The tablet's coat du jour

Atomic force microscopy has found a place in pharmaceutical process development.

BY JOHN T. THORNTON

Drug crystal growth, particle characterization, and tablet coatings are critical elements in the manufacture of solid dosage forms. Thus, microscopic examination is important for the design and evaluation of a pharmaceutical product after the steps in the drug formulation process are completed. Because atomic force microscopy (AFM) provides the ability to directly investigate surface structure at nanometer-to-subangstrom resolution in ambient and liquid environments, it has been applied to a wide range of pharmaceutical research, and it delivers a powerful complement to other common analytical techniques.

AFM is performed by drawing a sharp tip on the end of a flexible cantilever across a sample surface while maintaining a small, constant force. Contact mode and tapping mode are two commonly used AFM techniques of operation (*1*). Although AFM was initially used to produce high-resolution topographic images, several related techniques have been developed since to study the physical and material properties of sample surfaces. For example, phase imaging consists of mapping the phase lag of the oscillating cantilever with respect to the drive signal during tapping-mode imaging (*2*). This produces a topographic image along with a phase map that can differentiate areas on the basis of viscoelasticity, adhesion, hydrophobicity, and other properties.

Crystal growth

Therapeutic agents are commonly generated in crystalline forms, the three-dimensional surface morphology and crystal structure of which greatly affect the manufacture, ease of delivery, bioavailability, dissolution rate, and efficacy of the drug. To tailor the crystal growth process to fit desired behavior, growth parameters such as temperature, pH, concentration, and additive levels need to be optimized. In situ visualization of crystallization by AFM has been conducted to optimize the growth conditions to produce a desired morphology, as well as to study growth mechanisms and defect formation (*3, 4*).

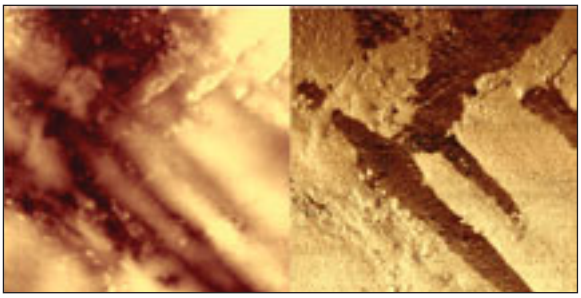

Figure 1. Topographic tapping-mode image (left) and phase imaging with hydrophilic probes (right). The phase image shows the distribution of A (dark) and B (light) polymorphs that are not evident in the tapping-mode image. The phase image contrast is due to differences in hydrophobicity. (4-µm scans.) (Images courtesy of Clive Roberts, University of Nottingham, U.K.)

For example, Christopher Yip and Michael Ward, then at the University of Minnesota (Minneapolis), used AFM to study in situ crystallization characteristics of several insulin forms. Because of the low forces needed to image the insulin surface, they used tapping-mode imaging directly in the crystallization liquor. The epitaxial growth of a screw dislocation was observed over 11 h on $Lys^{B28}Pro^{B29}$ insulin (*4*). Observing the crystallization behavior in situ made it possible to determine growth rates and observe defect formation in real time. Step advancement occurred at 2×10^{-6} µm/s, which corresponds to the attachment of approximately five unit cells (15 $Lys^{B28}Pro^{B29}$ hexamers) per second. Defects appeared to be caused by large insulin aggregates that were unable to align properly with the crystal structure because of poor mobility, thus forming dislocations and voids in the growing terraces.

These observations were conducted to study differences in growth characteristics between $Lys^{B28}Pro^{B29}$ insulin and wild-type porcine-bovine insulin. $Lys^{B28}Pro^{B29}$ insulin differs from wild-type porcine or bovine insulin by a sequence inversion at the C-terminus in the B-chain. This modification was designed to reduce the insulin monomer association for better dissolution properties. But the sequence inversion also produced differences in crystallization behavior, as observed by AFM.

$Lys^{B28}Pro^{B29}$ insulin was shown to have smaller attachment energies (ΔG_k), more rounded screw dislocations, larger terrace widths, and more persistent vacancies in the (001) plane. These differences can have a significant influence on the crystal quality, which affects the manufacture, ease of delivery, and bioavailability of the compound. Furthermore, if the drug has defects in the crystal structure, it might be a less effective therapeutic agent because of changes in and less control over the dissolution rate. Using AFM to study in situ crystallization also allows the pharmaceutical scientist to judge the quality of the growth process and then adjust the growth parameters (e.g., temperature, pH, concentration, and additives) to reduce the defects.

Polymorphism

The ability of a drug substance to form into more than one crystalline form is called polymorphism, and different polymorphs possess different physicochemical prop-

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erties, which affect solubility, dissolution, adsorption, melting point, and stability (*5*). Thus, polymorphic characterization is an important parameter in maintaining high product quality and reproducibility in the pharmaceutical industry.

In Yip and Ward's study, the polymorphic forms of insulin were identified by using AFM to map the molecular lattice spacing in all three dimensions. Clive Roberts and colleagues at the University of Nottingham and SmithKline Beecham Pharmaceuticals (Harlow, U.K.) used the advanced phase-imaging technique to identify and map the distribution of polymorphs of the drug cimetidine (*6, 7*). Force–interaction studies (amplitude–phase distance relationships) were then conducted to identify the polymorphs on the basis of differences in hydrophobicity. In Figure 1, cimetidine polymorphs A and B are not easily distinguished in the topographic image, but their distribution is easily characterized in the phase image. The contrast in the phase images is most likely due to the differences in hydrophobicity between the polymorphs, which produce a difference in the tip–sample interaction because of variations in capillary force. This contrast was investigated by conducting the experiment with hydrophilic and hydrophobic functionalized probes.

Particles

Production of solid dosage forms commonly begins with the formation of the drug into particles, typically within 0.1–10 µm. Characterization of these particles can be important before drug formulation because their morphology, size, and shape can provide information about the manufacturing process (*8*). Particle size has also been shown to influence dissolution rate, bioavailability, content uniformity, stability, texture, flow characteristics, and sedimentation rate, and thus has a significant effect on formulation and therapeutic efficiency.

Many methods are commonly used to investigate particles, such as dynamic light scattering and laser diffraction. But these techniques sample a large number of particles to provide a distribution of particle size or characteristics (*9*). There are often cases in which studying the particles individually becomes a key step in understanding a particle system. A common method of directly studying individual particles is transmission electron microscopy (TEM). But the sample preparation of small particles for TEM is often challenging and time-consuming.

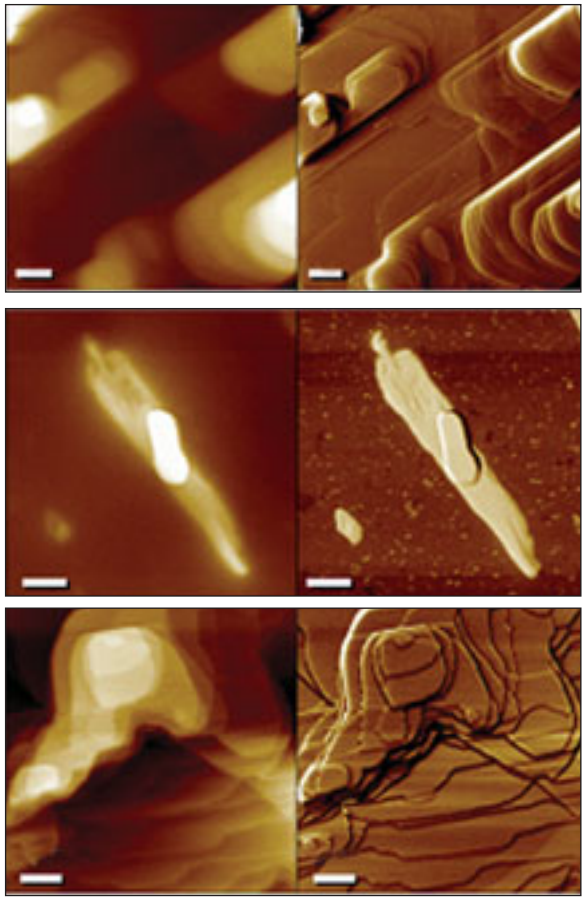

Figure 2. Height (left) and phase (right) images of Paracetamol formed into drug particles by micronization and SEDS. Top, raw starting material showing crystalline lamellae; middle, micronized particle showing rough, irregular structure; bottom, SEDS particles showing regular, smooth structure with 0.9-nm crystalline steps. The scale bar is 1 µm. (Images courtesy of Molecular Profiles and Bradford Particle Design, Ltd.)

AFM, however, has been used to examine pharmaceutical particles directly to correlate their morphology with the manufacturing process and behavioral properties more quickly and easily (*8, 10*). Figure 2 shows an example of using AFM to characterize drug particle morphology. Drug particles are traditionally formed by milling a drug crystal to particle sizes less than 10 µm by micronization or spray-drying techniques. However, problems can result from these techniques because of batch-to-batch variations, residual solvent, and statically charged particles that can affect powder stability and flow. Another method of particle formation, called solution-enhanced dispersion by supercritical fluids (SEDS), overcomes many of these problems and provides more control of the particle size, shape, and morphology (*10*).

The top AFM images in Figure 2 show the starting raw material of Paracetamol, in which crystalline terraces are seen. Particles formed by micronization and SEDS are shown in the middle and bottom images, respectively. The micronized particles vary in size, are irregular, and have a significant amount of surface roughness, whereas the SEDS particles have a regular shape and a size of approximately 10 µm and show less roughness than the raw starting material. As shown through AFM, the smoother surface and regular shape produced by SEDS should reduce the batch-to-batch variations and static-charge problems encountered with the micronized materials and improve the flow properties of the particles.

Granules

Once the drug is in particulate form, it is often formed into a granule by mixing the particles with binding agents, diluents, and disintegrating agents. The wet granulation process consists of adding a liquid binder or adhesive to the mixture, passing the wetted mass through a screen sieve of the desired mesh size, and drying the granules. The resulting granules are typically in the range of a few millimeters and show improved flow properties, as well as chemical and physical stability. AFM has been successful in characterizing the morphology and roughness of granules to correlate their surface structure with the underlying physicochemical and mechanical processes during manufacture (*8*).

Coatings

Many coatings may be applied to tablets to serve various purposes. Common uses of pharmaceutical coatings include protecting the drug from air and humidity, and controlling the dissolution behavior. Sugar coatings are commonly applied to tablets, as are polymer coatings, which are more durable, less bulky, and less time-consuming to apply. The polymer coatings are often designed to rupture in the intestinal tract to avoid stomach irritation and improve drug absorption. Coating granules and other substances are also key steps in the design of controlled-release and microencapsulated dosage forms.

AFM has been commonly used to correlate the surface of coatings and thin films to deposition parameters (such as temperature, rate, and composition) and performance (*11*). AFM is commonly used to evaluate coating surface morphology, roughness, hardness, porosity, surface area, and compositional distribution. Changes in these properties have also been studied with respect to aging and environment.

The bottom line

AFM provides pharmaceutical researchers and manufacturers with a wide variety of techniques to evaluate the steps of the drug formulation process. With high-resolution imaging in air and fluid environments, AFM is used to study dynamic processes, fabrication variables, component distribution, and structure–function relationships. With its capability for tapping-mode and phase-imaging techniques, AFM provides information that cannot be acquired by other analytical techniques, and thus, its use is increasing.

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