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Characterisation of adhesional properties of lactose carriers using atomic force microscopy

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Abstract

The atomic force microscopy (AFM) colloid probe technique was investigated as a method for the characterisation of adhesional properties of pharmaceutical powder surfaces. Lactose carriers used in dry powder inhaler (DPI) formulations were chosen for investigation since adhesion between the carrier surface and drug particles has been proposed to affect the dispersion of drug particles. Individual adhesion forces were determined by measuring the detachment forces in air between the colloid probe and the lactose particle surface. The colloid probe consisted of a silica sphere (10 μm diameter) attached to a V-shaped silicon nitride cantilever (spring constant, $k = 0.42$ N/m). Adhesion forces were calculated from individual force–distance curves using Hooke's Law. Individual forces measured at various adhesion sites were observed to be reproducible and stable over 10 min (coefficient of variation, CV below 5%). The adhesion force distribution determined from measurements at multiple sites ($n > 50$) on each sample followed a log–normal relationship (regression coefficient, r^2 ranged between 0.95 and 0.99). This enabled characterisation in terms of the geometric mean adhesion force and a geometric standard deviation (GSD). Significant differences ($P < 0.001$) in adhesion force were observed between samples, ranging from 37.47 ± 1.95 to 117.48 ± 2.20 nN. This study demonstrates the suitability of AFM as sensitive technique for the characterisation of adhesional properties of pharmaceutical particles.

Keywords: Adhesion force; Atomic force microscopy; Colloid probe technique; Dry powder inhaler; Lactose

1. Introduction

Particle interactions are of great importance within dry powder inhaler (DPI) formulations where the redispersion of drug particles from excipient carrier particles (usually lactose) is critical for lung deposition. In such preparations, the inspiratory force of the patient or external me-

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chanical forces supplied by the inhaler device must overcome the adhesion forces between drug and carrier particles and the cohesion forces between drug particles. Strong interparticulate forces within the powder formulation have been cited as the cause of the poor efficiency of DPI [1].

The force of particle adhesion is equivalent in magnitude to the force required for particle detachment [2]. Previously used techniques for the determination of interparticulate forces within powder systems include vibration [3], centrifugation [4,5] and impact separation [6]. These bulk detachment methods determine adhesion force by measuring the amount or number of drug particles detached from a surface at a given force.

The use of the atomic force microscope (AFM) with a colloid probe, consisting of a spherical colloid particle attached to the micro-cantilever, provides an alternative technique where the adhesion force is determined using single particle detachment. The advantage of this technique is that direct measurements are performed on an area comparable to the contact area of the drug particles. The AFM is a scanning probe microscope originally developed as a high-resolution imaging tool [7]. The colloid probe technique [8] allows direct force measurement between a single particle and a sample surface. The probe acts like a soft spring and detects interactive forces between the probe and the sample surface. Individual force measurements are obtained during an extension-retraction cycle whereby the AFM probe is brought into and out of contact with the sample surface. The adhesion force (F) is calculated according to Hooke's law [9,10]:

$$F = kx, \tag{1}$$

where k is the cantilever spring constant and x is the vertical displacement of the cantilever.

The colloid probe provides a well-defined geometry and enables reproducible contact between the AFM probe and the sample. The adhesion force between a rigid and incompressible sphere and a flat surface is given by [11]:

$$F = 4\pi\gamma R, \tag{2}$$

where γ is the interfacial energy between the two surfaces and R is the particle radius of the colloid probe. The colloid probe technique has been generally used to measure particle interactions in liquids [8,12], with some recent adhesion measurements performed in air [13,14]. Although surface characterisation by AFM has the potential to advance the understanding of the relationship between surface properties and drug delivery system functionality [9], adhesion force measurements on pharmaceutically relevant powders using the colloid probe technique have not yet been performed, to the knowledge of the authors.

The aim of this paper was to investigate the use of atomic force microscopy for the characterisation of adhesional properties of lactose carrier particles used in formulations for dry powder inhalers (DPI).

2. Experimental methods

2.1. Atomic force microscopy

A schematic diagram of an atomic force microscope (AFM) with a colloid probe is presented in Fig. 1. The sample mounted on a piezoscanner tube enables 3-dimensional movement (x, y and z) relative to the stationary probe. Interactive forces acting between the probe and sample surface cause vertical (z) displacement of the cantilever, which is monitored by the angle of reflection of

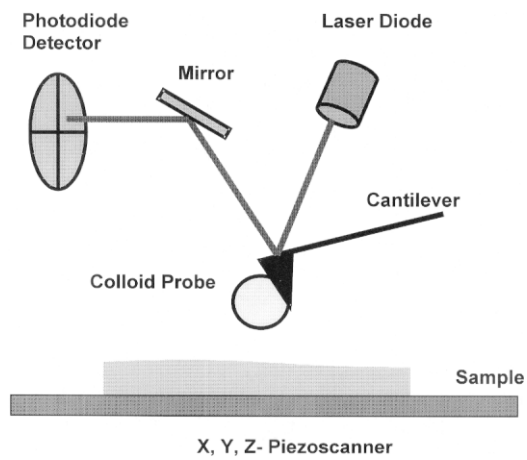

Fig. 1. Schematic diagram of an atomic force microscope (AFM).

the laser from the upper side of the cantilever using a split photodiode detector.

2.2. Sample preparation

Nine α-lactose monohydrate samples from two manufacturers were chosen for this study: Superfine (SF), 100 Mesh (100 M), Special Dense (Sp D) and Edible (Ed) (New Zealand Milk Products Pty. Ltd., Australia) and Pharmatose DCL11, 110 M, 125 M, 325 M and 450 M (DMV International, The Netherlands). The lactose particles were affixed to stainless steel sample holders using epoxy resin (Araldite Epoxy Resin 5 minute, Selleys Chemical Company Pty. Ltd., Australia). Excess particles were removed under gentle nitrogen flow, following overnight drying. Powder discs of α-lactose monohydrate were prepared by compression of 300 mg in a 10 mm diameter die (Graseby Specac, UK) using a hydraulic press (Carver Laboratory Press, NJ) with a load of 2.7 metric tonnes for 3 min. The powder discs were attached to a stainless steel sample stub with epoxy resin. In order to minimize possibility of surface contamination, the die was cleaned with ethanol prior to compression and all samples were stored in covered petri dishes.

2.3. Colloid probe preparation

A silica sphere (10 μm diameter, Kunishima Kikai Ltd., Japan) was attached to the apex of a V-shaped silicon nitride cantilever (Type NP-S, Digital Instruments, CA) (cantilever spring constant, $k = 0.42$ N/m) using epoxy resin (Araldite Epoxy Resin 24 h, Selleys Chemical Company Pty. Ltd., Australia) with the aid of a piece of tungsten wire under an optical microscope. Care was taken to prevent the spreading of epoxy resin around the sphere. The colloid probe was examined under an optical microscope to ensure successful attachment following overnight drying. The determination of the cantilever spring constant was performed using the unloaded resonant frequency [15].

2.4. Adhesion measurements

Adhesion measurements were performed in air at room temperature (20–25°C) and ambient humidity (40–50% RH) using an atomic force microscope (Dimensions 3100, Digital Instruments, CA). The applied load and contact duration used in this study were maintained constant for all force measurements. The vertical displacement of the cantilever (pull-off distance) following adhesion was determined from the force-distance plot of each extension-retraction cycle using AFM Force Curve Analysis (Patrick Hartley, CSIRO, Australia). The software requires the definitions of zero force and zero separation. The zero force was defined as the point where the cantilever deflection remained constant when the sample displacement was altered, otherwise known as the zero deflection line. The point of zero separation was defined as the onset of the constant compliance region, where increasing sample displacement produces an equivalent increase in cantilever deflection. This linear region occurred when the colloid probe was 'in contact' with the sample surface.

The reproducibility of adhesion forces was investigated at three individual sites on a lactose sample. Ten measurements, each measurement obtained 2 s apart, were performed at 2 min intervals for 10 min at each site. The mean adhesion forces and standard deviations at each adhesion site for each 2 min interval and overall mean adhesion force and coefficient of variation for the 10 min period were determined.

The adhesion force distribution for each sample was obtained from adhesion measurements at greater than 50 individual sites on at least three different particles. Regression analysis was subsequently performed on the log-transformed adhesion data using a least-squares method (Minitab, Minitab Inc., PA). The geometric mean was determined from the adhesion force at which under 50% of the population was distributed ($F_{50\%}$). The geometric standard deviation (GSD) was calculated from the ratio of $F_{50\%}-F_{16\%}$, where $F_{16\%}$ represented the adhesion force at which under 16% of the population was distributed. The $F_{50\%}$ and $F_{16\%}$, were determined from the adhesion force which corresponded to n-score values (Minitab) of zero and -1, respectively.

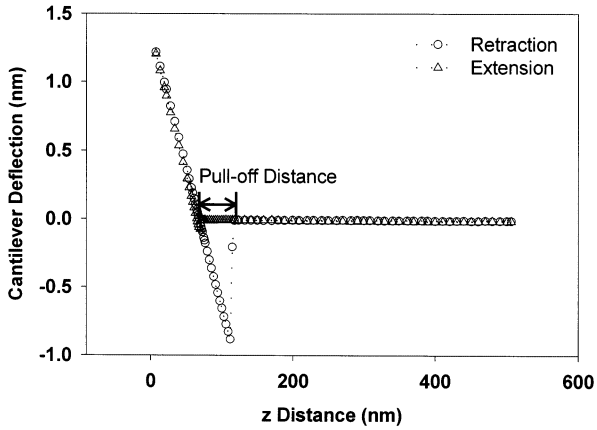

Fig. 2. A typical force–distance plot of an individual adhesion.

Comparison between lactose samples was performed on the log-transformed data using either a Mann–Whitney rank sum test or Kruskal–Wallis one-way analysis of variance on ranks (Sigmastat, Jandel Scientific, CA) at the 5% significance level. Multiple comparison between groups was performed at the 5% significance level overall using the Dunn's test (Sigmastat, Jandel Scientific). The 325 M lactose was used as the control since it was the only inhalation-grade lactose used in this study.

3. Results

3.1. Individual adhesion measurements

A typical force-distance plot of an individual adhesion is presented in Fig. 2, showing the cantilever deflection as a function of z-piezo position. Initially as the probe approached the sample surface (extension curve), the probe-sample distance was large with no probe-surface interaction. This non-contact region is known as the 'zero-deflection line' or baseline. At close proximity (less than 5 nm), the short-range van der Waals forces produced attraction, which resulted in a small downward deflection and contact between the probe and the sample. During sample contact, the AFM stage movement and cantilever deflection became coupled, causing an upward deflection of the cantilever. This linear region represented the zero separation distance and is known as the 'hard wall' or 'constant compliance' region.

As the probe retracted away from the sample surface to its original non-contact position (retraction curve), the adhesion between the probe and the sample produced a hysteresis observed as a downward deflection past the initial contact point. When the retraction force overcame the adhesion force, the probe and sample separated and the cantilever returned to the baseline. The magnitude of the adhesion force was determined from the vertical cantilever deflection prior to detachment from the sample. Larger adhesion forces produced larger vertical cantilever deflections.

3.2. Reproducibility of individual adhesion forces

The repeated adhesion forces on a 325 M particle sample measured over a 10 min period are presented in Fig. 3. The mean adhesion force and coefficient of variation obtained for the three individual sites were 33.85 nN (2.27%), 75.52 nN (3.67%) and 75.26 nN (2.59%). Significant differences in adhesion force between adhesion sites were observed ($P < 0.001$). The adhesion force

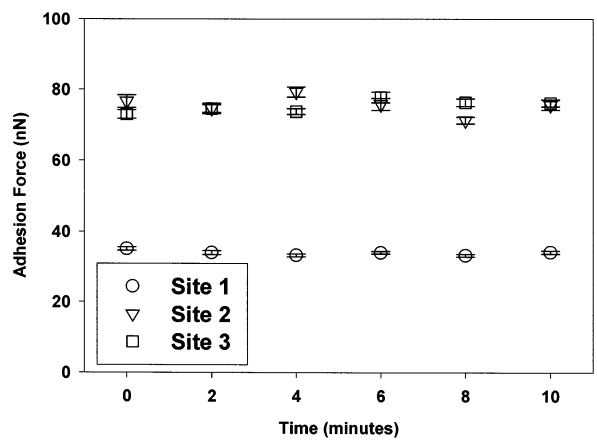

Fig. 3. Reproducibility of adhesion force at three individual sites over time (Arithmetic mean ± SD, $n = 10$).

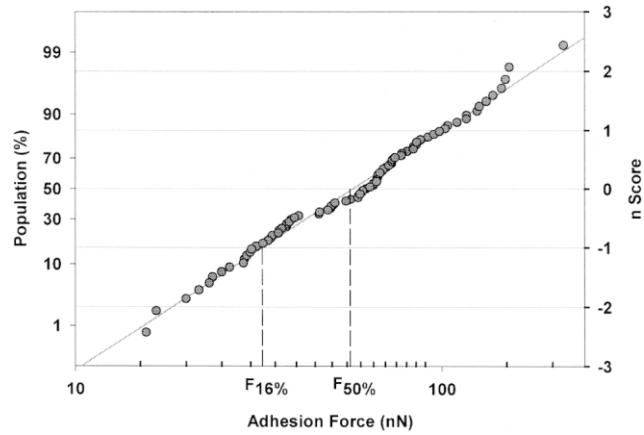

Fig. 4. Adhesion force distribution of 325 M lactose particles (Regression coefficient, $r^2 = 0.990$).

measured at site 1 was significantly lower ($P < 0.05$) than between sites 2 and 3, but no difference was detected between sites 2 and 3.

3.3. Adhesion force distributions

The distribution of adhesion forces obtained at multiple sites on the 325 M particle sample is presented in Fig. 4. The linear adhesion profile for data plotted as frequency on a probability scale (ordinate) versus logarithm of force (abscissa) indicated a log-normal distribution function. Confirmation was obtained from the regression coefficient ($r^2 = 0.990$) and an analysis of variance ($F = 8102.77$, $P < 0.001$). The log-normal adhesion distribution observed enabled characterisation using the geometric mean adhesion force and the geometric standard deviation (GSD) (57.01 ± 1.75 nN).

The adhesion force distributions of all samples followed a log-normal relationship and were characterised using the geometric mean and GSD (Table 1). Significant differences in adhesion force were observed between lactose carriers ($P < 0.001$). Higher adhesion ($P < 0.05$) was obtained from SF, Sp D, Edible and DCL11 carriers, compared with 325 M (control), while lower adhesion ($P < 0.05$) was obtained from 125 M, compared with 325 M.

3.4. Effect of surface morphology

The adhesion forces obtained from particles and compressed discs of 325 M and SF lactose samples are shown in Fig. 5. The adhesion forces determined from measurements on the compressed discs were significantly lower than those obtained from the lactose particles for both the 325 M and SF samples ($P = 0.006$ and $P < 0.001$, respectively). Significant differences in adhesion force were observed between 325 M and SF particles ($P < 0.001$) and compressed discs ($P = 0.017$).

4. Discussion

The AFM colloid probe technique enabled direct measurement of adhesion forces by detachment of a single particle from a sample surface. No surface treatment was required, which eliminated any mechanical influences that may alter the adhesional properties of the samples. The force–distance plots obtained for individual adhesion (Fig. 2) was concordant with those observed for particle interactions with hard surfaces [10]. The linear 'constant compliance' region observed during contact of the probe and sample indicated that the interaction between the silica sphere and lactose surface was elastic and no irreversible deformation occurred.

Table 1
Mean adhesion force of lactose carrier particles determined by AFM with a colloid probe

Lactose carrier	Geometric mean (nN)	Geometric standard deviation (nN)	Regression coefficient (r^2)	Sample size (n)	Statistical difference[a] ($P<0.05$)
SF	96.85	1.79	0.969	79	*
100M	73.03	1.62	0.966	52	
Sp D	117.48	2.20	0.978	72	*
Ed	97.33	2.34	0.957	77	*
DCL11	104.57	2.26	0.991	75	*
110M	50.52	1.52	0.990	56	
125M	37.47	1.95	0.985	53	*
325M	57.01	1.75	0.990	83	control
450M	42.84	1.85	0.985	75	

[a] Statistical difference compared with 325M (control).

The individual adhesion forces were observed to be both reproducible and stable over time, indicated by the low variability within each adhesion site (Fig. 3). Differences in adhesion between individual sites were observed. This indicated that various magnitudes of adhesion exist on the surface of the same sample, as observed by the distribution of adhesion forces for the various lactose samples. The reason for differing magnitudes of adhesion measured on the sample surface is unknown and needs to be examined further. Possible reasons include the dislocation of crystalline surface structure, chemical composition (α- or β-lactose), presence of fine particles or surface roughness of the adhesion site affecting contact area of adhesion.

The adhesion force distributions determined from multiple measurements at different adhesion sites along the sample surface were observed to fit a log–normal distribution, enabling characterisation in terms of the geometric mean and geometric standard deviation. These parameters were used to evaluate the adhesion characteristics of the carrier samples. The greater the geometric mean, the higher the degree of adhesion between the AFM colloid probe and the sample surface; and the greater the GSD, the larger the scatter or variation within the adhesion distribution. Significant differences in adhesion force were observed between lactose carriers ($P < 0.001$), illustrating the ability of the colloid probe AFM technique to distinguish between the adhesional properties of lactose carriers.

The mean adhesion forces ranged between 37.47 and 117.48 nN for the colloid probe (10 μm diameter silica sphere). The corresponding γ values (determined using Eq. (2)) of 0.60 and 1.87 mN/m suggested quite low adhesion energies. The median adhesion forces determined in previous studies using the centrifuge technique ranged from 0.64 to 2.75 nN for the adhesion of salmeterol particles (35.9 μm diameter) on coarse lactose particles [5] and 5090–11280 nN for micronised lactose particles (3–5 μm diameter) on compressed lactose disks [16]. The adhesion forces obtained in this study lie between these values. Differences in the magnitude of adhesion were expected and may be explained by differences in

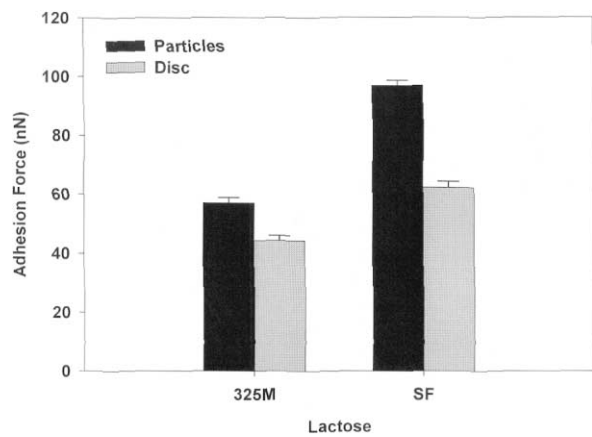

Fig. 5. Adhesion force of lactose particles and compressed discs (Geometric mean ± GSD, $n > 50$).

the specific properties of the drugs and surfaces, such as particle size, chemical composition of the detaching particle, surface roughness and contact geometry; and the specific processes involved in the formation of the initial particulate interaction used in the published experiments.

The differences in adhesion force observed between the lactose particles and compressed discs were attributed to the sample surface roughness. Compressed discs were expected to have a smoother surface compared to the lactose particles, resulting in smaller contact area and smaller adhesion, as indeed was observed.

An important finding from this study is the direct observation of a log-normal distribution of adhesion forces, as previously observed in studies using centrifugation [2,17]. Some researchers have previously interpreted high and low adhesion sites on the carrier surface in terms of 'active' and 'passive' sites, respectively [18,19]. Since a continuum of adhesion forces has been shown to exist, this 'active' and 'passive' sites approach appears simplistic. Although the assignment of a nominal dividing point on the force continuum may be employed to define 'active' and 'passive' sites, it is emphasized that a continuum of forces exists, rather than distinct low and high adhesion sites.

The study clearly shows that the AFM colloid probe technique is capable of direct measurement of the adhesion forces experienced by a single particle on the carrier surface. This enables characterisation of the adhesional properties of the carrier. Furthermore, the sensitivity of the technique is sufficient to differentiate between the adhesional properties of various samples. Since particle interactions between drug and carrier particles have been cited as important in the dispersion of DPI formulations [1], the use of AFM as a technique for characterising adhesional properties of carriers particles may provide useful information for DPI formulations. The significance of adhesion measurements in specific relation with the dispersion of drug particles from DPI formulations will be examined in future publications.

Whether these adhesional characteristics, determined by the spherical silica probe, are representative of the adhesional forces experienced by drug particles on the carrier surfaces is uncertain. The direct measurement of adhesion forces between a single drug particle and the carrier surface was not undertaken for reasons described below. While this is a limitation of the technique, the use of a standard spherical probe enabled reproducible adhesional characteristics of the surface to be mapped.

The use of a colloidal silica probe may not be fully indicative of drug-carrier interactions within DPI formulations for various reasons. The different chemical nature of the silica compared to a drug particle may influence the magnitude of adhesion due to the differences in van der Waals interaction energies [20]. Differences in elastic or plastic deformation may also affect the contact area of adhesion, though the constant compliance region suggests this should be minimal. Differences in moisture adsorption characteristics may affect capillary forces. The larger size of the AFM probe (10 μm diameter) compared to that of drug particles used in DPI formulations (< 5 μm diameter) may result in higher adhesion force, due to the corresponding increased contact area. Use of a colloid probe with a size comparable to micronised drug particles would be more appropriate. Adhesion measurements were undertaken by the sampling of adhesion sites on the lactose surfaces. There may be preferential adhesion of drug particles to particular adhesion sites on the carrier surface, which were not sampled by the probe.

Measurement of adhesion forces determined using a drug probe would provide further information on the drug-carrier interactions within powder mixtures for inhalation. However, many difficulties are associated with the use of drug probe. Technically, it is difficult to obtain a single micronised drug particle since micronised particles tend to be cohesive and exist as agglomerates. The large variations in particle size and shape of individual drug particles preclude the preparation of identical probes, which would limit the usefulness of such probes. The fragmented irregular shape of micronised particles is likely to produce varying contact geometry and adhesion forces depending on the contact orientation. Although adhesion is also dependent on the sample surface roughness, the spherical nature of the colloid probe enabled a

reproducible contact area. Drug probes may also be friable with the propensity to fragment during adhesional measurements exposing new surfaces and complicating the interpretation of adhesion measurements.

The degree of surface roughness on the sample surface may also affect adhesion by altering the contact area between the adhering particle and sample surface. The effect of surface roughness depends on the size of the asperities, the interval distances between asperity peaks and the relative size of the adhering particle [21]. Increased adhesion may occur when a particle fits snugly into pits or grooves of the surface; whereas decreased adhesion may occur when particles sit atop ridges or bumps on the surface [22]. In addition to the effect on contact area, surface roughness of the sample may reduce van der Waals forces by increasing the 'effective' separation between the adhering particle and surface [23].

The adhesion forces measured by AFM were determined by detachment of the probe at a normal angle to the sample surface. However, during inhalation of DPI formulations drug detachment occurs at all angles. Rolling and sliding of the drug particles over the carrier surface may occur prior to detachment. This may result in collision with other adhered particles enhancing detachment. Although the detachment forces of drug particles during inhalation were not fully examined using the AFM technique, this technique provided a direct measure of the adhesional characteristics of the lactose carriers against a standard control probe.

Other potential factors affecting particle adhesion include the applied load, contact duration, surface contamination and humidity conditions. Higher applied loads, controlled by the cantilever deflection setpoint, may result in higher adhesion due to increased contact area [13]. The influence of these factors was not investigated in this study.

5. Conclusion

Atomic force microscopy offers a new approach for the characterisation of adhesion forces on pharmaceutical particle surfaces. The colloid probe technique allowed adhesion force measurements to be undertaken from single particle detachment. Log-normal adhesion distributions were observed, enabling characterisation by the geometric mean force and geometric standard deviation. The technique allowed discrimination between samples and showed potential as a tool for screening the adhesional properties of lactose carriers used in dry powder inhaler formulations.

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