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Thermal insight of mechanically activated bile acid powders

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ABSTRACT

Mechanical activation of pharmaceutical materials presents an important but poorly understood phenomenon of milled molecular crystals. In this work, a strategy was followed in an effort to understand this phenomenon, cryo-milled of both crystalline and amorphous counterpart of bile acids materials were characterized by X-ray powder diffraction (XRPD) and differential scanning calorimetry (DSC). The XRPD results for the 30-min milled crystalline powders displayed a characteristic amorphous halo patterns for all compounds tested. The DSC thermograms exhibited the typical glass transition temperatures (T_g) associated with amorphous but only for two materials. For the remaining four milled compounds, a rather interesting behavior was manifested through a characteristic exothermal bimodal peak. The findings seemed to suggest that the occurrence of this event was not related to the (T_g), but likely to the melting temperature (T_m). The DSC results for the melt-quenched (amorphous) ursodeoxycholic acid after cryo-milling revealed that the material crystallized after the influence of the mechanical stress, and a bimodal peak was also observed similar to that of the cryo-milled crystalline material. It is contemplated that the response of the physical instability of the disordered phase could be explained either by the result of surface crystallization kinetics which is different from that of the bulk crystallization, or by the creation of supersaturated dislocated crystal prior to amorphization.

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1. Introduction

Mechanical milling is a common process in the pharmaceutical industry to reduce particle size of crystalline active pharmaceutical ingredients (APIs), to improve handling (Vendola and Hancock, 2008), to enhance dissolution rate (Florence et al., 1974) or for dry powder inhaler (DPI) formulations (Saleem and Smyth, 2008). This pharmaceutical manufacturing unit operation process not only reduces the size of drug particles, but also causes a partial or complete physical transformation to a new solid form (polymorph or amorphous form) (Crowley and Zografi, 2002; Gusseme et al., 2008). Many compounds are reported to become amorphous upon milling (Bates et al., 2006; Han et al., 1998; Mosharraf and Nyström, 2003; Otsuka and Kaneniwa, 1990; Price and Young, 2005; York et al., 1998), with solid-state properties different from those of the melt-quenched (Graeser et al., 2008) or spray-dried (Yonemochi et al., 1999b) samples. The physical transformation of API may result in drastic changes in dissolution rate (Terada et al., 2000) or chemical stability (De Villiers et al., 1992). In addition, mechanical stress during the milling process may produce mechanically activated powders. The mechanical activation of powders, where the milled material stores some of the energy imparted to it during milling, is an intrinsic aspect of milling pharmaceutical materials and presents an important but poorly understood phenomenon. Of particular interest is the understanding of the microstructure of mechanically activated materials. Mechanical activation is associated with decreased crystallinity of the material. It can take the form of crystal dislocations (Feng et al., 2008) or of full fledged (cooperative) amorphous regions (Johnson, 1986; Savolainen et al., 2007). Mechanical activation also affects the physical stability of powders, leading to physical transformations, such as polymorphic transformations and dehydration hydrated molecular crystals (Chieng et al., 2006; Shakhtshneider and Boldyrev, 1993; Wang et al., 2002; Willart et al., 2001; Yajima et al., 1997).

Therefore, milled materials should be carefully monitored and characterized during and after milling. To-date, several milling mechanisms have been proposed (Fecht, 1992; Wildfong et al., 2006), but up till now, a fundamental insight to explain the milling behavior of organic compounds is still needed. Pharmaceutical organic compounds are usually very anisotropic, have a wide variety of orientations and conformations that makes the task challenging.

The behavior of crystalline compounds during milling is governed by crystal stability, i.e. fragility or hardness in the initial step of pulverization, and then by physical stability of phase transformations, especially amorphization, have long been observed in

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mechanically milled pharmaceutical molecular crystals (Willart and Descamps, 2008; Bates et al., 2006). The crystal stability of organic compounds toward mechanical stress has been studied with respect to brittleness (Meier et al., 2009), slip planes (Sun and Kiang, 2008), dislocation density (Wildfong et al., 2006), heat capacity (Chamarthy and Pinal, 2008) and surface energetics (Chamarthy and Pinal, 2008; Otte and Carvajal, 2011).

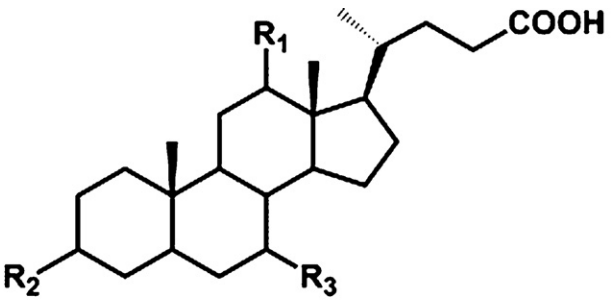
The benefit of the particle size reduction on dissolution is of interest but this is accompanied by detrimental effects on the physico-chemical properties and stability of the milled materials. Hence, it is important to assess the fundamentals, effects and consequences of milling, to interrogate materials subjected to milling in an effort to better understand the crystal integrity during drug development. The work of Feng et al. (2008) started to study in more detail the consequences of milling, the strategy was to compare thermal analyses of cryomilled vs. quench-melt griseofulvin, both samples gave XRPD pattern typical of amorphous. However, DSC thermograms of the milled crystalline sample, exhibited unusual bimodal exothermal peaks without a glass transition, whereas the T_g was obvious for the quench-melt sample. Yonemochi et al. (1999a) reported that milled ursodeoxycholic acid crystallized faster than the melt-quenched material over ethanol–water vapor environment where the milled sample might retain the local structure similar to the crystalline form whereas the molecular structures in quenched sample were distributed more randomly than that in the milled form. Descamps et al. (2007) stated that physical transformation or crystallization often occur at room temperature during grinding.

The rational, for choosing the compounds and experimental conditions in the study presented herein, was based on previous literature reports. Thus, the objective of this study was to investigate the effect of cryomilling on the thermal behavior using ursodeoxycholic acid and various similar bile acids derivatives. Cryomilling was chosen to avoid reaching high temperatures, thus it is reasonable to assume that any changes in the sample are attributed only to mechanical activation. It was hypothesized that after milling all compounds become mechanically activated but some store the energy prior to trigger amorphization. Hence, in this study, 'disorder' will be used for any state showing a halo pattern on its X-ray diffractogram, while amorphous will refer to a specific state exhibiting an XRPD halo pattern with the presence of glass transition on the DSC.

2. Materials and methods

2.1. Materials

The bile acids used in this study are displayed in Fig. 1. These compounds have in the chemical structure the same framework with changing functional groups. These are crystalline materials and used as received: dehydrocholic acid ("DEH" β-form, TCI America, Portland, OR, USA), lithocholic acid ("LIT", TCI America, Portland, OR, USA), cholic acid ("CHO", Sigma, St. Louis, MO, USA) and deoxycholic acid ("DEO", Spectrum, Gardena, CA, USA), ursodeoxycholic acid ("URS", generously supplied by Mitsubishi Tanabe Pharma Co., Osaka, Japan). The crystalline form of chenodeoxycholic acid ("CHE", Sigma, St. Louis, MO, USA) was metastable form (III) (Oguchi et al., 2003), the sample was heated at 140 °C for 1 h under dry nitrogen atmosphere, to obtain the most stable form (I). All samples were stored at 25 °C over desiccant prior to use. Glassy materials only for two compounds out of the six were chosen, these were prepared by quench melt procedure followed by cryomilling and were analyzed under similar conditions as the cryomilled crystalline counterparts.



	abbreviation	R_1	R_2	R_3
dehydrocholic acid	DEH	= O	= O	= O
ursodeoxycholic acid	URS	— H	··· OH	— OH
cholic acid	CHO	··· OH	··· OH	··· OH
lithocholic acid	LIT	— H	··· OH	— H
deoxycholic acid	DEO	··· OH	··· OH	— H
chenodeoxycholic acid	CHE	— H	··· OH	··· OH

Fig. 1. Structures and abbreviations of bile acids used in this study. All compounds have the same cholesterol scaffolds, but the functional groups in R_{1-3} positions are different.

2.2. Methods

2.2.1. Cryomilling procedure

Cryomilling was performed by using the reported method (Feng et al., 2008) with a cryogenic impact grinder (SPEX CertiPrep 6750, Metuchen, NJ, USA). Briefly, for the preparation of the processed materials, 1 g of crystalline cholic acids were ground with milling rate 10 (corresponding to 20 impacts/s) under liquid nitrogen temperature for 30 min. The samples were ground at different lengths of time, and immediately analyzed by XRPD and DSC.

For the assessment of the effect of milling time on recrystallization upon DSC analysis, 1 g of URS and DEO were cryomilled with milling rate 10 for 10, 30, 60 and 120 min for URS and 10, 30 and 60 min for DEO. A half gram of glassy URS and DEO was also milled with milling rate of 10; URS milling times were 10, 30 and 60 min and whereas for DEO were 10 and 30 min. Milled samples were collected in a glove box at room temperature under dry nitrogen atmosphere and stored over P_2O_5 at −20 °C. Samples were allowed to reach room temperature prior analysis.

2.2.2. Preparation of melt-quenched bile acids

Glassy bile acids were prepared by the melt-quench method reported by Feng et al. (2008). The crystalline sample was melted on a hot plate at a temperature about 10 °C higher than melting temperature and immediately submerged in liquid nitrogen. The resulting sample was collected and gently pulverized with a spatula to avoid excess mechanical stress and then stored over P_2O_5 at −20 °C. Samples were allowed to reach room temperature prior analysis.

2.2.3. X-ray powder diffraction (XRPD)

XRPD was measured by using Shimadzu XRD-6000 (Kyoto, Japan) X-ray powder diffractometer with Cu Kα radiation at 40 kV and 30 mA. The sample was scanned in steps of 0.04° (2θ/s) from 4 to 40° (2θ) with a silicon holder. A silicon standard was used to check the alignment of the instrument before carrying out all measurements of the milled substrate.