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The use of isothermal microcalorimetry in the study of small degrees of amorphous content of a hydrophobic powder

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Abstract

The crystallinity of a hydrophobic drug (L-365,260) has been investigated by X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC) and isothermal microcalorimetry. The crystallinity was assessed in the isothermal microcalorimeter by taking a ratio of the responses seen when an unknown sample and an amorphous standard were exposed to ethanol vapour. It was found that large amounts of the material (up to 75%) became amorphous with protracted micronisation. The XRPD, DSC and isothermal microcalorimetry methods could all be used to characterise the amorphous content for these highly disordered samples. When the drug was milled in a ball mill, considerably less of the sample mass became amorphous (less than 10% even for reasonably long milling times) and for such samples, only isothermal microcalorimetry was a suitable technique for quantifying the degree of disorder as no difference was observed by use of DSC or XRPD for materials with up to 10% amorphous content. Microcalorimetry is a suitable approach for crystallinity studies on hydrophobic powders, giving a lower limit of detection for amorphous content that is in the order of 1% or less, which is well below that seen for XRPD.

Keywords: Disorder; Microcalorimetry; X-ray diffraction; Differential scanning calorimetry; Milling; Hydrophobic solid

1. Introduction

It has been argued that the presence of amorphous material may have a very significant influence on the way that powders interact, and thus may influence the ease of processing, stability (chemical and physical), and use of the powder.

There are many techniques by which it is possible to probe the crystal properties of powdered materials. These include powder X-ray diffraction (XRPD), differential scanning calorimetry (DSC), solution calorimetry, spectroscopic techniques (infra red) and gravimetric water sorption. Saleki-Gerhardt et al. (1994) have recently compared the sensitivity of different techniques used to detect amorphous material in powders which are predominantly crystalline. It was noted that XRPD

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was unable to detect amorphous content if it amounted to less than 10% of the total mass. Similar lower detection limits were noted for density and heat of crystallisation determinations. Water vapour sorption (gravimetric) was deemed to be the most sensitive approach. Recently, isothermal microcalorimetry has been used to monitor amorphous content of powders by causing the amorphous material to recrystallise due to the presence of water vapour in the calorimeter cell. We are only aware of a small number of studies which have utilised isothermal microcalorimetry in this manner (Briggner et al., 1994; Sebhatu et al., 1994a; Sebhatu et al., 1994b; Aso et al., 1995; Buckton and Darcy, 1995; Buckton et al., 1995); although others (most recently Thompson et al. (1994) and Ward and Shultz (1995)) have used solution calorimetry. The samples which have been investigated are lactose (Briggner et al., 1994; Sebhatu et al., 1994a; Sebhatu et al., 1994b; Buckton and Darcy, 1995; Buckton et al., 1995), salbutamol sulphate (Buckton et al., 1994) and nifedipine (Aso et al., 1995); we are not aware of any reports on the investigation of materials with low aqueous solubility.

The purpose of this work is to investigate the possibility of using non-aqueous vapours to recrystallise amorphous contents of a powder with poor aqueous solubility, to determine whether isothermal microcalorimetry may have a more general application for precise studies on powder characterisation. A further aim is to utilise the fact that the experiment allows the process to be followed in real time, to see if any fundamental information is available concerning the recrystallisation event.

2. Materials and method

The drug used was L-365,260 (MSD, a novel compound being evaluated for the treatment of anxiety and panic disorders, which is (3*R*)-3[*N*'-(3-methylphenyl)ureido]-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one). The compound has a molecular weight of 398.5 it is soluble in ethanol to the extent of 11 mg ml^{-1}, but its solubility in water is < 0.0001 mg ml^{-1}.

Amorphous material was prepared by rapidly adding a solution of 1.1 g of drug in 100 ml of 96% ethanol to 300 ml of distilled water. The precipitate was collected by filtration and vacuum dried at 30°C, 200 mbar, for 48 h.

2.1. Isothermal microcalorimetry

The experiment involved weighing a sample of powder (normally 30 mg accurately weighed) into the bottom of a glass vial designed for use in the calorimeter. A small tube containing ethanol (96%) or ethanol:water mixtures was placed into the vial, such that the vapour could escape, but so that the powder was separated from the liquid. The vial was then crimp-sealed with a rubber stopper and aluminium over seal, in order to make an air-tight closure. The vial, powder and liquid reservoir were all pre-equilibrated separately to 25°C prior to assembly and sealing, and then were immediately presented to the equilibration position of an isothermal microcalorimeter (Thermal Activity Monitor, Thermometric AB), set at 25°C. After 30 min the vial was lowered into the measuring position. The calorimeter has a water bath which will achieve thermostation of about 10^{-4}°C, but which can detect heat changes as small as 10^{-6}°C due to a differential reading between a sample and reference cell. In this instance the reference cell was a freshly sealed empty vial, which allows a correction for heat effects due to stress relaxation in the rubber seal of the vial. The positioning of the vapour generation site and the powder both within the measuring cell means that evaporation of the vapour and sorption to the powder are almost equal and opposite in nature, thus the detected response for vapour phase wetting of the powder is minimal. If the vapour were generated remote from the cell and passed over the powder a large wetting event would be seen which may often mask any small thermal events due to changes in crystallinity within the sample.

2.2. X-ray powder diffraction

XRPD was undertaken using a Phillips PW3710 X-ray powder diffractometer. The operating conditions were 45 kV, 49 mA CuKα radiation with a scan speed of 2° 20 min^{-1}.

2.3. Differential scanning calorimetry

DSC was undertaken using a Perkin Elmer 7 series instrument scanning at 10 K min^{-1} from 313 to 523 K. Sample weights were approximately 4 mg, which were weighed accurately and sealed into aluminium pans.

2.4. Milling

Samples of drug were milled in a microniser (Glen Creston) for varying periods of time. The microniser is a very small scale high energy ball mill system. After preliminary experiments it was found that reproducibility was only obtained if the sample was milled for 2 min, then removed, mixed to homogenise the material and then remilled for a further period of 2 min. The data for different milling times are, therefore, composite milling times of sequential 2-min periods. This process was repeated with a vibrating ball mill (Fritsch) instead of the microniser.

3. Results and discussion

The XRPD pattern for the starting crystalline material is given in Fig. 1. The absolutes of crystallinity are not readily definable, but this material shows no evidence of an amorphous halo, and is being taken as a standard for the crystalline drug. It yielded a single melting endotherm, with a peak temperature (T_m) of 443.3 K and an enthalpy of fusion (ΔH_{fus}) of 43.3 J g^{-1} when investigated by DSC. There was no evidence of any recrystallisation event when exposed to ethanol vapour in the microcalorimeter.

The material which was rapidly precipitated out of solution shows all signs of being amorphous. The XRPD pattern (Fig. 1) showed a simple 'halo' effect without individual peaks. The DSC trace showed no melt, but a small peak with a T_m at 397.6 K which may well relate to a solid state transition, such as a glass transition temperature. There has been considerable discussion about the significance of glass transition temperature in terms of facilitating recrystallisation of materials (Hancock and Zografi, 1994). In this instance the transition is significantly above room temperature and as such it would not be expected that the material would spontaneously recrystallise to the thermodynamically stable form. This will have significance for processed materials, as changes induced during processing will tend to endure if recrystallisation is not possible at ambient conditions.

A typical response obtained from the microcalorimeter is shown in Fig. 2 for the situation where the precipitated (amorphous) material has been exposed to the ethanol vapour. The trace shows an initial exotherm due to the standard disruption of lowering the cell into the measuring site, combined with a small response for the net difference between the enthalpy of vaporisation of the ethanol and the enthalpy of sorption to the powder. This is followed after about 30 min by an endotherm, which in turn is followed by a large sharp exotherm (just before 1 h). The sharp exotherm is the response for the crystallisation of the amorphous material. The sharp peak is indicative of an extremely cooperative process, such that the material effectively all recrystallises at the same time (or within a very short time). These observations are totally in keeping with those seen by Briggner et al. (1994) (for example) who followed the recrystallisation of lactose with water vapour.

If the sample is removed after the major exotherm in Fig. 2 and reexamined by XRPD and DSC the material is seen to have indeed recrystallised. The DSC data reveal a single melting endotherm with mean T_m 442.7 K (n = 5 different samples from the microcalorimetry recrystallisation experiments) and mean ΔH_{fus} of 43.3 J g^{-1}. The XRPD of the sample as removed from the calorimeter (after crystallisation) was indistinguishable from the starting material. All materials removed after the recrystallisation event in the calorimeter gave the same XRPD pattern. The

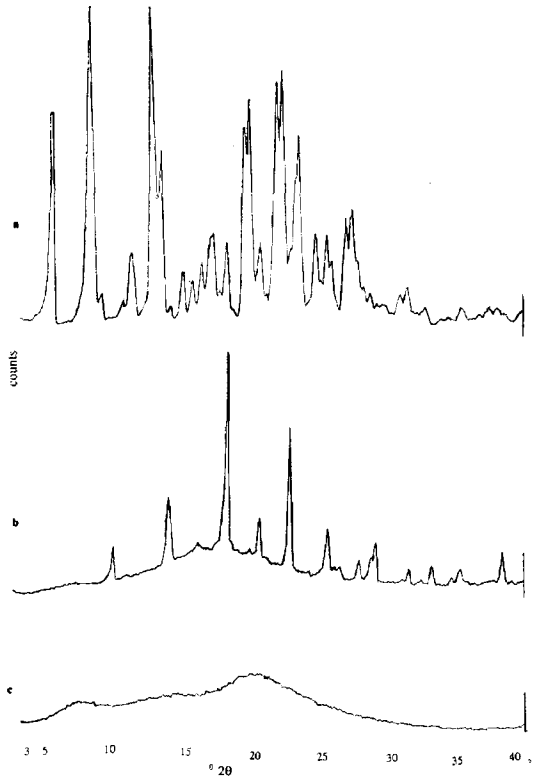

Fig. 1. XRPD pattern for the crystalline starting material (top trace); XRPD pattern of a sample which was removed from the calorimeter after being exposed to ethanol vapour up until the point where the endotherm takes place, before rapidly removing from the calorimeter cell (centre); XRPD of the amorphous sample (bottom).

mean (n = 5) area under the power-time curves obtained using the isothermal microcalorimeter for the recrystallisation event for five runs on the amorphous material has been calculated and equals 9.38 ± 1.56 J g^{-1}.

With the experiments undertaken with lactose it was found that the recrystallisation event could be slowed by use of lower relative humidities (Briggner et al., 1994). To investigate the analogous situation for non-aqueous vapour, the microcalorimetric experiment was repeated using alcohol-water mixtures. Typical microcalorimetry responses for recrystallisation of the amorphous samples using either a mixture of 75:25 or 50:50 of 96% ethanol:water are shown in Fig. 3, in comparison with the result using 96% ethanol alone. It can be seen that the lag time prior to recrystallisation is increased as the ethanol content in the liquid reservoir is decreased (4–6 h for 75:25 and up to 10 h for 50:50). The responses obtained at lower ethanol content also show clear signs of multiple peaks. These multiple peaks could be interpreted as a more gradual crystallisation (a less co-operative process), being representative of some element of differentiation between the onset of crystallisation (exothermic), followed by the expulsion of the ethanol vapour (endothermic) and then a superimposed repeat of this process and the condensation of the ethanol back into solution (exothermic). The total areas under the curves did not change due to these changes in fluid.

It may be postulated that the endotherm in Fig. 2, marked '*' between the initial sorption event and the major recrystallisation event reflects the point where the vapour has plasticised the material such that a solid state transition is possible. With care, it was possible to stop an experiment in this region, and rapidly remove the powder from the ethanol vapour. The XRPD for one such sample is shown in Fig. 1, in comparison with the XRPD patterns for the original (crystalline) and the amorphous materials, from which it can be seen that an element of structure is present in this

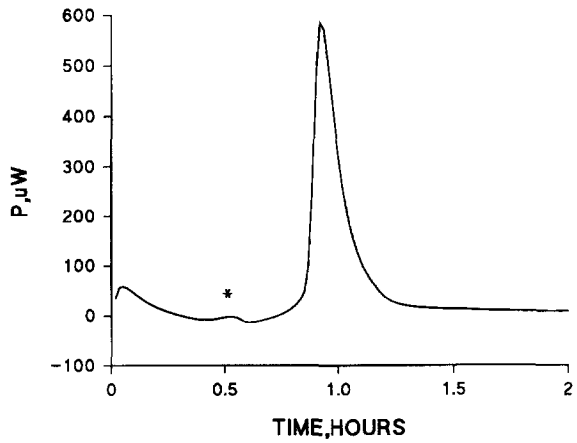

Fig. 2. A typical response (power as a function of time) obtained using the isothermal microcalorimeter where the amorphous sample has been exposed to ethanol vapour in a sealed vial.

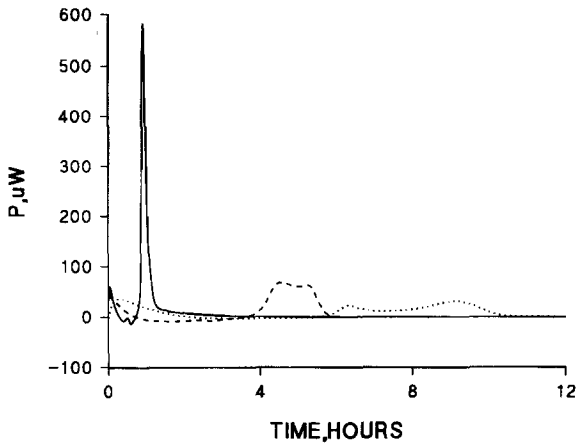

Fig. 3. Typical responses from the microcalorimeter for the recrystallisation of the amorphous material, using 96% ethanol (solid line), 96% ethanol:water in a 75:25 mix (dashed); 96% ethanol:water in a 50:50 mix (dotted).

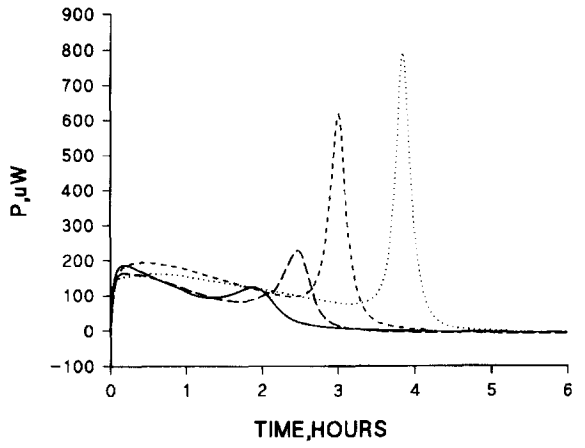

Fig. 4. Typical responses obtained in the microcalorimeter for samples micronised for 2 (solid); 5 (long dashed); 10 (short dashed) or 15 (dotted) min.

sample which has been removed after the early stage of the microcalorimetric experiment. However, the XRPD pattern for this material is very different to that of the original crystalline material. The DSC trace for this sample was, however, indistinguishable from the amorphous starting material, showing a small transition at 397.2 K, and no melting endotherm thereafter. A real probability is that the ethanol vapour has indeed plasticised the sample, but the act of drying off the ethanol has then caused the onset of a small degree of crystallisation into a different polymorphic form of the drug, an event which would probably not have occurred had the sample not been removed from the microcalorimeter.

3.1. Milling effects

3.1.1. Micronised samples

Samples of crystalline drug which were micronised for 2, 5, 10, 15 or 20 min were studied in the microcalorimeter (n = 3 or more) and the peak area for the main recrystallisation transition was compared with that for the mean peak area recorded for the precipitated amorphous sample. Typical responses are shown in Fig. 4, from which it can be seen that the traces are of different appearance to those observed for the precipitated amorphous material. The recrystallisation peaks in Fig. 4 occur superimposed on the down slope of the initial response. There is no evidence of the endothermic response that was seen for the highly amorphous samples in Fig. 2, this is true even for the 15-min milled sample for which the recrystallisation did not occur until after nearly 4 h (compared with ca. 7 h which was seen for the totally amorphous sample (Fig. 2)). The reproducibility of each response is good, with variability being due to the nature of the sample that is offered to the instrument, rather than a change in the crystallisation process (tested by mixing or not mixing the milled sample prior to recrystallisation). Typical reproducibility is shown by the three replicate determinations for samples milled for 10 min (Fig. 5), the areas under the curve for each of these recrystallisation events are 416, 480 and 514 mJ (each for 100 mg of sample), which when compared to the sample that was taken as the amorphous standard equate to 44, 51 and 55% amorphous nature, respectively. The samples which were exposed to longest micronisation times showed the greatest variability in response when recrystallised in the calorimeter. It is probable that this variability reflects the difficulty in obtaining exact control over the milling processing conditions over this longer period. This can be taken to imply that the longer and more arduous

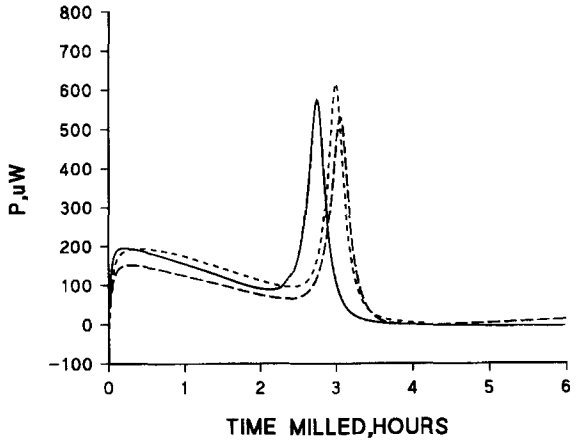

Fig. 5. Demonstration of reproducibility of the recrystallisation response in the microcalorimeter, showing three samples which had each been micronised for 10 min.

Fig. 6. The relationship between amorphous content induced during processing and time in the mill.

a milling process the harder it will be to produce a product of uniform properties. There is a clear relationship between time in the microniser and amorphous content of the samples (Fig. 6). It is clear that the microniser is making a large amount of the material amorphous, rising to 75% of the content after 20 min milling.

The DSC data for the micronised samples show a gradual loss of the melting endotherm and a corresponding increase in the peak at ca. 396 K (the T_g). The values for ΔH_{fus} are 37.3, 30.0, 15.0, 5.5 and 0.9 J g^{-1} after 2, 5, 10, 15 and 20 min, respectively. The T_g for the amorphous content is visible after 15 min and significant for the 20-min sample. The DSC of the samples that had been exposed to ethanol vapour and recrystallised in the microcalorimeter all showed one melting endotherm (ΔH_{fus} in the range 37–42 J g^{-1}). It would certainly be simple to differentiate between unmilled samples and those which had been milled for longer than 5 min. The XRPD of the micronised samples shows a significant change in crystallinity as the time of micronisation is increased (not shown). However, the XRPD is unable to detect the amorphous content in the sample that has been micronised for just 2 min (trace not shown). The microcalorimetric data for the micronised material indicate that the sample which has been micronised for 2 min is about 13% amorphous. The indications are that this is the lower detection limit for amorphous content for the XRPD system. This is the same order of magnitude as the figure of 10% amorphous content suggested as a lower detection limit by Saleki-Gerhardt et al. (1994) for studies on sucrose samples.

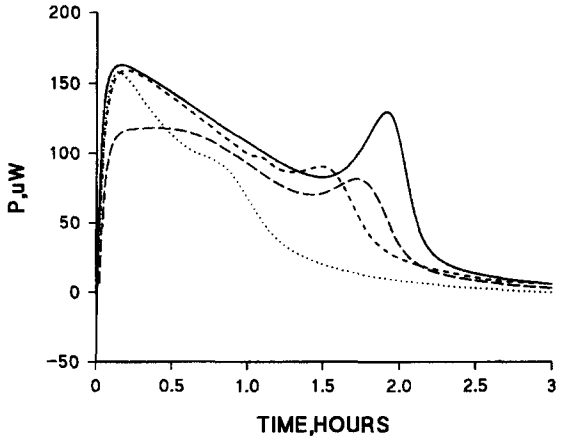

Fig. 7. Typical microcalorimetric traces for samples which had been ball milled for: 10 (dotted); 15 (Long dashed); 20 (short dashed); and 30 (solid) min, respectively.

3.1.2. Ball milled samples[1]

Ball milling is a less energetic process and is expected to cause less disruption to the sample. The crystalline powder was milled in a vibrating ball mill for either 10, 15, 20 or 30 min and then checked for the degree of crystallinity by exposing the samples to ethanol vapour in the microcalorimeter (Fig. 7). By comparing the areas under the recrystallisation peak to the amorphous standard the degree of crystallinity was determined and found to range from 3–12% (Fig. 6). The ball milled samples were investigated by DSC which showed each sample to have a single melting endotherm. In each case the melting point was identical, as was the enthalpy of fusion, thus the amorphous content was not detected by DSC. It follows that the microcalorimetric technique has a very significant sensitivity advantage over both XRPD and DSC for the detection of amorphous material.

4. Conclusions

Isothermal microcalorimetry can be used as a method of assessing crystallinity of hydrophobic, as well as hydrophilic materials, as long as a vapour phase exists which can cause the required transition. A potential advantage of the microcalorimetric technique is that the process can be followed in real time which allows the mechanism for the transition to be ascertained. In this instance it can be seen that the recrystallisation is by means of a rapid cooperative process which occurs after all the amorphous regions of the sample have been fully saturated with vapour. There is potential for understanding something of the mechanism of the processes which lead up to the main crystallisation event by following the endothermic pre-crystallisation transition. However, as with all thermal methods, isothermal microcalorimetry is non-specific and interpretation must be undertaken alongside as much additional information as possible, obtained from other techniques.

[1] Ball milled is being used here to define the lower energy milling process, even though the microniser was based in a ball mill principle with one ball in a small cylinder which was shaken at high speed. Thus a distinction is made between micronised (high energy mill) and ball milled (lower vibrational energy mill).

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