Journal of Thermal Analysis, Vol. 49 (1997) 991-998

A COMBINED DIFFERENTIAL SCANNING CALORIMETER-OPTICAL VIDEO MICROSCOPE FOR CRYSTALLIZATION STUDIES

J. M. Hey[1], *P. M. Mehl*[1] *and D. R. MacFarlane*[2]

[1]Transfusion and Cryopreservation Program, NMRI, Naval Medical Center, Bethesda, MD, 20889–5607, USA
[2]Department of Chemistry, Monash University, Wellington Road, Clayton, Victoria, 3168, Australia

Abstract

A simultaneous differential scanning calorimeter (DSC)-optical video microscope instrument has been developed. The instrument development included slight modifications to the sample head of a Perkin-Elmer DSC-7, along with the use of a CCD camera and magnifying lenses. The instrument permitted simultaneous following of optical and thermal events during isothermal and non-isothermal DSC experiments. The DSC curves were almost identical to those given by a standard DSC-7. The operational temperature range of the instrument is between −160 to 600°C. The capabilities of the DSC-video microscope are illustrated by examples of ice crystallization data in aqueous solutions of glycerol and dimethyl sulphoxide.

Keywords: cryo-microscopy, crystallization, DSC, ICE, microscopy

Introduction

A study of ice crystallization by Reid [1] has shown that the results from differential scanning calorimetry (DSC) experiments and optical cryomicroscopy can be correlated successfully, and that a combination of the two techniques can help in the identification of the processes being studied. Mehl has also used optical cryomicroscopy in conjunction with DSC analysis to investigate the crystallization of ice from various aqueous systems [2–4]. He found good agreement between the thermal events recorded by DSC, and the optical observations from the cryomicroscope. However, both of these investigators used separate instruments to obtain their thermal and optical data. It was the aim of this work to develop an instrument that would allow simultaneous recording of thermal and optical crystallization data on the same sample. Such an instrument would permit a clearer understanding of the physical origins of thermal events recorded and not always understood in the thermal trace. The paper details the design of the DSC-video microscope: experimental results from this instrument will be published elsewhere [5–7].

0368–4466/97/ $ 5.00

John Wiley & Sons Limited
Chichester

Experimental

The DSC-video microscope was based on a modified Perkin-Elmer DSC-7. The sample head of the DSC was mounted on a copper cold finger within a dry-box. The base of the cold finger was immersed in a 6 litre stainless steel dewar flask below the dry-box. A 1 mm thick disc of annealed pure silver was used as a heat transfer agent between the sample head and the cold finger.

Visual access to the sample was permitted by removing the aluminium cover that is normally used with the Perkin-Elmer DSC. Instead, glass coverslips were used to control the flow of gas around the sample head. One small coverslip (10 mm in diameter) was laid over each sample holder, resting on the head, and these were necessary to ensure a steady baseline. A 12 mm high teflon spacing ring was used to support a third, larger, glass coverslip above the sample holders. This then created a boundary which tended to fill with the dry helium gas flowing through the head, and prevented condensation forming on the lower coverslips when the instrument was used at low temperature. A teflon spacing ring of less than 12 mm permitted the upper glass coverslip to cool down sufficiently to allow any residual water vapour in the dry-box to condense on it in the form of ice. The arrangement of the coverslips on the sample head is shown diagrammatically in Fig. 1a. A ring of high density foam with the same outside diameter as the DSC sample head was placed around the teflon spacing ring for thermal insulation. The dry-box used to house the combined instrument was a flow-through design, with dry nitrogen as the purge gas. The dry-box volume was kept to a minimum so that the atmosphere changed regularly. Also, a small ante-chamber was used to provide access to the sample head without contaminating the atmosphere of the dry-box.

The flow of helium through the head of the DSC cell was controlled and monitored by the use of a flow meter. It was found that relatively small changes in gas flow did not significantly change the calibration of the instrument, but did result in large changes in the experimental baseline. Similarly, if the light source was turned on during an experiment, the resultant change in heat flow around the sample holder caused a large step in the baseline; a similar step (but opposite in direction) occurred if the light source was turned off again. However, the positions of the peaks resulting from the phase transitions that cyclohexane undergoes at –87°C and 6.5°C were found not to vary when heating thermograms were run with and without the light source switched on. This is important, since each time a sample is placed into the head of the instrument, one of the fibre-optic goosenecks is moved in order to gain access to the head.

Microscope and video details

A Pulnix TM-6CN black and white video camera connected to a series of magnifying lenses (Navitar Zoom 6000II) was mounted above the DSC sample head on a X-Y stage, this then acted as a video microscope system. The X-Y stage was connected by cables to levers on the outside of the dry-box so that the camera could be manipulated without entering the dry-box. The X-Y stage was set so that the camera could move vertically and in one plane horizontally. The vertical manipulation thus allowed the camera to be focused remotely, and the horizontal movement was

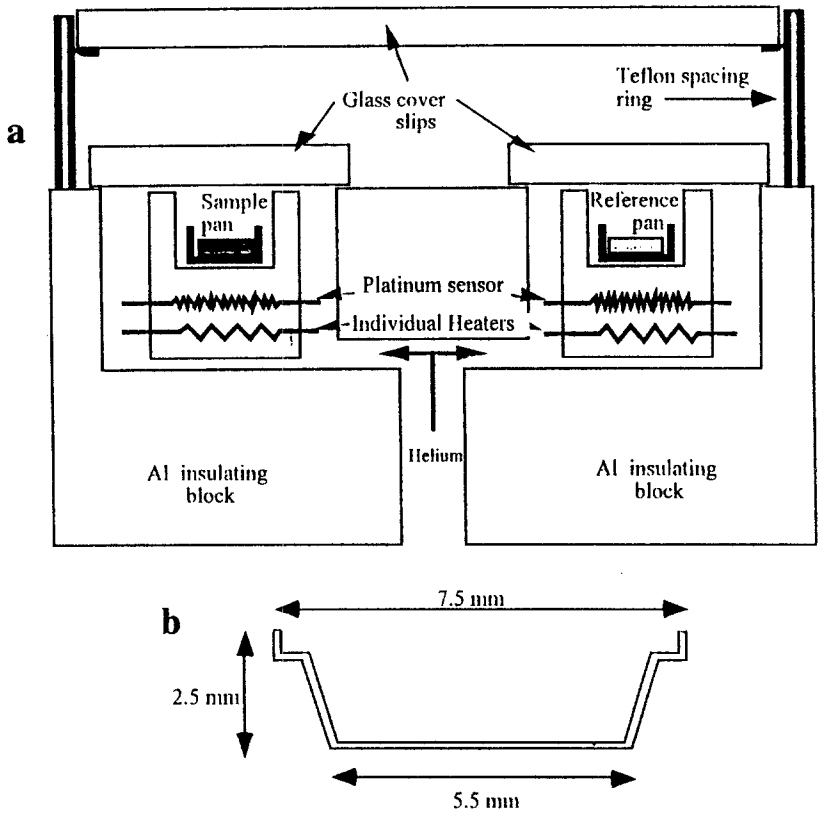


Fig. 1 a) Diagrammatic representation of the arrangement of the glass coverslips and teflon spacing ring on the sample head of the DSC-video microscope;
b) Design of sample pans for DSC-video microscope. The lip at the top of the pan is for placement and sealing of the glass coverslip

used to move the field of view across the sample, and was set up in such a way that the plane of movement was parallel to the front of the dry-box. A Volpi Intralux 6000 halogen light source with twin optical fibre goosenecks was used to illuminate the sample. A Panasonic NV FS90 S-VHS video recorder was used to videotape the crystallization experiments, and a Pro-video TD-100-S time/date generator labelled each video frame with the time, date and frame number. A black and white monitor was used to observe the experiments as they proceeded, and also showed when the sample was in focus. A Macintosh IIcx computer with a Mass Microsystems Quick-Image 24 video frame grabber card was then used to analyse the captured frames.

Stock solutions and sample sealing

Dimethyl sulphoxide (DMSO) (BDH and Aldrich) was dried by stirring over CaH_2 and then distilled under reduced pressure (0.5 mm Hg) before use. Glycerol

(Aldrich) was dried over 4A molecular sieves and then distilled under reduced pressure (0.5 mm Hg) before use. All solutions were made with doubly distilled water, and each solution was prepared gravimetrically. The solutions were made up as weight percentage solute of total solution weight (*w/w*). The sample masses varied between 35 and 50 mg.

Aqueous samples placed directly into the DSC sample holders without sealing were found to lose water vapour, which then condensed onto the colder sample head. This is due to the fact that even at low temperatures the vapour pressure of water is not zero, and the condensation of the vapour on to the colder regions of the head led to the water continually distilling from the sample. To prevent this, specially designed sample pans were made with a lip on their outer edge (Fig. 1b) so that a glass coverslip could be sealed over the sample. The sample was pipetted into the sample pan, a glass coverslip was placed over the solution, within the lip of the pan, and then a bead of fast curing epoxy resin (5 min Araldite) was used to seal the glass coverslip to the lip of the pan. Sample were weighed to assess the total mass, and then left for at least 12 h to allow the epoxy resin to reach maximum strength. Before use the samples were re-weighed to confirm that no leakage had occurred. The epoxy resin did not undergo any phase transitions in the temperature range of interest, and hence its use did not interfere with the DSC traces produced from these samples. Repeated cooling/warming cycles were found to have no adverse effects on the sample integrity. Generally, the sealed samples were found not to deteriorate within short periods of storage.

The samples prepared by the method described above usually contained a small air bubble which tended to sit at the edge of the sample, against the glass coverslip. Such air bubbles resulted from the preparation process, and were often difficult to avoid.

The sample pans were made from either aluminium or platinum and the DSC traces did not appear to vary with pan type. Under the microscope the base of each type of pan appeared speckled due to the relatively rough surface of the metal. In order to give a uniform background for each experiment, a portion of the base of each sample pan was painted with matt black enamel paint. The stability of the samples sealed in this way, along with the reproducibility of the DSC traces of each sample indicated that no reaction was occurring between the solution and the enamel paint. The presence of the paint did not affect the temperatures of the various transitions to a significant degree, however, it was noted that when the paint was used in a sample pan, there appeared to be a slightly greater tendency of the samples to form heterogeneously nucleated ice crystals on cooling.

DSC-Video microscope experimental procedures

The DSC-video microscope was calibrated using the crystal–crystal transition of cyclohexane at –87°C and the melting point of cyclohexane at 6.5°C. The isothermal experimental procedures used for the DSC-video samples were as follows. For both glycerol and DMSO solutions the sample was initially held at 0°C and then was quenched to the isothermal hold temperature at 150°C min^{-1}. The video recording was started as soon as the DSC had reached the isothermal hold temperature.

Frame capture and analysis of the video data

A Mass Microsystems QuickImage 24 video frame grabber card and the Mass Microsystems software package QuickImage were used to capture images from video tape. QuickImage allows capturing of single or multiple images. In most cases multiple frames of a crystallization video were captured. The number of frames captured, and the period of time between each captured frame depended upon the length of time over which the crystallization even took place. Captured images were saved in PICT format for analysis. The time-date generator printed the time and frame number (25 per second) on each video frame, and hence the time between each captured image could be ascertained accurately.

The NIH image analysis program Image was used to analyse all the frame grabbed crystallization data. In order to derive accurate crystal growth rates from the videotaped experiments, the Image software was spatially calibrated using the videotaped image of a 0.01 mm graticule. This spatial calibration was then propagated to each captured image. To determine crystal growth rates, two different analysis methods were employed depending on whether the measurement was of the linear growth of an ice front, or the area of a spherulite. For linear growth of ice fronts, a measurement line, normal to the direction of the ice crystal growth, was drawn on the first captured frame, this was then duplicated onto all frames to be analysed, ensuring that all measurements had a common origin point. Keeping the origin point of the line fixed, and moving the other end of the line to the ice/solution interface allowed the distance from the fixed origin to the interface to be measured for each frame. The growth of spherulitic crystals with time was measured somewhat differently, since the program Image contains software capable of measuring areas of binary (black and white) images. In the case of spherulites, the image was first converted to binary, and then the area measurement software was used to determine the area of each spherulite. Also, the Image software was also used to measure the change in opacity of samples which crystallized under conditions in which it was not possible to detect isolated crystals. For these measurements, the optical density of a section of each frame was measured using the Image software.

The estimated uncertainties for the DSC-video microscope thermal measurements were $\pm 1.0^{\circ}C$ for onset temperatures, and $\pm 0.75^{\circ}C$ for peak temperatures. The estimated uncertainty in the peak times for the isothermal measurements was estimated to be the same as for the conventional studies, i.e. ± 0.2 min. The uncertainty associated with the linear growth measurements was estimated to be ± 0.001 mm.

Results and discussion

The development of the combined DSC-video microscope required only slight modifications to the normal sample head arrangement of the Perkin-Elmer DSC. Although this modification of the instrument did not permit the use of the standard platinum sample holder covers, it was found that the thermal response of the instrument was almost identical to that of the standard DSC arrangement for both isothermal and non-isothermal experiments. Figure 2a shows a comparison between the

non-isothermal DSC curves of 50 *w/w%* glycerol samples run in the conventional DSC and in the DSC-video microscope. The temperature of each transition (T_g, devitrification and melting) was found to be the same, but the melting peak appeared

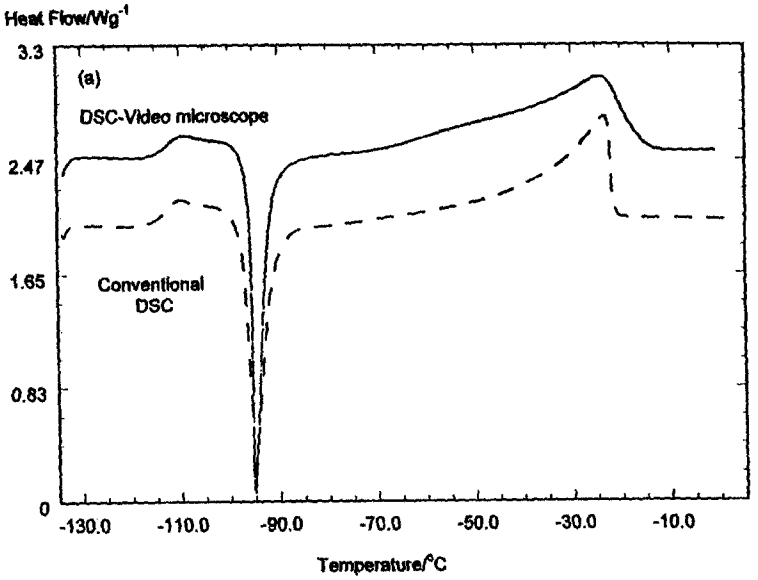


Fig. 2a Non-isothermal DSC traces of samples of 50 *w/w%* glycerol in conventional DSC and combined DSC-video microscope. Scanning rate = 10°C min^{-1}

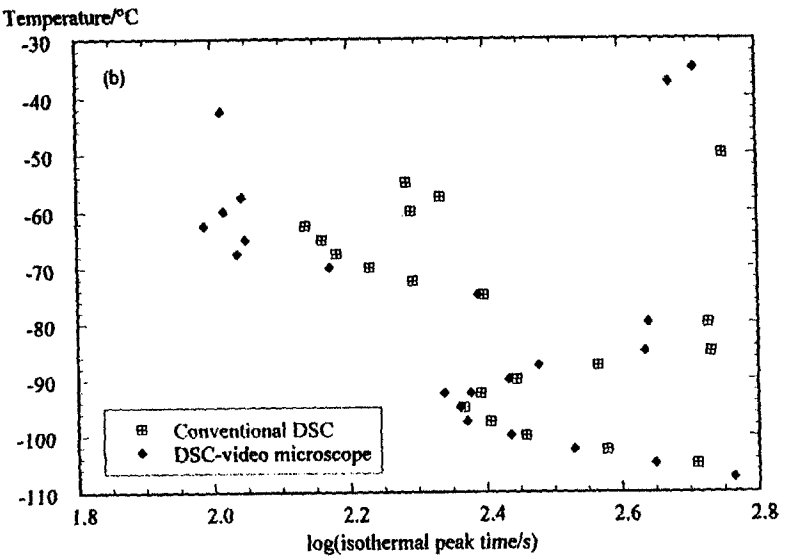
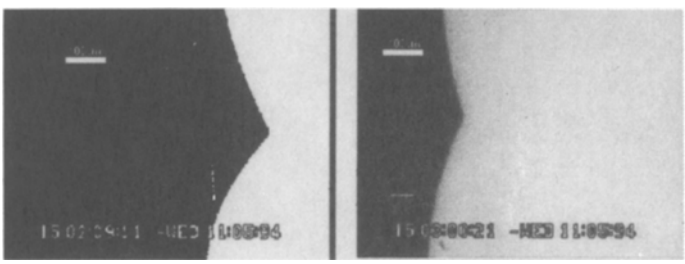


Fig. 2b Comparison of TTT curves for 50 *w/w%* glycerol solutions from the conventional DSC and the DSC-video microscope. Note: the TTT curve from the conventional instrument is taken from [8]

less sharp in the modified instrument, perhaps indicating that the heat transfer characteristics are not as rapid as in the standard DSC. This may be due to the sample pan design, since the top cover is made from a relatively poor thermal conductor, i.e. glass, leading to a slower transfer of heat from the instrument's heater to the sample (during melting), and thus results in a broader peak. DSC curves of samples contained in the glass-covered sample pans also exhibited broader melting peaks when tested in the conventional DSC heat, indicating that the major reason for this difference in peak shape was the sample pans themselves.

Isothermal traces from both the conventional and DSC-vide microscope sample heads are even more similar than the non-isothermal traces. TTT curves generated from isothermal peak crystallization times for 50 *w/w*% glycerol solutions from both instruments were found to be very close in the homogeneous nucleation temperature region [8] below −80°C, as shown in Fig. 2b.

Two distinctly different types of video data were generated from the isothermal DSC video experiments. At the higher temperatures, and in the experiments in which the solutions had not been passed through a temperature region where the nucleation rate is high, direct measurement of the crystal growth rate was possible. Above a certain temperature region, which depended upon the solution being studied, the nucleation of the ice occurred at a limited number of sites. In all of these cases the initial nucleation sites were at the point of contact of the air bubble and the glass cover slip. This small number of crystals then grew into the solution as large spherulites. An example of this type of ice crystal growth is shown in Fig. 3 for 45 *w/w*% DMSO at −55°C. Below this temperature region, but above the region of homogeneous nucleation, the ice crystals were found to form at apparently random sites, and grew into the solution as spherulites. At lower temperatures where the homogeneous nucleation rate was high, or when the solution had passed through its homogeneous nucleation temperature region, growth rate measurements on individual crystals or ice fronts was not possible due to the small size and large number of ice crystals present. Instead, measurements were made of the opacity of the solution as the crystals grew.



(a) (d)

Fig. 3 Micrographs of the growth of two adjacent ice solution interfaces in 45 *w/w*% DMSO at −55°C. Pictures (a) and (b) were frame-grabbed 51 seconds apart. Bar = 100 μm in each case

Conclusion

The addition of video microscopic equipment to a standard DSC creates a versatile instrument that allows simultaneous recording and direct correlation of optical and thermal data. The conversion is simple and relatively inexpensive. The temperature range for which this instrument is useful is the same as for the standard Perkin-Elmer DSC-7. Construction of a simultaneous instrument to be used only at above ambient temperatures would be much simpler than the instrument described in this paper, since many of the features were designed to overcome the problems encountered at sub-ambient temperatures.

* * *

Naval Medical Research and Development Command, Work Unit No. 61153 N. MRO 4120.001–1462 NM R&D. The opinions and assertions contained herein are the private ones of the writers and are not to be construed as official nor as representing those of Defense or of the Navy.

References

1 D. S. Reid, Cryobiology, 21 (1984) 60.
2 P. Mehl, Thermochim. Acta, 155 (1989) 187.
3 P. Mehl, Cryobiology, 30 (1993) 509.
4 P. Mehl, Thermochim. Acta, 203 (1992) 475.
5 J. M. Hey, Ph. D. Thesis, Monash University, 1994.
6 J. M. Hey and D. R. MacFarlane, to be submitted to Cryobiology.
7 J. M. Hey and D. R. MacFarlane, accepted for publication by J. Non-cryst. Solids.
8 J. M Hey and D. R. Macfarlane, Cryobiology, 33 (1996) 205.