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Rapid communication

Toward understanding the evolution of griseofulvin crystal structure to a mesophase after cryogenic milling

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ABSTRACT

The purpose of this research is to investigate the response of crystalline griseofulvin to mechanically induced stress through cryogenic milling. Crystalline griseofulvin was subjected to cryogenic milling for two different lengths of time. Following cryo-milling, the samples were immediately analyzed by differential scanning calorimetry (DSC) and X-ray powder diffraction (XRPD). The DSC thermograms of cryo-milled griseofulvin showed a complex exothermic event at around 65 °C for the 30 min cryo-milled sample and around 75 °C for the 60 min cryo-milled sample. A glass transition event was not observed for the cryo-milled samples. This is in direct contrast to the X-ray amorphous griseofulvin sample prepared through the quench melt method The XRPD patterns of cryo-milled griseofulvin show a loss of the crystalline Bragg peaks and a corresponding increase in diffuse scattering (halos). The disordered griseofulvin material produced through cryo-milling appears X-ray amorphous, yet different from the amorphous phase produced using the quench melt method. Both X-ray amorphous materials have distinctive DSC thermograms and X-ray powder patterns. These findings suggest that the evolution of the griseofulvin crystal structure during cryo-milling is not simply a crystal-to-amorphous transition but a transition to an intermediate mesophase.

1. Introduction

Most active pharmaceutical ingredients (API) are preferred in crystalline form for the development of pharmaceutical products. In many cases, the particle size of the starting crystalline material is not optimal for the intended dosage form. In consequence, the initial particle size of the crystalline material needs to be reduced by milling. The process of milling is one of the most commonly used unit operations in pharmaceutical manufacturing. The benefits of smaller particle size are apparent on the enhancement of the dissolution rate of solid dosage forms leading to higher bioavailability of the final pharmaceutical product (Macdonald and Himelick, 1948; Ober et al., 1958; Kraml et al., 1962; Kanig, 1963; Fincher, 1968; Parrott, 1975; Burt and Mitchell, 1981). It is well documented that the ball or jet milling process of crystalline materials induces damage to the crystal lattice leading to changes on both physical and chemical properties of pharmaceutical substances. These changes resulted in greater chemical instability (Huttenrauch et al., 1985; Shalaev et al., 2002), altered mechanical properties (Hiestand and Smith, 1984; Wildfong et al., 2006; Huttenrauch and Keiner, 1977), propensity to hygroscopicity (Huttenrauch, 1977; Ahlneck and Zografi, 1990), polymorph transformations (Otsuka et al., 1986a,b; Otsuka and Kaneniwa, 1986; Kaneniwa and Otsuka, 1985; Matsumoto et al., 1988) and dehydration (Otsuka et al., 1999). Qiu et al. (2005a,b) also found that milling could trigger the chemical reactivity on the solid-state Maillard reaction between metaclopramide hydrochloride and lactose. Most authors (Otsuka et al., 1986a,b, 1999; Qiu et al., 2005a,b) have linked these transformations to the formation of amorphous phase. The amorphous phase was presumably considered as the most immediate phase following mechanical activation. The present communication follows the recent resurgence of interest in the formation of crystal defects during dehydration and milling process (Bates et al., 2007; Feng et al., 2007). Organic crystals are generally imperfect in the structure of the crystal lattice by containing various types of crystal defects (Crowley, 1990). These crystal defects interrupt the long-range three-dimensional order in the crystal lattice. As a result, these are considered as disordered region with higher energy landscape (Zhang et al., 2006). Crystal defects could be formed when crystalline materials are subjected to mechanical and/or temperature stresses. Crystal defects have played an important role in the intermediate amorphous phase formation for metals and organic crystals (Burt and Mitchell, 1981; Huttenrauch et al., 1985; Shalaev et al., 2002). Subsequently, as part of an ongoing investigation,

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the influence of mechanical processing conditions on the crystal lattice is being studied in the context of defects or amorphous formation. This investigation was supported by using analytical probes that are sensitive to the disorder in the solid state. Thus this short communication reports the evaluation of the nature of the disordered material formed during cryo-milling of crystalline griseofulvin. The suitability of micro-structural characterization techniques such as thermal and diffraction has been investigated on the cryo-milled material to determine the differences between amorphous phase and defective crystal. Cryogenic milling (cryo-milling) was used to avoid excessive heat otherwise produced during milling process. Griseofulvin was selected as the model compound because of robustness of the crystal in the context of no polymorphs or solvates and reasonable chemical stability of the material.

2. Materials and methods

2.1. Materials

Crystalline griseofulvin was purchased from Sigma (St Louis, MO) and stored at 25 °C in desiccator over P_2O_5 before use.

2.2. Preparation of X-ray amorphous griseofulvin

X-ray amorphous griseofulvin was prepared by the quench melt method. An appropriate amount of crystalline griseofulvin was weighed and placed on an aluminum dish. The crystalline drug was heated to 220 °C in a temperature controlled heating plate and held at that temperature for approximately 1 min. The molten griseofulvin was transferred immediately to a mortar and gently ground with a pestle until a powdered glassy material was obtained. The resulting powdered griseofulvin sample was transferred and stored in a desiccator over P_2O_5 at 0 °C to ensure physical stability of the X-ray amorphous form (reported glass transition temperature (T_g) of griseofulvin is about 85 °C) (Elamin et al., 1994).

2.3. Cryogenic milling of griseofulvin

Cryogenic milling was performed using a SPEX CertiPrep 6750 cryogenic impact mill (Metuchen, NJ) and as described in a previous report (Feng et al., 2007). Briefly, the mill consisted of a cylindrical polycarbonate vessel, with stainless steel rod that vibrates by means of coil having a controlled oscillating magnetic field. The vessel and coil assembly house the samples to be milled while completely submerged in a bath of liquid nitrogen. Griseofulvin samples were milled for 30 min and 60 min periods followed by storing them into a desiccator over P_2O_5 at 0 °C prior to calorimetric and X-ray diffraction analyses.

2.4. Differential scanning calorimetry (DSC)

A Q10 DSC (TA Instruments) was used for thermal analysis. The DSC was calibrated for enthalpy using high purity indium in addition to the three-point temperature calibration was performed using indium, tin and benzophenone. Nitrogen was used as purging gas at a constant flow rate of 50 mL/min. Samples typically weighing about 3 mg were heated at 10 °C/min to temperatures in excess of the melting point of the drug (218 °C). Samples were analyzed in hermetic aluminum pans (TA Instruments). Data analysis was performed using the Universal Analysis software version 2.5H (TA Instruments). All DSC determinations were conducted in triplicates.

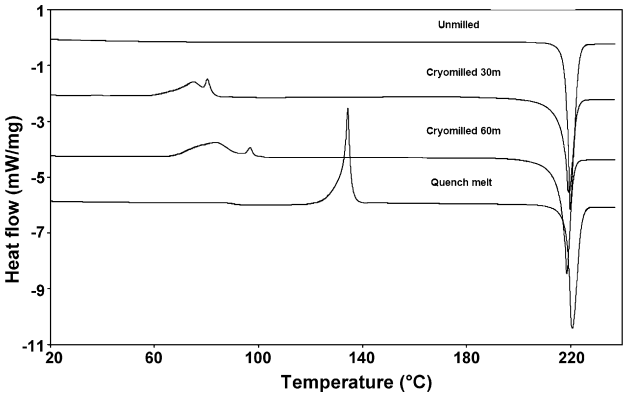

Fig. 1. DSC thermograms of unmilled, cryo-milled 30 min, cryo-milled 60 min and quench melt griseofulvin. The significant differences between cryo-milled and quench melt griseofulvin suggest that cryo-milling is not directly producing the same X-ray amorphous griseofulvin as produced via quench melt but some intermediate disordered state.

2.5. X-ray powder diffraction (XRPD)

Samples were analyzed using a Shimadzu XRD-6000 X-ray powder diffractometer equipped with a Bragg–Brentano optical setup. Cu Kα radiation was used with a long fine focus X-ray tube. The tube voltage and amperage were set to 40 kV and 30 mA, respectively. The divergence and scattering slits were set at 0.5° and the receiving slit was set at 0.15 mm. Diffracted radiation was detected by a NaI scintillation detector. Samples were scanned at 0.02 $2\theta/s$ from 5° to 60° 2θ. Scans of each sample were run in triplicate. All powder samples were front filled using a circular area aluminum holder. A silicon standard was analyzed to check the instrument alignment each time before sample measurements.

3. Results

3.1. Thermal behavior of disordered griseofulvin

The DSC thermograms for unmilled, quench melt and cryo-milled 30 and 60 min griseofulvin samples are overlaid in Fig. 1.

Both the cryo-milled 30 min and 60 min griseofulvin show exothermic doublet around 60 °C. While the X-ray amorphous sample shows glass transition around 90 °C and recrystallization onset around 120 °C. The exothermic heat for cryo-milled 30 min sample is 29.1 ± 2.5 J/g, while for cryo-milled 60 min sample is 57.6 ± 3.3 J/g. The heat of fusion for cryo-milled 30 min sample is 119.2 ± 4.9 J/g, while for cryo-milled 60 min sample is 121.9 ± 6.7 J/g. For unmilled griseofulvin, the heat of fusion is 132.4 ± 4.0 J/g.

The cryo-milled griseofulvin does not show a glass transition and its heat of fusion is somehow low compared to the crystalline sample. The significant differences of thermal behavior between the cryo-milled and X-ray amorphous griseofulvin suggest that the cryo-milled sample presents some type of disordered phase other than amorphous phase. As discussed in early work (Feng et al., 2007), those exothermic events at relatively low temperature were considered as the manifestation of crystal defects.

3.2. XRPD patterns of griseofulvin with cryo-milling

Fig. 2 shows the comparison of the evolution of XRPD spectra for cryo-milled and X-ray amorphous griseofulvin. By carefully analyzing the evolution of the Bragg peaks, no significant peak position changes were observed with cryo-milling. This suggests that cryo-milling did not result in any polymorph transformation of the initial

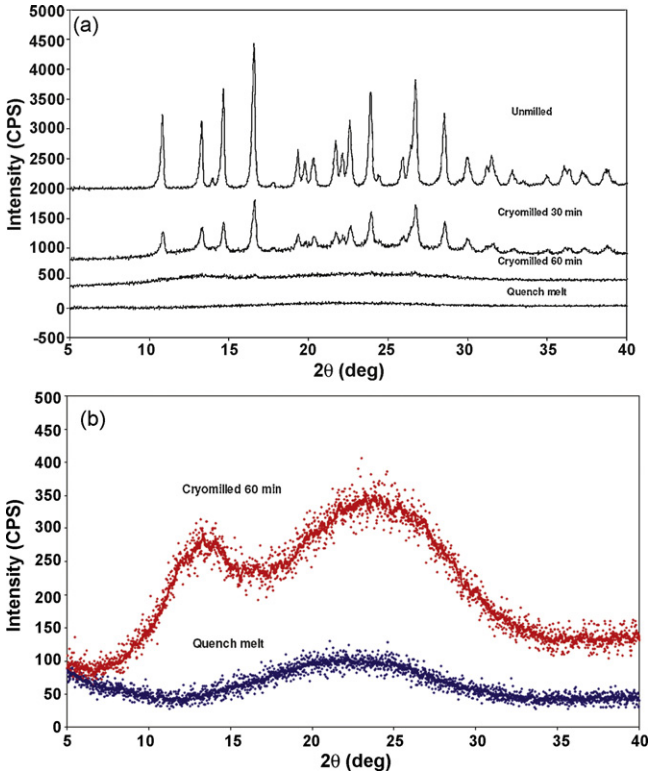

Fig. 2. XRPD patterns of unmilled, cryo-milled 30 min, cryo-milled 60 min and quench melt griseofulvin. (a) Overall XRPD patterns. (b) Scale-up comparison between cryo-milled 60 min and quench melt griseofulvin. Note with cryogenic milling the crystallinity of the sample decreases drastically.

crystal structure. The diffuse scattering was observed to increase with the cryo-milling until for the cryo-milled 60 min sample the diffuse scattering dominated the measured data with little evidence for residual crystallinity. The overlay between the XRPD data for the cryo-milled 60-min and amorphous samples (Fig. 2b) indicated significant differences between them. The powder pattern for the cryo-milled 60-min sample has two major broad peaks at 13° and 24°, while the X-ray amorphous powder pattern from the quench melt sample has only one major halo at 22°. These significant differences between the measured powder patterns indicate that the local structure is very different between the two X-ray amorphous phases and therefore the disordered phase produced by cryo-milling is different to the X-ray amorphous phase produced via quench melt (Fig. 1).

By examining the peak width of each Bragg reflection, no significant peak broadening was observed for the cryo-milled griseofulvin. Normally, when crystalline materials are subjected to mechanical stresses, the Bragg peaks broaden due to two effects on the microscopic structure of the materials: loss of crystalline correlation length (crystallite size reduction) and micro-strain. Micro-strain occurs when different parts of the powdered sample are subjected to different stress conditions leading to a distribution of strain values for different crystals in the powder.

The lack of Bragg peak broadening for the cyro-milled griseofulvin implies that the loss of crystallinity during cryo-milling process was not due to crystallite size reduction or micro-strain development. Typically preferred orientation would not be expected to significantly affect the crystallinity. The cryo-milled griseofulvin has also been analyzed and found no significant degradation after cryo-milling, which eliminates possibility of impurity effects on XRPD patterns. Thus the continuous build-up of the diffuse scattering intensity as the crystalline Bragg peak intensity diminishes is a fingerprint of increasing coherent strain or point defects within the crystal structure as a function of cryo-milling.

4. Conclusion

Cryogenic milling of crystalline griseofulvin produces a disordered X-ray amorphous material that differs in the physical characteristics from those of the amorphous phase formed via melt quench method. The thermal and X-ray diffraction measurements indicate that these differences may not be micro-structural in nature but somehow related to the local molecular packing (structure) present in the material. The two X-ray amorphous phases are likely "thermodynamically" different phases, which have significantly different thermal and diffraction signatures.

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