

## The evolution of hot-stage microscopy to aid solid-state characterizations of pharmaceutical solids

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### Abstract

A variety of techniques can be used to characterize the physical properties of pharmaceutical solids, including thermal analysis, hot-stage microscopy, X-ray powder diffraction, spectroscopic and micromeritic analysis. Comprehensive characterizations of the physical properties of pharmaceutical solids require a multi-disciplinary approach, since no single technique is capable of characterizing the materials completely.

The combination of traditional hot-stage microscopy with new technologies such as high-resolution micrography, image capture, storage manipulation, and presentation, have permitted more comprehensive physical property characterizations to be conducted. As a result of these technological advances, it is possible to present the results of these microscopic analyses, as they were initially collected by the microscopist, outside of the laboratory.

An evolutionary trail detailing the use of hot-stage microscopy in the Materials Science Group, from a simple melting point apparatus to the current hot-stage DSC microscopy instrument, will be presented. Examples of materials characterized using the hot-stage microscopy system will also be presented. © 1998 Elsevier Science B.V. All rights reserved.

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### 1. Introduction

Thermoptometry is a term used to describe “a family of thermoanalytical techniques in which an optical property of the sample is monitored versus time or temperature, while the temperature of the sample in a specified atmosphere, is programmed” [1]. Two examples of thermoptometry are thermomicroscopy (or hot-stage microscopy), in which the sample is observed while under a microscope, and thermoluminescence, in which the light emitted from a sample is monitored during the experiment [1].

Microscopy was first applied to chemical analysis in 1833 by Raspail [2], who suggested using crystal habits as a means of identifying chemical compounds. This idea was further developed by Wormley, as well as Behrens, Kley, Emich, and Chamot to form a method of analysis [3]. Various outgrowths from this early optical crystallography research included the work of Otto Lehman [4], Ludwig and Adelheid Kofler [5], Emile Chamot and Clyde Mason [6] and Walter McCrone [3]. Lehman proposed that the crystallization of an organic compound from its own melt was characteristic for that material, and the Koflers further identified the characteristics of organic compounds. Chamot and Mason investigated the physical and chemical methods of chemical microscopy, while

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McCrone presented a review of the applications and equipment of fusion methods. Later, Maria Kunhert-Brandstätter applied thermomicroscopy to an even narrower segment of chemical compounds, organic compounds with medicinal benefit [7].

Since the availability of chemical microscopy equipment was very limited, researchers were required to develop their own tools and equipment. As a result, several hot and cold stages, hot bars, etc., were developed. Much of the work conducted by the Koflers was developed utilizing the Kofler hot stage [3], which became available in 1940 [8]. The Mettler hot stage, introduced in 1967, greatly increased the use of hot-stage microscopy because of its ease of use and precision [9] enabling the development of thermomicroscopic techniques to be refined in the 1970s. As technological developments expanded the capabilities of analytical instrumentation with the advent of computers, improved hot stages, photographic equipment with associated hardware and sophisticated software, thermomicroscopists were able to conduct even more elegant thermomicroscopy experiments.

Hot-stage microscopy is an analytical technique which combines the best properties of microscopy and thermal analysis to enable the characterization of the physical properties of materials as a function of temperature. The importance of complementary thermal analysis techniques has been documented [10,11], and with the marriage of hot-stage microscopy to new technology, such as high-resolution color cameras and image manipulation software, video-enhanced microscopy [12] offers even greater possibilities for the characterization of materials.

Hot-stage microscopy in the pharmaceutical industry is used in a variety of ways to confirm transitions observed using other techniques. Hot-stage microscopy may be used for the solid-state characterization of bulk drugs [13], evaluation of crystal forms and hydrates [14–19], and other physio-chemical properties. Recently, the use of digital cameras [20], and the development of a photo visual and optical DSC [21,22] have expanded the visual collection capabilities for the characterization of materials using hot-stage microscopy.

The pharmaceutical industry utilizes a wide variety of disciplines to research, develop, and manufacture compounds which possess therapeutic value. The characterization of the physical properties of pharma-

ceutical solids is one of the disciplines utilized early in the drug development process. The characterization of these properties are a vital component in determining whether the component under development is a candidate for continued development as a drug product.

Compounds under development are generally characterized using the following techniques: differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), powder X-ray diffraction (XRD), hot-stage microscopy (HSM), solid-state nuclear magnetic resonance (NMR), infrared (IR), and Raman spectroscopy, moisture uptake, particle size, scanning electron microscopy (SEM), and micromeritic assays.

The initial characterization of compounds under development generally undergo DSC, TGA, hot-stage microscopic and XRD analyses. These four techniques are chosen since the data generated from them, when viewed collectively, amount to a relatively complete initial analysis of the physical properties of the compound. It is important to stress that no one technique is adequate to completely characterize the properties of the material. While XRD is essential in determining the X-ray diffraction pattern of the compound, DSC is required to collect information regarding the thermal properties of the material. DSC, however, is not absolute in characterizing these thermal events. TGA is required to confirm transitions such as volatilizations and decompositions, and a visual technique, such as hot-stage microscopy, is required to confirm transitions such as melts and recrystallizations. Depending on the information collected from these techniques, other techniques may be used to complete the characterization of the material.

This article chronicles the development of an integrated hot-stage photomicroscopy system to aid in the physical characterization of pharmaceutical compounds. The use of hot-stage microscopy in the characterization of three solid-state systems is also presented.

## 2. Experimental

DSC curves were collected using a TA Instruments 3100 thermal analysis system using a 2920 DSC with autosampler. Approximately 3 mg samples were placed into aluminium DSC autosampler pans, and the weight accurately recorded. The samples were heated at a rate of 10°C in open pan configurations

to a final temperature of 300°C. A nitrogen purge rate of 25 cc/min was utilized.

Relative humidity (RH) studies were conducted by placing material in a desiccator with a saturated NaCl solution. The RH generated by the solution was ~75% RH. Material was stored in the desiccator for two weeks and then analyzed.

TGA curves were obtained using a TA Instruments model 3100 thermal analysis system using a 2950 TGA with autosampler. Approximately 10 mg of sample was placed onto a platinum pan and inserted into the TGA furnace. These samples were heated at a rate of 10°C/min, up to a final temperature of 300°C. A nitrogen purge rate of 100 cc/min (60 cc/min for the furnace and 40 cc/min for the balance) was utilized.

X-ray powder diffraction (XRD) measurements were obtained on a Philips X'PERT model APD 3750 powder diffraction system, equipped with a vertical goniometer in the  $\theta/2\theta$  geometry. The X-ray generator was operated at 45 kV and 40 mA, using the copper  $K\alpha$  line at 1.544056 Å as the radiation source. Each sample was scanned between 2 and 32°2 $\theta$  and in step sizes of 2 $\theta$ =0.03°. Vendor software was used for all data collection and data analysis.

Hot-stage/DSC video microscopy analysis was conducted using a Mettler FP84HT hot stage with a Nikon Microphot SA optical light microscope equipped with super long working distance objectives. A few crystals of sample were placed into an open 7-mm quartz crucible and heated at a ramp rate of 10°C/min up to a final temperature of 300°C using magnifications from 100 to 400×. Crossed polarizers with a Red I compensator were also utilized. Data was imported into a computer for real-time observation and storage. Additional information regarding the configuration of the integrated hot-stage system is provided later.

### 3. Discussion

To present a synopsis of the evolution of the hot-stage photomicroscopy system that was developed in the Materials Science group, several phases in the development of this technique will be presented. As appropriate, sample photomicrographs and associated data will be presented to illustrate the important role a visual technique plays to confirm transitions, such as melts and recrystallizations.

#### 3.1. Fisher–Johns melting point apparatus

Initial material characterizations involved a multi-disciplinary approach, in which a number of analytical instruments are utilized to characterize various properties of a material. However, one could only utilize the equipment that was available at the time, that was provided for by R&D budgets, and which was recognized as a required tool for the materials scientist.

The Fisher–Johns melting point apparatus is a relatively inexpensive (@\$1000), elementary melting point determination apparatus, in which material is placed between glass cover slips and is placed onto an aluminum casting containing a heating element. During the experiment, the temperature is controlled by adjusting a powerstat, and viewing the material is done through a 10× magnifying eyepiece. While the accuracy of the heating element is only  $\pm 2^\circ\text{C}$ , for the simple requirement to determine whether a material became molten, the Fisher–Johns melting point apparatus is a useful tool.

This technique, along with TGA, was used to confirm the transitions observed in DSC curves. As experience with the melting point apparatus grew, so also did the knowledge that additional aspects of the analysis, such as the thermal data and photographic images from that particular sample were not being captured. This pointed to the need for an improved technique for the determination of melting point.

#### 3.2. Mettler FP82HT hot stage

The next step in the evolution of thermal microscopy began with the acquisition of a hot-stage accessory which would permit 'remote' observation of the thermal transitions. Remote meaning the use of a microscope to observe the thermal events without having to hover above a volatilizing solid sample and peer through a magnifying lens for an extended period of time. The use of color camera mounted on a microscope provided the medium through which the progressing thermal experiment could be viewed on a computer monitor. The Mettler FP82HT permitted programmed ramping experiments to confirm the suspect transitions apparent in the DSC curve, and the use of the microscope with the color camera provided the means by which the analysis could be viewed remotely.

Numerous experiments were conducted with this upgraded hot-stage microscopy system providing the confirmatory data to decipher the thermal profile of the compounds under evaluation. While this system was a great stride in controlling experiments by using ramping, iso-thermal, and oil-immersion experiments, the analyst was still required to closely monitor the experiment and record observations as they occurred. Additionally, while the experiment was viewed in color and in real time, the collection and storage of images electronically at various time points during the thermal experiments was still not possible due to lack of a computer.

### 3.3. Computer interfacing

Once the hot-stage system had been developed and used for a time, the attributes that were still thought to be advantageous were pursued. Upgrading the system with super long working distance objectives, a computer, image frame grabber, and a Mettler FP84HT hot stage/DSC, increased the capabilities of thermal microscopy system to a much greater level. The addition of these features provided a relatively intricate collection of equipment that was able to accurately characterize both the visual and thermal properties of the material.

Super long working distance objectives permitted observation up to 400 $\times$ , the computer and image frame grabber permitted collection of individual images at critical moments during the experiment, and the upgraded version of the Mettler hot stage collected the thermal information on the exact sample under visual examination. While the frame grabber permitted collection and storage of color images, the constraints of the image manipulation software required  $\sim 30$  s to collect a single image. This inevitably was during the time when the most interesting aspect of the analysis was occurring and forced the analyst to be very selective when the images were to be collected. Additionally, since each image file was nearly 500 KB, storage and manipulation of these color images were important issues. Again, while great strides were accomplished utilizing this design, several important aspects in the development of a comprehensive thermal microscopy analysis system were still lacking, namely an easily retrievable real-time representation of the micro-thermal experiment.

### 3.4. An integrated hot-stage photomicroscopic system

To achieve this real-time video capability, a new high-resolution camera, a computer with an upgraded frame grabber, along with associated hardware components and software were needed. Since the entire hot-stage photomicroscopy system was based upon the images of the microscopic crystalline particles, the new system was designed using high-resolution color images as the foundation to provide a real-time presentation of the temperature dependent transitions. Initially, a high-resolution digital color camera was investigated, however, due to the scanning mechanism utilized, the time required to collect the images ranged from  $\sim 30$  s to several minutes. Considering that a typical hot-stage experiment was proceeding at 10°C/min, the analyst could not afford several minutes to collect a single image.

The best alternative was to evaluate a high-resolution color video camera which would not only provide the required resolution, but collect images instantaneously. Once the image collection device was selected, the only component still required was a system to allow the analyst to quickly view what had just transpired during the thermal experiment. A high speed Pentium computer with a frame grabber and associated software, and high capacity optical drives would provide this ability. This new equipment would permit image collection of 1–30 frames per second, rather than four frames per experiment which was possible using the previous frame grabber. Additionally, the image manipulation software stored each frame as a separate file with a unique name so that individual 1MB frames could be selected for incorporation into reports.

The developed hot-stage microscopy system now encompassed an FP84HT DSC/hot stage, 400 $\times$  magnification, a high-resolution color camera, and computer hardware and software, which provided the ability to immediately view a real-time representative 'video' of the thermal experiment. While the image manipulation capabilities of this new system provided relatively instantaneous high quality results to view on the computer monitor, this data was not available to the scientists and engineers who had originally submitted the samples.

To provide the same high quality images and real-time presentation capabilities to those outside the laboratory, two avenues of image presentation were developed. A color digital printer with the ability to produce photographic quality images was purchased. Downloading of images into word processing programs and then printing the images on the digital printer, provided a very realistic visual representation of the interesting thermal transitions of the experiment. Additionally, a S-VHS video cassette recorder was connected to the computer which permitted copying of the real-time video experiment to videotape. This enabled a scientist to take a good quality copy of the video data to a project team meeting, providing the team with a greater understanding of the thermal properties of the compound under development.

## 4. Applications

### 4.1. Compound A

To provide an illustration of a compound exhibiting a simple melt, data from compound A is presented. Fig. 1 shows an overlay of the DSC and TGA data in which the DSC curve showed an endothermic

transition at 151.9°C, while the TGA curve indicated that decomposition occurred at the same temperature.

Figs. 2 and 3 are photomicrographs collected at 25° and 130°C at 200× under crossed polarizers with the Red I compensator, showing the material as a solid at ambient temperature, and at the melt onset of compound A, respectively. While compound A represents a simple system, confirmation of the endotherm at 151.9°C by a visual technique was required to confirm the melt transition.

### 4.2. Compound B

Some compounds under development often exhibit interesting properties under high relative humidities. Fig. 4 represents the X-ray powder diffraction patterns of compound B at initial and two weeks at 75% RH. This figure illustrates that at high relative humidity, compound B begins to convert from an amorphous material to a low crystallinity material over a two week period.

Fig. 5 presents an overlay of the DSC and TGA data from the material held at 75% RH for two weeks. The DSC curve exhibits an endothermic maximum at 121.4°C, an exothermic maximum at 195.5°C, and

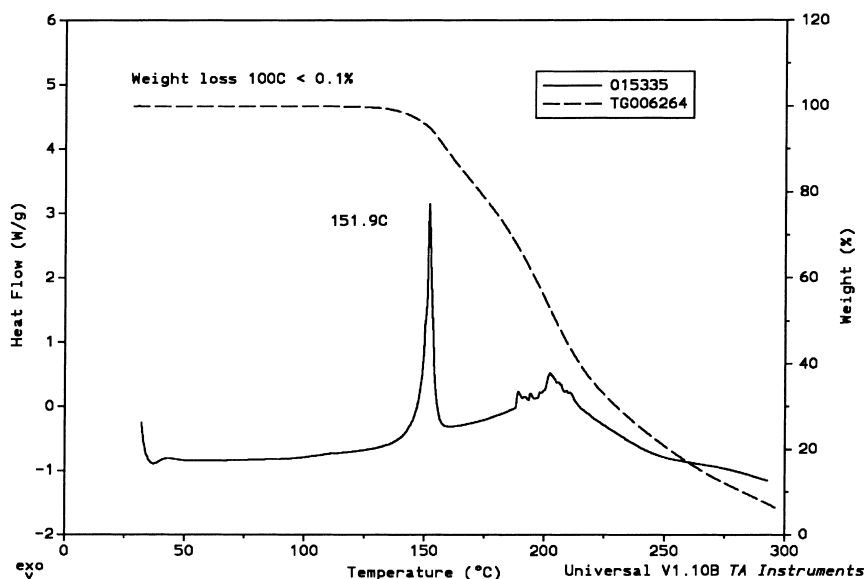


Fig. 1. Compound A overlay of DSC curve (bottom) exhibiting endothermic maximum at 151.9°C and TGA curve (top) exhibiting weight loss of <0.1% at 100°C.

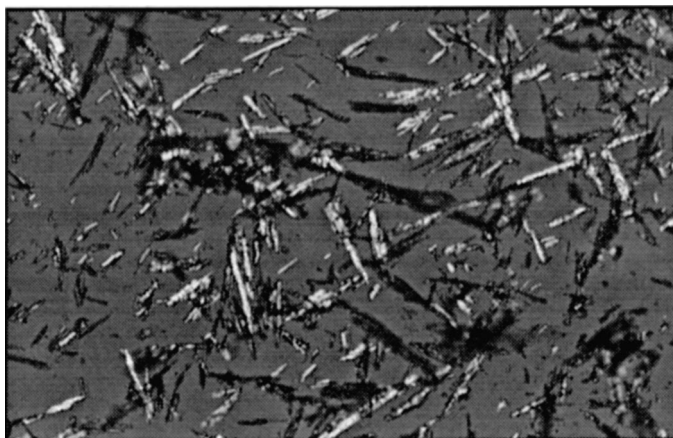


Fig. 2. Compound A photomicrograph, collected at 25°C under 200× magnification using crossed polarizers with the Red I compensator, showing the solid particles.

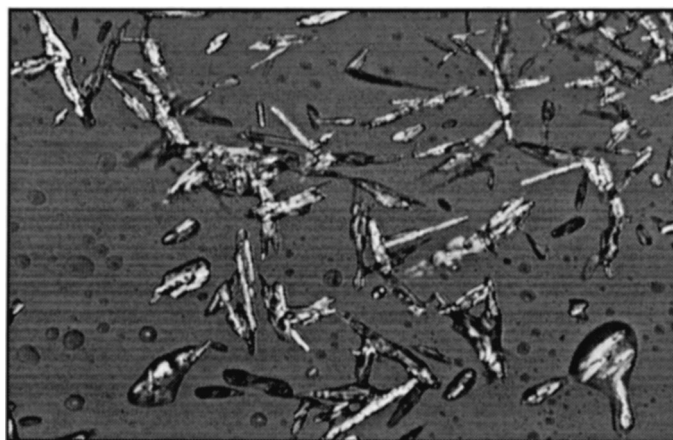


Fig. 3. Compound A photomicrograph, collected at 130°C under 200× magnification using crossed polarizers with the Red I compensator, showing the melt onset of the particles.

an endothermic maximum at 264.8°C while the TGA curve shows a 12.1% weight loss at 200°C. Since the material was amorphous initially, no hot-stage characterization was conducted. However, once the XRD indicated that the material had converted to a low crystallinity material, the hot-stage experiment was warranted.

The hot-stage experiment indicated that the endotherm near 121.4°C was a combination of volatile loss and melt of the material. The exotherm in the DSC curve at 195.5°C was explained using hot-stage microscopy as shown in Figs. 6 and 7. These figures,

collected at 208° and 230°C, respectively, at 400× with crossed polarizers with Red I compensator, show the material in a molten state showing various stages of the recrystallization. The hot-stage experiment also observed the melt of this recrystallized material near 250°C with confirmed the DSC endotherm at 264.8°C as a melting transition.

To complete the characterization of this system, further evaluation would be required: TG/IR or TG/MS to identify the volatiles lost near 100°C, and/or to confirm decomposition above 275°C; XRD to study the crystallinity changes at high RH with additional

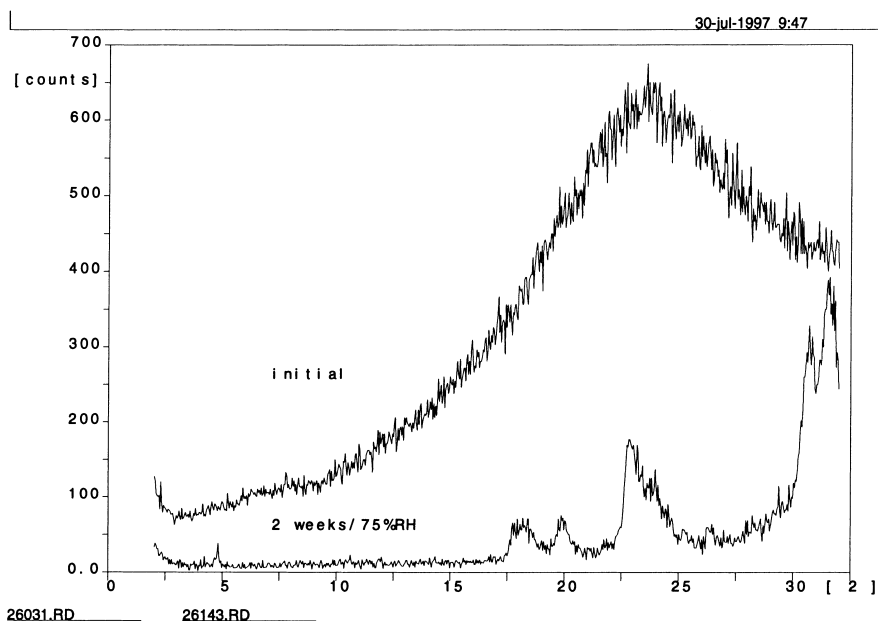


Fig. 4. Compound B XRD powder diffraction at initial conditions (top) exhibiting an amorphous pattern, and after two weeks at 75% RH (bottom) exhibiting a low crystallinity pattern.

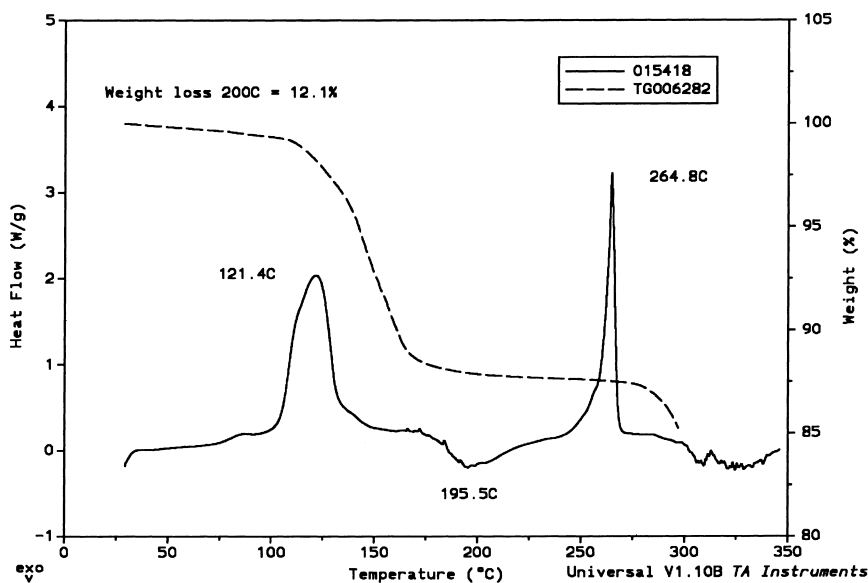


Fig. 5. Compound B overlay of DSC curve (bottom) exhibiting endothermic maxima at 121.4°C and 264.8°C, and an exothermic maximum at 195.5°C, and TGA curve (top) exhibiting weight loss of 12.1% at 200°C.

time; and VT-XRD and quench-cooling DSC experiments studies near 220°C to characterize the properties of the recrystallized material. In this second

example, the importance of using hot-stage microscopy is essential to confirming the thermal transitions of compound B.

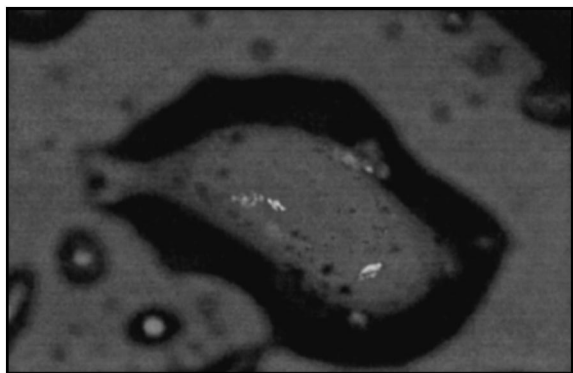


Fig. 6. Compound B photomicrograph, collected at 208°C under 400× magnification using crossed polarizers with the Red I compensator, showing the recrystallization onset in the molten pool.

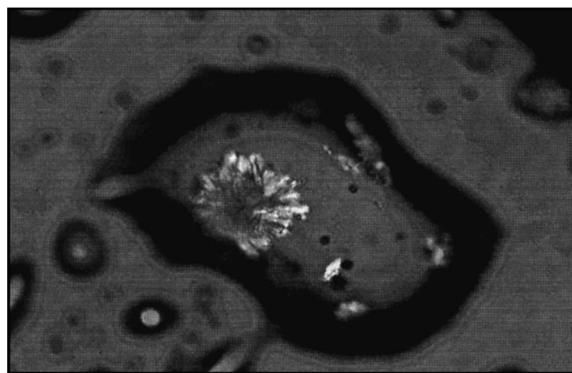


Fig. 7. Compound B photomicrograph, collected at 230°C under 400× magnification using crossed polarizers with the Red I compensator, showing the recrystallized onset in the molten pool.

#### 4.3. Compound C

Visual melting assays are extremely important especially with systems exhibiting unusual properties. Fig. 8 presents an overlay of DSC and TGA data from compound C.

An endothermic transition was observed at 134.6°C, followed by an endo/exothermic combination beginning near 181.6°C, and an additional

endothermic transition at 263.5°C. The TGA curve exhibited a 7.8% weight loss measured at 150°C which correlated with the 134.6°C endotherm. A large weight loss near 250°C, confirmed the 263.5°C endotherm as a decomposition. The conclusion made from this preliminary data set would be that the material possibly melts, and volatilizes at 134.6°C, possibly melts and recrystallizes at 181.6°C and then melts with decomposition at 263.5°C, how-

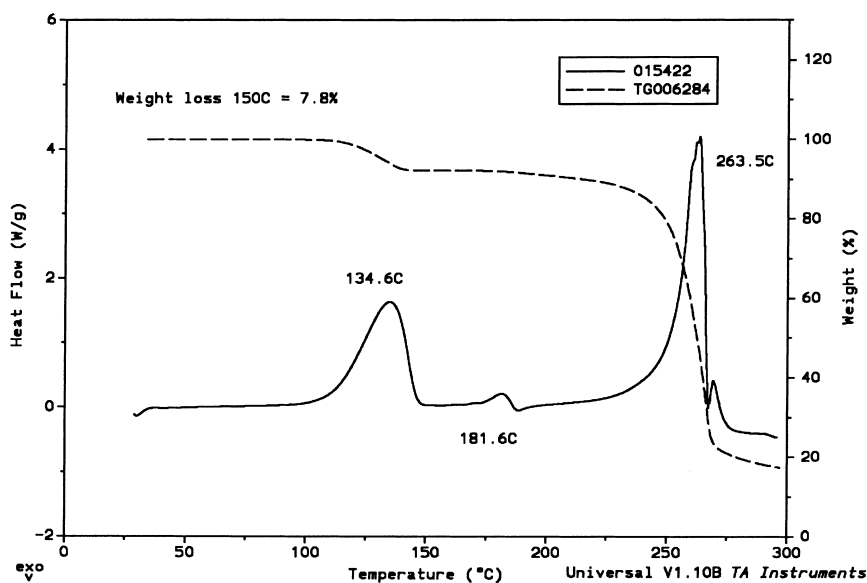


Fig. 8. Compound C overlay of DSC curve (bottom) exhibiting endotherm maxima at 134.6° and 263.5°C, and an endotherm/exotherm combination near 181.6°C, and TGA curve (top) exhibiting weight loss of 7.8% at 150°C.



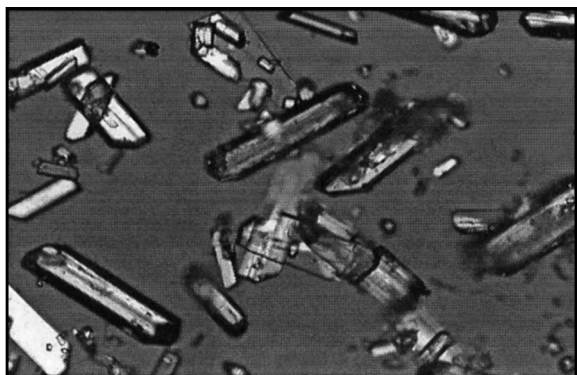


Fig. 9. Compound C photomicrograph, collected at 30°C under 200× magnification using crossed polarizers with the Red I compensator, showing the solid material.

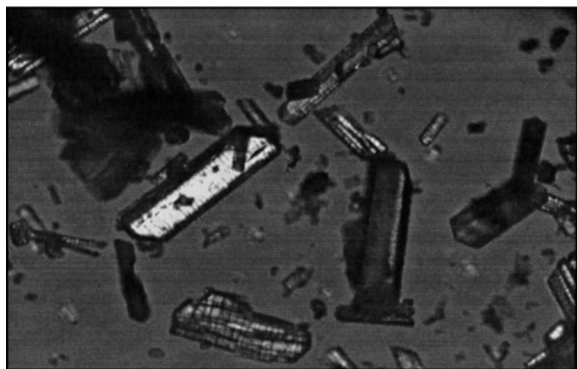


Fig. 10. Compound C photomicrograph, collected at 135°C under 200× magnification using crossed polarizers with the Red I compensator, showing the solid material following a volatilization and solid-state transformation.

ever, no visual confirmation of these transitions is available.

Figs. 9–11 present photomicrographs collected at 30°, 135° and 190°C, respectively, at 200× using crossed polarizers with the Red I compensator. These hot-stage images, along with the DSC and TGA data, indicate that compound C loses volatiles and undergoes a solid-state transformation near 134.6°C (since the particles did not exhibit a melt, but became fractured), undergoes a second solid-state transformation at 181.6°C. (since the particles became less birefringent), and then began to decompose near 190°C.

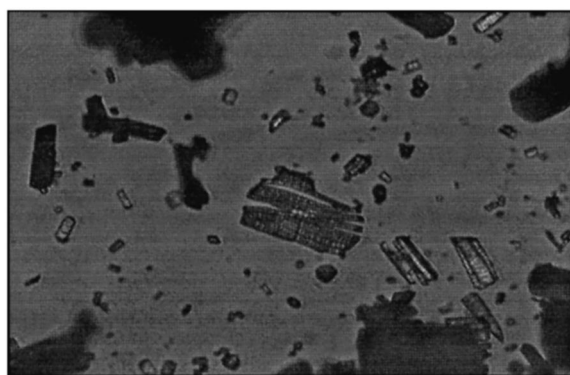


Fig. 11. Compound C photomicrograph, collected at 190°C under 200× magnification using crossed polarizers with the Red I compensator, showing the solid material following a second solid-state transformation.

To completely characterize this system, additional studies are required such as, variable temperature XRD and quench cooling DSC to study the material at elevated temperatures, and TG/IR, TG/MS or solids GC/MS for identification of decomposition products. It is important to state that the data provided by the hot-stage photomicrography experiment was essential in the characterization of compound C.

## 5. Conclusions

The comprehensive characterization of the physical properties of many materials, including pharmaceutical solids, requires a multi-disciplinary approach, since no single technique is capable of characterizing the materials completely. The use of any visual melting point apparatus provides vital information to the characterization of these materials. As the sophistication of these instruments increases, so does the value of the data it provides. A hot-stage photomicroscopy system with capabilities such as high-resolution micrography, image capture, storage, manipulation, and presentation, permit comprehensive physical property characterizations to be conducted. In addition, results from these hot-stage photomicrographic assays, in the form of reports containing photographic quality images and real-time video presentations, can be easily made available to other personnel interested in the physical properties of these materials.

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