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Supercritical carbon dioxide treatment as a method for polymorph preparation of deoxycholic acid

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Abstract

A new polymorph of deoxycholic acid (DCA) was formed by using a supercritical carbon dioxide treatment. Deoxycholic acid crystals were stored in a pressure vessel purged with carbon dioxide at 12 MPa, 60 °C for definite intervals. After storage for 1 h in supercritical carbon dioxide (SC–CO_2), new X-ray diffraction (XRD) peaks, not found in the bulk DCA crystal, were observed at $2\theta = 7.4°$, 9.7° and 14.0°. The intensities of the new diffraction peaks increased with an increase in storage time, whereas the intensities of the diffraction peaks due to bulk DCA crystal decreased. On the DSC curves, the crystals obtained showed an exothermic peak at around 155 °C followed by the melting peak of bulk DCA crystal at 175 °C. By the temperature-controlled powder XRD measurement, the crystals obtained were found to be a metastable form of DCA. The polymorphs of DCA have not been reported; therefore, the SC–CO_2 treatment would be a peculiar method to obtain a DCA polymorph.

Keywords: Supercritical carbon dioxide; Supercritical nitrogen; Deoxycholic acid; Polymorph

1. Introduction

Supercritical fluids have several advantageous properties with the high density, the low viscosity, and the high diffusion rate (Budich and Brunner, 2003; Turner et al., 2001). Supercritical carbon dioxide, a state of carbon dioxide at temperatures and pressures above its critical points (31.1 °C, 7.39 MPa), exists as a single phase with properties of both liquid and gas. For pharmaceutical applications, supercritical carbon dioxide is widely used due to the relatively low critical temperature and pressure and its non-toxic property (Shekunov and York, 2000; González et al., 2000). Recently, there are a number of applications using supercritical fluid in pharmaceutical industries including particle size reduction of medicinal, preparation of microspheres and microemulsions (Jung and Perrut, 2001; Moshashaée et al., 2000; Schreiber et al., 2002). Since many of devices which generate the supercritical carbon dioxide are the special one and are expensive, a relatively large cost should be demanded to start up the experiment.

As a method for generating the supercritical carbon dioxide atmosphere, Fukushima and Wakayama (1999) reported a very simple and an useful method, in which a stainless steel vessel was used for closed container, and the closed vessel was filled with CO_2 and kept in an oil bath. In these processes, they had

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synthesized porous silica fibers by templating activated carbon. This technique is a method for obtaining the supercritical carbon dioxide condition as a comparatively low-cost; therefore, we used this technique for estimating the change in crystal form of medicines in the supercritical carbon dioxide.

Deoxycholic acid (DCA) is a bile acid and has an ability to form inclusion complexes with a variety of organic compounds due to its flexible channel structure (Giglio, 1981; Bertolasi et al., 2001). We have reported that DCA could be formed in inclusion complexes with guest organic compounds during grinding process (Limmatvapirat et al., 1997, 1998), and we are interested in whether complexes of medicinal and DCA can be formed in the supercritical carbon dioxide fluid. The aim of this work was to investigate the effect of supercritical condition on molecular behavior of DCA. In the present study, we estimated the crystal form of DCA in the presence of supercritical carbon dioxide.

2. Materials and methods

2.1. Materials

DCA (Nacalai Tesque, Kyoto, Japan) of reagent grade was used without further purification. An ground sample of DCA was prepared by grinding DCA crystals (2.0 g) with a vibration mill (CMT TI-2000, Tochigi, Japan) made of alumina.

2.2. Sample preparation

Deoxycholic acid powders and solid carbon dioxide were placed together in a stainless steel vessel of 90 ml capacity (upper limit pressure: 20 MPa). The vessel was kept in the hot water bath of a constant temperature, and the temperature in the container was approximated with the temperature in the hot water bath. A supercritical state of carbon dioxide can be easily acquired by controlling the amount of solid carbon dioxide loaded, owing to the pressure in container changed in proportion to the amount of solid carbon dioxide loaded under the constant temperature. The inside pressure and temperature were controlled at 12 MPa and 60 °C, respectively, and the sample was stored in the vessel for various periods.

2.3. Powder X-ray diffractometry

Powder X-ray diffraction (XRD) was performed using a Rigaku Miniflex diffractometer (Tokyo, Japan). The measurement conditions were as follows: target, Cu; filter, Ni; voltage, 30 kV; current, 15 mA; scanning speed, 2°/min.

2.4. Temperature controlled X-ray powder diffraction (XRD)

To investigate polymorphic changes induced by heating, XRD was carried out at elevated temperatures using Philips X’Pert-MPD diffractometer (The Netherlands) equipped with Philips Japan TTK2-HC heat controller attachment model (Japan). Experimental condition was as follows: Cu Kα radiation; voltage, 40 kV; current, 50 mA; scanning speed, 0.1 s^{-1}; scanning angle (2θ), 3–40°; heating rate, 5 °C/min.

2.5. Differential scanning calorimetry (DSC)

A differential scanning calorimeter (EXSTAR6000 DSC6200, SEIKO Instruments, Japan) was used for thermal analysis. The operating conditions in the closed-aluminum pan system were as follows: sample weight, 3 mg; heating rate, 5 °C/min; without nitrogen gas flow.

2.6. Fourier-transform infrared (FT-IR) spectroscopy

Fourier-transform infrared spectrum was measured by the KBr disc method at a resolution of 2 cm^{-1} for 32 scans using a JASCO 230 FT-IR spectrophotometer (Tokyo, Japan).

2.7. Density measurement

The density of DCA was evaluated by using Micromeritics AutoPycnometer 1320 (Shimadzu, Japan). After properly preparing the sample with heat and/or vacuum, helium gas fills the sample chamber and the pressure was measured. The helium then enters another empty chamber and the pressure in both chambers was measured. The sample volume was calculated based on these pressures and then with the known weight of the sample, the density was calculated.

3. Results and discussion

3.1. Polymorphic transition of DCA in the presence of supercritical CO_2

Fig. 1 shows the changes in powder XRD patterns of DCA crystals after the storage with supercritical carbon dioxide (SC–CO_2) at 60 °C, 12 MPa for definite intervals. Characteristic diffraction peaks of intact DCA crystals were observed at $2\theta = 13.5°$ and 14.9° indicated as closed triangles. After the storage with SC–CO_2 for 1 h, new diffraction peaks, not found in the powder XRD patterns of DCA crystals, were observed at $2\theta = 7.4°$, 9.7° and 14.0° indicated as closed stars. The intensities of new diffraction peaks increased with storage time, whereas the intensities of intact DCA crystals decreased. This result suggests that bulk DCA crystals transformed to another crystal form in SC–CO_2 atmosphere and the polymorphic transformation had finished almost completely by SC–CO_2 treatment for 6 h. Since the existence of the polymorphism has not been reported for the DCA crystals, we propose the possibility to produce a new polymorph by SC–CO_2 processing.

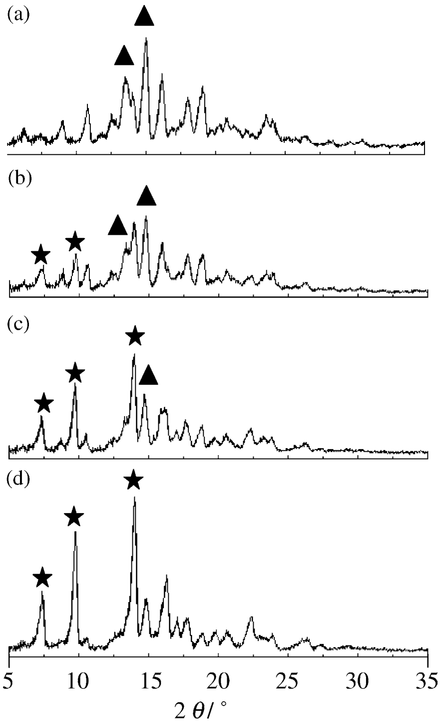

Fig. 1. Changes in powder X-ray diffraction (XRD) patterns of deoxycholic acid (DCA): (a) DCA crystals (intact), (b) DCA crystals after storage with supercritical carbon dioxide (SC–CO_2) at 60 °C, 12 MPa for 1 h, (c) DCA crystals after storage with SC–CO_2 at 60 °C, 12 MPa for 3 h, (d) DCA crystals after storage with SC–CO_2 at 60 °C, 12 MPa for 6 h.

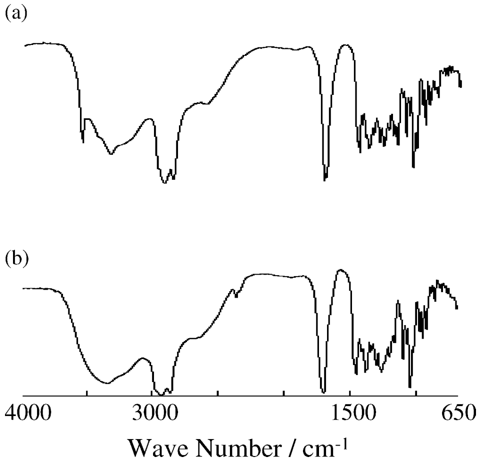

Fig. 2. FT-IR spectra of (a) intact DCA crystals and (b) DCA crystals after storage with SC–CO_2 at 60 °C, 12 MPa for 6 h.

To investigate a difference in molecular state between the intact DCA crystals and the new crystal form of DCA after SC–CO_2 processing, FT-IR spectroscopy was carried out, and the results are shown in Fig. 2. In the spectrum of intact DCA crystals, DCA exhibits a sharp peak due to free O–H stretching vibrations at 3552 cm^{-1}. For the new crystals of DCA, the O–H stretching band at 3552 cm^{-1} disappeared. It was reported that DCA possesses an ability to form complexes with many guest compounds and DCA crystal structures were divided into three groups: orthorhombic, tetragonal and hexagonal (Giglio, 1981). For the IR spectra of DCA–guest complexes, the peak derived from the free O–H stretching vibration of DCA disappeared in the DCA–menadione complex (Limmatvapirat et al., 1997) and that was shifted to 3552 cm^{-1} in the DCA–salicylic acid complex (Limmatvapirat et al., 1998). Since the DCA has a flexibility to form various crystal structures, the molecular interaction mode of OH groups in the new crystals might be different from those of intact DCA crystals. The DSC curves of the intact DCA crystals and its processed sample in SC–CO_2 are shown in Fig. 3. The intact DCA crystals demonstrated only the fusion

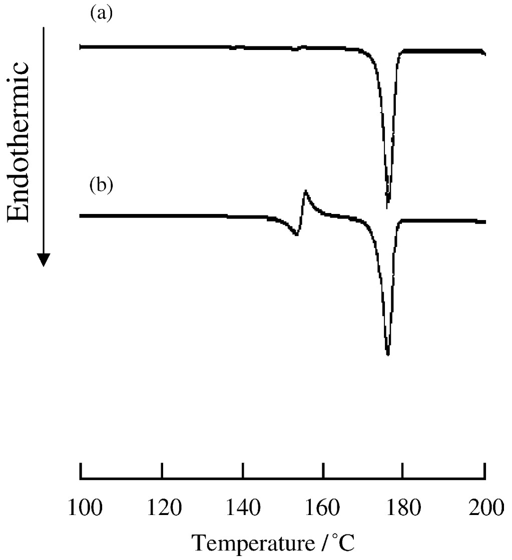

Fig. 3. DSC thermograms of (a) intact DCA crystals and (b) DCA crystals after storage with SC–CO$_2$ at 60 °C, 12 MPa for 6 h.

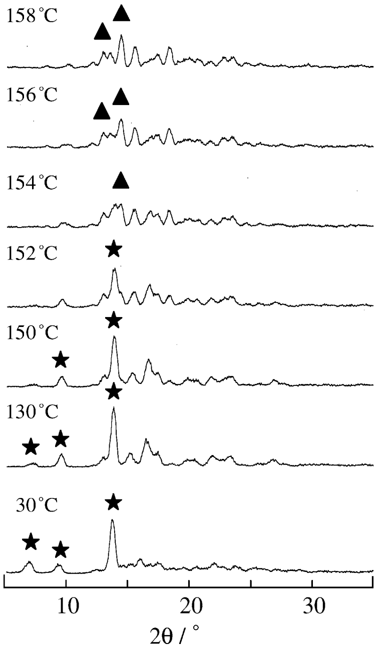

Fig. 4. Changes in powder XRD patterns of the treated sample of DCA by heating from 30 to 158 °C.

peak at 175 °C, whereas the sample after SC–CO$_2$ treatment showed a small endothermic peak and an exothermic peak at around 155 °C followed by the fusion peak at 175 °C. In order to elucidate the exothermic peaks at 156 °C, temperature controlled XRD was carried out. Changes in XRD pattern of the new form of DCA crystals when temperature was raised up to 158 °C are shown in Fig. 4. A decrease in the intensity of characteristic X-ray diffraction peaks of the new crystal form was found with an increase in heating temperature, and finally these peaks disappeared after heating at 152 °C. When the new form of DCA was heated at 152 °C, characteristic X-ray diffraction peaks of intact DCA appeared in the XRD pattern. The intensity of these peaks gradually increased as temperature increased. Since the diffraction pattern after heating at 158 °C was closely similar to that of intact DCA, the exothermic peak in Fig. 3 indicated a transformation from the new crystal form to the stable form of intact DCA. From the density measurement, the density for intact DCA crystals and the new form was calculated as 1.146 and 1.118 g/cm^3, respectively. If a polymorph has a lower density than another polymorph at room temperature, it maybe assumed that at absolute zero this form is metastable, because the energetically most favorable packing of molecules in a crystal has the strongest interactions between the molecules and hence the greatest density (Grunenberg et al., 1996). Therefore, the new crystal form of DCA, a crystal form after treatment with SC–CO$_2$, was considered to be a metastable form of DCA crystals.

In order to clarify the generation mechanism of the new polymorph by an SC–CO$_2$ processing, it should be considered the difference in solubility of both intact DCA crystals and the new crystal form of DCA in SC–CO$_2$. With a view to compare the solubility of two polymorphs of carbamazepine in supercritical dioxide, Bettini et al. (2001) showed that Form I and Form III of carbamazepine have different solubility in SC–CO$_2$ at 55 °C above 300 bar, and they reported that when the equimolar mixture of Form I and Form III of carbamazepine was treated under the previous condition, Form III was predominantly obtained due to a conversion of Form I via solubilization in SC–CO$_2$ followed by recrystallization of the less soluble polymorph. If the solubility of new form of DCA in SC–CO$_2$ was lower than that of intact DCA, it would be possible to discuss about the experimental result without contradiction. When the transformation from intact DCA to the metastable polymorphic form occurred during the processing with SC–CO$_2$, the polymorphic form would be generated by a nucleation from intact DCA dissolved in the supercritical carbon dioxide, followed by a crystal growth of the new form.

3.2. Crystallization behavior of amorphous DCA in supercritical fluids

Since an amorphous state is energetically unstable than crystals, it is known that crystallization of drugs from amorphous state is often caused by moisture and/or temperature (Andronis and Zografi, 2000). We showed a possibility that DCA was able to take two kinds of crystal forms in the preceding section. It would be interesting to investigate the changes in physicochemical properties of DCA amorphous when it was treated in SC–CO_2. An amorphous sample of DCA was obtained by 30 min grinding with vibrational mill and was stored with SC–CO_2 at 60 °C, 12 MPa for definite intervals. Fig. 5 shows the changes in powder XRD patterns of amorphous and crystal DCAs after the storage with SC–CO_2. The treatment for 5 min caused the change in X-ray diffraction pattern, which was due to the crystallization of the new polymorphic form of DCA. The intensity of these diffraction peaks remained unchanged though the processing time was extended for 30 min. It was noteworthy that crystallization from amorphous DCA to a new crystal form took place during a short processing time in the SC–CO_2. In the intact DCA system, the X-ray diffraction peaks derived from a new crystal form was hardly observed after the processing for a short period.

For a further understanding of this phenomenon, it is important to know the interaction mode between DCA molecules and SC–CO_2. For this purpose, processing by using supercritical nitrogen (SC–N_2) was performed for amorphous and crystalline DCAs. Liquid nitrogen in appropriate amount was enclosed in the stainless steel vessel and kept in a water bath at 60 °C so as to make a SC–N_2 atmosphere. Fig. 6 shows the change in powder XRD patterns of amorphous DCA after storage for 6 h in SC–N_2 atmosphere. Crystallization from the amorphous sample did not occur in SC–N_2 even after the processing for 6 h. It should be reasonable to consider that the crystallization from amorphous to the new crystal form did not happen only by pressure and the temperature conditions but was caused by the interaction of SC–CO_2 and DCA molecules.

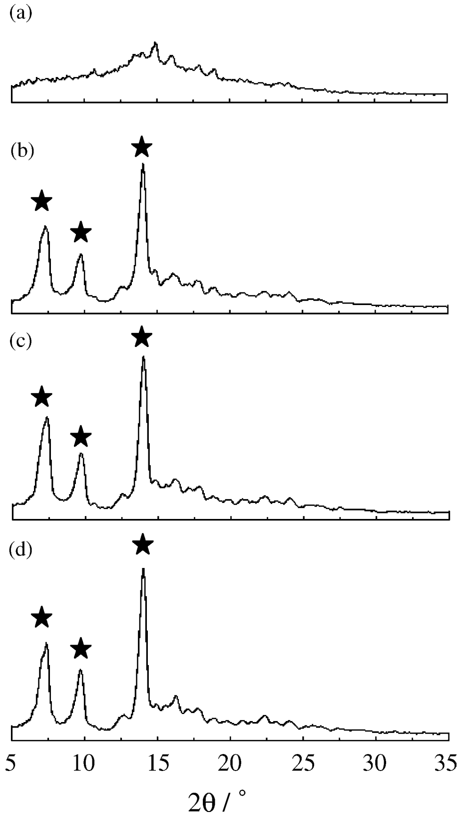

Fig. 5. Changes in powder XRD patterns of amorphous DCA after SC–CO_2 processing at 60 °C, 12 MPa for different intervals: (a) 30 min ground DCA, (b) stored in SC–CO_2 for 5 min, (c) stored in SC–CO_2 for 15 min, (d) stored in SC–CO_2 for 30 min.

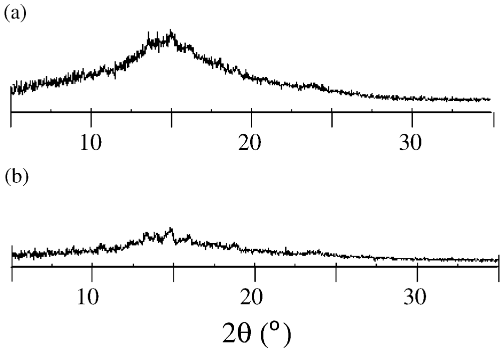

Fig. 6. Changes in powder XRD patterns of after supercritical nitrogen processing at 60 °C (a) amorphous DCA, (b) after supercritical nitrogen processing of (a) at 60 °C, 12 MPa for 6 h.

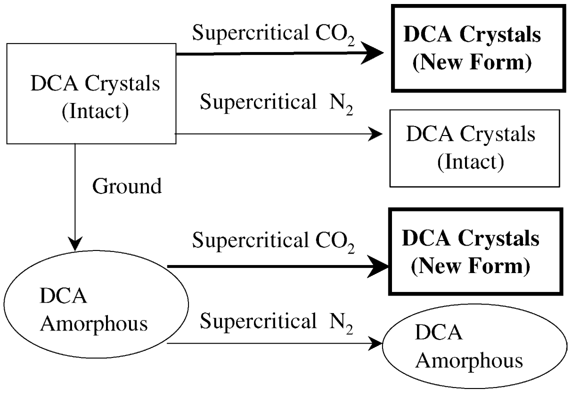

Fig. 7. Schematic representation of the changes in DCA crystals after treatment with different supercritical fluids.

Fig. 7 shows the schematic diagram of the consequence of this experiment. In conclusion, a new crystal form of DCA was obtained either in the DCA crystals or in the amorphous DCA systems after treatment with SC–CO_2 at 12 MPa, 60 °C, whereas no significant phase change of DCA was found for the treatment with SC–N_2 at 12 MPa, 60 °C. With respect to polymorphic interconversions, we should consider a possibility of solid–solid physical transformation of DCA crystals at the high-pressure condition. However, the processing in SC–N_2 (12 MPa, 60 °C) did not cause a polymorphic transition of DCA crystals; a solution-mediated physical transformation among polymorphs and/or a crystallization of the new form from intact DCA dissolved in the SC–CO_2 might be a plausible hypothesis for understanding these results in SC–CO_2. Polymorphism is one of the major problem in the pharmaceutical industry because different polymorphs exhibit significantly different physicochemical properties. The recent developments in computational chemistry allow the prediction of possible polymorphic forms based on the molecular structure of drugs (Paine et al., 1999). However, a predicted polymorph by using molecular simulations could not always be obtained by solvent method; a processing in a supercritical fluid would be one of the useful method for obtaining an unknown polymorph.

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