Evaluation of Amorphous Ursodeoxycholic Acid by Thermal Methods

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Purpose. The purpose of this study was to characterize the amorphous state of ursodeoxycholic acid (UDCA) samples by using isothermal microcalorimetry, X-ray diffraction, infrared (IR) spectroscopy and solid state carbon 13 nuclear magnetic resonance (^{13}C-NMR) spectroscopy, and to demonstrate the application of the thermal methods (microcalorimetry and differential scanning calorimetry (DSC)) for studying the amorphous state and clarifying the dissolution mechanism of UDCA.

Methods. Amorphous UDCA was prepared by grinding and rapid cooling of the melts. The heat of solution of UDCA was measured by an isothermal heat-conduction twin microcalorimeter at 25.0°C. Some physicochemical properties of amorphous UDCA were also studied.

Results. The intensities of X-ray diffraction peaks of crystalline UDCA decreased with an increase in grinding time. The heat levels of solution of crystalline UDCA and UDCA ground for 1 min were endothermic, and became exothermic with an increase in grinding time. A good correlation was obtained between the heat of solution and the heat of crystallization determined from the peak area in DSC. Although no significant difference was observed in X-ray diffraction patterns of amorphous UDCA prepared by the two methods, significant differences were recognized in DSC, IR and ^{13}C-NMR, and the heat of solution indicated different values among the two samples. The stability of amorphous UDCA samples stored under 74.5% relative humidity at 40°C was found to depend upon the preparation methods.

Conclusions. Different states of amorphous UDCA were obtained depending on the preparation method. The application of thermal methods to evaluate the amorphous state was demonstrated. The mechanism of dissolution of UDCA was discussed from the results of the heat of solution examination.

KEY WORDS: ursodeoxycholic acid; amorphous; powder X-ray diffraction; thermal analysis; ^{13}C-NMR.

INTRODUCTION

Solid state properties of drug substances, namely, polymorphs, hydrates, solvates and crystallinity, influence the pharmaceutical properties, dissolution, chemical stability, tabletting and bioavailability of solid dosage forms (1). In general, drugs in solid dosage forms are used in their crystalline state. The amorphous state, however, has become of interest in preparing solid dosage forms because it can improve the drug's dissolution behavior. Egawa *et al.* (2) reported that amorphous cefalexin prepared by pulverization and lyophilization showed different dissolution behaviors. Amorphous tri-O-methyl-β-cycrodextrin prepared by pulverization and rapid cooling of the melts showed different IR patterns and DSC thermograms (3). According to Yamaguchi *et al.* (4), the amorphous 16-membered macrolide compound prepared by different conditions of spray drying showed different dissolution behavior and physicochemical stability. It is thus important to study the physicochemical properties of a drug's amorphous state, prepared by different methods. Various methods are employed to study the amorphous state, such as powder X-ray diffraction, differential scanning calorimetry (DSC), infrared (IR) spectroscopy, photoelectron spectroscopy and dissolution testing. Isothermal calorimetry is commonly used to measure with accuracy and precision, the heat evolved or absorbed by a chemical or physical process (5–9). It is also used to study the inclusion phenomenon of cyclodextrins (10). Pikal *et al.* indicated that the heat of solution was a precise unambiguous measure of the relative crystallinity of β-lactam antibiotics (11). As little as 1% amorphous content in a powder could be discerned using microcalorimetry (12). Sebhatu *et al.* also indicated that only microcalorimetry was able to measure the disorder with high accuracy at lower degrees of disorder (below 10% disorder) (13). It was suggested in a study of albuterol sulfate micronized with air jet micronization that the differences in the solution enthalpy between micronized and unmicronized albuterol sulfate determined by microcalorimetry were probably related to the surface energy (14). The use of microcalorimetry for studying solid state properties appears to be useful when DSC or powder X-ray diffraction cannot distinguish the properties (11–17).

Ursodeoxycholic acid (UDCA) is administered to dissolve cholesterol gallstones (18–19). We have already reported that UDCA was amorphized by grinding and that amorphous UDCA had a faster dissolution rate (20). The purpose of the present study was to characterize the amorphous state of UDCA samples, which have different grinding times or are prepared by different methods, by determining the heat of solution and powder X-ray diffraction patterns, and by using DSC thermograms, IR spectra and solid state carbon 13 nuclear magnetic resonance (^{13}C-NMR). The stability of the amorphous state of UDCA was also studied.

MATERIALS AND METHODS

Materials

UDCA was of JPXIII grade, supplied by Tokyo Tanabe Co., Ltd. (Tokyo, Japan). Ethanol was of analytical reagent grade.

Preparation of Amorphous Solid

For grinding of UDCA, a Heiko Seisakusho model TI-200 vibration mill was used. The cell was made of aluminum oxide. The total weight of the specimen was 2.0 g. For preparing the quenching sample, 2.0 g UDCA was placed in glass tube and kept at 210°C to melt, then cooled rapidly by using liquid

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nitrogen. Solidified UDCA was pulverized using a mortar and pestle, then stored in a reduced pressure desiccator at room temperature.

X-Ray Diffraction Measurement

The X-ray powder diffraction patterns were obtained from a 2027 diffractometer (Rigaku Denki, Tokyo, Japan) using a scintillation counter, a Cu target X-ray tube with Ni filter (30 kV, 5 mA), and a symmetrical reflection goniometer scanned at 4°/min.

Determination of Heat of Solution

The heats of solution of UDCA samples were determined by using an isothermal heat-conduction twin microcalorimeter (model MPC-11; Tokyo Riko, Tokyo, Japan) at 25.0 ± 5 × 10^{-3}°C. The microcalorimeter had a break-ampoule-type attachment. The attachment had two vessels in which an ampoule was placed. A calibration heater was wrapped around the outer surface of the vessel holder at a sample side. Ethanol containing 4.0% distilled water was used as a dissolution medium, because UDCA is slightly soluble in water.

The particle size of UDCA powder was regulated between 125 and 150 μm. Two hundred mg of UDCA powder was placed in a glass ampoule and dried over phosphorus pentoxide under reduced pressure at 40°C for 24 h; the ampoule was then sealed. The glass ampoule was placed in one vessel of the break-ampoule-type attachment. Twenty ml of the dissolution medium was previously added to the vessel. The vacant ampoule, used as a reference, was placed in the other vessel, which had been filled with 20.0 ml dissolution medium. The attachment was placed in the calorimeter and was thermally equilibrated for 12h. After equilibrium was established, the ampoules were simultaneously broken to dissolve UDCA powder with a mixing rate of 50 rpm. The heat flow signal was monitored as a function of time. By integrating the heat flow curve over a specific time interval (3h), the heat evolved or absorbed was obtained. Exothermic signals were given negative values. Calibration was performed at each time of determination. The calibration data were obtained by electrical heating at a known power for different periods. The amount of the heat of solution of UDCA was calculated from the calibration curve.

Thermal Analysis

A differential scanning calorimeter (DSC), model TA9900 (Du Pont, New Castle, DE, USA), was used under a N_2 gas flow (60 ml/min) at a heating rate of 5°C/min.

Infrared (IR) Spectroscopy

A Nicolet model 5ZDX Fourier transformed IR spectrophotometer was used. Measurements were carried out by KBr disk method.

Solid State ^{13}C-NMR spectroscopy

A Chemagnetics model CMX-360 solid state NMR spectrometer was used. Hexamethylbenzene was used as an external standard.

Physicochemical Stability Study of Amorphous UDCA

One hundred and fifty mg of amorphous UDCA samples were stored in a desiccator under 74.5% relative humidity (RH) at 40°C. The powder X-ray diffraction patterns were determined at regular time intervals.

RESULTS AND DISCUSSION

Effects of Grinding on the Heat of Solution of UDCA

Thermograms for the dissolution of crystalline UDCA and ground UDCA samples are shown in Fig. 1. The heat of solution was found to change from endothermic to exothermic with an increase in grinding time. The endothermic heat of solution was 14.3 J/g for the crystalline UDCA and 7.3 J/g for the UDCA sample ground for 1 min. The exothermic heat of solution was −9.6, −16.3 and −22.4 J/g for UDCA ground for 3, 10 and 30 min, respectively. Figure 1 shows the trend in the change of the heat of solution, i.e., an abrupt change of the heat with the destruction of the crystal structure.

The heat of crystallization, as determined from the peak area in the DSC curves, was −3.7, −12.0, −18.5 and −19.7 J/g for UDCA ground for 1, 3, 10 and 30 min, respectively. The relationship between the heat of solution determined from microcalorimeter and the heat of crystallization determined by DSC is illustrated in Fig. 2. A linear correlation was observed between the heat of solution and the heat of crystallization of UDCA, with a correlation coefficient of 0.993. It is well known that there is a relationship between the crystallinity and the heat of crystallization, and that the lower the crystallinity, the larger the exothermic peak area on DSC curves (the heat of crystallization). The correlation between the heat of solution and the heat of crystallization suggests that it is possible to presume crystallinity and energy state from the heat of solution.

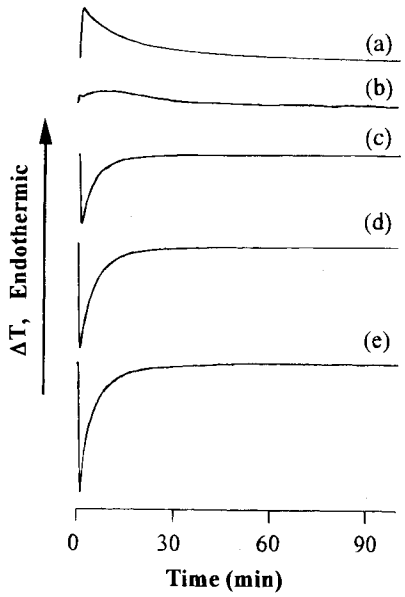


Fig. 1. Thermograms for dissolution of crystalline and ground ursodeoxycholic acid samples into ethanol containing 4.0% distilled water at 25.0°C. (a) Crystalline, (b) ground for 1 min, (c) ground for 3 min, (d) ground for 10 min, (e) ground for 30 min.

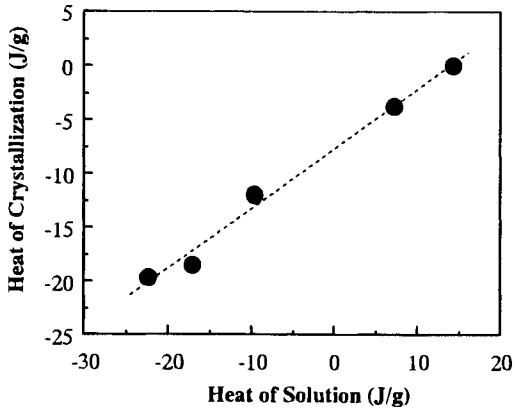
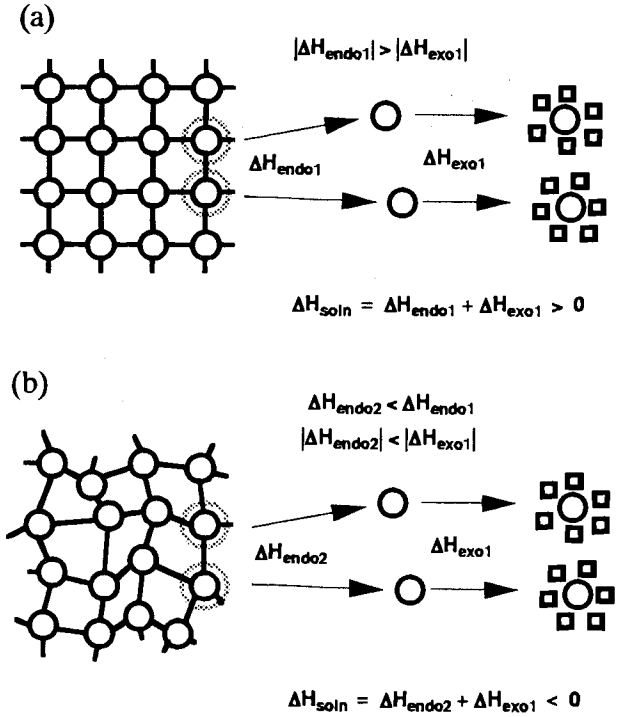


Fig. 2. Relationship between the heat of crystallization and the heat of solution of ursodeoxycholic acid.

In order to interpret the dissolution mechanism, a hypothesis was drawn from the above results. The proposed dissolution process of UDCA to ethanol containing 4.0% distilled water is shown in Scheme 1. The release of UDCA molecules from the crystalline lattice is followed by solvation by solvent molecules. Since the heat of release of the UDCA molecule from the crystalline lattice is endothermic (ΔH_{endo1}) and the heat of solvation of UDCA molecules is exothermic (ΔH_{exo1}), the heat of release could be greater than the heat of solvation ($|\Delta H_{endo1}| > |\Delta H_{exo1}|$), and the heat of solution will become endothermic (Scheme 1a).

The amorphous state is a higher energy state than the crystalline state because of its larger disorder of molecule arrangement and its higher molecular mobility. Accordingly, UDCA molecules are more likely to be released from the amorphous state than from the crystalline state. Therefore, the heat of release in the amorphous state (ΔH_{endo2}) is lower than that of the crystalline state (ΔH_{endo1}), while the heat of solvation is unchanged. Thus, we propose a ΔH_{endo2} decrease with an increase in the disorder of molecule arrangement, and if $|\Delta H_{endo2}|$ is lower than $|\Delta H_{exo1}|$, the heat of solution appeared to be exothermic (Scheme 1b).



Scheme 1. Schematic diagram of the speculated dissolution of ursodeoxycholic acid. (a) Crystalline state; (b) amorphous state; ○, Ursodeoxycholic acid molecule; □, solvent molecule.

Characterization of Amorphous UDCA Prepared by Various Methods

Amorphous UDCA was obtained by rapidly cooling the melt (quenching) and by the solvent evaporating method, as well as grinding (21). The effects of the preparation methods on the properties of amorphous UDCA were investigated.

The powder X-ray diffraction patterns of crystalline, ground, and quenched UDCA samples are shown in Fig. 3. No

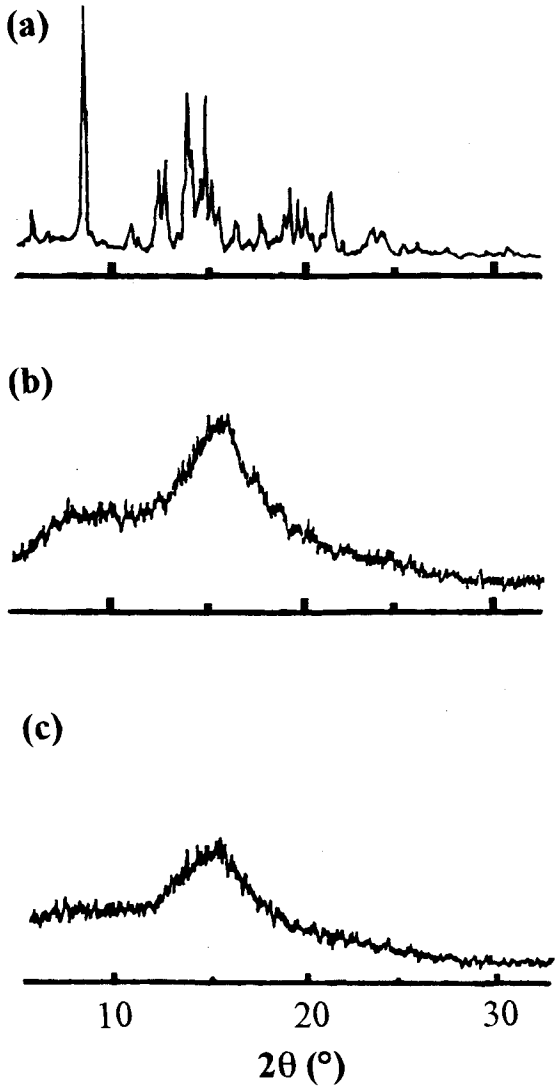


Fig. 3. Powder X-ray diffraction patterns of crystalline, ground and quenched ursodeoxycholic acid. (a) Crystalline, (b) ground for 30 min, (c) quenched.

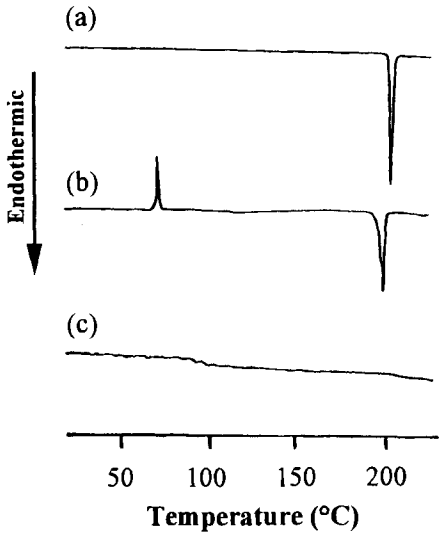


Fig. 4. DSC thermograms of crystalline, ground and quenched ursodeoxycholic acid. (a) Crystalline, (b) ground for 30 min, (c) quenched.

diffraction peaks of UDCA crystals were observed in ground and quenched samples. No significant differences in the diffraction patterns were observed among the two amorphous samples, except that the baseline of the diffraction patterns of ground UDCA was slightly higher than that of the other sample. This result appears reasonable, since powder X-ray diffraction, one of the more widely used methods for crystallinity quantitation, does not have the sensitivity required to conclusively distinguish among the two amorphous states.

The DSC curves of crystalline, ground, and quenched UDCA samples are given in Fig. 4. The thermograms of the ground sample showed both an exothermic peak at about 80°C due to crystallization of UDCA and an endothermic peak at about 203°C due to melting of the crystallized UDCA. The heat of crystallization determined from the peak area in the DSC curve was −19.7 J/g for ground UDCA. In contrast, no endothermic or exothermic peak was observed for the quenched sample. The DSC curve of the quenched sample exhibited a jump in heat capacity at 100°C, which was attributed to glass transition. We assume the discrepancy in the thermal behavior of each sample could be attributed to the differences in the amorphous states, such as the orientation of molecules or the degree of disorder.

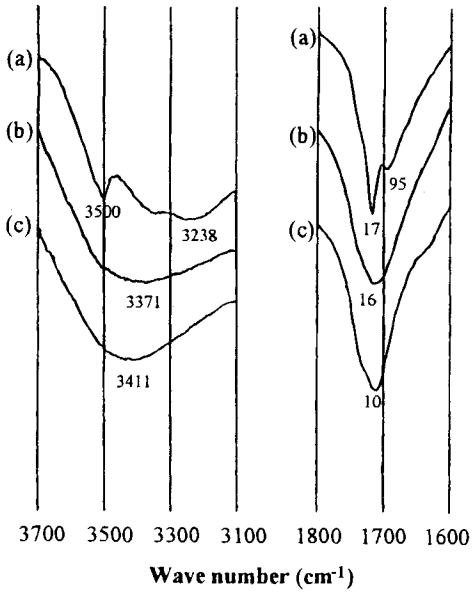


Fig. 5. IR spectra of crystalline, ground and quenched ursodeoxycholic acid. (a) Crystalline, (b) ground for 30 min, (c) quenched.

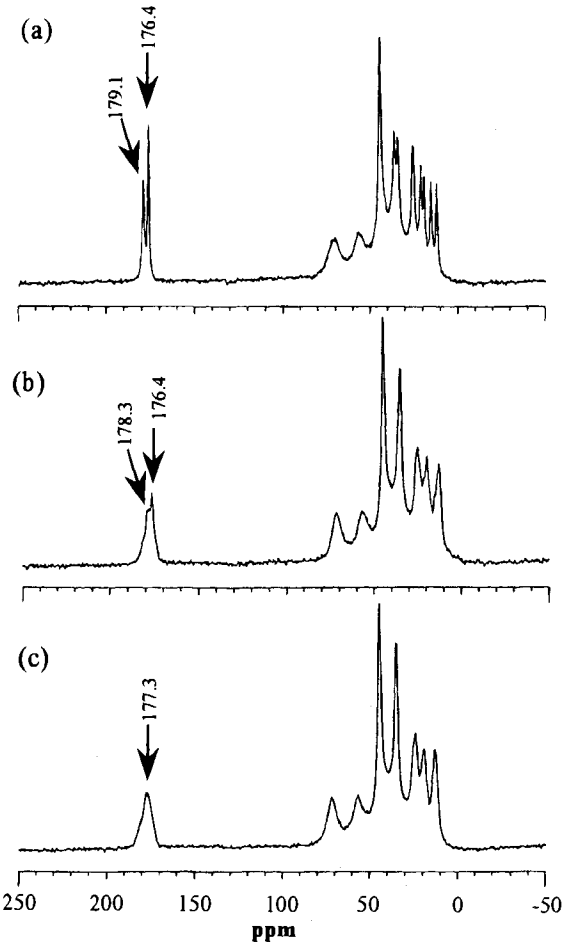


Fig. 6. Solid state ^{13}C-NMR spectra of crystalline, ground and quenched ursodeoxycholic acid; (a) crystalline, (b) ground for 30 min, (c) quenched.

The heats of solution of ground and quenched samples to ethanol containing 4.0% distilled water were determined to investigate the effects of preparation methods on the properties of amorphous UDCA. The heats of solution of two samples were exothermic, and they were determined as −22.4 and −27.1 J/g for ground and quenched samples, respectively. Significant differences in the enthalpy of solution were observed. The order, from highest to lowest energy, was quenched>ground UDCA. Bystrom and Briggner showed that as little as 1% amorphous content in a powdered sample could be discerned using microcalorimetry (12). Sebhatu *et al.* also indicated that microcalorimetry was only able to measure the disorder with high accuracy at lower degrees of disorder (below 10% disorder) (13). The discrepancy of the heat of solution in the two amorphous samples suggested a slight difference in the crystallinity of the samples.

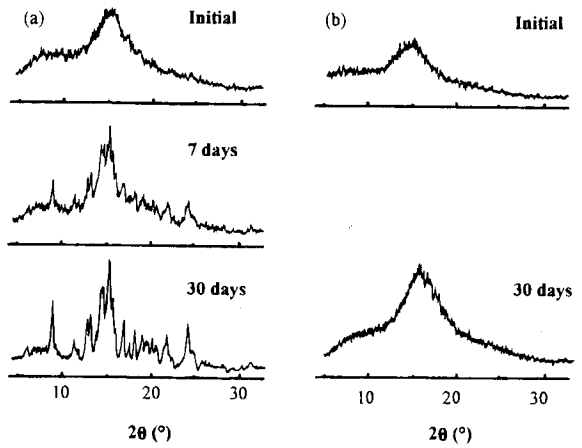


Fig. 7. Change in powder X-ray diffraction patterns of ground and quenched ursodeoxycholic acid samples stored under 74.5% RH at 40°C. (a) Ground for 30 min, (b) quenched.

Solution microcalorimetry proved to be useful in detecting the slight differences in amorphous states as well as DSC.

The IR spectra of crystalline, ground, and quenched UDCA are shown in Fig. 5. The IR spectra of crystalline UDCA show a broad peak at 3238 cm^{-1} and a sharp peak at 3500 cm^{-1} due to hydroxyl stretching, and two carbonyl stretching bands at 1695 and 1717 cm^{-1}. For the ground and quenched UDCA samples, the 3500 cm^{-1} band had disappeared, and a broad band at 3400 cm^{-1} was observed only in the hydroxyl stretching band region. The 1695 cm^{-1} band had also disappeared, and only 1710 cm^{-1} was observed in the carbonyl stretching band region. Higuchi *et al.* reported that two independent UDCA molecules were located in the crystalline lattice, and that the hydrogen bonding network was formed between all hydroxyl groups and carboxyl groups (22). Both the hydroxyl stretching and the carbonyl stretching bands observed in crystalline UDCA (Fig. 5a) are attributed to the different hydrogen bonding modes of two independent UDCA molecules. The different hydrogen bonding modes were, however, diminished in the amorphous state.

The ^{13}C-NMR spectra of crystalline, ground, and quenched UDCA are shown in Fig. 6. Two peaks due to carboxyl groups are found at 179.1 and 176.4 ppm in crystalline UDCA. The two peaks could be contributed to two non-equivalent molecules whose conformation were different in the crystal lattice. Peaks at 176.4 ppm and a shoulder at 178.3 ppm were observed for the ground sample, and a broad peak at 177.3 ppm was observed for the quenched sample. The ^{13}C-NMR spectra suggested that UDCA existed in two distinguishable forms in the ground sample as well as in the crystal, while in the quenched sample, UDCA molecules were homogeneously disordered. It was also suggested that the ground UDCA contained crystallite portion which was not detected by powder X-ray diffraction.

Physicochemical Stability of Amorphous UDCA

Ground UDCA sample was crystallized under heating as shown by the DSC curves of Fig. 4(b), but the quenched UDCA sample was not crystallized under heating, as shown in Fig. 4(c). Crystallization is triggered by temperature and/or moisture. The effects of moisture on crystallization were investigated. The changes in the powder X-ray diffraction patterns of ground and quenched samples stored under 74.5% RH at 40°C are shown in Fig. 7. The crystalline portion appeared at 2 days, and a remarkable peak was observed at 7 days for the ground UDCA sample. The amorphous state was maintained for over 30 days for the quenched UDCA sample. We suggested that the adsorption of water vapor and the subsequent dissolution of UDCA on the solid surface promoted the crystallization of UDCA (20). From these results we consider that the difference of preparation method for amorphous UDCA alternates the ability to adsorb water vapor by the solid surface of UDCA and the extent of UDCA nucleation promotes the crystal growth, and as a result, the stability of the amorphous state is different.

CONCLUSIONS

Amorphous UDCA samples prepared by several methods were characterized. The heat of solution was found to change from endothermic to exothermic with an increase in grinding time. A good correlation was obtained between the heat of solution and the heat of crystallization determined from the peak area in DSC. The amorphous UDCA samples differed by the heat of solution, DSC, IR spectra, and ^{13}C-NMR spectra, although no significant difference in powder X-ray diffraction patterns was observed. The heat of solution could clarify the difference of crystallinity which powder X-ray diffraction could not distinguish. It was suggested from ^{13}C-NMR spectra that ground UDCA still had ordered regions, like crystallite, but different from quenched UDCA. The amorphous samples showed the different features in water vapor adsorption as well as the frequency of UDCA nucleation promoting crystal growth.

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