Expert Opinion

Towards screening of inhalation formulations: measuring interactions with atomic force microscopy

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This review charts the progress of atomic force microscopy (AFM) to investigate particle interactions relevant to the performance of inhalers. AFM provides a unique opportunity to examine and quantify single particle behaviour of powdered drugs and excipients in a variety of environmental conditions. An introduction to AFM and particle interactions is given. Comparative experiments that rank adhesion between materials, and quantitative experiments that lead to the measurement of properties such as the work of adhesion and surface energy, are reviewed. The AFM has been widely used to investigate the effects of relative humidity and surface roughness on particle adhesion; these experiments are also reviewed. In the final section, the potential of this approach to screen formulations is discussed.

Keywords: atomic force microscopy, dry powder inhaler, inhalation drug delivery, particle interactions

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1. Introduction

Over the last 5 years, atomic force microscopy (AFM) has been increasingly used to study particle interactions relevant to inhalation drug delivery systems. This seems to be driven by an increase in the popularity of inhalation delivery in general, dry powder inhalers (DPIs) in particular, and a consequent need to study interactions on a single particle level. Elucidating the relationships between the various fundamental forces that contribute to particle interactions is helpful, both when assessing current formulations and, potentially, when screening new ones [1]. Traditional methods such as vibration, centrifugation, microbalance methods and aerodynamic techniques offer insight into bulk behaviour [2,3], whereas AFM allows measurement of the adhesion and cohesion of single particles under a variety of environmental conditions. This flexibility allows factors such as relative humidity (RH) to be studied and for experiments to be performed in liquids that simulate a metered dose inhaler (MDI) propellant.

1.1 Atomic force microscopy

AFM was invented in 1986 [4] and began as an imaging technique that 'feels' surfaces, allowing subnanometre resolution [5]. The basic principle is simple; a sharp tip at the end of a flexible microfabricated cantilever is raster-scanned across the surface, and the amount of deflection of the cantilever, monitored by a laser reflected onto a position-sensitive photodiode detector, is recorded as an indication of the surface topography (**Figure 1**). Typically, the cantilever is moved up and down (Z direction) and is raster-scanned by piezoelectric scanners (X-Y directions). For a good introduction to AFM see [6], and for a recent review of imaging techniques see [7].

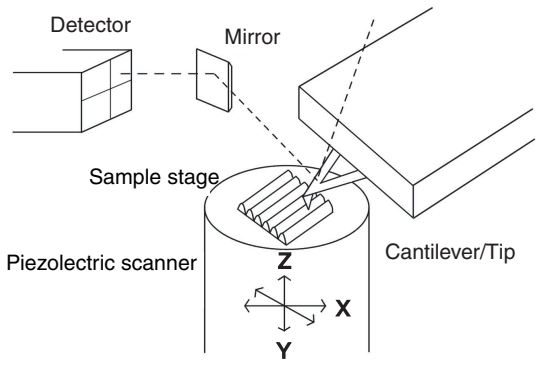

Figure 1. Basic atomic force microscopy set-up showing the main components of a typical system.

It was soon realised that the AFM is ideally suited to the measurement of surface and interfacial forces [8,9], and has since been used in a wide variety of applications, including xerography [10], the adhesion of biological cells [11] and even the adhesion of spiders feet [12].

When challenged to a substrate, the cantilever deflects due to an interaction between the tip and the surface. The magnitude of this deflection (x) can be related to the force acting (F) by the application of Hooke's law, treating the cantilever as a spring of stiffness (k);

$$F = -kx \qquad (1)$$

The force sensitivity of the instrument is very high, theoretically as high as 10^{-15} N, although practically, around 10^{-11} N [5]. AFM force data is recorded in the form of force–distance curves, measured by bringing the tip towards the surface until it contacts and then withdrawing. Plotting the force against the tip–sample separation during one such cycle gives a force–distance curve. A typical example is shown in **Figure 2**. For a detailed review of AFM force curves see [13].

When conducting force measurements with the AFM, the spring constant (k) needs to be known to a high degree of accuracy. As the manufacturer's supplied value is generally an estimate for a batch of cantilevers and is not considered accurate enough for quantitative measurements, several different methods have been developed to find k values [14].

The colloidal probe technique was developed to allow the study of colloidal particle interactions by attaching them to the end of an atomic force microscope cantilever and measuring force–distance curves [15,16]. These spherical probes simplify the contact geometry and allow comparison of data to various models. This method has been used to study a pharmaceutical system [17], although the use of such a probe has limited relevance.

A more powerful technique is to attach a 'real' particle to a cantilever and measure interactions. The first example of this for a pharmaceutical powder was published in 2000, in which Neuman's group reported differences in the adhesion of lactose particles to two gelatin DPI capsule surfaces [18]. An example scanning electron microscope (SEM) image is shown in **Figure 3**, where a drug particle ~ 10 µm in diameter is attached to a triangular atomic force microscope cantilever. This technique forms the basis of all AFM studies of inhalation particle interactions.

Attachment of particles to cantilevers is usually achieved either by the use of a micromanipulator [19,20] or by using AFM directly [21]. Both processes are time consuming and labour intensive and can provide a practical limitation to the amount of particles used in experiments [1].

AFM can be used to record topography and force curves simultaneously [22,23]. It is, therefore, possible to examine the spatial variation of adhesion, elasticity, friction and other properties across a surface and to gather many force curves in one preprogrammed routine. This technique is sometimes referred to as force–volume imaging and has been used in pharmaceutical applications [24,25]. For a review of AFM force mapping see [26].

1.2 Particle interactions

Particle interactions are complicated processes based on the interplay between different forces and factors. There are three main forces that cause particle interactions, the most important of these are van der Waals forces, which act between all surfaces under all conditions [6] and usually dominate interactions [27]. The force (F) between a particle of diameter (d) separated from a plane surface by distance (r) is given by;

$$F = \frac{\sqrt{A_{11}A_{22}}}{6r^2}d_1 \qquad (2)$$

where A_{11} and A_{22} are the Hamaker constants of the two materials.

Electrostatic or columbic forces can be the most significant intermolecular force [6]. This attractive or repulsive force scales as $1/r^2$ and is, therefore, generally long range [2]. The effect of electrostatic forces on particle adhesion has not been explicitly studied in a significant number of cases [28] (with the exception of [21,29]). It is thought that these forces are likely to be highly dependant on both the system and the environment, and that their main contribution is deposition and agglomeration during powder handling [2].

Capillary forces are caused by the condensation of water into the small gap between surfaces on contact. The formation of this liquid bridge can cause large adhesion forces (although repulsion effects are also possible) due to surface tension in the resulting meniscus [6]. These forces can sometimes dominate in ambient conditions [26] but can be removed experimentally by conducting measurements at low RH.

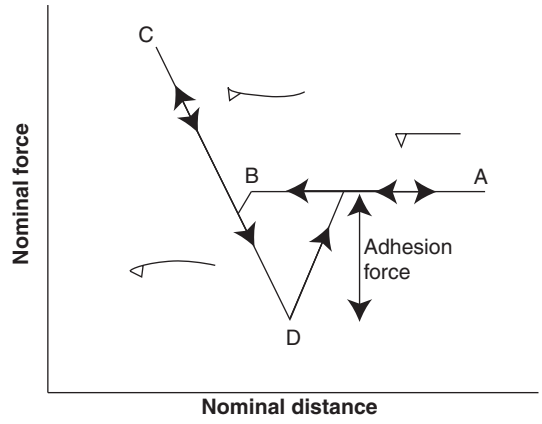

Figure 2. Example of a force–distance curve showing the principal features associated with adhesion measurements. A. "Free level", no surface forces are felt by the AFM probe; **B.** Tip jumps into contact with surface due to attractive forces; **C.** Cantilever bends whilst pressed into surface to a present maximum deflection; **D.** Tip adheres to the surface on retraction and bends back until it snaps free and returns to free level.
AFM: Atomic force microscopy.

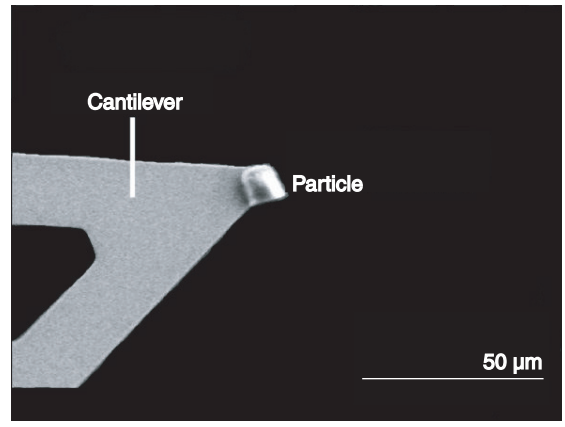

Figure 3. Scanning electron microscopy image of a single drug particle attached to an AFM cantilever.
AFM: Atomic force microscopy.

1.3 Factors influencing particle interactions

The way in which interparticle forces affect particle behaviour is determined by other particle and surface properties. Some of these are reviewed briefly below.

It is apparent from Equation 2 that the larger the particle, or asperity on the particle, the stronger the van der Waals forces will become. However, the balance between van der Waals and gravitational forces can often determine the behaviour of a powder [3]. Because gravitational forces increase as d^3, whereas van der Waals only increase as d, the behaviour of larger particles (~ >100 µm) will start to become dominated by gravity [2].

As mentioned above, humidity can lead to the formation of capillary forces, which can increase adhesion; studies on these effects are reviewed in Section 2.4. Humidity can also increase charge mobility and dissipation on particle surfaces, which can decrease the magnitude of electrostatic forces [3]. It is thought that capillary forces generally occur at RHs > 50% and will dominate at ~ 70%. The amount to which a surface is affected by humidity is controlled by its hygroscopicity.

The topology of both particles and surfaces can dominate the adhesion behaviour, since they determine the contact area of an interaction and hence, the strength of the forces acting [2]. As will be seen in Section 2.5, the effects of roughness are complicated, but generally rougher surfaces offer smaller contact areas and so give lower adhesion forces. This relationship is likely to be more significant for harder materials, as less deformation occurs [2]. The scale of the roughness is important, as when gaps between asperities are large enough, particles can fit in and adhesion will increase [2].

The chemistry of the surfaces in contact influences the strength of the van der Waals force as it determines the Hamaker constant and the surface free energy of particles and surfaces. The surface free energies of two bodies in contact are related to the work of adhesion (W) by the Dupré equation [2];

$$W = \gamma^a + \gamma^b - \gamma^{ab} \qquad (3)$$

where $\gamma^{a,b,ab}$ are the surface free energies of material a, b and the a–b interface, respectively. Particles adhere more strongly to materials with high surface energies [3]. For cohesive interactions, the situation is simpler [2];

$$W = 2\gamma \qquad (4)$$

The amount to which a particle or surface deforms on contact determines the contact area of the interaction. The Hertz theory can be used to estimate the contact radius of a spherical particle (R) as follows [30];

$$R^3 = \frac{3R_pF_{ON}}{4E^*} \qquad (5)$$

where R_p is the radius of the particle, F_{ON} is the press on force and E^* is the reduced Young's modulus, which is given by;

$$\frac{1}{E^*} = \frac{1-\nu_1^2}{E_1} + \frac{1-\nu_2^2}{E_2} \qquad (6)$$

where $v_{1,2}$ are the Poisson's ratios and $E_{1,2}$ the Young's modulus of the particle and substrate, respectively [31].

1.4 Models to describe particle adhesion

Two models, the Johnson-Kendal-Roberts (JKR) theory [32] and the Derjaguin-Muller-Toporov (DMT) theory [33], are commonly used in AFM experiments. They are both based on the Hertz theory and describe the behaviour of an elastic spherical particle when pressed onto a flat surface. The adhesion force, F_{ADH} is given by;

$$F_{ADH} = XWR\pi \qquad (7)$$

For the JKR theory, which assumes deforming forces act only inside the contacting area forming a neck at the interface, X = 3/2. The DMT theory, when X = 2, takes surface forces acting outside the area of contact into account and is generally more suited to the contact of hard materials with low surface energy or small contact radii [34]. The choice of which model is appropriate is aided by the calculation of the parameter (ϕ) as put forward by Tabor [35];

$$\phi = \left(\frac{W^2 R}{E^{*2} z_0^3}\right)^{\frac{1}{3}} \qquad (8)$$

where z_0 is the distance of closest approach (commonly taken as 0.3 nm [36]). If ϕ is > 0.3, then the JKR theory applies.

The JKR and DMT theories actually represent opposite ends of the same scale [37]. In a recent paper, Drelich *et al.* [34] claimed that these models are often chosen poorly and misused in the literature and recommend the use of another parameter, λ, as defined by Maugis [38];

$$\lambda = \frac{2.06}{z_0}\sqrt[3]{\frac{RW^2}{E^{*2}\pi}} \qquad (9)$$

JKR applies if $\lambda \geq 5$ and DMT applies if $\lambda \leq 0.1$. There is a corresponding model, known as the Maugis–Dugdale model, to describe the adhesion behaviour between the two extremes of JKR and DMT [38], which the authors propose is more appropriate for AFM adhesion data [34].

1.5 Inhalation formulations

The majority of inhalation single particle work with AFM has been focused on interactions relevant to DPI's, because these are becoming increasingly important as older MDI systems are withdrawn. This is mainly driven by the environmental need to move away from propellant-based systems [39,40], but also because pulmonary drug delivery is a good option for the administration of peptides, proteins and other drugs with low bioavailability through the oral route [41]. Following this trend in the literature, this review will focus on interactions that are relevant to DPI performance, although studies on MDI systems will also be covered.

A detailed explanation of DPI systems will not be given here; for a good introduction see [3], and for a more comprehensive review see [39]. A DPI generally consists of an interactive mixture of micronised drug particles, 1 – 5 µm in diameter, adhered to larger carrier particles, typically made of lactose [3]. The purpose of the carrier particle is to aid the removal of the smaller drug particles from the device by the air stream, and to act as dilutent excipients. Following removal, the drug becomes separated from the carrier and is free to deposit further down the airway. The quantities fine-particle dose (FPD) and fine particle fraction (FPF) are often used to describe the effectiveness of a DPI.

Even with a simple understanding of how a DPI functions, it is easy to comprehend the importance of particle interactions to its efficiency. The removal of the powder from the device, any subsequent adhesion to other device components and, of course, the removal of the drug from the carrier particles are all important interactions that can be investigated by single particle AFM techniques. These fundamental measurements could be used to improve device design and material choice or to screen potential formulations [1].

2. Atomic force microscopy studies of inhalation particle interactions

The remainder of this review will cover experiments performed using AFM to measure single particle interactions relevant to inhalation drug delivery. Links with other methods are highlighted wherever they have been made. A major limitation of using AFM to study single particle interactions is that the contacting area and its nature are unknown and no determination of properties such as surface energy can be made. As will be explained in the following sections, some authors have taken a direct approach to this and attempt to quantify the contact area, but most studies involve making comparisons using the same particle, where the contact area is assumed to remain the same. Two areas that have received particular attention are the effects of humidity and surface roughness, and these are reviewed in separate sections.

2.1 Comparative studies of particle–particle interactions

An interesting example of the comparative approach is the production of a 'cohesive–adhesive balance' (CAB) graph [42-44], which was used to study salbutamol sulfate-lactose and budenoside-lactose systems. The CAB graph is produced by plotting a graph of force of adhesion against force of cohesion, and identifying a series of linear relationships. The line of unity gradient represents where $F_{ADH} = F_{COH}$.

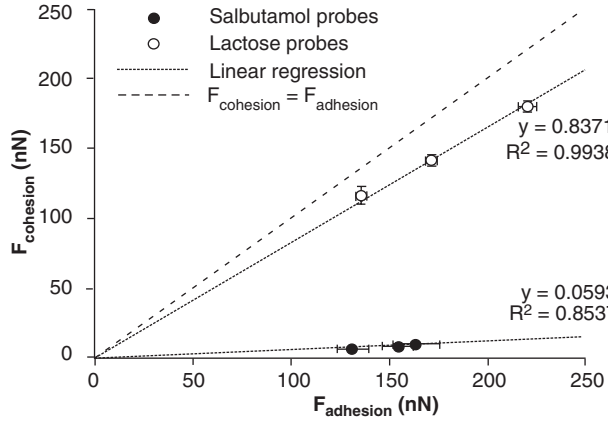

Figure 4. Cohesive–adhesive balance graph showing adhesive tendency of salbutamol sulphate–lactose system. Reproduced with permission from BEGAT P, PRICE R, STANIFORTH JN, MORTON DAV: The cohesive-adhesive balances in dry powder inhaler formulations I: direct quantification by atomic force microscopy. *Pharm. Res.* (2004) **21**(9):1591-1597 [44]. R: Correlation coefficient; F: Force.

Any system with a steeper line than this will be more cohesive than adhesive.

The results shown in **Figure 4** indicate that the salbutamol sulfate–lactose system shows a strong adhesive tendency and, therefore, would not require much mixing to give a uniform blend [44]; whereas budenoside–lactose (not shown) would appear to favour cohesion and would, therefore, prove difficult to blend. These predictions were confirmed by SEM observations, although a further study showed that the relationship with actual de-agglomeration measured with a twin-stage impinger is more complicated [42].

It has been shown that the amorphisation of a zanamivir drug crystal surface increases the adhesion of a lactose particle probe [45]. A reason as to why the adhesion is higher on the amorphous sample is not presented, but as it appears to have a lower surface energy, it is probably the larger contact area with the smoother crystalline form that causes the greater adhesion.

2.2 Comparative studies of particle–surface interactions

An early example used the colloidal probe technique to characterise the interactions between a 10-µm silica sphere and lactose substrates [17]. Although the relevance of this probe may be limited, the experiment raised some important points. A continuum of adhesion values were found at different sites, suggesting that the idea of 'active and passive sites' on carrier surfaces [46] may be too simple. The adhesion was found to be lower on compressed disks than on particles; the author's claim this occurs due to a lower contact area on the disk. This is an unusual observation as the smoother disk will almost certainly offer a larger contact area than a particle.

In another early series of papers, Neumann and colleagues measured the adhesion of lactose particles to gelatine capsules as used in DPI's [18,25,47]. They developed a technique of using force–volume scans to visualise the spatial variation of adhesion over a 10-µm area [25]. A difference in adhesion values and distribution was observed between two types of gelatin capsule.

A common technique in AFM experiments with pharmaceutical materials is to create a model surface by re-crystallisation or compression of powdered material to produce surfaces with relatively smooth topography.

Sindel *et al.* made smooth surfaces of lactose by pressing tablets at different pressures and measured the adhesion forces of rough lactose particles in force–volume mode [24]. It was then possible to link the topography to the shape of the force curves; data taken in valleys showed multiple pull-off points. AFM gave much lower adhesion forces than measurements from a shear cell and tensile tester. This is because the latter two methods are measuring average forces from multiple contacts, which highlights the difficulty of extrapolating single particle properties from bulk methods.

The difference in separation energies of a drug probe with three types of treated lactose surface has been ranked as unmodified > smoothed (process A) > smoothed plus magnesium stearate (process B) [48]. The FPD was measured with a twin-stage impinger and was shown to significantly increase as the adhesion values decreased, as shown in **Figure 5**.

The adhesion of small drug particles (< 2.5 µm) to steel device walls was found to be stronger than to lactose plates [20]. The authors believe the use of these particles (smaller than most used in AFM experiments) more accurately represents real DPI interactions. The difference was attributed to the higher Hamaker constant of steel than lactose, although the roughness of the two surfaces was not quantified. This result may imply that drug particles are more likely to stick to device walls than carrier particles.

The adhesion forces of salbutamol particles to various substrates relevant to a DPI formulation were ranked in a study by Eve *et al.* as glass > lactose particle > salbutamol particle > polytetrafluoroethylene (PTFE) [21]. The high adhesion to glass was attributed to its hydrophilic surface, leading to capillary forces. The variations in adhesion were greatest for the lactose and salbutamol particles (this was expected as these were the roughest surfaces). Significantly, salbutamol seemed to adhere more to lactose than itself, indicating lactose is an effective carrier, a similar result to that found elsewhere [43]. The adhesion to PTFE is low, presumably because of its low surface energy [49]; this suggests that it would make a good packaging material. It was noted by the authors that adhesion to the PTFE increased with repeated contacts due to tribocharging, as electrostatic forces became more important.

Although the majority of research has focused on DPI devices, some work on MDIs has been published and will be briefly reviewed here. Ashlayer [50] investigated the interaction

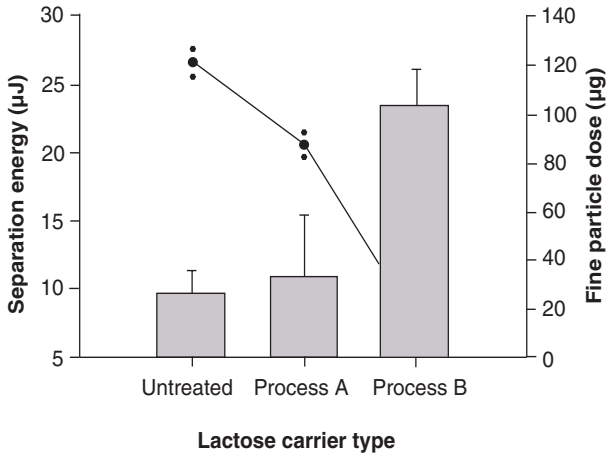

Figure 5. Correlation between median separation energy of a drug particle as determined by AFM (points) and FPD as determined by a twin-stage impinger (bars) on three lactose surfaces [48]**.** Process A: smoothed; Process B: smoothed with the addition of magnesium stearate. Reproduced from YOUNG PM, COCCONI D, COLOMBO P *et al*.: Characterisation of a surface modified dry powder inhalation carrier prepared by particle smoothing. *J. Pharm. Pharmacol.* (2002) **54**:1339-1344 [48] with permission of Journal of Pharmacy and Pharmacology published by Pharmaceutical Press, the Publications Division of the Royal Pharmaceutical Society of Great Britain.
AFM: Atomic force microscopy; FPD: Fine particle dose.

of drug particles with MDI components in a model propellant. They found that the addition of various polymers as stabilisers can reduce the strong attractive forces found between the drug and the components.

A similar study looked at the adhesion of salbutamol sulfate particles to parts of MDI canisters in a model propellant [49]. The authors found that the median separation energy values rank as glass > aluminium > PTFE, suggesting that PTFE is the best choice for the canister material.

2.3 Quantitative studies of particle interactions

Centrifuge studies have indicated that the real contact area for a particle interaction is < 15% of the apparent contact area (estimated from the size of the particle) [30]. A similar result was found using AFM by Sindel and Zimmerman, who estimated the contact area of a particle using the blind probe reconstruction algorithm of Villarrubia [24]. This method searches the image for the sharpest feature, from which the real shape of the probe can be estimated. They found that the radius of the contacting asperity of a 20 µm lactose particle is < 30 nm. It follows from these results that the contact area needs to be estimated from measurements of the surface asperities rather than the overall size.

An interesting approach to finding the contact area was presented by Beach *et al.* [52]. They pressed a drug particle onto a soft polymer film and then took AFM images of the resulting indent. For a particular particle, they showed that it only contacted the film with two small (< 0.5 µm wide) asperities, thus providing a useful insight to back up the observations above. However, due to the softness of the polymer film, it is not possible to estimate the contact area of the particle with a harder substrate, but an upper limit could be set.

Hooton *et al.* used a sample consisting of a grid of sharp spikes to directly image the contacting asperities of particles [31]. This method involves scanning the probe over the spikes and, as the surface features are sharper, the substrate effectively images the probe [52]. It is possible to estimate the effective radius of the contacting asperity, which can be related to the contacting radius using Hertz theory, Equation 5.

This technique was applied to the adhesion of two types of salbutamol particles, prepared by different methods, to a highly oriented pyrolitic graphite (HOPG) substrate in a model MDI propellant [31]. The force of adhesion was measured and the data normalised for the contact area of each particle. The authors then used the JKR theory, Equation 7, to derive the work of adhesion. The particles produced by a 'solution-enhanced dispersion by supercritical fluids' (SEDS™) process were found to have a lower work of adhesion than micronised particles; 4 mJm^{-2} and 19 mJm^{-2}, respectively. This compares well with the results from inverse gas chromatography (IGC) where the SEDS material has a lower surface energy.

The authors showed that it is possible to determine the surface energy of a particle in a later paper on the same material by applying either Equation 3 or 4 [53]. The measured surface energy from AFM was 10.8 mJm^{-2} for micronised salbutamol, which compares reasonably with the IGC value of 58.6 mJm^{-2}.

The same group went on to compare measured adhesion forces for the same two types of salbutamol particles with values predicted by the JKR and DMT models [54]. Using a measured contact radius and known values for the surface energy of the two materials, a predicted force of adhesion was calculated. These predictions were found to be too high in all cases and varied between factors of 1.2 to 7. The JKR predictions were closer to the observed forces in all cases and the authors propose this model is more suited for contacts where elastic deformations occur, as is likely to be the case for a pharmaceutical particle.

2.4 Effect of relative humidity

A significant amount of published work has included experiments to determine how RH changes the adhesion behaviour. This problem appears to be complex and depends on the surface chemistry and morphology of the particles; however, several general trends have become apparent.

An early study by Young *et al.* showed that as RH increased, the cohesion of a salbutamol sulphate particle to a compact surface also increased [19]. This increase in separation energy, by a factor of six as RH increased from 15 to 75%, was attributed to the growing influence of capillary forces. As salbutamol sulfate presents a hydrophobic surface, no changes in the surface morphology were observed over the range of humidities studied. Interestingly, the same authors suggested

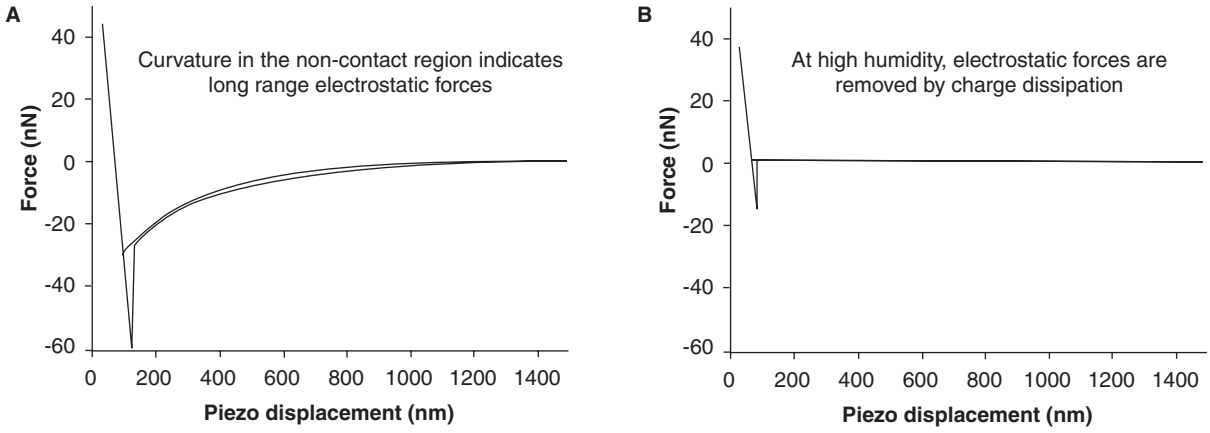

Figure 6. The effect of humidity on the force–piezo movement curve for triamcinolone acetonide cohesion showing decreased electrostatic contribution at high RH [29]**. A.** 15% RH; **B.** 75% RH. YOUNG PM, PRICE R, TOBYN MJ, BUTTRUM M, DEY F: The influence of relative humidity on the cohesion properties of micronised drugs used in inhalation therapy. *J. Pharm. Sci.* (2003) **93**(3):753-761 [29]; copyright © (2003) reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

in an impinger study on salbutamol sulfate that, at high humidities, amorphous regions on the surface can be recrystallised and 'fuse' to lactose particles [55].

The same group went on to investigate the influence of humidity on the cohesion of two further drugs [29]. They found that while disodium cromoglyate showed an increase in adhesion force with humidity, the other, triamcinolone acetonide showed the opposite trend. To explain this behaviour, the authors point to the decreasing electrostatic contribution, as evident in the non-contact region of the force curves (**Figure 6**). As the amount of water on the surface increases, the surface electron mobility also increases, leading to a dissipation of charge and a decrease in electrostatic forces.

Experiments have been performed with spherical drug particles < 2.5 µm in diameter, made using a novel aerosol crystallisation method [20]. The adhesion was found to significantly increase on both lactose plates and a steel surface with increasing humidity. They also noted that the effects of RH on adhesion appeared to be reversible.

Bérard *et al.* studied the effects of RH on the adhesion to amorphous and crystalline zanamivir and lactose surfaces [45]. With a bare SiO_2 AFM tip as the probe, the adhesion force decreases for both zanamivir surfaces at ~ 35% RH; this is said to be a consequence of its hydrophobic tendency. In contrast, the more hydrophilic lactose showed an increase in adhesion with RH. When using a lactose particle as a probe, the adhesion steadily increased for all three surfaces, with the amorphous zanamivir showing a greater relative rise than the crystalline.

In a similar study, the same group used a zanamivir crystal as a probe and found that the adhesion to a lactose surface increased with humidity [56]. They also noted that the roughness of the lactose surface decreased with exposure to humidity. Although not explicitly discussed, the observed increase in adhesion could be due, in part, to an increase in contact area, hence, extracting the true contribution of capillary forces to the total adhesion force from this information may not be possible.

A study using salbutamol particles prepared by micronisation and SEDS, on an HOPG substrate, found a lack of consistency in the adhesion behaviour with RH [54]. The morphology of the contacting asperities was found using an asperity imaging approach as described in Section 2.3. To explain this behaviour a model is proposed relating to the asperity shape and containing three different scenarios.

In the first scenario, shown in **Figure 7A**, there is a single point of contact due to one sharp asperity and the adhesion peaks at a certain value of RH. The second case (**Figure 7B**) has multiple asperities of similar scale to the first scenario and there is a similar peak in adhesion but it is less pronounced. In the last scenario (**Figure 7C**) there are many small asperities, giving one large contact area similar to a macroscopic contact; the adhesion continues to rise with RH. In this study, adhesion was measured at only four values of humidity, which means fitting one of these curves to four data points may be difficult.

In a series of studies using the centrifuge technique, Podczeck's group looked at how RH affects the adhesion of salmeterol xinafoate and lactose [57-59]. They found that for lactose, particle cohesion capillary forces appeared at 75% RH, whilst for the less polar salmeterol, the small increases in cohesion were more likely due to the plasticising effect of the water. Interestingly, this possible plasticising effect has not been reported in an AFM study, but could potentially be studied with localised mechanical measurements [60].

2.5 Effect of surface roughness

It is apparent in almost all the literature that particle or substrate roughness has a major or even dominating effect on adhesion. When considering the basic situation of a particle being challenged to a substrate, the simplest view is that surface roughness will decrease the contact area and hence,

decrease the short-range van der Waals interactions that lead to adhesion [2].

There are several experiments where roughness has been the dominant factor. Tsukada *et al.* studied the adhesion of small (< 2.5 µm) drug particles to two lactose plates and a steel surface of varying roughness [20]. They found that the adhesion to smooth surfaces was consistently higher and had a narrower distribution, but unfortunately no attempts were made to quantify the roughness. Surface roughness was also concluded to be the dominant factor affecting the adhesion of salbutamol particles to salbutamol and lactose [21].

In some cases, roughness appears to lower the adhesion of a particle to a surface. A drug probe was challenged to various lactose surfaces, which had undergone particle smoothing processes to lower the roughness; the smoother samples showed lower separation energies [48]. Interestingly, the authors attribute the decrease in adhesion to a decrease in contact area due to nanometre asperities; this result, therefore, indicates the importance of scale when considering roughness. Although some samples appeared to be smoother when the roughness was measured from a 10 × 10 µm image, on the scale of the particle interaction they appear to be rougher.

A similar result was found in a study that ranked the separation energies of salbutamol sulfate particles on various MDI canister materials as glass > aluminium > PTFE [49]. The roughness values were ranked as aluminium > PFTE > glass. This shows that although aluminium is rougher than PTFE, it has a stronger adhesion to the particles; in this case the lower surface energy of the PTFE is the dominant factor.

Some work has been done to try and model the effect of roughness on particle adhesion. The work of Rumpf [61], and later Rabinovich [36,64], has been applied to pharmaceutical particles.

Beach *et al.* measured the adhesion of a range of particles, including lactose and drug probes, and compared this to the predictions from the Rabinovich model [51]. The adhesion to a number of DPI canister materials was measured and was found to be the lowest on a surface of 194 nm root mean square (rms) roughness. Interestingly, although many smoother samples gave a higher adhesion, so did one that was rougher, presumably because the particle could fit into gaps between the asperities increasing the contact area.

The authors predicted adhesion forces by applying Rabinovich's model, which requires measurements of roughness on two length scales, in the form of Equation 10 below [36];

$$F_{AD} = \frac{AR}{6z_0^2}\left[\frac{1}{1+\left(\frac{58R(rms_2)}{\lambda_2^2}\right)} + \frac{1}{\left(1+\frac{58R(rms_1)}{\lambda_1^2}\right)\left(1+\frac{1.82(rms_2)}{z_0}\right)^2}\right] \quad (10)$$

where $\lambda_{1,2}$ represents the distance between asperities, and $rms_{1,2}$ is the root mean square roughness on large and small scales, respectively. Values for the Hamaker constants were taken from the literature. This model was found to underestimate adhesion forces for all systems, although it was considerably closer for particles known to resist deformation, such as lactose and glass, than for particles that may deform (i.e., peptide and polystyrene).

Hooton *et al.* took a similar approach in the calculation of the surface energy of SEDS and micronised salbutamol particles [53]. A similar experimental process to that reported in Section 2.3 was used, where the contacting asperities are imaged to estimate the contact area [31]. Cohesive forces were measured between particles and compressed disks of the materials, and compared with the predictions of the Rabinovich model by applying Equation 10 [36]. The forces predicted were always lower than the measured forces by factors varying from 2 to 13.

To calculate the surface energy of the particles, another equation was used based on the JKR model, which accounts for deformation [36];

$$F_{AD} = \frac{3WRr_2\pi}{2(r_2+R)} + \frac{\frac{AR}{6z_0^2}}{\left(1+\frac{58R(rms_1)}{\lambda_1^2}\right)\left(1+\frac{1.82(rms_2)}{z_0}\right)^2} \quad (11)$$

where r_2 is the asperity radius. This was rearranged to give W, and the surface energy follows from Equation 4. It was found that these surface energy values were considerably higher than the ones calculated without roughness compensation (by a factor of 6.5 for micronised and 1.4 for the SEDS sample). Importantly, the authors emphasise that the calculations are greatly dependant on the scale at which the roughness parameters are measured.

3. Conclusion

In a short period of time, AFM has shown its use as a tool to investigate particle behaviour in inhalation formulations. These studies broadly fall into two types. The first is to make comparative studies using the same particle; in this way, the adhesion of a particle to different surfaces can be ranked [21,49]. Experiments of this type have been able to provide potentially useful formulation information such as, whether a particle shows stronger adhesion to one container surface or another, and if a drug is more likely to be cohesive than to adhere to a carrier particle. The second approach involves attempting to normalise the data for contact area and to characterise the contacting region [24,31,51]. By doing this meaningful comparison of adhesion forces between particles, experiments and methods can be made, and properties such as the work of adhesion and surface energy can be calculated [31,53]. The process of measuring the contact area is experimentally difficult, but these studies have revealed that at loads accessed by AFM, contact occurs only at several small asperities [24,31,51].

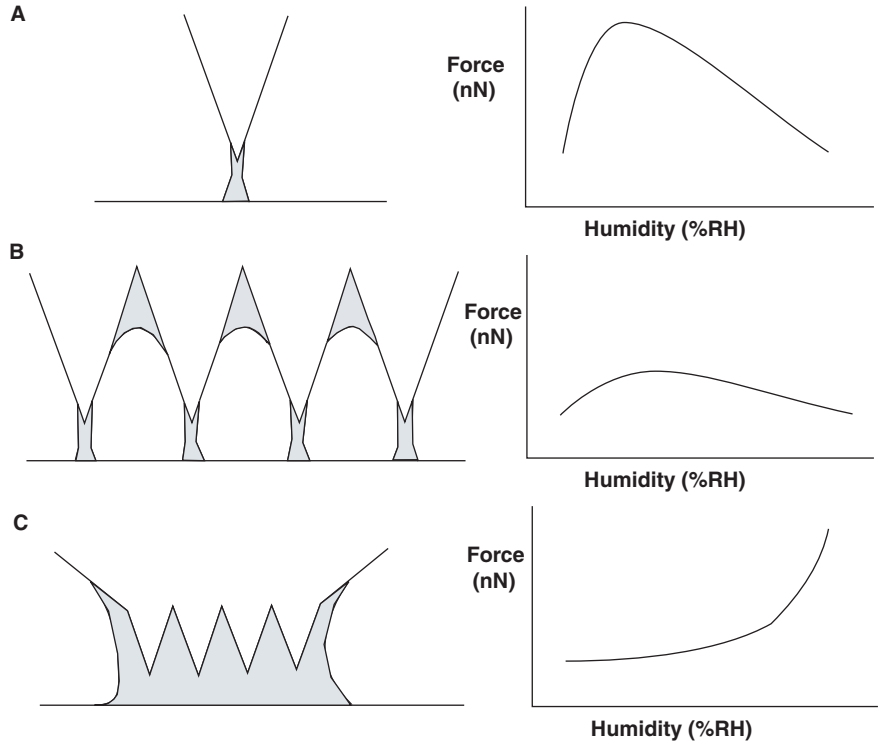

Figure 7. Proposed relationships between adhesion and RH for three scenarios of contact morphology. A. Single asperity contact; **B.** Multiple nanoscale asperities; **C.** Saturation of asperities. Reproduced with permission from HOOTON JC, GERMAN CS, ALLEN S *et al.*: An AFM study of the effect of nanoscale contact geometry and surface chemistry on the adhesion of pharmaceutical particles. *Pharm. Res.* (2004) **21**(6):952-960 [54].
RH: Relative humidity.

Investigations into the effects of RH have shown that, generally adhesion increases at high values due to the formation of capillary bridges. RH can also affect electrostatic contributions by changing charge dissipation [29]. It has been shown that exposing pharmaceutical surfaces, such as lactose, to high humidity can alter its topology [56], which may make it difficult to quantify capillary forces because the contact area may change.

Studies of surface roughness have revealed that, generally, a rougher surface will decrease particle adhesion due to the reduction in contact area. In some cases, however, the opposite is true because particles are able to interpose into the larger gaps between asperities. The scale at which roughness parameters are measured needs careful consideration. There have been some promising developments in modelling the effects of roughness, particularly using the model proposed by Rabinovich [36,64], which has been applied to pharmaceutical systems [51,53].

4. Expert opinion: towards screening of inhalation formulations

The key to developing AFM single particle studies as a screening tool may lie with increasing the links with bulk methods. For the comparative type AFM studies, it would be interesting to consider if the ranking of adhesion forces can be replicated and, hence, validated by a bulk testing method. For the quantitative AFM experiments, how do measured quantities such as particle surface energy compare with results from established bulk techniques such as contact angle measurements?

Experiments concerning the effects of RH or other appropriate environmental challenges could be used to provide information about shelf-life, storage and preparation conditions. An intriguing parallel can be drawn with established accelerated stability testing methods with the clear advantage that AFM-based methods would require very little material and, therefore, could be executed much earlier within a development programme.

AFM single-particle studies are, by their nature, limited in the sense that the area of the particle involved in any interaction is, by necessity, a very small proportion of the whole. Hence, care must be exercised in drawing general conclusions if, for example, there are different regions of high and low adhesion sites on particles [46]. In such cases AFM does provide the opportunity – given that sufficient data is recorded, to characterise and spatially locate such heterogeneous behaviour, avoiding the average view that would be produced from a bulk approach. For force experiments with particles adhered to AFM probes, there is currently no control over which asperity or exposed crystal face will contact a surface, and,

therefore, a random cross section of adhesion forces is collected. The resulting range of data would reflect any heterogeneity in potential interactions, whereas IGC, for example, is believed to produce data based only on the high surface energy regions [63,64].

The amount of AFM force data that can be collected is currently limited by the time-consuming process of particle attachment and instrumental limits to the speed of data collection. As a relatively new field, however, it is not unreasonable to believe that more rapid protocols for particle attachment and orientation will be developed. In addition, improvements to AFM technology are likely. Possibilities that would enhance data acquisition rates include the introduction of multi-cantilever systems [65] or the use of pulsed-force mode type methods [66].

AFM provides possibilities to measure other quantities, and not just the particle adhesion force. Recently, it has been shown that nano-tribological insights can be gained by studying single-particle friction using the AFM in lateral force mode [67]. This is interesting, as measuring the adhesion force only represents a special case (the case where removal forces act in the normal direction only) of what happens during 'real' particle interactions, which are likely to involve forces acting in all directions. AFM can also be used to measure the nano-mechanical properties of particles or surfaces to give quantities such as the Young's modulus, which could be important in particle behaviour [60,68,69].

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