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RESEARCH PAPER

Thermal Behavior of Ursodeoxycholic Acid in Urea: Identification of Anomalous Peak in the Thermal Analysis

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ABSTRACT

The objective of this study was to clarify the thermal behavior of ursodeoxycholic acid (UDCA) in mixtures with urea. Physical mixtures of UDCA and urea in various ratios were prepared, and the thermal analysis of these sample mixtures was investigated using conventional differential scanning calorimetry (DSC) and variable-temperature powder X-ray diffractometry (VTXRD). The hot-stage microscopy (HSM) and powder X-ray diffractometry (PXRD) were used as complementary techniques. From the DSC results of all sample mixtures, it was found that there was no endothermic peak at the melting temperature of intact UDCA crystals. The DSC thermograms of each ratio showed only the endothermic peak at about 136°C due to the melt of urea and the anomalous endothermic peak at about 155°C–157°C. The VTXRD study revealed that the crystals of urea completely disappeared at a temperature of 140°C. At this temperature, it was identified that the VTXRD pattern obtained was of UDCA crystals. The crystalline peaks gradually decreased in intensity at a temperature of 150°C. When the temperature was up to 160°C, the identical crystalline peaks of UDCA crystals completely disappeared. It was concluded that the anomalous endothermic peak at 155°C–157°C was the peak due to the dissolution of UDCA crystals in the surrounding melted urea.

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Key Words: *Differential scanning calorimetry; Endothermic; Hot-stage microscopy; Powder X-ray diffraction; Urea; Ursodeoxycholic acid*

INTRODUCTION

Ursodeoxycholic acid (UDCA) is a white, odorless, crystalline powder with a bitter taste. It has been used for the dissolution of radiolucent gallstones formed predominantly from cholesterol (1–3). It is also useful in the treatment of other liver diseases, such as primary biliary cirrhosis, chronic hepatitis, and biliary pain (4–6). Besides these, the use of UDCA in the treatment of dyspeptic symptoms has been reported (7–9).

Unfortunately, UDCA has pharmaceutical problems of water solubility. Owing to its low solubility, the in vitro studies have shown that intestinal absorption, and consequently the bioavailability, of the drug are generally poor (10), and more than 50% is lost in the stool after a single oral dose of 300 mg (11). It is well known that the amorphous form of the drug can improve its solubility. Thus, various trials have been undertaken to produce amorphous UDCA, such as grinding (12), spray-drying (13), and solid dispersion (14). Grinding and spray-drying are generally carried on a single drug substance, whereas the solid dispersion technique is usually performed with at least two substances, a drug and a carrier. The physicochemical properties of drug might be significantly altered due to the drug-carrier interaction. In our previous study (14), when we attempted to improve the dissolution rate of UDCA by solid dispersion with urea, the anomalous endothermic peak occurred between 150°C and 160°C during the DSC runs.

In this study, our objective was to clarify this anomalous peak and to evaluate the influence of urea on the solid state of UDCA by using conventional differential scanning calorimetry (DSC) and variable-temperature powder X-ray diffractometry (VTXRD). Hot-stage microscopy (HSM) and powder X-ray diffractometry (PXRD) were used as complementary techniques.

EXPERIMENTAL

Materials

The UDCA used was JP 13 grade (Tokyo Tanabe Co., Ltd., Tokyo, Japan). Urea was purchased from Nacalai Tesque Incorporated (Japan). Both UDCA and urea were used as received.

Preparation of Samples

The UDCA and urea were accurately weighed to obtain the definite weight ratio of 1 : 1, 2 : 1, 3 : 1, and 4 : 1 and then mixed by light trituration in a mortar.

Differential Scanning Calorimetry

A differential scanning calorimeter (DSC), model TA 9900 (Du Pont, New Castle, DE) was used under N_2 gas flow of 60 ml/min. A 3.0-mg sample was heated in a sealed aluminum pan at a heating rate of 5°C/min. The temperature was calibrated with pure indium, with a melting point of 156.60°C. An empty pan was used as a reference.

Variable Temperature Powder X-ray Diffractometry

The VTXRD (Phillips) was used to identify the transition that occurred in the DSC. The samples were subjected to a continuous controlled temperature program over the range of 30°C–170°C at a heating rate of 5°C/min. The VTXRD patterns were obtained by exposure to CuK_α radiation (30 kV, 15 mA) in a wide angle over the 2θ range of 5°–40°. During the VTXRD runs, the samples were maintained under isothermal conditions at the selected temperatures.

Powder X-ray Diffractometry

The PXRD was carried out on a Rigaku Denki diffractometer (CuK_α, 30 kV, 5 mA, scanning speed 4°/min). A scintillation detector was used to scan over the 2θ range of 5°–35°.

Hot-Stage Microscopy

A 100× optical microscope with polarizing optics fitted to a hot stage was used to observe the crystallographic properties and phase transitions of the

samples. A small amount of sample mixture (approximately 1 mg of each) was placed on a glass slide with a coverslip; this was placed on a sample stage and heated from room temperature to 250°C at a heating rate of 5°C/min.

RESULTS AND DISCUSSION

Thermal Behavior of Ursodeoxycholic Acid—Urea Mixtures

After UDCA was mixed physically with urea in definite weight ratios of 1:1, 2:1, 3:1, and 4:1, the samples were subjected to DSC at a heating rate of 5°C/min. The results as DSC thermograms are shown in Fig. 1. The DSC data of endothermic peaks and corresponding ΔH values are listed in Table 1.

On the DSC curves, the melting point of pure UDCA and urea crystals appeared at 205.8°C and 136.9°C, respectively. The DSC thermograms for each mixture of UDCA-urea showed two endothermic peaks at around 136°C–137°C, which was due to the melting of urea, and the anomalous endothermic peak around 155°C–157°C. Although the DSC was run until a temperature of 220°C, there was no endothermic peak at the temperature near the melting point of UDCA crystals. It was suspected that the anomalous endothermic peak was due to some phase transition of UDCA or other novel complex compound.

When the sample mixtures were heated at 5°C/min to 170°C, then cooled rapidly to room temperature; the heating was repeated to 170°C again at the same heating rate of 5°C/min. The cooling and heating was repeated again for a third run.

Figure 2 shows the DSC thermogram of the second and third runs of the sample mixtures. It was shown that, during the second and third runs, there was an exothermic peak that occurred in the temperature range of about 40°C–100°C. The exothermic peak trended to shift to the higher temperature when the concentration of UDCA was higher. However, around the temperature range 117°C–124°C, there appeared to be two overlapping endothermic peaks. These peaks were more clearly discernible when the amount of UDCA in the mixtures was less. The first endothermic peak was at about 117°C–119°C, and the second peak was around 122°C–124°C. These profiles revealed that there were at least two states of solid crystals in the system.

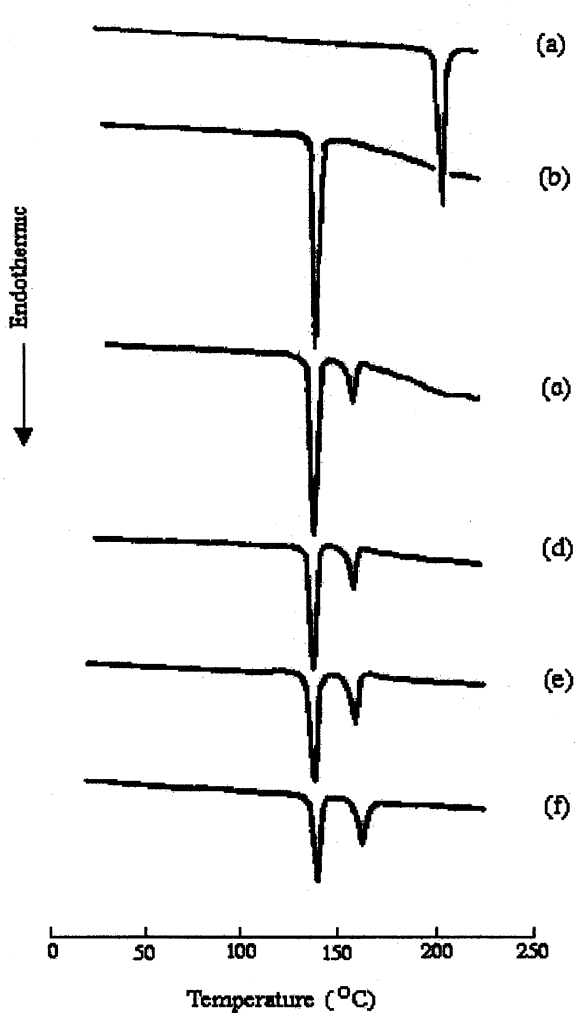

Figure 1. DSC thermograms of (a) UDCA intact, (b) urea intact, (c) UDCA-urea 1:1 mixture, (d) UDCA-urea 2:1 mixture, (e) UDCA-urea 3:1 mixture, and (f) UDCA-urea 4:1 mixture.

Crystalline Characteristics Studies

PXRD is a powerful technique for identification of the solid phase. Figure 3 exhibits the PXRD patterns of pure UDCA and urea over the range 5°–35°. Identical crystalline peaks for UDCA were obtained at 2θ of 9°, 13°, 15°, and 24°, whereas the identical peaks of urea were at 2θ of 22°, 29°, and 32°. Since VTXRD permits crystalline patterns to

Table 1

Endothermic Peaks and Enthalpy Values

Substances	Endothermic Peak (°C)	ΔH (J/g)
UDCA	205.8	83.8
Urea	136.9	227.0
UDCA-urea (1 : 1)	136.9	100.2
	155.9	24.1
UDCA-urea (2 : 1)	137.2	75.0
	157.2	27.3
UDCA-urea (3 : 1)	137.2	82.3
	156.8	34.2
UDCA-urea (4 : 1)	136.4	49.6
	157.4	38.7

UDCA, ursodeoxycholic acid.

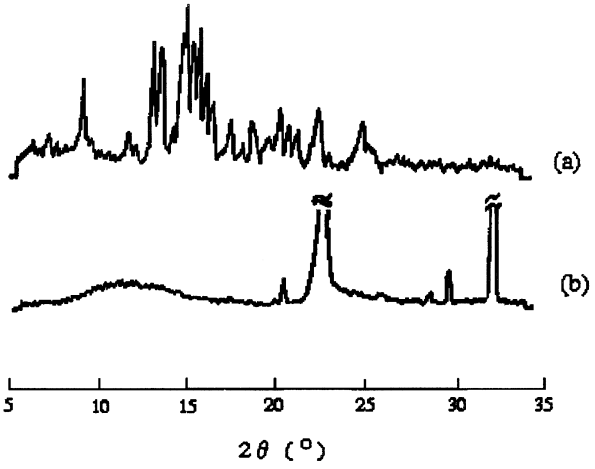

Figure 3. PXRD patterns of (a) UDCA intact and (b) urea intact.

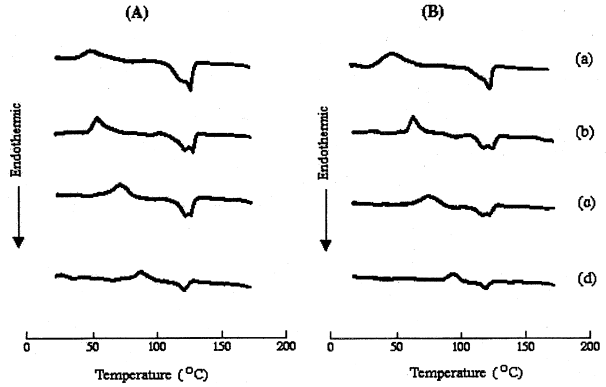

Figure 2. DSC thermograms of (A) the second run and (B) the third run of UDCA-urea mixtures of (a) 1 : 1, (b) 2 : 1, (c) 3 : 1, and (d) 4 : 1.

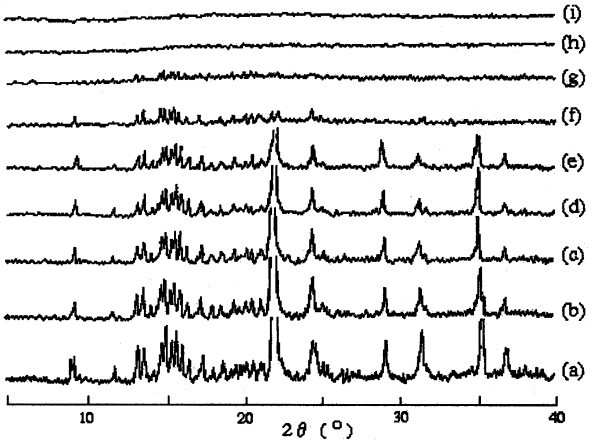

Figure 4. VTXRD patterns of UDCA-urea 1 : 1 mixture at (a) 30°C, (b) 100°C, (c) 110°C, (d) 120°C, (e) 130°C, (f) 140°C, (g) 150°C, (h) 160°C, (i) and 170°C.

be obtained as a function of temperature, it is an excellent complement to DSC.

VTXRD patterns of UDCA-urea mixtures in the ratios of 1 : 1 and 2 : 1 are shown in Fig. 4 and Fig. 5, respectively. The VTXRD patterns of both 1 : 1 and 2 : 1 mixtures obtained at 30°C–130°C were virtually identical due to UDCA and urea crystals. However, at 140°C, an abrupt change was observed. Identical peaks due to urea crystals disappeared. This result indicated that, at 140°C, there should be no crystal of urea. This result was in good agreement with the DSC results that urea completely melted at a temperature around 137°C. The crystalline peaks exhibited at 140°C were identical due to UDCA crystals. At 150°C, a poorly crystalline phase of UDCA was observed. From 160°C, crystalline UDCA was not detectable. Therefore, UDCA crystals completely disappeared between 150°C and 160°C. In the DSC, the endothermic peak was observed at a temperature around 155°C–157°C. Thus, according to the results from VTXRD, this endothermic peak was likely to be the melting of UDCA crystals or the dissolution of UDCA into the melted urea.

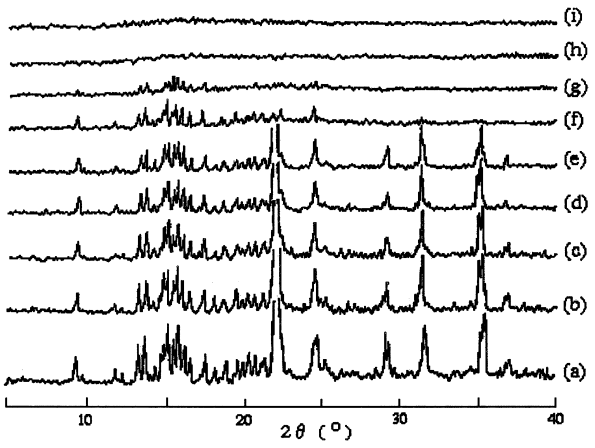

Figure 5. VTXRD patterns of UDCA-urea 2 : 1 mixture at (a) 30°C, (b) 100°C, (c) 110°C, (d) 120°C, (e) 130°C, (f) 140°C, (g) 150°C, (h) 160°C, and (i) 170°C.

Hot Stage Microscopic Studies

To clarify that the endothermic peak that appeared around 155°C–157°C in the DSC thermogram could be responsible for the melting of UDCA crystals or the dissolution of UDCA into the melted urea, polarizing optical HSM was used. The particle morphologies of UDCA and urea at room temperature were also investigated. The urea crystal appeared as a needle shape, whereas the UDCA crystal exhibited a small granular shape. When the sample mixtures were heated at a heating rate of 5°C/min from room temperature to a temperature of about 135°C, it was found that the urea crystals gradually melted. At a temperature of 137°C, all of the urea crystals observed were completely melted and transformed into the liquid phase. At this temperature, the crystals of UDCA still appeared in the same morphology as their original condition at room temperature. When the sample was continuously heated to 152°C, it was found that the crystals of UDCA suspended in the liquid urea gradually disappeared. At a temperature of about 158°C, all UDCA crystals were completely disappeared.

From these results and based on the information deduced from the DSC thermograms and the VTXRD patterns, it was possible to identify the endothermic anomalous peak that appeared in the first run at about 155–157°C as the dissolution of UDCA crystals in the liquid phase of urea.

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