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In situ forming microparticle system for controlled delivery of leuprolide acetate: Influence of the formulation and processing parameters

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ARTICLE INFO

Article history:

Received 10 June 2005

Received in revised form 23 August 2005

Accepted 4 September 2005

Available online 21 October 2005

Keywords:

In situ forming microparticle systems (ISM-systems)

Leuprolide acetate

Initial release

Polymer concentration

Drug loading

ABSTRACT

The objective of present study was to control the delivery of leuprolide acetate using in situ forming microparticle (ISM) systems. A solution of leuprolide acetate and poly(lactide-co-glycolide) (PLGA RG 503H) or poly(lactide) (PLA R 202H) in *N*-methyl-2-pyrrolidone (NMP) was emulsified into an external oil phase using a two-syringe/connector system. After injection into an aqueous environment, NMP diffusion led to polymer precipitation and microparticle formation in situ. ISM-systems were characterized with respect to particle morphology and the influence of formulation and processing parameters on the in vitro release. ISM from RG 503H showed a high initial release (approximately 40%), which could be attributed to the high porosity of microparticles. The initial release could be reduced by increasing the polymer concentration, increasing the amount and viscosity of the oil phase, and decreasing the drug loading. ISM-systems from R 202H had a much lower initial release (approximately 9%) compared to that from RG 503H, which was followed by a slow and continuous drug release. In comparison to conventional microparticles prepared by a solvent evaporation method, ISM from R 202H showed a lower initial release and a more linear continuous release.

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1. Introduction

Leuprolide acetate is a synthetic superagonist of the luteinizing hormone-releasing hormone (LH-RH). It has been used in treatment of sex hormone-dependent diseases, such as prostate cancer. As a water-soluble nona-peptide, leuprolide acetate has very poor peroral absorption and a short half-life after i.v. or s.c. injection (Okada, 1997). Initially, leuprolide acetate was administered by daily injection. To improve the patient compliance and therapeutic efficacy, various biodegradable polymer poly(lactide-co-glycolide) (PLGA)-based parenteral depot formulations have been developed.

Leuprolide acetate loaded PLGA microparticles prepared by a water-in-oil-in-water (W/O/W) emulsion solvent evapora-

tion technique was commercialized (Lupron Depot®) (Okada et al., 1991, 1994). These depot formulations provide a constant drug release for 1, 3, or 4 months after one administration.

Because of various disadvantages of classical microencapsulation method (e.g., complicated processes, difficult scale-up, solvent toxicity, reproducible release profiles and encapsulation efficiencies), in situ forming drug delivery systems have been developed as alternatives (Hatefi and Amsden, 2002). In situ forming implant systems based on PLGA for 1, 3, 4, or 6 months delivery of leuprolide acetate were developed (Eligard®) (Dunn et al., 1990; Ravivarapu et al., 2000b,c). These systems consist of a solution of the drug and PLGA dissolved in a biocompatible solvent such as *N*-methyl-2-pyrrolidone (NMP). After injection in the body, NMP diffuses into the tis-

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doi:10.1016/j.ejps.2005.09.002

sue fluid, leading to polymer precipitation and formation of a solid implant. In situ forming implant systems avoid the complex fabrication process of microparticles, have no drug loss during the preparation, and easy scale-up; however, they also have some limitations. The high viscosity of the PLGA solution may lead to a painful injection; the surface area of the resulting implant, controlling the drug release, may be variable depending on the injection technique and site; in addition, a high initial release may occur because of the formation of highly porous implants.

To address these drawbacks, **in situ forming microparticle (ISM)** systems have been prepared recently (Bodmeier, 1997; Kranz et al., 2001). ISM-systems are based on an emulsion of an internal drug-containing PLGA solution and a continuous oil or aqueous phase. After injection, the inner polymer phase hardens upon contact with body fluids and thus forms in situ microparticles. The advantages of ISM-systems include a lower viscosity of the emulsion when compared to the pure polymer solution and thus a reduced pain during injection; a reduced initial rapid release because of the presence of an external oil phase; in addition, ISMs are multiparticulates and could thus minimize the variation of single unit implant morphology and provide a more consistent and reproducible drug release.

In the present study, leuprolide acetate-containing PLGA (RG 503H) and PLA (R 202H) ISM-systems were prepared with an external oil phase. The influence of various formulation and processing parameters on the drug release was investigated.

2. Materials and methods

2.1. Materials

The following chemicals were used as received: poly(lactide-co-glycolide) (PLGA, 50:50) Resomer[®] RG 503H, poly(lactide) (PLA) Resomer[®] R 202H (Boehringer Ingelheim Pharma KG, Ingelheim, Germany), leuprolide acetate (leuprolide, Lipotec S.A. Barcelona, Spain), *N*-methyl-2-pyrrolidone (NMP) (Pharmasolve) (ISP Customer Service GmbH, Köln, Germany), sesame oil, peanut oil, soybean oil, aluminum monostearate, sorbitan monooleate (Span[®] 80), polyethylene sorbitan monooleate (Tween[®] 80) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), polyvinyl alcohol (PVA, Mowiol 40-88, Clariant GmbH, Frankfurt am Main, Germany), methylene chloride, methanol, sodium hydroxide, sodium chloride and sodium azide (Merck KGaA, Darmstadt, Germany).

2.2. Preparation of in situ forming microparticles and conventional microparticles

ISM-systems were prepared by a two-syringe/connector system and the standard formulation was as follows: a 1 ml syringe (single-use syringe, B. Braun Melsungen AG, Melsungen, Germany) containing the internal polymer phase (a solution of 5 mg leuprolide acetate and 95 mg RG 503H in 221 mg NMP, or a solution of 12 mg leuprolide acetate and 108 mg R 202H in 252 mg of NMP) was coupled with a connector (inner diameter: 1.4 mm) to another 1 ml syringe containing the same

weight of external continuous phase (peanut oil containing 2%, w/w, Span 80 and 2%, w/w, aluminum monostearate or sesame oil containing 2% Span 80 and 2.5% aluminum monostearate). Emulsification was achieved by pushing the internal and external phases forward and backward 50 times (cycles) at a speed of 2 cycles/s. The resulting O/O-emulsion was injected into the release medium (phosphate buffer, 1/30 M, pH 7.0) and microparticles formed in situ.

2.3. Conventional microparticles prepared by the cosolvent solvent evaporation method

574 mg PLA and 100 mg leuprolide acetate were dissolved in a solvent mixture of 4.0 g methylene chloride and 0.8 g methanol. This solution was emulsified into 1300 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 organic solvents. The solidified microparticles were recovered by filtration and vacuum-dried for 1 day at room temperature.

2.4. In vitro release

2.4.1. ISM-systems prepared with RG 503H

Approximately 0.2 g emulsion was injected into a dialysis bag (6 cm long and 2.2 cm wide, molecular weight cut-off 12–14,000 Da, Medicell International Ltd., London, UK). The dialysis bags were placed into 10 ml phosphate buffer (1/30 M, pH 7.0, 0.01% sodium azide) at 37 °C in an incubation shaker (GFL 3033, Gesellschaft für Labortechnik GmbH & Co. KG, Burgwedel, Germany) at 85 rpm ($n=3$). 8 ml release medium was collected and replaced at predetermined time points. The leuprolide concentration was determined by UV (UV-vis scanning spectrophotometer 2101 PC, Shimadzu, Kyoto, Japan) at 279 nm. Leuprolide in the release medium at 37 °C was stable at least for 7 days (longest sampling span) confirmed by reverse phase high-performance liquid chromatography RP-HPLC (data not shown).

2.4.2. ISM-systems and conventional microparticles prepared with R 202H

Formulations prepared with R 202H were targeted to a 4 months drug release period. Because leuprolide degraded in the release medium, leuprolide left in the polymer matrix rather than in the release medium was analyzed for all sampling points after 30 days.

Approximately 0.15 g emulsion (ISM-systems) was injected into 10 ml phosphate buffer (1/30 M, pH 7.0, 0.05%, w/w, sodium azide), or, in the case of conventional microparticles, 13 mg microparticles were suspended in 6 ml of the same buffer. The mixtures were incubated at 37 °C in an incubation shaker (85 rpm). During the first 30 days, 8 ml (ISM-systems) and 5 ml (conventional microparticles) release medium was separated and replaced. The leuprolide concentration was analyzed by UV at 279 nm ($n=3$). After 30 days, at each time interval, microparticles were separated from

the release medium and washed with hexane and water, and filtered. Thereafter, they were suspended in a mixture of methylene chloride (2 ml) and phosphate buffer (1/30 M, pH 7.0, 0.05%, w/w, sodium azide, 8 ml) ($n=3$). After shaking overnight, the leuprolide concentration in the aqueous solution was analyzed by RP-HPLC (SCL-10A VP, Shimadzu, Japan), C18 Eurospher-100 column (150 mm \times 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).

2.5. ISM hardening and morphology of the resulting microparticles

ISM emulsions were injected into 0.1% (w/w) Tween 80 containing phosphate buffer (1/30 M, pH 7.0) under stirring. After 5 h of stirring, the formed microparticles were filtered and vacuum-dried.

Scanning electron microscopy (SEM) was used to image the surface and internal morphology of the microparticles. To investigate the inner structure, the microparticles were dispersed in a solvent-free glue UHU® (UHU GmbH & Co. KG, Baden, Germany). After drying in a desiccator, the hardened matrix was frozen in liquid N₂, followed by cutting with a razor blade. Samples were sputtered 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

In ISM-systems, a solution of leuprolide acetate and biodegradable polymer in NMP is dispersed in a continuous oil phase. Upon contact with body (release) fluid, NMP diffuses from the polymer solution droplets into the aqueous environment either directly or through the oil, which leads to polymer precipitation and microparticles solidification in situ. Two polymeric carriers, PLGA RG 503H and PLA R 202H were used to get different leuprolide delivery periods. RG 503H was planned for 1 month and PLA R 202H for 4 months leuprolide release periods.

3.1. ISM-systems prepared with RG 503H

3.1.1. Morphology and in vitro release

The in situ forming microparticles were spherical and had a smooth surface but a porous inner structure (Fig. 1). Upon contact with the release medium, the completely water-miscible NMP diffused rapidly into the aqueous environment, leading to a rapid PLGA precipitation and formation of porous microparticles (Herrmann and Bodmeier, 1995; Graham et al., 1999).

The leuprolide release from ISM occurred in two phases (Fig. 2). A high initial release (40% of drug released during the first day) was followed by an almost constant and slower release phase over 45 days. The initial release from conventional microparticles is commonly attributed to the release of drug close to the microparticle surface and the inner poros-

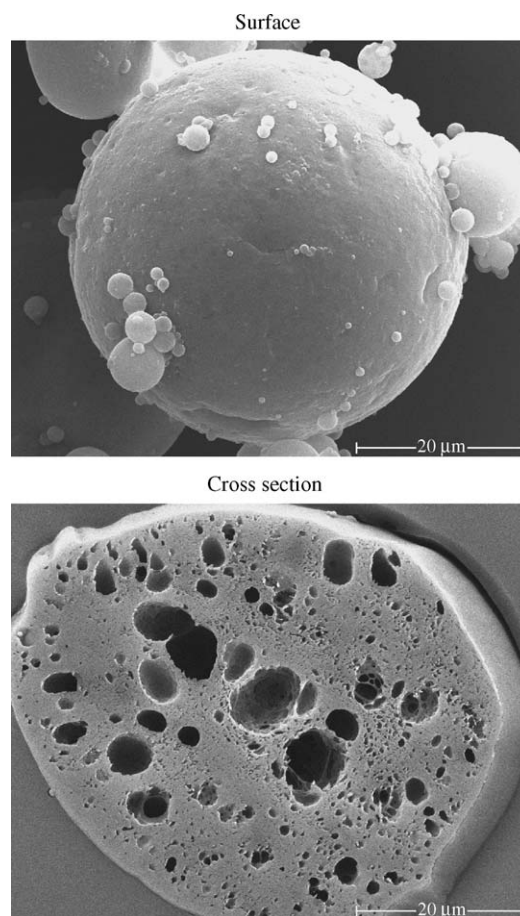


Fig. 1 – Scanning electron micrographs of in situ forming microparticles (RG 503H, standard formulation).

ity (Cohen et al., 1991; Pitt, 1990). For ISM-systems, the initial release could be caused by two effects: (i) ISM-systems were injected into the release medium as an emulsion of polymer solution droplets in oil. The lag time prior to PLGA solidifi-

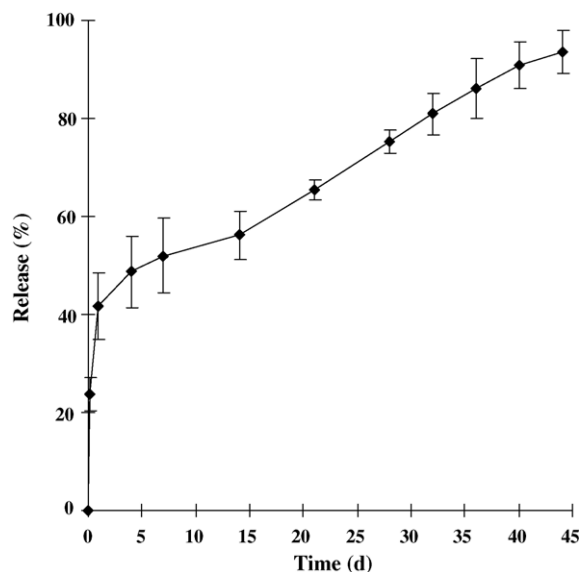


Fig. 2 – Leuprolide release from in situ forming microparticles (RG 503H, standard formulation).

cation might lead to a drug loss and (ii) the initial release could also be attributed to the porous microstructure of the microparticles (Fig. 1).

The proper control of the initial release is a crucial issue in designing leuprolide acetate controlled delivery systems. No initial release might lead to a delayed suppression of testosterone; however, an undesirable high initial release may exhaust the encapsulated drug from the formulation and even cause toxicity problems.

3.1.2. Parameters affecting the initial release

To reduce the undesired high initial release, various formulation and processing parameters were investigated. In each experiment, the standard formulation was repeated as a reference and only one parameter was varied. The leuprolide release was monitored over a 14-day period since only the initial release was of interest.

The polymer concentration plays an important role in the drug release from in situ forming systems. A decrease in the drug release from in situ forming implant systems with the increasing polymer concentration was already reported (Graham et al., 1999; Lambert and Peck, 1995). A higher polymer concentration led to a more viscous solution, which delayed the polymer precipitation and resulted in a less porous polymer matrix with a slower drug release. In ISM-systems, the initial release decreased dramatically from 62.7 to 43.7 and 11.7% with an increasing polymer solution concentration of 20, 30 and 40% (w/w), respectively (Fig. 3). The effect of polymer concentration on the second release phase (after initial release) was marginal. ISM-systems prepared with 40% RG 503H formed lumps during the emulsification into the external oil phase due to the high viscosity of the inner polymer solution and fast diffusion of NMP into the oil phase. Therefore, the polymer concentration was kept at 30% during the following experiments.

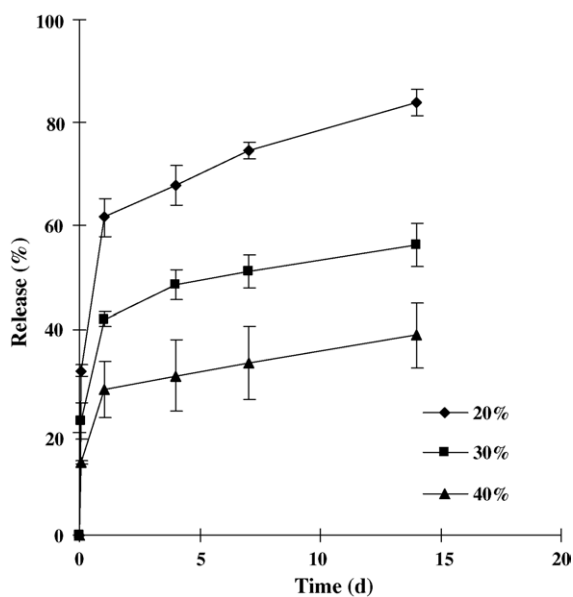


Fig. 3 – Influence of the polymer concentration (w/w) on leuprolide release from in situ forming microparticles (RG 503H).

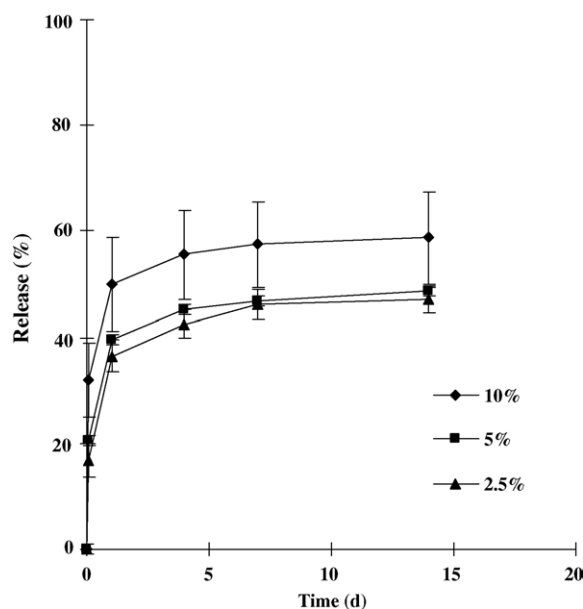


Fig. 4 – Influence of the drug loading (w/w) on leuprolide release from in situ forming microparticles (RG 503H).

In terms of injection volume, a high drug loading (resulting in a smaller injection volume at the same dose) is favored. However, an increase in the drug loading resulted in a higher initial release (Fig. 4), which could possibly attribute to more drug loss before microparticle solidification. The increase in the initial release with increasing drug loading has also been reported in other depot formulations (Lambert and Peck, 1995; Ravivarapu et al., 2000a). Again, the effect of drug loading on the second release phase was insignificant.

A decrease in the internal polymer to the external oil phase ratio (1:1 to 1:2.5, w/w) led to a decreased initial release (41.6–27.0%) (Fig. 5). More oil decreased the direct contact area between the inner leuprolide-polymer phase and the release medium and increased the diffusion pathway of the drug/droplets to the oil/release medium interface, thus resulting in a lower initial release.

The initial release increased with increasing surfactant (Span 80) concentration in the oil phase (w/w) (Fig. 6), which could possibly be explained with the smaller particle size at the higher surfactant concentration because of a reduced interfacial tension between the polymer solution and the oil (Lamprecht et al., 2002; Sanghvi and Nair, 1991).

Aluminum monostearate was added as a viscosity-increasing agent to the oil. The high viscosity of the oil could avoid the lump formation during emulsification with the polymer solution. The initial release of the formulation decreased with the addition of aluminum monostearate in the oil (Fig. 7), which could be attributed to the increased viscosity of the external oil phase, with a similar role like increasing the oil amount (reducing the drug participation into the release medium).

The oil type (peanut oil, sesame oil and soybean oil) or the number of mixing cycles (25, 50 and 75) did not affect the leuprolide release from ISM-systems (data not shown). An increase in mixing speed from 1 to 2 cycles/s led to a slight

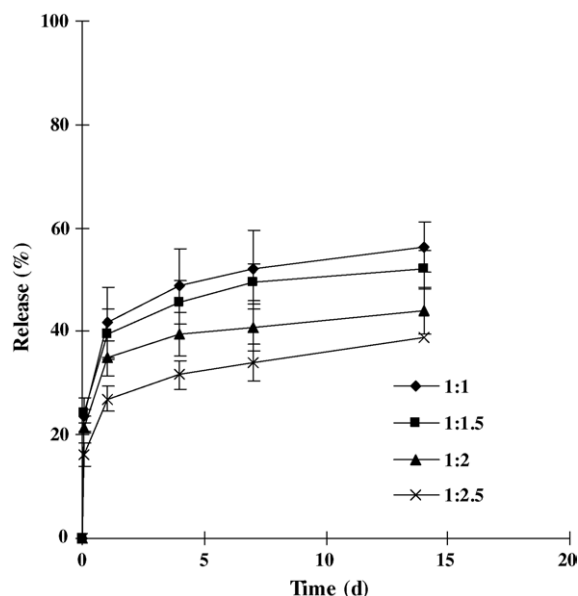


Fig. 5 – Influence of the internal:external phase ratio (w/w) on leuprolide release from in situ forming microparticles (RG 503H).

increased initial release, which could be attributed to a smaller particle size (data not shown).

3.2. ISM prepared with R 202H

A prolonged therapeutical duration is favorable to reduce the administration frequency. For ISM-systems, this could be achieved by choosing a biodegradable polymer with a longer biodegradation time span. An increase in the lactide-content in the PLGA generally decreases the polymer degradation

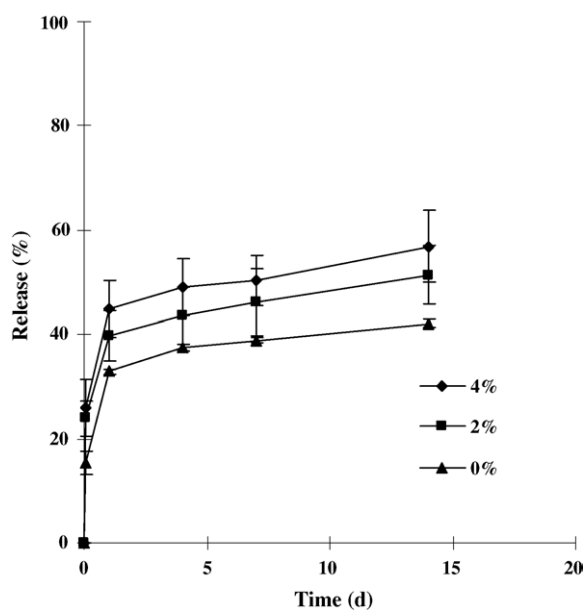


Fig. 6 – Influence of Span 80 concentration (w/w) on leuprolide release from in situ forming microparticles (RG 503H).

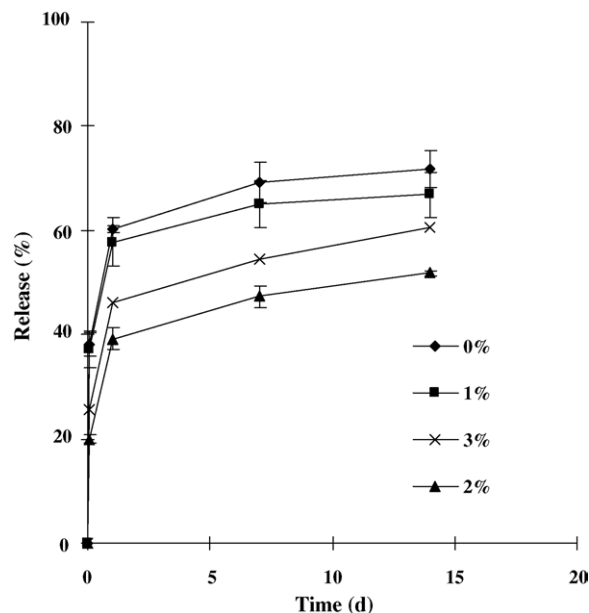


Fig. 7 – Influence of the aluminum monostearate concentration (w/w) on leuprolide release from in situ forming microparticles (RG 503H).

(Göpferich, 1996; Li, 1999). The homopolymer PLA R 202H was selected as the polymer of choice for the targeted 4 months release period, because conventional microparticles based on R 202H and prepared by a solvent evaporation method showed a high potential for a 4 months suppression of the testosterone in rats (Woo et al., 2001).

3.2.1. In vitro release and morphology

As described above with the copolymer RG 503H, two critical formulation parameters, drug loading and polymer concentration, which are closely related to the drug release and injection volume, were varied to study their influence on the leuprolide release (Fig. 8). ISM prepared with a 30% (w/w) polymer concentration but different drug loading (10 and 15%) had similar release profiles. An initial release of approximately 9% during day 1 was followed by a fast release phase until day 14. Thereafter, a continuous and slow drug release was observed until day 150, on which the experiment was terminated. In contrast, ISM with a 10% drug loading and a 40% polymer concentration showed a different release behavior. As expected, the initial release decreased from 8.7 to 2.6% with an increase in polymer concentration from 30 to 40%. Interestingly, ISM with 40% polymer concentration had an almost linear leuprolide release from days 2 to 150. After 150 days of incubation, approximately 90% of leuprolide was released from all three formulations (Fig. 8).

ISM prepared with 30% polymer concentration showed a very porous surface and inner structure (Fig. 9). The formulations with 10 and 15% drug loading did not show significant differences in morphology (data not shown). The porosity of the microparticles decreased significantly with an increase in polymer concentration from 30 to 40% (Fig. 9), which explained the slower drug release with ISM prepared with a 40% polymer concentration. The less porous microparticles at the higher

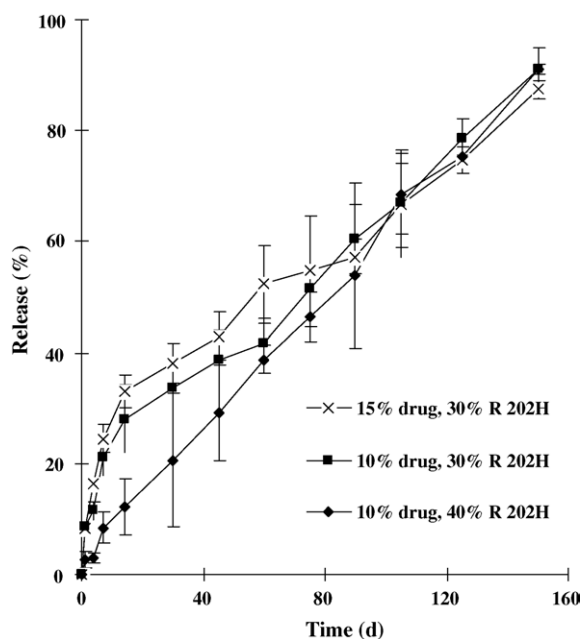


Fig. 8 – Leuprolide release from in situ forming microparticles (R 202H, 10 or 15%, w/w, drug loading, and 30 or 40%, w/w, polymer concentration).

polymer concentration could be attributed to a slower polymer precipitation caused by a higher viscosity of the polymer solution (Graham et al., 1999). A 40% R 202H solution in NMP was less viscous and did not result in lump formation during mixing with peanut oil because of the lower molecular weight of R 202H compared to RG 503H.

In comparison to RG 503H, R 202H led to a much lower initial leuprolide release (40% versus 9%) (Fig. 2 versus Fig. 8). This could possibly be explained with the higher carboxylic acid content of the lower molecular weight polymer R 202H (acidic number 10 mg KOH/g versus 4 mg KOH/g for RG 503H). The ionic interaction between the carboxylic acid groups in PLA and arginyl and histidyl residues of leuprolide acetate in a water-in-oil emulsion has already been reported in preparation of the leuprolide-loaded microparticles (Okada, 1997). In ISM-system, leuprolide acetate and PLGA or PLA were dissolved in the polar solvent NMP. The existence of ionic interactions between the polymer and drug was therefore also possible and a stronger interaction in the case of R 202H could impede the drug loss from the polymer solution and thus led to a lower initial release.

3.2.2. ISM versus conventional microparticles

Conventional microparticles were prepared by a solvent evaporation (cosolvent) method. The encapsulation efficiency was 70.8% (actual drug loading 10.5%) and a further optimization was not performed.

In comparison to conventional microparticles, ISM (40% polymer concentration, 10% drug loading) showed a lower initial release (2.6% versus 7.5%) (Fig. 10). Additionally, after the

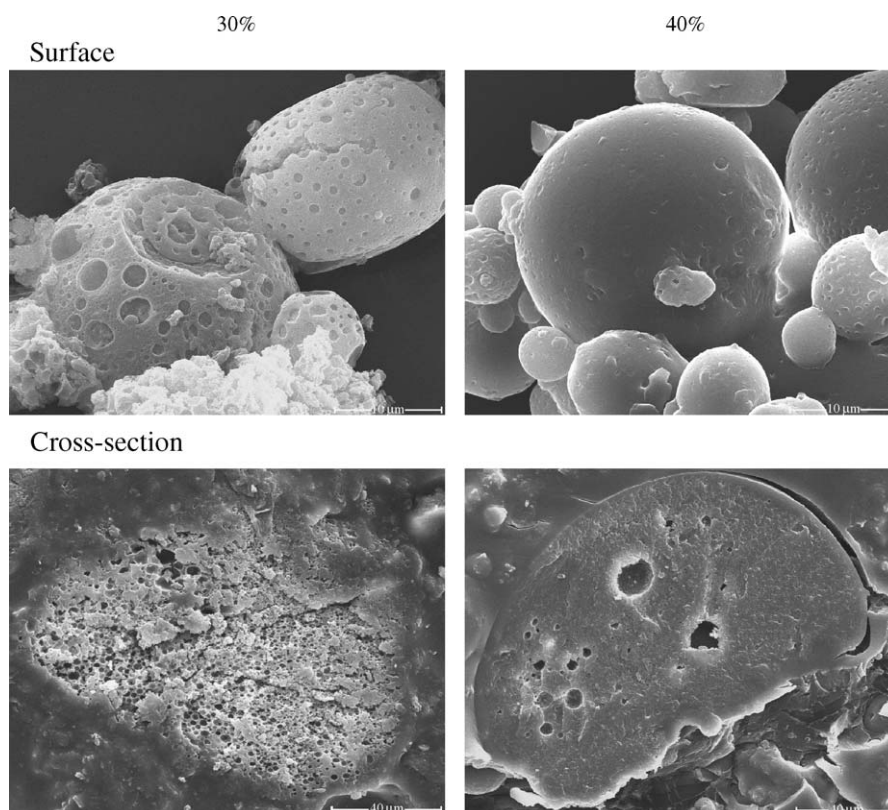


Fig. 9 – Scanning electron micrographs of in situ forming microparticles (R 202H, 10%, w/w, drug loading, and 30 or 40%, w/w, polymer concentration).

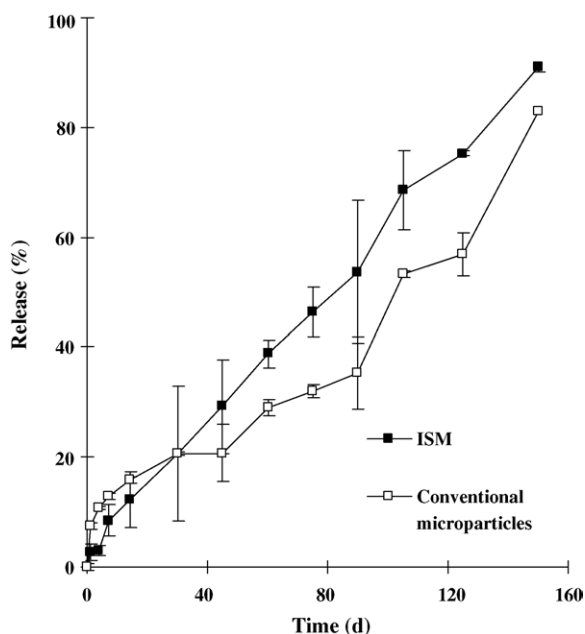


Fig. 10 – Leuprolide release from in situ forming and conventional microparticles (PLA R 202H, ISM: 10% drug loading, 40% polymer concentration; conventional microparticles: 10.5% actual drug loading, encapsulation efficiency 70.9%).

initial release phase, ISM showed a more linear release than conventional microparticles. Over 150 days, 91 and 83% of drug were released from ISM and conventional microparticles, respectively. This ISM formulation is therefore a good candidate for future animal studies.

Acknowledgement

This research was partially supported by a grant of the Deutsche Forschungsgemeinschaft (BO 1766/1-2).

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