Available online at www.sciencedirect.com
SCIENCE DIRECT®

International Journal of Pharmaceutics 253 (2003) 81–88

international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Differentiated thermal crystallization from amorphous chenodeoxycholic acid between the ground specimens derived from the polymorphs

Toshio Oguchi [a,b], Nahomi Sasaki [a], Tetuya Hara [a],
Yuichi Tozuka [a], Keiji Yamamoto [a,*]

[a] *Graduate School of Pharmaceutical Sciences, Chiba University, 1-33 Yayoicho,*
Inage-ku, Chiba 263-8522, Japan
[b] *Department of Pharmacy, Faculty of Medicine, University Hospital,*
University of Yamanashi, 1110 Shimokato, Tamaho-cho,
Nakakoma-gun, Yamanashi 409-3898, Japan

Received 27 May 2002; received in revised form 2 November 2002; accepted 29 November 2002

Abstract

Crystallization behavior of amorphous chenodeoxycholic acid (CDCA) was studied using X-ray diffraction (XRD), infrared (IR) spectroscopy and differential scanning calorimetry (DSC). The two polymorphs of CDCA, *form I* (mp 166 °C) and *form III* (mp 119 °C), were ground with a vibrational mill. The ground samples of both crystal forms showed halo X-ray diffraction patterns. DSC curves of the amorphous samples derived from the form I and form III showed exothermic peaks due to the crystallization to the form I at 120 and 147 °C, respectively. When the ground *form III* was mixed with the ground *form I*, the crystallization temperature shifted to a lower temperature as the content of the ground *form I* increased. In the case of co-ground sample of *form I* and *form III*, the crystallization to *form I* crystals proceeded by two different modes. These results indicate that the physicochemical state was different among the ground samples of the *form I* and *form III*, and that the crystal nuclei played an important role on the crystallization process of the amorphous CDCA.

Keywords: Chenodeoxycholic acid; Amorphous; Grinding; Crystallization; Polymorph

1. Introduction

Since dissolution properties of organic drug powders usually determine the rate of gastrointestinal absorption, the poor solubility of a drug may result in low bioavailability after oral administration. Many novel drugs developed recently were found to have low aqueous solubility (Serajuddin, 1999). Thus, the enhancement of drug solubility is often an important step to be considered in pharmaceutical manufacturing. Amorphization had been attempted to improve drug solubility together with other methods such as solid dispersion using water-soluble polymers (Chutimaworapan et al., 2001), adsorption onto porous materials (Tozuka et al., 2000), complexation with cyclodextrins (Latrofa et al., 2001), micronization (Reverchon et al., 2001). Amorphous solids can commonly be prepared by condensation

---

[*] Corresponding author. Tel.: +81-43-290-2937; fax: +81-43-290-2939.
E-mail address: yamamotk@p.chiba-u.ac.jp (K. Yamamoto).

0378-5173/02/$ – see front matter © 2002 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0378-5173(02)00675-0

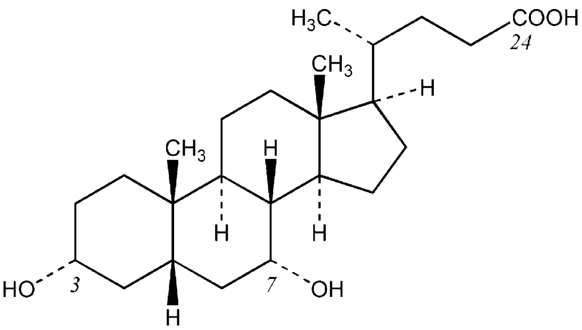

Fig. 1. Chemical structure of chenodeoxycholic acid (CDCA).

from the vapor state, quenching of the melt (Lu and Zografi, 1997), mechanical milling of a crystalline mass (Ohta et al., 2000), freeze-drying and spray drying (Ueno et al., 1998). Amorphous solids of ursodeoxycholic acid (UDCA) prepared by melt quenching and milling methods showed a different thermal behavior (Yonemochi et al., 1997).

Chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholanoic acid, CDCA, Fig. 1) is a 7α-hydroxyl epimer of UDCA and has been used as a cholesterol gallstone dissolving agent. The dissolution rate of cholesterol increased proportionally with the increased concentration of CDCA (Tateyama and Matsushiro, 1981) and therefore the solubility of CDCA can be considered as an important factor to control the dissolution rate of cholesterol. Guiseppetti and Paciotti (1978) reported the three polymorphic forms of CDCA, *form I*, *form II* and *form III*, whose melting points were 168, 138 and 119 °C, respectively. The stable polymorph is *form I*. *Form III*, a low melting point polymorph, was obtained by crystallization of CDCA from a chloroform solution followed by the removal of chloroform from solvate crystals and should be regarded as a “desolvated solvate” (Stephenson et al., 1994). The crystal structures were determined for *form I* (Lindley et al., 1980) and *form III* (Rizkallah et al., 1990) polymorphs but no information could be found for *form II* polymorph.

There are only a few reported studies on the characteristics of amorphized polymorph drugs prepared by the grinding method (Crowley and Zografi, 2002). In this paper, we report the characteristics and crystallization behavior of two amorphous CDCA forms prepared by the grinding method.

2. Materials and methods

2.1. Materials

Chenodeoxycholic acid (CDCA) of *form I* crystals (mp 165–166 °C) and *form III* crystals (mp 119 °C) were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Nacalai Tesque (Kyoto, Japan), respectively. All chemicals were of analytical reagent grade and used without further purification.

2.2. Preparation of amorphous samples

Amorphous CDCA samples were prepared by grinding 4.0 g of CDCA with a TI-200 vibrational rod mill (CMT Co., Japan) for 45 min. The ground *form I* and ground *form III* were physically mixed at various weight ratios to prepare different amorphous mixtures. For the preparation of the co-ground samples, the CDCA crystals were mixed at various weight ratios and then ground using the vibrational rod mill.

2.3. Powder X-ray diffraction

Powder X-ray diffraction patterns were determined with Cu Kα radiation at ambient temperature using a Rigaku Miniflex diffractometer (Tokyo, Japan). The measurement conditions were as follows: voltage 30 kV, current 15 mA, scanning speed 4° min^{-1} and range (2θ) 4–40°.

2.4. Temperature-controlled powder X-ray diffraction

Powder X-ray diffraction patterns were determined with Cu Kα radiation using a Philips X’Pert-MPD PW3050 diffractometer (Tokyo, Japan) with a TKK model type temperature controllable attachment. Measurements were preformed after maintaining the sample for 1 min at each temperature. The measurement conditions were as follows: voltage 40 kV, current 50 mA, scanning speed 0.08° s^{-1}, heating rate 2 °C min^{-1} and range (2θ) 4–40°.

2.5. Infrared spectroscopy

Infrared (IR) spectra were determined using JASCO 230 FT-IR spectrometer (Tokyo, Japan). The measurements were carried out using the KBr method.

2.6. Differential scanning calorimetry (DSC)

A Perkin-Elmer Pyris 1 differential scanning calorimeter (Connecticut, USA) was used. A sample, accurately weighed, of approximately 3 mg was loaded in a crimped aluminum pan and measured at a heating rate of 2 °C min^{-1} under nitrogen gas flow (20 ml min^{-1}).

3. Results and discussion

The two polymorphs of CDCA, *form I* and *form III*, were individually ground for 45 min. Fig. 2 shows the powder X-ray diffraction patterns of the unprocessed and ground samples for each CDCA polymorph. With regard to the unprocessed samples, clear differences in the diffraction patterns were observed between the *form I* and *form III*. When the *form I* crystals were ground for 15 min, the diffraction patterns revealed a halo pattern. In the case of the *form III* crystals, small X-ray diffraction peaks were observed even after the grinding for 15 min. After grinding for 45 min, these X-ray diffraction peaks of crystals disappeared and the diffraction patterns showed a broad hump at around $2\theta = 15°$. These results indicate that CDCA was changed from the crystalline state into an amorphous state by the grinding process.

To investigate the molecular state of the unprocessed and ground CDCAs, FT-IR spectroscopy was carried out, and the results are shown in Fig. 3. In the IR spectrum of the *form I* crystals, OH-stretching vibrations were observed as sharp bands at 3579, 3545 and 3393 cm^{-1} and a C=O stretching vibration band at 1714 cm^{-1}. In the spectrum of the *form III*, OH-stretching vibrations were observed at 3455 and 3305 cm^{-1} and C=O stretching vibrations at 1703 cm^{-1}. The crystal structures of *form I* and *form III* samples had been reported by Lindley et al. (1980) and Rizkallah et al. (1990), respectively. The

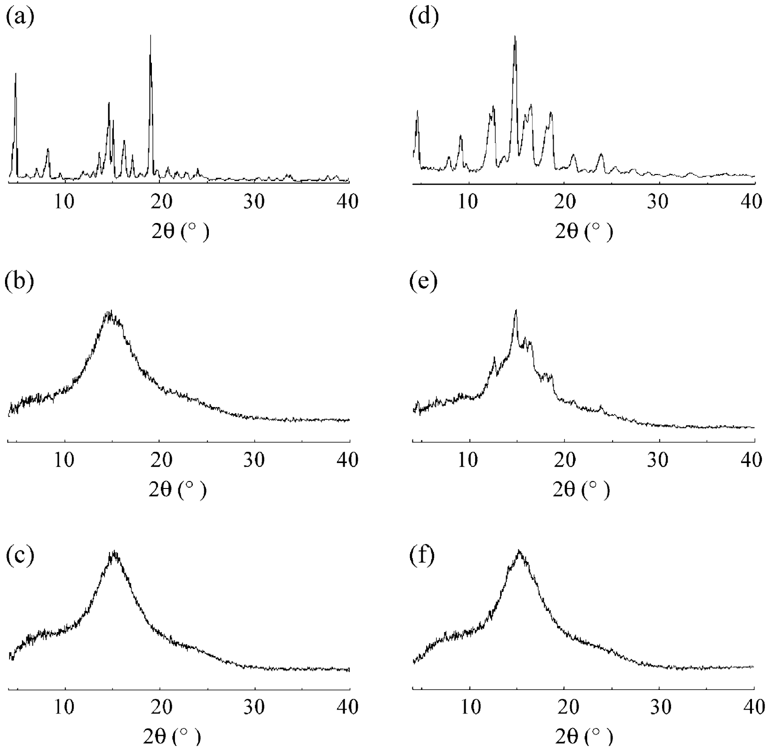

Fig. 2. Powder X-ray diffraction (XRD) patterns of the intact and ground CDCA. (a) *Form I* crystals, (b) ground sample of *form I* (ground for 15 min), (c) ground sample of *form I* (ground for 45 min), (d) *form III* crystals, (e) ground sample of *form III* (ground for 15 min), (f) ground sample of *form III* (ground for 45 min).

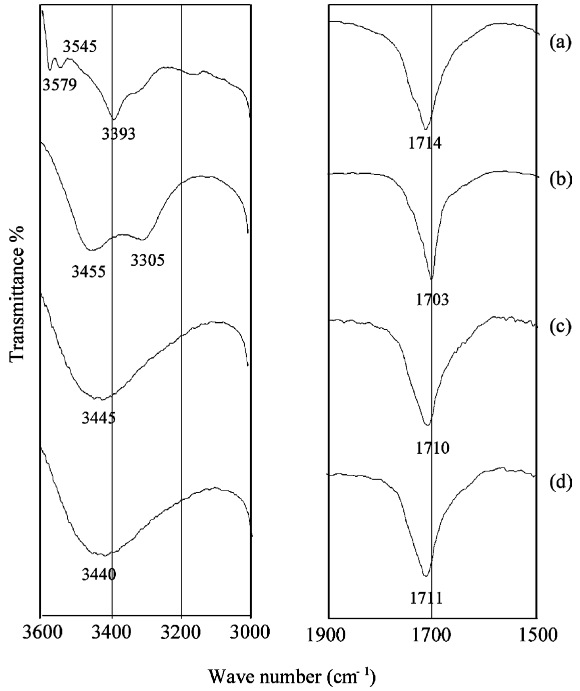

Fig. 3. IR spectra of the intact and ground CDCA. (a) *Form I* crystals, (b) *form III* crystals, (c) ground sample of *form I* (ground for 45 min), (d) ground sample of *form III* (ground for 45 min).

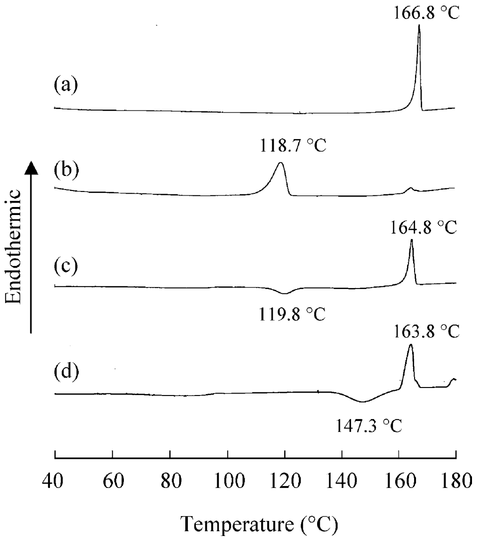

Fig. 4. Differential scanning calorimetry (DSC) curves of intact and ground CDCA (heating rate; 2 °C min^{-1}). (a) *Form I* crystals, (b) *form III* crystals, (c) ground sample of *form I* (ground for 45 min), (d) ground sample of *form III* (ground for 45 min).

form I crystals contained two non-equivalent CDCA molecules of A and B in the crystal lattice and these molecules were approximately perpendicular to one another. Hydrogen bonds existed between adjacent molecules A, between adjacent molecules B and also between molecules of A and B (Lindley et al., 1980). In contrast, for case of *form III* crystals, the hydrogen bond network was not as well developed as that in *form I* crystals (Rizkallah et al., 1990). The differences in IR spectra between *form I* and *form III* crystals were considered to reflect such hydrogen-bonding modes in their respective crystal structures. With regard to the ground samples of the *form I* and of *form III*, the sharp OH-stretching vibration peaks disappeared and a new broad band appeared around 3445 cm^{-1}. There was no significant difference between the ground samples.

The DSC curves of the *form I* and *form III* crystals and their 45 min ground samples are shown in Fig. 4. The *form I* crystals showed an endothermic peak due to melting at 166.8 °C, while the *form III* crystals showed an endothermic peak at 118.7 °C. Rizkallah et al. (1990) reported that the structure of the *form I* crystals was like a double sheet structure with well-packing, while the *form III* crystals bore a hexagonal canal-type structure with channels (Rizkallah et al., 1990). The crystal densities were reported as 1.16 and 0.89 g cm^{-3} for *form I* and *form III* crystals, respectively. This significant difference in the crystal structure could explain the large difference in the melting points of the two polymorphs. On the other hand, the ground sample of *form I* crystals showed an exothermic peak at about 120 °C as well as an endothermic peak at 164.8 °C, while the ground *form III* showed exothermic and endothermic peaks at about 147 and 164 °C, respectively. Clearly, DSC measurements differentiated the amorphous samples originated in *form I* and *form III* crystals although no significant difference was observed in powder X-ray diffraction nor IR spectroscopy between the two amorphous samples as described above.

To investigate the exothermic peaks observed in the amorphous samples, temperature-controlled powder X-ray diffraction was employed. The results for the ground samples derived from *form I* and *form III* crystals are shown in Figs. 5 and 6, respectively. After the ground samples of *form I* and *form III* crystals

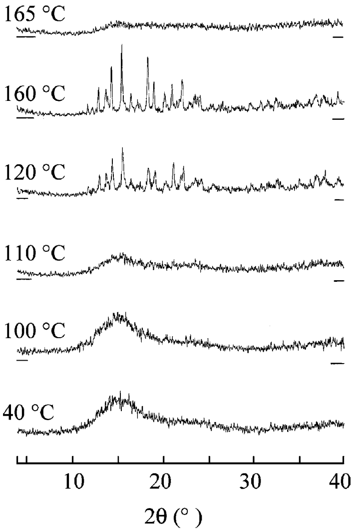

Fig. 5. Powder XRD patterns of the ground sample of the *form I* (ground for 45 min) measured at various temperatures.

were heated to the temperature at which each exothermic peak was observed, the powder X-ray diffraction patterns showed identical patterns to that of *form I* crystals ($2\theta = 17.1, 19.0°$). This indicated that the exothermic peaks observed on the DSC curves of both ground samples (at 119.9 and 147.3 °C for the ground *form I* and the ground *form III*, respectively) were due to the crystallization of amorphous CDCAs to the *form I* crystals. The endothermic peak which appeared around 164 °C was considered to be due to the melting of the *form I* crystals.

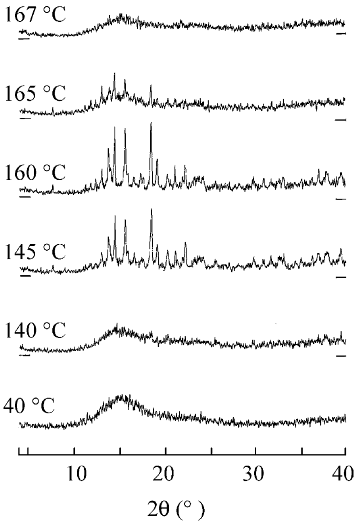

Fig. 6. Powder XRD patterns of the ground sample of the *form III* (ground for 45 min) measured at various temperatures.

Yonemochi et al. (1999a) reported that the amorphous samples prepared by the grinding process caused the loss of the long range order of molecular arrangements but from microscopic observations, crystal-like ordering still remained even after grinding. Therefore, it should be reasonable to consider that the ground *form I* and ground *form III* included crystalline nuclei which were too small to be detectable by powder X-ray diffraction. In the case of the ground *form I*, the crystal nuclei acted as seeds and promoted crystallization to *form I* crystals at around 120 °C. This result also suggests that CDCA molecules acquired mobility for crystal growth when the temperature reached 120 °C. In case of the ground *form III*, potential nuclei of the *form III* crystals could exist in the ground sample and the necessary temperature for the crystal growth could be around 120 °C as well. The crystal nuclei should melt at a temperature below the melting point of *form III* crystals (118 °C) and thus, promotion of the crystallization of *form III* crystals no longer existed at 120 °C. Alternatively, when *form III* crystals was in a desolvated solvate state, it may be more appropriate to consider that the crystal structure of *form III* could not be developed by thermal activation in the solid state. The crystallization into *form III* crystals could not take place at 120 °C and the amorphous state lasted until the nucleation process for the stable *form I* crystals occurred at a higher temperature of 147 °C accompanied by the prompt crystallization to the *form I* crystals.

In addition, the physical mixture of the two ground samples derived from *form I* and *form III* crystals, which will be referred as “amorphous mixtures” hereafter, and the co-ground samples were prepared and characterized by thermal analysis. Fig. 7 shows the DSC curves for the amorphous mixtures at various weight ratios of ground *form I* and ground *form III* crystals. The amorphous mixtures showed a single exothermic peak due to crystallization to *form I* crystals. When 1% of the ground *form I* was added to ground *form III*, the exothermic peak shifted from 147 to 138 °C (a, b). With the increase of the ground *form I* content, the exothermic peak shifted to a lower temperature (c, d). In the case of amorphous mixtures including small amount of ground *form I*, the crystal

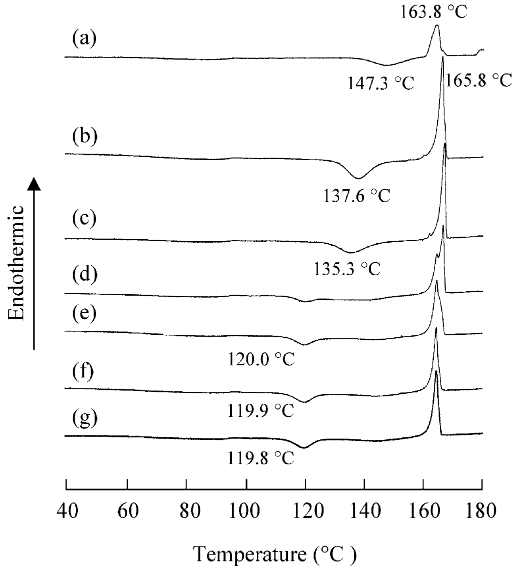

Fig. 7. DSC curves of physical mixture of ground *form I* and ground *form III* of CDCA: mixing weight ratio of *form III*/*form I*. (a) 100/0, (b) 99/1, (c) 95/5, (d) 50/50, (e) 20/80, (f) 1/99, (g) 0/100.

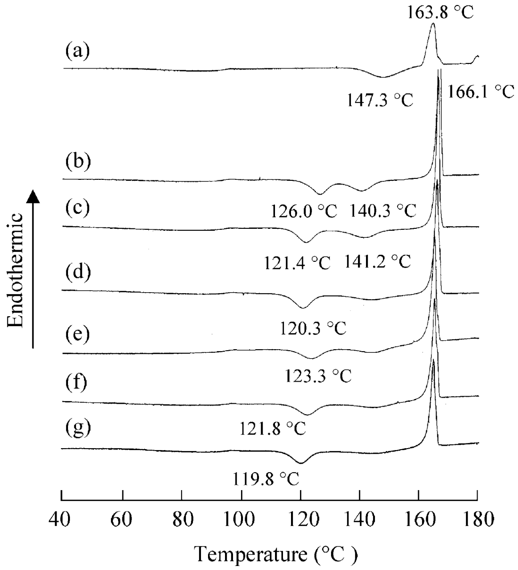

Fig. 8. DSC curves of co-ground sample of the *form I* and *form III*: mixing weight ratio of *form III*/*form I*. (a) 100/0, (b) 99/1, (c) 95/5, (d) 50/50, (e) 5/95, (f) 1/99, (g) 0/100.

nuclei of *form I* crystals were assumed to promote crystallization of the ground *form III* to *form I* crystals. Similar phenomenon was observed when 1% of *form I* crystals was added to ground *form III*. That is, the exothermic peak due to the crystallization of ground *form III* to *form I* crystals was observed at 136 °C which was lower enough compared to intact ground *form III* crystallization temperature.

Fig. 8 shows the DSC curves of the co-ground samples prepared at various weight ratios of *form I*/*form III*. Amorphization in all the co-ground samples was confirmed by powder X-ray diffraction. When 99–95% of the *form III* crystals were co-ground with 1–5% of *form I* crystals, two exothermic peaks at ca. 120 and 140 °C were observed on the DSC curves (b, c). The exothermic peak at 140 °C became small as the content of *form I* crystals increased. In order to characterize the two exothermic peaks observed at a low mixing ratio of *form I*/*form III*, temperature-controlled powder X-ray diffraction was carried out for the ground mixture of a mixing ratio of 5:95 (*form I*:*form III*). The results are shown in Fig. 9. The powder X-ray diffraction pattern indicates that crystallization of the *form I* crystals when heat treatment at 120 °C was attempted. It should be noted that diffraction

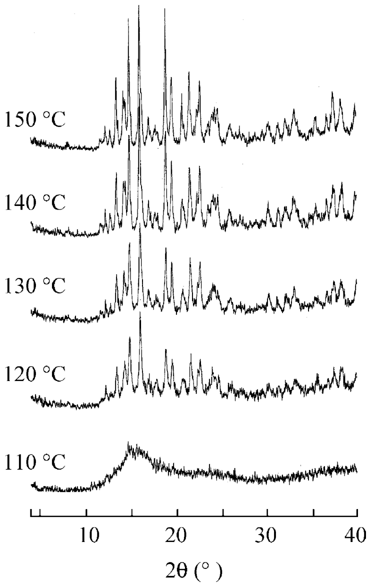

Fig. 9. Powder XRD patterns of the ground sample of the ground mixtures (ground for 45 min) measured at various temperatures: mixing weight ratio of *form III*/*form I* = 95/5.

peaks due to *form I* crystals increased in intensities when the temperature was increased to 140 °C. The results indicate that both the exothermic peaks on the DSC curves resulted from the crystallization of *form I* crystals. Interestingly, crystallization was found to proceed independently at different temperatures.

As described above, no significant difference between the amorphous mixture and co-ground sample was observed using powder X-ray diffractometry but differences were seen in DSC curves. In the case of amorphous mixtures, the existence of crystal nuclei of *form I* crystals did not induce the ground *form III* to crystallize as *form I* crystals at 120 °C. This could be explained by assuming that almost all fraction of the amorphous CDCA in the amorphous mixture is contacted with the *form I* nuclei not at the microscopic (molecular) level but only in the physical level. When the amorphous mixture was heated up to 135–140 °C, the ground *form III* would crystallize via the nucleation process of *form I* crystals which would be affected to some extent by the existence of *form I* nuclei even when not in contact. In the case of the co-ground samples, the microscopic contact should be achieved between the *form I* nuclei and amorphous CDCA. The crystallization to *form I* crystals was considered to occur by the two different modes. One mode was when the fraction of ground *form III* powder closely surrounded the *form I* crystal nuclei and was transformed by crystal growth to *form I* crystals at around 120 °C. The other mode was that the fraction of the ground *form III* did not crystallize at 120 °C but crystallized out at 140 °C without any influence of the *form I* crystal nuclei. Consequently, the two exothermic peaks were observed from the independent crystallization processes.

Previously, we found that the amorphous sample prepared by grinding showed two-peak exotherm of crystallization on the DSC thermogram (Yonemochi et al., 1999b), but the interpretation of the phenomena had not been adequately explained. The discussions in this paper provide a better understanding of this phenomena.

4. Conclusion

Physicochemical characteristics of amorphous CDCA samples derived from the two different crystal forms, *form I* and *form III* were compared. Both powder X-ray diffraction and FT-IR spectroscopy were not able to distinguish between ground *form I* from ground *form III* but significant differences in the crystallization behavior were observed when DSC thermograms were studied.

The thermal crystallization from amorphous CDCA to *form I* crystals was considered to proceed by two methods which were clearly distinguished by the crystallizing temperature. From the studies using amorphous mixtures and co-ground samples of the two polymorphs, we believed that these crystallization behaviors can be explained by assuming the following: (1) crystal growth in which the *form I* crystallites in the ground sample act as crystal nuclei, and (2) crystallization in which nucleation process is intrinsically necessary in the absence of the *form I* crystallites. The crystallization from the ground *form I* and ground *form III* were assumed to take place in the former and latter processes, respectively. The higher mobility of CDCA molecules is required for the nucleation process because of the activation energy to be overcome, in comparison with “spontaneous” crystal growth process. This proposed mechanism can also explain the difference in crystallization temperatures between ground *form I* and ground *form III*.

Acknowledgements

This work was supported in part by Grant-in Aid for Scientific Research (c) from the Ministry of Education, Culture, Sports, Science and Technology, Japan (12672085). We would like to thank Dr. Paul W.S. Heng for checking the English.

References

Chutimaworapan, S., Ritthidej, G.C., Oguchi, T., Yamamoto, K., 2001. Controlled release of nifedipine from coevaporates prepared with Eudragit and poloxamer. J. Pharm. Sci. Technol. Jpn. 61, 21–33.

Crowley, K.J., Zografi, G., 2002. Cryogenic grinding of indomethacin polymorphs and solvates: assessment of amorphous phase formation and amorphous phase physical stability. J. Pharm. Sci. 91, 492–507.

Guiseppetti, G., Paciotti, M., 1978. Polymorphism of chenodeoxycholic acid. Farmaco. Edu. Sci. 33, 64–72.

Latrofa, A., Trapani, G., Franco, M., Serra, M., Muggironi, M., Fanizzi, F.P., et al., 2001. Complexation of phenytoin with some hydrophilic cyclodextrins: effect on aqueous solubility, dissolution rate, and anticonvulsant activity in mice. Eur. J. Pharm. Biopharm. 52, 65–73.

Lindley, P.F., Mahmoud, M.M., Watson, F.E., Jones, W.A., 1980. The structure of chenodeoxycholic acid, $C_{24}H_{40}O_4$. Acta Cryst. B36, 1893–1897.

Lu, Q., Zografi, G., 1997. Properties of citric acid at the glass transition. J. Pharm. Sci. 86, 1374–1378.

Ohta, M., Tozuka, Y., Oguchi, T., Yamamoto, K., 2000. Evaluation of physicochemical stability of amorphous cefditoren pivoxil, using modulated-temperature differential scanning calorimetry. J. Pharm. Sci. Technol., Jpn. 60, 160–165.

Reverchon, E., Della Porta, G., Pallado, P., 2001. Supercritical antisolvent precipitation of salbutamol microparticles. Powder Technol. 114, 17–22.

Rizkallah, P.J., Harding, M.M., Lindley, P.F., Aigner, A., Bauer, A., 1990. Structure of a low-temperature polymorph of chenodeoxycholic acid, $C_{24}H_{40}O_4$, determined with synchrotron radiation. Acta Crystallogr. B46, 262–266.

Serajuddin, A.T.M., 1999. Solid dispersion of poorly water-soluble drugs: early promises, subsequent problems, and recent breakthroughs. J. Pharm. Sci. 88, 1058–1066.

Stephenson, G.A., Stowell, J.G., Toma, P.H., Dorman, D.E., Greene, J.R., Byrn, S.R., 1994. Solid-state analysis of polymorphic, isomorphic, and solvated forms of dirithromycin. J. Am. Chem. Soc. 116, 5766–5773.

Tateyama, T., Matsushiro, T., 1981. Bile acid composition affecting cholesterol dissolution rate: a use of multiple regression analysis. Tohoku J. Exp. Med. 133, 467–475.

Tozuka, Y., Yonemochi, E., Oguchi, T., Yamamoto, K., 2000. Molecular states of 2-naphthoic acid in solid dispersions with porous crystalline cellulose, as investigated by fluorescence spectroscopy. Bull. Chem. Soc. Jpn. 73, 1567–1572.

Ueno, Y., Yonemochi, E., Tozuka, Y., Yamamura, S., Oguchi, T., Yamamoto, K., 1998. Characterization of amorphous ursodeoxycholic acid prepared by spray-drying. J. Pharm. Pharmacol. 50, 1213–1219.

Yonemochi, E., Ueno, Y., Ohmae, T., Oguchi, T., Nakajima, S., Yamamoto, K., 1997. Evaluation of amorphous ursodeoxycholic acid by thermal methods. Pharm. Res. 14, 798–803.

Yonemochi, E., Inoue, Y., Buckton, G., Moffat, A., Oguchi, T., Yamamoto, K., 1999a. Differences in crystallization behavior between quenched and ground amorphous ursodeoxycholic acid. Pharm. Res. 16, 835–840.

Yonemochi, E., Kitahara, S., Maeda, S., Yamamura, S., Oguchi, T., Yamamoto, K., 1999b. Physicochemical properties of amorphous clarithromycin obtained by grinding and spray drying. Eur. J. Pharm. Sci. 7, 331–338.