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SHORT COMMUNICATION

A slow cooling rate of indomethacin melt spatially confined in microcontainers increases the physical stability of the amorphous drug without influencing its biorelevant dissolution behaviour

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Abstract Amorphous indomethacin was prepared by melting the γ-form of indomethacin, spatially confined within microcontainers (inner diameter of 223 μm), followed by cooling of the melt at a rate of 14, 23 or 36 K/min. The physical stability of the amorphous indomethacin within microcontainers was investigated using Raman microscopy. Furthermore, the dissolution behaviour of confined amorphous indomethacin was evaluated in biorelevant intestinal media at pH 6.5. After 30 days of storage, 10.3±1.2 % of the amorphous indomethacin cooled at 14 K/min and confined within microcontainers was found to be crystalline. When the melt of indomethacin was cooled at 23 or 36 K/min, 20.7±1.5 and 31.0±2.6 % of the indomethacin were found to be crystalline after storage for 30 days. Scanning electron microscopy showed a smooth surface of amorphous indomethacin within the microcontainers when cooling the melt at 14 K/min, whereas cracks and an uneven surface were observed when cooling at rates of 23 and 36 K/min. The uneven surface is hypothesised to be the main reason for the lower physical stability, as the cracks could act as nucleation sites for crystal growth. The rate of cooling was not seen to have any effect on the dissolution of amorphous indomethacin from the microcontainers.

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Introduction

When administering a drug orally, drug delivery systems can be useful not only in the protection of a drug from harsh conditions such as those encountered in the stomach, but also to facilitate the controlled release of drugs. Microcontainers are micro devices consisting of a flat polymer base with a walled reservoir, which have shown promising properties as oral drug delivery systems [1, 2]. The microcontainers show advantages compared to traditional spherical particles for oral drug delivery, as it is easy to control the size and shape of the microcontainers. This allows for ease in optimisation processes, for instance in order to maximise the contact area between the microcontainers and the intestinal wall [3].

An increasing number of marketed drugs are poorly water soluble, and hence, drug solubility and dissolution properties in the gastrointestinal fluids can become rate limiting for oral drug absorption [4]. One of the most common interventions to improve the dissolution characteristics of a drug is to convert the drug to its amorphous form [5, 6]. However, the amorphous form is in a thermodynamically unstable state and will spontaneously convert to a metastable or stable crystalline form over time [5, 7–9]. It is therefore necessary to stabilise the amorphous form and inhibit or delay spontaneous crystallisation, so that the solubility and dissolution advantages of an amorphous formulation can be utilised. An amorphous form will crystallise when sufficient molecular mobility exists; various efforts have therefore been made to decrease

molecular mobility and thereby achieve physical stabilisation of the amorphous form [6]. Surface crystallisation of the amorphous form can initiate heterogeneous nucleation and accelerate crystal growth, an occurrence which can be prevented by coating or uniformly dispersing the drug in a polymer [10, 11]. A coating of only a few nanometres in thickness is necessary to reduce molecular mobility and thereby inhibit surface crystallisation [10]. In literature, it has been reported that a 3–20-nm coating of a polyelectrolyte or a 10-nm gold coating can inhibit surface crystallisation of amorphous indomethacin [10]. Previously, microcontainers have also been used to stabilise an amorphous drug, specifically indomethacin, by spatially confining the drug. In this case, the potential for dissipation of crystallinity is hindered [12]. Confinement of amorphous indomethacin in microcontainers has previously shown to lead to a 25 and 43 % higher physical stability compared to bulk indomethacin when microcontainers of 223 and 174 μm inner diameter, respectively, are employed [12].

Indomethacin is a nonsteroidal anti-inflammatory drug and is practically insoluble in water at pH 7 (0.94 mg/L). It has four polymorphic forms and one amorphous form [13, 14]. The most stable polymorph is the crystalline γ-form, while the α-form is reported as a metastable form of indomethacin [15, 16]. Amorphous indomethacin can be prepared using various methods, with the most frequently used method involving melting of γ-indomethacin, followed by cooling of the melt [17]. Cooling of the indomethacin melt should occur at a faster rate than the molecules can rearrange themselves into a crystalline lattice, thereby fixing the molecules in a disordered (amorphous) form [13, 18]. In literature, it has been demonstrated that even small variations in preparation conditions can greatly influence the physical stability of amorphous drugs [19]. Different preparation techniques for the same drug can result in variations in the molecular mobility and will influence the recrystallisation process substantially [18, 20]. Certainly, amorphous indomethacin prepared by melting and quench-cooling has been shown to crystallise to either the α- or γ-form depending on the specifics of the preparation method and also on the storage conditions employed [21, 22]. It has also been shown previously that rapid cooling of the melt prevented a full development of nuclei, thereby enhancing the glass stability of the amorphous form [6]. Appropriate cooling rates of the melt of indomethacin have been suggested to be as low as 0.2–1.2 K/min [20, 22–24]. Karmwar et al. showed that different cooling rates of indomethacin (varying from 1.2 to 30 K/min) resulted in differences on the molecular level and that variations in the crystallisation process were regulated by the number of nuclei formed [17, 20]. In accordance with the previously mentioned studies, indomethacin cooled at 30 K/min was seen to crystallise much more slowly than amorphous drug cooled at 3 and 1.2 K/min. The differences in physical stability observed as a result of employing different cooling rates did not, however, result in differences in the dissolution rate or dissolution behaviour of the amorphous indomethacin [20, 25].

Previously, amorphous indomethacin spatially confined within microcontainers was prepared by melting and quench-cooling of γ-indomethacin [12]. This preparation method created an uneven drug surface, with cracks clearly apparent in the amorphous indomethacin. It was speculated that these cracks provide nucleation sites for crystal growth, and by avoiding the formation of these cracks, a further physical stabilisation of the amorphous indomethacin could possibly be obtained [12]. The aim of this study was therefore to investigate if varying the processing conditions (cooling rates) used in the preparation of microcontainer-confined amorphous indomethacin could have an effect on drug physical stability and biorelevant dissolution behaviour. The effect of varying cooling rates was assessed using Raman microscopy.

Materials and methods

Materials

Indomethacin (Ph.Eur quality, γ-form) was obtained from Fagron (Copenhagen, Denmark). Ethanol (analytical grade) was purchased from Merck (Darmstadt, Germany). Potassium acetate (99 % purity) was sourced from Ajax Finechem (Auckland, New Zealand). Silicon wafers (4 in.<100>n-type) were purchased from Okmetic (Vantaa, Finland). SU-8 2075 and SU-8 Developer were supplied by Microresist Technology GmbH (Berlin, Germany). Chromium masks were designed using L-Edit® from Tanner EDA (Monrovia, CA, USA) and supplied by Delta Mask B.V. (GJ Enschede, the Netherlands). Taurocholic acid sodium salt hydrate (sodium taurocholate) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Phosphatidylcholine (Lipoid S PC, purity ≥98 %) was purchased from Lipoid AG (Ludwigshafen, Germany). Sodium hydroxide (NaOH), chloroform and sodium chloride were obtained from Merck (Darmstadt, Germany), as were sodium azide and potassium dihydrogen phosphate. Ultra-purified water was obtained freshly in each instance from a SG Ultra Clear water system (SG Water USA, LLC, Nashua, NH, USA).

Methods

Preparation of microcontainers

The microcontainers were fabricated as described previously with two steps of photolithography employing the negative epoxy-based photoresist SU-8 [12, 26]. The microcontainers had an inner diameter of 223±3 μm and a volume of

10.6±0.5 nL. The silicon wafers supporting the fabricated microcontainers were cut into squares of 12.8×12.8 mm^2 resulting in arrays of 25×25 containers with a pitch of 450 μm being contained on each square.

Preparation of amorphous indomethacin confined within microcontainers

Indomethacin (γ-form) was filled into 100 microcontainers on an array. The filled microcontainers were placed on a heating plate at 200 °C for 10 s in order to facilitate melting of the indomethacin. The indomethacin melt confined in the microcontainers was then cooled immediately by employing one of three cooling methods—plunging the microcontainers into liquid nitrogen, placing the microcontainers on ice or cooling at room temperature. The cooling rates were measured using an infrared thermometer and determined in each of the methods illustrated in Table 1. Preparation and cooling of amorphous indomethacin were repeated three times for each cooling rate.

Scanning electron microscopy

The morphology of the filled microcontainers was examined on the day of preparation using scanning electron microscopy (SEM). The investigations were carried out using a Nova 600 NanoSEM from FEI (Eindhoven, the Netherlands). Imaging was performed in low-vacuum mode at a pressure of 0.6 mbar and an operation voltage of 5 kV. Prior to examination, the samples were mounted onto metal stubs and were tilted by 15–30°.

Storage

Filled, cooled microcontainers were stored in a desiccator at 30.1±0.2 and 23.5±0.5 % relative humidity (RH) in order to investigate the effect of cooling rate on drug stability. A level of 23.5 % RH was obtained by placing a saturated solution of potassium acetate into the desiccator (equilibrium was achieved after 3 days). The RH was measured initially and regularly thereafter using a hygrometer (Testo 608-H1, Smorum, Denmark). The stability of indomethacin within microcontainers was then investigated as detailed below.

Table 1 Cooling methods and rates used for cooling the melt of indomethacin in order to produce the amorphous form

Cooling method	Cooling rate (K/min)
Room temperature (23 °C)	14
Ice	23
Liquid nitrogen	36

Physical stability of amorphous indomethacin confined within microcontainers

The physical stability of amorphous indomethacin confined within microcontainers was investigated using a similar approach to that described earlier [12]. Raman spectra of indomethacin confined within the microcontainers were gathered with a dispersive Raman microscope (Omnic, Thermo Fisher Scientific, Copenhagen, Denmark) controlled by Omnic software version 8.3 (Thermo Fisher Scientific). The Raman signal was generated using a 785-nm excitation wavelength and 10-mW power. A resolution of 9–18 cm^{-1} and an aperture of 50 μm were used. A ×10 objective was used for visualisation. Each Raman spectrum was collected for 5 s with two co-additions. The spectra were collected from 90 to 3,200 cm^{-1}, and the form of indomethacin in the microcontainers was identified by characteristic differences in the spectra of crystalline and amorphous indomethacin in the wave number range from 1,500–1,800 cm^{-1} [12].

A total of 100 microcontainers containing amorphous indomethacin and prepared at each of the three investigated cooling rates were examined using Raman microscopy. Containers were examined on day 0 to confirm the presence of pure amorphous indomethacin and further after 15 and 30 days of storage to determine if the confined indomethacin remained amorphous or had crystallised. Indomethacin confined within each microcontainer was determined to be of the α-form, γ-form or amorphous form by comparing obtained Raman spectra with reference spectra of the three forms.

Preparation of simulated biorelevant media

A simulated intestinal medium was prepared in accordance with a study in literature (Nielsen et al., submitted). The medium contained 5 mM of sodium taurocholate as a bile salt and 1.25 mM of phosphatidylcholine as a phospholipid and was utilised to investigate the release of indomethacin from microcontainers. The required volume of a stock solution of phosphatidylcholine in chloroform was measured out, and the chloroform was evaporated to form a lipid film. The volume of a stock solution of phosphate buffer/sodium azide required to give a concentration of 20/0.6 mM was then added, together with a quantity of sodium chloride stock solution necessary to achieve a constant osmolarity of 270 mOsm. The medium was stirred overnight at 37 °C, following which the pH was adjusted to 6.5, and the medium was made to volume with ultra-purified water.

Release of amorphous indomethacin from microcontainers

At day 0 of preparation, the release of amorphous indomethacin from microcontainers prepared using each of the three cooling rates was investigated on a μ-Diss Profiler (*p*ION

INC, Woburn, MA). Before each release experiment, a standard curve was first constructed in each channel of the apparatus, using in situ UV probes with a path length of 10 mm. Aliquots of a stock solution of indomethacin (in methanol) were added to simulated intestinal media, followed by measurement of the resulting UV spectrum. Addition of aliquots and spectral measurement were repeated eight times in order to produce a standard curve covering the entire linear absorbance range. For the experiments, arrays of microcontainers (which had been weighed before and after filling in order to allow accurate determination of the weight of contained amorphous indomethacin) were attached to the cylindrical magnetic stirring bar and covered with 10 mL of simulated intestinal media. The in situ UV probes scanned the samples every 30 s for 1,000 min at 100 rpm, and data were analysed in a wavelength range between 310 and 350 nm. Experiments were carried out at 37 °C, and five to six replicates for each cooling rate were performed.

Statistical analyses

In all cases where possible, results are expressed as mean ± standard deviation (SD). Statistical analysis was performed using ANOVA and carried out using SAS JMP® version 10.0.0.

Results and discussion

SEM images showing microcontainer morphology

The SEM images in Fig. 1 show the morphology of microcontainers filled with crystalline indomethacin that was then melted and cooled at each of the three employed cooling rates. In previous work, amorphous indomethacin confined within microcontainers was prepared by melting the crystalline drug in the containers in an oven followed by cooling with liquid nitrogen. This led to the formation of cracks in the amorphous indomethacin within the microcontainers, which were suggested to provide potential nucleation points for crystal growth [12]. In the current study, the indomethacin within the microcontainers was melted on a heating plate rather than in an oven and cooled with liquid nitrogen (36 K/min), on ice (23 K/min) or at room temperature (14 K/min) in an attempt to form an even surface of the amorphous indomethacin and, in doing so, reduce the formation of nucleation sites. Melting of indomethacin on a heating plate resulted in the surface of indomethacin being smoother compared to melting in an oven when comparing to the previous conducted study [12].

The SEM image in Fig. 1a, b shows the appearance of some cracks in the surface of the amorphous indomethacin after quench-cooling (indicated with the black arrows). The high cooling rate leads to a significant risk of surface cracking. Even though the surface appears smoother as a result of melting on a heating plate rather than in an oven [12], nucleation sites will still be able to form in the cracks present which could significantly influence the physical stability of the confined amorphous indomethacin. The SEM image of spatially confined indomethacin cooled at a rate of 23 K/min (Fig. 1c, d) shows indications of the formation of some small cracks (indicated with black arrows) which again could be a starting point for crystallisation. The slowest cooling rate of 14 K/min resulted in the formation of a smooth indomethacin surface, without indications of cracks or surface unevenness (Fig. 1e). This could potentially translate to a higher stability of the slowest cooled melt of indomethacin as there are no immediately visible nucleation sites at the surface.

Physical stability of amorphous indomethacin confined within microcontainers

In a previous study, we have described how the physical stability of amorphous indomethacin confined within microcontainers can be determined without the need of a standard curve using Raman microscopy [12]. Reference Raman spectra of the crystalline γ- and α-forms and of amorphous indomethacin are shown in Fig. 2. Clear differences can be observed between the spectra in the wave number range between 1,579 and 1,698 cm^{-1} (marked), allowing this area to be used to distinguish between the three forms of

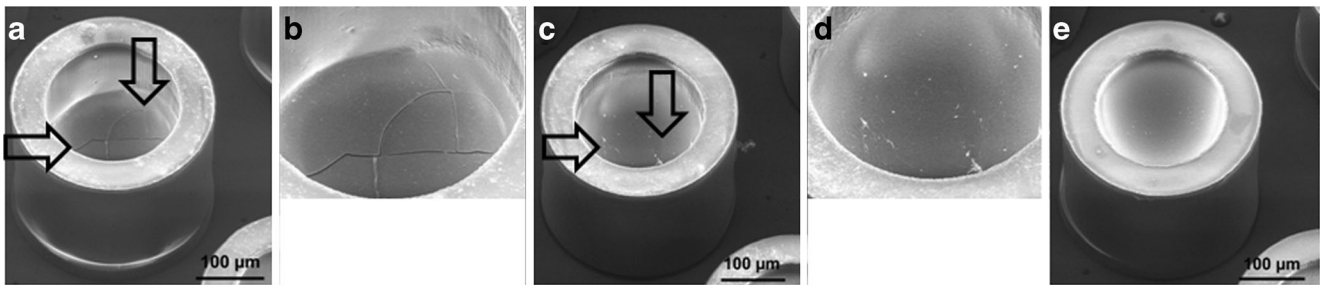

Fig. 1 SEM images of microcontainers loaded with amorphous indomethacin. The melt was cooled at rates of **a** 36 K/min (**b** enlargement of image **a**), **c** 23 K/min (**d** enlargement of image **c**) and **e** 14 K/min. The *arrows* in image **a** and **c** indicate the appearance of the cracks in the amorphous indomethacin

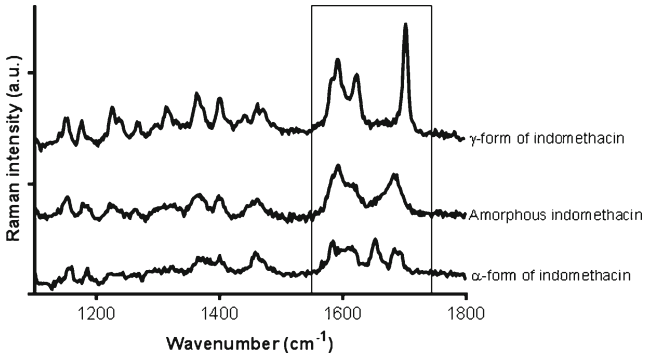

Fig. 2 Reference Raman spectra of the crystalline γ- and α-forms of indomethacin and of amorphous indomethacin. The *marked area* shows the wavenumber region used to distinguish between the different solid forms of indomethacin

indomethacin. Differences in the recorded Raman spectra within this region may further be used to determine the numeric crystallisation of amorphous indomethacin (to either the α-form or γ-form) over time [12]. In the current work, the Raman microscope was manually focused on each microcontainer to ensure that the best-quality spectra were obtained at each time point, as the filling height of indomethacin varied from microcontainer to microcontainer (also seen in Fig. 1). One Raman spectrum was measured in each microcontainer, and the numeric crystallisation was obtained at day 15 and 30 as a measure for the physical stability of the amorphous indomethacin. The Raman spectrum obtained from each microcontainer at each time point was compared with the three reference spectra shown in Fig. 2, and thereby, the form of indomethacin within each microcontainer was determined.

At day 0, all indomethacin confined within the microcontainers was amorphous, regardless of the cooling rate employed (data not shown). After 15 and 30 days of storage at 30 °C and 23 % RH, however, it was clearly observed that there were differences in the physical stability of amorphous indomethacin related to the cooling rate of the melt (Fig. 3).

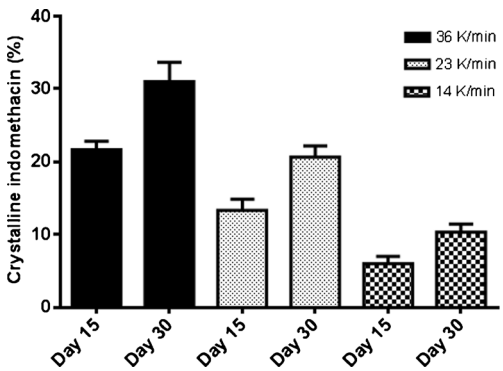

Fig. 3 Number of microcontainers containing crystalline indomethacin on days 15 and 30 of storage. Data represent the melt of indomethacin cooled at 36, 23 and 14 K/min in order to obtain the amorphous form of indomethacin. Data correspond to $n=3\pm SD$

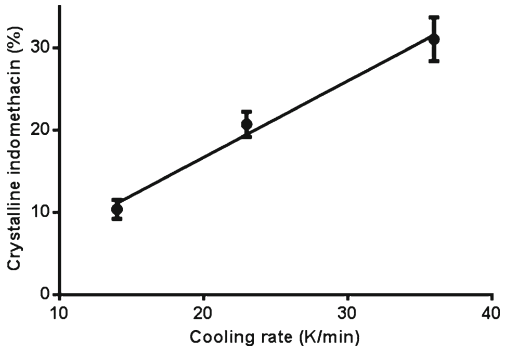

Fig. 4 Relationship between employed cooling rate and the percentage of microcontainers containing crystalline indomethacin after 30 days of storage. Data represent $n=3\pm SD$

After 15 days of storage, the fastest cooled indomethacin (36 K/min) had a crystallisation number (the number of microcontainers containing crystalline indomethacin; see [12] for further information) of 21.7±1.2 %, and this number increased to 31.0±2.6 % after 30 days of storage. The melt of indomethacin cooled at 23 K/min had a numeric crystallisation of 13.3±1.5 % after 15 days of storage, which was significantly lower compared to the numeric crystallisation from the 36-K/min cooled indomethacin (p value<0.0001). After 30 days of storage, 20.7±1.5 % of crystalline indomethacin was found in microcontainers cooled at 23 K/min, which was again significantly lower than the crystallisation number for containers cooled at 36 K/min after the same storage period (p value<0.0001). Even lower crystallisation numbers were observed when cooling of the melt had been performed at a rate of 14 K/min—crystallisation numbers of 6.0±1.0 and 10.3±1.2 % were seen after 15 and 30 days of storage of these microcontainers, respectively. These numbers were both significantly lower than the crystallisation numbers of microcontainers prepared at the two higher cooling rates (p value<0.0001).

In this study, an increased physical stability of the amorphous indomethacin spatially confined in microcontainers was observed as the cooling rate was lowered. The ranking of amorphous indomethacin physical stability as a function of

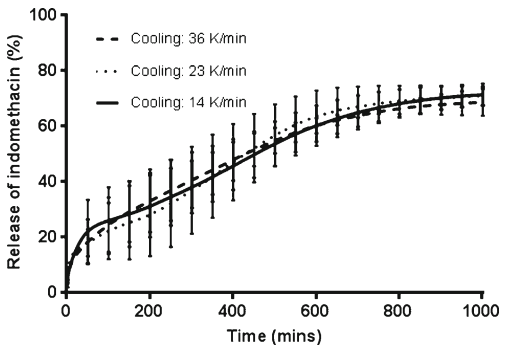

Fig. 5 Release of amorphous indomethacin (at day 0) from microcontainers prepared at three different cooling rates of the melt: 36, 23 and 14 K/min. Data represent mean ± SD of five to six replicates

cooling rate was found to be in the order 14>23>36 K/min. A relationship between the crystalline indomethacin and the cooling rates was found (Fig. 4). It can be observed that the percentage of crystalline indomethacin increases with increasing cooling rate as stated above. This observation was in contrast with the ranking found by Karmwar et al., where a higher physical stability of amorphous indomethacin was seen with an increase in cooling rate [20]. In the current study, it was found that the cracks in the surface of the amorphous indomethacin have a large influence on the crystal growth. It is speculated that upon cooling of the drug melt in microcontainers, the presence or absence of cracks has more influence on the crystallisation number than the cooling rate. Using the slowest cooling results in a smooth surface, and therefore, no cracks, where crystal growth can be initiated, are formed. Earlier, it has been reported that micro-cracks were formed after compression of amorphous indomethacin, and these were found to act as nucleation sites that facilitated crystallisation [22]. It is also found in this study that even a small number of cracks (as seen in Fig. 1c, d) can greatly influence the physical stability of amorphous drug. In the current study, the slowest cooling rate of 14 K/min resulted in an even surface and, therefore, the best conditions for amorphous indomethacin within the microcontainers from a physical stability point of view.

Previously in literature, it has been shown that slowly cooled indomethacin (5 K/min) crystallised predominantly to the γ-form, whereas rapidly cooled (liquid nitrogen) amorphous indomethacin converted mainly to the α-form [21, 27]. In the current study, no trend could be observed with regard to which crystalline form the amorphous indomethacin converted to. At all three cooling rates, the amorphous indomethacin crystallised to both the α-form and γ-form, with the proportion of each form not noted to vary in a clear manner as a function of the cooling rate employed. It has previously been found that amorphous indomethacin confined within 223-μm microcontainers, as in this study, only converted to the γ-form [12]. The confined amorphous indomethacin was prepared in a similar way to that cooled at 27 K/min in the current study, but the γ-form of indomethacin was melted in an oven instead of on a heating plate followed by cooling with liquid nitrogen. This was seen to give a different morphology of the resulting amorphous indomethacin (Fig. 1a, b). In the current study, the amorphous indomethacin had an even surface with cracks, whereas the melting in an oven resulted in the formation of particles at the container surface [12]. The difference in melting methods therefore seemed to affect both the morphology of amorphous indomethacin as well as the form to which the amorphous indomethacin converted.

The release profiles (Fig. 5) of amorphous indomethacin in biorelevant intestinal media of pH 6.5 from microcontainers prepared at the three different cooling rates were found to be not significantly different (p value 0.0052). This is in agreement with a previous study where it has been shown for indomethacin powder that different cooling rates give rise to molecular variations in produced amorphous drug, but that no differences in drug dissolution characteristics could be observed [25].

In agreement with earlier studies, it can be observed that small differences in the preparation of amorphous indomethacin can lead to differences in the physical stability [17]. It is therefore necessary and important to consider every preparation step carefully when preparing the amorphous form for drug delivery as it can have great influence on the physical stability.

Conclusion

It can be concluded that employing different cooling rates when preparing amorphous indomethacin confined within microcontainers has a significant influence on the physical stability of the drug. The use of a slower cooling rate resulted in a higher physical stability of confined drug—10.3±1.2 % of the amorphous indomethacin within microcontainers crystallised within 30 days when indomethacin was cooled at 14 K/min, whereas when cooling at 36 K/min, 31.0±2.6 % had crystallised within the 30 days of storage. The three different cooling rates of 36, 23 and 14 K/min had no influence on the release behaviour of the amorphous indomethacin from the microcontainers. The current work therefore illustrates the importance of careful consideration when deciding on preparation techniques used to produce amorphous drug, as the choice of technique parameters can lead to great differences in drug physical stability.

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Conflict of interest All the authors declare that they have no conflict of interest.

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