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Research Paper

Nanoscale Characterisation and Imaging of Partially Amorphous Materials using Local Thermomechanical Analysis and Heated Tip AFM

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Purpose. The purpose is to investigate the use of thermal nanoprobes in thermomechanical and heated tip pulsed force modes as novel means of discriminating between amorphous and crystalline material on a sub-micron scale.
Materials and methods. Indometacin powder was compressed and partially converted into amorphous material. Thermal nanoprobes were used to perform localised thermomechanical analysis (L-TMA) and heated tip pulsed force mode imaging as a function of temperature.
Results. L-TMA with submicron lateral spatial resolution and sub-100 nm depth penetration was achieved, allowing us to thermomechanically discriminate between amorphous and crystalline material at a nanoscale for the first time. The amorphous and crystalline regions were imaged as a function of temperature using heated tip pulsed force AFM and a resolution of circa 50 nm was achieved. We are also able to observe tip-induced recrystallisation of the amorphous material.
Discussion. The study demonstrates that we are able to discriminate and characterise amorphous and crystalline regions at a submicron scale of scrutiny. We have demonstrated the utility of two methods, L-TMA and heated tip pulsed force mode AFM, that allow us to respectively characterise and image adjacent amorphous and crystalline regions at a nanoscale.
Conclusions. The study has demonstrated that thermal nanoprobes represent a novel method of characterising and imaging partially amorphous materials.

KEY WORDS: amorphous; atomic force microscopy; glass transition; indometacin; microthermal analysis.

INTRODUCTION

The interfacing of atomic force microscopy (AFM) with thermal (1) and spectroscopic (2) methods has led to the development of a new generation of techniques that allows characterisation of specific regions of complex samples. These site-specific thermal techniques have been referred to under the umbrella terms of microthermal analysis when referring to localised thermometric, thermomechanical, and calorimetric measurements. In addition the same probes may be used in scanning mode (scanning thermal microscopy) so as to obtain images base on thermal properties.

In its original form, microthermal analysis involved replacing the conventional AFM probe by a Wollaston wire, the apex of which is etched away to reveal the platinum filament (3). By applying a voltage to the tip, the filament may be selectively heated, allowing thermal measurements to be made on selected regions of complex samples. More specifically, the heated probes allow a range of measuring modes to be used. These include localised thermomechanical analysis (L-TMA) whereby the sensor position is measured as a function of tip temperature, thereby allowing transition temperatures to be detected via the penetration of the tip into the sample at the softening point (melting or glass transition). This method has recently been used to perform three dimensional imaging of multicomponent samples (4) via the possibility of simultaneously identifying (via the transition temperature) and measuring the thickness (via measuring the probe displacement) of the individual components. Other modes include heat flow measurements (i.e. using the system in a manner analogous to differential scanning calorimetry) (5), temperature measurements (6) and heated tip pull-off force measurements (7). All of the above involve one-point determinations on a specific region or regions of sample, hence it is necessary to perform a series of measurements in order to develop a profile across the sample surface.

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ABBREVIATIONS: AFM, atomic force microscopy; L-TMA, localised thermomechanical analysis; MTA, microthermal analysis; PFM, pulsed force mode.

A major limitation to this family of approaches to date has been the size of the probes available, resulting in typical spatial resolutions of the order of ten microns. We have explored the use of higher resolution micromachined probes for imaging and local thermal analysis (6) but these probes have not passed into routine use as they were insufficiently robust for repeated use. A recent parallel development within the thermal probe field has been pursued by IBM and is referred to as the millipede. In brief, this consists of a series of high resolution probes which use a thermal signal for data storage applications. The group of King has developed similar thermal probes that are capable of nanoscale analysis (8, 9) and their utility as a means of nanocharacterisation has been demonstrated (10). However, the tip height of these probes is typically less than 1 micron and this limits their application to very flat samples. Here we present the first results using much larger tips (in the sense of tip height) that are suitable for more topographically complex 'real world' samples such as those studied here. In brief, the probes are composed of boron- or phosphorus-doped silicon that renders the probe electrically conductive. The free end of the cantilever is doped with a lower level of contaminant than the legs, hence it has a higher electrical resistance allowing selective heating at the tip end. Figure 1a and b show images of the tips in question.

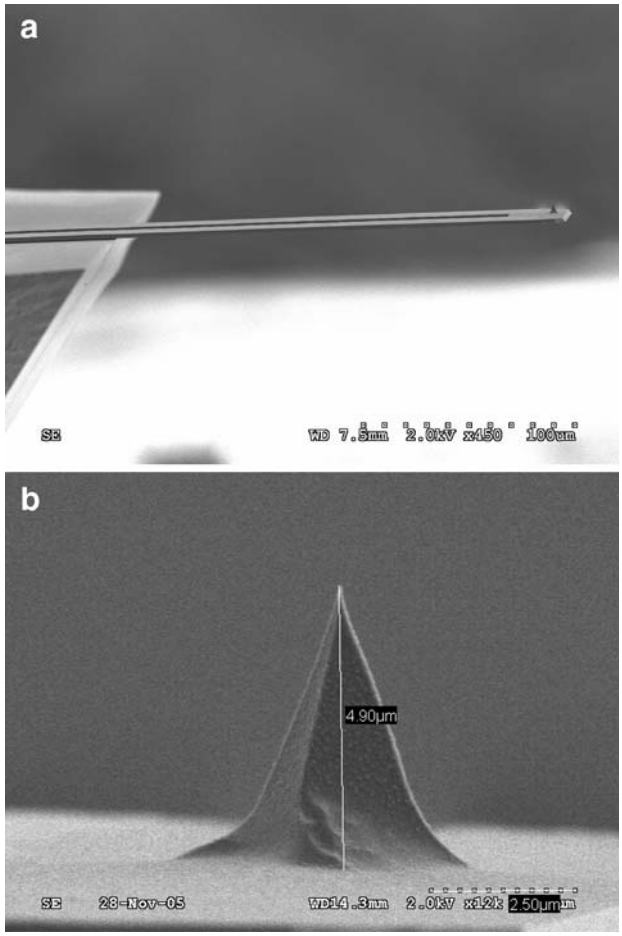

Fig. 1. a and b: SEM images of nanoprobes designed by King (9) at two magnifications. **a** Shows the two legs of the thermal probe along which current is passed to heat the tip. **b** Shows the tip which can be seen to have a sharp tip and a height comparable to conventional AFM probes. Images provided by Anasys Instruments (previously unpublished).

Our early studies using MTA indicated that the method may be used as a means of discriminating between amorphous and crystalline regions of indometacin based on localised thermomechanical analysis (11). Such discrimination is of very considerable practical application as it is now well accepted that partial amorphicity may be responsible for a range of performance issues (12–14). In brief, it was demonstrated that by landing the probe on a specific region of a partially amorphous sample and applying a heating signal to that probe it was possible to measure the softening temperature of the sample associated with the glass transition and melting for the amorphous and crystalline regions respectively. In this manner it was possible to obtain a series of one-point determinations which could in theory map out the amorphous and crystalline regions on the sample surface. However there were two limitations to this early approach. As stated above, the scale of scrutiny of the probes was in the region of tens of microns which is larger than one may wish for in a practical setting. Secondly, only analysis of selected points was possible, hence constructing an image on the basis of LTA measurements posed significant practical difficulties.

In this study we attempt to overcome both these drawbacks by the use of the new thermal probes which we propose will allow L-TMA to be performed on a much smaller scale of scrutiny. We also introduce the novel technique of heated-tip pulsed force mode AFM whereby we obtain scanning images based on local thermomechanical or thermorheological properties as a function of tip temperature, thereby allowing us to examine and image the behaviour of the surface in relation to the glass transition of the indometacin.

MATERIALS AND METHODS

Indometacin powder (Sigma, St Louis, MO, USA) was compressed into 13 mm diameter tablets using an IR press. Amorphous indometacin was prepared using a standard melt-cooling protocol. The thermal nanoprobes (AN nanoprobes) were developed by King working in collaboration with Anasys Instruments and supplied by the latter. Localised thermomechanical analysis (L-TMA) was performed using a heating rate of 25°C/s and a probe force equivalent of 3nA using an Anasys Instruments Nano-TA system; the sensor signal (V) reflects the cantilever deflection. Temperature calibration was carried out using the manufacturer supplied melting point standards, polycaprolatone, polyethylene and polyethyleneterepthalate. Pulsed force mode AFM imaging was performed using the same probes using a Witec PFM module coupled to a TA Instruments μTA 2990 Micro Thermal Analyser. A scanning speed of 0.5 Hz was used at a resolution of 300 lines. The probe was subjected to a modulation of 500 Hz and approximate amplitude of 130 nm. Each image was obtained over an approximately 20 min period. The technique allows for a measurement to be made that is related to the resistance the probe encounters on indentation (which can be related to sample stiffness if the probe is of sufficiently low spring constant) and for a measurement that is related to the pull-off force (sometimes referred to as a measure of adhesion). Both of these measurements should be able to detect glass transitions

but we have found in preliminary work that the pull-off force is by far the more sensitive indicator of the property changes that occur at T_g. This is not because the work of adhesion changes significantly but because the material becomes more fluid and so able to wet the tip causing the surface to be 'sticky.' The necking and rupture behaviour that occurs as the tip is withdrawn from the surface is related to the sample's extensional rheology hence this can be viewed as a thermo-rheological measurement.

RESULTS

Localised Thermomechanical Analysis of Crystalline and Amorphous Indometacin

In the first instance we have studied the spatial resolution that may be obtained for the compacted samples using the probes for local thermomechanical analysis (L-TMA). This is indicated in Fig. 2a showing the 'hole' generated by the

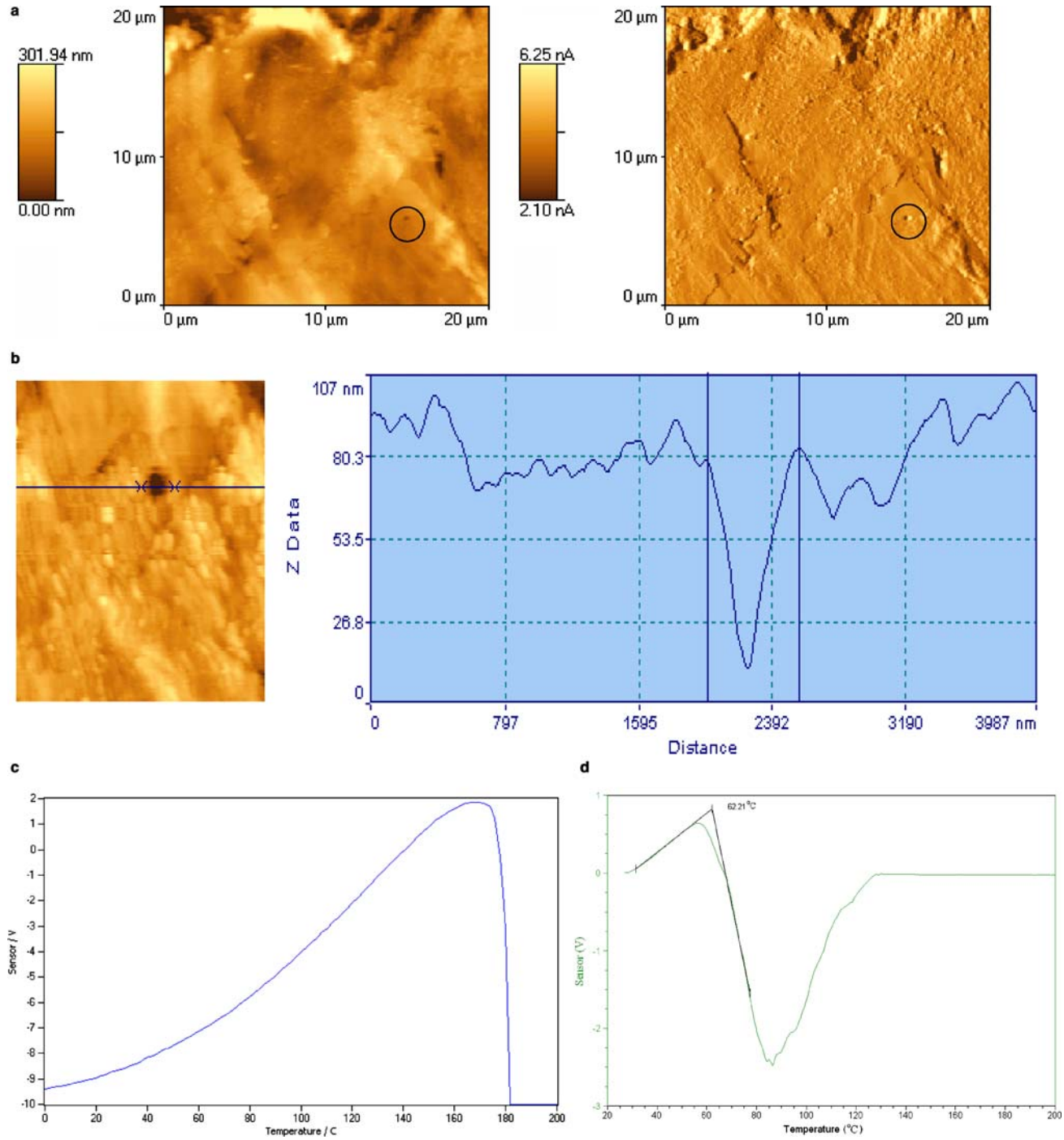

Fig. 2. Localised thermomechanical analysis of crystalline indometacin **a** post-analysis topographic (*left*) and internal sensor (*right*) image of the indometacin surface, showing a typical indentation caused by the measurement (circled), **b** dimensional analysis of the indentation, **c** localised thermomechanical analysis of the crystalline indometacin surface, showing initial expansion followed by probe penetration on melting **d** localised thermomechanical analysis of the amorphous indometacin surface.

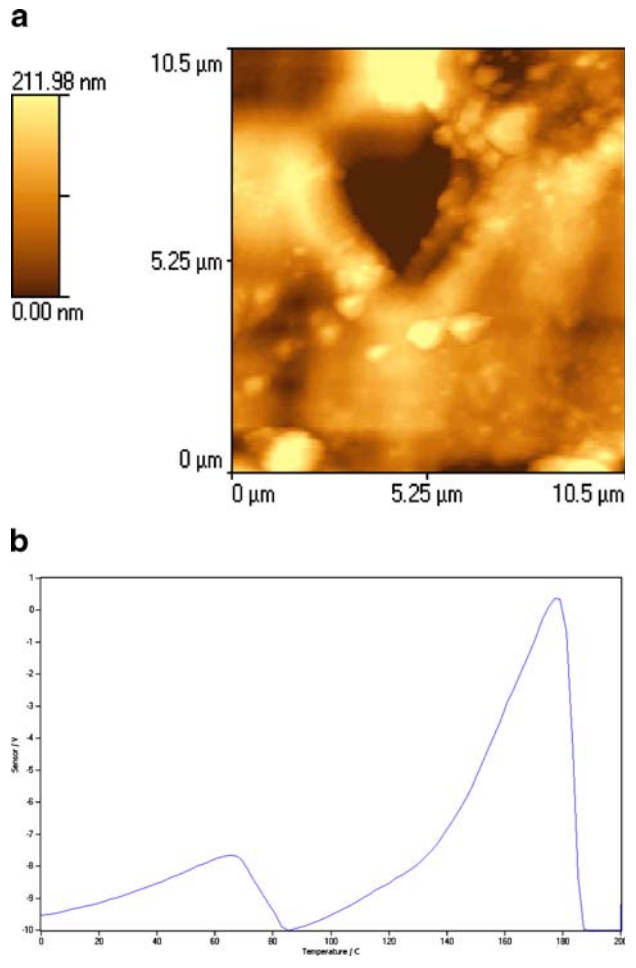

Fig. 3. **a** Topographic image of crater caused by local heating with the thermal probe **b** L-TMA data for material inside the crater, showing responses corresponding to amorphous and underlying crystalline material.

localised thermomechanical experiment. Subsequent dimensional analysis of the indentation using the same probes in contact AFM mode indicated a maximum width of approximately 560 nm and a depth of circa 65 nm. This represents an improvement in *x*,*y* specificity of over an order of magnitude compared to the value of circa 20 µm previously reported (11). The L-TMA response is shown in Fig. 2c, indicating a region first of thermal expansion followed by penetration into the sample at the melting point (T_m). The value for T_m obtained here (158°C) is in reasonable agreement with the value obtained by DSC (161°C). Similarly, studies on amorphous indometacin (Fig. 2d) showed probe penetration at circa 62°C which is in good agreement with the softening point identified using Wollaston wire probes (11). It is of interest to note that a higher temperature pull-in effect is apparent, whereby the probe is drawn into the liquid surface before returning to the baseline position. This has been studied in detail in a previous investigation and is ascribed to high affinity between the sample and probe tip (7). In this study the focus is on the transition temperature as indicated by the extrapolated onset of the penetration measurement, hence the pull-in effect is not considered further here.

Discrimination Between Crystalline and Amorphous Regions Using L-TMA

A model surface that contains both crystalline and amorphous material was then generated using the probe itself; a region was melted using a nanothermal probe followed by abrupt withdrawal of that tip and hence rapid cooling (a similar approach using the Wollaston probes has been described by Ward *et al.* (15)). This resulted in the appearance of a crater, the topographic image of which is shown in Fig. 3a. The centre was analysed using nano-L-TMA

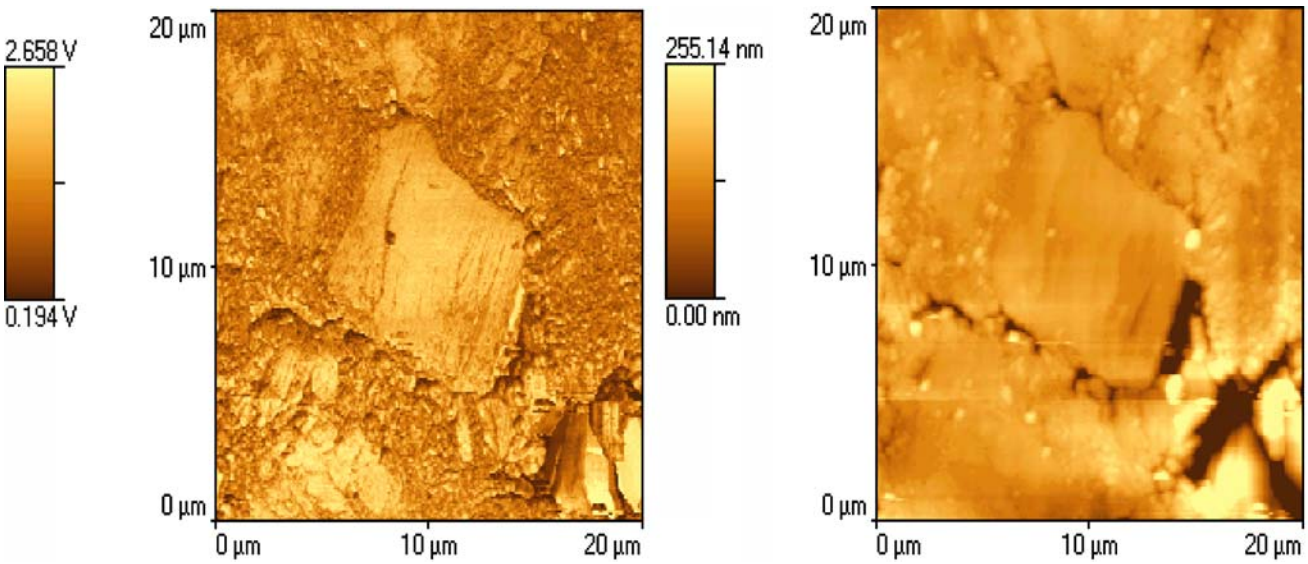

Fig. 4. Pulsed force mode AFM adhesion (*left*) and topographic (*right*) images of the untreated indometacin surface, showing what appears to be two distinct regions (smooth, high adhesion shown as a light region on the PFM image; rougher, low adhesion region shown as a darker region on the PFM image).

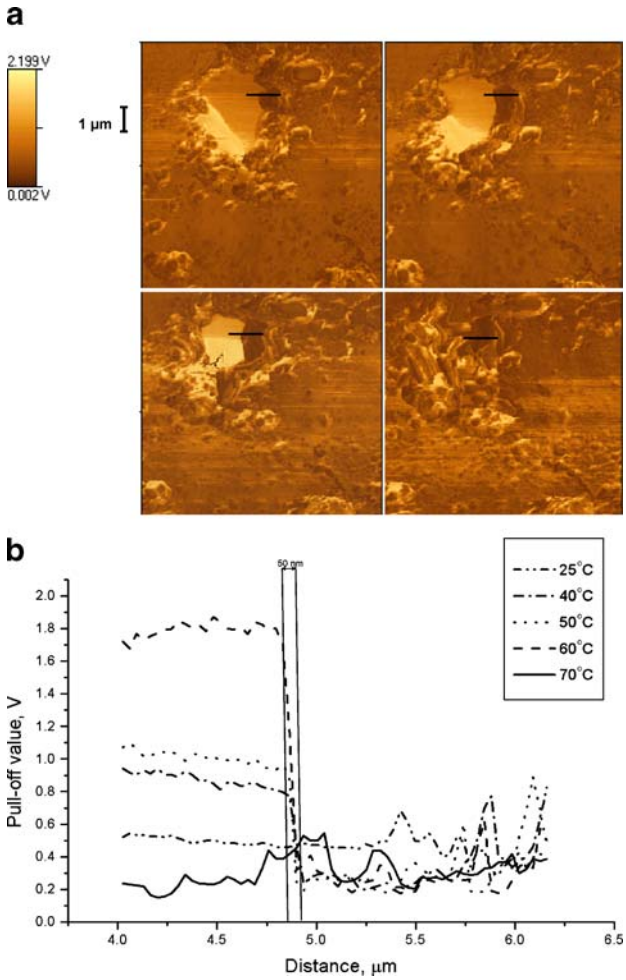

Fig. 5. **a** Pulsed force mode pull-off force images with heated nanotip of the indometacin sample with an amorphous region created by heating with the thermal probe (just as is shown if Fig. 3), *top left* 40°C, *top right* 50°C, *bottom left* 60°C, *bottom right* 70°C, **b** pull-off force line-scans as indicated in **a** by back horizontal lines plus data at room temperature for comparison (taken from the same region). The two vertical back lines on **b** are 50 nm apart.

(Fig. 3b), confirming the presence of an amorphous layer on top of the underlying crystalline material. The temperature of the first softening event was in good agreement with that published previously for amorphous indometacin using the Wollaston probes (11) and that measured on the 'pure' amorphous indometacin surface (see Fig. 2d). It is also interesting to note that this measurement, with suitable calibration, could provide a measure of the depth of the amorphous layer. This can then lead to 3D mapping via measurement of depth of penetration at a temperature at which one component in a multicomponent system softens (4).

We noted when using conventional pulsed force mode adhesion studies that the surface of the untreated material appeared to be biphasic; this is demonstrated in Fig. 4. We were able to show, using the nanoprobes in L-TMA mode, that this is an entirely topographic (or more specifically roughness) effect as both regions showed softening points corresponding to the expected γ polymorph. This is in itself an important finding as it demonstrates the ability of the nanoprobes to discriminate by local thermal analysis between surface morphological effects and 'true' differences in inherent structure and that topography and mechanical/rheological property images alone can be misleading.

Heated Tip Pulsed Force Mode AFM

The observations made above confirm that L-TMA provides a highly useful means of one-point characterisation; however it is less convenient for mapping of different physical regions as this requires a grid of numerous measurements. Here we extend preliminary work with a low spatial resolution flat ended pyramid tip (16) to demonstrate high spatial resolution thermal property imaging based on pulsed force mode mapping using a heated nanotip. In Fig. 5a the pull-off force images are shown for a series of tip temperatures, along with accompanying profile analysis (Fig. 5b) that indicates resolution of approximately 50 nm at the border between the crystalline and amorphous material. This then represents an improvement of an order of magnitude over what is possible on this sample using local thermal analysis. Whether this is the upper limit that can be achieved is an open question that will be the subject of future work.

It should be noted that the accepted value of T_g for amorphous indometacin is circa 43°C (11) as measured by DSC taking the mid-point of the transition. At 40°C a significant increase in the pull-off force in the region of the amorphous material previously created by the probe can already be seen thus the heated-tip PFM measurement is sensitive to the onset of the glass transition. At 50°C (i.e. above the T_g of the drug) little change in the surface morphology was apparent, whereas there was a further increase in pull-off force compared to the surrounding (crystalline) region. At 60°C the region within the crater showed still higher pull-off force while the amount of amorphous material was seen to reduce as it started to crystallise. At a temperature of 70°C virtually all of the amorphous material crystallised; L-TMA studies using the nanoprobes confirmed that the melting point (158°C) corresponded to the stable γ form of indometacin. We noted that an equivalent sample crystallised at circa 50°C when using a hot stage (data not included) thus the changing mechanical or rheological properties of the surface as a function of temperature could not be followed as it is accompanied by a complete change in the structure. It should be emphasised that the L-TMA experiments are conducted over timescales that are orders of magnitude faster than the isothermal mapping experiments, hence the recrystallisation is not seen for the former. This duality is a key advantage of the approach as the L-TMA allow reliable identification of the phase present at the beginning and end of the experiment while the mapping studies allows spatial assignment of those phases. It should also be noted that 50°C is the stage temperature; the surface temperature of the compact at the point of contact of the tip is much more difficult to ascertain. The hot-tip approach therefore enables a window to be accessed where thermally induced changes in properties for an individual phase can be monitored without influence from the surrounding material. Similarly, within this spatial

window, phases can be mapped and studied without significantly changing the structure of the sample as a whole.

DISCUSSION

The study has shown that it is now possible to perform thermal analysis on a nanoscale and that such studies may be used to characterise and differentiate between adjacent physical (and potentially chemical) regions. We believe that this may have considerable significance not only for component mapping in pharmaceuticals but also for a wide variety of complex materials that contain multiple physical or chemical phases. In this respect it is useful to consider the data in the context of the identified strengths and limitations of the approaches used and the manner in which this data relates to that obtainable using existing techniques.

The L-TMA approach allows quantitative information to be obtained regarding the thermal transitions of the region of interest. It may be convincingly argued that the two-dimensional scale of scrutiny we have achieved here (circa 500 nm) puts the technique into a new category of utility compared to the Wollaston approach as most pharmaceutical multicomponent systems have regions of interest of circa 10–100 μm, hence this level of scrutiny now allows these regions to be interrogated in isolation from the surrounding material. Of even greater interest is the heated tip pulsed force approach as that now allows high resolution images to be obtained as a function of temperature which may be selected to maximise differentiation between components. Earlier studies using conventional AFM have allowed mapping of topographically distinct material in semicrystalline samples (17–19) but this by itself does not allow convincing identification of the nature of the regions identified other than by implication based on the topographic differences. The advantage of the thermal approach is that it allows both characterisation and imaging, hence it negates the need to make assumptions with regard to the relationship between topography and composition. Similarly, variable temperature AFM has been utilised for a range of samples (20); this method involves the use of a heated stage to allow temperature control of the sample. However the method has two disadvantages; firstly the temperature measurement is reliant on the assumption of thermal equilibration through the sample so that the tip is experiencing the same temperature as the thermocouple which is located within the stage itself and secondly the method involves heating the whole sample. As we have already demonstrated, this may lead to the structure of the sample changing dramatically thus the as-received structure, which is typically the subject of interest, is lost. The use of the heated tip (as opposed to the stage) overcomes both these difficulties as the calibration procedure allows a high degree of certainty as to the temperature of the material immediately beneath the tip (thus the material that is being interrogated) and the heated tip can measure changes in properties that occur due to a transition without significantly changing the samples structure. Furthermore, although at higher temperatures the region that is imaged is changed, the rest of the sample is unaffected and is available for further study.

CONCLUSION

The study has demonstrated the use of nanoscale localised thermomechanical analysis and heated tip pulsed force imaging as a novel means of characterising and imaging partially crystalline systems. The resolution of the two approaches appears at present to be circa 500 and 50 nm respectively, thereby allowing measurements to take place within the scale of scrutiny typically needed for pharmaceutical systems. We present an argument that the key advantage of this approach is that it allows both characterisation and imaging, thereby allowing the operator to assign specific regions to different components or physical forms with greater confidence than is possible using standard techniques.

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We wish to declare that Mike Reading is a director of Anasys Instruments who are marketing the probes designed by Bill King.

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