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Assessment of disorder in crystalline powders—a review of analytical techniques and their application

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Abstract

The need to be able to measure amorphous contents in crystalline powders is now recognised. In this review, calorimetric and gravimetric methods are reviewed in a way that should alert workers in the field to the theoretical, and practical considerations which are important to understanding how best to study crystalline samples which contain low levels of amorphous material. It is shown that vapour sorption techniques are very powerful as long as serious consideration is given to the choice of environmental conditions and the exact experimental methodology. As the amount of published work in this field grows, it becomes increasingly necessary to describe experimental and data manipulation methods in great detail. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Amorphous; Microcalorimetry; Gravimetric sorption; Inverse phase gas chromatography; Near infra-red; Glass transition; Collapse

1. Introduction

In recent years, there has been a growing realisation that 'crystalline' powders can have varying amounts of disorder in their structures. The presence of amorphous materials is known to be of great significance for reasons which will be outlined briefly below. However, the purpose of this review is not to demonstrate the importance of the amorphous content of powders (see Hancock and Zografi, 1997 and Byrn et al. 1995), but rather to give a critical review of some of the techniques which can be used to characterise partially amorphous materials. Thus, the aim of this review is to provide workers with a greater understanding of what is measured by the different techniques, so that they can be used in an appropriate manner.

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2. Why is amorphous content important?

Amorphous regions in crystals are generally thermodynamically unstable, i.e. they are in a higher energy state than the crystalline form. Within amorphous regions, there will be substantial absorption of water vapour (as will be discussed in detail below), which can cause physical and chemical transitions to occur. This means that the amorphous regions are reactive 'hot spots' in which physical changes and/or chemical degradation can be initiated. The amorphous material is formed either during crystallisation or subsequent processing. It is argued that the processing induced disruption is mostly at the powder surface, and as such a small amount of disorder (by weight of the sample) can be a very substantial amount of the powder surface. If the powder surface is essentially all amorphous, then the interaction between the powder and other phases will be different to that which was observed when the material was in the crystalline state. Alterations to interfacial interactions can affect many aspects during the production, storage and use of products. As processing induced disruption is seldom a deliberate and controlled event, it tends to lead to batch-to-batch variations, and as the disruption is unstable, recovery to the crystalline form will result in a change in properties with time after processing. Overall, there is an urgent need to characterise materials for amorphous content and to understand how this relates to the functionality of the material in the subsequent processes and in the product for which it is to be used.

3. Many bulk techniques are inappropriate

Bulk analytical techniques, such as differential scanning calorimetry, powder X-ray diffraction and IR spectra, will measure the properties of the sample as a whole. The detection limits for amorphous content with such techniques can vary, but will generally have a lower cut off of 5–10% (Saleki-Gerhardt et al., 1994). This detection limit is due to the fact that these techniques measure the entire sample, thus, the amorphous content becomes a small part of the total signal, and consequently it is difficult to detect with confidence. It may be more appropriate to preferentially investigate the properties of the powder surface only, where amorphous material may predominate. A powerful way of investigating surface properties is by use of vapour sorption studies, which coincidentally also preferentially probe amorphous (over crystalline) regions due to absorption behaviour. Hancock and Zografi (1997) describe water sorption studies as the 'preferred means of studying pharmaceutical systems containing low levels (of amorphous material)'. The remainder of this review will concentrate on how such studies should be performed, and how the data can be used.

4. The amorphous to crystalline transition

For many materials the amorphous form is thermodynamically unstable. Any unstable system has to have a mechanism by which it can transfer to its stable (lowest energy) state. The activation barrier which needs to be passed in order to move to the stable state will determine whether the transition is spontaneous. The transition from the amorphous to the crystalline form will depend upon the mobility of the molecules. When below the glass transition temperature (T_g), the molecules lack sufficient mobility to allow spontaneous crystallisation (at least within the time scale of observation of in situ experiments). However, this does not mean that materials do not crystallise below T_g; there is simply an issue of the duration over which the system is observed and the activation energy needed to allow crystallisation to occur. For example, Yoshioka et al. (1994) reported crystallisation of indomethacin within a few weeks when stored at 30°C below T_g, but stability in excess of 1 year when stored at $\sim$ 50°C below T_g. Hancock et al. (1995) calculated that for all significant (i.e. capable of affecting a shelf-life) mobility to cease, indomethacin needed to be stored at least 50°C below T_g.

At the point when T_g drops to the experimental temperature (T), most amorphous (glass) materials have a viscosity of 10^{12}–10^{14} Pa S (Levine and

Slade, 1988). In reality, this viscosity is too high to allow crystallisation to occur rapidly, thus, it is more usual to see crystallisation when T_g has been reduced below the experimental temperature. The first observation for particulate materials as T_g drops below T will be a collapse in the structure. Shalaev and Franks (1995) have described the softening temperature as being the point at which collapse of structures under gravity will occur within a short period of time. The collapse occurs (at a rapid rate) when the viscosity drops to $\sim 10^8$ Pa S, which for carbohydrates is when $T_g/T = 0.9$. Further significance of this collapse will be discussed in detail below. Subsequent to the collapse event, the material will crystallise. Again the rate of onset of crystallisation is dependent upon the extent to which T_g has been reduced below T.

The difference between T_g and T can be adjusted either by adding a plasticiser to lower T_g, or by heating the sample to raise T. Many amorphous structures absorb water, which then acts as a plasticiser in order to lower T_g. However, certain hydrophobic materials may require alternative small molecules to absorb and lower T_g (these could include the vapours of ethanol or chloroform for example) (Ahmed et al., 1996). The use of absorbed vapours to lower T_g and hence cause collapse and crystallisation will be discussed below. The increase of T to cause collapse and crystallisation would be typical of what is observed when differential scanning calorimetry is used to study glass systems. Shalaev and Franks (1995) have reported DSC data for freeze dried sucrose showing the effects of thermocycling (Fig. 1). Here, the sample of amorphous sucrose was heated and cooled through the temperature cycle 280–343–280–345–280–365–280–370 K. Two transitions are observed T_1 (the glass transition) is reversible and is seen on each scan, whereas T_2 (collapse) is seen in the first scan to 365 K, but is absent on the second scan to such temperatures. Shalaev and Franks (1995) note that unlike the T_g, the collapse response in the DSC is not a true transition in the sense of a change in physical state, i.e. the collapse is not necessarily associated with a change in heat capacity of the sample, but more likely a consequence of the change in sample geometry and conductivity.

The DSC response of amorphous lactose differs depending on whether the sample has previously been allowed to collapse by exposure to increased RH. Fig. 2 shows typical responses for pre-collapsed and collapsed samples. The collapsed sample was prepared by exposure to water vapour, and as such the impact of that trapped water will be a major reason for the differences in the DSC response.

5. Isothermal microcalorimetry

Isothermal microcalorimetry is now widely available within the pharmaceutical sciences, both in academic and industrial laboratories. The calorimeter is a non-specific monitor of heat changes which occur within a small measuring site of the instrument. The calorimeter water bath maintains a temperature control of $\sim 10^{-4}$°C and by using the difference between the sample and reference cell, it is possible to detect temperature changes as small as 10^{-6}°C. The lack of specificity means that the calorimeter can be used to monitor almost any biological, chemical or

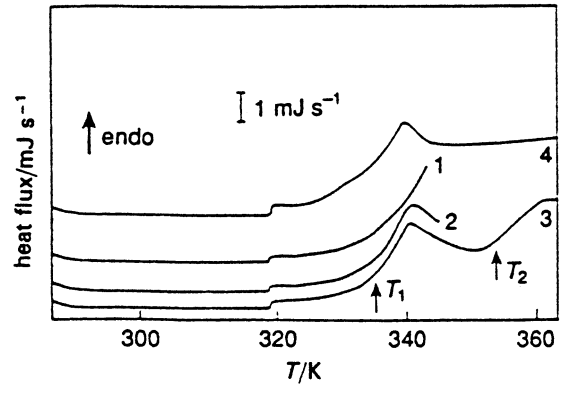


Fig. 1. DSC of collapse (reprinted from J. Chem. Soc. Faraday Trans., 91, E.Y. Shalaev and F. Franks, Structured glass transitions and thermophysical processes in amorphous carbohydrates and their supercritical solutions, pp. 1511–1517, Copyright (1995), with permission from Elsevier Science). (1) First upscan to 343 K; (2) Second upscan to 345 K; (3) Third upscan to 365 K; (4) Fourth upscan to 370 K; the first transition occurs on each occasion, the second transition (collapse) only occurs on scan 3, i.e. collapse does not occur at T_g, and is irreversible as it does not occur on scan 4.

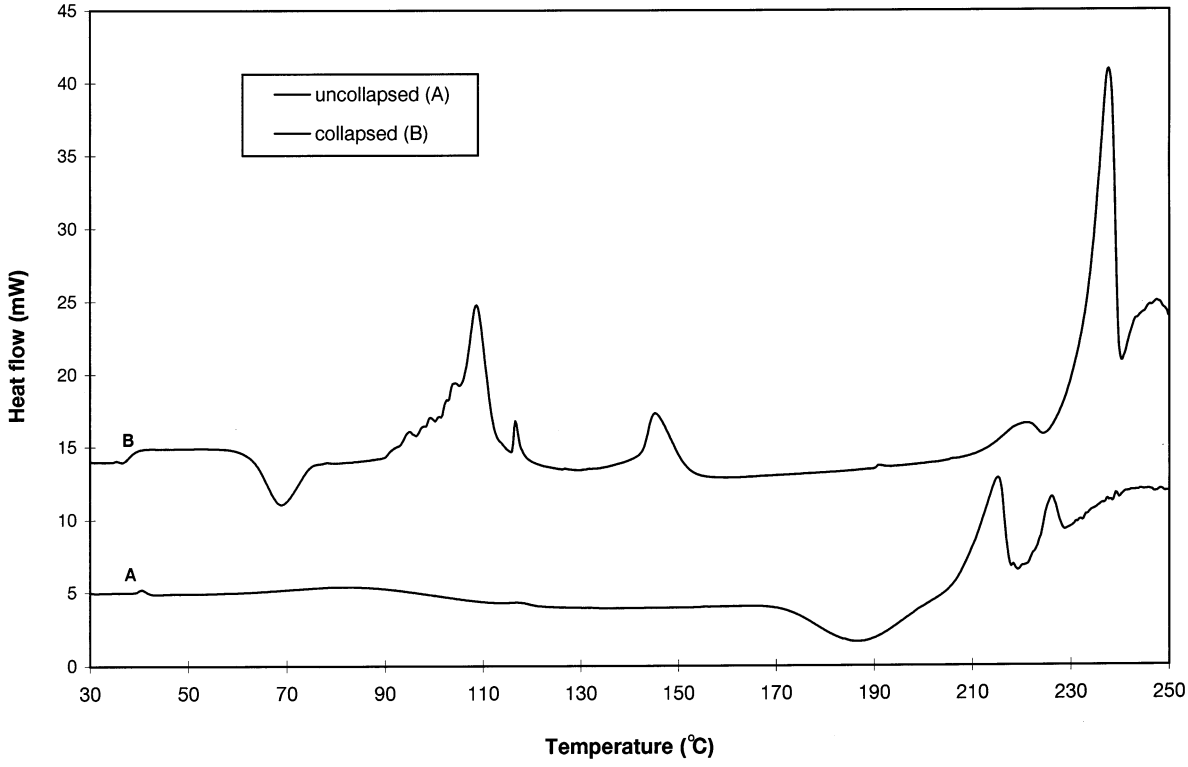


Fig. 2. DSC of amorphous lactose before and after collapse.

physical process (conceptually) and this gives a great advantage of versatility. The disadvantage is that the response of interest may well be masked by, or associated with, responses which are not of interest. The skill of the operator is therefore needed to devise experiments which limit artifacts due to parallel reactions.

Perhaps the simplest and most used calorimetric method to study amorphous material in powders, is to seal the powder in a glass ampoule with a small tube containing a saturated salt solution (Fig. 3). Saturated salt solutions will yield a defined relative humidity (RH) at any set temperature (Nyqvist, 1983)

5.1. How to interpret the shape of the response for the sealed ampoule experiment

A typical response for amorphous lactose equilibrated at 25°C 75% RH (saturated sodium chloride solution) is shown in Fig. 4. Fig. 4 has two distinct sections, an initial small protracted response followed by a large sharp peak. It is possible to delay the onset of the large sharp peak, to a certain extent, by either increasing the sample mass or by decreasing the RH (to a lower critical point, which is that RH which will allow sufficient absorption to cause crystallisation in a measurable time). Aso et al. (1995) have shown that the temperature and humidity used in the calorimetric experiment alter the rate of crystallisation, and they concluded that this was due to changes in matrix viscosity affecting the molecular motion of the drug under study (nifedipine).

For samples with lower amorphous contents, the large sharp peak is often seen to split into two separate regions. There is considerable debate concerning the significance of the different sections of the response and if logical decisions are to be made on how to interpret the data it is important to consider the processes which occur.

The initial slow peak (Fig. 4) is not due to crystallisation, as it is possible to stop the experiment at the end of this phase and show that the sample is still amorphous (by X-ray diffraction, and DSC). It has been argued (Sebhatu et al., 1994) that this region represents a vapour phase wetting of the powder (exotherm) minus a response for the generation of the humid air from the saturated salt solution (endotherm), such that the net response is small. It seems likely that this is too simplistic a description. Fig. 5 shows blank experiments (i.e. no powder load, just a freshly sealed empty ampoule as the reference and a freshly sealed ampoule containing a saturated salt solution in the sample side) for different saturated salt solutions. From the data in Fig. 5 it can be seen that changes in the salt solution have a significant contribution to the shape of the initial peak. These changes may reflect wetting of the cell, evaporation from the salt solution, and crystallisation of the salt following evaporation. These data raise the first key issue, which is what should be loaded as the reference cell? If the effect of different saturated salt solutions needs to be removed, then it is essential that the reference cell is a freshly sealed ampoule containing an equal amount of the salt solution in a tube of identical geometry to that in the sample cell. In any event it is essential when reporting these experiments to make it clear what was used as a reference, and to understand that changes in the choice of how to set up the reference cell will result in different measured responses.

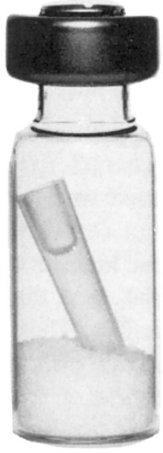


Fig. 3. An ampoule with saturated salt solution, allowing powders to be controlled at a desired humidity (Photograph supplied by Thermometric).

As well as contributions from wetting of the powder and the blank response in the cell, it is probable that transitions in the powder also make a contribution. Buckton and Darcy (1996) have shown that collapse occurs during this early stage of the response. Indeed a sample which had been collapsed (by exposure to 50% RH) did not show a response during this early period. Unfortunately, the process by which collapse was induced will result in retained absorbed water, thus it cannot be proved as to whether this initial response is due to the collapse process, the water sorption or (most probably) both. In conclusion, the initial response is a composite of wetting, collapse, and changes in the vapour space and the saturated salt solution. Thus an apparently simple thermal event is in fact a complex mixture of numerous different responses.

The large peak in Fig. 5 includes the response for crystallisation. This can be proved by showing that the sample is amorphous before, and crystalline after, this peak. It is noticeable that this peak is extremely sharp, i.e. a rapid rise in heat followed by a rapid decline. In Fig. 6, the shape of this crystallisation response is plotted with the decay of an electrical calibration signal. The electrically generated heat was switched off at the point when it matched the heat generated during crystallisation. It can be seen that the decay from the electrical heat is much faster than the decay for the crystallisation response. However the decay for the crystallisation event is fast compared to that which would be expected for an ongoing reaction, thus the crystallisation occurs rapidly once initiated. This peak is made up of at least two processes i.e. the crystallisation exotherm and the water desorption endotherm, however, it is probable that there will be further contributions from within the salt solution reservoir, and potentially some condensation of the desorbed water in the ampoule.

The fact that the measured crystallisation response is often split into two regions causes further confusion. For example, Sebhatu et al. (1994)

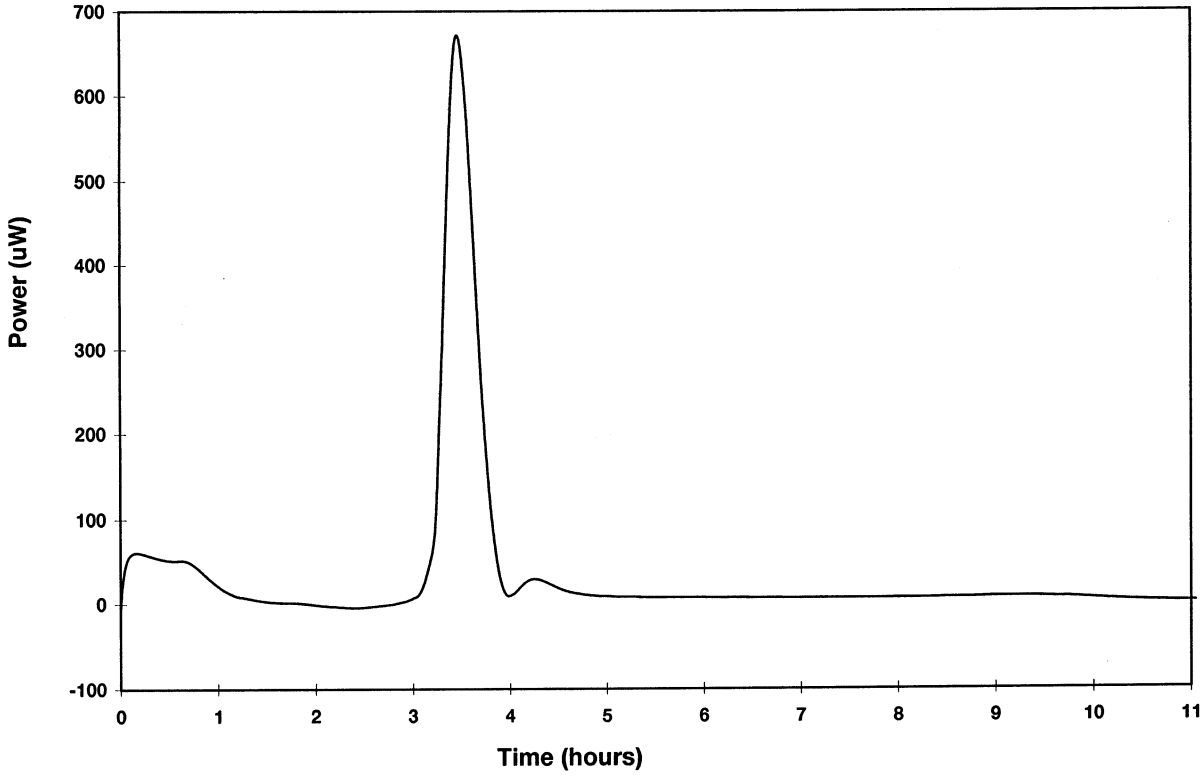


Fig. 4. Typical crystallisation response measured for an ampoule experiment in an isothermal microcalorimeter (75% RH 25°C) for amorphous lactose.

show a schematic version of the calorimetric response for the crystallisation of lactose (Fig. 7) and state that Part II is crystallisation and Part III may be mutarotation from β to α-lactose. They assess crystallinity by using the area under the curve for Part II, which equates to ~ 32 J/g. However, Briggner et al. (1994) and subsequent publications from our group, use the area under what would be Parts II and III to describe the heat change for the crystallisation process. This gives a measured value of ~ 48 J/g for the crystallisation of amorphous lactose. Work in our laboratory is currently directed to measuring the extent of mutarotation during crystallisation to ascertain what contribution this makes to the measured response. It should be noted that for samples which do not exhibit mutarotation, there is very clear evidence for two distinct peaks during crystallisation. This is very obvious during the crystallisation of salbutamol sulphate (Buckton et al., 1995), where a distinct endotherm is seen in the first half (before the peak reaches a maximum) of the crystallisation exotherm. Given that the contribution of mutarotation to the overall response is not yet proved, it cannot be certain as to which is the best way to treat the lactose data, however, the evidence from the salbutamol example makes it probable that Peaks II and III in Fig. 7 are both part of the crystallisation response.

Irrespective of whether the crystallisation response should be taken as Parts II and III or just Part II for lactose (see Fig. 7 and discussion above), it is clear that the crystallisation response is reduced in size due to the endotherm for water desorption. Recently Buckton and Darcy (1998) have shown that the measured calorimetric crystallisation peak, plus the enthalpy of vaporisation for an estimate of the amount of water desorbed (i.e. the quantity of water needed to plasticise T_g below T minus the quantity of water which is

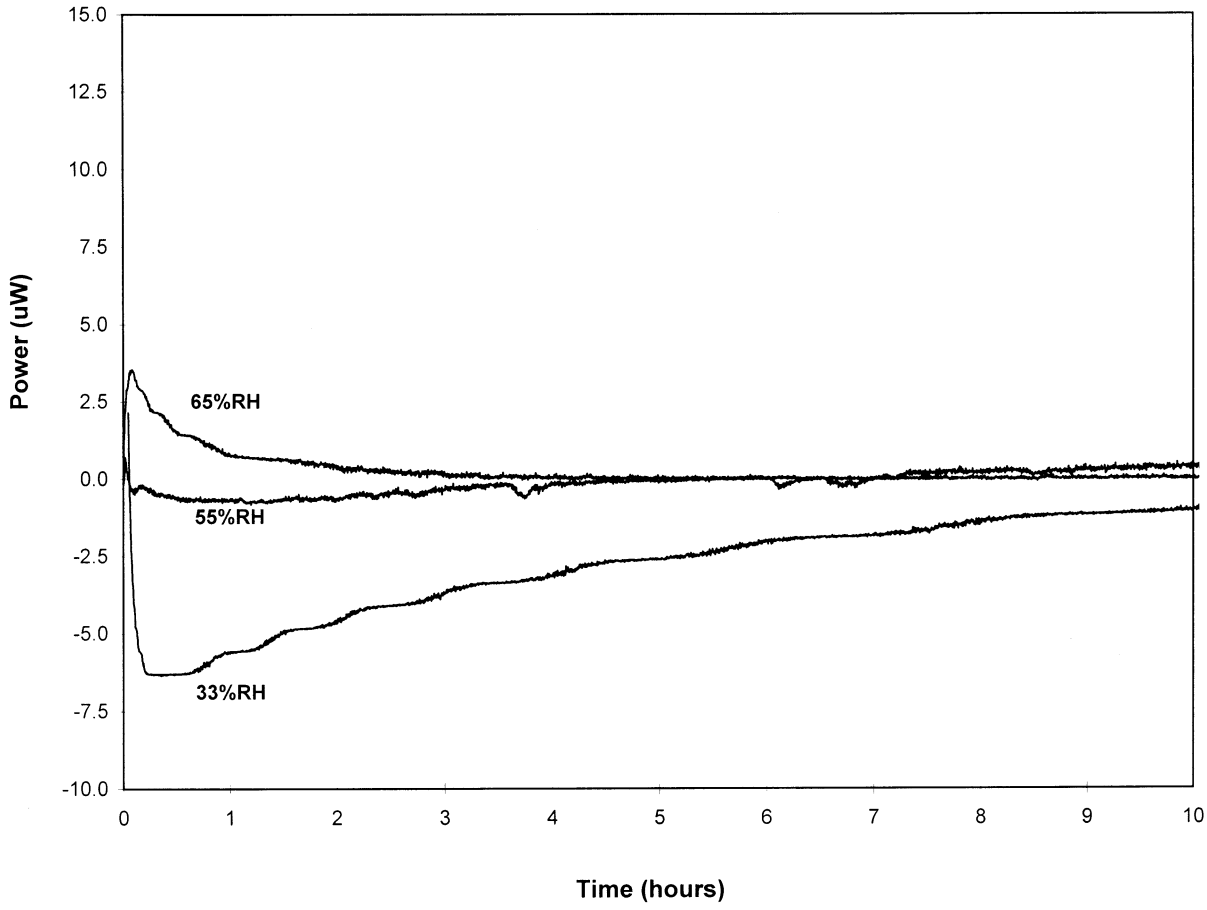


Fig. 5. Examples of blank responses for different saturated salt solutions being lowered into an isothermal microcalorimeter. The reference cell was a freshly sealed empty ampoule.

retained as a hydrate) approximates to the combined endotherms for hydrate loss and melting which are measured in a DSC for the crystallised lactose. This balance of heats would indicate that the contributions within the saturated salt solution are relatively small during the crystallisation response. It was concluded by Buckton and Darcy (1998) that because the net exotherm in the microcalorimeter was the same at each RH studied at 25°C, then the water desorption must have been similar at each RH. This means that the amount of water sorbed at each RH was the minimum quantity needed to cause rapid crystallisation. This would be the case if the supply of water vapour were slow, due to slow diffusion in the cell and the small surface area of the saturated salt solution. It can be shown that if the supply of water vapour is rapid and plentiful, then the amorphous lactose will equilibrate to a different water load at each RH, prior to crystallisation (see gravimetric sorption below). Bearing this in mind, it can be considered fortuitous that the experimental design resulted in a balance of kinetics for which the area under the curve at each RH was essentially identical for crystallisation at 25°C. This fortuitous situation held true for both amorphous lactose (Briggner et al., 1994) and amorphous salbutamol sulphate (Buckton et al., 1995). Buckton and Darcy (1998) went on to show that at higher temperatures there was a difference in the net area under the curve for different humidities (Table 1). This is due to the

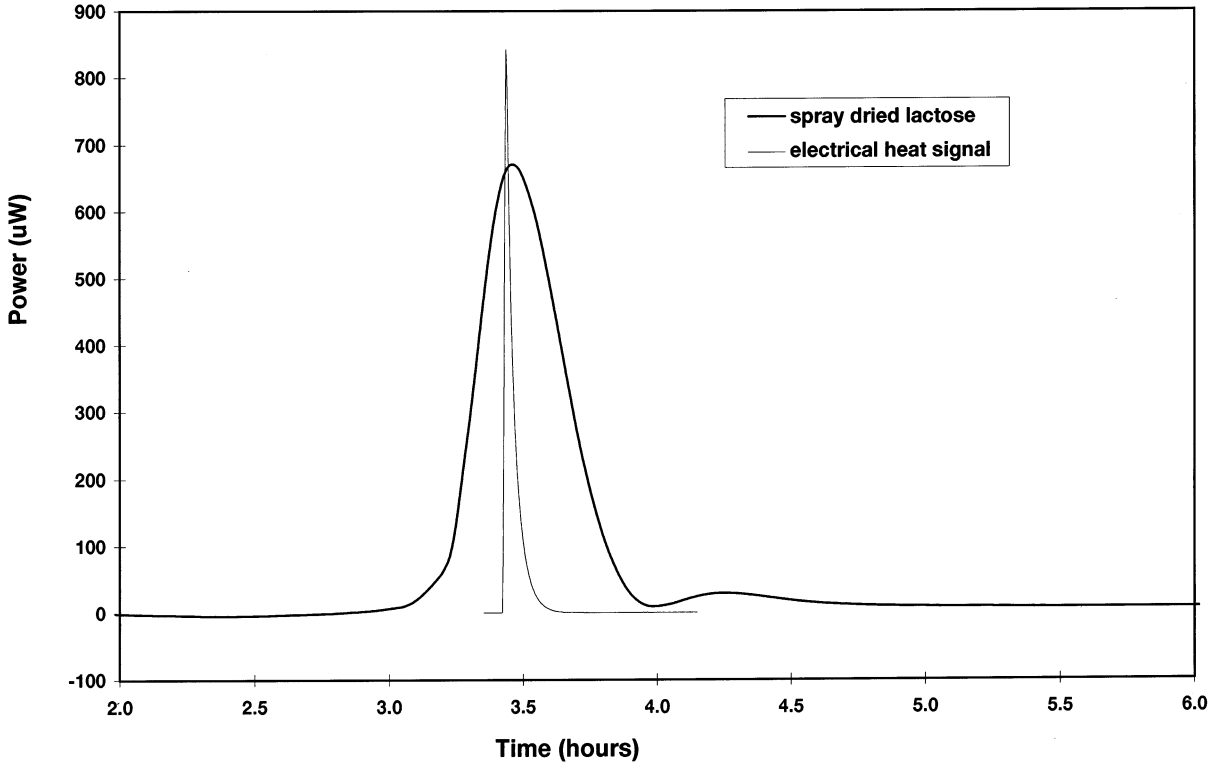


Fig. 6. The crystallisation response for 20 mg of amorphous lactose exposed to 75% RH compared with an electrically generated heat of the similar magnitude.

fact that the amount of water needed to lower T_g below T is less when T is higher, and also that the rate of evaporation and diffusion of the water vapour will increase with T. It is extremely important to realise that the calorimetric response for crystallisation contains this substantial balance of exotherm(s) and endotherm(s). If the data are to be used in a quantitative manner it is vital that the impact of changes in environmental conditions (temperature and/or RH) is understood, and that any comparison between data at different temperatures and humidities is undertaken with great care. It should be noted, for example, that the fact that lactose has a constant net area under the crystallisation peak(s) for any RH at 25°C is a consequence of the rate of supply of the water vapour and the viscosity/temperature relationship for lactose (which will determine the point at which crystallisation will occur rapidly).

A further difficulty with quantification of the degree of crystallinity is the choice of calibration standard. Calibration is carried out via a sample which is completely amorphous, which when mixed with a completely (within all reasonable limits) crystalline sample gives a linear relationship between measured area under the crystallisation response and the percentage amorphous material present. However, real samples will have individual particles which are each partially crystalline and partially amorphous. There are serious uncertainties as to whether the two state calibration model is the same as the one state mixed particle sample. Indeed there is good evidence to suggest that the responses are different, if one compares the milled samples reported by Briggner et al. (1994) to mixtures then the responses are of different shapes. With particles which have been processed to make them partially amorphous it seems to be impossible to separate the crystallisa-

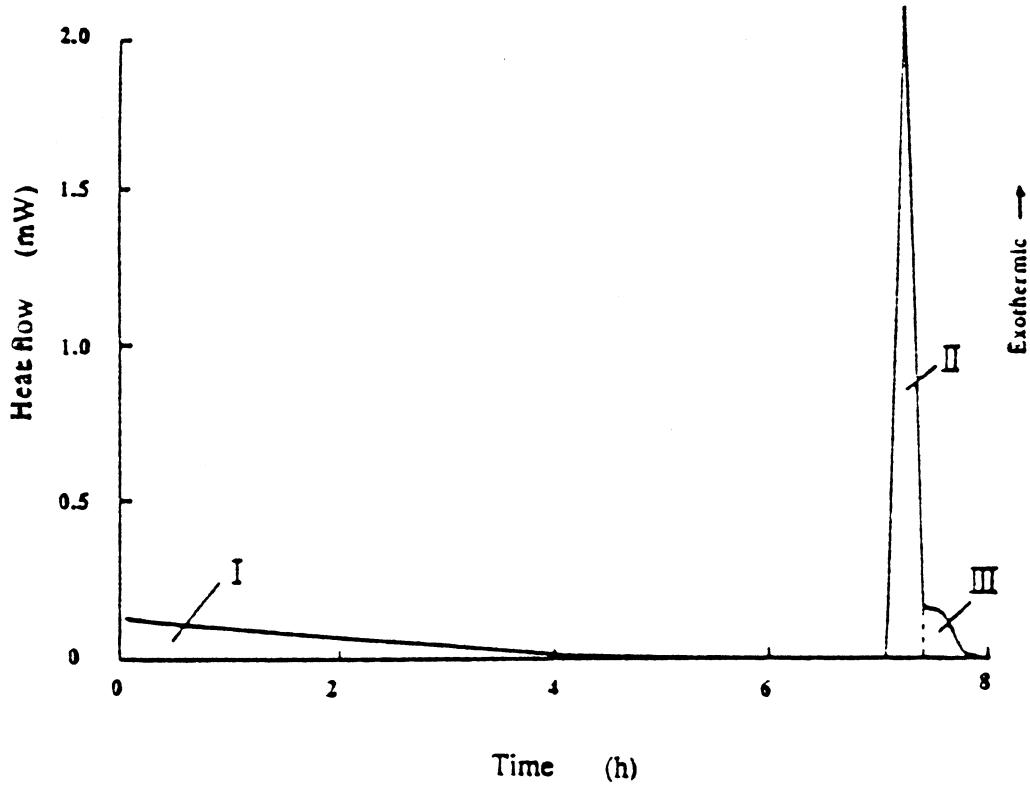


Fig. 7. Schematic microcalorimetric heat flow curve for crystallisation of amorphous lactose reproduced from Sebhatu et al., 1994, with permission.

tion event from the initial response (Part I, Fig. 7). As such it seems impossible to halt the crystallisation process for the milled samples once collapse has started, whereas there is a clear lag phase between these two for completely amorphous lactose, or mixtures of amorphous and crystalline powders. The merging of the crystallisation response with the initial response for processed samples makes it harder still to decide how to measure the area under the curve for crystallisation. The choices include taking the total area (which is certain to include contributions which are not part of the crystallisation event) or estimating the area for the crystallisation peak over and above the underlying wetting/collapse/environmental equilibration peak. Neither approach is perfect and it is probable that workers will have to adapt the approach to suit their sample needs. This means that it becomes important to give a clearer indication of how the area under the curve was determined in all future published work.

Table 1
Net area under the curve for the crystallisation of amorphous lactose at different temperatures and humidities (adapted from Buckton and Darcy 1998)[a]

Temp.	Sodium chloride	Sodium nitrite	Magnesium nitrite
25	48.9	47.9	48.2
35	53.0	47.1	59.4
45	62.1	63.2	66.5

[a] Sodium chloride, 75% RH at all temperatures studied; sodium nitrite, 65% RH at 25°C, 62% at 35°C and 60% RH at 45°C; magnesium nitrite, 53% RH at 25°C, 50% RH at 35°C and 47% RH at 45°C.

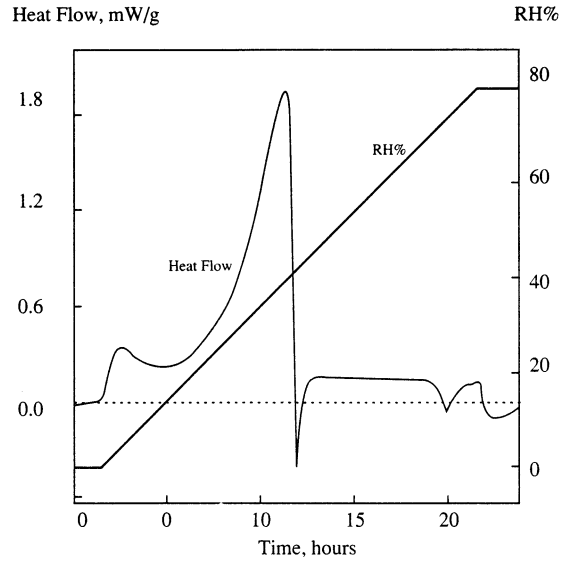


Fig. 8. A micronised drug exposed to a RH ramp. The trace shows vapour wetting the powder, then a very large exotherm (crystallisation) at 10 h, followed by a sharp endotherm (water loss). Reproduced from Briggner (1993) with permission of Thermometric.

6. Other calorimetric approaches

6.1. Gas-flow

This approach uses a calorimeter cell in which the powder is housed, which has been constructed to allow a constant flow of humid air. The humidity can be altered in steps or as a ramp. As the powder crystallises, there will be a clear exotherm and also an endotherm associated with the water loss, these can be seen in Fig. 8 for the crystallisation of amorphous lactose. All water sorption experiments on thermodynamically unstable systems present difficulties in defining an equilibrium. This is because there can be a long lag time between establishing an environmental condition and any change in state of the solid. This lag period can easily be longer than the time for which the sample is allowed to wait at that set condition. As such a new environmental condition is established, at which point the sample may change state, giving the impression that this was the key environmental condition to cause the change. However, it could well be that longer storage at the previous condition could have caused the change to occur. This presents a real problem in defining how long to run any experiment for. The ramp experiments, where RH is continually changed at a defined rate, which have been used by Briggner (1993) are especially at risk from this failure to reach equilibrium. This is true even if the RH ramp is sufficiently slow to ensure that the extent of water sorption is essentially at equilibrium (as water sorption may be much faster than the physical changes in the solid which result as a consequence of that sorption). Having registered this reservation, it is probable that the onset of crystallisation will be seen at about the same point for repeat experiments on the same mass of the same sample. Such ramp experiments have been used by Jakobsen et al. (1997) to study the deliquescence of pharmaceutical materials. A number of studies on the use of these gas flow cells in calorimeters are now being published (e.g. Sheridan et al., 1995, Puddipeddi et al., 1996, and Sokoloski and Ostovic, 1997).

6.2. Solution calorimetry

Solution calorimetry has been used to a small extent to assess powder crystallinity. This approach works on the basis that the heat of solution will be different for the crystalline and the amorphous states of a sample. Ward and Shultz (1995) showed clear differences in heat of solution between micronised and crystalline salbutamol sulphate samples. These differences were attributed to changes in surface energy, however, it is yet to be proved how large a contribution will be made to the heat of solution by changes in the immersion component of the response. It is intuitively more likely that changes in lattice energy were the major contribution to the different measured responses for the amorphous and crystalline materials. However, Hollenbeck et al. (1978) have shown that immersion of (insoluble) microcrystalline cellulose can yield valuable information on its physical state, which must be due to changes in 'wetting'. Studies of materials such as microcrystalline cellulose are not possible with the ampoule style calorimetric experiments, as the vapour does not cause the cellulose to crystallise, as such only

vapour flow experiments and immersion experiments can reasonably be expected to show differences.

Thompson et al. (1994) have shown that solution calorimetry can be an effective way of differentiating between drug samples with different degrees of crystallinity. They (Thompson et al., 1994) also show good agreement between heat of solution and thermal activity measured in an isothermal microcalorimeter over the range 0–100% crystallinity. Salvetti et al. (1996) have demonstrated that different physical forms of carbohydrates can be differentiated by measuring heat of solution. Pikal et al. (1978) used heat of solution measurements to correlate the extent of crystallinity to the chemical stability of antibiotics.

In certain circumstances, solution calorimetry may be more appropriate than vapour sorption approaches as a tool to assess powder crystallinity. For example, solution calorimetry would be more appropriate if there were any serious fear that the amorphous material could not be accessed by the vapours, or that there are no suitable vapours to induce the crystallisation response. The potential disadvantage of the solution calorimetry approach is that both the response for the amorphous and crystalline material is measured, and thus there is a need for a substantially different heat of solution between the two if small amounts of amorphous material are to be detected. A further difficulty with solution calorimetry can be in finding a suitable solvent system which will achieve complete solution in a rapid time. Thus solution calorimetry works as a bulk technique, and measures the response for the entire sample, whereas the vapour sorption works by simply detecting the crystallisation response for the amorphous material, with little or no interfering response from the crystalline component. This fundamental difference in approach may mean that on some occasions solution calorimetry will be the preferred option, whilst on others it would be not as good as the vapour sorption approaches. More work is required to check the versatility and detection sensitivity of the solution calorimetry approach.

7. The gravimetric approach

As has been described above, many amorphous materials are able to crystallise by the process of water absorption, which lowers T_g and gives sufficient molecular mobility to allow crystallisation to proceed rapidly. The availability of microbalance assemblies with good temperature and humidity control (examples include the Dynamic Vapour Sorption apparatus from Surface Measurement Systems, and the VTI MB300G) makes it rather simple to detect changes in crystallinity gravimetrically.

Water sorption has been used in the study of many amorphous and partially amorphous powders. A few examples in the pharmaceutical literature include sucrose (Saleki-Gerhardt et al., 1994), lactose (Buckton and Darcy, 1995), raffinose (Saleki-Gerhardt et al., 1995) and salbutamol sulphate (Ward and Shultz, 1995). There are few studies on drugs in the public domain, however the fact that gravimetric sorption instruments are now commonly used by the major pharmaceutical companies world-wide indicates that many samples have been studied, even if the data have not been released for publication.

The data in Fig. 9 show a typical isotherm for a partially amorphous sample. The lower lines are the adsorption responses for crystalline lactose, and the upper line is for a sample with 0.5% amorphous material added. It can be seen that the weight increase in the early part of the isotherm is much greater for the amorphous material than the crystalline. The adsorption response for crystalline samples is due to the gradual build up of a few layers of water molecules on the surface. If water molecules are evenly distributed across the surface as a thin layer, there will be no changes in the product (an adsorbed layer will not have the properties of liquid water). The only way in which there can be a risk of the material changing would be if excessive condensation occurred in a pore or at a point contact between two particles (giving the potential for some dissolution of the surface for example), or if high local concentrations of water exist in amorphous regions. It can be seen from Fig. 9 that the sample with 0.5% amorphous lactose has a large increase in water content over

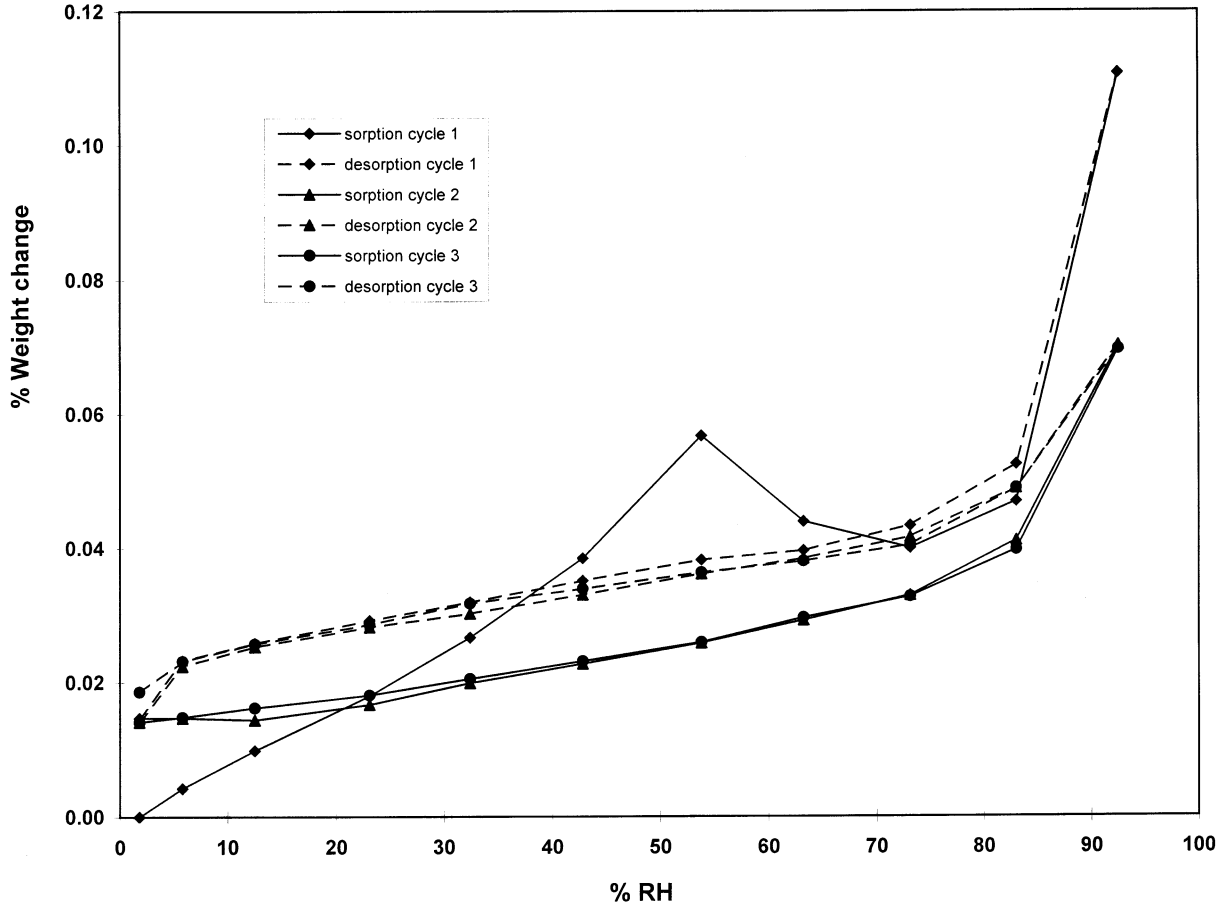


Fig. 9. Water sorption isotherm for a sample containing 0.5% amorphous lactose (Reproduced from Buckton and Darcy (1995) with permission).

the crystalline sample at the low RH values, and it follows that this water is all in the amorphous region. It can be shown that the water content can be an equal mass ratio to the amorphous content. As water has a lower molecular weight than the solid, there are more moles of water than solid present in the amorphous regions. This excess of water, combined with the thermodynamically unstable state of the solid, is what makes the amorphous region a reactive hot spot. This amplification of the influence of water, due to the high local concentration, makes it incorrect to conclude that a material with an apparently low water content is a priori not at risk. If the water is almost all in one small region then the sample is indeed at risk of undergoing physical and chemical changes.

At a certain critical RH (being when T_g has been lowered well below T), the amorphous material crystallises, and in so doing, the absorbed water is lost. This is seen as a clear weight loss at the 50% RH point in Fig. 9. Subsequent to the crystallisation, the higher RH points on the isotherm equate to adsorption to, and potentially hydrate formation in, the now crystalline material.

7.1. Quantification of amorphous content

With the calorimetric methods described above, it was possible to attempt quantification by use of an amorphous standard and the assumption that

a linear relationship exists between the measured heat and the amorphous content. Using gravimetric methods it can be somewhat harder to quantify the amorphous content with accuracy. Saleki-Gerhardt et al. (1995) were able to show a good correlation between residual water content (i.e. remaining amount of hydrate water) and amorphous content for raffinose. However the relationship between the amount of water sorbed and the amorphous content showed deviation from the calculated linear relationship at both low and high amorphous contents (Saleki-Gerhardt et al., 1995). Ward and Shultz (1995) were able to show that partially amorphous salbutamol samples had a different shape of isotherm to the crystalline material (just as seen in Fig. 9 for lactose), however, they did not attempt to use this difference to quantify the amorphous content. In our own studies on lactose (Buckton and Darcy, 1995) we were able to show a correlation between the amorphous content and the increase in hydrate water (i.e. the increase in residual water at the end of the desorption cycle, given that desorption for short times at low RH and 25°C does not cause loss of hydrate water for lactose). This relationship held for the concentration range 0.05–0.5% amorphous content (Buckton and Darcy, 1995) however, subsequent to that publication it has become clear that when larger amounts of amorphous content are present not all of the sample forms the hydrate (in fact from calorimetric studies it is clear that not all of the sample crystallises to the α-lactose form). This observation has not yet been published, so supporting data are presented in Table 2. It follows that the quantification through calculation of the amount of hydrate which forms will only be possible if all the sample immediately forms the hydrate, which is not the case for lactose.

Table 2
The theoretical increase in weight of lactose samples containing various amorphous contents after water sorption (causing crystallisation) and desorption (to remove absorbed water, but not the hydrate water)[a]

Amorphous content of mix (%)	Theoretical increase assuming 5% hydrate	Measured increase
100	5.26	1.43
10	0.526	0.258
1	0.053	0.029
0.500[b]	0.026	0.025
0.250[b]	0.013	0.012
0.125[b]	0.007	0.010
0.050[b]	0.003	0.005

[a] All values expressed as % W/W.
[b] Data from Buckton and Darcy (1995), other data are previously unpublished.

The discussion above shows that gravimetric studies are extremely useful for determining the existence of an amorphous material (as long as a vapour is used which will cause crystallisation). They are also valuable to demonstrate if hydrates are forming during humidification. However, quantification of the amorphous content from gravimetric studies may be rather difficult.

7.2. Studies of collapse

The fact that amorphous material will collapse under gravity when its viscosity gets sufficiently low has been described above. Gravimetric methods are a good way of assessing collapse. In Fig. 10, the weight changes due to exposure of amorphous lactose to 40 and 50% RH, respectively (25°C) are shown. Exposure to 40% RH does not result in sufficient water uptake to cause T_g to drop below T, however at 50% RH, T_g does drop below T. The desorption (at 0% RH 25°C) from these two samples is very different, being rapid and complete for the one which was equilibrated to 40% RH and being very slow from the one which was at 50% RH. The difference in desorption is because the sample which was stored at 50% RH collapsed, whilst the one at 40% did not. With prolonged storage at 0% RH, the sample which had been exposed to 50% RH air will subsequently crystallise, whereas the one which had been equilibrated at 40% RH remains in the amorphous state when dried.

The collapse process can be visualised by attaching a video microscope near the measuring pan in the gravimetric sorption apparatus. This is shown for lactose in Fig. 11, where it can be seen that at the collapse point the material has densified (micrograph B compared with A). The water desorption at the onset of crystallisation is

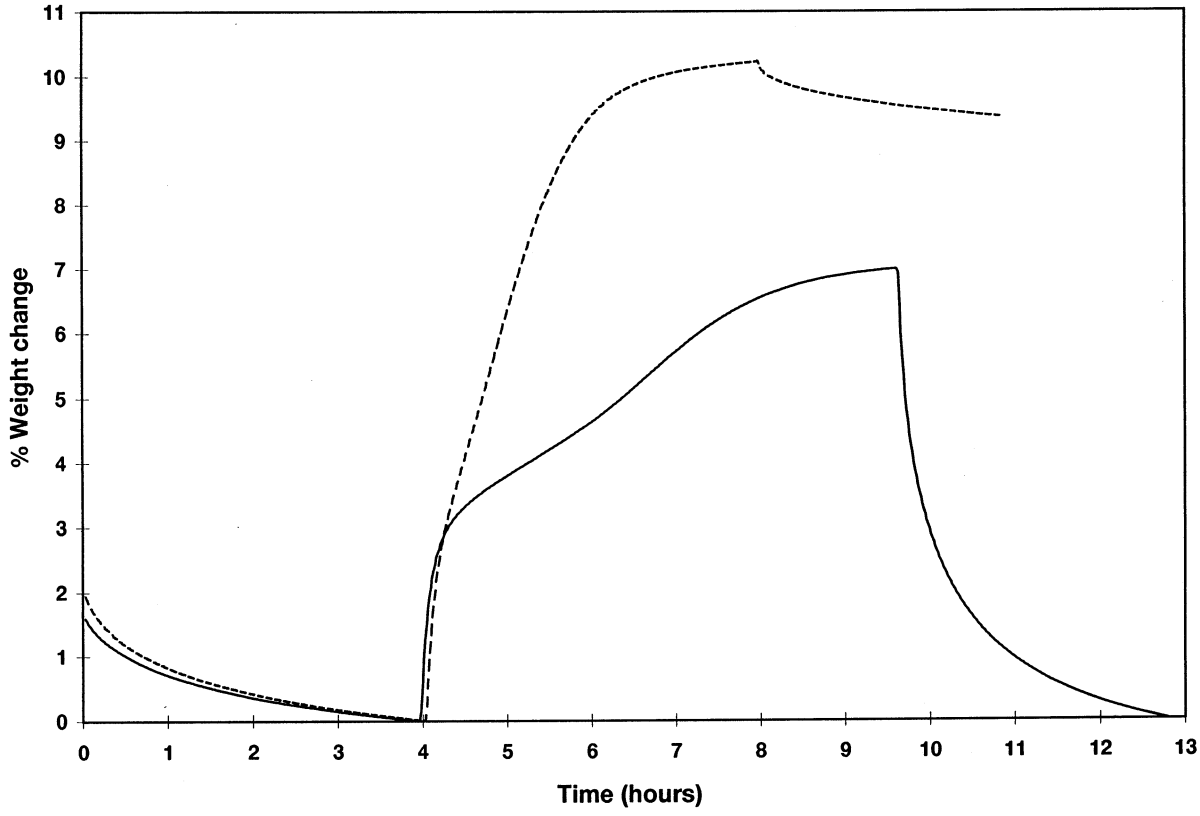
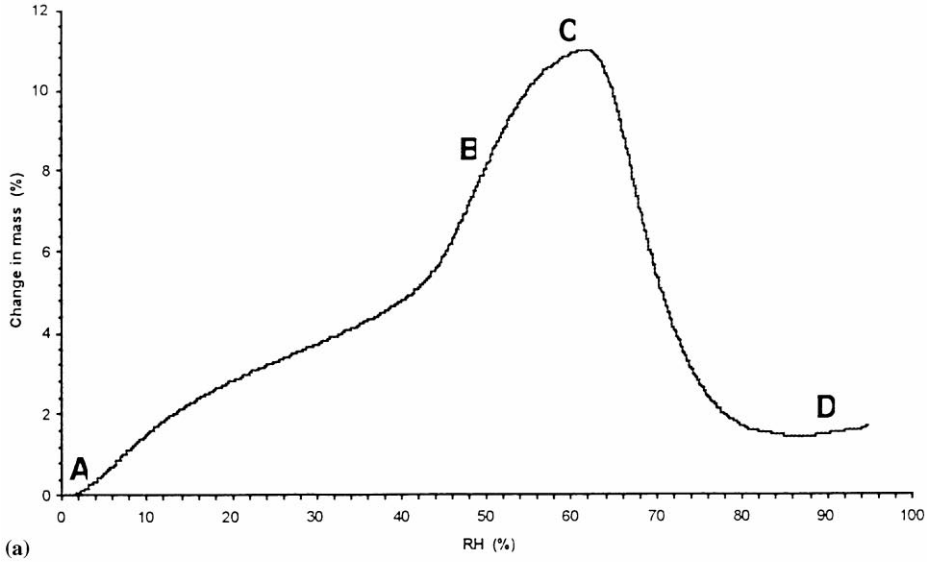


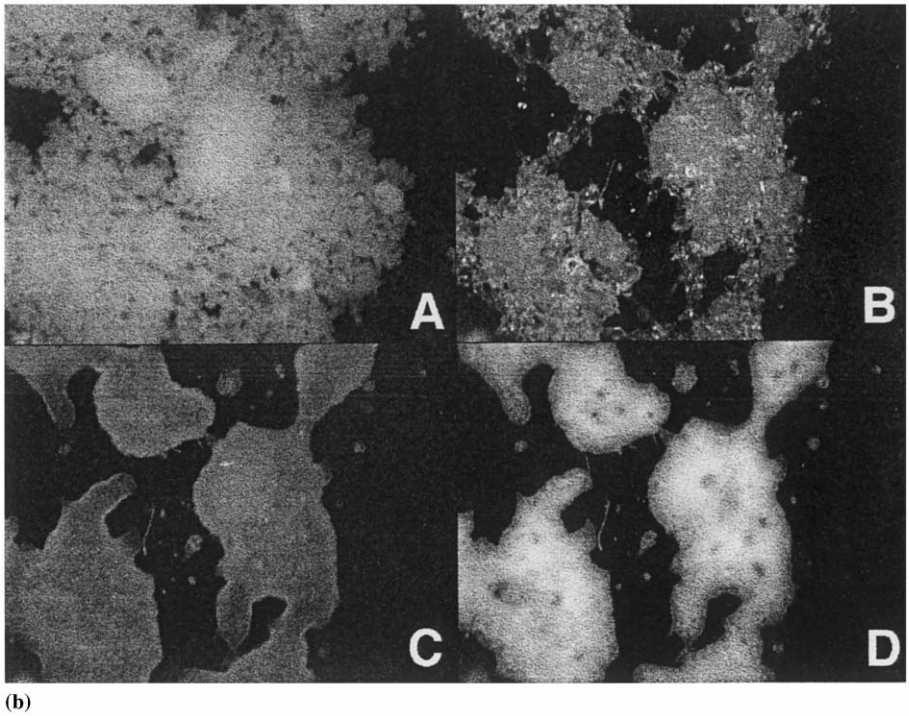
Fig. 10. Water sorption at 50% RH (4–8 h) and 40% RH (4 h–9.5 h) at 25°C, for amorphous lactose, followed by desorption at 0% RH. (Reproduced for Buckton and Darcy (1996))

obvious in micrograph C (Fig. 11(b)) where the sample looks very wet. Collapsed material must be considered when using 'loss on drying' to judge whether a pharmaceutical is dry. It has been shown (Fig. 10) that water desorption from collapsed material is very slow, this would mean that a product (such as a granulation) which contained substantial amounts of water trapped in collapsed material would not show any significant weight loss in a 'loss on drying', and as such may be assumed to be dry. If this situation occurs the amorphous material will eventually crystallise (irrespective of the storage conditions, which will simply alter the rate at which the sample crystallises) resulting in a situation as seen in micrograph C in Fig. 11(b). This will result in the sample, which had appeared dry, becoming noticeably wet, the extent of the problem will depend upon the amount of amorphous material involved. The amorphous material may be drug or excipient or both. Whilst we are not aware of published reports of 'dry' samples becoming wet in such a manner, discussions with industrialists reveal that this is something which does occur from time to time in many companies.

The collapse of amorphous particles is not an 'all or nothing' response (Fig. 12), as it is obvious that the length of time for which the sample is held at 50% RH affects the rate at which water desorbs (this being a monitor of the amount of collapse, as rapid desorption is from pre-collapsed and slow desorption is from collapsed material). Under the conditions used, 75 min at 50% RH results in approximately half of the sample collapsing, whilst 3 h at 50% RH is necessary for total collapse.



(a)



(b)

Fig. 11. (a) Weight increase as a function of humidity, where humidity was changed at a constant rate with time, for amorphous spray-dried lactose at 25°C. Photographs were taken at the following points: A, dry amorphous material; B, the point where T_g has dropped well below T, when collapse occurs, but the sample remains amorphous; C, when crystallisation begins and the absorbed water starts to be expelled; D, when the sample has completely crystallised; (b) photo-micrographs taken at points A–D during the isotherm produced in (kindly supplied by Surface Measurement Systems).

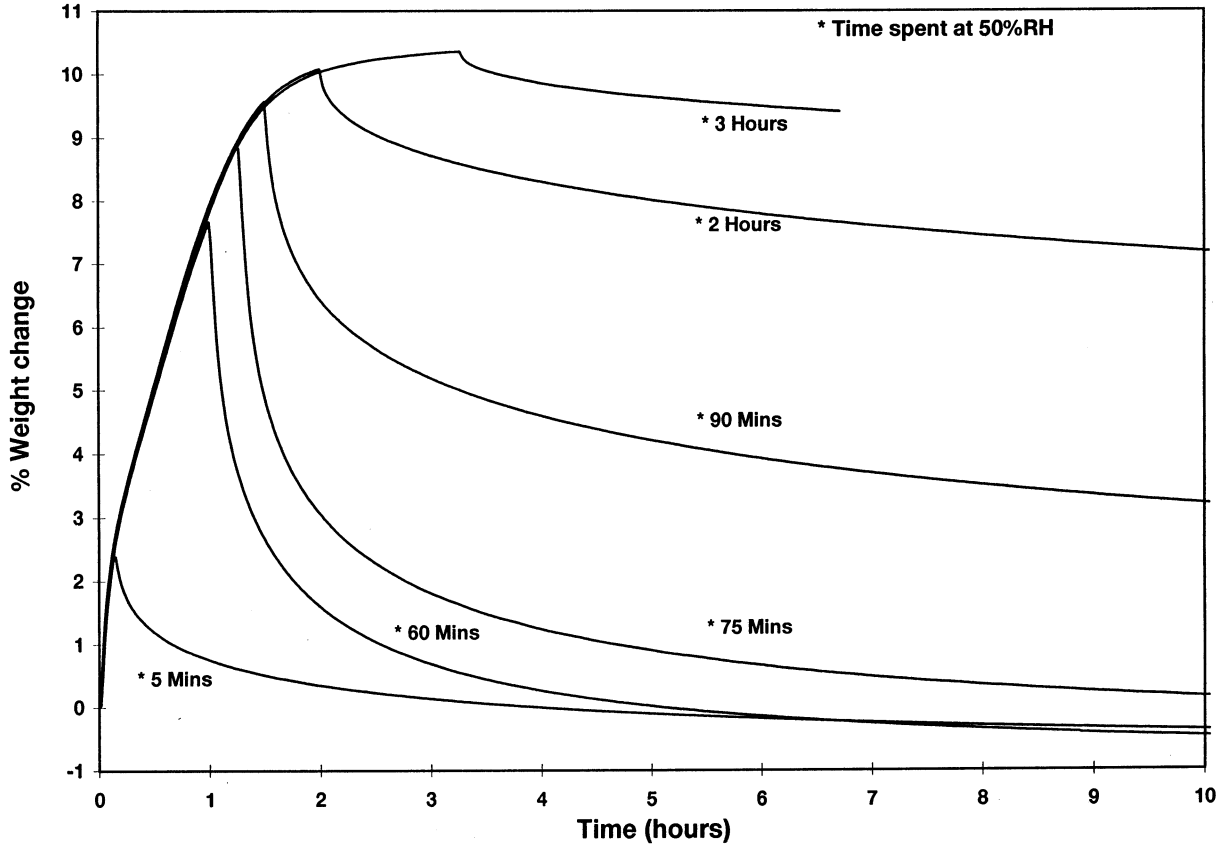


Fig. 12. Sorption of water to amorphous lactose at 50% RH/25°C for different durations, followed by desorption at 0%RH. It can be seen that rapid desorption occurs prior to collapse and that the extent of collapse increases with longer periods at 50% RH. Reproduced from Buckton and Darcy (1996) with permission.

8. Inverse phase gas chromatography (IGC)

IGC is a vapour sorption technique in which the powder is packed in a column and known vapours (usually at infinite dilution in a carrier gas) are injected. From the retention times of the probes it is possible to assess the surface nature of the material in the column. In keeping with the calorimetric and gravimetric techniques, it is to be expected that this vapour sorption approach will also be able to detect differences in samples due to small amounts of amorphous content. For example, Ticehurst et al. (1994) reported on surface differences between two different batches of salbutamol sulphate (although this was not described as being due to changes in crystallinity), and our own (unpublished) data indicates that changes in crystallinity are detectable by this method.

9. Near infra-red spectroscopy

Recent studies (Seyer et al., 1997) have indicated that second derivative NIR spectra are able to quantify small amounts of amorphous materials in crystalline samples. Work in our laboratories would indicate that this technique is extremely good at showing the state of water in the sample, but is possibly not as good as calorimetric methods in quantifying the amorphous content. However the use of this technique is in its infancy for such applications and much more work is needed to show the detection cut off.

10. Solid state NMR

Recently, Lusting et al. (1997) have shown that solid state NMR has a similar detection limit for amorphous lactose ($\sim 0.5\%$) to that which has been claimed for calorimetric techniques. This may prove to be an alternative method by which to study samples with small amounts of processing induced disorder.

11. Conclusion

It is increasingly important to characterise materials in order to allow better quality products to be produced. It is acknowledged that amorphous material can be present in many samples which are believed to be crystalline. Detecting small amounts of amorphous material in crystalline solids can be difficult with bulk analytical techniques (although solution calorimetry shows promise and NIR and solid state NMR require further investigation). Vapour probes are extremely useful in this field due to the tendency of amorphous material to absorb vapours, and in some cases to subsequently change physical state. In order to use the data obtained from vapour sorption techniques to best effect, it is necessary to understand exactly what is being measured.

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