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Amorphization of different furosemide polymorphic forms during ball milling: Tracking solid-to-solid phase transformations

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ABSTRACT

Ball milling is used, not only to reduce the particle size of pharmaceutical powders, but also to induce changes in the physical properties of drugs. In this work we prepared three crystal forms of furosemide (forms I, II, and III) and studied their solid phase transformations during ball milling. Powder X-ray diffraction and modulated differential scanning calorimetry were used to characterize the samples after each milling time on their path to amorphization. Our results show that forms I and III directly converted into an amorphous phase, while form II first undergoes a polymorphic transition to form I, and then gradually loses its crystallinity, finally reaching full amorphousness. During ball milling of forms I and II, the glass transition temperature (T g) of the amorphous fraction of the milled material remains almost unchanged at 75 °C and 74 °C, respectively (whilst the amorphous content increases). In contrast, the T g values of the amorphous fraction of milled form III increase with increasing milling times, from 63 °C to 71 °C, indicating an unexpected phenomenon of amorphous-to-amorphous transformation. The amorphous fraction of milled forms I and II samples presented a longer structural relaxation (i.e., lower molecular mobility) than the amorphous fraction of milled form III samples. Moreover, the structural relaxation time remained the same for the increasing amorphous fraction during milling of forms I and II. In contrast, the structural relaxation times were always shorter for the amorphous fraction of form III, but increased with increasing amorphous content during milling, confirming amorphous-to-amorphous transformation.

1. Introduction

It has been estimated that about 40% of the approved active pharmaceutical ingredients (APIs) and as high as 90% of low molecular weight drug candidates are insufficiently soluble in water for the intended dose (Babu and Nangia, 2011). The pharmaceutical industry is therefore investing considerable efforts into searching for strategies to improve the solubility and bioavailability of APIs. Non-crystalline (amorphous) solid forms offer a promising solution to the solubility problem. Amorphous materials represent a higher energy solid form, and although the present low physical stability (i.e., amorphous forms may easily convert to their respective crystalline state), this often coincides with a higher solubility that for its crystalline counterparts (Laitinen et al., 2013; Liu et al., 2021). These non-crystalline solid materials, also called glasses, have a unique thermal behavior, characterized by the presence of a glass transition temperature (T g) at which, upon cooling from the melt, a transition from a viscous state (known as the super-cooled melt state) to the amorphous form occurs (Debenedetti and Stillinger, 2001; Varshneya and Mauro, 2019).

Other than by rapidly cooling a melt, amorphization can also be achieved (intentionally or unintentionally) by direct transformations from crystalline to amorphous forms during processes such as mechanical grinding, micronization, compression, granulation and drying (Bezzon et al., 2022; Feng et al., 2008; Modhave et al., 2020). Mechanical grinding by means of vibrational ball milling is a common procedure for particle size reduction (Michalchuk et al., 2021). During ball milling, collision of the milling balls with the powder particles inside the milling jar not only changes the size and thus the specific surface area of the particles (which in turn increases the dissolution rate), but can also induce polymorphic transformations and even amorphization of the API (Mah et al., 2014; Pazesh et al., 2017). Feng et al. reported that milling can induce defects in the crystalline structure of griseofulvin without causing its amorphization (Feng et al., 2008). In contrast, complete amorphization was achieved after ball milling the α, γ and δ polymorphs of indomethacin (Crowley and Zografi, 2002). In case of the occurrence of amorphous forms during milling, it is generally believed

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that the continuous input of mechanical energy can either increase the number of defects in the crystal lattice eventually to full amorphization or cause melt quenching in specific zones of the material as a consequence of a temperature increase after the impact or shearing effects of the milling balls on the powder (Fecht, 1992; Willart and Descamps, 2008). To understand the specific pathway of transitions from a crystalline to an amorphous form, it is necessary to monitor those transformations during the milling process. This is important as milling to achieve amorphization (following the so-called kinetic path) is fundamentally different from the use of melt or solvent based techniques (following the so-called thermodynamic path) (Bates et al., 2006; Blaabjerg et al., 2017; Rades et al., 2013). As stated above, using the kinetic path, the amorphization progresses through the induction of crystal defects (Zimper et al., 2010a; Zimper et al., 2010b) or localized melting, and thus the nature of the used material, for example the polymorphic form of the API may have an influence on the amorphization path. In contrast, following the thermodynamic path, the original nature of the crystalline form of the material (e.g., the exact polymorphic form) is of no direct consequence for the amorphization, as the drug is either molten or in solution at the start of the amorphization process.

The most common methods used to monitor solid-to-solid transformations are based on both in-situ and ex-situ powder X-ray diffraction (PXRD), and differential scanning calorimetry (DSC) as an orthogonal technique, not only for an accurate characterization of crystallinity in the samples (as for PXRD), but also to allow direct detection of the amorphous forms (e.g., by determining the $T_g$ values of partly amorphous forms) at different milling times (Linberg et al., 2023; Martins et al., 2021; Martins and Emmerling, 2021; Mazzeo et al., 2023; Zheng et al., 2019). The structural relaxation properties of different amorphous forms can be determined using isothermal microcalorimetry as this method allows a direct detection of the rate of energy loss during enthalpy of relaxation (Kawakami and Pikal, 2005; Kawakami and Ida, 2003).

Different $T_g$ values of the amorphous form of the water insoluble API furosemide (FUR) obtained by different preparation methods have been reported. In early work, two amorphous forms of FUR with $T_g$ values of 44.2 °C and 54.4 °C were obtained when using different inlet temperatures during a spray drying process (Matsuda et al., 1992). Similar $T_g$ values (44.2 °C) were also obtained for amorphous FUR prepared by cryo-milling (Mishra et al., 2019). Nielsen et al. found another $T_g$ value for amorphous FUR of 61.8 °C when preparing the amorphous form by spray-drying (Nielsen et al., 2013). Later, Ruponen et al. reported a $T_g$ value of 71.1 °C for amorphous FUR obtained after in situ melt quenching in a DSC (Ruponen et al., 2021). With a difference of 5 °C, Jensen and Wu found a $T_g$ value of 76 °C for amorphous FUR prepared by ball milling (Jensen et al., 2015; Jensen et al., 2016; Wu et al., 2018). These reports indicate that the $T_g$ of amorphous FUR is strongly affected by the preparation and analytical conditions, such as the preparation method (use of spray-drying with different inlet temperatures and different solvents, cryo ball milling or ball milling at room temperature), different DSC parameters used to determine the $T_g$ (different heating rates and implementation or not) of an extra heating cycle to remove possible moisture content in the sample that may strongly affect the $T_g$ values, and also possible degradation of FUR upon milling which can also interfere with the measured $T_g$ values (Adrjanowicz et al., 2011).

Herein we will present an investigation on the $T_g$ variation of FUR amorphous forms prepared by ball milling different crystalline phases (polymorphs I-III) at different milling times as well as on the structural relaxation of the amorphous fractions of the different starting FUR polymorphs during the ball milling process. For this, we have used consistent preparative and analytical parameters, such as the same milling conditions and implementation of the same heating rates and modulation parameters in the DSC experiments, in order to determine the $T_g$ values under comparable conditions.

## 2. Materials and methods

### 2.1. Material

FUR form I (purity > 99%) was purchased from sigma-Aldrich (St. Louis, Mo, USA). FUR form II was obtained by cooling form I from a n-butanol solution (2.00 g of FUR form I dissolved in 100 g of n-butanol at 80 °C) to 5 °C. FUR form III was prepared by fast evaporation of the acetone solvent (at 25 °C under a pressure of 100 bar) from a solution containing 4.00 g of FUR form I in 80 mL of acetone. Acetone and n-butanol were of analytical grade and used without further purification. The chemical structure of FUR and the overlaid structures of FUR conformations in the three different polymorphs (forms I, II and III), are shown in Fig. 1.

### 2.2. Sample preparation

Samples were prepared by oscillatory ball milling of 200 mg of the respective FUR polymorph (form I, II and III) at different temperature points of 5, 10, 15, 30, 45, 60, 90, 120 min. Milling was performed in a cold environment (6 °C) to reduce the risk of recrystallization caused by sample heating during the milling process (Jensen et al., 2015). Form I ball milled for 5 min and 120 min are referred to as form I-5 min and form I-120 min, respectively. The same description is applied for forms II and III and other milling times. All independent milling experiments were performed in triplicates at a frequency of 30 Hz in 5 mL stainless steel jars containing one stainless steel ball with a diameter of 7 mm (Mixer mill MM400, Retech GmbH & Co., Hann, Germany).

### 2.3. Powder X-ray diffraction (PXRD)

PXRD patterns were recorded in Bragg Brentano mode with an X Pert PRO X-ray diffractometer (PANalytical, B. V., Almelo, The Netherlands) using Cu Kα radiation ($\lambda = 1.54187$ Å) at ambient temperature. All scans were performed at a voltage of 45 kV and current of 40 mA, from 5 to 35° 2θ, with a scan speed of 0.067°/s and a step size of 0.026. Data was collected and analyzed using the software X’Pert Data Collector 2.2i (PANalytical B. V., Almelo, The Netherlands).

### 2.4. Modulated differential scanning calorimetry (MDSC)

MDSC measurements were conducted on a Discovery DSC (TA Instruments, New Castle, DE, USA) in aluminum pans closed with punched aluminum lids under a nitrogen gas flow of 50 mL/min. $T_g$ values were determined for the samples obtained from the first two of the three milling experiments (SI, Figures S1-S4, S6-S9 and S11-S14) using procedure A (one heating cycle): samples were heated in modulated temperature mode from −20 to 180 °C with an applied linear heating rate of 2 °C/min, modulation amplitude of 0.2120 °C, and a period of 40 s. An additional experiment using procedure A with unpunched aluminum lids under nitrogen gas flow at 25 mL/min with a linear heating rate of 5 °C/min and modulation amplitude of 1.0 °C was also performed.

![Fig. 1. (a) Chemical structure of FUR; (b) Overlay structures of different FUR conformations in each polymorph. Green and light green conformations correspond to form I, while the purple and orange conformations correspond to forms II and III, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)](image-url)
lids was conducted for selected ball milled samples of crystalline form III (form III-5 min and form III-120 min).

Procedure B (two heating cycles) was applied to the samples obtained from the third milling experiment (SI, Figures S5, S10 and S15) and used to confirm if procedure A would give similar T$_g$ values without the need of using a second heating cycle. In procedure B, samples were equilibrated at −20 °C, and then heated to a temperature lower than the recrystallization temperature (62 °C for forms I and II and 60 °C for form III) of the amorphous fractions of the samples (values taken from procedure A) and kept isothermal for 1 min to remove moisture (first heating cycle). Subsequently, samples were equilibrated to −20 °C for ca. 5 min and reheated to 180 °C (second heating cycle). During procedure B, the heating rate and modulation parameters used were the same as for procedure A. MDSC data of samples form II-60 min, form II-90 min, form III-15 min, form III-30 min and form III-45 min measured by procedure B are not shown due to complete recrystallization at the milling endpoint (120 min).

The data was analysed by Trios software (TA Instruments-Waters LLC, New Castle, DE, USA). The amorphous content of the samples during the ball milling process was determined by the ratio of the recrystallization enthalpy of the sample at the milling endpoint (120 min). The samples at the milling endpoint were considered as fully amorphous. The T$_g$ values were calculated as the onset of the T$_g$ events. This was done because the T$_g$ of the ball milled samples overlaps with recrystallization events. The noise caused by the separation of the reversible heat flow from the total heat flow did not allow for a complete discrimination of the full T$_g$ event (SI, Figures S1-S15).

2.5. Thermogravimetric analysis (TGA)

Absorbed moisture in the samples was determined using a Discovery thermogravimetric analyser (TA instruments, New Castle, DE). Approximately 10 mg of FUR milled samples were heated from 10 to 300 °C at a heating rate of 10 °C/min.

2.6. High performance liquid chromatography (HPLC)

HPLC measurements were performed using an Agilent 1260 Infinity HPLC instrument (Agilent, Santa Clara, CA, USA) equipped with an Agilent 1290 Diode Array Detector. An Agilent C18 (250 mm × 4.6 mm, 5 μm) column was used. Acetonitrile and acetic acid (0.1%) at a ratio of 80:20 (v/v) were used as the mobile phase. The signal was monitored at a wavelength of 230 nm. It has been reported that FUR powder may gradually decompose during cryo-milling (Adrianovic et al., 2011). In our work, when ball milling FUR at low temperature, the powder becomes yellow as a result of the successful transformation from crystal-line to amorphous form. Yet no degradation was detected after a milling time of 120 min as no additional peaks were found in the HPLC chromatograms when compared to the raw materials (SI, Figures S16-S18).

2.7. Fourier-transform infrared (FTIR) spectroscopy

FTIR spectra were determined by using a Bruker alpha ATR platinum FTIR instrument (Bruker, Berlin, Germany) in the wavenumber range of 4000 to 400 cm$^{-1}$, with a resolution of 2 cm$^{-1}$ at ambient conditions.

2.8. Physical stability study

Amorphous forms of ball milled FUR after 120 min (form I-120 min, form II-120 min and form III-120 min) were kept in a desiccator over phosphorous pentoxide and activated silica for 3 days. PXRD data was collected daily for all samples.

2.9. Isothermal microcalorimetry (IMC)

Isothermal microcalorimetry was used to directly measure the structural relaxation time of amorphous samples. This technique collects the rate of enthalpy relaxation as a function of time during annealing, shown by a power-time curve. In order to reduce the effect of thermal history on sample relaxation, each sample, around 115 mg, was freshly prepared and placed in a 4 mL disposable crimp-sealed ampoule and immediately measured at 25 °C using a thermal activity monitor (TAM III, TA Instruments, New Castle, England). Before loading the samples, the instrument had acquired the initial baseline value corresponding to a temperature of 25 °C. The obtained power-time curves were fitted with the derivative form of the modified stretched exponential (MSE) equation to obtain the parameters $\tau_0$, $\tau_1$ and $\beta$ (Blugra et al., 2006; Liu et al., 2002).

$$P = \frac{\Delta H_f(\infty)}{\tau_0} \left[1 + \left(\frac{\tau_1}{\tau_0}\right)^{\beta}\exp\left[-\left(\frac{\tau_1}{\tau_0}\right)^{\beta^{-1}}\right] \right]$$

(1)

where $P$ is the power (in μW/g), $t$ is the measurement time (in hours). 277.8 is a numerical factor due to conversion of units, $\tau_0$ and $\tau_1$ are the relaxation time constants, and $\beta$ reflects the distribution of independently relaxing states (0 < $\beta$ < 1). $\Delta H_f(\infty)$ is the enthalpy relaxation at time infinity obtained by equation (2).

$$\Delta H_f(\infty) = (T_g - T) \times \Delta C_p$$

(2)

where $T_g$ is the glass transition temperature. $\Delta C_p$ is the heat capacity change at $T_g$ and $T$ is the temperature of annealing. The structural relaxation time ($\tau_f$) can then be calculated by equation (3).

$$\tau_f = \left(\tau_0^{\beta}(\tau_1^{\beta})^{\frac{1}{\beta}}\right)^{\gamma}$$

(3)

3. Results and discussion

3.1. Characterization of FUR polymorphs

The successful preparation of FUR Form I, II and III was confirmed by comparing the experimental PXRD patterns with the calculated ones from the reported single crystal structures deposited at the CSD database (refcode FURSEM03, FURSEM16, and FURSEM14, respectively) (Allen, 2002). The various patterns are shown in Fig. 2a. The experimental PXRD pattern of FUR form II shows some differences when compared to the simulated data (FURSEM16), but excellent agreement with a previously published experimental PXRD of FUR form II (Tang et al., 2019). These differences between the simulated and experiments PXRD are likely to be due to preferred orientation, as the crystals of form II are known to be small needles. Furthermore, the structure from FURSEM16 was obtained at lower temperature (100 K) and therefore the simulated PXRD data may show differences when compared with the experimental data (Tang et al., 2019). The FTIR spectra of all FUR polymorphs were also collected and are presented in Fig. 2b. Distinct differences in the absorption band region of 3500–3000 cm$^{-1}$ attributed to the stretch vibration of the sulfonamide group and the secondary amine N–H were detected in all FUR polymorphs. Specifically, the lack of the absorption band region of 3500–3000 cm$^{-1}$ resulting from the vibration of the sulfonamide N–H is a characteristic feature of FUR form III (Tang et al., 2019). The sulfonamide N–H stretching vibration of FUR form I is found at the lower wavenumbers of 3397.8 cm$^{-1}$ and 3281.8 cm$^{-1}$ compared with 3406.0 cm$^{-1}$ and 3285.1 cm$^{-1}$ for FUR form II. These major shifts can be related to differences occurring in intermolecular interactions among FUR molecules and molecular conformations in the different polymorphic forms (Doherty and York, 1988).
3.2. Conversion pathway of FUR form I during ball milling

The obtained PXRD data of FUR form I ball milled at different milling times are presented in Fig. 3a. A decrease in the Bragg diffraction peaks of FUR form I is observed after 30 min of ball milling. During this time period, no detectable polymorphic phase transitions were observed, and thus FUR form I directly converts from a crystalline form into an amorphous form. MDSC is a useful method to attempt to separate $T_g$ events from other overlapping thermal events, allowing the detection of accurate $T_g$ values, even when the sample is not fully amorphous (Xiville et al., 2015).

Fig. 2. (a) PXRD patterns of the prepared FUR polymorphic forms (Form I, II, and III) compared to the respective calculated patterns extracted from CCDC database (refcodes FURSEM03, FURSEM16, and FURSEM14); (b) FTIR spectra of the three FUR polymorphic forms.

Fig. 3. (a) PXRD patterns; (b) MDSC total heat flow curves; (c) MDSC reversing heat flow curves; and (d) onset temperature, $T_g$ values and amorphous content of form I ball milled at different milling times. The data in Fig. 3d shows the average values measured by MDSC procedure A for the first two milling experiments (n = 2, see experimental section). The green dashed line shows the constant trend in the $T_g$ values. The black and red curves show the development of the onset temperature of the recrystallization peak and the amorphous content. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
et al., 2012). Figures S1 and S3 demonstrate the presence of a wide dehydration peak, where the loss of moisture content is followed by a $T_g$ event accompanied by an enthalpy relaxation and a recrystallization peak. The shifted recrystallization peaks towards higher temperature evidence an increase in the thermal stability of the samples (Fig. 3b) (Luisi et al., 2012; Otte et al., 2012). The reversing heat flow was extracted to show the $T_g$ values of the amorphous forms at different milling times (Fig. 3c). Both the onset temperature of the recrystallization and the amorphous content increased during the milling process (Fig. 3d). The $T_g$ values were found to be the same, ca. 75 °C, for all amorphous samples collected at different milling times (Fig. 3d). Although the removal of moisture occurs before the $T_g$ (SI, Figures S1 and S3), a second heating cycle (MDSC procedure B) was used on ball milled FUR form I, to determine if the $T_g$ values obtained by both procedures were similar, i.e., if the moisture content would be completely removed before reaching the $T_g$ when using MDSC procedure A or if it would be necessary to use MDSC procedure B (SI, Figure S5). After performing the first heating cycle, no broad dehydration peak was observed during the second heating of MDSC procedure B (SI, Figure S5). The detected $T_g$ values were ca. 76 °C similar to those determined by procedure A (SI, Figures S1 and S3). This suggests that procedure A is reliable to determine the $T_g$ values.

3.3. Conversion pathway of FUR form II during ball milling

When ball milling FUR form II for 5 min, two concomitant phenomena were detected in the PXRD data: a polymorphic conversion occurs from the metastable form II to the stable form I (Matsuda and Tatsumi, 1990) and amorphization of form I starts to occur, as detected by the decrease in the degree of crystallinity (Fig. 4a). The presence of a recrystallization peak and a $T_g$ value after 5 min of milling confirmed the presence of an amorphous form (Fig. 4b-c). The onset temperature of the recrystallization peaks increases during the amorphization pathway of FUR form II in a similar way as observed for FUR form I. The detected onset temperature of the recrystallization events increases from 76.0 °C to 89.6 °C over the milling time, while the recrystallization temperatures of ball milled FUR form I increased from 76.8 °C to 90.8 °C (Fig. 4d). The $T_g$ values were detected at ca. 74 °C and did not considerably change during the milling process. It may be speculated that the decrease by 1 °C of the $T_g$ value of ball milled FUR form II (74 °C), compared to the $T_g$ of 75 °C of ball milled FUR form I, may be a result of the presence of a small amount of amorphous content produced from the direct amorphization of FUR form II, which may be different from that obtained by milling FUR form I, but this needs to be investigated further.

In a similar way as for the amorphization of FUR form I, the $T_g$ values of ball milled FUR form II were determined by both MDSC procedures A and B (see experimental section). In both procedures, the obtained $T_g$ values were very similar (ca. 74 °C, SI, Figures S6-S10), thus excluding any influence of moisture content on the determined $T_g$ values.

3.4. Conversion pathway of FUR form III during ball milling

A direct conversion of FUR form III into an amorphous form was observed during ball milling (Fig. 5a). In contrast to the thermal

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Fig. 4. (a) PXRD patterns; (b) MDSC total heat flow curves; (c) MDSC reversing heat flow curves; and (d) onset temperature, $T_g$ values and amorphous content of FUR form II ball milled at different times. The data in Fig. 4d shows the average values measured by MDSC procedure A for the first two milling experiments ($n = 2$, see experimental section). The green dashed line shows the constant trend in the $T_g$ values. The black and red curves show the development of the onset temperature of the recrystallization peak and the amorphous content. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
behaviours observed when ball milling FUR forms I and II, two exothermic recrystallization peaks were detected for milling times shorter than 90 min (Fig. 5b). However, after 90 min of ball milling, only one recrystallization peak was detected at ca. 89.7°C (Fig. 5b). The presence of two recrystallization peaks can be explained by three effects; different molecular mobility at the surface and in the bulk of the amorphous form, different activation energies necessary to nucleate and/or grow crystals, or a combination of both (Otte et al., 2012; Trasi et al., 2010; Zhu et al., 2008). Variable temperature PXRD measurements were performed before and after the bimodal recrystallization peaks of FUR form III ball milled for 45 min at 60°C, 77°C, 82°C, 89°C and 100°C. The measurements showed the same PXRD patterns, excluding the possibility of recrystallization of different crystal forms (SI, Figure S19). Interestingly, the T_g values gradually increased with the increase in amorphous content (i.e. increasing milling time), from ca. 63°C to a final value of ca. 71°C, and stayed constant at this maximum value even when milling longer than 120 min (150 min) (Fig. 5c-d). As a considerable difference of the T_g values was observed for these samples, MDSC procedure B was also tested to assure the absence of moisture content that could potentially influence the determined T_g values (SI, Figure S15). A high agreement between the values determined by both MDSC procedures was found (SI, Figures S11-S15), thus again excluding any moisture content being present in the sample (anymore) that could potentially influence the position of the T_g values obtained by procedure A.

TGA measurements were performed and the moisture content for form III-5 min and form III-120 min was determined to be 1.21% and 1.46% respectively. Furthermore, an additional MDSC experiment on form III-5 min and form III-120 min, using procedure A but with unpunched aluminum lids to avoid the release of moisture, was also performed. The determined T_g values for these two samples were found to be 37.7°C and 33.6°C, respectively (SI, Figure S20). This suggests that the moisture content in the samples greatly decreased the T_g values, when it cannot escape. Thus, when using procedure A, the moisture is already completely removed and the observed changes on T_g values, during milling, are considered a real effect of the presence of different amorphous forms at different milling times (SI, Figures S11-S14).

The T_g value of form III-120 min is lower by 4°C and 3°C than the ones found in form I-120 min and form II-120 min. This indicates that form III-120 min is different from form I-120 min and form II-120 min, and might present distinct molecular conformations or molecular arrangements. Figure S21 shows similar FTIR spectra and PXRD patterns of form I-120 min, form II-120 min and form III-120 min, which demonstrates the amorphous forms obtained at the milling endpoint are similar whereas the MDSC data suggests that they are not the same.

We have searched for possible effects that could explain the peculiar T_g behavior of amorphous FUR upon ball milling form III. Looking at the lattice energy of each FUR polymorph we could found that they are fairly similar for all polymorphs (−41.65 kcal.mol$^{-1}$ for form I, −41.78 kcal.mol$^{-1}$ for form II and −41.53 kcal.mol$^{-1}$ for form III). Moreover, from the calculation of the surface map for each polymorph and found that the conformational energy in form I was higher (4.5 kcal.mol$^{-1}$) than that of forms II and III (0.7 and 0.0 kcal.mol$^{-1}$).
3.5. Determination of the structural relaxation time below the $T_g$

The relaxation process in amorphous materials is characterized by the energy difference between unstable and metastable glass states, reflecting the rate of molecular diffusion (Groel et al., 2021; Shamblin et al., 2006). The structural relaxation time ($\tau_D^{\beta}$) was used to evaluate the rate of structural relaxation and degree of molecular mobility of all FUR amorphous forms obtained after ball milling FUR form I, II and III for 45 min, 60 min, 90 min and 120 min, respectively (SI, Figures S22-S24 and Table S1). A smaller value of $\tau_D^{\beta}$ suggests a faster structural relaxation due to a higher degree of molecular mobility (Abdul-Fattah et al., 2007; Groel et al., 2021; Luthra et al., 2008). The heat flows of form I-120 min, form II-120 min and form III-120 min are taken as an example and are shown in Fig. 6. The fast decrease of the initial heat flow is attributed to the structural relaxation, which subsequently decays towards a baseline. The derivative form of the MSE equation, represented by the red solid line, fits very well the experimental data, $R^2 > 0.999$ (SI, Table S1). Only parameters for samples with milling times higher than 45 min were calculated (Table 1). Insufficient enthalpy loss data was collected for the samples ball milled for times shorter than 45 min due to the fast crystallization of the material. $\tau_D^{\beta}$ of form I-120 min has a similar value than for form I-120 min, suggesting that they have similar molecular mobility. However, $\tau_D^{\beta}$ of form II-120 min is smaller than those obtained for form I-120 min and form II-120 min (Fig. 6). This indicates that form III-120 min has a higher molecular mobility than forms I-120 min and II-120 min. Moreover, the PXRD results from the short physical stability study also revealed a similar tendency as of $\tau_D^{\beta}$, i.e., form III-120 min presented a shorter physical stability (recrystallized to polymorph I after 2 days) than form I-120 min and form II-120 min (recrystallized to polymorph I after 3 days). Not only do all ball milled samples of form I and form II, at similar milling times show similar $\tau_D^{\beta}$ values, but they also have similar $T_g$ values (Table 1). This indicates that the molecular arrangements of ball milled form I and form II may be similar, and eventually, two similar fully amorphous forms of form I-120 min and form II-120 min were obtained. The $\tau_D^{\beta}$ value of ball milled form III however, increases over the milling time, consistent with the changes seen in the $T_g$ values. This suggests that the amorphous fractions of ball milled form III samples show a decrease in molecular mobility during ball milling, albeit never reaching values obtained for FUR forms I and II.

### Table 1

<table>
<thead>
<tr>
<th>Milling time (min)</th>
<th>Form I</th>
<th>Form II</th>
<th>Form III</th>
</tr>
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<tr>
<td>$\tau_D^{\beta}$</td>
<td>$T_g$/°C</td>
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<td>45</td>
<td>15.66</td>
<td>75.6</td>
<td>15.47</td>
</tr>
</tbody>
</table>

4. Conclusion

In the present work we have explored the conversion pathway of

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Fig. 6. Isothermal microcalorimetry heat flow as a function of time for (a) form I-120 min, (b) form II-120 min, and (c) form III-120 min. Red lines represent the curves fitted by equation (1). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
crystalline polymorphic forms I, II and III of the API FUR during ball milling using PXRD and MDSC, and isothermal microcalorimetry. Three amorphization pathways were observed: i) crystalline form I converts directly to an amorphous form; ii) crystalline form II converts into crystalline form I after 5 min of ball milling while at the same time form I and possibly form II (a small portion that did not convert to form I) progress towards complete amorphization during ball milling; and iii) crystalline form III shows an unusual transition from one amorphous form (\(T_g = 63.4 \, ^\circ C\)) to another one (\(T_g = 71.6 \, ^\circ C\)) (amorphous-to-amorphous transformation). Structural relaxation times \(\tau_g\) showed a positive correlation with the \(T_g\) values, i.e., a higher \(T_g\) value corresponds to a lower molecular mobility. This study provides insights into the solid state transformations during ball milling of FUR, highlighting how different starting polymeric forms of an API and the resulting \(T_g\) values during amorphization are affected upon using mechanical force to prepare amorphous materials following the kinetic path of amorphization.

CRediT authorship contribution statement

Mengwei Wang: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. Junbo Gong: Supervision, Conceptualization, Methodology, Formal analysis, Writing – review & editing, Funding acquisition, Project administration. Thomas Rades: Supervision, Conceptualization, Methodology, Formal analysis, Resources, Writing – review & editing, Funding acquisition, Project administration. Inés C.B. Martíns: Supervision, Conceptualization, Methodology, Formal analysis, Investigation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2023.123573.

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and possibly form II (a small portion that did not convert to form I)


