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Research paper

Modification of the tri-phasic drug release pattern of leuprolide acetate-loaded poly(lactide-co-glycolide) microparticles

Xiaosong Luan, Roland Bodmeier *

College of Pharmacy, Freie Universität Berlin, Berlin, Germany

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Abstract

Leuprolide acetate-loaded poly(lactide-co-glycolide) (PLGA RG503H) microparticles prepared by the solvent evaporation method had a tri-phasic drug release pattern over a duration of up to 2 months. An initial release was followed by a slow drug release phase and a final rapid drug release. The objective of this study was to identify parameters, which shift the release profile from the tri-phasic to a more continuous release profile. Varying formulation and processing parameters (e.g., drug loading, volume of the external aqueous phase, using low molecular weight PLGA, different microparticle drying methods) affected the initial release (burst) but did not influence the drug release thereafter. The addition of the hydrophilic polymer polyvinylpyrrolidone (PVP) led to the formation of more porous microparticles. This influenced the initial release but did not change the tri-phasic drug release pattern. The inclusion of medium chain triglycerides (MCT) successfully shifted the tri-phasic pattern to a continuous release profile. MCT accelerated the leuprolide release in the second, slow release phase and reduced it in the final rapid release phase. MCT led to the formation of microparticles with an irregular surface and a highly porous inner structure. Differential scanning calorimetry (DSC) revealed a high encapsulation efficiency of MCT (88–105%) in the microparticles and an unchanged glass transition temperature (T_g) of PLGA.

Keywords: Tri-phasic release; Initial release; Leuprolide acetate; Polyvinylpyrrolidone; Medium chain triglyceride

1. Introduction

The drug release from PLGA microparticles often has a tri-phasic pattern in vitro [1–4] as well as in vivo [5]. A fast initial release phase (burst) followed by a second slow release phase lasting days or weeks and a third rapid release phase is seen in particular with peptide- and protein drugs. Except for vaccines [6], a tri-phasic drug release is generally not desirable for most drug therapies. Insufficient drug may be delivered to maintain the desired pharmacological effect in the slow release phase and toxicity problems may occur during the rapid initial and third release phases because of too high drug levels.

The initial release (burst) is commonly attributed to drug being released from the surface of the microparticles [7,8] or to easily accessible drug, for example, in the case of highly porous microparticles [9,10]. Thereafter, a diffusion-controlled slower release phase follows. Finally, when the molecular weight of PLGA approaches a certain lower threshold, the weight of the microparticles decreases rapidly and an erosion-controlled rapid release phase occurs [11]. Recently, the formation of a nonporous film around the microparticles after incubation in the release medium has been reported [4]. The decreased surface porosity of the microparticles led to a reduced drug permeability and resulted in the slow release phase.

Leuprolide acetate is a synthetic superagonist of the luteinizing hormone-releasing hormone (LH-RH) for the treatment of prostate cancer. Leuprolide acetate-loaded PLGA microparticles have been prepared to deliver the drug over time spans of 1–6 months [12–14]. The administration

* Corresponding author. College of Pharmacy, Freie Universität Berlin, Kelchstr. 31, D-12169 Berlin, Germany. Tel.: +49 30 83850643; fax: +49 30 83850692.
E-mail address: bodmeier@zedat.fu-berlin.de (R. Bodmeier).

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of these formulations leads to a typical initial testosterone elevation and a subsequent testosterone suppression. The initial rise in testosterone level is due to the stimulation of the pituitary LH-RH receptor and the gonadotropin secretion. However, a continuous high dose of leuprolide produces antagonistic inhibitory effects on the pituitary gonadotropin secretion and testicular steroidogenesis (chemical castration) [15]. The transient stimulatory phase of testosterone may persist for several weeks in humans before chemical castration is achieved. During this time, an accelerated tumor growth may be observed [16]. We speculated that an increased leuprolide release in the slow release phase may accelerate its inhibitory effect and thus shorten the undesired transient stimulatory phase.

The objective of this study was thus to prepare leuprolide acetate-containing microparticles with a more continuous and not tri-phasic release pattern by a solvent evaporation (cosolvent) method.

2. Materials and methods

2.1. Materials

The following chemicals were used as received: poly(D,L-lactide-co-glycolide) (PLGA, 50:50), Resomer® RG 503H and RG 502H (inherent viscosity of a 0.1% polymer solution in chloroform at 25 °C is 0.19 and 0.38 dl/g, respectively, Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany), leuprolide acetate (leuprolide, Lipotec S.A. Barcelona, Spain), polyvinylpyrrolidone (PVP) Kollidon® 12 PF and 17 PF (average molecular weight of 2000–3000 and 7000–11,000, respectively, BASF AG, Ludwigshafen, Germany), medium chain triglyceride (MCT) (Miglyol 812 N®, Synopharm GmbH, Barsbüttel, Germany), polyvinyl alcohol (PVA) (Mowiol40-88, Clariant GmbH, Frankfurt am Main, Germany), methylene chloride, methanol, sodium chloride (Merck KGaA, Darmstadt, Germany), polyethylene sorbitan monooleate (Tween® 80), and mannitol (Sigma–Aldrich Chemie GmbH, Steinheim, Germany), acetonitrile (Rotisolv® HPLC Gradient Grade, Carl Roth GmbH + Co, Karlsruhe, Germany).

2.2. Preparation of microparticles by the cosolvent method

Three hundred and fifty milligrams of PLGA and the 88 mg leuprolide acetate were dissolved in a solvent mixture of 2.5 g methylene chloride and 0.5 g methanol. This solution was emulsified into 800 ml of 0.25% w/w PVA aqueous solution (external phase) using a homogenizer (Ultra-Turrax T 25, Janke & Kunkel IKA, Labortechnik, Staufen, Germany) at 8000 rpm. The dispersion was stirred at 400 rpm for 2 h with a magnetic stirrer (Variomag®Electronicrührer, Multipoint HP 6, H+P Labortechnik GmbH, Oberschleissheim, Germany) to extract and evaporate the organic solvents. The solidified microparticles were recovered by filtration and vacuum-dried for 1 d at room temperature or freeze-dried (microparticles prepared with PVP or MCT addition) [13,14].

The standard formulation was kept as 20% drug loading based on PLGA plus drug, PLGA RG 503 H as polymer carrier, 800 ml external aqueous phase, and vacuum-dried unless specifically mentioned.

The additives, PVP (K12 and K17) and MCT, were added to the leuprolide-containing polymer solution. The theoretical content (%, w/w) was calculated based on the weight of polymer and leuprolide.

2.3. Freeze-drying of an aqueous microparticle suspension

After filtration, microparticles (≈450 mg) were suspended in 900 mg of 16% (w/w) (20% in the case of the MCT addition) mannitol solution. The suspension was frozen at −40 °C for 2 h and freeze-dried (primary drying: chamber pressure 0.01 mbar over 24 h with a shelf temperature of −15 °C, second drying: chamber pressure 0.01 mbar with a shelf temperature of 20 °C for 12 h) (Gamma 2-20, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany).

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

Leuprolide acetate-containing PLGA microparticles (≈10 mg) were suspended in a mixture of 10 ml phosphate buffer (1/30 M, pH 7.0) and 2 ml methylene chloride. The suspension was shaken for 24 h at ambient temperature on a horizontal shaker (HS 501 Digital, Janke & Kunkel, IKA-Labortechnik, Staufen, Germany). After centrifugation, the aqueous supernatant was collected. The concentration of leuprolide in the supernatant was analyzed by RP-HPLC (SCL-10 A VP, Shimadzu, Japan), C18 Eurospher-100 column (150 × 4 mm, Knauer GmbH, Germany) (mobile phase: phosphate buffer (1/30 M, pH 7), acetonitrile 70:30 v/v; flow rate: 1.2 ml/min; UV detection at 280 nm) ($n = 2$). The percent encapsulation efficiency is calculated as (actual drug loading/theoretical drug loading) * 100%. With formulations containing PVP or MCT, the encapsulation efficiency was calculated based on 100% PVP and MCT entrapment.

2.5. In vitro drug release

The in vitro release was determined by suspending the microparticles (≈10 mg) in 6 ml phosphate buffer (1/30 M, pH 7.0, 0.01% w/w Tween 80, and 0.01% w/w sodium azide). The suspensions were incubated in glass test tubes at 37 °C in an incubation shaker (GFL 3033, Gesellschaft für Labortechnik GmbH & Co. KG, Burgwedel, Germany). The samples were centrifuged at predetermined time points. Five milliliters of supernatant was collected and replaced. The leuprolide concentration was determined by UV (UV–vis scanning spectrophotometer 2101 PC, Shimadzu, Kyoto, Japan) at 279 nm ($n = 2$). In the case of PVP-containing

microparticles, the leuprolide concentration was determined by RP-HPLC (method described in Section 2.4). UV-absorbance of leuprolide solutions at 37 °C did not change during sample intervals up to 7 d (longest sampling span) confirmed RP-HPLC (data not shown).

2.6. Particle size distribution

Microparticles were suspended in 0.1% (w/w) Tween 80 aqueous solution and the size distribution was determined by the laser diffractometry (LD) (LS 230, Beckman Coulter GmbH, Krefeld, Germany).

2.7. Morphology of the microparticles

Scanning electron microscopy (SEM) was used to image the surface and interior morphology of the microparticles. Freeze-dried microparticles were washed once with distilled water to remove mannitol and then vacuum-dried. To investigate the inner structure, the particles were dispersed in a solvent-free glue (UHU®, UHU GmbH, Baden, Germany). After drying in a desiccator, the hardened matrix was cooled in liquid N_2 and then cut with a razor blade. Samples were sputtered under an argon atmosphere with gold to a thickness of 8 nm (SCD 040, Bal-Tec GmbH, Witten, Germany), and were then observed with a scanning electron microscope (S-4000, Hitachi High-Technologies Europe GmbH, Krefeld, Germany).

2.8. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was used to quantitate MCT in the microparticles and to determine the glass transition temperature (T_g) of the PLGA.

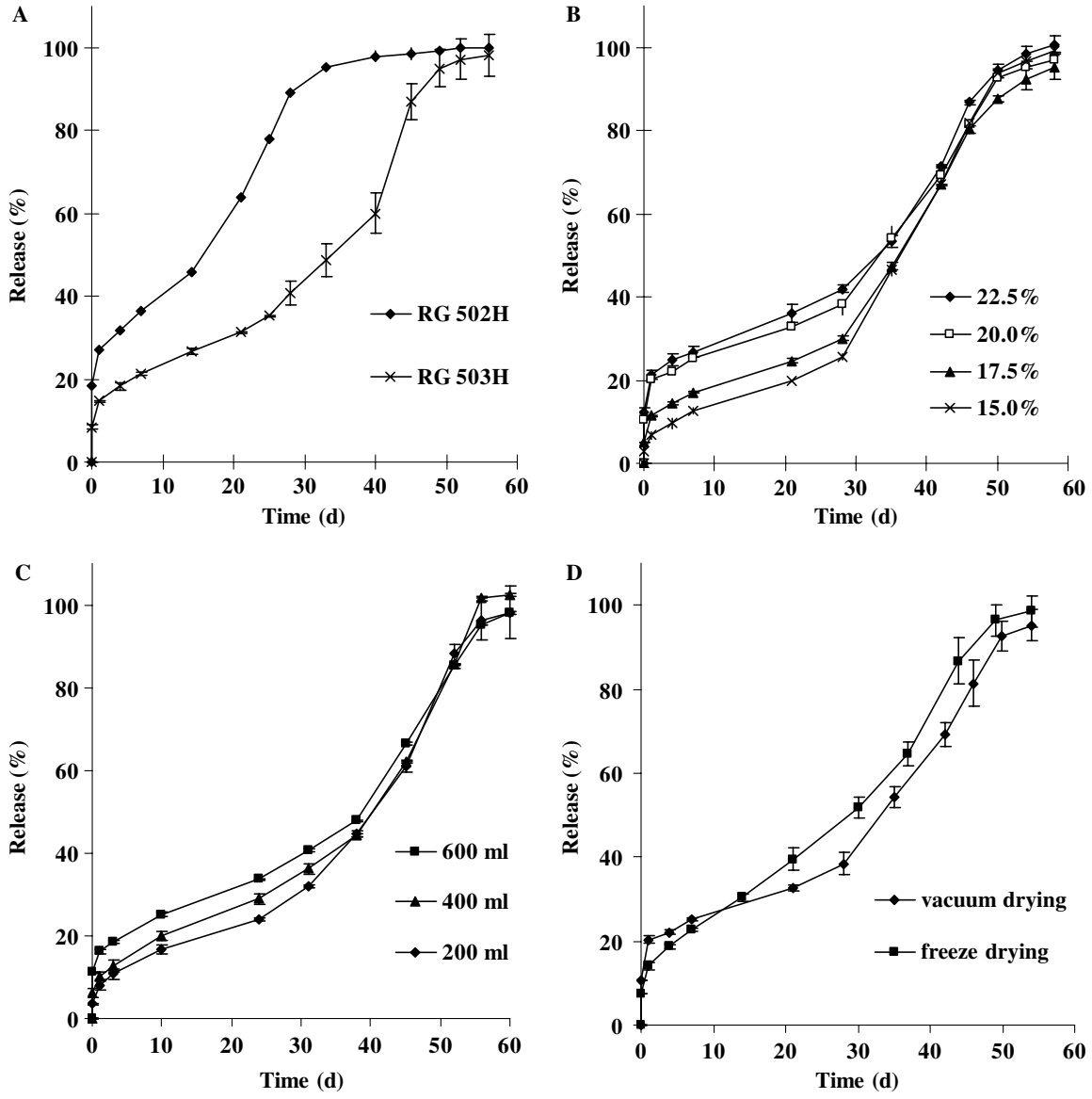

Fig. 1. Influence of formulation and process parameters on the leuprolide release from microparticles. (A) Type of the PLGA, (B) theoretical drug loading, (C) volume of the external phase, and (D) drying method.

The sample (≈5 mg, MCT standard or microparticles) was weighed in an aluminum pan (Al-Crucibles, 40 μl) and then analyzed (DSC 821, Mettler Toledo AG, Giessen, Germany). In a typical analysis, 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 constant rate of 10 °C/min. The T_g was determined during the second heating run.

The amount of MCT in the microparticles was quantified from the melting enthalpy of MCT, which was ≈92.2 J/g. MCT entrapment was calculated by comparing the melting enthalpies of MCT-containing microparticles with that of pure MCT ($n = 2$).

3. Results and discussion

The objective/strategy of this study was to increase the drug release in the second slow release phase of microparticles 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.

Table 1
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.

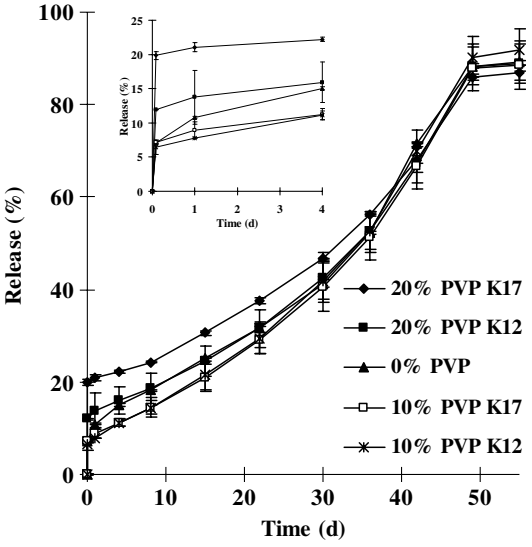

Fig. 2. Influence of the PVP K12- and K17-addition on the leuprolide release from PLGA microparticles.

3.1. Influence of various formulation and processing parameters on the release pattern

Varying the molecular weight of PLGA (Fig. 1A), the drug loading (Fig. 1B), the volume of the external phase

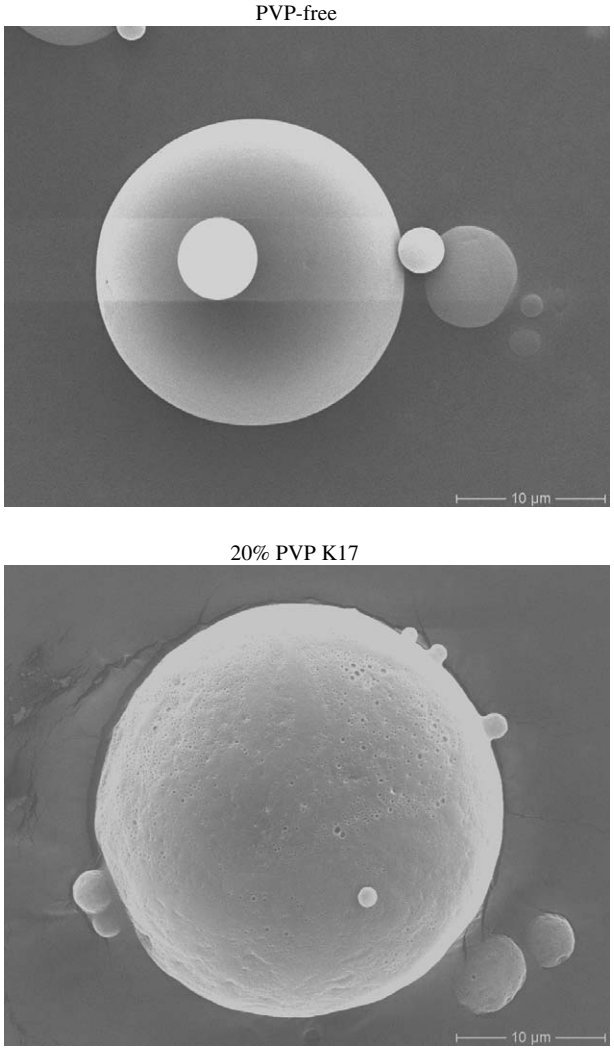

Fig. 3. Scanning electronic micrographs of microparticles prepared without or with 20% PVP K17.

Table 2
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.

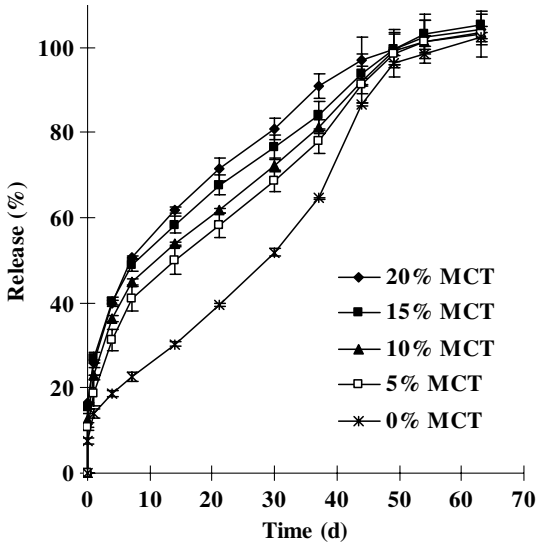

Fig. 4. Influence of the addition of medium chain triglycerides on the leuprolide release from microparticles.

(Fig. 1C), and the drying method (Fig. 1D) 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 [17,18]. Vacuum-drying led to a slightly higher initial release than freeze-drying.

These variations did not significantly affect the drug release after the initial release phase (Figs. 1B–D). The tri-phasic release profile was still obtained with all formulations. For RG 503H, the initial release during the first 24 h was dependent on the investigated parameters and was in the range of 7–21%. It was followed by a slow release phase over a period of ≈4 weeks, during which ≈30% drug was released. The final rapid release phase lasted for ≈3 weeks with a release of ≈50–60% of drug released. The theoretical drug loading, the volume of the external phase, and the drying method did not significantly affect the encapsulation efficiency; however, the low molecular weight PLGA (RG 502H) led to a lower encapsulation efficiency.

3.2. 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 [19]. The drug release from lysozyme-loaded in situ forming implant system was also increased by PVP-addition [17].

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 1). Ten percent of PVP slightly

Table 3
Quantification of the encapsulation efficiency of medium chain triglycerides (MCT) in 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

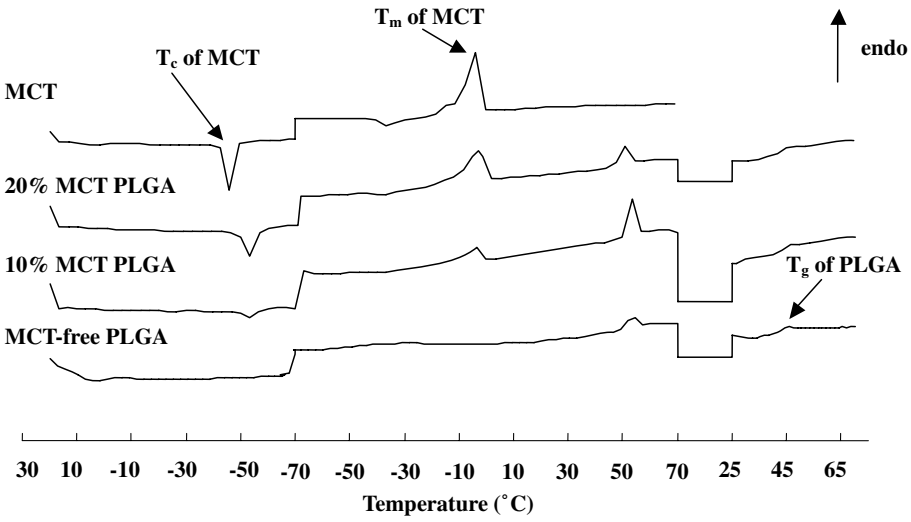

Fig. 5. DSC thermograms of pure medium chain triglycerides (MCT) and PLGA microparticles (0%, 10%, and 20% MCT), scanning process: the sample was cooled to −70 °C, followed by heating to 70 °C, cooling to 25 °C, and finally reheating to 70 °C at a rate of 10 °C/min.

decreased the initial release (Fig. 2), which could be attributed to the slight decrease in the actual drug loading (Table 1). 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

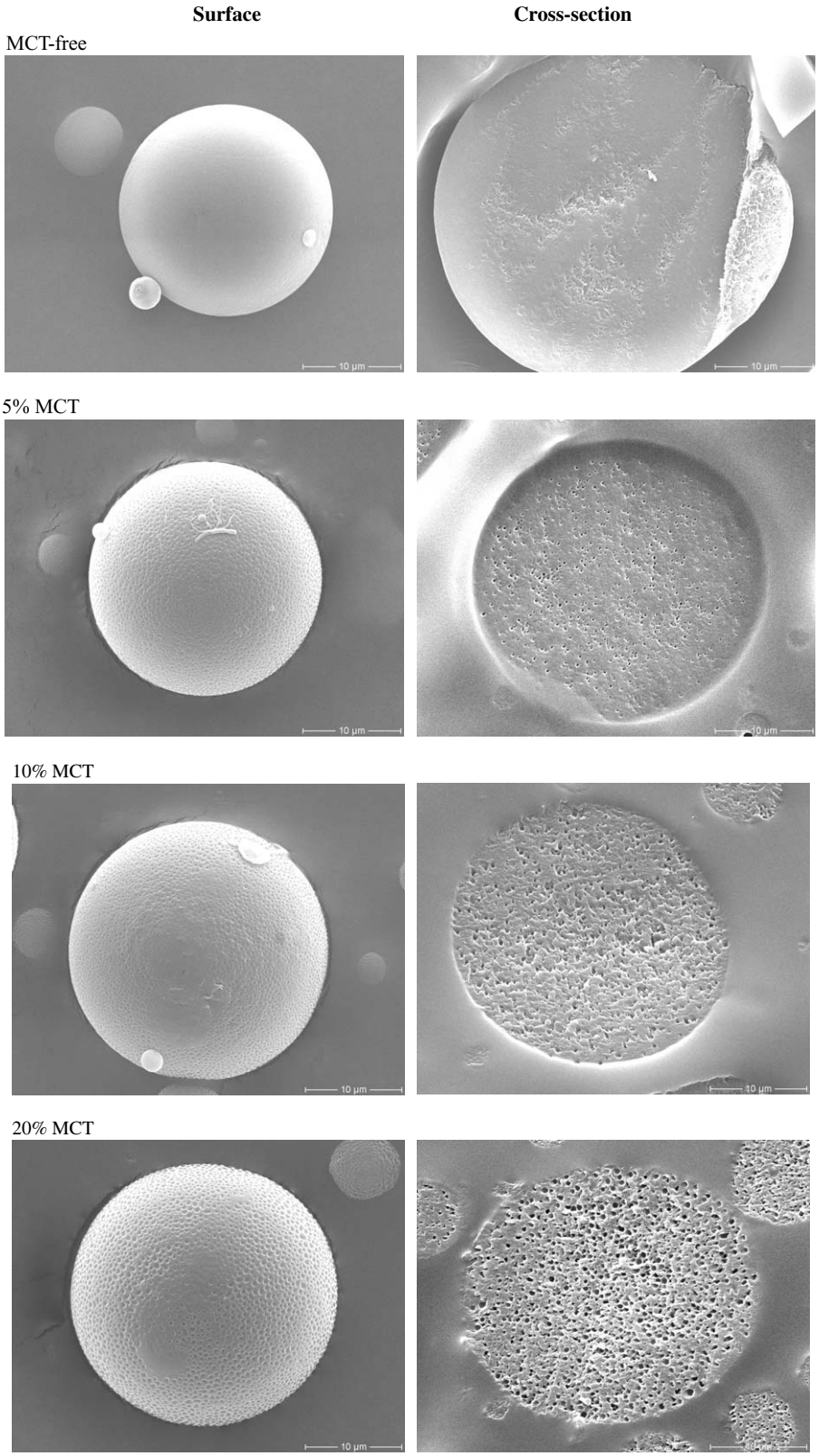

Fig. 6. Scanning electron micrographs of medium chain triglycerides (MCT)-free and -containing microparticles.

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. 3). 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. 2) despite the more porous structure of microparticles. This might be due to the nonporous film formation on the surface of microparticles after incubation [4]. 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 [9].

3.3. Influence of medium chain triglycerides on the release pattern

Medium chain triglycerides (MCT) are glycerol esters of medium chain fatty acids ranging from 6 to 12 carbon atoms. They are widely used for parenteral nutrition. MCT are water-immiscible and are poor solvents (or non-solvents) 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) [20] or a faster drug release (fatty acid esters) [21].

The MCT-addition did not influence the size of the microparticles (mean diameter: 27–29 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 2). 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. 4). 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. 4). 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 (Figs. 1B and C).

Differential scanning calorimetry (DSC) was used to quantify MCT in the microparticles (Fig. 5). Upon cooling of pure MCT (liquid at room temperature), a crystallization exotherm (T_c) occurred at −44 °C, which corresponded to the less orderly α-polymorph [22]. During the subsequent heating, a melting endotherm (T_m) was visible at −3 °C. With MCT-containing microparticles, the T_c shifted from −44 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% encapsulation efficiency for 10% and 20% MCT loading) (Table 3). MCT did not partition into the external aqueous phase but remained in the microparticles because it was insoluble in water [23].

The influence of MCT on the glass transition temperature (T_g) of PLGA was also analyzed by DSC (Fig. 5). In the first heating scan, PLGA showed an overlay peak around the T_g known as kinetic overshoot [24]. 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 ≈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.

The inclusion of MCT had a strong effect on the microstructure of the particles (Fig. 6). 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

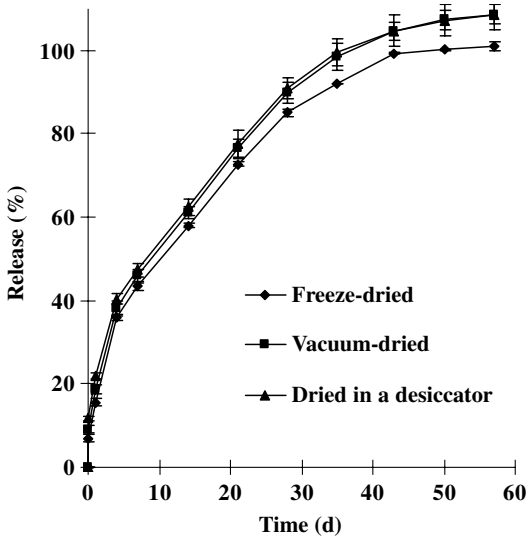

Fig. 7. Influence of drying on the leuprolide release from microparticles (20% medium chain triglycerides).

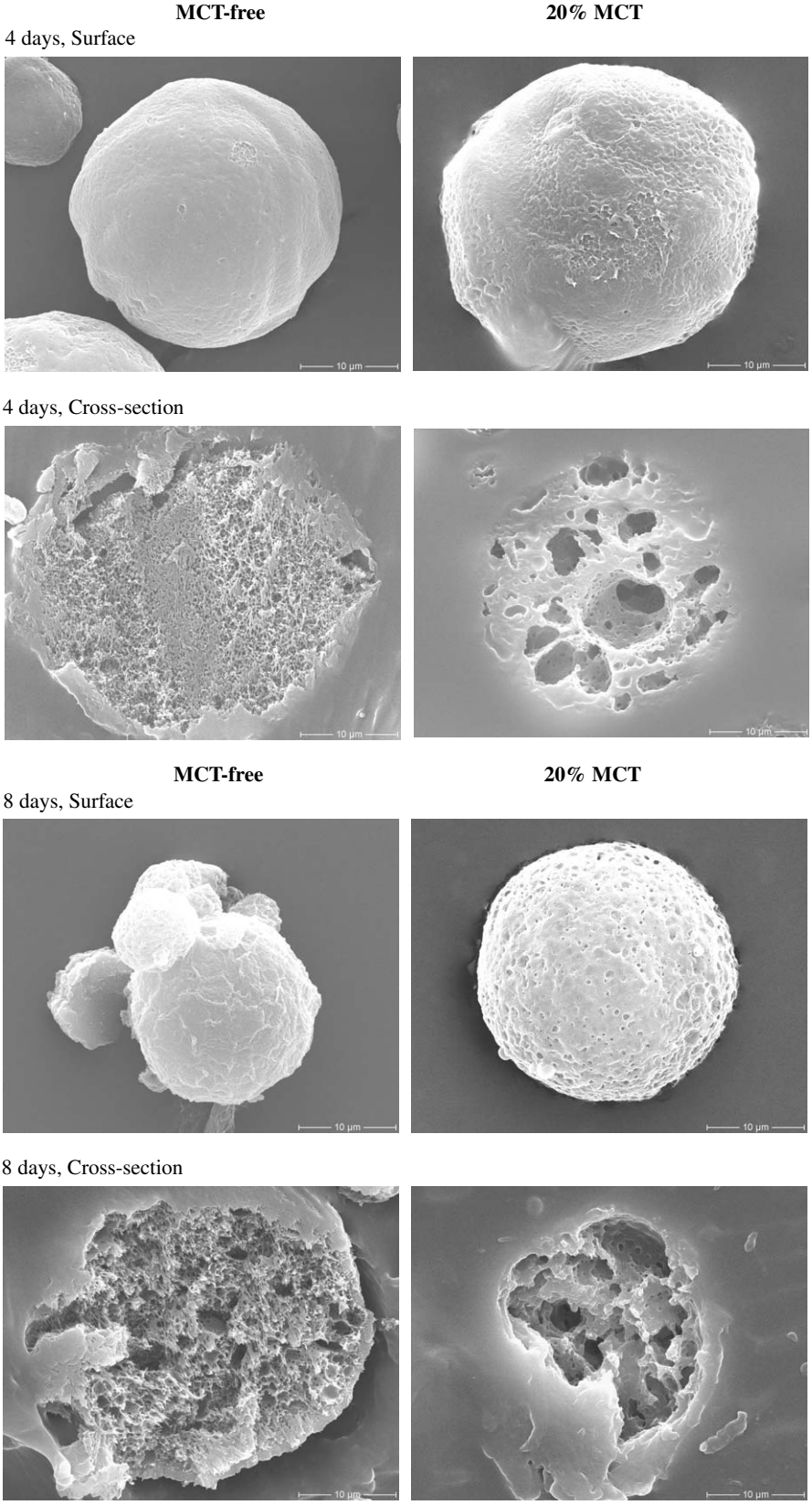

Fig. 8. Scanning electron micrographs of microparticles (without or with 20% medium chain triglycerides) after incubation in the release medium at 37 °C for 4 or 8 days.

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. 7). Therefore, evaporation of MCT under vacuum was not critical with respect 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 [17,25]. 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 poor solvent/nonsolvent for PLGA, which is water-immiscible but miscible with methylene chloride) might form MCT-rich regions (droplets) within 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 the microparticles after incubation in the release medium at 37 °C (Fig. 8). 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. 6). This led to the high water accessibility and thus a fast destruction/swelling of the polymer matrix.

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 release pattern of leuprolide acetate-loaded microparticles to a more continuous release in vitro. The impact of MCT addition on the in vivo release needs to be further investigated.

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