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International Journal of Pharmaceutics 274 (2004) 35–40
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Rapid communication

The potential of high speed DSC (Hyper-DSC) for the detection and quantification of small amounts of amorphous content in predominantly crystalline samples

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Received 21 August 2003; received in revised form 7 January 2004; accepted 12 January 2004

Abstract

The purpose of this study was to explore whether it is possible to use hyper differential scanning calorimetry (HDSC) to detect and quantify low levels of amorphous content in samples that are mostly crystalline. HDSC uses scan rates that are much faster than conventional DSC, and consequently results in greater sensitivity. It was found that with every increase in scan rate it became easier to detect the glass transition (T_g) response. Scanning at 500 °C/min was possible and this gave such great sensitivity that very low sample mass (ca. 1 mg) could be used without any loss in detection of T_g. Mixtures of crystalline and amorphous (spray dried) lactose were prepared and scanned at 500 °C/min. It was observed that the T_g response was easily detected even for samples that contained 1.5% amorphous content when using very low samples mass. The view held at present is that DSC is not well suited to studies of amorphous content if the sample contains 10% or less of the amorphous material. The data generated here show that much better detection sensitivity is possible when using the rapid scan rates. As well as being able to detect the presence of very low amorphous contents it was also possible to obtain a quantification, as a linear response was obtained for the step height change in heat flow as a function of amorphous content. It was concluded that HDSC provides a method of obtaining a very fast assessment of the presence of amorphous form, the possibility to quantify this and the need to use a very low sample mass. The combination of speed, low sample mass and sensitivity makes this a very valuable technique for studies on partially amorphous samples.

Keywords: Amorphous content; Crystalline samples; Sensitivity; High sensitivity DSC; Lactose

1. Introduction

Differential scanning calorimetry (DSC), although used frequently for investigations of phase behaviour, compatibility and polymorphism, is not frequently reported in the field of characterising amorphous content. It has often been difficult to quantify very low levels of amorphous content using DSC (below 10% w/w), because of the small energy changes associated with the measurement of the glass transition (T_g) at these low levels (Saklatvala et al., 1999).

High speed or high performance DSC (Hyper-DSC), has been reported to have high sensitivity while operating at extremely high scan rates, in the 100–

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0378-5173/$ – see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.ijpharm.2004.01.018

500 °C/min range (Pijpers et al., 2002). No workers have yet applied such high heating rates to pharmaceutically significant amorphous material in order to characterise (detect and/or quantify) samples with low amorphous content.

Lactose was selected as the test material for the present study as it is one of the most frequently used pharmaceutical excipients. The following methods can all detect/quantify amorphous lactose in crystalline samples to 1% (or better) amorphous content: near infrared, gravimetric vapour sorption, a combined gravimetric—near IR method (Hogan and Buckton, 2001), isothermal microcalorimetry (Briggner et al., 1994; Buckton et al., 1995), solution calorimetry (Hogan and Buckton, 2000), inverse gas chromatography (Newell et al., 2001) and Raman spectroscopy (Taylor and Zografi, 1998). All of the methods described above provide advances in detection, quantification and understanding of amorphous content in largely crystalline samples, however, they all have potential limitations, including; interference of formulation components in spectroscopic and solution calorimetry methods, difficulties in selecting a probe vapour to absorb into (and cause crystallisation for) hydrophobic materials, uncertainty about quantification for isothermal microcalorimetry (Darcy and Buckton, 1998) and inverse gas chromatography experiments. Consequently, there is still a need for a reliable method to test for low levels of amorphous content in powders, which ideally should use a small sample mass (as often very limited quantities of material exist) and should be rapid (to increase confidence that the amorphous content has not been lost during the test and to allow for fast sample screening).

The aim of this study was to evaluate the potential of Hyper-DSC to characterise amorphous and low amorphous content pharmaceutical powders and to test its potential as a screening tool.

2. Materials and methods

α-Lactose monohydrate (batch 144018, Lactochem) was used as received, after the structure was confirmed using powder X-ray diffraction. The feed solution for the spray dryer was 25 g of α-lactose monohydrate dissolved in 250 ml of distilled water. The spray drier (Büchi B-191, Büchi, Switzerland) was operated as described by Buckton et al. (1997) in order to produce amorphous lactose (stored in a desiccator above silica gel at room temperature). Samples with a range of amorphous contents from 100 to 1% w/w were mixed. The amorphous sample was found to give a halo in the X-ray diffractometer (absence of detectable crystallinity). For X-ray experiments, approximately 50 mg of sample was placed onto the sample holder and gently compressed into a disc, ensuring that there were no cracks or defects on the disc surface. The sample holder was then loaded into a Philips PW3710 powder X-ray diffractometer and run under the following conditions: Cu 45 kV, 30 mA current 2–50° 2θ, step size: 0.02° time at each step 5 s. All measurements were performed at ambient temperature and relative humidity.

Hyper-DSC experiments were carried out using a diamond DSC (Perkin-Elmer, Shelton, CT), using a helium purge gas with a rate of flow of 30 ml/min. 50-μl aluminum pin-holed pans were used for scans that proceeded to the melting point of lactose, hermetically sealed pans were used for samples that were only scanned as far as T_g. Two reference pans were used in order to increase the baseline reproducibility. Samples masses were 1–3 mg calibration was with indium and zinc.

3. Results and discussion

It was important to test whether linear control of heating rate could be achieved at rapid scan rates. To test for this, plots of temperature (measured directly under the sample pan) were obtained as a function of time (not shown), and all demonstrated the linear control of the heating rate for all scan rates used in this study (after the first 10 s of the experiment).

The DSC scans of amorphous lactose revealed a glass transition, a crystallisation and a melt region (showing alpha and beta melts). For this study the area of interest was the glass transition region. The T_g responses for amorphous lactose are shown in Fig. 1 for different scan rates. It was observed (Fig. 1) that the size of the DSC response increased substantially as the scan rate was increased (change in heat capacity (W/g) was ca. 1 at 100 °C/min, 3 at 250 °C/min, 5 at 400 °C/min and 10 at 500 °C/min). DSC output is measured in mW (J/s), at fast scan rates the same

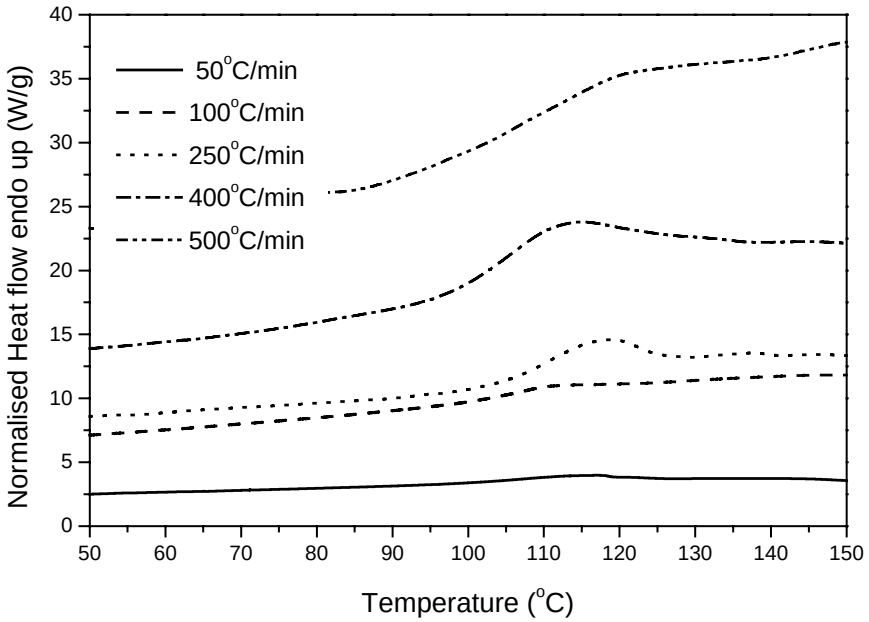

Fig. 1. The DSC response showing the increase in detection sensitivity of the T_g as scan rate is increased.

heat flow occurs over a shorter time frame and therefore the thermal event becomes larger. This allows extremely low-energy transitions, which will not even be seen using conventional DSC scan rates, to be identified and measured with ease and also allows the measurement of much smaller samples, down to a few micrograms. For the amorphous sample T_g was easily observed close to the literature value of 116 °C (Shergill, 2003). A surprising result was that crystallisation exotherms were observed at heating rates up to 500 °C/min. Pijpers et al. (2002) did not observe crystallisation in amorphous samples of polyethylene terephthalate heated at the same rates, and it was expected that the scan rate would be too fast for crystallisation of lactose to occur in the time scale of the experiment. This difference indicates the increased mobility of small molecules, for example lactose, when compared to polymers. Investigating the relationship between crystallisation and heating rate may offer an opportunity to characterise, or at least rank order, the mobility within de-vitrified amorphous pharmaceuticals.

The T_g responses for mixtures of amorphous and crystalline lactose are shown in Fig. 2 using a *y*-axis scale that shows most of the T_g values clearly. The T_g response for the 100% amorphous sample is too large to be recorded fully on this axis scale, furthermore it is easy to rescale to show the responses more clearly for the lower amorphous content (e.g. 1.5%). The onset of T_g was not affected by scan rate (80 °C at all rates), but was much lower than the accepted value of lactose T_g, probably due to the fact that efforts were not taken to prevent water absorption into the sample during mixing. The aim of the work here was to demonstrate the explore the utility of Hyper-DSC to study samples rapidly as a screening tool, and not to try to get the most accurate value of T_g for a perfectly dry sample (which is more about sample loading than instrument method). There was no change in T_g as a function of scan rate, as hermetically sealed pans were used resulting in retention of water within the pan. For scan rates of 500 °C/min it was very easy to see the detail of the T_g for the sample with 1% amorphous content. The fact that at high scan rates it is easy to detect the T_g of these samples is contrary to the established knowledge. Saleki-Gerhardt et al. (1994) first demonstrated that a sample had to have around 10% amorphous content in order to be detected by conventional DSC and this figure has not been challenged to any meaningful extent since that time. It has

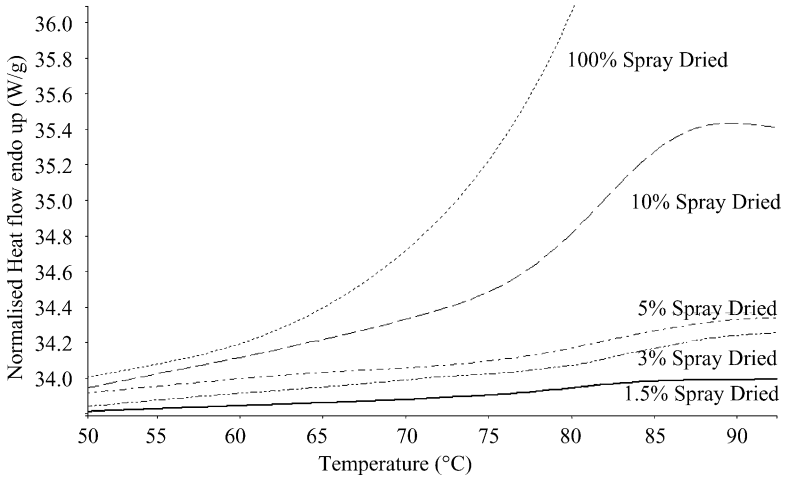

Fig. 2. DSC responses for the T_g region for various mixtures of amorphous and crystalline lactose (% = amorphous content).

been shown that modulated temperature DSC is able to detect approximately 1% w/w amorphous content (Guinot and Leveiller, 1999). However, the data presented here show that with fast scan rates it is simple to rapidly (much more rapdily than with slow modulated temperature experiments) detect T_g for samples with very low amorphous contents. There is obviously a great advantage in being able to detect small amorphous contents, and given that the amorphous form is not thermodynamically stable there is further great advantage to being able to detect it quickly and so minimise the chance of the amorphous form being lost during the experiment (even though crystallisation is most rapid above T_g it is also possible for materials to crystallise, albeit more slowly, below T_g, and to suffer relaxation if low underlying heats rates are applied). The change in the heat flow signal at T_g was measured as the step height change from the onset to the maximum height for the sample, giving an indication of the change of the specific heat of transition for the T_g. The results are shown in Fig. 3 for the range 0–100% and Fig. 4 for 0–10% amorphous content. These data produce a linear relationship from 0 to 100% amorphous content, and it is clear that detection is possible to less than 1% amorphous content. Following the method described in the United States Pharmacopoeia and by Miller and Miller (1993), it is possible to determine the theoretical limit for detection and quantification.

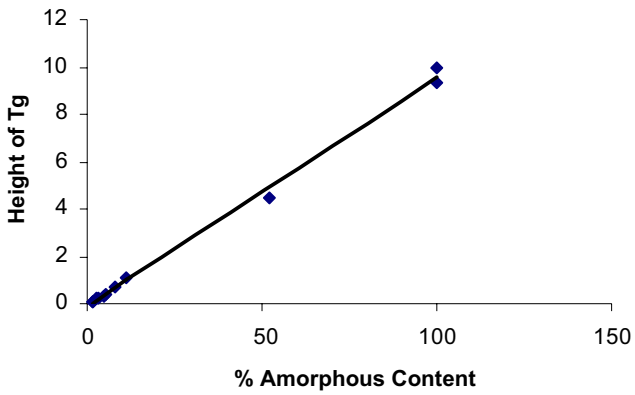

Fig. 3. Relationship between amorphous content and height of the T_g response, measured at 500 °C/min.

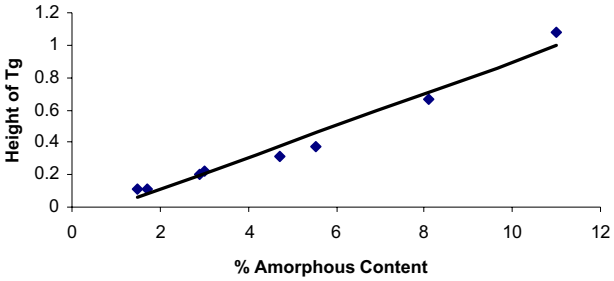

Fig. 4. Relationship between amorphous content and height of the T_g response, measured at 500 °C/min, showing only the responses in the region 0–10% amorphous content, that being the area that has not been thought to be measurable using DSC.

The equation for the line is

$$\text{height change (W/g)} = 0.039 + 0.043 \times \text{concentration}$$

the $r = 0.9999$ ($n = 8$). Based on these data the theoretical limit of detection is 0.57% and the limit of quantification = 1.89% amorphous content. The commonly accepted definitions were used to determine these values, and these are as follows; the limit of detection is the analyte concentration giving a signal equal to the blank or zero response, plus three standard deviations of this value; whereas the limit of quantification is the analyte concentration giving a signal equal to the blank or zero response, plus 10 standard deviations of this value. In the present study, the standard deviation of the intercept from the linear relationship given above, 0.008, was used as the standard deviation of the blank response, this is accepted practice in such calculations (Miller and Miller, 1993).

4. Conclusion

There are increasing numbers of methods by which it is possible to determine the amorphous content of samples with small amorphous contents. All existing methods have some limitations and it remains important to find rapid methods that require very small same masses.

Hyper-DSC is able to provide valuable information, rapidly and on small samples opening a new area for research in amorphous materials.

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