Characterization of a surface modified dry powder inhalation carrier prepared by ''particle smoothing''

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Abstract

Atomic force microscopy (AFM) was used to investigate drug–carrier interactions between beclometasone dipropionate (BDP) and a series of untreated and modified lactose surfaces. This quantitative information was correlated with bulk characterization methods and an in-vitro study. Modified lactose surfaces were prepared using a proprietary process referred to as ''particle smoothing'' to obtain smooth carrier surfaces with or without the presence of magnesium stearate. The engineering of lactose carrier surfaces using the particle smoothing process resulted in significant differences in surface morphology when compared with the "as supplied" starting material. The energy of separation, between BDP and lactose samples, determined by AFM suggested similar lognormal distributions with a rank decrease in median separation energy ($e_{0.5}$) (26.7, 20.6 and 7.7 μJ for untreated, particle-smoothed and particle-smoothed with magnesium stearate, respectively). A series of in-vitro twin stage impinger studies showed good correlation with the AFM separation energy measurements. The mean fine particle dose increased for the two processed lactose samples, with a significant increase for the lactose processed with magnesium stearate, 102.0 ± 16 μg compared with 24.2 ± 10.7 μg for the untreated lactose. Thus, the AFM presents as a possible pre-formulation tool for rapid characterization of particle interactions.

Introduction

The delivery of micron sized (sub 5 μm) dry powder particulates to the respiratory tract, using dry-powder inhalers (DPI), has become commonplace in the prophylactic treatment of asthma and other bronchial related diseases. Drug particulates are often blended with larger (50–200 μm), inert crystalline carrier materials to form ordered mixes that, on inhalation, are liberated from the carrier to penetrate the lung. The efficacy of such a system is dependent on the interactive forces between the drug particulates and carrier. There are essentially three primary forces present in a dry powder inhalation system: van der Waals, electrostatic and capillary interactions. The magnitude of each of these forces, the total interaction, and subsequent aerolization efficiency will be dependent on the surface characteristics of the drug and carrier, and the environmental conditions in which they are stored and delivered.

Previous investigations reported that the carrier morphology directly affects the aerolization efficiency from a DPI (Kawashima et al 1998; Podczeck 1998; Larhrib et al 1999; Zeng et al 2000). In general terms, a decrease in roughness is believed to improve aerolization efficiency of a drug–carrier blend. However, an important balance between the morphologies of both the drug and carrier can exist. For example, a tabular, atomically smooth carrier material and tabular micron sized drug particulate would most likely have a high carrier–drug interaction owing to an increased contact area. In contrast, a material with a roughness parameter slightly less than that of the drug would potentially lead to a decreased contact area and subsequent drug–carrier interactions. Another factor to consider is the surface free energy of the carrier. The use of low surface free energy materials, such as magnesium stearate or leucine (commonly used as lubricants in the tableting industry), has been reported as a possible means of increasing the aerolization efficiencies of such systems (Staniforth 1997).

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The direct measurement of drug particulate–carrier interactions could provide invaluable data, useful in the pre-formulation step of DPI development. Previously, fundamental measurements of drug–carrier interactions have been limited to bulk analysis techniques such as centrifugal studies (Podczeck 1998; Clarke et al 2002). Although these techniques provide a useful insight into the efficacy of a DPI formulation, direct measurement of the drug–carrier interactions may not be possible owing to stratifying variables.

As part of an ongoing investigation to quantify drug–carrier interactions, the atomic force microscope (AFM) (Binnig et al 1986) was used to directly measure the energy required to separate a drug particulate from a carrier surface. In addition, the effect of carrier morphology on drug–carrier interactions was also investigated by AFM, using a proprietary "particle smoothing" process.

Briefly, such AFM separation measurements are achieved by mounting a colloidal drug onto a microfabricated AFM cantilever of known spring constant, before conducting contact separation measurements between the drug and a substrate (in this case modified lactose surfaces), as a function of separation distance. Accurate measurement of the particle substrate forces and subsequent separation energy is calculated by measuring the deflection of the cantilever (by laser reflection) in relation to sample movement (sub angstrom piezoelectric crystal movement). The AFM colloidal probe technique in relation to the measurement of adhesion force and separation energy is described in more detail elsewhere (Ducker et al 1991; Mizes et al 2000; Price et al 2002).

Previous investigations, using similar techniques, have demonstrated the AFM to be a powerful tool in determining the interactions between pharmaceutical systems (Ibrahim et al 2000; Louey et al 2001; Berard et al 2002). However, recent investigations (Eve et al 2002; Young et al 2002) have begun to focus on the interactions between micronized particulates and surfaces of direct relevance to DPI technology.

Micronized beclometasone dipropionate (BDP), a corticosteroid commonly used for the prophylactic treatment of asthma, was used as a model drug, as it is commonly formulated as a dry powder for inhalation. The interaction between BDP and modified lactose surfaces was investigated using the AFM colloidal probe technique. Finally, a series of in-vitro studies was undertaken to examine the relationship between the fundamental AFM measurements and performance of the surface modified lactose in a DPI system.

Materials and Methods

Materials

Lactose monohydrate crystals (90–150 μm sieve fraction) were supplied by Meggle (Wasserburg, Germany). Micronized BDP and magnesium stearate was supplied by Chiesi Farmaceutici S.p.A (Parma, Italy).

Preparation of lactose surfaces

Modified lactose surfaces were prepared using a proprietary process (Caponetti et al 2001), referred to as particle smoothing. Lactose samples (750 g) were agitated (450 rev min^{-1}) and dried under vacuum for 15 min at 50°C, by a high-speed mixer (Roto J; Zanchetta, Lucca, Italy) in the presence of a small amount (40 mL) of wetting solvent (water/ethanol, 5:3). Samples were reprocessed 10 times with equivalent volumes of wetting solvent, resulting in a lactose sample referred to as process A. In addition, a suspension of magnesium stearate (0.25% w/w) added to the wetting solvent throughout the process resulted in a lactose sample referred to as process B.

Physical characterization

The particle size distributions of the three lactose samples were determined by laser light scattering (Mastersizer 2000; Malvern, UK). Samples were dispersed in 0.1% w/w lecithin/cyclohexane before analysis. Specific surface area was measured by a BET adsorption method (Gemini; Micromeritics Ltd, USA) using nitrogen gas.

Particle morphology was investigated using scanning electron microscopy (SEM) (Jeol 6400; Jeol, Japan) at 10 KeV. Samples were mounted on carbon sticky tabs and carbon-coated before imaging.

Detailed topographical information of the lactose samples was investigated using AFM (Multimode SPM with Nanoscope III controller; DI instruments, UK). Samples were mounted on carbon sticky tabs and imaged using tapping mode with a high aspect ratio silicon probe (OTESP; DI instruments), over 10 μm × 10 μm areas at a scan rate of 0.7 Hz. Root mean square roughness (R_{rms}) values for each sample were calculated from the AFM height data as follows:

$$R_{rms} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} y_i^2}$$

where n is the number of points in topography profile and y_i is the distance of a point i from the centre line.

Separation measurement using AFM

The method used for drug probe preparation and AFM separation energy measurement followed that of Young et al (2002). Briefly, a BDP particulate drug probe (approx. diam. 2 μm) was mounted onto a V-shaped tipless silicon nitride cantilever (0.58 Nm^{-1} spring constant, NP-O20; DI instruments) using a custom-built multistage micromanipulation technique. Variations in spring constant were minimized by obtaining a wafer of tipless cantilevers (> 500) as this eliminated batch-to-batch variations. In addition, randomly chosen cantilevers from across the wafer had a spring constant variance of less than 14% using the thermal method (Hutter & Bechhoefer 1993).

Multiple force–distance curves (n = 4096) were determined between the BDP drug probe and sample lactose surfaces by AFM in force volume mode (Multimode SPM with Nanoscope III controller; Digital Instruments, UK)

at 45% relative humidity and 25°C. Measurements were conducted over a 10 μm $\times$ 10 μm area on a representative crystal surface of the original lactose and both processed lactose samples with the following settings: approach–retraction cycle 2 μm, cycle rate 8.14 Hz, and constant compliance region 60 nm. The area under each of the force–distance curves was integrated using custom-built batch conversion software and exported to give a block of 64 $\times$ 64 separation energy values corresponding to the x and y position of each force distance curve. In addition, this allowed offline comparison between individual force distance curve data and specific separation energy values.

In-vitro aerosolization

The aerolization of BDP–lactose blends was investigated using apparatus A (British Pharmacopoeia), the twin stage impinger (TSI) (Copley Instruments Ltd, Nottingham, UK), containing 7 mL of dilution solvent in stage one and 30 mL of dilution solvent in stage two, which at 60 L min^{-1} produces a cut-off mass median aerodynamic diameter of 6.4 μm between the two stages (Hallworth & Westmoreland 1987). Three powder blends, BDP–original lactose, BDP–lactose process A and BDP–lactose process B were prepared in 0.8:99.2 ratios using a Turbula mixer (Bachofen, Basel, CH, Switzerland) and loaded into a multidose DPI (Pulvinal; Chiesi Farmaceutici S.p.A, Parma, Italy). The loaded DPI was tested at 60 L min^{-1} for 10 s using a solenoid valve timer (Copley Instruments Ltd). The deposited drug fractions were collected from the TSI stages using a suitable wash solvent and analysed using high-performance liquid chromatography. All experiments were performed 14 times.
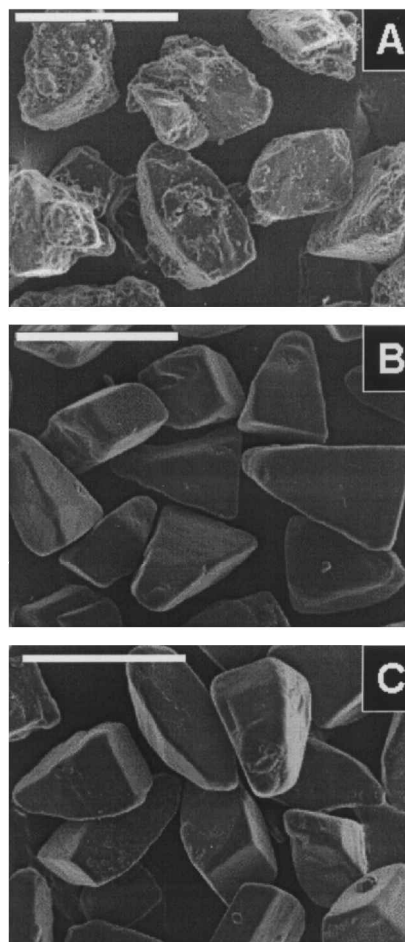
Results and Discussion

General physical characterizations

In order to fully understand the effect of particle smoothing on the aerosolization of micronized BDP from modified lactose surfaces, samples were first characterized for par-

Figure 1 Scanning electron micrographs of untreated lactose monohydrate (A), lactose treated with process A (B), and lactose treated with process B (C). Scale bars indicate 200 μm.

ticle size and sample morphology. Mean particle diameters and percentage volume distribution for the three lactose samples are shown in Table 1. Although statistically significant, the median particle diameter ($d_{0.5}$) of the three lactose

Table 1 Particle size and surface roughness of untreated and processed lactose, and separation energy and aerolization efficiency of beclometasone dipropionate with the different carriers.

Lactose sample	Particle size (μm)[a]			Surface roughness (nm)[b]	Separation energy (μJ)[c]			In-vitro deposition[d]	
	$d_{0.1}$	$d_{0.5}$	$d_{0.9}$	R_{rms}	$e_{0.1}$	$e_{0.5}$	$e_{0.9}$	ED (μg)	FPD (μg)
Original lactose	16.4$\pm$0.5	117.6$\pm$0.6	204.0$\pm$1.2	108.0$\pm$36.8	5.3	26.7	133.7	179.8$\pm$31.9	24.2$\pm$10.7
Lactose process A	71.9$\pm$0.1	116.4$\pm$0.1	184.7$\pm$0.3	26.5$\pm$7.4	3.8	20.6	111.5	204.1$\pm$22.0	28.9$\pm$24.3
Lactose process B	77.5$\pm$0.2	122.5$\pm$0.3	178.8$\pm$0.4	12.2$\pm$2.9	2.5	7.7	32.0	174.7$\pm$29.4	102.0$\pm$16.0

[a]Mean$\pm$s.d. (n = 3). [b]Mean$\pm$s.d. (10 $\times$ 10 μm areas) (n = 6). [c]Median separation values obtained from cumulative separation energies (n = 4096). [d]Mean values$\pm$s.d., fine particle dose (FPD) of drug collected from stage two of the twin stage impinger (mass median aerodynamic diameter < 6.4 μm) (n = 14).
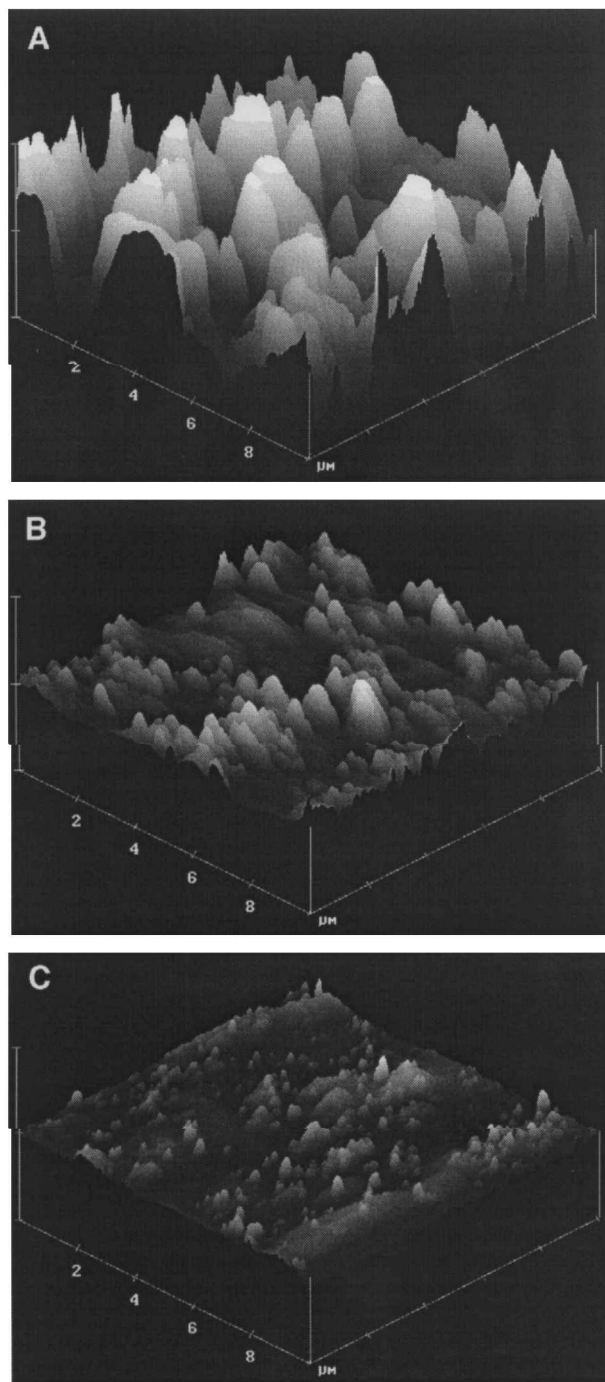
Figure 2 Representative atomic force microscope height images of untreated lactose monohydrate (A), lactose treated with process A (B), and lactose treated with process B (C). x, y and z axes are 10 μm, 10 μm and 0.5 μm, respectively.

samples changed relatively little, which was reflected in the mode diameter. The 10th percentile values, however, showed a large increase in value, suggesting that the wetting solvent solubilized fine particulates present in the lactose samples. In addition, a small, but statistically significant,

decrease in the 90th percentile volume undersize was recorded, suggesting that particle smoothing of asperities present on the lactose surface led to a small reduction in particle diameter.

Gemini specific surface area measurements of 0.5516, 0.2766 and 0.2829 $m^2\,g^{-1}$ for untreated, process A and process B lactose samples, respectively, suggested the particle smoothing technique significantly decreased ($P <$ 0.05, analysis of variance) the surface area. Again, this is possibly attributable to a decrease in lactose fines and surface irregularities.

Representative SEM photomicrographs of the untreated lactose, lactose process A and lactose process B samples are shown in Figure 1. Photomicrographs of all three samples suggested a crystalline tomahawk shape, with approximate particle length and width of $< 200\ \mu$m and $< 150\ \mu$m, respectively, which is in agreement with the sieve fraction used (90–150 μm). In addition, representative photomicrographs of process A and process B lactose samples (Figure 1B and C) suggested a smooth surface structure, in comparison with the irregular surface morphology of the untreated lactose (Figure 1A).

Representative AFM topography images of the untreated lactose, lactose process A and lactose process B materials are shown in Figure 2. All topographical data were adjusted by a plane-fit algorithm, which compensated for the curvature of the AFM piezo-electric scanner, and allowed for comparative analysis.

In qualitative terms, all three batches of lactose appeared different in morphology, with an apparent decrease in rugosity when comparing the untreated and processed materials. Furthermore, significant differences ($P < 0.05$, analysis of variance) in the surface roughness (R_{rms}) of the three lactose batches were recorded (n = 6) and are shown in Table 1. Both process A and process B resulted in large statistically significant decreases in R_{RMS} (Table 1), and were ranked in order of roughness: process B < process A < untreated lactose.

Separation energy measurement using AFM

Separation energy measurements between a BDP drug probe and three lactose samples were investigated using AFM and are summarized in Table 1. Integration of force–distance curves (n = 4096) measured over 10 μm $\times$ 10 μm areas on each of the lactose batch surfaces produced lognormal separation energy distributions, which can be represented as cumulative log distributions (Young et al 2002). In general, the untreated and process A lactose samples produced similar, lognormal separation energy distributions of comparable magnitude, which is in contrast to the much smaller energy distribution observed for the process B material. In addition, the median separation energy values ($e_{0.5}$) shown in Table 1 suggest significant differences (CI = 0.95) in the drug–lactose batch interactions, with a rank decrease: untreated $>$ process A $>$ process B lactose. Offline analysis of the individual force–distance curves, over the scan area, indicated large variations in separation energy with respect to point of contact, but

showed no direct trend related to the order in which each measurement was taken. This is most likely due to the irregular topography of the surface leading to large variations in particle lactose contact geometry. It would be logical to conclude therefore, that the degree or scale of roughness will directly affect such measurements. Furthermore, separation energy measurement geometric standard deviations (GSD) of 3.5 for untreated, 3.7 for process A, and 3.0 for process B lactose batches suggested that particle smoothing affected the separation energy spread. For example, although a significant decrease in the separation energy between untreated lactose and process A lactose was observed ($e_{0.5}$ 26.7 to 20.6 μJ), the GSD increased (GSD 3.5 to 3.7). This may be related to an increase in nanometre-asperities on the lactose sample surface (Figure 2) leading to a decreased contact area between the drug and carrier. Furthermore, the addition of magnesium stearate to the wetting solvent resulted in a significant decrease in separation energy ($e_{0.5}$ 26.7 to 7.7 μJ) and GSD (GSD 3.5 to 3.0) between untreated and process B samples. This is possibly attributable to a decrease in both surface roughness and surface free energy, as such a hydrophobic glidant may lower the separation energy barrier between a drug particulate and carrier surface. In addition, the presence of magnesium stearate on the lactose surface may alter the capillary force contribution to the system. The investigation was conducted at 45% relative humidity, which previous studies have indicated may have a dominant effect on DPI particle–particle interactions (Young et al 2002). It would be envisaged, therefore, that the presence of a hydrophobic glidant may reduce such capillary interactions. Furthermore, it is unclear whether the interactions between BDP and process B lactose are BDP–lactose, BDP–magnesium stearate or magnesium stearate–magnesium stearate measurements. It is likely that magnesium stearate would transfer onto the BDP drug probe, as the interfacial energies between magnesium stearate and the lactose/BDP is of a higher value than the interfacial energy between the magnesium stearate (Bolhuis & Holzer 1995). Although this phenomenon cannot be directly observed, it clearly warrants further investigation as it may have a significant impact on drug bioavailability.

The possibility of magnesium stearate drug probe contamination was circumvented by conducting the BDP process B lactose sample measurements last. Subsequent SEM images taken of the drug probe did not indicate any obvious evidence of contamination.

Particle smoothing technology essentially results in a decrease in separation energy, which may improve the aerolization of a drug from the carrier surface. It is important to note, however, that separation energy measurements were conducted between one drug probe and individual lactose crystals, making interpretation of bulk powder behaviour difficult. Previous investigations have shown significant differences between AFM drug probe number and separation measurement, but have shown consistent rank correlations as a function of material or environmental conditions (Eve et al 2002; Young et al 2002). Such observations are most likely owing to the variations in morphology and subsequent contact geometry

of the micronized drug probe. Investigation into the effect of lactose processing on separation energy, using different BDP drug probes, again indicated this to be the case.

Separation energy measurements between an additional two BDP drug probes were investigated with randomly chosen processed lactose samples. As expected, a rank decrease was observed between the untreated and processed lactose, with the largest decrease in median separation energy being between the untreated and process B lactose (10.9 μJ to 2.9 μJ for BDP drug probe 2, and 8.6 μJ to 6.5 μJ for BDP drug probe 3).

Another issue to consider is how the immobilization of a drug probe onto an AFM cantilever with glue may affect the surface characteristics of the drug. Utmost care was taken during drug probe preparation to limit the amount of drug–glue contact. A quick setting epoxy resin was chosen to reduce the possibility of low molecular weight polymer creep. Images of the BDP drug probes, taken by SEM post investigation, indicated the BDP particulate to be proud of the epoxy resin and of similar size (approx. 5 μm).

In-vitro aerosolization

The aerolization efficiency of BDP from blends of untreated and processed lactose carrier, investigated using a TSI, are summarized in Table 1, with graphical comparisons to the AFM separation energy data in Figure 3. An increase in mean fine particle dose (FPD) between the untreated lactose and both processed lactose blends was observed indicating that particle smoothing had an effect on DPI aerolization efficiency. However, it is important to note that statistical analysis (analysis of variance, Fisher pairwise comparison, $P < 0.05$) of the in-vitro data indicated that only process B improved DPI efficacy. In addition, the relative standard deviation (RSD) for the FPD of process A appeared substantially larger (RSD 84% in
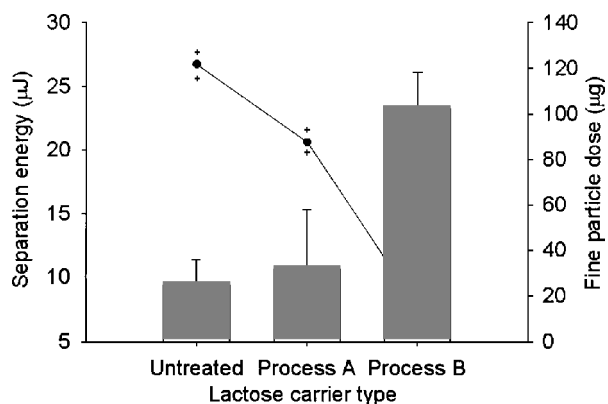

Figure 3 Median separation energy values (data points) with corresponding fine particle dose (bars) (FPD < 6.4 μm) for beclometasone dipropionate drug probe interactions with untreated lactose, process A lactose and process B lactose. Separation energy errors indicate 95% confidence intervals; FPD errors indicate standard deviations.

comparison with 44 and 15% for the untreated lactose and process B lactose blends, respectively). These observations correlated with the large GSD values recorded by AFM for the process A lactose. Possible sources of these variations may be related to the presence of nanoscale asperities on the surface of the process A material leading to limited drug–surface contact, affecting drug liberation during aerolization and/or blend stability in the DPI. It is important to note, however, that the energy required to separate drug particulates from a carrier during patient use of a DPI will depend on many additional factors other than carrier morphology. These include drug type, DPI type, DPI components, patient inhalation profile and environmental conditions.

Conclusion

The engineering of lactose carrier surfaces using the particle smoothing process resulted in significant differences in surface morphology when compared with the ''as supplied'' starting material. In addition, BDP–carrier blends using the process B lactose (containing magnesium stearate) resulted in improved aerolization efficiency from a commercially available DPI. These observations correlated with separation energy measurements obtained by AFM, indicating a rank decrease in separation energy between a BDP drug probe and modified lactose crystals: untreated > process A > process B. The AFM thus presents as a possible pre-formulation tool for rapid characterization of particle interactions, allowing the collection of fundamental data that may make DPI development a little easier.

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