Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 300 (2005) 13–21
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Calorimetric determination of amorphous content in lactose: A note on the preparation of calibration curves

Rita Ramos, Simon Gaisford, Graham Buckton *

The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, UK

Received 22 March 2005; received in revised form 15 April 2005; accepted 20 April 2005
Available online 11 July 2005

Abstract

Calorimetric methods (isothermal or solution calorimetry) offer the ability to detect amorphous contents to 0.5% (w/w) or better in processed pharmaceuticals and calorimetric data are becoming more widely accepted in regulatory submissions. However, both methods require the construction of calibration curves, prepared using quantitative physical mixtures of entirely amorphous and entirely crystalline material. If the sample under investigation exists in two or more isomers or polymorphs, and the enthalpy of solution (solution calorimetry) or the enthalpy of crystallisation (isothermal calorimetry) are different for the isomers or polymorphs, then it must be ensured that the batch of material used to prepare the calibration samples has the same isomeric or polymorphic composition as the (processed) material to be tested. Here, we demonstrate the problems that may arise using lactose as a pharmaceutically important model substance. Calibration curves were prepared from solution calorimetry and isothermal gas perfusion calorimetry data using two batches of lactose (one predominantly anhydrous α-lactose and one predominantly β-lactose). The calibration curves are shown to be significantly different for the two batches, and it is shown that quantification of the amorphous content of a processed sample of unknown isomeric composition is impossible, unless the calibration curve is prepared from the same batch of material as the processed sample. In addition, some of the other problems inherent in using isothermal gas perfusion calorimetry for amorphous content determination, such as wetting issues and the preparation of calibration standards that mimic processes samples, are discussed.

Keywords: Solution calorimetry; Isothermal gas perfusion calorimetry; Lactose; Amorphous content quantification; Processed pharmaceuticals

1. Introduction

Understanding the effects of processing on solid pharmaceuticals is an essential prelude to attaining process control because many manufacturing steps, such as spray-drying, milling or compression, can induce the formation of (thermodynamically unstable) amorphous regions in what are often presupposed to be crystalline materials. The effects of even small (on the order of 0.5–1%, w/w) amorphous contents can be considerable, because these regions frequently exist on the surface of materials and are thus ideally posi-

* Corresponding author. Tel.: +44 20 7753 5858; fax: +44 20 7753 5942.
E-mail address: graham.buckton@ulsop.ac.uk (G. Buckton).

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2005.04.022

tioned for interaction. Many potential processes, which have been well-documented elsewhere (Hancock and Zografi, 1997; Hancock and Parks, 2000), can occur if a material that is amorphous (or partially amorphous) is used in a formulation. The detection, quantification and management of small amorphous contents therefore play a central role in the implementation of automated process control (APC) during manufacturing.

We have long advocated the use of calorimetric techniques to analyse such samples, principally because modern instruments are extremely sensitive (routinely detecting nano-Watt signals, Gaisford and Buckton, 2001). Two principal calorimetric methods can be employed: solution calorimetry (Gao and Rytting, 1997) or isothermal calorimetry (Ramos et al., 2005). In the former, the enthalpy of solution is measured when the solid sample dissolves in a liquid reservoir, and in the latter, the enthalpy of crystallisation is recorded as the amorphous regions are induced to crystallise (usually by exposure to a plasticiser, often humidity). The methodology in either case is simple; a range of samples of varying amorphous contents is prepared (usually by proportional mixing of entirely amorphous and entirely crystalline material); the measured enthalpy of solution or enthalpy of crystallisation is quantitatively proportional to the amorphous content, which allows the construction of a calibration curve.

Using these methodologies, we have shown previously that it is possible to detect amorphous contents to ±0.5% (w/w) with both solution calorimetry (Hogan and Buckton, 2000) and isothermal calorimetry (Dilworth et al., 2004). Of course, as with any other similar analytical approach, the construction of the calibration curve is critical if the results of any subsequent investigation are to have any value. For calorimetric methods, if the study material has more than one isomer or polymorph, and the enthalpies of solution or crystallisation of the isomers or polymorphs are different; then, it is essential that the material used to prepare the calibration curve matches the isomeric or polymorphic composition of the unknown (processed) sample. The effect is likely to be greatest for polymorphic samples. Furthermore, it is virtually impossible to prepare calibration standards that mimic the type of partially amorphous material formed during processing (i.e. crystalline particles which are partially amorphous on the surface); this necessitates the use of a number of assumptions during data analysis. It is the purpose of this paper to discuss these issues and to demonstrate the problems that may arise during construction of a calibration curve using a model example, lactose (which exists in α and β isomeric forms), as a pharmaceutically important example.

2. Materials and methodology

Two batches of lactose were used in this study, obtained from Borculo Whey Products (Cheshire, UK) and from Sigma–Aldrich (UK). From this point forwards, the Borculo Whey Products batch will be referred to as α-lactose and the Sigma–Aldrich batch as β-lactose.

The precise isomeric composition of each batch of lactose was determined by gas chromatography (GC, data shown in Table 1; data are quoted throughout with a standard deviation (S.D., σ_{n-1}) which represents a confidence limit of 68%), using the protocol devised by Dwivedi and Mitchell (1989). Briefly, a CP 9001 Gas Chromatograph (Chrompack) with a CPsil-5CB capillary column (0.25 mm (i.d.) × 10 m length) (Varian) and a flame ionisation detector was employed. Lactose samples were derivatised prior to GC analysis to prevent mutarotation from the β-form to the α-form once in solution. Dry lactose (1 mg) was dissolved in a derivatisation mixture (comprising 22% trimethilsilylimidazole (Fluka), 19.5% dimethyl sulphoxide (Acros Organics) and 58.5% pyridine (Acros Organics), 2.25 mL) and vortexed for 2 min. Samples (1 μL) were then injected onto the column (the following

Table 1
The isomeric compositions of the lactose batches as supplied and post spray-drying, as determined by GC ($n = 3$)

Supplier	Crystalline material		Spray-dried material	
	α-Lactose (%)	β-Lactose (%)	α-Lactose (%)	β-Lactose.) (%)
Borculo	94.4 ± 1.2	5.6 ± 1.2	66.2 ± 1.4	33.8 ± 1.4
Sigma–Aldrich	14.0 ± 0.9	86.0 ± 0.9	65.4 ± 2.0	34.2 ± 2.0

operating conditions were used: injector port temperature, 300 °C; detector temperature, 250 °C; column outlet temperature, 260 °C; column heating rate, 10 °C min^{-1}; carrier gas, helium at 55 kPa). Data were collected using the Prime Multichannel data station for windows (HPLC Technology, Ltd.). The relative areas under the α- and β-lactose peaks were used to calculate the percentage of each isomer in the mixture. Experiments were conducted in triplicate.

Amorphous lactose samples were prepared from a 10% (w/v) solution (Büchi 190 mini spray-drier), using the operating conditions described previously (Chidavaenzi et al., 1997); spray-dried samples were stored in a desiccator over phosphorus pentoxide. Confirmation of the amorphous nature of the yield was obtained using PXRD (data not shown). The isomeric compositions of the amorphous samples were also determined by GC (Table 1). It can be seen that irrespective of the isomeric composition of the starting material the amorphous material produced had the same composition.

2.1. Solution calorimetry

The partially amorphous samples used to construct the calibration plots were prepared by directly weighing proportional masses of the appropriate crystalline and amorphous materials into glass crushing ampoules. The mass of the crystalline component was kept constant in all the mixtures (200 ± 0.01 mg) and an appropriate amount of spray-dried material was added to make 1, 3 and 5% (w/w) amorphous samples, respectively. The ampoules were wax-sealed using the method described by Hogan and Buckton (2000), loaded into the solution calorimeter (2225 Precision Solution Calorimeter, Thermometric AB, Järfälla, Sweden) and allowed to reach a temperature offset of −180 mK from the thermostatting bath temperature (TAM, 25 °C). Data capture was initiated using the dedicated software package Software for Solution Calorimeter Systems v.1.2. Following an electrical calibration, the ampoule was broken into solvent (deionised water, 100 mL); complete dissolution was ensured by stirring the mixture at 600 rpm. A second electrical calibration was performed after the break. Data were analysed using the dedicated software package. The performance of the calorimeter was checked periodically using the dissolution of a certified reference material (CRM) sample of KCl (NIST, Gaithersburg, USA). Experiments were performed in triplicate.

2.2. Isothermal calorimetry

The partially amorphous samples used to construct the calibration plots were prepared by directly weighing proportional masses of the appropriate crystalline and amorphous materials into the calorimetric ampoule. The mass of the crystalline component was kept constant in all the mixtures (50 ± 0.01 mg) and an appropriate amount of spray-dried material was added to make 1, 3 and 5% amorphous samples (this methodology allowed two methods of data analysis—discussed below). All lactose samples were passed through a sieve stack prior to weighing and only the <125 μm fraction was used for experimentation. The mixtures were made reasonably homogenous in the calorimetric ampoule by tapping and rotating the ampoule a number of times before loading onto the perfusion apparatus (an alternative method would have been to sample from a larger batch of material that had been mixed in a turbula mixer, while this method reduces the weighing error, there is the likelihood that the amorphous component will adhere to the mixing vessel walls which, at such low amorphous contents, is the more significant error). Calibration curves were then constructed for each of the isomeric samples listed in Table 1 by plotting the measured heat of crystallisation (these were determined using two different analysis routines, discussed below) versus the known amorphous content.

Calorimetric data were recorded using a 2277 Thermal Activity Monitor (TAM, Thermometric AB, Järfälla, Sweden) at 25 °C equipped with a gas perfusion unit. Briefly, the unit controls the relative humidity (RH) of a carrier gas flowing over the sample by proportional mixing of two gas lines (0 and 100% RH) using mass-flow controllers. This allows freshly loaded samples to be held under a dry atmosphere, preventing the onset of crystallisation and allowing the apparatus to reach thermal equilibrium before the commencement of data capture. Data were recorded every 10 s with an amplifier range of 3000 μW using the dedicated software package Digitam 4.1. The RH programme started at 0%, switched to 90% after 5 h and returned to 0% after 15 h. Data were recorded in triplicate. Peak anal-

ysis was performed using Origin (Microcal Software Inc., USA).

3. Results and discussion

3.1. Solution calorimetry

Solution calorimetry was first applied to pharmaceutical systems by Pikal et al. (1978), and several excellent discussions of its principles are available (for instance, Gao and Rytting, 1997; Yff et al., 2004). The measured parameter is the enthalpy of solution ($\Delta_{sol}H$); for the dissolution of a pure substance, the value obtained reflects contributions from bond breaking (endothermic) as the crystal lattice breaks apart ($\Delta_{lattice}H$) and bonds formed (exothermic) as the molecules are solvated ($\Delta_{solvation}H$) and can be endothermic or exothermic, depending upon the relative values of the two components. Its utility in quantifying small amorphous contents is predicated on the relationship shown in Eq. (1), which holds true for binary mixtures of non-interacting species (Gao and Rytting, 1997);

$$\Delta_{sol}H_{obs} = X_a\Delta_{sol}H_a + X_b\Delta_{sol}H_b \quad (1)$$

where $\Delta_{sol}H_{obs}$ is the observed enthalpy of solution for a binary mixture of substances a and b, and $\Delta_{sol}H_a$ and $\Delta_{sol}H_b$ are the enthalpies of solution for the pure substances a and b, respectively. As has been well-documented (Motooka et al., 1969; Kishimoto et al., 1973; Pikal et al., 1978), an amorphous material by definition has no lattice enthalpy so its heat of solution reflects $\Delta_{solvation}H$ only. This usually differs significantly from the enthalpy of solution of its crystalline counter-part(s) and provides the necessary sensitivity to allow quantification of small amorphous contents. Previous studies (using α-lactose monohydrate) have shown that amorphous contents in lactose can be quantified to ±0.5% (w/w) using solution calorimetry (Hogan and Buckton, 2000).

Fig. 1 shows the raw power output from the solution calorimeter for the dissolution of a 5% (w/w) amorphous sample of α-lactose. The solution calorimeter used here operates on a semi-adiabatic principle, meaning the raw data recorded are of the form temperature offset versus time. Data are converted to power–time

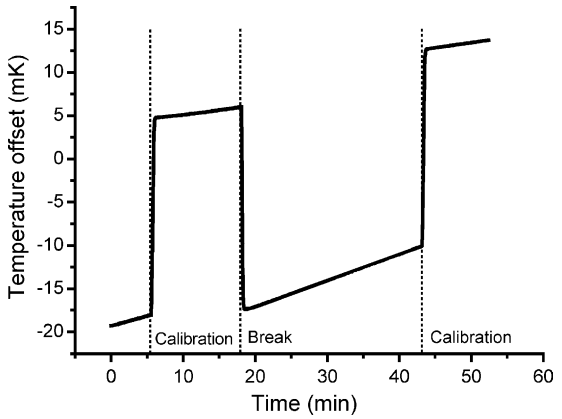

Fig. 1. Raw data from the solution calorimeter showing the dissolution of a 5% (w/w) amorphous sample of α-lactose into water and the two electrical calibrations.

using a series of heat-balance equations (discussed previously; Yff et al., 2004), and integrated to obtain the heat of solution ($\Delta_{sol}H$). Fig. 2 shows the calibration curves constructed for both batches of lactose using the enthalpy of solution data obtained; the raw data are also given in Table 2.

Two important observations can be made. Firstly, it is immediately apparent that there are significant differences between the two batches. Using entirely crystalline samples, the enthalpy of solution of the α-lactose batch was determined to be $57.1 \pm 0.2\,J\,g^{-1}$ while that of the β-lactose batch was $6.5 \pm 0.2\,J\,g^{-1}$, corresponding to the *y*-intercepts of the two calibration

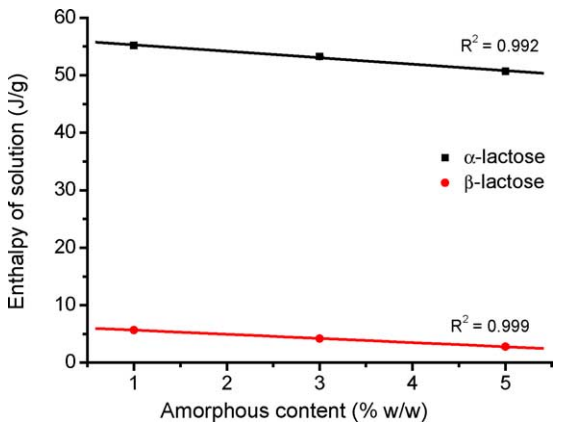

Fig. 2. Calibration curves for the two lactose batches prepared from enthalpy of solution data. The lines shown are linear regression fits; error bars are plotted, but fall within the symbols.

Table 2
Enthalpy of solution data (±S.D.) for the two batches of lactose ($n = 3$)

% Amorphous content	$\Delta_{sol}H$ (J g^{-1})	
	α-Lactose	β-Lactose
1	55.2 ± 0.1	5.7 ± 0.1
3	53.3 ± 0.1	4.2 ± 0.1
5	50.7 ± 0.1	2.8 ± 0.3

plots. For both batches, the measured heats of solution decrease as the amorphous content increases, reflecting the fact that the endothermic contribution from $\Delta_{lattice}H$ is decreasing. Of course, one of the major concerns regarding the preparation of calibration curves for amorphous content determination is now clear; measurement of the enthalpy of solution of a sample of lactose that is partially amorphous would result in two different estimates of amorphous content and, without prior knowledge of its isomeric composition, it would be impossible to know which the correct value was.

Secondly, the error limits are much smaller (the greatest S.D. in the measurements being ±0.3 J g^{-1}) in this study than those reported by Hogan and Buckton (2000) using the same equipment. It is likely that this is a result of the different methodologies used to prepare the ampoules. Here, we opted to weigh the appropriate masses of the crystalline and amorphous materials directly into the crushing ampoule, while in the previous study, large batches of partially amorphous material were prepared by mixing (in a turbula mixer) from which samples were taken and loaded into the crushing ampoules. In the former method, a weighing error is introduced (50 ± 0.01 mg or R.S.D. 0.02%) but this is likely to be much lower than the errors inherent in mixing and sampling. It, therefore, appears that for quantification of small (0.5%, w/w) amorphous contents, the methodology reported here is preferable.

3.2. Isothermal calorimetry

A common approach to crystallise partially amorphous samples in an isothermal calorimeter is to use an elevated RH (maintained through the use of a mini-hygrostat located in the sample ampoule). The sample absorbs humidity, is plasticised and, after a time period that varies in proportion to the amorphous content and RH selected, crystallises. While this experiment is simple to run, when lactose is the sample the data are complex and contain (usually) up to five phases. We have recently discussed the likely causes of these phases (Dilworth et al., 2004). Two of the principal problems of the mini-hygrostat method are that hydration is initiated externally from the calorimeter (and, hence, the initial wetting, and possibly some crystallisation, data are lost) and that there are imbalances in the rate of water evaporation and condensation. To a large extent, the water evaporation/condensation issue is negated, if net heats are determined, but the problem of initiating reaction externally from the calorimeter is an issue for samples that crystallise over a short (1–2 h) time span.

In this study, we opted to use RH perfusion to initiate crystallisation because it obviates this problem; however, it also requires a different approach to data analysis. This is because, once the RH has been elevated, all the internal surfaces of the ampoule, as well as the sample, are wetted, which produces a large exothermic heat signal that often occurs over a time-period that is longer than the time required for the sample to crystallise. In effect, the crystallisation signal is obscured by the wetting response; an example of this is shown by the response of a 5% (w/w) amorphous sample of α-lactose, which is represented in Fig. 3. There are four ways to ameliorate the data:

(i) Assume that the wetting response is uniform in all samples and measure the total heat released once the RH is increased.

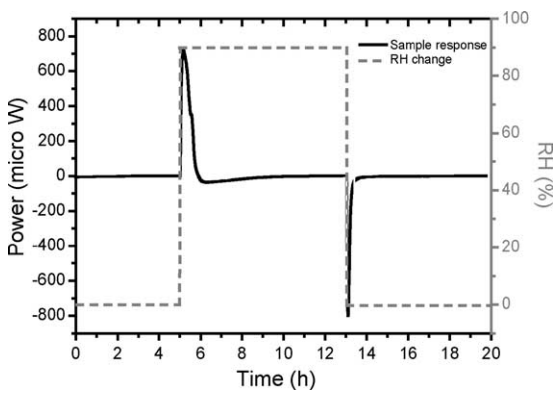

Fig. 3. The wetting and drying calorimetric response of a 5% (w/w) amorphous sample of α-lactose.

(ii) Determine the wetting response of the empty ampoule in a separate experiment and subtract this value from the experimental data.
(iii) Determine the wetting response of an equivalent mass of the crystalline material in a separate experiment and subtract it from the experimental data.
(iv) Return the system to 0% RH after crystallisation and subtract the drying response from the wetting response.

Method (i) does not allow a quantitative assessment of the enthalpy of crystallisation, while method (ii) does not compensate for the wetting response of the sample, which may become significant with larger sample masses; these methods were therefore discarded. Method (iii) compensates for the wetting response of the crystalline component of the sample (and ignores the wetting of the amorphous material) while method (iv) compensates for the wetting response of the entire sample (although it is noted that the sample that wets, partially amorphous, differs slightly from that which dries, crystalline, and the method does not compensate for any water used to form the monohydrate); in both cases, it is assumed that the errors are negligible which is reasonable for the standards used to prepare the calibration plots but may be an issue for processed samples—this point is discussed more fully below.

Accordingly, calibration curves were prepared using methods (iii) and (iv). Fig. 4 shows a typical wetting response of a crystalline sample of α-lactose. Subtraction of these data from those shown in Fig. 3 resulted in the peak shown in Fig. 5. The area under this peak was used in the construction of a calibration plot in accordance with method (iii) (Fig. 6). Alternatively, subtraction of the wetting and drying peaks shown in Fig. 3 resulted in the peak shown in Fig. 7; the area under this peak was used in the construction of a calibration plot in accordance with method (iv) (Fig. 8). Calibration plots were constructed using these methods for both of the lactose batches. The data are summarised in Tables 3 and 4.

As in the case of the solution calorimetry data, the calibration plots shown in Figs. 6 and 8 represent lactose samples that are either predominately α-lactose or predominately β-lactose and are clearly different. Since the enthalpies of crystallisation of the two forms differ (α-lactose, ~169 J g^{-1} and β-lactose,

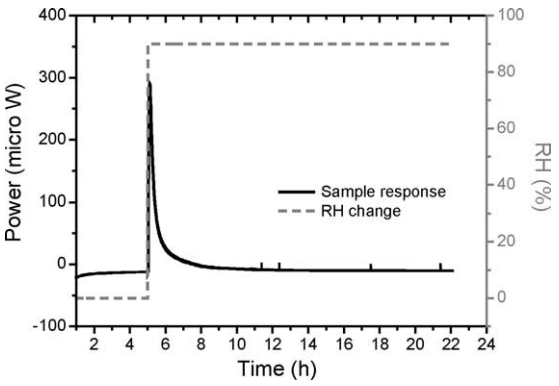

Fig. 4. Power–time data for the wetting response of a crystalline batch of α-lactose.

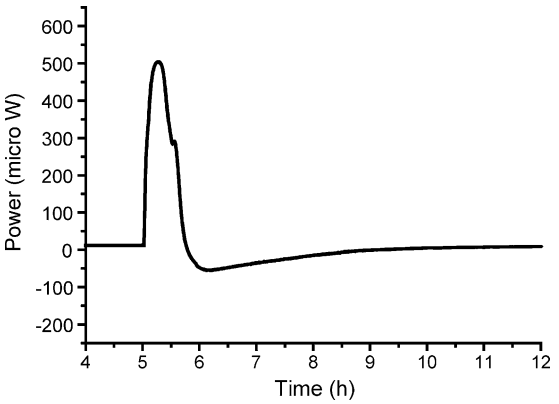

Fig. 5. The power–time data obtained via method (iii) (subtraction of the crystalline wetting response from the wetting response of the partially amorphous sample) for a 5% (w/w) sample of amorphous α-lactose.

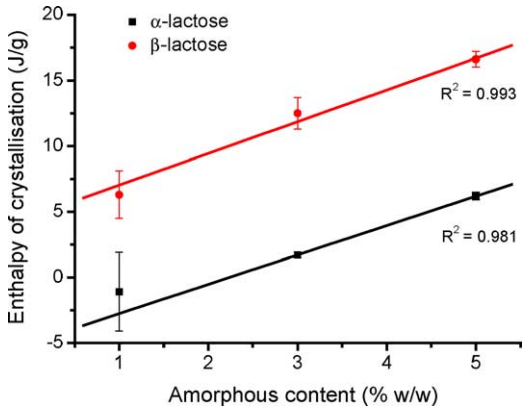

Fig. 6. Calibration curves for the two lactose batches prepared using method (iii). The lines shown are linear regression fits.

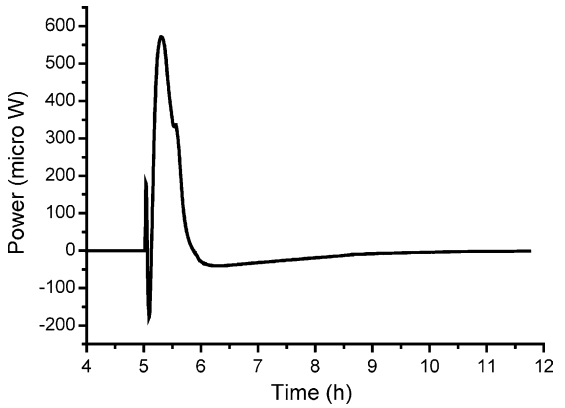

Fig. 7. The power–time data obtained via method (iv) (subtraction of the drying response from the wetting response of the partially amorphous sample) for a 5% (w/w) amorphous lactose sample (predominately, α-lactose).

∼197 J g^{-1}; both estimated by DSC, Dilworth et al., 2004), it is likely that the differences in the calibration plots arise because the samples crystallise to different forms (the isomeric compositions of the spray-dried batches being the same within error, Table 1). It has been suggested that complete crystallisation of lactose cannot happen below an RH of 94% (Price and Young, 2004), if crystallisation occurs via primary nucleation. However, in the case of the data presented here there is a large amount of crystalline seed material present; so it is likely that secondary nucleation is the overriding mechanism. This would allow complete crystallisation to happen even though an RH of lower than 94% was

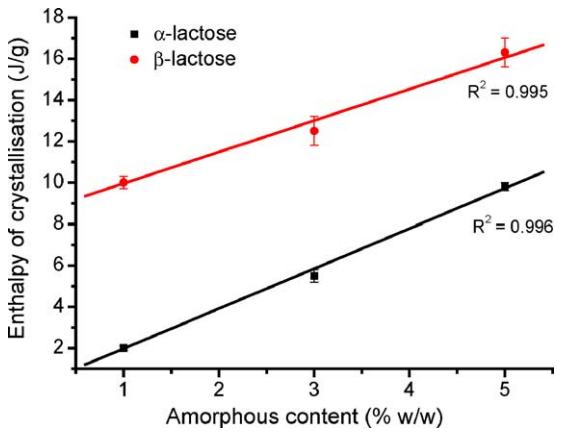

Fig. 8. Calibration curves for the two lactose batches prepared using method (iv). The lines shown are linear regression fits.

Table 3
Enthalpy of crystallisation data (±S.D.) for the two batches of lactose determined using method (iii) ($n = 3$)

% Amorphous content	$\Delta_{sol}H$ (J g^{-1})	
	α-Lactose	β-Lactose
1	−1.1 ± 3.0	6.3 ± 1.8
3	1.7 ± 0.2	12.5 ± 1.2
5	6.2 ± 0.3	16.6 ± 0.6

used (previous experiments have shown that samples removed from the TAM post crystallisation have been entirely crystalline (Briggner et al., 1994)). It would appear that the ratio of α- to β-lactose formed is governed to a large extent by the isomeric nature of the crystalline seed material, the α-lactose batch forcing crystallisation mainly to α-lactose monohydrate, and the β-lactose batch forcing crystallisation mainly to β-lactose.

A more interesting feature of the calibration curves is that those for the β-lactose batch do not tend to a *y*-intercept value of zero with no amorphous material present, the effect being more dramatic when method (iv) is used to construct the curve. Although this observation initially seems counter-intuitive, one explanation for this discrepancy may be the fact that β-lactose is known to mutarotate to α-lactose monohydrate under elevated RH conditions, an event that is exothermic (Angberg et al., 1991). This being so, mutarotation of the sample during measurement would result in a net exothermic addition to the measured response during wetting. This event would not be cancelled out at all using method (iv) and, assuming mutarotation occurs faster in amorphous material than crystalline material because of its faster uptake of water, would only be partially compensated for using method (iii). This would lead to the behaviour noted for the β-lactose batches in Figs. 6 and 8, although further experimentation would be required to confirm this theory.

Table 4
Enthalpy of crystallisation data (±S.D.) for the two batches of lactose determined using method (iv) ($n = 3$)

% Amorphous content	$\Delta_{sol}H$ (J g^{-1})	
	α-Lactose	β-Lactose
1	2.0 ± 0.1	10.0 ± 0.3
3	5.5 ± 0.3	12.5 ± 0.7
5	9.8 ± 0.2	16.3 ± 0.7

As before, measurement of the crystallisation response of a partially amorphous lactose sample of unknown isomeric composition would give different results from the two calibration plots. The best way to resolve this problem, therefore, is to ensure that any calibration standards are prepared from the same batch of material as that to be tested during processing. It is also not sufficient to quote an enthalpy of crystallisation for a material without also defining its isomeric state. This, in part, may be one of the factors that contributes to the range of stated enthalpies of crystallisation for lactose (we have published an overview of these values previously, Dilworth et al., 2004), although these discrepancies also arise in part through the use of different integration strategies.

Comparison of the standard deviations listed in Tables 3 and 4 reveals that method (iv) is the most precise, and would thus be recommended for future studies of this type.

An additional problem with isothermal calorimetric methodologies is that the nature of the amorphous standards used to prepare the calibration plot do not mimic the physical nature of processed materials (i.e. the standards comprise particles that are either wholly amorphous or wholly crystalline while a processed material is likely to comprise of smaller particles (and hence have a greater surface area) consisting of crystalline cores with an outer corona of amorphous material). This raises a number of concerns. Firstly, the differences in surface area mean that the rate of water absorption will be different in a processed material than from the calibration standards; necessarily, this will manifest itself as a change in the kinetic response of the samples during analysis although, as net areas are measured, should not affect the enthalpy obtained.

Secondly, a greater problem is likely to be that the wetting response of a processed sample will (in effect) be that of a wholly amorphous material while that of a standard approximates to the wetting response of a crystalline material. This is important because, using method (iv), the drying response (of the now crystalline material) is subtracted from the wetting and crystallisation response (of the processed material). Thus, if the wetting enthalpies of the amorphous and crystalline forms are different, it is likely that the amorphous content predicted from the calibration plot will have a (small) systematic error.

Finally, the amorphous material in a processed material is sited directly on top of a crystalline substrate, which acts as a seed, which means that secondary nucleation predominates. In the material used for calibration, the amorphous and crystalline particles are discreet entities, which means that primary nucleation may occur. As noted above, this requires an almost saturated vapour space presumably because more water needs to be absorbed to plasticise the sample sufficiently to induce crystallisation. To a large degree, the data presented above suggest that the crystalline particles do act as a seed, allowing secondary nucleation, because at such low amorphous contents, they considerably outnumber the amorphous particles; this effect will, however, diminish as the proportion of amorphous material increases.

Since it is difficult to conceive of a method by which calibration standards that mimic processed materials could be prepared, method (iv) appears to be the best currently achievable.

4. Summary

This work discusses two calorimetric approaches that may be used to quantify small amorphous contents. Both solution calorimetry and isothermal calorimetry can quantify small (of the order of 0.5%, w/w) amorphous contents in processed pharmaceuticals although, from the data presented here for lactose, solution calorimetry appears to give the more precise results. However, both methods require the construction of a calibration plot using standard materials with known amorphous contents and we have demonstrated that care must be taken when using these approaches if the sample to be studied has more than one isomeric form (and each form has a different heat of solution or crystallisation). In the specific case of lactose, calibration plots prepared from material that comprised mainly α-lactose monohydrate or mainly β-lactose were significantly different; the use of each plot to quantify the amorphous content of an unknown sample would result in drastically different answers. As such, it is recommended that calibration plots be prepared from the same batch of material as that to be processed. It is further noted that in the case of isothermal calorimetric experiments, because the nature of a processed material differs from that of the calibration standards,

and it is not clear how calibration standards could be prepared that mimic processed materials, a small systematic error may be inherent to such data that should be accounted for during subsequent data interpretation.

References

Angberg, M., Nyström, C., Castensson, S., 1991. Evaluation of heat-conduction microcalorimetry in pharmaceutical stability studies. Part III: crystallographic changes due to water vapour uptake in anhydrous lactose powder. Int. J. Pharm. 73, 209–220.

Briggner, L.-E., Buckton, G., Byström, K., Darcy, P., 1994. The use of isothermal microcalorimetry in the study of changes in crystallinity induced during the processing of powders. Int. J. Pharm. 105, 125–135.

Chidavaenzi, O.C., Buckton, G., Koosha, F., Pathak, R., 1997. The use of thermal techniques to assess the impact of feed concentration on the amorphous content and polymorphic forms present in spray dried lactose. Int. J. Pharm. 159, 67–74.

Dwivedi, S.K., Mitchell, A.G., 1989. Gas chromatographic analysis of anomeric composition of lactose. J. Pharm. Sci. 78, 1055–1056.

Dilworth, S.E., Buckton, G., Gaisford, S., Ramos, R., 2004. Approaches to determine the enthalpy of crystallisation, and amorphous content, of lactose from isothermal calorimetric data. Int. J. Pharm. 284, 83–94.

Gaisford, S., Buckton, G., 2001. Potential applications of microcalorimetry for the study of physical processes in pharmaceuticals. Thermochim. Acta 380, 185–198.

Gao, D., Rytting, J.H., 1997. Use of solution calorimetry to determine the extent of crystallinity of drugs and excipients. Int. J. Pharm. 151, 183–192.

Hancock, B.C., Parks, M., 2000. What is the true solubility advantage for amorphous pharmaceuticals? Pharm. Res. 17, 397–404.

Hancock, B.C., Zografi, G., 1997. Characteristics and significance of the amorphous state in pharmaceutical systems. J. Pharm. Sci. 86, 1–12.

Hogan, S.E., Buckton, G., 2000. The quantification of small degrees of disorder in lactose using solution calorimetry. Int. J. Pharm. 207, 57–64.

Kishimoto, K., Suga, H., Seki, S., 1973. Calorimetric study of the glassy state. Part VIII: heat capacity and relaxational phenomena of isopropylbenzene. Bull. Chem. Soc. Jpn. 46, 3020–3031.

Motooka, I., Hashizume, G., Kobayshi, M., 1969. Studies of the grinding of condensed phosphates. Part VI: the effect of grinding on the heat of solution of sodium trimetaphosphate. J. Phys. Chem. 73, 3012–3013.

Pikal, M.J., Lukes, A.L., Lang, J.E., Gaines, K., 1978. Quantitative crystallinity determinations for β-lactam antibiotics by solution calorimetry: correlation with stability. J. Pharm. Sci. 67, 767–772.

Price, R., Young, P.M., 2004. Visualisation of the crystallisation of lactose from the amorphous state. J. Pharm. Sci. 93, 155–164.

Ramos, R., Gaisford, S., Buckton, G., Royall, P.G., Yff, B.T.S., O'Neill, M.A.A., 2005. A comparison of chemical reference materials for solution calorimeters. Int. J. Pharm. 299, 73–83.

Yff, B.T.S., Royall, P.G., Brown, M.B., Martin, G.P., 2004. An investigation of calibration methods for solution calorimetry. Int. J. Pharm. 269, 361–372.