International Journal of Pharmaceutics 399 (2010) 163–172

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Solubility of Ketoprofen in colloidal PLGA

Johannes Kluge[a], Marco Mazzotti[a,*], Gerhard Muhrer[b]

[a] ETH Zurich, Institute of Process Engineering, Sonneggstrasse 3, 8092 Zurich, Switzerland
[b] Novartis Pharma AG, Chemical and Analytical Development, 4002 Basel, Switzerland

ARTICLE INFO

Article history:
Received 4 June 2010
Received in revised form 11 August 2010
Accepted 13 August 2010
Available online 20 August 2010

Keywords:
PLGA
SFEE
Ketoprofen
Solid solubility
Supercritical

ABSTRACT

The successful design and development of pharmaceutical drug–polymer composites requires detailed information about the phase behavior of the drug–polymer binary system. This study presents an extended investigation of the phase equilibrium established between the chiral anti-inflammatory drug Ketoprofen (KET) and the bio-compatible and biodegradable polymer poly(lactic-co-glycolic) acid 5050 (PLGA). Equilibration experiments were carried out in aqueous suspensions of KET crystals together with PLGA in the form of spherical amorphous nanoparticles obtained by supercritical fluid extraction of emulsions (SFEE). The influence of temperature was studied in the range between 0 °C and 50 °C, while the effect of KET chirality was investigated by using two different crystalline forms of KET, namely enantiopure S-KET and a racemic compound, RS-KET, in equilibration experiments. It was found that the level of KET established in PLGA at equilibrium increases with temperature, e.g. from 6.9 wt.% at 20 °C to 25.8 wt.% at 40 °C for the case of S-KET. At each temperature level, the solubility of KET in PLGA was lower for equilibration with RS-KET, significantly higher for equilibration with S-KET, and the highest for simultaneous equilibration with both crystalline species. Experimental solubility data of KET in PLGA were also described in a model based on the Sanchez–Lacombe equation of state. For experiments carried out at 10 °C or below, an equilibrium state could not be reached even after a prolonged equilibration period, presumably because the polymer phase had undergone a transition into the glassy state. For this temperature range, where an experimental equilibration is not any more possible, the model may be used to estimate the solubility of KET in PLGA by extrapolation.

1. Introduction

The microencapsulation of active pharmaceutical ingredients into polymeric drug delivery systems is a promising and widely used method in drug formulation technology, enabling a number of interesting novel pharmaceutical delivery concepts. For instance, in controlled drug release applications, encapsulation enhances and prolongs the effectiveness of active ingredients, while in drug targeting, polymeric particles are used as carrier vehicles for the targeted delivery of the drug to a specific site of action.

The entrapping of an active ingredient into polymeric microparticles may be achieved by different techniques. Among the conventional processes, the most prominent and widely applied are spray drying, emulsion processes with subsequent solvent extraction or evaporation, and anti-solvent processes such as coacervation. A number of new manufacturing methods are based on the application of supercritical fluids, i.e. mostly $scCO_2$, as anti-solvent, aerosol propellant and solvent-extracting agent (Yeo and Kiran, 2005). In this context, the process called supercritical fluid extraction of emulsions (SFEE) is a relatively new technique that has been particularly successful in the formulation of micro- and nanoparticles of water-insoluble pharmaceutical polymers such as poly(lactic-co-glycolic) acid (PLGA) (Kluge et al., 2009a).

In many cases, the co-formulation of drug and polymer aims at producing a solid solution where the drug is dispersed in the amorphous polymer matrix at a molecular level. However, the entrapment of the active ingredient into the polymeric material may be thermodynamically unfavorable, especially if high drug loadings are desired, or if the mutual affinity between drug and polymer is low. Such co-formulations would bear the risk of reduced long-term stability and shelf-life, representing a major hurdle with respect to potential applications (Vasanthavada et al., 2005). Hence, the design and development of such co-formulations could strongly benefit from a priori information about the compatibility of drug and polymer, in order to select a promising polymer excipient forming stable co-formulations and allowing for maximal drug load. However, there is still a lack of such data, as well

* Corresponding author. Tel.: +41 44 6322456; fax: +41 44 6321141.
E-mail address: marco.mazzotti@ipe.mavt.ethz.ch (M. Mazzotti).

0378-5173/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2010.08.016

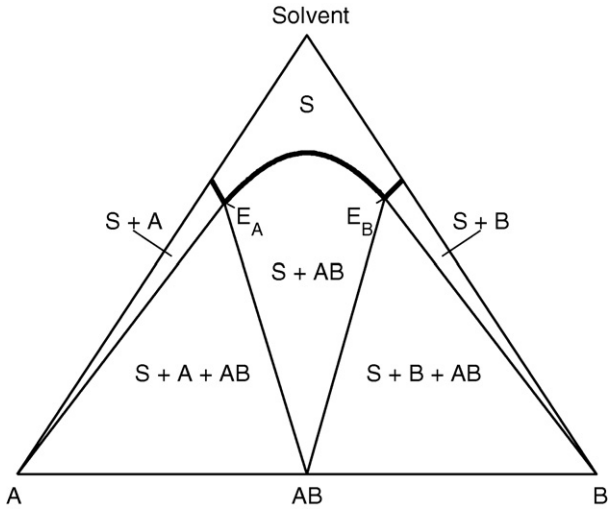

Fig. 1. Scheme of a ternary phase diagram for the solution of a compound-forming chiral system such as KET. (A) S-KET, (B) R-KET, (AB) the crystalline compound RS-KET and (S) the solution. The bold line represents the solubility of the relevant solid form of KET in PLGA. Eutectic points are designated as E_A and E_B, respectively.

as of robust and reliable experimental methods to generate such information in an accurate and efficient manner.

A crucial step in this direction is the determination of the level of drug present in the polymer phase if the latter is fully equilibrated with the pharmaceutical compound in the crystalline state, i.e. the solubility of the drug in the polymeric matrix, which in this case represents the solvent. In practice, equilibration is hardly achieved in short times since it is based on the slow diffusion of molecules inside the polymer phase. However, the equilibration process may be facilitated by using polymeric nanoparticles, where diffusion distances are short, and consequently, equilibration is fast. In this context, a previous study has successfully demonstrated that solvent-free PLGA nanoparticles as obtained by SFEE processing may be used to investigate the equilibration of PLGA with crystalline Ketoprofen (KET), a chiral molecule belonging to the class of non-steroidal anti-inflammatory drugs (NSAIDs) (Kluge et al., 2009b).

In order to further underline the potential that arises from SFEE as a viable manufacturing technique and in order to highlight a novel application of and opportunity for this process, this work presents the results of an extended study, investigating the temperature dependence of the phase equilibrium between KET and PLGA in the range between 0 °C and 50 °C, as well as the effect of KET chirality. While it may be anticipated that the solubility of KET in PLGA increases with the temperature, the expected effect of chirality on equilibrium needs to be elaborated in more detail. As a chiral molecule, KET exists in nature as two mirror-symmetric enantiomers, S-KET and R-KET. In the solid state KET forms a racemic compound, i.e. a crystal structure with a one to one ratio of S-KET and R-KET, which is crystallographically different from the enantiopure crystals of S-KET and R-KET (Lu et al., 2009). The characteristic symmetric behavior of such a system in solution is shown qualitatively in Fig. 1. At temperatures below the melting point, there are three thermodynamically stable solids of KET, namely crystalline S-KET (A), crystalline R-KET (B), and the racemic crystalline compound further referred to as RS-KET (AB). Depending on the ratio of the two enantiomers in the solution, equilibrium is always established with the relevant solid. Only in the transition from one domain to the other, the solution is simultaneously in equilibrium with two solids, i.e. with one of the enantiopure crystals and with the racemic compound. The fixed composition of the solution in these transition points is referred to as the eutectic composition, and is designated with E_A and E_B in Fig. 1.

Racemic RS-KET as well as enantiopure S-KET are commercially available, and have been used for the preparation of seed crystals. Hence, if PLGA is equilibrated with crystals of S-KET or RS-KET, solutions of enantiopure or racemic composition, respectively, are obtained. If PLGA is equilibrated with both crystalline forms simultaneously, a solution with eutectic composition is attained.

2. Experimental

2.1. Materials

Carbon dioxide (CO_2; 99.9%, from PanGas, Schlieren, Switzerland), poly(lactic-co-glycolic) acid 5050 DLG 5A (PLGA; Lakeshore Biomaterials, Birmingham, AL, United States), poly(vinyl alcohol) 4-88 (PVA), racemic Ketoprofen (RS-KET), enantiopure S-Ketoprofen (S-KET), dimethylsulfoxide (DMSO), trifluoroacetic acid (TFA), ethyl acetate (all from Sigma–Aldrich, Buchs, Switzerland), ethanol (analytical grade, Scharlau, Sentmenat, Spain), and n-hexane (Merck KGaA, Darmstadt, Germany) were used as received.

2.2. Emulsion preparation and SFEE processing

The organic solutes, i.e. PLGA, RS-KET or S-KET, or specified mixtures thereof, were dissolved at 10 wt.% in ethyl acetate saturated with water. A 1 wt.% solution of PVA was prepared in water saturated with ethyl acetate. The organic and aqueous solutions were used at a weight ratio of 1:4 to form an oil-in-water (o/w) emulsion upon ultrasonication with a Branson Sonifier 450 (Skan AG, Basel, Switzerland). Using 100 ml of emulsion, a sonication time of 2 min was applied at maximal power output while cooling the emulsion on ice.

Directly after emulsion preparation, the organic solvent was extracted from the dispersed micelles using $scCO_2$ as extracting agent in the supercritical fluid extraction of emulsions (SFEE) process. A scheme of the experimental setup used for the SFEE experiments as well as a detailed description of the process can be found in a previous study (Kluge et al., 2009a).

When only PLGA was used for the preparation of the emulsion and for SFEE processing, the procedure led to the formation of pure PLGA nanoparticles, which were then used for the impregnation experiments described in Section 2.4. Otherwise, if KET was directly dissolved in the organic micelles together with PLGA, SFEE processing led to KET–PLGA composite particles, as they were used for the de-supersaturation experiments described in Section 2.5.

2.3. Preparation of Ketoprofen crystals

Crystals of the racemic compound RS-KET and of enantiopure S-KET were prepared as follows. In a first step, either RS-KET or S-KET was used for the preparation of an emulsion and subsequent SFEE processing as described above. In all cases, this step led to a suspension of solvent-free amorphous precursor particles, and these product suspensions were stored in 40 ml aliquots that were allowed to recrystallize for 2 days. After recrystallization, each aliquot contained approximately 1 g of crystals, i.e. either the compound, RS-KET, or enantiopure crystals of S-KET. In order to remove all crystal fragments too small for sedimentation, the crystals were repeatedly centrifuged at 4000 rpm (Eppendorf Centrifuge 5810R) and resuspended in pure water until the supernatant was clear to the eye and considered free of suspended solids. The obtained fraction of large crystals of either RS-KET or S-KET was resuspended in water and used for equilibration experiments. Fig. 2 allows for a comparison of seed crystal morphology as compared to the corresponding raw material. It can be seen that the recrystallized

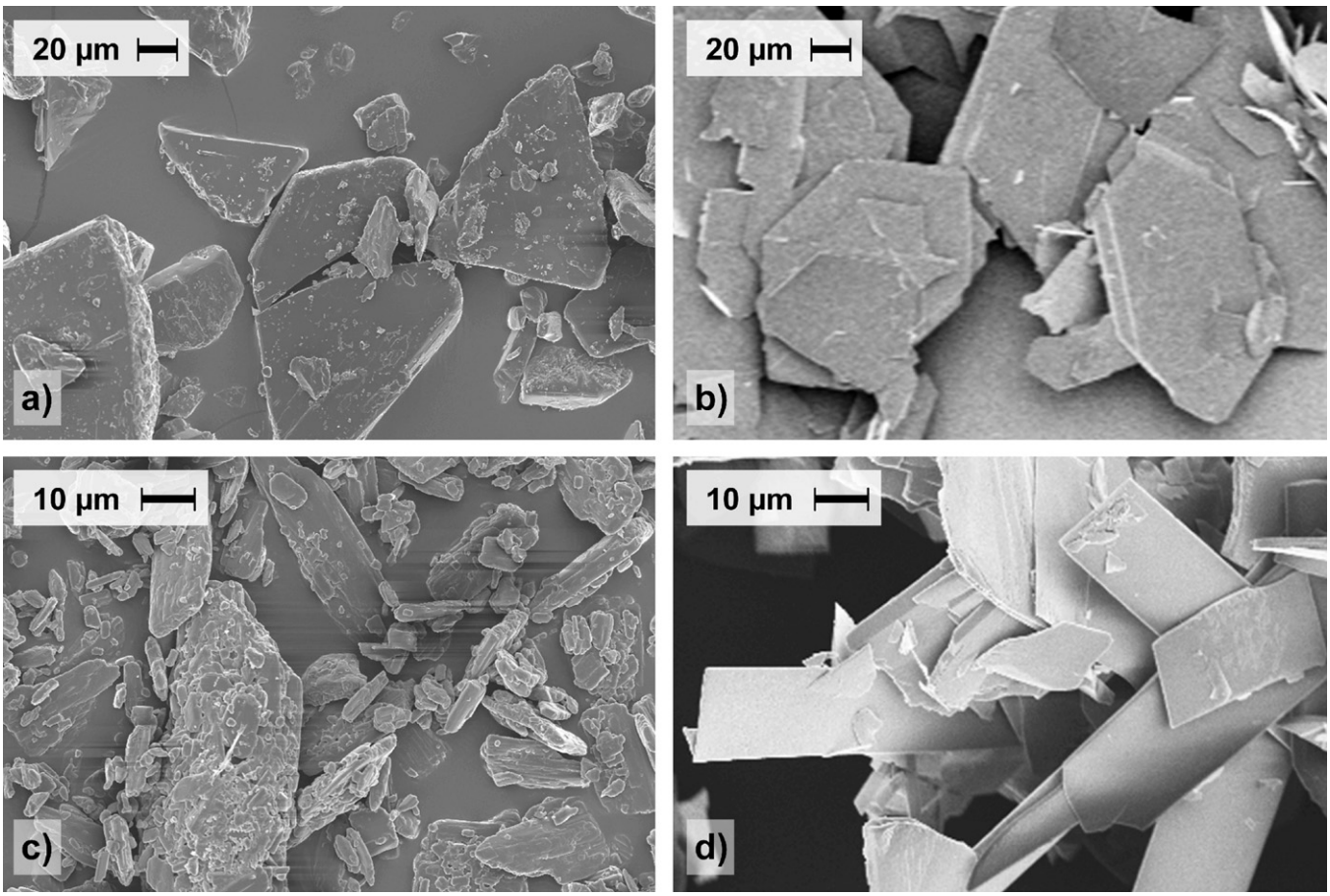

Fig. 2. Raw materials (left) respectively recrystallized seed crystals (right) of S-KET (top) and RS-KET (bottom). The recrystallized seeds are generally larger and of more regular structure as compared to the raw material.

material consists of larger and well-developed crystals that have a much more regular shape as compared to the raw material.

2.4. Equilibration-impregnation experiments

Experiments for equilibration by impregnation were carried out in a thermostated shaking water bath (GFL 1086, GFL, Burgwedel, Germany). Pure PLGA nanoparticles were mixed and equilibrated in suspension with Ketoprofen crystals, namely with RS-KET, S-KET and also with mixtures thereof. Three 200 ml samples of PLGA product suspension, each containing about 2.5 g PLGA as suspended nanoparticles, were mixed with 40 ml aliquots containing KET crystals while being kept on ice, i.e. with RS-KET, S-KET or both RS-KET and S-KET, respectively. For equilibration, the mixed suspensions were put in the shaking water bath and kept at a constant temperature of 0 °C for 1 day before samples were taken to analyze the amount of KET uptaken by PLGA through impregnation. The temperature was then increased to 50 °C in steps of 10 °C each. Samples were always taken after at least 1 day of equilibration at constant temperature.

2.5. Equilibration-de-supersaturation experiments

Supersaturated KET–PLGA nanoparticles were produced in three separate SFEE experiments, using each 2.5 g PLGA as solute, and 0.5 g RS-KET or 0.5 g S-KET or a mixture of 0.3 g S-KET and 0.2 g RS-KET, respectively. The selection of these KET:PLGA ratios was based on previous experiences from impregnation experiments at 20 °C. Equilibration-de-supersaturation experiments were carried out in the thermostated shaking water bath. The obtained product suspensions, i.e. three 200 ml samples each containing about 3 g of supersaturated KET–PLGA co-formulation in the form of suspended particles, were mixed with 40 ml aliquots containing KET crystals while being kept at 20 °C, i.e. with RS-KET or S-KET or both RS-KET and S-KET, respectively. For equilibration, the mixed suspensions were then put in the shaking water bath and kept at a constant temperature of 20 °C for 4 days before samples were drawn to analyze the amount of KET remaining in PLGA after equilibration by de-supersaturation. The temperature was then decreased to 10 °C and 0 °C. Samples were taken as specified, i.e. after at least 4 days of equilibration at constant temperature. Also, part of the samples was kept at constant temperature in a separate vessel, in order to monitor further concentration changes in the polymer phase during the following days.

2.6. Product characterization

2.6.1. Sample preparation

The recovery of equilibrated PLGA particles was carried out in two steps. First, all KET crystals present were isolated from the suspension by applying 10 min centrifugation at the medium rotation speed of 4000 rpm twice (Eppendorf Centrifuge 5810R, Vaudaux-Eppendorf AG, Basel, Switzerland), thus sedimenting all crystals, but leaving polymeric nanoparticles suspended in the crystal-free supernatant. Throughout the entire process, all samples were carefully kept at the equilibration temperature of the corresponding sample. After centrifugation, the crystalline sediment was recycled to the equilibration experiment, and the crystal-free supernatant

Table 1
Overview of the results of product analysis. Weight fractions given in roman numbers are assumed to represent equilibrium data, whereas weight fractions in *italic* do not correspond to equilibrium state, as explained in the text.

	Temperature (°C)	Equilibration time (days)	RS-KET content (wt.%)	S-KET content (wt.%)	RS-KET + S-KET	
					content [wt.%]	composition [% S-KET]
Impregnation	Initial	–	*0.00*	*0.00*	*0.00*	N.A.
	0	1	*0.83*	*1.24*	*1.37*	89.8
	10	1	*1.29*	*2.27*	*2.42*	93.2
	20	1	3.30	6.88	7.41	93.6
	30	1	5.07	11.53	12.43	93.5
	40	1	9.13	25.83	30.24	92.6
	50	1	15.25	*37.22*	*44.96*	89.3
De-supersaturation	Initial	–	*11.12*	*10.03*	*11.62*	81.6
	20	4	3.63	6.83	7.06	–
		8	3.44	6.56	6.80	94.1
	10	4	*3.20*	*5.15*	*5.93*	–
		21	*3.21*	*5.09*	*5.97*	94.2
	0	7	*3.10*	*5.08*	*5.91*	–
		14	*3.12*	*5.08*	*5.85*	94.0
		30	*3.04*	*5.04*	*5.77*	–

was subjected to 15 min of ultra-centrifugation at 20,000 rpm (Avanti J-20, Beckman, USA) in order to sediment the polymeric nanoparticles, while the excess of the PVA surfactant could be discarded with the particle-free supernatant. Finally the nanoparticles were resuspended in pure water and subsequently freeze-dried for further analysis (FlexiDry, FTS Systems, USA).

2.6.2. SEM photomicrographs

In order to assess the morphology and the size of the nanoparticles, SEM photomicrographs were obtained from dried samples sputter-coated with about 5 nm platinum using a Zeiss Gemini 5 1530 FEG scanning electron microscope.

2.6.3. KET content by UV spectroscopy

A known amount of freeze-dried polymeric nanoparticles containing approximately 10 mg Ketoprofen was dissolved in 10 ml of DMSO. A volume of 100 ml of the solution was pipetted into a cuvette containing 2 ml DMSO, and the concentration of KET was determined by UV spectroscopy (Beckman Coulter, DU520). For calibration, five independent solutions containing 10 mg KET were analyzed as described. At a wavelength of 300 nm, a molar extinction coefficient of 1217.01 AU/(mol/l) was determined and used for the conversion of absorption units into molar concentrations. The described method, which could not distinguish between the two enantiomers of KET, was used for the determination of KET content in all samples.

2.6.4. Enantiomeric composition by HPLC

For PLGA samples equilibrated with both RS-KET and S-KET crystals, the enantiomeric composition was determined by High Performance Liquid Chromatography (HPLC). Liquid samples were obtained by extracting both KET enantiomers from a small amount of dried product using ethanol. Enantioseparation of KET in the extract was carried out on a 4.6 mm × 250 mm chiral chromatographic column Chiralpak AD (Daicel Chemical Industries Ltd., Japan). The mobile phase was a mixture of 20% ethanol and 80% n-hexane (v/v) containing 0.01% TFA, and a flow rate of 0.7 ml/min was used. Depending on the sample concentration, amounts between 2 μl and 50 μl were injected, and injections were repeated three times. Monitoring of the outlet stream by UV spectroscopy at 260 nm showed that baseline separation was successfully achieved in all cases. At the conditions of the analysis R-KET had a retention time of 7.6 min, while S-KET was the more retained species, and was eluted after 9.7 min. The enantiomeric composition in the sample was directly calculated from the two peak areas, assuming linear UV response.

2.6.5. Crystallinity by X-ray powder diffraction (XRPD) analysis

The crystalline sediment obtained during sample preparation in the first centrifugation step was freeze-dried and investigated using XRPD in order to determine the structure of the crystalline residual.

3. Results and discussion

In order to determine the influence of temperature as well as of KET chirality on the equilibrium solubility of KET in PLGA, the equilibration of amorphous PLGA nanoparticles with different crystalline forms of KET was studied in aqueous suspension. Both KET and PLGA are virtually insoluble in water, which indicates that the degree of physicochemical interaction between water and both solids is small. Hence, the presence of water is assumed to have negligible influence on the equilibrium established between KET and PLGA.

Equilibration may be achieved from initial concentrations of the PLGA phase either below or above the solubility limit. The two cases differ only with respect to the direction of KET flux. In the first case, the polymer phase is initially undersaturated, and is equilibrated by impregnation, i.e. by KET dissolving from the crystalline phase, and diffusing through the aqueous phase into the polymer matrix, until the polymer phase is saturated. In the second case, where the polymer phase is initially supersaturated, equilibration is achieved by supersaturation depletion, i.e. by KET diffusing out of the polymer matrix, through the aqueous phase until it is consumed by growth of KET crystals.

A proper combination of impregnation and de-supersaturation experiments as outlined above is a reliable way to investigate whether or not equilibrium has indeed been achieved. For example, it has been demonstrated for the case of RS-KET at ambient conditions that an equivalent level, i.e. the equilibrium solubility of KET in PLGA, is ultimately attained by following either procedure (Kluge et al., 2009b). On the other hand, if equilibrium is not reached, impregnation and de-supersaturation experiments still provide a lower and an upper bound, respectively, for the solubility of KET in PLGA.

In that sense, this work represents an extension of our previous investigation on the equilibrium between PLGA and KET to the temperature range between 0 °C and 50 °C, covering relevant con-

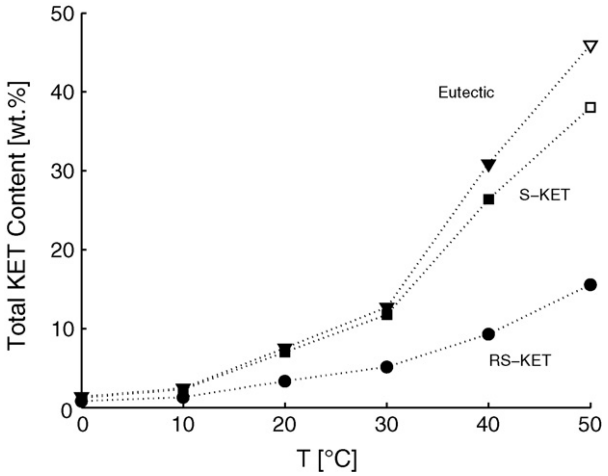

Fig. 3. Total KET content (sum of both enantiomers) of PLGA equilibrated at different temperatures with crystals of RS-KET (●), S-KET (■) and with both crystal forms together (▲). Open symbols: experiments at 50 °C where equilibrium has not been reached due to complete dissolution of crystals.

ditions of long-term storage at refrigerated or ambient conditions as well as physiological temperatures encountered during application of the drug product. Moreover, a broader range of feasible KET solid states has been investigated, namely enantiopure S-KET, racemic RS-KET and a combination thereof. The work is structured as follows: first, the results of the equilibration experiments carried out both by impregnation and by de-supersaturation are presented, then experimental results are integrated in a mathematical model describing the solubility of a chiral compound in a polymeric solvent, and finally conclusions are drawn by comparing experimental results with model predictions.

3.1. Equilibration-impregnation experiments

Equilibration of PLGA by impregnation has been studied in three experiments using enantiopure S-KET, racemic RS-KET or a mixture of the two solids, respectively, and varying the temperature between 0 °C and 50 °C. Experiments started with initially pure PLGA nanoparticles at 0 °C, and the temperature was increased in steps of 10 °C. Assuming that the equilibrium solubility increases with temperature, this means that at each temperature level, PLGA particles are initially undersaturated, and that equilibration is always attained by impregnation of KET into PLGA. A previous study carried out at ambient temperatures has shown that the uptake of KET by impregnation takes place immediately after mixing. Although the experiment has been continued during the following week, a further increase of the impregnated drug content was not observed (Kluge et al., 2009b). Based on this experience, an impregnation time of 1 day has been used in the present study, and we presume that equilibrium between KET and PLGA is reliably established within this time frame.

Experimental results are summarized in Table 1, and illustrated in Figs. 3–5. It can be readily observed that in all three experiments, the level of KET impregnated into PLGA increases with increasing temperature, starting from levels around 1 wt.% at 0 °C, and reaching a peak value of above 40 wt.% at 50 °C. At each temperature level, the concentration of KET impregnated into PLGA is lower for equilibration with RS-KET, significantly higher for equilibration with S-KET, and the highest for simultaneous equilibration with both crystalline species. For samples that had been equilibrated simultaneously with RS-KET and S-KET, also the eutectic composition has been determined at each temperature level. The composition was found to remain rather constant, i.e. with S-KET at around 93% and R-KET at around 7%, except for the samples at 0 °C and 50 °C, which exhibit a lower content of S-KET.

During storage at 50 °C it was observed that the crystalline residual of S-KET disappeared completely, hence equilibrium could no longer be established since solid S-KET had been completely consumed through dissolution and impregnation. The situation was different for RS-KET, where crystals were still present, thus preserving the equilibrium. In the case of simultaneous equilibration with S-KET and RS-KET, XRPD analysis of the crystalline residual showed that S-KET crystals had completely dissolved whereas RS-KET was still present. Hence, PLGA was equilibrated with RS-KET, but not with S-KET, and indeed the corresponding sample is characterized by a lower content of S-KET, i.e. around 89%, as compared to the other samples exhibiting the eutectic composition of roughly 93%. In Fig. 3, the two experiments at 50 °C where equilibrium was not reached are indicated by open symbols, and they are obviously not considered in the further analysis and discussion of KET–PLGA equilibrium. Nevertheless, the KET loadings measured at 50 °C are clearly higher as compared to those determined at 40 °C, thus proving that at 40 °C the amounts of crystalline KET present were still sufficient to enable equilibration in all impregnation experiments.

Co-formulation particles obtained at different temperature levels by impregnation of PLGA with S-KET are shown in Fig. 4a–c, whereas particles obtained at 20 °C by impregnation of PLGA using the different crystalline morphologies of KET available are shown in Fig. 4d–f. In agreement with previous results (Kluge et al., 2009b), it is noticed that particles exhibiting a high KET content, such as those displayed in Fig. 4c, appear generally softer and more blurred, and are also characterized by a rougher surface. Otherwise, polymeric particles exhibit in all cases a very similar morphology. Co-formulations equilibrated at 40 °C and 50 °C were observed to recrystallize promptly upon storage at ambient conditions, obviously because they were highly supersaturated at room temperature. Important product properties such as the overall KET content and the enantiomeric composition of the samples were not affected by recrystallization, and could still be determined. Indeed, these recrystallization events underline the significance of determining equilibrium data for the manufacturing of long-term stable drug–polymer composites.

3.2. Equilibration-de-supersaturation experiments

Equilibration of PLGA by de-supersaturation has been studied in three experiments using enantiopure S-KET, racemic RS-KET or a mixture of the two solids, respectively, and by investigating the temperature range between 0 °C and 20 °C. Experiments were initiated by mixing crystals and nanoparticles of previously prepared KET–PLGA composites at 20 °C. After at least 4 days, the level of KET in PLGA was determined and subsequently the equilibration temperature was decreased in steps of 10 °C. Thus, each experiment at a new temperature starts with PLGA particles that are initially supersaturated, hence equilibration is always achieved by diffusion of KET out of the supersaturated PLGA particles. A previous 1-week study investigating the equilibration of PLGA and KET at ambient temperatures has shown that de-supersaturation is significantly slower as compared to impregnation (Kluge et al., 2009b). Based on experiences from that study, an equilibration time of at least 4 days was selected, and part of the samples was kept at constant temperature in a separate vessel, in order to check and exclude further concentration changes.

Experimental results are summarized in Table 1, and illustrated in Fig. 6. The level of KET in the polymeric nanoparticles is initially above 10 wt.% in all three experiments, and therefore supersaturated with respect to the solubilities expected at the initial temperature of 20 °C. Thus, equilibration at 20 °C leads to a considerable decline in KET content in all three experiments,

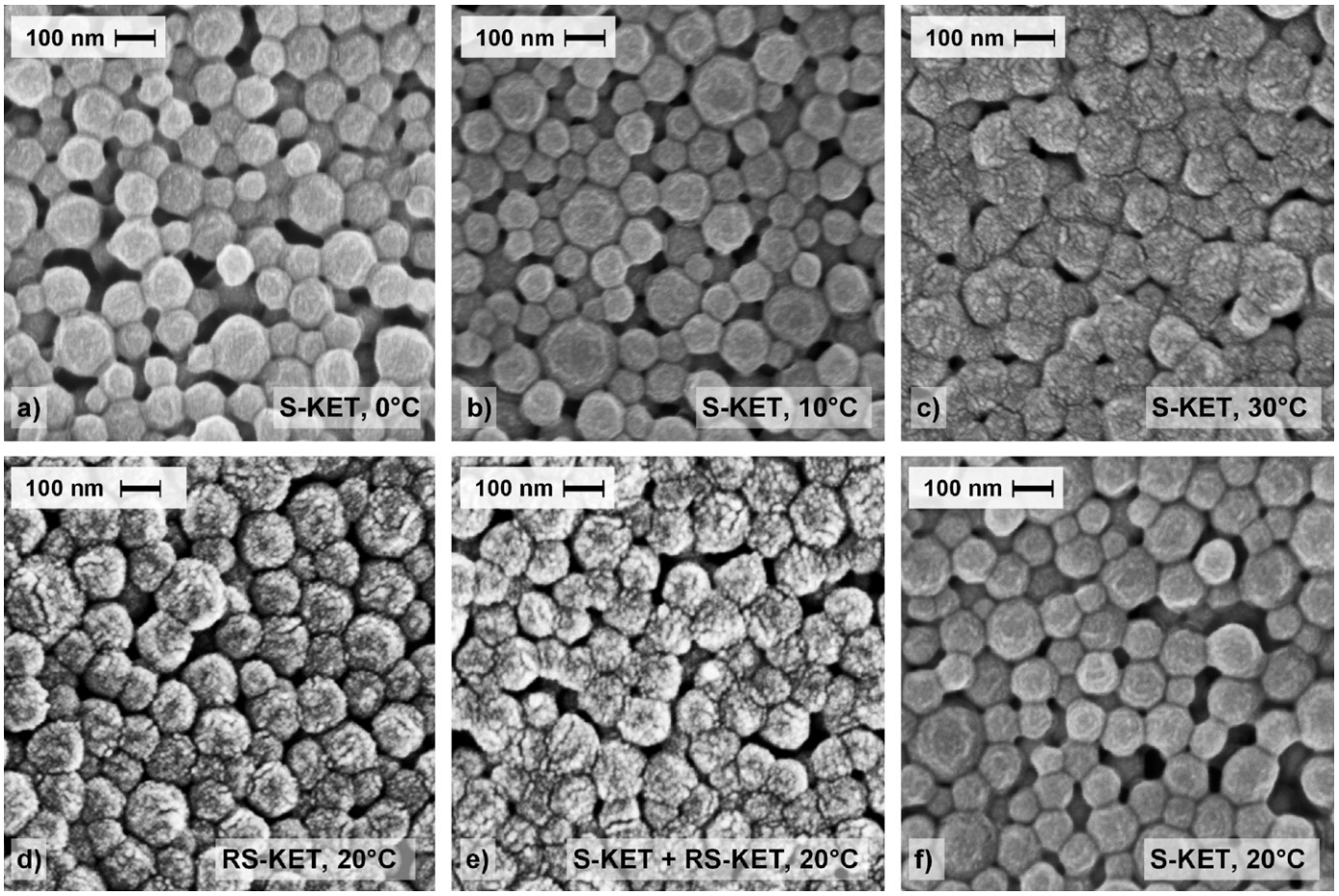

Fig. 4. (a–c) Co-formulation particles obtained at different temperature stages by impregnation of PLGA with S-KET. (d–f) Co-formulation particles obtained at 20 °C by impregnation of PLGA using different crystalline morphologies of KET.

and the KET levels measured after 4 days of equilibration are indeed very close to the levels attained by impregnation at the same temperature. This indicates that at 20 °C, both impregnation and de-supersaturation lead to complete equilibration, and measured concentrations correspond to the solubility of KET in PLGA that is attained in equilibrium with the relevant KET solid states. Since equilibration depends on temperature dependent kinetic phenomena such as diffusion and crystal dissolution and growth,

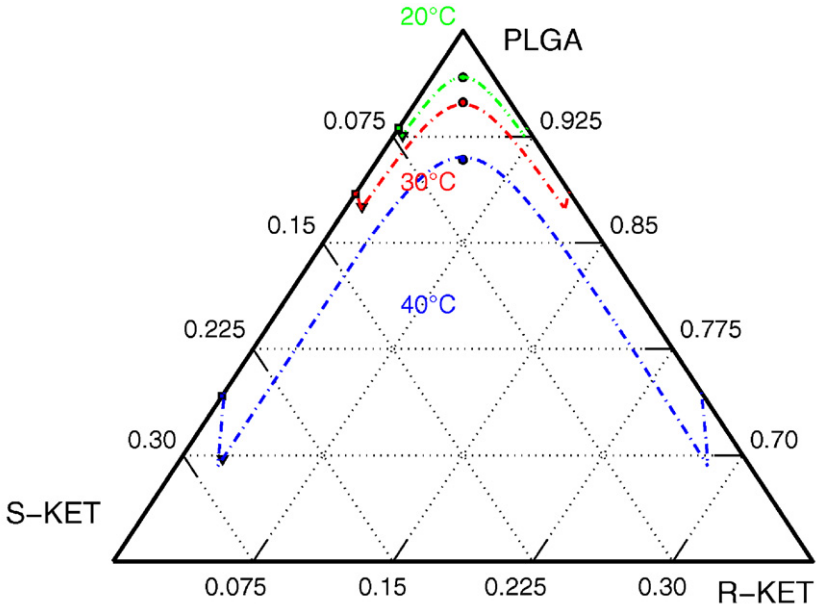

Fig. 5. Comparison of experimental data points obtained by impregnation at 20 °C (green), 30 °C (red) and 40 °C (blue) to the liquidus lines obtained from the SL model at these temperatures. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 2
Physico-chemical and material properties of KET and PLGA and pure component parameters used in the Sanchez–Lacombe equation of state.

Property	Units	RS-KET	S-KET	PLGA	Source
MW (number av.)	(g/mol)	254.3	254.3	33 400	(Dwan'Isa et al., 2007)/supplier
T_m	(K)	367.7	348.6	–	(Lu and Ching, 2004)
T_G	(K)	270.2	270.2	319.4	(Di Martino et al., 2004; Blasi et al., 2007)/supplier
ΔH^f	(kJ/mol)	27.38	22.79	–	(Lu and Ching, 2004)

Parameter	Units	S-KET	R-KET	PLGA	Source
P^*	(MPa)	662.3	662.3	572.7	(Liu and Tomasko, 2007; Coimbra et al., 2006; Gauter and Heidemann, 2000)
T^*	(K)	806.0	806.0	649.6	
ρ^*	(kg/m^3)	1169	1169	1452	

respectively, i.e. processes that are generally faster at elevated temperatures, it may be concluded that de-supersaturation and impregnation lead to complete equilibration also at temperatures above 20 °C.

In further de-supersaturation experiments carried out at lower temperatures, i.e. 10 °C and 0 °C, only small decreases in the KET content have been observed, as shown in Fig. 6. In all cases, the measured KET contents remained clearly above the concentration levels obtained in impregnation experiments at the same temperature. As shown in Table 1, even changes in the drug content upon longer equilibration times are negligibly small as compared to the gap remaining between the outcome of de-supersaturation and impregnation experiments. Therefore it is concluded that either impregnation or de-supersaturation – or presumably both – do not lead to complete equilibration at lower temperatures, since the kinetics of equilibration are too slow at 10 °C and below. Also, it is worth pointing out that the average standard deviation observed in the evaluation of 0 °C samples taken at different points in time is smaller than 0.1 wt.%. This suggests that the accuracy of experimental methods used in sample preparation and analysis is high enough to detect even small concentration changes.

Summarizing, de-supersaturation experiments yielded equilibrium data at 20 °C only. From the major disagreement between the outcome of impregnation and de-supersaturation experiments at 10 °C and 0 °C, it is concluded that these measurements do not represent equilibrium data, but should be seen as lower and upper bounds, respectively, for the corresponding equilibrium states.

3.3. Modeling equilibrium between KET and PLGA

In this section, a model is introduced to describe the equilibria of Ketoprofen, as a compound-forming chiral system, and PLGA, as a polymeric solvent. The description of equilibrium between solid KET and amorphous, polymeric PLGA includes three different equilibrium situations, namely equilibrium of the polymer either with the racemic solid, RS-KET, or with the enantiopure solid, S-KET, or with both solids at the same time. In the following section, the species S-KET, R-KET and PLGA are indicated by subscripts 1, 2 and 3, respectively. Subscripts *RS* and *S* refer to the two crystalline forms RS-KET and S-KET, respectively.

Equilibrium of PLGA with an enantiopure solute such as S-KET at a temperature T may be described using the Schröder–van Laar Equation (Lorenz and Seidel-Morgenstern, 2002; Worlitschek et al., 2004):

$$\ln(a_1^s) = -\frac{\Delta H_S^f}{RT_S^m}\left(\frac{T_S^m}{T} - 1\right) \tag{1}$$

where T_S^m is the melting temperature of S-KET and ΔH_S^f is its enthalpy of fusion; a_1^s is the activity of solute S-KET in a polymer solution that is in equilibrium with crystalline S-KET. On the other hand, equilibrium between solid racemic RS-KET and a ternary solution of both enantiomers in PLGA is described by the Prigogine–Defay Equation (Lorenz and Seidel-Morgenstern, 2002; Worlitschek et al., 2004):

$$\frac{1}{2}\ln(4a_1^s a_2^s) = -\frac{\Delta H_{RS}^f}{RT_{RS}^m}\left(\frac{T_{RS}^m}{T} - 1\right) \tag{2}$$

where T_{RS}^m is the melting temperature and ΔH_{RS}^f the enthalpy of fusion of the racemic compound RS-KET; a_1^s and a_2^s are the activities of S-KET and R-KET, respectively, in a polymer solution that is in equilibrium with RS-KET. Thus, the right-hand sides of Eqs. (1) and (2) are formally comparable, and since they contain only solute parameters they can be calculated using the material properties of either S-KET or RS-KET as given in Table 2. The activities are functions of temperature and composition, and are described using the Sanchez–Lacombe (SL) equation of state. This equation is based on a lattice fluid theory, and has found a broad use in the modeling of phase equilibria involving polymers (Pini et al., 2008). The SL equation of state is given by (McHugh and Krukonis, 1994):

$$\tilde{\rho}^2 + \tilde{P} + \tilde{T}\left[\ln(1-\tilde{\rho}) + \tilde{\rho}\left(1 - \frac{1}{r^0}\right)\right] = 0 \tag{3}$$

Here, $\tilde{\rho}$, $\tilde{P}$ and $\tilde{T}$ are the reduced density, pressure and temperature, respectively. The variables are reduced using the so-called characteristic quantities:

$$\tilde{\rho} = \frac{\rho}{\rho^*} = \frac{\rho r^0 v^*}{M_W}, \qquad \tilde{P} = \frac{P}{P^*} = \frac{Pv^*}{\varepsilon^*}, \qquad \tilde{T} = \frac{T}{T^*} = \frac{TR}{\varepsilon^*} \tag{4}$$

where ρ^*, P^* and T^* are the characteristic density, pressure and temperature. These are in turn functions of the Sanchez–Lacombe lattice parameters, namely of v^*, the volume of one mole of lattice sites, r^0, the number of lattice sites per molecule, and ε^*, the interaction energy per lattice site. R is the ideal gas constant and M_W is the molecular weight, i.e. the number average molecular weight in the case of polymers. Hence, three of the characteristic quantities are needed to completely characterize a pure fluid. For PLGA 5050, SL characteristic parameters have been reported previously (Liu and Tomasko, 2007) and for KET, they may be estimated from the critical parameters (Coimbra et al., 2006) using a set of equations proposed elsewhere (Gauter and Heidemann, 2000). Numerical values of the pure component characteristic parameters used in this study are reported in Table 2.

Mixing rules according to the so-called van der Waals 1 theory have been applied in order to extend these equations to the case of a mixture (McHugh and Krukonis, 1994; Prausnitz et al., 1986). A first mixing rule describes the volume of lattice sites in a ternary mixture:

$$v^* = \sum_{i=1}^{3}\sum_{j=1}^{3}\Phi_i\Phi_j v_{ij}^*, \qquad v_{ij}^* = \frac{v_i^* + v_j^*}{2}(1-\eta_{ij}) \tag{5}$$

where i and j are component indices and the parameters η_{ij} correct for deviations from the arithmetic mean. We assume that there is no such deviation for mixing of the two enantiomers (1 and 2), and

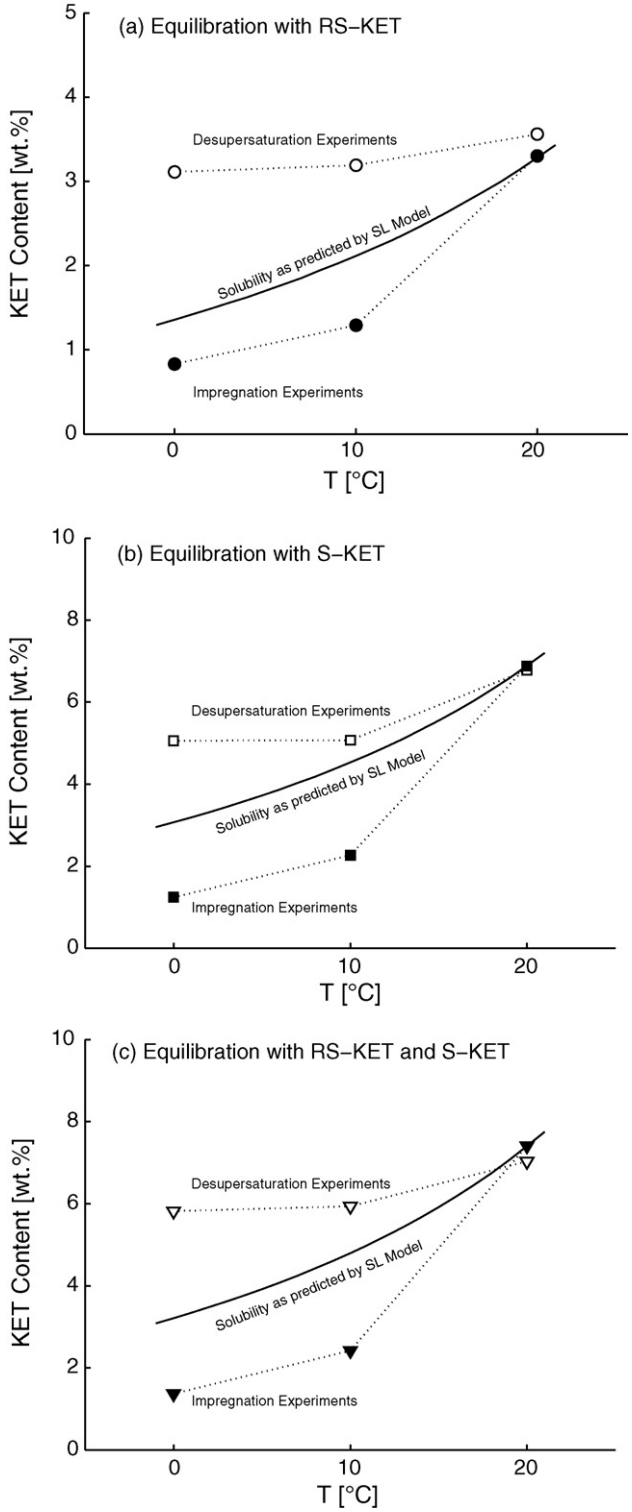

Fig. 6. Experimental data points obtained by equilibration through impregnation and de-supersaturation using (a) crystalline RS-KET, (b) crystalline S-KET and (c) a mixture thereof at temperatures of 0 °C, 10 °C and 20 °C as compared to the corresponding liquidus lines obtained by extrapolation of the SL model. The average standard deviation associated with the procedure of sample preparation and analysis was found to be smaller than 0.1 wt.%.

that it is the same for mixing of the KET enantiomers (1 or 2) with PLGA (3):

$$\eta_{12} = \eta_{21} = 0, \qquad \eta_{13} = \eta_{23} = \eta_{31} = \eta_{32} \tag{6}$$

The variable Φ_i is the volume fraction of the ith component in the mixture and is related to its weight fraction w_i:

$$\Phi_i = \frac{w_i/\rho_i^* v_i^*}{\sum_{j=1}^{3}(w_j/\rho_j^* v_j^*)} \tag{7}$$

A second mixing rule defines the characteristic interaction energy per lattice site, ε^*, for the case of a mixture:

$$\varepsilon^* = \frac{1}{v^*}\sum_{i=1}^{3}\sum_{j=1}^{3}\Phi_i\Phi_j\varepsilon_{ij}^* v_{ij}^*, \qquad \varepsilon_{ij}^* = \sqrt{\varepsilon_i^*\varepsilon_j^*}(1-k_{ij}) \tag{8}$$

where k_{ij} is a binary interaction parameter. Here, we assume interactions both between the two enantiomers (1 and 2) and between either of the enantiomers (1 or 2) and achiral PLGA (3):

$$k_{12} = k_{21}, \qquad k_{13} = k_{23} = k_{31} = k_{32}. \tag{9}$$

Finally, the number of sites occupied by one mole of the mixture, r, is calculated using the third mixing rule:

$$\frac{1}{r} = \sum_{i=1}^{3}\frac{\Phi_i}{r_i}, \qquad r_i = \frac{r_i^0 v_i^*}{v^*} \tag{10}$$

Hence the number of lattice sites occupied by one mole of component i in the mixture, r_i, differs from the one in the pure fluid, r_i^0.

At equilibrium, temperature, pressure and the chemical potentials of all species are equal in both phases. Using the set of mixing rules presented above, the chemical potential of component i in the mixture is:

$$\begin{aligned}\mu_i = RT\Bigg[&\ln\ \Phi_i + \left(1-\frac{r_i}{r}\right)\Bigg] \\ &+ r_i\left\{-\tilde{\rho}\left[\frac{2}{v^*}\left(\sum_{j=1}^{3}\Phi_j\varepsilon_{ij}^* v_{ij}^* - \varepsilon^*\sum_{j=1}^{3}\Phi_j v_{ij}^*\right) + \varepsilon^*\right]\right\} \\ &+ r_i\left\{\frac{RT}{\tilde{\rho}}\left[(1-\tilde{\rho})\ln(1-\tilde{\rho}) + \frac{\tilde{\rho}}{r_i}\ln\ \tilde{\rho}\right] + \frac{P}{\tilde{\rho}}\left[2\sum_{j=1}^{3}(\Phi_j v_{ij}^*) - v^*\right]\right\}\end{aligned} \tag{11}$$

For KET, also the standard chemical potential of the pure subcooled liquid state is required, and can be obtained by solving Eq. (11) for pure species 1:

$$\begin{aligned}\mu_1^{\ominus} = \mu_2^{\ominus} = r_1^0\Bigg\{&-\tilde{\rho}_1\varepsilon_1^* + \frac{RT}{\tilde{\rho}_1}\left[(1-\tilde{\rho}_1)\ln(1-\tilde{\rho}_1) + \frac{\tilde{\rho}_1}{r_1^0}\ln\ \tilde{\rho}_1\right] \\ &+ \frac{P}{\tilde{\rho}_1}v_1^*\Bigg\}\end{aligned} \tag{12}$$

Using Eqs. (11) and (12), the activities of species 1 and 2 in the mixture can be calculated:

$$\ln\ a_1 = \frac{1}{RT}(\mu_1 - \mu_1^{\ominus}), \qquad \ln\ a_2 = \frac{1}{RT}(\mu_2 - \mu_2^{\ominus}) \tag{13}$$

3.4. Results

At fixed temperature, there are three independent parameters that need to be assigned to characterize equilibrium in the ternary system of the two enantiomers of KET and the polymeric solvent PLGA, namely η_{13}, k_{12} and k_{13}. As it may be expected for a system containing polar species, these mixture parameters are dependent

on temperature (McHugh and Krukonis, 1994), and are therefore expressed using linear functions of T:

$$\eta_{13} = c_1 T + c_2, \qquad k_{12} = c_3 T + c_4, \qquad k_{13} = c_5 T + c_6. \tag{14}$$

For the experiments where equilibrium has been reached (see Table 1), the activities as given by Eq. (13) should be equal to the activities in equilibrium, as given by Eqs. (1) and (2). For this set of experiments, the coefficients c_1 to c_6 in (14) have been obtained by optimization using a Nelder–Mead Simplex algorithm, solving the set of Eqs. (1)–(14), further called the SL model, while minimizing an objective function for which the following contribution has been considered at each temperature level:

$$\Psi(T) = \left(\frac{\ln\ a_1 - \ln(a_1^s)}{\ln(a_1^s)}\right)^2\Bigg|_{\text{S-KET}} + \left(\frac{\ln(4a_1a_2) - \ln(4a_1^s a_2^s)}{\ln(4a_1^s a_2^s)}\right)^2\Bigg|_{\text{RS-KET}} + \frac{1}{2}\left[\left(\frac{\ln\ a_1 - \ln(a_1^s)}{\ln(a_1^s)}\right)^2 + \left(\frac{\ln(4a_1a_2) - \ln(4a_1^s a_2^s)}{\ln(4a_1^s a_2^s)}\right)^2\right]\Bigg|_{\text{Eutectic}} \tag{15}$$

The numerical values of the coefficients obtained upon optimization are:

$$\begin{aligned} c_1 &= 1.085 \times 10^{-3}\ \text{K}^{-1}, & c_2 &= -0.358; \\ c_3 &= 6.86 \times 10^{-4}\ \text{K}^{-1}, & c_4 &= -0.220; \\ c_5 &= -4.98 \times 10^{-5}\ \text{K}^{-1}, & c_6 &= 0.0525. \end{aligned} \tag{16}$$

Using these coefficents and assuming equilibrium between the polymer solution and the relevant solid phase, i.e. $a_1 = a_1^s$ and $a_2 = a_2^s$, the SL model has been used to calculate the range of compositions corresponding to the liquidus line of the polymer solution in the range of 20–40 °C. These are shown in Fig. 5 together with experimental data points obtained at these temperatures. It can be seen that the model is able to describe the effect of temperature rather well, and also the influence of chirality on the solubility of KET in PLGA.

An extrapolation of the model to lower temperatures of 0 °C and 10 °C is shown in Fig. 6, together with the corresponding data points obtained by impregnation and de-supersaturation experiments. It can be seen that at these conditions the model predictions are always above the experimental values obtained by impregnation, and below the levels obtained by de-supersaturation. This is in agreement with our previous findings, namely that the results from impregnation and de-supersaturation experiments at 10 °C and 0 °C represent lower or upper bounds for the corresponding equilibrium states rather than equilibrium states, respectively. We conclude that the model can also be used in a predictive manner to estimate equilibrium under conditions where its experimental determination is difficult.

3.5. Equilibration and glass transition temperature

A major remaining question is why equilibration is so much slower at temperatures below 10 °C as compared to temperatures above 20 °C. This may be explained by looking at the interplay of the operating conditions and of the glass transition temperature T_G that is expected for the polymeric particles.

Upon vitrification, i.e. the transformation of the polymer from the rubbery liquid state to the glassy state induced by cooling below the glass transition temperature, foreign molecules trapped in the polymer matrix loose their mobility, thus leading to a decrease in their diffusivity by several orders of magnitude (Foss et al., 2009). Hence the glass transition is expected to have a strong influence on the kinetics of equilibration, and might explain the observed behavior. Now pure PLGA has a T_G around 45 °C, i.e. far above the observed transition. However, there are two additional effects having a diminishing influence on T_G. First, PLGA is not pure, but contains KET that is dissolved at a molecular level. The effect of KET on the glass transition of PLGA has been investigated by Blasi et al. (2007), and a decrease of roughly 0.83 °C and 1.49 °C per wt.% KET has been determined experimentally using two different types of PLGA, respectively. Secondly, equilibration is carried out in aqueous suspension, and also the presence of water has a diminishing effect on T_G that has been assessed experimentally by Blasi et al. (2005). Depending on the duration and temperature of incubation in water, wet PLGA samples exhibited glass transition temperatures in the range between 26.7 °C and 32.7 °C, i.e. significantly below the T_G observed for dry PLGA (45 °C). Assuming that both effects are additive, the expected T_G of KET–PLGA co-formulations is shown in Fig. 7 as a function of the KET content, together with the range of possible temperature dependent equilibrium concentrations of KET in PLGA as determined from the SL model presented in Section 3.3.

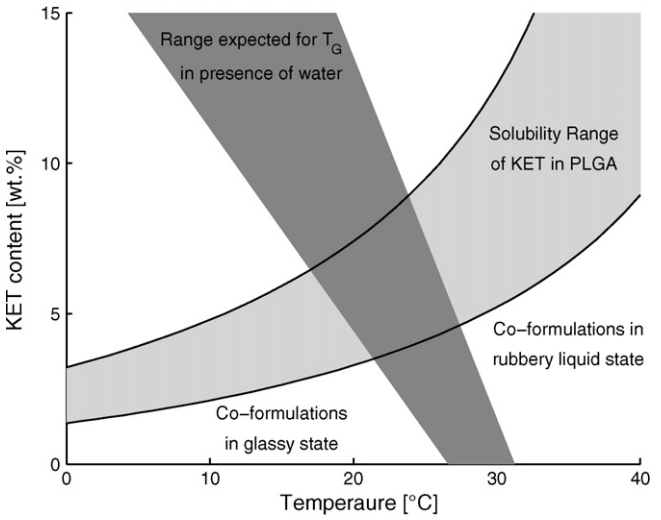

Fig. 7. Solubility range of KET in PLGA as calculated using the SL model (light grey zone) together with the expected range of the glass transition temperature of KET–PLGA composites suspended in water as a function of the KET content (dark grey zone). The glass transition separates the region of glass state (left) from the region of rubbery liquid state (right).

Fig. 7 illustrates how the equilibrated polymer phase is clearly in the rubbery liquid state for temperatures of 30 °C and above, leading to facilitated diffusion of KET molecules into the polymer matrix and to rapid equilibration of the polymer phase with its surroundings. On the other hand, if equilibration is carried out at 10 °C or below, the polymer phase is clearly in the glassy state, and consequently the movement of KET molecules within the polymer matrix is largely inhibited so as the polymer phase does not easily equilibrate with its surrounding. Judging from Fig. 7, the situation is less clear for equilibration at 20 °C, leading to compositions that are located in the transition region. However at 20 °C, i.e. ambient conditions, it is evident from this work as well as from previous experimental results (Kluge et al., 2009b) that complete equilibration is achieved during the selected experimental times by impregnation as well as by de-supersaturation.

4. Conclusion

The solubility of the chiral non-steroidal anti-inflammatory drug Ketoprofen in PLGA, i.e. one of the pharmaceutically most relevant polymer systems, has been determined experimentally and modeled using standard thermodynamic methods. The presented results are interesting from a fundamental point of view, since equilibrium data involving a chiral crystalline drug and a biocompatible polymer have been determined in detail and presented for the first time. Further, the present findings allow for a number of practically relevant conclusions with respect to potential

applications of PLGA as drug delivery system for KET, such as the level of drug that may be loaded into the polymer to achieve long-term stable co-formulations with acceptable shelf-life. Further, it has been demonstrated that the capacity of PLGA for S-KET is more than twice as high as compared to the racemic compound, RS-KET. Considering that the pharmaceutical activity of KET resides in S-KET whereas R-KET is inactive (Lu et al., 2009), the application of enantiopure S-KET allows stable drug loadings that are more than four times higher than the loadings achieved with racemic RS-KET. The presented approach to determine drug–polymer equilibrium seems also suitable for the comparison and the evaluation of different polymers with respect to their suitability as drug delivery systems for KET.

Experimental findings show that drug–polymer equilibrium is temperature dependent, and may be described using the Sanchez–Lacombe equation of state, i.e. a thermodynamic standard model that is typically applied in the description of equilibria involving polymers. It has been demonstrated that equilibration is fast for temperatures above the glass transition temperature of the polymer phase, but becomes very slow if the temperature of the equilibration process is lowered below T_G. Consequently, the experimental investigation of equilibrium for a polymer in glassy state seems to be more difficult as compared to a polymer in liquid-rubbery state. There are two conclusions about how to handle this problem. First, if a model is available, equilibrium for temperatures below T_G may be estimated by extrapolation of the model, as is shown in Fig. 6. Second, the experimental investigation of equilibrium may be extended and facilitated by lowering the T_G of PLGA, which can be achieved for instance by applying a high-pressure CO_2 atmosphere (Pini et al., 2008). It is also interesting whether or not the presence of an additional T_G-lowering component in the system, such as water in this case, has a strong influence on the investigated equilibrium.

Acknowledgement

The financial support by the Swiss Innovation Promotion Agency (Project KTI 7783.1) is gratefully acknowledged.

References

Blasi, P., D'Souza, S.S., Selmin, F., DeLuca, P.P., 2005. Plasticizing effect of water on poly(lactide-co-glycolide). J. Control. Release 108, 1–9.
Blasi, P., Schoubben, A., Giovagnoli, S., Perioli, L., Ricci, M., Rossi, C., 2007. Ketoprofen poly(lactide-co-glycolide) physical interaction. AAPS PharmSciTech 8.
Coimbra, P., Duarte, C.M.M., de Sousa, H.C., 2006. Cubic equation-of-state correlation of the solubility of some anti-inflammatory drugs in supercritical carbon dioxide. Fluid Phase Equilibr. 239, 188–199.
Di Martino, P., Joiris, E., Gobetto, R., Masic, A., Palmieri, G.F., Martelli, S., 2004. Ketoprofen–poly(vinylpyrrolidone) physical interaction. J. Cryst. Growth 265, 302–308.
Dwan'Isa, J.P.L., Rouxhet, L., Preat, V., Brewster, M.E., Arien, A., 2007. Prediction of drug solubility in amphiphilic di-block copolymer micelles: the role of polymer–drug compatibility. Pharmazie 62, 499–504.
Foss, W.R., Anderl, J.N., Clausi, A.L., Burke, P.A., 2009. Diffusivities of dichloromethane in poly(lactide-co-glycolide). J. Appl. Polym. Sci. 112, 1622–1629.
Gauter, K., Heidemann, R.A., 2000. A proposal for parametrizing the Sanchez–Lacombe equation of state. Ind. Eng. Chem. Res. 39, 1115–1117.
Kluge, J., Fusaro, F., Casas, N., Muhrer, G., Mazzotti, M., 2009a. Production of PLGA micro- and nanocomposites by supercritical fluid extraction of emulsions. I. Encapsulation of lysozyme. J. Supercrit. Fluids 50, 327–335.
Kluge, J., Fusaro, F., Muhrer, G., Mazzotti, M., 2009b. Production of PLGA micro- and nanocomposites by supercritical fluid extraction of emulsions. II. Encapsulation of ketoprofen. J. Supercrit. Fluids 50, 336–343.
Liu, D.H., Tomasko, D.L., 2007. Carbon dioxide sorption and dilation of poly(lactide-co-glycolide). J. Supercrit. Fluids 39, 416–425.
Lorenz, H., Seidel-Morgenstern, A., 2002. Binary and ternary phase diagrams of two enantiomers in solvent systems. Thermochim. Acta 382, 129–142.
Lu, Y.H., Ching, C.B., 2004. Physicochemical properties, binary and ternary phase diagrams of ketoprofen. Chirality 16, 541–548.
Lu, Y.H., Wang, X.J., Ching, C.B., 2009. Application of preferential crystallization for different types of racemic compounds. Ind. Eng. Chem. Res. 48, 7266–7275.
McHugh, M.A., Krukonis, V.J., 1994. Supercritical Fluid Extraction—Principles and Practice, 2nd edition. Butterworth-Heinemann, Newton, MA, pp. 120–123.
Pini, R., Storti, G., Mazzotti, M., Tai, H.Y., Shakesheff, K.M., Howdle, S.M., 2008. Sorption and swelling of poly(DL-lactic acid) and poly(lactic-co-glycolic acid) in supercritical CO_2: an experimental and modeling study. J. Polym. Sci. B: Polym. Phys. 46, 483–496.
Prausnitz, J.M., Lichtenthaler, R.N., Gomes de Azevedo, E., 1986. Molecular Thermodynamics of Fluid Phase Equilibria, 2nd edition. Prentice-Hall Inc., Englewood Cliffs, NJ.
Vasanthavada, M., Tong, W.Q., Joshi, Y., Kislalioglu, M.S., 2005. Phase behavior of amorphous molecular dispersions. II. Role of hydrogen bonding in solid solubility and phase separation kinetics. Pharm. Res. 22, 440–448.
Worlitschek, J., Bosco, M., Huber, M., Gramlich, V., Mazzotti, M., 2004. Solid–liquid equilibrium of troger's base enantiomers in ethanol: experiments and modelling. Helv. Chim. Acta 87, 279–291.
Yeo, S.D., Kiran, E., 2005. Formation of polymer particles with supercritical fluids: a review. J. Supercrit. Fluids 34, 287–308.