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Approaches to determine the enthalpy of crystallisation, and amorphous content, of lactose from isothermal calorimetric data

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Abstract

Amorphous lactose will crystallise rapidly if its glass transition temperature is reduced below its storage temperature. This is readily achieved by storing samples at ambient temperature and a relative humidity (RH) of greater than 50%. If the sample is monitored in an isothermal microcalorimeter as it crystallises, the heat changes associated with the event can be measured; indeed this is one of the methods used to quantify the amorphous content of powders and formulations. However, variations in the calculation methods used to determine these heat changes have led to discrepancies in the values reported in the literature and frequently make comparison of data from different sources difficult. Data analysis and peak integration software allow the selection and integration of specific areas of complex traces with great reproducibility; this has led to the observation that previously ignored artefacts are in fact of sufficient magnitude to affect calculated enthalpies. In this work a number of integration methodologies have been applied to the analysis of amorphous spray-dried lactose, crystallised under 53 or 75% RH at 25 °C. The data allowed the selection of a standard methodology from which reproducible heat changes could be determined. The method was subsequently applied to the analysis of partially amorphous lactose samples (containing 1–100% (w/w) amorphous content) allowing the quantification limit of the technique to be established. It was found that the best approach for obtaining reproducible results was (i) to crystallise under an RH of 53%, because this slowed the crystallisation response allowing better experimental measurement and (ii) to integrate all the events occurring in the ampoule, rather than trying to select only that region corresponding to crystallisation, since it became clear that the processes occurring in the cell overlapped and could not be deconvoluted. The technique was able to detect amorphous contents as low as 1% (w/w), using this integration strategy, although it was observed that the calibration plot constructed showed a negative deviation from linearity. It is suggested that such non-ideal behaviour results from the formation of varying ratios of α-lactose monohydrate, anhydrous α-lactose and anhydrous β-lactose.

Keywords: Amorphous spray-dried lactose; Isothermal microcalorimetry; Crystallisation; Determination of amorphous content

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1. Introduction

One result of the mechanical processing of solid-state pharmaceuticals may be the accidental formation of amorphous regions in what was previously an entirely crystalline material. The presence of amorphous material can have significant effects on material properties, including increased chemical instability (Pikal et al., 1978) and hygroscopicity (Kontny et al., 1987). Despite the amorphous material occupying only a few percent of the bulk mass, its location on particle surfaces gives it a disproportionate control over the surface interactions of what is a predominantly crystalline powder (Newell et al., 2001a,b). The reversion of the unstable amorphous material to the lower energy state crystalline form can also have a dramatic effect due to particle aggregation. The development of techniques that allow a quantitative determination of low (∼1%, w/w) amorphous contents is therefore of considerable importance.

One approach to determine amorphous contents is to measure the heat changes that result when a partially crystalline material is exposed to a specific vapour (often humidity) using isothermal calorimetry (for example, Byström, 1990; Briggner et al., 1994). The simplest methodology is to place a powder in an air-tight glass ampoule with a small glass tube (a Durham tube or mini-hygrostat) containing a saturated salt solution to control the local RH. An alternative approach is to use gas perfusion calorimetry, whereby the RH of a carrier gas is precisely maintained as it flows over a sample. The major difference between these two techniques is that with the gas perfusion method the air-flow is humidified remotely from the calorimeter measuring site, whereas for the mini-hygrostat the air is humidified (by evaporation from the saturated salt solution) within the measuring site of the calorimeter. The advantage of the mini-hygrostat method is that the response for the wetting of the powder by the vapour is mostly compensated for by the (almost equal and opposite) response for the generation of the vapour, making it much easier to see just the response due to transitions within the powder sample. RH perfusion allows a sample to be held under dry conditions until thermal equilibrium has been attained and allows the investigation of the effects of a range of RHs, which are both advantageous, however the masking of physical changes with vapour phase wetting responses can influence ease of data interpretation. It is clear that each approach will result in a different thermal response; this work focuses solely on the use of the mini-hygrostat method.

Isothermal calorimetry has been widely used to determine the amorphous contents of many pharmaceuticals, such as salbutamol sulphate (Buckton et al., 1995b), nifedipine (Aso et al., 1995) and lactose (Briggner et al., 1994; Sebhatu et al., 1994; Angberg, 1995; Buckton et al., 1995a). Lactose, in particular, is an interesting subject, both as a model system and because it is a very commonly used pharmaceutical excipient. This method has given rise to different reported values for the net enthalpy of the response related to lactose crystallisation (Table 1; Note that the crystallisation process is a net exothermic event. Conventionally, these heats would therefore have a negative sign; however, because most calorimeters record these changes as positive, literature values recorded for lactose crystallisation are positive and we will adhere to this convention). A major reason for the differences seen in Table 1 has been differences in the section of the calorimetric power-time response that was integrated, because of the fact that the response arises from a large number of competing exothermic and endothermic processes, giving a composite response that may well be seen as consisting of several peaks or shoulders on peaks. The availability of data analysis and peak integration software means it is possible to select and integrate specific areas of complex traces with great reproducibility. It has also led to the observation that previously ignored artefacts are in fact of a magnitude to affect calculated enthalpies significantly. A further reason for the differences in Table 1 is that it has been observed that changes in experimental conditions can have an impact upon the observed response in the calorimeter, because the balance of events occurring is altered. For instance, experiments conducted at 60 °C resulted in a larger crystallisation net measured enthalpy, because less plasticising water needed to be absorbed,

Table 1
Reported apparent enthalpies of crystallisation for lactose using isothermal microcalorimetry at 25 °C

Reference	Enthalpy (J/g)
Sebhatu et al. (1994)	32
Briggner et al. (1994)	45–48
Chidavaenzi et al. (1997)	50
Darcy and Buckton (1998)	48

and subsequently expelled (Darcy and Buckton, 1998).

It is essential, in order to use calorimetric data to determine quantitatively small amorphous contents, that there is an accepted value for the net apparent enthalpy of crystallisation of 100% amorphous lactose (or any other material that is used). For this to be derived there needs to be a standard methodology by which start- and end-points can be assigned, allowing reproducible peak areas to be determined. It is the purpose of this work to demonstrate the variation in values obtained by using a number of approaches for determining peak areas for lactose re-crystallisation and to recommend a standard approach for data analysis. The methodology is then applied to the analysis of partially amorphous samples in order to determine the quantitative sensitivity of the technique for assessment of amorphous content in processed pharmaceuticals.

2. Materials and methods

Lactose monohydrate was supplied by Borculo Whey Products (Cheshire, UK). Sodium chloride and magnesium nitrate were purchased from BDH and were used as received. Amorphous spray-dried lactose was prepared from a 10% (w/v) solution (Büchi 190 mini spray-drier) as discussed previously (Chidavaenzi et al., 1997). Confirmation of the amorphous nature of the yield was obtained using XRPD. Partially amorphous lactose samples were prepared by combining accurately weighed quantities of sieved (<425 μm) crystalline and amorphous spray-dried lactose (∼15 g in a 30 ml amber glass container) and mixing in a Turbula mixer for 20 min. Samples were stored under vacuum over phosphorous pentoxide.

Calorimetric data were recorded using a 2277 Thermal Activity Monitor (TAM, Thermometric AB, Järfälla, Sweden) at 25 °C. Samples (∼30 mg) were weighed into glass ampoules (3 ml volume). The sample and reference ampoules both contained a mini-hygrostat containing a small quantity of saturated salt solution (NaCl which maintains an RH of 75% and $Mg(NO_3)_2{\cdot}6H_2O$ which maintains an RH of 53%, both at 25 °C). Ampoules were sealed with a crimped metal cap; a rubber sealing disc ensured an air-tight seal. Because of the need to reduce the thermal equilibration time as much as possible, all materials were pre-stored at 25 °C for 30 min. The instrument was calibrated periodically using an electrical substitution method. Data were recorded using the dedicated software package Digitam 4.1. Data analysis was performed using Microcal Origin.

DSC data were recorded on a Perkin-Elmer Pyris 1. Samples (∼1–2 mg) were crimped into non-hermetic aluminium pans. A blank aluminium pan was used as a reference. Data were recorded from −30 to 260 °C at a scan rate of 200 °C min^{-1}. The instrument was calibrated using indium and zinc standards. Data were recorded and analysed using the dedicated Pyris software package.

3. Results and discussion

3.1. Power-time responses described in the literature

A typical thermal response generated by amorphous lactose under high RH conditions (>50%) is represented in Fig. 1 (in this case, a 33 mg sample of amorphous lactose under 75% RH at 25 °C). The sample has several distinct regions, denoted A–E. An initial sharp exotherm, which results from friction of lowering of the ampoules and does not form part of the thermal response of the sample, is followed by a more prolonged exotherm lasting for almost 1 h (region A). This results from evaporation of water from the hydrating reservoir in order for the atmosphere in the ampoule to reach the desired RH (endothermic), wetting of the lactose as it absorbs water (exothermic) and, possibly, structural collapse of the sample. It is likely that the rates of water evaporation and absorption are slightly out of balance which explains both the presence of the exotherm (region A; absorption predominates) and the subsequent endothermic baseline (region B; evaporation predominates) before the commencement of crystallisation. It should be noted that during region B it is usually assumed that the processes of water evaporation and absorption are considered to be equal in rate and, thus, generate no heat response (Angberg et al., 1992a,b) but close inspection of the data reveals this not to be the case. In the work described here, an equivalent saturated salt solution is placed in the sample and reference of the microcalorimeter. This will blank out some of the response associated with generating the water vapour in the sealed ampoules; however, the presence

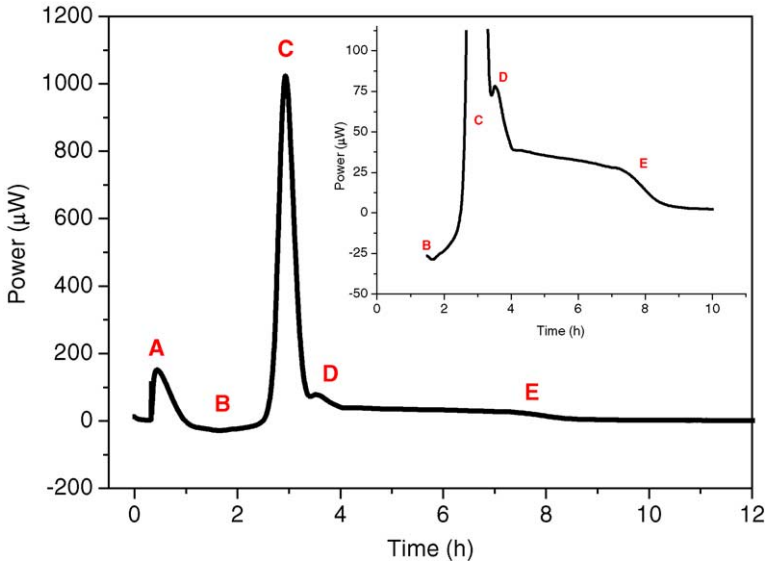

Fig. 1. A typical power-time response for a sample of amorphous lactose held at 25 °C under an RH of 75%. The data comprise five main regions, denoted A–E (discussed in the text). The inset graph shows an expanded view of regions B–E between −30 and 120 μW.

of amorphous lactose will cause a greater generation of water vapour in the test side as the lactose effectively desiccates the air space. It is clear therefore that the "blank" in the reference site is not a complete blank. It is also clear that the data generated using this methodology will differ from those obtained when an empty ampoule (no saturated salt solution reservoir) is used as the reference.

After approximately 2.5 h the sample crystallises (region C, Fig. 1). This lag time has been shown to relate to the mass of amorphous material, the RH generated by the saturated salt solution and the surface area of the tube in which the salt solution is stored (Briggner et al., 1994; Hogan, 2001). Previous work has shown that samples removed immediately after this exothermic response are crystalline (Briggner et al., 1994) and physical inspection of the sample reveals a hard, fused, solid mass instead of a free-flowing powder. It has been observed that following crystallisation, carbohydrates expel the previously absorbed plasticising water (Makower and Dye, 1956). The same effect can be followed using dynamic vapour sorption apparatus (Buckton and Darcy, 1996). While crystallisation will be an exothermic event, the expulsion of water will be endothermic and the calorimetric data will represent the balance of these heat changes. The crystallisation response (region C) shows a clear shoulder (region D). Sebhatu et al. (1994) suggested that this shoulder should not be attributed to the expulsion of water following crystallisation since that event should be cancelled out by the exothermic condensation of that water back into the hydrating reservoir, in a manner similar to that suggested by Angberg et al. (1992a,b) to explain the lag phase before crystallisation. However, since it has been shown that the lag phase is, in fact, an endothermic event, a similar imbalance of events could also explain this shoulder. Sebhatu et al. (1994) suggested that the shoulder represented incorporation of water into the anhydrous α-lactose formed immediately after crystallisation to form α-lactose monohydrate. Briggner et al. (1994) attributed the event to mutarotation of β-lactose to α-lactose monohydrate, which could be possible as Angberg et al. (1991) showed that such mutarotation occurs to a significant extent at relative humidities above 94% (which would be achieved as large quantities of water desorb from the crystallising sample).

Finally, for the first time we report a long, slow exothermic process which is observed after crystallisation (region E). Since this has not previously been noted, no explanations for its existence have been proposed. It is possible that this event arises from water movement within the ampoule, either between the sample and the hydrating reservoir or within the sample

itself, or may represent mutarotation. Angberg et al. (1991) found that complete mutarotation occurred over a period of 112 days at 94% RH. This indicates that mutarotation is a slow process so it would be surprising if it went to completion over the few hours that region E extends.

It is clear that the crystallisation of lactose is a complex event, comprising a number of exo- and endothermic processes, and that the calorimeter records only the balance of these events. It is therefore not a trivial matter to determine the enthalpy of crystallisation of amorphous lactose, since it is difficult to assign start- and end-points to the event, and this has led to ambiguity in the literature.

3.2. Assessment of various integration methodologies

The regions (A–E) in Fig. 1 will be considered to attempt to define a reproducible methodology. In order to ensure consistent reporting of data, start- and end-points for integration need to be defined. Since an endothermic region precedes the crystallisation response, the start point of region C will be negative, although the precise value varies between samples (again, indicating its likely cause being an imbalance of events). It must be decided whether the area should be measured from the minimum inflection of this endotherm or, perhaps, from the point at which region C first crosses the $y = 0$ axis. If the area of the endotherm is ignored, it may be the case that an event which sometimes occurs during the main crystallisation region, and hence contributes to the measured heat change, is being omitted. This would lead to an over-estimation of the enthalpy of crystallisation.

The long exothermic region following crystallisation is observed in all experiments and gives rise to a difficulty in assigning the end-point of the area to be integrated. Without a greater understanding of the nature of the processes occurring during this phase it is not possible to know if this region should, or should not, be regarded as an extension of the crystallisation region. As mentioned above, it seems likely that water movement, mutarotation or a combination of both are responsible for this signal. However, inclusion of the area under region E can have a huge impact on the measured heat change, depending upon the integration method (see below). To omit it raises a further question regarding the assignment of the end-point to some coordinate following the main crystallisation region. This is difficult because the return to baseline after region D is masked by the onset of region E. It must be decided if the area is to be integrated to $y = 0$ or to a sloping baseline (in other words, it must be judged whether the heat capacity of the sample changes in going from an amorphous or partially amorphous form to the crystalline form).

In order to determine the most reproducible method for quantifying the heat of crystallisation, 12 combinations of start- and end-points were selected:

Method 1A	Determination of enthalpy based upon the inclusion of region C only. Integration from $y = 0$
Method 1B	As method 1A but with the inclusion of region D (if no shoulder was observed, it was assumed that it was included in the main crystallisation response, region C)
Method 1C	As method 1A but with the inclusion of regions D and E
Method 2A	Integration of peak C only, using a sloping baseline from the minimum inflection of region B to the end-point of region C
Method 2B	As method 2A but with the inclusion of region D (region C only if no shoulder present)
Method 2C	As method 2A but with the inclusion of regions D and E
Method 3A	Integration of region C only, using a flat baseline from the minimum inflection of region B
Method 3B	As method 3A but with the inclusion of region D (region C only if no shoulder present)
Method 3C	As method 3A but with the inclusion of regions D and E
Method 4A	Integration of regions B and C from $y = 0$
Method 4B	As method 4A but with the inclusion of region D (region C only if no shoulder present)
Method 4C	As method 4A but with the inclusion of regions D and E

Each method was then applied to 18 different crystallisation responses of 100% amorphous lactose. The results are presented in Table 2.

It is evident from the data in Table 2 that the use of the different integration methodologies results, unsurprisingly, in a wide range of crystallisation heats (36.2–69.2 J/g). It is also evident that the errors associated with some of the integration methods are considerable and are thus disregarded. It must also be remembered that the response of wholly amorphous material may not be the same as that of partially amorphous samples (which, as a consequence, may not exhibit all five regions) and, hence, ease of use of the method is of importance. For these reasons, methods 1B and 4C were selected to be used for the analysis of partially amorphous samples. It should be noted that method 1B represents an attempt to integrate solely the crystallisation response (and results in an average value of 45.4 J/g, very close to other reported values (see Table 1)) while method 4C represents the determination of the net heat change for all the processes occurring during the course of the experiment (and, hence, results in a larger value of 53.4 J/g).

3.3. Quantification of amorphous content

Isothermal microcalorimetric data can be used to quantify amorphous contents because it is assumed that there is a linear relationship between amorphous content and the measured heat of crystallisation. Lactose samples containing between 1 and 75% (w/w) amorphous content were crystallised at 75% RH and the heat changes recorded for each were determined using integration methods 1B and 4C. The data were used to calculate percent amorphous content by using the heat change obtained for the wholly amorphous sample, using each respective method, as a reference enthalpy.

As mentioned in the methodology section, the partially amorphous samples were prepared by mixing appropriate quantities of amorphous (spray-dried) and crystalline lactose in a glass container; this method was chosen because it is relatively gentle compared with other mixing methodologies (such as blend-sieve-blend for instance) although the possibility exists that the forces exerted on the sample during mixing could cause some of the amorphous material to crystallise, thus affecting the magnitude of the subsequently recorded experimental data. However, analysis of the heat output from the crystallisation of two samples of the same batch of spray-dried lactose, one of which had been subjected to 20 min in the turbula mixer, resulted in the same measured heat (data not shown), indicating that mixing was unlikely to affect the amorphous nature of the samples. Equally, analysis of crystalline material that had been tumbled in the mixer did not show any evidence of amorphous material being formed.

The known amorphous contents versus calculated amorphous contents determined using method 1B are shown for amorphous contents between 1 and 10% (w/w) in Fig. 2 and for amorphous contents between 5 and 75% (w/w) in Fig. 3. It can be seen that in all cases the repeatability of measurement is poor and at most amorphous contents the average calculated values are lower than the known values, indicating the likely presence of a systematic error with this integration method, possibly associated with the difficulty in assigning an end-point to region D. In particular, the 1–10% (w/w) data exhibited a very poor linear regression fit (R^2 = 0.769), although this improved in the 5–75% (w/w) data (R^2 = 0.986). The gradients of the plots also give an indication of the discrepancy between calculated and known amorphous contents, with a value of 1 indicating a perfect correlation. The 1–10% (w/w) data exhibited a very poor correlation (slope = 0.48) although again this improved for the 5–75% (w/w) data (slope = 0.78).

Similar data sets are presented in Figs. 4 and 5 for amorphous contents determined using method 4C. In this case good linear regression fits were obtained for

Table 2
Enthalpy of crystallisation for 100% amorphous lactose (at 25 °C, 75% RH) determined using the 12 integration methodologies discussed in the text (n = 18)

Integration method	Enthalpy of crystallisation (J/g)											
	1A	1B	1C	2A	2B	2C	3A	3B	3C	4A	4B	4C
Average	41.3	45.4	53.8	36.2	42.8	55.7	43.5	46.8	69.2	39.4	45.1	53.4
S.D.	2.8	3.3	4.3	3.3	3.4	9.1	2.9	5.0	11.8	2.8	3.1	3.7
Error (%)	6.9	7.2	8.1	9.0	8.0	16.3	6.6	10.6	17.0	7.2	6.9	6.9

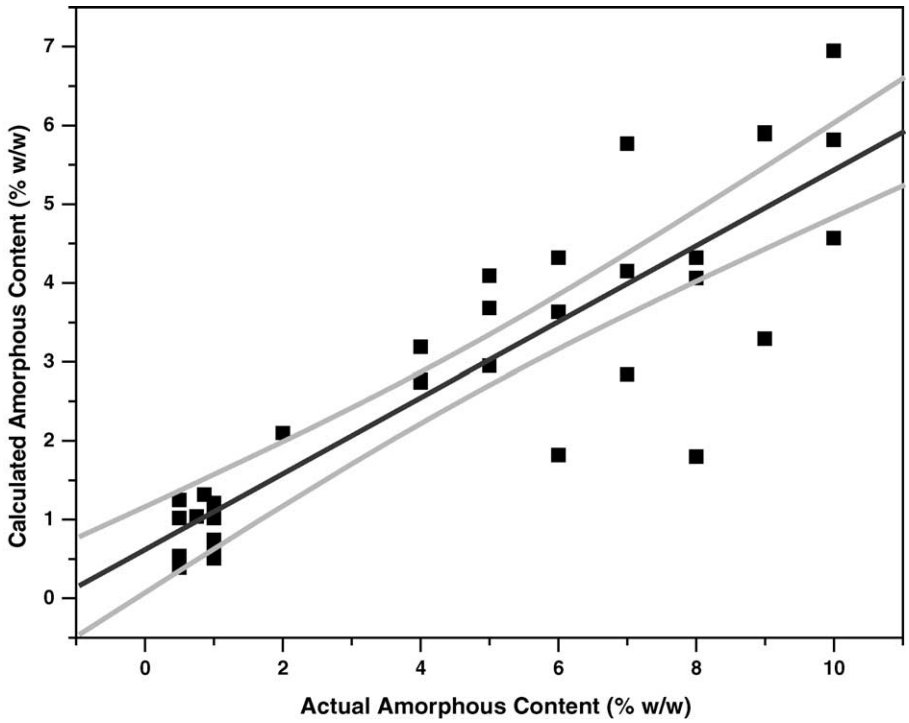

Fig. 2. The calculated vs. known amorphous contents for samples containing between 1 and 10% (w/w) amorphous content, determined by integration of power-time data (at 25 °C and 75% RH) using method 1B, showing the linear fit to the data and the 95% confidence limits.

both plots (1–10% (w/w), R^2 = 0.974; 5–75% (w/w), R^2 = 0.984) although, as above, calculated amorphous contents were consistently lower than the actual values. The slopes of the lines also indicated a better correlation using this integration approach (1–10% (w/w) data, slope = 0.73; 5–75% (w/w) data, slope = 0.88). Possible reasons for these discrepancies are discussed below.

One possible reason for the negative deviation could be that the amorphous material is lost during mixing, perhaps by preferential adsorption to the mixer. This was checked by running one sample in which the amorphous and crystalline material were weighed directly into the TAM ampoule and then stirred with a spatula in the ampoule. The result for this experiment was in keeping with the results for the mixtures, in that there was deviation from the expected linear plot.

The crystallisation response of a sample containing a very low amorphous content is clearly much smaller than that of an entirely amorphous sample and will occur over a much shorter time span (because less water needs to be absorbed to initiate crystallisation). Indeed, crystallisation may often occur before a baseline has been achieved after the ampoules have been lowered into the instrument. One consequence of this is that it is difficult reproducibly to determine crystallisation enthalpies because the starting point of the event may be lost. One way to mitigate this effect is to use a larger sample mass, although this usually impedes water absorption and expulsion, leading to multiphase crystallisation peaks. An alternative approach is to lower the RH to which the sample is exposed. In this work, amorphous samples were crystallised under an RH of 53%, since previous data have shown that this provides sufficient water to induce crystallisation but noticeably increases the lag time (Briggner et al., 1994).

It is notable here that recent studies of lactose crystallisation using electro-chemical atomic force microscopy (EC-AFM) have suggested that primary nucleation cannot occur in samples maintained under an RH of 58% (Price and Young, 2004) and that under low RH atmospheres lactose does not fully crystallise; it

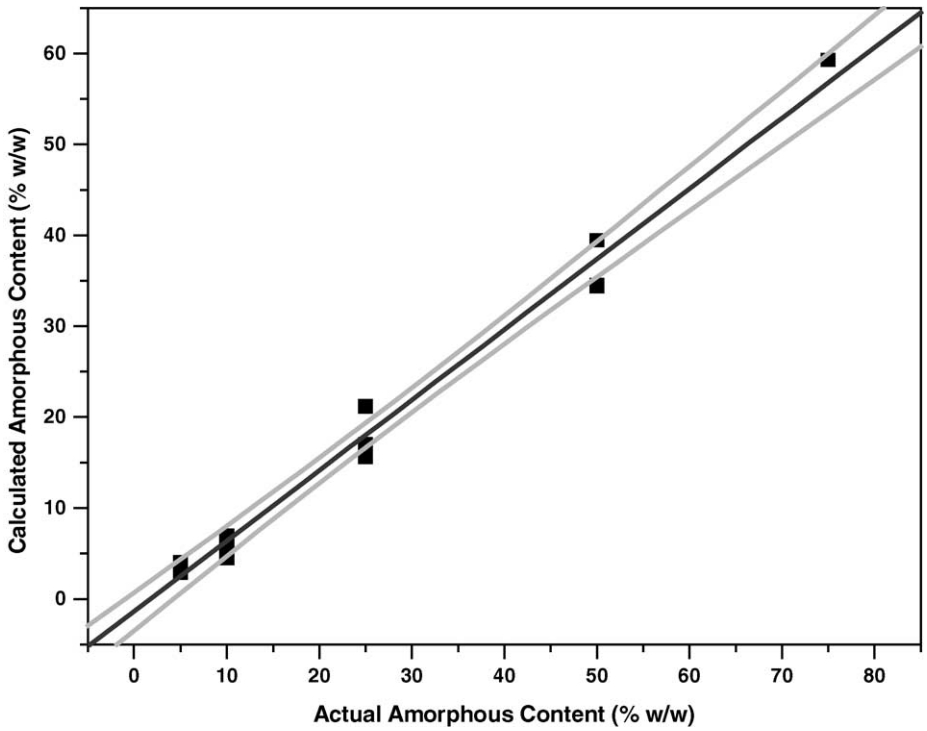

Fig. 3. The calculated vs. known amorphous contents for samples containing between 5 and 75% (w/w) amorphous content, determined by integration of power-time data (at 25 °C and 75% RH) using method 1B, showing the linear fit to the data and the 95% confidence limits.

was suggested that complete crystallisation requires an RH of 94% or greater. In this work, all samples removed from the TAM (once the heat-flow had returned to zero) were completely crystalline (by DSC, data not shown), irrespective of the RH under which they were maintained. Clearly, the presence of crystalline material in the ampoule will promote secondary nucleation and it may be the case that a small quantity of crystalline material exerts a disproportionate effect on the observed behaviour of the system; furthermore, it is likely that the expulsion of water following crystallisation is an extremely rapid process, resulting in the (temporary) formation of a saturated vapour space which will force the complete crystallisation of the sample.

The thermal response of amorphous lactose crystallised at 53% RH is shown in Fig. 6. Integration using method 4C resulted in a heat change of 57.3 J/g, which is slightly higher than that calculated at 75% RH. Darcy and Buckton (1998) noted that changes in temperature resulted in different heat changes but did not note this effect of RH at 25 °C. A plot of known versus calculated amorphous content between 1 and 100% (w/w) is shown in Fig. 7. As in the case of experiments conducted at 75% RH, the method showed a negative deviation from ideality, returning calculated amorphous contents that were lower than the known contents.

Gravimetric data have shown that the mass increase following crystallisation of amorphous lactose is of the order of 3% (w/w) (Hogan, 2001). If the sample crystallised totally to α-lactose monohydrate, a mass increase of approximately 5% (w/w) would be expected. These data therefore suggest that amorphous lactose crystallises to a mixture of α-lactose monohydrate and anhydrous β-lactose and it may be the case that the ratio of the two species formed varies as a function of RH, resulting in the enthalpy difference noted above. DSC data for pure α-lactose monohydrate show a dehydration peak centred at 155 °C and a melt centred at 222 °C, which gives an enthalpy of fusion of ∼169 J/g, Fig. 8. Pure β-lactose is difficult to prepare, so it was not possible to record a DSC trace for this material

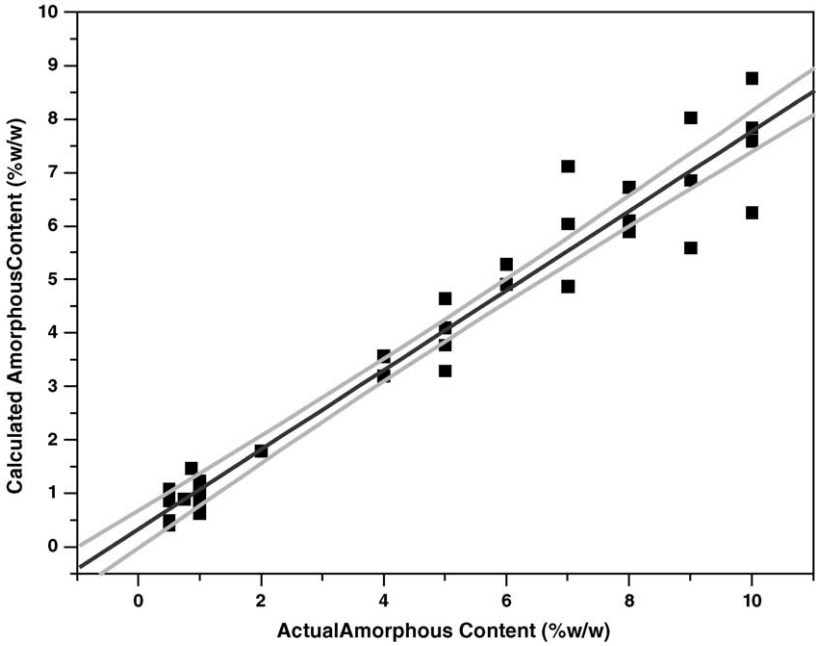

Fig. 4. The calculated vs. known amorphous contents for samples containing between 1 and 10% (w/w) amorphous content, determined by integration of power-time data (at 25 °C and 75% RH) using method 4C, showing the linear fit to the data and the 95% confidence limits.

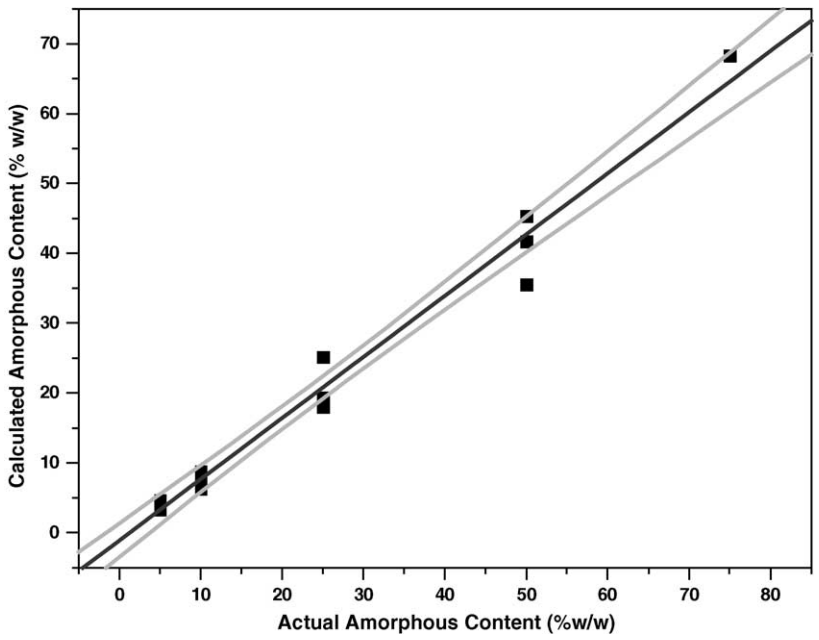

Fig. 5. The calculated vs. known amorphous contents for samples containing between 5 and 75% (w/w) amorphous content, determined by integration of power-time data (at 25 °C and 75% RH) using method 4C, showing the linear fit to the data and the 95% confidence limits.

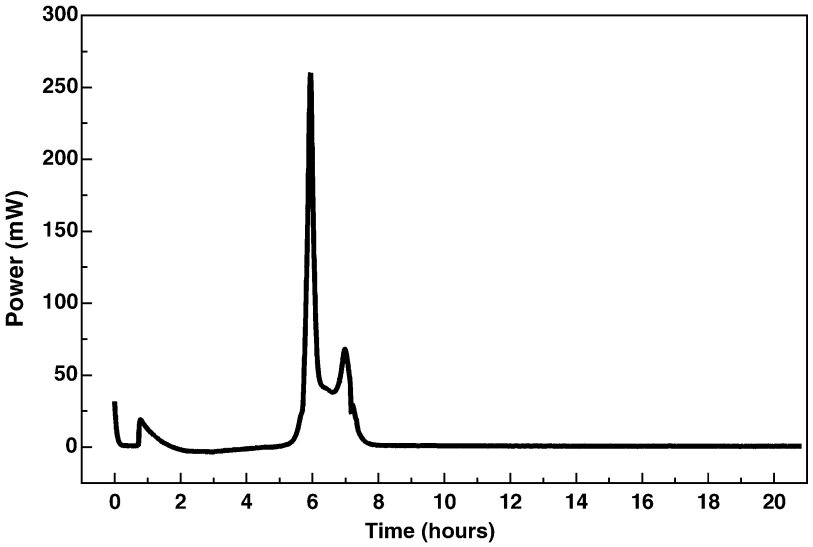

Fig. 6. A typical power-time response for a sample of amorphous lactose held at 25 °C under an RH of 53%.

directly. However, amorphous lactose crystallises to a mixture of α-lactose monohydrate and anhydrous β-lactose (Fig. 8) and from these data it is possible to estimate the enthalpy of fusion for the β-form. The β-lactose melt is centred at ∼248 °C, and gives an enthalpy of fusion of ∼197 ± 19 J/g; it is difficult to quantify this heat change accurately because it overlaps with the α-melt and the subsequent degradation (∼255 °C onwards). Areas for the β-peak were determined by fitting the α- and β-peaks to a double Gaussian model, allowing their separation. The DSC traces for crystallised mixed amorphous:crystalline samples clearly show a change in the ratio of α- to β-lactose formed (Fig. 9). Since the aim of this work was to assess the use of the microcalorimetric method to determine percent amorphous content, we have not studied this phenomenon further, but such a study will form the basis of future work. We simply note here that this effect

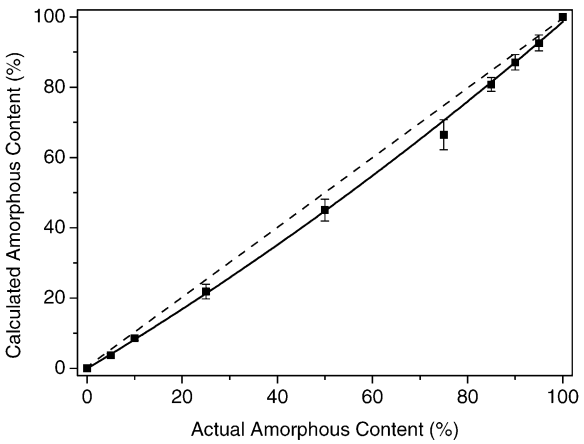

Fig. 7. The calculated vs. known amorphous contents for samples containing between 1 and 100% (w/w) amorphous content, determined by integration of power-time data (at 25 °C and 53% RH) using method 4C.

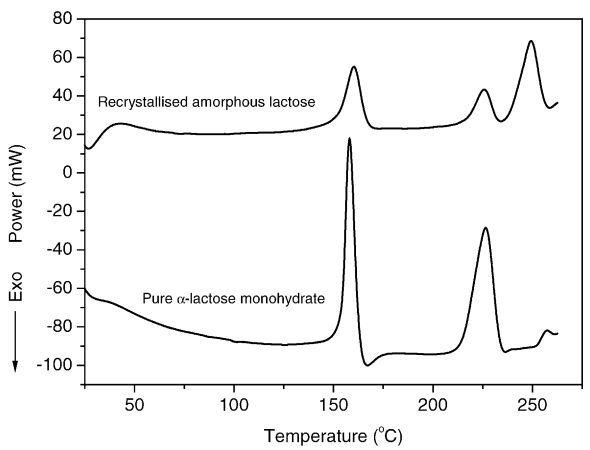

Fig. 8. DSC traces recorded for re-crystallised spray-dried lactose (top) and pure α-lactose monohydrate (bottom).

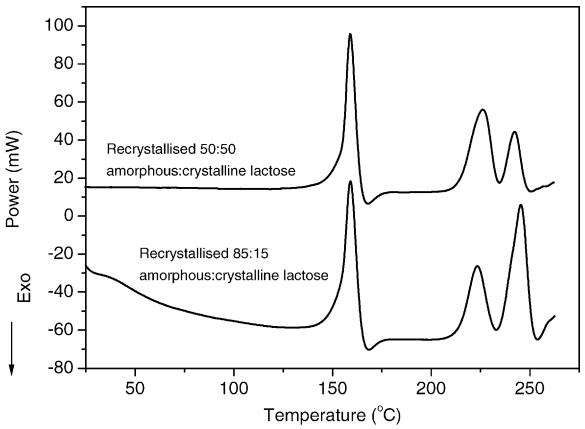

Fig. 9. DSC traces recorded for samples removed from the TAM following re-crystallisation; 50:50 amorphous:crystalline (top) and 85:15 amorphous:crystalline (bottom).

may well account for the negative deviation from ideality observed. The slow exothermic event following crystallisation (region E in Fig. 1) is absent in samples crystallised at 53% RH, suggesting it derives from an event occurring only at higher RHs, such as the mutarotation suggested by Angberg et al. (1991).

4. Conclusion

The data presented here have highlighted the complexity of, and number of potential processes involved in, lactose crystallisation. Analysis of the data for amorphous lactose using different integration methodologies has suggested that the best approach for obtaining reproducible results is to integrate all five regions of the trace, since it is clear that the processes occurring in the cell overlap and cannot be distinguished from each other. It is therefore difficult reproducibly to omit one or more of them. Integration of the net heat change does not guarantee completely reproducible results, but it does ensure that all processes are included in the measurement, suggesting that any differences between data sets reflect an alteration of the balance of events occurring, whether this is caused by batch-to-batch sample variability or changes in experimental conditions. We determined a net heat change of 53.4 J/g at 75% RH and 57.3 J/g at 53% RH.

The second purpose of this work was to see if a standard integration method would allow the quantification of amorphous content in a partially crystalline sample. The data did allow the construction of a calibration plot, but this plot was not linear. However, the heats measured in the calorimeter, at any particular amorphous content, were reproducible. This implies that calorimetric data can be used to quantify amorphous contents in partially amorphous lactose samples, although it should be noted that these data were recorded using mixtures of wholly amorphous and wholly crystalline particles; the response of a sample containing particles which are partially amorphous and partially crystalline (such as would be produced during processing) may be significantly different. The most precise results were obtained when samples were crystallised under an RH of 53% and the net heat change was determined (method 4C); the technique was able to detect amorphous contents as low as 1% (w/w).

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