3. Results and Discussion

3.1. Microparticles prepared by the solvent evaporation (cosolvent) method

3.1.1. Key parameters affecting the initial release and encapsulation efficiency

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.

3.1.1.1. 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. 11). 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; Herrmann and Bodmeier, 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 (0.05M 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. 11). The presence of 0.05M 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., 2000d; Bodmeier and McGinity, 1987; Bodmer et al., 1992). To verify this assumption, microparticles

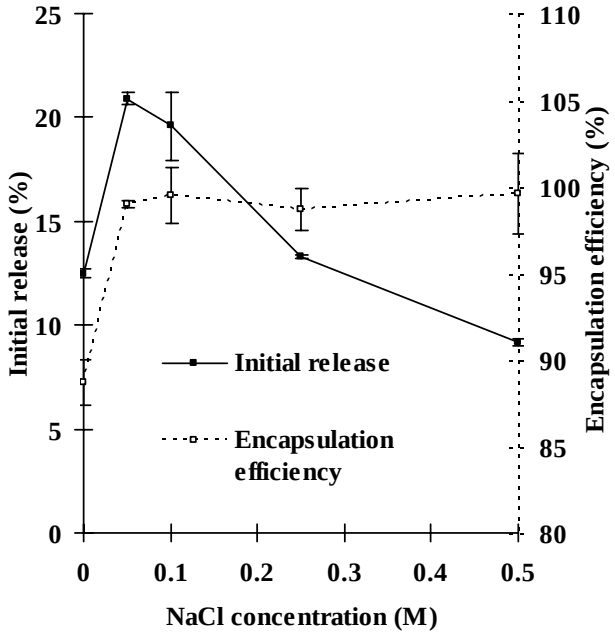


Fig. 11. Influence of NaCl addition to the external phase on the encapsulation efficiency and initial release of leuprolide acetate-loaded microparticles (drug loading 20%, external phase 800 ml containing 0.25% PVA).

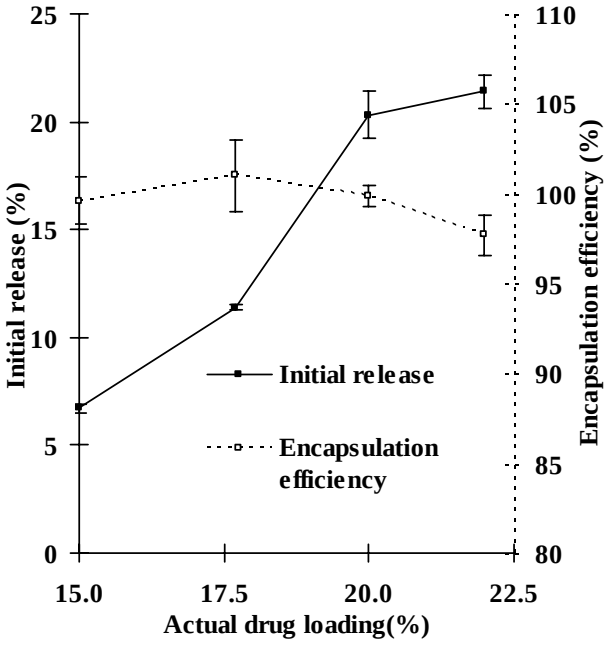


Fig. 12. Influence of the actual drug loading on the encapsulation efficiency and initial release of leuprolide acetate-loaded microparticles (external phase 800 ml containing 0.1M NaCl and 0.25% PVA).

with different leuprolide acetate loading were prepared. As expected, the initial release increased with increased actual drug loading (Fig. 12). Especially, 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.

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. 13). Microparticles prepared without NaCl addition showed a porous surface and inner structure, while microparticles prepared with 0.5M 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. 11).

3.1.1.2. Addition of ethanol to the external phase

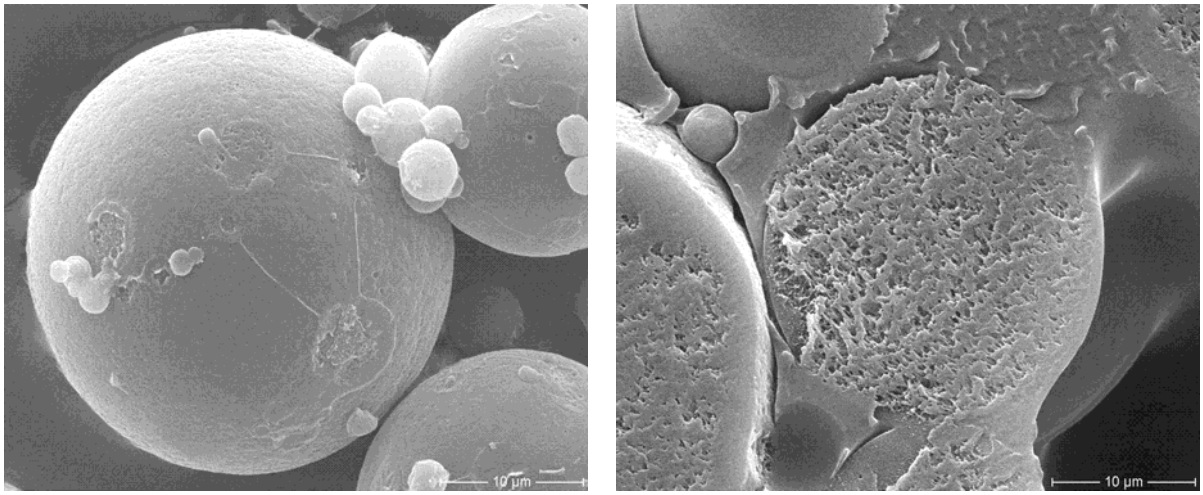
NaCl at high concentration in the external phase reduced the affinity between the PLGA solvent methylene chloride and the nonsolvent water and led to a slower polymer precipitation and formation of nonporous microparticles with increased encapsulation efficiency and reduced initial release. To further 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 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. 14). The increase in the initial release could be attributed to the increased porosity of the microparticles obtained with the addition of ethanol (Fig. 15).

The miscibility of solvents is strongly related to their polarity, which is reflected by the dielectric constant (ε). Methylene chloride, a non-polar solvent (ε=9.5), has a low miscibility with the polar solvent water (ε=80). The addition of a semi-polar solvent, such as ethanol (ε=25)

Surface **Inner structure**

Without NaCl



0.5M NaCl

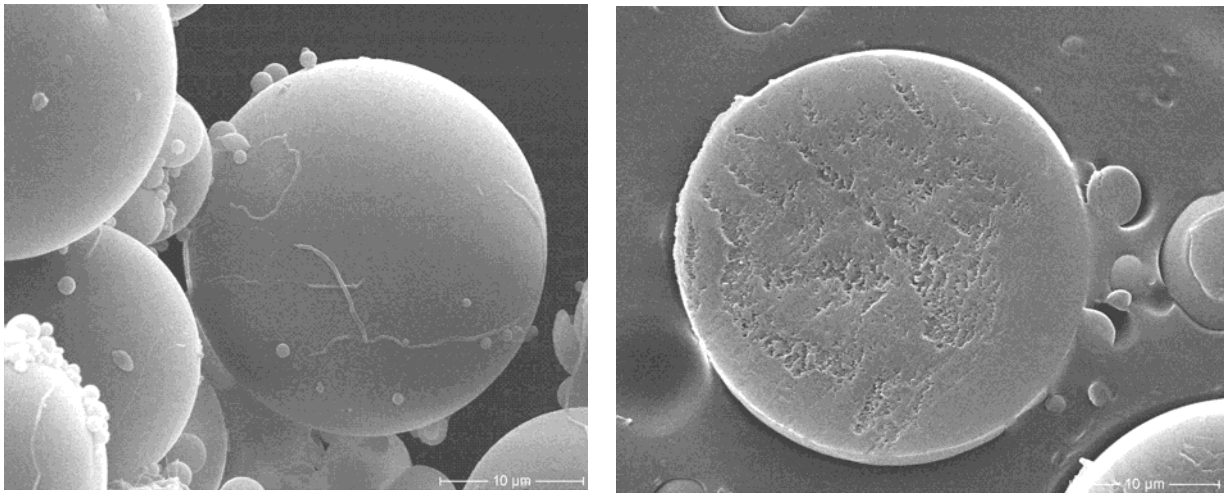


Fig. 13. Scanning electron micrographs of microparticles prepared without or with NaCl addition to the external phase.

reduced the gradient in dielectric constant between methylene chloride and the aqueous phase, thus increased 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. 16. The drug release profile was characterized by a rapid initial release followed 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; 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.

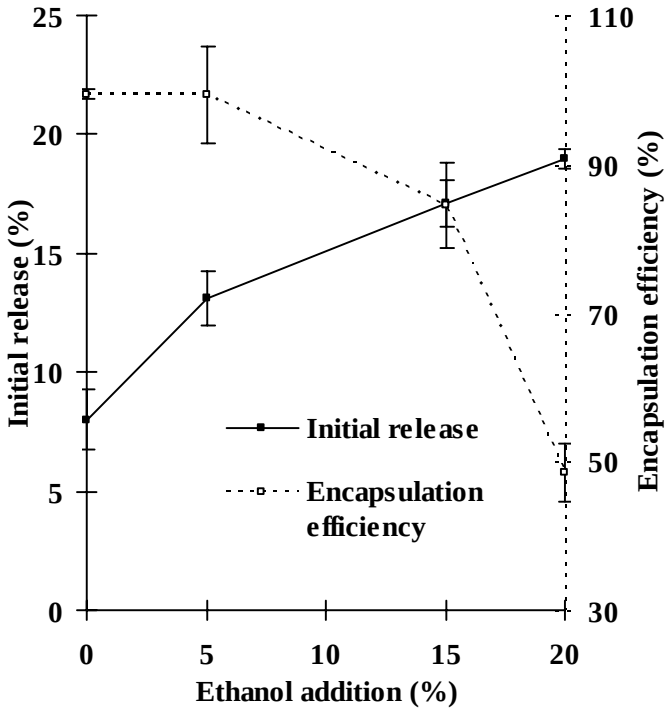
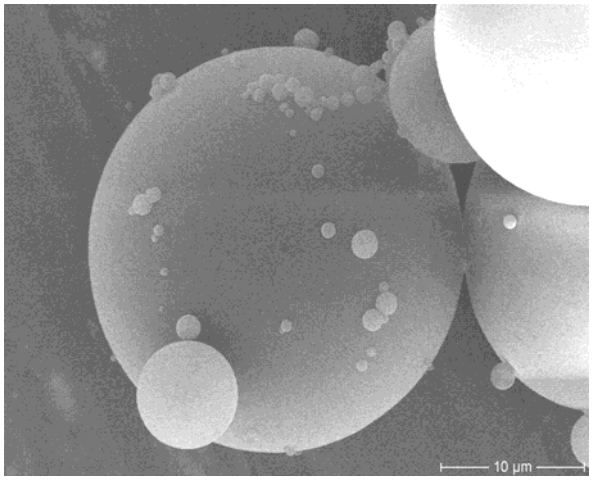
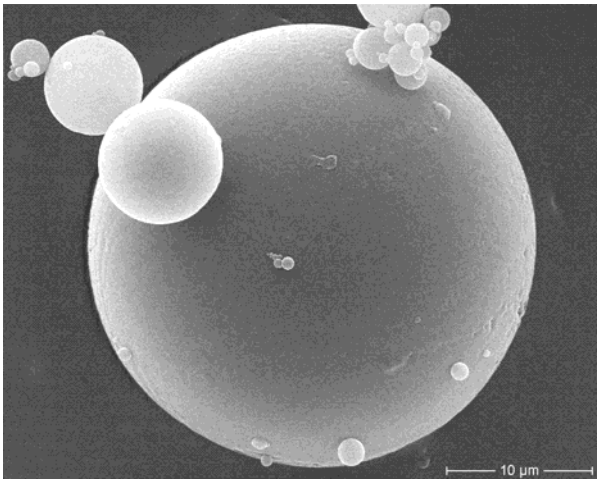


Fig. 14. Influence of ethanol addition to the external phase on the encapsulation efficiency and initial release of leuprolide acetate-loaded microparticles (drug loading 20%, external phase 200 ml containing 0.1M NaCl and 0.25% PVA).

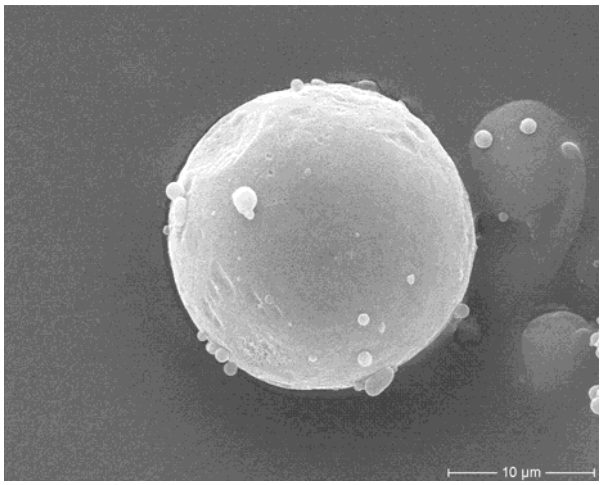
Without ethanol



5% ethanol



10% ethanol



20% ethanol

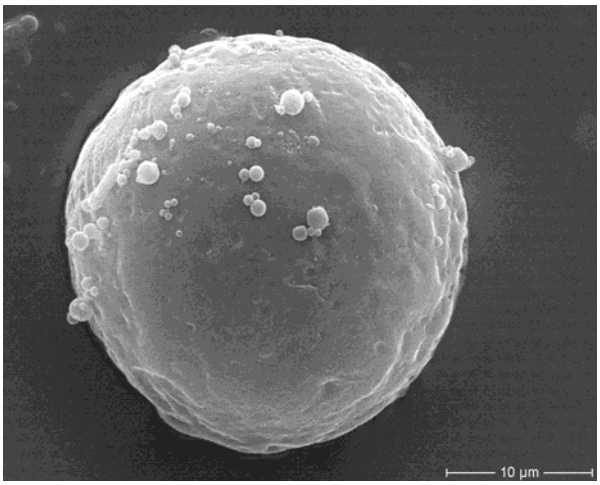


Fig. 15. Scanning electronic micrographs of microparticles prepared with different concentrations of ethanol in the external phase.

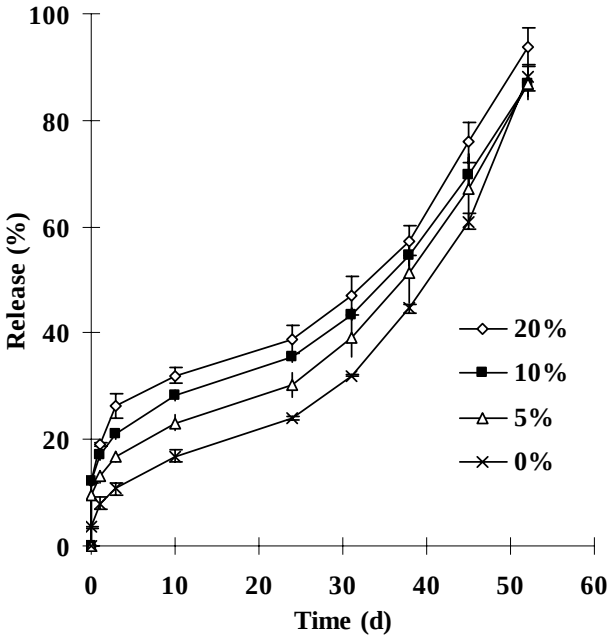


Fig. 16. Influence of ethanol addition to the external phase on leuprolide release from microparticles (drug loading 20%, external phase 200 ml containing 0.1M NaCl and 0.25% PVA).

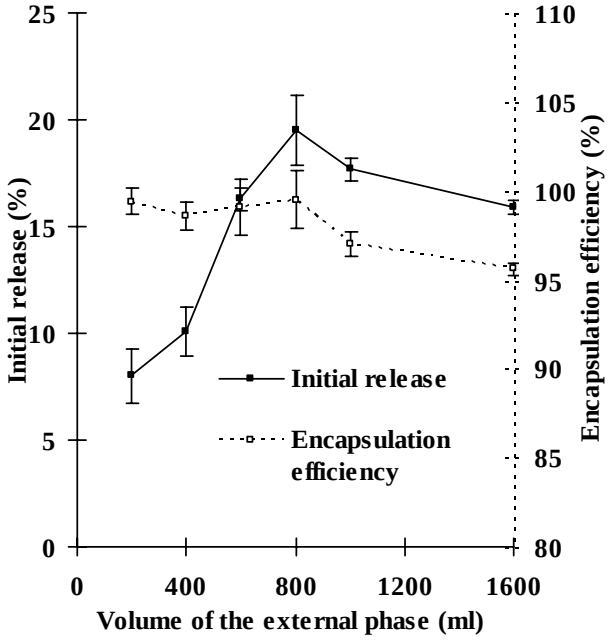


Fig. 17. Influence of volume of the external phase on the encapsulation efficiency and initial release of leuprolide acetate-loaded microparticles (drug loading 20%, external phase containing 0.1M NaCl and 0.25% PVA).

3.1.1.3. 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. 17). The encapsulation efficiency was not affected significantly by the volume of the external aqueous phase (Fig. 17).

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. 17). 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).

3.1.1.4. 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 5). 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 to 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 5). 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 microparticles. After 24 h stirring, the microparticles showed a slight increase in the initial release regardless of open or closed system.

Table 5 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*	
	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

* 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.

3.1.1.5. Other variables

The effect of some other process variables on the initial release and encapsulation efficiency were summarized in Table 6. 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/120s) 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.

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 sec 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 precipitation, 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.

Table 6 Influence of various variables on the encapsulation efficiency and initial release of leuprolide acetate-loaded microparticles (drug loading, 20%; external phase, 800 ml containing 0.1M NaCl and 0.25% PVA).

Variables		Encapsulation Efficiency (%)	Initial release (%)
Duration of injecting organic phase into the external phase	10 sec	98.8±2.9	21.2±0.8
	30 sec	99.8±0.9	21.8±1.1
	60 sec	97.6±1.2	20.3±0.4
	120 sec	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 sec	98.8±2.9	21.2±0.8
	30 sec	99.7±0.9	21.8±1.2
	60 sec	97.6±1.2	20.3±0.4
	120 sec	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

3.1.1.6. 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 7). In term of drug release, no significant difference was observed at scale-up factor of 25 (data not shown).

Table 7 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 (4000ml), factor 25 (20000 ml), external phase containing 0.1 M NaCl, 0.25% PVA, freeze-dried).

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

***** Volume size distribution. 50% of total microparticles have a diameter smaller than the mentioned value.

3.1.2. Modification of the tri-phasic release pattern

The objective/strategy of this study was to increase the drug release in the second slow release phase in order to change the tri-phasic release pattern to a more continuous release pattern. A higher depletion of the drug from the microparticles in the intermediate release time period would also lower the release during the final rapid release phase. Variables, which increase the drug release, such as increased drug loadings, the use of low molecular weight PLGA, the addition of a hydrophilic polymer (pore-former) or of oil, were investigated.

3.1.2.1. Influence of various formulation and processing parameters

Varying the molecular weight of PLGA (Fig. 18A), the drug loading (Fig. 18B), the volume of the external phase (Fig. 18C) and the drying method (Fig. 18D) affected primarily the initial release of the microparticles. Microparticles prepared from the lower molecular weight PLGA RG 502H had a higher initial release than microparticles prepared from the higher molecular weight RG 503H. The initial release increased with increasing drug loading and volume of the external phase. An increased drug loading led to a higher absolute amount of drug located close to surface of microparticles and thus an increased initial release. A higher volume of external phase results in a faster diffusion of the organic solvent methylene chloride from the internal polymer phase to the external aqueous phase and thus a faster polymer precipitation and more porous microparticles with a higher initial release (Graham et al., 1999, Ravivarapu et al., 2000c). Vacuum-drying led to a slightly higher initial release than freeze-drying, the reason is not clear so far.

These variations did not significantly affect the drug release after the initial release phase (Fig. 18B-D). The tri-phasic release profile was still obtained with all formulations. For 503 H, the initial release during the first 24 h was dependent on the investigated parameters and was in the range of 7 to 21%. It was followed by a slow release phase over a period of approx. 4 weeks, during which approx. 30% drug was released. The final rapid release phase lasted for approx. 3 weeks with a release of approx. 50 to 60% of drug released. With respective to encapsulation efficiency, the variants, drug loading, the volume of the external phase, and the drying method did not exhibit the significantly influence, however, using low molecular weight PLGA (RG 502H) led to lower encapsulation efficiency.

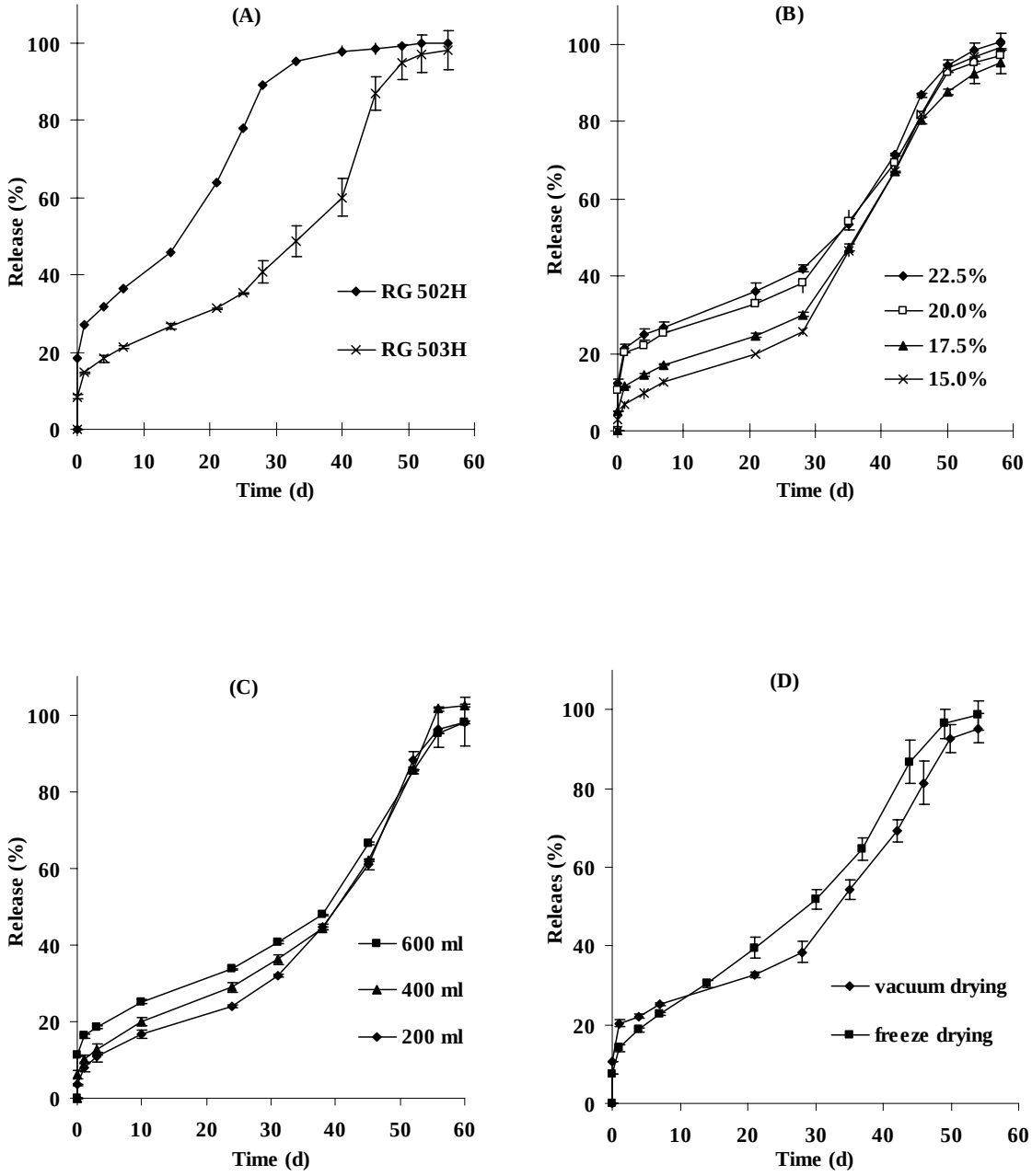


Fig. 18. Influence of formulation and process parameters on the leuprolide release from microparticles. A) Type of the PLGA, B) Drug loading, C) Volume of the external phase, D) Drying method.

3.1.2.2. Influence of PVP on the release pattern

PVP, a hydrophilic polymer, has been used as a pore-former to give more porous polymer structures after contact with release medium, resulting in an increase in drug release (Verma et al., 2003). The drug release from lysozyme-loaded in situ forming implant system was also increased by PVP-addition (Graham et al., 1999).

The addition of PVP (K12 and K17 grades evaluated) to the microparticle formulation did not significantly affect the encapsulation efficiency and the particle size of the microparticles (Table 8). 10% PVP slightly decreased the initial release (Fig. 19), which could be attributed to the slight decrease in the actual drug loading (Table 8). In contrast, 20% PVP K12 and K17 increased the initial release from 11% (PVP-free) to 13% and 20%, respectively. Upon emulsification of the organic polymer solution into the external aqueous phase, the presence of PVP (hydrophilic polymer) in the polymer solution might lead to a faster water penetration into the polymer solution resulting in a faster polymer precipitation and more porous microparticles with a higher initial release. The increased porosity of PVP-containing microparticles was confirmed by SEM (Fig. 20). PVP-free microparticles had a smooth surface, while microparticles with 20% PVP K17 had a porous surface. In addition to porosity effects, PVP/PLGA blends are more permeable than pure PLGA.

The addition of PVP also did not have a strong effect on the leuprolide release during the second and third release phase (Fig. 19) despite the more porous structure of microparticles. This might be due to the nonporous film formation on the surface of microparticles after incubation (Wang et al., 2002). Herrmann and Bodmeier reported that the change in the microstructure of somatostatin-loaded PLGA microparticles affected primarily the initial release but did not significantly change the following drug release (Herrmann and Bodmeier, 1995b).

3.1.2.3. Influence of medium chain triglycerides (MCT) on the release pattern

MCT are glycerol esters of medium chain fatty acids ranging from 6 to 12 carbon atoms. MCT are water-immiscible and are nonsolvents for PLGA. Other lipophilic additives, such as cyclohexane and fatty acid esters, have been added into PLA microparticles, leading to the formation of highly porous microstructures with less residual organic solvent (cyclohexane) (Spenlehauer et al., 1986) or a faster drug release (fatty acid esters) (Urata et al., 1999).

Table 8 Influence of PVP K12- and K17- addition on the encapsulation efficiency and particle size of microparticles.

PVP-type, %	Theoretical drug loading (%)	Encapsulation efficiency [a] (%)	Particle size [b] (μm)
0	19.9	90.7 ± 1.1	27.6
K12, 10	18.1	94.1 ± 2.2	24.3
K12, 20	16.6	91.5 ± 3.2	25.5
K17, 10	18.1	91.9 ± 2.4	25.6
K17, 20	16.4	87.9 ± 2.6	23.4

[a] Calculated based on 100% PVP entrapment.

[b] Mean diameter based on the volume.

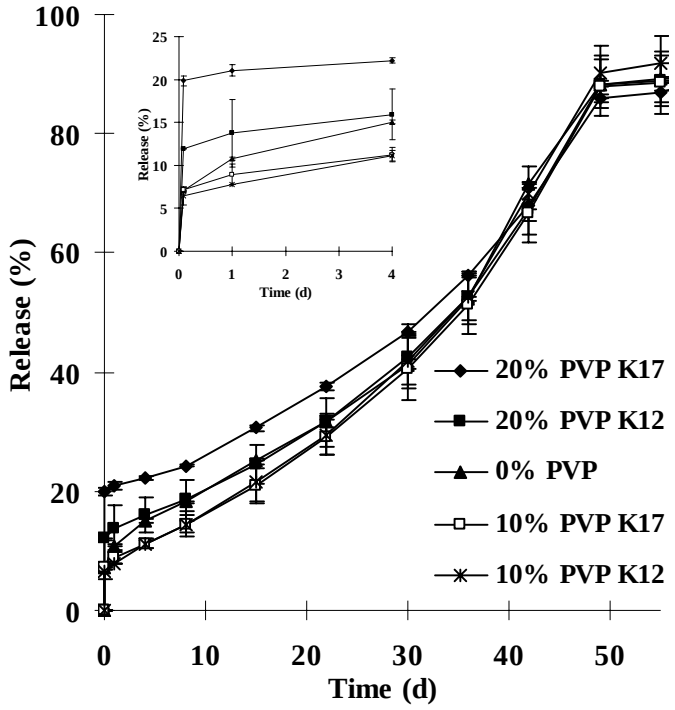
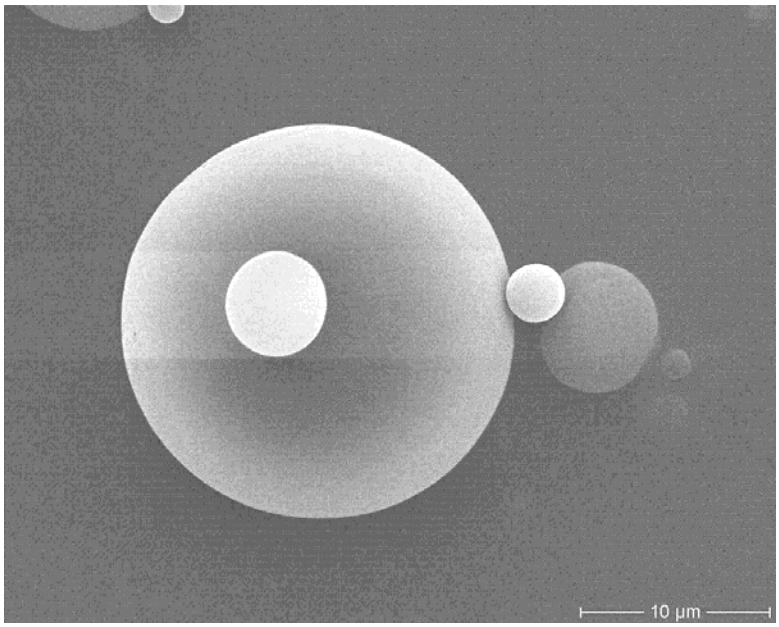


Fig. 19. Influence of the PVP K12- and K17-addition on the leuprolide release from PLGA microparticles. Inset picture was the amplified initial release.

PVP-free



20% PVP K17

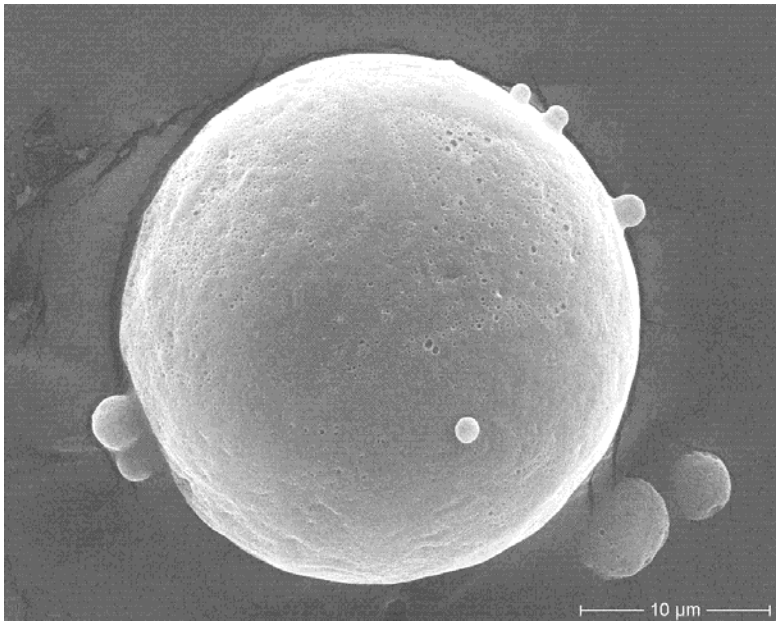


Fig. 20. Scanning electronic micrographs of microparticles prepared without or with 20% PVP K17.

The MCT-addition did not influence the size of the microparticles (mean diameter: 27 to 29 µm) and had also no major effect on the encapsulation efficiency; only the addition of 20% MCT led to a slight decrease in the encapsulation efficiency from 94% (MCT-free) to 88% (Table 9). This could possibly be attributed to the decreased solubility of leuprolide acetate in the internal organic phase at the higher amount of MCT. The internal organic phase was clear up to 15% MCT, but slightly turbid with 20% MCT.

Interestingly, the addition of MCT changed the tri-phasic drug release pattern to the desired more continuous drug release profile. The MCT-addition significantly increased the drug release in the second slow release phase and reduced the drug release in the final rapid release phase (Fig. 21). 5, 10, 15, and 20% MCT-addition led to an increased drug release (day 2 to day 21) from 25% (MCT-free) to 39, 39, 41 and 45%, respectively. Thereafter, the drug release (day 22 to day 49) was reduced from 57% (MCT-free) to 40, 37, 32, and 28%, respectively. The initial release increased with increasing MCT addition (14.0% for MCT-free versus 25.5% for 20% MCT-containing microparticles) (Fig. 21). This undesired higher initial release might be reduced by adjusting other formulation variables, for example by reducing the drug loading or the volume of the external phase (Fig. 18 B, C).

Differential scanning calorimetry (DSC) was used to quantify MCT in the microparticles (Fig. 22). Upon cooling of pure MCT (liquid at room temperature), a crystallisation exotherm (T_c) occurred at –44 °C, which corresponded to the less orderly α-polymorph (Jenning, 1999). During the subsequent heating, a melting endotherm (T_m) was visibly at –3 °C. With MCT-containing microparticles, the T_c shifted from -44 °C to -54 °C and the T_m remained unchanged. The shift in T_c might be explained with the change in the polymorphic of MCT or residual organic solvent present. The entrapment of MCT in the microparticles, which was calculated from the melting enthalpy of MCT, was high (88 and 105% for 10 and 20% MCT addition, respectively) (Table 10). MCT did not partition into the external aqueous phase but remained in the microparticles because it was insoluble in water (Traul et al., 2000).

The influence of MCT on the glass transition temperature (T_g) of PLGA was also analyzed by DSC (Fig. 22). In the first heating scan, PLGA showed an overlay peak around the T_g known as kinetic overshoot (Hausberger and DeLuca, 1995). After eliminating the thermal history of the polymer with the first heating and cooling process, the T_g of the PLGA RG 503H was at approx. 46 °C. Microparticles prepared without or with 10 and 20% MCT had the identical kinetic overshoot and T_g. Thus, MCT did not affect the T_g of PLGA and did not act as plasticizer. This is

advantageous with regard to storage of the microparticles, because a reduction in T_g would have resulted in more "sticky" microparticles and possible agglomeration during storage.

Table 9 Influence of the addition of medium chain triglycerides (MCT) on the encapsulation efficiency and particle size of PLGA microparticles.

MCT (%)	Theoretical drug loading (%)	Encapsulation efficiency [a] (%)	Particle size [b] (μm)
0	20.1	93.9 ± 0.9	29.1
5	19.3	94.0 ± 1.3	29.1
10	18.3	93.3 ± 0.7	27.7
15	17.3	92.9 ± 4.4	26.9
20	16.7	88.0 ± 0.5	28.7

[a] Calculated based on 100% MCT entrapment.

[b] Mean diameter based on the volume.

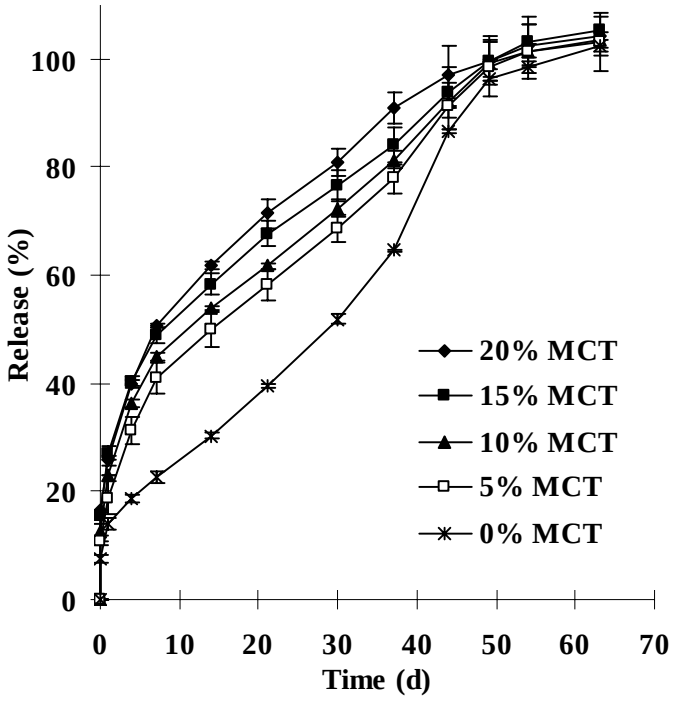


Fig. 21. Influence of the addition of medium chain triglycerides on the leuprolide release from microparticles.

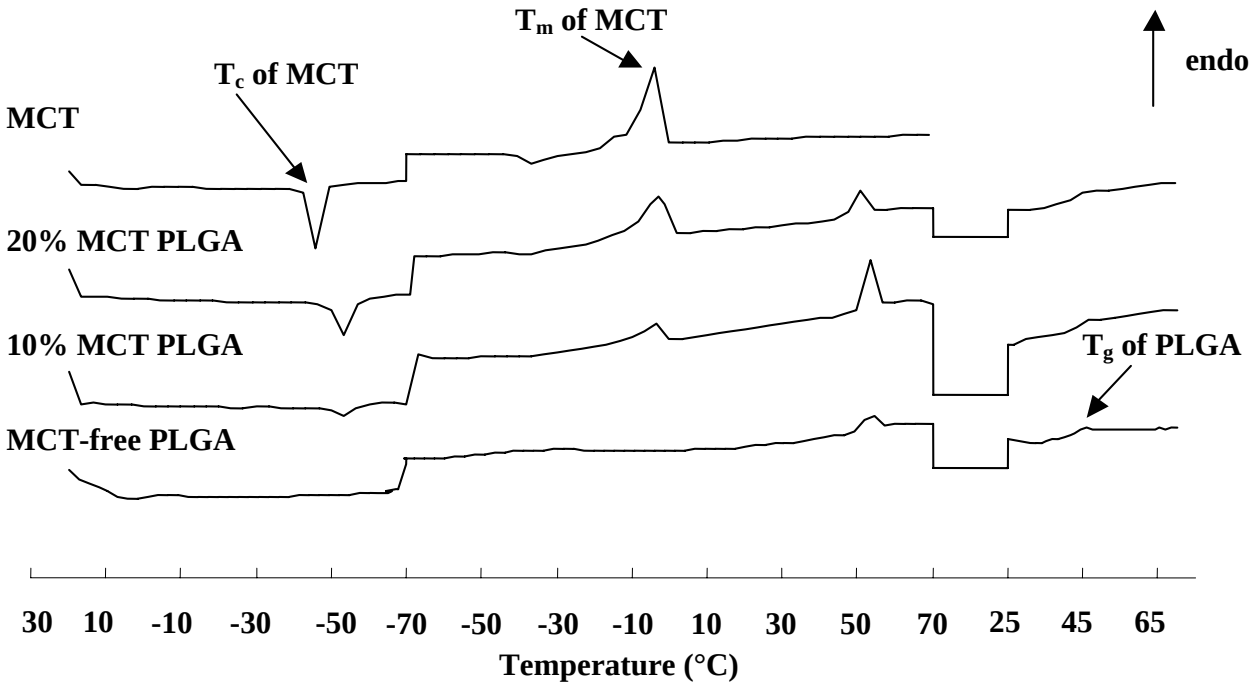


Fig. 22. DSC thermograms of pure medium chain triglycerides (MCT) and PLGA microparticles (0, 10, 20 % MCT), scanning process: the sample was cooled to –70 °C, followed by heating to 70 °C, cooling to 25 °C, and finally reheating to 70 °C at a rate of 10 °C/min.

Table 10 Quantification of the encapsulation efficiency of medium chain triglycerides (MCT) in the microparticles.

Sample	Melting peak (°C)	Melting enthalpy (J/g)	MCT- Encapsulation efficiency (%)
MCT	-3.4	92.2 ± 1.8	-
Microparticles, 10% MCT	-3.3	7.4 ± 0.3	88 ± 3
Microparticles, 20% MCT	-3.0	16.0 ± 0.4	105 ± 4

The inclusion of MCT had a strong effect on the microstructure of the particles (Fig. 23). MCT-free microparticles had a smooth surface and a dense inner structure, while increasing amounts of MCT led to a more porous surface and inner structure. As shown in previous results, the microstructure of microparticles primarily affected the initial release. This increasing porosity at higher MCT-contents thus explained the increased initial release with MCT-containing microparticles. Initially, the porous microstructure was speculated to be the result of the evaporation of MCT under vacuum. To verify this assumption, microparticles with 20% MCT were prepared and dried by different techniques (vacuum-dried, freeze-dried, or dried in a desiccator). SEM micrographs showed an identical porous structure regardless of the drying techniques (data not shown) and the drug release was also similar (Fig. 24). Therefore, evaporation of MCT under vacuum was not critical with respective to the formation of the porous structure and the modified drug release pattern. In addition, the DSC-results described above verified, that MCT was almost completely entrapped in the microparticles.

The porosity/microstructure of the microparticles depends strongly on the precipitation kinetics of the polymer (Graham et al., 1995, Schlicher et al., 1997). Upon contact with the external aqueous phase, methylene chloride (a solvent for PLGA) diffuses out of the PLGA solution droplets; simultaneously, aqueous solution penetrates into the droplets, leading to polymer precipitation. In this process, MCT (a nonsolvent for PLGA, which is water-immiscible but miscible with methylene chloride) might form MCT-rich regions (droplets) within in the polymer matrix. Methylene chloride might diffuse rapidly into the MCT-rich regions, leading to an interior acceleration of the PLGA precipitation and thus a porous inner structure. Methylene chloride trapped in the MCT might then be removed by evaporation out of the PLGA matrix during the drying process.

The MCT-addition also led to a more porous microstructure of microparticles after incubation in the release medium at 37 °C (Fig. 25). After 4 or 8 d, MCT-free microparticles had a slightly deformed, nonporous surface; cross-sections revealed a fairly dense matrix after 4 d and a more porous inner structure after 8 d. In contrast, microparticles with 20% MCT had a highly porous surface and an almost hollow inner structure. The highly porous microstructure of MCT-containing microparticles after incubation could be attributed to their originally higher porosity (Fig. 23), which caused a high water accessibility and a fast destruction of the polymer matrix.

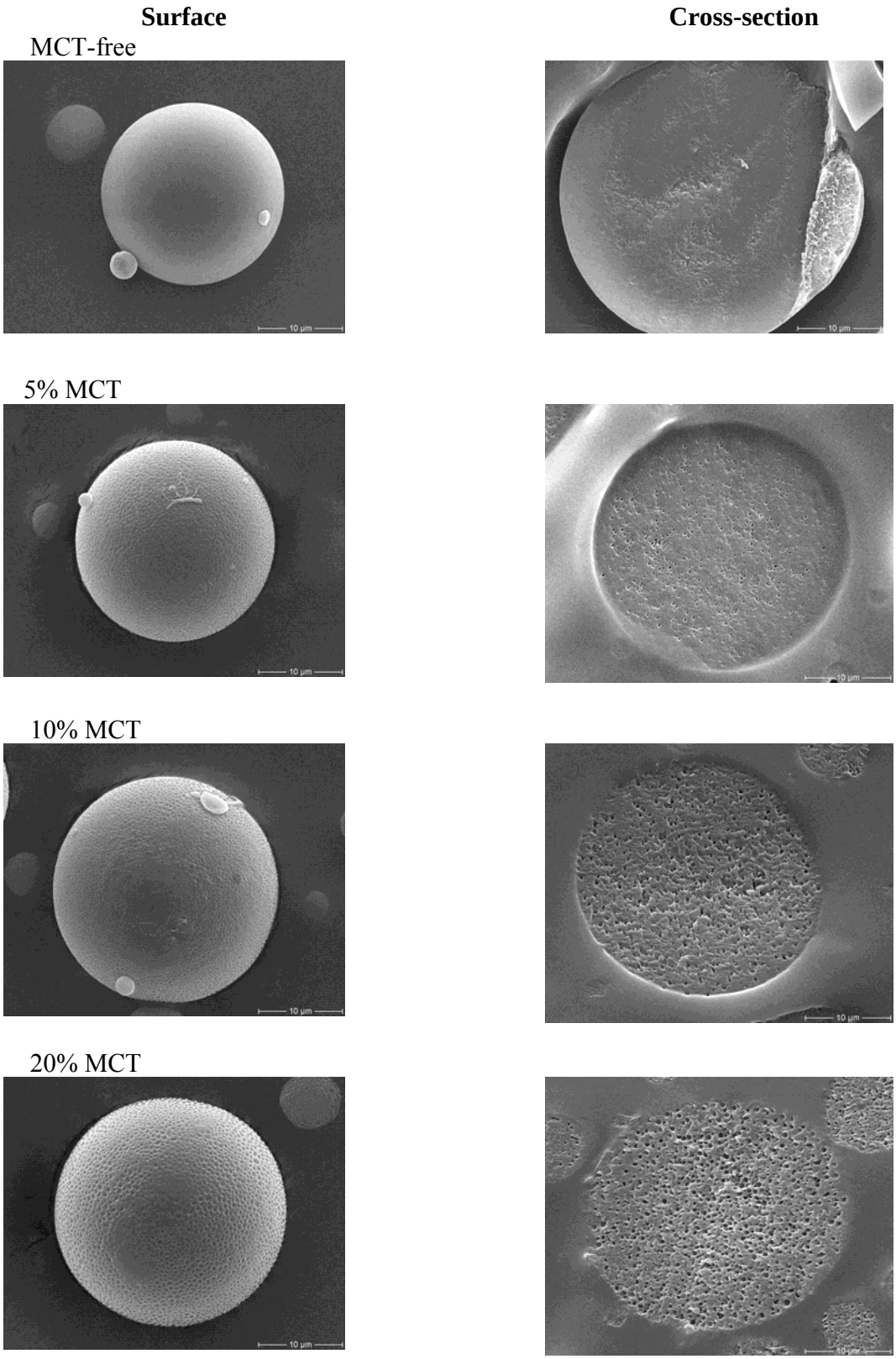


Fig. 23. Scanning electronic micrographs of medium chain triglycerides-free and -containing microparticles.

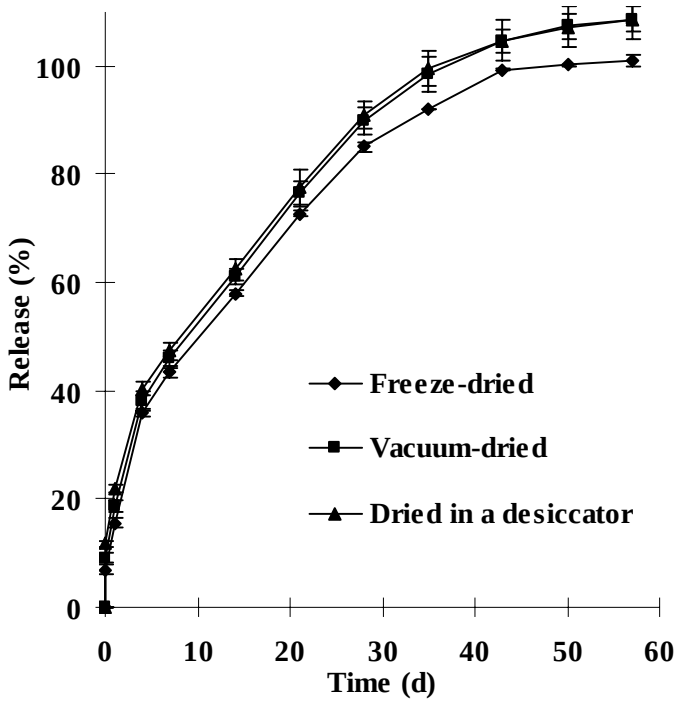


Fig. 24. Influence of drying on the leuprolide release from microparticles (20% medium chain triglycerides).

With microparticles showing a tri-phasic release behavior, a slower diffusion-controlled drug release period takes place after depletion of the outer and inner surface-associated drug during the initial release phase. During this period, a low porosity of microparticles impedes the diffusion of drug from PLGA matrix and results in a slow drug release. In contrast, MCT-containing microparticles had a more porous structure after incubation in the release medium and thus resulted in a faster drug release. The lower amount of drug present in the microparticles at the time of the erosion-controlled rapid release phase could explain the reduced drug release in the final release phase.

In conclusion, the addition of medium chain triglycerides modified the tri-phasic drug release pattern of leuprolide acetate-loaded microparticles to a more continuous release.

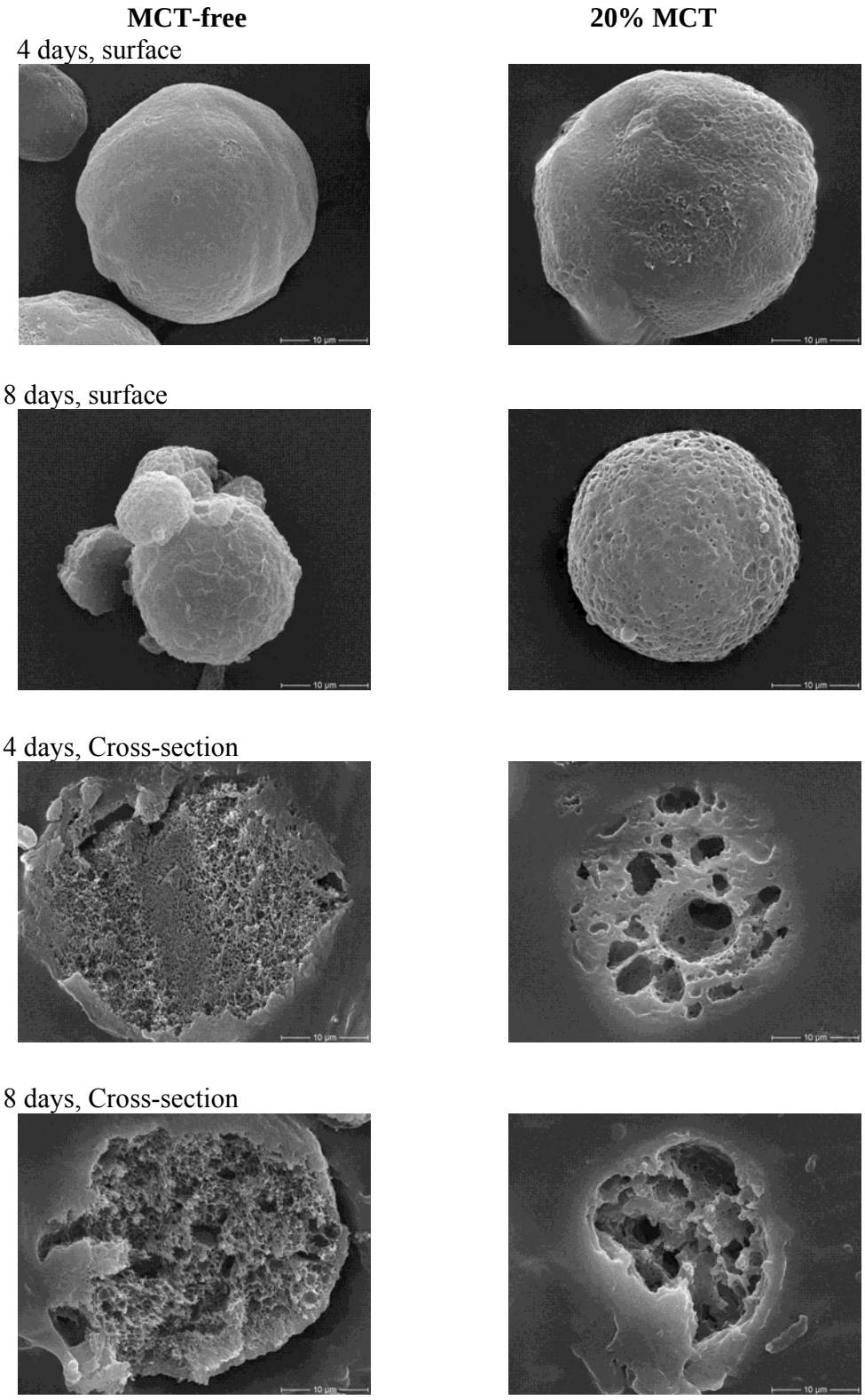


Fig. 25. Scanning electronic micrographs of microparticles (without or with 20% medium chain triglycerides) after incubation in the release medium at 37 °C for 4 or 8 days.

3.2. In situ forming microparticle (ISM) systems

3.2.1. Emulsion formation and morphology of resulting microparticles

A prerequisite for the ISM systems is to form emulsion of polymer solution with external continuous phases. The objectives of this study are threefold: 1. Investigate the influence of polymer solution and external continuous phase on the emulsion formation using a two-syringe system. 2. Elucidate the mechanism of such effects. 3. Study the morphology of in situ formed microparticles prepared by different polymer solutions.

30% (w/w) PLGA solution was used as the internal polymer phase. Oil with Span 80 or water with Lutrol F68 consisted of the external continuous phase. Mixing the different internal and external phases, either emulsion or lump was obtained (Table 11). When mixing with aqueous solution, PLGA solutions in propylene carbonate (PC), triacetin (TA), and triethyl citrate (TEC) formed emulsions; in contrast, PLGA solutions in *N*-methyl-2-pyrrolidone (NMP), dimethyl sulfoxide (DMSO) and 2-pyrrolidone (2P) formed lump, which could be attributed to the high water miscibility of these solvents. When mixing with oils, PLGA/PC solution formed emulsions, PLGA/DMSO solution only could not form emulsion with castor oil, PLGA/2P solution formed emulsion with sesame oil, soybean oil, safflower oil, and peanut oil, PLGA/NMP solution only formed emulsion with peanut oil with 2% aluminum monosterate as viscosity increasing agent; whereas PLGA solution in TA and TEC could not form emulsion will all investigated oils, which seemed correlated with their extremely high viscosity (Table 11).

3.2.1.1. Influence of the polymer solution viscosity

The polymer concentration was varied to get different viscose solution with the same solvent. Not surprisingly, the viscosity of the PLGA solution in NMP and TA increased with increasing the polymer concentration (Table 12). Mixing the PLGA/NMP solutions (polymer concentration of 20, 30, and 40%) with water, ethyl oleate, MCT, and castor oil all formed big lumps. When mixing with soybean oil, safflower oil, sesame oil, and peanut oil, 20% solution of low viscosity formed emulsion; in contrast, 30 and 40% solution of higher viscosity formed lump and the lump size increased with increasing viscosity (Table 12). Similar result was obtained in the case of TA. Mixing 20% PLGA/TA solution with all oils except ethyl oleate and MCT formed

emulsions; in contrast, lumps were obtained when using 30% solution of higher viscosity (Table 12). Therefore, less viscose polymer solution was favorable to the emulsion formation.

Table 11 Resulting products from mixing 30% w/w PLGA solutions with oils with 2% Span 80 or water with 1% Lutrol F68

		PLGA solution in					
		DMSO	NMP	PC	2P	TA	TEC
	Viscosity (mPas)	1336	1358	2251	19581	59356	108706
Water	0.9	- - -	- - -	+	- - -	+	+
Ethyl Oleate	3.1	+	- - -	+	- - -	- - -	- - -
MCT	17.2	+	- - -	+	- - -	- - -	- - -
Soybean oil	38.0	+	- -	+	+	- - -	- - -
Safflower oil	40.0	+	- -	+	+	- - -	- - -
Sesame oil	46.4	+	- -	+	+	- - -	- - -
Peanut oil	59.2	+	-	+	+	- - -	- - -
Peanut oil 2% AM	115.3	+	+	+	+	- - -	- - -
Castor oil	669.3	-	- - -	+	- - -	- - -	- - -

\+ Emulsion

\- Emulsion and lumps up to 1 mm

\- - Emulsion and lumps up to 5 mm

\- - - Lumps larger than 5 mm

Dimethyl sulfoxide (DMSO); *N*-methyl-2-pyrrolidone (NMP); propylene carbonate (PC); 2-pyrrolidone (2P); triacetin (TA); triethyl citrate (TEC); aluminum monostearate (AM).

Table 12 Influence of viscosity of the polymer solution on the emulsion formation

	Polymer solution in				
	NMP			TA	
	20%[a]	30%	40%	20%	30%
Vis[b] / EP[c]	165	1358	7858	4903	59356
Water	- - -	- - -	- - -	+	+
Ethyl oleate	- -	- - -	- - -	- -	- - -
MCT	- - -	- - -	- - -	- -	- - -
Soybean oil	+	- -	- - -	+	- - -
Safflower oil	+	- -	- - -	+	- - -
Sesame oil	+	- -	- -	+	- - -
Peanut oil	+	-	- -	+	- - -
Peanut oil 2% AM	+	+	- -	+	- - -
Castor oil	- - -	- - -	- - -	+	- - -

a Polymer concentration (w/w)

b Viscosity (mPas)

c External phase

\+ Emulsion

\- Emulsion and lumps up to1 mm

\- - Emulsion and lumps up to 5 mm

\- - - Lumps larger than 5 mm

3.2.1.2. Influence of the solvent diffusion into the external continuous phase

The volume loss of the PLGA solution after shaking with oils was used to determine the solvent diffusion rate from the PLGA solution (Fig.10). When shaking with aqueous solution, solvents such as NMP, 2P, and DMSO diffused so fast into water that PLGA precipitated immediately and the volume loss was not measurable. In the case of oils, the solvent diffusion was much slower. The volume of the PLGA solution decreased gradually and two phases presented a clear and flat interphase surface. The volume loss was related to the diffusion rate of solvent. In this model experiment, the long shaking time was employed to compensate the larger surface area of polymer solution droplets during mixing in a two-syringe system.

Due to the high viscosity and presence of oil insoluble solute (PLGA) in the polymer solution, the imbibition of oil into polymer solution might be negligible. This was confirmed by ^{1}H nuclear magnetic resonance (NMR) spectrums. After 24 hours of shaking with peanut oil, No presence of peanut oil in PLGA/NMP solution was observed; on the contrary, the diffusion of NMP into peanut oil was prominent shown by their specific peaks (Fig. 26).

The diffusion rate of the solvents, NMP, 2P, DMSO, and PC from their 20% (w/w) PLGA solution into peanut oil was compared using this method (solutions of TEC and TA were exclude due to their high viscosity) (Fig. 27A). The diffusion of NMP into peanut oil was much faster than that of other 3 solvents. After 41 hours of shaking, PLGA/NMP solution reduced its volume by 37%. For other 3 solvents, the volume reduced by less than 7%.

NMP solution was of quite low original viscosity (Fig.27B). After shaking with peanut oil, the fast diffusion of NMP led to a significant increase in the solution viscosity (0.17 to 3.60 Pas), which could explain the lump formation when mixing PLGA/NMP solution with peanut oil (Table 11). In the case of other 3 solvents, slower solvent diffusion rate led to less prominent increase in the solution viscosity (Fig. 27B), which correlated with the emulsion formation (Table 11).

After 41 hours of shaking with aqueous solution, the viscosity of PLGA/PC solution increased slightly from 0.29 to 0.38 Pas (Fig. 27B). In the case of NMP, DMSO, and 2P, polymer precipitated immediately upon contact with aqueous solution. This indicated a slower diffusion rate of PC and could be used to explain the emulsion formation when mixing PLGA/PC solution with aqueous solution (Table 11).

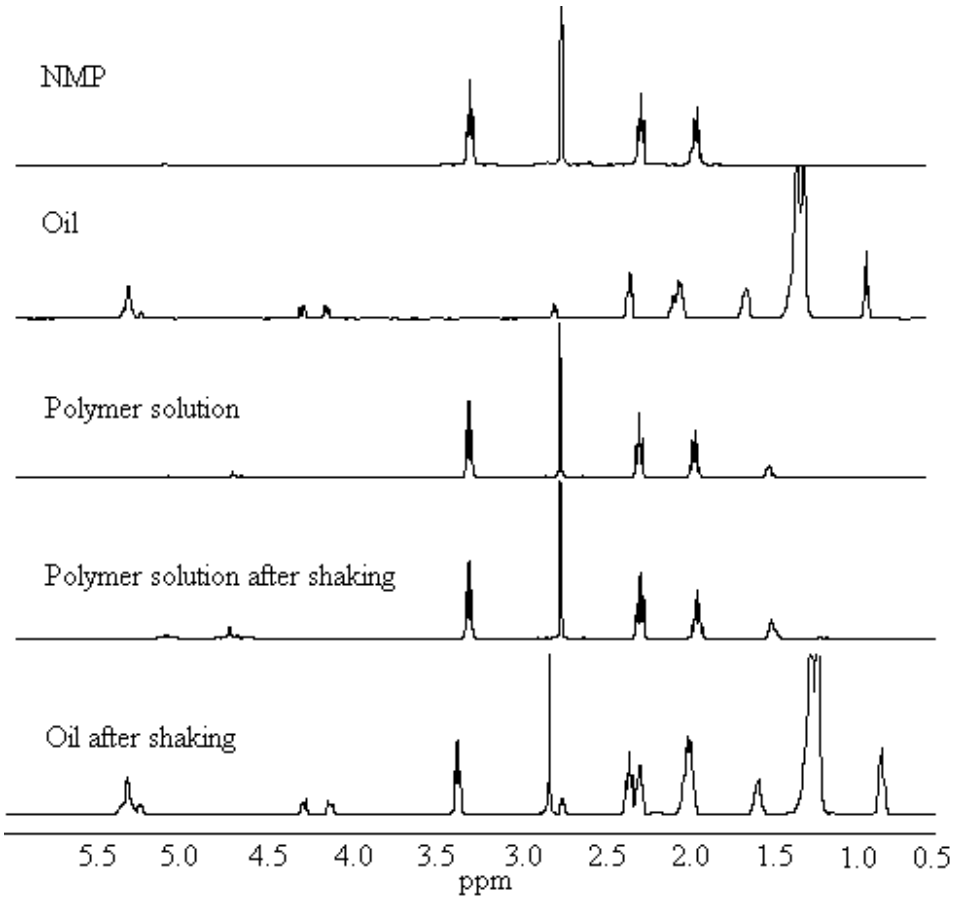


Fig. 26. ^{1}H nuclear magnetic resonance (NMR) spectrums of 20% w/w PLGA/NMP solution before and after shaking 24 hour with peanut oil

Influence of the external continuous phases on the solvent diffusion rate was also investigated (Fig. 28A, B). The diffusion rate of NMP from PLGA/NMP solution into external continuous phases ranked as water > castor oil > MCT > ethyl oleate > safflower oil > soybean oil > sesame oil > peanut oil > peanut oil with 2% aluminum monostearate (AM). (Fig. 28A). Upon contact with aqueous solution, PLGA/NMP solution precipitated rapidly. After 41 hours of shaking with castor oil, PLGA/NMP solution reduced its volume by 67.9%; in contrast, for peanut oil with 2% AM, PLGA/NMP solution reduced its volume by 26.6%.

The driving force for the solvent diffusion from the internal polymer solution phase into the external oily or aqueous phase is the chemical gradient in two phases. The higher chemical affinity between solvent and external continuous phase leads to faster diffusion. NMP is a hydrophilic solvent. It diffuses fast into a hydrophilic medium. The highest hydrophilicity of water leads to the fastest NMP diffusion into aqueous solution. Castor oil (presence of polar hydroxyl group) and MCT (short chain length) are relative more hydrophilic and thus results in faster NMP diffusion. In the case of ethyl oleate, the small molecular size and low viscosity may favor the NMP diffusion. The relative faster NMP diffusion into safflower oil may due to the high unsaturation of safflower oil that increases its hydrophilicity.

The faster the solvent diffused, the higher viscosity of the remaining solution was. The viscosity of PLGA/NMP solutions after shaking with various oils was in agreement with the sequence of solvent diffusion rate (Fig. 28B). In the case of aqueous solution and castor oil, the polymer solution solidified and the viscosity was not measurable.

A clear relationship between the emulsion formation and the solvent diffusion rate were shown. The faster NMP diffusion into water, castor oil, MCT, and ethyl oleate led to highly viscous polymer solution after mixing and resulted in lump formation. As the solvent diffusion rate decreased, the resulted lump size also decreased (Fig. 28B, Table 11). The addition of 2% aluminum monostearate increased the viscosity of peanut oil (59.2 to 115.3 mPas) and thus impeded the NMP diffusion, which resulted in a less viscose polymer solution after mixing and the emulsion formation (Fig. 28B, Table 11).

Other factors such as concentration of Span 80 (0, 2 and 4%) in oil, temperature (4, 25 and 40 °C) of the internal polymer and external continuous phase, and the diameter of the connectors (1.0, 1.4, 1.9 mm) showed no clear effects on the emulsion formation when mixing 30% (w/w) PLGA solution in NMP and TA with various external continuous phases.

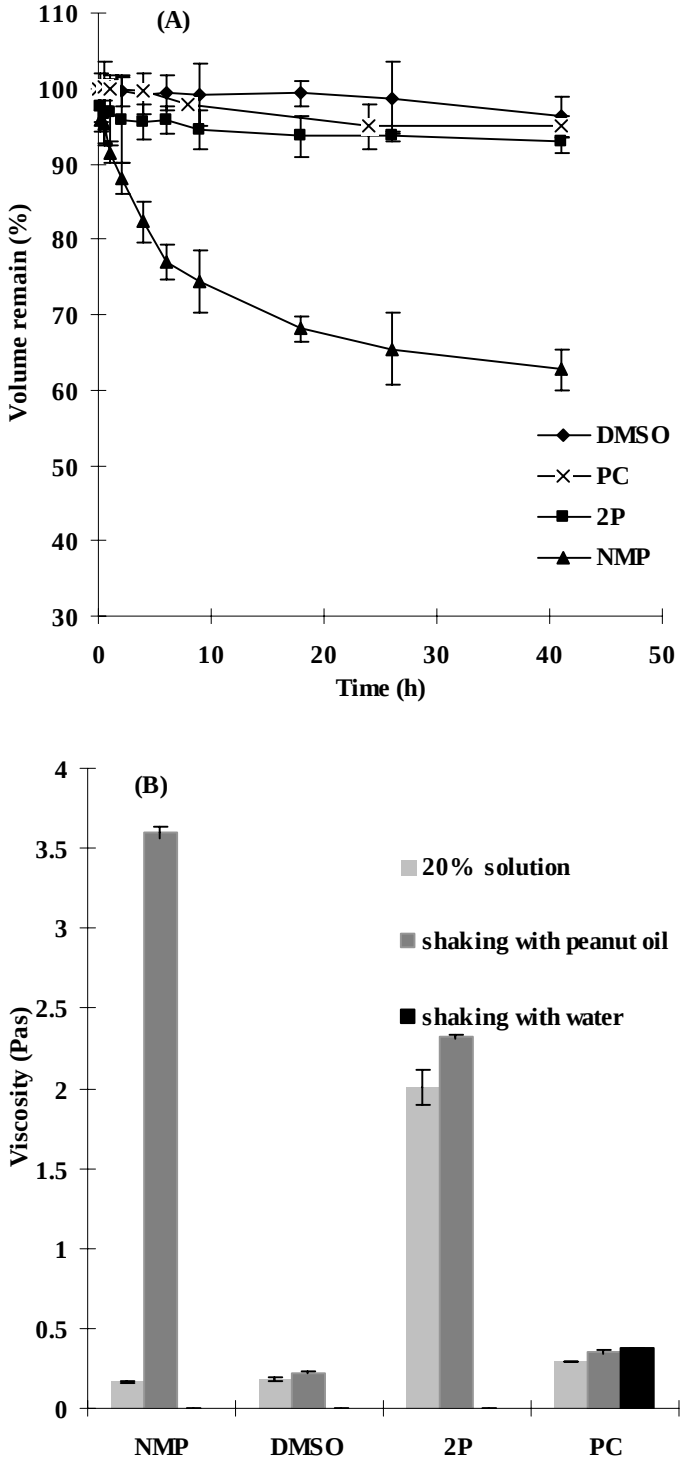


Fig. 27. Volume remain of the PLGA solution in different solvents after shaking with peanut oil (A), and the viscosity of the remaining solutions (B)

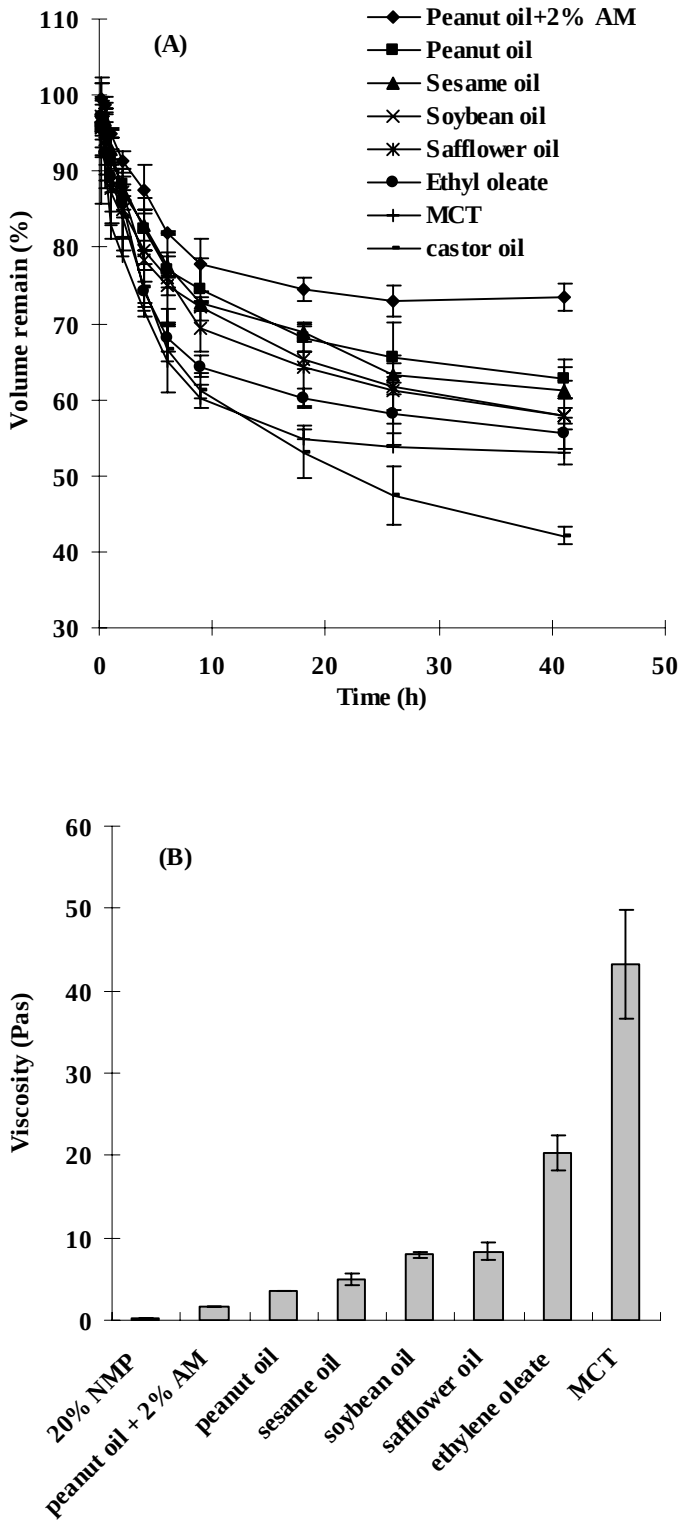


Fig. 28. Volume remain of the PLGA/NMP solution after shaking with investigated oils (A), and the viscosity of the remaining solutions (B)

During emulsification process in ISM systems, the solvent diffuses into the external continuous phase, which leads to an increase in the polymer solution viscosity. The increased surface area as decreased polymer solution droplet size facilitates the solvent diffusion. Therefore, the original low viscosity of the polymer solution and the slow solvent diffusion into the external continuous phase benefit the emulsion formation.

3.2.1.3. Morphology of the in situ forming microparticles

After in vitro hardening, the in situ forming microparticles from mixing PLGA solution in DMSO or NMP with peanut oil showed porous surface and inner structure; in contrast, the microparticles from mixing polymer solution in TA or TEC with aqueous solution had an irregular surface but a dense inner structure with certain voids (Fig. 29). After injection of emulsion into the release medium, the solvent diffuses into aqueous environment and release medium (nonsolvent) penetrates into the surface of the polymer droplets. The precipitation kinetics of the polymer droplets determines the microstructure of the solidified microparticles. Faster solvent diffusion in the case of DMSO and NMP (water miscible solvent) led to rapid polymer precipitation and results in porous polymer matrix, on the contrary, slow diffusion in the case of TA and TEC (partial water miscible solvent) led to slow polymer precipitation and resulted in less porous microparticles (Graham et al., 1999). The different structure characteristic of the in situ forming microparticles could used to modify the release behavior of the ISM after loading with drugs.

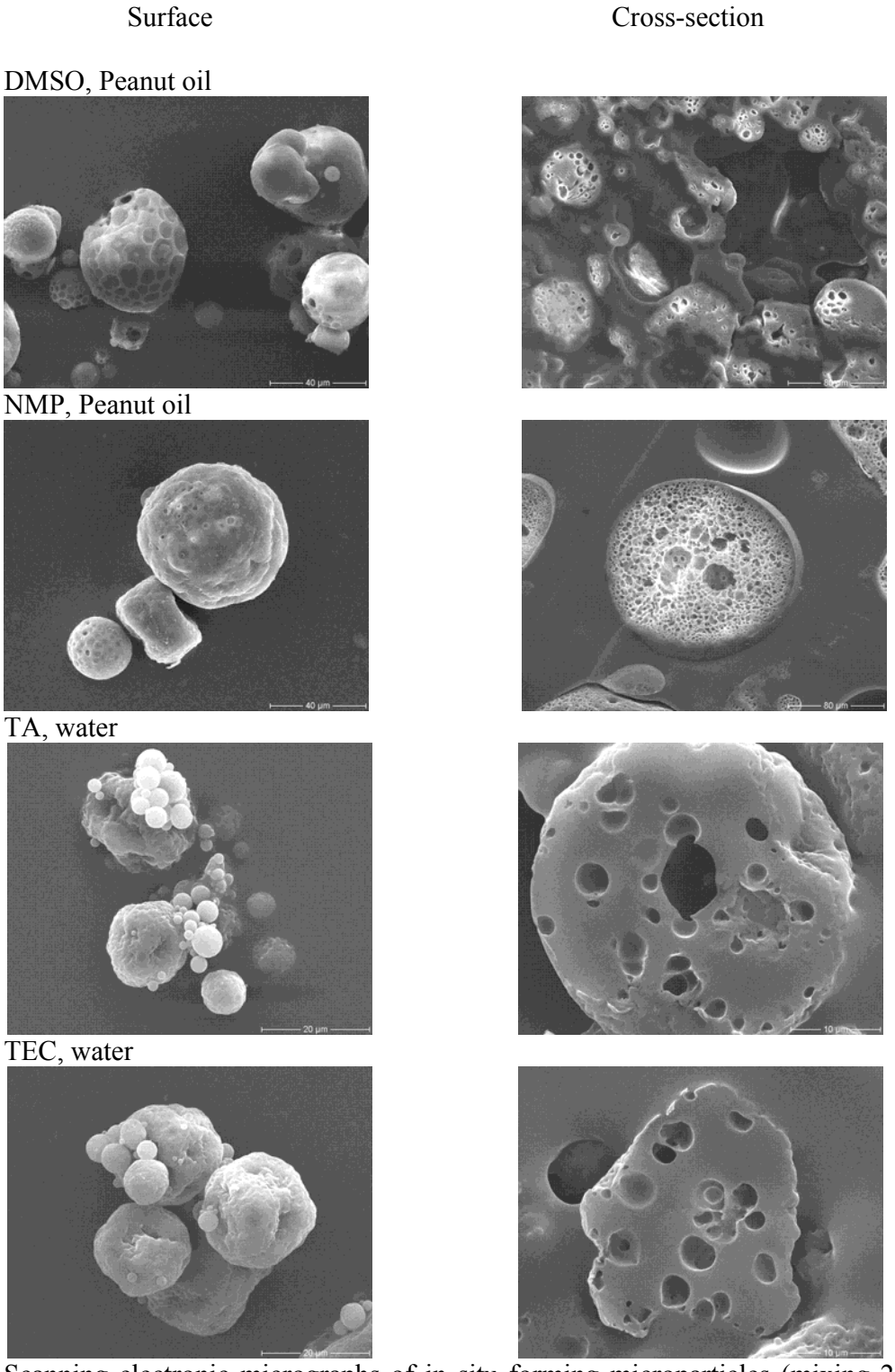


Fig. 29. Scanning electronic micrographs of in situ forming microparticles (mixing 20% w/w PLGA solution in DMSO, NMP, TA, or TEC with peanut oil containing 2% Span 80 or water containing 1% Lutrol F68)

3.2.2. In vitro evaluation: influence of the parameters

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. In this study, influence of the formulation and processing parameters on the drug release from ISM-systems was investigated. Two polymeric carriers, PLGA R 503H and PLA R 202H were used to get different leuprolide delivery periods. R 503H was planned for 1 month and PLA R 202H for 4 months leuprolide release periods.

3.2.2.1. ISM-systems prepared with RG 503H

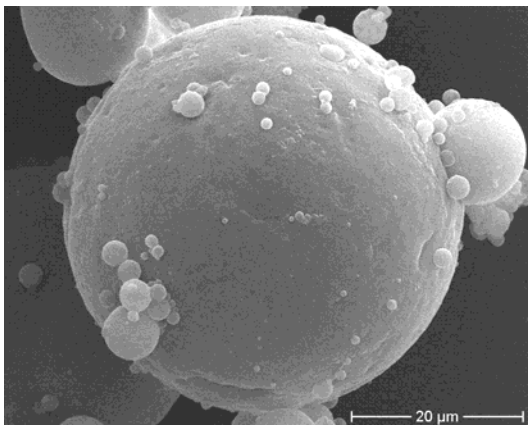
3.2.2.1.1. Morphology and in vitro release

The in situ forming microparticles were spherical and had a smooth surface but a porous inner structure (Fig. 30). 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, R. Bodmeier, 1995b; Graham et al., 1999).

The leuprolide release from ISM occurred in 2 phases (Fig. 31). 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 porosity (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 solidification might lead to a drug loss; (ii) the initial release could also be attributed to the porous microstructure of the microparticles (Fig. 30).

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.

Surface



Cross-section

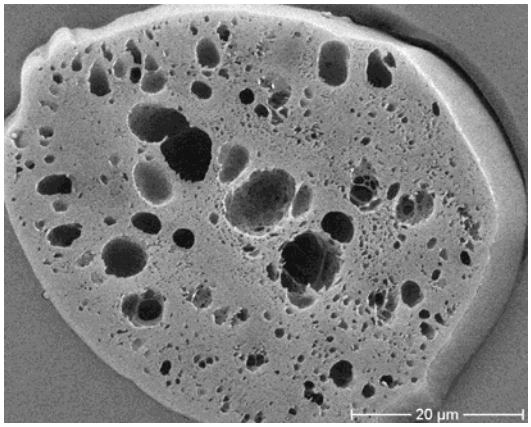


Fig. 30. Scanning electron micrographs of in situ forming microparticles (RG 503H, standard formulation).

3.2.2.1.2. 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 (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%, respectively (Fig. 32). 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.

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. 33), which could possible attributed 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). 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) led to a decreased initial release (41.6% to 27.0%) (Fig. 34). 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, resulting in a lower initial release.

The initial release increased with increasing surfactant (Span 80) concentration in the oil phase (Fig. 35), 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 (Sanghvi and Nairn, 1991)

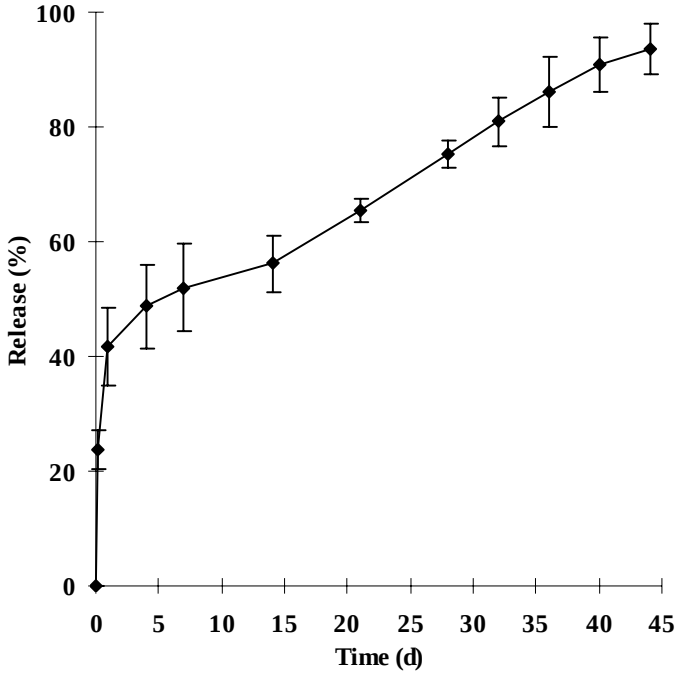


Fig. 31. Leuprolide release from in situ forming microparticles (RG 503H, standard formulation).

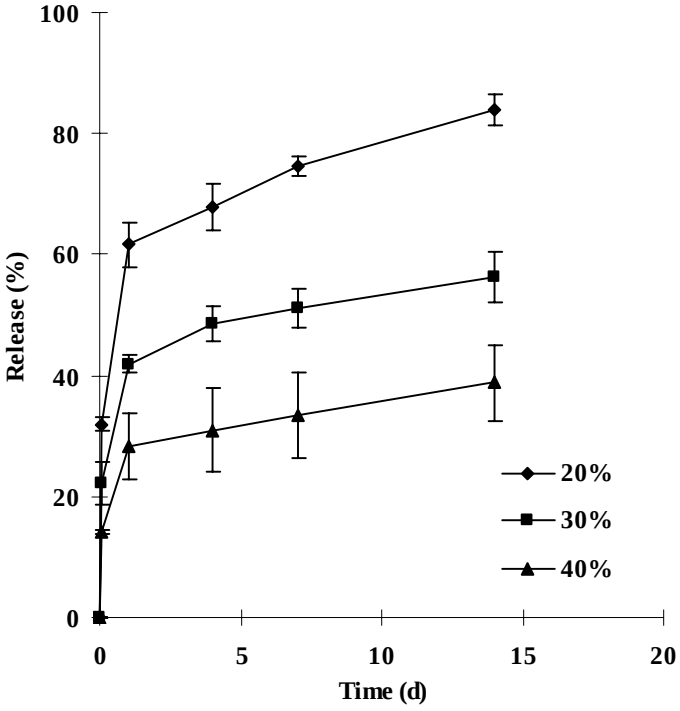


Fig. 32. Influence of the polymer concentration w/w on leuprolide release from in situ forming microparticles (RG 503H).

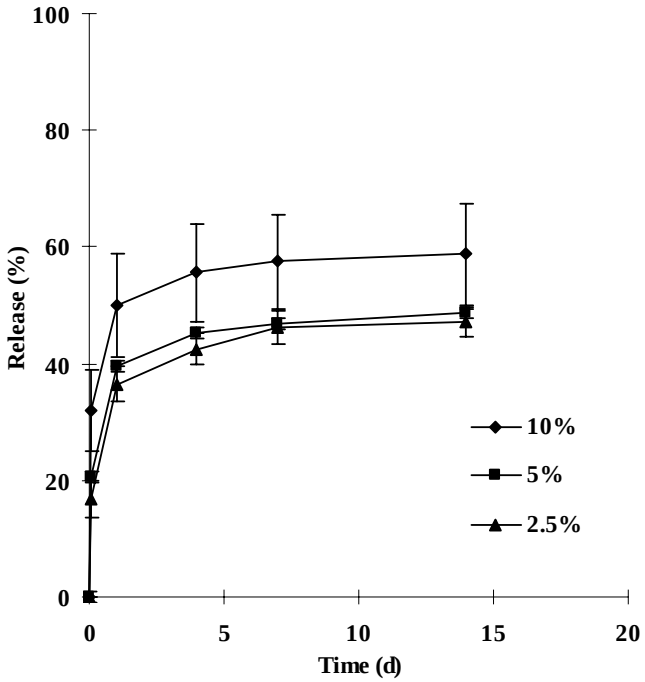


Fig. 33. Influence of the drug loading w/w on leuprolide release from in situ forming microparticles (RG 503H).

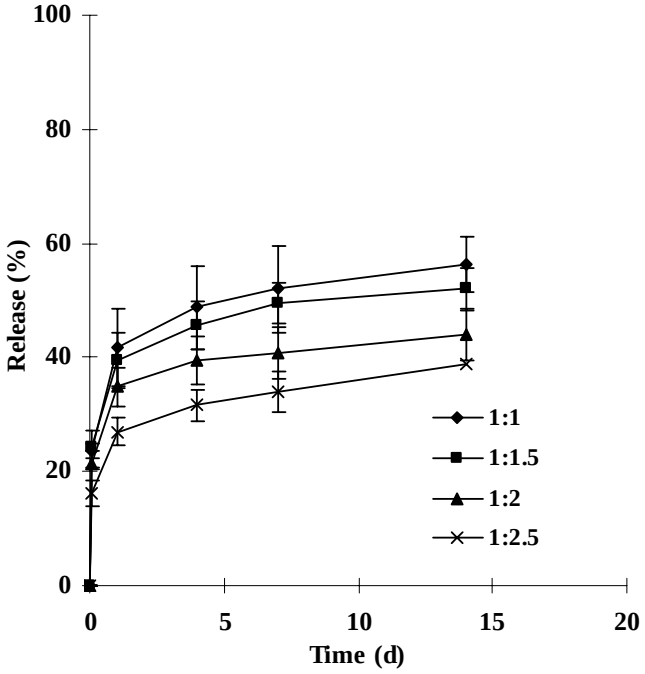


Fig. 34. Influence of the internal:external phase ratio w/w on leuprolide release from in situ forming microparticles (RG 503H).

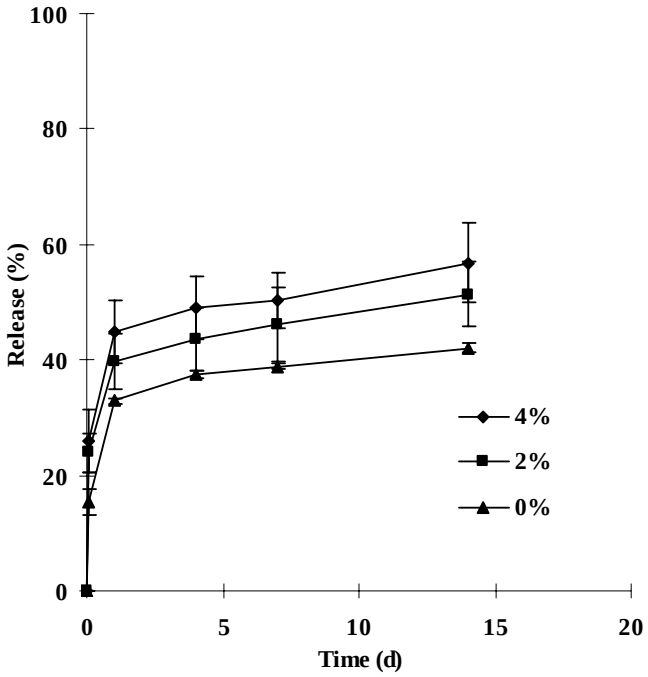


Fig. 35. Influence of Span 80 concentration w/w on leuprolide release from in situ forming microparticles (RG 503H).

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. 36), 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) (data not shown) or the number of mixing cycles (25, 50, and 75) (Fig. 37A) did not affect the leuprolide release from ISM systems. An increase in mixing speed from 1 to 2 cycles per second led to a slight increased initial release, which could possible be attributed to a smaller particle size (Fig. 37 B).

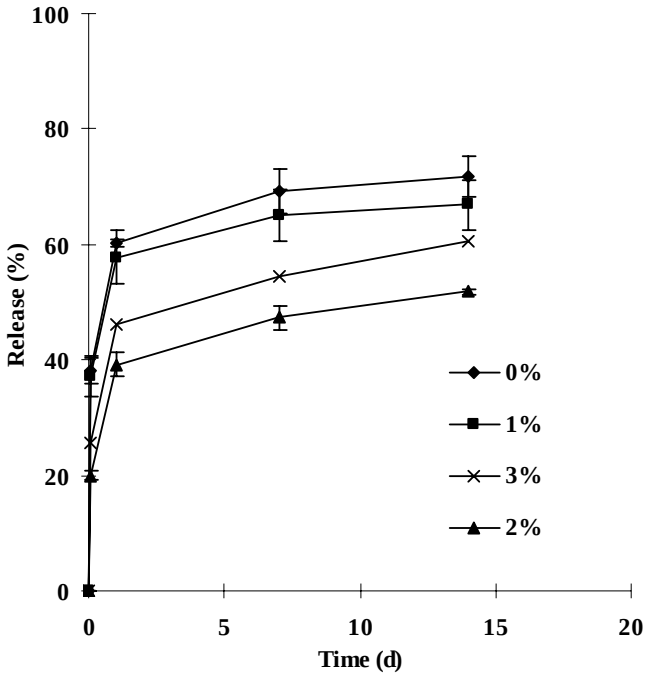


Fig. 36. Influence of the aluminum monostearate concentration w/w on leuprolide release from in situ forming microparticles (RG 503H).

Comparison of ISI and ISM

When comparing the susceptibility of the two in situ systems (in situ implants versus in situ microparticles (ISM)) to the injection techniques, In situ implants presented a significant variation in release with respective to different injection techniques (Fig. 38). A high initial release (71.5%) was observed when the formulation was injected fast with 24 G needle; in contrast, A lower initial release (10.1%) was achieved with using 20 G needle and being injected slowly. This difference in the release linked to the different morphology of the resulted implants. The fast injection with a small needle resulted in a thread-like implant with large surface area and hence a higher initial release; however, the slow injection with big size needle led to the formation of spherical implant with small surface area and a lower initial release. As comparison, ISM showed high consistency in release in different injection testes.

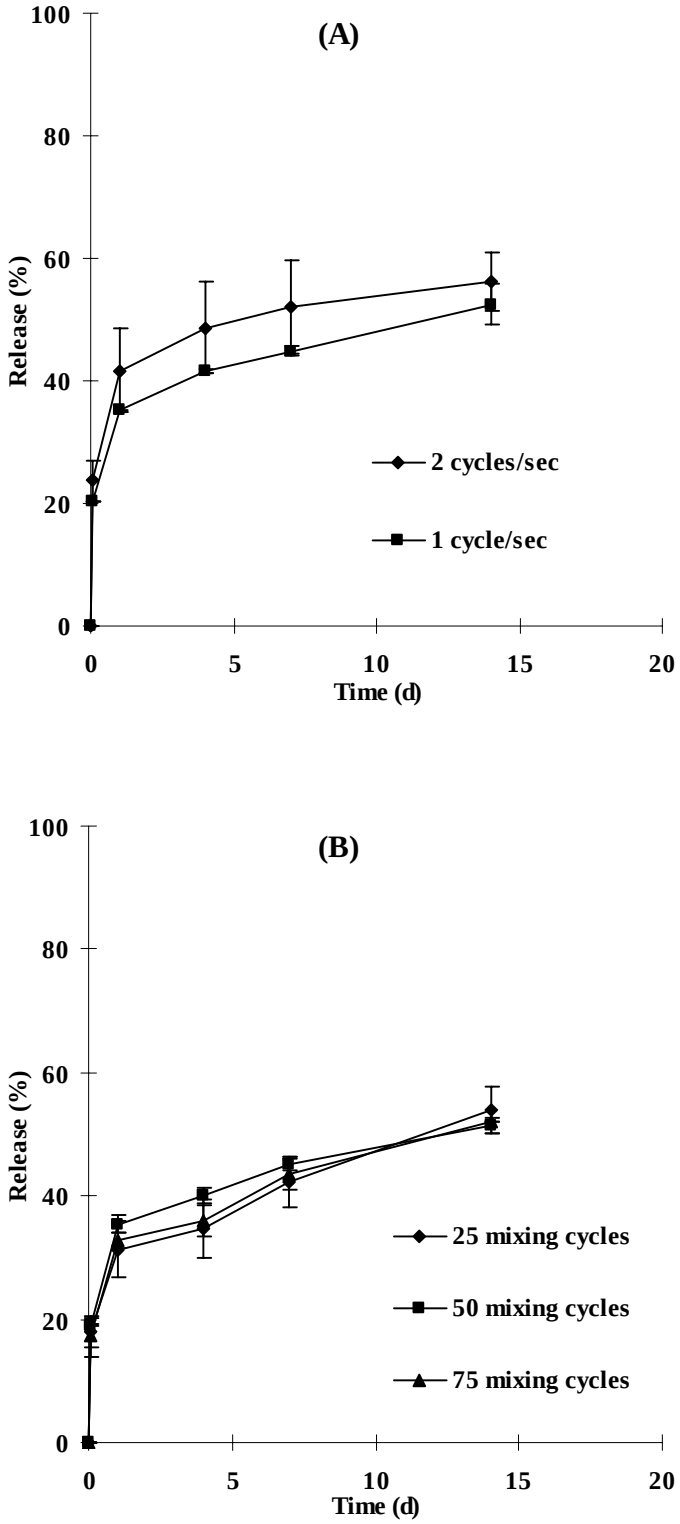


Fig. 37. Effect of the mixing speed (A) and mixing cycles (B) on leuprolide release from ISM prepared with PLGA RG 503H.

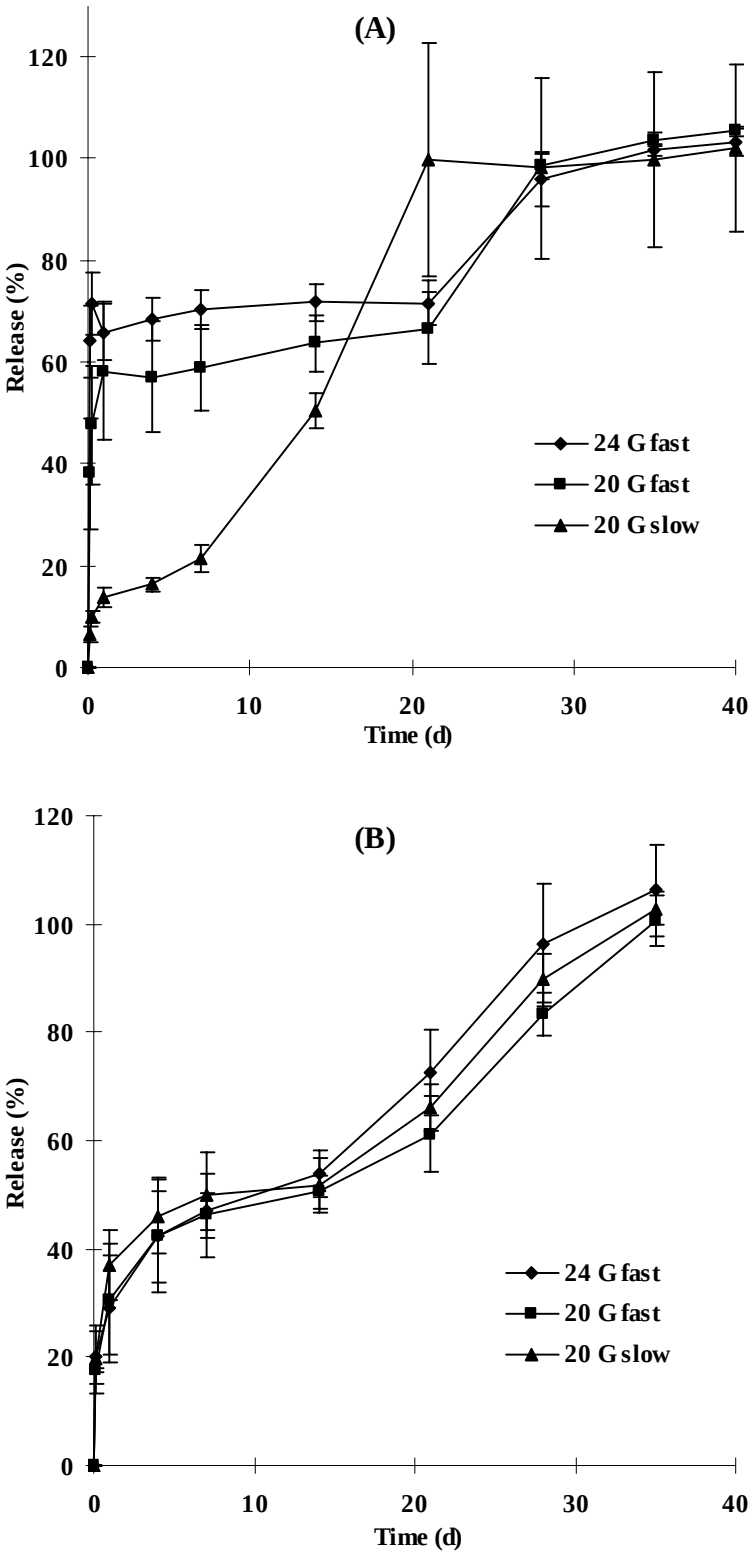


Fig. 38. The influence of injection techniques (size of needle 24 G or 20G, injection speed fast or slow) on the in vitro release of the ISI (A), and ISM (B).

3.2.2.2. 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 (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).

3.2.2.2.1. 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. 39). ISM prepared with a 30% polymer concentration but different drug loading (10% and 15%) had similar release profiles. An initial release of approx. 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 day 2 to day 150. After 150 days of incubation, approx. 90% of leuprolide was released from all 3 formulations (Fig. 39).

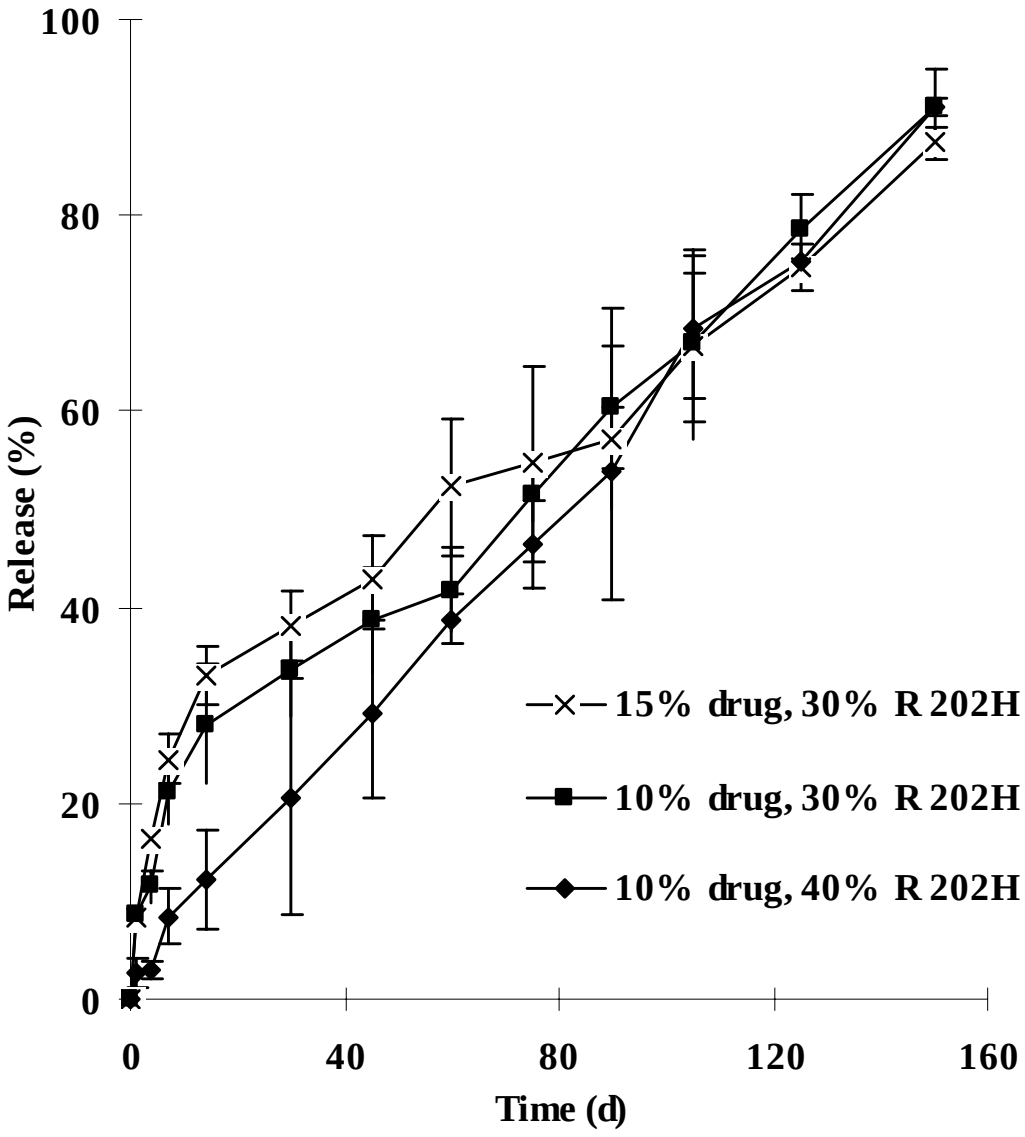
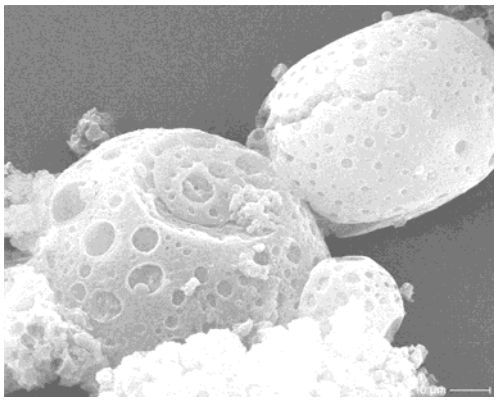
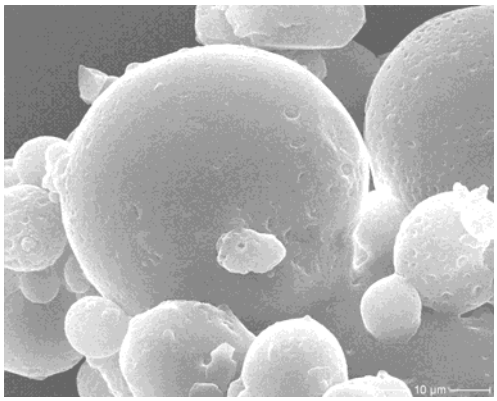


Fig. 39. Leuprolide release from in situ forming microparticles (R 202H, 10 or 15% drug loading, and 30 or 40% polymer concentration)

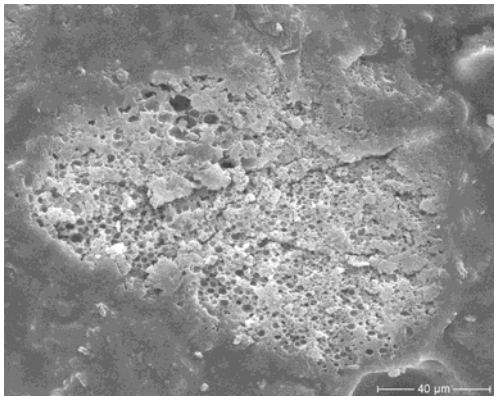
30% 40%

Surface





Cross-section



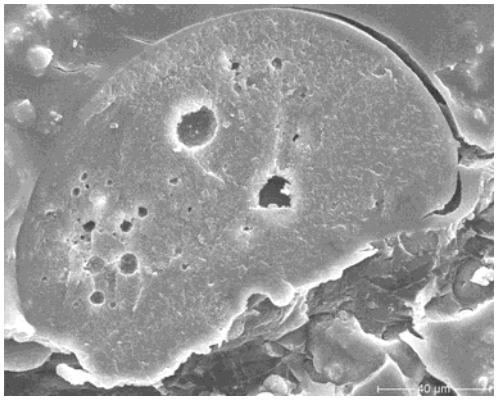


Fig. 40. Scanning electron micrographs of in situ forming microparticles (R 202H, 10% drug loading, and 30 or 40% polymer concentration)

ISM prepared with 30% polymer concentration showed a very porous surface and inner structure (Fig. 40). 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. 40), which explained the slower drug release with ISM prepared with a 40% polymer concentration. The less porous microparticles at the higher 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. 31 versus Fig. 39). This could possibly be explained with the higher carboxylic acid content of the lower molecular weight polymer R 202H (acidic number 10 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.

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

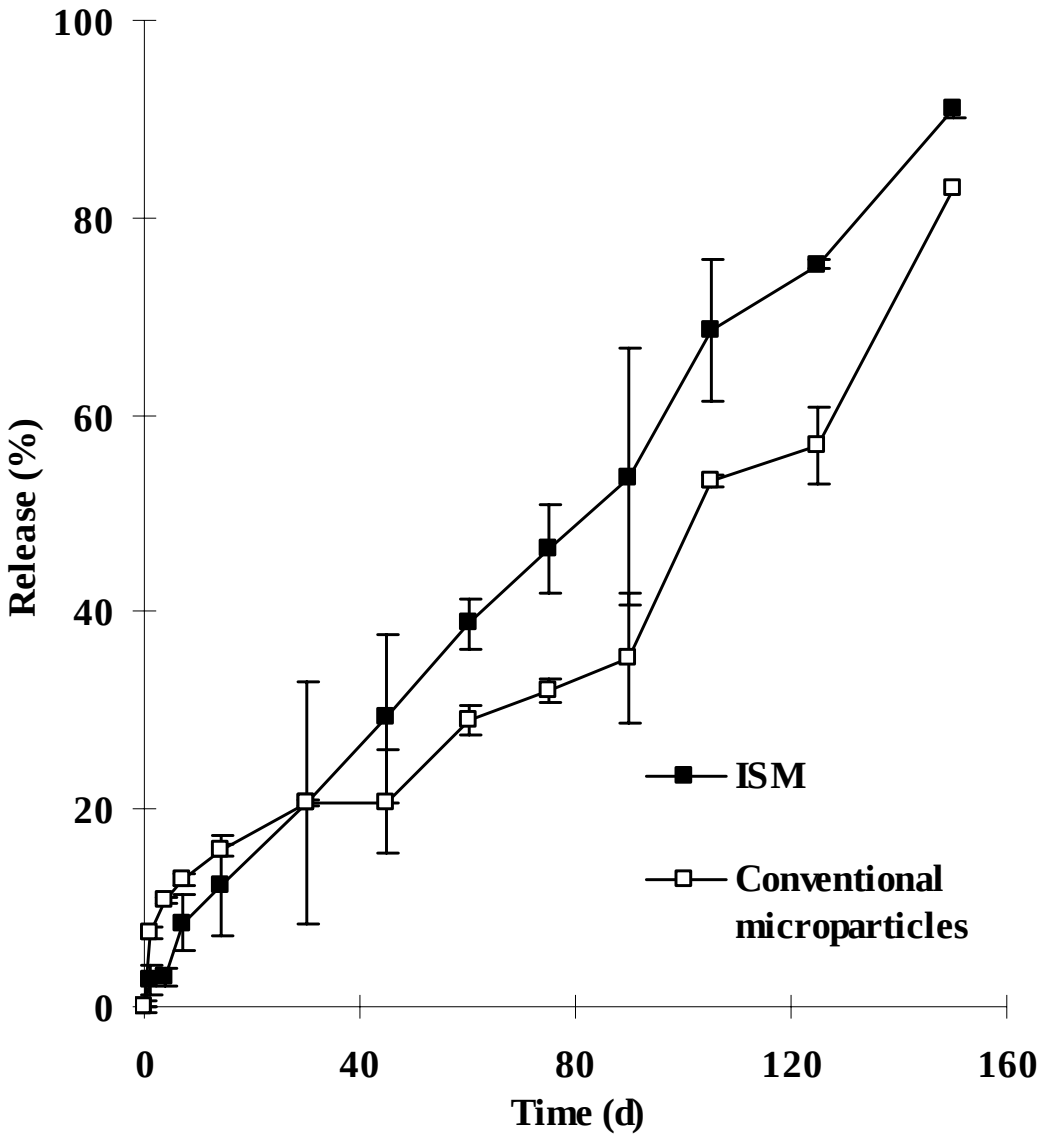


Fig. 41. Leuprolide release from in situ forming and conventional microparticles (R 202H) (ISM: 10% drug loading, 40% polymer concentration; conventional microparticles: 10.5% actual drug loading, encapsulation efficiency 70.9%)

3.2.3. Reduction of the initial release with cosolvent

NMP was selected as solvent for PLGA because it has been widely used in in situ implant systems. The release profile of leuprolide from ISM prepared with NMP was biphasic (Fig. 42). A high initial release (burst effect, approx. 40% of drug released during the first day) was followed by a slower continuous release phase. A high initial release is often unwanted because of a too rapid drug depletion of the system or even toxic drug levels. The type of organic solvent strongly affects the precipitation of the polymer and thus the microparticle morphology and drug release. The objective of this study was to control the initial drug release phase through a proper choice of solvent/solvent mixtures. Partially water-miscible solvents reduced the initial release because of a slower polymer precipitation and the formation of a less porous polymer matrix with in situ implants (Brodbeck et al., 1999a; Brodbeck et al., 1999b; Wang et al., 2003). Leuprolide is soluble in NMP but much less soluble in the partially water-miscible solvents triacetin or benzyl benzoate. A solvent mixture of NMP and a partially water-miscible cosolvent was thus used to dissolve the drug and PLGA. The influence of cosolvent addition on the leuprolide release from ISM-systems was investigated.

3.2.3.1. Use of NMP and triacetin in ISM

Adding triacetin (water miscibility 7%) as a cosolvent to NMP resulted in a slower initial leuprolide release (Fig. 42). Increasing the triacetin concentration from 0 to 10 to 20% (w/w, based on total solvents) decreased the initial release from 42.7 to 27.1 to 16.9%, respectively. A further increase to 30 or 40 % triacetin caused a less significant reduction in initial release. Triacetin also showed a concentration-dependent effect on the second continuous release phase. At a concentration of 10 and 20%, triacetin did not significantly affect the continuous release; however, at 30 and 40%, triacetin clearly reduced the drug release during the period between days 2 to 14 and increased the drug release thereafter. The leuprolide release changed from a bi-phasic to a tri-phasic pattern. An initial release was followed by a lag time of almost no drug release until PLGA degradation and sufficient mass loss occured to initiate the final erosion-controlled rapid drug release phase.

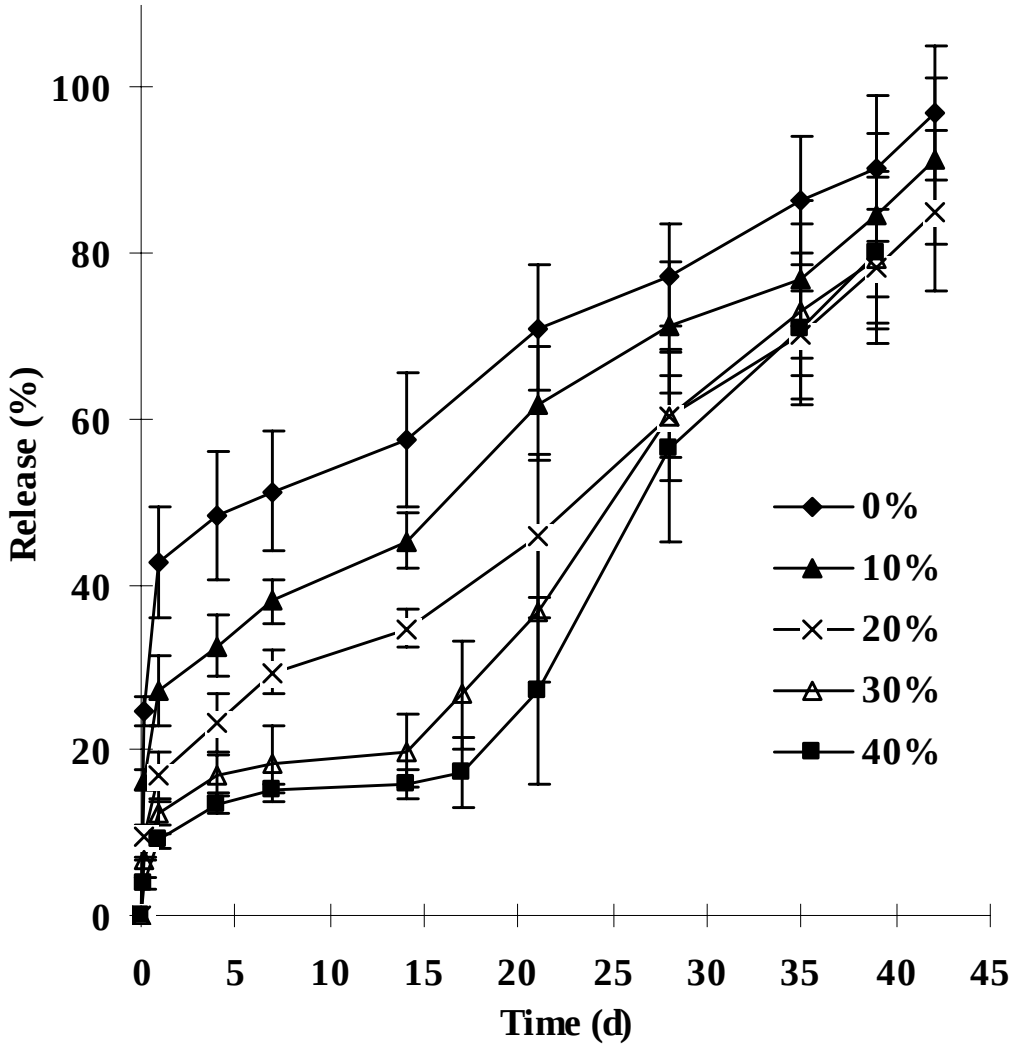


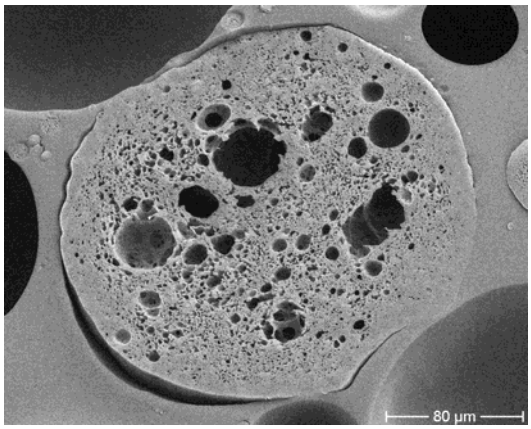
Fig. 42. Influence of triacetin addition on the leuprolide release from in situ forming microparticles (5% leuprolide loading, RG 503H, 30% polymer concentration, solvent mixture: NMP and triacetin)

The addition of triacetin reduced the porosity of the ISM (Fig. 43). ISM prepared with NMP showed a very porous inner structure (0% triacetin). In contrast, 20% triacetin resulted in ISM with a much denser inner structure. The less porous microparticles reduced the accessibility of the drug to the release medium and thus resulted in a lower initial release. The influence of triacetin on the ISM surface morphology was less significant; both formulations had a smooth surface (data not shown).

In ISM-systems, the solvent diffusion from the internal polymer phase of the o/o emulsion involves two steps: (i) the solvent diffuses into the external oil phase during emulsification of the polymer solution into the oil phase and (ii) the solvent diffuses into the aqueous medium (e.g., release medium) after injection of the emulsion into the aqueous medium. The kinetics of both steps could influence the drug release. During the first step, a rapid and significant extent of solvent diffusion may lead to a concentration of the polymer solution and thus an increase in solution viscosity, resulting in a less porous polymer matrix with a slow drug release after injection. During the second step, a fast and significant extent of solvent diffusion may lead to a rapid polymer precipitation, resulting in a porous polymer matrix with a fast drug release (Brodbeck et al., 1999a, Graham et al., 1999).

In order to elucidate the effect of the solvent on the drug release and microstructure of the ISM, the above described two solvent diffusion steps were investigated in more detail. The influence of triacetin on the solvent diffusion into the oil phase was evaluated by measuring the volume loss and viscosity change of the PLGA solution after shaking with peanut oil (Fig. 44A and B). The volume loss of the NMP/triacetin solvent mixture was reduced with increasing triacetin concentration (Fig. 44A), which indicated a delayed solvent diffusion into the oil in the presence of triacetin. This result was confirmed by the viscosity change of the PLGA solution after shaking with peanut oil (Fig. 44B). The presence of 20% triacetin led to a less prominent viscosity increase when compared to NMP only. At 40% triacetin, the viscosity increase was similar to NMP despite a lower a total solvent loss in the oil phase. This was caused by the higher solution viscosity of a PLGA/triacetin solution when compared to a PLGA/NMP solution at the same polymer concentration (59356 versus 1358 mPas at 30% w/w polymer concentration). The slower solvent diffusion with triacetin might be as a result of a lower affinity of triacetin to peanut oil in comparison to NMP. The lower porosity of microparticles prepared with triacetin as cosolvent was therefore not due to concentration of the polymer solution prior to precipitation.

Triacetin-free



20% triacetin

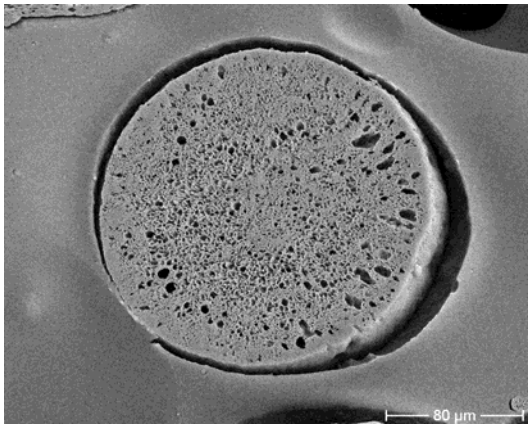


Fig. 43. Scanning electronic micrographs of the cross-section of in situ forming microparticles prepared in the absence or presence of 20 and 40% triacetin

Next, the influence of triacetin on the NMP diffusion (Fig. 45A) and solvent mixture (NMP plus triacetin) diffusion (Fig. 45B) into the aqueous release medium was evaluated by measuring the solvent concentration in the release medium by RP-HPLC. Triacetin addition showed insignificant impact on the NMP diffusion in the first 3 h after contact with release medium and led to a reduced NMP diffusion afterwards (Fig. 45A), which may be due to the denser structure of the microparticles (Fig. 43). To the total solvent mixture, solvent diffusion rate decreased with increasing triacetin concentration as a result of its low water miscibility (Fig. 45B). The slower solvent diffusion led to a slower polymer precipitation and thus resulted in less porous microparticles with a slower initial leuprolide release.

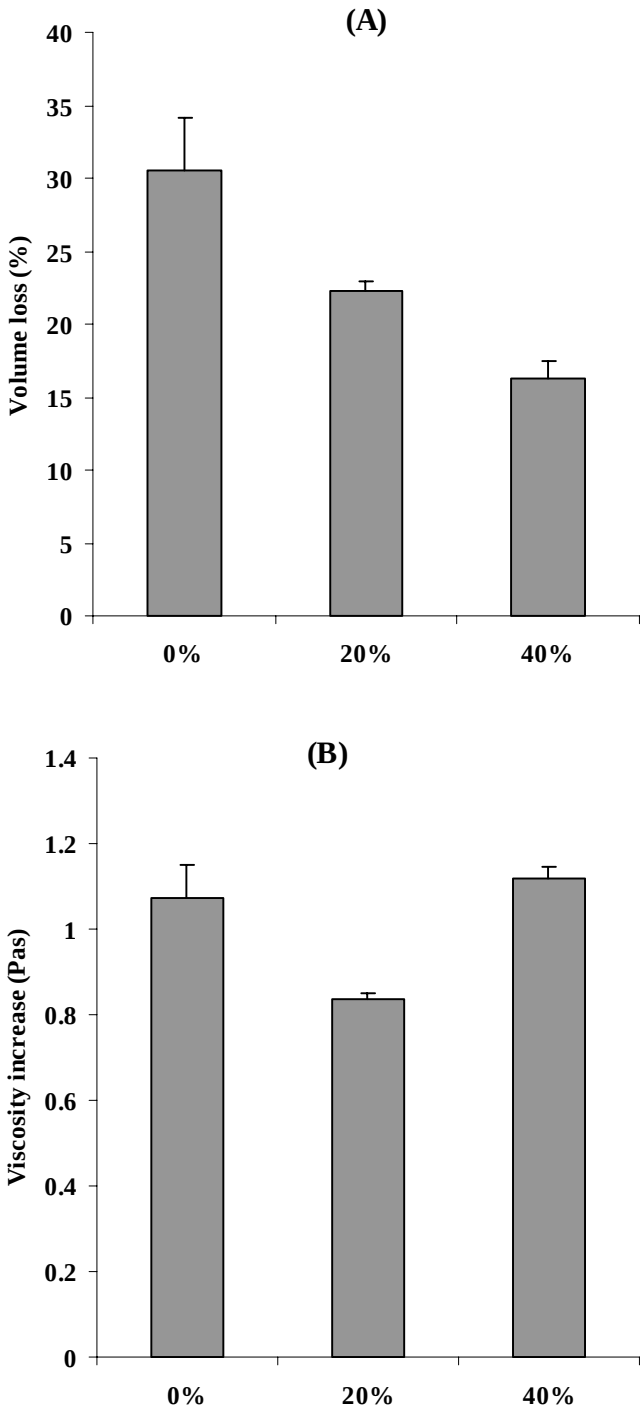


Fig. 44. Influence of triacetin addition on the volume loss (A) and viscosity increase (B) of PLGA solution (RG 503H, 20% polymer concentration) after shaking with peanut oil.

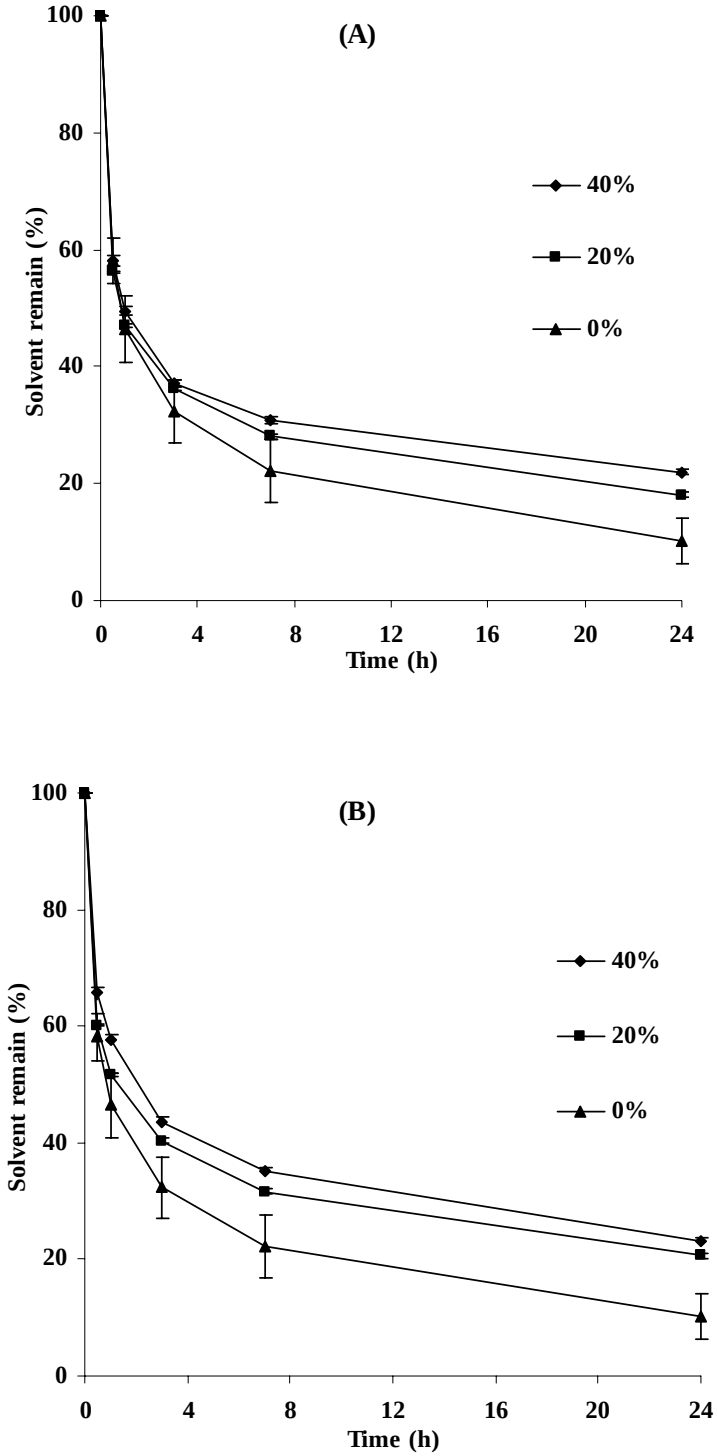


Fig. 45. Influence of triacetin addition on the NMP (A) and solvent mixture (NMP plus triacetin, B) diffusion from PLGA solution (5% leuprolide loading, RG 503H, 30% polymer concentration) into the release medium.

3.2.3.2. Use of NMP and solvents with varying water miscibility in ISM

Solvent mixtures containing 80% w/w NMP and 20% w/w the second solvent with varying water miscibility were evaluated in ISM-systems. Influence of the second solvent on the polymer solution viscosity and the initial leuprolide release from ISM were shown in Table 13. PEG 400 and benzyl benzoate significantly increased the viscosity; however, no clear effect of such changes on the release from ISM was observed.

Table 13 Water miscibility and influence of the second solvent (20% w/w and 80% w/w NMP) on the viscosity of the polymer solution (30% w/w RG 503H) and the initial release (first day release) from in situ forming microparticles.

The second solvent	Water miscibility	Viscosity (mPas)	Initial release (%)
100% NMP	Completely miscible[1]	1358	42
PEG 400	Completely miscible[1]	3333	46
Propylene carbonate	22%[2]	1571	20
Triacetin	7%[3]	2071	15
Benzyl alcohol	4%[1]	1894	18
Benzyl benzoate	Immiscible[1]	2472	12

1 Value from Merk Index, twelfth edition, 1996.

2 Value at 20 °C was from Lexikon der Hilfsstoffe, 1996

3 Value at 20 °C was from Handbook of pharmaceutical excipients, 1994

In general, the initial release from ISM (Fig. 46) decreased with decreasing water solubility of the second solvent (Table 13) in the release order of PEG 400 > NMP > propylene carbonate > triacetin > benzyl alcohol > benzyl benzoate. This could be explained by a slower polymer precipitation and consequently a less porous polymer matrix. However, there were two exceptions: (i) ISM prepared from a solvent mixture of NMP and PEG 400 (both are completely water-miscible) showed a higher initial release than ISM prepared with NMP only and (ii) benzyl alcohol (4% water solubility) led to a slightly higher initial release than triacetin (7% water solubility). Most second solvents did not show a significant effect on the continuous release phase. Only 20% w/w benzyl benzoate (water-immiscible) reduced the drug release from day 1 to day 14 (Fig. 46) and increased the release thereafter. This was similar to the effect of 30 and 40% triacetin (Fig. 42).

The presence of the second solvent influenced the solvent diffusion into the oil phase (Fig. 47A). The solvent mixtures contained the same amount of NMP; the differences in solvent diffusion rate thus reflected essentially the diffusion rate of the second solvent, which was in the sequence of benzyl benzoate > NMP > benzyl alcohol > propylene carbonate > triacetin > PEG 400. This result was confirmed by a similar trend in viscosity increase of the polymer solution after shaking with peanut oil (Fig. 47B). An unexpected lower viscosity increase in the case of propylene carbonate could be explained by the low viscosity of its PLGA solution compared to the other solvents (Table 13).

As discussed before, the diffusion of solvent into the oil played a role in the drug release behavior of ISM. In the case of completely water-miscible solvents, NMP diffused faster into peanut oil than PEG 400; this resulted in a concentration of the polymer solution and thus a slower initial release. In the case of partially water-miscible solvents, two overlapping effects might take place: (i) a faster solvent diffusion led to a higher polymer concentration a lower initial release and (ii) a faster solvent diffusion also led to a decreased amount of partially water-miscible solvent in the polymer solution, which could cause a faster polymer precipitation after injection into the aqueous release medium resulting in porous microparticles with a faster drug release. With benzyl alcohol or triacetin, the second effect might be more prominent resulting in a lower initial release in the case of triacetin (higher water miscibility but slower diffusion rate into peanut oil).

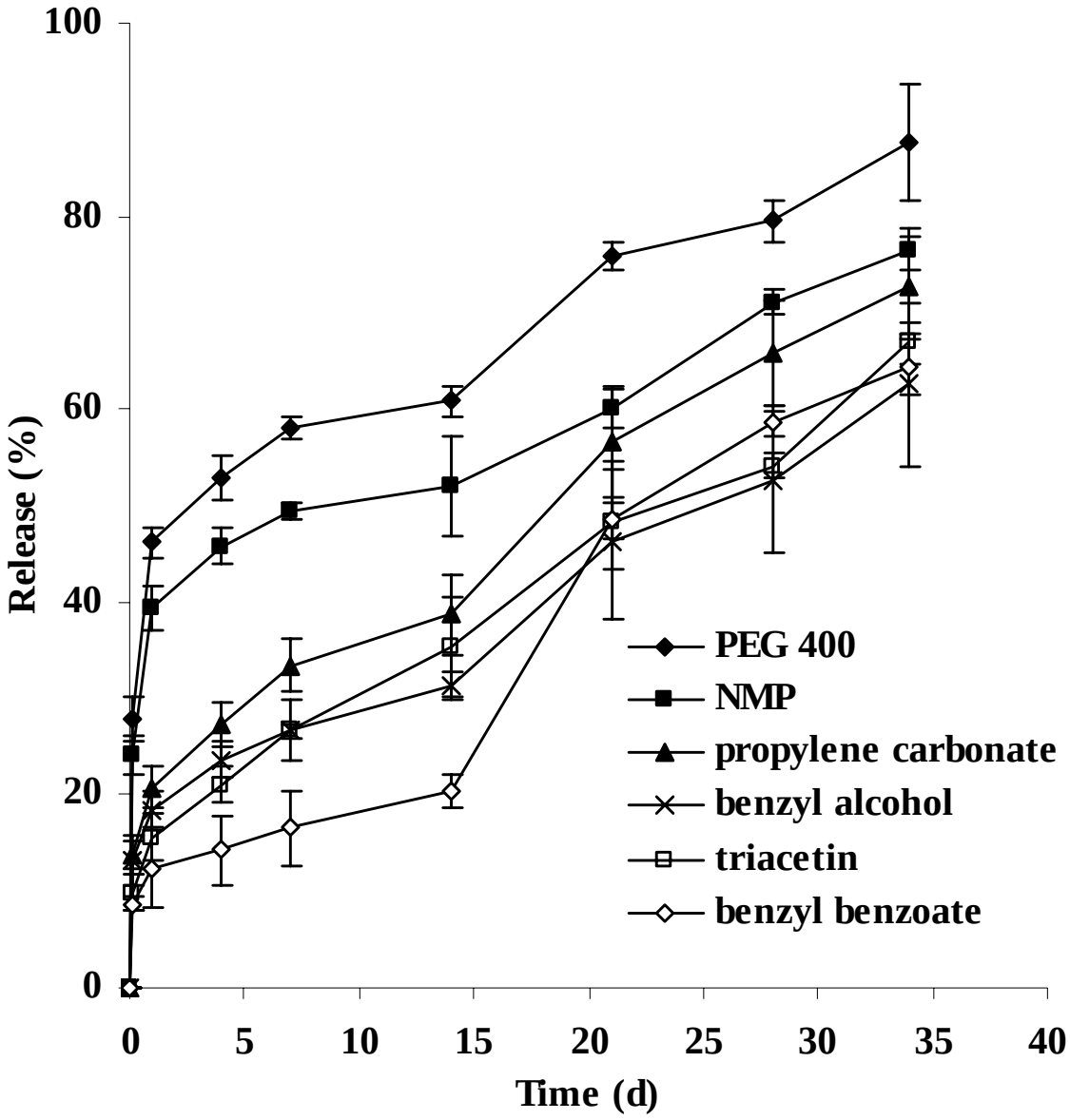


Fig. 46. Influence of the second solvent addition on the leuprolide release from in situ forming microparticles (5% leuprolide acetate, RG 503H, 30% polymer concentration, solvent mixture: 80% w/w NMP and 20% w/w second solvent).

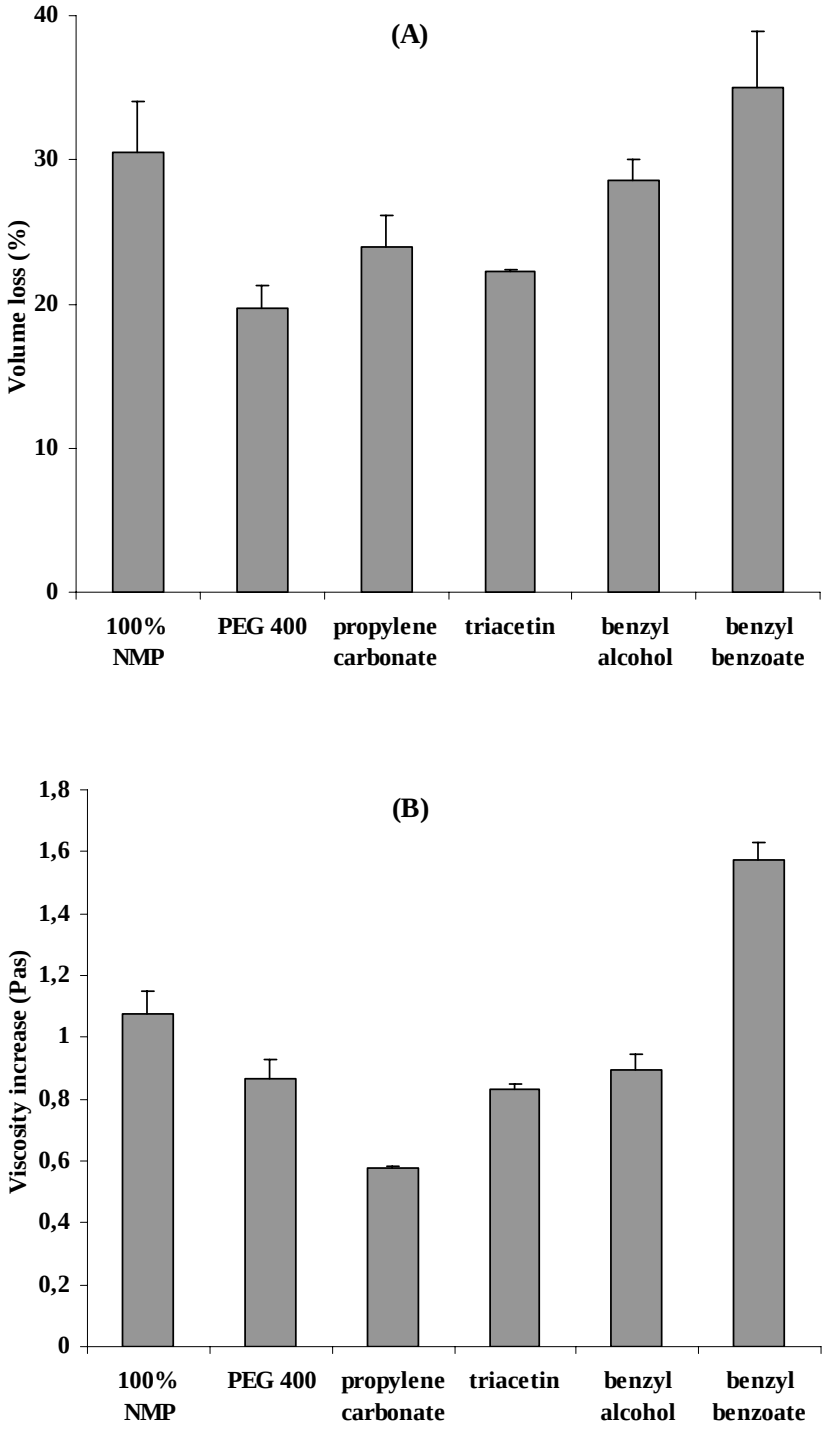


Fig. 47. Influence of the second solvent addition on the volume loss (A) and viscosity increase (B) of PLGA solution (RG 503H, 20% polymer concentration, solvent mixture: 80% w/w NMP and 20% w/w cosolvent) after shaking with peanut oil.

3.2.3.3 In vivo testing of NMP/triacetin-based ISM

The preparation of leuprolide acetate-loaded ISM with PLGA RG 503H as biodegradable polymer was targeted for a one month suppression of testosterone. After a single subcutaneous administration of ISM (5% leuprolide w/w, based on PLGA plus leuprolide), 30% PLGA (w/w, based on solvent plus polymer), 80% NMP (w/w, based on NMP plus triacetin), 20% triacetin (w/w, based on NMP plus triacetin), polymer phase and oil phase 1:1) in rabbits (leuprolide acetate 75 µg/Kg/day), the testosterone levels of rabbits increased to 11 ng/ml immediately (Fig. 48). Thereafter, the testosterone level was suppressed to less than 1 ng/ml at day 12 up to day 29. After 30 days, the testosterone levels approached their normal value.

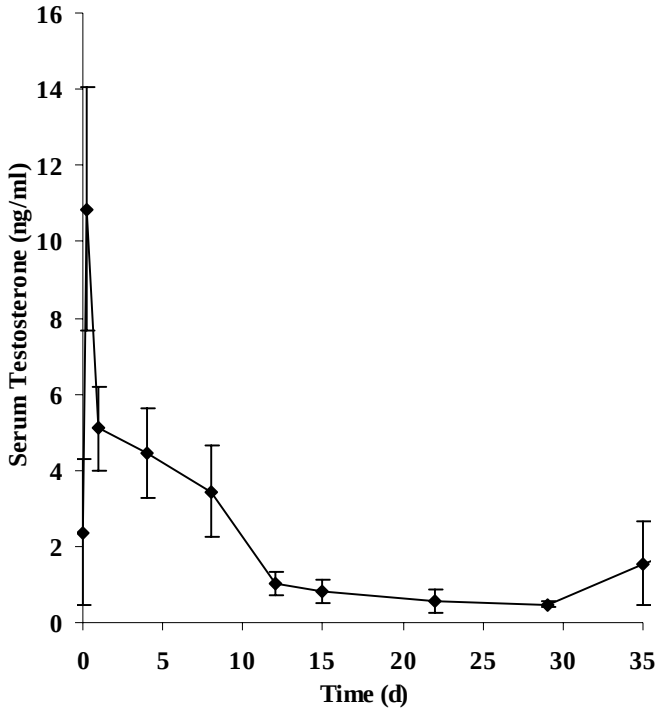


Fig. 48. In vivo testosterone suppression (n=5) with in situ forming microparticles (5% leuprolide loading, RG 503H; 30% polymer concentration, solvent mixture: 80% w/w NMP and 20% w/w triacetin)

3.2.3.4. NMP/triacetin in ISM-systems prepared with PLA R 203H

PLA R 203H was chosen as polymer to formulate ISM-system for a 6 months controlled delivery of leuprolide acetate. Conventional microparticles based on the same polymer and prepared by a solvent evaporation method showed a 6 months suppression of the testosterone in rats (Woo et al., 2002). For a 6 months preparation, drug depletion through a high initial release is unwanted in order to maintain sufficient drug levels in the later release periods.

ISM prepared with NMP as the only solvent had a high initial release (57.4% during the first day); with triacetin, the initial release could be significantly reduced to only 17.2% (Fig. 49). Triacetin did not affect the release thereafter during the 30 day release period.

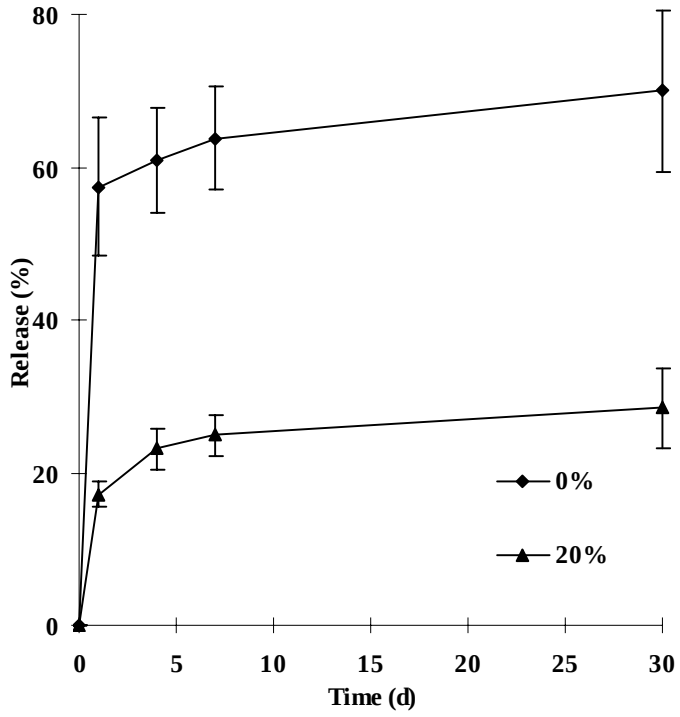


Fig. 49. Influence of triacetin addition on the leuprolide release from in situ forming microparticles from PLA R 203H (15% leuprolide loading, 30% polymer concentration, solvent mixture: NMP and triacetin).

3.2.4. Influence of the PLGA type

The effect of different PLGA properties such as molecular weight, lactide/glycolide ratio, and terminal functional groups on the drug release have been extensively investigated with conventional PLGA systems. PLGA with a lower molecular weight generally leads to a faster polymer degradation (Park, 1994; Jalil and Nixon 1990a) and a more rapid drug release (Ravivarapu et al., 2000c). An increase in the lactide content decreases the polymer degradation rate and results in a slower drug release (Göpferich, 1996; Li, 1999). Uncapped PLGA with free carboxyl termini is more hydrophilic and has higher hydrolysis rate than its end-capped species with esterified carboxyl termini. However, the carboxyl termini may interact with encapsulated drug (such as BSA and L-asparaginase) leading to a slower drug release (Blanco and Alonso, 1997; Gaspar et al., 1998).

The objective of present study was to investigate the effect of PLGA type on the drug release from the leuprolide acetate loaded ISM systems. The polymer used in the present study was GMP grade PLGA with different molecular weight (RG 503H, 503 with higher molecular weight than RG 502H, 502).and endgroup functionality (H-series with uncapped [free] carboxyl termini and non-H-series with capped ester termini).

3.2.4.1. Influence of the PLGA molecular weight

The leuprolide release from ISM prepared with RG 503H, RG 502H, and their blends and using NMP as polymer solvent was shown in Fig. 50. ISM made of the higher molecular weight RG 503H had a high initial release (first day, 37.2%), followed by a slow and continuous drug release. In contrast, ISM made of the lower molecular weight RG 502H showed a much lower initial release (5.7%), followed by a slow drug release until day 7 and thereafter a rapid drug release. ISM made of blends of two PLGA had a decreased initial release and an increased release in the later release stages with increasing RG 502H content.

Replacing NMP with other hydrophilic solvents, dimethyl sulfoxide (DMSO) or 2-pyrrolidone also led to a lower initial release with RG 502 H ISM than with RG 503H ISM (Fig. 51A and B); the 1:1 polymer blend resulted in an intermediate release. Thus, blending with low molecular weight PLGA provided a tool to reduce the initial drug release from ISM.

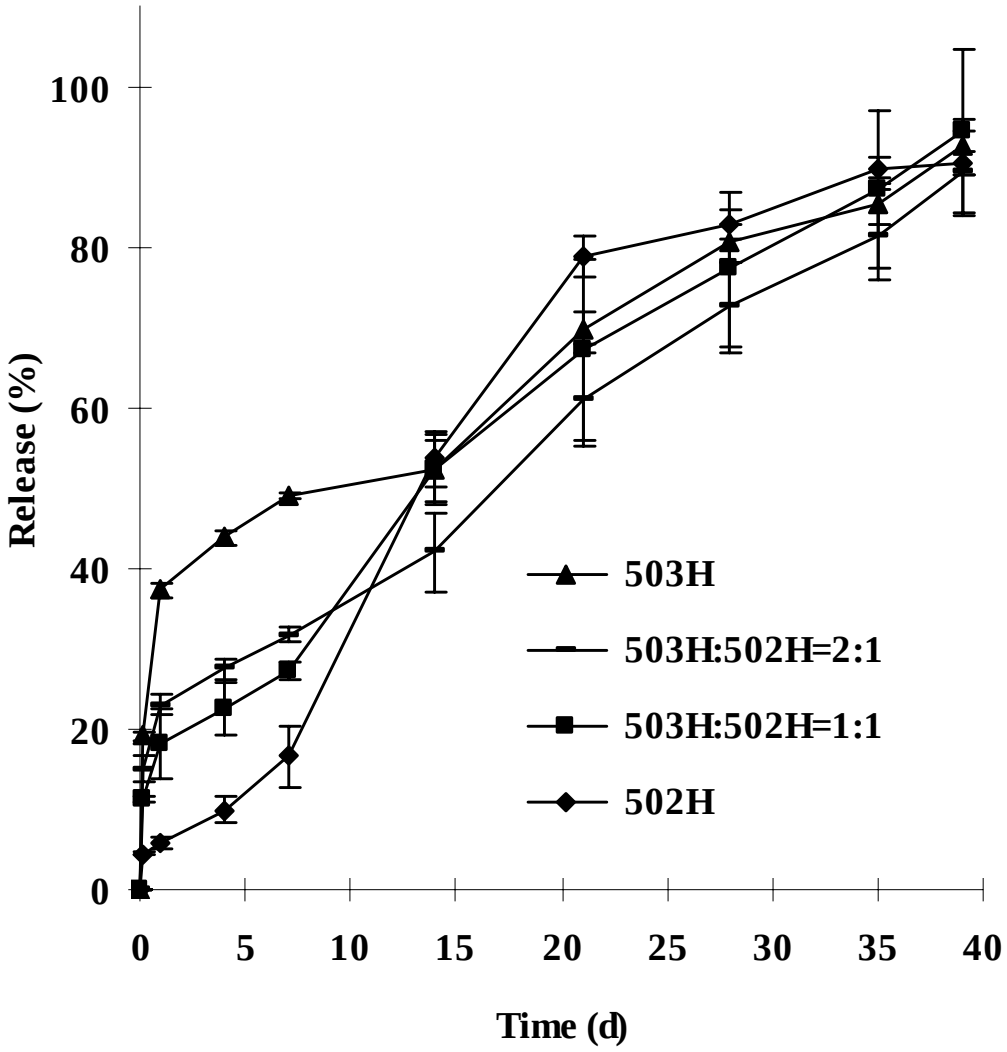


Fig. 50. Influence of PLGA type (RG 503H, RG 502H, and their 2:1, 1:1 blends) on the leuprolide release from ISM (drug loading 5%, polymer concentration 30%).

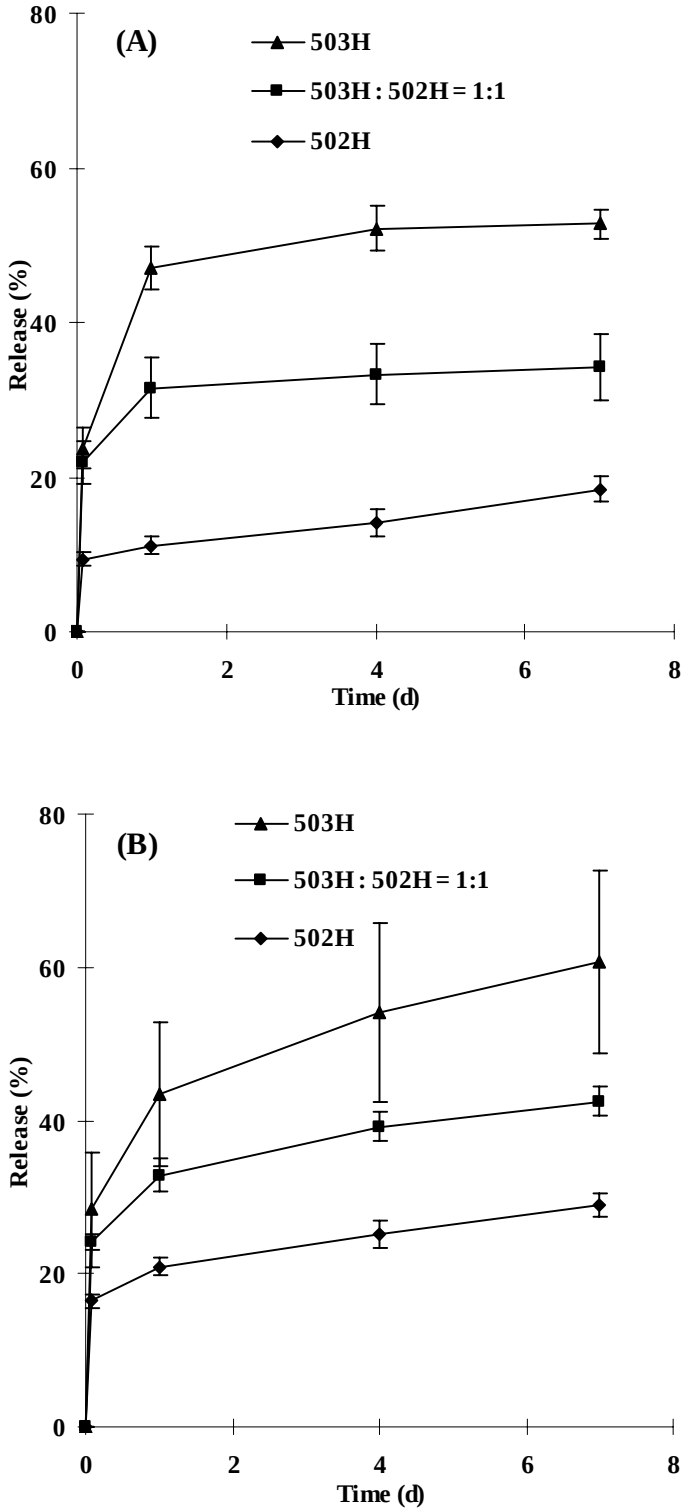


Fig. 51. Influence of PLGA type (RG 503H, RG 502H, and their 1:1 blend) on the leuprolide release from ISM (drug loading 5%) prepared with (A) DMSO, (B) 2-pyrrolidone.

For comparison, conventional microparticles were prepared by the solvent evaporation method. Interestingly, an opposite effect of the two PLGA polymers on the initial leuprolide release was observed. RG 502H led to a significant higher initial drug release than RG 503H (26.9 versus 14.6%) (Fig. 52). Microparticles prepared from blending two polymers resulted in release profiles between the profiles of microparticles prepared from the pure polymer; the initial release increased with increasing RG 502H content. The microparticles showed a tri-phasic drug release, namely, an initial release followed by a slow release phase and a final rapid release phase. Due to the faster degradation of RG 502H, the final rapid release phase occurred earlier with RG 502H.

RG 502H has a lower inherent viscosity than RG 503H (0.19 versus 0.38 dl/g). This led to a less viscous polymer solution and consequently to a smaller particles size in both ISM and microparticles. To explain the opposite effect of the PLGA polymers on the release from ISM and conventional microparticles and to eliminate particle size effects, the release from in situ implants and dried PLGA films was studied.

The release results with in situ implant system were in good agreement with the results obtained with ISM; RG 502H led to a much lower initial release than RG 503H (18.8 versus 48.1%) (Fig. 53). The initial release for a 1:1 blend was close to that for RG 502H (18.0%). After the initial release, the leuprolide release increased with increasing RG 502H content due to its faster degradation rate. This result was in agreement with the results on in situ implants, whereby a lower molecular weight PLGA led to a slower drug release (Tipton et al., 1991). The release from PLGA films was not strongly affected by the PLGA molecular weight; the release trends were similar to the microparticles prepared with the solvent evaporation method (Fig. 54).

The initial drug release from conventional biodegradable systems (implants and microparticles) is normally attributed to the surface-associated drug. For in situ forming systems (in situ implants and in situ microparticles), the initial drug release is affected by two factors: (i) the in situ systems are administrated in liquid form (solution or emulsion). Prior to polymer solidification, the drug may diffuse out of the system in parallel with the diffusion of the solvent; this happens in particular with drugs, which are soluble in solvent, and with solvents, which rapidly diffuse in the release medium (Brodbeck et al., 2000); (ii) after the polymer solidification, the drug associated to the surface of polymer matrix or drug entrapped in highly porous polymer matrix is rapidly released.

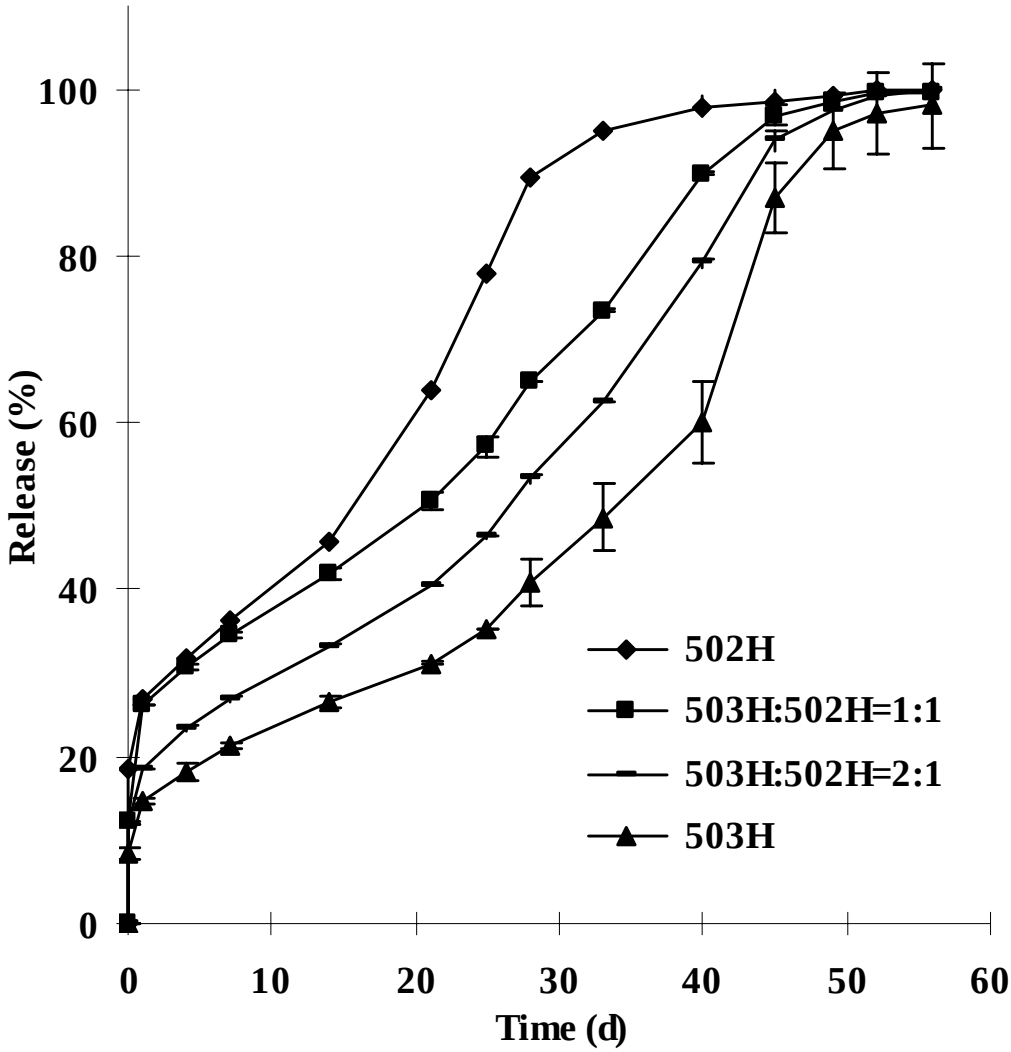


Fig. 52. Influence of PLGA type (RG 503H, RG 502H, and their 2:1, 1:1 blends) on the leuprolide release from microparticles prepared by solvent evaporation method (drug loading 20%, encapsulation efficiency 97% for 503H, 97% for 2:1 blend, 94% for 1:1 blend, and 84% for 502H).

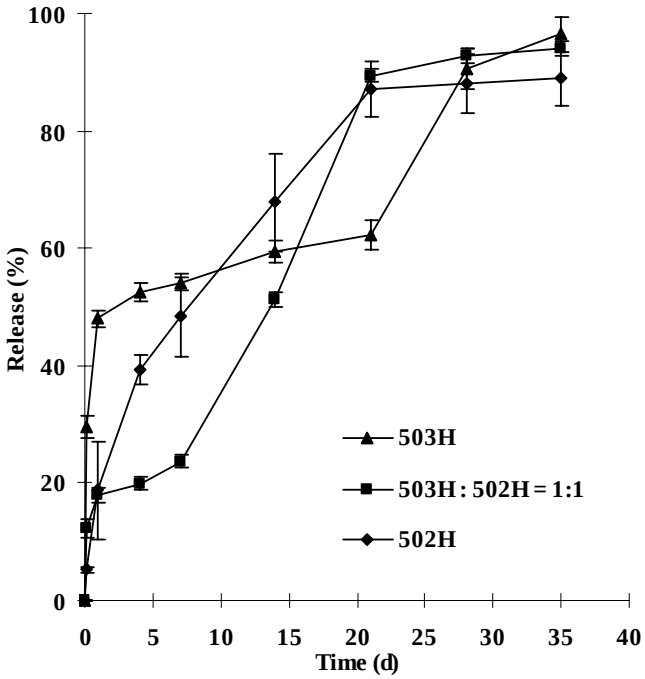


Fig. 53. Influence of PLGA type (RG 503H, RG 502H, and their 1:1 blend) on the leuprolide release from ISI (drug loading 5%, polymer concentration 30%).

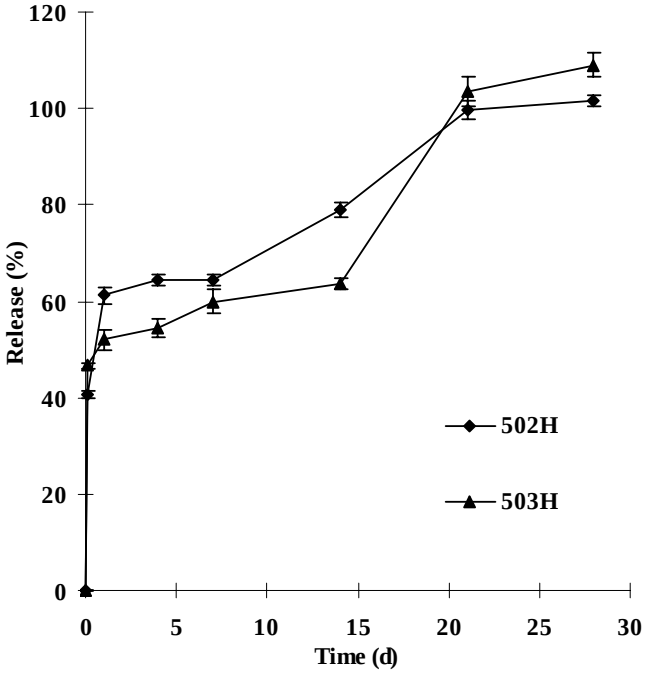


Fig. 54. Influence of PLGA type (RG 503H and RG 502H) on the leuprolide release from films (drug loading 5%).

Thus, the diffusion rate of the organic solvent from the polymer solution (in situ implants) or polymer solution droplets (ISM) into the aqueous release medium plays an important role in the solidification of the liquid polymer phase into implants or microparticles and therefore for the initial drug release. A rapid solvent diffusion leads to a rapid drug loss from the polymer solution and to a fast polymer precipitation, resulting in the porous polymer matrix (Graham et al., 1999); both favor a high initial release.

The lower initial release from ISM made of RG 502H correlated well with a slower NMP diffusion rate from the system compared to ISM prepared with the high molecular weight RG 503H (Fig. 55). This could possibly be attributed to the stronger interactions between polar solvent NMP and the more hydrophilic polymer RG 502H (smaller molecular size and more carboxyl endgroups, when compared to RG 503H) (Fig. 55).

The slower solvent diffusion rate resulted in a denser microparticle structure with the lower molecular weight RG 502H (Fig. 56). ISM from RG 503H had a very porous inner structure. The influence of the two PLGAs on the surface structure of the resulting microparticles was less significant (data not shown).

An ionic interaction between the terminal carboxylic endgroups of PLGA and the basic amino acids (arginine and histidine) of leuprolide acetate was reported by Okada et al. (Okada et al., 1994). This interaction hindered the drug diffusion and thus led to an increase in the encapsulation efficiency of microparticles prepared by a w/o/w solvent evaporation method. In in situ forming systems, wherein leuprolide acetate and PLGA are dissolved in hydrophilic solvents such as NMP or DMSO, interaction between leuprolide acetate and the carboxylic endgroups possibly occur. RG 502H has more terminal carboxyl groups (acidic number, 11 mg KOH/g vs. 4 mg KOH/g for RG 503H), which could lead to a stronger interaction and impede the drug diffusion from the system. This interaction could be a second reason contributing to the lower initial release besides the above described slower solvent diffusion rate and denser microparticle structure.

In conventional systems (microparticles and films), leuprolide acetate and PLGA were dissolved in a much less polar solvent mixture (methylene chloride and methanol). The above mentioned two interactions (hydrophilic-hydrophilic interaction between polymer solvent and PLGA, as well as the ionic interaction between leuprolide and PLGA) were less expected. RG 502H with smaller molecular weight led to a less viscous polymer solution than RG 503H, which

could play a similar role as decreasing the polymer concentration, resulting in formation of smaller and more porous microparticles with a faster drug release (Schlicher et al., 1997; Li, et al., 1995).

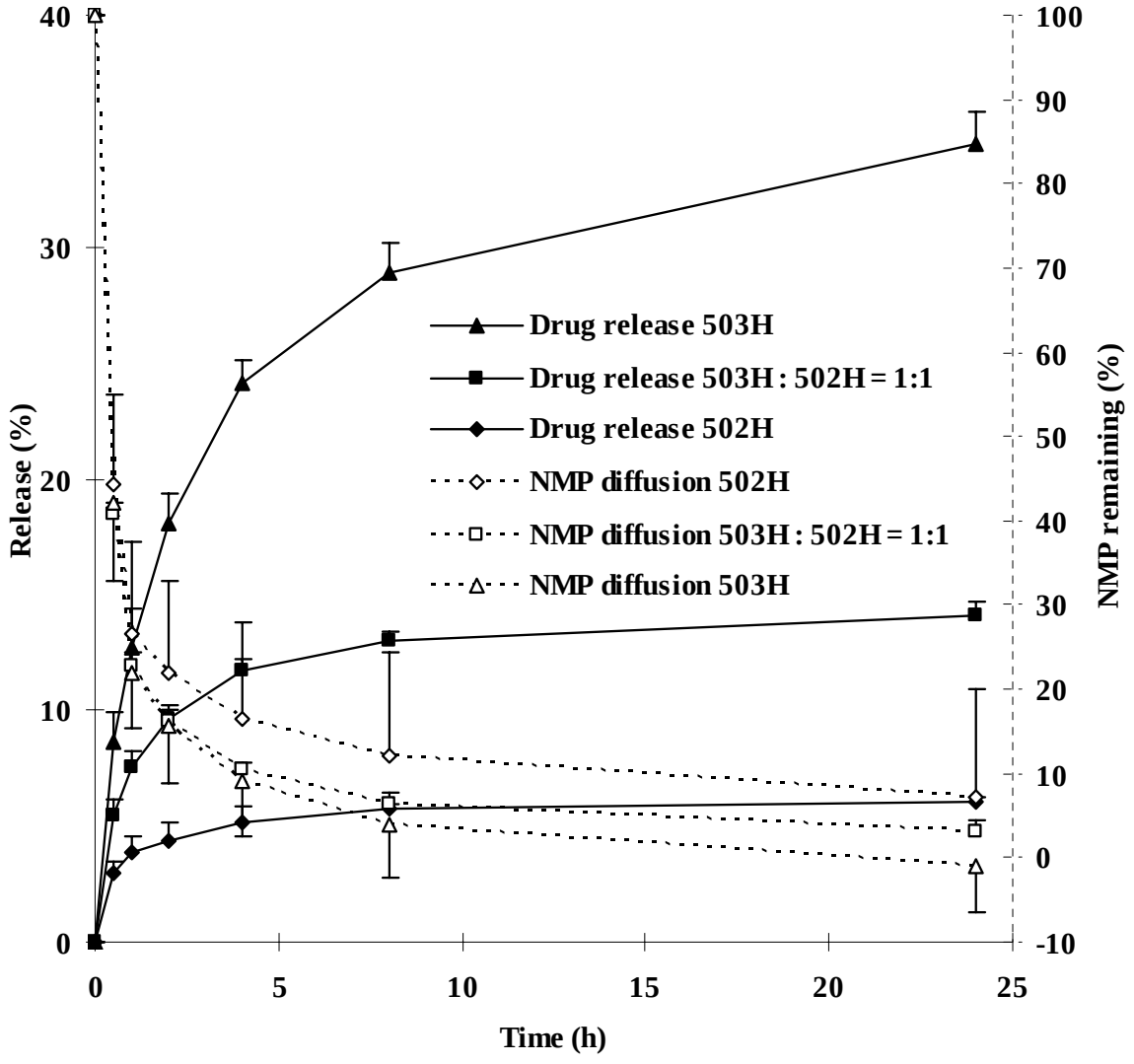
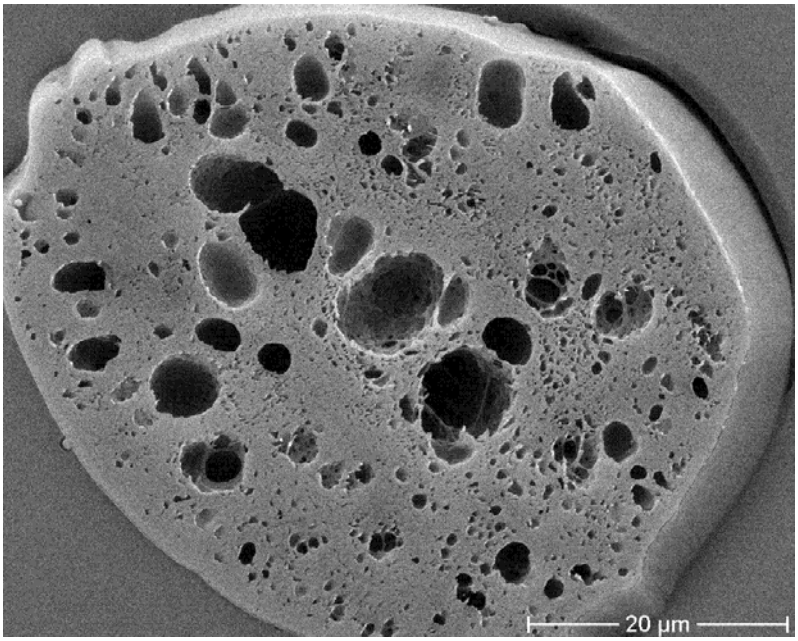


Fig. 55. Leuprolide release and NMP (solvent) diffusion from the ISM prepared with RG 503H, RG 502H, and their 1:1 blend (5% drug loading, 30% polymer concentration).

Cross-section of ISM from 503H



Cross-section of ISM from 502H

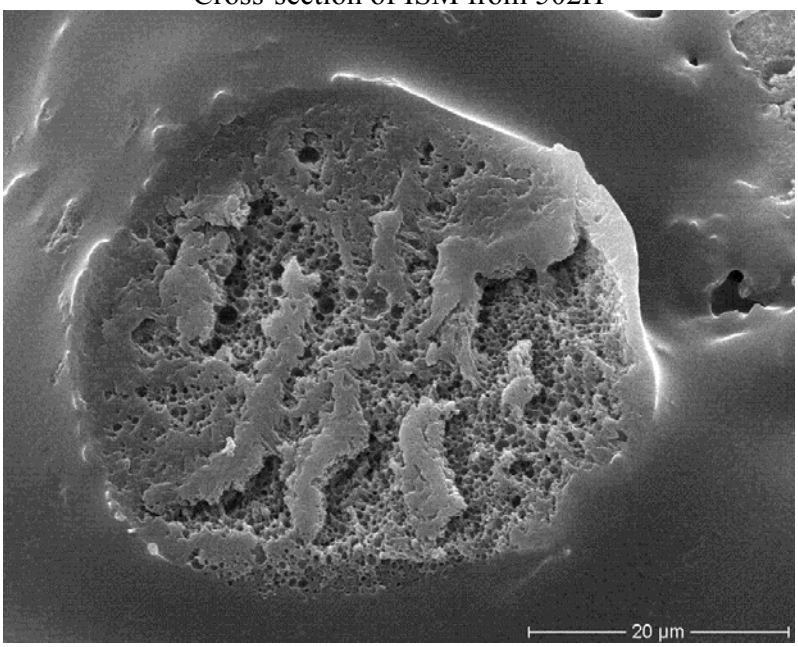


Fig. 56. Scanning electron micrographs of ISM prepared with RG 503H and RG 502H.

3.2.4.2. Influence of the polymer endgroup functionality

Having a similar molecular weight, RG 502 ISM led to a faster initial leuprolide release than RG 502H ISM (initial release for RG 502: 32.0%; RG 502H: 7.2%) (Fig. 57). Similar results were obtained with the higher, but similar molecular weight RG 503 with RG 503H (initial release 50.9 vs. 39.8%). RG 502 and 503 (end-capped) have terminal residues functionalized as esters; in contrast, RG 502H and 503H (uncapped) have terminal residues existing as free carboxylic acids. The presence of terminal carboxylic acids might result in interactions with leurpolide acetate, leading to a slower drug release. In addition, the more hydrophilic nature of the uncapped polymer might lead to a higher affinity to the hydrophilic solvent NMP, resulting in a slower solvent diffusion from the polymer solution and polymer precipitation and hence denser microparticles and a slower initial release.

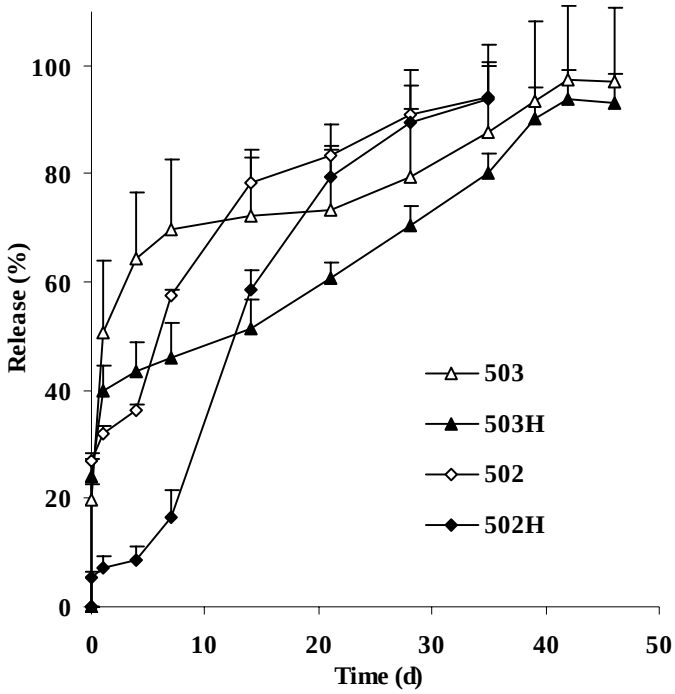


Fig. 57. Influence of PLGA type (end-capped polymer RG 503, RG 502, uncapped polymer RG 503H, RG 502H) on the leuprolide release from ISM (5% drug loading, 30% polymer concentration).

In agreement with the result from uncapped PLGA and for the same reasons, RG 502 also led to a lower initial release than RG 503 (30.2 vs. 50.9%) (Fig. 57), which could be attributed to the different polymer molecular weight.

3.2.4.3. Blending poly(D,L-lactide) R 203H with R 202H

Based on results on PLA R 203H microparticles prepared by a solvent evaporation method, which managed to suppress the testosterone level in rats for 6 months (Woo et al., 2002), ISM-systems were formulated with 203H for a targeted 6 months controlled delivery of leuprolide.

ISM from PLA 203H exhibited a high initial release (57.4% in the first day), followed by a continuous slow drug release of approximately 25% leuprolide until day 190, on which the experiment was terminated (Fig. 58). As expected, ISM prepared from the lower molecular weight R 202H had a much lower initial release (8.7%) due to its smaller molecular size and the presence of more terminal carboxylic groups. After the first day, the drug release from R 202H ISM was faster and approximately 83% of drug was released until day 150.

To reduce the high initial release of R 203H ISM and to prolong the drug release for R 202H ISM, ISM were prepared with a 1:1 blend of the two PLA grades. This formulation had a 20% initial drug release, followed by a continuous release of 65% drug until day 190.

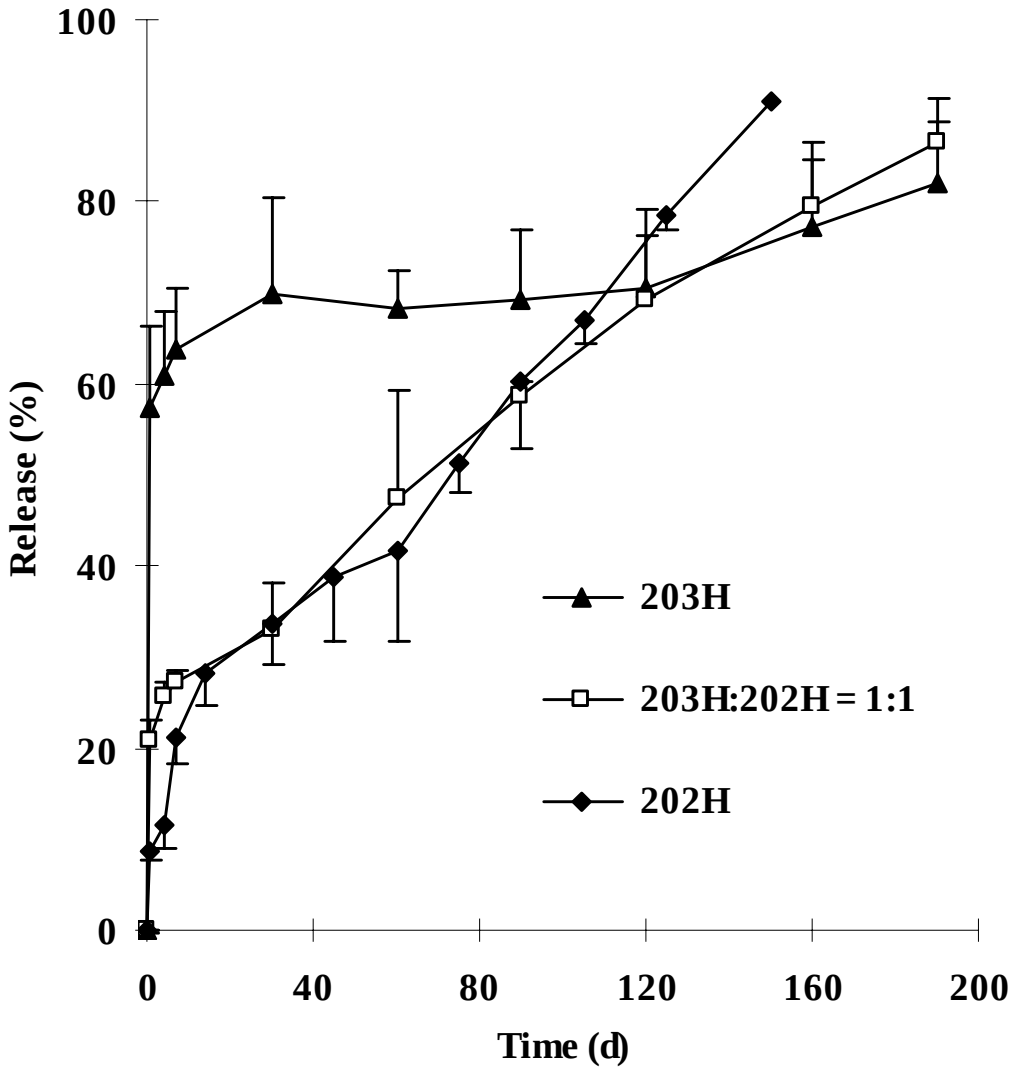


Fig. 58. Blending PLA R 203H and R 202H at ratio of 1:1 to reduce the initial release of ISM (drug loading 15%, polymer concentration 30%).