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Quantification of low levels (< 10%) of amorphous content in micronised active batches using dynamic vapour sorption and isothermal microcalorimetry

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Abstract

During the processing of pharmaceutical solids (e.g. milling, spray drying, tablet compaction, wet granulation and lyophilisation), various degrees of disorder in the form of crystal defects and/or amorphous regions may be generated. Even relatively low levels of amorphous material (< 10%) may have a detrimental impact on the stability, manufacturability and dissolution characteristics of the formulated drug product. In this paper an isothermal heat conduction microcalorimetry and dynamic vapour sorption technique have been evaluated for the quantification of low levels (< 10%) of amorphous material within a crystalline active. Both techniques were able to detect a 0.5% amorphous content, and in each case the limit of detection may be further lowered by increasing the sample size. The impact of micronisation on the crystallinity of a batch of active was evaluated using the two methods. The isothermal microcalorimetry and dynamic vapour sorption data showed excellent agreement (± 0.2% amorphous content) and indicated that the amount of amorphous material generated is extremely sensitive to small changes in the operating conditions of the microniser. The techniques described in this paper have been developed at a very early stage of the actives development program such that the impact of small quantities of amorphous material on the quality attributes of the formulation can be fully assessed. The methods can be applied to any active, the only criteria is that the amorphous material will recrystallise on exposure to moisture or solvent vapours, and no hydrates or solvates are formed.

Keywords: Isothermal microcalorimetry; Dynamic vapour sorption; Amorphous; Micronisation; Glass transition temperature; Moisture sorption

1. Introduction

During the development of a pharmaceutical solid dosage form it is often discovered that the particle size distribution of the active can not be controlled and/or optimised by the chosen crys-

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tallisation route. Consequently, the majority of actives are milled to improve their dissolution and/or mixing characteristics in the chosen formulation. Significant quantities of energy can be generated during these milling operations resulting in various degrees of disorder in the form of crystal defects, or if this disorder is more extensive, amorphous regions within the crystal structure (Dialer and Kuesner, 1973; Saleki-Gerhardt et al., 1994; Ward and Schultz, 1995) are produced.

From a thermodynamic perspective these amorphous regions are metastable with respect to their crystalline counterparts, and as such render the solid with different properties when compared with its crystalline state. The presence of amorphous regions can be beneficial in terms of an enhanced dissolution rate and increased bioavailablity (Chiou and Kyle, 1979), however, many of the property changes (e.g. enhanced chemical and physical instabilities Otsuka and Kaneniwa, 1990; Kontny et al., 1987, changes in processing properties Sebhatu et al., 1994a), are undesirable.

The amount of amorphous material generated during milling is a function of many factors including the particle size distribution of the unmilled active, the solid state properties of the active, the type of milling technology and the milling conditions used. Even if the milled active contains relatively low levels of amorphous material ($< 10\%$), as these regions are most likely to be located at the surface (Elamin et al., 1994), they have the potential to have a major impact on the drug product performance.

Amorphous character in highly crystalline solids can be difficult to detect using traditional analytical techniques such as Powder X-Ray Diffraction (PXRD) and Differential Scanning Calorimetry (DSC), as the limit of detection is 5–10% (Giron et al., 1997). Over recent years a number of papers have been published using isothermal microcalorimetry to quantify low levels of amorphous content (Briggner et al., 1994; Sebhatu et al., 1994b; Buckton et al., 1995). The majority of these studies used the miniature humidity/solvent chamber technique (Angberg, 1995) where a sample under investigation is placed in a sealed ampoule under conditions which allow the transition to the thermodynamically stable state to take place. One disadvantage of this technique is that recrystallisation of the amorphous solid may not be detected, either because of the time required to equilibrate the sample, or because the vapour pressure/temperature conditions are not optimised. A number of studies have been published that describe the use of moisture sorption for the detection of low levels of amorphous material (Saleki-Gerhardt et al., 1994; Buckton and Darcy, 1999). The majority of these studies, however, describe the use of the technique in a qualitative rather than quantitative manner.

The aim of this work was to develop a method for the quantification of low levels of amorphous content ($< 10\%$) in a micronised active using both a vapour perfusion isothermal microcalorimetry and a dynamic vapour sorption technique.

2. Materials and methods

The compound used in this study is a development candidate for Pharmacia. It is a benzyl ether derivative and for the remainder of this paper it will be given the abbreviation BED.

2.1. Preparation and characterisation of amorphous and crystalline standards

Samples of '100%' amorphous BED were prepared by ball milling in a Wig-L-Bug (Crescent) for 20 min. The amorphous samples were analysed by PXRD, moisture sorption and DSC.

Samples of '100%' crystalline BED were prepared by storing a micronised sample of the material at 90% relative pressure of acetone and then drying the material. This was to ensure that any amorphous material in the sample was recrystallised.

All other chemicals and reagents were HPLC grade and were used as received.

2.2. Methods

2.2.1. Dynamic vapour sorption analysis

The studies were undertaken using a Dynamic Vapour Sorption apparatus (DVS, Surface Mea-

surement Systems, London, UK). The apparatus consists of a Cahn microbalance housed inside a temperature-controlled cabinet. All experiments were performed at 25 °C. The DVS was used to characterise the amorphous standard (water) and to quantify the amorphous content (acetone). Dry nitrogen was bubbled through acetone or water to give 100% relative pressure of the solvent. The relative pressure of acetone or water flowing past the sample is controlled via a computer program which sets the appropriate flow to the wet (100% relative pressure acetone or water) and dry side (dry nitrogen).

2.2.1.1. Amorphous quantification. Samples (50 ± 5 mg) were prepared by weighing known quantities of amorphous and crystalline BED directly into the DVS sample cup which was loaded onto one side of the twin pan balance. The samples were exposed to the following method: dry nitrogen for 3 h, 30% relative pressure acetone for 4 h, dry nitrogen for 4 h, 85% relative pressure acetone for 3 h, dry nitrogen for 3 h then finally 30% relative pressure acetone for 3 h.

2.2.1.2. Moisture uptake. Samples (5–10 mg) were weighed directly into the DVS sample cup. The samples were exposed to the following method: dry nitrogen for 3 h, 10, 20, 30, 40, 50% RH for 1 h, 60, 70, 80 and 90% RH for 2 h.

2.2.2. Isothermal microcalorimetry

The heat flow studies were performed using a heat conduction microcalorimeter (TAM 2277, Thermometric AB, Jarfalla, Sweden), equipped with four 20 ml vapour perfusion units. All experiments were performed at 25 °C. Dry nitrogen was bubbled through acetone to give 100% relative pressure of the solvent. The relative pressure of acetone flowing into the sample ampoule is controlled via a computer program, which switches the flow to the wet (100% relative pressure acetone), or dry side (dry nitrogen) to generate the required relative pressure of acetone.

Samples (500 ± 5 mg) were prepared by weighing known quantities of amorphous and crystalline BED directly into the vapour perfusion ampoules. The samples were then exposed to the following method: 0% RH for 3 h, 30% relative pressure acetone for 3 h, 90% relative pressure acetone for 4 h, 0% RH for 3 h then finally 30% relative pressure acetone for 3 h.

2.2.3. Milling

The active was micronised using a pilot plant scale jet mill (Fluid Energy Aljet, Model 8). The feed rate (200–800 g/min) and grinding pressures (25–40 psig) were varied to give five different sets of milling parameters and therefore five different particle size distributions (PSD) of the active.

2.2.4. Particle size analysis

Micronised samples were analysed in the dry state using a laser light diffraction particle size analyser (Sympatec, Inc, Princeton, NJ). The diffracted light is captured by a 64-channel photodiode array detector, and Fraunhofer theory is used to calculate the sample's volume particle size distribution from the energy distribution. Using a feed rate of 35%, approximately 500 mg of sample was fed into the dispersion system. Dispersion of the particles was achieved with a pressure of 3 bar and an evacuation pressure of 98 mBar. The samples were analysed using the R2 (50 mm) lens, which has a working range of 0.25–87.5 μm.

3. Results and discussion

3.1. Characterisation of amorphous and crystalline BED standards

When developing a method to quantify the amorphous content of a micronised active, one of the most important areas to consider is the validity of the 100% amorphous and crystalline standards. A number of different techniques should be evaluated for producing the amorphous standard (e.g. quenching the melt, rotary evaporation, milling, freeze drying, etc.), and the samples characterised using a variety of techniques that can differentiate between the amorphous and crystalline states. The crystalline standard should ideally have the same physical properties (i.e. size, shape, surface features) as the micronised active. In this study the crystalline standard was micro-

nised BED which was stored under conditions known to recrystallise the amorphous component (i.e. 90% relative pressure acetone).

During this work only milling and rotary evaporation produced samples which exhibited a halo pattern, typical for an amorphous substance, when analysed by PXRD. To investigate the lot-to-lot variability of the amorphous standards produced by the milling and rotary evaporation technique, a number of different samples were produced and characterised by PXRD, DSC and moisture uptake.

Both milling and rotary evaporation consistently produced amorphous standards, which exhibited a halo pattern when analysed by PXRD.

The DSC data proved inconclusive in terms of characterising the lot-to-lot variation of amorphous BED. On close inspection of the DSC traces, an endothermic shift was evident at about 185 °C, which could represent the glass transition temperature (Tg) of amorphous BED. The Tg gives an indication of the stability of the amorphous material. In this case since it is significantly above room temperature the amorphous material would be expected to be relatively stable. However, the plasticising effects of residual solvents, sorbed water and other additives/impurities make this stability prediction less certain. In addition, studies have shown significant molecular mobility in amorphous pharmaceutical solids up to 50 °C below their Tg (Hancock et al., 1995). As such although it is very useful to know the Tg for an amorphous compound, additional studies covering a variety of environmental conditions (e.g. humidity, temperature), need to be performed to determine the stability of the amorphous standard. In the case of amorphous BED it was found to be extremely stable under ambient environmental conditions such that the amorphous standard did not have to be freshly prepared prior to analysis.

Moisture sorption was found to be the most sensitive technique for investigating the variation in the amorphous content of the samples prepared by milling and rotary evaporation. Fig. 1 shows the respective moisture sorption isotherms for a sample of 100% crystalline and 100% amorphous BED. The difference in moisture uptake is dramatic, with amorphous BED sorbing 22% moisture at 90% RH compared with 0.9% moisture for its crystalline counterpart. The difference in uptake is due to the amount of moisture than can be absorbed into the amorphous structure compared with that adsorbed onto the surface of the tightly packed and highly organised crystalline lattice.

From Fig. 1 it was evident that amorphous BED showed no signs of recrystallisation with moisture. The effect of sorbed vapour on the depression of the Tg of an amorphous compound may be explained by the Gordon–Taylor equation (Gordon and Taylor, 1952). Assuming perfect volume additivity with no specific interaction between the components, the glass transition temperature of the mixture, Tg_{mix} is given by:

$$Tg_{mix} = \phi_1 Tg_1 + \phi_2 Tg_2 \quad (1)$$

where ϕ is the volume fraction and the subscripts represent the two components. The Gordon–Taylor equation can be rearranged for weight fractions giving:

$$Tg_{mix} = \frac{(w_1 Tg_1) + (K w_2 Tg_2)}{w_1 + (k w_2)} \quad (2)$$

where w_1 and w_2 are the weight fractions of the two components and K is represented by:

$$K = \frac{(\rho_1 Tg_1)}{(\rho_2 Tg_2)} \quad (3)$$

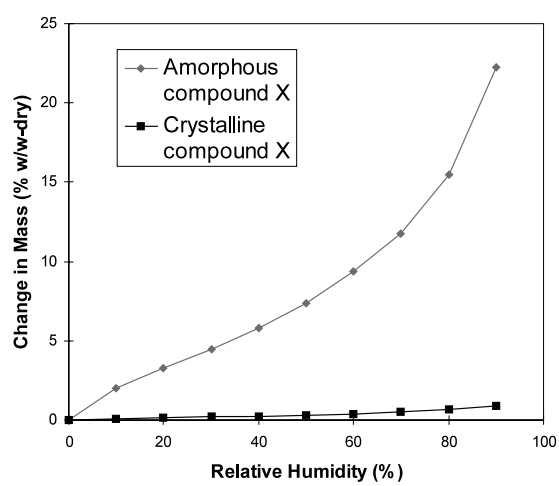

Fig. 1. Moisture sorption profiles for amorphous and crystalline BED.

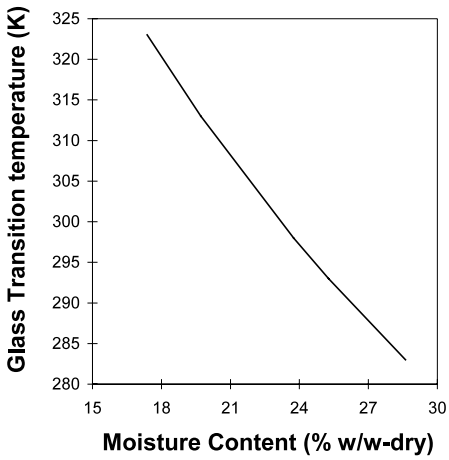

Fig. 2. Relationship between moisture content and the Tg for amorphous BED as determined by the Gordon–Taylor equation.

where ρ_1 and ρ_2 are the densities of the two components.

The published value for the Tg of water is 135 K (Sugisaki et al., 1968) which compares to 458 K for BED from DSC. From Eq. (2), water will act as a plasticiser for amorphous BED, with the depression of Tg related to the weight fraction of sorbed moisture. Fig. 2 shows the effect of sorbed moisture on the depression of the Tg for amorphous BED as calculated by the Gordon–Taylor equation.

From the data shown in Fig. 2, a sample of amorphous BED would have to sorb in excess of 24% (w/w-dry) moisture before it would start to collapse and recrystallise at 25 °C. From the DVS moisture sorption profile (Fig. 1), it is clear that at 90% RH the amorphous sample of BED had only sorbed 22% moisture and as such showed no signs of recrystallisation.

The moisture sorption isotherms were not used to quantify the amount of amorphous material as the total amount sorbed is a combination of moisture adsorbed onto the surface and absorbed into the amorphous regions. Variations in moisture uptake could be due to either differences in the amorphous content or the surface properties of the sample. Whilst the amount of adsorbed moisture is negligible compared with absorbed moisture in the 100% amorphous samples, for micronised active batches with relatively low levels of amorphous material, the difference in surface adsorbed moisture could lead to large errors in quantifying the levels of amorphous material. However for characterising the lot to lot variability in the amorphous standards produced by milling and rotary evaporation, moisture sorption proved to be an extremely valuable technique.

During the characterisation of the amorphous standards, the DVS was used to select a solvent, which would recrystallise the amorphous content of micronised BED. Fig. 3 shows the interaction of acetone with amorphous BED. Amorphous BED sorbs approximately 9.7% acetone up to a partial pressure of 60%. Above 60% relative pressure the sample has sorbed sufficient acetone for the Tg of the amorphous BED to fall below the experimental temperature. At this point the amorphous BED starts to recrystallise and the sorbed acetone is expelled from the crystal lattice, which shows up as a characteristic sharp decrease in weight on the DVS. In contrast to water, acetone, which has a significantly lower Tg value (93–100 K Angell et al., 1978), has the ability to lower the Tg of amorphous BED below 25 °C and cause it to recrystallise.

After reviewing the results from the characterisation study, milling was selected as the technique for generating amorphous standards of BED as it produced samples which consistently had a PXRD halo, exhibited the greatest moisture sorption profile, and was the same method by which the amorphous material was generated in the micronised samples.

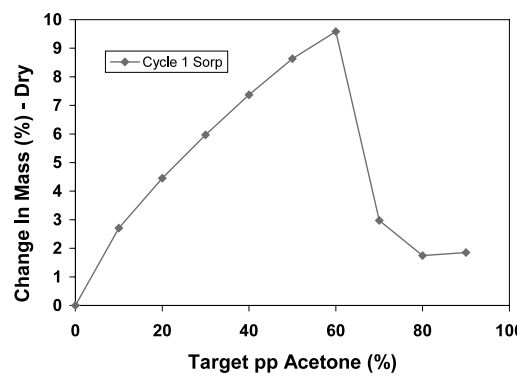

Fig. 3. Acetone sorption profile for amorphous BED.

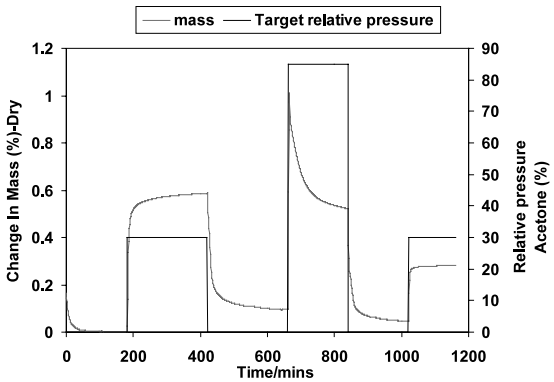

Fig. 4. DVS change in mass profiles for a sample of BED with a 4.8% amorphous content.

3.2. Dynamic vapour sorption analysis

A typical DVS response is shown in Fig. 4 representing the acetone uptake for a sample of BED with a 4.8% amorphous content. The method rationale was to measure the acetone uptake of the crystalline/amorphous sample at 30% relative pressure, recrystallise the sample at 85% relative pressure, and then measure the acetone uptake of the resultant crystalline sample at 30% relative pressure again. The difference between the first and second sorption profiles at 30% relative pressure is directly related to the amorphous content of the sample. The uptake at 30% relative pressure was chosen as a reference point for the method because at this partial pressure amorphous BED would not recrystallise but at the same time the uptake of acetone was significant enough to quantify low levels of amorphous material.

The DVS profile can be divided into four different sections.

0–3 h: the sample is exposed to dry nitrogen to ensure all water/solvents are removed.

3–7 h: the sample is exposed to 30% relative pressure acetone. The mass uptake of acetone at 30% relative pressure is a combination of acetone adsorbed onto the surface and absorbed into the amorphous regions.

7–14 h: the sample was dried and exposed to 85% relative pressure acetone. After 5–10 min the amount of absorbed acetone reaches a critical point at which time the Tg of the amorphous BED has been reduced below the operating temperature of the experiment. The sharp decrease in weight corresponds to the expulsion of the absorbed acetone as the amorphous BED recrystallises.

14–20 h: the sample is dried again and then exposed to 30% relative pressure acetone for a second time. This time the mass uptake only represents adsorption of acetone onto the surface as the sample is 100% crystalline.

By subtracting the second mass uptake of acetone at 30% relative pressure from the initial uptake value, the amount of absorbed acetone can be calculated, which is directly proportional to the amorphous content of the sample.

During preliminary work on the method, it was noted that following exposure to 85% relative pressure the sample retained acetone when it was dried. To investigate at what point this acetone became trapped, an additional drying step was added following the initial exposure of the sample to 30% relative pressure. It is evident from Fig. 4 that a small amount of acetone is trapped as soon as the sample is exposed to an acetone atmosphere, and that the amount trapped remains fairly constant throughout the experiment and as such does not affect the results. It should be noted at this point if the active under investigation forms solvates or hydrates, then this method is unlikely to be suitable for quantifying the amorphous content.

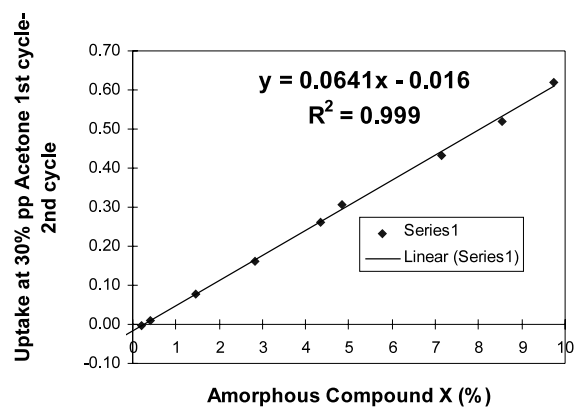

Fig. 5. Dynamic uptake of acetone versus amorphous content for mixtures of amorphous and crystalline BED.

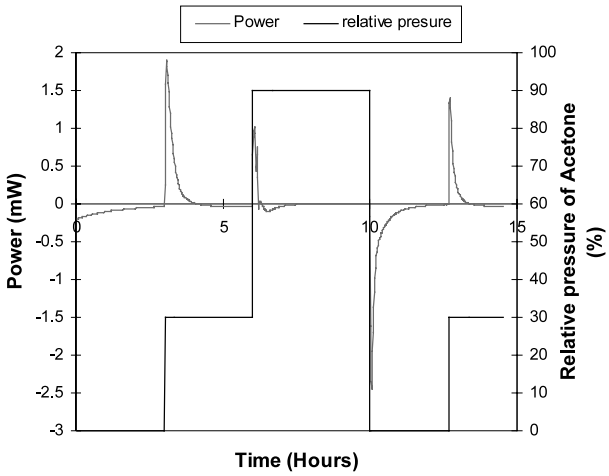

Fig. 6. Heat response from the TAM for the interaction of acetone with a sample of BED with an amorphous content of 4.4%.

Fig. 5 depicts the difference in mass uptake of acetone from the first and second cycles versus the amorphous content of BED samples and demonstrates excellent linearity over the range studied (0.2–9.8% amorphous content, $R2 = 0.999$).

3.3. Isothermal microcalorimetry analysis

Essentially the same method as described for the DVS analysis was used on the thermal activity monitor (TAM). A TAM response is shown in Fig. 6 representing the heat response from the calorimeter for the interaction of acetone with a sample containing 4.4% amorphous BED. The trace can be divided into five different sections.

0–3 h: an initial response is observed during the drying cycle as the sample loses ad/absorbed moisture and the sample equilibrates.

3–6 h: an exothermic response, which represents the heat of adsorption by crystalline BED and absorption by the amorphous BED, is obtained when the sample is exposed to 30% relative pressure acetone.

6–10 h: a second exothermic response is obtained after exposing the sample to 90% relative pressure acetone representing a number of different events including heats of ad/absorption, recrystallisation of the amorphous material and expulsion of the absorbed acetone.

10–13 h: an endothermic desorption response is observed on exposure to dry nitrogen.

13–16 h: a third exothermic response is obtained at 30% relative pressure acetone which this time only represents the heat of adsorption by crystalline BED.

As in the dynamic sorption method, by subtracting the area under the third exothermic response from the first exothermic response the heat associated with the amount of absorbed acetone can be calculated, which is directly proportional

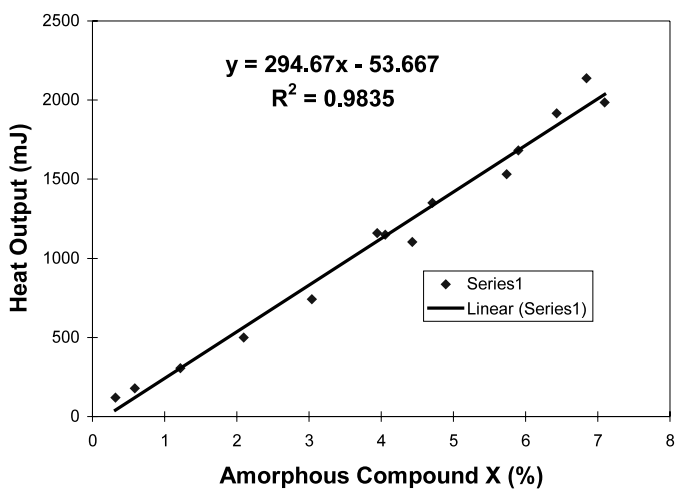

Fig. 7. Heat output from the TAM versus amorphous content for mixtures of amorphous and crystalline BED.

Table 1
Comparison of the amorphous content of five micronised lots of BED by TAM and DVS analysis

Batch Number	Amorphous content (%)			
	TAM analysis			DVS analysis
	1	2	Mean	
1	2.0	2.0	2.0	2.1
2	0.8	0.9	0.9	1.1
3	2.8	2.8	2.8	2.8
4	2.3	2.2	2.3	2.5
5	2.7	2.7	2.7	2.9

to the amorphous content of the sample. Fig. 7 depicts the heat associated with the absorption of acetone versus the amorphous content of BED samples and again showed excellent linearity over the range studied (0.3–7.1% amorphous content, $R2 = 0.984$).

It should be noted that in generating the data in Figs. 5 and 7, seven different lots of amorphous BED and two of crystalline BED were used. The linearity achieved was therefore not only a measure of the instrument precision and the method used, but also of the consistency of the 100% amorphous and crystalline samples of BED.

Although both techniques were evaluated for a specific sample size, 50 mg for the DVS and 500 mg for the TAM, the calibration data generated in Figs. 5 and 7 is still valid for a larger sample size, although the x-axis would have to be modified to account for the increase.

3.4. Micronisation trials

As part of the milling strategy for this active, part of a batch (15 kg) was micronised into five sub lots using pilot plant scale equipment. Each sublot was generated using different milling parameters such that the impact of the PSD of the active on the quality attributes of the formulated drug product could be evaluated. The amorphous content of the sublots was analysed using both the DVS and TAM techniques. The data in Table 1 indicates there is excellent agreement between the two techniques and demonstrates the ability of either technique to quantify low levels of amorphous material in the micronised active.

The PSD of each of the lots was analysed using the Sympatec method described in Section 2.2.4. Fig. 8 shows the correlation between the sympatec D90 results and the amorphous content of micronised lots of BED using the TAM data in Table 1. It is evident that the amount of amorphous material is extremely sensitive to small changes in the milling conditions as measured by the PSD of the active.

4. Conclusions

Both the DVS and TAM techniques were able to detect an amorphous content of 0.5% in the micronised active batches. This lower limit of detection is entirely dependent on the sample size and for both techniques it should be possible to further lower this limit by increasing the sample size of the micronised active being analysed. The correlation coefficient for the DVS was marginally better than for the TAM technique, although the data from both techniques was very accurate and reproducible. In addition there was more scope to increase the sample size, and hence the lower limit of detection, on the TAM than the DVS

A major advantage of the DVS and TAM over other more traditional techniques for determining

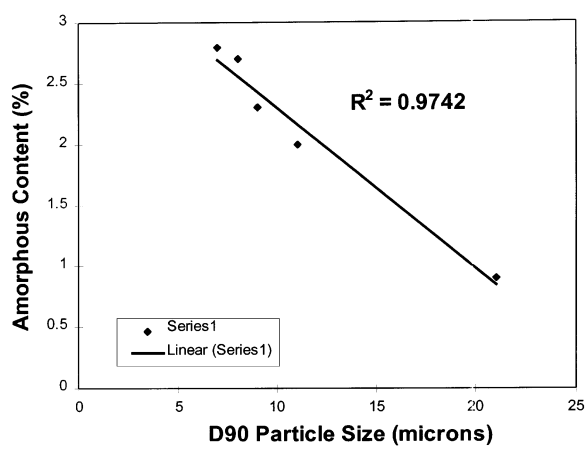

Fig. 8. Relationship between the D90 particle size distribution of micronised batches of BED and their amorphous content.

the amorphous content of micronised drugs (e.g. PXRD, IR, Raman), is that it is not necessary to achieve a completely homogenous mixture of the crystalline/amorphous standards prior to analysis. For techniques such as PXRD a homogenous mixture is required as only a portion of the entire sample is analysed. Preparing homogenous mixtures of a micronised active on a small scale is very difficult and often provides the most significant source of error for techniques that require this approach. In contrast the DVS and TAM techniques do not require a homogenous mixture to be prepared as the entire sample is analysed and the results are entirely independent of the recrystallisation kinetics of the amorphous material as long as the method has been developed to allow the sample to reach equilibrium at each partial pressure.

It has been suggested that the most important cause of variability in powder properties is the change in extent of the crystallinity of powder surfaces (Buckton, 1995). The techniques described in this paper have been developed during the early stages of the compound's development cycle such that the impact of small quantities of amorphous material on the processability, stability and bioavailability of the formulation can be fully investigated. On transferring the technology to the commercial sites or whenever a change to the micronisation process is made (e.g. type of mill, scale, operating conditions etc), the amount of amorphous material being generated should be re-evaluated and the impact on the quality attributes of the formulation re-assessed.

The general principle of the techniques as described can be applied to any active, with the minimum of development. The only criteria is that the amorphous material can be recrystallised by exposure to moisture or solvent vapours and that it does not form solvates or hydrates.

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