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Following mechanical activation of salbutamol sulphate during ball-milling with isothermal calorimetry

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ABSTRACT

Formulation of actives for pulmonary delivery with dry powder inhaler devices frequently requires a particle size reduction step. The high-energy forces imparted to a material during milling, as well as reducing particle size, can cause a significant change in physicochemical properties, in particular mechanical activation of the surface (manifested as generation of amorphous regions) which can affect formulated product performance. It is not clear whether particle size reduction occurs prior to, or concomitantly with, generation of amorphous content. In this study the formation of amorphous content with time in crystalline salbutamol sulphate was quantified with isothermal gas perfusion calorimetry as the sample was ball-milled. The data showed that the most particle size reduction occurred initially ($d_{0.5}$ dropping from 12.83 ± 0.4 to 4.2 ± 0.4 within 5 min). During this time period, no detectable amorphous content was observed. Between 5 and 15 min milling time the particle size distribution remained relatively constant but the amorphous content increased non-linearly with time. After 20 min milling time the particle size increased slightly. The data suggest that particle size reduction occurs initially upon application of a force to the crystal. Once maximum particle size reduction has occurred the crystal absorbs the force being applied and the crystal lattice becomes disordered. After extended milling the conditions in the ball mill (heat and/or humidity) may cause crystallisation of some of the amorphous material resulting in particle–particle fusion. It would appear that the ball-milling process could be optimised to achieve the desired particle size distribution but without any loss of crystalline structure.

1. Introduction

Delivery of active principals via the pulmonary route is an increasingly popular strategy, but presents a number of unique formulation challenges, in particular the fact that only small particles (between 2 and 5 μm) can be successfully deposited in the lower respiratory tract. Powders with such a small particle size distribution can be difficult to aerosolise because of their intrinsic cohesiveness, caused by their large surface area to mass ratio, irregular morphology, disordered surface chemistry, electrostatic charge and the fact that gravitational forces acting on particles of this size are not as dominant as other physical forces.

One approach is to formulate actives for delivery with a dry powder inhaler (DPI) device. Here, the small drug particles are located on the surface of a larger, crystalline carrier (typically lactose), bound by a force of adhesion. The aggregates, by virtue of larger physical dimensions, have better flowability and are easier to aerosolise. When the patient inspires, the turbulent air-flow causes deaggregation of the drug and carrier. The large carrier particles impact the back of the throat while the micronised drug enters the airways (Telko and Hickey, 2005).

Such an approach is convenient, because the inhaler device is breath actuated and hence the aerosolisation step is coordinated with inspiration (a common problem with pressurised metered dose inhalers (pMDI)). However, product performance is critically dependent upon the force of adhesion, which is in turn dependent upon the surface properties, mentioned above, of the active and carrier. It follows the method by which the active is prepared can control final product consistency (Chow et al., 2007). Particle engineering approaches such as antisolvent precipitation (Murnane et al., 2008), solution atomisation and crystallisation by sonication (SAX, Pitchayajittipong et al., 2009), supercritical fluid processing (Schiavone et al., 2004) and spray-freeze-drying (Amorji et al., 2007) have been employed, but these can be complex to design and difficult to scale to commercial batch manufacture.

A more generally used approach is milling, wherein a force is applied to large crystalline particles to achieve particle size reduction. Ball mills or air-jet mills are common designs. In the former

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case ceramic or metal balls are placed in a container with the sample and whole apparatus is rotated; the size, weight and number of balls can be varied as can the number of revolutions per minute and the total milling time. In the latter case compressed air is used to agitate particles, causing size reduction by particle attrition; the pressure of the air can be varied and centrifugal force determines when the particles are ejected from the mill. Since milling is a high-energy process, crystalline particles may become partially disordered (or amorphous) during size reduction, particularly at their surfaces (so called mechanical activation, Brodka-Pfeiffer et al., 2003a). While milling is easier to scale to industrial manufacture, the processes that lead to particle size reduction, and their effect on the physicochemical properties of the milled material, in particular at the surface, are not totally understood. On a commercial scale, this means it may well be the case that the milling process is optimised on particle size parameters alone, when surface factors may in fact be more important in ensuring consistency of product performance (Jones et al., 2008). As a consequence, milled material may often be 'conditioned' prior to formulation (with humidity, time and/or temperature) as this is seen empirically to produce a more consistent product (Brodka-Pfeiffer et al., 2003b).

While it is not possible to prevent the changes in surface chemistry caused by milling, quantifying the changes that occur is the first step in understanding, and ultimately controlling, these surface effects. Here, we show with isothermal microcalorimetric data, how some of the physicochemical properties of a model inhalation drug, salbutamol sulphate, change with increasing milling time and correlate the results with particle size distribution data to construct a simple model of the processes that occur during milling.

2. Materials and methods

Salbutamol sulphate (SS) was obtained from Micron Technologies Ltd (UK). Acetone (ACS grade) was purchased from Aldrich (UK). Aqueous solutions were prepared in deionised water.

Because the SS sample arrived micronised, it was recrystallised prior to commencement of experimentation, both to remove any amorphous content and to increase the particle size distribution. SS (35 g) was dissolved in water (110 mL) at 25 °C with continuous stirring and the solution was cooled to 0 °C in an ice bath. Acetone (2000 mL) was then poured slowly into the solution to precipitate SS crystals. The crystals were filtered, washed with acetone and dried in a vacuum oven at 40 °C for 1 week. Thermogravimetric measurements (Pyris 6, Perkin-Elmer Ltd) were performed on the SS crystals to confirm attainment of complete dryness during this time period (data not shown). The crystallinity of the sample was confirmed with powder X-ray diffraction (PXRD, Philips PW1730/10), Fig. 1, which showed the existence of the stable Form I. Scanning electron micrographs (Phillips XL-30) of the initial and recrystallised SS samples also confirmed the existence of Form I crystals (Fig. 2).

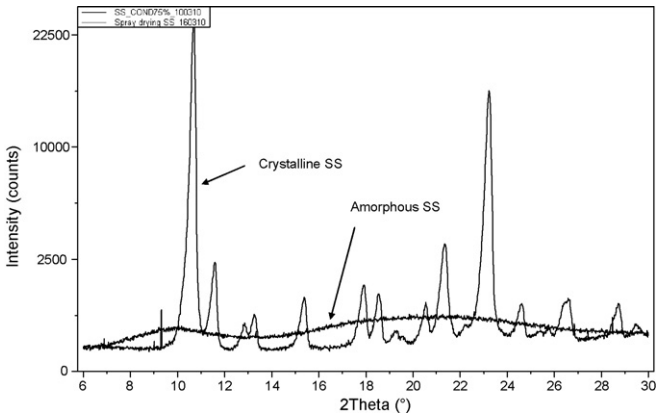

Fig. 1. PXRD patterns of recrystallised SS, showing the presence of Form I and spray-dried SS.

Amorphous SS was prepared by spray-drying. An aqueous solution of SS (5%, w/v) was spray-dried (Buchi B-191 mini-spray-drier) in accordance with the methodology of Columbano et al. (2002). XRPD measurements confirmed that the sample was amorphous, Fig. 1.

Extent of disorder was quantified with isothermal calorimetry (IC, TAM, TA Instruments UK). Samples (20 mg) were loaded into the ampoule (4 mL total volume) and allowed to equilibrate at 25 °C under 0% relative humidity (RH). The RH was then increased to 90% for a period of 8 h before being reduced to 0% for a further 8 h. Data were recorded with the dedicated software package Digitam 4.1. The instrument was calibrated with the electrical substitution method prior to use and operated on an amplifier setting of 1000 μW. The reference channel contained an empty stainless steel ampoule. A calibration curve was prepared by mixing crystalline and amorphous SS in appropriate ratios. All experiments were repeated in triplicate.

Ball-milling of SS was performed with a Fritsch Planetary Ball mill, Pulverisette 5 (Idar-Oberstein, Germany). The mill consisted of a ceramic jar (300 mL volume) containing ceramic balls (10) of diameter 2 cm. Drug (500 mg) was weighed and poured into the jar in which the balls had already been placed. Samples were milled at 100 rpm for various periods of time up to 20 min. Immediately following milling, the milled SS was analysed with IC for amorphous content quantification.

Particle size distribution was determined by laser diffraction (Mastersizer S, Malvern Instruments UK, equipped with a small volume stirred cell and 100 mm lens). Samples (ca. 5 mg) were suspended in a cyclohexane-lecithin solution (0.1%, w/v) and sonicated with a PUL 55 Sonicator (Kerry Ultrasonics, UK) at 50 Hz for 1 min prior to drop-wise addition to the sample cell to achieve a laser

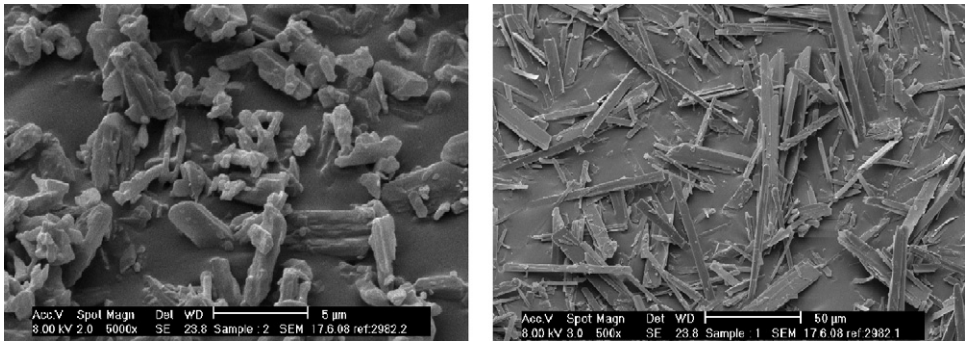

Fig. 2. SEM images of SS as received (left) and post-crystallisation (right).

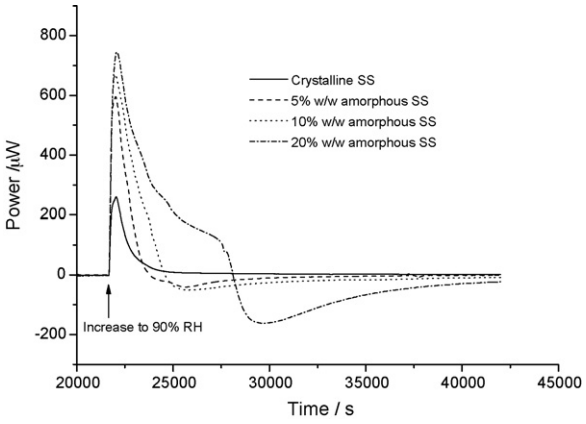

Fig. 3. Power-time data showing response of crystalline and partially amorphous salbutamol sulphate samples under 90% RH.

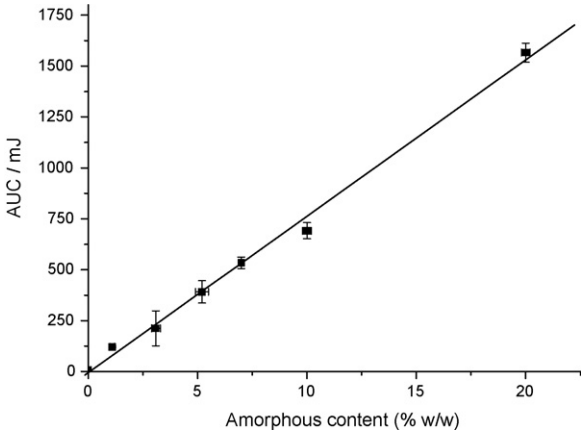

Fig. 4. Calibration plot of amorphous content versus heat of crystallisation for salbutamol sulphate, determined with IC (fit values; $y = -5.75 + 76.8x$, $r^2 = 0.9974$).

obscuration of approximately 22%. The particle size of five aliquots taken from different parts of the powder bed of each sample were measured in this way and the data combined and analysed by the instrument software to give a distribution by volume.

3. Results and discussion

Typical calorimetric data that represent crystallisation of salbutamol sulphate with increasing amorphous contents are shown in Fig. 3. Upon an increase in RH to 90% there is a sharp exotherm, followed by an endothermic peak. The form of these data is slightly different to those reported earlier (Columbano et al., 2002; Buckton et al., 1995); in these cases there is no obvious endotherm in the data, although the method of wetting the sample was different. Columbano et al. (2002) also followed the crystallisation with dynamic vapour sorption and noted that water movement occurred in the sample for a considerable time following crystallisation. We thus reason that the endotherm present in our data correlates with water movement and hence we integrated only the sharp exothermic peak associated with crystallisation. The area under the crystallisation peak varied linearly with amorphous content, Fig. 4. It was then possible to use the data to quantify extents of disorder in milled SS samples (representative calorimetric data are shown in Fig. 5). It should be noted that the use of this type of calibration curve has some limitations (Gaisford and Ramos, 2007; Ramos et al., 2005). Principally, the material used to prepare the calibration curve (a mixture of wholly amorphous and wholly crystalline particles) is physically distinct from the study material, which in this

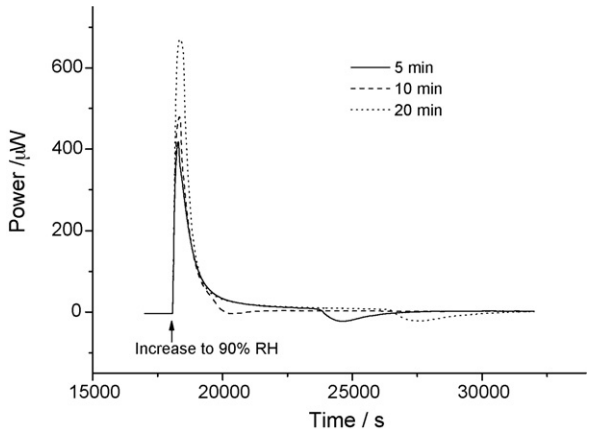

Fig. 5. Power–time data for salbutamol sulphate, ball-milled for various time periods, from 5 to 20 min.

case will have disordered regions (that is, a combination of crystal dislocations and amorphous areas) forming a corona around an otherwise crystalline substrate. The magnitude of this effect will vary according to the sample studied. Since the calibration curve was prepared with totally amorphous material (because it was spray-dried), the values reported below for milled SS samples are expressed in amorphous content (%, w/w) but it is noted that this term really means extent of disorder and accounts for crystal dislocations as well as true amorphous regions. Note also that the forms of the calorimetric data for the ball-milled samples (Fig. 5) are slightly different from those of the calibration samples (Fig. 3), again a likely result of the differences in the physical constitutions of the materials.

SS samples were milled for various time periods up to 20 min. Samples were subsequently removed from the mill and analysed for particle size and amorphous content immediately (to reduce the risk of relaxation or recrystallisation increasing the particle size or reducing amorphous content value). The average particle size (both $d_{0.5}$ and $d_{0.9}$) and extent of disorder data are given in Table 1 and plotted in Figs. 6 and 7.

Over short milling times (1–3 min) there is a sharp reduction in particle size, with no increase in amorphous content detected with the calorimeter. Between 5 and 15 min milling time, the particle size distribution remains relatively constant and there is a concomitant increase in extent of disorder, although this rise is not linear. After 20 min milling time there is a slight increase in particle size, seen in both the $d_{0.5}$ and $d_{0.9}$ data.

Since milling is a high-energy process it is likely that a crystalline material will lose some of its structural order as it reduces in particle size, resulting in the formation of amorphous regions (Brodka-Pfeiffer et al., 2003b). These metastable forms can affect both the stability of the active (Brodka-Pfeiffer et al., 2003a) as well as formulated product performance. As has been well docu-

Table 1
Amorphous content and particle size data as a function of milling time.

Milling time (min)	Amorphous content (%, w/w)	Particle size	
		$d_{0.5}$ (μm)	$d_{0.9}$ (μm)
0	0 ± 0.50	12.83 ± 0.4	43.13 ± 3.2
1	0 ± 0.49	8.9 ± 1.1	34.41 ± 1.3
3	0 ± 1.27	5.3 ± 0.4	13.0 ± 0.5
5	5.2 ± 3.34	4.2 ± 0.4	10.39 ± 0.4
10	10.9 ± 3.4	3.0 ± 0.6	9.43 ± 0.6
15	16.63 ± 3.91	2.7 ± 0.2	10.51 ± 0.8
20	22.34 ± 9.84	3.1 ± 0.1	12.72 ± 2.0

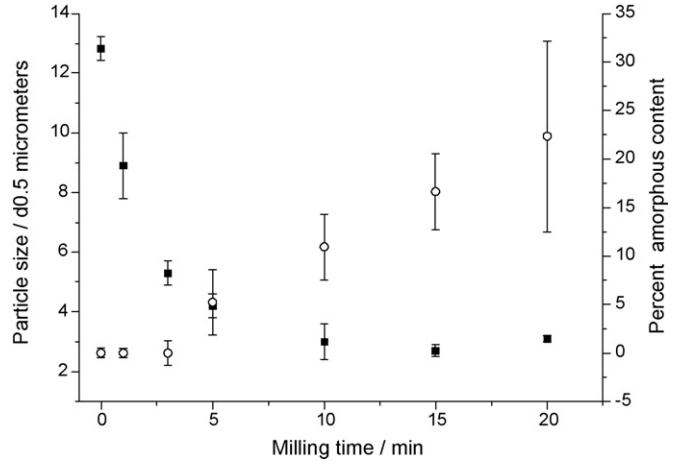

Fig. 6. Particle size ($d_{0.5}$ solid squares) and amorphous content (open circles) data as a function of milling time for ball-milled salbutamol sulphate.

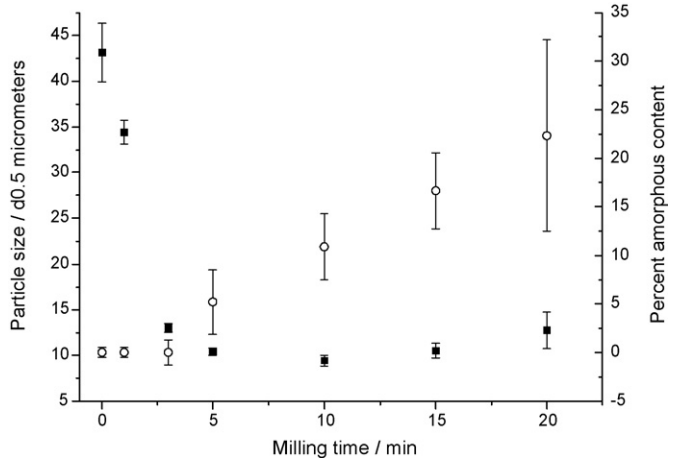

Fig. 7. Particle size ($d_{0.9}$ solid squares) and amorphous content (open circles) data as a function of milling time for ball-milled salbutamol sulphate.

mented elsewhere (Feeley et al., 1998; Hogan and Buckton, 2000), the amount of amorphous material formed in this way can be quite small (of the order of a few percent, w/w). Gorny et al. (2007) measured an amorphous content of ca. 6% (w/w) for air-jet milled salbutamol sulphate, although the residence time in an air-jet mill cannot be extended over long time periods as it can for ball-milling. The nature of the force applied (impaction) means that the amorphous material must necessarily be located on the surface of the milled material and, hence, although the milled material is predominantly crystalline it behaves as if it were entirely amorphous. Newell et al. (2001) published some inverse phase gas chromatography (IGC) data that convey this point. They show that the surface energy of ball-milled lactose (ca. 1%, w/w amorphous) was actually greater than the surface energy of the spray-dried amorphous reference material. It is inevitable that this amorphous material will crystallise, probably over a relatively short time period as it is located on a crystalline substrate (that can act as a seed). Prior to crystallisation, the amorphous regions will relax, losing energy in the process. Both processes act to alter the surface energy of the material.

The data in Figs. 6 and 7 allow a simple model of the processes involved in milling to be visualised. The mechanical forces imparted result initially in a reduction in particle size, as the material fractures along natural fault lines and crystal defects. Eventually there comes a point at which the bulk material can no longer fracture and maximum particle size reduction has been achieved. However, mechanical forces are still being applied and must be absorbed and dissipated by the sample; these processes result in mechanical activation of the sample, initially by creation of surface dislocations and then by generation of amorphous regions.

The data clearly show that particle size reduction occurs before generation of amorphous content. The immediate benefit of this is that it appears to be feasible to control the micronising process to produce a product with both a defined particle size distribution *and* a crystalline surface. Although we did not attempt to make similar measurements on a larger scale, there would seem to be no reason why this outcome would not apply to larger ball-milling apparatus; the break-point time at which maximum particle size reduction is achieved would simply change.

One final hypothesis relates to the particle size data that show a slight increase after 20 min milling time (while the increase in amorphous content with time is not linear). This is that the conditions in the ball mill (heat and/or humidity) might cause recrystallisation of some of the amorphous material—this would act both to lower the total amount of amorphous material observed as well as cause particle–particle fusion.

4. Summary

The data show that milling of a crystalline pharmaceutical results initially in a reduction in particle size with no measurable surface disorder. Continued milling does not reduce particle size further; rather, the surface of the material becomes disordered. This disorder can act to change the surface energy and, as a direct consequence, may alter DPI product performance. Controlling this process starts with being able to monitor the formation of these disordered regions. The study has shown that isothermal calorimetry has the potential to accomplish this.

Acknowledgements

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