Rapid Pre-formulation Screening Of Formulation Components Using The Atomic Force Microscope.

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Aim

The delivery of dry powders to the respiratory tract has become an essential part of current asthma therapy. In order to achieve a therapeutic dose the drug particulates require a diameter of less than 5μm to avoid impaction and sedimentation in the upper airways. However, particles with micron size are naturally cohesive/adhesive due to a high surface area to mass ratio, thus making formulation and delivery to the patient problematic. The degree of cohesion/adhesion is dependent upon a number of factors including; surface chemistry, morphology and environmental factors such as humidity. Here we demonstrate the use of atomic force microscopy (AFM) as tool capable of constructing cohesion profiles, at specific humidities for three asthma drugs; salbutamol sulphate (SS), disodium cromoglycate (DSCG) and triamcinolone acetonide (TAA). In addition, the AFM data was compared with conventional in-vitro performance tests.

Atomic Force Microscopy

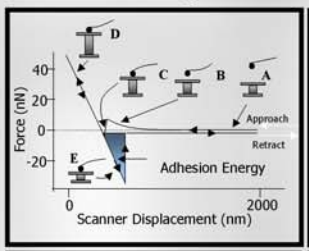

Figure 2: Diagrammatic representation of an ideal force-distance curve

At large tip sample separation there is no cantilever deflection

A *As the tip approaches the sample, it is subjected to electrostatic forces (in this example repulsive) causing a tip deflection.*

B *The van der Waals forces overcome the cantilever spring constant and the tip jumps to contact.*

C *Scanner displacement results in a linear cantilever response due to the tip and sample being in contact. The tip is compressively loaded to a pre-set value.*

D *Upon retraction a linear response is observed until the tip sample contact is broken.*

E *The difference between the minimum point on the retraction curve and the point of disconnection is directly related to the adhesion force. With the area under the curve being equal to the separation energy.*

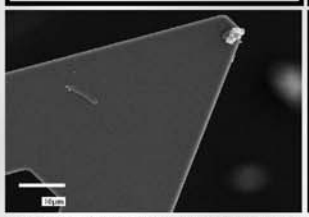

Figure 1: Scanning electron - microscope image of A, salbutamol sulphate drug probe immobilised on the tip of a pyramidal AFM cantilever (2000 X magnification).

Cohesion measurements conducted by AFM are achieved by ramping a drug probe, mounted on a micro-fabricated cantilever, towards, in contact with and away from a substrate surface. Measurement of the cantilever deflection can be directly related to the forces acting on the probe (using Hooke's law). By integrating the area under the resultant retraction curve a separation energy can be calculated (Figure 1).

Micronised SS, DSCG and TAA particulates were mounted onto tipless V shaped AFM cantilevers (Figure 1) (0.58N/m spring constant, DNP-020, DI, Cambridge, UK) using a micromanipulation process. Model compacts of each were prepared by direct compression and were used as substrates for separation energy measurements. Separation energy measurements were conducted using a Nanoscope IIIa AFM (DI, Cambridge, UK) in force volume mode. This allowed the collection of 4096 individual separation energy measurements over a 10x10μm area of the corresponding model compact. Relative humidity during the AFM analysis was controlled using a custom-built perfusion apparatus. Separation energy measurements between each drug probe and corresponding drug surface was conducted at 15, 30, 45, 60 and 75% RH (n=5)

Analysis of the force curve data for each drug probe indicated a log-normal energy distribution. This was to be expected, however, since measurements were conducted on model compacts of the drugs (thus resulting in a wide spread in energy values). In general, an increase in cohesion was observed for both SS and DSCG as humidity was increased, while a decrease in cohesion for TAA was observed across the same range (15-75% RH). The relative differences between the cohesion profiles for each drug can be related to the physical and chemical properties of each. For example, DSCG adsorbs ~20%$^{w/w}$ moisture at 75 % RH and is effectively a liquid crystal, while TAA is hydrophobic and known to readily tribo-charge during processing. Therefore it is likely DSCG is strongly effected by capillary interactions at higher humidity while TAA would be influenced by electrostatic interactions at low humidity. This can be seen directly when analysing the individual force curves for TAA at 15% RH and 75% RH (shown in Figure 4)

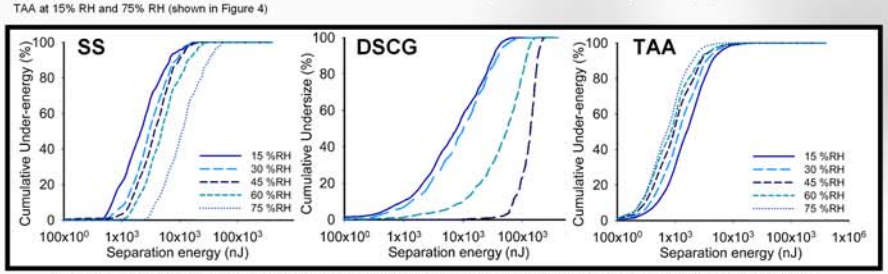

Figure 3. Separation energy profiles (cohesion) for SS, DSCG and TAA drug probes on model compacts at specific RH Single drug probe measurements over 10μm X 10μm area. (n=4096)

In-vitro Correlation

The aerosolisation efficiency of the drug powders was investigated using apparatus A (British Pharmacopoeia), the twin stage impinger (TSI) (Copley Instruments Ltd, Nottingham, UK). All testing was conducted inside an environmental test chamber, (Termarks 6350, Copley Instruments Ltd. Nottingham, UK) capable of maintaining an environment of 10-95% RH (±0.2%) at 25°C. Approximately 20mg samples of the micronised drugs were stored on open pans at the analysis humidity, in the environmental chamber for 12 hours prior to loading. Approximately 2mg of the humidity equilibrated pure micronised drug was precisely weighed onto the plastic metering disk of a modified dry powder inhaler (DPI) Turbohaler™, containing no desiccant. The DPI and assembled TSI were equilibrated at the test conditions for a further 60 minutes before testing at 60Lmin^{-1} for 5 seconds. At 60Lmin^{-1}, recovered drug mass from stage 2 of the TSI represents particles with an aerodynamic diameter of <6.4μm.

The deposited drug fractions were collected from the DPI and TSI stages using a suitable wash solvent and analysed using high performance liquid chromatography (Waters Alliance, Waters Ltd, UK). Aerosolisation efficiency was calculated from the deposited drug in stage 2 of the TSI as a fraction of the loaded dose (fine particle fraction of the loaded dose (FPF). All experiments were preformed in triplicate at 15,30,45,60 and 75% RH at 25ºC

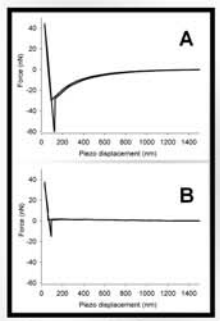

Figure 4. Representative force curve of TAA cohesion at A. 15% RH and B. 75% RH

The influence of humidity on the FPF (n=3) of each drug is shown in Figure 5 with the median cohesion energy (n=5 probes for each drug) measured by AFM. In general, good correlation between the FPF and separation energy was observed. With an increase in cohesion resulting in a concomitant decrease in FPF or aerosolisation performance.

Conclusions

The atomic force microscope is a powerful tool capable of determining fundamental separation energy measurements, in environments specific to pharmaceutical sciences., allowing the rapid screening of drugs and/or formulation components prior to in-vitro testing.

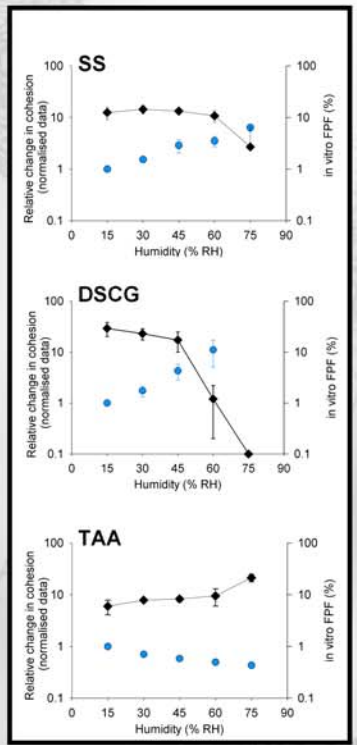

Figure 5. Relative change in median separation energy (circles) (n=5 probes) plotted alongside in-vitro FPF. (diamonds)

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