



## Research paper

## A detailed view of microparticle formation by in-process monitoring of the glass transition temperature

Kerstin Vay<sup>a,\*</sup>, Wolfgang Frieß<sup>b</sup>, Stefan Scheler<sup>a</sup><sup>a</sup>Sandoz GmbH, Sandoz Development Center Austria, Kundl, Austria<sup>b</sup>Department of Pharmacy, Pharmaceutical Technology and Biopharmaceutics, Ludwig-Maximilians-Universitaet Muenchen, Munich, Germany

## ARTICLE INFO

## Article history:

Received 21 October 2011

Accepted in revised form 29 February 2012

Available online 9 March 2012

## Keywords:

Glass transition temperature

Microspheres

Solvent evaporation

PLGA

Drug release

## ABSTRACT

Biodegradable poly(D,L-lactide-co-glycolide) microspheres were prepared by a well-controlled emulsion solvent extraction/evaporation process. The objective of this study was to investigate how drug release can be modified by changing the morphology of the polymer matrix. The matrix structure was controlled by the preparation temperature which was varied between 10 and 35 °C, thus changing the 4 weeks release pattern from almost linear kinetics to a sigmoidal profile with a distinct lag phase and furthermore decreasing the encapsulation efficiency. By monitoring the glass transition temperature during the extraction process, it was shown that the preparation temperature determines the particle morphology by influencing the time span in which the polymer chains were mobile and flexible during the extraction process.

Further factors determining drug release were found to be the molecular weight of the polymer and the rate of solvent removal. The latter, however, has also influence on the encapsulation efficiency with slow removal causing a higher drug loss. A secondary modification of the outer particle structure could be achieved by ethanolic post-treatment of the particles, which caused an extension of the lag phase and subsequently an accelerated drug release.

© 2012 Published by Elsevier B.V.

## 1. Introduction

By now a variety of biodegradable poly(lactide-co-glycolide) microspheres for the sustained release of several drug substances are commercially available. As many new drugs are peptides, proteins, or small molecules with low solubility and permeability and thus poor oral bioavailability research on these dosage forms is still of growing interest [1]. Biodegradable depot formulations offer numerous benefits. For example, they can help to reduce side effects and to enhance the therapeutic compliance and efficiency.

There is a variety of different preparation techniques to encapsulate an active ingredient into a PLGA matrix including coacervation, spray drying, melting techniques, methods using supercritical fluids, and emulsion solvent removal techniques. The latter is the oldest and most popular technique, especially for active ingredients with poor water solubility [2–4]. The influences of numerous process parameters on the characteristics and the drug release profile of the resulting microspheres have been extensively studied [5–8]. It was shown that drug release from these dosage forms de-

pends strongly on the molecular order, the amorphous, or crystalline state of the active ingredient and how it is embedded in the polymer matrix. Furthermore, the morphology of the surrounding polymer matrix is crucial for the resulting drug release rates.

Most of these studies concerned drug substances with good aqueous solubility and thus the results are not valid for drugs with poor water solubility. Furthermore, the majority of the experiments were performed on a laboratory batch scale without exact determination of the solvent removal rate and temperature of the external phase. We examined the structure formation mechanism of PLGA microspheres in a well-controlled emulsion solvent extraction/evaporation process on a 5 l batch scale by monitoring the glass transition temperature during solvent removal. The encapsulated drug was 4-[2-(4-(6-fluorobenzo[d]isoxazol-3-yl)-1-piperidyl)ethyl]-3-methyl-2,6-diazabicyclo[4.4.0]deca-1,3-dien-5-one, a substance with poor aqueous solubility.

The glass transition temperature ( $T_g$ ) of the polymer, which marks the change between the rigid, glassy, and the more flexible, rubbery state, is closely correlated to the amount of solvent in the polymer matrix. As the latter decreases in the course of the extraction step, the  $T_g$  should rise during the microsphere preparation process. It can be assumed that the morphology of the particle structure is only formed in the rubbery state, as long as  $T_g$  is below the process temperature. As the extraction rate is also controlled

\* Corresponding author. Sandoz GmbH, Sandoz Development Center Austria, Biochemiestrasse 10, A-6250 Kundl, Austria. Tel.: +43 5338 200 2835; fax: +43 5338 200460.

E-mail address: [kerstin.vay@sandoz.com](mailto:kerstin.vay@sandoz.com) (K. Vay).

by the process temperature, it can be expected that the mobility of the polymer chains and the duration of the rubbery phase are determined by the extraction temperature in a complex way [9]. We investigated the change of  $T_g$  during processing in relation to the applied extraction temperature and its influence on the particle morphology and functional characteristics.

As the  $T_g$  of the forming microparticles is strongly dependent on the solvent content in the polymer matrix, we assumed that the modification of the solvent removal rate should result in a change of the particle properties. These factors are supposed to be in close interaction with the molecular weight of the polymer, which was also varied in this study. Similarly and as a third factor investigated subsequent suspension of the resulting microspheres in a second solvent or solvent–water mixture should also change the particle morphology and thus alter the drug release profile.

Firstly, however, for a better understanding of the process data, we examined the influence of the solvent concentration on the glass transition temperature of the polymer in the presence and absence of the drug substance and correlated the experimentally derived  $T_g$  values with the theoretical ones predicted by the Gordon–Taylor equation.

## 2. Materials and methods

### 2.1. Materials

Poly(D,L-lactide-co-glycolide) 75:25 with different molecular weight were purchased from Boehringer Ingelheim, (Ingelheim, Germany): Resomer 753S (36,510 Da), Resomer 755S (57,670 Da), and Resomer 756S (107,200 Da) (Table 1). Methylene chloride analytical grade was obtained from Merck (Darmstadt, Germany), and TRIS (Tris(hydroxymethyl)aminomethane) from AppliChem (Darmstadt, Germany). 4-[2-4-(6-fluorobenzo[d]isoxazol-3-yl)-1-piperidyl]ethyl]-3-methyl-2,6-diazabicyclo[4.4.0]deca-1,3-dien-5-one was purchased from Jubilant Organosys (Mysore, India) and PVA 18-88 from Kuraray Europe GmbH (Frankfurt, Germany).

### 2.2. Microparticle preparation

An emulsification solvent extraction/evaporation technique was employed to prepare PLGA microparticles. For plain particles (free of drug) 4.8 g PLGA, for all other particle batches 3.8 g active agent and 5.1 g PLGA were dissolved in 46.1 g of methylene chloride and the solution was emulsified with 500 ml of 0.5% (w/v) povidone in 0.1 M Tris buffer (pH 9.0) (aqueous phase). The emulsion was fed into a 5 l jacketed glass reactor containing additional 3.5 l of the aqueous phase. By stirring for 5 h, the droplets were hardened by solvent extraction and evaporation with an air flow of 10 l/min (exactly regulated by a mass flow controller (red-y smart meter, Vögtlin instruments AG, Aesch, Switzerland)) and a stirring speed of 260 rpm (curved blade paddle-type stirrer). The obtained particles were separated by filtration and dried under vacuum in a desiccator. Different particle batches were produced by varying the extraction temperature between 10 and 35 °C. For further modifications, the air flow was reduced to 1.5 l/min and the stirring speed to 180 and 220 rpm. In another experiment, after collection on a filter, the particles were resuspended in 25% ethanol at 25 or 40 °C for 1 or 2 h, collected and dried as described above.

### 2.3. Analytical methods

#### 2.3.1. Thermal analysis – differential scanning calorimetry (DSC)

The glass transition temperature ( $T_g$ ) was determined by differential scanning calorimetry (DSC) using a DSC 823e/500 calorimeter (Mettler Toledo (Greifensee, Switzerland)). For in-process

**Table 1**

Characteristics of the different types of PLGA (75:25) used.

PLGA 75:25	Weight average molecular weight (Mw in Da)	Polydispersity (Mw/Mn)	End group	Inherent viscosity (ml/g)
RG 753 S	36,510	1.6	Alkyl	0.39
RG 755 S	56,020	1.6	Alkyl	0.56
RG 756 S	109,200	1.6	Alkyl	0.98

measurements, about 50 ml of the suspension was sampled (after 10, 30, 60, 120, 180, 240 min of processing and at the end of the process) and centrifuged at 4000 rpm for 2 min. Approximately 10 mg was weighed into 40 µl aluminum pans and hermetically sealed. As a reference, an empty aluminum pan was used. Samples were cooled down to –40 °C and then heated up to 80 °C at 10 °C/min to eliminate any sample history, cooled to –10 °C and then heated again up to 200 °C at 10 °C/min. For  $T_g$  determination, the data were analyzed using the STAR software (Mettler Toledo, Switzerland) and the midpoint of the corresponding glass transition was evaluated. The  $T_g$  was determined in duplicate at every time point of sampling.

#### 2.3.2. Particle size – single particle optical sizing (SPOS)

The particle size distribution was determined by single particle optical sizing (SPOS) with an Accusizer 780 particle sizing system (Anasysta, Santa Barbara, CA). Approximately 20 mg of microspheres was suspended in an aqueous solution of polysorbate 80 and deagglomerated by sonication. Per measurement, a minimum of 10,000 particles were sized.

#### 2.3.3. Particle morphology characterization – scanning electron microscopy (SEM)

The morphological structure of the particles was examined by scanning electron microscopy (JEOL JSM – 5310LV; JEOL Ltd., Tokyo, Japan). To study the internal structure, the particles were frozen in liquid nitrogen and cut with a razor blade. The specimens were sputtered with gold.

#### 2.3.4. Drug distribution – chemical imaging

The drug distribution inside the microspheres was analyzed by IR-imaging of cross-sections of the microspheres. To produce even cross-sections, the microspheres were as first step embedded in an epoxy resin, afterward cooled in liquid nitrogen, and cut with a high precision milling cutter (Leica EM Rapid, Leica Microsystems GmbH, Wetzlar, Germany).

From this cross-section, an IR image of 150 × 150 µm area with a pixel size of 1.56 µm was subsequently obtained with a Perkin Elmer Spectrum&Spotlight 400 IR-NIR (PerkinElmer LAS, Rodgau-Jügesheim, Germany) imaging system. The IR spectra were obtained between 4000 and 750 cm<sup>-1</sup> with a spectral resolution of 4 cm<sup>-1</sup> and 2 spectra averaged per pixel. To display the distribution of PLGA and the drug substance within the obtained image, the adsorption intensity images at 1643 cm<sup>-1</sup> (characteristic for the drug substance) and 1747 cm<sup>-1</sup> (characteristic for PLGA) were selected.

#### 2.3.5. Drug loading and in vitro dissolution studies – RP-HPLC

The drug load of the microspheres was determined by dissolving 20 mg microspheres in 25 ml acetonitrile using sonication and filling up to 200 ml with 0.1 M HCl. The drug concentration was determined by HPLC with a DAD detector at 235 nm and evaluated with the Chromeleon 6.7 software (Dionex, USA). A RP 18 column (20 × 2.1 mm) column was used with a flow rate of 1 ml/min and an injected volume of 50 µl. The mobile phase consisted

of a 75:25 (v/v) mixture of 0.25 M phosphate buffer (pH 8.5) and acetonitrile. A membrane filtered (0.45  $\mu\text{m}$  hydrophilic cellulose filter) clear test solution was analyzed.

For the *in vitro* release studies, the microspheres (17 mg) were placed in 100 ml of a 10 mmol phosphate buffer solution (pH 7.4) in a screw cap bottle and incubated in electrically heated aluminum blocks with drill holes on an orbital shaker (rotating speed  $200 \pm 10$  rpm) at 37 °C. At predefined time points, 0.2 ml samples were taken and analyzed by HPLC as described above.

### 2.3.6. Molecular weight of PLGA – SE-HPLC

30 mg microparticles were dissolved in 5 ml tetrahydrofuran. The molecular weight was determined by gel permeation chromatography with refractive index detection. Three columns (30  $\times$  8 mm) filled with a stationary phase of styrene–divinylbenzene copolymers with different pore sizes (0.1, 10, 100  $\mu\text{m}$ ) were connected in series for the size separation. THF was used as mobile phase, stabilized with 0.025% of butylhydroxytoluene.

## 3. Results and discussion

### 3.1. Factors influencing the glass transition temperature of the polymer

In microparticle formation, the structure of the polymer matrix is a result of the arrangement of the polymer chains and is thus strongly determined by the mobility of the chains before they become fixed in their final positions. This stage is achieved when the  $T_g$  falls below the process temperature and the polymer changes from a rubbery to a glassy state. During the preparation of PLGA microspheres, a variety of formulation and process parameters affect the  $T_g$  of the polymer and thus influence the microparticle formation [10]. One of them is the type of organic solvent utilized and its concentration profile during extraction. We investigated this effect by monitoring both the concentration of methylene chloride and the  $T_g$  of the hardening microspheres.

A significant decrease of the methylene chloride concentration in the microspheres took place in the first hour of processing (Fig. 1). During this period, most of the solvent is extracted. The further removal of the remaining solvent from the particles occurs slowly. Depending on the extraction temperature, a fraction between 1% and 3.5% of methylene chloride remains in the particles. The most effective removal of the solvent is achieved with a process temperature of 35 °C. The lower the process temperature the higher is the amount of residual solvent in the microspheres.

In order to analyze whether the plasticizing effect of the solvent is the only factor determining the  $T_g$  and, otherwise, to detect addi-

tional parameters of influence, the solvent concentrations were plotted against the measured  $T_g$  and regressed using the Gordon–Taylor equation (Eq. (1)).

$$T_{gm} = \frac{w_p T_{gp} + k w_s T_{gs}}{w_p + k w_s} \quad (1)$$

The Gordon–Taylor equation, which has been developed to calculate the glass transition temperature of polymer blends, has proved to be also applicable to predict the  $T_g$  of polymers which are plasticized by solvents, i.e., to predict the glass transition temperature of polymer/solvent mixtures ( $T_{gm}$ ). In equation 1,  $T_{gp}$  and  $T_{gs}$  are the glass transition temperatures of the polymer and the solvent while  $w_p$  and  $w_s$  are the weight fractions of the two components.  $k$  is a constant. In a first step, the relationship between solvent concentration and  $T_{gm}$  was investigated with placebo PLGA microspheres (Fig. 2). As expected, the  $T_{gm}$  decreases with increasing amount of residual solvent. The  $T_{gm}$  values range between 22.5 and 32.5 °C for amounts from 4.2% to 0.9% of methylene chloride (the amount of methylene chloride was calculated referring to the undried sample weight). Regressing the glass transition temperature on the amount of residual methylene chloride, a value of 36.0 °C was calculated for  $T_{gp}$  ( $T_g$  of the polymer with 0% methylene chloride in a fully hydrated state), which is consistent with the literature [11]. For  $T_{gs}$  a value of  $-173.0$  °C was obtained (corresponding to 100% methylene chloride), which also meets the literature values for the  $T_g$  of methylene chloride ranging between  $-170$  and  $-174$  °C [12]. Identical results are also obtained by using the Kelley–Bueche equation (Eq. (2)) instead of the Gordon–Taylor equation.

$$T_{gm} = \frac{\alpha_p V_p T_{gp} + \alpha_s (1 - V_p) T_{gs}}{\alpha_p V_p + \alpha_s (1 - V_p)} \quad (2)$$

where  $V_p$  is the volume fraction of the polymer,  $\alpha_p$  and  $\alpha_s$  are the volumetric expansion coefficients of the polymer and the solvent, respectively. The other symbols have the same meaning as before. In this case also, the expansion coefficients are close to values given in literature:  $\alpha_p = 5.3 \times 10^{-4} \text{ } ^\circ\text{C}^{-1}$  (Lit.: thermal expansion coefficient for polylactic acid (PLA) =  $7.4 \times 10^{-4} \text{ } ^\circ\text{C}^{-1}$  [13],  $\alpha_p$  for PLGA is assumed to be close to  $\alpha_p$  for PLA) and  $\alpha_s = 8.6 \times 10^{-4} \text{ } ^\circ\text{C}^{-1}$  (Lit.:  $1.4 \times 10^{-3} \text{ } ^\circ\text{C}^{-1}$ ) [14].

The samples were taken from the aqueous suspension and the polymer matrix was therefore fully hydrated, reducing the  $T_g$  of the polymer from 54 °C in the dry state to 36 °C. Methylene chloride has a much stronger impact on the glass transition temperature than the water molecules, reflected by a decreasing  $T_g$  with rising methylene chloride concentrations. Overall, for the placebo

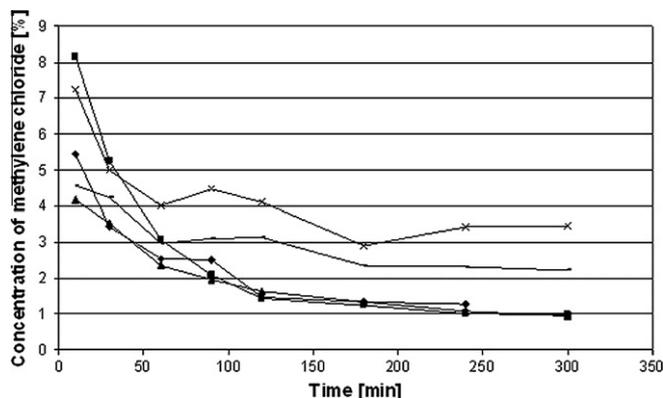


Fig. 1. Concentration of methylene chloride in PLGA microspheres during solvent extraction (referring to solvated and hydrated particles) at 10 °C (x), 20 °C (---), 27.5 °C (-▲-), 30 °C (-■-), and 35 °C (-◆-).

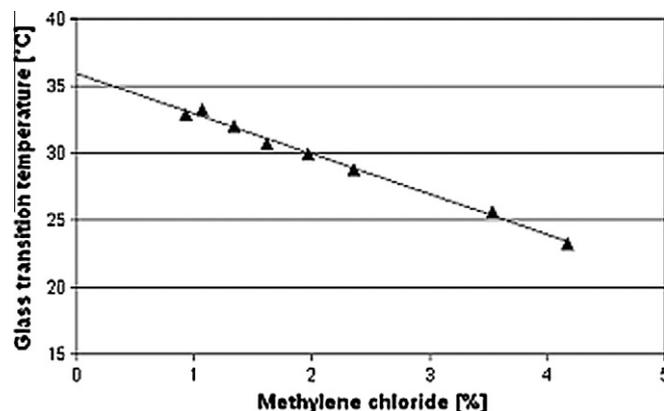


Fig. 2. Correlation between concentration of methylene chloride and  $T_g$  for plain microspheres: measured values (▲) and regression line calculated by the Gordon–Taylor equation.

microparticles the experimentally determined glass transition temperatures at different methylene chloride concentrations are in good agreement with the calculated values and hence other factors co-influencing the  $T_g$  can be excluded.

At the beginning of the process, the polymer is fully solvated by methylene chloride. During processing, the methylene chloride is extracted from the hardening particles and in return water diffuses into the particles. Both methylene chloride and water have a plasticizing effect on the  $T_g$  of the PLGA. If poly(lactide-co-glycolide) copolymers undergo the transition between their glassy and rubbery state, the molecular relaxation time changes. The polymer chains get more mobile and molecular deformation occurs [15–17]. The small solvent molecules embed themselves between the molecules of the amorphous solid and increase the spacing and free volume of the sample, resulting in an increased degree of molecular mobility. An increase of the  $T_g$ , called antiplasticization, can for example occur by the addition of a drug substance to the polymer matrix [18].

In case of drug loaded PLGA microspheres, a similar correlation was found. However, for these particles, experimentally determined  $T_g$  values deviated stronger from the theoretical  $T_g$  values (Fig. 3). Starting with about the same  $T_1$  value for the fully hydrated microparticles as in case of placebo microparticles, the  $T_g$  decrease is steeper with rising methylene chloride concentration. As can be seen in SEM, the active ingredient is deposited in the polymer matrix mainly in crystalline form and only a very small portion is molecularly dispersed [19]. The crystalline fraction is not active with respect to the  $T_g$  depression. When the methylene chloride in the particles increases, more drug becomes dissolved, which obviously causes a substantial additional plasticizing effect.

### 3.1.1. Influence on the particle morphology

Previous experiments showed that particles with different drug release and morphology, specifically different pore size distribution, can be obtained when the extraction temperature was varied between 10 and 35 °C [19]. In order to understand the role of the process temperature on the structure formation, the extraction process was monitored. In a series of microparticle preparation processes at 10, 20, 27.5, 30, 32.5, and 35 °C, both the glass transition temperature of the polymer and the amount of residual methylene chloride within the particles were measured in intervals between 10 and 60 min. All other process variables like stirring speed or air flush through the reactor were kept constant. Fig. 4 shows the  $T_g$  changes during solvent extraction. In all experiments,

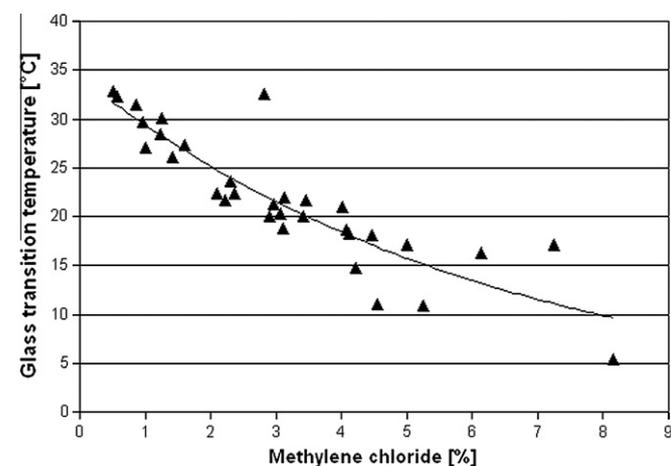


Fig. 3. Correlation between concentration of methylene chloride and  $T_g$  depression for drug loaded particles: measured values ( $\blacktriangle$ ) and regression line calculated by the Gordon–Taylor equation.

the first sample, which was withdrawn 10 min after the emulsion was fed into the reactor, had a  $T_g$  between 8 and 17 °C. In the further course of the preparation process, the samples showed an increase of the  $T_g$ . In all cases, the  $T_g$  did not increase at a constant rate. After an initial fast phase, the increase slowed down and in some cases came to a hold. The substantial change in  $T_g$  during the first 90 min of processing corresponds to the loss of methylene chloride from the microspheres (Fig. 1). Depending on the applied process temperature, the final  $T_g$  value ranged from 22 °C for 10 °C (Fig. 4a) to 34 °C (Fig. 4f) for 35 °C process temperatures. Except for the experiment at 10 °C, the  $T_g$  tended to approach the process temperature.

At the beginning of the process, the  $T_g$  of the microspheres was below 17 °C due to the high concentration of methylene chloride in the polymer matrix and rose with decreasing solvent concentration. Usually, mass transfer processes start with a high rate when the concentration gradient is high and decelerate when the reservoir depletes. This should be reflected in the  $T_g$  vs. time profiles. During the first 60 min (30 min in case of the 27.5 °C experiment) of the studied solvent evaporation process, a slight acceleration of the  $T_g$  increase could be observed. We assume that the solvent transfer from the particles into the extraction medium may be superimposed by another mass transfer within the particles which is caused by phase separation. Phase separation occurs when a solvated polymer precipitates and a polymer-rich phase separates from a solvent-rich phase. This process accelerates the desolvation of the polymer. Because of its poor solubility in PLGA, the drug will partition mainly into the solvent phase. However, if the volume of the solvent phase shrinks due to a proceeding solvent extraction, the drug is forced to redistribute into the polymer matrix where it intensifies the plasticizing effect of the remaining methylene chloride. This process could explain the temporary drop of the  $T_g$  at about 90 min which occurs only in drug loaded but not in plain microparticles (data not shown).

Overall, a higher variance at the first sampling time point may be due to the fact that the  $T_g$  is more difficult to analyze with high amounts of residual solvents, because the change of the DSC signal is small and prolonged. With decreasing amount of methylene chloride, the transition becomes more distinct.

The process temperature also influences the amount of residual solvent in the microspheres at the end of the process (Fig. 1). For this reason, there is no uniform final  $T_g$  value. The particles prepared at 10 °C with the highest amount of residual solvent (3.46%) show the lowest  $T_g$  (21.6 °C) at the end of the process and the particles prepared at 35 °C with only 0.93% residual solvent exhibit the highest  $T_g$  of 34 °C after 5 h.

The variation of the process temperature strongly determines the properties of the resulting microsphere. This is attributed to the flexibility of the polymer chains during processing. After 10 min, the  $T_g$  of the particles prepared at 10 °C is already above the process temperature. Thus, the polymer matrix becomes rigid and immobile and its structure is fixed within the first minutes of the process, resulting in a sponge-like morphology of the microspheres and a porous surface (Fig. 5a). A higher preparation temperature prolongs the time span, in which the process proceeds above the glass transition temperature. By applying a preparation temperature of 20 °C, the polymer is in a rubbery state for the first 50 min of the process and this time span becomes more and more extended for the batches prepared at higher process temperatures. At 35 °C, the  $T_g$  does not exceed the preparation temperature at all during the total 5 h of processing, leading to microspheres with a dense outer layer, a smooth surface, and a fine porous structure inside (Fig. 5b). This does not agree with the findings of Fu et al., who observed the opposite effect, i.e., a highly porous structure at high temperatures and a smooth surface at low temperatures [20]. In case of other ingredients, like a low molecular PLGA as used by

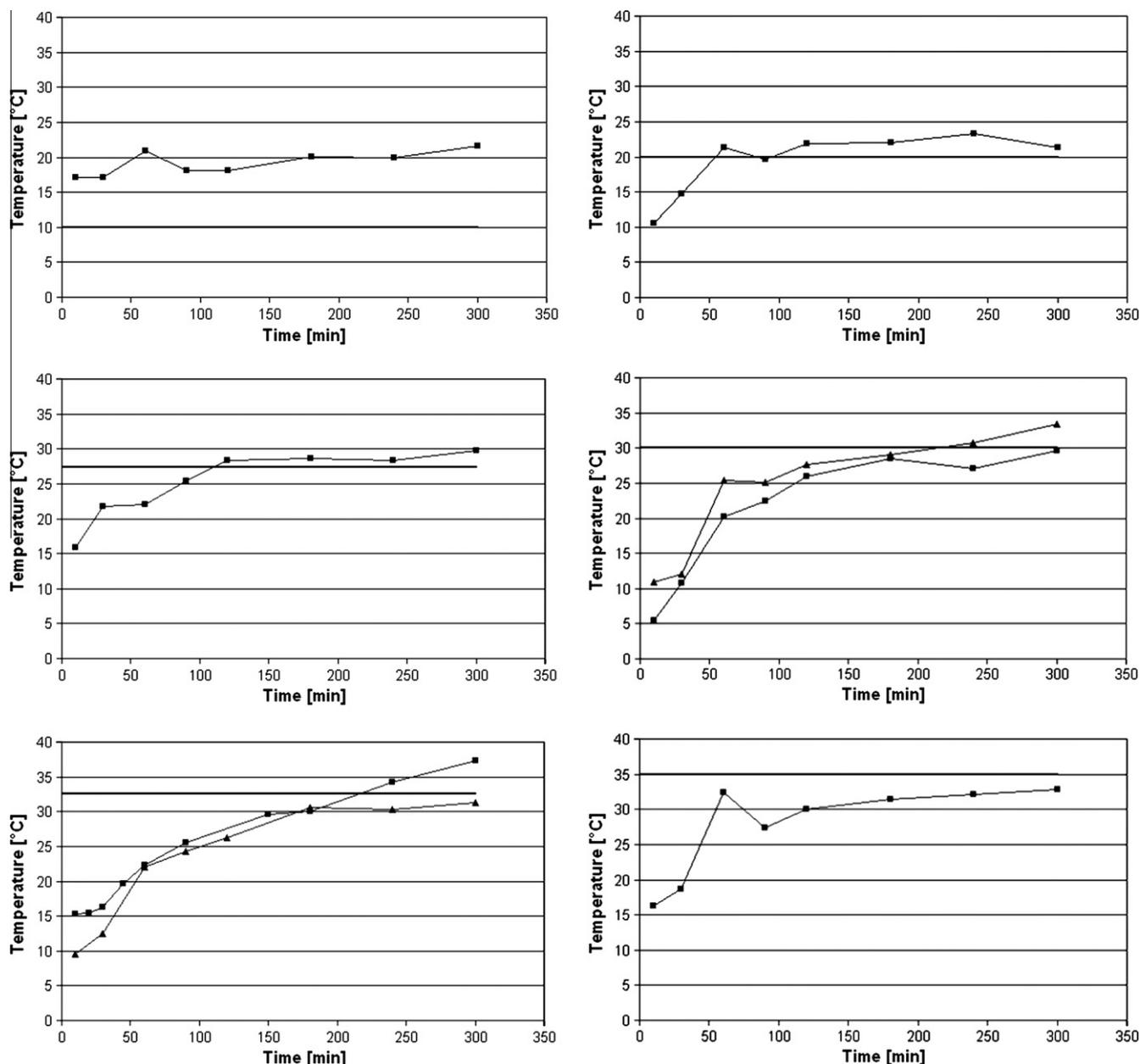


Fig. 4. Change of the  $T_g$  (—■—) depending on the applied preparation temperature (—) of 10 °C (a), 20 °C (b), 27.5 °C (c), 30 °C (d), 32.5 °C (e), and 35 °C (f).

Fu et al., these conditions might lead to other morphological properties.

### 3.1.2. Influence of the process temperature on the encapsulation efficiency and molecular weight

In this investigation, the highest drug encapsulation efficiency was found at low process temperatures of 10 and 20 °C and decreased with rising temperature of the external phase (Fig. 6a). Graves et al. found that the encapsulation efficiency is significantly influenced by the rate of polymer precipitation [21] and thus also by the applied preparation temperature. A fast solidification rate is considered to be beneficial for a high entrapment of drug substance. Whereas Yang et al. found the best encapsulation efficiency for the lowest and highest formation temperatures [22], we obtained the best encapsulation efficiency between 93% and 96% of the introduced drug substance only at low process temperatures. With increasing process temperature from 10 to 35 °C, we ob-

served a sharp drop of the encapsulation efficiency; however, after passing a minimum of 80% at 32.5 °C, the drug load started to increase again at 35 °C.

Not only the temperature of the external phase but also the phase ratio of dispersed and continuous phase is an important parameter for the fast precipitation of the polymer. As the amount of the dispersed phase is very low (1:100), the solidification rate is very fast even for low preparation temperatures. Soon after mixing both phases, the  $T_g$  of the polymer exceeds the process temperature leading to a slower diffusion rate of the dissolved drug substance in the polymer matrix and thus favoring the drug entrapment. By applying a higher temperature, the polymer molecules stay flexible and the drug molecules can diffuse through this soft matrix into the external phase resulting in reduced encapsulation efficiency.

Besides the encapsulation efficiency, the polymer molecular weight of the resulting microspheres is influenced by the applied

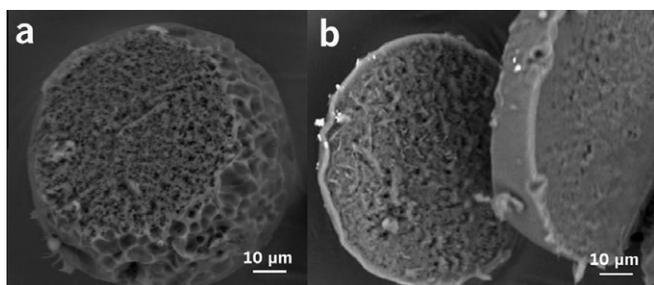


Fig. 5. SEM micrographs of microspheres prepared at 10 °C (a) and 35 °C (b).

process temperature (Fig. 6b). As expected, it decreased with increasing process temperature, as the hydrolysis of PLGA is accelerated by rising temperature [23]. No significant temperature dependence of the degradation rate could be detected between 10 and 27.5 °C. Within this temperature range, the molecular weight drops during the process from the initial value of 56.0 kDa by an average of approximately 2.0 kDa. A sharp further decline occurs between 30 and 35 °C with a decrease by up to 5.5 kDa which is about 10% of the initial value. The degree of polymer degradation correlates with the time span during which the process temperature exceeds  $T_g$ . In other studies, even more dramatic weight losses during the time of process temperature above the  $T_g$  of the polymer have been reported [24] and own preliminary experiments at higher temperatures (not shown here) revealed an about linear decrease of the molecular weight from 56 to 35 kDa between 30 and 50 °C. Thus, the process temperature not only affects the glass transition temperature and structure formation but also clearly leads to differences in the molecular weight. For all

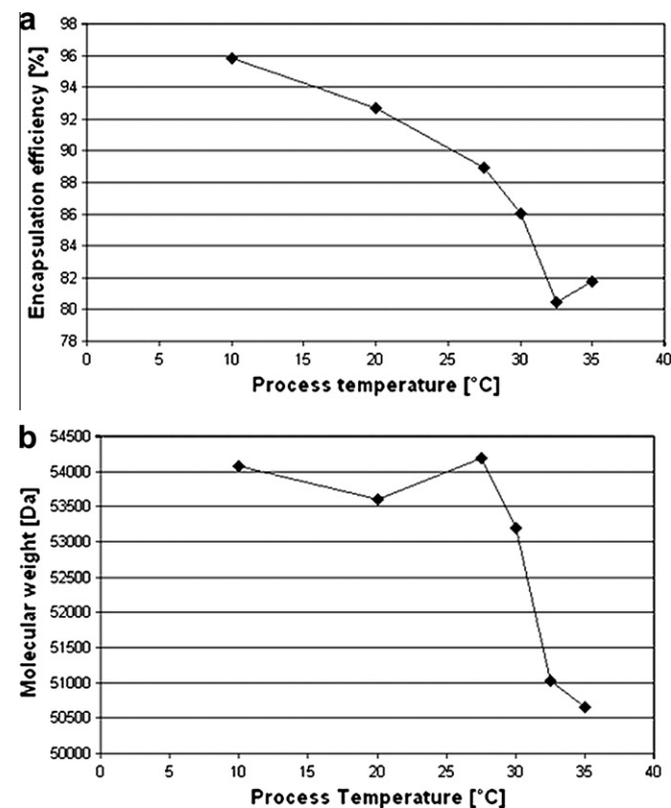


Fig. 6. Influence of the preparation temperature on the encapsulation efficiency (a) and the molecular weight (b) of the resulting microspheres.

these factors, the process temperature and the process time below and above the  $T_g$  appear to be crucial.

### 3.1.3. Influence of the process temperature on the drug release rate

The drug release from PLGA microspheres is influenced by a variety of process parameters in an emulsion solvent removal process [25,26] and by the resulting morphology of the microspheres [27]. These factors include the homogenization speed, the molecular weight of PLGA and PVA, and the PLGA concentration. Su et al. studied the influence of these parameters on the drug release profiles of microspheres at room temperature [28]. However, especially the process temperature during formation and hardening of the microspheres can be shown to have an outstanding impact on the structural properties and the drug release profiles. For this reason, in our study, special emphasis was laid on the extraction temperature. Fig. 7 shows the release profiles of microspheres which were prepared at different process temperatures.

The particles manufactured at 10 °C exhibited an almost linear release of the drug substance. Process temperatures of 20 °C and above caused more and more sigmoidal profiles. They start with a lag phase without any significant drug release, followed by a second phase with an accelerated release rate. The higher the applied process temperature the more pronounced is the lag phase and the sigmoidal drug release profile. At a process temperature of 30 °C, the lag phase reaches a maximum of 15 days, which was prolonged by applying higher temperatures.

As discussed before, at a preparation temperature of 10 °C the polymer matrix is not exposed to a temperature above its  $T_g$  resulting in microspheres with a sponge-like porous structure without any visible shell (Fig. 5a). As a result, water can rapidly penetrate into the microspheres and dissolve the drug substance out of the polymer matrix. Simultaneously, degradation of the polymer matrix starts throughout the whole particle. This causes a bulk erosion of the microspheres as described by Burkersroda et al. [29]. From a certain point in time, the drug release is a result of both drug diffusion and erosion of the polymer matrix. Because of the open-porous structure, the degradation products of the polymer hydrolysis diffuse out of the particles into the surrounding medium. This impedes the accumulation of these acidic hydrolysis products inside the microspheres, which otherwise would cause an autocatalytic acceleration of the degradation process [30]. For this reason, the drug release does not achieve the rate of the particles with a denser structure.

When the solvent extraction is performed at 20 °C, the process temperature exceeds the  $T_g$  of the microspheres for about 50 min. Thus, a more compact structure is formed and the drug release

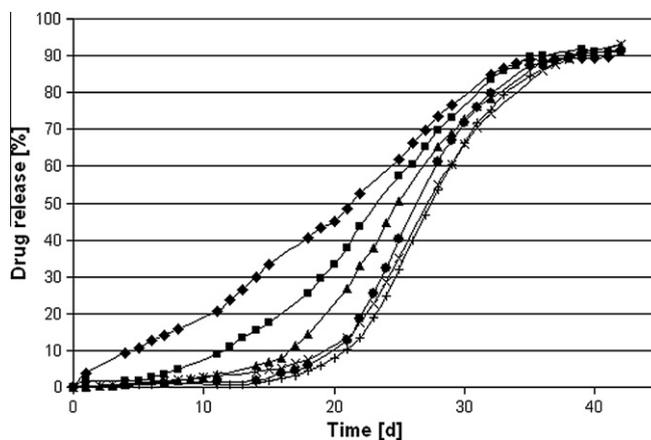


Fig. 7. Drug release profiles of the microspheres prepared at 10 °C (◆-), 20 °C (■-), 27.5 °C (-▲-), 30 °C (-x-), 32.5 °C (-+-), and 35 °C (-●-) (37 °C and pH 7.4).

does not start before day 5. The higher the applied preparation temperature, the longer the polymer matrix is exposed to a temperature above its  $T_g$ . In case of the batch prepared at 35 °C, the  $T_g$  does not reach the preparation temperature for the entire process. The polymer chains are flexible and mobile, and the polymer matrix remains in a rubbery state. A dense structure is formed during extraction, which has a crucial impact on the lag phase and the release characteristic of the resulting microspheres (Fig. 5b). The small water molecules can diffuse through the dense matrix into the microspheres and hydrolysis of the polymer chains starts. As the resulting fragments of the polymer chains cannot diffuse out of the particles, their accumulation leads to a significant drop of the pH in the interior of the spheres [30]. This drop of the pH accelerates the polymer degradation, and at a certain molecular weight, mostly between 10,000 and 15,000 Da the drug release rate strongly increases [31]. Furthermore, the pH drop leads to a better solubility of the drug substance. The drug applied in this study is only poorly soluble at neutral pH (0.06 mg/ml), but its solubility increases considerably with decreasing pH to 10.91 mg/ml at pH 3. Thus, not only the diffusion coefficient within the polymer is increased by acidic degradation but also the concentration of the saturated drug solution in the core of the particles rises as a result of the acidification. According to Fick's first law of diffusion, both factors increase the diffusion flux of drug out of the particles. For these reasons, a dense structure is essential for a sigmoidal drug release profile with a lag phase and subsequent fast drug release, whereas a porous outer surface of the microspheres leads to an almost linear release profile from the beginning of the dissolution testing. Plotting the lag-time against the time interval in which the process takes place above the  $T_{g(tT_p > T_g)}$  suggests that a distinct change in microparticle morphology, which is caused by treatment at temperatures above  $T_g$ , determines the length of the lag phase (Fig. 8).

In addition to the time span which the process takes place above the  $T_g$  of the polymer, also the difference between the applied process temperature and the  $T_g$  at a certain point in time might influence the resulting morphology of the particles. For this reason, the area between the curves for the process temperature and the  $T_g$  was calculated by integration. The larger this area, the more pronounced is the difference between the applied process temperature and  $T_g$  of the microspheres. Fig. 9 shows the correlation between the integral of the temperature by which  $T_p$  exceeds  $T_g$  ( $\int(\Delta T)dt$ ) and the lag-time before drug release. The diagram reveals a monotonically increasing relationship between the integrated temperature difference and the lag-time.

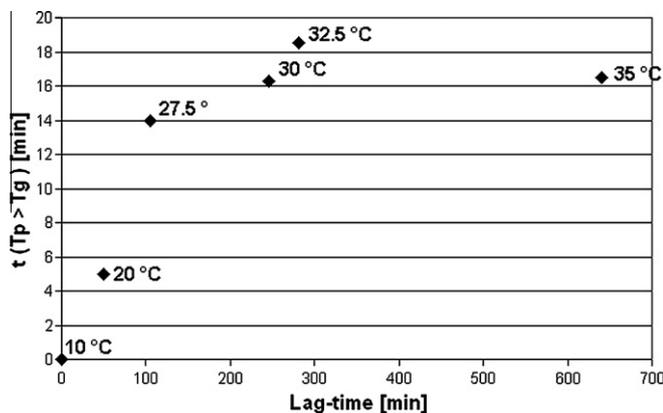


Fig. 8. Lag-time of drug release vs. period of time during which processing takes place above the  $T_g$  of the polymer ( $t_{T_p > T_g}$ ).

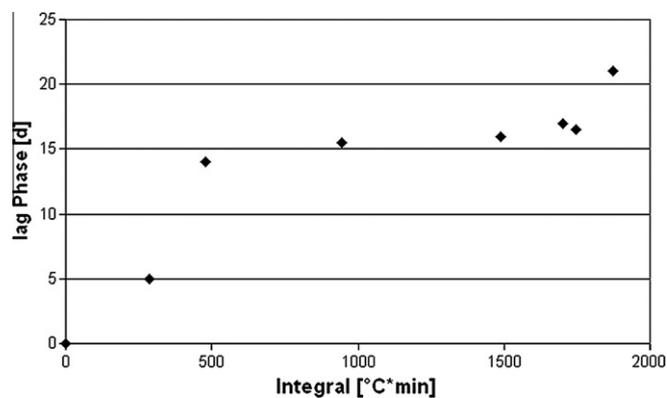


Fig. 9. Lag-time depending on the integral of  $T_p$  and  $T_g$ .

### 3.1.4. Influence of the polymer chain length

The molecular weight of the employed PLGA has a strong influence on the encapsulation efficiency and the drug release rate of microspheres [32]. Su et al. found that the encapsulation efficiency increased with higher molecular weight of the polymer [28]. In other cases, depending on the drug to be encapsulated, a low molecular weight polymer could be more appropriate for a high drug load [20]. As the length of the polymer chains has an impact on the resulting morphology of the polymer matrix, the molecular weight has also an influence on the drug distribution and release rate from the microspheres. Fu et al. found that a high molecular weight leads to a high initial burst and a subsequent slow release, whereas the use of a low molecular weight PLGA resulted in a fast drug release. This effect occurs in addition to the impact that the polymer degradation itself has on drug release.

We investigated the influence of the molecular weight on the solidification rate of the polymer matrix measured by the change of the glass transition temperature during particle formation.

Although the molecular weight of the applied polymers ranged from 36.5 to 109.2 kDa, no differences in the  $T_g$  vs. time profiles at a manufacturing temperature of 35 °C could be observed (Fig. 10). However, there is a significant influence of the molecular weight on drug release. The fastest release was obtained for the polymer with the lowest molecular weight (Fig. 11).

The drug release starts after 13 days for the 36.5 kDa PLGA and thus about 4 days earlier compared to the batch prepared with 58.3 kDa PLGA. This might be due to the fact that the low molecular weight PLGA exhibits enhanced water permeation and that a critical degree of polymer degradation is achieved earlier if the

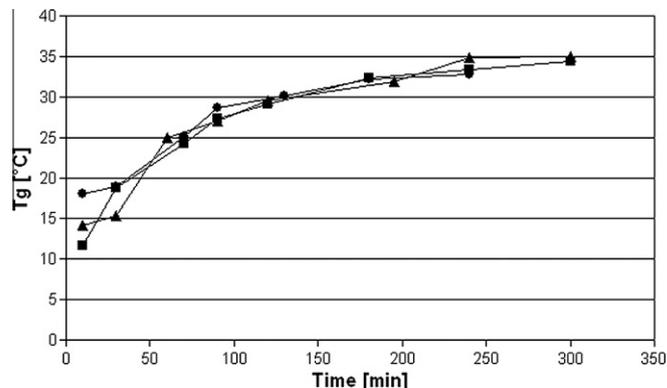


Fig. 10.  $T_g$  vs. time profiles from particle preparations using PLGA types of different molecular weight (36,510 Da (—▲—), 58,300 Da, (—■—), 109,200 Da (—●—)) at a process temperature of 35 °C.

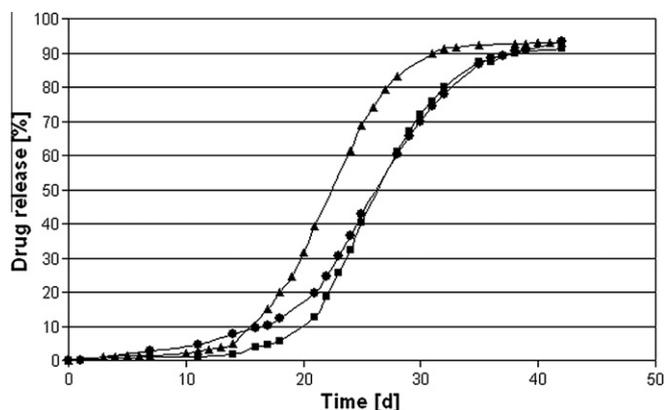


Fig. 11. Drug release profiles of the microspheres prepared at 35 °C with PLGA types of different molecular weights (36,510 Da (-▲-), 58,300 Da (-■-), 109,200 Da (-●-)).

polymer degradation starts with a lower initial molecular weight. The lag-time is followed by a steep incline of the curve during the erosive phase of the drug release. The release profile of the microspheres prepared with 109.2 kDa PLGA differs only with respect to its diffusive phase compared to the batch prepared with 58.3 kDa PLGA. Because of the more pronounced degradation of the high molecular weight polymer during processing (109.2 kDa → 70.5 kDa vs. 58.3 kDa → 54.7 kDa), the difference of the final particles' molecular mass is smaller as might be suspected from the employed polymers. A higher initial drug loss usually indicates a less tightly closed matrix which is in this case presumably a consequence of the more rigid and less densified structure of the higher molecular polymer.

### 3.1.5. Influence of solvent removal rate

As mentioned above, the flexibility of the polymer chains during processing has a strong influence on the resulting particle morphology and it depends, inter alia, on the solvent content of the polymer phase. The higher the amount of solvent in the polymer matrix, the lower is its  $T_g$ . A decelerated solvent evaporation can be achieved by reducing the stirring speed and the air flow through the reactor (Table 2). At a process temperature above 30 °C, such conditions lead to a larger time integral ( $\int(\Delta T)dt$ ) of the difference between  $T_g$  and the extraction temperature (Fig. 12).

The change of the glass transition temperature in case of slow solvent removal was significantly decelerated compared to fast evaporation. The particles prepared at 32.5 °C and slow solvent evaporation showed an almost linear increase of the  $T_g$  from 8 °C at the beginning to 32 °C after 5 h of processing.

At 35 °C, the slow solvent extraction leads to a marked increase of the polymer degradation during processing (Table 2). The encapsulation efficiency was hardly affected in case of the batch prepared at 35 °C, whereas in the case of the batch prepared at 32.5 °C only half of the drug substance was encapsulated, if the evaporation was slowed down. The microscopic pictures revealed

Table 2  
Process parameters and properties of microspheres prepared at 35 and 32.5 °C with fast and slow solvent evaporation.

Process temperature (°C)	Stirring speed (rpm)	Air flow (l/min)	Encapsulation efficiency (%)	Molecular weight end of process (kDa)
35	260	10	81.8	50.7
35	180	1.5	79.3	44.9
32.5	260	10	80.4	51.0
32.5	200	1.5	44.7	51.5

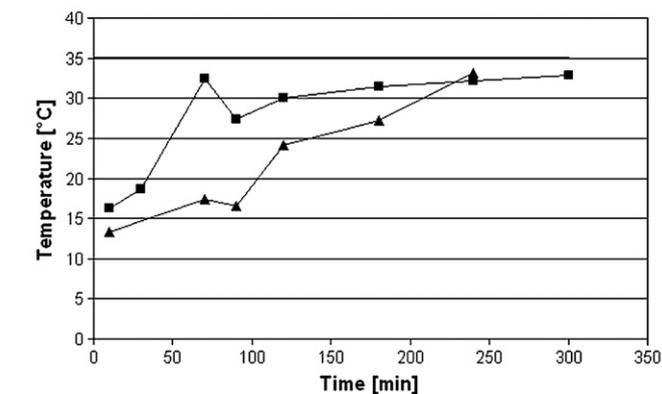
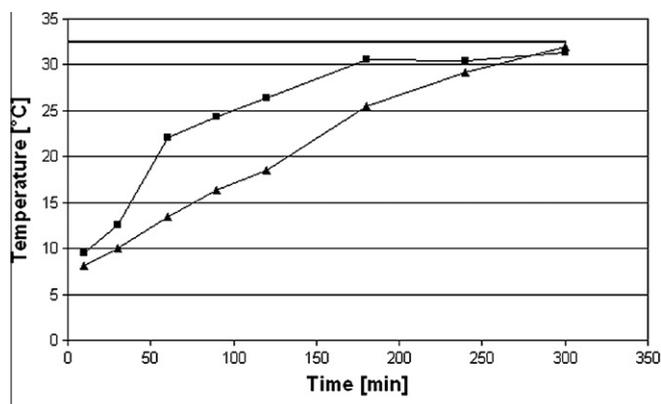


Fig. 12.  $T_g$  vs. time profiles during processing at 32.5 °C (left) and 35 °C (right) with fast (-■-) and slow (-▲-) solvent extraction according to Table 2.

that no spherical but irregular shaped and broken particles were obtained under these process conditions. With a very slow removal of the organic solvent and thus a delayed skin formation on the particle surface, the preparation process can become unstable. During processing, the organic solvent diffuses from the liquid emulsion droplets or hardening particles into the surrounding extraction medium. Consequently, solidification starts from the surface of the particles and at the beginning of the process a highly viscous outer shell is formed, which covers the liquid core of the particle. This outer shell is still fragile and with a rising vapor pressure of the solvent the shell can be ruptured, releasing parts of the enclosed liquid into the surrounding medium. This causes a high drug loss during processing. In former experiments, even the precipitation of drug crystal needles could be observed (data not shown). Thus, a certain rate of particle solidification is essential to minimize the length of this vulnerable stage of the solidification process and to obtain intact microspheres with a high drug load.

### 3.1.6. Influence of a post-treatment of the particles with ethanol

In order to gain more knowledge on how structural changes influence particle properties and behavior, we studied the effect of a terminal ethanolic resuspension step on the resulting particle characteristics. Ahmed et al. showed that the treatment of the wet microparticles with an organic solvent/water mixture and adding solvents into the external aqueous phase changed the microstructure of the particles [33]. It caused the reduction of pores and thus reduced burst release. In our own experiments, solvent exposure was implemented as a post-treatment step. After 5 h of extraction at 35 °C the microspheres were filtrated, immediately suspended in an ethanol/water mixture (25:75 (v/v)), and heated up to 25 °C or 40 °C, respectively. Ethanol is a non-solvent for PLGA and only a poor solvent for the encapsulated drug. By diffusing into the polymer matrix, it can act as a plasticizer and lower the  $T_g$  of the

**Table 3**

Preparation parameters and characteristics of microparticles treated with ethanol/water mixture (25:75) at different temperatures.

Temperature (°C)	Duration (min)	Encapsulation efficiency (%)	Molecular weight (Da)	Lag phase (d)
40	60	60.50	48,930	17
40	120	61.44	45,390	17
25	60	80.27	49,750	14

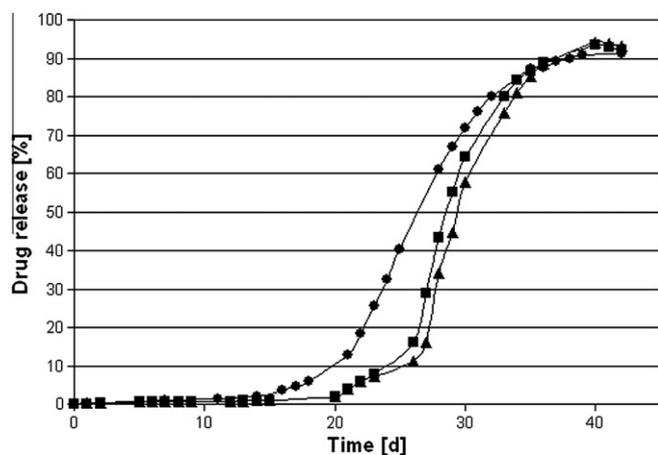
polymer. This was confirmed experimentally as a slight reduction of  $T_g$  from 33 °C to 29 °C after 1 h was measured. Thus, depending on the applied preparation temperature, a further change in the structure of the polymer matrix can be expected.

In all samples which were treated with ethanol, the degradation of the polymer was more pronounced in consequence of the prolonged processing time (Table 3). The treatment of the microparticles with 25% ethanol at 40 °C caused an extreme drug loss. Only about 60% of the drug substance employed was encapsulated. By contrast, particles treated at 25 °C, a temperature at which even the ethanol-solvated particles are in a glassy state, showed an encapsulation efficiency of more than 80%, comparable to the microspheres without ethanol treatment.

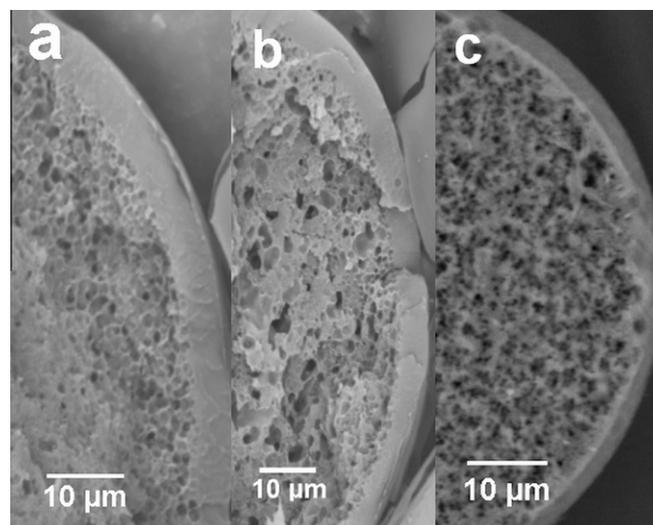
The microspheres showed a lag phase of 14 days when treated with ethanol for 1 h at 25 °C. Incubation at 40 °C caused a change to a triphasic pattern. A lag phase of 17 days is followed by a slightly increased drug release up to 10% and finally a very fast release after 18 or 26 days, respectively (Fig. 13). The particles treated with ethanol at 25 °C had a similar shaped drug release profile as particles without this post-treatment. Due to the decreased molecular weight (49,750 Da), the profile lies between the curves of non-incubated particles made from a 36,510 Da PLGA and those made from a 58,300 Da PLGA. At 25 °C, the  $T_g$  was not exceeded by the process temperature and thus, besides the increased polymer degradation, no structural changes could occur.

Compared to the untreated microspheres, those particles treated with ethanol at 40 °C showed a thick and dense outer shell (Fig. 14). Chemical imaging revealed that this shell was almost completely free of drug (Fig. 15).

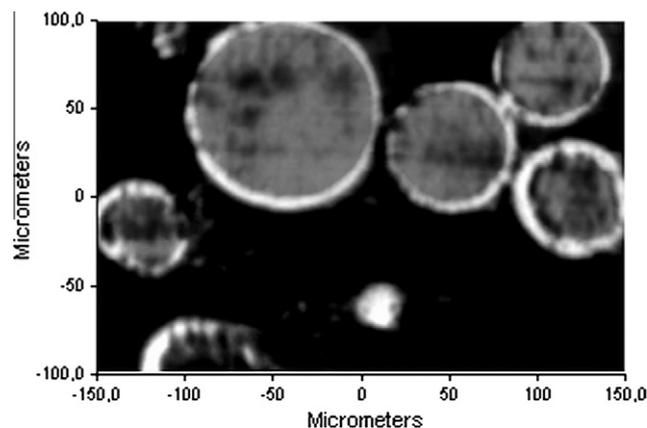
Hindrance of diffusion by this low porous shell is to be considered as the cause of the extended lag phase after ethanol incubation at elevated temperature.



**Fig. 13.** Drug release profiles of the microspheres treated with ethanol–water mixtures for 1 h at 40 °C (■), 2 h at 40 °C (◆), and 1 h at 25 °C (●).



**Fig. 14.** SEM micrographs of microparticles after treatment with ethanol (25%) at 40 °C after 1 (a) and 2 h (b) and without treatment (right).



**Fig. 15.** Chemical imaging of the microparticles: distribution of PLGA in the particles (white colored area = high adsorption at 1747  $\text{cm}^{-1}$  (characteristic band for PLGA)).

#### 4. Conclusions

The effects of different process parameters on the characteristics of PLGA microspheres were investigated with a focus on the processing temperature. The  $T_g$  of the polymer was used to measure the flexibility of the polymer chains during processing. The  $T_g$  depends on the amount of organic solvent in the polymer matrix and can also be affected by other molecules like water or drug substance. A good correlation, well fitted by the Gordon–Taylor or by the Kelley–Bueche equation, can be found for the concentration of organic solvent and the decrease of the  $T_g$  if no drug substance is present. However, in case of drug loaded particles these correlations cease to apply, indicating a synergistic effect of the solvent and the drug substance dissolved in the polymer phase.

The encapsulation efficiency and the drug release can be distinctly modified by changing the preparation temperature. This is due to the fact that particles prepared at 10 °C show an open-porous structure, whereas a higher temperature leads to the formation of a dense matrix and a smooth surface, impeding a diffusive drug release at the beginning of the dissolution testing. Consequently, the degradation products cannot diffuse out of the microspheres.

This induces a significant pH drop inside the particles with the occurrence of autocatalytic effects and enhanced drug solubility.

The time span during which the polymer chains remain flexible, which can be recognized by a  $T_g$  higher than the process temperature, can be modified by a slower removal of the organic solvent. If the solvent removal is carried out too slowly, the process becomes instable resulting in irregularly shaped and broken microspheres. A certain rate of solidification is essential to obtain spherical microspheres with efficiently encapsulated drug substance.

The release profile of a poorly soluble drug substance from a PLGA matrix is influenced by the chain length of the applied polymer. However, apart from formulation parameters, the formation process plays a crucial role since essential structural properties of the polymer matrix are strongly determined by operating factors. For this reason a good control of the  $T_g$  during the process is essential, as its change has a major influence on the resulting microsphere morphology. A precise control of the process temperature and the solvent removal rate are necessary which is often difficult on a laboratory batch scale. Determined by the process time during which the polymer chains remain flexible and mobile, the obtained polymer matrix can vary within a large range between a coarse- and open-porous structure and a fine-porous texture with a more or less dense outer shell. Thereby the drug release ranges from an almost zero-order rate to a sigmoidal type with a distinct lag-time.

## References

- [1] C. Wischke, S.P. Schwendeman, Principles of encapsulating hydrophobic drugs in PLA/PLGA microparticles, *International Journal of Pharmaceutics* 364 (2008) 298–327.
- [2] M. Cavalieri, J.P. Benoit, C. Thies, The formation and characterization of hydrocortisone-loaded poly((+/-)-lactide) microspheres, *Journal of Pharmacy and Pharmacology* 38 (1986) 249–253.
- [3] A. Smith, I.M. Hunneyball, Evaluation of poly(lactic acid) as a biodegradable drug delivery system for parenteral administration, *International Journal of Pharmaceutics* 30 (1986) 215–220.
- [4] K. Suzuki, J.C. Price, Microencapsulation and dissolution properties of a neuroleptic in a biodegradable polymer, poly(D,L-lactide), *Journal of Pharmaceutical Sciences* 74 (1985) 21–24.
- [5] B. Conti, I. Genta, T. Modena, F. Pavanetto, Investigation on process parameters involved in polylactide-co-glycolide microspheres preparation, *Drug Development and Industrial Pharmacy* 21 (1995) 615–622.
- [6] P.B. O'Donnell, J.W. McGinity, Preparation of microspheres by the solvent evaporation technique, *Advanced Drug Delivery Reviews* 28 (1997) 25–42.
- [7] T. Sato, M. Kanke, H.G. Schroeder, P.P. Deluca, Porous biodegradable microspheres for controlled drug delivery. 1. Assessment of processing conditions and solvent removal techniques, *Pharmaceutical Research* 5 (1988) 21–30.
- [8] Y.H. Cheng, L. Illum, S.S. Davis, A poly(D,L-lactide-co-glycolide) microsphere depot system for delivery of haloperidol, *Journal of Controlled Release* 55 (1998) 203–212.
- [9] W.I. Li, K.W. Anderson, R.C. Mehta, P.P. Deluca, Prediction of solvent removal profile and effect on properties for peptide-loaded PLGA microspheres prepared by solvent extraction/evaporation method, *Journal of Controlled Release* 37 (3) (1995) 199–214.
- [10] D.M. Lynn, M.M. Amiji, R. Langer, PH-responsive polymer microspheres: rapid release of encapsulated material within the range of intracellular pH, *Angewandte Chemie – International Edition* 40 (2001) 1707–1710.
- [11] G. Reich, Use of DSC to study the degradation behavior of PLA and PLGA microparticles, *Drug Development and Industrial Pharmacy* 23 (12) (1997) 1177–1189.
- [12] R.F. Fedors, A universal reduced glass transition temperature for liquids, *Journal of Polymer Science – Polymer Letters Edition* 17 (1979) 719–722.
- [13] D.E. Henton, P. Gruber, J. Lunt, J. Randall, Poly(lactic acid) technology, in: A.K. Mohanty, M. Misra, T. Drzal (Eds.), *Natural Fibers, Biopolymers and Biocomposites*, Taylor & Francis, Boca Raton, FL, USA, 2005, pp. 527–577.
- [14] Product information methylene chloride, Dow Chemicals, Midland, MI, USA.
- [15] P. Blasi, S.S. D'Souza, F. Selmin, P.P. Deluca, Plasticizing effect of water on poly(lactide-co-glycolide), *Journal of Controlled Release* 108 (2005) 1–9.
- [16] B.C. Hancock, P. York, R.C. Rowe, The use of solubility parameters in pharmaceutical dosage form design, *International Journal of Pharmaceutics* 148 (1997) 1–21.
- [17] N. Passerini, D.Q.M. Craig, An investigation into the effects of residual water on the glass transition temperature of polylactide microspheres using modulated temperature DSC, *Journal of Controlled Release* 73 (2001) 111–115.
- [18] H. Okada, M. Yamamoto, T. Heya, Y. Inoue, S. Kamei, Y. Ogawa, H. Toguchi, Drug-delivery using biodegradable microspheres, *Journal of Controlled Release* 28 (1994) 121–129.
- [19] K. Vay, S. Scheeler, W. Friess, New insights into the pore structure of poly(D,L-lactide-co-glycolide) microspheres, *International Journal of Pharmaceutics* 402 (2010) 20–26.
- [20] X. Fu, Q. Ping, Y. Gao, Effects of formulation factors on encapsulation efficiency and release behaviour in vitro of huperzine A-PLGA microspheres, *Journal of Microencapsulation* 22 (2005) 57–66.
- [21] R.A. Graves, T. Freeman, S. Pamajula, N. Praetorius, R. Moiseyev, T.K. Mandal, Effect of co-solvents on the characteristics of enkephalin microcapsules, *Journal of Biomaterials Science – Polymer Edition* 17 (2006) 709–720.
- [22] Y.Y. Yang, T.S. Chung, X.L. Bai, W.K. Chan, Effect of preparation conditions on morphology and release profiles of biodegradable polymeric microspheres containing protein fabricated by double-emulsion method, *Chemical Engineering Science* 55 (12) (2000) 2223–2236.
- [23] M. Hakkarainen, A.C. Albertsson, S. Karlsson, Weight losses and molecular weight changes correlated with the evolution of hydroxyacids in simulated in vivo degradation of homo- and copolymers of PLA and PGA, *Polymer Degradation and Stability* 52 (1996) 283–291.
- [24] F. Alexis, Factors affecting the degradation and drug-release mechanism of poly(lactic acid) and poly[(lactic acid)-co-(glycolic acid)], *Polymer International* 54 (2005) 36–46.
- [25] A.S. Zidan, R. Ziyaur, A.K. Mansoor, Online Monitoring of PLGA Microparticles Formation Using Lasentec Focused Beam Reflectance (FBRM) and Particle Video Microscope (PVM), AAPS, 2010.
- [26] Y. Yeo, K.N. Park, Control of encapsulation efficiency and initial burst in polymeric microparticle systems, *Archives of Pharmacal Research* 27 (2004) 1–12.
- [27] S.E. Bae, J.S. Son, K. Park, D.K. Han, Fabrication of covered porous PLGA microspheres using hydrogen peroxide for controlled drug delivery and regenerative medicine, *Journal of Controlled Release* 133 (2009) 37–43.
- [28] Z.X. Su, F.Y. Sun, Y.N. Shi, C.J. Jiang, Q.F. Meng, L.R. Teng, Y.X. Li, Effects of formulation parameters on encapsulation efficiency and release behavior of risperidone poly(D,L-lactide-co-glycolide) microsphere, *Chemical & Pharmaceutical Bulletin* 57 (2009) 1251–1256.
- [29] F.v. Burkersroda, L. Schedl, A. Göpferich, Why degradable polymers undergo surface erosion or bulk erosion, *Biomaterials* 23 (2002) 4221–4231.
- [30] J. Siepmann, K. Elkharraz, F. Siepmann, D. Klose, How autocatalysis accelerates drug release from PLGA-based microparticles: a quantitative treatment, *Biomacromolecules* 6 (2005) 2312–2319.
- [31] W. Friess, M. Schlapp, Modifying the release of gentamicin from microparticles using a PLGA blend, *Pharmaceutical Development and Technology* 7 (2002) 235–248.
- [32] R.A. Graves, S. Pamajula, R. Moiseyev, T. Freeman, L.A. Bostanian, T.K. Mandal, Effect of different ratios of high and low molecular weight PLGA blend on the characteristics of pentamidine microcapsules, *International Journal of Pharmaceutics* 270 (2004) 251–262.
- [33] A.R. Ahmed, M. Ciper, R. Bodmeier, Reduction in burst release from poly(D,L-lactide-co-glycolide) microparticles by solvent treatment, *Letters in Drug Design & Discovery* 7 (10) (2010) 759–764.