Physicochemical Properties of Amorphous Ursodeoxycholic Acid Obtained by Grinding

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The crystallinity and disorder parameters of intact and ground ursodeoxycholic acid were determined by the X-ray diffraction method based on Ruland's theory. An increase in grinding time caused a decrease in crystallinity, while the disorder parameters were independent of the grinding time. Dissolution rates were measured for ursodeoxycholic acid with different crystallinities, and the recrystallization behavior of the amorphous samples was investigated during storage in a vapor of water, *n*-hexane, *n*-octane and *n*-decane. The amorphous ursodeoxycholic acid showed enhanced dissolution in ethanol, and was stable only under dry conditions.

Keywords crystallinity; ursodeoxycholic acid; amorphous; dissolution; recrystallization

Crystallinity is an important parameter which affects the pharmaceutical properties, dissolution, chemical stability, tabletting and bioavailability of solid dosage forms.[2-8] A number of methods have been reported to determine the degree of crystallinity: the X-ray diffraction method, thermal method, density method and infrared method.[9] Among these methods, Ruland's method, one of the X-ray methods, is theoretically rigorous and simultaneously gives the degree of crystallinity and the lattice disorder parameters. The purpose of the present study is to evaluate the crystallinity and disorder parameters of ursodeoxycholic acid (UDCA) and to investigate their influence on its pharmaceutical properties. The crystallinity and disorder parameters were determined by the powder X-ray diffraction method based on Ruland's theory.[10]

Experimental

Materials UDCA was of JP grade. Ethanol was of analytical grade.

Grinding Method A Heiko Seisakusho model TI-200 vibration mill was used, and the cell was made of tungsten carbide. The total weight of the specimen was 2.0 g.

X-Ray Diffraction Measurement The X-ray diffractograms were obtained from a 2027 diffractometer (Rigaku Denki) using a scintillation counter, a Cu target X-ray tube with a Ni filter (30 kV, 10 mA), and a symmetrical reflection goniometer scanned at 2°/min. For measuring the diffraction intensity at a definite diffraction angle interval, the step scanning method was used under the condition of a step width of 0.1°, a preset time of 4 s, and a diffraction angle range of $2\theta = 150$–5°.

Determination of Crystallinity and Disorder Parameters The determination method was the same as reported in the previous paper.[11]

Thermal Analysis A differential scanning calorimeter, DSC 1B (Perkin Elmer), was used under N_2 gas flow at a scanning rate of 4°C/min.

Dissolution Measurements The dissolution rate of UDCA was determined by the beaker method at 25, 37 and 50 °C and a rotating speed of 100 rpm. A compressed UDCA tablet, 10 mm in diameter, was placed at the center of the bottom of a 500 ml beaker, then 250 ml of ethanol was added. The amount of UDCA dissolved was determined by a color reaction method.[12]

Stability Study of Amorphous UDCA 150 mg of the amorphous UDCA was stored in a desiccator under definite relative humidities (RHs; 0, 31 and 79%) or saturated vapors of *n*-hexane, *n*-octane and *n*-decane at 40 °C.

Results and Discussion

Crystallinity of UDCA Figure 1 shows the X-ray diffraction patterns of UDCA after various grinding times. The intensities of the crystalline peaks of UDCA decreased with an increase in grinding time, and after 30 min a halo pattern was observed. The values of crystallinity and disorder parameters calculated by Ruland's method are shown in Fig. 2. The crystallinity of UDCA decreased with the increase in grinding time, while the disorder

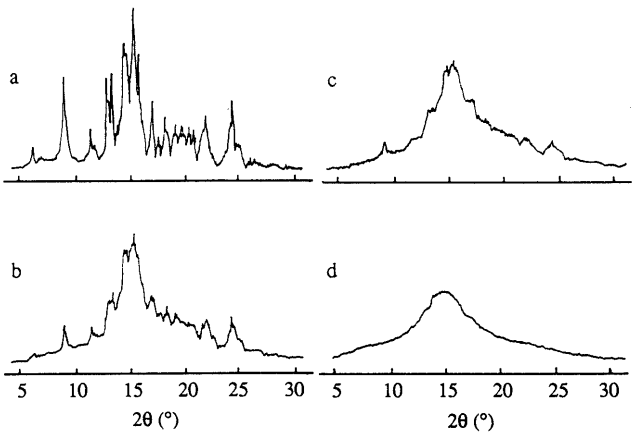

Fig. 1. Effects of Grinding on X-Ray Diffraction Patterns of UDCA
a, before grinding. Grinding time: b, 40 s; c, 2 min; d, 30 min.

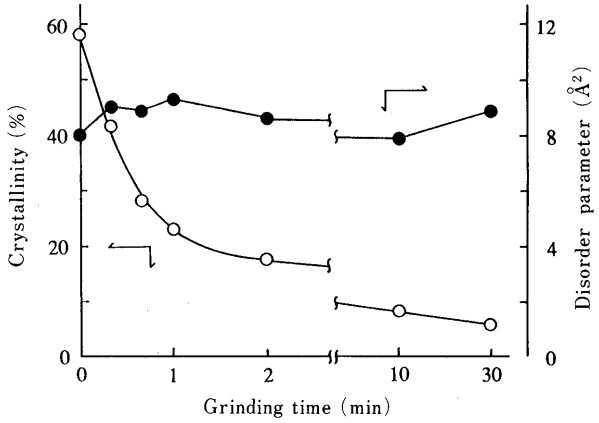

Fig. 2. Relationship between Grinding Time and Crystallinity and Disorder Parameter Values of UDCA
○, crystallinity; ●, disorder parameters.

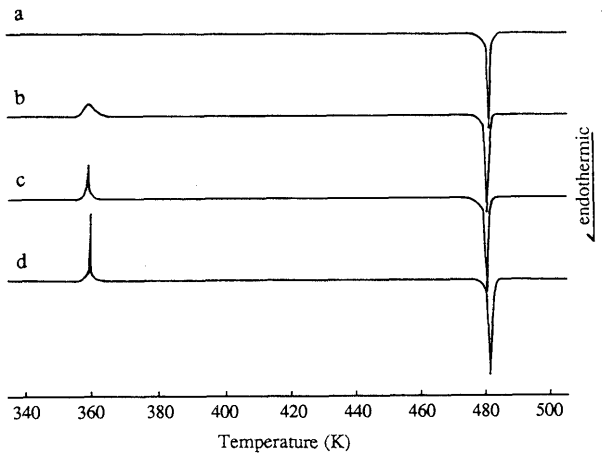

Fig. 3. Effects of Grinding on DSC Curves of UDCA
Grinding time: a, 0; b, 1 min; c, 2 min; d, 30 min.

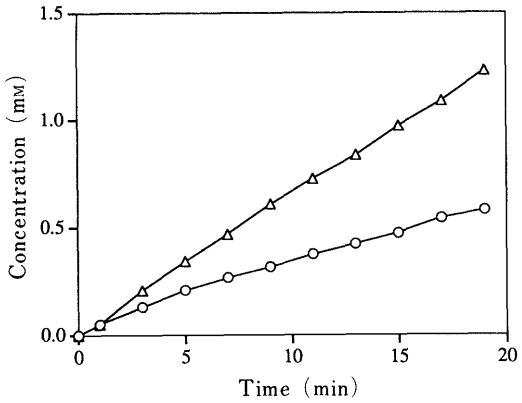

Fig. 4. Effects of Crystallinity on Dissolution of UDCA in Ethanol at 25 °C
Crystallinity: ○, 59%; △, 9%.

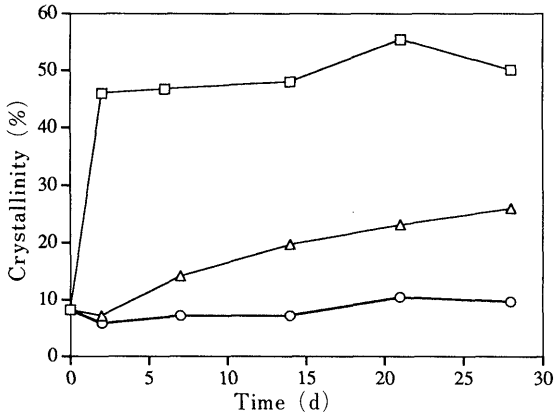

Fig. 5. Effects of RH on Crystallization of Amorphous UDCA at 40 °C
RH: ○, 0%; △, 31%; □, 79%.

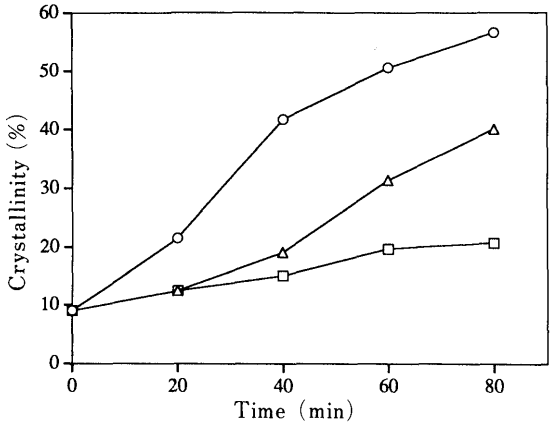

Fig. 6. Changes in UDCA Crystallinity during Storage in a Vapor at 40 °C
○, *n*-hexane, △, *n*-octane, □, *n*-decane.

parameters showed a little variation. After 30 min of grinding, the values of crystallinity and disorder parameters were calculated as 5.9% and 8.9 Å^2, respectively; even in the X-ray diffraction pattern, no diffraction peaks of crystalline UDCA were observed. It was impossible to detect diffraction peaks for disordered UDCA crystals. The disorder parameters comprised the thermal motion and the lattice imperfections of the crystals. By assuming a constancy of the thermal motion on the disorder parameter during grinding, a small variation in the disorder parameter also showed no progress of the lattice imperfection by grinding.

Figure 3 shows DSC curves of UDCA powders with different crystallinities. The melting point of UDCA crystal was observed to be 480 K. The thermograms of low crystallinity samples showed an exothermic peak at 360 K which can be attributed to the recrystallization of UDCA. Since decreasing crystallinity caused an increase in the exothermic peak area which was proportional to the heat of crystallization, the calculated crystallinity seemed to be reasonable.

Dissolution Study When a poorly soluble drug was administered orally, the amount and the rate of drug absorption (bioavailability) were usually affected by the dissolution rate of the drug. The transformation of a crystalline drug to an amorphous state is a practical method for enhancing the dissolution rate. Because of the poor solubility of UDCA in water, ethanol was used for the dissolution measurement. Figure 4 shows a comparison of the dissolution patterns of crystalline and amorphous UDCA in ethanol at 25 °C. A linear relationship between the dissolved amount and the dissolution time was observed for both UDCA samples. The amorphous UDCA showed a dissolution rate 2.2 times faster than that of the crystalline UDCA. The dissolution studies were also carried out at 37 °C and 50 °C. The activation energies calculated for the UDCA dissolution were 20.4 and 24.4 kJ/mol for the crystalline and amorphous UDCA, respectively. The frequency factors, however, were 11 times different from each other. The difference of the dissolution rate of both UDCA samples was related to the difference in crystallinity. It was suggested that the dissolution measurement in ethanol better clarified the difference of the interaction.

Stability of Amorphous UDCA Amorphous UDCA was recrystallized under heating as shown on the DSC curves of Fig. 3. The effect of RH on the crystallization was investigated. Figure 5 shows the recrystallization of amorphous UDCA stored at RH 0%, 31% and 79% at 40 °C. At 79% RH, the recrystallization occurred im-

mediately, while the amorphous UDCA was fairly stable at 0% RH. The crystallization proceeded gradually at 31% RH. These results suggest that the adsorption of a water vapor and the subsequent dissolution of UDCA on the solid surface promotes the crystallization of UDCA. Figure 6 shows the changes in crystallinity during the storage of amorphous UDCA under a saturated vapor of *n*-hexane, *n*-octane and *n*-decane at 40 °C. The crystallization rate of amorphous UDCA in these vapors was significantly greater than that in a water vapor. The vapor pressure of each solvent was determined to be 352, 31 and 4.0 mmHg, respectively.[13] The crystallinity rapidly increased in the *n*-hexane vapor, which had the highest vapor pressure among these solvents. The UDCA was slightly insoluble in *n*-hexane, while it was almost totally insoluble in water. Once the adsorption of a vapor occurred on the UDCA surface, the dissolution of UDCA at the surface liquid layer may cause the crystallization of UDCA.

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References and Notes

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2) R. C. Rowe, A. G. McKillop, D. Bray, *Int. J. Pharmaceut.*, **101**, 169 (1994).
3) M. L. Shively, S. Myers, *Pharm. Res.*, **10**, 1071 (1993).
4) M. Landin, R. M.-Pacheco, J. L. G.-Amoza, C. Souto, A. Concheiro, R. C. Rowe, *Int. J. Pharmaceut.*, **91**, 123 (1993).
5) K. Takeshima, N. Sunagawa, S. Nagata, K. Hirano, Y. Takagishi, *Yakugaku Zasshi*, **112**, 203 (1992).
6) T. Yamaguchi, M. Nishimura, R. Okamoto, T. Takeuchi, K. Yamamoto, *Int. J. Pharmaceut.*, **85**, 87 (1992).
7) Y. Nakai, K. Yamamoto, K. Terada, A. Kajiyama, *Chem. Pharm. Bull.*, **33**, 5110 (1985).
8) M. Morita, Y. Nakai, E. Fukuoka, S. Nakajima, *Chem. Pharm. Bull.*, **32**, 4076 (1984).
9) L. E. Alexander, "X-Ray Diffraction Methods in Polymer Science," Kagaku Dojin, Tokyo, 1973, p. 125.
10) W. Ruland, *Acta Cryst.*, **14**, 1180 (1961).
11) Y. Nakai, K. Yamamoto, K. Terada, A. Kajiyama, *Yakugaku Zasshi*, **105**, 580 (1985).
12) Y. Sakai, T. Tanimura, Z. Tamura, *Anal. Chem.*, **47**, 34 (1975).
13) The Chemical Society of Japan (ed.), "Kagaku Binran Kisohen," 3rd ed., Maruzen, Tokyo, 1984, p. II-118.