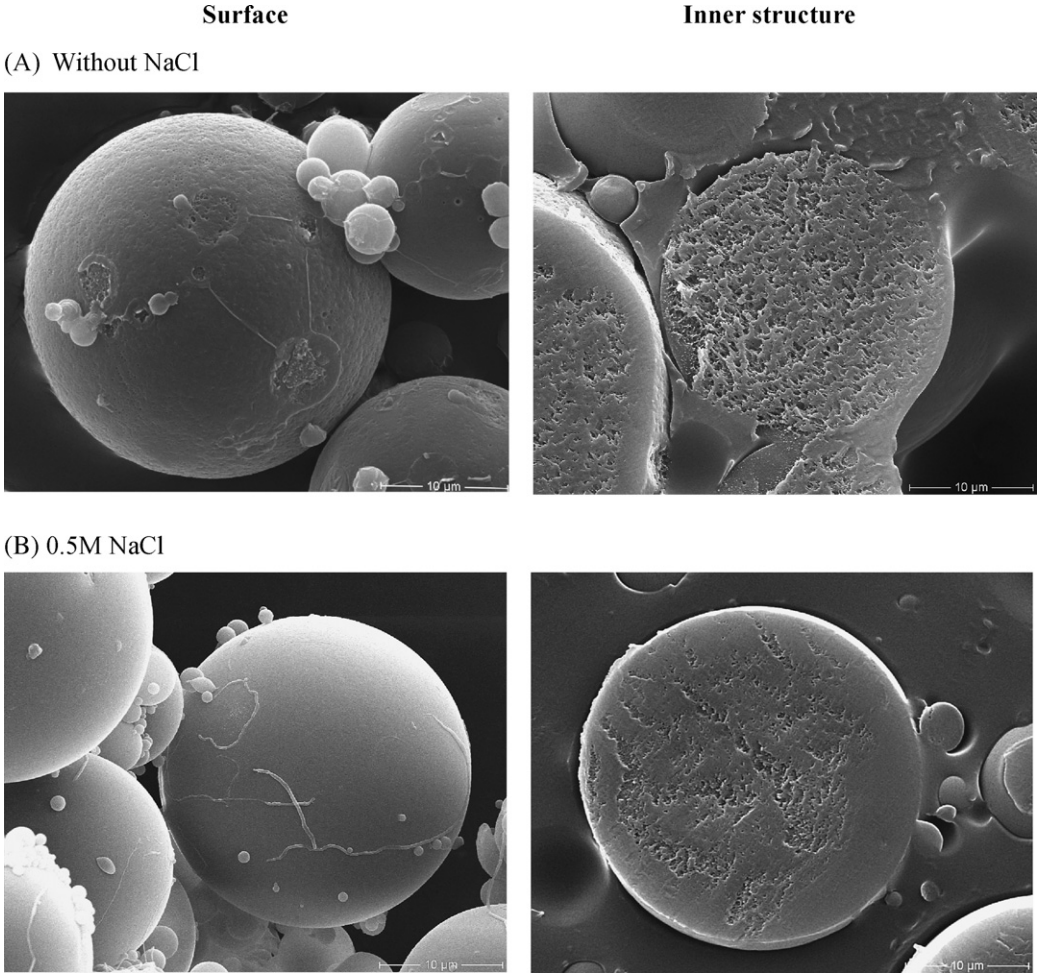

Fig. 3. Scanning electron micrographs of microparticles prepared without or with NaCl addition to the external phase. (A) Without NaCl; (B) 0.5 M NaCl.

polymer solvent (methylene chloride) and nonsolvent (external aqueous phase).

Increasing the ethanol concentration in the external phase increased the initial release increased from 8.0% (ethanol-free) to 19.0% (20% ethanol) and led to a dramatic decrease in the encapsulation efficiency (Fig. 4). The increase in the initial release could be attributed to the increased porosity of the microparticles obtained with the addition of ethanol (Fig. 5).

The miscibility of solvents is strongly related to their polarity, which is reflected by the dielectric constant (ε). Methylene chloride, a non-polar solvent ($\varepsilon = 9.5$), has a low miscibility with the polar solvent water ($\varepsilon = 80$). The addition of a semi-polar solvent, such as ethanol ($\varepsilon = 25$) reduced the gradient in dielectric constant between methylene chloride and the aqueous phase, thus increasing the solubility of methylene chloride in the external aqueous phase. The increased solvent/nonsolvent affinity then led to a faster polymer precipitation and more porous microparticles. The increase in the organic solvent diffusion out of the polymer solution might lead to a faster penetration of the aqueous solution (external phase) into the polymer solution, which resulted in a higher drug loss to the external phase and consequently lower encapsulation efficiency.

The effect of the addition of ethanol to the external phase on the complete release profile is shown in Fig. 6. The drug release profile was characterized by a rapid initial release fol-

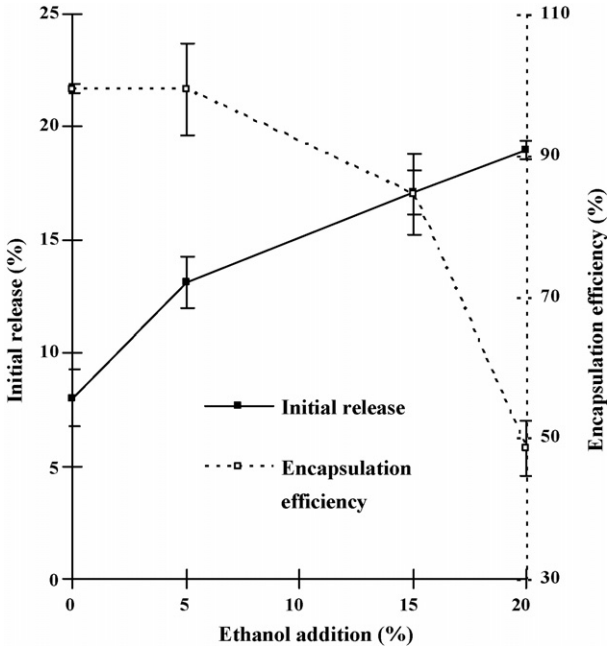

Fig. 4. Influence of ethanol addition to the external phase on the encapsulation efficiency and initial release of leuprolide acetate-loaded microparticles (drug loading 20%, external phase 200 ml containing 0.1 M NaCl and 0.25% PVA).

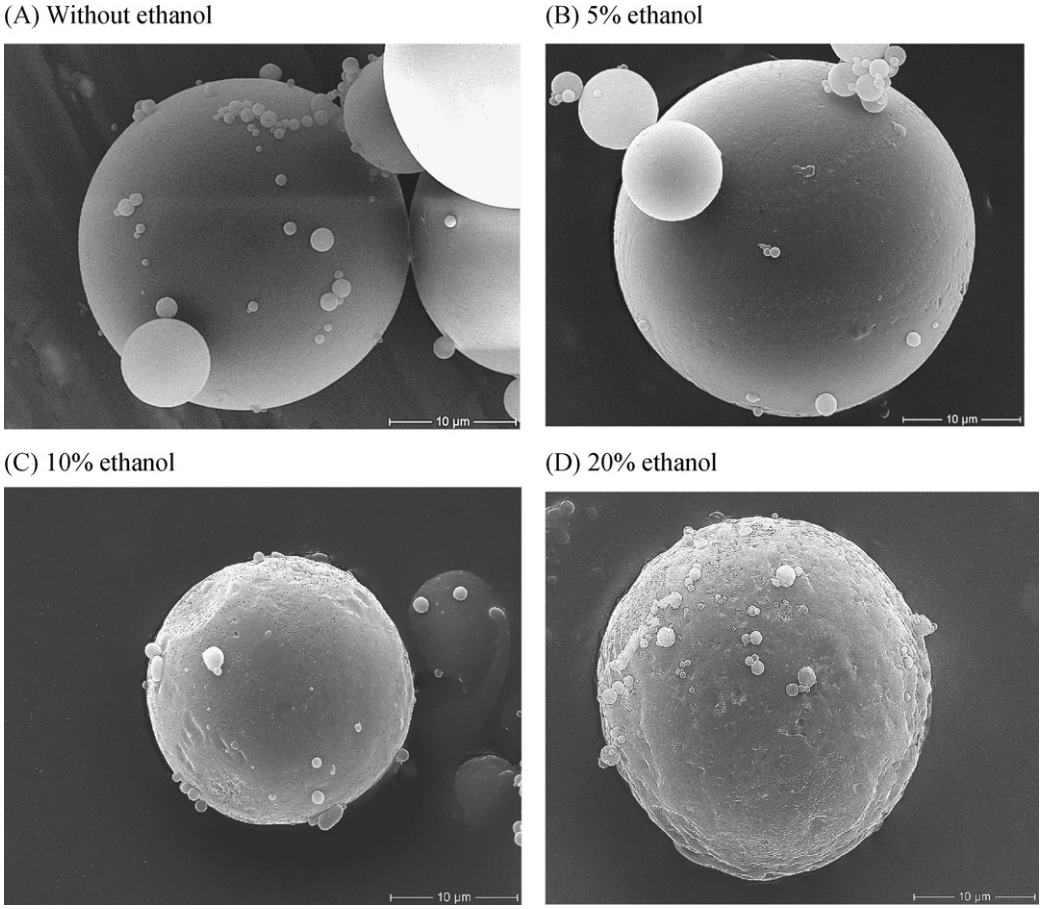

Fig. 5. Scanning electronic micrographs of microparticles prepared with different concentrations of ethanol in the external phase. (A) Without ethanol; (B) 5% ethanol; (C) 10% ethanol; (D) 20% ethanol.

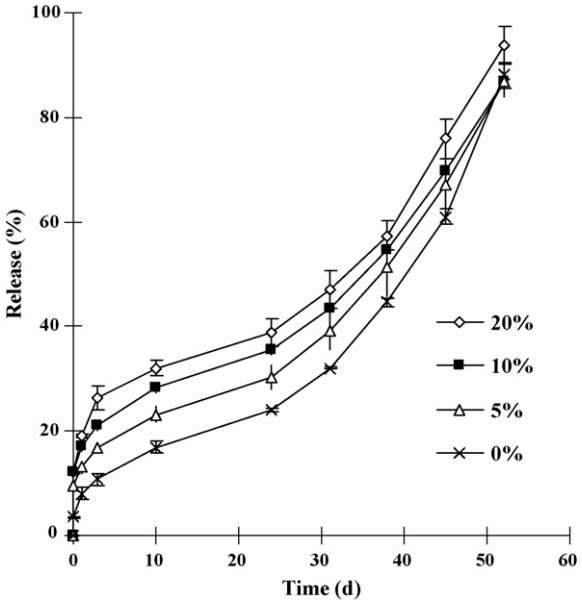

Fig. 6. Influence of ethanol addition to the external phase on leuprolide release from microparticles (drug loading 20%, external phase 200 ml containing 0.1 M NaCl and 0.25% PVA).

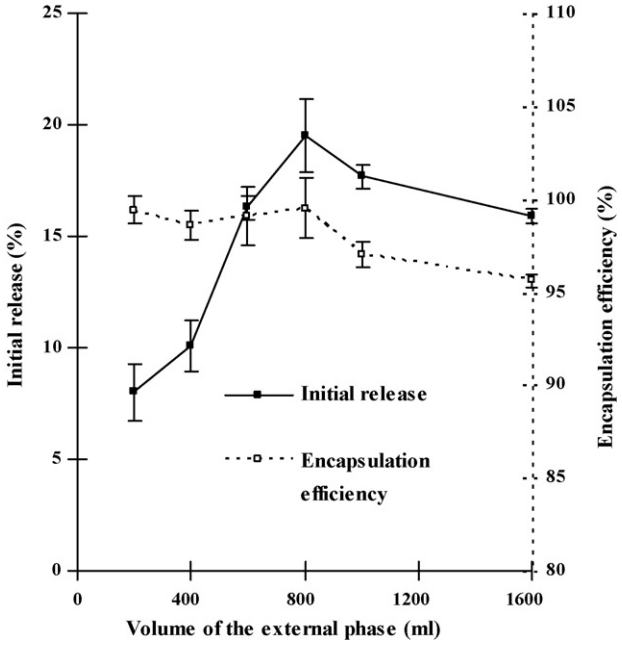

Fig. 7. Influence of volume of the external phase on the encapsulation efficiency and initial release of leuprolide acetate-loaded microparticles (drug loading 20%, external phase containing 0.1 M NaCl and 0.25% PVA).

lowed by a slow release phase and, after approximately 4 weeks, another rapid release phase caused by the erosion of PLGA. This tri-phasic pattern is typical for PLGA microparticles (Ruiz and Benoît, 1991; Igartua et al., 1998; Pitt, 1990). The addition of ethanol in the external phase thus resulted in a higher initial release but did not significantly affect the following release phases. Therefore, a change in the microstructure (porosity) of the microparticles showed primarily an effect on the initial release.

3.3. Volume of the external phase

The precipitation kinetics of the polymer solution droplets will not only be affected by the affinity between methylene chloride and the external phase, but also by their phase ratio. Increasing the volume of the external phase from 200 to 800 ml almost tripled the initial release; however, a further increase to 1600 ml slightly decreased the initial release (Fig. 7). The encapsulation efficiency was not affected significantly by the volume of the external aqueous phase (Fig. 7).

These results might be explained by two overlapping effects: (i) volume below 800 ml: an increase in the volume of external aqueous phase leads to an increased diffusion rate of methylene chloride into this phase and, thus, a faster polymer precipitation, resulting in more porous microparticles with a higher initial release; (ii) volumes above 800 ml: the faster methylene chloride diffusion may lead to a more rapid polymer nonsolvent (external aqueous phase) penetration into the polymer solution, which results in a high drug loss and a low encapsulation efficiency (Fig. 7). The low actual drug loading then leads to a decrease in the initial release.

In comparison to the addition of ethanol to the external phase, an increasing external phase volume had a less significant effect on the microstructure of the microparticles (SEM data not shown).

3.4. Solvent evaporation (closed versus open beaker) and stirring time

Methylene chloride diffuses into the external aqueous phase and then evaporates into air during microparticle preparation. Variables, which are related to the organic solvent evaporation, could also influence the polymer precipitation and thus the morphology and properties of the microparticles.

The rate of solvent removal was varied by preparing microparticles with an open (standard) or closed beaker set-up. The initial release was more rapid from microparticles, which were prepared in an open beaker when compared to those prepared in a closed beaker (Table 1). Methylene chloride evaporated more rapidly from the open beaker; the microparticles hardened faster, resulting in a more porous microparticle structure when compared to the closed system, which had a slower methylene chloride evaporation rate.

The stirring time in the external phase provides the time span to harden the microparticles. A reduction in encapsulation efficiency with prolonged stirring time was observed previously (Freytag et al., 2000). In this study, stirring times between 0.5 and 2 h did not have a strong effect on the encapsulation efficiency and initial release; however, a longer stirring time of 24 h resulted in a significantly higher encapsulation efficiency with the closed than with the open system (Table 1). This can be attributed to the slower organic solvent evaporation, resulting in a slower polymer precipitation and thus formation of microparticles with low porosity and reduced water imbibition into the

Table 1
Influence of solvent evaporation (open vs. covered beaker) and stirring time on the encapsulation efficiency and initial release of leuprolide-loaded microparticles (drug loading, 20%; external phase, 800 ml containing 0.1 M NaCl and 0.25% PVA)

Stirring time (h)	Open beaker		Covered beaker[a]	
	Encapsulation efficiency (%)	Initial release (%)	Encapsulation efficiency (%)	Initial release (%)
0.5	96.8 ± 0.6	15.3 ± 0.1	97.3 ± 0.1	11.5 ± 0.1
1	96.3 ± 0.4	16.7 ± 1.8	97.3 ± 0.3	11.1 ± 0.5
2	97.1 ± 1.6	17.1 ± 0.9	96.1 ± 0.3	12.6 ± 0.4
24	80.4 ± 0.3	20.5 ± 0.6	96.1 ± 2.6	16.9 ± 0.4

[a] The beaker was covered with aluminum foil, Parafilm and a Petri dish on top. This significantly hindered the evaporation process, which was confirmed by the strong smell of methylene chloride after 24 h of stirring.

microparticles. After 24 h stirring, the microparticles showed a slight increase in the initial release regardless of open or closed system.

3.5. Other variables

The effect of some other process variables on the initial release and encapsulation efficiency were summarized in Table 2. The organic phase (leuprolide acetate and PLGA in organic solvents) was added to the external phase via injection through a needle. The speed at which the organic phase was added to the external aqueous phase (ranging from 3 ml/10 s to 3 ml/120 s) did not significantly affect the encapsulation efficiency or the initial release.

Increasing the homogenization speed from 8000 to 9500 rpm did not significantly affect the encapsulation efficiency, but increased the initial release. This was caused by the smaller particle size of the microparticles at the higher homogenization speed.

Table 2
Influence of various variables on the encapsulation efficiency and initial release of leuprolide acetate-loaded microparticles (drug loading, 20%; external phase, 800 ml containing 0.1 M NaCl and 0.25% PVA)

Variables	Encapsulation efficiency (%)	Initial release (%)
Duration of injecting organic phase into the external phase		
10 s	98.8 ± 2.9	21.2 ± 0.8
30 s	99.8 ± 0.9	21.8 ± 1.1
60 s	97.6 ± 1.2	20.3 ± 0.4
120 s	97.1 ± 1.4	22.6 ± 0.1
Homogenization speed and drying method		
8000 rpm, freeze-drying	99.7 ± 1.8	17.3 ± 0.8
8000 rpm, vacuum-drying	99.7 ± 1.8	20.1 ± 0.3
9500 rpm, freeze-drying	98.4 ± 0.8	22.4 ± 1.2
9500 rpm, vacuum-drying	98.4 ± 0.8	26.2 ± 0.1
Homogenization time		
10 s	98.8 ± 2.9	21.2 ± 0.8
30 s	99.7 ± 0.9	21.8 ± 1.2
60 s	97.6 ± 1.2	20.3 ± 0.4
120 s	97.0 ± 1.4	22.6 ± 0.1
PVA-concentration in external phase		
0.25%	99.8 ± 0.6	20.3 ± 1.1
0.50%	99.2 ± 1.1	16.5 ± 0.2
1.00%	99.1 ± 1.3	26.1 ± 1.0

Table 3
Influence of scale-up on the encapsulation efficiency and initial release of leuprolide acetate-loaded microparticles (drug loading, 20%; external phase: factor 1 (800 ml), factor 5 (4000 ml), factor 25 (20000 ml), external phase containing 0.1 M NaCl, 0.25% PVA, freeze-dried)

Scale-up factor	Particle size[a] (μm)	Encapsulation efficiency (%)
1×	19.4	99.5 ± 1.6
5×	19.4	98.6 ± 1.1
25×	19.5	99.8 ± 2.3

[a] Volume size distribution. Fifty percent of total microparticles have a diameter smaller than the mentioned value.

Microparticles dried under vacuum showed a higher initial release than freeze-dried microparticles. The mechanism is so far still unclear. Varying the homogenization time from 10 to 120 s did not show a significant effect on the initial release and encapsulation efficiency. Increasing the PVA (stabilizer) concentration in the external phase from 0.25 to 0.5% led to a decreased initial release from 20.3 to 16.5%, however, a further increase to 1% resulted in an increased initial release of 26.1%. A possible explanation might be an increase in the osmotic pressure of the external phase at low PVA concentration. This delays the diffusion of methylene chloride and leads to a slower PLGA pre-

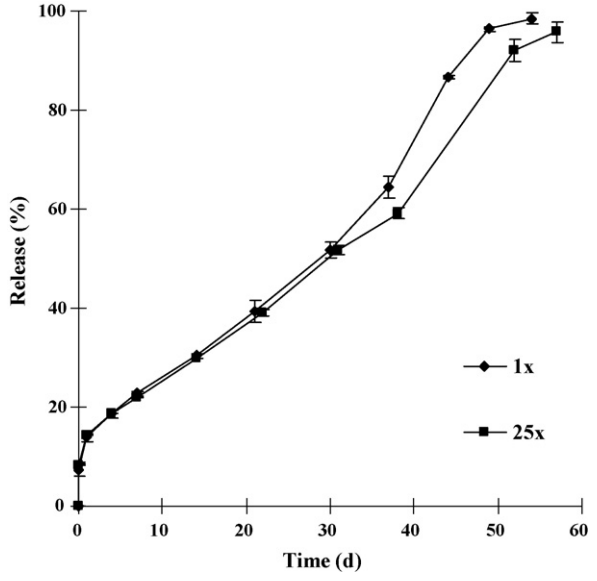

Fig. 8. Influence of scale-up on the drug release of leuprolide acetate-loaded microparticles.

cipitation, resulting in less porous microparticles with a lower initial release; a further increase in PVA concentration may result in micelle formation in the external phase resulting in an increase in the methylene chloride solubility in the external phase and thus in more porous particles with an increased initial release.

3.6. Scale-up

After investigation the influence of variables on the initial release and encapsulation efficiency, the laboratory size was scaled-up in a linear fashion. All the component quantities were multiplied by the respective scale-up factor. The encapsulation efficiency and particle size were not affected by a scale-up of the lab batch size by a factor of 5 and 25 (Table 3). In term of drug release, no significant difference was observed at scale-up factor of 25 (Fig. 8).

References

Batycky, R.P., Hanes, J., Langer, R., Edwards, D.A., 1997. A theoretical model of erosion and macromolecular drug release from biodegrading microspheres. J. Pharm. Sci. 86, 1464–1477.

Bodmeier, R., Chen, H., 1989. Preparation and characterization of microspheres containing the anti-inflammatory agents, indomethacin, ibuprofen, and ketoprofen. J. Control. Release 10, 167–175.

Bodmeier, R., McGinity, J.W., 1987. The preparation and evaluation of drug-containing poly(D,L-lactide) microspheres formed by the solvent evaporation method. Pharm. Res. 4, 465–471.

Bodmer, D., Kissel, T., Traechslin, E., 1992. Factors influencing the release of peptides and proteins from biodegradable parenteral depot systems. J. Control. Release 21, 129–137.

Boury, F., Marchais, H., Proust, J.E., Benoît, J.P., 1997. Bovine serum albumin release from poly(α-hydroxy acid) microspheres: effects of polymer molecular weight and surface properties. J. Control. Release 45, 75–86.

Brodbeck, K.J., DesNoyer, J.R., McHugh, A.J., 1999. Phase inversion dynamics of PLGA solutions related to drug delivery. Part II. The role of solution thermodynamics and bath-side mass transfer. J. Control. Release 62, 333–344.

Choi, S.H., Park, T.G., 2000. Hydrophobic ion pair formation between leuprolide and sodium oleate for sustained release from biodegradable polymeric microspheres. Int. J. Pharm. 203, 193–202.

Cohen, S., Yoshioka, T., Lucarelli, M., Hwang, L.H., Langer, R., 1991. Controlled delivery systems for proteins based on poly(lactic/glycolic acid) microspheres. Pharm. Res. 8, 713–720.

Dunne, M., Bibby, D.C., Jones, J.C., Cudmore, S., 2003. Encapsulation of protamine sulphate compacted DNA in polylactide and polylactide-*co*-glycolide microparticles. J. Control. Release 92, 209–219.

Freytag, T., Dashevsky, A., Tillman, L., Hardee, G.E., Bodmeier, R., 2000. Improvement of the encapsulation efficiency of oligonucleotide-containing biodegradable microspheres. J. Control. Release 69, 197–207.

Graham, P.D., Brodbeck, K.J., McHugh, A.J., 1999. Phase inversion dynamics of PLGA solutions related to drug delivery. J. Control. Release 58, 233–245.

Herrmann, J., Bodmeier, R., 1995a. Somatostatin containing biodegradable microspheres prepared by a modified solvent evaporation method based on W/O/W-multiple emulsions. Int. J. Pharm. 126, 129–138.

Herrmann, J., Bodmeier, R., 1995b. The effect of particle microstructure on the somatostatin release from poly(lactide) microspheres prepared by a W/O/W solvent evaporation method. J. Control. Release 36, 63–71.

Herrmann, J., Bodmeier, R., 1998. Biodegradable, somatostatin acetate containing microspheres prepared by various aqueous and non-aqueous solvent evaporation methods. Eur. J. Pharm. Biopharm. 45, 75–82.

Igartua, M., Hernández, R.M., Esquisabel, A., Gascón, A.R., Calvo, M.B., Pedraz, J.L., 1998. Stability of BSA encapsulated into PLGA microspheres using PAGE and capillary electrophoresis. Int. J. Pharm. 169, 45–54.

Jain, R.A., 2000. The manufacturing techniques of various drug loaded biodegradable poly(lactide-*co*-glycolide) (PLGA) devices. Biomaterials 21, 2475–2490.

Kim, H.K., Park, T.G., 2001. Microencapsulation of dissociable human growth hormone aggregates within poly(D,L-lactic-*co*-glycolic acid) microparticles for sustained release. Int. J. Pharm. 229, 107–116.

Kimmerle, K., Strathmann, H., 1990. Analysis of the structure-determining process of phase inversion membranes. Desalination 79, 283–302.

Okada, H., Doken, Y., Ogawa, Y., Toguchi, H., 1994. Preparation of three-month depot injectable microspheres of leuprorelin acetate using biodegradable polymers. Pharm. Res. 11, 1143–1147.

Pitt, C.G., 1990. The controlled parenteral delivery of polypeptides and proteins. Int. J. Pharm. 59, 173–196.

Ravivarapu, H.B., Burton, K., DeLuca, P.P., 2000a. Polymer and microsphere blending to alter the release of a peptide from PLGA microspheres. Eur. J. Pharm. Biopharm. 50, 263–270.

Ravivarapu, H.B., Lee, H., DeLuca, P.P., 2000b. Enhancing initial release of peptide from poly(D,L-lactide-*co*-glycolide) (PLGA) microspheres by addition of a porosigen and increasing drug load. Pharm. Dev. Technol. 5, 287–296.

Ruiz, J.M., Benoît, J.P., 1991. In vivo peptide release from poly(D,L-lactic acid-*co*-glycolic acid) copolymer 50/50 microspheres. J. Control. Release 16, 177–186.

Schlicher, E.J.A.M., Postma, N.S., Zuidema, J., Talsma, H., Hennink, W.E., 1997. Preparation and characterization of poly(D,L-lactic-*co*-glycolic acid) microspheres containing desferrioxamine. Int. J. Pharm. 153, 235–245.

Strathmann, H., Kock, K., 1977. The formation mechanism of phase inversion membranes. Desalination 21, 241–255.

Wang, J., Wang, B.M., Schwendeman, S.P., 2002. Characterization of the initial burst release of a model peptide from poly(D,L-lactide-*co*-glycolide) microspheres. J. Control. Release 82, 289–307.

Woo, B.H., Kostanski, J.W., Gebrekidan, S., Dani, B.A., Thanoo, B.C., DeLuca, P.P., 2001. Preparation, characterization and in vivo evaluation of 120-day poly(D,L-lactide) leuprolide microspheres. J. Control. Release 75, 307–315.