Differences in Crystallization Behavior Between Quenched and Ground Amorphous Ursodeoxycholic Acid

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Purpose. To study the crystallization of ground and quenched ursodeoxycholic acid (UDCA) and to characterize their amorphous states.
Methods. Amorphous UDCA was prepared by grinding and also by rapid cooling of the melt. These samples were characterized by powder X-ray diffraction (XRD), near IR spectra and dynamic water sorption. The heat associated with crystallization was measured in an isothermal microcalorimeter at 25°C at various relative humidities (RH) (50%–100%) and, in the presence of the vapour from a mixed solvent of ethanol and water (ethanol conc. 10%–100%). The specific surface area was calculated from krypton adsorption. Contact angles were measured by using a Wilhelmy plate to calculate the surface energy of the samples.
Results. Ground and quenched samples yielded amorphous XRD patterns. Differential scanning calorimetry thermographs of the milled sample revealed that crystallization occurred at around 80°C, whereas the quenched sample did not crystallize. Exposure to humid air did not result in crystallization of either amorphous sample during the microcalorimetric experiments. In the presence of ethanol vapour, the ground sample did, but the quenched sample did not, crystallize. The amount of water sorption into the quenched sample was larger than that of the ground sample at low RH. The surface energy of the quenched material was different to that of the ground. Peak shifts were observed in the NIR spectra at around 1450, 2100 nm, allowing differentiation between the ground and quenched samples.
Conclusions. It can be concluded that different molecular states of amorphous UDCA were obtained depending on the preparation method. The crystallisation of amorphous UDCA was related to the molecular state of disorder.

KEY WORDS: ursodeoxycholic acid; amorphous; recrystallization; microcalorimetry; near infrared; water sorption.

INTRODUCTION

It is well known that a significant increase in dissolution and bioavailability can be achieved by creating an amorphous product (1–3). However, the amorphous forms of many drugs can transform to the thermodynamically stable crystalline form on storage. Therefore, pharmaceutical product development with amorphous drugs can be difficult unless one can guarantee that transformation to the crystalline form will not occur during storage. Consequently, studies of crystallization from the amorphous state are needed to better understand the underlying mechanisms.

We have already reported that ursodeoxycholic acid (UDCA) was made amorphous by grinding and by rapid cooling of the melt (quenching) (4,5). These amorphous UDCA samples had differences in their heats of solution, DSC, IR spectra, and ^{13}C-NMR spectra, although no significant difference in XRD was observed.

Near-infrared (NIR) spectroscopy has been used extensively in the food and agricultural industries for the past twenty years. NIR spectroscopy in the diffuse reflectance mode may be used directly for solid samples such as tablets, pellets in capsules, or bottles containing lyophilized substances. Advantages of NIR spectroscopy are that it is non-destructive and penetrates further into the sample than mid-infrared. Currently the major use of NIR is for chemical identification studies, although its application in the field of physical characterisation is developing. We have already reported that it is possible to use NIR spectroscopy to monitor changes in amorphous lactose during humidification (6,7).

The purpose of this report is to study the crystallization of amorphous samples that were prepared by grinding and quenching, and characterize their amorphous state by using XRD, NIR spectroscopy, dynamic water sorption analysis, and dynamic contact angle measurement.

MATERIALS AND METHODS

Materials

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

Preparation of Amorphous Solid

Samples of amorphous UDCA were prepared by grinding and rapid cooling of the melt as described previously (5). It is possible for materials to undergo substantial decomposition on melting, so an HPLC assay was conducted. No decomposition products were seen in either of the amorphous samples.

Powder XRD Measurement

The powder XRD patterns were measured with CuKα radiation at 5–35° (2θ) using a Rigaku Desk top X-ray powder diffractometer (MiniFlex).

Isothermal Microcalorimetry

The samples (30 mg) were loaded into a glass ampoule with a small tube containing either a saturated aqueous salt solution (controlled RH), or a mixture of water and ethanol (ethanol concentration 0–100%, to give vapours with different partial pressures of ethanol). The heat responses were measured by an isothermal microcalorimeter (Thermal Activity Monitor,

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Thermometric) at 25°C. The exact experimental method was as reported previously (2).

To investigate whether crystallization would be aided if seed crystals were added to the amorphous samples, different UDCA powders were mixed using a vortex mixer for 1min. Mixtures of crystalline and amorphous materials and mixtures of the quenched and milled amorphous materials were studied.

Water Vapour Sorption Isotherm Measurement

The water vapour sorption isotherms of samples were measured by use of a temperature and humidity controlled microbalance system (Dynamic Vapour Sorption system (DVS), Surface measurement systems) at 25 and 40°C.

Specific Surface Area Determination

The adsorption of krypton gas to the sample was measured using a Shimadzu Accusorb 2100E. The specific surface area was calculated from the Kr isotherm using the BET equation.

Surface Energy Determination

Surface energies of the samples were calculated from contact angle data measured using a Cahn Dynamic Contact Angle Analyzer. The powder was stuck to a thin glass slide using spray adhesive (Spray Mount 3M), this was then used as a Wilhelmy plate. Water, ethylene glycol, and formamide were used as the probe liquids (8).

Near Infrared Spectroscopy

Samples (2 g) of the amorphous UDCA were placed into flat bottom clear glass jars. This jars were placed on the lens of Rapid Content Analyzer module attached to a NIRSystems 6500 spectrophotometer. The NIR instrument recorded the mean spectrum of 32 scans of each sample, over the wavelength region 1100–2500 nm.

RESULTS AND DISCUSSION

Attempts to Crystallize Ground and Quenched UDCA

As shown in Fig. 1 (5), ground and quenched samples yielded similar amorphous XRD patterns.

It was well known that the crystallization of amorphous materials is triggered by increases in temperature and/or the addition of a plasticizer. The effect of an increase in temperature is to increase the molecular mobility of the amorphous material. As the temperature rises above the glass transition temperature (Tg) the mobility is greatly enhanced, and this continues as further increases in temperature cause the viscosity of the material to drop. We have already studied the thermal stability of the amorphous UDCA samples by using differential scanning calorimetry (DSC). The DSC thermograph for the ground sample revealed an exotherm at around 80°C, which was due to crystallization, and a melt at 203°C. For the quenched sample neither a crystallization exotherm nor a melting endotherm were observed for the quenched sample. In keeping with the DSC data, no crystallization was observed in the quenched sample on a hot stage microscope during heating, but gradual liquefaction was observed above Tg (100°C).

As mentioned above, the alternative to raising the temperature above Tg is to lower Tg below room temperature, by use of a plasticiser. For many materials water acts as a good plasticiser, although for especially hydrophobic molecules it may be that alternative low molecular weight absorbates are needed. The effect of moisture on the stability of amorphous UDCA was measured in an isothermal microcalorimeter at 25°C at various relative humidities (RH; range 50%–100%). Exposure to humid conditions at 25°C for the 24 h duration of the calorimetric experiment did not cause either amorphous sample to crystallize. However, when the milled sample was sealed in an ampoule with a solution containing a 50:50 mixture of ethanol:water crystallization started immediately (i.e., it was being recorded immediately when the sample was lowered into the measuring site of the calorimeter, this being up to 30 minutes after initiation of the experiment due to the need to reach temperature equilibration). When exposed to the vapour from a solution containing 10% ethanol in water, the milled sample crystallized after a 120 min lag time (Fig. 2), making this a good choice of experimental conditions to study the crystallization event. Whilst the milled sample crystallizes, the quenched sample remains in the amorphous state when exposed to ethanolic vapours.

The fact that the quenched sample does not crystallize (on heating, or on exposure to either ethanol or water vapours), shows that it is in a more stable state than the amorphous material which was produced by milling. As the stable state is the crystalline material it is thermodynamically desirable for both the milled and quenched material to crystallize. The fact that there is a difference between the milled and quenched samples could be due to the molecules being more disordered in the quenched sample than in the milled sample. For the more disordered quenched sample, the probability of nucleation would be lower than that for the milled sample.

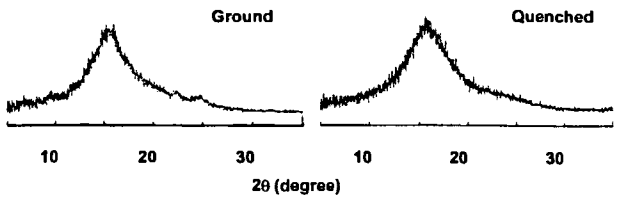

Fig. 1. XRD patterns of ground and quenched UDCA.

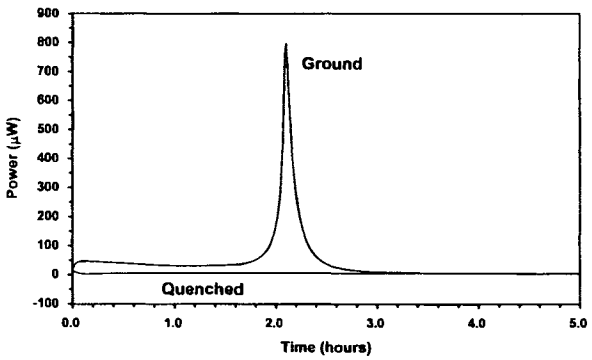

Fig. 2. Isothermal microcalorimetry traces of ground and quenched UDCA at 25°C sample size, 30 mg; ethanol concentration, 10%.

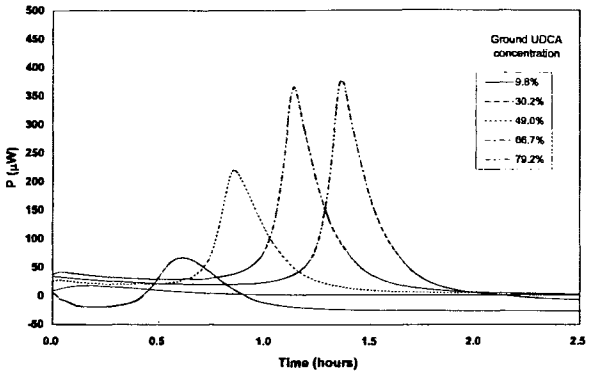

Fig. 3. Isothermal microcalorimetry traces of the mixture of ground and crystalline UDCA at 25°C total sample size, 30 mg; ethanol concentration, 10%.

Seeding Studies

An alternative reason for changes in the ability to crystallise could be that certain seed crystals remain in the milled sample but not in the quenched. The presence of seed crystals accelerates the rate of crystallization of amorphous materials. In order to explore the effect of seed crystals small amounts of crystalline material were added to the amorphous samples. Figure 3 shows the isothermal microcalorimetry traces for mixtures of ground UDCA and crystalline UDCA (seed crystals) exposed to the vapour of a 10% ethanol solution at 25°C. Different ratios of amorphous:crystalline material resulted in crystallization onsets at different times, however, this could simply be due to the different quantities of amorphous material present, rather than being due to the seed crystals speeding the process (this effect could be investigated further, but is not the main subject of this study). Mixtures of quenched and crystalline UDCA did not produce a crystallization response following exposure to humid or ethanolic air. It follows that there was no interaction between quenched and crystalline UDCA, meaning that the seeding crystals could not be a nucleus.

AS well as mixing amorphous and crystalline material, mixtures of the milled and quenched amorphous samples were also prepared. The isothermal microcalorimetry traces for the mixtures of ground and quenched UDCA at 25°C are shown in Fig. 4. The heat associated with crystallization and the lag time before crystallization increased with an increase in the ground UDCA content. These samples were completely amorphous, however, the heats associated with crystallization were less than that of 100% ground sample. In Fig. 5 the heat associated with crystallization per mg of the milled amorphous material is plotted for the mixtures of both the ground with crystalline, and ground with quenched material. The heat recorded per mg of ground material was the same as that recorded for 100% ground material for each of the mixtures, except for that with the lowest amorphous content. The reason for the low reading for the low amorphous content mixture was probably because part of the evolved heat was not measured, as the crystallization started before the ampoule could be loaded into the measuring site of the calorimeter (samples with low amorphous contents usually crystallize most rapidly). However, for the mix of quenched and milled amorphous material (Fig. 6), rather than inducing the crystallization of the quenched material, the effect of mixing the two different amorphous forms, in the presence of ethanolic vapour, was to provide a partial stabilization of the milled amorphous form. It can be seen that the heat output is substantially lower than that which would be expected for the crystallization of the milled sample alone. This is a very surprising observation, as even if the sample is monitored in the calorimeter for 24 hours the observed heat output is not equivalent to that which would be expected if the milled content crystallized. The mixtures of the two amorphous forms were removed from the ampoules following exposure to ethanolic air in the microcalorimeter and examined using DSC. It was found that a crystallization peak was observed in the DSC, which is believed to be crystallization of the remaining amorphous milled (but not the quenched) material. The protection offered by the quenched material, whilst not fully understood, is probably related to the competition for the ethanol vapour, which will be absorbed into the quenched sample.

In order to obtain a better understanding of the absorption properties of the two amorphous forms, water vapour sorption isotherms of UDCA samples were prepared at 25 and 40°C (Fig. 6). The isotherm for the crystalline sample exhibits negligible sorption because UDCA crystals have no (very limited) hygroscopicity. The isotherms for the ground and quenched samples showed sorption of water which is far more than could be accommodated by adsorption. At 25°C (Fig. 6a) the water uptake was greater at low RH for the quenched material, which would be indicative of a more hydrophilic sample. However, in the range from 20% to 95% RH, the water vapor uptake of the ground sample was greater than that of the quenched sample.

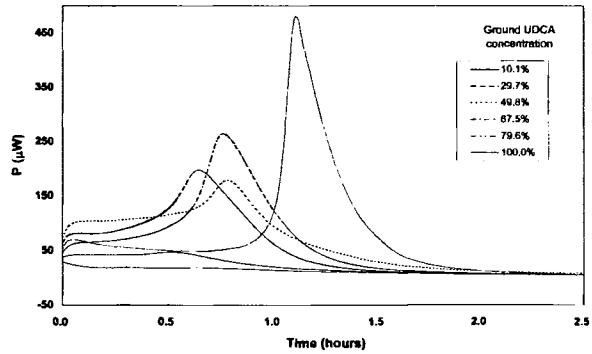

Fig. 4. Isothermal microcalorimetry traces of the mixture of ground and quenched UDCA at 25°C total sample size, 30 mg; ethanol concentration, 10%.

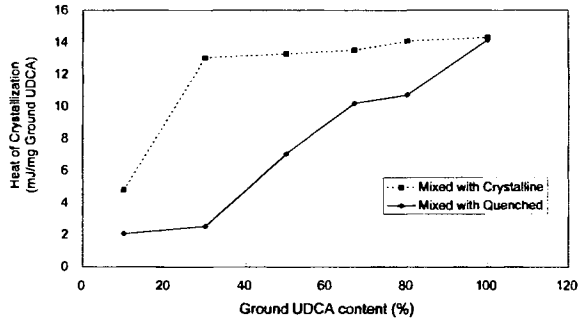

Fig. 5. Relationship between ground UDCA content of sample and heat of crystallization.

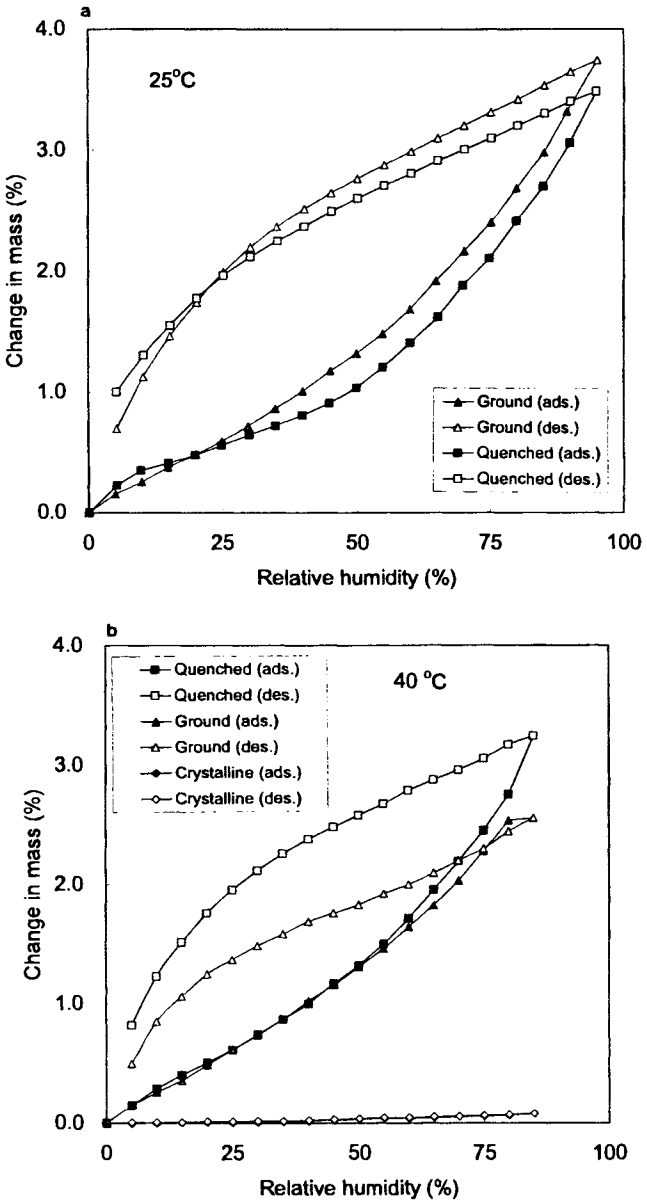

Fig. 6. Water vapor sorption isotherms of UDCA samples. (a) 25°C (b) 40°C.

At 40°C, the water uptake of the quenched sample was greater than that of ground sample above 50% RH, and crystallization of the ground sample was observed during the sorption process.

The specific surface area of the crystalline sample was 2.13 m^2/g, and that of ground and quenched samples were 5.99 and 0.16 m^2/g, respectively. As expected the surface areas do not correlate with the water sorption behaviour, as the water uptake was by absorption into the bulk of the amorphous forms.

The data show that the lack of crystallization seen for the quenched sample is not due to an inability to allow sorption of vapour.

A Change in Surface Character

The data described above show that the two amorphous forms have different stability, possibly due to a disruption of molecular packing of the quenched sample. The water sorption data at low RH may indicate that the quenched material is more hydrophilic than the milled. A possible reason for a more hydrophilic surface for the quenched material would be the fact that the quenched material should be more disordered than the milled one. Increasing the disorder of surface may decrease the probability of nucleation. If this were so it could well explain the apparent increased activation energy needed to induce nucleation. To test this hypothesis, contact angles were measured on the different samples, and surface energies were calculated (Table 1). It can be seen that the surface nature of the milled material is identical to that of the crystalline sample (a relatively hydrophobic surface), whereas the surface of the quenched material is much more polar, and very different from the other two. It can be concluded that the surface of the quenched material has been well disrupted, thus exposing more hydrophilic functional groups, whereas the ground sample must have the same balance of functional groups exposed as in the crystalline state.

A further interest is whether it is just the surface of the quenched material that is different or is there a substantial change in the packing, compared with the milled sample. This is somewhat difficult to prove, however spectroscopic techniques offer some help.

The Bulk Character of the Amorphous Forms

The NIR spectra for these sample are shown in Fig. 7a. It can be seen that all the samples have differences around 1450 and 2100 nm. However, no significant spectral difference can be seen in the C-H related peaks that are located around 1100, 1750, and 2300 nm. The crystalline sample has peaks due to the first overtone O-H at 1452 nm and the C-O stretching/O-H deformation combination at 2088 nm (10). The first overtone O-H peak intensity of crystalline sample was decreased by grinding and quenching. The peak observed at 2088 nm for the crystalline sample was also present in the ground sample, however it was replaced by a peak at 2060 nm after quenching. In the crystalline state, there is hydrogen bonding between each UDCA molecule. The spectral difference between these samples could be related to a change in hydrogen bonding. The NIR spectra of the UDCA samples after storage at 75% RH for 1 month are shown in Fig. 7 b. No significant difference was observed in the crystalline sample because of the lack of hygroscopicity of the crystals. A peak due to absorbed water was observed in both the ground and quenched samples at around 1450 nm. A further peak related to water sorption was observed in the ground sample at 1930 nm; this peak was at a lower wavelength for the quenched sample. This reveals a different water-UDCA interaction when water is absorbed into the two amorphous samples. This change must be a consequence of structural differences between the two amorphous solids.

Table 1. Surface Energy Component Values of UDCA Samples

	γ_s^{TOT}	γ_s^d (mJ/m^2)	γ_s^p
Crystalline	19.2	13.9	5.3
Ground	22.9	15.1	7.8
Quenched	23.6	9.3	14.3

Note: TOT, total; d, dispersion; p, polar component.

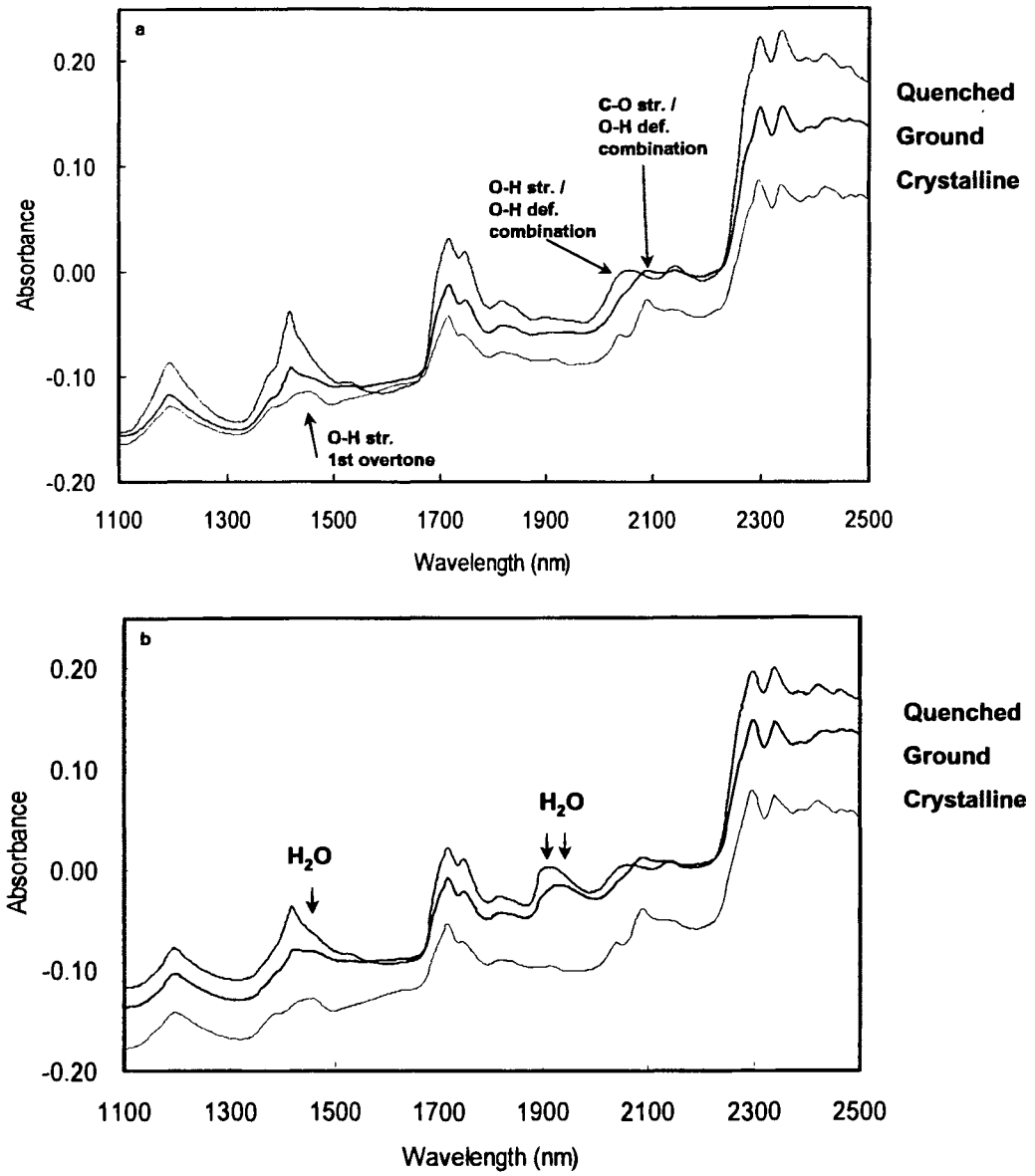

Fig. 7. NIR spectra of UDCA samples. (a) before storage, and (b) after stored at 75% RH, 20°C for 1 month.

Whilst the NIR data show that there are substantial differences between the quenched and milled amorphous forms, it is not possible to conclude whether these differences are just at the surface or throughout the sample.

CONCLUSIONS

UDCA was made amorphous by milling and by quenching into ice from the melt. The two methods resulted in different molecular states of amorphous UDCA. Only the ground sample crystallised when heated or when exposed to ethanol vapour. Seeding with crystalline material did not cause the quenched form to crystallise. At present we have been unable to obtain crystals from the quenched form through a solid state transition, however, when dissolved in aqueous solution results show that some crystals are formed.

The two amorphous forms had differences in hygroscopicity, and the quenched material had a much more polar surface. NIR revealed that the two amorphous forms had spectral differences in the dry state, probably due to changes in internal hydrogen bonding. There were also differences in the way the two amorphous forms bond with absorbed water, which again would relate to changes in internal structure.

It has been shown that two amorphous forms of the same drug have very different physical properties. The best explanation for the differences would be a due to substantial disruption for the quenched sample, which has not happened for the milled sample, and which makes the quenched sample less like the crystalline state. Our hypothesis is that the milled sample has lost long range order, but retains molecules in similar positions to that seen in the crystalline form, whereas the quenched sample has molecules distributed much more randomly than in the milled form.

The fact that two amorphous forms have different physical stabilities and different physical properties raises important

issues for drug substances and makes it important not to regard the amorphous state as if it described just one type of material with one type of physical properties. This work has shown that it is conceptually possible to form amorphous material with increased physical stability, which may be advantageous if it is to be included in dosage forms.

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