Journal of Microscopy, Vol. 198, Pt 2, May 2000, pp. 77–81.
Received 29 September 1999; accepted 6 March 2000

SHORT COMMUNICATION

Discrimination of polymorphic forms of a drug product by localized thermal analysis

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Key words. Drug polymorphism, localised thermal analysis, pharmaceuticals, scanning thermal microscopy.

Summary

In chemical processing, it is important to distinguish between and identify polymorphic forms. We demonstrate the novel use of scanning thermal microscopy (SThM) and localized thermal analysis to distinguish and identify polymorphic forms of the drug cimetidine. These forms cannot be resolved by classical bulk thermal analysis. SThM reveals a sample consisting of a 50 : 50 mixture of the polymorphs contains regions of different thermal conductivity, corresponding to the different polymorphs. Localized thermal analysis of small volumes of pure polymorphic samples (approximately 50 μm^3) shows that the origin of the thermal conductivity contrast lies, at least in part, with the presence of a surface water layer on the more hydrophilic polymorph.

Introduction

Polymorphism, the existence of a substance in more than one crystal form, is increasingly a topic of ubiquitous importance within the pharmaceutical industries and necessitates routine and early characterization (Lian *et al.*, 1998). Polymorphic variations affect density, morphology and mechanical behaviour, which can be important in manufacture and packaging. More importantly, different polymorphic forms display varying physicochemical properties, such as solubility and stability, and hence the form has a significant impact upon drug efficacy and therapeutic value. An additional complication is that polymorphs may transform to another structure whilst in storage.

An inability to fully characterize drug polymorphic forms has led to a number of problems in the past (Halebian & McCrone, 1969). For example, recently the formation of a different polymorph (crystalline form II) of the protease inhibitor Ritonavir in the manufacturing stage led to a significant delay in the supplies of the capsule form (Simpson, 1998). Here we demonstrate that scanning thermal microscopy (SThM) (Dinwiddie *et al.*, 1994; Stopka *et al.*, 1995; Gmelin *et al.*, 1998) combined with localized thermal analysis (Reading *et al.*, 1998; Pollock *et al.*, 1998) can be successfully employed to distinguish between two polymorphs (types A and B) of the drug cimetidine.

Cimetidine, *N''*-cyano-*N*-methyl-*N'*-{2-[[(5-methyl-1H-imidazol-4-yl)methyl]thio]ethyl}guadine (Shibata *et al.*, 1983), is a specific competitive histamine H_2-receptor antagonist (Hall, 1997). It has seven known polymorphic forms that can be produced using a variety of well-established crystallization conditions (Hegedus & Gorog, 1985), the anhydrous modifications A, B, C and D and the monohydrate modifications M1, M2 and M3. In pharmaceutical formulations only modifications A and B are used; A in tablets and B in suspensions (British Patent Specification, 543 2381976). Form A is obtainable in a pure crystallographic state but form B has been reported to also contain an amount of amorphous material (Bauer-Brandl, 1996). Previous methods employed to differentiate the polymorphic forms have included light microscopy, X-ray

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crystallography and IR and Raman spectroscopy (Tudor *et al.*, 1991; Bauer-Brandl, 1996) and, more recently, atomic force microscopy (AFM) operating in phase imaging mode (Danesh *et al.*, 2000). However, thermal methods, such as differential scanning calorimetry or thermomicroscopy, have thus far proved unable to identify the different cimetidine polymorphs owing to the similarity in their bulk thermal behaviour (Bauer-Brandl, 1996).

SThM operates in a manner analogous to AFM but with a miniature resistance thermometer acting as the probe (Dinwiddie *et al.*, 1994; Pylkki *et al.*, 1994; Hammiche *et al.*, 1996a; Zhou *et al.*, 1997). The probe can be heated by passing a current through it whilst it is rastered over the sample under conventional AFM force feedback. By monitoring the current required to maintain a constant probe temperature, images whose contrast is determined by the apparent surface thermal conductivity can be obtained simultaneously with the usual surface topographical information. For localized thermal analysis, the probe is placed at a selected point on the sample surface (using the topographic and thermal images as a guide) and the temperature of the probe ramped as in conventional thermal analysis. The *z*-axis deflection of the probe is monitored as a function of probe temperature. Spatially resolved thermal expansivity measurements can be made and the identification of softening temperatures can be performed. This technique represents the microscopical equivalent (Price *et al.*, 1999a) of thermomechanical analysis (Riga & Neag, 1991). By comparing the power required to make the probe follow the temperature program with that of a reference probe isolated from the sample, spatially resolved calorimetric information regarding the nature of transitions can be obtained. An AC temperature modulation can also be applied during the heating ramp and the changes in power required to keep the modulation amplitude constant (Hammiche *et al.*, 1996b; Fryer *et al.*, 1998) can be measured, thus providing the microscopical analogue of modulated temperature differential scanning calorimetry (Reading, 1993).

Materials and methods

Sample preparation

Discs of cimetidine of polymorphic types A and B were prepared by placing approximately 200 mg of the pure polymorphic material (SmithKline Beecham, Harlow, U.K.) in a press dye under vacuum at a pressure of 10 tonnes for 5 min. Near-field infrared Fourier transform Raman spectroscopy of the discs confirmed that no polymorphic transition had occurred as a result of the pressing process (Tudor *et al.*, 1991). Discs were formed of the pure polymorphs and of a 50 : 50 mixture of the two forms.

Scanning thermal microscopy and localized thermal analysis

Scanning thermal microscopy and localized thermal analysis were undertaken using a μTA 2990 Micro-Thermal Analyser (TA Instruments Inc., New Castle, DE, U.S.A.; ThermoMicroscopes, Sunnyvale, CA, U.S.A.). This comprises a ThermoMicroscopes Explorer scanning probe microscope adapted for scanning thermal imaging and localized thermal analysis. ThermoMicroscopes Wollaston process wire probes were employed.

All images were obtained using a probe temperature of 50 °C and a scan rate of 1 Hz. Localized thermal analysis was performed with a temperature ramp rate of 10 °C s^{-1}. Such high heating rates can be employed because of the small thermal mass of the probe and limited contact area with the sample. The probe was cleaned between acquiring each thermal scan by ramping the temperature up to 450 °C in order to burn off any contaminants. A second probe placed away from the sample functioned as a reference probe.

In both cases, the probes were calibrated by measuring the probe resistance at room temperature and the melting point of zone refined benzoic acid using a linear temperature extrapolation.

Results

Typical SThM images of two different areas of a pressed disc of a 50 : 50 mixture of cimetidine polymorphs A and B are illustrated in Fig. 1. This figure displays the topographic (a and c) and corresponding thermal (b and d) images. The topographic images are consistent with those expected from such a disc. There is a reduction in resolution compared to AFM, with the maximum resolution expected being of the order of 100 nm under ideal conditions. This is a result of the much less sharp probes employed by the SThM; the apex of the thermocouple probe has a radius of curvature of the order of 100 nm, whereas typical AFM imaging probes have radii of curvature in the region of 5–10 nm.

Figure 2a displays results of localized calorimetry of two different points on a sample of pure polymorph B. The plot shows two different types of data: the micro-temperature differential thermal analysis (solid lines I and III) and the corresponding thermomechanical measurements (dotted lines II and IV). Figure 2b displays similar localized thermal analysis data of a disc of pure A polymorph. The data show both calorimetric and thermomechanical analysis of two typical points taken on the disc.

Discussion

The thermal images in Fig. 1 show features arising from two separate sources: topographic contrast due to varying tip–sample contact areas and true thermal contrast arising from differing thermal conductivity in the sample. At

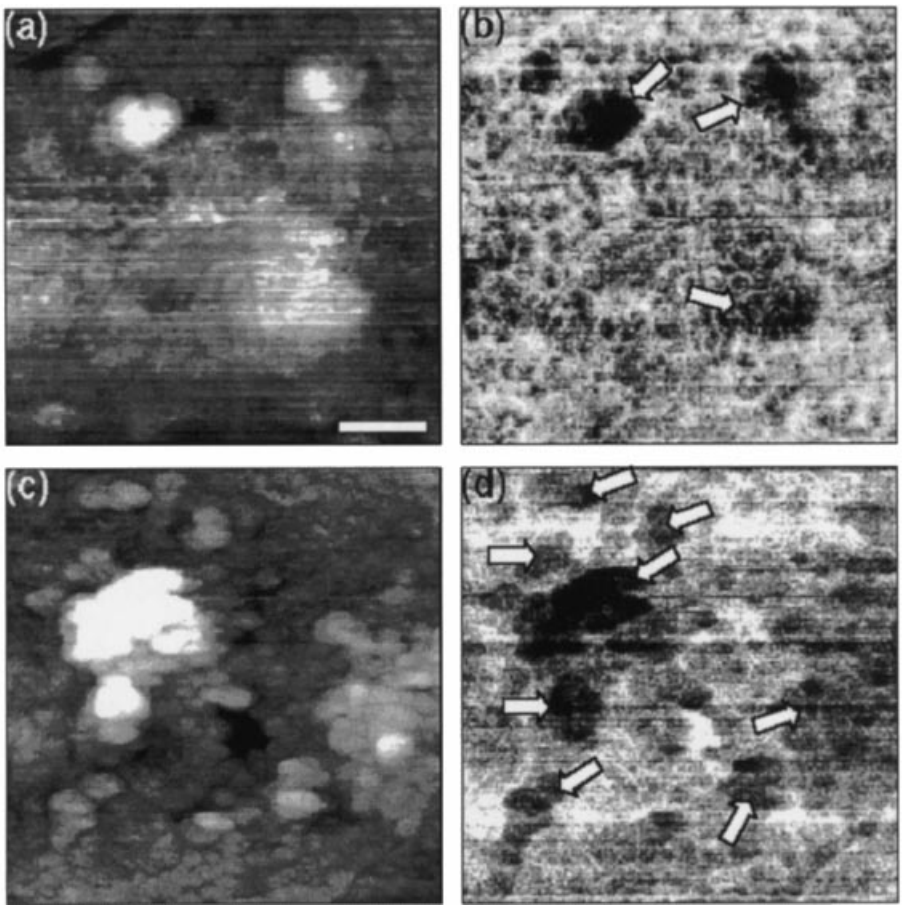

Fig. 1. 50 × 50 μm images of a pressed disc comprising a 50 : 50 mixture of the A and B polymorphs of cimetidine (scale bar 10 μm): (a) and (c) show topographical images of two different areas of the disc and (b) and (d) show the corresponding thermal conductivity images.

present, it is difficult to completely separate thermal and topographical artefacts in the images except for the case of an ideal (flat) sample (Riuz *et al.*, 1998). Such cross-talk occurs because it is not possible to completely separate motion of the SThM probe induced by changes in topography from those which result from changes in thermal properties.

In general we believe that the higher resolution thermal information seen in both images is artificial (for example, regions of higher conductivity, which correspond to edges of microcrystallites seen in the topographic images). However, features can be seen in both sets of images that we propose result from differing thermal behaviour. For example, where no significant changes in topography occur but contrast is seen in the thermal data (features marked with arrows in Figs 1b and 1d), the contrast observed is probably a result of differing surface thermal conductivity of the two polymorphic forms of cimetidine. Support for this approach can be drawn from similar discussions concerning AFM imaging on mixed polymorph cimetidine discs (Danesh *et al.*, 2000) which demonstrate that topographic imaging alone is unable to unequivocally identify the polymorphic A and B forms of cimetidine and that, in this case, additional data from phase imaging were also required.

It is apparent from the data in Fig. 1 that the features highlighted in (b) and (d) correspond to a significant extent with a particular type of large 'aggregate' morphology. It is not unreasonable to suggest that this morphology is related to one of the polymorphic forms of cimetidine and that this fact results in the thermal contrast observed. However, the origin of the differing surface thermal conductivity of the A and B polymorphs of cimetidine is not initially clear, as they display very similar bulk thermal behaviour. Local thermal analysis of pure discs of each polymorph allowed elucidation of the contrast mechanism and the identification of the polymorphic forms, which correspond to the dark and light regions observed in Figs 1(b) and (d).

The data in Fig. 2(a) from the pure B form of cimetidine show a sharp endothermic peak indicative of melting. This is also seen in the thermomechanical data, which display a rapid penetration of the probe into the surface at the melting point. It is worth noting that for polymorph B these data do not necessarily overlay, as might be expected from thermal analysis of pure material. This may be due to varying ratios

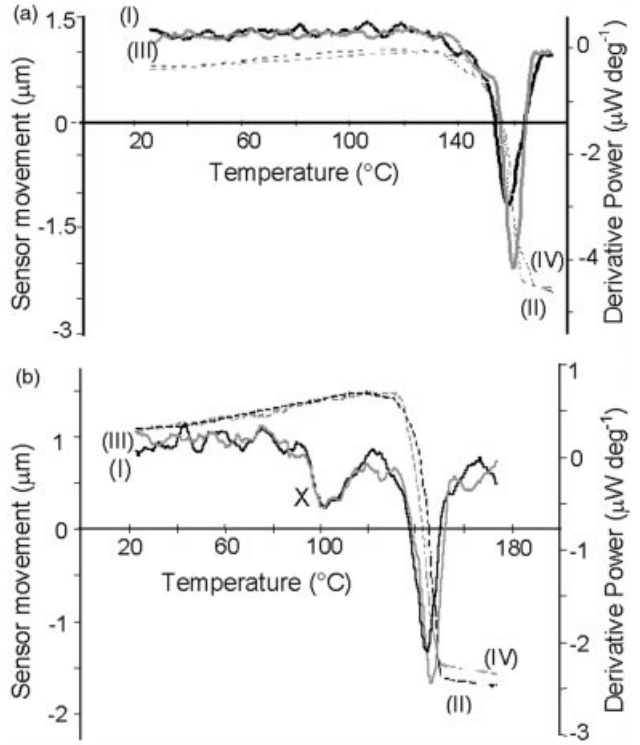

Fig. 2. Localized thermal analysis of a disc of pure cimetidine of (a) B and (b) A. Lines denoted (I) show the calorimetric data for a single point and dashed lines (II) show the movement of the sensor during the measurement, corresponding to thermomechanical analysis. Lines (III) and (IV) show the similar data for other points on the samples.

of amorphous and crystalline material (Bauer-Brandl, 1996; Danesh *et al.*, 2000). Generally, such behaviour would not be observed with bulk thermal analysis, where a peak corresponding to a statistical average of the varying crystalline and amorphous content of the material would be seen.

In the polymorph A case (Fig. 2b), the peaks observed do not vary considerably in position or peak width from point to point on the sample. This corroborates previous findings that polymorph A of cimetidine is of a pure crystalline nature (Bauer-Brandl, 1996; Danesh *et al.*, 2000). Slight variations in the depth of the peaks arise from a number of factors including varying contact areas and packing density of material under the probe. The melting point seen by the localized thermal analysis of polymorph A (141–143 °C) lies within the range seen for polymorph B (140–146 °C), which clearly illustrates why bulk thermal analysis is not adequate for the differentiation of these two polymorphs.

The most surprising feature of the data seen for polymorph A is the endothermic peak seen just above 100 °C (marked X in Fig. 2b). No corresponding transitions are seen in the localized TMA curves, which suggests that this feature is not a result of change of polymorphic state or phase. This feature is seen in neither the bulk thermal analysis of form A nor the localized thermal analysis of form B. This would suggest that this feature is a result of the increased surface sensitivity of the localized measurements, and this endothermic peak is likely to be a result of adsorbed surface water. This postulation has been substantiated by independent contact angle measurements (which show form A to be more hydrophilic than form B) and scanning probe phase–distance data, which also display behaviour indicative of adsorbed water at the surface (Danesh *et al.*, 2000).

The localized thermal analysis therefore allows us to conclude that the origin of the contrast in Fig. 1 is a result of the differing surface thermal conductivity. A contributory factor to this variation in conductivity is the adsorbed surface water on polymorph A. Localized thermal analysis on mixed polymorph samples provides thermal data similar to the pure discs and identifies the darker regions observed in Fig. 1 as being polymorph A, and the lighter regions polymorph B. However, it should be noted that the area melted by the thermal probe is on the order of several micrometres in all directions. The volume melted can be approximated through scanning probe analysis of the sample after localized thermal analysis, where pits corresponding to the melted points are seen. Hence, regions of a particular polymorph in a mix smaller than or similar to this size could not be conclusively identified. However, with improved thermal probe technology (Mills *et al.*, 1998) arising from increased miniaturization and semiconductor/metal-based probes, such sub-micrometre resolution is likely to be accessible.

Conclusions

In chemical processing, it is important to distinguish between and identify polymorphic forms. In the early stages of drug development, it is often necessary to perform such tasks with a minimal amount of sample (Lian *et al.*, 1998). The data presented here, and other work (Price *et al.*, 1999b), show that localized thermal analysis is a powerful tool capable of such characterization. The miniature probe used in these measurements allows for rapid heating and faster measurements than traditional thermal techniques. It also allows for minute masses of material to be analysed – a typical experiment melts less than a 5 μm × 5 μm × 2 μm volume, corresponding to a mass of less than 0.125 μg (Price *et al.*, 1999b).

SThM revealed that a sample consisting of a 50 : 50 mixture of two cimetidine polymorphs contains regions of different thermal conductivity, corresponding to the different polymorphs. Localized thermal analysis of the pure polymorphic samples demonstrated that the origin of the thermal conductivity contrast lies, at least in part, with the presence of a surface water layer in the case of type A that is not present in type B. This represents the first time that these polymorphs have been successfully differentiated by thermal methods and demonstrates the advantages of such

a surface selective technique in the speed and resolution of polymorphic discrimination.

Acknowledgements

G.H.W.S. thanks the BBSRC for funding and A.D. acknowledges SmithKline Beecham and the EPSRC for the provision of his studentship.

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