A p p l i c a t i o n s o f A t o m i c F o r c e M i c r o s c o p y i n P h a r m a c e u t i c a l R e s e a r c h

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I N T R O D U C T I O N

There is an increasing demand to develop - and hence analyse - pharmaceuticals and biomedical devices which have architectures and complex chemistries that can be controlled and manufactured at the nanoscale. For example, the need to design delivery systems capable of targeting specific sites in the body, or to formulate poorly soluble drugs is frequently addressed with nanoscale-based solutions. Such systems require analysis capable of minimal disruption and the ability to operate in environments consistent with standard regulatory testing and in-vivo conditions. These demands often exceed the capacity of analytical approaches used in the sector to provide an effective understanding of this new generation of therapeutics.

The role of atomic force microscopy (AFM) and other related scanning probe microscopies (SPMs) in the qualitative and quantitative analysis of pharmaceuticals has steadily increased in the last decade. AFM as a technique is based upon the measurement of forces as low as 10 pN using a flexible micro-cantilever bearing a sharp probe which contacts the sample under study. Its spatial resolution derives from the size of the contacting area of the probe, which is typically less than a few nanometres in diameter. Imaging is achieved by moving the sharp probe over a surface whilst monitoring its position and the forces it experiences. These microscopes have enabled the study of medical devices, drug particles and formulations with minimal pretreatment in both air and liquids at the nanoscale level. The potential for SPMs, and AFM in particular, has now developed to the point where their exploitation as an integral part of formulation development, in both academic and industrial research, is taking place.

A F M A S A F O R C E M E A S U R E M E N T T O O L I N P H A R M A C E U T I C A L S

The majority of pharmaceuticals are produced as solid dosage forms (e.g. tablets, capsules, inhalable powders) and hence their manufacture involves the handling and manipulation of powdered materials. These powders can have particles ranging in size from the nanoscale to many hundreds of micrometres. An understanding of how the mechanical properties of these particles, and the forces between them, influences processability, formulation stability and delivery is important. For example, in some cases the final form of a drug (and other materials in the medicine – the excipients) is as a loose powder, as for instance in a dry powder inhaler (DPI), the successful delivery of the drug to the lung during inhalation demands an intimate understanding of particle properties and their interactions.

The ability to study single particle interactions and the forces involved has become an important application of AFM. To achieve these measurements, typically a single particle (e.g. drug) is attached to an AFM cantilever and brought into contact with a substrate of interest. The force required to separate these two materials provides a measure of their adhesion. The ability of AFM to work in a variety of environments, such as controlled humidity and in liquids, is significant for pharmaceutical applications, for example to allow an in-situ assessment of particle adhesion in solvents.
used in pressurized metered-dose inhalers (pMDIs). The first example of this approach being used for a pharmaceutical powder examined the differences in the adhesion of lactose particles to two gelatin dry powder inhaler capsule surfaces [1]. It is important when attaching such individual drug particles to an AFM cantilever that their contacting region remains free from any adhesives employed, or damage during attachment.

An example scanning electron microscope (SEM) image of a drug particle attached to an AFM cantilever is shown in Figure 1a. The accessible particle size is typically in the range between 0.5 µm and 50 µm. For very small and/or cohesive particles (1 µm or less) more than one may become attached to the lever. However, as long as only one comes into contact with the surface to be challenged, this is acceptable. Figure 1b illustrates a schematic of a force curve and the main stages in acquiring such single particle adhesion data. If such data is to be quantified in terms of force then the spring constant of the AFM lever needs to be determined. To ease comparisons between different particle adhesion measurements it is important to control key environmental factors such as humidity and to keep instrument parameters such as maximum press-on force, contact time, particle approach and retract speeds constant.

Many researchers have now exploited this approach to explore particle interactions between drug and excipient particles and the components of delivery devices. Louey et al. [2] used this approach to measure pull-off forces between a model colloidal silica probe and lactose particles suitable for use as carriers of the smaller drug particles in a DPI. Using AFM in this way has also revealed the effect of inducing amorphous content on the surface of particles of the drug zanamivir, where an increase in its affinity with a lactose carrier was observed [3]. Such surface amorphous material is important from a pharmaceutical perspective, as this will be more soluble and probably less stable than a crystalline form of the drug, and hence can cause undesirable effects in terms of increased amounts of drug delivered on administration and stability problems in a medicine.

The effect of relative humidity on the forces between particles is an important consideration in pharmaceuticals that has been studied by AFM, as this factor can strongly influence the stability of a medicine through the mediation of solution based processes and can cause aggregation due to capillary forces [4]. The ability to record force data in controlled environments has been extended to work in liquids, for example to quantify drug-device interactions in model propellants to simulate the environment within a pMDI [5].

These and other studies can broadly be divided into two types: those that rank relative particulate interactions, and those that make a quantitative comparison. Ranking studies address, for example, how drug-drug cohesion compares to drug-excipient particle and drug-device adhesion. Since particulate interactions are dominated by aspects such as surface mor-

Figure 2: (a) Schematic of AFM particle friction setup. Position 1: low friction surface causes very small lateral force on cantilever. Position 2: high friction surface causes large lateral force and cantilever twists. (b) Processed data showing friction on all surfaces for three lactose particles (denoted A, B, C) on the different substrates. Adapted from reference [8]. Reproduced with the permission of Elsevier.

Figure 3: (a) AFM images of crystalline sorbitol (topographic height and phase images). (b) AFM images within a quench-cooled amorphous domain of sorbitol. (c) Nanoindentation curves for the crystalline and amorphous domains. (d) Load dependence of Young’s modulus. Adapted from reference [9]. Reproduced with permission of Springer Science and Business Media.
Mchanical Properties from Single-Particle Measurements

Consideration of the schematic force distance curve in Figure 1b reveals that if the region of contact is considered, where the probe (without a particle) is pressed into a surface, then information on the mechanical properties of the sample can be obtained. Such nanoscale contact data are similar to traditional indentation measurements more typically measured on a micrometre or greater scale to determine the elastic modulus and hardness. There are, however, a number of limitations of the AFM data that should be considered. Firstly, as standard AFM cantilevers are typically very flexible (so that they are sensitive to nanonewton forces) they are incapable of deforming surfaces beyond an elastic modulus of approximately 10 GPa. Secondly, unlike a traditional indenter, an AFM probe does not approach or deform a surface completely normal to that surface, due to the need to have the lever at a slight angle to ensure that the probe apex contacts the sample first. This causes lateral deformation to the need to have the lever at a slight angle to ensure that the probe apex contacts the sample first. This causes lateral deformation errors in the data, which are only negligible with relatively small indent depths. Despite these issues, many groups have employed AFM to determine the elastic, inelastic and hardness properties of materials. Such behaviour of pharmaceutical materials is known to affect properties of materials. Such behaviour of pharmaceutical materials is known to affect drug crystal growth, morphology and surface properties from erodible materials and crystallisation phenomena. It is widely known that pharmaceutical additives and impurities in drug formulations can affect drug crystal growth, morphology and potential drug efficacy. This may be achieved deliberately to control crystal habit or an undesirable consequence of contamination. By understanding the degree to which impurities affect these critical formulation parameters, drug delivery systems can be developed more effectively. The AFM has the capability to study drug crystal changes in real time as demonstrated by Thompson et al. [11] in the study of paracetamol in the presence of impurities, acetanilide and metacetamol using AFM in liquid. AFM images of the (001) face of a paracetamol crystal during incubation with 4 mol % acetanilide over time are shown in Figure 5. The defects in the crystal surface form weak points leading to deepened holes (one highlighted by a black arrow) over 12 hours, indicating that the dissolution occurs both laterally and towards the crystal core.

From the consideration of AFM as a force measurement tool, it is clear that the probe is sensitive not only to topography but also to the magnitude and nature of the forces experienced by the probe. The use of phase imaging alongside topographical imaging in tapping mode has proved particularly useful for

The application of AFM to the measurement of dynamic rheological properties of formulations has also been explored. For example, the viscoelastic properties (loss tangent, tan δ, storage G′, and loss shear moduli G″) of intergranular material bridges for granules formed from lactose and polymer binders has been determined by introducing a high frequency (50-1000 Hz), but low amplitude (≈3 nm) oscillation to the cantilever (with granule) during force distance measurements (Figure 4) [10].

In general, it can be seen therefore that AFM provides a very flexible force-measurement platform, adaptable to a range of experimental geometries and conditions with real pharmaceutical materials.

AFM IMAGING BASED STUDIES

As a microscopy, AFM is perhaps better known for its ability to image surfaces at nanometre resolution in a variety of environments than as a force measurement tool. Since the material under study can be exposed to environmental stresses (temperature, humidity, solvents, etc), it can often be in a dynamic state and hence, as long as the kinetics are not too rapid, AFM is able to provide a dynamic view of surface mediated processes. This approach has found effective applications in pharmaceuticals in the study of environmental stress on the surface properties of formulations, drug release from erodible materials and crystallisation phenomena.

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Figure 4:
G′, G″ and tan δ profiles of an adhesion event at 1% RH, between a spherical cantilever probe oscillating at 500 Hz and the surface of a lactose/PVP granule as a function of probe-sample separation distance. Adapted from reference [10]. Reproduced with permission of Springer Science and Business Media.

Figure 5:
A sequence of 10 µm x 10 µm AFM images of the (003) face of a paracetamol crystal in paracetamol/4 mol% acetanilide solution. Image (a) was taken 11.5 min after addition of paracetamol/4 mol% acetanilide solution. Images (b–f) were taken at the regular intervals over the next 12 min [11].

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A sequence of 10 µm x 10 µm AFM images of the (003) face of a paracetamol crystal in paracetamol/4 mol% acetanilide solution. Image (a) was taken 11.5 min after addition of paracetamol/4 mol% acetanilide solution. Images (b–f) were taken at the regular intervals over the next 12 min [11].
studying drug polymorphs and phase-separated systems, as it can be used to map, at the nanoscale, materials with different mechanical and physicochemical properties. For example, AFM imaging and force analysis of stents coated with either poly(lactic acid) (PLA) or PLA/everolimus has been performed under ambient conditions [12]. Such drug-coated stents are implantable medical devices used to treat the narrowing of the coronary artery. Everolimus is an anti-proliferation drug used to overcome scarring and the need for stent replacement after implantation. Temperature-controlled force measurements on PLA only coated stents in air and in a 1% Triton surfactant solution showed a significant drop of the Young’s modulus in solution at 36°C. Hence, in vivo the Tg of the polymer is around body temperature which has significant consequences on drug release [12]. The advent of further imaging modes which can rapidly and quantitatively map multiple surface properties (Figure 6), such as Harmonix [13], will considerably expand the potential of AFM in such work.

**MICRO AND NANO THERMAL CHARACTERIZATION WITH SPM**

Classically, one of the main tools used to identify the form a drug takes within a formulation and the interactions between its components is calorimetry. However, such bulk approaches cannot reveal the spatial distribution of these components.

An adaptation of AFM that can achieve this is the scanning thermal microscope (SThM). Here the normal silicon cantilever is replaced with a Wollaston wire probe brought to a sharp apex in the form of a cantilever. Such probes have demonstrated the ability to distinguish and identify phase separated material in solid dispersions and to indentify drug polymorphs, but only at resolution lower than around a micrometre. However, the recent development of a commercially fabricated doped-silicon cantilever with a heated probe has enabled this problem to be overcome and for nanoscale thermal events to be probed with nanometre precision [14].

An example of using such a local thermal analysis probe is shown in Figure 7, where a lactose monohydrate crystal within a blend has been studied. The resultant indent due to local melting is apparent in the image. The corresponding cantilever deflection trace reveals the loss of water from the monohydrate and the melt of the anhydrous form.

**CONCLUSIONS**

As the barriers to developing medicines become ever greater, due to new challenges in delivery, greater regulation and the scarcity of ‘easy’ molecules to develop into medicines, the role of scanning probe microscopes within the range of analytical tools employed in pharmaceutical developments is set to increase. The advantages of nanoscale resolution, minimal sample preparation and the non-invasive imaging capabilities of atomic force microscopy in particular, make this an ideal tool to bring new approaches to the investigation of the properties of drug materials.