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Surface Analysis

Use of In Situ Atomic Force Microscopy to Follow Phase Changes at Crystal Surfaces in Real Time**

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Atomic force microscopy can be used to identify and observe phase changes at crystal surfaces where the transformation is accompanied by a change in the spacing between layers of molecules. The conversion of a metastable polymorph of the caffeine–glutaric acid cocrystal to the thermodynamically stable form was analyzed continuously in situ using intermittent contact-mode atomic force microscopy (IC-AFM), allowing the mechanism by which molecules move during the transformation to be determined.

Polymorphism, as defined by Haleblian and McCrone[1] is "the ability of a given element or compound to crystallize in more than one distinct crystal species." As a result of the variability in solid-state structure, polymorphs of a substance may have different physicochemical properties[2] such as solubility, melting point, stability, bioavailability, and compaction behavior. For this reason, polymorphism is important in industrial sectors such as the pharmaceutical industry.[3] The phenomenon of polymorphism applies equally to multicomponent systems such as cocrystals, salts, and solvates.[4]

Atomic force microscopy,[5] a type of scanning probe microscopy, is a useful tool for imaging and measuring features at the nanoscale. In recent years, atomic force microscopy has been used extensively to study various aspects of molecular crystals such as the mechanism of crystal growth,[6] additive-induced growth inhibition,[7] crystallization,[8] and dissolution.[9] Also, nanoindentation studies within the AFM have been used to estimate the Young's modulus and hardness of different pharmaceutical materials[10] including polymorphs,[10c,d] salts,[10e] and cocrystals.[10f] This work has been summarized recently in reviews by Ward[11] and Chow et al.[12]

The three main imaging modes available with AFM are contact mode (C-AFM), where the applied force to the cantilever is fixed to a certain value within the constant compliance region, intermittent contact mode, where the base of the cantilever is allowed to oscillate slightly below its resonance frequency, and non-contact mode (NC-AFM). A limitation of non-contact mode is that ultrahigh vacuum conditions are required, making it unsuitable for in situ studies. Generally, intermittent contact mode is a suitable imaging technique for soft organic samples as it is less destructive to sample surfaces than contact mode.

Caffeine and glutaric acid form two cocrystal polymorphs which can be prepared by liquid-assisted grinding and solution crystallization.[4e] The stability of these forms under different relative humidity conditions was studied as part of a larger investigation into caffeine–dicarboxylic acid cocrystals where certain cocrystals were found to increase the stability of caffeine to hydrate formation.[4f,13] The two polymorphs of caffeine–glutaric acid showed notable variation in stability to high humidity with Form I transforming to Form II within 24 h, and Form II being stable for over three days under these conditions before undergoing conversion to caffeine hydrate. Herein, we show that atomic force microscopy can be further used to distinguish the two polymorphs of the caffeine–glutaric acid cocrystal on the basis of the thickness of molecular layers of the two forms and use this difference to monitor changes at the crystal surfaces during the transformation of Form I to Form II.

As part of this study, single-crystal X-ray diffraction analysis of Form I was performed at ambient temperature (295 K), and revealed subtle differences when compared to the previously reported structure which had been collected at 180 K[4e] (Table 1). At room temperature, the space group is $P2_1/m$, rather than $P2_1/c$, and one of the unit cell dimensions is halved corresponding to caffeine and glutaric acid molecules sitting on a mirror plane with occupancy values of 0.5. These observations indicate that a structural modulation occurs in Form I between room temperature and 180 K. It is suggested that the room-temperature structure is referred to as Form I and the low-temperature structure as Form I′.

The cocrystal polymorphs (Form I and Form II) both have a 1:1 stoichiometry of caffeine and glutaric acid with similar hydrogen-bond synthons, and consist of linear O–H···O hydrogen-bonded tapes established between the hydroxy

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Table 1: Crystallographic data for caffeine–glutaric acid Form I and Form I′.

	Form I′ [180 K][a]	Form I [295 K]
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/m$
a [Å]	13.0129(5)	8.6480(5)
b [Å]	6.6017(2)	6.7296(6)
c [Å]	17.1427(8)	13.0336(8)
β [°]	97.836(1)	97.951(5)
V [Å^3]	1458.93(10)	751.23(9)

[a] Reported by Trask et al.[4e]

groups of one glutaric acid molecule and the carbonyl of the next. The second carboxylic O-H group of glutaric acid is hydrogen bonded to the imidazole ring of caffeine through an O–H···N hydrogen bond (Figure 1). The hydrogen-bonded tapes stack to form layers. The orientations of molecules

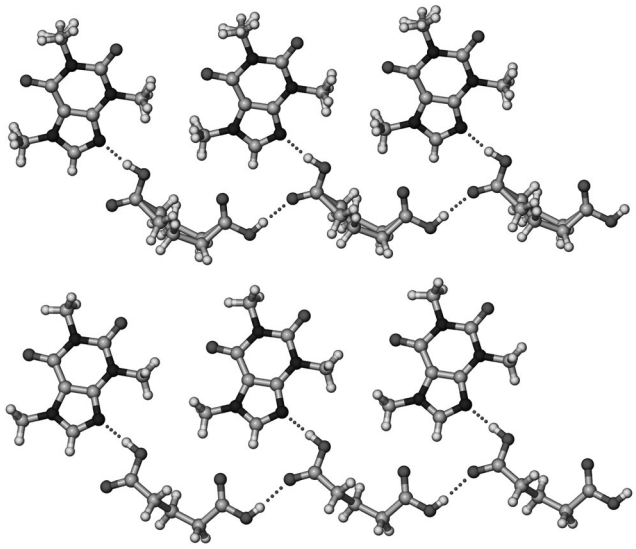
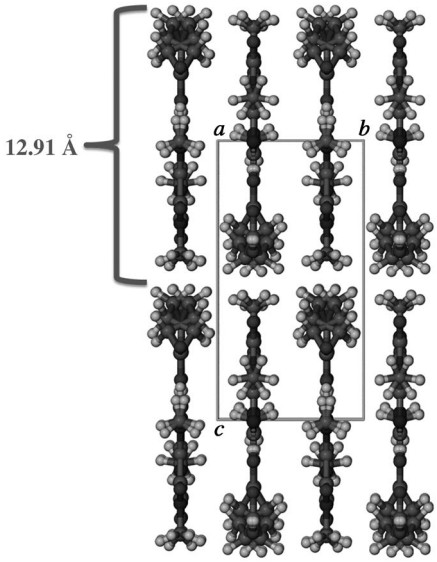


Figure 1. Hydrogen bonding present in the two conformational polymorphs of the caffeine–glutaric acid cocrystal: Form I (top) viewed along the *b* axis and Form II (bottom) viewed along the *a* axis.



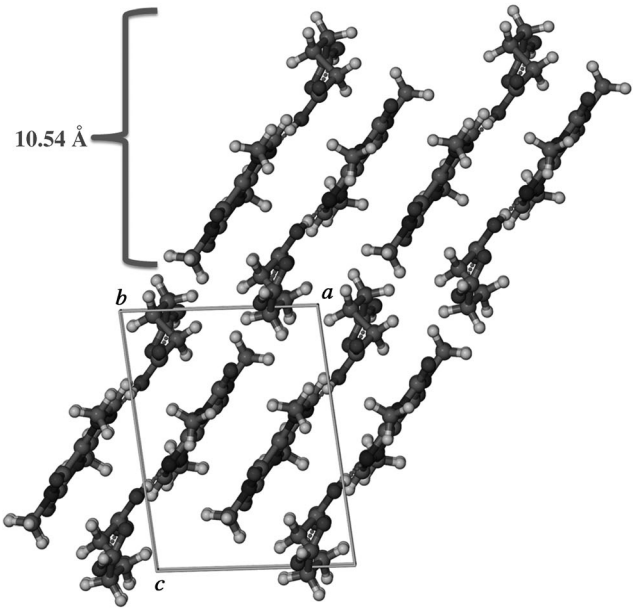


Figure 2. Packing difference between the layers of caffeine–glutaric acid cocrystal structures in Form I (top) viewed along the *a* axis and Form II (bottom) viewed along the *b* axis.

within these layers are slightly different in the two polymorphs. Form I consists of parallel tapes of caffeine and glutaric acid molecules stacked in an alternating fashion that are connected through weak van der Waals interactions to give a flat layer, whereas the tapes in Form II are tilted and form into a slightly corrugated layer (Figure 2). The major difference in packing between the two forms is a result of changes in the conformation of the alkyl chain of the glutaric acid molecules. The morphologies of the two polymorphs were quite different making them easily distinguishable under an optical microscope—lath-shaped crystals for Form I, block-shaped crystals for Form II.

The IC-AFM analysis was performed on crystals of Forms I and II obtained from solution crystallization, along with face indexing to determine which crystal face was studied. The face indexing of a lath-shaped crystal of Form I revealed that the dominant face is the large, molecularly flat (001) face, which is in agreement with the predicted morphology calculated using the Bravais, Friedel, Donnay, and Harker model (BFDH model; see Figure S3 in the Supporting Information). IC-AFM images of the (001) face show a layered structure (Figure 3 and Figure S1 in the Supporting Information) and step heights were measured to be 13.0 Å (with a standard deviation of 0.14 Å), a value in agreement with the spacing between layers of molecules in the crystal structure of Form I, 12.91 Å, suggesting that each step corresponds to one supramolecular layer (Figure 2).

A block-shaped crystal of Form II was face indexed, with the dominant face identified as the (001) face, in agreement with BFDH predictions (see Figure S4 in the Supporting

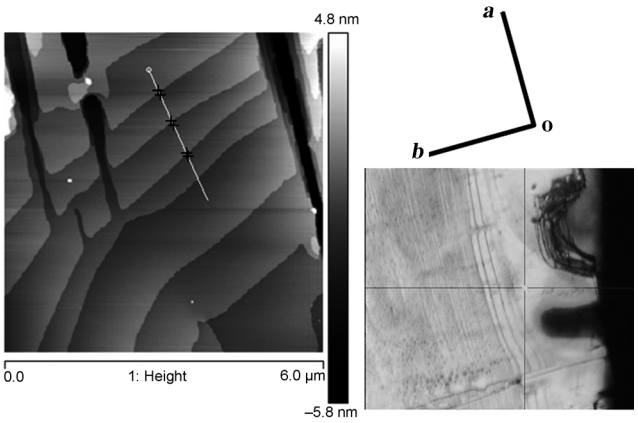


Figure 3. IC-AFM height image of the (001) surface of a Form I crystal showing steps corresponding to individual supramolecular layers. An optical image displaying the orientation of the crystal is also shown.

Information). IC-AFM images of the (001) surfaces of crystals of Form II obtained from solution crystallization show rounded features protruding from the crystal surface (see Figure S5 in the Supporting Information), and individual crystal layers were not evident. After cleaving the crystals along the (001) plane, however, predominantly flat (001) surfaces with widely spaced parallel steps were observed (Figure 4 and Figure S2). The heights of the steps were measured to be 10.5 Å (with a standard deviation of 0.19 Å), in good agreement with the interlayer spacing in the Form II crystal structure of 10.54 Å.

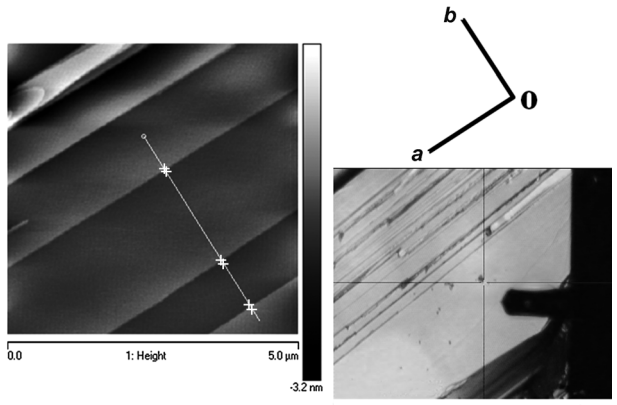


Figure 4. IC-AFM height image of the cleaved (001) surface of a Form II crystal showing steps corresponding to individual supramolecular layers. An optical image displaying the orientation of the crystal is also shown.

AFM images of the surfaces of Form I crystals and cleaved Form II crystals show strong similarities, with both being substantially flat and showing parallel steps. Importantly, however, it was possible to distinguish the two polymorphs using AFM on the basis of the heights of the supramolecular layers.

As outlined above, under high humidity conditions Form I converts to Form II. Changes at the surface of a Form I crystal during this conversion were investigated by performing an analysis on a 3 μm^2 area at ambient temperature and 70% relative humidity for 1 day using real-time IC-AFM (Figure 5). There is a clear change in the appearance of the

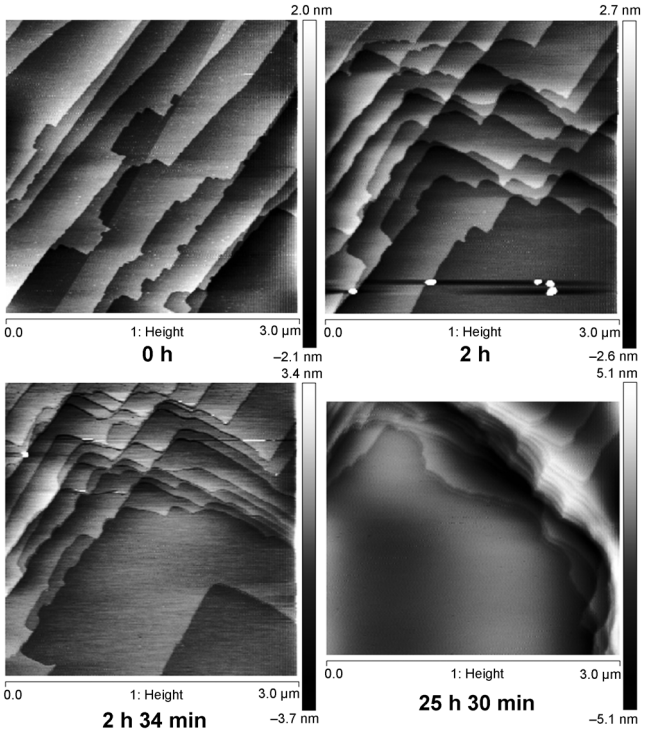


Figure 5. Real time in situ IC-AFM images of the movement of layers at the surface of a caffeine–glutaric acid coccrystal undergoing a phase change from Form I to Form II at ambient temperature and 70% relative humidity. Images were recorded over a 26 hour period, with the phase change occurring in all surface layers at the 2 hour 34 minutes time point.

surface between the initial measurement and that after two hours. In the initial image steps marking the edges of molecular layers can be seen running primarily in the [010] direction, from the bottom left to top right of the image. After two hours the edges of layers run in both the [010] and [100] directions. A key observation was that this step movement occurred before any changes in step height were observed, and so before the crystal converted from Form I to Form II. Importantly, this means that the re-arrangement of the surface layers is caused by the interaction of water with molecules at the surface of Form I, rather than being associated with the form change itself. The form change was observed after approximately 2 h and 30 minutes, as evidenced by a change in the heights of surface layers from that characteristic of Form I (about 12.9 Å) to one of 10.5 Å characteristic of Form II (there is no crystal plane of caffeine hydrate which has a d-spacing consistent with this measured value of 10.5 Å), and occurred rapidly throughout the 3 μm^2 area under analysis (the change occurred across the whole area within the time taken to record one frame: approximately five minutes). By comparison, powder X-ray diffraction measurements of the form change in crystals as a whole

(under similar conditions) showed approximately 41 % conversion after 24 h as measured by quantitative Rietveld analysis (see Figure S6 in the Supporting Information), suggesting that the transformation occurs initially at the crystal surface before propagating into the crystal. The form change appears to be a single-crystal to single-crystal transformation, with the (001) plane common to both structures and each layer in Form I remaining as a single layer in Form II. It can therefore be concluded that during the phase transformation molecules in each of the parallel layers of Form I are tilted by 38° in a concerted manner resulting in a decrease in step height by 2.5 Å to that corresponding to Form II (Figure 6). The form change occurs without the

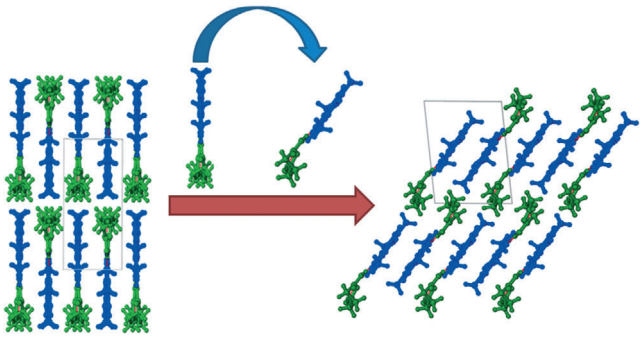


Figure 6. Phase transformation of Form I to Form II involves tilting of the layers by 38° in a concerted manner resulting in a decrease in step height by 2.5 Å.

formation of cracks at the crystal surface. Interestingly, a similar concerted, layer-by-layer polymorphic transformation mechanism has recently been hypothesized on the basis of molecular dynamics simulations for the β to α transition in DL-norleucine.[14]

In conclusion, we believe for the first time, AFM height images have been used to distinguish different crystal forms of a material (Form I and Form II of the caffeine–glutaric acid cocrystal). Step movement and surface rearrangements which occurred both prior to and during the polymorphic phase transformation of Form I to Form II under high humidity conditions were studied using in situ IC-AFM height images. This work shows that AFM height images may be an alternative technique to AFM nanoindentation and phase imaging[15] for distinguishing polymorphs of a substance with an AFM instrument and be a key tool for investigating the surfaces of samples undergoing polymorphic changes thereby giving important insights into conversion mechanisms.

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