Drug Dissolution Studies with Atomic Force Microscopy

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Introduction

The ability of atomic force microscopy (AFM) to resolve structures on the nanometer-to-subangstrom scale in ambient and fluid environments has made it a particularly useful tool in pharmaceutical research. AFM has been used with great success to evaluate many of the steps in the drug fabrication process, including studies of drug interactions, gene delivery vehicles, crystal growth, and particle formation.[1–4] Once a drug is formed, its dissolution properties have a direct effect on its absorption in the body. In addition to the wide range of uses in drug fabrication, AFM has also been successfully utilized to study the dynamics of the dissolution process, and has added to our overall understanding of this process at the molecular scale. This application note discusses the utility of AFM in dissolution studies and details some example studies on acetaminophen and aspirin surfaces.

AFM Methods

AFM is performed by scanning a sharp tip on the end of a flexible cantilever across a sample surface, while maintaining a small, constant force. Tip sizes and materials vary depending on application requirements, but they typically have an end radius of 5 to 10 nanometers. In a basic AFM setup, a piezoelectric tube scanner scans the tip in a raster pattern over the sample (Figure 1). The resulting tip-sample interaction is monitored by reflecting a laser off the back of the cantilever onto a split-photodiode detector. Over the past several decades, a variety of scanning modes have been developed to control how the tip scans the sample. Contact mode and TappingMode are two of the more commonly used AFM techniques of operation.

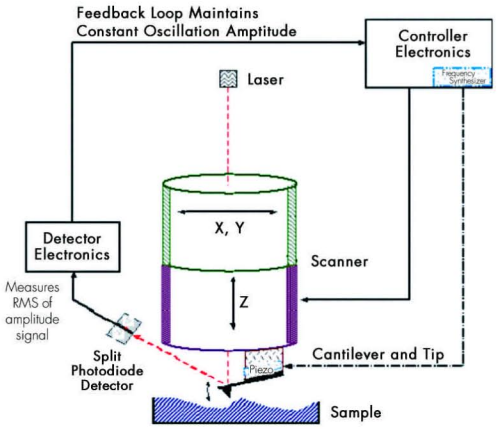

Figure 1. Schematic of the major components of an atomic force microscope, showing the feedback loop for TappingMode operation.

In contact mode AFM, a constant cantilever deflection is maintained by a feedback loop that moves the scanner vertically (Z) at each lateral (X,Y) data point to form the topographic image. By maintaining a constant deflection during scanning, a constant vertical force is maintained between the tip and sample. Applied forces during imaging typically range between 0.1 and 100 nanonewtons. Although contact mode has proven useful for a wide range of applications, it can damage relatively soft samples, and thus is often not the ideal method for biological and other sensitive material applications.

On the other hand, TappingMode AFM consists of oscillating the cantilever at its resonance frequency (typically about 300 kilohertz) and scanning across the surface with a constant, damped amplitude. The feedback loop maintains a constant root-mean-square (RMS) amplitude by moving the scanner vertically during scanning, which correspondingly maintains a constant applied force to form a topographic image (Figure 1). The main advantage of TappingMode is that it typically operates with a lower vertical force than contact mode, and it eliminates the lateral, shear forces that can damage some samples. Thus, TappingMode has become the preferred technique for imaging soft, fragile, adhesive, and particulate surfaces, like those found in pharmaceutical applications.

AFM in Drug Dissolution Studies

Once a drug has been produced in its appropriate dosage form (tablets, capsules, powders), it must be able to dissolve at the proper location, under specific environmental conditions

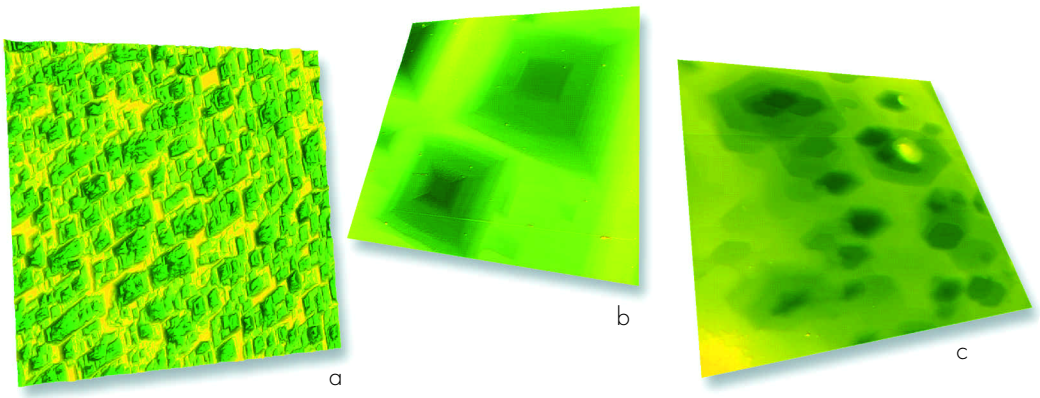

Figure 2. AFM images of the (010) crystal surface of acetaminophen single crystals after partial dissolution by a) water, b) acetone, and c) pyridine. The resulting surface texture provides information about the dissolution mechanisms of acetaminophen and how the dissolution is related to its crystal structure[7]. 60µm scans. Images courtesy T. Li, K.R. Morris, and K. Park, Purdue University

(temperature, pH), and at an appropriate rate for the drug to be effectively absorbed. Dissolution rate and behavior have been shown to be affected by surface area, temperature, crystal structure, pH and the nature of the solvents.[5] Thus, proper characterization of these factors has a very important role in a drug's overall efficacy.

Traditionally, dissolution testing has been carried out at the macroscale by constant surface area or particulate dissolution methods to examine the behavior of drug products under different conditions. However, there are several advantages of studying the dissolution processes by AFM. Dissolution is commonly tested at 37±0.5°C, which is made possible with AFM by heating the fluid environment during experimentation. Since the AFM can operate in air and fluid environments, the operator has the choice of how to conduct the experiment. It can be conducted *in-situ*, i.e. by imaging the changing surface structure in the dissolution medium which provides information about the dynamics of the process. Or it can be conducted *ex-situ*, by viewing the resulting surface topography at different stages of the etching process by removing the sample from solution and conducting the imaging in air. Since the AFM provides this information at the nanometer-to-micron scale, it can provide information about the dissolution mechanisms at the molecular scale. For these reasons, it is increasingly being utilized by pharmaceutical researchers and manufacturers.[6–9]

Dissolution Studies

The influence of different solvents on acetaminophen single crystals was investigated by AFM to gain a better understanding of the dissolution mechanisms at the molecular level.[7] Partial dissolution tests were conducted on the (010) crystal surface with water, dichloroethane, pyridine, acetone, ethyl acetate, and acetic anhydride. Dissolution patterns were produced that are related to the interaction between the solvent molecules and the crystal structure.

As an example, Figure 2 shows parallelogram, square, and hexagonal dissolution patterns formed by water, acetone, and pyridine, respectively. In many cases, the thickness of the steps seen in these images is approximately 10 angstroms, which is consistent with the dissolution of a layer of unit cells. The dissolution process produced etching patterns that are related to the crystal structure, and the surface interaction between the solvent and acetaminophen molecules in the diffusion layer. These results were compared to computer simulations of the same process to gain a better understanding of how the resulting surface texture provides information about the dissolution mechanisms of acetaminophen. In the cases of water, acetic anhydride, and pyridine, the etch pits were formed parallel to the *a* and *c* crystallographic axes of the crystal. However, the etching patterns formed by acetone, dichloroethane, and ethyl acetate were not clearly understood and did not match the structure expected from the computer simulations. It is believed that in these cases, the etching process was disrupted by the absorption of solvent molecules on the crystal surface, resulting in unexpected etching patterns.

This hypothesis was further tested by observing the etching patterns produced by water with the tailor-made additives, acetanilide and 4-methyl acetanilide.[8] Drastically different patterns resulted from small amounts of these additives. This effect is believed to be caused by the absorption of additive molecules that diffuse into the crystal lattice and disrupt the original supramolecular interaction network, changing the etch pits from parallelograms (in pure water) to rectangular, square, or circular forms.

In another example, AFM has also been applied to gain an understanding of the differences in dissolution behavior of the (001) and (100) crystal surfaces of aspirin.[9] Dissolution studies have shown a 50 percent larger dissolution rate for the (100) crystal plane with respect to the (001) surface. TappingMode images show a smooth (001) surface with 7.3-angstrom molecular steps (Figure 3). The (100) surface was much rougher with 50- to 100-angstrom

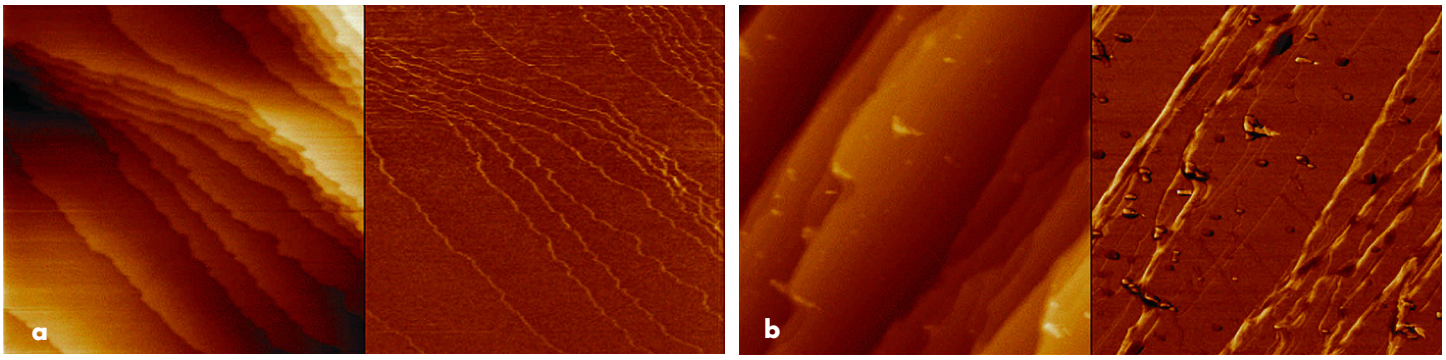

Figure 3. TappingMode images of aspirin crystal surface. a) (001) crystal surface showing 7.3Å monomolecular steps, b) (100) crystal surface irregular steps ranging from 50 to 100Å[9]. 4 µm height (left) and Phase (right) images. Images courtesy C.J. Roberts, Univ. of Nottingham, UK.

steps. Thus, the (100) surface has a greater surface area, which may contribute to the faster dissolution rate.

Further investigation of these surfaces was conducted by the functionalization of the AFM probes with –CH3 terminated groups and –COOH terminated groups to produce hydrophobic and hydrophilic sensors, respectively. Using functionalized probes to study the chemical interactions with the surface is commonly referred to as Chemical Force Microscopy (CFM)[10]. By using amplitude-phase distance relationships between the crystal planes and the functionalized probes, it was observed that the hydrophobic –CH3 terminated probes had a strong attractive relationship with the (001) surface, whereas the hydrophilic –COOH terminated probe had a strong attractive relationship with the (100) surface. Thus, the strong hydrophilic (100) surface would lead to easier wetting in an aqueous environment resulting in a faster dissolution rate.

The dynamics of the dissolution process of aspirin (001) and (100) crystal planes were further tested by conducting real-time AFM dissolution studies in a 0.05M HCl solution.[6] By conducting the imaging during dissolution, the dissolution mechanisms and rate were studied. For the (001) plane, dissolution occurred by the recession of molecular step edges, resulting in a dissolution rate of 0.45 nm/s (Figure 4). For the (100) plane, dissolution occurred by the crystal terraces sinking at a rate of 2.93nm/s. Intrinsic dissolution rates were determined to be 1.35×10^{-7} g s^{-1} cm^{-2} and 8.36×10^{-7} g s^{-1} cm^{-2} for the (001) and (100) planes, respectively. Thus, the (100) plane dissolved at a rate six times greater than the (001) plane.

This difference in the dissolution rate could be contributed partially to the molecular orientation of the crystal faces. The (001) crystal face has benzene rings at the surface, which are not easily removed in the 0.05M HCl solution, whereas, the ester groups are exposed as the terraces on the (001) face, which are more easily removed, resulting in dissolution of the receding step edges. The exposure of many ester groups at the surface of the (100) crystal face leaves all points on the surface susceptible to removal during dissolution, resulting in a faster dissolution rate and a different mechanism for material removal. Also, as discussed in the previous example, the increased surface roughness and the hydrophilic nature of the (100) surface contribute to a faster dissolution rate as well.

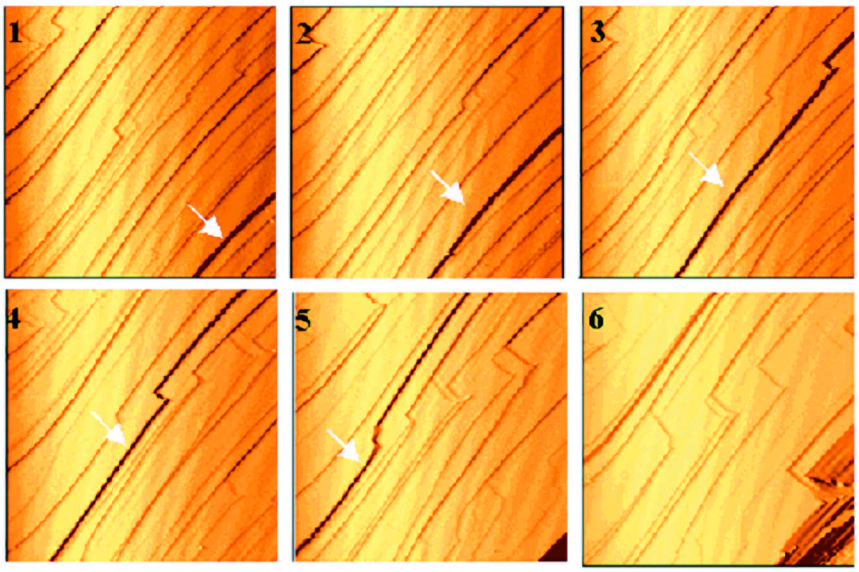

Figure 4. *In situ* dissolution of the (001) in 0.05M HCl by receding step edges. The white arrow shows the progression of the same crystal terrace in each images at times of a) 0, b) 66, c) 132, d) 197, e) 263, and f) 329 seconds.[6] 10µm scans. Images courtesy C. Roberts, University of Nottingham, UK.

Conclusion

These examples highlight some of the advantages atomic force microscopy (AFM) provides researchers in evaluating the conditions of dissolution with respect to different solvents and crystal orientations. AFM dissolution studies may be conducted in two ways: 1) by removing the drug from the dissolution medium to study the resulting structure of the crystal face, or 2) by imaging during the dissolution process to provide insight into the dissolution mechanism and dissolution rate from real-time observations.

AFM in air provides clues to dissolution behavior in samples that have been partially dissolved by examining the patterns formed on the remaining surface. As shown, AFM elucidated how the molecules were removed in steps from a sample surface under different conditions. These steps were molecular in scale, and would be difficult or impossible to see by other techniques. Scanning electron microscopy (SEM) has the resolution to see molecular scale features, but seeing the atomic steps would be very challenging since there is little topography to provide contrast in the Z direction. In addition, SEM requires conductive samples, which are rare in pharmaceutical materials.

Perhaps the most exciting aspect of using AFM for dissolution studies is its ability to image *in-situ*, in fluid, and, with an AFM heater, at 37°C. This allows the three-dimensional monitoring of dissolution behavior as it is occurring, at molecular resolution and in near-physiological conditions. Since the dissolution behavior of a drug determines how effectively it can be absorbed into the body, AFM's detailed visualization is a huge advantage for researchers. Other techniques cannot match these benefits. High-resolution SEM requires a vacuum environment, and optical techniques lack the necessary resolution.

Atomic force microscopy has the potential of greatly increasing our understanding of the dissolution process and drug absorption, particularly when used in collaboration with various macroscale and chemistry tests. It provides another piece to the dissolution puzzle that cannot be elucidated with any other technique. Future AFM dissolution studies should help increase overall drug efficacy and help enable ever greater scientific advances.

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