Bifonazole: a missing case of polymorphism

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Abstract

Bifonazole, an imidazole-based antifungal drug, can be easily amorphized and crystallizes upon reheating at moderate scanning rate. Driven by recent results in literature reporting for this compound the appearance of polymorphism under different thermal histories, the present study presents an extensive calorimetric characterization of bifonazole in its crystalline and amorphous state. A rich scenario in crystallization behaviour has been found, implying that the behaviour observed on reheating scans can strongly depend on the previous thermal history and on the distribution in number and size of crystalline regions. In particular, the decrease in melting enthalpy during reheating, related to the increase of the heating rate, can be stated to be the result of a partial crystallization of the sample. Additionally, the temperature intervals more favourable to crystal nucleation and growth, respectively, have been determined, thanks to the choice of ad hoc time–temperature profiles. Finally, also the nucleation occurrence in time at high temperature has been investigated using a suitable DSC protocol. The overall results show how complex can be the cold-crystallization behaviour of a molecular liquid.

Keywords Calorimetry · Crystallization · Partial crystallization · Pharmaceuticals · Bifonazole

Introduction

Pharmaceutical materials have attracted scientists' attention from many years thanks to the huge number of physical phenomena shown. Among them, the most important are crystallization/melting and polymorphism.

Physico-chemical treatment of drugs can lead to fully or partially crystalline compounds. It is important to note that pharmaceuticals can be treated in several ways, among them we mention, as example, thermal, chemical and mechanical treatment. Partial crystallization can alter physico-chemical properties of the drug from what is expected from crystalline material. The same properties can be altered by the crystalline phase in the case of polymorphism. In fact, it has been largely shown that the same compound can spatially pack in different ways (polymorphism) [1, 2] and knowing how to obtain and stabilize one form with respect to another is important for some key parameters, like bioavailability and dissolution rate [3, 4]. Moreover, different crystalline structures have different thermodynamical properties, like for example Gibbs free energy [5]. On the other hand, crystallization is still challenging in its understanding because, despite many theories appeared in literature [6], there is still the lack of a unified theory able to predict the behaviour of a compound. Among the theories present in literature, classical nucleation is probably the most used. In this framework, crystallization is thought as the interplay between two steps: nucleation and growth. In the first step, nanoscopic crystalline nuclei are formed, whereas in the second one they progressively grow, until the sample is fully crystallized. Both steps are influenced by the temperature: in particular, nucleation is ruled by the supercooling degree, and the second, dominated by its diffusive nature, is governed by molecular mobility [6, 7].

Bifonazole (BIF) is an imidazole-based antifungal drug, used as ointment. According to Baird et al. [8, 9], it is a class

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II pharmaceutical, meaning that it can be easily amorphized but crystallizes upon reheating at moderate scanning rate.

Recently, there has been the appearance of a study on bifonazole [10] providing some insights in the physical behaviour of BIF. The aim of the current study is to provide new thermodynamical information on this compound, thanks to an extensive calorimetric characterization of BIF behaviour in its crystalline and amorphous states.

Experimental

Materials

Bifonazole, 1-(p,α-Diphenylbenzyl)imidazole, with molecular formula $C_{22}H_{18}N_2$, molecular mass 310.39 Da, CAS 60628-96-8, was provided by Sigma-Aldrich, with a purity of 98.9%. The sample was used without further purification.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) measurements have been performed with a PerkinElmer DSC model 8500, equipped with an Intracooler III as refrigerating system reaching approximately 150 K and allowing experiments down to 173 K.

Sample has been loaded in a 40 µl aluminium pan, and then hermetically sealed: mass ranges between 5 and 10 mg. Temperature and enthalpy scales have been calibrated with indium. Nitrogen has been used as purge gas with a flow rate of 20 mL min⁻¹.

Powder X-rays diffraction

Powder X-rays diffraction (PXRD) measurements have been done with two instruments. The choice of two diffractometers is motivated by the availability of the instruments.

The as-received form has been studied with a STOE Stadi P diffractometer operating in Debye–Scherrer geometry and equipped with Cu-$K\alpha1$ radiation ($\lambda = 1.5406$ Å), a Ge (111) Johansson monochromator from STOE & Cie and a MYTHEN2 1 K detector from Dectris. The sample was loaded in a borosilicate glass capillary (0.8 mm external diameter), and data were acquired in the range 2–50° 2θ with an interval of 0.015° between consecutive points.

The thermally recrystallized samples have been investigated with a Bruker D2 Phaser diffractometer operating at 30 kV and 10 mA in Bragg–Brentano geometry (θ–θ scan mode) and equipped with a one-dimensional Lynxeye detector. Ni-filtered Cu-$K\alpha$ radiation was used. The sample was dispersed on a Si low-background sample holder, and data were collected in the range 4–65° 2θ with a step scan of 0.01°.

Results and discussion

Differential scanning calorimetry

The as-received sample was first investigated to check if we were able to obtain values in agreement with [10]: thus, a heating/cooling/heating cycle at 10 K min⁻¹ was performed. Results are displayed in Fig. 1.

As expected from a class II compound, BIF does not crystallize upon cooling, but during the reheating scan. The melting temperature and enthalpy of the first heating at 10 K min⁻¹, i.e. of the as-received crystalline sample, are $T_m = 422.3$ K and $\Delta H_m = 122.6$ J g⁻¹, in good agreement with $T_m = 421.9$ K and $\Delta H_m = 124$ J g⁻¹ reported in Ref. [10]. Additionally, in Ref. [10], during the second heating, it was observed a melting with significantly different ΔH_m and a slightly different T_m: this phenomenon was explained in term of the formation of a new crystalline structure, different from the as-received one, namely polymorphism.

Interestingly, our thermodynamical parameters during the reheating scan disagree with Ref. [10]: in our experiment, at 10 K min⁻¹, we found $T_m = 423.1$ K and $\Delta H_m = 65.2$ J g⁻¹, whereas Ramos et al. [10] at 2.5 K min⁻¹ found $T_m = 421.8$ K and $\Delta H_m = 107.9$ J g⁻¹. Such slight difference in $T_{m,s}$ can be explained by the fact that in our measurement the cold crystallization extends up to the start of the melting, making difficult the exact definition of T_m. The same argument, on the other hand, cannot explain the fact that in Ref. [10] the value of enthalpy is almost double with respect to the one found in our experiment.

We made a further measurement replicating Ramos et al. time–temperature profile, i.e. using a reheating scan of 2.5 K min⁻¹, and we obtained results in good agreement with Ref. [10] (data not shown). Summarizing, the increase
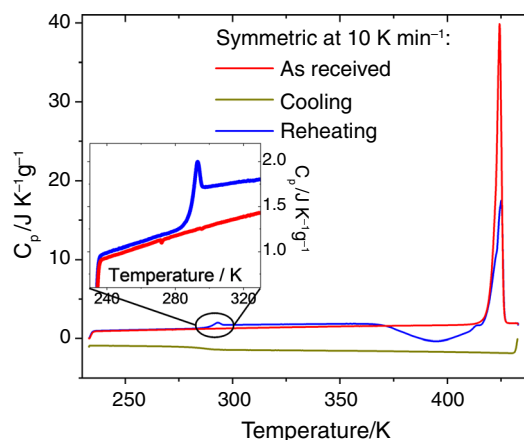

Fig. 1 Heating/cooling/heating cycle of bifonazole at 10 K min⁻¹. Inset: zoom into the glass-to-liquid transition interval

of the heating rate during reheating leads to a decrease in the melting enthalpy. Such a situation of similar $T_{m,s}$ but different $\Delta H_{m,s}$ has already been reported in literature for polymorphism of, e.g. indomethacin and ROY [11, 12], but polymorphism itself does not explain why on increasing the heating rate the ΔH_m decreases but the T_m remains unchanged. Thus, we decided to make another measurement in which we stopped the heating scan right after the end of the crystallization peak, i.e. at 388 K, and quenched the sample immediately after. In fact, thanks to the quenching, we could freeze the sample right after the crystallization was completed and, with the subsequent reheating, we can check the state of the sample. The results of this measurement, together with the adopted time-temperature profile, are displayed in Fig. 2.

As it is possible to note in Fig. 2, upon reheating (green curve in Fig. 2, right panel), above 400 K, there is a small exothermic peak, meaning that there is still a small portion of the sample crystallizing. Moreover, if we zoom in on the temperature interval of the glass transition peak (Fig. 2, inset in right panel), we can note a small endothermic peak, signature of the glass transition. The peak and the jump in heat capacity at T_g are very small but distinguishable from the uncertainty of the instrument, suggesting a very small amorphous fraction. From enthalpies calculation, it emerges that the melting enthalpy is $\Delta H_m^{\text{as received}} = 122.6$ J g^{-1} for the as-received sample, while the reheated sample shows $\Delta H_m^{\text{reheat}} = 117.5$ J g^{-1}, with a crystallization enthalpy of $\Delta H_{\text{cryst}}^{\text{reheat}} = 8.5$ J g^{-1} which, within the error on enthalpy estimation of 5%, tells us that the sample is now fully crystallized. Thus, by direct comparison of the melting enthalpies of the as-received sample and the crystallization enthalpy during reheating, we can infer that the amorphous fraction is of around 7%.

We suggest that such difference from [10], i.e. the decrease in melting enthalpy experienced during reheating observed in both works, can be attributed to partial crystallization: indeed, if it was due to another polymorph, no difference in melting enthalpies would have been found with the heating rate, but only a shift in $T_{m,s}$ should have been revealed.

To further demonstrate our thesis, we decided to perform PXRD measurement. To demonstrate that upon recrystallization the crystalline structure is the same as the as-received sample, we compared the diffractograms of the as-received sample with the one of the samples crystallized with the time–temperature profile reported in Fig. 2. Moreover, we have added a third sample: in Ref. [10] the authors say to have crystallized bifonazole by keeping it in the supercooled state at constant temperature (in their case 363 K) for a time long enough. Thus, we produced a crystalline bifonazole specimen grown from its supercooled liquid at 363 K within 2 hours. In Fig. 3, we display the diffractograms of the as-received sample, the sample crystallized during the heating
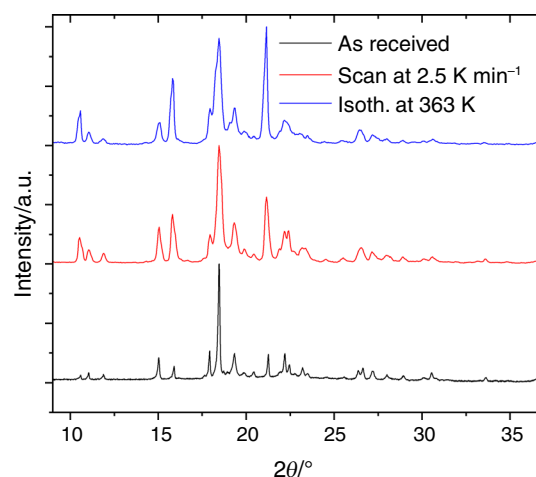

Fig. 3 PXRD scans of crystalline bifonazole in its as-received form (black line), recrystallized upon heating scan at 2.5 K min^{-1} (red line) and recrystallized upon isothermal holding for 2 hours at 363 K (blue line)

Fig. 2 Left: Time–temperature profile adopted. Scan rate reported in figure. $T_m = 422.3$ K. Right: Experimental results on BIF obtained upon heating (colours indicate the different ramps as indicated in the left panel). Inset: zoom in on the glass transition interval of the reheated scan after partial heating (green curve)
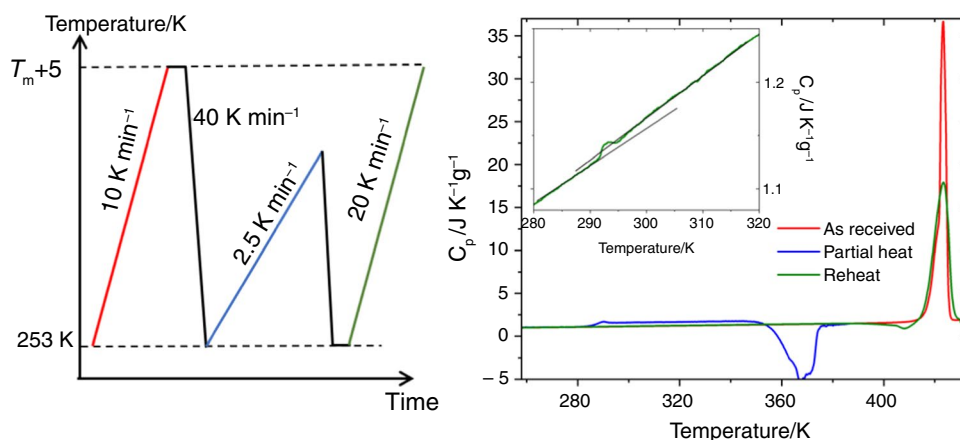
Fig. 4 Left: Time–temperature profile adopted. Scan rate reported in the figure. $T_m = 422.3$ K. Right: Experimental results obtained on reheating. Curves have been vertically shifted for the sake of clarity
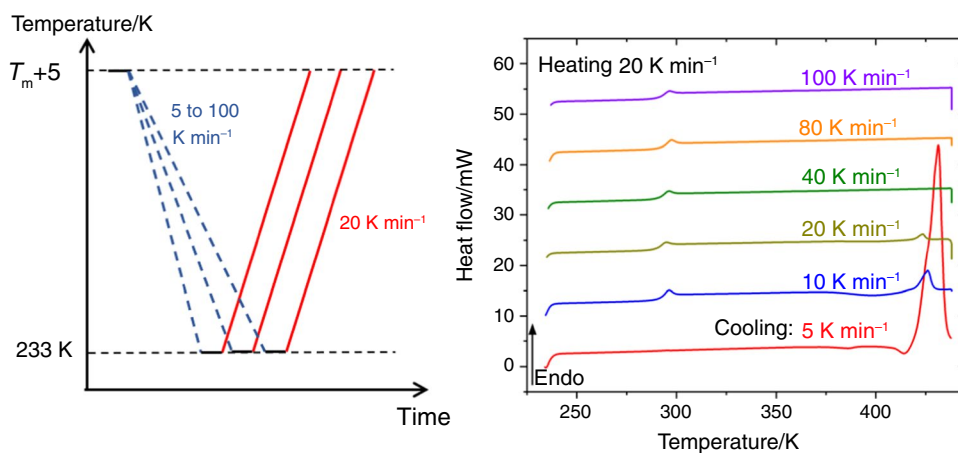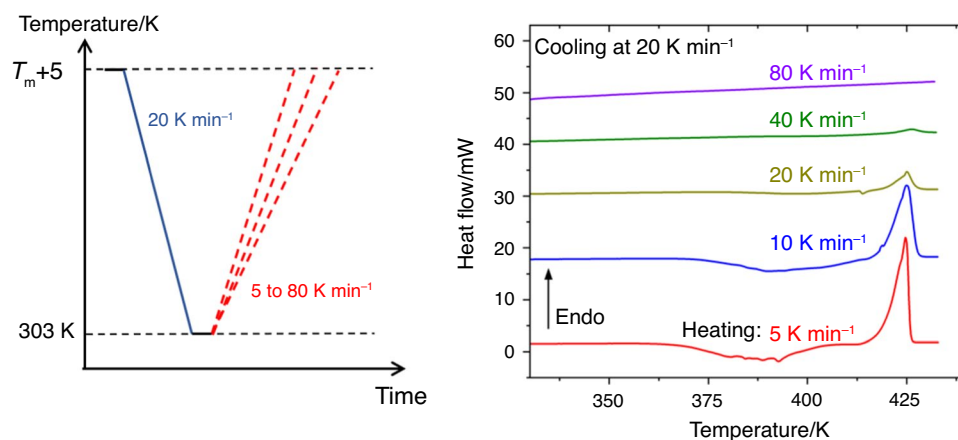
Fig. 5 Left: Time–temperature profile adopted. Scan rate reported in the figure. $T_m = 422.3$ K. Right: Experimental results obtained on reheating: curves have been vertically shifted and only the temperature range where crystallization could occur is reported for the sake of clarity

scan, and the sample crystallized during the isotherm at 363 K.

As it is possible to note from Fig. 3, the three X-ray diffraction patterns agree quite well among them, apart for small differences in the peak shape and intensities. These can be attributed to differences in the instrumental angular resolution and to possible different degree of preferential orientation of crystallites in the studied samples, respectively. We can conclude that the crystal structure of the recrystallized bifonazole, independently on the way it crystallizes, remains the same of the as-received one; thus, bifonazole does not exhibit polymorphism under thermal treatment.

To characterize the occurrence of partial crystallization, there is the need for ad hoc measurements. The main goal is to demonstrate that BIF crystallization depends on the time–temperature profile adopted. Thus, we designed two protocols: in the first, we varied the cooling rate and fixed the heating rate, and in the second protocol we did the opposite. Results of this investigation are displayed in Figs. 4 and 5, respectively.

Figures 4 and 5 show a common trend, i.e. BIF crystallization can be progressively inhibited by increasing the heating and/or cooling rate. In particular, the crystallized
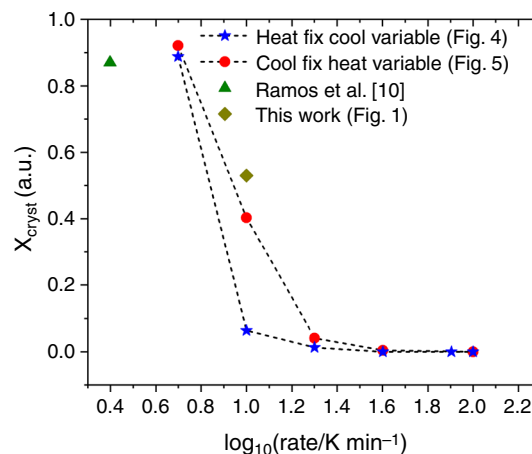
Fig. 6 Normalized melting enthalpies as a function of the scan rate varied in the measurement

fraction (X_{cryst}) has been estimated by computing the melting enthalpies reported in Figs. 4 and 5 and divided for the melting enthalpy of the as-received sample. In Fig. 6 we report the results of this analysis, together with the

crystallized fraction obtained upon reheating in this work (see Fig. 1) and in [10].

From Fig. 4, it is possible to note that there is a change in the melting temperature as a function of the cooling rate. This behaviour can be explained by considering the crystal mosaicity, i.e. the presence of multiples crystalline domains connected to each other inside the sample. In fact, by changing the cooling rate, we change the number of nuclei formed: the latter act as starting point for crystal growth. Thus, the higher the number of nuclei, the higher the number of domains in the sample. As demonstrated in literature, both experimentally [13] and theoretically [14], a higher mosaicity leads to a decrease in the melting temperature. Contrarily to Fig. 4, in Fig. 5 the melting temperature does not change: indeed, the cooling rate is fixed, so the mosaicity of the sample remains constant, and the same applies to the T_m; what changes is the extent of the crystal grown upon heating.

In conclusion, we can ascribe the decrease in melting enthalpy during reheating to a partial crystallization of the sample. Moreover, the difference in the melting enthalpies found in this work and in Ref. [10] can be explained by the different scanning rates used, respectively, 10 and 2.5 K min^{-1}, creating a different crystalline fraction in the recrystallized sample.

Finally, it is possible to understand the fact that the melting temperature remains almost unchanged during reheating, a fact quite strange if we were in the presence of a new polymorph: in fact, different crystalline structures not only have different $\Delta H_{m,s}$ but also different $T_{m,s}$ [5].

After demonstrating the tendency of BIF to partially crystallize upon reheating, we decided to investigate the crystallization behaviour of the compound under exam: in particular, we determined the temperature intervals more favourable to crystal nucleation and growth, respectively, at least on the time scale imposed by the scanning rate which, in a first approximation, can be considered equal to the reciprocal of the scan rate itself. Thus, we exploited symmetrical heating and cooling cycles at 20 K min^{-1}, by changing the lowest reached temperature. Results are shown in Fig. 7.

From Fig. 7 it is possible to infer that the interval 273–313 K is the most favourable to nucleation on the time scale 1 K/(0.33 K s^{-1}) ~ 3 s. By looking at the melting peaks, it is possible to note that the maximum in their area is at 303 K, whereas at higher and lower temperatures the area is progressively less, see Fig. 8 for the results of this analysis.

The symmetric distribution of crystallization area around 303 K resembles the fact that the crystallization process is the result of the interplay between two steps: nucleation and growth [6]. In particular, the nuclei formation rate is ruled by the degree of supercooling, namely the difference between T_m and the actual temperature; thus the higher the degree of supercooling, the higher the rate. On the other hand, growth rate is inversely proportional to the molecular mobility; consequently, it will become faster at higher temperatures where viscosity is lower and so the internal mobility is faster [6]. As shown experimentally [5, 15], the maxima of these two quantities fall at different temperatures. It is important to
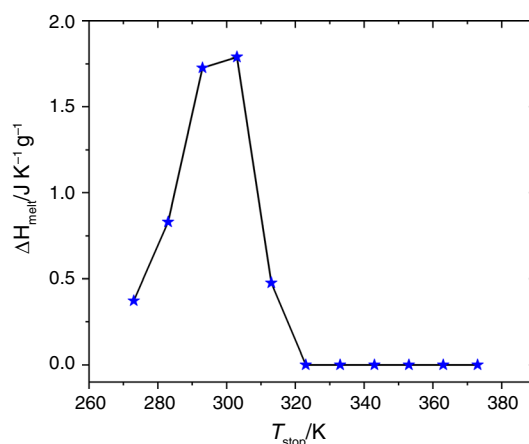
Fig. 8 Behaviour of melting enthalpy as a function of the lowest reached temperature (T_{stop}) during the measurement

Fig. 7 Left: Time–temperature profile adopted. $T_m = 422.3$ K. Right: Results obtained. Data have been vertically shifted for the sake of clarity. Lowest temperature reached is included in the graph
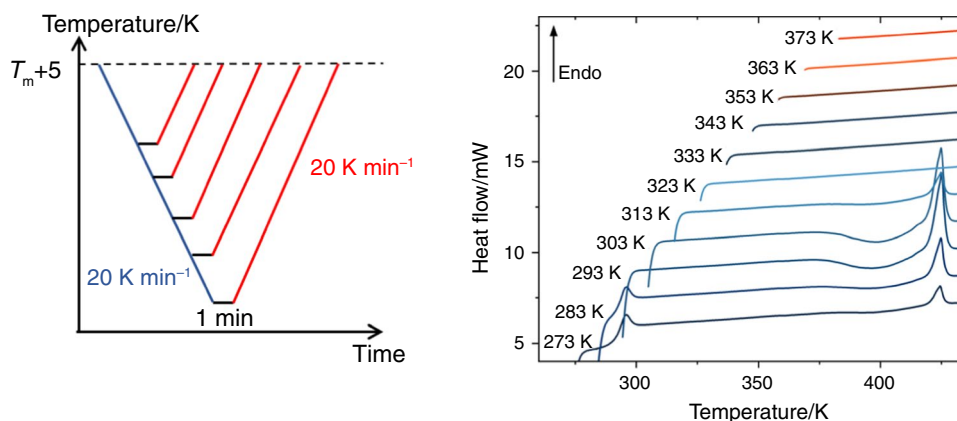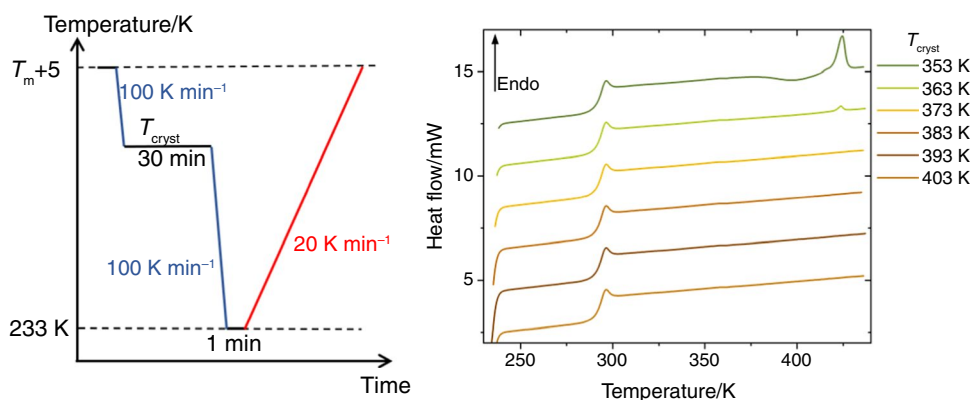
Fig. 9 Left: Time–temperature profile adopted. $T_m = 422.3$. Right: Measured scan after 30 min at constant temperature. Crystallization temperatures are indicated in the legend. Data have been vertically shifted for the sake of clarity

keep in mind that both quantities, i.e. nuclei formation rate and growth rate, have a bell-shaped behaviour around their maximum: this trend can be recognized in Figs. 7 and 8 by the fact that the maximum in the crystallization area falls in the middle of the interval where crystallization takes place. From these considerations, we can infer that the interval 272–313 K is more favourable to nucleation, whereas at higher temperatures crystal growth is favoured. As noted by Van Eerdenbrugh and co-workers [16], in the crystalline form the molecules interact via a C–H⋯N hydrogen bonds, forming chains and these chains are further stabilized by a three-dimensional network by C–H⋯π stacking. Molecularly speaking, the observed partial crystallization can be attributed to the lack of formation of the long-range order, i.e. the C–H⋯π stacking, which is also responsible of the chains stabilization.

Nevertheless, nucleation is possible even in an unfavourable temperature interval, i.e. where crystal growth is more favoured, if we give enough time to the sample to form nuclei. In fact, even if the nuclei formation rate at high temperature is low, it is still different from zero: thus, by waiting enough time, we can have the formation of few crystalline nuclei which can be grown during the subsequent heating. To make this statement stronger, we decided to apply a high cooling rate from the molten state to the targeted crystallization temperature (T_{cryst}), to better suppress the probability of nuclei formation on cooling (see left panel of Fig. 9): indeed, the process of nuclei formation takes a certain amount of time and starts just below T_m; thus, only by arriving quickly at the crystallization temperature we can avoid formation of nuclei during cooling. In this way, the nuclei formation only during the isotherm at the desired high temperature can be probed. Thus, we have quickly cooled BIF from the melt and made it nucleate isothermally for 30 min at different T_{cryst}. Results are shown in Fig. 9.

Figure 9 clearly shows that nucleation can happen even in an unfavourable temperature region if enough time elapses. Crystallization after nucleation at 363 K is expected to arrive at completion if more time is given to the sample to nucleate:

in fact, the highest the number of nuclei, the less the time required to make them fully grown. The same applies to temperatures higher than 363 K.

Conclusions

The thermal and structural properties of bifonazole have been systematically investigated with DSC and PXRD, showing that bifonazole undergoes to a partial crystallization during reheating, and that the crystallized fraction depends on the time–temperature protocol: in particular, the higher the cooling/heating rate, the lower the crystalline fraction. It is notable that both heating and cooling rate influence the crystallization process, by progressively inhibiting crystallization when their value is increased.

Within this investigation we have highlighted the influence of the temperature on BIF crystallization, in terms of nucleation and growth processes: in particular, we have found the temperature intervals more favourable to each process. We have also shown that isothermal crystallization can take place even at temperatures unfavourable for nuclei formation.

In conclusion, bifonazole has shown no signs of polymorphism under different thermal treatments but a rich scenario of partial crystallization depending on the time–temperature history applied.

As a final remark, regarding the practical applications of our findings, it is possible to hypothesise that BIF dissolution properties can be slightly varied by tuning the amorphous fraction inside the sample. This statement is true only if the amorphous fraction remains constant during the storage, but this aspect, that is worthwhile to be explored, is outside the scope of the present paper.

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