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The quantification of small degrees of disorder in lactose using solution calorimetry

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Abstract

There is a realisation that small quantities of amorphous material can have a significant impact on the properties of crystalline solids. Consequently there is a growing interest in quantifying the amount of amorphous material that is present in "crystalline powders". Success has been reported when using isothermal microcalorimetry and vapour sorption techniques, however, the use of solution calorimetry has largely been ignored. In this study the enthalpies of solution of mixtures of amorphous and crystalline lactose are reported concentrating on the range 0–10% w/w amorphous content. It was found that there was a possible error due to water vapour penetration into the ampoule, resulting in crystallisation of the amorphous content, however this was overcome by double sealing the ampoules with wax. Subsequently there was a good correlation between the enthalpy of solution and the amorphous content, which was not adversely affected by stirring rate used during the experiment. Over the range from 0 to 10% amorphous content, quantification of the amorphous content of an unknown would be good to ± 0.5%. The effects of residual moisture retained within a sample were also investigated. Storage at 33% or 43% RH resulted in a much reduced wetting (exothermic) response compared with that seen for completely dry samples, which in turn led to a higher net enthalpy of solution. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

It has been widely accepted that pharmaceutical processing is often responsible for the induction of small amounts of amorphous material in crystalline samples. Although the amorphous content may be a small percentage of the total mass, and thus apparently insignificant, it is now acknowledged that it is capable of affecting that material's behaviour. This phenomenon has been explained on the basis that processing induced disorder results in the amorphous material residing primarily on a particle surface thus making the surface entirely amorphous and consequently altering interfacial interactions.

The accurate quantification of amorphous material in 'crystals' has been the goal of many

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workers. Long-standing techniques such as X-Ray Powder Diffraction (XRPD) and Differential Scanning Calorimetry (DSC) are known to be capable of quantifying amorphous contents at 10% w/w and above (Saleki-Gerhardt et al., 1994). However, processing-induced material regularly comprises only a fraction of that amount, and as such, techniques with much lower detection limits have been sought. Isothermal Microcalorimetry (IMC) has been investigated to this end.

When providing amorphous or partially amorphous samples with the environment in which they are capable of crystallising rapidly, the thermal event related to the crystallisation can be monitored, from which an apparent enthalpy of crystallisation may be calculated. This technique, which has become known as the 'mini-hygrostat' approach as it is usual to expose the sample to a defined humidity in the sealed ampoule, has proved useful in detecting amorphous contents below 0.5% w/w (Buckton and Darcy, 1999), however, there has been no great success in using this method to quantify the amorphous content. There are two basic problems with the use of the 'mini-hygrostat' isothermal microcalorimetry method. The first is the difficulty in achieving full equilibration of the sample before crystallisation commences. Despite increasing the sample mass to the maximum which can be contained within the 3 ml ampoule, and ensuring that pre-equilibration of the ampoule components and sample to the temperature at which crystallisation will occur is carried out, those samples of approx. 5% w/w amorphous content or less have proved extremely difficult to quantify. The second problem is that more than one process will make a major contribution to the calorimetric response. Darcy and Buckton (1998) have noted that the extent of water desorption during the crystallisation event will contribute to the overall magnitude of the measured response. Consequently, there is a need for a better technique when the quantification of amorphous content is required. Possible alternatives for identification and characterisation of amorphous contents include gravimetric vapour sorption and near IR spectroscopy. The use of these techniques has been discussed by Buckton and Darcy (1999). One method that has not been studied to any great extent is solution calorimetry.

Solution calorimetry offers an alternative thermal analysis technique with which to attempt the quantification of these problematic partially amorphous samples. First applied pharmaceutically in the 1970s (Pikal et al., 1978), the principle of the technique is to create a system within which very small enthalpy changes in a liquid system due to dispersion or dissolution may be recorded as a function of time. The theory behind the technique was explained in Gao and Rytting (1997). The enthalpy of solution of any particular solute in one of its solvents can be measured. The two-phase system comprises 100 ml of solvent and a known amount of solute (solid or liquid), which are housed together in the reaction vessel and equilibrated to the temperature at which the reaction is to take place. The solute is sealed in a small fragile glass ampoule and this is immersed into the solvent. The glass ampoule prevents dissolution until thermal equilibrium has been established. Following equilibration the glass ampoule may be broken, thus initiating the reaction and allowing the enthalpy of solution to be measured.

Solution calorimetry is a suitable technique for the study of liquid–liquid and solid–liquid interactions. It has already been shown capable of quantifying the crystallinity of sucrose between 0–100% w/w (Gao and Rytting, 1997). However, the suitability of this technique for the quantification of small amounts of amorphous content has yet to be reported. For the purposes of this work, the solution enthalpies of samples of less than 10% w/w amorphous content were studied in order to establish the resolution which solution calorimetry offers in studying materials of small degrees of disorder. Furthermore, the impact of sorbed water on the measured enthalpy of solution was also investigated.

2. Materials and method

2.1. Preparation of amorphous lactose

α-Lactose monohydrate was supplied by Borculo Whey Products UK Ltd, Cheshire, UK. Production of amorphous lactose was achieved by spray-drying a 10% w/v aqueous lactose solution in a Buchi 190 spray drier, following the parameters set out in Chidavaenzi et al. (1997). The yield was collected immediately and desiccated over phosphorous pentoxide (Avocado, UK) at 20°C. Confirmation that the yield was in fact entirely amorphous was obtained from XRPD (Philips PW3710 X-ray powder diffractometer).

2.2. Preparation of amorphous lactose mixes

Quantities of dried amorphous and crystalline lactose were sieved to produce a particle size of less than 425 μm and then accurately weighed and combined to produce powder mixes of 0–10% w/w amorphous lactose, increasing in increments of 1%. The samples were subsequently mixed in a Turbula mixer for 20 min to ensure a homogeneous amorphous content. Sample storage was at 0% relative humidity (RH) in a desiccator over phosphorous pentoxide, at room temperature (20°C).

2.3. Solution calorimetry

Enthalpies of solution of lactose mixes of various amorphous contents were measured using the Thermometric 2225 Precision Solution Calorimeter (Thermometric AB, Sweden), an instrument designed for use in conjunction with a Thermometric 2277 Thermal Activity Monitor (TAM). Data were collected by measuring a temperature offset (°C) from a predefined zero point (actually the temperature of the air bath of the TAM, in this case 25.000 ± 0.0001°C). Before and/or after the dissolution reaction takes place, the system is calibrated electrically, i.e. a known amount of energy is added to the system to match the anticipated thermal energy accompanying a reaction (Fig. 1). The small heat exchange between the environment and the vessel during the reaction and the heat arising from stirring are adjusted mathematically using the information obtained from the baseline temperature changes before and after the experiment.

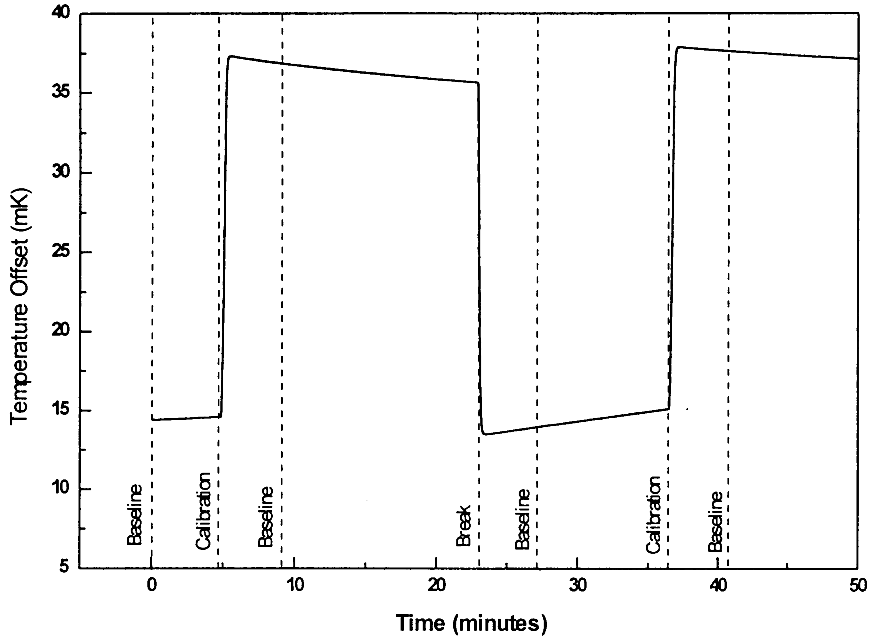

Fig. 1. Plot of temperature offset against time for a 5% w/w amorphous lactose sample, with a stirring rate of 600 rpm. This trace shows the calibration sections of the experiment, which occur before and after the ampoule is broken, as well as the baseline periods, which aid calculations to adjust for any heat lost to or gained from the water bath, as well as the heat generated by the stirrer. Following the breaking of the ampoule, a drop in temperature is seen, implying that the response in this case is endothermic.

The outer part of the calorimetric vessel is a stainless steel cylinder permanently mounted in a precision water bath. The actual calorimeter consists of a thin-walled 100 ml pyrex-glass reaction vessel fitted with a thermistor for sensing temperature and a heater for calibration and equilibration purposes. It is a semi-adiabatic calorimeter with temperature resolution, after noise reduction, close to 1 μK, which corresponds to a heat resolution of 1–4 mJ in a 100 ml reaction vessel. The time constant of the system is 2.22 h.

A glass ampoule containing approx. 200 mg of sample (accurately weighed) was sealed with a silicone bung and bees wax and loaded into the stirrer unit of the solution calorimeter. This was then inserted into the glass vessel containing exactly 100 ml of solvent (distilled water), the glass ampoule holding the solute isolated from the solvent. The combined unit was then lowered into the solution calorimeter channel of the TAM, initially being held in the equilibration position of the channel, to maintain contact with the water bath of the TAM. Upon equilibration to approx. 25°C, the unit was lowered into the experimental position, in contact with the air bath, and allowed to further equilibrate. Upon completion, an electrical calibration was undertaken and then the ampoule was broken, releasing the lactose into the water and allowing dissolution. The ensuing heat change was detected by a thermistor within the vessel, enabling measurement of the enthalpy of solution.

2.4. Vapour sorption study

Four different storage conditions were employed in order to investigate the effect of residual moisture content on the enthalpy of solution obtained. Two supposedly dry conditions were used — in a vacuum oven (Sanyo-Gallenkamp) at 50°C and at room temperature in a desiccator over phosphorous pentoxide. In addition two elevated RH environments were created by storage in a desiccator at room temperature over saturated salt solutions — 33% RH was produced by Magnesium Chloride (Aldrich); 43% RH was produced by Potassium Carbonate (Aldrich). In all cases the storage period was for 72 h. The filled ampoules were weighed (along with a sealing bung) immediately before storage and then at the end of storage the bung was inserted to seal the ampoule and the sample was weighed again to monitor the weight change. Subsequently the bung was double sealed with bees wax.

3. Results and discussion

3.1. Characterisation of enthalpic response

It can be seen from the results obtained in this study that the enthalpy of solution increases in a linear fashion with decreasing amorphous content (see discussion below). The heat of solution of 100% amorphous lactose exhibited an exothermic response (− 56.50 J/g), whilst the heat of solution of 100% crystalline lactose was endothermic (56.2 J/g). When a mixture containing 50% w/w amorphous and 50% w/w crystalline lactose was dissolved, an initial exothermic response was observed, followed by an endothermic response. This was due to the fact that amorphous lactose is more readily soluble in water than the crystalline form, so the exotherm for dissolution of the amorphous form is seen before the endotherm for the dissolution of the crystalline material. The bulk of this study relates to samples with an amorphous content in the range 0–10% and for these samples only a net endothermic response was observed.

The net measured response for the enthalpy of solution is a summation of numerous component parts. The first stage will be wetting of the powder, followed by dissolution, which will involve the disruption of the bonding between the solid molecules and the formation of bonds between the solute and the solvent. There will also be rearrangement of bonding within the solvent in order to accommodate the solute. Consequently the single number for the enthalpy of solution must be viewed with some caution.

3.2. Sealing of ampoules

Preliminary observations showed that the results followed two trends (data not shown). One trend was the expected notable decrease in enthalpy of solution with increasing amorphous content in the sample. However, some samples did not fit to this trend, and tended to be closer to the enthalpy of solution of the crystalline form, this type of response was more common in samples that had been equilibrated in the calorimeter overnight. There was no indication of penetration of liquid water into the ampoule even after overnight storage. However, it was observed that samples, especially if the sealed ampoule had been equilibrated in the water for a prolonged time, were tending to crystallise. This was attributed to water vapour passing into the ampoule through the wax covered sealed hole of the ampoule. It follows that the response observed for these samples was in fact that of a 100% crystalline sample. Subsequently all ampoules were sealed with wax twice, in order to eliminate water vapour penetration. The first layer of wax was allowed to set hard before the second layer was applied, resulting in satisfactory sealing. All data reported below were collected using this double sealing method.

3.3. Adjusting for additional heat generation

The significance of the effect of the heat generated by the stirrer was studied. It was observed that when the stirrer was operating at 300 rotations per minute (rpm) the equilibration temperature of the vessel in the air bath was 25.0000 ± 0.0001°C, but if the stirrer rate was increased to 600 rpm, that equilibration temperature was higher, at 25.0259 ± 0.0001°C. The impact of this raised equilibrium temperature was investigated by using both stirring rates on the same samples. There was no difference between the enthalpies of solution that resulted from increasing the rate from 300 to 600 rpm (data not shown). This indicates that the calibrations and baselines included in the method were capable of adjusting for the additional heat generated by the stirrer. As expected the dissolution occurred much more quickly at the higher stirring speed (the average time of dissolution was approx. 8 min at 300 rpm and < 3 min at 600 rpm).

3.4. Effect of sample mass

The effect of sample size has also been investigated. The normal fill mass of approx. 200 mg was halved in order to ascertain the influence of fill mass and/or volume on the enthalpies recorded. No effect at all was detected, indicating that as long as the solute is freely soluble in the solvent in the quantities being used, uniformity of sample size is not paramount. Obviously, if the solute were found to be sparingly soluble, this issue would be of greater importance.

4. Effect of water sorption on the enthalpy of solution

All calorimetric experiments have the potential to measure processes other than the one which is the intended subject of the investigation. In the case of enthalpy of solution measurement, there is inevitably a contribution due to the initial wetting of the powder surface prior to dissolution. It is indeed possible to use a non-solvent system in order to measure the enthalpy of immersion (powder wetting) in a solution calorimeter. Hollenbeck et al. (1978) have shown that the measured enthalpy of immersion of microcrystalline cellulose changes depended on the extent of sorbed water in the sample prior to sealing the ampoule. The sorption of water by a powder is the first stage of wetting, indeed it is argued that the sorption of the first few layers of molecules on the surface is the greatest part of the overall wetting response.

Amorphous lactose is able to sorb substantial amounts of water and this would be expected to alter the initial wetting (immersion) response and thus change the net enthalpy of solution value. It is important to know to what extent the enthalpy of solution may change as a function of water content, as this will influence the confidence in data that are generated for partially amorphous samples, that potentially have different water contents.

Individual ampoules were filled with 100% amorphous material that had been stored desiccated over phosphorous pentoxide at 20°C. The filled ampoules, each with an identified weight of amorphous lactose, were then stored for a further

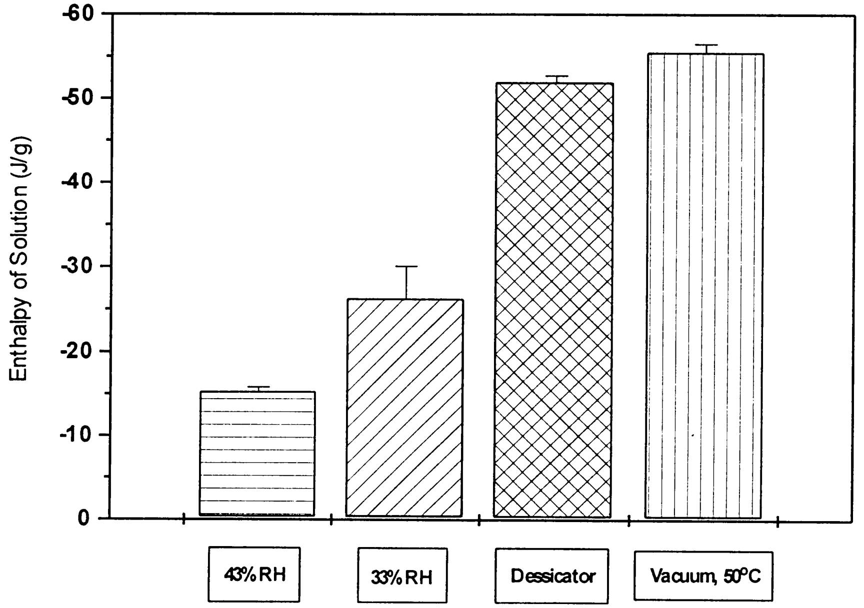

Fig. 2. Enthalpies of solution for samples of 100% amorphous lactose stored under different conditions.

period of 72 h either in a vacuum oven at 50°C, desiccated over phosphorous pentoxide at 20°C, or in desiccators at either 33% RH or 43% RH at 20°C. These humidities were selected as they were high enough to cause substantial water sorption into an amorphous material, but not high enough to induce crystallisation over the time of storage used (for the given sample mass in the given container geometry). Some ampoules were then rapidly double sealed with wax as described previously and their enthalpies of solution were then recorded (Fig. 2). The effect of water sorption was seen as a dramatic increase in the enthalpy of solution, the trend of which was in keeping with the sample having been progressively more wetted during storage at the more elevated humidities. Wetting is an exothermic response and in a sample that has already sorbed some moisture from the environment, the enthalpy of solution will be significantly different. Sorption of water prior to enthalpy of solution measurement results in a less exothermic response than would be observed for a completely dry sample.

Upon storage in the vacuum oven for 72 h, samples were seen to have undergone a reduction in mass of approx. 0.91%, which would indicate that the vacuum oven displaced more water than storage in a desiccator. Re-weighing samples after storage at elevated RH facilitated the quantification of sorbed water under these conditions. At 33% RH, a 4.87% w/w increase was observed, whilst at 43% RH, a 7.08% w/w mass gain was noted. The relationship between water content of the amorphous sample and the measured enthalpy of solution is shown in Fig. 3. There is approximately a 5 J/g reduction in the net response for each 1% of water that is absorbed.

It is often assumed that the wetting of amorphous materials by vapours is reasonably similar to the enthalpy of condensation of water. The enthalpy of vapourisation of water (numerically the same as the enthalpy of condensation, except with a changed sign) is 40.66 kJ/mol (CRC Handbook of Chemistry, 1999). The total enthalpy change expected for sorption of 7.08% water content in 1 g of powder (0.0708 g or 0.0040 mol of water) would be -163 J. As the total change in enthalpy of solution following the sorption of 7.08% water was 40.1 J/g between the dry and the most wetted sample, this is about one quarter of the equivalent enthalpy of condensation. This apparent difference requires further investigation.

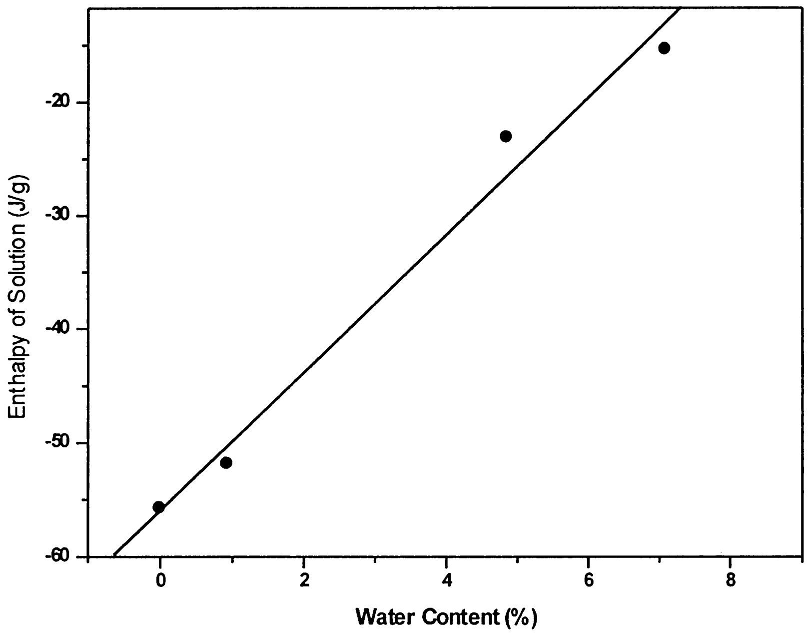

Fig. 3. Water content of 100% amorphous lactose stored under different conditions, against enthalpy of dissolution.

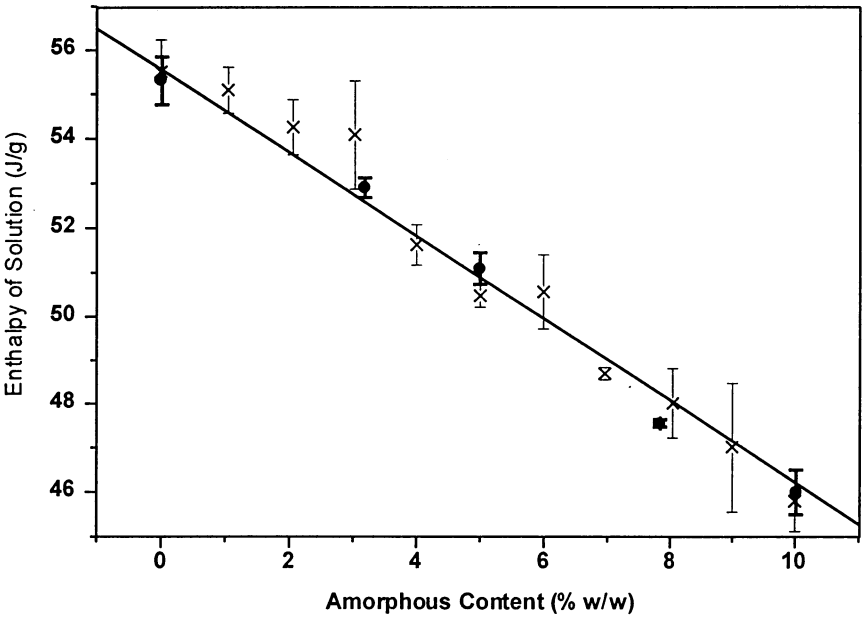

Fig. 4. Average enthalpies of solution and standard deviations for samples stored under two conditions: solid circle = under vacuum at 50°C; × = desiccated over phosphorous pentoxide at 20°C. The line represents the best fit for the vacuum-stored data, with a correlation of $R = 0.991$.

5. Quantification of the amorphous content

The data in Fig. 4 show that it is possible to differentiate between the enthalpies of solution for samples with amorphous contents ranging from 0 to 10%. To calculate the accuracy of this technique for amorphous content quantification, the standard deviation for enthalpies of solution were calculated for both the vacuum-dried and desiccated samples (Fig. 4). The enthalpy of solution of the upper and lower limits of each error bar was then used to calculate the amorphous content that would correspond to that enthalpy of solution on the best fit line for the vacuum oven dried samples. The differences between the amorphous content of the mixtures and the best fit line were then averaged to give a mean error, which is taken to be the limit of reliability for the technique in determining amorphous content. Based upon the results obtained the amorphous content of samples stored desiccated was accurate to within 1.0% w/w as opposed to 0.5% w/w for those stored under vacuum. The increased variability in those results of the desiccated samples can be attributed to the higher level of residual moisture contained within them. It should be remembered, however, that a proportion of the error in this low amorphous content region could relate to weighing and preparing a uniform mix containing such small amounts of the amorphous material

The quantification limits calculated here for lactose will not be the same for all materials, as the sensitivity will depend upon the difference in enthalpy of solution between the amorphous and the crystalline forms. Obviously the larger the difference the greater will be the sensitivity for quantification and vice versa.

6. Conclusion

Solution calorimetry provides a good method by which to detect and quantify the amorphous composition of materials that have small amorphous contents. In addition, it has been shown capable of differentiating between samples of different moisture content and providing an indication of how moisture sorption contributes to the thermodynamics of the solution process.

The results obtained have illustrated that sample storage is capable of impacting greatly upon the enthalpy of solution obtained. It is clear that hygroscopic materials such as lactose are liable to produce erratic results if storage conditions are not controlled. Residual water is capable of distorting results, and the greatest accuracy in quantification of amorphous content can only be achieved if samples have been maintained in an extremely dry environment.

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