Modulated Temperature Differential Scanning Calorimetry for Examination of Tristearin Polymorphism: I. Effect of Operational Parameters

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ABSTRACT: The transformations of tristearin were examined by modulated temperature differential scanning calorimetry (MTDSC) in order to study the effect of operational parameters on the nature of information obtained from this technique. Tristearin has been used as a model polymorphic system showing metastable phases and complicated transformation routes occurring at relatively slow rates. The parameters examined were underlying heating/cooling rates and the amplitude of modulation. The first conclusion is that MTDSC enables overlapping α-melting and β-crystallization events to be separated, thus increasing the information obtained compared to normal thermal analysis. Other general conclusions are that observation of reversible processes is strongly influenced by the underlying heating rate; low to moderate heating rates are recommended. Amplitude of modulation has a complicated effect on the phenomenon being studied; when studying systems that exhibit metastable or polymorphic transitions, it is recommended that a range of amplitudes be tested to enable confirmation of whether an observed "recrystallization" effect is a new phase or the same phase as the one melting. Cooling with modulation disturbs the crystallization process, possibly leading to smaller or imperfect crystals; however, the phases obtained are not different compared to normal DSC. The usefulness of MTDSC in analyzing these types of complicated systems is primarily qualitative at the moment. Some recommendations have been made as to the combinations of parameters for studying such systems.

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KEY WORDS: Differential scanning calorimetry, metastable forms, MTDSC, modulated temperature, oscillating, phase behavior, polymorphism, triglycerides, tristearin.

Lipids are important excipients in the pharmaceutical industry, finding use as matrix formers, binders, lubricants, etc. The major components of these commercial excipients obtained from natural fats are blends of triglycerides. Pure monoacid triglycerides can serve as simple models for studying these blends.

The physical properties of triglycerides are mostly determined by their complex polymorphism. Generally, triglycerides exhibit three different polymorphic forms characterized by particular chain packing and thermal stability: alpha (α), beta-prime (β′), and beta (β). The transformations are monotropic and theoretically take place from α to β′ to β (1).

The different polymorphic forms of triglycerides have been extensively studied (1–6), and the transformations are summarized in Figure 1. The melt crystallizes primarily into the α form on cooling, although special thermal treatment or very slow cooling can lead to the formation of the β′ or β form, respectively (7,8). The transformation from the metastable α form to the most stable β form may occur in the solid state through aging of the α form at temperatures below its melting point. Heating the α form slowly can also lead to its transformation to the β form; the amount or extent of β′ formation during this process depends on the fatty acid chain-length. The existence of two (or even more) forms of the β′ form is debated as to whether there are two distinct phases (7) or whether the β'_2 consists simply of imperfect β′ crystals (8).

Differential scanning calorimetry (DSC) is a powerful tool in the study of the polymorphic transformations in these substances (5,8). The recently developed technique of modulated temperature DSC (MTDSC), also known as modulated DSC or oscillating DSC, has been cited as having several advantages in this area (9). These include the ability to improve separation of reversible and irreversible thermal events (over the time scale of the experimental parameters), and improved resolution of closely occurring or overlapping events. The theoretical background of this technique has been presented elsewhere (9,10) and is briefly summarized below.

Crystallization of a stable phase from a liquid can be regarded as a reversible event at the equilibrium temperature. Melting of a metastable phase is normally an irreversible

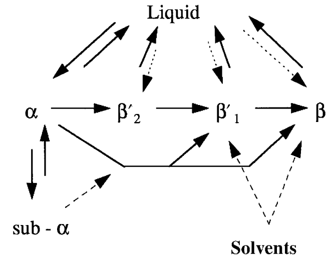

FIG. 1. Schematic of polymorphic phase transitions in saturated monoacid triglycerides. Dotted lines represent transitions that occur *via* special thermal treatments only. Adapted from Reference 4.

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event, while melting of a stable form can be a combination of reversible and nonreversible events. The transformation of a metastable phase into a more stable phase (solid–solid cold crystallization) is an irreversible event; if the metastable phase melts before recrystallizing into the more stable phase, we have a combination of two irreversible events (11). However, in MTDSC, reversibility/irreversibility must be considered on the time scale of the experiment. Separation of the "total heat flow" into "reversing or c_p" and "nonreversing or kinetic" components is not always based on "thermodynamic reversibility" but on changes occurring in the heat capacity of the sample within the experimental time scale (12).

In this work we have studied the polymorphism of a pure monoacid triglyceride (tristearin) by MTDSC. The primary objective of the work is to examine the relevancy of this technique to the analysis of a complicated polymorphic system. Of the substances studied to date by this technique, the overwhelming majority are polymers (13–16). Some work has appeared on substances such as amorphous lactose (17), polyethylene glycol and griseofulvin (18), inorganic glasses and glass–polymer composites (19), and aqueous solutions (20), but none on the analysis of lipidic materials. Few studies in the literature, such as those of Aldén *et al.* (21) and Lujan *et al.* (22), present a systematic study of the operational parameters for which MTDSC gives reproducible and relevant results. As emphasized by Coleman and Craig (23) in their review, more critical studies are required to explore, exploit, and expose the full advantages of this technique for pharmaceutical systems.

We have looked at the influence of operational parameters on MTDSC results and have attempted to interpret the results on the basis of what is known about tristearin polymorphism (1,8,24–28).

THEORY

The response of an ideal DSC, where no temperature gradients exist, can be expressed (13)

$$\frac{dQ}{dt} = c_{p,t}\frac{dT}{dt} + f(t,T) \qquad [1]$$

where Q is the amount of heat absorbed by the sample, $c_{p,t}$ is the thermodynamic heat capacity, T is the absolute temperature, and t is time; $f(t,T)$ is a function of time and temperature that governs the kinetic (irreversible) response of a process.

The temperature program in MTDSC can be expressed as

$$T = T_0 + At + B\sin(\omega t) \qquad [2]$$

where T_0 is the starting temperature, A is the underlying heating rate, B is the amplitude of temperature modulation, and ω is the frequency in radians, or $\omega = 2\pi f$, where f is the frequency in Hz. Over a small temperature interval, the kinetic process can be approximated as linear.

Equation 1 can then be rewritten as

$$\frac{dQ}{dt} = c_{p,t}\,[A + B\omega\cos(\omega t)] + f'(t,T) + C\sin(\omega t) \qquad [3]$$

where $f'(t,T)$ is the average underlying kinetic function once the effect of the sine wave oscillation has been subtracted, and C is the amplitude of the kinetic response to the sine wave oscillation. A heat flow signal in response to a cyclic temperature modulation will thus contain a cyclic component dependent on the values of B, ω, and C. The kinetic contribution $f'(t,T) + C\sin(\omega t)$ occurs only during irreversible enthalpic processes, while the heat capacity contribution $c_{p,t}\,[A + B\omega\cos(\omega t)]$ is always present. For many kinetically controlled processes, C may be approximated to zero such that the response to the cyclic perturbation originates from the thermodynamic heat capacity contribution alone.

A discrete Fourier transform is used to separate the cyclic (modulating) heat flow component from the underlying heat flow signal. The cyclic heat flow component is termed the reversing or c_p component. The nonreversing (kinetic) heat flow component is then obtained by subtraction of the reversing component from the calculated total heat flow.

Based on the foregoing equations, Aldén *et al.* (21) suggested a dimensionless weighting parameter for the cyclic component in the heat flow equations, called the degree of oscillation, defined as

$$\text{Degree of oscillation} = \frac{B\omega}{2\pi A} \qquad [4a]$$

$$= \frac{Bf}{A} \qquad [4b]$$

MATERIALS

Tristearin ($C_{18:0}$) (1,2,3-trioctadecanoylglycerol) was obtained from Sigma Chemical Co., St. Louis, MO (T5016), 99% pure, and was used without further purification.

METHODS

Apparatus. MTDSC analyses were performed on a Seiko DSC 220C (Seiko Instruments, Inc., Japan) instrument with an SSC 5300 analysis system. Temperature and heat calibrations were performed with indium, tin, and gallium to provide constants that were used in the whole study. The use of these calibration constants at different operational settings is not of critical importance for the qualitative results that are obtained. A variation in the material constant, c_p, might influence the quantitative data, but such a variation will not influence the qualitative trends to any great extent. The slope of the baseline was calibrated using two empty aluminum pans with a mass difference of less than 0.1 mg. A purge gas flow of nitrogen (80 mL/min) was used in the cell, which was cooled by a liquid nitrogen system. Solid tristearin samples weighing between 3 and 4 mg were analyzed in covered aluminum sample pans with an empty pan as reference.

The experiments conducted can be divided into three main parts, as described below. The basic DSC cycle used in all the experiments involved a first heating to melt and remove all thermal history from the tristearin sample (90°C for 3 min). The samples were then cooled under various run conditions, and the resulting phase transformations analyzed by reheating. New samples were used for each new DSC cycle.

Quantitative evaluation. We have chosen not to emphasize the quantitative aspects of MTDSC in this work, since some of these remain to be fully understood (13). However, in order to show the importance of the parameters, some enthalpy results have been discussed.

Effect of operational parameters. The following experiments were performed to determine the effect of the underlying heating/cooling rates and amplitude of temperature modulation on the information obtained.

Experiment I: Variation of underlying heating rate and amplitude of modulation. The following cycle was used:

$$22°\text{C} \xrightarrow{a} 90°\text{C} \xrightarrow{b} 22°\text{C} \xrightarrow{c} 90°\text{C}$$

Scan *a* at 2.5°C/min was used to remove the thermal history of the sample. Cooling scan *b* at 5°C/min generated the metastable/stable phases, which were then analyzed in the heating scan *c*. Scan *c* was performed at various heating rates between 1 and 5°C/min, while the amplitudes of temperature modulation used were 0.1, 0.2, and in some cases 0.4°C. The frequency was fixed at 0.02 Hz (≡ 50 s/period). Effect of amplitude was tested by varying the amplitude between 0.2 and 6°C, using a heating rate of 2.5°C/min and frequency 0.02 Hz. Temperature modulation was active over all three scans *a*, *b*, and *c*. The parameter combinations used for scan *c* result in both "heat-only" and "heat–cool" scans. ("Heat-only" scans are those in which the modulation is such that the overall heating rate of the sample never becomes negative; this effect is mainly achieved at the low amplitudes.)

Experiment II. Variation of cooling cycle/rate. The following cycle was used:

$$22°\text{C} \xrightarrow{a} 90°\text{C} \xrightarrow{b} \underset{30\ \text{min hold}}{0°\text{C}} \xrightarrow{c} 90°\text{C}$$

Scan *a* was as in Experiment I. Cooling rates of 1, 15, 30, ~60, and ~90°C/min were used in scan *b* and combined with a 30-min hold at 0°C. Reheating scan *c* was performed at 2.5°C/min. Modulation parameters (active during *all* scans) were an amplitude of 4°C and a frequency of 0.02 Hz.

RESULTS AND DISCUSSION

MTDSC thermogram of tristearin. The typical thermal behavior of tristearin in MTDSC is illustrated in Figure 2. We will describe this plot in some detail as an example.

During the first heating scan a fresh sample, when heated, shows an endotherm in the total heat flow corresponding to the melting of the β phase at 73°C. The reversing (c_p) and nonreversing (kinetic) components also follow this endotherm (*a*, Fig. 2). Melting of a stable polymorph represents a mixture of kinetic and thermodynamic effects and thus manifests itself in both the components (12). Keeping in mind that the direct liquid-to-β transition in tristearin occurs only under extremely slow cooling conditions, the melting of β should properly be regarded as an irreversible transition in the time scale of the experiment. However, since modulation results in a wide range of heating rates in a "heat–cool" scan, some part of the melt crystallizes (into α and/or β-form) and remelts, leading to a signal in the reversing component.

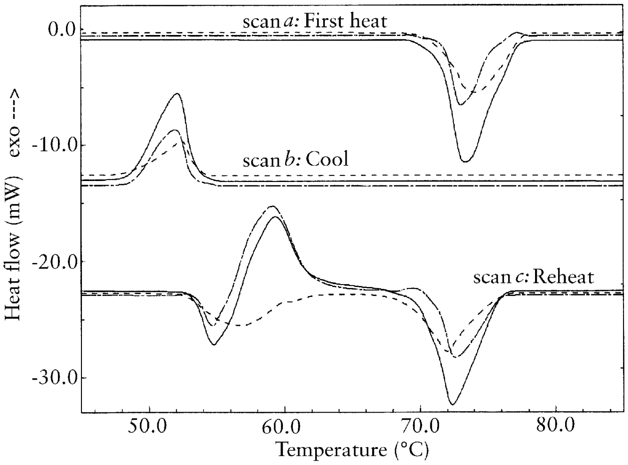

FIG. 2. Typical thermal behavior of a fresh tristearin sample in MTDSC analysis including first heat scan *a*, cooling scan *b*, and reheating scan *c*. The total heat flow curve (——), the c_p component (-----), and the kinetic component (—·—) are shown for each scan performed at 2.5°C/min, 0.02 Hz, 2°C.

On cooling from the melt (cooling scan), the α phase crystallizes out at approximately 52°C, showing an exotherm. This is normal for tristearin, since only extremely slow cooling rates give rise to the β phase directly from the melt. The c_p and kinetic components also show exotherms (*b*, Fig. 2). However, we have also obtained an exothermic c_p and an endothermic kinetic component for this step when using low amplitudes (e.g. 0.2°C; data not shown).

The reheating scan, in which the sample is reheated after crystallization from the melt, shows multiple phase transitions (*c*, Fig. 2). Melting of the α form is represented by an endothermal peak (55°C) in the total heat flow curve, a few degrees above its onset crystallization temperature. The melt converts rapidly to the stable β phase, the crystallization being represented by an exotherm. It is apparent from this total heat flow curve that the melting endotherm and the crystallization exotherm overlap. The reversing component of the heat flow shows a broad melting endotherm extending from 51°C to 64.5°C (i.e., even under the crystallization event). Melting of a metastable phase is generally an irreversible event, but since the transition liquid ⇔ α is facile, this melting can be regarded as completely reversible within the time scales examined here. The kinetic component shows an exotherm over this range. The crystallization of the α melt to β solid is an irreversible event, since the melting point of the generated phase (β) is much higher (73°C; see also Fig. 1); however, a small bump is seen at 60°C in the c_p component too, which can be a result of heat capacity changes on crystallization (see Fig. 3b).

It is interesting to compare these curves to the "cryothermograms" of Perron *et al.* (29), who analyzed various triglycerides including tristearin with differential thermal analysis (DTA). After overlaying cooling and heating thermograms, they "deduced" the individual curves that resulted in the observed overlapping thermal effect around α melting. These deduced endothermic melting and exothermic crystallization curves, which together made up the actual observed composite, resemble greatly the reversing and nonreversing curves obtained here.

Effect of operational parameters. The importance of choosing the correct operational parameters in MTDSC has been stressed. The reported experience in the field is limited, but the few guidelines that are available emphasize the following. Frequency and overall heating rate should be selected so as to obtain at least five cycles over the temperature range of the event to be studied. Reading *et al.* also recommend that melting phenomena be preferably studied by "heat-only" scans (13,30). Another possible approach is to give a certain weight to the cyclic component of the overall heating rate. This has been done by Aldén *et al.* (21), by defining a combination of variables called the degree of oscillation given in equation [4]. The authors recommend a certain level of this parameter in order to obtain reproducible quantitative data.

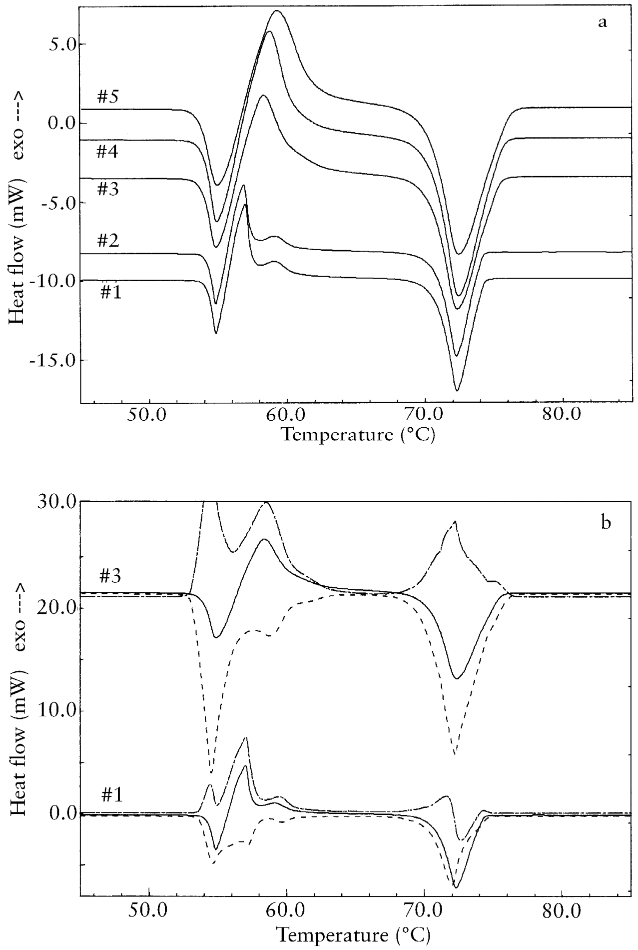

FIG. 3. Effect of heating rates during the reheating scan *c* on (a) the total heat flow thermogram (——) and (b) selected deconvoluted thermograms showing total heat flow (——), c_p component (-----), and kinetic component (-·-·-). #1: 1°C/min, 0.1°C; #2: 1°C/min, 0.2°C; #3: 1.5°C/min, 0.2°C; #4: 2°C/min, 0.2°C; #5: 2.5°C/min, 0.2°C. Frequency: 0.02 Hz in all scans. Note that all except #2 are "heat-only" scans. Samples had been cooled at 5°C/min under temperature modulation.

Experiment I. Variation of underlying heating rate. The effect of varying underlying heating rate during scan *c* is shown in Figure 3; all the runs except #2 are "heat-only" scans.

The total heat flow thermograms in Figure 3a show that increasing the rate results in broader and higher peaks with loss of resolution as in normal DSC. A small exotherm at 59.5°C is seen in the thermograms obtained at 1°C/min; it is absent in the others. This probably represents the conversion of the β′ form to β (27). The β′ form is normally formed only by (isothermally) holding the α phase just above its melting point. However, modulation on an underlying rate of 1°C/min will produce instances with extremely slow heating rates such that near-isothermal holding conditions are obtained and some β′ crystals are formed from the α melt.

The reversing (c_p) components of the heat flow corresponding to some of the above scans are plotted in Figure 3b. The region between 53 and 64°C shows three distinct though overlapping endotherms, especially at the low heating rates (54.8, 56.9, 59.6°C in #1, Fig. 3b). The second and third endotherms are more sensitive to heating rate and shift to higher temperatures with higher heating rates (55.1, 58.0, 61.9°C in #3, Fig. 3b). The first of these can be taken to represent α melting. The second and third are likely to be reversible components involved in the crystallization of the β and β′ forms as identified above, partly as a result of heat capacity changes on crystallization (31). A general point to note is that the c_p component peaks in these runs become increasingly deeper and wider than the total heat flow curves with increasing heating rates; the size of the peaks is a clear indication of a mismatch of parameters.

In the nonreversing (kinetic) component curves, three overlapping exotherms are seen in the region 53–64°C, at the low heating rates in Figure 3b. It is apparent that the first endotherm in the c_p curve has an onset at a lower temperature and is shifted slightly to the left as compared to corresponding peaks in the total heat flow curve. The first peak seen in the nonreversing thermogram is therefore an exotherm, since this curve is a difference between the total and c_p curves. We will discuss later the possible reasons for this observation of an "M-shaped" peak. The second (and third, where apparent) peak in the range 52–65°C, are assigned as β (and β′) crystallization. The kinetic component β melt region in #1 also shows two exothermic peaks with a particular "M" shape.

We conclude from the above discusssion that addition of modulation and the subsequent separation of heat flow components allows the overlapping phenomena to be resolved. It is seen clearly that melting and crystallization (of the α phase) in tristearin occur simultaneously over a wider range of temperature than is apparent from just the total heat flow curve.

The influence of underlying heating rate on the MTDSC results is confounded with the effect this rate has on the phenomenon being studied itself. Observation of reversible processes is particularly influenced, since reversibility as implied in MTDSC is related to the experimental time scale (crystallization events are more sensitive than melting events). Low heating rates give more time for the process to occur which may thus strengthen the c_p signal. Low heating rates give higher resolution and allow for more modulation cycles to be accommodated per unit temperature. In tristearin, where melting and crystallization events are simultaneous and the temperature ranges over which the events occur are narrow (5–10°C wide at baseline), we obtain approximately 3–6 modulations at 2.5°C/min and 0.02 Hz which, is at the limit of acceptability. However, it is known that the α to β transformation occurs increasingly *via* a solid–solid route as the heating rate is decreased, thus bypassing the melt (24,25); thus, a different phenomenon may be observed at the very low heating rates.

The enthalpies of melting of the α and β phases, as obtained from both the total heat flow curve and the reversing component, were tentatively evaluated. While the total heat flow enthalpies are not significantly influenced by the underlying heating rate, the c_p component enthalpies are much more sensitive to this parameter, increasing rapidly with heating rate (data not shown). A deeper interpretation of the enthalpies will not be attempted at this moment; however, it is theoretically possible that with the proper selection of operation parameters, a "true" melting enthalpy for the α phase can be obtained from the c_p component, in contrast to the normal DSC, where observation of the melting process is confounded by the crystallization (25).

Experiment I. Variation of amplitude of modulation. The first heating scan (scan *a*) involves simple melting of the stable β phase (Fig. 4a). However, the effect of varying amplitudes of modulation on this melting is not straightforward. The melting range is approximately 11°C, which gives an acceptable 6 modulations over the event. An amplitude of 0.2°C, however leads to an initial exotherm in the kinetic component, which shows an M-shaped peak, again because the c_p component is shifted to the left of the total heat flow curve as discussed previously. This effect disappears at an amplitude of 2°C, leading to endotherms in all components. An exotherm in the kinetic component is normally indicative of some recrystallization phenomenon as is discussed below.

The c_p component melt enthalpies for this (β) phase as a function of amplitude (plot not shown, but resembles Fig. 5) decrease rapidly between amplitudes 0.1 and 2°C, and become less sensitive to amplitudes larger than about 3°C although decreasing continuously. This may be a result of the sample not being able to respond to the rapidly modulating temperature, and thus the signal is increasingly seen as a nonreversing component. At low amplitudes, c_p component melting enthalpies much larger than the total are obtained. We do not normally expect any overlapping exothermic (recrystallization) phenomenon to occur during the melting of a stable β phase. It is possible that during the early stages of melting, some recrystallization occurs because of the heating modulation, with the crystallites present serving as nuclei. However, it is quite surprising that for the low amplitudes this effect is so highly magnified, leading to c_p-enthalpies one to three times the total heat flow enthalpy (21,22). A fairly constant total heat flow enthalpy is obtained for β melt over all amplitudes.

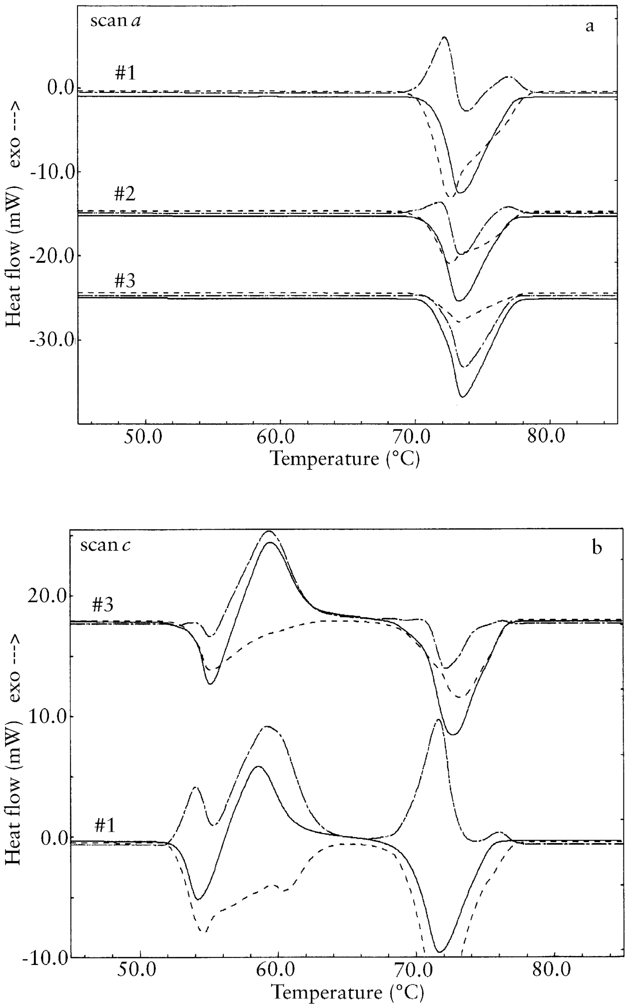

FIG. 4. Effect of amplitude of modulation (a) on melting of β phase during first heating scan *a*, and (b) on reheating scan *c*. #1: 0.2°C; #2: 0.4 °C; #3: 2°C. The total heat flow curve (——), the c_p component (-----), and the kinetic component (·——·) thermograms are shown. Heating rate is 2.5°C/min, frequency 0.02 Hz. Scan #1 is a "heat-only" scan. Samples had been cooled at 5°C/min under temperature modulation prior to scan *c*.

Shown in Figure 4b are the results of some experiments in which the amplitude of modulation was varied in the reheating scan *c*. The total heat flow themograms are not significantly affected by the amplitude; some slight broadening is seen at the high amplitudes. The reversing heat flow components in Figure 4b show that the overall features seen at the various amplitudes are similar. The low amplitude (0.2°C) shows more detail in the region 52–65°C than does amplitude 2°C, although the second endotherm around 60°C can be discerned even at higher amplitudes.

The nonreversing component at low amplitudes in Figure 4b again shows an initial exotherm (M-shaped peak) due to the reversing endotherm starting before the total heat flow curve, for both the α-melt and β-melt regions. This effect disappears at amplitudes around 2°C. As observed earlier, this effect seems to occur only at the low amplitudes, irrespective of heating rate. The possible occurrence of recrystallization during melting with modulation could lead to an exothermic effect of this type, although the enormous enthalpic effects at the low amplitudes (Fig. 5) do not seem physically reconcilable. The implications are that when studying systems that exhibit metastable or polymorphic transitions, it is recommended that a range of amplitudes be tested, giving both "heat-only" and "heat–cool" cycles. The "heat-only" scans with low amplitudes show details that may be lost at the higher amplitudes. The larger amplitudes will, however, confirm whether any recrystallizations seen are truly due to new phases being formed or simply recrystallizations of the same phase that is melting (and thereby simply an effect of the temperature modulation and not a property of the material being tested). Lujan *et al.* (22) made the same observation in their study of $KNiPO_4$ crystal phase transitions and judged them to be pure artifacts arising from deviations from the ideal conditions required in an MTDSC experiment or from the presence of temperature gradients in the crystal.

Summing up, it appears that amplitudes up to 2°C lead to thermograms that can be interpreted best without unacceptable loss of detail. Some ambiguities seem to arise at the low amplitudes in the c_p and kinetic components. The effect of varying heating rates and amplitudes on this system show that for systems in which the thermal transformations are comparably slow processes, the combined requirements of "degree of oscillation ≥ 1" (21) and "at least 5–6 modulations over the event" (13) may be a better guide to the choice of operating parameters than each condition by itself.

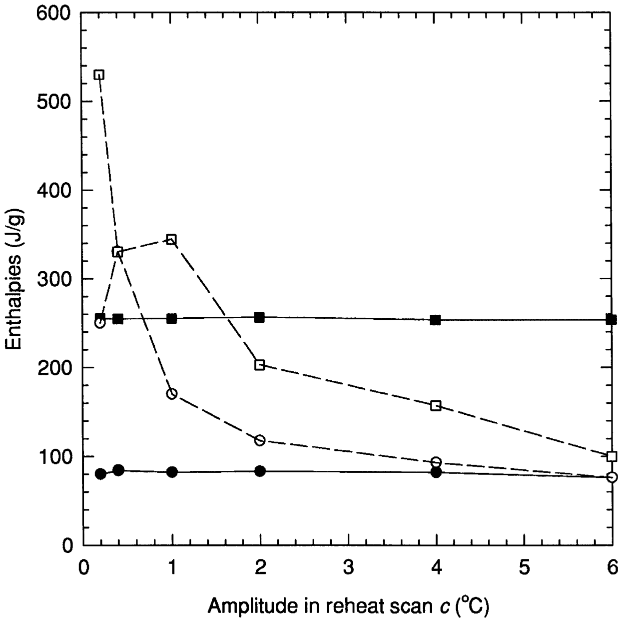

FIG. 5. Total (= filled symbols) and c_p (= open symbols) component melting enthalpies for α (—●—,--O--) and β (—■—,--□--) phases as a function of amplitude of modulation in the reheating scan *c*. Data was obtained at 0.02 Hz and a heating rate of 2.5°C/min. Samples had been cooled at 5°C/min under temperature modulation (see Figure 4b).

Experiment II. Variation of cooling cycle/rate. In this experiment, the samples were cooled at various rates in the apparatus, and the amplitude was increased to 4°C to see whether the crystallization of the α phase in scan *b* is disturbed. The results of heating scan *c* show that the temperature modulation during cooling has no effect on the total heat flow curve. The reversing thermograms are also not influenced significantly; the curve for cooling rate 1°C/min shows the melting of the β′ phase most clearly. Thermograms for these runs are not shown.

The β phase melting enthalpy is not significantly influenced by the cooling rate, as is to be expected, since a majority of this phase is formed during the reheating scan itself. The effect on the α phase c_p component enthalpy is, however, significant when the cooling rate is changed from 15 to 30°C/min. Slow cooling with large modulation leads to melting–recrystallization phenomena and could result in a larger number of smaller or imperfect crystals; nucleation is favored over crystal growth. The c_p component enthalpy is thus lower at the low cooling rates (0–15°C/min), where this effect would be the strongest. At cooling rates larger than 30°C/min, the small effect of modulation is not significant, and a higher but constant c_p enthalpy is obtained. The relative levels of the c_p component enthalpy as a function of the cooling rate thus reflects the kinetics of the recrystallization process (32). The total melting enthalpy of the α phase, however, does not reflect this effect, being unaffected by cooling rate. Although more work needs to be done to understand these effects, these studies provide further evidence for the utility of the MTDSC measurements.

To summarize, since the temperature modulation has an effect on the nature of the crystals obtained during the cooling step, the option of turning off the modulation must be considered during the cooling stage.

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