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A study of single drug particle adhesion interactions using atomic force microscopy

J.K. Eve [a], N. Patel [a,*], S.Y. Luk [a], S.J. Ebbens [a], C.J. Roberts [b]

[a] *Molecular Profiles Ltd., 1 Faraday Building, Nottingham Science and Technology Park, University Boulevard, Nottingham NG7 2QP, UK*

[b] *Laboratory of Biophysics and Surface Analysis, School of Pharmaceutical Sciences, The University of Nottingham, Nottingham NG7 2RD, UK*

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Abstract

This paper aims to use Atomic Force Microscopy (AFM) to characterise the interaction forces between micronised salbutamol particles, an active ingredient frequently used in metered dose inhalers, and also to glass, lactose and a fluoropolymer. The methodology used involves challenging a salbutamol functionalised AFM tip to the surfaces of interest and measuring the force experienced by the cantilever as a function of tip–sample separation. Analysis of this force–distance data allows quantification of the particle–substrate adhesion. This study yields a ranking of adhesion as glass > lactose > salbutamol > polytetrafluoroethylene (PTFE). An increase in the interaction force between the salbutamol particle and PTFE on repeated contact due to tribocharging is also observed. © 2002 Published by Elsevier Science B.V.

Keywords: Adhesion; Atomic force microscopy; Inhalation devices

1. Introduction

Inhalation as a route for drug administration has long been used for the treatment of lung and respiratory diseases. Recently, however, there has been growing interest in the use of the lung as a delivery point for systemic drugs (Schulz, 1998; Gonda, 2000; Sanjar and Matthews, 2001). The formulation of therapeutic aerosols, and effects on drug delivery are consequently of great interest. Two commonly used delivery devices to produce aerosols for inhalation are pressurised packages and dry powder generators. In a pressurised 'powder aerosol' the active ingredients are suspended in a propellant gas, usually a fluorinated hydrocarbon, as solid micronised particles. The formulation may also contain additives such as sorbitan trioleate and oleic acid. Dry powder generators contain suitably sized particles for respiration (i.e. less than 5 μm), sometimes with excipients such as lactose, contained within a capsule or blister.

Deposition of drug in the lungs has been found to be highly dependent on the dry powder formu-

* Corresponding authors. Tel.: +44-115-951-7070; fax: +44-115-951-7071.

E-mail address: nikin@molprofiles.co.uk (N. Patel).

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lation in both pressurised and dry powder inhalers (Steckel and Müller, 1997; Concessio et al., 1999; Olsson, 1995). Consequently, the adhesion and interactions between the active ingredient, excipients and container walls is of great importance. The components are frequently micronised to ensure efficient deposition. Micronisation leads to the formation of particulates with a high surface area, which can form unstable cohesive systems that readily agglomerate or adhere to surfaces. These factors lead to potentially serious problems with dosage control, dispersion and precipitation in dispersions or suspensions often used to produce an aerosol for inhalation. In dry powder inhalers adhesion to the walls of the container and formation of large agglomerates must be avoided. The adhesion forces also control powder flow, which is important for dosage form manufacturing and dose sampling. The principal interactions determining the adhesion between particles and surfaces are attractive Van der Waal forces, and in the case of charged particles, electrostatic. Numerous factors have been found to influence particle adhesion, these include, the elastic properties of the surfaces, surface morphology and roughness (Staniforth, 1995) and surface hydrophobicity. Environmental factors such as humidity and temperature also have a large influence on adhesion.

Atomic Force Microscopy (AFM) has found applications in pharmaceutical characterisation by virtue of its ability to determine high-resolution surface morphology. One example is the measurement of surface roughness of a range of lactose carrier particles (Heng et al., 2000). AFM also provides a method of investigating particle adhesion, by virtue of its ability to sense intermolecular forces with high sensitivity. In such experiments the deflection of an AFM cantilever is recorded as it approaches and is withdrawn from the surface. With knowledge of the cantilever stiffness, cantilever deflections can be converted to forces using Hookes law. This allows the adhesion force to be determined. For example, AFM tips comprising well-defined silica colloidal particle have been used to measure the adhesion of lactose carriers (Louey et al., 2001). An alternative approach is to attach a particle of interest to the AFM tip. Using this approach, maps of the adhesion between an individual lactose particle and gelatin capsules have been obtained (Wiling et al., 2000). Here we extend this particle probe method to study the adhesion between a salbutamol particle immobilised on an AFM tip and micronised salbutamol particles, lactose monohydrate, a fluoropolymer and glass.

Salbutamol is a drug commonly used to treat asthma, and as such is most commonly delivered respiratorily, both in dry powder and pressurised inhalers. Fluoropolymers are frequently used to coat the inside of pressurised containers, to minimise adhesion to the container wall. Lactose monohydrate is used in dry powder formulations either as a diluent to increase dose accuracy, or as a carrier where it serves to increase effective particle size, improving flow properties and decreasing agglomeration of the active ingredient. Work carried out by others indicates that surface roughness is an important criteria in determining the efficiency of the lactose as a carrier particle. One study suggests that a smooth lactose particle surface is beneficial (Ganderton, 1992), while there is also evidence for an optimum surface roughness at which the release of carrier from inhaler, and subsequent detachment of drug particles is maximised (Heng et al., 2000). Reduction in carrier particle size also produces an improvement in performance (French et al., 1996; Steckel and Müller, 1997).

Particle–particle interactions underpin the effectiveness of these delivery systems. Until now, tests in vivo, on 'electronic lungs' and using Andersen–Cascade Impactors have been the key approaches to determining delivery efficiency of formulations. Adhesion and friction between particles have also been measured using a centrifuge-based approach (Podczeck and Newton, 1995). These macroscopic methods however, can only derive information from the ensemble of particles. This information has been extrapolated to the single particle level in order to determine more effective packaging and particle carrier size and shape, but this extrapolation includes assumptions that are not necessarily valid. Consequently, there is a need for a more detailed understanding of these forces and interactions. The ability of the

AFM to measure force interactions between a single particle and a surface demonstrated for model systems (Ducker et al., 1991; Veeramasuneni et al., 1996), provides an excellent opportunity to further understand and characterise such pharmaceutical systems.

2. Materials and methods

2.1. Sample preparation

The fluoropolymer sample used, polytetrafluoroethylene (PTFE) (PTFE tape, BASF, UK), was immersed in acetone and thoroughly dried in a stream of dry nitrogen prior to use, to ensure surface cleanliness. The glass (standard microscope slide, BDH, UK) sample was immersed in 4:1 H_2SO_4 (97.5 + %, Aldrich, UK)–H_2O_2 (27.5%, Aldrich, UK) solution, rinsed with ultrapure (minimum restitivity of 18 MΩ cm) water and acetone, then thoroughly dried in a stream of dry nitrogen. Salbutamol sulphate (99%, Sigma, UK) and lactose monohydrate (97%, Aldrich, UK) were micronised using a fluid energy mill. Lactose and salbutamol dry powders were prepared for imaging and force–distance measurements immediately before use by immobilising on an AFM stub using double-sided adhesive tape. Excess loose powder was removed using a stream of dry nitrogen.

2.2. Imaging and force measurements

All images and force–distance measurements were recorded in air (25 °C, 25% RH). Images of all substrates were obtained using a DI Nanoscope IIIa in TappingMode™ with a J-scanner (Digital Instruments, Santa Barbara, CA, USA). Tapping mode images were acquired using etched silicon cantilever probes, with resonance frequency 200–400 kHz (Digital Instruments, Santa Barbara, CA, USA). Typical drive amplitude voltages were 99–150 mV, which generated a free cantilever vibration amplitude of 100 nm. Images are displayed as grey-scale representations, with the lowest points as dark pixels and highest points as light pixels.

All SEM images were acquired on a Phillips XL-30 instrument at a probe beam acceleration voltage of 10 keV.

Particle attachment to the cantilever is performed using the AFM. To effect tip modification, a sacrificial AFM tip was used to draw out a very thin line of adhesive from a small glue droplet placed on an AFM stub. The tip to be functionalised is then positioned above the glue line, approached to the surface using the microscopes force feedback loop, and then withdrawn. This results in the attachment of a small glue globule to the AFM tip. This sticky tip is then positioned above a single particle of salbutamol (< 10 μm) (identified by high resolution optical microscopy) and the engage/withdraw procedure is repeated. The tip is then exposed to UV radiation to cure the glue and secure the attached particle. A SEM image of a cantilever prepared in this way is shown in Fig. 1. Fig. 1A shows an overhead view of a modified tip, and Fig. 1B a side-on view (different tip). Fig. 1B clearly shows that the particle will be the first point of contact with the surface.

Force–distance data was obtained as a plot of deflection signal of the cantilever against vertical distance moved by the base of the cantilever (scanner displacement). The cantilever deflection can be converted into force using Hooke's Law if the spring constant of the cantilever is known. The spring constant for each cantilever used was determined using the thermal method, first described by Hutter and Bechhoefer (1993). These were found to vary from 50 to 70 Nm^{-1}, depending on the mass and positioning of the attached particle. Capillary forces from surface water are significant for cantilevers with this stiffness level, and so the experiments were performed at a constant humidity level.

Force distance measurements were performed in randomly chosen locations on the glass and PTFE substrates. For the micronised substrates, optical microscopy was used to locate the drug probe on top of well-defined individual particles.

A tip coated with cured glue served as a reference and was used to generate force curves with each substrate. As an additional control the interaction between a drug particle and the adhesive tape used for immobilisation was measured.

3. Results

3.1. Images of samples

Prior to recording force–distance measurements between functionalised tips and substrates, each sample substrate was imaged using AFM. Knowledge of the topography of the surface is an advantage in choosing a suitable site for measurements. The scale over which images were acquired was chosen to be of a similar magnitude to the dimensions of the salbutamol particle functionalised onto the AFM tip. Typical images are presented in Fig. 2. The height-images show glass (Fig. 2A) to have a regular, uniform morphology, with smooth rounded protrusions (RMS roughness from region depicted in image = 2.1 nm). The surface of PTFE (Fig. 2B) also shows regular features. These are elongated and predominantly aligned in a preferential direction and most likely represent polymer fibrils. The PTFE exhibits a larger vertical variation (RMS roughness from region depicted in image = 43.0 nm) than the glass.

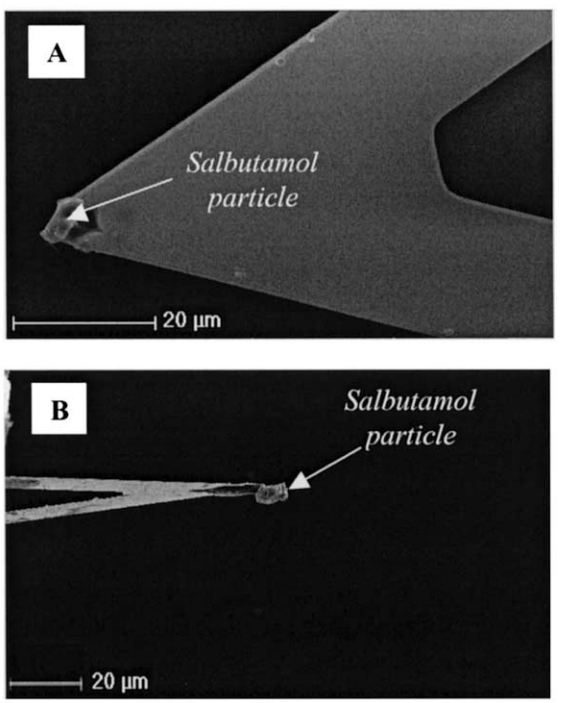

Fig. 1. Scanning Electron Micrographs of modified cantilevers: (A) A × 3200 magnification micrograph. A particle is covering the microfabricated tip, thus when this cantilever contacts the surface the particle will be the first point of contact, becoming the surface probe rather than the tip. (B) A × 1600 magnification side view micrograph of a different modified tip. It is clear that the particle is proud of the rest of the cantilever, and so will become the surface probe.

Images of large clusters of powdered micronised lactose and salbutamol are shown in Fig. 2C and D, respectively. Individual particles were not imaged, because these smaller particles do not provide good images over the required scale. These two substrates both exhibit a similar morphology of rounded globular features. The similarity of morphology is consistent with both samples having been produced in the same manner (i.e. solvent cast and micronised). The globular features have many aspersions, and these probably result from small crystallites clumped together. The dimensions of these features are similar in both images, but the salbutamol powder exhibits greater vertical displacements.

SEM images of the lactose and salbutamol samples used for the force–distance measurements are shown in Fig. 3A and B, respectively. From these images it is apparent that a single layer of particles has been achieved, ensuring reliable interpretation of the force–distance data.

3.2. Force–distance measurements

Typical force-distance curves obtained from each substrate are shown in Fig. 4. Fig. 4A shows a typical force–distance curve obtained from the interaction of a salbutamol functionalised tip with clean glass. At the start of each approach-retract cycle, the tip is withdrawn a fixed distance from the surface. Should the cantilever feel a long-range attractive (or repulsive) force in the non-contact region, as indicated in Fig. 4A, during approach it will deflect downwards (or upwards) before making contact with the surface, giving rise to a curved, not straight line. The noise observed in this part of the curve is a result of the thermal oscillations of the free cantilever. At the region indicated as 'jump-to-contact', the tip experiences an attractive force, and has jumped into contact with the surface. For the glass substrate this oc-

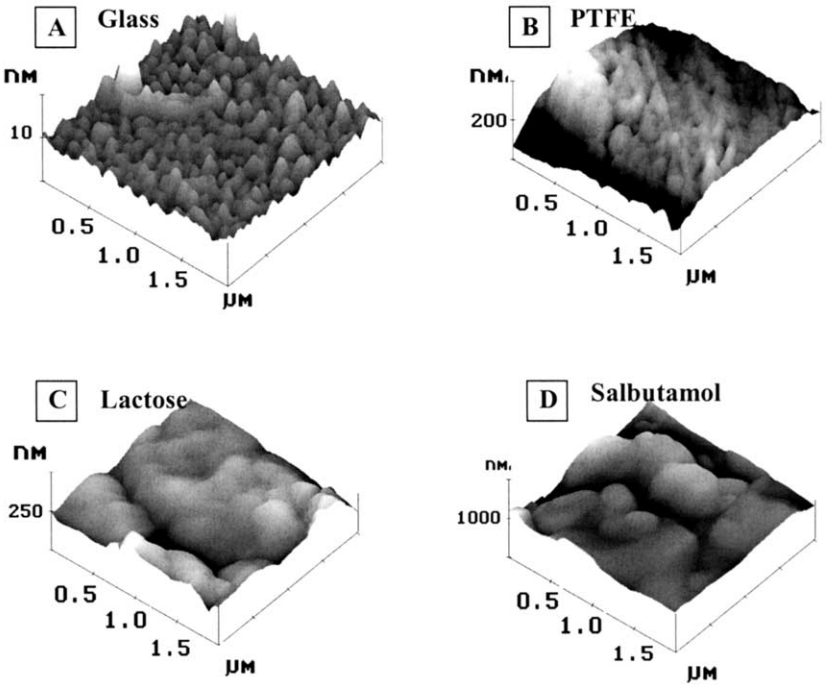

Fig. 2. Typical tapping mode AFM images (size 2000 × 2000 nm) of the surface topography of substrates used in this study. (A) Glass; (B) PTFE; (C) lactose monohydrate powder; (D) salbutamol sulphate powder.

curs at a distance of approximately 20 nm from the surface. Once the tip is in contact (marked as the contact region) with the surface, cantilever deflection will increase as the fixed end of the cantilever is brought closer to the sample. After loading the cantilever to a pre-determined force value, the direction of the scanner travel is reversed. As the cantilever is withdrawn, adhesion forces cause the cantilever to adhere to the sample some distance past the point at which contact was made during approach. The vertical distance between the point at which the cantilever comes free from the surface (marked in Fig. 4B), and the non-contact level is used to measure the particle–substrate interaction. For off-scale data, such as that obtained on a glass substrate, the gradient of the retract trace is extrapolated to the point of maximum adhesion. Such off-scale data may have been avoided by the use of a stiffer cantilever, however, this reduces the sensitivity to particle–surface interactions. Another solution to this problem is to functionalise the tip with smaller salbutamol particles, reducing the contact area and adhesion force. However, this would increase the possibility of contaminating the particle with glue. The raw data obtained is a plot of cantilever deflection as a function of scanner displacement. Here, the data is presented with force (nN) as a function of tip–sample separation, with the data corrected using the method outlined by Stevens et al. (Stevens et al., 2000; Pope et al., 2001).

A typical force-curve obtained from the interaction of a salbutamol-functionalised tip with PTFE is shown in Fig. 4B. The form of the curve is very similar to that obtained from the interaction with glass, except that less force is required to pull the tip free from the surface. The tip jumps into contact with the surface from a distance of approximately 20 nm.

A typical force-curve obtained from the interaction of a salbutamol-functionalised tip with lactose is shown in Fig. 4C. The adhesion force between salbutamol and lactose is greater than that between salbutamol and PTFE. The form of

the salbutamol–lactose curve has a linear non-contact region, and the tip does not come into contact with the surface until a distance approximately 5 nm from the surface.

Fig. 4D shows a typical force-curve recorded from between a salbutamol-functionalised tip and -substrate. The curve is similar to those obtained from the interaction with the glass and PTFE substrates in that no curvature of the non-contact part of the curve is observed, indicating no significant long-range interactions. However, in this case the tip jumps into contact very close (~ 5 nm) to the surface, suggesting that the interaction is of a shorter range than that between salbutamol and glass or PTFE.

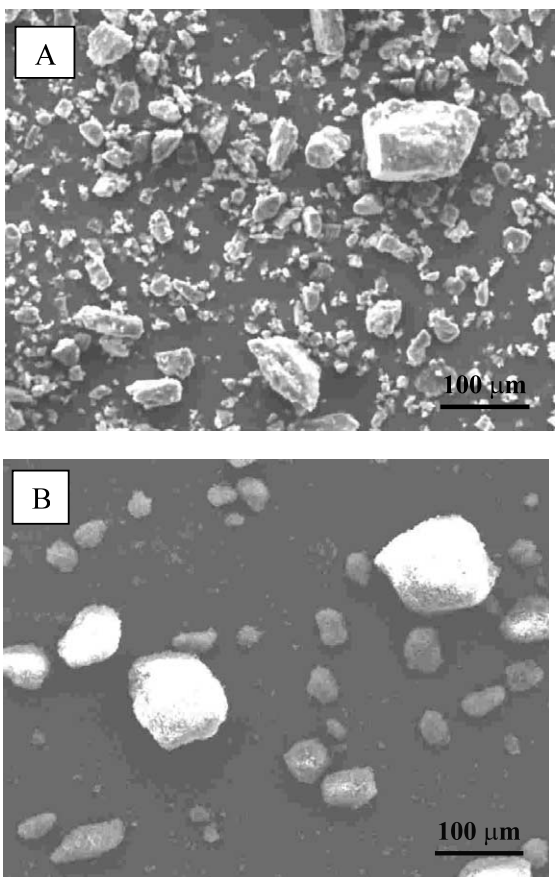

Fig. 3. Scanning Electron Micrographs of the powder surfaces: (A) lactose monohydrate; (B) salbutamol sulphate.

Although, the exact value of the adhesion force will vary between tips because of differences in contact area and geometry, the ranking of interaction between an immobilised salbutamol particle and each substrate should be consistent for each modified tip. For a given tip, 10 adhesion measurements were obtained from each substrate in turn, and this cycle was repeated until at least 100 measurements for each substrate were obtained. No systematic variations between the groups of measurements obtained for a particular substrate were observed, indicating that the tip was remaining intact and uncontaminated during the course of the experiment. This procedure was repeated for three different functionalised tips. Histograms of the maximum adhesion force obtained between tip and substrate are displayed in Fig. 5A. A consistent ranking of strength of interaction in the order glass > lactose > salbutamol > PTFE is displayed. The values for both lactose and salbutamol are most variable, which is consistent with the rough nature of the surfaces coming into contact.

For reference purposes, a tip that had been dipped in glue and cured, but had no particle attached, was prepared, and the adhesion to the surfaces under study was obtained. The results are displayed in Fig. 5B. The adhesion values show a different trend from that observed for the functionalised tips. This confirms that particle–particle and particle–surface (and not particle–glue) interactions have actually been measured. The adhesion between a piece of adhesive tape used to immobilise the powder substrates, and an AFM tip was also investigated. A full force distance measurement was not possible, as the tip stuck fast to the surface, and could only be removed manually, proving that particle–adhesive tape interactions do not contribute to the dataset.

4. Discussion

The results presented consistently show that the adhesion interaction between a salbutamol particle immobilised on an AFM tip and the substrates under study can be ranked in the order glass >

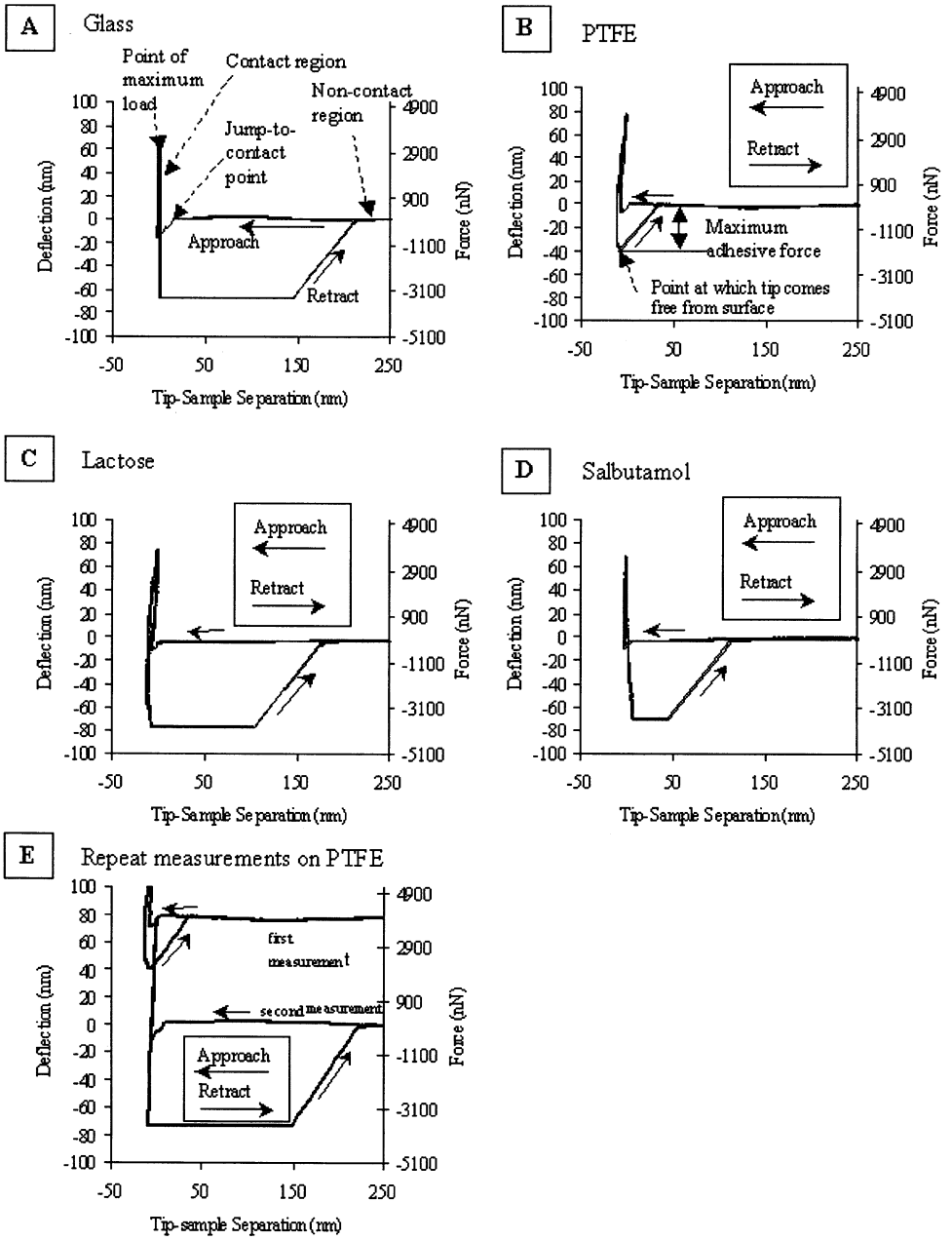

Fig. 4. Typical force–distance curves obtained from the interaction of a salbutamol–functionalised tip with: (A) glass; (B) PTFE; (C) lactose monohydrate powder; (D) salbutamol sulphate powder; (E) repeat measurements at the same point on a PTFE substrate. Data is presented showing tip–sample separation as a function of both cantilever deflection and the calculated associated force.

lactose > salbutamol > PTFE. The contributing factors to the total particle–surface interaction are: electrostatic forces, capillary forces, surface chemistry and contact area, all of which play a role, of varying importance in the interactions under study here.

Capillary forces arise from the thin layer of water that covers most surfaces under ambient air conditions. This film can form a capillary bridge between the tip and sample, which generates a large adhesion force. The thickness of this layer of water depends on the hydrophilicity (or hydrophobicity) of the surface as well as the humidity. The chemistry of the surfaces in contact also plays an important part in determining the adhesion between them. Surface chemistry and hydrophilicity are together described by the surface free energy. A large capillary force will cause the approaching tip to 'jump-to-contact', as the tip approaches the water layer, and a large adhesion force on retraction.

Electrostatic forces arise from the differences in charge between tip and substrate. These are likely to be consistent throughout the experiment for all substrates except for PTFE. PTFE is very high in the triboelectric series (Schein, 1996), and contact (such as by an AFM tip) has been shown to charge the surface according to the triboelectric series (Gady et al., 1998). PTFE retains stored charge very well, so an adhesion force measurement on a previously contacted area will be higher than on an uncontacted area. This accounts for the occasional exceptionally large force measurement observed during experiments, for example as shown in Fig. 4E (these were not included in the adhesion calculations). Electrostatic forces are long-range, so if electrostatic forces are significant the tip will jump to contact from a larger distance than for a short-range interaction and/or exhibit significant repulsion.

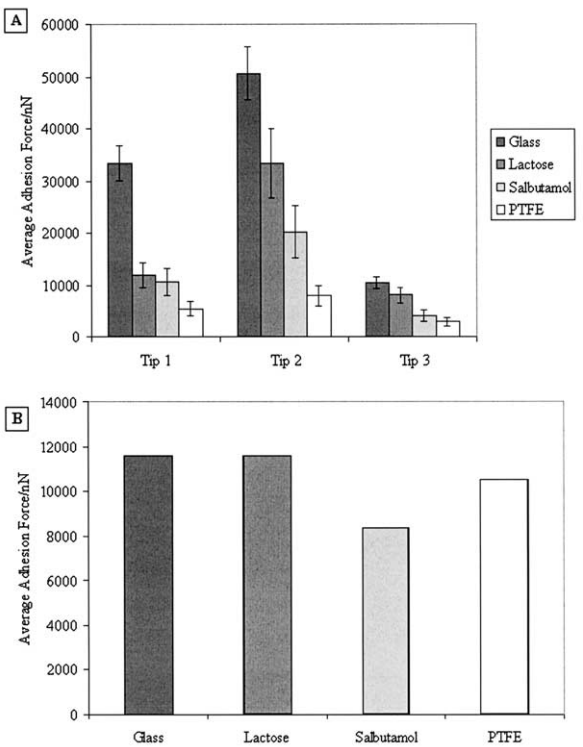

Fig. 5. Chart showing the average adhesion force to each substrate for: (A) salbutamol-functionalised tips; and (B) a glue reference tip.

The area of contact between two objects is a key factor in determining the force of adhesion between them. The smaller the contact area the smaller the adhesion force. With a very rough substrate only a very small area may come into contact, resulting in a small adhesion force. The contact area at each sampling point will also vary more with a rougher surface, resulting in a wider spread of data and larger standard deviation. The dependence of adhesion force on contact area is the reason that the absolute values of the pull-off force cannot as yet be determined. In this type of experiment, the area of contact on each occasion is unknown. Hence absolute forces have not been compared, but instead the strength of adhesion has been ranked by surface. Probe deconvolution methods may in the future allow rapid determination of contact geometry and area (Kitching et al., 1999).

All these factors must be considered when rationalising the observed order of adhesion to a salbutamol particle. Examination of the approaching force–distance curves obtained indicates that interactions such as those resulting from electrostatic or capillary forces are more important for PTFE and glass substrates than for the particulate substrates. This is indicated by the significant jump-to-contact point observed for both PTFE and glass. Glass presents a hydrophilic surface, so will attract a relatively thick layer of water, with correspondingly large adhesion to the probe tip. Although, electrostatic interactions and contact area also influence the

adhesion of a tip to glass, the large hydrophilicity suggests that the dominant factor for glass is most probably the capillary force. PTFE will exhibit a much lower capillary interaction, due to its hydrophobicity. Here, the attraction observed between a salbutamol-functionalised tip and PTFE most likely arises from electrostatic forces.

The micronised powders expose a random selection of surfaces, with the result that the exposed surface is not a defined crystal plane with known surface chemistry. The lactose crystal contains one water of crystallisation per unit cell (Beevers and Hanse, 1971) and there are many –OH groups in various directions. Therefore, cleavage along any plane will reveal surface –OH groups, providing a hydrophilic surface that presents many opportunities for hydrogen bonding. In comparison, salbutamol sulphate crystals contain one sulphate per two unit cells (Leger et al., 1978) and one end of the salbutamol moiety is more hydrophilic than the other end. Consequently a randomly exposed surface of a salbutamol sulphate crystal is likely to be less hydrophilic than a similarly randomly exposed surface of a lactose crystal. However, it is probable that the difference in adhesion to a salbutamol particle exhibited by lactose and salbutamol may not stem wholly from small differences in hydrophilicity. As the AFM images show (Fig. 2C and D), the surfaces of both powders are rough, having a much greater height variation than either PTFE or glass. Although PTFE shows corrugation, the surfaces are smooth, whereas those of the particles are rough, with many asperities. The area of contact between tip particle and substrate will therefore vary considerably depending on the point at which the tip contacts the surface.

Inspection of the images displayed in Fig. 2 indicates that the salbutamol powder contains crystallites varying more in size than the lactose powder. Additionally, the topographical scale of the salbutamol surface is larger, suggesting that the surface is more corrugated. The data from both salbutamol and lactose substrates shows a large amount of spread, suggesting that surface roughness is indeed an important factor in the adhesion forces observed, and is most likely the dominant factor in determining the tip–surface interaction.

The results presented here show, that interparticle adhesion forces are predominantly affected by surface roughness. This is in agreement with work performed at the macroscopic level in which it has been found that adhesion of drug particles to carrier particles increases with surface roughness (Podczeck, 1998a). It has previously been determined that carrier particle size and rugosity (Wong and Pilpel, 1990) plays an important role in drug distribution. Previous work has found that the relationship between particle physical properties and aerodynamic behaviour is complicated, although a relationship between particle–particle adhesion and drug loss between inhaler and lung has been reported (Podczeck, 1998b), and linked to particle shape. The distribution effectiveness and deposition profiles have also been linked to carrier particle size (Zeng et al., 1999). Recent work has attempted to quantify surface roughness by modelling the fractal morphology, and has found that this is affected in suspension by the presence of surfactants (Bower et al., 1995).

Podczeck et al. have investigated the adhesion of salmeterol, another drug delivered by inhalation, to surfaces using the centrifuge technique (Podczeck et al., 1996), and found that the order of surface adhesion could be accounted for by considering the surface free energy of the substrates. Surface free energy is defined as the work required to increase the area of a substance by 1 cm^2. This is related to both surface chemistry and hydrophobicity, with hydrophobic surfaces having a lower free energy than hydrophilic ones. However, it was also noted that increased roughness decreased the strength of adhesion. This technique involves pressing the particle against the test surface, thus potentially deforming surface asperities. Hence the contact area, compared to that which the AFM technique would measure, may be larger. Another study by Podczeck found that the adhesion of salbutamol sulphate to the 'flat' surface of materials commonly used in inhalers increased with increased roughness of the surface and this was a key factor in determining the strength of adhesion (Podczeck, 1998b), although surface free energy was not considered.

It has been suggested that increased surface roughness can increase the force of adhesion between particles and surfaces if the particles are small, as they are able to slip into the valleys between individual asperities (Zimon, 1982). This surface roughness effect depends on the ratio of distance between asperities to their height and may also be enhanced by the application of force, as this would deform asperities thus enabling particles to slip in between them. Using the AFM technique to measure interaction forces, this would not be a factor, as the adhesion force is measured as soon as any contact, however small, is made with the surface. In addition the forces involved are much smaller.

The results presented here demonstrate that AFM can successfully and statistically–significantly rank the adhesion interaction of a drug particle with pharmaceutically relevant materials. The adhesion of a salbutamol particle to substrates has been ranked, from strongest to weakest in the following order glass > lactose > salbutamol > PTFE. This result shows that lactose is acting as an effective carrier in that salbutamol adheres more strongly to lactose than to itself thus preventing formation of salbutamol agglomerates. In contrast salbutamol adheres more strongly to glass, suggesting that contact of the formulated drug/carrier mixture with glass surfaces will cause drug loss, as the adhesion of the drug to glass is greater than that to the carrier particle. On first contact salbutamol also adheres more strongly to lactose than to PTFE, suggesting that contact of the formulated drug/carrier mixture with PTFE surfaces will not cause drug loss. However, the increase in adhesion force attributed to an electrostatic charge effect observed when PTFE is contacted with an AFM tip is worthy of further investigation. In an aerosol formulation, this type of charging does not take place, but it may be a significant factor in dry powder inhalers, rendering the inside of the delivery chamber more attractive than might be expected from other consideration, such as hydrophobicity and surface roughness.

The technique used here has considerable potential for screening excipients and packaging for formulation suitability, as significant adhesion interactions may be measured between small amounts of material.

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