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Differential scanning calorimetry and scanning thermal microscopy analysis of pharmaceutical materials

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Abstract

Micro-thermal analysis (μTA) by scanning thermal microscopy is being used increasingly for the analysis of pharmaceutical dosage forms. However, there is currently little evidence to show that μTA data can compare directly with that from the established approach of differential scanning calorimetry (DSC). This work compares DSC and μTA data from an active vitamin B6 analogue, pyridoxal hydrochloride, and two commonly used pharmaceutical excipients, Mannitol and Avicel™ which are used in its formulation. It is found that μTA provides precise and accurate micro-thermal analytical data with 0.1 K thermal sensitivity, which is comparable to that obtained by DSC measurements of bulk samples. It is also shown that μTA offers the opportunity to study single particles and the interfacial region between particles, data which is currently inaccessible through the DSC technique.

Keywords: DSC; μTA; Excipient; Particle

1. Introduction

Thermal methods such as differential scanning calorimetry (DSC) are commonly used in the pharmaceutical industry for the characterisation of the physiochemical properties of powders and to aid the rational development of new chemical entities (NCEs) (Giron, 1998; Perkin–Elmer Technical Note). The advent of the scanning thermal microscope (SThM) has resulted in the recent use of micro-thermal analysis (μTA) for the measurement of thermal properties of materials (Pillay and Fassihi, 1999; Price et al., 1999; Hassler and zur Muhlen, 2000; Xie et al., 2001), including recently, those used in the pharmaceutical industry (Royall et al., 1999, 2001). Currently however, there is little evidence to suggest that these two very different methods provide directly complementary thermal data, and so to date, the SThM has predominantly been used as a research tool and for qualititative analysis.

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DSC can be used for a wide range of pharmaceutical applications ranging from the characterisation of raw materials to the investigation of the interactions between phospholipids and drugs (Giron, 1998). The reason why it is so widely used is that most phase transitions are accompanied by a change in heat; be it a chemical reaction, melting, absorption or mixing (Giron, 1998). When a sample is submitted for a typical DSC analysis, a few milligrams of the sample are sealed in an aluminium pan and placed in the instrument alongside an empty aluminium pan (the 'reference' pan). Each pan is then heated at a pre-determined and constant rate. The pan containing the sample will require a different amount of power to heat it at the same rate as the reference, and it is this difference in amount of power to heat the sample which is measured. The resulting plot is one of heat flow against temperature, which can give information about both first order transitions (such as melting and crystallisation) and second order transitions (such as the glass transition temperature in polymer samples) (Perkin–Elmer Technical Note). An example of the current use of DSC for the study of pharmaceutical dosage forms is the work by Wissing et al., 2000 where they have investigated incompatibilities of magnesium stearate and stearic acid with aspirin. Malan et al., 1997 performed a similar experiment but compared the results from DSC to the data obtained by isothermal stress testing followed by HPLC assay. He concluded that DSC could identify destabilising interactions which were not detected by HPLC.

Mahot (2000) highlighted the common use of temperature modulated DSC, photo-DSC and DSC-thermocalorimetry at production sites of large pharmaceutical/chemical companies. Moreover, Mathot claimed that the potential of thermal analysis on a micrometre/nanometre scale would expand analytical support for thermal analysis in a major fashion, concerning not only in-line processes, but particularly for 'the combination of time- and position-resolved analysis of materials'. It would therefore seem likely that the combination of thermal analysis with scanning probe technology should be an exciting and productive tool for the thermal analysis of sample surface features and sample heterogeneity. It would allow the analysis of samples of size and geometries previously inaccessible to bulk DSC, such as the analysis of thin films and specific layers in a multicomponent formulation such as a tablet. The inability of DSC to investigate these areas is a major limitation of the technique.

Whilst the data provided by the DSC is well understood, it is only able to give information about the thermal properties of the bulk sample under study. In contrast, the SThM is able to provide simultaneous topographical and thermal information about a surface and to some extent the near surface region, of the studied sample (Williams and Wickramasinghe, 1986). Specifically, the sample surface may first be imaged, then a specific area identified for further thermal analysis (localised thermal analysis (LTA)), for example, a chosen step on the face of a drug crystal.

The SThM is one of the family of techniques known as the scanning probe microscopes (SPMs), the most well known of which is the atomic force microscope (AFM) (Binnig et al., 1986). These approaches depend upon the measurement of a surface related property using a sharp proximal probe either in contact with or close to the surface. The AFM typically utilises a deflecting cantilever feedback system, which comprises of a silicon nitride tip on the end of a cantilever, which deflects according to the amount of attraction or repulsion between the tip and sample. The SThM developed from AFM by replacing the silicon nitride tip with a small thermal probe, the most common type of which is a resistive probe made from a Wollaston process wire. This thermal probe is currently manufactured by bending a small Wollaston wire (a platinum core (diameter 5 μm) surrounded by silver (diameter 75 μm)) to form a loop attached to the cantilever. The silver at the end of the loop is then etched away to leave a platinum based thermal tip with a thermal sensitivity of 0.1 K (Gmelin et al., 1998). At the tip end, the small wire diameter and the bend in the

platinum core results in a high ohmic resistivity whereby electric current flowing through the wire will then generate heat in the tip (Hassler and zur Muhlen, 2000).

When the tip is in contact with the sample, it acts as an AFM in contact mode (Binnig et al., 1986), which relies on repulsive forces between the tip and sample. Raster scanning the sample relative to the tip generates a topographic image, although the resolution of this image tends to be lower than that obtained by conventional AFM due to the larger size of the probe tip (100 nm for the thermal tip compared to 20–60 nm for a standard AFM tip). Thermal properties of the substrate can be investigated simultaneously during imaging by monitoring the power required to maintain the tip at a pre-determined temperature. The heat flow between tip and sample varies with the thermal properties of the sample and so changes in local thermal conductivity can be matched with the topographical image. From the produced topography and thermal conductivity images, a particular area of interest can then be identified, over which the tip may be held and LTA performed (Hammiche et al., 1996). In such measurements, the tip is heated at a specific rate and both the deflection of the cantilever and the variation of power supplied to the tip are recorded as a function of time (note the similarity to DSC) (Xie et al., 2001).

This technique is increasingly being used in the study of pharmaceutical systems. For example, Sanders et al. (2000) studied a mixture of two crystal polymorphs of the drug cimetidine with the SThM and were able to distinguish between them, confirming their identities by localised thermal analysis. Interestingly such polymorphic distinction of cimetidine is not possible by DSC. μTA has also been used by Royall et al., 1999 to differentiate between components in a model tablet formulation by mapping the thermal variation of the surface and performing μTA on selected regions of the sample. This increasing use of SThM suggests that a direct comparison to DSC data is timely.

Here we describe the results of experiments, which directly compare and contrast DSC and μTA obtained for the pharmaceutical compounds, pyridoxal hydrochloride, Avicel™ and mannitol.

2. Materials and methods

The samples investigated were a vitamin B6 analogue as a model drug substance and two commonly used pharmaceutical excipients. Pyridoxal hydrochloride (Fluka, Dorset, UK) was employed as the model drug. The excipients used were Mannitol (Roquette, Lestrem, France) and Avicel™ PH101 (Honeywill and Stein Ltd. Sutton, UK).

DSC analysis was performed using a Perkin–Elmer DSC 7 (Perkin–Elmer, Norwalk, USA) which was calibrated with an indium calibration sample (Perkin–Elmer, Norwalk, USA). A few milligrams of sample were hermetically sealed into aluminium pans (Perkin–Elmer, Norwalk, USA) and heated under argon between −30.00 and 220.00 °C at a rate of 10.00 °C/min. Results are presented in the baseline subtracted form, endotherm upwards.

For SThM analysis, sample discs were prepared as follows: approximately 200 mg sample was placed into a die and pressed at 10 Tons for 2 min to form discs of 1 cm diameter. These discs were then mounted onto metal sample stubs using double sided tape.

SThM analysis was performed using an Explorer AFM system (ThermoMicroscopes, Sunnyvale, USA) with a thermal tip (ThermoMicroscopes, Sunnyvale, USA). The thermal response of the tip was first calibrated using anisic acid crystals of melting point 182 °C (Sigma Aldrich) (Sigma, Dorset, UK). Topography images of the disc were first obtained in contact mode (imaged with a scan rate of 0.5 Hz, and 512×512 pixel resolution) and particles on the surface were then selected for LTA. LTA was performed between room temperature and 220.0 °C at 10.0 °C/s in ambient conditions. The tip was cleaned between analyses by heating to 500 °C. The thermal data is obtained as a power vs. time plot, but for ease of identification of melting transitions it is shown here as the derivative of power (endotherm upwards for ease of comparison to DSC data). The cantilever movement data is obtained as sensor response vs. time, but is also shown in its derivative form for ease of identification of sensor movement (i.e. cantilever deflection) events. It is

worth noting the different scan rates for DSC and LTA of 10 °C/min and 10 °C/s, respectively. These rates are typical for each technique and are in accordance with the respective calibration methods of the instruments. The slower rate of the DSC is compensated in the DSC software for lag temperature based on the Indium calibration curve (Kedwood et al., 2000). It is generally recognized for DSC that increasing the heating rate improves sensitivity at the loss of resolution of closely spaced thermal transitions. The loss in resolution at high heating rates is due to an increased time for reequilibration to be established between the sample and the reference (Coleman and Craig, 1996). Such an understanding of the effect of heating rates in SThM analysis has yet to be established. However, it is important to note that with SThM higher heating rates can be employed without sacrificing resolution due to the much smaller mass being heated and the speed of response of the SThM probe.

Higher resolution images were also obtained using an Explorer AFM (ThermoMicroscopes, Sunnyvale, USA). All images were acquired in intermittent contact mode under ambient conditions, using silicon TESP tips (Vecco, California, USA). AFM images were acquired with cantilevers oscillating just below their resonant frequencies (approximately 300 kHz). All images were taken at a scan rate of 2 Hz, with a 512×512 pixel resolution.

3. Results and discussion

Initially, the sample discs of the various materials were imaged using the SThM so that relevant areas could be selected for LTA. Fig. 1a–c show typical topography images obtained of the mannitol, Avicel™ and pyridoxal hydrochloride, respectively. No evidence of sample deformation or tip degradation was observed during such imaging. The images clearly show the surface to be composed of closely packed particles, enabling the easy identification of areas suitable for LTA study.

Fig. 2a–c show representative images obtained using standard AFM tips displaying the topography of the same surfaces with higher resolution. This lower resolution apparent in the SThM image as compared to the AFM image, is due to the larger radius of the thermal tip (Giron, 1998) as described in the introduction, but is still sufficient for the clear identification of particles for LTA.

Fig. 3a shows a DSC plot for Avicel™, in which an endothermic peak can be seen at approximately 198 °C. This is in excellent agreement with the work by Ntawukulilyayo et al. (1995) who reported the melting of Avicel at approximately 200 °C.

Fig. 3b shows results of LTA analysis of Avicel™ from point X in image Fig. 1a. The derivative of power signal is used to aid data analysis since it aids the identification of exothermic and endothermic events. Here it shows constant heat flow until point 1 where the slope of this line rapidly changes to form a downwards peak, indicative of an exothermic event. Such events are typically seen for crystallisation or water loss from the sample. This exothermic event ends at point 2, after which the heat flow between tip and surface is constant. At point 3, the beginning of an endothermic event is seen, characteristic of melting. This ends at point 4, when the heat flow between tip and sample again remains at a constant rate. Here, an exothermic peak can clearly be seen at approximately 150 °C and an endothermic peak at 199 °C. We propose that the observed exothermic peak at 150 °C results from an endothermic loss of water from amorphous regions of the sample, which is accompanied by a dominant exotherm from the subsequent crystallisation process (Adrizzone et al., 1999; Durig and Fassihi, 1991; Buckton and Darcy, 1999). The overall change in energy hence being exothermic. The peak at 199 °C is typical of the melting of crystals of Avicel™, and agrees very well with both the data obtained in our own DSC experiments and with literature values (Ntawukulilyayo et al., 1995). It is apt to note that the peak seen by SThM at $\sim$150 °C was not detected by DSC. Such differences previously observed between DSC and SThM have been related to the bias of SThM towards surface related phenomena. For example, Sanders et al. (2000) found that the more hygroscopic A-polymorph of the drug

cimetedine displayed a peak due to water loss at its surface (not seen by DSC) which was not seen for the more hydrophobic B-polymorph. We suggest that the lack of an observed peak at 150 °C in DSC, results from the amorphous content of Avicel™ mostly residing at the surface of the sample. Similar observations have been made previously in a general sense by Buckton and Darcy, 1999.

Fig. 4a shows a DSC plot for mannitol, showing an endothermic peak at 167 °C, with the onset of the melting at approximately 160 °C. This corresponds well to the value of 166–168 °C given by the Merck Index (Budavari). Fig. 4b

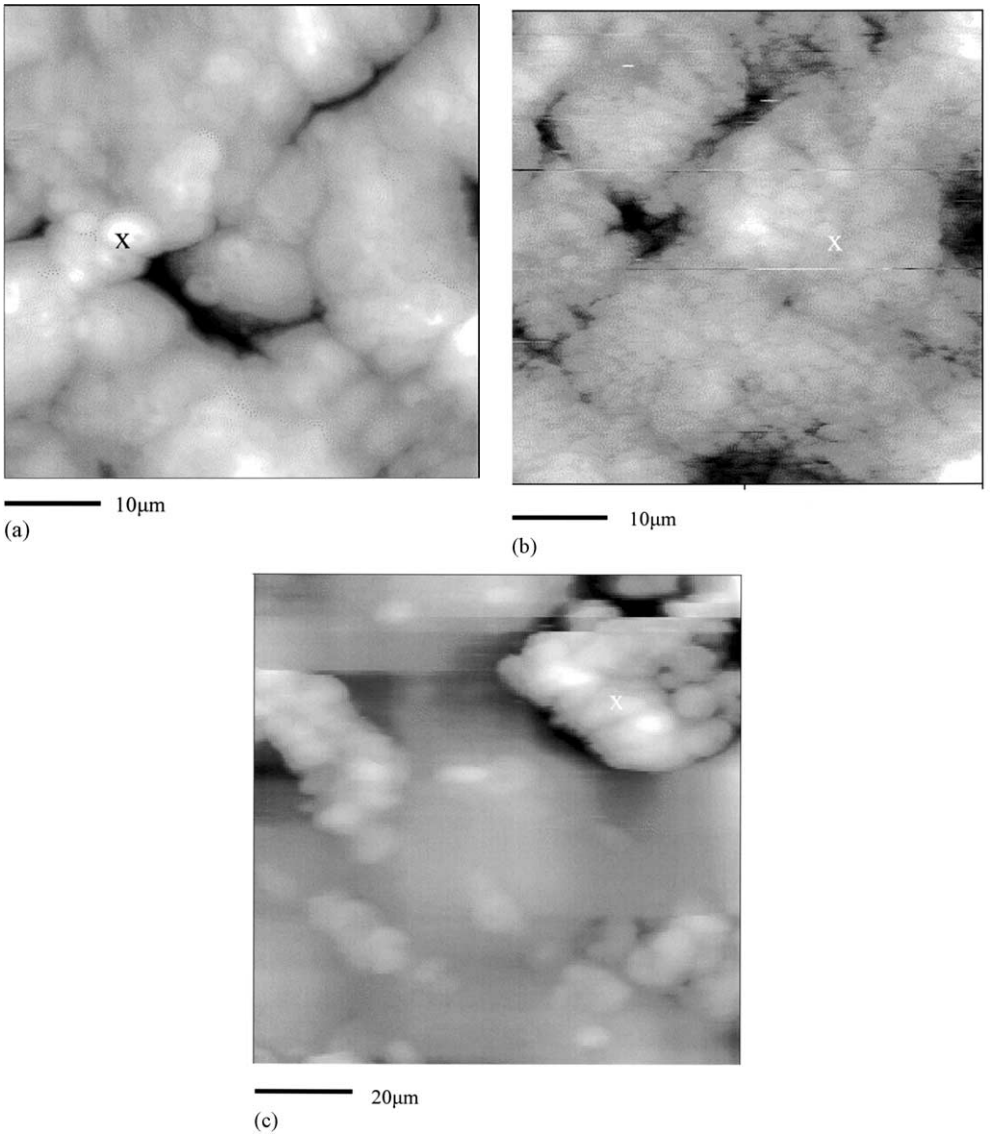

Fig. 1. 50 × 50 μm^2 topographical images recorded using the thermal probe of: (a) Avicel™ disc surface (height range = 0–1.31 μm); (b) mannitol disc surface (height range = 0–254 nm); and (c) a 100 × 100 μm topographical images recorded using the thermal probe of pyridoxal hydrochloride disc (height range = 0–4.70 μm). For all images X marks the site of thermal analysis for data shown in subsequent figures.

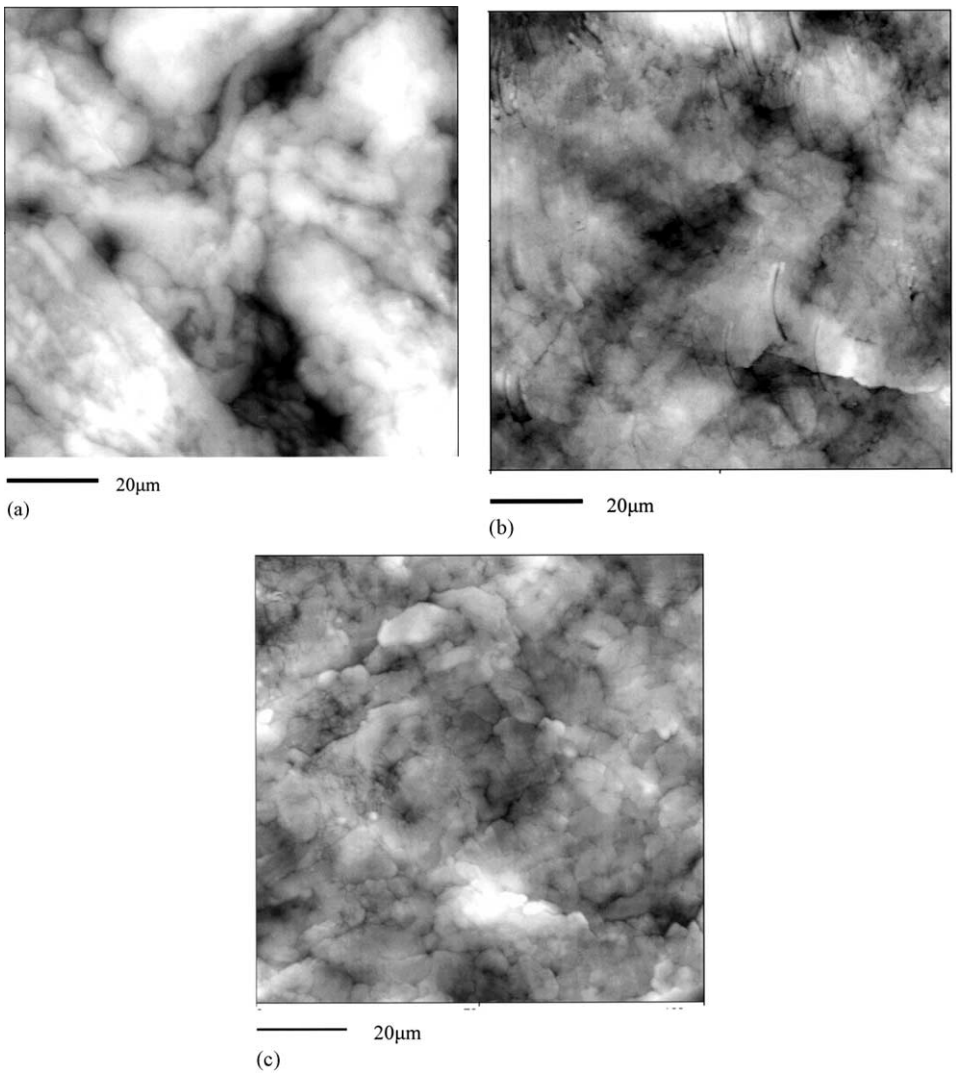

Fig. 2. 100×100 μm^2 topographical image recorded using a standard AFM tip of: (a) Avicel™ disc (height range = 0–2210 nm); (b) mannitol disc (height range = 0–345 nm); and (c) pyridoxal hydrochloride disc (height range = 0–595 nm).

shows the LTA of the mannitol disc surface, taken from point X in image Fig. 1b. The data from the derivative of power signal, when combined with data from the derivative of the sensor signal, shows melting between 163 and 168 °C which also corresponds well with our DSC values and the literature values (Budavari). The plots are presented in the derivative form to help highlight significant peaks. The plot describing the sensor may be explained as follows: between points 1 and 2, the probe remains at a constant height in contact with the sample surface. At 2, the sample begins to deform as a result of the heating, and so the probe moves downwards, until point 3, when the melting has ended and the probe now stays at a constant height buried in the melted compound. This process also highlights the importance of cleaning the tip between sample analyses.

Fig. 5a shows a DSC plot of pyridoxal hydrochloride. It is known that two polymorphs of pyridoxal hydrochloride exist (Durig and Fassihi, 1991) and that these have distinctly different melting points of ~172 and ~187 °C. The DSC in Fig. 4a shows endothermic peaks at ~175 °C and ~200 °C, and an exothermic peak at ~180 °C. The thermal profile is again consistent with that seen in Merck Index (Budavari). This profile is characteristic of the presence of forms II (metastable) and I (stable) of pyridoxal hydrochloride with melting points 175 and 200 °C, respectively. In our experiments, it may therefore be suggested that one of three possible scenarios has occurred. Either:

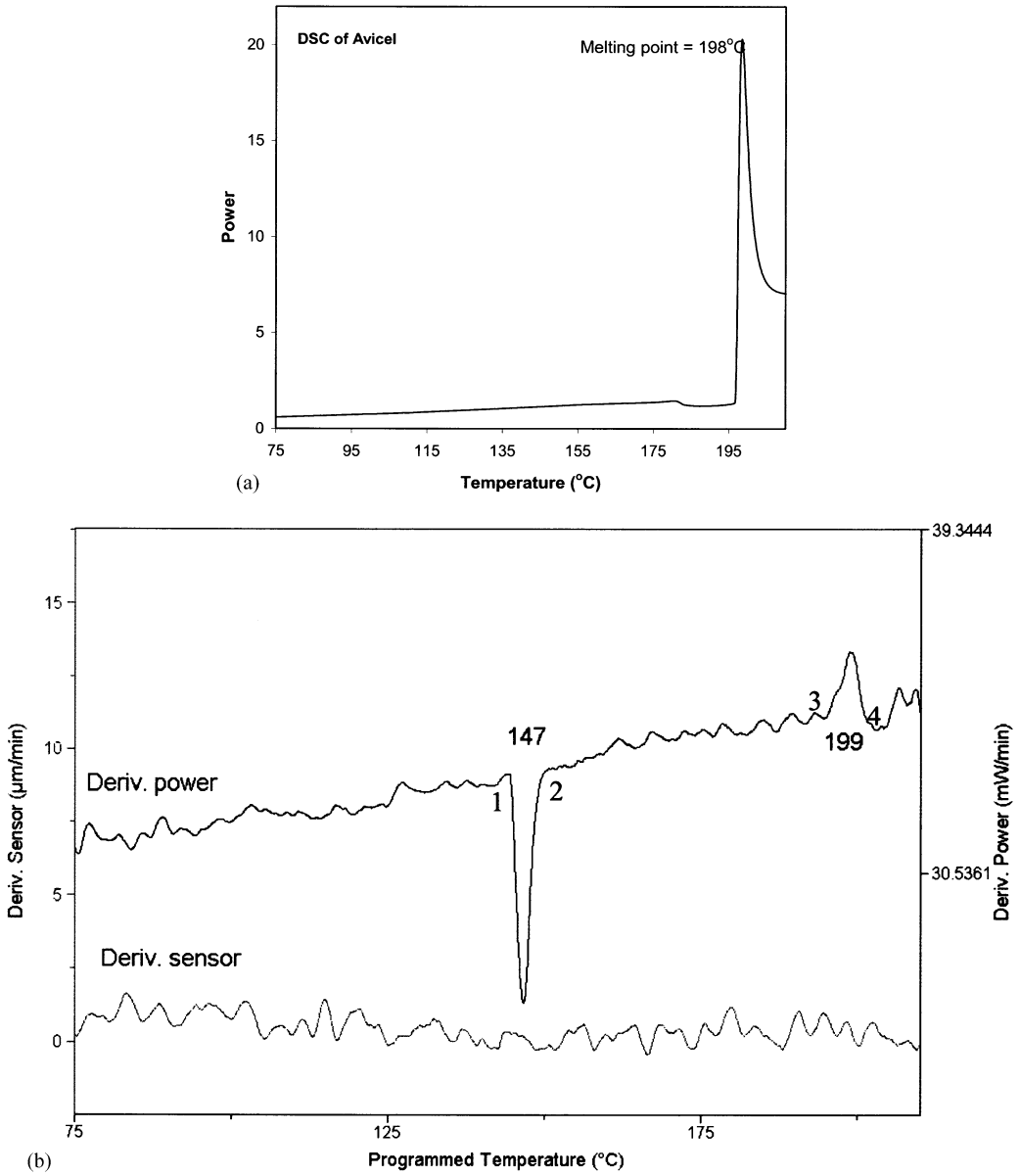

Fig. 3. (a) DSC thermogram of Avicel™. (b) SThM LTA analysis of Avicel™. Plot shows derivative of power and sensor data.

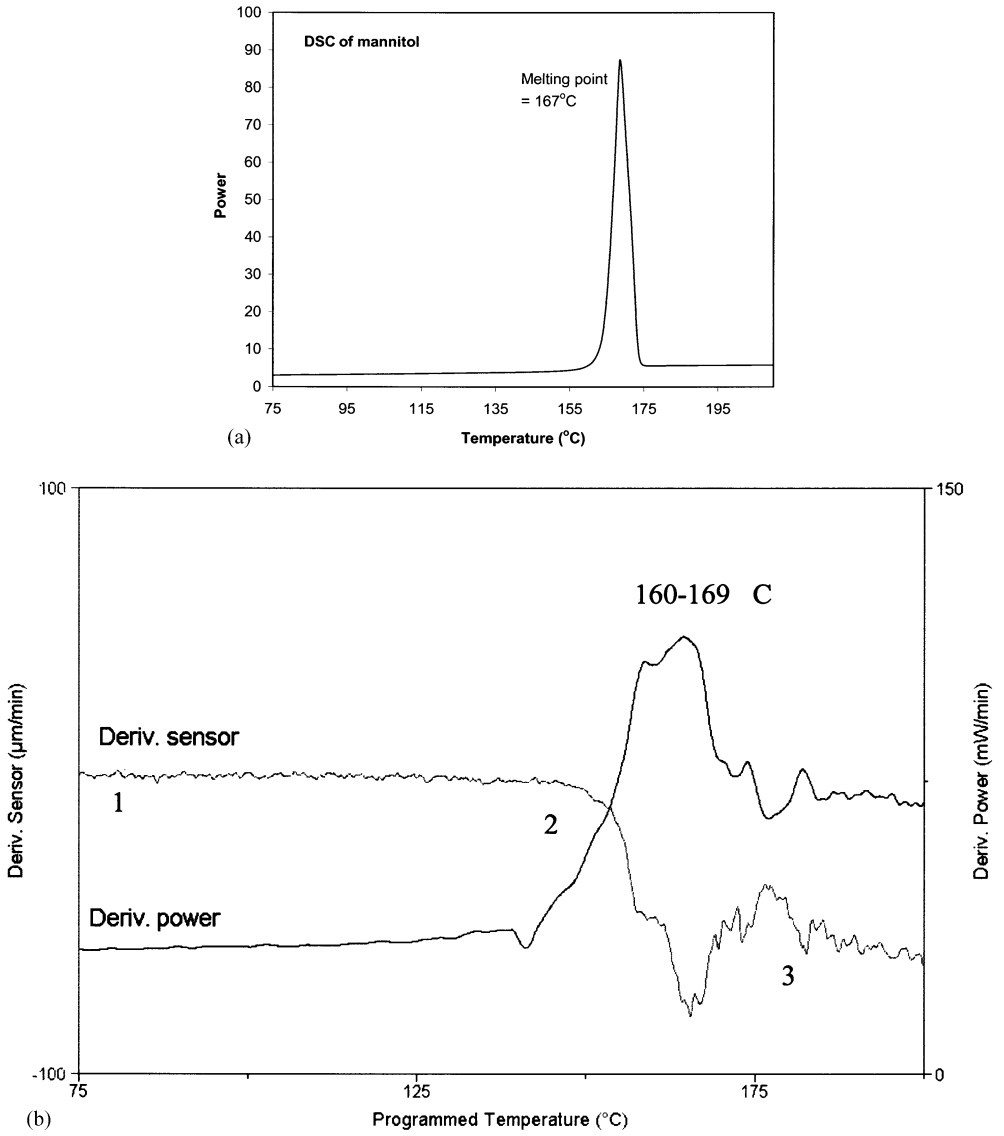

Fig. 4. (a) DSC thermogram of mannitol. (b) SThM LTA analysis of mannitol. Plot shows derivative of power and sensor data.

1. both forms II and I were initially present in the sample;
2. the metastable from II melted and recrystallised into form I, or;
3. both 1 and 2 have occurred.

Fig. 5b shows the LTA for a single crystal in the sample. The resulting melting point at 200 °C identifies this crystal to be polymorph form I. Fig. 5c shows results of the LTA when the thermal probe was inserted at the interface between two particles. The presence of the two melting peaks indicate that the particles were different polymorphs. The melting points again agreed well with the DSC plot. LTA analysis seen in Fig. 5c, taken at point X in image Fig. 1c, indicates that initially, there are both forms present in the sample, agreeing with previous studies which have shown both polymorphs to be present in the sample (Durig and Fassihi, 1991). The presence of two crystal forms may also be confirmed by AFM

phase images taken of the sample as shown in Fig. 6. Phase imaging has identified a two component system which is consistent with the presence of two polymorphs. It has previously been used to determine the presence of polymorphic mixtures of pharmaceutical compounds (Danesh et al., 2000), and has been used to this effect here, where the identity of the two species as polymorphs has been confirmed by thermal analysis. LTA would be unable to detect scenario 2, since after melting, the probe is embedded into the compound, and so any recrystallisation would not be detected.

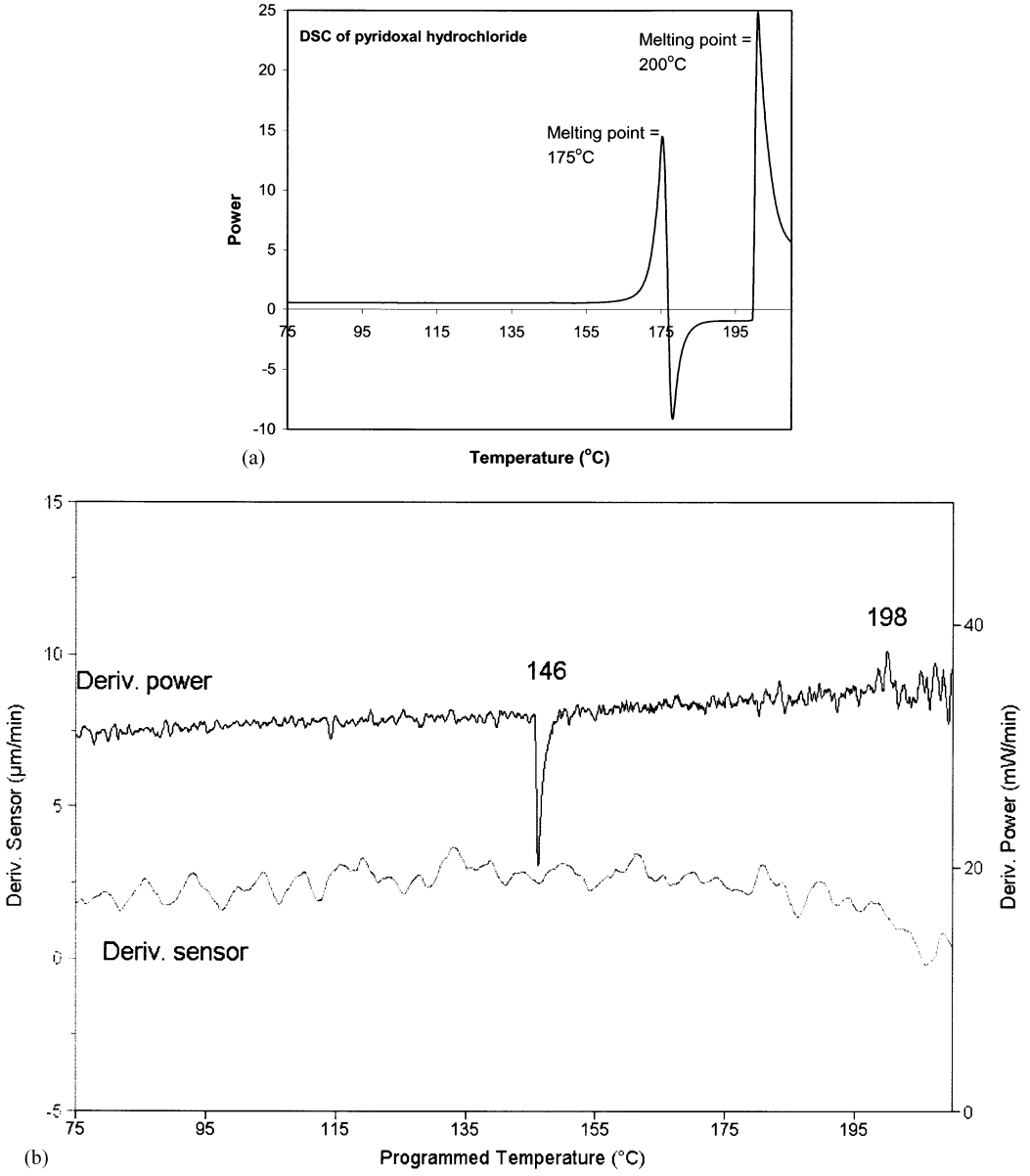

Fig. 5. (a) DSC thermogram of pyridoxal hydrochloride. (b) SThM LTA analysis of a single crystal of pyridoxal hydrochloride. (c) SThM LTA analysis at an interface between two crystals of pyridoxal hydrochloride. Plots shows derivative of power and sensor data.

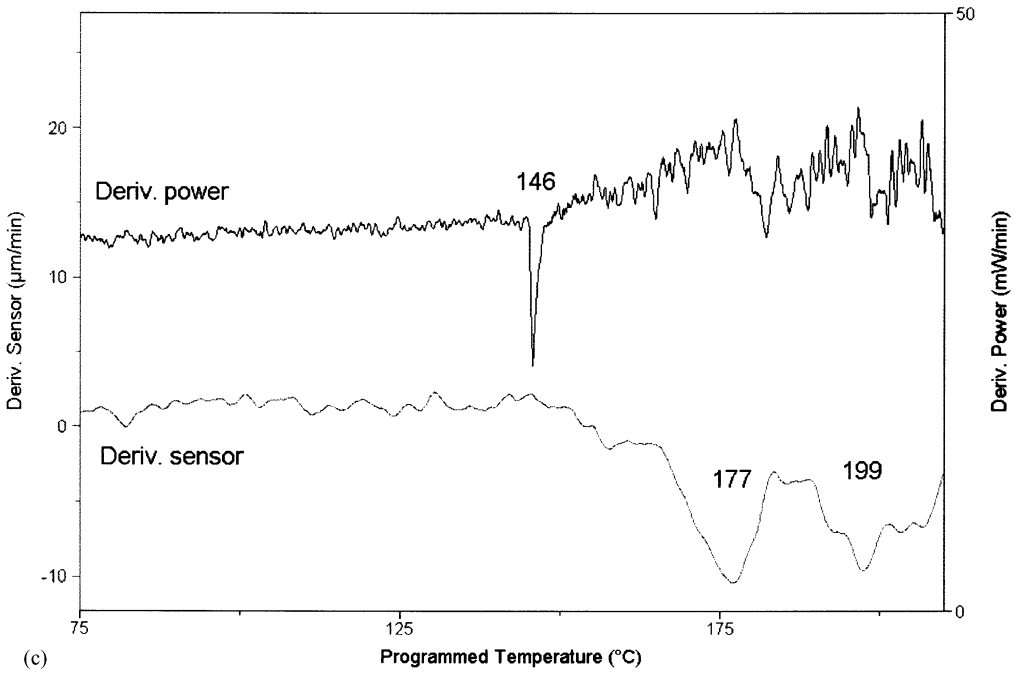

Fig. 5. (*Continued*)

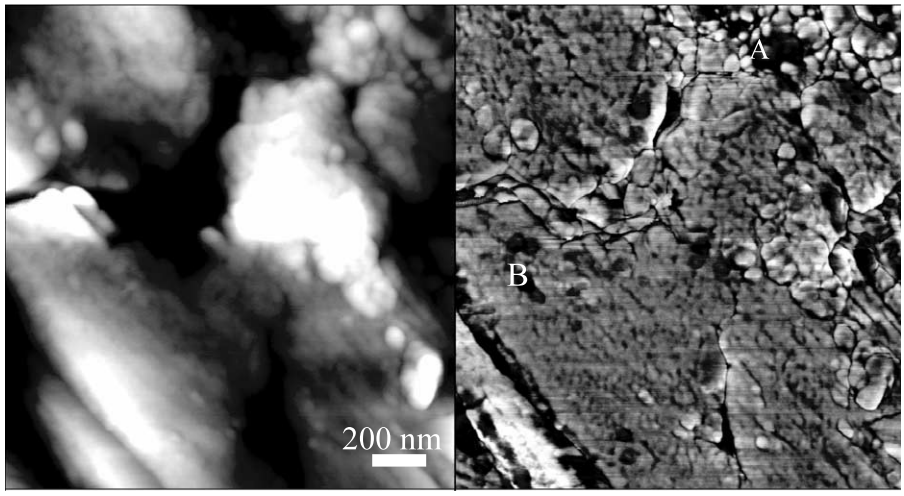

Fig. 6. Tapping mode AFM image of a pyridoxal hydrochloride pressed disc ((left) topography, (right) phase). Points A and B are areas of phase contrast in the image which are not attributable to topographical features.

Table 1 compares all the data obtained which indicates that there is excellent agreement between techniques. Data obtained from Fig. 5b and c show the ability of the SThM to analyse specific points on a surface and its ability to analyse interfacial regions, whilst Fig. 3b highlights the ability of SThM to gain information unobtainable by DSC.

Table 1
A comparison of all melting point data obtained from DSC and SThM

Compound	DSC peak (°C)	DSC range	SThM peak (°C)	SThM range
Pyridoxal hydrochloride	175	170–178	172	166–178
Pyridoxal hydrochloride	202	200–208	198	194–201
Mannitol	168.5	155–175	164	157–168
Avicel	198	195–205	199	197–200

4. Conclusions

This work has shown how LTA using the SThM may provide comparable thermal data to that obtained by DSC analysis, and has highlighted significant advantages i.e. that it is a more rapid technique and may perform thermal analysis on a single crystal. It also demonstrates the ability of the SThM to analyse specified areas on a surface with a good degree of precision and accuracy, and how thermal analysis of a single particle and of the interface between particles may be achieved using this technique.

It should be emphasised that the calibration of this equipment is a vital part of the experimental process for both techniques, but in particular for gaining meaningful data from the SThM.

There are other potential applications for this technique in the pharmaceutical industry outside the realm of drug and excipient crystals. For example, polymer materials are increasingly being found in complex heterogenous formulations and this technique could lead to an increased understanding of the behaviour of the polymers at interfaces in situ within a dosage form, and of thin films present in some formulations.

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