

Characterization of Amorphous Ursodeoxycholic Acid Prepared by Spray-drying

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Abstract

The objectives of this study were to characterize the amorphous state of ursodeoxycholic acid (UDCA) samples prepared by spray-drying, and to demonstrate the applicability of thermal and water-vapour-adsorption techniques for studying the material.

Amorphous UDCA was prepared by spray-drying a solution of the compound in a mixture of ethanol and dichloromethane. The amorphous material was characterized by powder X-ray diffraction, infrared (IR) spectroscopy, isothermal microcalorimetry, differential scanning calorimetry (DSC) and water-vapour adsorption. When the inlet-air temperature of the spray drier was increased beyond 140°C, the intensity of X-ray diffraction peaks from crystalline UDCA decreased and the IR bands in the hydroxyl-stretching and carboxyl-stretching regions changed. Dissolution of intact and spray-dried samples of UDCA prepared at 60 and 100°C was an endothermic process but the dissolution became exothermic with increasing inlet-air temperature. UDCA samples differing in crystallinity were obtained, depending on the inlet-air temperature. A good correlation was obtained between the heat of solution and the heat of crystallization determined from DSC peak area. A good correlation was also obtained between the heat of solution and the crystallinity determined by Ruland's method from X-ray diffraction patterns. The amount of water vapour adsorbed on UDCA samples increased with increasing inlet-air temperature, indicating hydrogen bonding between water molecules and the hydroxyl groups or the carboxyl groups of amorphous UDCA.

These results indicate that measurement of adsorption of water vapour and thermal analysis can both be used to evaluate the crystallinity of solid substances.

It is well known that the physicochemical properties of drug substances influence the properties of solid dosage forms (Byrn et al 1995). Solid drugs are generally comminuted to improve dissolution from solid pharmaceutical preparations, but amorphization cannot be used when the physical stability of the amorphous state is insufficient. Nevertheless, the amorphous state has gained much interest in the preparation of solid dosage forms because it can usually improve drug-dissolution behaviour. Several methods are used to prepare the amorphous state, including grinding, solvent evaporation, freeze-drying and spray-drying. Spray-

drying is widely used in the chemical, food, pharmaceutical, cosmetic and paint industries. In the pharmaceutical field, spray-drying is used in the preparation of spherical particles of drugs and excipients; and in granulating, microencapsulation, complex formation, solid dispersion, and drug-drug complex formation (Broadhead et al 1992). Spray-drying is also used to enhance the dissolution rate of poorly soluble drugs (Kai et al 1996; Yano et al 1997). This usually occurs as a result of a polymorphic change from a crystalline form to an amorphous form or a metastable crystalline form. Matsuda et al (1992) reported that two forms of frusemide spray-dried at 50 and 150°C had different glass-transition temperatures (T_g) and different stability profiles. Yamaguchi et al (1992) reported that the amorphous 16-membered macrolide compound prepared by spray-drying at different temperatures had different dissolution behaviour and

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physicochemical stability. Kristmundsdottir et al (1996) studied the preparation of diltiazem HCl microparticles by spray-drying with Eudragit RS and Eudragit RL as coating materials, and reported that the choice of solvent determined the structure of the resultant microparticles. Spray-drying using dichloromethane as the solvent resulted in microspheres and the drug was distributed in the coating polymer matrix, whereas spray-drying using toluene furnished microcapsules with the drug coated by the polymer. Thermal analysis showed that diltiazem HCl was amorphous in microspheres but was crystalline in microcapsules. It is therefore possible to prepare the different crystalline forms of drugs by changing the method of preparation.

Various methods are employed to study the amorphous state: powder X-ray diffraction, differential scanning calorimetry (DSC), infrared (IR) spectroscopy, photoelectron spectroscopy and dissolution testing. Isothermal microcalorimetry is commonly used for measurement of the heat evolved or absorbed by a chemical or physical process (Blackett & Buckton 1995; Joshi & Wilson 1995; Pudipeddi et al 1995). Isothermal calorimetry has also been used to study the amorphous state (Yonemochi et al 1997). In addition, an accurate humidity- and temperature-controlled microbalance system has been developed and widely used to investigate the water vapour-adsorption and -desorption properties of crystalline and amorphous drugs (Buckton & Darcy 1995; Ward & Schultz 1995).

Ursodeoxycholic acid (UDCA) is administered to dissolve cholesterol gallstones (Sugata & Shimizu 1974; Makino et al 1975). We have already reported that UDCA can be amorphized by grinding and that amorphous UDCA has a faster dissolution rate (Yonemochi et al 1994). The purpose of the current study was to characterize amorphous UDCA samples prepared by spray-drying at different temperatures by determination of the heat of solution, and study of powder X-ray diffraction patterns, DSC thermograms, IR spectra and water vapour-adsorption and -desorption isotherms.

Materials and Methods

Materials

Ursodeoxycholic acid of JPXIII grade was supplied by Tokyo Tanabe (Tokyo, Japan). Ethanol and dichloromethane were of analytical reagent grade.

Preparation of spray-dried UDCA

A solution (50 mg g⁻¹) of UDCA in ethanol-dichloromethane, 1:1, was spray-dried with an ML-12 spray-dryer (Ogawara, Yokohama, Japan)

with inlet-air temperatures between 60 and 200°C. The rate of supply of the solution was 100 mL min⁻¹. The rotation speed of the atomizer used to form the droplets of UDCA solution was 15 000 rev min⁻¹.

X-ray diffraction measurement

X-ray powder diffraction patterns were obtained with a RINT 2000 diffractometer (Rigaku Denki, Tokyo, Japan) using a scintillation counter, a Cu target X-ray tube with Ni filter (50 kV, 180 mA), and a symmetrical reflection goniometer scanned at 2° min⁻¹ over a 2θ range between 5 and 35°.

Infrared spectroscopy

Fourier-transform infrared (FTIR) spectrophotometry of samples as KBr disks was performed with a Perkin-Elmer (Yokohama, Japan) model 1725X instrument.

Determination of heat of solution

The heats of solution of UDCA samples at 25 ± 0.005°C were determined by use of a Tokyo Riko (Tokyo, Japan) model MPC-11 isothermal heat-conduction twin microcalorimeter. The microcalorimeter was equipped with a break-ampoule-type attachment which had two vessels in which an ampoule was placed. A calibration heater was wrapped around the outer surface of the vessel holder on the side of the sample. Ethanol containing 4.0% distilled water was used as dissolution medium, because UDCA is only very slightly soluble in water.

The particle size of UDCA powder used was between 125 and 150 μm. UDCA powder (200 mg) 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 of the vessels of the break-ampoule-type attachment and a vacant ampoule, used as a reference, was placed in the other vessel. Each vessel had previously been filled with dissolution medium (20 mL). The attachment was placed in the calorimeter and was left to equilibrate thermally for 12 h. After equilibrium was established the ampoules were simultaneously broken and the UDCA powder was released to dissolve in the dissolution medium; the mixing rate was 50 rev min⁻¹. The heat flow signal was monitored as a function of time. Integration of the heat flow curve over a specific time interval (3 h) enabled calculation of the heat evolved or absorbed. Exothermic signals were given negative values. Calibration was performed at each time of determination. The calibration data were obtained by electrical heating at a specified

voltage for different periods. The heat of solution of UDCA was calculated from the calibration curve.

Thermal analysis

A Du Pont (New Castle, DE) model TA9900 differential scanning calorimeter (DSC) was used with N_2 gas flow (60 mL min^{-1}) at a heating rate of 5°C min^{-1} . The temperature and heat-flow calibrations had previously been performed with indium as standard.

Determination of crystallinity of UDCA samples

The crystallinity of crystalline and spray-dried UDCA was determined by Ruland's method (Ruland 1961). Determination by computer was performed using software developed by Yamamura et al (1997).

Water vapour-adsorption and -desorption analysis

A VTI (Hialeah, FL) model MB300G microbalance was used for the determination of water-vapour-adsorption and -desorption isotherms at 25.0°C . The particle size of intact and spray-dried UDCA samples was between 125 and $150 \mu\text{m}$. UDCA samples were placed on the microbalance pan, surrounded by a thermal jacket used for controlled isothermal scanning. All samples were dried under vacuum at 30°C until equilibrium was reached (i.e., less than 3 mg change in weight over 5 min for three consecutive measurements). Water vapour was then introduced to the sample at increments of 5% relative humidity up to 95%. All moisture levels were maintained until the sample became gravimetrically equilibrated, as described above. Variations in weight, relative humidity and

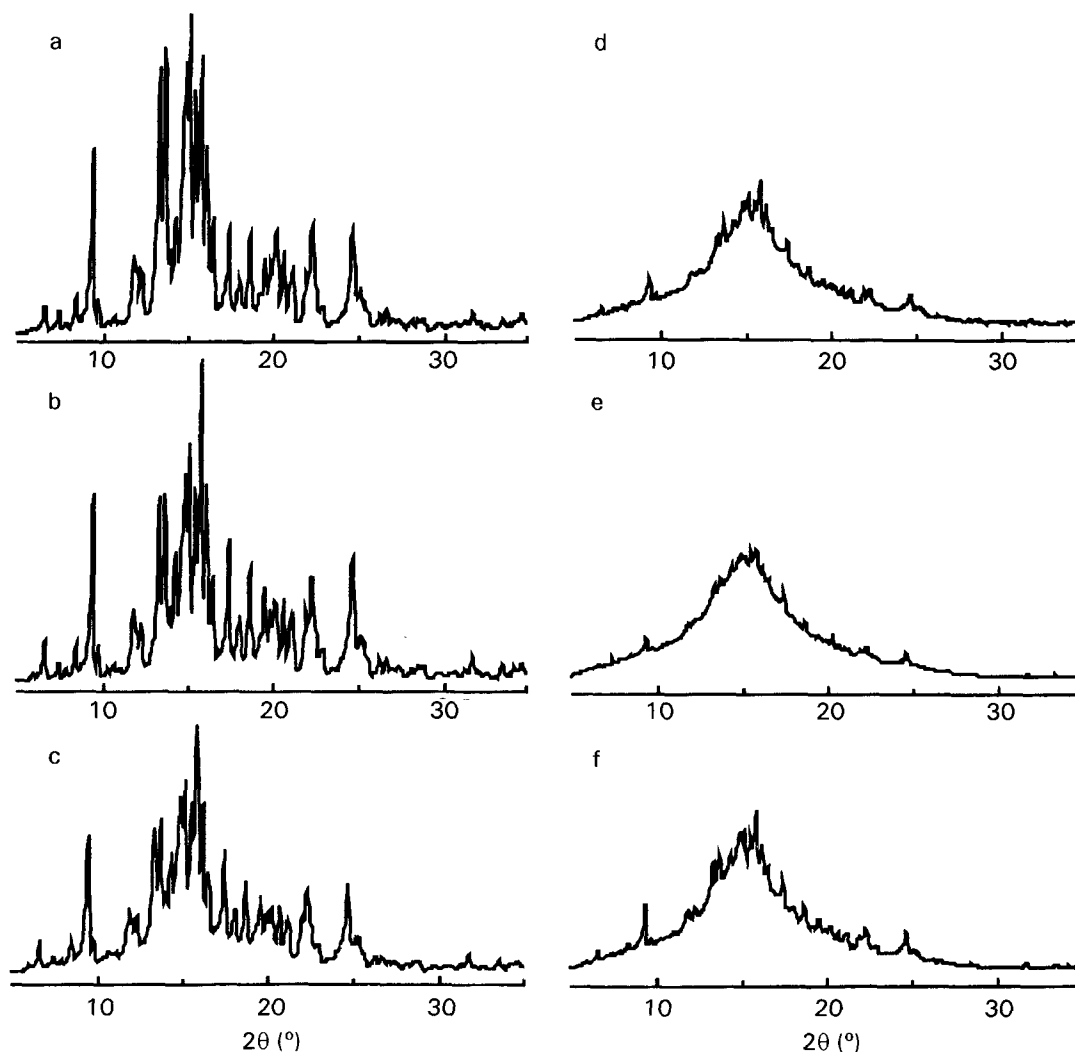


Figure 1. Changes in the powder X-ray diffraction patterns of ursodeoxycholic acid upon spray-drying. a, intact; b, spray-dried at 60°C (SD-1); c, spray-dried at 100°C (SD-2); d, spray-dried at 140°C (SD-3); e, spray-dried at 180°C (SD-4); f, spray-dried at 200°C (SD-5).

temperature were recorded simultaneously throughout each sample determination. The measurements were performed in duplicate.

Results and Discussion

Effects of inlet-air temperature on the crystallinity of UDCA

The spray-dryer was used with inlet-air temperatures of 60, 100, 140, 180 and 200°C; the spray-dried samples were designated SD-1 to SD-5 in order of increasing inlet-air temperature. The powder X-ray diffraction patterns of intact UDCA and spray-dried UDCA samples are shown in Figure 1. X-ray diffraction peaks from crystalline UDCA were obtained for SD-1 and SD-2. When the inlet-air temperature was above 140°C (SD-3, SD-4 and SD-5) intensities of X-ray diffraction peaks from crystalline UDCA decreased and the X-ray diffraction patterns closed to a halo pattern. Although SD-5 was spray-dried at the highest temperature (200°C), X-ray diffraction peaks were clearly observed. The intensity of powder X-ray diffraction of SD-5 was similar to that of SD-3. The explanation for this phenomenon might be that immediately after spray-drying at 200°C UDCA was amorphous, but that some of the amorphous UDCA immediately crystallized, because of high temperature of the spray-drying apparatus, whereas the rest of the spray-dried UDCA moved from the drying chamber to the sample trap container.

The IR spectra of intact and spray-dried UDCA samples are shown in Figure 2. Intact UDCA gave a broad peak at approximately 3251 cm⁻¹ and a sharp peak at 3504 cm⁻¹, arising from the hydroxyl-stretching vibration, and two carbonyl-stretching bands at 1695 and 1718 cm⁻¹. The band near 3500 cm⁻¹ was not present in the spectra of samples of spray-dried UDCA prepared at temperatures above 140°C (SD-3, SD-4 and SD-5), and the only band observed in the hydroxyl-stretching region was a broad band at approximately 3400 cm⁻¹. The 1695 cm⁻¹ carbonyl-stretching band also disappeared, only that at 1716 cm⁻¹ being observed in the carbonyl stretching region. The same phenomenon had been reported for ground and quenched UDCA (Yonemochi et al 1994). Higuchi et al (1985) reported that the crystalline lattice contained two non-equivalent UDCA molecules and that a hydrogen-bonding network was formed between all the hydroxyl and carboxyl groups of the UDCA molecules. The two hydroxyl-stretching and two carbonyl-stretching bands observed in intact UDCA (Figure 2a) are attributed to two non-equivalent UDCA molecules. It was

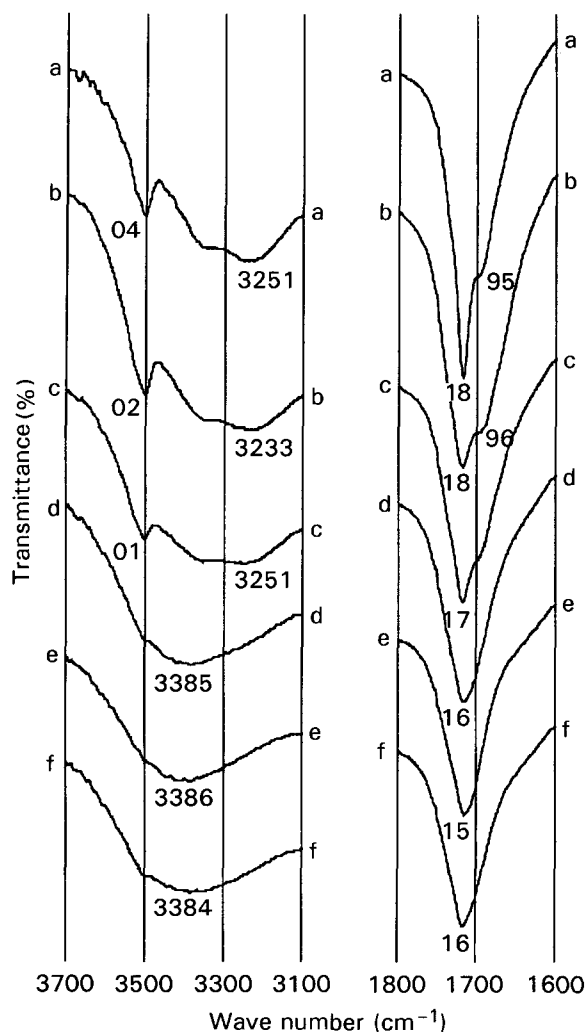


Figure 2. Changes in the infrared spectra of ursodeoxycholic acid upon spray-drying. a, intact; b, spray-dried at 60°C (SD-1); c, spray-dried at 100°C (SD-2); d, spray-dried at 140°C (SD-3); e, spray-dried at 180°C (SD-4); f, spray-dried at 200°C (SD-5).

considered that UDCA molecules became equivalent and that the hydrogen-bonding network of UDCA molecules changed in the spray-dried amorphous UDCA samples.

The DSC curves of crystalline and spray-dried UDCA samples are given in Figure 3. The thermograms of intact UDCA and SD-1 had an endothermic peak at approximately 203°C owing to the melting of UDCA. The thermograms of UDCA spray-dried samples prepared at temperatures >140°C (SD-3, SD-4 and SD-5) had an exothermic peak between 100 and 120°C, as a result of the crystallization of amorphous UDCA, and an endothermic peak at 203°C owing to the melting of crystallized UDCA. The heat of crystallization determined from the peak areas in the DSC curves were -26.0, -30.9 and -28.9 J g⁻¹ for SD-3,

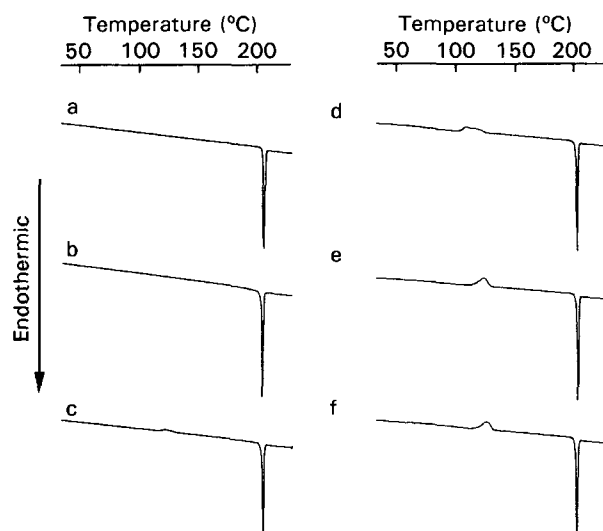


Figure 3. DSC thermograms of intact and spray-dried ursodeoxycholic acid. a, intact; b, spray-dried at 60°C (SD-1); c, spray-dried at 100°C (SD-2); d, spray-dried at 140°C (SD-3); e, spray-dried at 180°C (SD-4); f, spray-dried at 200°C (SD-5).

SD-4 and SD-5, respectively (Table 1). We deduced that the variation of the heat of crystallization was because of the different crystalline states of the spray-dried UDCA. The thermograms of SD-2, which gave X-ray diffraction peaks of crystalline UDCA and no change of IR spectra, had an exothermic peak at approximately 113°C as a result of crystallization of amorphous UDCA and an endothermic peak at 203°C owing to melting of crystallized UDCA. The heat of crystallization determined from the peak area in the DSC curve of SD-2 was only -5.48 J g^{-1} , smaller than that of the other samples (SD-3, SD-4 and SD-5). It was thought that the powder X-ray diffraction pattern and the IR spectra did not show the changing crystallinity of UDCA because of the small amount of disorder of SD-2, whereas the DSC could detect the lowering of crystallinity of SD-2.

The heats of solution of intact and spray-dried UDCA are shown in Table 1. The heat of solution was found to vary from endothermic to exothermic

as the inlet-air temperature was increased. The endothermic heats of solution were 19.7, 20.8 and 12.6 J g^{-1} for intact, SD-1 and SD-2, respectively. The exothermic heats of solution were -6.84 , -15.6 and -10.1 J g^{-1} for SD-3, SD-4 and SD-5, respectively. It was considered that the decreasing crystallinity of spray-dried UDCA led to the change in the heat of solution. The same phenomenon was observed for ground UDCA by Yonemochi et al (1997), who discussed the mechanism of dissolution of UDCA in ethanol containing 4.0% distilled water. The mechanism for ground UDCA was considered to be similar to that for spray-dried UDCA.

The relationship between the heat of crystallization and the heat of solution is illustrated in Figure 4. A good linear correlation was found (correlation coefficient 0.996). It is well known that there is a relationship between crystallinity and heat of crystallization, and that the lower the crystallinity, the larger the exothermic peak area on DSC curves (the heat of crystallization). Therefore, from the correlation between the heat of solution and the heat of crystallization, it is reasonable to presume the crystallinity and the energy state from the heat of solution.

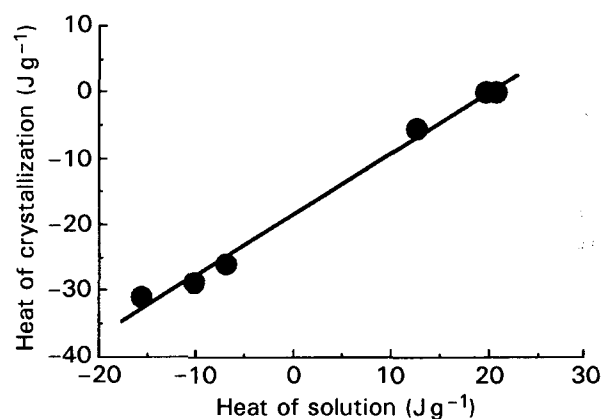


Figure 4. Relationship between the heat of solution and the heat of crystallization of ursodeoxycholic acid.

Table 1. Heat of crystallization, heat of solution and degree of crystallinity of intact and spray-dried ursodeoxycholic acid.

Sample	Heat of crystallization (J g^{-1})	Heat of solution (J g^{-1})	Crystallinity (%)
Intact	0	19.7	71.5
Spray-dried at 60°C (SD-1)	0	20.8	66.2
Spray-dried at 100°C (SD-2)	-5.48	12.6	47.5
Spray-dried at 140°C (SD-3)	-26.0	-6.84	20.6
Spray-dried at 180°C (SD-4)	-30.9	-15.6	12.9
Spray-dried at 200°C (SD-5)	-28.9	-10.1	21.3

To determine the quantitative relationship between the heat of solution and the crystallinity of spray-dried UDCA, the degree of crystallinity of intact and spray-dried UDCA was determined by Ruland's method. The degrees of crystallinity of intact and spray-dried samples of UDCA are also shown in Table 1. The degree of crystallinity of SD-2, which was partly apparent from the X-ray diffraction peaks and from the exothermic peak that appeared on the DSC curve, was determined to be 47.5%. The crystallinity of SD-5 was high compared with that of SD-4, because the intensity of the X-ray diffraction peaks of SD-5 was higher than for SD-4 (Figure 1). The crystallinity of SD-5 was found to be similar to that of SD-3 (Table 1). The reason SD-5 was more crystalline than SD-4 could be explained as follows. From DSC results, the spray-dried UDCA crystallized at approximately 100–120°C. UDCA spray-dried at 200°C was amorphous, however, some amorphous UDCA immediately crystallized because of the high temperature of the spray-drying apparatus.

The relationship between the crystallinity and the heat of solution is illustrated in Figure 5. A linear correlation was observed; the correlation coefficient was 0.983. From this relationship and the correlation between the heat of solution and the heat of crystallization, it is possible to derive the crystallinity of samples by determining the heat of solution.

Water vapour-adsorption and desorption

Water vapour-adsorption and -desorption isotherms of intact and spray-dried UDCA samples are shown in Figure 6. Intact UDCA hardly adsorbed water vapour, because UDCA is a hydrophobic compound. But adsorption of water vapour was observed for the spray-dried UDCA samples with low crystallinity (SD-2, SD-3, SD-4 and SD-5) and

as the crystallinity of spray-dried UDCA samples decreased the amounts of water vapour adsorbed by the samples increased. Hysteresis was also observed on the water vapour-adsorption and -desorption isotherms for spray-dried UDCA. The relationship between crystallinity and the amount of adsorbed water is illustrated in Figure 7. A negative correlation was observed with a correlation coefficient of 0.985.

Higuchi et al (1985) reported that the crystalline lattice of UDCA contained two non-equivalent

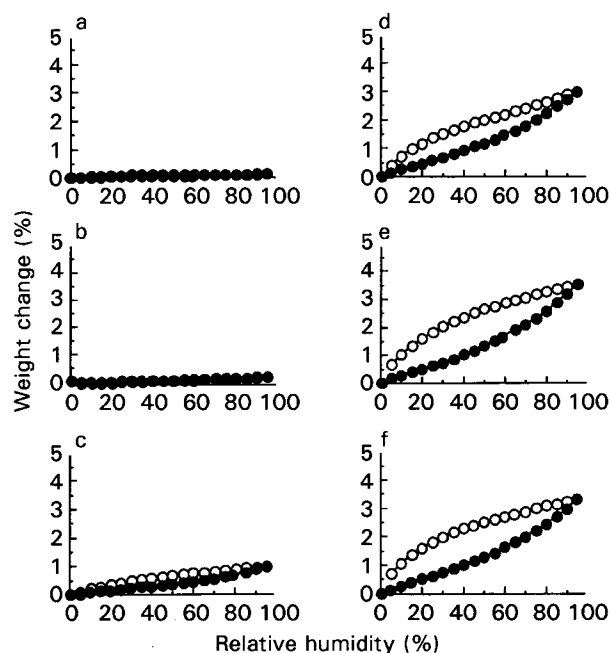


Figure 6. Water-vapour-adsorption and -desorption isotherms of intact and spray-dried ursodeoxycholic acid. a, intact; b, spray-dried at 60°C (SD-1); c, spray-dried at 100°C (SD-2); d, spray-dried at 140°C (SD-3); e, spray-dried at 180°C (SD-4); f, spray-dried at 200°C (SD-5). ●, adsorption; ○, desorption.

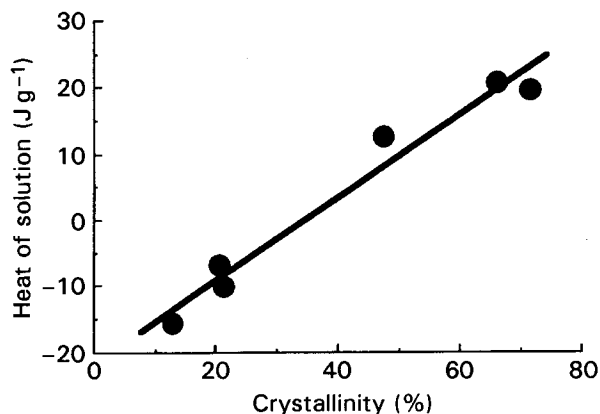


Figure 5. Relationship between the degree of crystallinity and the heat of solution of ursodeoxycholic acid.

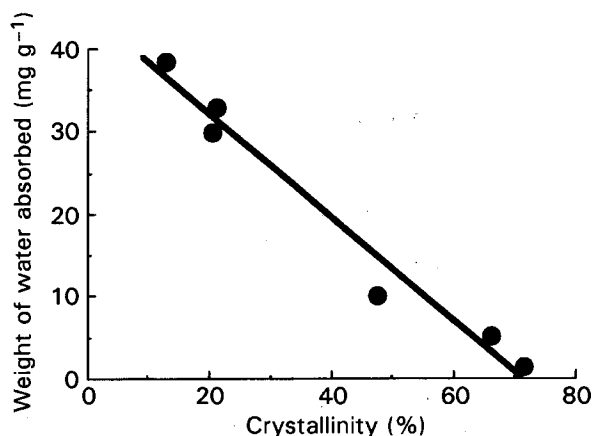


Figure 7. Relationship between the degree of crystallinity and weight of water absorbed by ursodeoxycholic acid.

molecules and that a hydrogen-bonding network was formed from all the hydroxyl and carboxyl groups of the UDCA molecules. It was considered from IR spectra that in amorphous UDCA samples the hydrogen-bonding network of UDCA molecules was disrupted and it was, therefore, easy for the water molecule to form a hydrogen bond with the hydroxyl or carboxyl groups of the UDCA molecule. More water molecules were taken up by amorphous solids than by the crystalline form of the same chemical entity (Nakai et al 1977; Pikal et al 1978). It was mentioned that in contrast to adsorption, when the amount of water taken up depends on the surface area available, uptake by amorphous solids was predominantly proportional to the total mass of amorphous solid (Ahlneck & Zografi 1990). As water vapour was barely absorbed by the surface of crystalline UDCA, it was thought that the amount of water vapour absorbed by UDCA was determined by the disordered regions of amorphous UDCA. Therefore, it might be concluded that both measurement of water vapour-adsorption and thermal analysis could be used to evaluate the crystallinity of solid substances.

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