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# The effect of particle microstructure on the somatostatin release from poly(lactide) microspheres prepared by a W/O/W solvent evaporation method

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**Abstract**

Somatostatin acetate-containing poly(lactide) microspheres were prepared by a W/O/W multiple emulsion solvent evaporation method. The resulting microspheres were characterized with respect to encapsulation efficiency, drug release and morphological properties (scanning electron microscopy). The addition of various buffers (pH 2.2, 3.0, 4.0 or 5.0) or salts (NaCl or $CaCl_2$) to the internal aqueous and/or external aqueous phase affected the osmotic pressure gradient between the two aqueous phases and the solvent/water flux during the microsphere formation. Addition to the internal aqueous phase promoted the influx of water from the external aqueous phase. This resulted in more porous microspheres. Reversing the osmotic pressure gradient by adding salts to the external aqueous medium resulted in the formation of a dense and homogeneous polymer matrix. Intermediate structures were obtained through variations in the salt concentration gradient. The drug release profiles consisted of a rapid drug release phase followed by a slow release phase. The initial peptide release from the microspheres could be controlled through changes in the microstructure of the microspheres, with the peptide being released faster from the more porous microspheres. Lower encapsulation efficiencies were obtained with the more porous microspheres.

*Keywords:* Biodegradable polymer; Microencapsulation; Microsphere; Peptide delivery system; Poly(lactide); Somatostatin; Solvent evaporation method

## 1. Introduction

Peptides such as thyrotropin releasing hormone [1], a somatostatin agonist [2], several LH-RH analogues [3–6] and proteins, such as porcine somatotropin [7] and Interleukin-2 [8] have been encapsulated within biodegradable polymers to prolong their duration of action and to improve their therapeutic efficacy. The biodegradable polymers are usually based on poly(lactide) and its glycolide copolymers; these materials have a proven record of good biocompatibility.

The most popular methods for the preparation of biodegradable microparticles are solvent evaporation and phase separation techniques. In the solvent evaporation method, the drug is dissolved, dispersed or emulsified into an organic polymer solution, which is then emulsified into an external aqueous or oil phase [9–12]. The microspheres are formed after solvent diffusion/evaporation and polymer precipitation. In the phase separation method, the polymer is precipitated

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around a dispersed drug phase through the addition of a non-solvent, an incompatible polymer or a temperature change [5,6]. This method results in the formation of microcapsules (core-shell structure) vs. microspheres (matrix structure) formed by the solvent evaporation method. More recently, biodegradable microparticles have also been prepared by spray-drying and by supercritical fluid technology [13–16]. Both techniques are potentially suitable for the encapsulation of peptide and protein drugs.

The drug release from biodegradable microparticles is governed by various properties of the polymer, drug and carrier system [17]. Polymer dependent factors include the molecular weight and molecular weight distribution, the co-polymer ratio and distribution, and the crystallinity. Important drug dependent parameters are the solubility of the drug in dissolution or biological fluids, the molecular weight and possible polymer-drug interactions. Carrier system dependent factors comprise the type of microparticle (microsphere vs. microcapsule), the drug loading, the physical state of the drug in the polymer matrix (dissolved vs. dispersed), the particle size and particle size distribution, and the porosity and internal structure of the microparticles [18].

Although release modifiers such as plasticizers or hydrophilic additives (polymers, sugars, salts) are quite common with controlled release dosage forms, only few papers have described their use to modify the release from biodegradable microspheres [19]. The objective of this study was to investigate the effect of the addition of buffers or salts as release modifiers to the internal and external aqueous phases during microsphere preparation on the encapsulation efficiency, on the drug release and on the morphological characteristics of poly(lactide) microspheres prepared with a modified solvent evaporation method (W/O/W multiple emulsion technique). In addition, buffering the internal and/or aqueous phase could also affect the ionization of the drug and polymer and hence potential drug-polymer interactions. Somatostatin (somatotropin release inhibiting factor), a cyclic pentadecapeptide with high therapeutic potential, but a short in vivo half-life ($t_{1/2}$ = 1.1-3.0 min after intravenous injection [20]), was the peptide drug used.

## 2. Materials and methods

The following materials were used as received: somatostatin acetate (free base 1638 D; Dr. Willmar Schwabe Arzneimittel, Karlsruhe, Germany), poly(D,L-lactide) (PLA, M.W. 6,000 D, Resomer R202, Boehringer Ingelheim, Ingelheim, Germany), poly(vinyl alcohol) (PVA, 88 mol% hydrolyzed, M.W. 125 000 D, Polysciences Inc., Worthington, PA), acetonitrile, methylene chloride, phosphoric acid (EM Science, Gibbstown, NJ), citric acid, sodium chloride, sodium hydroxide, sodium phosphate heptahydrate (Fisher Scientific Co., Fair Lawn, NJ), calcium chloride dihydrate, sodium azide (Sigma Chemical Company, St. Louis, MO).

The microspheres were prepared by a modified solvent evaporation technique based on the formation of a W/O/W-multiple emulsion [4,21]. An aqueous solution of somatostatin acetate (somatostatin acetate equivalent to 3.0 mg somatostatin base dissolved in 0.1 ml 10 mM pH 5.9 acetate buffer) was emulsified into a solution of polymer (150 mg) in methylene chloride (3.0 ml) by sonication for 30 s under ice-cooling (sonicator, Heat Systems, Ultrasonics Inc., Plainview, NY). The resulting W/O emulsion was emulsified into the external phase (800 ml PVA-solution, 0.25% w/v) with a magnetic stirrer (Corning PC-351 hot plate stirrer, Corning, Inc., NY). After 30 min, the microspheres were collected by filtration, rinsed with 30 ml water, vacuum dried and sieved (U.S. Standard Sieve Series, Dual MFG CO., Chicago, IL); the 75–150 $\mu$m particle size fraction was used for further studies. The pH of the internal and/or external aqueous phase was adjusted to pH 2.2, 3.0, 4.0, and 5.0 with a 0.1 M citric acid/HCl buffer. Various water-soluble additives (NaCl or $CaCl_2$ 2 $H_2O$) were dissolved in the internal and/or external aqueous phase to study their effect on the encapsulation efficiency, and the release and morphological characteristics of the resulting microspheres.

For the analysis of the drug content, approx. 3 mg microspheres were accurately weighed and dissolved in 1.0 ml methylene chloride, followed by the addition of 1.0 ml 1 M acetic acid and agitation of the two phase system for 12 h at room temperature (peptide recovery from peptide containing films with known amounts of drug: 101.5% $\pm$ 4.6%, $n$ = 4). The low pH of the aqueous phase had no detrimental effect on the drug stability

[22]. The drug concentration of the aqueous phase was determined with a HPLC method: LC-600-HPLC-pump, SIL-9A autoinjector, SPD-6A UV-detector, CR-601 integrator (Shimadzu, Kyoto, Japan); ET 250/8/4, Nucleosil 300-5 C18 column (Macherey and Nagel, Düren, Germany); Vydac I-218TP guard column (Vydac, Hesperia, CA); mobile phase: 75.5% (v/v) water, 24.0% (v/v) acetonitrile, 0.5% (v/v) phosphoric acid; flow rate 0.9 ml/min; UV detection at 210 nm). Somatostatin solutions of known concentrations (0.01–0.15 mg/ml) in the same solvent system were used to generate calibration curves.

In vitro drug release profiles (up to seven days) were obtained by incubating the microspheres (3–4 mg) in 1.0 ml isotonic pH 7.4 phosphate buffered saline (containing 0.05% (w/v) sodium azide as the preservative) at 37°C in freeze-drying vials (type I glass vials) in a horizontal shaker (Lab-Line Instruments, Inc., Melrose Park, IL) ($n=2$). Due to the instability of the peptide in the release medium and the small amounts released with certain formulations, the amount of drug remaining in the microspheres rather than the amount of drug released was determined in order to characterize the drug release. At each time interval, the microsphere suspension was centrifuged and the supernatant was removed. The microspheres were then dried overnight at 37°C and extracted as described above. The peptide extracted from the microspheres after drug release was intact; no degradation products were detected in the extraction solution with the stability indicating HPLC method.

To study the internal structure of the particles, the microspheres were dispersed in a glue (Testor Corporation, Rockford, IL) followed by cutting of the dried matrix with a razor blade. The cross-sections were coated for 70 s with gold palladium (Pelco Model 3 Sputter Coater) and observed with a scanning electron microscope (SEM, Jeol JSM 35C).

## 3. Results and discussion

Somatostatin acetate, a water-soluble peptide, was encapsulated within PLA microspheres by a modified solvent evaporation method. The classical solvent evaporation method, in which the drug is dissolved/dispersed in the organic polymer solution followed by emulsification into an external aqueous phase to form an O/W emulsion did not result in acceptable encapsulation efficiencies. Because of its high water solubility, the drug partitioned into the external phase during microsphere preparation, thus resulting in microspheres with low drug loadings. Recently, modified solvent evaporation methods based on an external aqueous phase have been developed for the encapsulation of water-soluble drugs [9]. The micronized drug (dispersion method) or an aqueous drug solution (W/O/W multiple emulsion method) is dispersed in the organic polymer solution followed by emulsification into the external phase. The key parameter for the successful entrapment of the drug is its insolubility in the organic polymer solution; the polymer solution separates the dispersed drug crystals or the internal aqueous phase from the external aqueous phase and thus prevents the drug from partitioning and hence drug loss into the external aqueous phase.

In this study, the W/O/W multiple emulsion method was used to prepare somatostatin acetate containing microspheres. An aqueous solution of the peptide was emulsified into a solution of PLA in methylene chloride to form a primary W/O emulsion, which was then emulsified into an external aqueous phase to form a W/O/W emulsion. The organic solvent diffused from the emulsion droplets into the external aqueous phase and evaporated at the air/water interface; the microspheres formed after polymer precipitation.

Due to the high potency of the drug, low theoretical loadings were used (1.9% w/w). High encapsulation efficiencies were obtained, however, the drug release was very slow. Less than 10% of the drug were released after 7 days. The slow drug release could be caused by the low drug loading and possibly by interactions of the cationic peptide drug with the polymer. Interactions or complex formation between the charged carboxyl groups of low molecular weight PLA and oppositely charged cationic drugs have been reported to result in a significant reduction in drug release [23]. For many drugs, it is desirable to develop delivery systems having an initial rapid release phase followed by a slow release phase. Effective ways to increase the drug release from microspheres are to increase the drug loading or to decrease the molecular weight of the polymer. Since it was not feasible to increase the drug loading and since the molecular weight of the polymer was already low (6000 Da), we attempted to influence the drug release through pH changes in the internal and external aque-

Table 1
Effect of the pH and composition of the internal and external aqueous phase on the encapsulation efficiency of the microspheres (theoretical drug loading = 1.9%, citric acid, HCl buffer system)

| pH of the internal aqueous phase | pH of the external aqueous phase | Encapsulation efficiency (%) |
|---|---|---|
| Standard | PVA-solution | 85 |
| 2.2 | 2.2 | 91 |
| 3.0 | 3.0 | 93 |
| 4.0 | 4.0 | 96 |
| 5.0 | 5.0 | 84 |
| 2.2 | PVA-solution | 69 |
| 3.0 | PVA-solution | 73 |
| 5.0 | PVA-solution | 35 |

ous phase with the intent to eliminate possible peptide-polymer interactions and through the addition of salts to the internal and external aqueous phases to affect the microstructure and porosity of the microspheres.

The degree of ionization of the peptide and of the polymer and hence the potential ability to form a complex depends on the pH of the internal and aqueous phase used during the preparation of the microspheres. A citric acid/HCl buffer system was used to adjust the pH of the internal aqueous phase and/or the external aqueous phase to pH 2.2, 3.0, 4.0 or 5.0. These pH values affect the degree of ionization of the polymer but not of the peptide. The dissociation constant of the carboxyl groups of a poly(lactide-glycolide)-polymer was reported to be approximately 4.0 [24] and should be similar for PLA. The preparation of microspheres at a pH above the isoelectric point of the peptide (the peptide would be negatively charged) was not possible, because somatostatin is very unstable in aqueous solutions above pH 8 [22]. Somatostatin is a basic peptide containing two lysine amino acid residues (isoelectric point of somatostatin >9). It was therefore positively charged under all experimental conditions applied in this study.

The effect of the pH of the internal and external aqueous phase on the encapsulation efficiency is shown in Table 1. High encapsulation efficiencies in excess of 90% (with the exception of pH 5 buffer) were obtained when the pH of both the internal and external aqueous phase were adjusted to the same value. Interestingly, the encapsulation efficiencies were significantly lower when only the pH of the internal aqueous phase was adjusted. In this case, the pH-adjusted primary W/O emulsion was emulsified into the standard, buffer-free PVA solution. A gelled precipitate was formed when the peptide solution in pH 5.0 buffer was added to the solution of the polymer in methylene chloride prior to the emulsification step. This phenomenon was probably responsible for the lower encapsulation efficiencies of the microspheres prepared at pH 5.0. High encapsulation efficiencies were also obtained when only the external aqueous phase was buffered (e.g. with pH 2.2 citric acid buffer as the external phase, an encapsulation efficiency of 87% was obtained). Explanations for the different encapsulation efficiencies are given below together with the findings on the drug release and the morphological properties of the microspheres.

The drug release from three batches of microspheres prepared with pH 2.2 buffer (pH 2.2, external aqueous phase; pH 2.2, internal and external aqueous phases; pH 2.2, internal aqueous phase) is shown in Fig. 1.

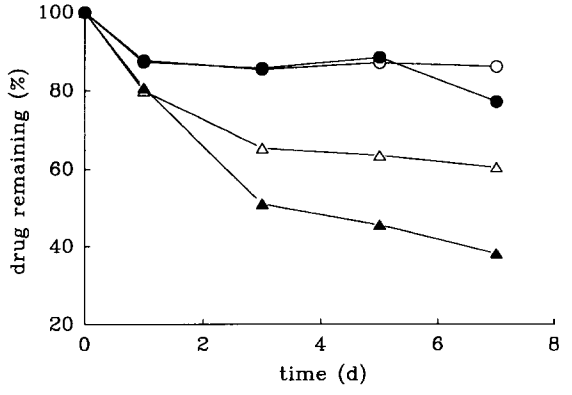



Fig. 1. Effect of addition of 0.1 M pH 2.2 citric acid/HCl buffer to the internal and/or external aqueous phase during the preparation of PLA microspheres on the drug release (encapsulation efficiencies given in Table 1). (○) standard preparation without additives; (●) pH 2.2 buffer, external aqueous phase; (△) pH 2.2 buffer, external and internal aqueous phase; (▲) pH 2.2, internal aqueous phase.

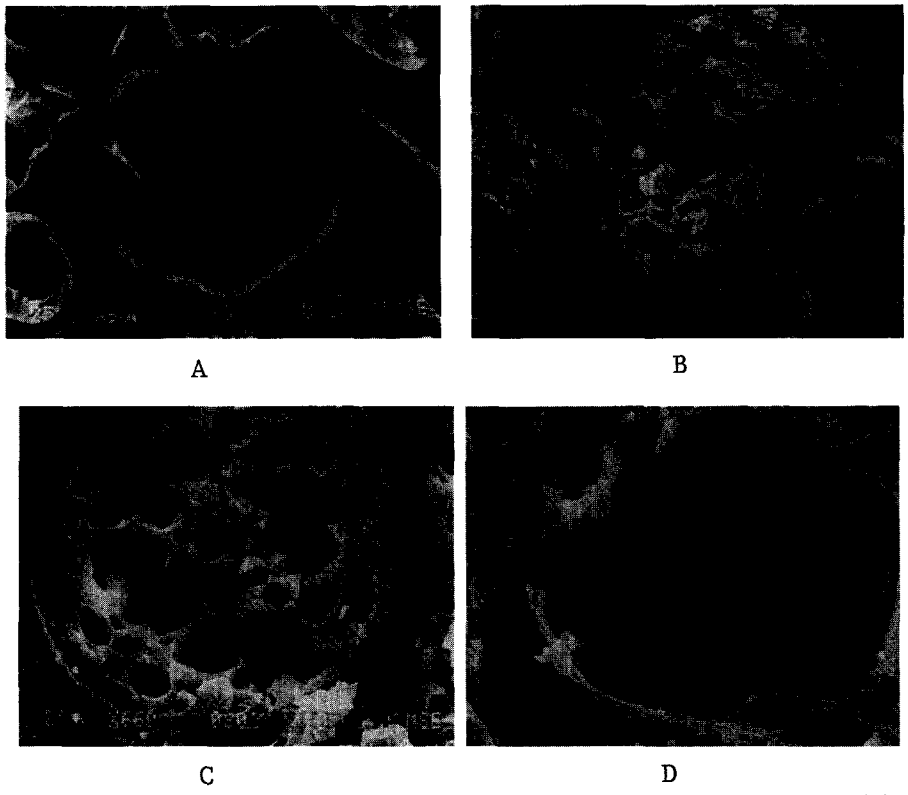



Fig. 2. Scanning electron micrographs of cross-sections of somatostatin containing PLA microspheres prepared with a citric acid/HCl buffer in the internal and/or external aqueous phase: (A) pH 2.2, external aqueous phase; (B) pH 2.2, internal and external aqueous phase; (C) pH 2.2, internal aqueous phase; (D) pH 4.0, internal and external aqueous phase.

The drug release was slowest from microspheres prepared with buffer in the external aqueous phase, followed by microspheres prepared with buffer in both aqueous phases, and by microspheres with buffer only in the internal phase. The observed release behavior and the encapsulation efficiencies could be explained with the internal structure of the microspheres (Fig. 2). Microspheres prepared with buffer in the external aqueous phase had a dense matrix structure (Fig. 2A), while microspheres prepared with buffer in both the internal and the external phase were porous (Fig. 2B) and microspheres prepared with buffer in only the internal aqueous phase were highly porous (Fig. 2C). The standard microspheres also had a dense, non-porous structure (not shown). The structure of the microspheres correlated well with the drug release, with the peptide being released faster from the more porous microspheres. It is well known, that an increase in matrix porosity enhances the drug release because of the easier accessibility of the drug by dissolution fluids.

The differences in the microstructures of the microspheres and hence also the drug release behaviour and the different encapsulation efficiencies could be explained qualitatively with the effect of the buffer salts on the precipitation of the polymer during the preparation of the microspheres. The microstructure or porosity of the microspheres depended primarily on the rate of precipitation of the polymer and the amount of solvent and water (nonsolvent) present at the time of precipitation. The addition of buffer salts affected the solvent-water exchange. Addition to the internal aqueous phase promoted the influx of water from the external aqueous phase due to a difference in osmotic pressure. This resulted in a more porous microsphere structure (Fig. 2C), faster drug release and lower encapsulation efficiencies. Reversing the osmotic pressure gradient by using a buffer as the external aqueous

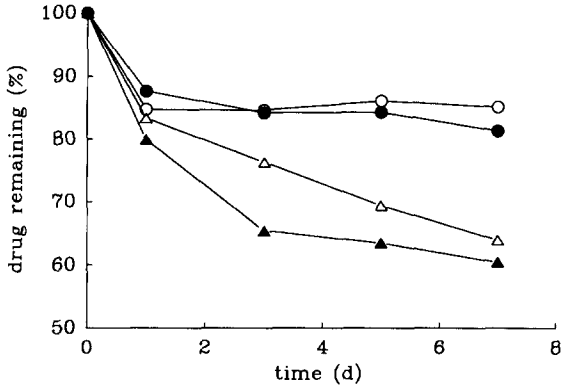



Fig. 3. The effect of the pH of the internal and external aqueous phase during the preparation of PLA microspheres on the drug release (encapsulation efficiencies given in Table 1). (○) pH 3.0, (●) pH 4.0, (△) pH 5.0, (▲) pH 2.2.

medium resulted in the formation of a dense and homogenous polymer matrix (Fig. 2A), a slower drug release and high encapsulation efficiencies. A less porous structure with an intermediate drug release pattern was formed when buffer salts were used in both aqueous phases (Fig. 2B).

The drug release from microspheres prepared with both aqueous phases adjusted to different pH values was in the following order: pH 2.2 > pH 5.0 > pH 3 or 4 (Fig. 3). The microspheres prepared at pH 5.0 could not be directly compared with the other batches because of the precipitation of the drug during microsphere preparation (see above), since this probably leads to different release properties. The faster drug release from the microspheres prepared with pH 2.2 buffer could be explained with the absence of interactions between the peptide and the polymer or with differences in the microstructure of the microspheres. A complex formation due to electrostatic interactions could be ruled out for microspheres prepared at pH 2.2 because of the unionized state of the carboxyl groups of the polymer. Assuming a pKa around 4, ionized carboxyl groups at pH 3 or 4 could interact and form an insoluble complex with the cationic peptide, resulting in a slower drug release. However, although the carboxyl groups were primarily unionized at pH 2.2 during the preparation of the microspheres, they should be ionized during release studies at the pH of the dissolution medium (pH 7.4). In addition, it could be argued, that the drug release from microspheres prepared at pH 3.0 or 4.0 in comparison to microspheres prepared at pH 2.2 should be faster because of the more hydrophilic character of the polymer due to the higher degree of dissociation of the carboxyl groups. The more likely explanation for the faster drug release from microspheres prepared at pH 2.2 vs. microspheres prepared at pH 3 or 4 was again based on a comparison of the microstructures of the microspheres. While microspheres prepared at pH 2.2 had a porous structure (Fig. 2B), cross-sections of microspheres prepared at pH 3.0 and 4.0 revealed a dense matrix structure (as exemplified with microspheres prepared at pH 4.0 in Fig. 2D) and hence a slower drug release. The more porous structure of microspheres prepared with pH 2.2 buffer vs. the dense structure of microspheres prepared with pH 3 or 4 buffer could possibly be explained with the more hydrophobic character of poly(lactic acid) at pH 2.2 due to the small number of ionized carboxyl groups. The more hydrophobic character of the polymer resulted in a more rapid polymer precipitation and hence in a more porous structure. Another considerations involved the particle size of the primary W/O-emulsion. The droplet size of the primary emulsion could be influenced by the type of buffer used; this could also affect the microstructure of the microparticles.

Since it was apparent that the differences in encapsulation efficiency, drug release, and microstructure were caused by the presence of buffer salts as a result of the pH adjustment of the aqueous phases, various salts (NaCl or $CaCl_2$) were added to the external and internal aqueous phases with the intent to influence the drug release through changes in the microstructure of the microspheres.

The effect of the addition of the salts to the internal and external aqueous phases on the encapsulation efficiency is shown in Table 2. The same trends as seen with microspheres prepared with the buffer salts were obtained. High encapsulation efficiencies (between 94 and 97%) were obtained when the salts were added to both the internal and the external aqueous phase. The encapsulation efficiencies were significantly lower with the salt being added to only the internal aqueous phase. Increasing the internal salt concentration, as exemplified with $CaCl_2$, further reduced the encapsulation efficiency. Control experiments with salts being added to only the external phase resulted in dense microspheres and high encapsulation efficiencies.

The addition of salts also significantly affected the drug release from the microspheres (Fig. 4). The drug

Table 2
Effect of the addition of NaCl or $CaCl_2$ to the internal and external aqueous phase on the encapsulation efficiency of the microspheres (theoretical drug loading = 1.9%, external phase, 0.25% PVA)

| Salt added to internal aqueous phase | Salt added to external aqueous phase | Encapsulation efficiency (%) |
|---|---|---|
| NaCl, 0.38 mol/l | NaCl, 0.38 mol/l | 94 |
| NaCl, 0.38 mol/l | None | 80 |
| $CaCl_2$, 0.38 mol/l | $CaCl_2$, 0.38 mol/l | 97 |
| $CaCl_2$ mol/l | None | 86 |
| $CaCl_2$, 1.52 mol/l | None | 51 |

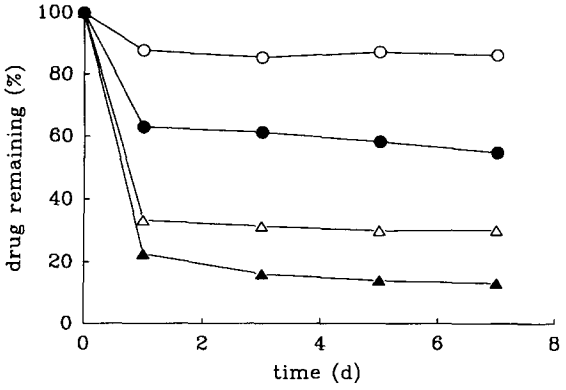
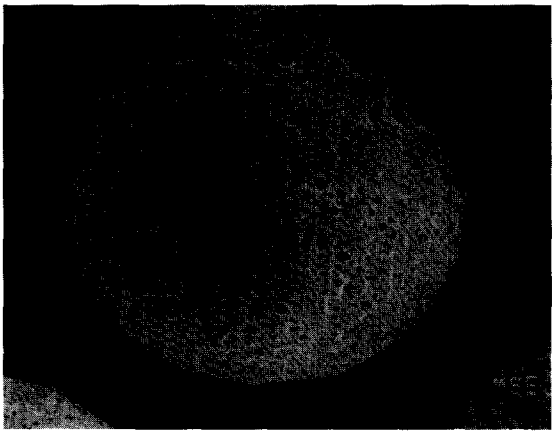



Fig. 4. The effect of the addition of $CaCl_2$ to the internal and/or external aqueous phase during the preparation of PLA microspheres on the drug release (encapsulation efficiencies given in Table 2). (○) standard preparation without additives; (●) 0.38 mol/l $CaCl_2$, both aqueous phases; (△) 0.38 mol/l $CaCl_2$, internal aqueous phase; (▲) 1.52 mol/l $CaCl_2$, internal aqueous phase.

release profiles were characterized by a rapid initial drug release phase followed by a slow release phase. When the salt was added to only the internal aqueous phase, 50–65% of the encapsulated somatostatin was released within 24 h for all salts. Increasing the amount of calcium chloride further increased the initial drug release (burst effect) (Fig. 4). Microspheres prepared with sodium chloride or calcium chloride added to both the internal and external aqueous phase had a larger burst effect than control microspheres prepared without salt. The presence of the salts in the polymer matrix could have resulted in faster penetration of the dissolution medium and hence a faster drug release.

The structure of the microspheres prepared with salts present in the aqueous phases correlated well with their encapsulation efficiency and their release behavior. Increasing the osmotic pressure difference between the internal and external aqueous phase increased the surface porosity, as shown with microspheres prepared with $CaCl_2$ (Fig. 5). When the same concentration of salt was added to the external aqueous phase to counterbalance the osmotic pressure difference, dense



A

B

Fig. 5. Scanning electron micrographs of somatostatin containing PLA microspheres prepared with $CaCl_2$ in the internal and/or external aqueous phase: (A) 0.38 mol/l $CaCl_2$, internal aqueous phase; (B) 0.38 mol/l $CaCl_2$, internal and external aqueous phase.

microspheres with very few and small surface pores were formed (Fig. 5B). Microspheres prepared with high internal $CaCl_2$ concentrations were hard and too brittle to be cut to obtain cross-sections for scanning electron micrographs. Very similar structures were found with microspheres prepared with sodium chloride as the additive (photographs not shown).

As observed with many matrix-type drug delivery systems, the drug release profiles could be divided into a rapid release phase representing drug release by diffusion through fluid filled pores and a slow release phase representing the release of the peptide by diffusion through the biodegradable polymer matrix. The microstructure (porosity) of the microspheres affected primarily the initial drug release phase. In conclusion, the drug release from somatostatin acetate containing PLA microspheres prepared by a W/O/W multiple emulsion solvent evaporation method could be controlled through changes in the microstructure of the microspheres as a result of the addition of various salts to the internal and/or external aqueous phases during the preparation of the microspheres.

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