



Polymeric nanoparticles – Influence of the glass transition temperature on drug release



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ARTICLE INFO

Article history:

Received 14 October 2016

Received in revised form 8 December 2016

Accepted 10 December 2016

Available online 13 December 2016

Keywords:

Drug delivery system

Poly (DL-lactic-co-glycolic acid)

Poly (lactic acid)

Differential scanning calorimetry

Release profile

ABSTRACT

The physico-chemical characterisation of nanoparticles is often lacking the determination of the glass transition temperature, a well-known parameter for the pure polymer carrier. In the present study the influence of water on the glass transition temperature of poly (DL-lactic-co-glycolic acid) nanoparticles was assessed. In addition, flurbiprofen and *m*THPP as model drugs were incorporated in poly (DL-lactic-co-glycolic acid), poly (DL-lactic acid), and poly (L-lactic acid) nanoparticles. For flurbiprofen-loaded nanoparticles a decrease in the glass transition temperature was observed while *m*THPP exerted no influence on this parameter.

Based on this observation, the release behaviour of the drug-loaded nanoparticles was investigated at different temperatures. For all preparations an initial burst release was measured that could be attributed to the drug adsorbed to the large nanoparticle surface. At temperatures above the glass transition temperature an instant drug release of the nanoparticles was observed, while at lower temperatures less drug was released. It could be shown that the glass transition temperature of drug loaded nanoparticles in suspension more than the corresponding temperature of the pure polymer is the pivotal parameter when characterising a nanostructured drug delivery system.

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1. Introduction

The encapsulation of drugs into colloidal systems has become a very popular method to achieve drug targeting and enhance drug efficiency (Torchilin, 2007). Polymer based nanoparticles are a prominent representative of colloidal systems besides liposomes and drug-polymer conjugates (Petros and DeSimone, 2010). Different natural or synthetic materials are used for nanoparticle preparation. Especially poly (lactic acid) (PLA) and its copolymer with glycolic acid, poly (DL-lactic-co-glycolic acid) (PLGA), are commonly used for particle preparation due their distinct biodegradability and biocompatibility (Alexis, 2005).

The characterisation of such colloidal systems most often includes the determination of particle diameter and size distribution via dynamic light scattering as well as further analytics concerning the morphology, i.e. scanning electron microscopy (SEM). Additionally, in many cases the determination of surface charge is performed in order to estimate colloidal stability and aggregation tendency (Peltonen and Hirvonen, 2008). However, the determination of the glass transition temperature (T_g) of

nanoparticles in aqueous dispersion is less established, although T_g represents an important parameter of the pure polymer carrier. In most cases when T_g of the resulting polymeric nanoparticle system is quantified, a solid sample is analysed with the focus on the physical state of the embedded drug in terms of drug crystallisation or the formation of a solid solution (Dillen et al., 2004; Pamujula et al., 2004; Sant et al., 2005). Nevertheless, the relevance of T_g in aqueous nanoparticle dispersions is often underestimated. Especially for drug-loaded nanoparticles where a drug substance is incorporated in a polymeric matrix there are manifold interactions between the polymer chains and the drug due to their physical closeness.

Another characteristic of drug-loaded polymeric nanoparticles is the release profile. There are several factors that may influence drug release from the nanoparticulate system, e.g. drug solubility, diffusion, polymer biodegradation, particle size, and drug loading. For instance, there is a direct correlation between drug loading and initial burst release as well as subsequent release rate of the encapsulated amount (Kumari et al., 2010). Furthermore, the effect of different emulsifiers or steric stabilisers used in various concentrations for particle preparation also contributes to the release profile. For example the use of 0.5% instead of 5% poly (vinyl alcohol) (PVA) during nanoparticle preparation leads to an

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increased release of encapsulated bovine serum albumin (BSA) (Sahoo et al., 2002). Furthermore, the liberation of paclitaxel from PLGA nanoparticles is decreased when using 1,2-dipalmitoylphosphatidylcholin (DPPC) as an emulsifier instead of PVA (Feng and Huang, 2001). In addition, Budhian et al. described that the ratio of lactic to glycolic acid in the polymeric matrix additionally affects the burst release from haloperidol-loaded PLGA nanoparticles (Budhian et al., 2008). Moreover, other authors addressed the influences of the release medium on drug release from PLGA microspheres (Faisant et al., 2006). The release of 5-fluorouracil is strongly dependent on the osmolarity, buffer concentration, pH value, and temperature of the chosen incubation medium. However, when considering these studies a correlation between Tg of drug-loaded nanoparticles and temperature dependent release behaviour is not described so far.

The present study is focussed on a relationship between Tg influencing parameters and release profile kinetics. Glass transition temperatures of unloaded PLGA nanoparticles in dry form and in aqueous suspension were examined to reveal possible effects of the used emulsifier and stabilisers. In a second step, drug-loaded PLGA nanoparticles were characterised for drug loading, Tg, and physical state of the incorporated model drugs flurbiprofen and 5,10,15,20-tetra(*m*-hydroxyphenyl)porphyrin (*m*THPP) (Fig. 1). Both drugs were chosen because of their poor water solubility in combination with an insufficient transport across biological barriers which necessitates suitable drug formulation strategies such as the incorporation in colloidal dosage forms (Meister et al., 2013; Grünebaum et al., 2015). The release behaviour of the model drugs from PLGA nanoparticles was compared to nanoparticles based on DL-PLA and L-PLA, in order to compare typical biodegradable and approved starting materials for nanoparticle preparation. By using different temperature profiles the release behaviour could be correlated to Tg properties of the respective nanoparticle formulation.

2. Materials and methods

2.1. Materials

PLGA (Resomer[®] RG502H, inherent viscosity 0.16–0.24 dl/g), DL-PLA (Resomer[®] R203H, inherent viscosity 0.25–0.35 dl/g), and L-PLA (Resomer[®] L206S, inherent viscosity 0.8–1.2 dl/g) were obtained from Evonik Industries AG (Darmstadt, Germany). *m*THPP was kindly provided from biolitec research GmbH (Jena, Germany). Flurbiprofen (FBP), human serum albumin (HSA), poly (vinyl alcohol) (PVA), and mannitol were purchased from Sigma Aldrich (Steinheim, Germany). The purity of the drugs was $\geq 98.5\%$ according to the specifications of the suppliers. All other reagents were of analytical grade and used as received.

2.2. Nanoparticle preparation

For nanoparticle preparation an emulsion diffusion method was used. To obtain unloaded PLGA nanoparticles 125 mg of the polymer was dissolved in 2.5 mL ethyl acetate and 5 mL of an aqueous solution containing 1% (w/v) PVA was added. After homogenisation at 15,000 rpm for 5 min (Ultra Turrax[®], S25NK-19G, IKA, Staufen, Germany), the emulsion was diluted with 7.5 mL 1% (w/v) PVA solution. After evaporation of ethyl acetate by stirring the emulsion over night at room temperature, the nanoparticles were washed once by centrifugation and redispersion in purified water.

For drug-loaded PLGA nanoparticles 100 mg polymer and 2.5, 5, 10, 20, and 30 mg flurbiprofen, respectively, or 10 mg *m*THPP were dissolved in 1 mL ethyl acetate. After addition of 2 mL of 1% (w/v) PVA solution, this mixture was homogenised under cooling for 30 min at 24,000 rpm (Ultra Turrax[®], S25N-10G, IKA, Staufen, Germany). The emulsion was subsequently diluted with 8 mL 1% (w/v) PVA solution. The removal of the organic solvent and purification was performed as described for unloaded PLGA-NP.

The preparation of DL-PLA and L-PLA nanoparticles was carried out using a solution of 100 mg polymer and, in the case of flurbiprofen-loaded nanoparticles, 10 mg flurbiprofen in 2 mL methylene chloride. For *m*THPP-loaded PLA nanoparticles 10 mg drug and polymer were dissolved in a mixture of 1 mL ethyl acetate and 1 mL methylene chloride, respectively. The addition of 6 mL 1% (w/v) PVA solution was followed by homogenisation under cooling for 30 min at 24,000 rpm (Ultra Turrax[®], S25NK-19G, IKA, Staufen, Germany) and subsequent dilution with another 6 mL 1% (w/v) PVA solution. Further solvent removal and purification was performed as described before.

2.3. Lyophilisation and reduction of residual moisture

All prepared nanoparticle suspensions were freeze dried in the presence of mannitol using a single chamber-system (Epsilon 2–4, Martin Christ, Osterode am Harz, Germany).

For unloaded PLGA-NP a concentration of 10% (w/v) mannitol was added to the samples. The freeze drying process was performed as follows: Freezing at -40°C for 7 h, a primary drying step at -34°C and a vacuum of 0.05 mbar for 40 h, followed by a secondary drying phase at 20°C and 0.025 mbar for 11 h. Further extraction of residual water in the freeze dried product was achieved by storage in a desiccator at an elevated temperature of 40°C for 2–72 h.

For flurbiprofen-loaded PLGA nanoparticles mannitol was used in a reduced concentration of 3% (w/v) to enable DSC analysis of the drug in the particle samples. The freeze drying process was

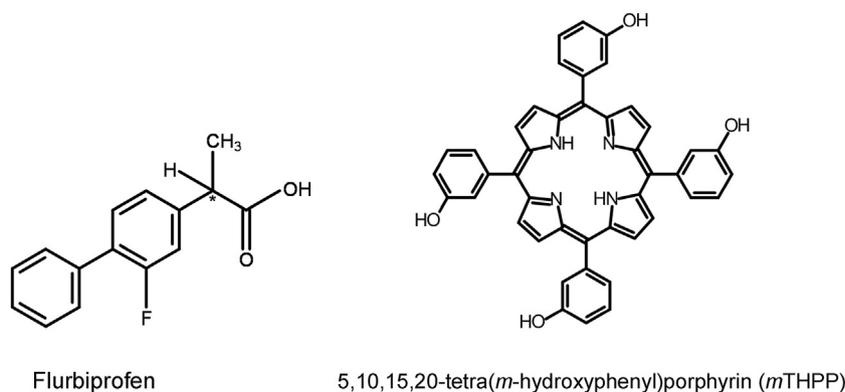


Fig. 1. Molecular structure of the drugs flurbiprofen and 5,10,15,20-tetra(*m*-hydroxyphenyl)porphyrin (*m*THPP).

adapted by a shorter freezing step of 3 h and a shorter primary drying of 24 h.

2.4. Nanoparticle diameter and size distribution

Nanoparticle diameter and polydispersity was measured using dynamic light scattering with a Zetasizer Nano S (Malvern Instruments GmbH, Herrenberg, Germany) at 22 °C and a backscattering angle of 173°. An appropriate volume of the nanoparticle suspension was diluted with 2 mL purified water right before every measurement. Particle size was calculated from the intensity of the scattered light as the mean hydrodynamic diameter of the particles (*Z*-average). The polydispersity index (PDI) was determined for the nanoparticles and is considered monodisperse in this study when it is less than 0.1.

2.5. Nanoparticle yield

The nanoparticle yield in the prepared suspensions was quantified gravimetrically. Therefore an aliquot of 20.0 µL of each sample was transferred to a weighing dish and dried to constant mass at a temperature of 80 °C.

2.6. Differential scanning calorimetry measurement

The glass transition temperature of the nanoparticles was analysed by differential scanning calorimetry (DSC) using a DSC Q 2000 (TA Instruments, New Castle, USA). The instrument was calibrated for heat flow and temperature using an indium reference. All samples were purged with pure nitrogen at a flow rate of 50 mL/min. T_g was obtained by taking the inflection point of the slope. Individual methods are described separately.

2.6.1. Pure PLGA, physical mixture, solid solution, and freeze dried PLGA-NP

An accurately weighted amount of each sample was placed in hermetically or non-hermetically sealed aluminium pans, respectively. The temperature ramp ranged from –30 °C to +100 °C with a heating rate of 20 °C/min. The first heating cycle was performed to eliminate the thermal history of the material and in the case of non-hermetically sealed pans to remove residual moisture content. All T_g values were evaluated in the second to fourth heating cycle.

2.6.2. Nanoparticle suspensions

The nanoparticle dispersions were concentrated to a particle content between 100 and 150 µg/µL. For that purpose an aliquot (200 µL) of the purified nanoparticle suspension was centrifuged, the supernatant was removed and the nanoparticle pellet was redispersed in 30 µL of the supernatant. An aliquot (20 µL) of this highly concentrated nanoparticle suspension was placed in hermetically sealed aluminium pans. The heating rate was set to 20 °C/min with the temperature varying between –17 °C and +80 °C. Only the second to fourth heating cycle was analysed for T_g as described above.

2.6.3. Pure flurbiprofen, physical mixture and freeze dried FBP-loaded-PLGA-NP

Non-hermetically sealed pans were used for these measurements. An accurately weighted amount of each sample was placed in the pans and prior to analysis the sample was stored at 80 °C to remove residual moisture and to eliminate thermal history. After four heating cycles ranging from –30 °C to +150 °C at a heating rate of 10 °C/min, T_g of PLGA and the melting point of flurbiprofen were determined.

2.7. Residual moisture content

The residual moisture content of the freeze dried PLGA-NP was quantified by Karl-Fischer titration. A weighted amount was placed into the Karl-Fischer titrator (V20 Compact KF Volumeter, Mettler Toledo, Gießen, Germany) and analysis was performed according to the instruction of the manufacturer.

2.8. Drug release studies from nanoparticulate systems

Release kinetics were determined using phosphate buffer pH 8.0 for flurbiprofen-loaded nanoparticles and 5% (w/v) HSA solution for *m*THPP-loaded nanoparticles. The samples for each time point were prepared as follows: Nanoparticle formulations (1 mg) were incubated in 1 mL of the release medium at 10 °C, 37 °C, or 55 °C for 24 h. At defined time points the whole sample was centrifuged (20,000g for 10 min) and the supernatant was collected. Additionally, the release kinetics were also determined by altering the incubation temperature from 10 °C to 37 °C, 37 °C to 55 °C or vice versa after 24 h.

2.9. Quantification of flurbiprofen and *m*THPP

The entrapment efficiency of drug-loaded nanoparticles was determined by dissolving 1 mg nanoparticles in 1 mL acetone. For the quantification of unbound flurbiprofen or *m*THPP the supernatant of 5 mg nanoparticles in aqueous dispersion was collected after sample centrifugation (20,000g, 5 min).

The amount of released, entrapped, and unbound flurbiprofen or *m*THPP was quantified by HPLC (HPLC1200 series, Agilent Technologies GmbH, Böblingen, Germany). A reversed phase column (Gemini[®] 5 µm NX-C18 110 Å, 250 × 4.6 mm, Phenomenex Inc., Aschaffenburg, Germany) was used. After injection of 10.0 µL for *m*THPP samples and 20.0 µL for flurbiprofen samples, the elution was performed at a flow rate of 1 mL/min at 30 °C over 8 min. The mobile phase consisted of 57.5% acetonitrile and 42.5% trifluoroacetic acid 0.1% in water. The detection of flurbiprofen was performed with a diode array detector at 245 nm. *m*THPP was detected with a fluorescence detector at an excitation wavelength of 350 nm and emission at 654 nm. A calibration curve in the range of 2.5–100 µg/mL flurbiprofen in phosphate buffer pH 8.0 and 0.5–100 µg/mL *m*THPP in ethanol was used for quantification.

2.10. Solubility of flurbiprofen and *m*THPP in the corresponding release medium

For the determination of the solubility of flurbiprofen in phosphate buffer pH 8.0 a saturated solution was prepared and incubated at 10 °C, 37 °C, and 55 °C for 24 h. After the removal of undissolved flurbiprofen via membrane filtration (0.2 µm), the dissolved fraction was analysed by HPLC as mentioned above.

A stock solution of *m*THPP in ethanol (20 mg/mL) was prepared to quantify the solubility in 5% (w/v) HSA solution. An aliquot of 100 µL stock solution was injected in 1 mL HSA solution. Further analysis was performed as described for flurbiprofen using the *m*THPP HPLC method.

3. Results

In the present study nanoparticles based on the polymers PLGA, DL-PLA, and L-PLA were prepared in combination with the lipophilic drugs flurbiprofen and *m*THPP (Fig. 1). The different nanoparticle samples were analysed with regard to the relationship between T_g influencing parameters and release profile kinetics. The design of the study is outlined in Fig. 2.

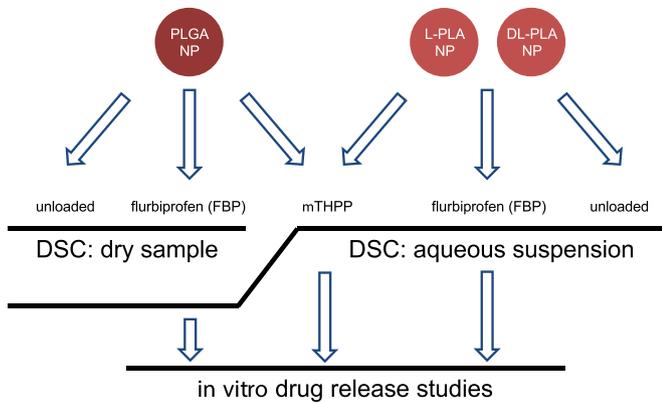


Fig. 2. Study design chart: PLGA, DL-PLA, and L-PLA nanoparticles were prepared in combination with the drugs flurbiprofen and *m*THPP. The glass transition temperature (T_g) of unloaded and flurbiprofen loaded PLGA nanoparticles was determined in dry state as well as in aqueous suspension whereas all PLA based nanoparticles were analysed in aqueous suspension. All of the drug loaded aqueous nanoparticles suspensions were used for temperature dependent *in vitro* drug release studies.

Prior to the DSC analysis of the nanoparticle samples, the thermal stability of both drugs was assessed. Flurbiprofen as well as *m*THPP showed no signs of instability over the temperature range from -30°C to $+150^\circ\text{C}$ as verified by DSC measurements of the pure drugs (data not shown). In the case of flurbiprofen only a melting event could be observed at about $+115^\circ\text{C}$ whereas for *m*THPP no thermal events could be detected even up to a temperature of $+300^\circ\text{C}$.

3.1. Preparation of unloaded PLGA-NP and T_g characterisation

For the unloaded PLGA-NP a particle diameter of 189 nm and a PDI <0.1 was achieved (Table 1). Using hermetically sealed pans a T_g of 32.1°C was detected for PLGA nanoparticles in suspension. After conducting a freeze drying process particle diameter and PDI were preserved while T_g increased by 3°C (Table 1). However, in comparison to T_g of the pure PLGA with 38.6°C , T_g of freeze dried PLGA-NP was decreased by 3°C , which is probably due to a higher water content in the freeze dried sample compared to the pure polymer. Regarding physical mixtures or solid solutions of the three components PLGA, PVA, and mannitol in the same concentrations as used for manufacturing of nanoparticles, no significant shift of T_g due to PVA or mannitol could be observed compared to the pure PLGA (one way ANOVA; $p > 0.05$). An experiment using different PVA concentrations during nanoparticle preparation was also conducted to reveal possible changes in T_g . Residual PVA contents between 0.1 mg PVA/mg PLGA-NP and 2 mg PVA/mg PLGA-NP showed T_g values of 31.9°C and 32.1°C , respectively, which was comparable to the standard formulation.

DSC measurements on pure PLGA using non-hermetically sealed pans gave a T_g of 45.4°C . For the freeze dried PLGA-NP a comparable T_g of 47.2°C was obtained when measuring under

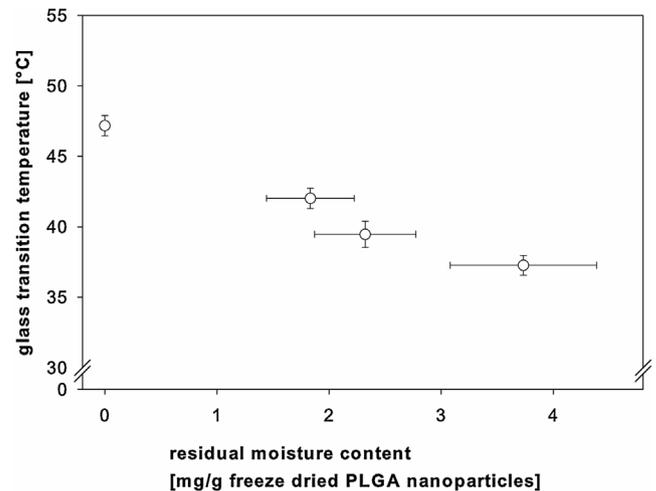


Fig. 3. Effect of residual moisture content on T_g of freeze dried unloaded PLGA-NP (mean \pm SD; $n = 3$).

moisture removing conditions (Table 1). For a decreasing residual moisture content from 3.7 ± 0.7 mg/g to 0 mg/g freeze dried nanoparticles, Fig. 3 displays an increasing T_g from $37.3 \pm 0.7^\circ\text{C}$ to $47.2 \pm 0.7^\circ\text{C}$.

3.2. Preparation of flurbiprofen-loaded PLGA nanoparticles and T_g influence

The incorporation of the model drug flurbiprofen in PLGA-NP was successful and nanoparticles at a particle diameter of about 220 nm and a PDI <0.1 were obtained (Table 2). It was possible to encapsulate a maximum amount of $210 \mu\text{g}$ flurbiprofen/mg NP for particle systems prepared with 30 mg flurbiprofen per 100 mg PLGA (FBP-PLGA-NP-30). In this context it is noteworthy that a linear correlation between drug added during particle preparation and drug incorporated into the PLGA nanoparticles could be observed, representing a constant loading efficiency. This is probably due to a constant partitioning of flurbiprofen between the aqueous and organic phase during the emulsion diffusion process of particle preparation.

Regarding T_g of FBP-PLGA-NP-2.5 in suspension, for the lowest used amount flurbiprofen, a decrease of T_g compared to unloaded PLGA-NP in suspension was observed. The encapsulated amount of $17 \mu\text{g}$ flurbiprofen/mg NP led to a decrease in T_g by 3°C (Table 2). Raising the encapsulated amount flurbiprofen up to $210 \mu\text{g}/\text{mg}$ NP for FBP-PLGA-NP-30 the T_g decreased progressively to 20°C , about 12°C below T_g of unloaded PLGA-NP. As outlined in Table 2, T_g decreased steadily when increasing the embedded amount of flurbiprofen in PLGA-NP.

For the determination of the physical state of flurbiprofen within the polymer matrix FBP-PLGA-NP were freeze dried using mannitol as a bulking agent. Due to the pre-treatment of the samples a quantification of T_g in all four DSC measurement cycles

Table 1

Nanoparticle diameter, PDI, and T_g for unloaded PLGA-NP, T_g of pure PLGA and a physical mixture as well as a solid solution of the corresponding pure materials (mean \pm SD; $n = 3$). T_g hermetical were measured under moisture retaining conditions in contrast to T_g non-hermetical.

	particle diameter [nm]	PDI	T_g hermetical [°C]	T_g non-hermetical [°C]
PLGA, starting material	–	–	38.6 ± 1.6	45.4 ± 0.6
physical mixture	–	–	40.0 ± 1.7	–
solid solution	–	–	40.0 ± 4.6	–
PLGA-NP freeze dried	189.5 ± 2.0	0.056 ± 0.017	35.5 ± 1.2	47.2 ± 0.7
PLGA-NP in suspension	189.0 ± 5.1	0.038 ± 0.018	32.1 ± 0.7	–

Table 2

Nanoparticle diameter, PDI, Tg of nanoparticles in aqueous suspension, and drug load for FBP- and *m*THPP-PLGA-NP (mean \pm SD; n = 3).

	particle diameter [nm]	PDI	drug load [μ g/mg NP]	Tg [$^{\circ}$ C]
FBP-PLGA-NP-2.5	226.8 \pm 6.8	0.078 \pm 0.029	16.9 \pm 1.0	28.8 \pm 0.6
FBP-PLGA-NP-5	224.2 \pm 5.3	0.059 \pm 0.014	32.9 \pm 1.8	26.9 \pm 0.5
FBP-PLGA-NP-10	222.8 \pm 4.8	0.060 \pm 0.021	71.8 \pm 6.2	25.3 \pm 1.1
FBP-PLGA-NP-20	216.0 \pm 3.8	0.045 \pm 0.017	142.0 \pm 6.3	22.4 \pm 1.5
FBP-PLGA-NP-30	223.3 \pm 11.7	0.085 \pm 0.037	209.7 \pm 5.4	19.9 \pm 1.6
<i>m</i> THPP-PLGA-NP	237.4 \pm 9.1	0.099 \pm 0.022	72.3 \pm 6.0	32.4 \pm 1.1

was feasible. In Table 3 the Tg values of FBP-PLGA-NP with different drug loadings are listed for the first as well as for the second to fourth measurement cycle. A monotonic decrease in Tg with increasing drug loading was confirmed. Comparing the glass transition temperatures of the first and fourth cycle only small but insignificant changes (one way ANOVA; $p > 0.05$) could be observed with the exception of FBP-PLGA-NP-30 resulting in a 6 $^{\circ}$ C decrease comparing the different cycles. In Fig. 4 the four heating cycles of (a) a physical mixture of mannitol, PLGA, PVA, and flurbiprofen, (b) FBP-PLGA-NP-10, and (c) FBP-PLGA-NP-30 are displayed. In the first heating cycle of the physical mixture the melting of flurbiprofen at about 115 $^{\circ}$ C was detected. The melting peak disappeared in the second to fourth heating cycle accompanied by a shift in Tg of PLGA to lower temperatures. For the FBP-PLGA-NP-10 no melting peak or shift in Tg was detected. Both, the physical mixture and FBP-PLGA-NP-30, showed a decrease in Tg from the first to the second to fourth heating cycle and a thermal event at 85 $^{\circ}$ C to 115 $^{\circ}$ C was determined for the FBP-PLGA-NP-30.

3.3. Preparation of unloaded and flurbiprofen-loaded PLA nanoparticles and Tg characterisation

The usage of DL-PLA and L-PLA as alternative polymeric matrices for nanoparticle preparation led to unloaded and flurbiprofen-loaded nanoparticles with a particle diameter of approximately 230 nm and a PDI < 0.1 (Table 4). An incorporation efficiency of 69.7 μ g flurbiprofen/mg NP and 66.7 μ g flurbiprofen/mg NP was achieved for DL-PLA and L-PLA, respectively. The Tg of FBP-DL-PLA-NP and FBP-L-PLA-NP was decreased by about 8 $^{\circ}$ C compared to the corresponding unloaded particle system.

3.4. Preparation of *m*THPP-loaded nanoparticles and Tg characterisation

The preparation of *m*THPP-PLGA-NP led to nanoparticles with a particle diameter of 237 nm and a PDI of 0.1 (Table 2). An incorporation of 72.3 μ g *m*THPP/mg NP was achieved. Tg remained unchanged for unloaded PLGA-NP and *m*THPP-PLGA-NP with 32.4 $^{\circ}$ C.

Switching to DL-PLA as a different polymeric matrix resulted in a particle diameter of 214 nm and a PDI < 0.1 (Table 4). Tg measured

Table 3

Tg of freeze dried FBP-PLGA-NP measured in non-hermetically sealed pans after a pre-treatment at 80 $^{\circ}$ C for 2 h with increasing content of flurbiprofen used for the nanoparticle preparation (mean \pm SD; n = 3).

	Tg (1. cycle) [$^{\circ}$ C]	Tg (2. – 4. cycle) [$^{\circ}$ C]
FBP-PLGA-NP-2.5	44.7 \pm 0.6	43.9 \pm 0.3
FBP-PLGA-NP-5	43.0 \pm 1.1	42.3 \pm 1.1
FBP-PLGA-NP-10	40.1 \pm 1.6	39.4 \pm 1.4
FBP-PLGA-NP-20	39.9 \pm 2.0	38.0 \pm 2.6
FBP-PLGA-NP-30	38.5 \pm 0.3	32.7 \pm 1.1

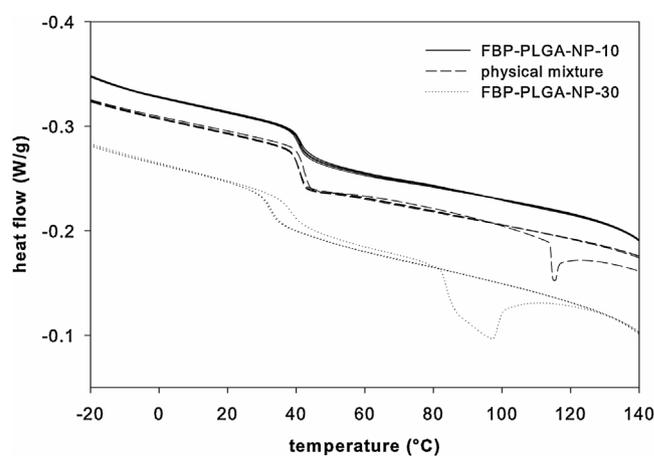


Fig. 4. DSC thermograms of freeze dried nanoparticles and corresponding solid pure materials: (a) physical mixture of mannitol, PLGA, PVA, and flurbiprofen, (b) FBP-PLGA-NP-10, and (c) FBP-PLGA-NP-30. The measurements were performed in non-hermetically sealed pans after a pre-treatment step at 80 $^{\circ}$ C for 2 h.

in nanoparticle suspension was only slightly higher (44.0 $^{\circ}$ C) for drug loaded nanoparticles compared to unloaded DL-PLA-NP (42.5 $^{\circ}$ C). The embedded amount of *m*THPP was comparable to *m*THPP-PLGA-NP. Additionally *m*THPP nanoparticles prepared with L-PLA represented a Tg of 53.9 $^{\circ}$ C compared to a Tg of 52 $^{\circ}$ C for unloaded L-PLA-NP. An encapsulated drug amount of 77.3 μ g *m*THPP/mg NP was obtained in combination with a particle diameter of 234 nm and a PDI of 0.1.

3.5. Release kinetics of flurbiprofen

For every nanoparticle formulation the release kinetics was performed with a temperature shift, one temperature below and one temperature above Tg. The release kinetics of flurbiprofen from FBP-PLGA-NP-10 was conducted at 10 $^{\circ}$ C and 37 $^{\circ}$ C in phosphate buffer pH 8.0. Within the first few hours about 93% of flurbiprofen was released from FBP-PLGA-NP-10 at 37 $^{\circ}$ C (Fig. 5A). Shifting incubation temperature to 10 $^{\circ}$ C a decreased amount of 70% flurbiprofen was released after 24 h incubation time. Further investigations with a prolonged incubation time of 48 h were performed with an alteration in temperature from 10 $^{\circ}$ C to 37 $^{\circ}$ C and vice versa. An instant release of flurbiprofen from 70% to 93% could be observed when increasing the temperature from 10 $^{\circ}$ C to 37 $^{\circ}$ C. When decreasing the temperature of the release medium from 37 $^{\circ}$ C to 10 $^{\circ}$ C the released amount flurbiprofen remained constant at approximately 90%.

Furthermore, the release of flurbiprofen from FBP-DL-PLA-NP was analysed. The experiments were also carried out in phosphate buffer pH 8.0 and the temperature set-up and temperature change was carried out as before. As previously observed for FBP-PLGA-NP-10 an immediate burst release of 90% flurbiprofen occurred above Tg (Fig. 5B). In contrast only 40% flurbiprofen was released within 24 h when the temperature was kept at 10 $^{\circ}$ C. Increasing the temperature from 10 $^{\circ}$ C to 37 $^{\circ}$ C after 24 h the released amount drug rapidly reached 90%. In the case of a temperature gradient from 37 $^{\circ}$ C to 10 $^{\circ}$ C after 24 h the liberated amount drug stayed constant at 90%.

Regarding FBP-L-PLA-NP the release behaviour was performed at 37 $^{\circ}$ C and 55 $^{\circ}$ C in phosphate buffer pH 8.0. The temperature was also altered after an incubation time of 24 h. In Fig. 5C the release profile of flurbiprofen is depicted with about 74% drug release after 24 h from the nanoparticle system at 37 $^{\circ}$ C. Increasing the temperature of the release medium to 55 $^{\circ}$ C, a temperature just above Tg, an instant release of flurbiprofen to 95% was observed.

Table 4Nanoparticle diameter, PDI, Tg of nanoparticles in aqueous suspension, and drug load for unloaded-, FBP-, and *m*THPP-loaded DL-PLA-NP and L-PLA-NP (mean \pm SD; n = 3).

	particle diameter [nm]	PDI	drug load [μ g/mg NP]	Tg [$^{\circ}$ C]
unloaded DL-PLA-NP	226.2 \pm 2.4	0.058 \pm 0.023	–	42.5 \pm 0.7
FBP-DL-PLA-NP	238.1 \pm 4.3	0.041 \pm 0.023	69.7 \pm 2.4	34.1 \pm 0.6
<i>m</i> THPP-DL-PLA-NP	213.7 \pm 5.0	0.048 \pm 0.021	78.8 \pm 8.2	44.0 \pm 0.4
unloaded L-PLA-NP	232.2 \pm 4.8	0.053 \pm 0.021	–	52.0 \pm 0.5
FBP-L-PLA-NP	236.2 \pm 2.6	0.060 \pm 0.022	66.7 \pm 2.6	43.5 \pm 1.3
<i>m</i> THPP-L-PLA-NP	234.2 \pm 7.3	0.103 \pm 0.024	67.3 \pm 6.4	53.9 \pm 0.3

Starting the experiment with temperatures of 55 $^{\circ}$ C an immediate release of 95% was confirmed. Even when decreasing the temperature of the release medium to 37 $^{\circ}$ C after 24 h the released amount flurbiprofen remained constant at 95%. For all temperatures the solubility of flurbiprofen in phosphate buffer pH 8.0 was determined. Solubilities of 3.1 mg/mL, 3.5 mg/mL, and 3.8 mg/mL were achieved at 10 $^{\circ}$ C, 37 $^{\circ}$ C, and 55 $^{\circ}$ C, respectively.

The determination of free flurbiprofen in the nanoparticle suspension prior to the release experiment revealed only small drug amounts confirming leak-tightness of the nanoparticle system in water. For FBP-PLGA-NP-10, FBP-DL-PLA-NP, and FBP-L-PLA-NP a free drug amount of 1.2 μ g flurbiprofen/mg NP, 2.8 μ g flurbiprofen/mg NP, and 2.6 μ g flurbiprofen/mg NP was found in the supernatant, respectively.

3.6. Release kinetics of *m*THPP

Regarding the *m*THPP nanoparticle systems the release was performed in HSA solution at different temperatures below and above Tg of the respective nanoparticle formulation. For *m*THPP-PLGA-NP 10 $^{\circ}$ C and 37 $^{\circ}$ C was chosen and the temperature was altered after 24 h. As could be seen in Fig. 5A, only 20% of the embedded *m*THPP was liberated at 10 $^{\circ}$ C after 24 h incubation time, whereas at 37 $^{\circ}$ C an amount of 55% *m*THPP was released nearly immediately within the same time frame. In addition, an instant release of *m*THPP was observed when increasing the temperature from 10 $^{\circ}$ C to 37 $^{\circ}$ C after 24 h. As already described for flurbiprofen, no shift in the released drug amount was observed when decreasing the temperature from 37 $^{\circ}$ C to 10 $^{\circ}$ C after 24 h.

When changing the polymer used, the release kinetics for *m*THPP loaded DL-PLA-NP and L-PLA-NP was conducted at 37 $^{\circ}$ C and 55 $^{\circ}$ C. In Fig. 6A the release profile of *m*THPP-DL-PLA-NP is shown. In the first 24 h an amount of 26% drug was liberated at 37 $^{\circ}$ C and about 68% at 55 $^{\circ}$ C. Changing the temperature from 37 $^{\circ}$ C to 55 $^{\circ}$ C additional *m*THPP was released and reached 58% after a complete incubation time of 48 h. When starting at 55 $^{\circ}$ C and decreasing the temperature from 55 $^{\circ}$ C to 37 $^{\circ}$ C after 24 h a reduction in the released amount drug to 55% was determined. After further 24 h incubation at 37 $^{\circ}$ C only 63% released *m*THPP was detected.

Furthermore, the liberation of *m*THPP from L-PLA-NP was analysed and the results are shown in Fig. 6B. Only 20% *m*THPP was released after 24 h incubation time at 37 $^{\circ}$ C. In contrast 70% drug substance was found in the release medium using an incubation temperature of 55 $^{\circ}$ C. An immediate release could be detected when increasing the temperature from 37 $^{\circ}$ C to 55 $^{\circ}$ C. A drop in the released amount *m*THPP was determined when changing temperature from 55 $^{\circ}$ C to 37 $^{\circ}$ C.

The determination of free *m*THPP in the nanoparticle suspension prior to the release experiment revealed only small drug amounts confirming leak-tightness of the nanoparticle system in water. For *m*THPP-PLGA-NP, *m*THPP-DL-PLA-NP, and *m*THPP-L-PLA-NP a free drug amount of 0.2 μ g/mg NP, 0.4 μ g/mg NP, and 1.1 μ g/mg NP was quantified, respectively, confirming minimal

leakage of the used nanoparticle systems. At all investigated temperatures a *m*THPP solubility in HSA solution (5% w/v) of about 1.4 mg/mL was determined.

4. Discussion

The glass transition temperature is a well defined parameter for the pure polymer of nanoparticle preparation. Amorphous polymers are characterised by their Tg indicating the temperature range within the polymer changes from being hard and brittle to a more soft and plastic manner. However, Tg of the resulting polymeric nanoparticle matrix is not necessarily the same as Tg of the pure polymer and is often unknown or not regarded. The close examination of Tg of different PLGA-, DL-PLA-, and L-PLA-based polymeric nanoparticles and the subsequent correlation to a temperature dependent release behaviour of two model drugs from nanoparticles is the subject of the present study.

4.1. Glass transition temperature of unloaded PLGA-NP

Differences in Tg between PLGA-NP in aqueous suspension, freeze dried PLGA-NP, and the pure PLGA were observed. A decrease in Tg of freeze dried PLGA-NP compared to the polymeric carrier was determined as well as a lowered Tg of PLGA-NP in suspension compared to the freeze dried nanoparticles. In order to exclude an influence of excipients such as PVA or mannitol, physical mixtures and solid solutions of the three components in the same composition present in the nanoparticles were prepared and analysed. A shift in the Tg of the polymer due to the presence of PVA or mannitol could not be detected (Table 1). Another experiment using different PVA concentrations during nanoparticle preparation did not show an antiplasticising effect of PVA compared to the standard nanoparticle formulation. These findings are in contrast to the mild antiplasticising effect of PVA previously reported by Rouse et al. (Rouse et al., 2007). However, when comparing the non-hermetically measured Tg of the PLGA starting material (45.4 $^{\circ}$ C) with the Tg of freeze dried PLGA-NP (47.2 $^{\circ}$ C) an antiplasticising effect of the excipients PVA or mannitol may be assumed.

According to the literature, PLGA (copolymer ratio 50:50) is characterised by a Tg of about 45 $^{\circ}$ C (Mu and Feng, 2003a). This value could only be confirmed when measured under moisture removing conditions using non-hermetically sealed pans. In Fig. 3 the correlation between residual moisture content in freeze dried PLGA-NP and the corresponding Tg is presented. With decreasing residual moisture Tg increased indicating that water appears to have a plasticising effect on the polymer (Passerini and Craig, 2001; Zweers et al., 2004; Blasi et al., 2005). Water might interact with polar polymer chain groups like the carboxyl group existing in PLGA or interfere with intermolecular polymer chain bonds with the result of increased polymer chain mobility and decreased Tg. Small amounts of water up to 3.7 mg/g freeze dried nanoparticles were able to decrease the Tg of PLGA-NP efficiently (37.3 $^{\circ}$ C) in comparison to Tg of the completely dried nanoparticles (47.2 $^{\circ}$ C). In

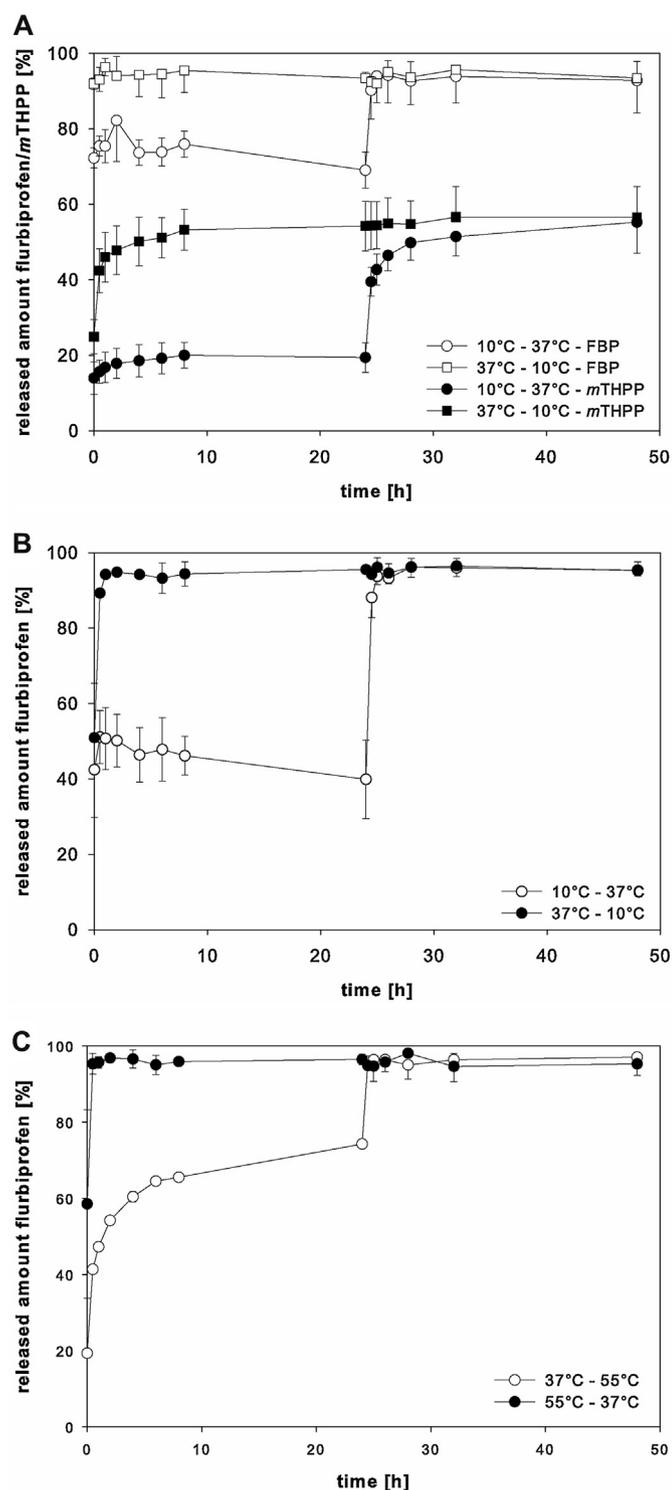


Fig. 5. Release profiles of flurbiprofen and mTHPP from (A) PLGA-NP at 10 °C and 37 °C in different media, temperature was altered after 24 h from 10 °C to 37 °C and vice versa. Release of flurbiprofen from (B) DL-PLA-NP at 10 °C and 37 °C in phosphate buffer pH 8.0, temperature was altered after 24 h from 10 °C to 37 °C and vice versa, (C) L-PLA-NP at 37 °C and 55 °C in phosphate buffer pH 8.0, temperature was altered after 24 h from 37 °C to 55 °C and vice versa (mean \pm SD; n = 3).

the literature three different types of water are described: Water closely associated to the polymer matrix is so called “non-freezing water” because of the absence of a thermal event in DSC measurements. “Freezing bound water” is the less closely associated water fraction which undergoes melting and

crystallisation, but different to that of free water. If the melting and crystallisation of water is not significantly different from those of normal (bulk) water, it is denoted as “freezing water” (Hatakeyama and Hatakeyama, 1998). The thermograms of freeze dried PLGA-NP did not show thermal events assigned to melting or crystallisation of water. The residual moisture seems to be attributed to “non-freezing water” whereas PLGA-NP in aqueous suspension also contained “freezing bound water” since melting and crystallisation of water were visible in the thermograms showing supercooling. It seems that only the closely associated “non-freezing water” was responsible for the plasticising effect on PLGA-NP whereas an excess of water did not lead to increased polymer chain mobility (Blasi et al., 2005).

4.2. Flurbiprofen-loaded nanoparticles

The incorporation of flurbiprofen in the three different polymeric matrices PLGA, DL-PLA, and L-PLA was successful obtaining monodisperse particle size distributions and a sufficient drug load. Compared to the corresponding unloaded nanoparticles the Tg of the drug-loaded nanoparticles was decreased. It has to be assumed that incorporated flurbiprofen acts as a plasticiser within the polymeric matrices as formerly described for ketoprofen in polymeric films. The interaction occurs mainly via the hydrogen of the hydrophilic carboxyl group of flurbiprofen and the carbonyl groups along the polymer backbone (Blasi et al., 2007). The hydrophilic carboxyl group of flurbiprofen might also interact with additional water molecules. This water co-localisation might also enhance the polymer chain mobility.

As shown in Table 2, Tg of FBP-PLGA-NP in suspension decreases steadily with increasing amounts of flurbiprofen. A higher drug content seems to be able to enhance polymer chain mobility resulting in lowered Tg. The shift in Tg suggests the formation of a solid solution during nanoparticle preparation since the Tg of the polymer is only affected when the drug is incorporated in a solid solution (Pamujula et al., 2004). To verify this assumption freeze dried FBP-PLGA-NP were analysed regarding glass transition temperature of the polymer and melting temperature of flurbiprofen. Flurbiprofen exhibited a melting peak at 115 °C, so a measurement in aqueous suspension was not possible and consequently a bulking agent for freeze drying without a thermal event at this temperature was chosen. As expected, Tg of the freeze dried FBP-PLGA-NP was lowered with increasing flurbiprofen loading (Table 3) due to the higher polymer chain mobility as previously shown for the suspended nanoparticles. Because of the pre-treatment at 80 °C for 2 h before the DSC measurements, residual moisture was removed and could not influence the Tg of the nanoparticles. There were no melting peaks visible in the thermogram of FBP-PLGA-NP-2.5, FBP-PLGA-NP-5, and FBP-PLGA-NP-10 in comparison to FBP-PLGA-NP-20 and FBP-PLGA-NP-30 (data not shown for FBP-PLGA-NP-2.5, 5 and 20). In Fig. 4 a broad melting peak at 85 °C to 115 °C was present in the thermogram of FBP-PLGA-NP-30. This effect is probably due to a part of flurbiprofen that is incorporated in crystalline state (Panyam et al., 2004). The broadening of the peak can be attributed to interactions between crystalline drug and polymer due to the nanoparticle preparation process. The absence of a melting peak in the second to fourth heating cycle suggests that previously crystalline flurbiprofen is from now on embedded in form of a solid solution into the polymeric matrix after melting which was not possible during nanoparticle preparation. A shift in the Tg of the polymer to lower temperatures in the second to fourth heating cycle supported this assumption. Further explanations like the presence of an enthalpy relaxation peak due to the thermal history could be excluded because of the pre-treatment of freeze dried FBP-PLGA-NP which were heated above the corresponding Tg

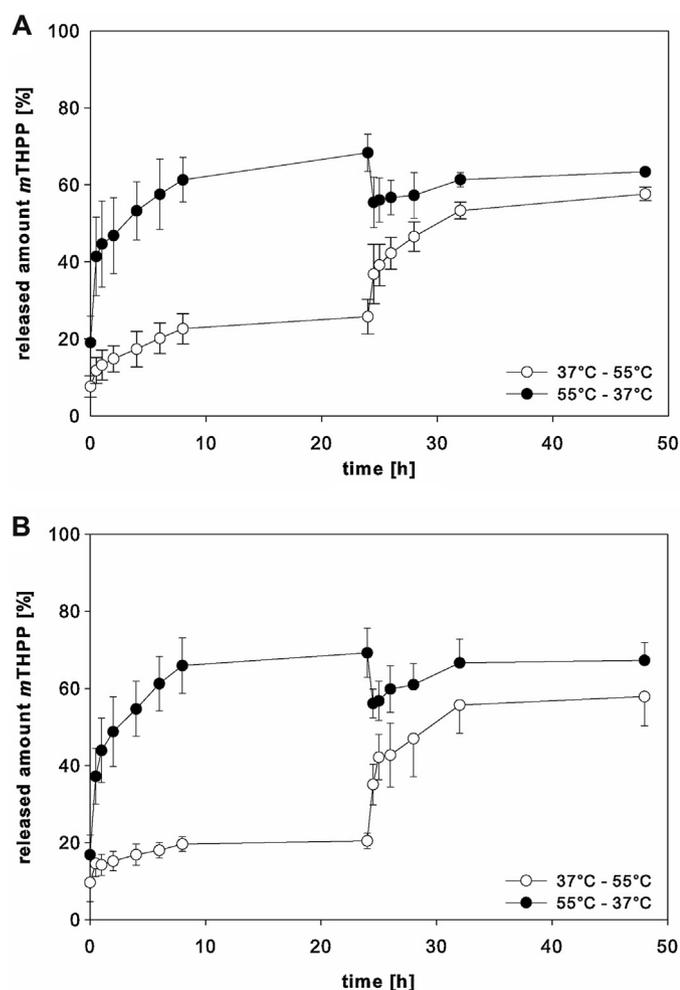


Fig. 6. Release profiles of *m*THPP from (A) DL-PLA-NP and (B) L-PLA-NP at 37 °C and 55 °C in HSA solution (5%, w/v), temperature was altered after 24 h from 37 °C to 55 °C and vice versa (mean \pm SD; n = 3).

(Rouse et al., 2007). The same effect could also be shown for the physical mixture of mannitol, PLGA, PVA, and flurbiprofen which was obtained by intensive blending of the solid pure materials. The sharp melting peak of crystalline flurbiprofen in the first heating cycle disappeared accompanied by a shift in T_g in the second to fourth heating cycle indicating the formation of a solid solution (Pamujula et al., 2004). For FBP-PLGA-NP crystalline drug was detectable starting at a minimum drug load of 140 μ g/mg assuming a limited miscibility of flurbiprofen and PLGA in the nanoparticle matrix.

4.3. *m*THPP-loaded nanoparticles

The more lipophilic compound *m*THPP was chosen as a second model drug. As already described for flurbiprofen, the photosensitizer *m*THPP was embedded in polymeric PLGA, DL-PLA, and L-PLA nanoparticles. Using an emulsion diffusion method nanoparticles with a monodisperse particle size distribution and a sufficient drug load were achieved (Tables 2 and 4). The T_g of *m*THPP-loaded nanoparticles was almost comparable to the T_g of the corresponding unloaded nanoparticle system. The molecular weight of *m*THPP (678.8 g/mol) is in comparison to flurbiprofen (244.3 g/mol) three times higher and the molecular structure of *m*THPP is sterically demanding and due to this the drug is not able to associate closely to the polymer for increasing polymer chain mobility. Moreover, *m*THPP is a more lipophilic drug and a co-localisation with water is

unlikely. Consequently, no plasticising effect of *m*THPP could be observed in any polymeric system under investigation.

4.4. Release kinetics of flurbiprofen-loaded nanoparticles

Drug release from colloidal systems may depend on various effects like diffusion in the solid particles, solubilisation by penetrating solvents in the matrix followed by drug diffusion through water-filled pores, or degradation of the polymer (Washington, 1990; Streubel et al., 2003; Vilar et al., 2012). The drug release from polymeric nanoparticles in this study was conducted over a maximum period of 48 h. In this time frame degradation processes are negligible, since PLGA nanoparticles are described to degrade over a period of several weeks (Zweers et al., 2004).

A burst release of about 93% flurbiprofen from FBP-PLGA-NP-10 was observed at an incubation temperature of 37 °C (Fig. 5A). At the lower temperature of 10 °C only about 70% drug was released within 24 h. Assuming that a part of the drug is not incorporated into the nanoparticle matrix, but adsorbed at the large particle surface, the burst release can be explained independently from the temperature (Soppimath et al., 2001; Kumari et al., 2010). Due to the small amount of flurbiprofen detected in the supernatant of nanoparticle suspensions (1.2 μ g flurbiprofen/mg NP), a significant release prior to the experiment can be excluded. The drug amount not liberated within the burst release at 10 °C is probably tightly incorporated into the nanoparticle matrix so that the release of this drug portion mainly depends on the polymer chain mobility (Faisant et al., 2006). With the choice of an incubation temperature above T_g , the polymer chain mobility increases thus creating new free volume in the polymeric matrix which is a prerequisite for a mainly diffusion controlled drug delivery (Mu and Feng, 2003b; Streubel et al., 2003). Altering the experimental temperature after 24 h from 10 °C, which is below T_g of FBP-PLGA-NP-10, to 37 °C thus above T_g an instant flurbiprofen release from 70% to 93% could be observed underlining the presented assumption of a T_g influenced release rate (Fig. 5A). The vice versa change in incubation temperature from 37 °C to 10 °C resulted in no shift in the released flurbiprofen amount demonstrating no temperature dependent drug solubility. An experimental setup under sink conditions was confirmed by the determination of the solubility of flurbiprofen in phosphate buffer at pH 8.0. At 10 °C and 37 °C the drug solubility was 43-fold and 50-fold higher than the released flurbiprofen concentration from the nanoparticle system.

The release kinetics of FBP-DL-PLA-NP are shown in Fig. 5B. With a T_g of 34.1 ± 0.6 °C, incubation temperatures of 10 °C and 37 °C, below and above T_g , were chosen. As described before for FBP-PLGA-NP-10, at temperatures above T_g (37 °C) a burst release of approximately 90% incorporated drug was determined for FBP-DL-PLA-NP. At the lower temperature (10 °C) only 40% flurbiprofen was liberated in the same time frame. This flurbiprofen represents the drug fraction that is associated with the surface, leading to a large burst effect. The other part of flurbiprofen is incorporated in the nanoparticle matrix, which was liberated when increasing the temperature above T_g .

In the case of FBP-L-PLA-NP incubation temperatures were set to 37 °C and 55 °C due to the higher T_g of 43.5 ± 1.3 °C. The same temperature dependent release behaviour was observed as previously shown for FBP-PLGA-NP-10 and FBP-DL-PLA-NP (Fig. 5C) underlining the presented correlation between T_g and release behaviour.

4.5. Release kinetics of *m*THPP-loaded nanoparticles

Due to the limited solubility of *m*THPP in aqueous buffer systems, the release study of the three different drug loaded

nanoparticle preparations was performed in 5% (w/v) HSA solution. Under these conditions the solubility of *m*THPP was about 17.5 fold higher compared to the maximum released *m*THPP concentration and therefore sink conditions were retained.

In the case of *m*THPP-PLGA-NP at 10 °C only 20% *m*THPP was released during 24 h in contrast to 55% liberated drug at 37 °C. This temperature dependent effect can be attributed to an increased polymer chain mobility and therefore increased diffusion possibilities of the embedded *m*THPP at higher temperatures. This assumption was confirmed by an immediate release of *m*THPP up to 55% when increasing the temperature from 10 °C to 37 °C. In contrast to flurbiprofen over the time period of 48 h no quantitative *m*THPP release was observed. This can be attributed to an insufficient degradation of the nanoparticle matrix over 48 h, which is required for a widespread release of larger molecules such as *m*THPP.

The burst release for *m*THPP-PLGA-NP was about 20% at 10 °C suggesting that this part of the drug is adsorbed onto the nanoparticle surface, while the rest is incorporated into the polymeric matrix with temperature dependent release behaviour. A very low amount *m*THPP was found in the supernatant of the nanoparticle suspensions (0.2 µg *m*THPP/mg NP) so that only negligible drug release occurred prior to the release experiment. In comparison to flurbiprofen the incorporation of *m*THPP in a polymeric nanoparticle matrix seems to be more effective with a much lower burst release and less liberation over 48 h. Hence, in the case of *m*THPP a higher solubility in PLGA might lead to a lower drug release (Streubel et al., 2003; Panyam et al., 2004). Interactions between hydrophobic drug molecules and the PLGA polymer matrix via hydrophobic binding forces are described in the literature underlining this assumption (Wischke and Schwendeman, 2008). Additionally, *m*THPP is more lipophilic due to a log P of 9.4 in contrast to flurbiprofen with a value of 2.9, respectively (Bouchard et al., 2003; Kirejev et al., 2014). However, the higher solubility of *m*THPP in PLGA showed no effect on Tg of the particle system. On the first sight this is unexpected, but can potentially be attributed to the sterically demanding *m*THPP structure, impeding a close association to the polymer as mentioned above.

Increased incubation temperatures of 37 °C and 55 °C were chosen for *m*THPP-loaded DL-PLA-NP and L-PLA-NP because of Tg values of 44.0 ± 0.4 °C and 53.9 ± 0.3 °C, respectively. At 37 °C, a temperature below Tg, a smaller drug amount was released than at 55 °C, a temperature above Tg. An instant release of *m*THPP was observed when the temperature was raised above Tg after 24 h assuming temperature dependent release behaviour for these nanoparticle systems. The alteration of temperature from 55 °C to 37 °C led to a slope in the released drug content which could not be fully balanced over the next 24 h. Additional release profiles in 1% (w/v) PVA solution were performed with a less pronounced drop after the temperature change (data not shown). These findings suggest a protein-associated phenomenon which could be attributed to the HSA content in the release medium.

Although Resomer[®] polymers are intended for controlled or extended drug release, the present study shows that the release kinetics of Resomer[®]-based nanoparticles depends mainly on the physico-chemical properties of the respective drug and the temperature of the experimental release setup. More lipophilic drugs such as *m*THPP in combination with a temperature below Tg of the polymer are released from the nanoparticles in a controlled manner whereas less lipophilic drugs at a temperature above the polymers' Tg are characterized by a short-term burst release.

5. Conclusion

The present study investigated the influencing factors on Tg of different polymeric nanoparticles. The importance of water as a

plasticising agent on amorphous polymers could be verified. Furthermore, the drug dependent influences on nanoparticles Tg were evaluated. It has to be noted that the molecular size, structure, and hydrophilicity of the used drug are relevant parameters with a crucial influence on Tg of the resulting nanoparticle system. Small and more hydrophilic molecules like flurbiprofen lead to a decrease in the Tg of nanoparticles whereas lipophilic molecules such as *m*THPP with a sterically demanding structure show no effect on Tg.

Additionally, the Tg dependent release behaviour of drug-loaded nanoparticles was examined. For a total of six different nanoparticle systems consisting of the polymers PLGA, DL-PLA, and L-PLA in combination with the model drugs flurbiprofen and *m*THPP, a temperature dependent release behaviour was evaluated. For all formulations a nanoparticle Tg below the temperature of the release medium led only to a burst release of the surface adsorbed drug substance whereas a temperature above the Tg of nanoparticles led to a release of the polymer entrapped drug amount. This effect could be useful for the selection of a suitable storage temperature for nanoparticle suspensions as well as for the choice of a suitable polymeric carrier for nanoparticle preparation. One consequence is that a rational combination of drug and carrier polymer should exhibit a Tg above the physiological temperature of 37 °C in order to prevent burst release directly after application and therefore enable a stable long-term drug transport to the diseased organ or tissue.

However, in this study this phenomenon was observed for polymers based on glycolic acid and lactic acid. In the future further studies are necessary to verify this effect for other polymeric nanoparticles.

Declaration of interest

The authors report no declarations of interest.

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