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International Journal of Pharmaceutics 332 (2007) 107–114
INTERNATIONAL JOURNAL OF PHARMACEUTICS
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A novel *in situ* forming drug delivery system for controlled parenteral drug delivery

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Received 19 June 2006; received in revised form 15 September 2006; accepted 21 September 2006
Available online 26 September 2006

Abstract

The objective of this study was to investigate the *in vitro* drug (diltiazem hydrochloride and buserelin acetate) release from different *in situ* forming biodegradable drug delivery systems, namely polymer solutions (*in situ* implants) and *in situ* microparticle (ISM) systems. The drug release from ISM systems [poly(D,L-lactide) (PLA) or poly(D,L-lactide-*co*-glycolide) (PLGA)-solution dispersed into an external oil phase] was investigated as a function of the type of solvent and polymer, polymer concentration and internal polymer phase:external oil phase ratio and was compared to the drug release from *in situ* implant systems and microparticles prepared by conventional methods (solvent evaporation or film grinding). Upon contact with the release medium, the internal polymer phase of the ISM system solidified and formed microparticles. The initial drug release from ISM systems decreased with increasing polymer concentration and decreasing polymer phase:external oil phase ratio. The type of biocompatible solvent also affected the drug release. It decreased in the rank order DMSO > NMP > 2-pyrrolidone. In contrast to the release of the low molecular weight diltiazem hydrochloride, the peptide release (buserelin acetate) was strongly dependent on the polymer degradation/erosion. One advantage of the ISM system when compared to *in situ* implant systems was the significantly reduced burst effect because of the presence of an external oil phase. ISM systems resulted in drug release profiles comparable to the drug release of microparticles prepared by the solvent evaporation method. Therefore, the ISM systems are an attractive alternative to existing complicated microencapsulation methods.

Keywords: Biodegradable polymers; Sustained-release depot; Peptide; Microparticles; Poly(D,L-lactide); Poly(D,L-lactide-*co*-glycolide); *In situ*

1. Introduction

Various intramuscular or subcutaneous controlled drug delivery systems (DDS) in the form of implants or microparticles have been developed based on biodegradable polymers. These biodegradable drug delivery systems mostly use polyesters such as PLA or PLGA as release-controlling materials (Brannon-Peppas, 1995).

Various microencapsulation or extrusion techniques are available to form drug–polymer composites in the form of implants or microparticles (Benoit et al., 1996; Rothen-Weinhold et al., 1999). Implants are formed by blending the drug, polymer and additives followed by melt extrusion, melt compression or injection molding to obtain the desired implant size or shape (Rothen-Weinhold et al., 1999). Problems of implants include elevated process temperatures, poor content uniformity (especially with low dose drugs), and often the requirement of surgery. Biodegradable microparticles (multiple unit dosage forms) have been developed to overcome problems associated with implants. They are prepared by solvent evaporation, organic phase separation, spray-drying or supercritical fluid technology (Benoit et al., 1996).

The preparation of implants and especially of microparticles is based on complicated, multiple step processes with many process and formulation parameters to be controlled (Jalil and Nixon, 1990). As an alternative to solid implant or microparticle formulations, liquid drug–polymer formulations have been developed, which form implants *in situ* upon injection and contact with body fluid through the precipitation of the polymer (Dunn et al., 1990; Graham et al., 1999; Hatefi and Amsden, 2002; Wang et al., 2003; Packhaeuser et al., 2004). PLA- or PLGA-polymers are dissolved in water-miscible solvents, such as *N*-methyl-2-pyrrolidone (NMP) or dimethylsulfoxide (DMSO). The polymer solution solidifies upon injection and forms an implant. This technology has been utilized for low

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doi:10.1016/j.ijpharm.2006.09.033

and high molecular weight drugs (Duysen et al., 1993; Shah et al., 1993; Lambert and Peck, 1995; Ravivarapu et al., 2000). Disadvantages of these *in situ* implant systems (polymer solutions) are the initial rapid drug release prior to solidification of the polymer, the difficulty to inject the highly viscous polymer solution and questions regarding the myotoxicity of the organic solvents used (Kranz et al., 2001). To circumvent the high initial drug release (burst-effect) of the *in situ* implants prepared with water-miscible solvents, solvents with low solvent/water affinity (PLGA in triacetin or ethyl benzoate) have been proposed (Brodbeck et al., 1999). However, these systems are still single-unit drug delivery devices based on high amounts of organic solvents.

As an alternative to microparticles or *in situ* implants, a novel *in situ* forming microparticle system (ISM) was developed (Bodmeier, 1997; Kranz and Bodmeier, 1998). These ISM systems consist of an internal, drug containing polymer-solvent phase (polymer phase) emulsified into an external phase (for example an oil phase). Upon injection of this emulsion, the internal polymer phase releases the drug in a controlled release fashion. Solvents for the polymers are for example NMP, DMSO and 2-pyrrolidone, which are able to form highly concentrated polymer solutions. Peanut oil, an oil for injection, can be used as a biocompatible external oil phase. The ISM systems have a significantly reduced myotoxicity and a lower viscosity (the viscosity is primarily controlled by the external oil phase and not by the internal polymer phase). Therefore, these systems are easier to inject when compared to the viscous *in situ* implants (Jain et al., 1998; Kranz and Bodmeier, 1998). In addition, the preparation process for ISM is simple when compared with classical techniques for the preparation of microparticles. The manufacturing of the ISM systems can be done aseptically including a final filtration step. Alternatively, terminal sterilization by using gamma radiation can be used.

The objective of this study was to investigate the *in vitro* drug release of a highly water-soluble low molecular weight model drug (diltiazem hydrochloride) and a peptide (buserelin acetate) from polymer solutions, ISM systems, and microparticles prepared by conventional methods (solvent evaporation or film grinding). Scanning electron microscopy (SEM), laser diffraction measurements (LD) and size exclusion chromatography (SEC) were performed in order to relate the drug release to the surface properties, particle size and polymer degradation/erosion of the biodegradable polymers.

2. Experimental section

2.1. Materials

The following chemicals were obtained from commercial suppliers and used as received: poly(D,L-lactide) (PLA, R 203, M_w 25,700), poly(D,L-lactide-*co*-glycolide) (PLGA 50:50, RG 502, M_w 16,000; RG 503, M_w 42,800; RG 504, M_w 56,500) (Boehringer Ingelheim, Ingelheim, Germany), dimethylsulfoxide (DMSO), acetone, ethanol, methylene chloride, sodium azide, sodium chloride, sodium hydroxide, tetrahydrofuran, triethylamine (Merck, Darmstadt, Germany), diltiazem hydrochloride (Gödecke, Freiburg, Germany), buserelin acetate (Aventis, Frankfurt, Germany), 2-pyrrolidone (Soluphor®), Pluronic F 68 (BASF AG, Ludwigshafen, Germany), peanut oil (Henry Lamotte GmbH, Bremen, Germany), aluminum monostearate (Fluka Chemie AG, Buchs, Switzerland), Tween 80, *N*-methyl-2-pyrrolidone (NMP, Sigma–Aldrich Company, St. Louis, USA), polyvinyl alcohol (Mowiol 40-88, Clariant GmbH, Frankfurt, Germany). All chemicals were at least reagent grade.

2.2. Methods

2.2.1. Preparation of the in situ forming drug delivery systems

In situ implants (polymer solutions) were prepared by mixing PLA or PLGA with the solvents (2-pyrrolidone, NMP or DMSO) in glass vials until the formation of a clear solution. For the *in situ* implants the polymer concentration was kept constant at 40% (w/w, based on the amount of solvent and polymer). Diltiazem hydrochloride or buserelin acetate were dissolved in the polymer solution (10% (w/w), based on the polymer).

The ISM systems were prepared by emulsifying the drug-containing polymer solutions (PLA or PLGA in 2-pyrrolidone, NMP or DMSO) (polymer phase) into a peanut oil phase (oil phase) at a polymer to oil phase ratio of 1:1, 0.5:1, 0.25:1 and 0.1:1 by probe sonication (Bandelin Sonopuls HD 200, Bandelin electronic, Berlin, Germany) for 30 s under ice cooling. The polymer concentration was varied between 0% and 40% PLA or PLGA (w/w, based on amount of solvent and polymer). Pluronic F 68 (1% (w/w), based on the amount of the total formulation) was dissolved in the polymer phase and aluminum monostearate (2% (w/w), based on peanut oil) in the oil phase to increase the stability of the emulsions. The active agent (10% (w/w), based on the weight of the polymer) was dissolved in the polymer phase for the preparation of the diltiazem hydrochloride- or buserelin acetate-containing ISM systems.

2.2.2. Preparation of microparticles by film grinding

The active agent (10% (w/w) diltiazem hydrochloride, based on the polymer) was dissolved in ethanol (0.5 ml) and added to a solution of PLA in acetone (350 mg R 203 in 6.5 ml acetone). The films were prepared by casting the drug-containing polymer solutions into Teflon molds. Dried films were removed from the Teflon surface, cut into 4 cm × 4 cm test sections, and stored for 48 h in a desiccator prior to further experimentation. Microparticles were then prepared by grinding the drug-loaded films in a ball mill (Retsch Schwingmühle MM 2000, Retsch, Haan, Germany) cooled with liquid nitrogen. The microparticles were sieved and the size range of 50–100 μm was used for further experiments.

2.2.3. Preparation of microparticles by the solvent evaporation method (W/O/W)

An aqueous solution of buserelin acetate (30 mg drug, 3 ml distilled water) was emulsified with a sonicator (Bandelin Sonopuls HD 200, Bandelin electronic, Berlin, Germany) into a solution of PLA (300 mg R 203) in methylene chloride (20 g) to form a W/O emulsion (sonication time = 2 min). The primary

emulsion was added to the external aqueous phase (800 ml water, 0.25% (w/w) PVA, 0.25 mol/l sodium chloride) with a propeller stirrer (1500 rpm, Heidolph Elektro KG, Kelheim, Germany) to form the microspheres. The microspheres were collected, rinsed with water and sieved into various particle size fractions. Microparticles in the size range of 50–100 μm were used for further experiments.

2.2.4. Preparation of oily drug suspensions

The active agent (diltiazem hydrochloride or buserelin acetate) was micronized in a ball mill (Retsch Schwingmühle MM 2000, Retsch, Haan, Germany) under nitrogen cooling. The obtained drug powder particles (particle size <10 μm) were dispersed in peanut oil (10% (w/w) drug, based on the oil).

2.2.5. Drug release studies

ISM systems, *in situ* implants and microparticles were placed into dialysis bags (Medicell International Ltd., London, England; M_w cut off 12,000–14,000 Da) ($n = 3$). The amount of diltiazem hydrochloride or buserelin acetate released (5 mg, respectively) was kept constant. The bags were placed into 50 ml 0.1 M phosphate buffer pH 7.4 (for the buserelin acetate release studies, 0.05% (w/v) sodium azide was added as a preservative) at 37 °C in a horizontal shaker (GFL 3033, Gesellschaft für Labortechnik, Burgwedel, Germany). At predetermined time intervals, 2 ml samples (which were replaced with fresh medium) were withdrawn and assayed. After 48 h, the complete medium was withdrawn and replaced by fresh medium at each sampling point. Diltiazem hydrochloride was analyzed UV-spectrophotometrically at 237 nm (UV–vis scanning spectrophotometer 2101 PC, Shimadzu, Kyoto, Japan). The buserelin acetate content was measured with a computer connected Shimadzu-HPLC system (SCL-10A System Controller, LC-10A pump, DGU-3A degasser, SIL-10A auto injector, SDS-10AV UV-detector, Class-LC 10 software, Shimadzu, Kyoto, Japan). A 50 μl volume was injected onto a LiChrospher-100 RP 18.5 μm vertex column (Knauer GmbH, Berlin, Germany) using as mobile phase a mixture of 26 ml acetonitrile and 70 ml 0.1 mol/l phosphoric acid; the mixture was adjusted to pH 2.5 with triethylamine; flow rate: 0.8 ml/min; UV-detection at 220 nm.

2.2.6. Solubility of the drugs

Excess amount of diltiazem hydrochloride and buserelin acetate was placed in 1 ml 0.1 M phosphate buffer pH 7.4. The samples were shaken for 48 h at 37 °C. The saturated drug solutions were filtered and then assayed as described above after appropriate dilution. The final pH of the saturated solutions in the phosphate buffer was adjusted to pH 7.4 by adding 0.1 M sodium hydroxide ($n = 3$).

2.2.7. Optical microscopy

Droplet sizes of the ISM-emulsions were analyzed under an optical microscope (Axioskop, Zeiss, Jena, Germany) with transmitted light directly after the preparation of the ISM systems.

2.2.8. Scanning electron microscopy (SEM)

The dried *in situ* microparticles or implants were coated for 70 s under an argon atmosphere with gold–palladium (SCD 040, Balzers Union, Lichtenstein) and then observed with a scanning electron microscope (PW 6703/SEM 515, Philips, Eindhoven, Netherlands) in order to examine the surface morphology after incubation in the buffer medium at predetermined time periods.

2.2.9. Particle size and particle size distribution

Size distribution of the ISM was evaluated by dispersing about 1 g of the ISM emulsion into 50 ml 0.1 M phosphate buffer pH 7.4 containing 0.1% (w/w) Tween 80. For particle hardening the ISM were shaken for approximately 2 h in a horizontal shaker (GFL 3033, Gesellschaft für Labortechnik, Burgwedel, Germany). The particle size distribution of microparticles obtained by the *in situ* method was determined by laser diffraction measurements (Coulter LS 230, Coulter Corporation, Hialeah, USA) ($n = 3$). The obtained LD data were evaluated using the volume distribution, being a sensitive tool to detect even a few larger particles. No differences between particle sizes or particle size distributions were observed when shaking the ISM for 1–3 h.

2.2.10. Size exclusion chromatography (SEC)

Weight average molecular weights (M_w) were determined by size exclusion (SEC) chromatography ($n = 2$). The ISM were prepared by injection of 1 g ISM emulsion (40% polymer in 2-pyrrolidone, polymer:oil phase ratio 1:4) into 50 ml 0.1 M phosphate buffer pH 7.4, stored for different time periods at 37 °C (PLA-ISM for 1, 3, 7, 14, 21, 28, 35, 40 days and PLGA-ISM for 1, 3, 7, 14, 21, 28, 35 days), filtered, washed with hexane and dried under vacuum for 3 days. Microparticles prepared by the solvent evaporation method as described above were included as control. The ISM or microparticles were dissolved in tetrahydrofuran (1 mg/ml) containing toluene as internal standard. A 100 μl volume was injected into a HPLC system using a LC 10A, Shimadzu pump (Shimadzu, Kyoto, Japan) connected to two columns (Eurogel GPC 1000, 5 μm, 300 mm × 7.7 mm; Eurogel GPC 100000, 5 μm 300 mm × 7.7 mm, Knauer GmbH, Berlin, Germany). The eluting peak was detected by a refractive index detector (Rid-10A, Shimadzu, Kyoto, Japan). The SEC software (PSS GPC-Software, Polymer Standards Service GmbH, Mainz, Germany) was calibrated using polystyrene standards (Knauer GmbH, Berlin, Germany).

3. Results and discussion

For the preparation of the *in situ* forming DDS, biodegradable water-insoluble polymers, such as PLA or PLGA were dissolved in the water-miscible solvents NMP, 2-pyrrolidone or DMSO. These solvents were able to form concentrated polymer solutions in order to achieve high drug entrapment and suitable release profiles. In addition, NMP, DMSO and 2-pyrrolidone were chosen, because they have an intravenous LD_{50} of higher than 2 ml/kg (Merck Index, 1996). *In situ* implant formulations (polymer dissolved in either NMP or DMSO) were also investigated in rhesus monkeys and considered acceptable for use as injectable implant systems (Royals et al., 1999). The potential *in vitro* and *in*

vivo myotoxicity of different *in situ* forming biodegradable drug delivery systems, namely *in situ* microparticle systems and polymer solutions has been investigated in detail elsewhere (Kranz et al., 2001). Within these studies 2-pyrrolidone was found to have a better compatibility with skeletal muscle compared to NMP or DMSO.

The amorphous PLA (R 203) and PLGA (RG 502, RG 503, and RG 504) polymers used in this study had a relatively low molecular weight. The degradation in aqueous medium was expected within 1 and 4 months for PLGA and PLA, respectively. Diltiazem hydrochloride was chosen as a low molecular weight model drug, highly soluble in pH 7.4 phosphate buffer (521 mg/ml), but insoluble in oils such as peanut oil. The luteinizing hormone-releasing hormone (LHRH) agonist, buserelin acetate, was investigated as a pharmaceutically relevant peptide drug with high therapeutic and commercial potential. The solubility of buserelin acetate in pH 7.4 phosphate buffer was 10.5 mg/ml. Comparable to diltiazem hydrochloride, the nonapeptide was insoluble in peanut oil.

Upon injection of the polymer solutions (PLA or PLGA in NMP, DMSO or 2-pyrrolidone) into the release medium the polymer solidified as the solvent dissipated into the buffer medium pH 7.4 and formed implants. Irrespectively of the type of solvent, the implants, which were collected 24 h after exposure to the aqueous medium, had a porous surface (Fig. 1) (photographs for NMP and DMSO are not shown). Injection of the novel ISM system (40% polymer in 2-pyrrolidone; polymer:oil phase ratio 0.25:1) into the phosphate buffer medium led to the formation of microparticles, irrespectively of the type of solvent used (Fig. 2) (photographs for NMP and DMSO are not shown). Twenty-four hours after injection into the buffer

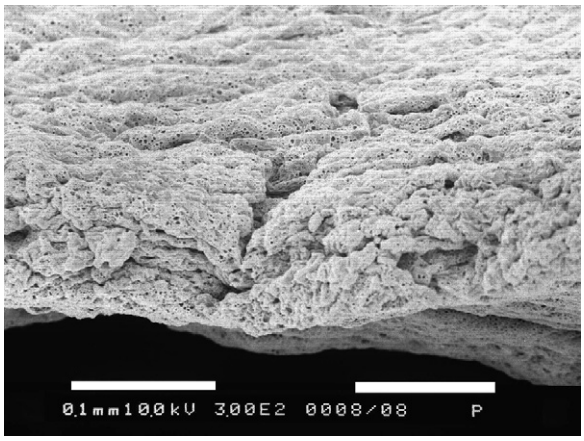

Fig. 1. Scanning electron micrograph of a polymer solution [*in situ* implant, 40% PLA (R 203) in 2-pyrrolidone] after injection into buffer medium pH 7.4.

medium, PLGA (RG 503) microparticles obtained by the ISM method showed a smooth, non-porous surface (Fig. 2A). Fourteen days after incubation of the PLGA (RG 503) microspheres into the buffer medium, small pores were observed distributed all over the matrix (Fig. 2B). After 28 days, the microparticles appeared highly eroded (Fig. 2C). In contrast, PLA (R 203) microparticles obtained by the *in situ* method did not show a significant mass erosion after 28 days (Fig. 2D).

The diltiazem hydrochloride-containing polymer solutions (40% PLA in NMP) showed a high initial drug release (burst-effect) with 72.6% diltiazem hydrochloride being released within 20 h (Fig. 3). With the ISM systems (polymer phase: 40% PLA in NMP), the initial release of the active agent decreased

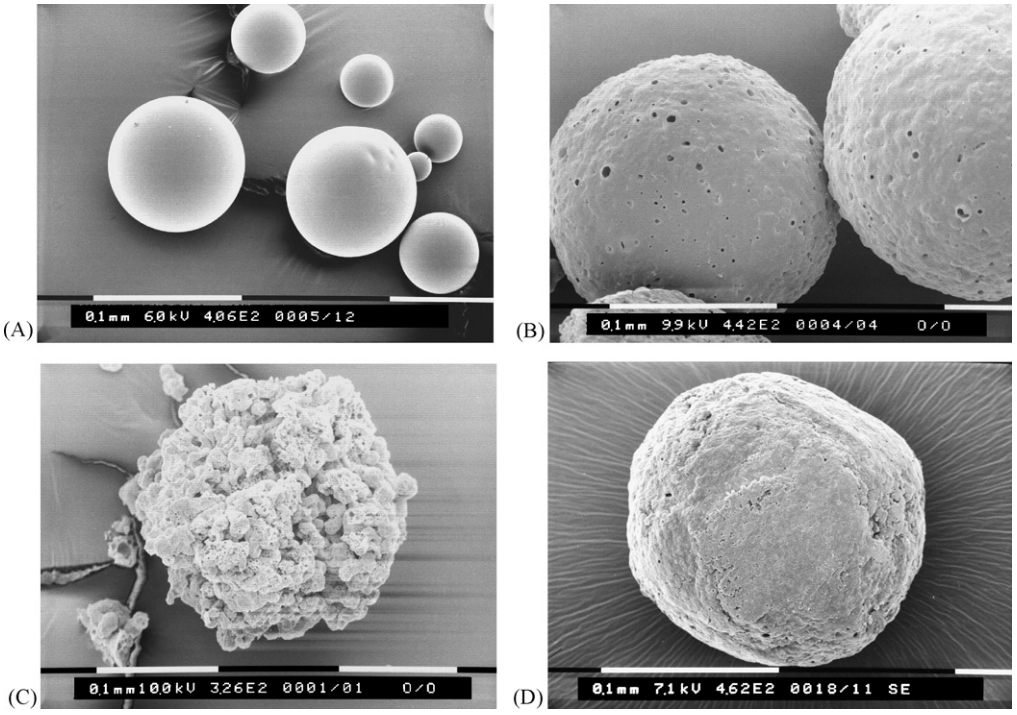

Fig. 2. Scanning electron micrographs of ISM [polymer:oil phase ratio 0.25:1, 40% PLGA (RG 503) in 2-pyrrolidone] after (A) 24 h (B) 14 days and (C) 28 days storage in buffer medium pH 7.4. (D) ISM system prepared with 40% PLA (R 203) in 2-pyrrolidone (polymer:oil phase ratio 0.25:1) after 28 days in buffer pH 7.4.

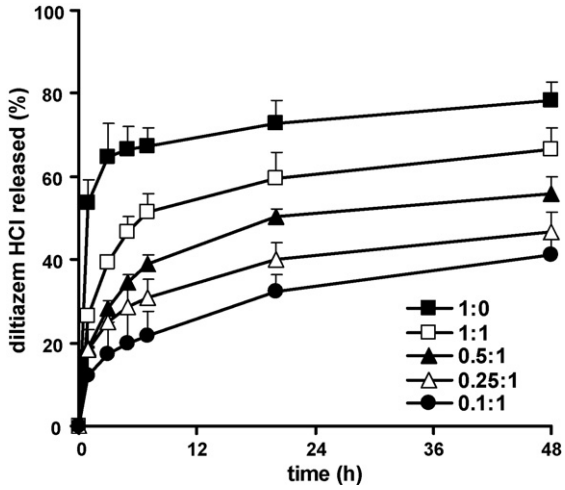

Fig. 3. Effect of the polymer phase:oil phase ratio on the diltiazem hydrochloride release from ISM systems containing 40% PLA (R 203) solutions in NMP as the inner polymer phase in comparison to a 40% PLA (R 203) solution in NMP (1:0, *in situ* implant).

with decreasing polymer phase:oil phase ratio. After 20 h 59.4% drug were released from ISM systems with a polymer:oil phase ratio of 1:1. This initial burst decreased to 39.9% and 32.3% for formulations with a polymer:oil phase ratios of 0.25:1 and 0.1:1, respectively. A similar high initial drug release in comparison to the ISM was observed for polymer solutions in NMP and DMSO (data not shown). The high initial drug release from *in situ* forming implants based on water miscible solvents is in good agreement with the literature (Brodbeck et al., 1999). Due to the loose network of interconnecting pores (Fig. 1), the active agent diffuses rapidly into the aqueous medium. This morphology has been described typically for a rapid phase inversion system (Graham et al., 1999). A reduced initial burst effect has been described when the aqueous affinity of the depot solvents was reduced (Brodbeck et al., 1999). In contrast to the polymer solutions, the polymer phase of the ISM systems was emulsified into an oil, which formed a partial barrier (increasing with decreasing polymer:oil phase ratio) between the aqueous medium and the internal polymer solution (Fig. 4). The low solubility of the active agent in the external oil phase caused

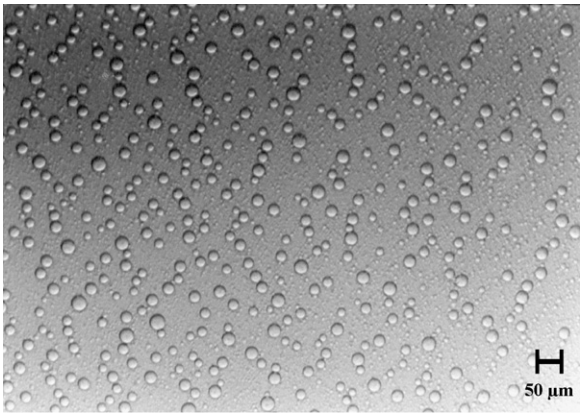

Fig. 4. Photograph of an ISM emulsion [polymer:oil phase ratio 0.25:1, 40% PLA (R 203) in 2-pyrrolidone].

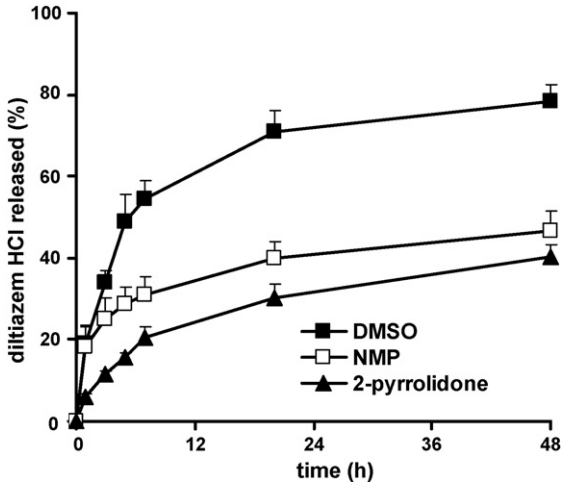

Fig. 5. Effect of the type of solvent on the diltiazem hydrochloride release of ISM [40% PLA (R 203), polymer:oil phase ratio 0.25:1].

the drug to stay in the inner polymer phase as it was encapsulated within the precipitated microparticles. In addition, the *in situ* microparticles had a less porous particle surface compared to the *in situ* implants (Figs. 1 and 2), this being a further possible explanation for the reduced initial drug release from ISM.

The initial drug release from ISM systems (40% (w/w) PLA based on the solvent and polymer, polymer:oil phase ratio of 0.25:1) prepared with different solvents decreased in the rank order of DMSO > NMP > 2-pyrrolidone (Fig. 5). After 20 h, 70.8% drug were released from ISM systems prepared with DMSO. This initial burst decreased to 39.9% and 30.2% for the ISM systems prepared with NMP and 2-pyrrolidone, respectively. The particle size of the ISM decreased in the rank order of 2-pyrrolidone > NMP > DMSO (Fig. 6). According to the Noyes–Whitney relation:

$$\frac{dM}{dt} = \frac{AD(c_s - c_t)}{h}$$

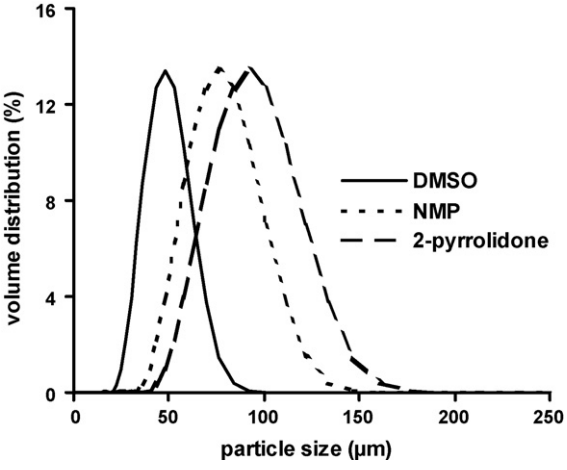

Fig. 6. Effect of the type of solvent on the particle size distribution of ISM systems [40% PLA (R 203), polymer:oil phase ratio 0.25:1] after immersion into phosphate buffer 7.4 at 37 °C.

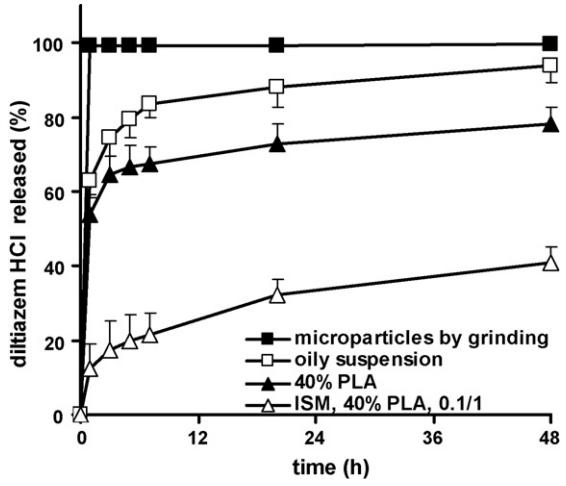

Fig. 7. Diltiazem hydrochloride release of different conventional PLA (R 203) drug delivery systems in comparison to the drug release of a 40% PLA (R 203) solution in NMP (*in situ* implant) and ISM [polymer phase: 40% PLA (R 203) in NMP].

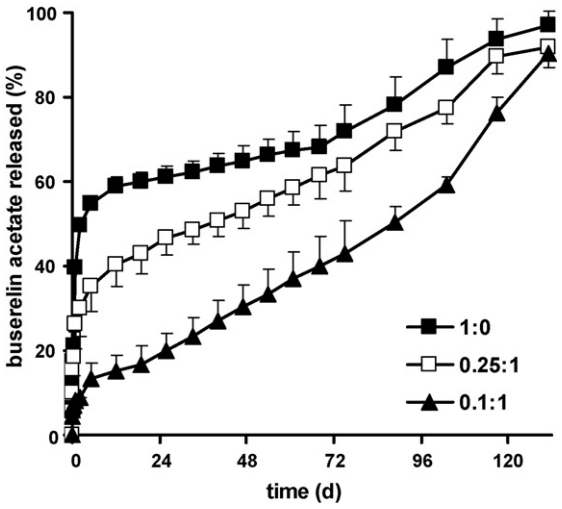

Fig. 8. Effect of the polymer phase:oil phase ratio on the buserelin acetate release of ISM systems containing 40% PLA (R 203) solutions in 2-pyrrolidone as the inner polymer phase in comparison to a 40% PLA (R 203) solution in 2-pyrrolidone (1:0, *in situ* implant).

where dM/dt is the dissolution rate, A the specific surface area of the drug particle, D the diffusion coefficient, h the diffusion layer thickness, c_s the saturation solubility and c_t is the instantaneous drug concentration the drug release rate increases with increasing surface areas. Smaller particles lead to higher specific surface areas. Therefore, the rank order of particles sizes (DMSO < NMP < 2-pyrrolidone) is in good agreement to the rank order of drug release (DMSO > NMP > 2-pyrrolidone).

The diltiazem hydrochloride release from ISM systems was compared to the release from microparticles prepared by film grinding and to an oily drug suspension (Fig. 7). After 1 h, 99.1% and 62.9% of the drug were released from microparticles prepared by film grinding and from the oily drug suspension, respectively. In contrast, the initial drug release from the ISM system was low. Only 12.3% of the active agent were released from ISM prepared with 40% PLA in 2-pyrrolidone at a polymer:oil phase ratio of 0.1:1 after 1 h. The high initial drug release rates from microparticles prepared by the film grinding method were in good agreement to other studies (El-Kharraz et al., 1998). The release of chlorpheniramine maleate, a highly water-soluble drug, from ethylcellulose microparticles prepared from different viscosity grade polymer samples was very rapid, irrespective of the viscosity grade of the polymer. This can be explained with the highly accessible surfaces after grinding. Microparticles prepared with other methods usually have a skin on the surface, which acts as a diffusion barrier. Oily drug suspensions have been reported to be a possible alternative for the prolonged delivery of lipophilic active agents (Zuidema et al., 1994). However, the oily drug suspension was not an alternative for the controlled delivery of a highly water-soluble model drug such as diltiazem hydrochloride.

Based on the results with the low molecular weight model drug, studies with buserelin acetate were performed with selected formulations (Figs. 8–11). 2-Pyrrolidone was chosen as solvent because of the potential to reduce the initial drug release and its lower myotoxic potential compared to NMP and

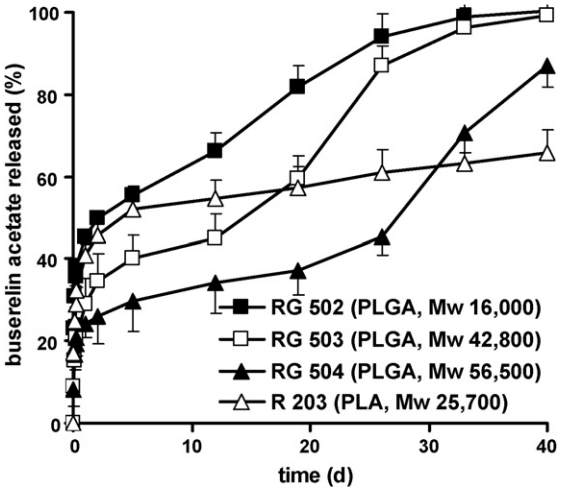

Fig. 9. Effect of the type of polymer on the buserelin acetate release from ISM (polymer:oil phase ratio 0.25:1; 20% polymer in 2-pyrrolidone).

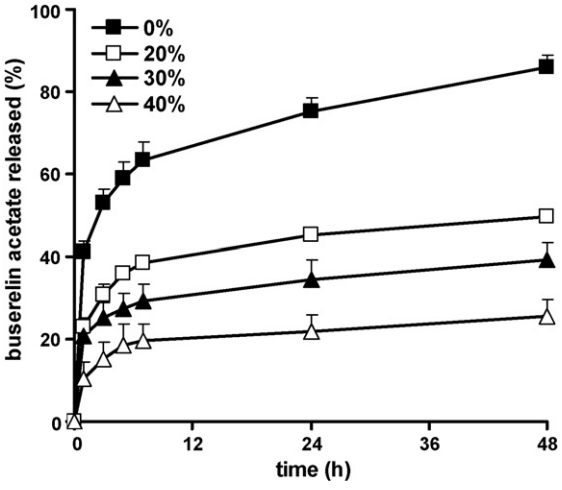

Fig. 10. Effect of the PLGA (RG 502) concentration in 2-pyrrolidone on the buserelin acetate release of ISM systems with a polymer:oil phase ratio of 0.25:1.

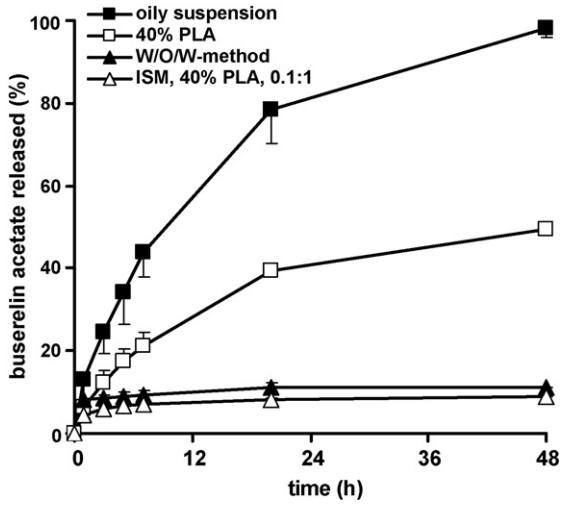

Fig. 11. Buserelin acetate release of different conventional PLA (R 203) drug delivery systems in comparison to the drug release of a 40% PLA (R 203) solution in 2-pyrrolidone (*in situ* implant) and ISM [polymer phase: 40% PLA (R 203) in 2-pyrrolidone].

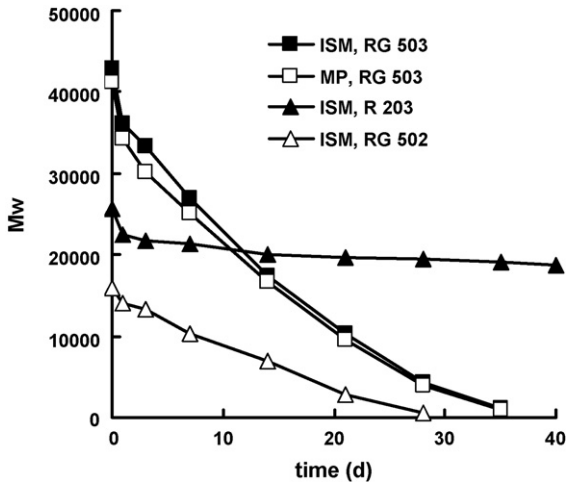

Fig. 12. Effect of the type of polymer on the molecular weight (M_w) of ISM prepared with 40% polymer in 2-pyrrolidone (polymer:oil phase ratio 0.25:1) and microparticles (denoted as MP) upon exposure to buffer medium pH 7.4 at 37 °C.

DMSO (Kranz et al., 2001). The stability of the peptide in the buffer medium pH 7.4 and within the precipitated microparticles was checked with a stability-sensitive HPLC assay. No degradation of the peptide was observed for the investigated time periods.

The *in vitro* release of buserelin from PLA solutions (*in situ* implants) and ISM systems was triphasic (Fig. 8); an initial burst release (prior to polymer hardening), a slower drug release over 72 days, followed by an increased drug release. Again, buserelin acetate containing polymer solutions had the highest initial drug release. After 24 h, 39.5% peptide were released from the polymer solutions. For the ISM systems, the initial drug release decreased with decreasing polymer:oil phase ratio. Decreasing the polymer:oil phase ratio of the ISM systems from 0.25:1 to 0.1:1 decreased the initial drug release after 24 h from 26.2% to 8.1%, respectively. In comparison to the initial diltiazem hydrochloride release, the peptide release was slower for all the investigated formulations. This can be explained with the lower solubility of the peptide in pH 7.4 phosphate buffer medium. The following period of slow peptide release and the increased release after 72 days can be explained as follows: the degradation of PLA and PLGA occurs in two steps—first chain cleavage of the biodegradable polymers as indicated by a decreasing molecular weight, and second, polymer erosion as indicated by a loss of matrix weight. Whereas the low molecular weight drugs can diffuse, by a simple partition-dependent process, through intact polymers, polymer erosion is an important factor influencing the release of higher molecular weight drugs such as peptides. Depending on the ratio of lactide:glycolide and the molecular weight of the PLA/PLGA, there is an induction period prior to mass loss or polymer erosion. High molecular weight polymers degrade to lower molecular weight fractions, which still remain water-insoluble until a critical molecular weight of approximately 15,000 Da is reached (Rothen-Weinhold et al., 1998; Li and McCarthy, 1999). The PLA (M_w 25,700) used in this study is known to degrade to water-soluble polymer fragments within approximately 2–3 months (Li and McCarthy, 1999), explaining the increased peptide release after 72 days.

In order to obtain suitable release profiles within 1 month, different ISM formulations with PLGA (lactide:glycolide ratio of 50:50) of different M_w at a polymer to oil phase ratio of 0.25:1 and a polymer content of 20% (w/w, based on the solvent and polymer) were tested (Fig. 9). Decreasing the M_w of PLGA from 56,500 to 16,000 Da resulted in constant release profiles after the initial burst. For RG 502, the critical M_w of approximately 15,000 Da for the water-soluble polymer fragments was almost reached; therefore, there was no induction period prior to mass loss. In contrast, RG 503 (M_w 42,800) and RG 504 (M_w 56,500) degraded to water-soluble fragments prior to the polymer erosion, thus explaining the triphasic release pattern. ISM systems prepared with PLA (R 203) did not degrade to soluble polymer fragments within 1 month, explaining the missing increase in peptide release. The initial burst after 24 h increased from 24.1% (RG 504), 28.9% (RG 503) to 49.3% (RG 502) for ISM formulations prepared with 20% polymer. This can be attributed to the decreased viscosity of the low M_w PLGA. However, increasing the polymer content to 40% PLGA (40% RG 502 in 2-pyrrolidone, polymer to oil phase ratio of 0.25:1) decreased the initial drug release to 25.4% after 24 h (Fig. 10).

Next, the drug release from ISM and polymer solutions was compared to conventional drug delivery systems (Fig. 11). In contrast to the oily drug suspension and the *in situ* implant, the ISM system had an initial peptide release comparable to microparticles prepared by the W/O/W solvent evaporation method.

In order to verify the effect of possible molecular weight changes on drug release, the M_w was determined by SEC (Fig. 12). A tremendous difference in the M_w-decrease was seen between PLA and PLGA microparticles prepared by the ISM method but no difference in the degradation behavior could be observed when compared to the microparticles prepared by the solvent evaporation method. PLGA microparticles (RG 502 and RG 503) showed a dramatically faster decrease in the M_w com-

pared to the PLA microparticles (R 203). After 28 days, almost complete polymer degradation was observed for ISM microparticles prepared with RG 502 and RG 503. In contrast to the PLGA, the PLA *in situ* microspheres degraded more slowly. After 28 days the weight average M_w of this type of microspheres was 18,000 Da. The degradation of PLA- and PLGA-microparticles is strongly dependent on the M_w and on the ratio of lactide to glycolide. Lower M_w and lactide contents lead to a faster polymer degradation, due to a faster polymer hydration. This explains the difference between PLA- and PLGA-microparticles, with the PLGA having a glycolide content of 50%.

In conclusion, the *in vitro* drug release from *in situ* forming drug formulations was dependent on the polymer to oil phase ratio, polymer concentration, and the type of solvent and polymer. One advantage of the ISM system when compared to *in situ* implant systems (polymer solutions) was the significantly reduced burst effect because of the presence of an external oil phase. These simple drug carrier formulations in liquid form (ISM) are also an attractive alternative to conventional microparticles.

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