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Note

The use of atomic force microscopy to study the conditioning of micronised budesonide

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Abstract

Patent literature describes "conditioning" techniques which employ organic vapours to recrystallise amorphous regions in micronised particles, with the aim of improving their processability and physico-chemical stability. This report describes a preliminary study investigating the efficacy of PhaseImaging™ atomic force microscopy (AFM) for the investigation of such processes. AFM phase images demonstrated variation in mechanical properties across the surface of milled budesonide particles, which diminished upon exposure to ethanol vapour. No variation was seen in phase images of unmilled budesonide. Dynamic vapour sorption confirmed the presence amorphous material in the milled sample and its subsequent recrystallisation following exposure to ethanol vapour under the same conditions as those used in the AFM experiment. It was therefore hypothesised that variation in the phase images indicated the presence of amorphous regions which were subsequently conditioned. PhaseImaging™ AFM may therefore be a useful method for the study of conditioning techniques, enabling the efficacy and kinetics of the process to be observed.

Keywords: Amorphous; Atomic force microscopy; PhaseImaging™; Conditioning; Micronised budesonide; Recrystallisation

The milling of materials to produce micronised particles for dry powder inhaler applications is known to induce amorphous regions in particles, especially on their surface, which can contribute towards high dose variability and poor aerosolisation (Malcolmson and Embleton, 1998). This is due to the fact that the storage conditions of milled materials have a dramatic effect on their behaviour. For example, small changes in humidity and/or temperature may cause increased molecular mobility in amorphous regions, resulting in recrystallisation and the potential for particle fusion (Malcolmson and Embleton, 1998; Ward and Schultz, 1995).

In order to address this issue, a number of patents describing processes to decrease the amount of amorphous material on the surface of micronised anti-asthma drugs have been filed (Trofast and Briggner, 1995; Trofast et al., 1992). These so-called "conditioning" techniques employ organic vapours, typically of ethanol, to promote the controlled recrystallisation of amorphous regions, thus producing more stable and less cohesive powders for formulation.

PhaseImaging™ atomic force microscopy (AFM) is a technique which examines surface mechanical properties in addition to surface topography (Garcia et al., 2007). A number of reports describe the use of this technique to examine the surface of milled particles. These have suggested that the contrast seen in phase images of the surface of such materials may be related to the presence of amorphous regions (Begat et al., 2003; Young and Price, 2004). It has also been reported that such areas of contrast diminish upon exposure to humid conditions likely to promote recrystallisation, lending weight to the hypothesis that

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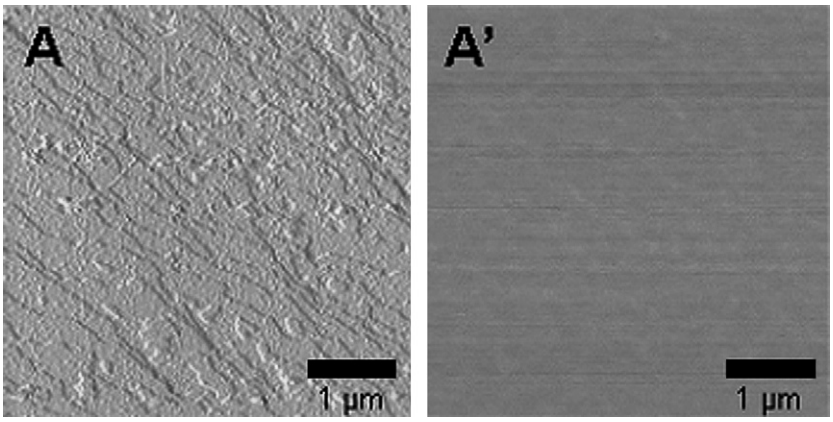

Fig. 1. Representative AFM amplitude (left pane) and phase (right pane) images of the surface of unmilled budesonide.

such images indicate the presence of amorphous material (Price and Young, 2005).

PhaseImaging™ AFM may, therefore, be a useful technique for the investigation of organic vapour conditioning techniques similar to those discussed above. This report describes a preliminary study carried out to investigate this hypothesis.

Crystalline budesonide was produced by precipitation from a saturated methanol solution with water. The precipitated material was filtered and dried at 40 °C. A sample of this material was milled using a Retsch ultracentrifuge mill (model ZM 100, Retsch GmbH and Co. KG, Haan, Germany) to produce freshly micronised material (volume median diameter = 7.0 μm by laser diffraction), which was immediately transferred to a tightly sealed container containing phosphorus pentoxide (0% RH). X-ray powder diffraction and conventional differential scanning calorimetry both suggested that these processes had not resulted in bulk modification of the budesonide crystal structure, although as the limit of amorphous detection for both these techniques is ∼10% (Buckton, 1997), the presence of small amounts of surface amorphous material could not have been detected.

The influence of the micronisation and subsequent exposure to conditioning ethanol vapour on the surface properties of the budesonide was investigated using PhaseImaging™ AFM (Multimode AFM with J-type scanner and Nanoscope IIIa controller, all from DI, Cambridge, UK). Topographical data were collected in TappingMode™, at a scan rate of 1.0 Hz using a high aspect ratio AFM probe (type OMCL-AC240TS, Olympus, Japan). The intermittent contact force produced as a result of tip oscillation was minimised by maintaining a low drive amplitude and relative set point. Height, amplitude and phase data were collected simultaneously. *In situ* ethanol vapour concentrations were controlled using a custom built perfusion apparatus described elsewhere (Young et al., 2003). Both milled and unmilled budesonide particles were investigated under dry nitrogen and during subsequent exposure to ethanol vapour. The relative partial pressure of ethanol achieved during these experiments could not be directly measured. However, the perfusion apparatus was used with settings known to give 70% relative humidity with water and as this was achieved by mixing dry and saturated nitrogen in variable proportions, it is reasonable to assume that an ethanol relative partial pressure of ∼0.7 was reached.

Representative AFM images (amplitude and phase) of the unmilled budesonide are shown in Fig. 1, while similar images of the freshly micronised budesonide before and after exposure to ethanol vapour for 60 min are shown in Fig. 2.

Clear variation in morphology between the milled and unmilled samples was observed. Amplitude images of the unmilled budesonide (Fig. 1) suggested an ordered crystalline state with multiple surface irregularities present across the surface. Phase images of the unmilled budesonide indicated little variation in mechanical properties across the surface (Fig. 1).

In comparison, the morphology and surface properties of the milled budesonide before and after exposure to ethanol vapour for 60 min suggested that micronisation and subsequent exposure to organic vapour had a dramatic effect on surface characteristics. Inspection of the amplitude and topographical data (Fig. 2A and B) indicated that, as expected, micronisation resulted in an increase in surface irregularities, which were decreased upon subsequent exposure to ethanol vapour. The corresponding phase images for the same budesonide sample (Fig. 2A′ and B′) suggested that milling induced the presence of large variations in the mechanical properties of the surface, which were reduced upon exposure to ethanol vapour.

As discussed, such physico-mechanical variations in the surfaces of milled drugs have previously been attributed to the presence of amorphous regions introduced by this highly energetic processing technique (Begat et al., 2003; Price and Young, 2005; Young and Price, 2004). One possible explanation for these observations, therefore, is that micronisation introduced amorphous regions into the previously crystalline surface of the budesonide, which subsequently recrystallised after exposure to ethanol vapour.

To investigate this hypothesis, the sorption of ethanol by the freshly micronised budesonide was examined using dynamic vapour sorption (DVS). Approximately 20 mg was weighed into the sample pan of a DVS-1 (Surface Measurement Systems Ltd., London, UK) and subjected to two ethanol relative partial pressure cycles similar to that used in the AFM (0.0 p/p_0 to 0.7 p/p_0 in a single step). Equilibrium sorption at each relative partial

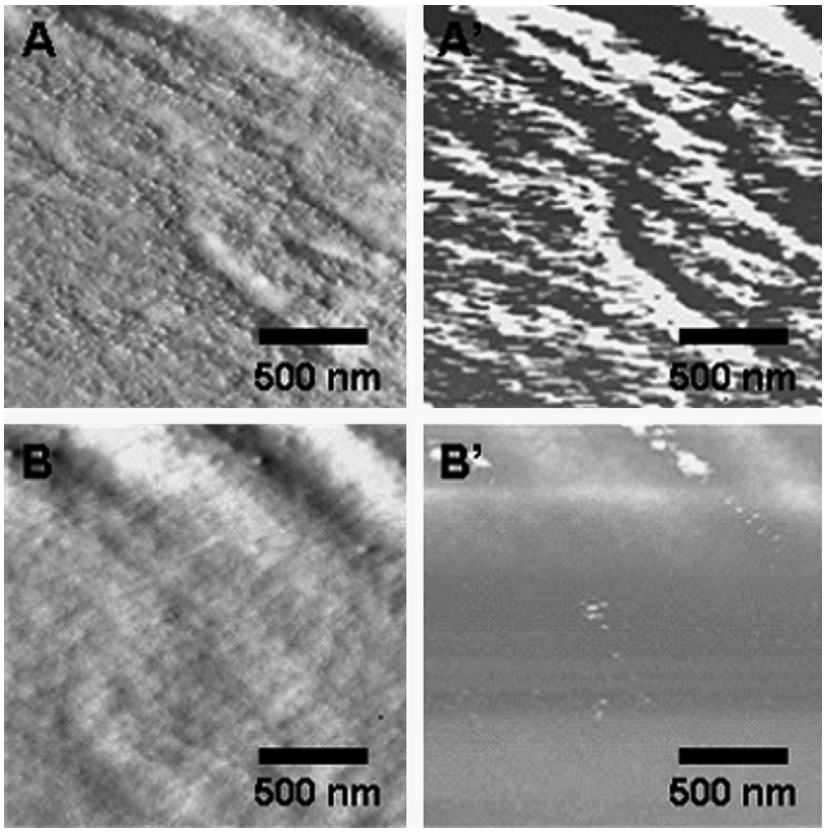

Fig. 2. Representative AFM amplitude (left pane) and phase (right pane) images of the same area of the surface of a milled budesonide particle before (A and A′) and after (B and B′) exposure to ethanol vapour for ∼60 min.

pressure was determined by a change in mass to time ratio of <0.0001% dm/dt.

The relationship between the percentage mass change and ethanol partial pressure for freshly micronised budesonide during a representative DVS experiment is shown in Fig. 3. Similar results were obtained from repeat experiments. It can be seen that the exposure of the sample to ethanol vapour resulted in a rapid mass gain which was followed by a mass reduction before reaching equilibrium. Classically, this suggests the absorption of vapour into amorphous regions followed by recrystallisation and expulsion of solvent molecules (Buckton, 1997). The subsequent lowering of the relative vapour pressure to zero resulted in a mass reduction (equilibrium at 0.07%), presumably due to the desorption of ethanol from the surface of the sample. During the second cycle, the introduction of ethanol vapour resulted in a similar equilibrium mass gain, but this was achieved directly, without an initial peak in mass, thus suggesting that complete recrystallisation had occurred during the first cycle. Once again, the removal of ethanol during the second cycle led to a loss of mass, to the same equilibrium as in the first cycle (0.07%).

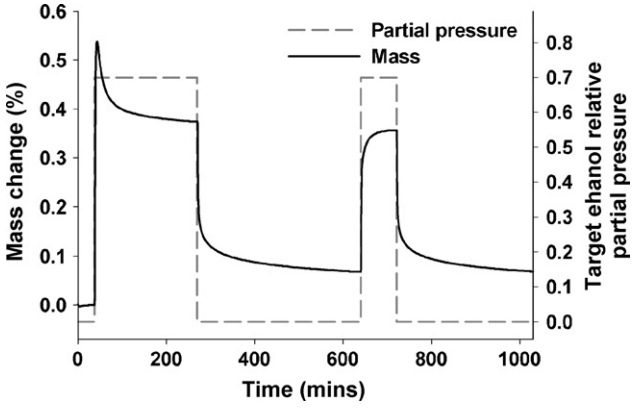

Fig. 3. Representative DVS trace showing the adsorption and absorption of ethanol vapour into milled budesonide at relative partial pressures of 0 and 0.7.

These observations suggest that amorphous material is present, presumably on the surface, in the freshly micronised budesonide, at a level greater than the lower limit of detection by DVS, which is approximately 0.5% (Buckton, 1997). Additionally, and importantly, it also suggests that this amorphous material may be recrystallised at 25 °C by the introduction of ethanol vapour at a relative partial pressure of 0.7, with the majority of recrystallisation occurring within 1 h. The DVS experiment therefore supports the explanation presented for the AFM results, as it demonstrates that exposure to ethanol vapour may cause recrystallisation of amorphous regions within the freshly micronised budesonide at approximately the same partial pressure and in the same time scale as that used in the AFM method.

The equilibrium mass change following the removal of ethanol vapour was 0.07%, indicating that exposure to ethanol

and subsequent crystallisation had increased the sample mass. This suggests that following recrystallisation, some ethanol may have remained trapped in the recrystallised budesonide, possibly in the form of a fused mass (as has previously been observed for salbutamol sulphate and water (Columbano et al., 2002)) or as a budesonide-ethanol solvate.

In conclusion, the results presented suggest that PhaseImaging^{TM} AFM can be developed into a useful tool for the study of particle conditioning processes, as amorphous areas on particle surfaces can be observed during exposure to different environmental conditions. The efficacy of a conditioning technique and the rate at which it occurs could therefore be monitored, giving a greater understanding of the complex processes involved and aiding the rational selection of the optimum conditions to apply to a given material.

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