Considerations when Quantifying Amorphous Contents in Milled Powders with Calorimetry

Interpretation of calorimetric data is tricky. The author suggests 10 questions that should be asked of any calorimetric method, along with the rationales behind them.

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The dry powder inhaler (DPI) is an increasingly popular delivery device, used to deliver a wide variety of drugs to the lung for either local or systemic action. With many originator DPI formulations reaching the end of their period of patent protection, the development of DPI products for the generic market is an area with much potential. Unlike other pharmaceutical delivery devices, the DPI device is as important as the powder blend it contains; it is simply not the case that a powder blend can be formulated that matches the originator, because its properties must be matched to the device that aerosolizes it (1, 2). A product will only achieve the desired fine particle fraction (FPF) if the two elements are considered together. This fact has particular relevance to the generics market in cases where the patent covering the device that generates an aerosol expires at a different time to any patents covering the active principal or powder blend it contains.

Particle size reduction

A critical parameter for successful DPI delivery is particle size; powders need to be between 2–4 μm in diameter to reach the alveoli and deposit (3). For this reason, the active is usually milled (or micronized) prior to formulation such that it has the required particle size distribution. Milling, being a high-energy process, has the potential to fundamentally change the physical form of the sample in addition to reducing its particle size. Changing physical form (usually an amorphous state is produced) necessarily changes (and usually increases) the surface energy of a material and, therefore, impacts its cohesiveness and adhesiveness. There has been much research over the

past 20 years into the nature of the forces that influence how powder blends aerosolize, and it is now well established that forces of adhesion and cohesion are critical (3). The determination of the amorphous content of milled powders is, therefore, crucial; it is usually possible to show that amorphous content correlates directly to FPF. For this reason, any submission to the US FDA for a DPI product usually requires an analytical method to quantitate amorphous content, as well as limits for batches of milled material that may be used in commercial batches.

Of the myriad of techniques available to quantitate amorphous content, those based on calorimetry are particular useful. The amorphous state is higher in energy than any crystalline counterpart and calorimetry measures energy directly. The two most popular calorimetric methods are solution (ampoule-breaking) and gas perfusion (4) (see Sidebar). The former uses heat of solution to indicate amorphous content and the latter uses heat of crystallization. These methods are excellent and are becoming widely used. However, interpretation of calorimetric data is tricky because heat is a ubiquitous property and if a calorimetric experiment is not constructed properly, the data are easily misinterpreted.

Ten questions to ask

This article suggests 10 questions that should be asked of any calorimetric method, along with the rationales behind them, so that any reader designing, undertaking, or reviewing experiments to quantitate amorphous contents by calorimetry can do so with critical thought. Before these questions are raised, it is worth briefly explaining the principles on which the two methods operate.

Gas perfusion calorimetry: In this method, a plasticizer (usually water or ethanol) is perfused over the sample in an inert carrier gas. The sample will adsorb and absorb the plasticizer, crystallize, and then expel any absorbed solvent. The calorimeter measures these heats as the sample is held at a constant temperature (4). Since all of these processes will occur with a change in heat, they will all contribute to the final measured heat change. It is essential that the method corrects for these values if the heat of crystallization

is needed. Typical power-time data for a partially amorphous material are shown in Figure 1.

Solution (ampoule-breaking) calorimetry: In this method, the sample is contained within a sealed ampoule and a reservoir of solvent (up to 100 mL) is allowed to reach thermal equilibrium. The ampoule is then broken and the powdered sample is exposed to the solvent. Rapid dissolution should ensue, and the heat of solution is measured (5). The technique is particularly suited to amorphous content quantification because crystalline materials typically have an endothermic heat of solution while amorphous materials have an exothermic heat of solution. Typical power-time data for a partially amorphous material are shown in Figure 1.

1) Is the particle size of the sample the same each time and/or the same as the material used for the calibration curve?

Particle size is most important where gas perfusion calorimetry is used. If the particle size of the sample is different from that of the material used to generate the calibration curve, or varies from batch to batch, then the proportion of the total heat change arising from adsorption will vary. This heat change? must be corrected for, or an incorrect heat of crystallization will be measured (see question 5). If solution calorimetry is used, changing particle size will change the rate of dissolution of the material, which will alter the shape of the power-time data but will not affect the total heat.

2) Does the sample exhibit polymorphism?

Different polymorphs will have different heats of solution or crystallization. Therefore, it does not matter which calorimetric method is used, the calibration curve *must* be generated with material of the same polymorphic form as that being tested. A good check, in the case of gas perfusion experiments, is to remove the sample after the experiment and to check the polymorphic form of the sample with X-ray diffraction, differential scanning calorimetry (DSC), or any other suitable method. Obviously, if a material is

amorphous, it has no crystal structure, but most milled powders have only a small (<5% w/w) amorphous fraction, with the remainder being crystalline. The form to which it crystallises, or the form that dissolves, is therefore critical.

3) Is the material anomeric?

Sugars are anomeric (i.e., they differ in conformation in their cyclic ring structure at either the C1, C2, or C5 carbon atoms). For instance, α lactose and β lactose are anomers of each other. The heat of crystallization or solution of each anomer is different, so as in the case of polymorphic materials, the calibration curve *must* be generated with material of the same anomeric form (or ratio) as that being tested. Ramos et al. (6) demonstrated the effect of the anomeric composition of lactose on amorphous calibration curves prepared by solution and gas perfusion calorimetry (shown in Figure 2). Importantly, unlike polymorphism, the anomeric structure is retained in the amorphous form because it is an intra-molecular property, not an inter-molecular property. A further complication is that anomeric sugars will frequently mutarotate under conditions of high humidity, meaning they change anomeric ratio. Finally, α lactose forms a monohydrate while β lactose is anhydrous. All of these processes will occur with a change in heat, or will cause a change in heat of solution.

4) What plasticizer or solvent is being used?

The use of a plasticizer applies only to gas perfusion experiments. Many small organic molecules will plasticize pharmaceuticals. Water might even plasticize a poorly water-soluble active sufficiently to cause recrystallization. However, the rate at which crystallization occurs, as well as the heats of adsorption and absorption, will vary with the plasticizer. Therefore, the plasticizer should (a) be optimized for the sample being studied and (b) be the sample for processed samples and those used to generate the calibration curve.

The use of solvents applies only to solution calorimetry experiments. The heat of solution of a compound will vary depending on the solvent into which it is dissolving. One of the benefits of solution calorimetry is that if the compound being studied is poorly water-soluble, an organic solvent can be used instead. It is crucial, however, that the same solvent is used for processed samples as well as those used to generate the calibration curve. It must also be remembered that many organic solvents are volatile, and may well evaporate from the calorimeter during the measurement. This process is endothermic and will, therefore, contribute to the measured heat signal.

5) Is the method a comparison of wetting responses?

This question applies only to gas perfusion experiments. The quickest way to assess a milled sample is to expose it to a plasticizer and measure the heat. However, as noted above, the heat measured this way includes contributions from adsorption, absorption, and expulsion of the plasticizer. It has been argued that the heats of absorption and expulsion should be equal and opposite, and so cancel each other out. However, the heat of adsorption remains and the only way to correct for it is to dry the sample and then re-expose it to plasticizer. This approach not only provides the heat of adsorption, it also unequivocally demonstrates that the sample underwent an irreversible phase transition during the initial exposure to plasticizer (reaffirming the material was partially amorphous). Figure 3 shows the power-time data for amorphous and crystalline samples of salbutamol sulphate when exposed to two phases of elevated humidity (90%). There are several important points to note:

- the response of the amorphous material to humidity is initially much larger than that of the crystalline material (which is why gas perfusion is so sensitive to small amorphous contents), but the data are complex and contain many phases—it is not easy in this case to determine which area to measure
- upon exposure to humidity for a second time, the response of the amorphous material has reduced significantly—this observation

indicates that an irreversible event occurred during the initial exposure to humidity, and it also suggests that the powder mass has fused during crystallization, reducing its surface area to less than that of the crystalline reference sample.

6) Can the sample form a hydrate or solvate?

If the sample can form a hydrate or solvate with the plasticizer, then the power signal may become complex in shape, especially where formation occurs over an extended time period. This makes determination of the heat of crystallization either difficult (because it is not easy to determine which section of data to integrate) or time-consuming (because conversion must progress to completion).

Further complicating factors to consider in these cases include:

- whether the sample could have formed a hydrate or solvate prior to or after milling
- whether the samples used to make the calibration curve were hydrates or solvates
- if a hydrate or solvate is formed directly on crystallization of the amorphous fraction, the heats of absorption and expulsion of the plasticizer will not cancel out (less plasticizer is expelled).

Ideally, a plasticizer would be selected with which the sample cannot form a hydrate or solvate. Returning to the example of salbutamol sulphate shown in Figure 3, the data are complex because the drug absorbs water and crystallizes to a mono-hydrate (7). The situation for salbutamol sulphate becomes more complicated if some crystalline drug is also present, because the anhydrate form is most stable. In this case, the amorphous fraction crystallizes to a monohydrate, but then slowly dehydrates to form the anhydrate with time (see Figure 4). Again, care must be taken when devising the experimental methodology for samples such as this, to ensure the heat measured is actually proportional to the amorphous fraction and does not result from some additional process.

7) Is the milled material sourced from different suppliers?

Milling is a high-energy process, and the way the energy is imparted to the sample can do more than simply reduce particle size. In principle, large particles will initially fracture when the mill imparts energy, but once their size has reduced sufficiently, they will reach their brittle-ductile point, at which further size reduction is unlikely. Any energy provided after this point will not cause size reduction, but must still be dissipated by the sample—most likely by disrupting crystal structure, which is why milled materials are usually partially amorphous. The data in Figure 5 illustrate this point for salbutamol sulphate. The parameters for milling are usually adjusted to optimize particle size distribution of the milled powder only; no consideration is given to the physical form of the material. Hence, powders with similar particle size distributions produced from different mills may well have appreciably different amorphous contents. Consequently, they will not process or blend in the same way, and DPI performance will be affected.

8) Are samples analyzed at the same time after milling?

The amorphous state is simple to define (lack of long-range crystal structure) but poorly understood. This complexity? is because it is a high-energy state and, unlike crystal lattices, an infinite number of arrangements of molecules is possible. Hence, the energy of an amorphous material will lie in a range of values above any crystalline forms. With time, the molecules in an amorphous matrix will move and reorient (i.e., the process of relaxation). Because spontaneous molecular rearrangement can only progress in the direction of increasing order, as an amorphous material relaxes, it will lose energy as its molecular structure changes to that of the nearest crystalline form (and, indeed, many amorphous materials will eventually crystallize). As the performance of a DPI formulation is critically dependent on the forces of adhesion and cohesion, relaxation of a milled powder prior to blending can significantly affect the FPF achieved. This is, in fact, the reason many milled powders are conditioned prior to blending, either under humidity or left for a period of time. It follows that the time period between milling and

measurement of amorphous content is critical—with longer time, more relaxation will have occurred and a lower amorphous content will be measured. A good protocol is to define a maximum time period permissible between milling and amorphous content quantification (three days, for example) and ensure all milled samples are stored in sealed, desiccated containers.

9) *How was the amorphous reference material prepared?*

The definition of an amorphous material as lacking long-range molecular order does not preclude the possibility that there is appreciable short-range structuring. The degree of short-range order in an amorphous material is affected by the method of production. Consequently, amorphous materials of the sample compound prepared by, for example, spray-drying, lyophilization (freeze-drying), and quench-cooling will often behave very differently. In simplistic terms, the faster the method of production, the less time the molecules in a sample have to orient. Hence, a calibration curve prepared with amorphous material will often be different from a calibration curve prepared with freeze-dried material, because the energy contents of the two amorphous materials will be different. A further consideration is that irrespective of the method used to make the amorphous standards, it is likely that the short-range order imparted by milling will be different and so, in the opinion of the author, no calibration curve is representative of the sample being tested.

10) Is percent amorphous content actually needed?

Determining the percent of amorphous content needed is a complicated concept, related to the concepts of relaxation and the problem of amorphous reference standards as discussedpreviously. The actual value determined by either solution or gas perfusion calorimetry is a heat, which can be considered as the excess energy of any amorphous fraction of a milled sample. While it is clearly possible to define some fraction of a sample as crystalline and the rest amorphous (and so also to use percentages), hopefully, it is apparent that as

any amorphous fraction of a milled material relaxes, its excess energy will reduce. If the value of the excess energy is then converted to a percent amorphous content, by reference to a calibration curve, it will appear to reduce with time. However, the actual proportion of the material that is crystalline has not changed. It is simply the case that the energy of the amorphous fraction is reducing. The situation is further complicated when one remembers the problems in choosing appropriate amorphous reference standards for the calibration curve. In the opinion of the author, it is a better concept to correlate excess energy with FPF than percent amorphous content, because ultimately, it is the energy of the material that influences forces of adhesion and cohesion. An additional benefit of this approach is that a calibration curve for percent amorphous content is not needed.

Conclusion

In summary, calorimetric techniques are excellent choices for identifying, characterizing, and quantifying partially amorphous materials. There is little doubt that if the physical properties of a material have changed during processing, calorimetric analysis will show a difference. The issue relates to interpretation of the data, because heat is a universal indicator of physical change. If the experimental design is not well constructed or the nature of the material being investigated is not thoroughly understood, it is very easy to misinterpret the data. Hopefully, by asking some or all of the questions raised in this article, readers will be better informed when quantifying small amorphous contents with calorimetric methods.

References

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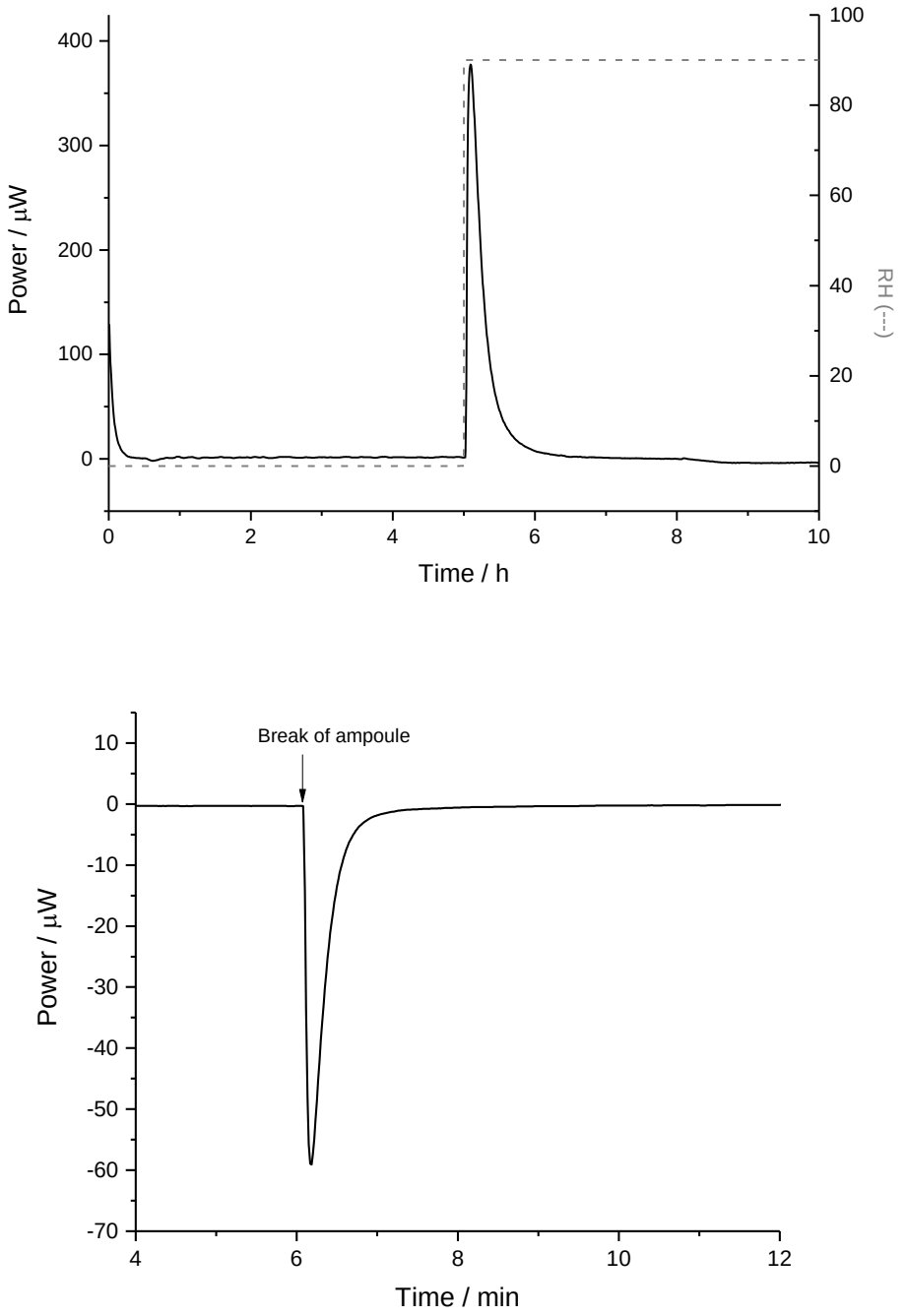

Figure 1. Schematic representations of typical power-time data for responses of a partially amorphous sample to increased humidity (top) and to dissolution (bottom). In both cases, integration of the area under curve (AUC) gives the heat of the process.

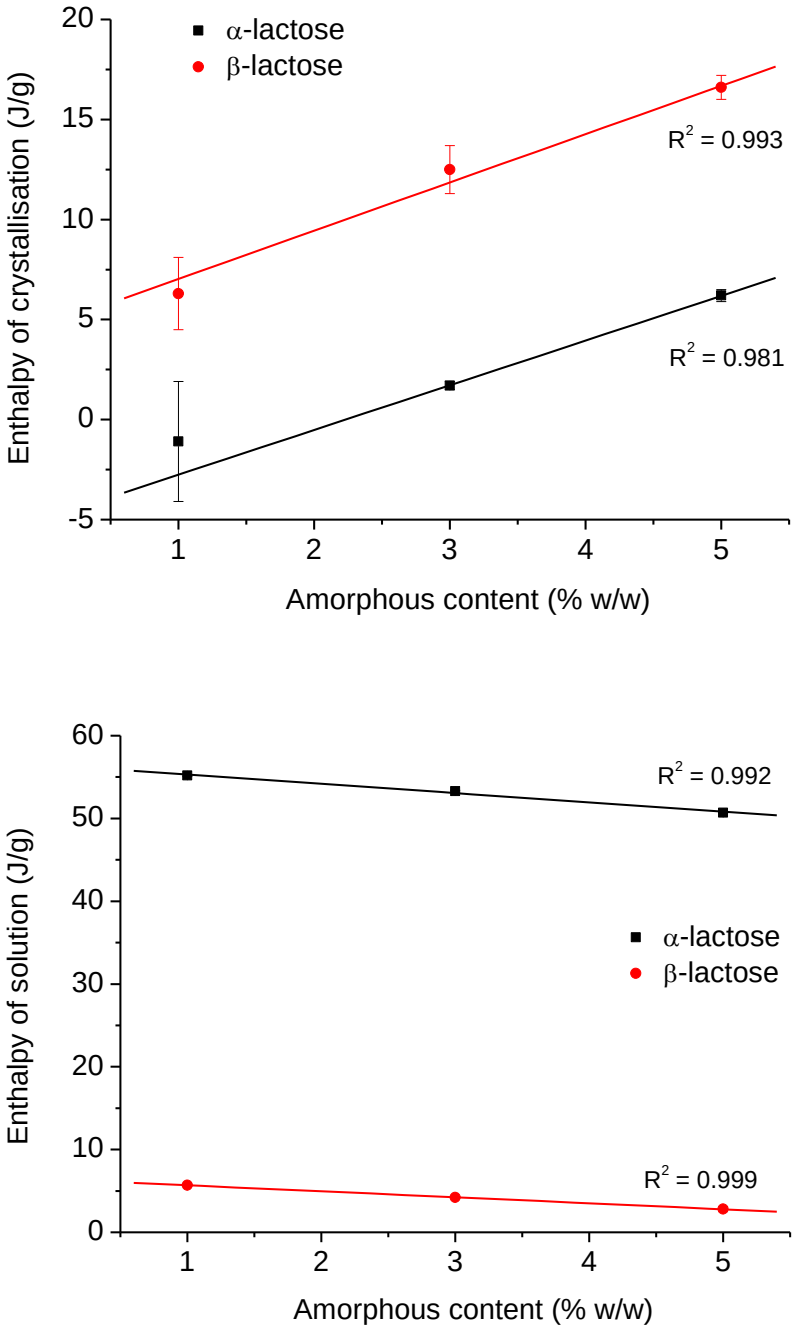

Figure 2. Calibration curves of amorphous content versus heat of crystallization (top) and heat of solution (bottom), showing the effect of the anomeric form of the sample.

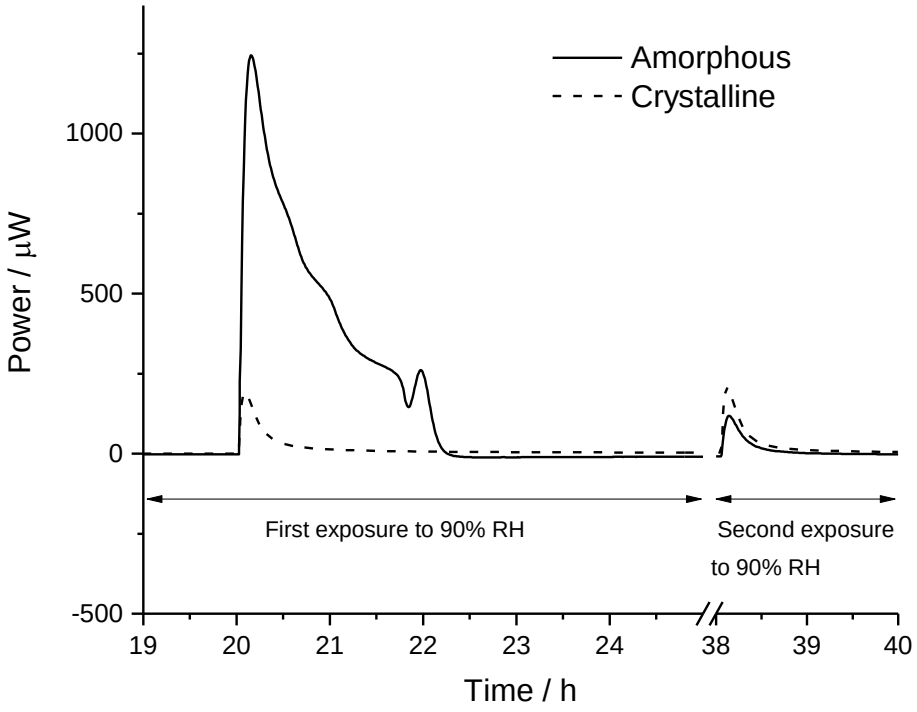

Figure 3. Power–time data for salbutamol sulphate (both amorphous and crystalline materials are shown) when exposed to successive environments of elevated relative humidity. The response of the amorphous material is initially much greater, as it crystallizes to a monohydrate, but is actually smaller than the crystalline material in the second phase, because particle fusion has reduced its surface area.

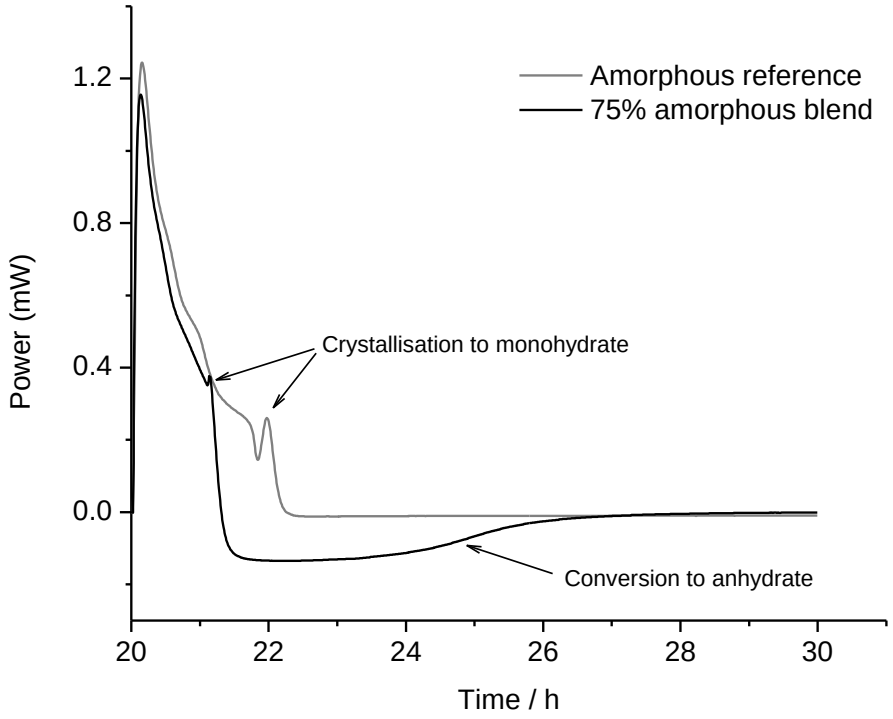

Figure 4. When amorphous salbutamol sulphate is exposed to elevated (90%) humidity it crystallizes to form a monohydrate (grey line) but if some crystalline salbutamol sulphate is present, the monohydrate slowly converts to the anhydrate form with time (endothermic, black line?).

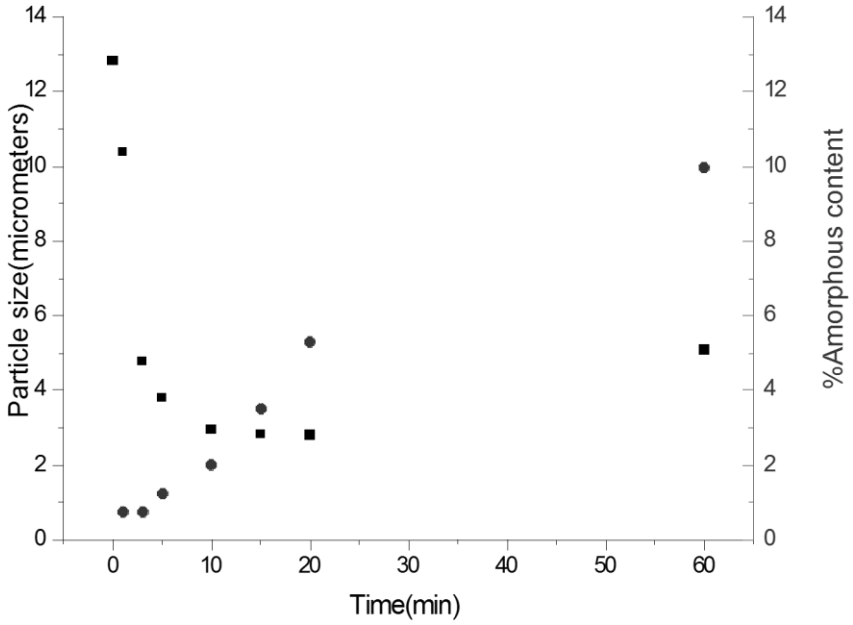

Figure 5. A plot showing the reduction in particle size (squares) when salbutamol sulphate is milled in a ball mill and the corresponding amorphous content measured by gas perfusion calorimetry (circles). It is apparent that initially particle size reduction occurs rapidly and without generation of amorphous material, but with time no further reduction in particle size is seen and the energy imparted by the mill causes disruption of crystal structure.