The Discrimination of Drug Polymorphic Forms from Single Crystals Using Atomic Force Microscopy

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Received February 22, 2000; accepted April 11, 2000

KEY WORDS: tapping mode; atomic force microscopy; cimetidine; polymorphs; phase imaging; amplitude-phase, distance.

INTRODUCTION

The potential of a compound to exhibit polymorphism (1) produces an important challenge to pharmaceutical and chemical industries, who wish to synthesise drugs and chemicals of consistent quality (2). History has demonstrated that changing of crystal polymorphic forms during any stage of drug development is not uncommon and can have severe implications (1). A recent example is the case of anti-AIDS drug "Ritanovir," where polymorphic change during late stages of production led to different dissolution and possibly different absorption profiles (3).

There are a number of techniques available to enable polymorphic characterization, including X-ray crystallography, infrared (IR) spectroscopy, thermal analysis and electron microscopy (1,2). In general, different compounds may often require a combination of different strategies (2) for their adequate characterization.

Previously, we have demonstrated that phase imaging in tapping mode (TM) atomic force microscope (AFM) can be used to discriminate between two polymorphs of the drug cimetidine in the form of pressed disks (4). However, the abundance of pressure-induced polymorphic transitions amongst many compounds (5) limits the applicability of this approach and necessitates evaluating its use on single crystals. Also, from a pharmaceutical point of view, analysing single crystals is ideal, since often only very small amounts of a substance are available for initial analysis. We believe the information provided by AFM can at an early stage help predict important information about the behaviour of the drug on large-scale operations.

In this paper we extend the technique to single crystals, to demonstrate the potential applicability to other polymorphic systems and discuss the advantages of the proposed method.

Cimetidine, N″-cyano-N-methyl-N′-{2-[[(5-methyl-1H-imidazol-4-yl)methyl] thio] ethyl} guanidine has seven known polymorphic forms, that can be produced using a variety of well established crystallization conditions (4). The only polymorphic forms used pharmaceutically are polymorphs A and B. Polymorph A is easier to handle, particularly in large scale operations due to good flow properties, and lack of adherence making it most suitable for manufacturing tablets (4). Polymorph B however, has poor flow properties and is more appropriate for preparing suspensions (4).

Since the inception of the AFM by Binnig *et al.*, (1986) (6), it has become an important tool for imaging the topography of a variety of surfaces (7,8) and for direct measurement of discrete intermolecular forces (9,10). In more recent years the development of TM-AFM (11) has generally overcome the problems of sample damage, which can occur when imaging soft or weakly adsorbed samples in contact mode AFM. In addition to acquiring topography data, the phase angle of cantilever oscillation relative to the driving oscillation is measured and recorded as a "phase image" (11). Further surface related properties can also be investigated by measuring the amplitude and/or phase lag of cantilever oscillation against tip-sample distance (11,12,13). We refer to such data as amplitude-phase, distance (a-p,d) measurements (4).

MATERIALS AND METHODS

Cimetidine samples were used as supplied by SmithKline Beecham Pharmaceuticals. Polymorph A was crystallised at room temperature from 2-propanol (IPA) and consists of platelet shaped crystals 9 μm–100 μm. Polymorph B was crystallised from a hot solution of 10% IPA in water and consists of needle shaped crystals 200 nm–35 μm. Pure crystal samples of each polymorph were prepared by sprinkling the crystalline powder onto double-sided adhesive tape with one side fixed onto an AFM sample stub. This resulted in isolated single crystals being available for investigation.

AFM measurements were performed using a Nanoscope IIIa MultiMode AFM (Digital Instruments, Santa Barbara, USA). All measurements were acquired in air using tapping mode with the E-type scanner (range 10 μm × 10 μm × 2 μm) and silicon TESP tips (Digital Instruments). These are 125 μm long rectangular shaped cantilevers with a nominal force constant and resonant frequency of approximately 50 N/m and 300 kHz respectively.

To ensure consistent interactions, a-p,d measurements were performed on the same crystal face. Scanning electron microscope (SEM) images of disk samples (results not shown) revealed that the largest faces of platelet shaped polymorph A and needle shaped polymorph B crystals are exposed at the surface. Therefore, when performing a-p,d measurements on single crystals an online optical microscope was used to select the position of measurements. This procedure resulted in consistent and reproducible a-p,d curves for both samples.

The effect of hydrophobic functionalization of AFM

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ABBREVIATIONS: R.H., relative humidity; TM-AFM, Tapping mode Atomic Force Microscopy; APDES, 3-aminopropyl dimethylethoxysilane; a-p,d amplitude-phase, distance.

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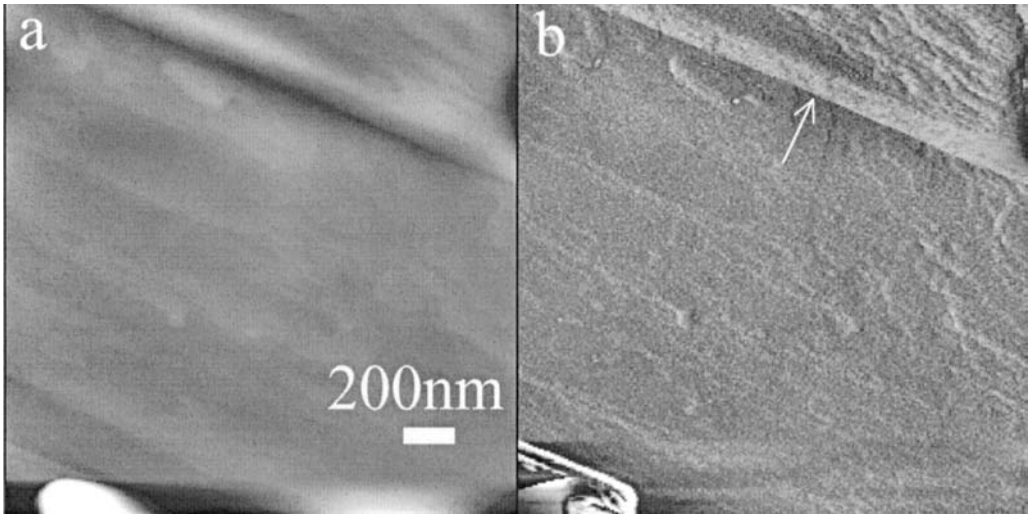

Fig. 1. 2 μm × 2 μm TM-AFM image from the surface of cimetidine polymorph A single crystal (a) height image (Z range = 300 nm), (b) phase image (Z range = 90 degrees).

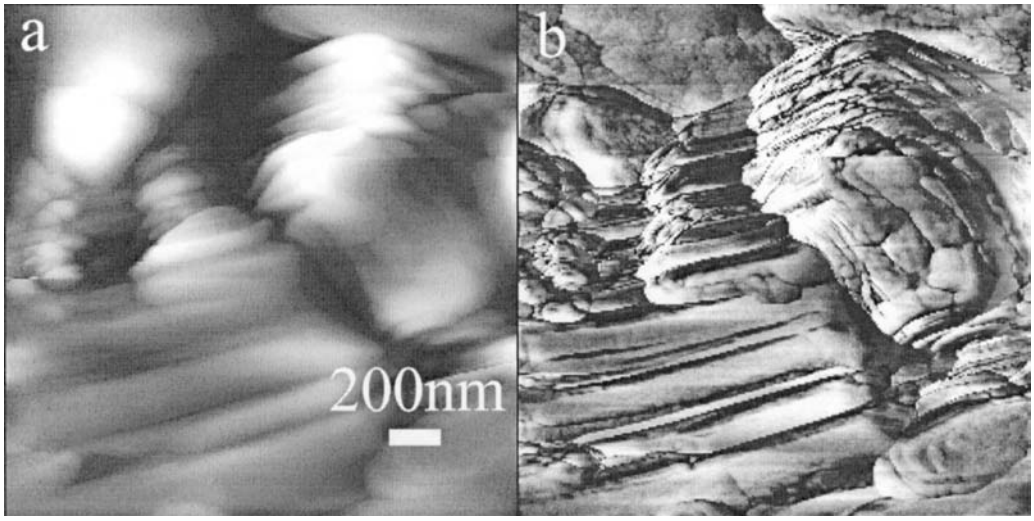

Fig. 2. 2 μm × 2 μm TM-AFM image from the surface of cimetidine polymorph B single crystals (a) height image (Z range = 300 nm), (b) phase image (Z range = 90 degrees).

probes on a-p,d curves was investigated by incubating the probe under 3-aminopropyl dimethylethoxysilane (APDES) for two hours following a 20 seconds treatment with oxygen plasma at 10 watts (RF Plasma Barrel etcher PT7100, Bio-Rad). The probes were then rinsed using methanol, isopropyl alcohol and water consecutively.

In order to allow the comparison of obtained results, several contributing factors need to be monitored. For example, the phase curve profile is very sensitive to the driving frequency relative to the cantilever's oscillation resonance and the phase contrast is not only sensitive to set point, but also to the oscillating cantilever's amplitude (15). Here, we keep the drive frequency at the cantilever's oscillation resonance, which is monitored at tip-sample distance below 100 nm. To confirm results, the experiments were repeated with different tips, but the data used for comparison purposes are obtained using the same tip.

RESULTS AND DISCUSSION

Images (2 μm × 2 μm) from the surface of polymorphs A and B single crystals are presented in Figs. 1 and 2, respectively. Figure 1 demonstrates a relatively flat surface where a step edge (indicated by an arrow) can be seen. Figure 2 illustrates a 2 μm × 2 μm image of several needle shaped crystals of polymorph B. The possibility of tip convolution effects, generating multiple images of individual prominent features can be discounted since the features are different distances apart.

The a-p,d measurements acquired from single crystals of cimetidine polymorphs A and B are illustrated in Fig. 3. The a-p,d curves are in good agreement with corresponding a-p,d curves obtained previously from the pressed disks (4). A-p,d measurements from polymorph A (Fig. 3a), have a larger attractive section compared to polymorph B (Fig. 3b). This can be explained by the more hydrophilic nature of polymorph A,

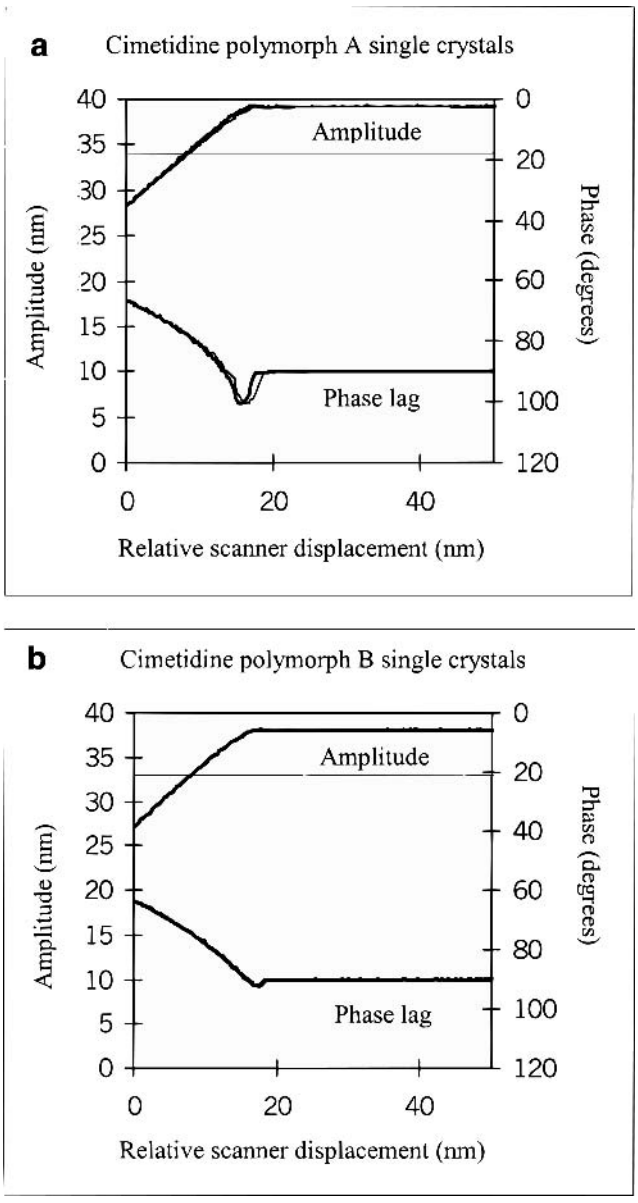

Fig. 3. A-p,d curves from the surface of (a) polymorph A single crystals and (b) polymorph B single crystals.

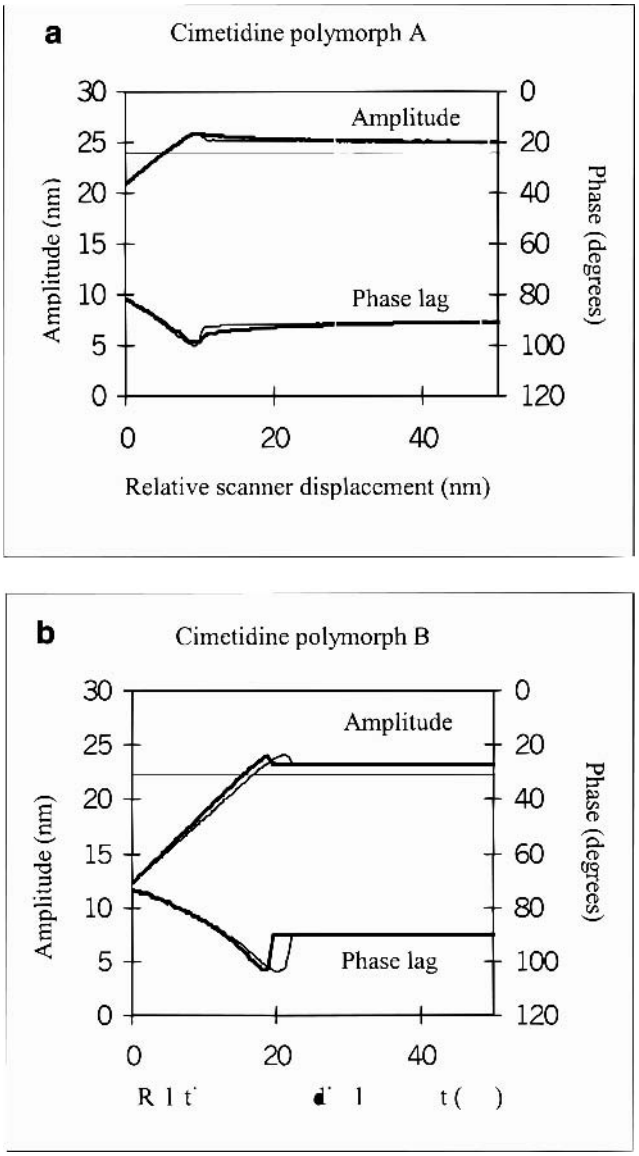

Fig. 4. A-p,d curves obtained using alkylsilane functionalised probes (a) polymorph A and (b) polymorph B.

resulting in a stronger capillary force (4). The influence of topographic variations on the observed a-p,d curves is minimal as the contact area of the AFM probe utilised is likely to be less than 50–100 nm^2 (at its apex), smaller than the crystal planes studied.

The a-p,d curves obtained using alkylsilane functionalised probes are displayed in Fig. 4. Here, the profile of a-p,d curves have reversed due to the stronger hydrophobic interactions between the functionalised tip and polymorph B (Fig. 4b). Thus, the chemical manipulation of the surface of the AFM probes has enabled the enhancement or diminution of the contrast obtained and may be used for selective detection of surface properties.

These results demonstrate how TM-AFM can be used to identify different polymorphs as well as characterising their surface properties. The differences in hydrophilic/hydrophobic properties of the two polymorphs observed here, in conjunction with known particle size, size range and shape contributes to our understanding and prediction of the differences in their formulation suitability's and physicochemical properties. Polymorph B consists of needle shaped crystals, exhibiting poor flow properties due to hindrance caused by crystal shape and as a result of adhesion and cohesion effects making polymorph B a poor candidate for tablet manufacture. Whereas, polymorph A has platelet shaped crystals with better powder flow and a more hydrophilic nature which leads to lower cohesion effects and a greater suitability for tableting. The hydrophilic character of polymorph A also allows easier wetting of its surface, which may be a contributing factor that leads to its higher solubility than polymorph B (15). When comparing a-p,d curves from different samples the possible effects of crystal faces studied must be considered. As detailed, variations arising from measurements on different faces of the crystals of polymorphs A

and B were avoided here. The consistency of results obtained verifies that the steps taken were appropriate for the scope of this study.

However, undoubtedly a contributing factor for variations in physicochemical properties, is the expression of different functional groups at the different crystal faces. The resolution of TM-AFM combined with functionalised AFM probes to delineate the surface chemistry as shown here, has the potential to be extended to the study of variations between individual crystal planes of a single crystal (16).

CONCLUSION

Investigations of the single crystals of the two cimetidine polymorphs have proved the versatility of TM-AFM for polymorphic characterisation. Single point amplitude-phase, distance (a-p,d) analysis, demonstrates that these curves can be used to unequivocally distinguish each polymorph. Tailoring surface hydrophobicity of the AFM probes can be used to promote or diminish the level of interaction observed between the probe and samples.

The application of TM-AFM in characterising polymorphs could prove of particular use, because of its ability to combine polymorphic discrimination and surface property differentiation through two means of investigation; phase imaging as well as single point a-p,d measurements. In addition, the ability of the proposed method to rapidly analyse single crystals of the material with minimal preparation, may warrant its use as a complementary tool to other techniques such as I.R. and thermal analysis in polymorphic characterisation. We therefore, propose that with suitable development TM-AFM could provide a powerful tool in pharmaceutical scientist's armoury for polymorphic discrimination and characterisation.

ACKNOWLEDGMENTS

The authors thank SmithKline Beecham and the EPSRC for the provision of a studentship for AD. GWHS and XC acknowledge funding from the BBSRC.

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