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Two solid forms of tauroursodeoxycholic acid and the effects of milling and storage temperature on solid-state transformations

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ABSTRACT

Two phase-pure solid forms of tauroursodeoxycholic acid (TUDCA) were prepared and characterized by thermal analysis, vibrational spectroscopy, X-ray diffraction, solid-state nuclear magnetic resonance, and morphological analysis. All solid forms can be produced from solvents and also can be obtained by mechanically and non-mechanically activated polymorph conversion. Near-infrared (NIR) spectroscopy, in combination with chemometrical techniques, was used for the quantitative monitoring of the polymorph conversion of TUDCA in milling process and at different storage temperatures. The NIR spectra in the range of 7139–5488 cm^{-1} were considered for multivariate analysis. Results demonstrated that the NIR multivariate chemometric model can predict the percentage of crystal and amorphous TUDCA with the correlation coeffcient of 0.9998, root mean square error of calibration of 0.740%, root mean square error of prediction of 0.698%, and root mean square error of cross-validation of 1.49%. In the milling process of crystal TUDCA (Form I), a direct transformation from crystal to glass was observed in 4 h. Moreover, the impact of different storage temperatures on the stability of amorphous TUDCA was investigated, and the rate of polymorph transformation was found to be accelerated with increasing temperature.

1. Introduction

Mechanical milling is frequently used in the pharmaceutical industry to reduce the particle size of drugs and excipients. Milling is often applied to improve the dissolution properties and bioavailability of drugs by increasing surface area, facilitate the compression of tablets in pharmaceutical manufacturing, and obtain more accurate dosing of drugs during administration (Lin et al., 2010; Ng et al., 2010; Willart et al., 2013). However, the high mechanical energy generated during milling may change the structural state of milled materials, such as changes in crystal morphology, increased crystal defects, alteration in chemical stability, polymorphic transformation, and partial or complete amorphization (Brittain, 2002; Hu et al., 2013a,b; Willart and Descamps, 2008). To date, solid-state polymorphic conversion and amorphization induced by milling have been reported for many pharmaceutical products such as gabapentin (Lin et al., 2010), sulfathiazole (Hu et al., 2013a,b), linaprazan (Willart et al., 2013), ranitidine hydrochloride (Chieng et al., 2006), and fananserine (De Gusseme et al., 2008). Diverse solid forms, polymorphs, pseudo polymorphs (solvates and hydrates), salts, co-crystals, and amorphous solids, may possess unique physicochemical properties and can remarkably affect the solubility, bioavailability, hygroscopicity, melting point, stability, compressibility, and other performance characteristics of pharmaceutical products (Braun et al., 2009; Byrn et al., 1999; Martínez et al., 2014). Given the larger surface area and higher surface activity, amorphous drugs may have higher bioavailability and display better medicine efficacy than other solid forms, so numerous studies have been performed to use the amorphous form of a drug, instead of its crystalline form (Caron et al., 2011; Zidan et al., 2012). However, the intrinsic tendency of the amorphous state to recrystallize may affect the stability of the amorphous drug and produce difficulties to maintain the therapeutic characteristics of drug during storage,

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which potentially have negative impacts on the medicine efficacy of active pharmaceutical ingredients (Bhugra and Pikal, 2008; Uchida et al., 2010). Therefore, determining the physicochemical stabilities of different drug forms and the mechanism of their transformation at various conditions is essential, and special attention should be given to the possible polymorph conversion of drugs induced by milling.

The tauroursodeoxycholic acid (TUDCA) is an endogenous hydrophilic bile acid that is normally produced at very low levels in humans and a commercially available bile acid derivative (Gaspar et al., 2013). It can attenuate the endoplasmic reticulum (ER) stress (Cha et al., 2014), and is widely used to treat cholelithiasis and cholestatic liver disease (Hofmann, 1984; Kars et al., 2010). Recently, numerous studies have demonstrated the pharmacological effects of TUDCA that showed its potential for therapeutic strategy for the prevention and treatment of various diseases. According to literature, TUDCA could provide a novel and effective treatment in patients with Huntington's disease by neuroprotective action (Keene et al., 2002), reverse type 2 diabetes in obese mice by decreasing ER stress (Ozcan et al., 2006), treat insulin resistance by improving protein folding and ameliorating ER stress (Kars et al., 2010), and possess a novel anti-inflammatory action with therapeutic implications for the inflammatory diseases of the centralnervoussystem (Yanguas-Casás et al., 2014). Polymorphism of drugs can significantly affect the clinical efficacy, toxic side effects, and quality of medicines. Hence, investigating the polymorphism and polymorph transformation in TUDCA is very meaningful. However, to date, only one solid form of TUDCA (referred to as Form I in this paper) has been obtained, the crystal structure of which was characterized by single-crystal X-ray dffraction (SXRD) (Sanctis et al., 2000). The study of polymorphs and polymorph transformation of TUDCA has not been reported. Meanwhile, the effects of mechanical and non-mechanical treatments by milling and storage conditions on the polymorph transformations of TUDCA are not investigated at present.

A wide range of analytical techniques, including X-ray diffraction, infrared spectroscopy, Raman spectroscopy, differential scanning calorimetry (DSC), and solid-state nuclear magnetic resonance (SS-NMR), have been employed to characterize the polymorphs and monitor the formation and transformation of polymorphs (Dempah et al., 2013; Lu and Rohani, 2009; Skrdla et al., 2001; Willart et al., 2013; Zeidan et al., 2013). X-ray powder diffraction (XRPD) is considered as the standard technique for the analysis of polymorphic mixtures and quantification of the degree of crystallinity (Byrn et al., 1999; Davis et al., 2003; Varasteh et al., 2009). However, XRPD has several drawbacks such as differences in particle size (Padden et al., 1999), preferred orientation (Macfhionnghaile et al., 2014), and overlap of peaks (Tanninen and Yliruusi, 1992), which seriously affect the accuracy of quantitative analysis, and this technique is not always suitable for the evaluation of lower contents of target crystal because of its unsatisfactory sensitivity (Li et al., 2005). Vibrational spectroscopic techniques, especially near-infrared (NIR) spectroscopy and Raman spectroscopy, have attracted more attention for solid-state analysis and has been successfully applied for the qualitative and quantitative analysis of polymorphic pharmaceuticals (Beer et al., 2009; Koradia et al., 2011; Willart et al., 2013). NIR spectroscopy combined with multivariate analysis, which is a fast and non-destructive analytical technique, has been successfully used for the solid-state analysis of tacrolimus (Zidan et al., 2012), sulfathiazole polymorphs (Hu et al., 2013a,b; MacFhionnghaile et al., 2013), carbamazepine (Terra and Poppi, 2014), and co-crystallization of furosemide and adenine (Sarraguca et al., 2014).

In this study, the polymorphism and polymorph transformation in TUDCA were first investigated using several analytical methods. The two solid-state forms of TUDCA were prepared and characterized by various analytical techniques. The solid-state transformation of TUDCA during the milling process and under different storage temperatures was investigated in detail. In the procedure of quantitative monitoring of the solid-state transformations of TUDCA, the strong preferred orientation during the XRPD determination of physical mixture with different mass percentages of form I badly interfered with the establishment of the model for the monitoring of polymorph conversion of TUDCA. However, NIR spectroscopy efficiently remedied this deficiency.

2. Materials and methods

2.1. Materials

TUDCA (98% pure) was purchased from Meryer Co., Ltd. (Shanghai, China). Ultrapure water (18 MΩ resistivity from a Millipore system) was used throughout the experiment. Methanol, 2-propanol and acetone were purchased from Kelong Co., Ltd. (Chengdu, China). All reagents were of analytical grade.

2.2. Methods

2.2.1. Preparation of TUDCA polymorphs

The Form I (single crystals) was obtained by slow crystallization from an aqueous solvent of TUDCA at 4 °C. The aqueous solvent was produced by stirring 6 g of TUDCA in 10 mL of water at 50 °C for at least 1 h until TUDCA completely dissolved. Meanwhile, crystallization experiments showed that Form I can also be obtained by crystallization from solvents such as 2-propanol and acetone. The amorphous TUDCA was prepared by drying viscous high-concentration of TUDCA methanol solution in a vacuum oven at 50 °C for 2 h. The viscous TUDCA methanol solution was obtained in advance by heating and stirring 20 mL of 0.3 g/mL TUDCA methanol solution in a thermostat water bath with magnetic stirring at 50 °C for 2 h. In addition, amorphous TUDCA can also be obtained by mechanically milling commercial TUDCA for certain duration.

2.2.2. Physical mixture preparation of TUDCA solid forms

To eliminate the effect of particle size when establishing the NIR multivariate chemometric model, TUDCA purchased (Form I) and amorphous TUDCA obtained from methanol and pulverized by ball mill were sieved using a 200 mesh screen to obtain uniform particle size, and the particle size fraction that passed through the screen was used for the study. To establish the model for monitoring the polymorph conversion of TUDCA, physical mixtures with different mass percentages of Form I (i.e., 10%, 25%, 40%, 55%, 70% and 85%) were prepared by adequately blending form I and amorphous TUDCA on a vortex for 30 min.

2.2.3. Milling experiments

Milling experiments were performed using a planetary mill (LNMN-QM 0.4L, Heishan Xinlitun Agate Handicrafts Co., Ltd. China) at room temperature. Four agate ball milling jars of 50 cm^3 with ten balls ($\varnothing$ = 10 mm) of the same material were used. Each milling was performed with 2 g of powder to ensure a homogeneous milling and reproducible results. The rotation speed of the solar disk was set to 400 rpm, and alternate milling periods (typically 10 min) with pause periods (typically 2 min) were applied to limit the mechanical heating of the sample. Samples were milled for 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0 h to evaluate the effect of milling on the TUDCA Form I. The milled samples have been analyzed as soon as possible after the end of the milling process. Each sample was measured in triplicate, and the mean value was used for final analysis.