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Application of modulated-temperature DSC to the analysis of enantiotropically related polymorphic transitions

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Abstract

Modulated-temperature DSC analysis of enantiotropically-related polymorphic transitions are presented. The transitions of furosemide and tolbutamide were detected by endothermic peaks as have been reported in literature. However, those peaks were reproduced only in the reversible heat flow, suggesting that there were no enthalpy jumps during the transitions. These polymorphic transitions were attributed to of the second-order. In the case of sulfamerazine, the transition peak could be explained mainly by the enthalpy jump. This transition was elucidated as being of the first-order. Some enantiotropically related polymorphs are known to be kinetically reversible, while others are not. This difference can most likely be explained by the difference in the order of the transition. The applicability of fractal analysis for quantifying the disorder of the Lissajous figure is also discussed.
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1. Introduction

Many compounds can exist in more than two crystalline states with different molecular arrangements and/or conformations. Crystalline solids that are the same compound but have different crystalline forms are called polymorphs or modification. Different crystalline forms may appear during the crystallization process, depending on conditions such as solvent type, temperature, and addition of seeds. If the obtained form is not the most stable one, polymorphic transition may occur spontaneously or after receiving various kinds of stimulation such as heat. Therefore, knowing the physical stability relationship is very important for controlling the characteristics of solid materials. Rules to examine the stability order were first summarized by Burger and Ramberger [1,2], among which methods using thermal analysis proved to be the most useful. Applicability of the heat

of transition rule, which basically correlates the endothermic transition with the enantiotropic relationship and the exothermic one with monotropic transition, was examined to be more than 90%. The heat of fusion rule, which distinguishes the transition mechanisms using the heat of fusion, has also been recognized as useful. In addition, the accurate measurement of the heat capacity was recently demonstrated as helpful to construct the energy–temperature diagram of polymorphs [3]. Solubility measurement is also a powerful method to determine the stability order of the polymorphs [4–6], because the most stable form exhibits the lowest solubility due to the lowest free energy. However, this method fails if there is a difference in crystallinity between the polymorphs. Polymorphic transition or solvation in testing medium may also spoil this experiment. Moreover, an extensive survey on the solubility ratio between pharmaceutical polymorphs showed that it is usually between 1 and 2 with an averaged value of 1.4 [7], making it difficult to rank the observed solubilities in a statistically meaningful manner. The solvate-mediated transformation method [8] is another useful method for examining the stability order. However, the

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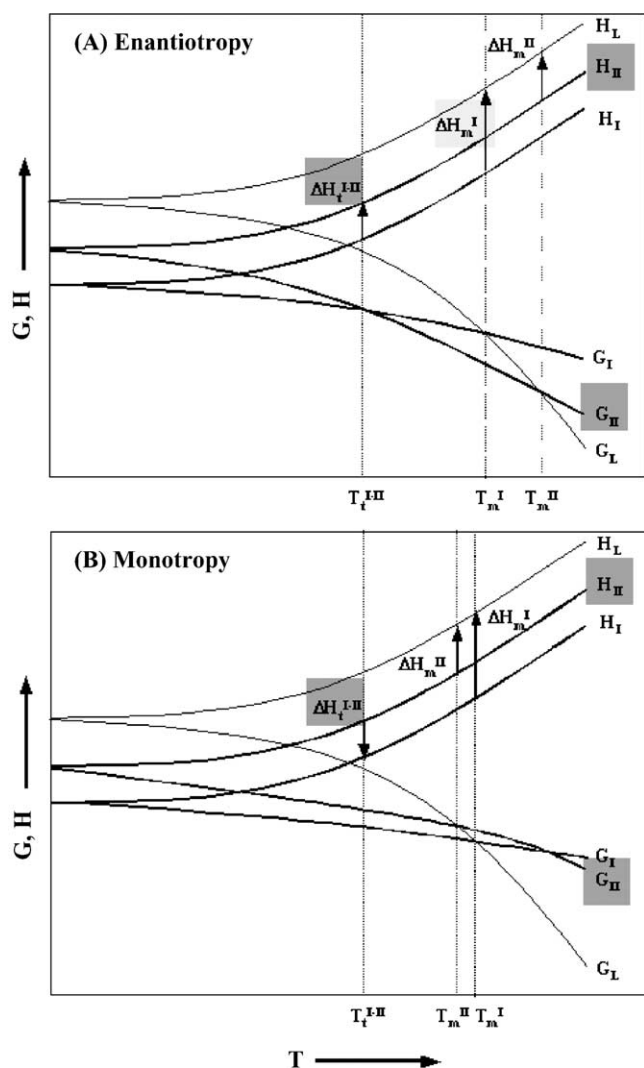*E-mail address:* kohsaku.kawakami@shionogi.co.jp (K. Kawakami).

Fig. 1. Free energy (*G*), enthalpy (*H*)–temperature (*T*) diagrams of enantiotropically (A) or monotropically (B) related compounds. Superscript or subscript I, II, I–II, and L denote form I, form II, forms I–II transition, and liquid state, respectively. ΔH_t and ΔH_m are the enthalpies of polymorphic transition and fusion. T_t and T_m are the transition and the melting temperature.

selected conditions do not always induce polymorphic transitions. Besides, solvates are frequently obtained. In sum, there is no integrated method for determining the stability relationship of polymorphs. Thus, studies on polymorphism require much knowledge and experience using many experimental techniques. Recently, the importance of polymorphism research for pharmaceutical compounds is widely recognized [4,9,10]. Although the difference in the characteristics of polymorphs is usually not significant, it sometimes causes serious problems, as represented by the famous Ritonavir case [9].

Fig. 1 shows the energy/temperature diagrams of polymorphs. In both figures, the most stable form at ambient temperature is defined as form I. When the polymorphs are enantiotropically related (Fig. 1(A)), their free energies be-

come equal at the transition temperature T_t^{I-II}. Because the enthalpy of form I is usually lower than that of form II at that temperature, this transition is an endothermic process. However, the observed transition temperature may be higher than the free energy cross point, if the energy barrier for the transition is sufficiently high. This is a kinetic effect depending on the heating rate used in the DSC experiment. It is even possible that form I melts without transformation. In this case, the enthalpy of fusion of form I is usually larger than that of form II as depicted in Fig. 1(A). When the polymorphs are monotropically related, the stability relationship does not depend on the temperature below their melting points. Heating of metastable form II may or may not cause polymorphic transition to form I. If it occurs, this transition is usually an exothermic process as described in Fig. 1(B). If form II does not undergo transformation, it melts with a smaller enthalpy of fusion than that of the stable form I. These knowledge indicate that thermal analysis can offer great insight into the relationship of polymorphs.

In this paper, we describe the application of modulated-temperature DSC (MTDSC) to the investigation of polymorphic transitions. According to Fig. 1, the slope of the enthalpy (i.e. heat capacity) is not continuous at the transition point. Therefore, the transition endotherm or exotherm may be observed due to the heat capacity change as well as the enthalpy jump in the DSC experiments. Because the heat capacity change and the enthalpy jump are observed in the reversible and irreversible heat flows, respectively, MTDSC should be a promising tool for separating these contributions. Also discussed in this paper is the quantification of the Lissajous figure, which has been used to judge the validity of modulation conditions [11] but with ambiguous criteria. We employed fractal analysis [12,13] to quantify the disorder of the plot. This new methodology may become useful for setting the modulation conditions.

2. Materials and methods

2.1. Materials

Furosemide (FRS), tolbutamide (TLB), and sulfamerazine (SLM) were obtained from Nacalai Tesque (Kyoto, Japan), Sigma–Aldrich (St. Louis, MO, USA), and ICN Biomedicals (Aurora, OH, USA), respectively. Acetonitrile (HPLC grade) was from Kanto Chemical (Tokyo, Japan). All materials were used as supplied.

2.2. Crystal forms of drugs

All of the crystal forms were characterized using powder X-ray diffraction.

FRS was supplied as the stable form I, which is known to transform into form IV at 136 °C enantiotropically [14]. The supplied drug was used as received and

enantiotropic transition into form IV was observed in this study.

TLB was supplied as the stable form at ambient temperature. It is known to transform into the stable form at higher temperatures at around 40 °C [15]. The supplied drug was used as received and the enantiotropic transition at around 40 °C was observed in this study.

SLM was supplied as form I, which is the stable form at higher temperatures. To prepare form II, which is the stable form at ambient temperature, form I was ball-milled at 500 rpm for 2 h to obtain a mixture of forms I and II, followed by suspension in acetonitrile at 60 °C for 1 h to obtain form II. The apparent transition temperature from form II to form I lies at around 175 °C [16]. However, the true transition temperature is implicated to be 51–54 °C [16]. The transition at around 175 °C was investigated in this study.

2.3. Modulated-temperature DSC

Modulated-temperature DSC measurements were performed on TA Q1000 (TA Instruments, DE, USA), which was calibrated with indium and sapphire. Five milligrams (±0.5) of sample was carefully loaded in a sealed aluminum pan to a uniform thickness. When the sample powder was coarse, it was ground in a mortar beforehand. The weight of the pans was between 22.0 and 23.5 mg with the weight difference between the sample and the reference pans being kept within 0.2 mg. When the temperature was raised at 2 °C/min with a 60 s period and 1 °C amplitude, the condition was presented as (2, 60, 1). The measurement was repeated three times and the representative figure is presented for non-isothermal experiments. In the quasi-isothermal experiments, the condition of (0, 60, 1) was employed. This modulation was continued for 30 min, and the latter half (i.e. 15 min) of the data was used for the analysis. The measurement was repeated three times to obtain average values with standard deviations. Dried nitrogen of purity higher than 99.99% was used as the inert gas at the flow rate of 50 ml/min.

2.4. Fractal analysis of Lissajous figure

The Lissajous figure (modulated heat flow as a function of time derivative of modulated temperature) has been employed to check the validity of the experimental condition of MTDSC measurements [11]. To obtain quantitative information on the plot, fractal analysis was employed using the box counting method [13]. Briefly, the figure was divided into squares of its axis length r. The squares, of which a part is occupied by the plot, were counted and the number was denoted as N. The fractal dimension D was obtained using the next relationship.

$$N(r) \propto r^{-D} \tag{1}$$

In the non-isothermal measurements, lines drawn just above the transition peak was always the disturbing factor of the figure. Thus, the fractal dimension D was evaluated for two ranges: below the peak top and the whole range investigated. All the D values are shown as averaged values of three measurements with standard deviations.

3. Results and discussion

3.1. Optimization of the modulation condition

First, the modulation condition to investigate the polymorphic transition was optimized by plotting the Lissajous figures. Fig. 2 shows the Lissajous figures obtained during the polymorphic transition of FRS. The most appropriate condition seemed to be (2, 60, 1), although the figure still showed slight distortion. The distorted lines mainly originated from the rapidly decreasing part of the heat capacity just above the transition, that is, the latter half of the peak. In this temperature region, the phase angle of the heat flow did not seem to follow the temperature change perhaps because of the rapid change in the heat capacity. Table 1 shows the fractal dimensions D for each figure. Comparison was made for the entire temperature range investigated (100–165 °C) and in the temperature range below the peak top (100–136 °C). As expected, the D values were smaller for the latter analysis. The validity of the modulation condition seems to be at the same level for (2, 60, 1), (2, 60, 2), and (2, 40, 1). We employed (2, 60, 1) for the analysis of FRS. The same procedure was repeated for SLM and TLB to optimize the modulation conditions. Quasi-isothermal measurement was also performed, for which the Lissajous figures are shown in Fig. 3. These figures indicate that the heat capacity values obtained from quasi-isothermal measurements should be used to validate the experimental condition of the non-isothermal measurements, because the figures were neat for all the temperatures. The quasi-isothermal experiments provided D values lower than 1.30 as shown in the figures.

3.2. Kinetically reversible transition

Fig. 4 shows the thermogram of FRS of the non-isothermal experiment, which has been known to undergo enantiotropic transition during the DSC analysis. The total heat flow exhibited an endothermic transition peak at 136 °C when the temperature was raised. Although the precise determination of the "apparent" heat of transition was difficult because of

Table 1
Fractal dimension D of the Lissajous figures in the analysis of FRS

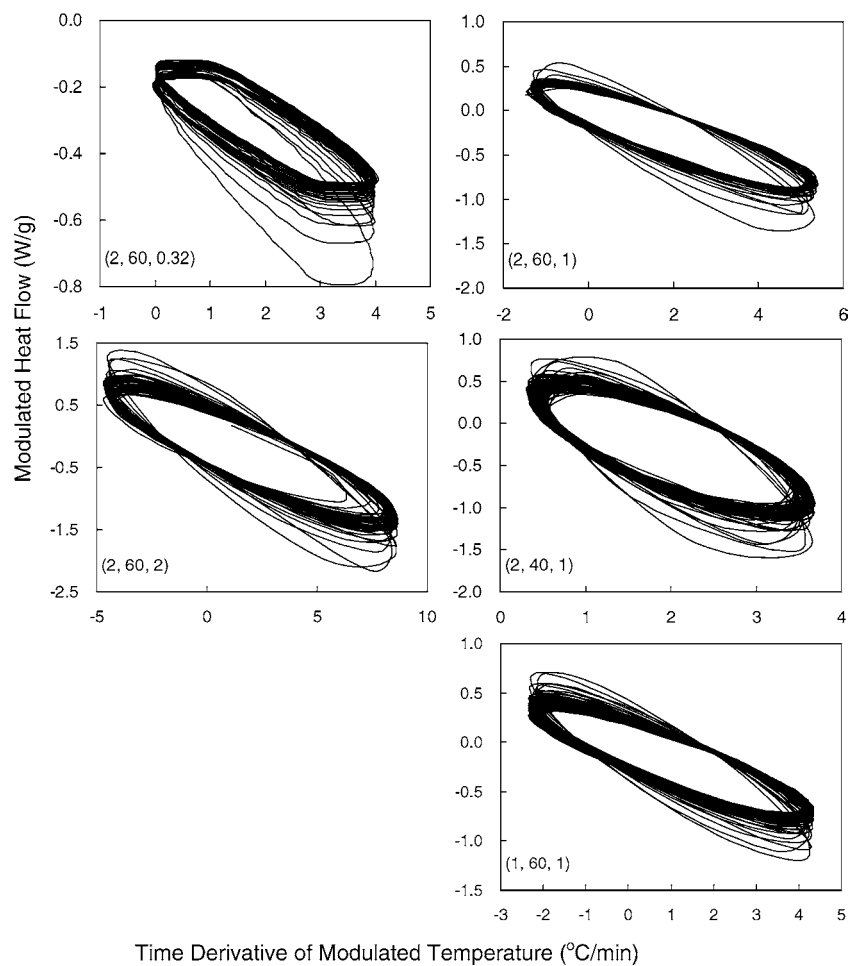| Condition	100–136 °C	100–165 °C
(2, 60, 0.32)	1.52 ± 0.03	1.63 ± 0.02
(2, 60, 1)	1.47 ± 0.05	1.54 ± 0.05
(2, 60, 2)	1.49 ± 0.06	1.52 ± 0.04
(2, 40, 1)	1.47 ± 0.04	1.55 ± 0.01
(1, 60, 1)	1.54 ± 0.03	1.55 ± 0.05

Fig. 2. Lissajous figures of non-isothermal measurements of FRS in the temperature range of 100–165 °C. The modulation conditions are shown in the figures.
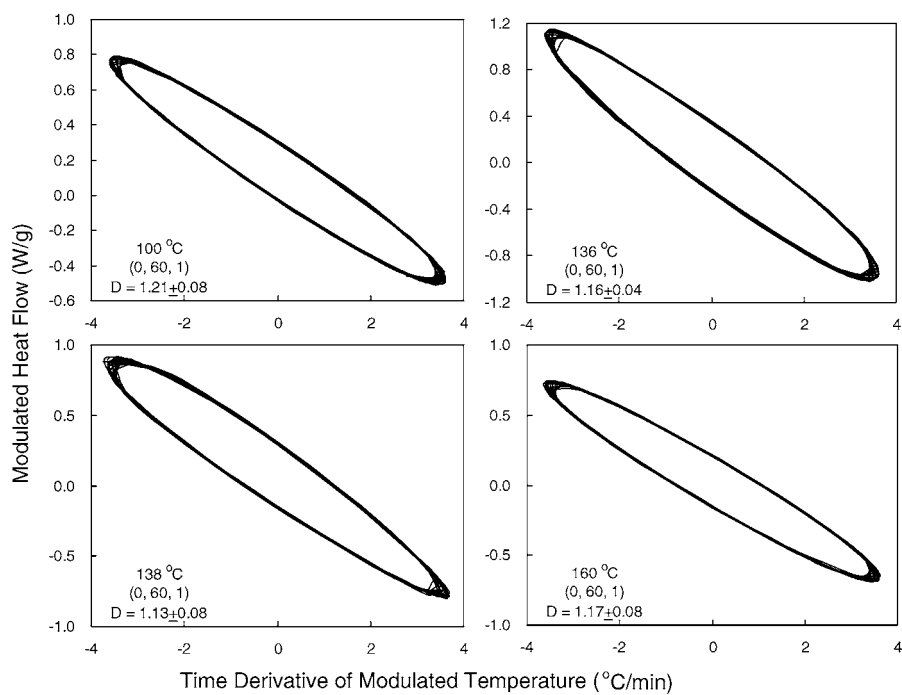

Fig. 3. Lissajous figures of quasi-isothermal measurements of FRS. The temperatures and fractal dimensions calculated are shown in the figures.
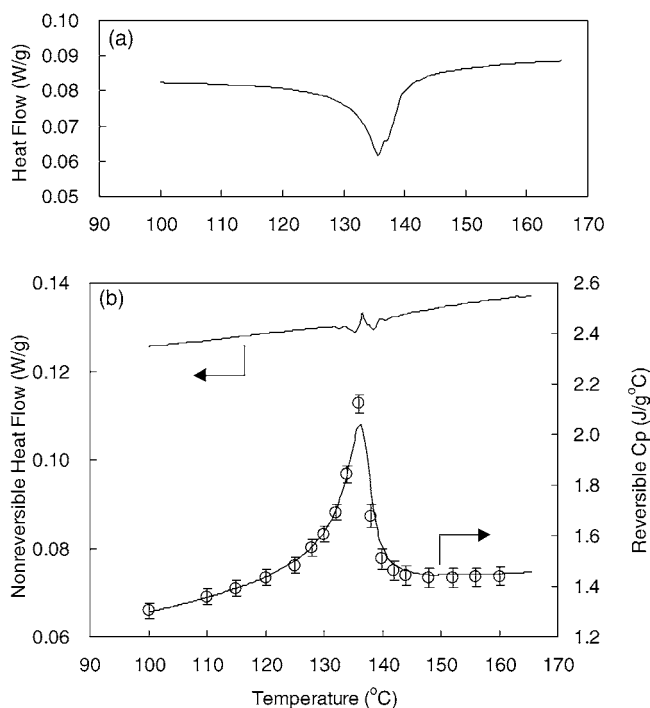
Fig. 4. Non-isothermal measurement of FRS: (a) total heat flow and (b) separated heat flows. The modulation condition was (2, 60, 1). The cooling process was investigated but omitted from the figure, because it agreed completely with those obtained in the heating process (except the direction of the heat flow for (a)). Open circles are the heat capacity values obtained in the quasi-isothermal experiments (see text). The standard deviations of three runs were indicated by error bars.
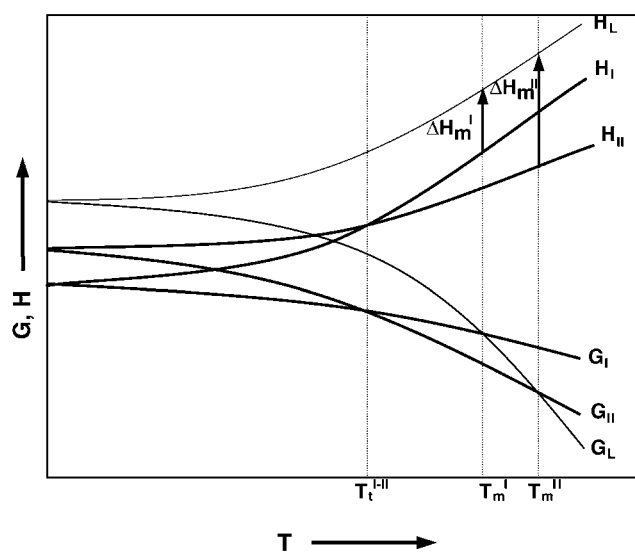

Fig. 5. Proposed free energy (G), enthalpy (H)–temperature (T) diagrams of enantiotropically related compounds of FRS type.

reversible peak is not an enthalpy jump but simply a result of the phase angle shift between the ramp and the heat flow [18,19], although no modulation conditions, thus far tested, could eliminate this peak. The best modulation condition was determined as (2, 60, 0.75). This is most likely due to the

the broadness of the peak, it was in the range of 7–8 J/g, which agreed with the reported value [14]. This can be interpreted as the transition from form I to form IV. The transition peak was reproduced only in the reversible heat capacity (shown as reversible Cp), and no enthalpy jump was observed. The heat capacity values obtained from the quasi-isotherm experiments were consistent with this result as shown in the figure, suggesting that the modulation condition employed provided the correct heat capacity. Due to the absence of the enthalpy jump and the λ-shape of the heat capacity [17], this transition could be attributed to the second-order. The phase diagram for this case could be modified as Fig. 5. The free energy and the enthalpy should be continuous at the transition temperature. This should be a reason for the kinetically reversible transition regardless of the ramp direction [14].

Fig. 6 shows another example of the enantiotropic transition obtained by TLB. The peak temperature and the "apparent" heat of transition were 39 °C and 9–10 J/g, respectively. Both values agreed with the reported values [15]. In this case, the endothermic peak in the total heat flow was mostly reproduced for the reversible heat capacity, but a small peak was also observed in the nonreversible heat flow. When the temperature was decreased, the peak was reproduced in the same direction, that is, as the endothermic one. Therefore, this ir-
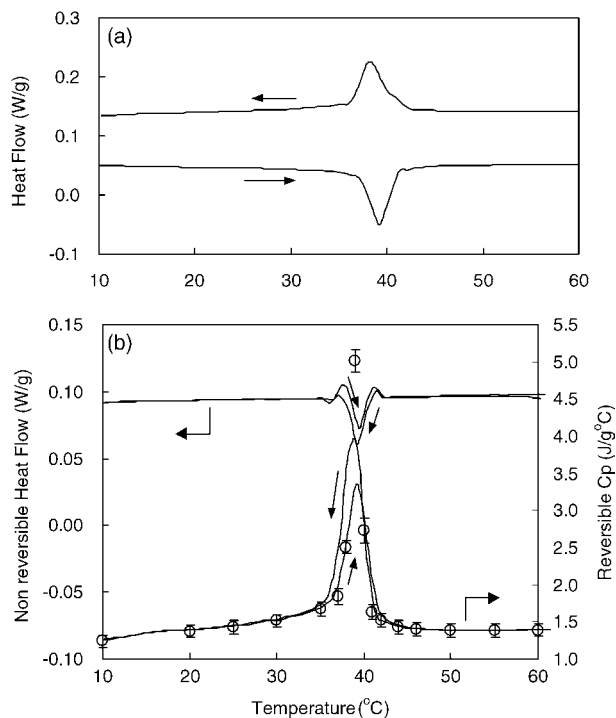

Fig. 6. Non-isothermal measurement of TLB: (a) total heat flow and (b) separated heat flows. The modulation condition was (2, 60, 0.75). After reaching 70 °C, the temperature was held for 3 min and then decreased to 10 °C. Open circles are the heat capacity values obtained in the quasi-isothermal experiments (see text). The standard deviations of three runs were indicated by error bars.
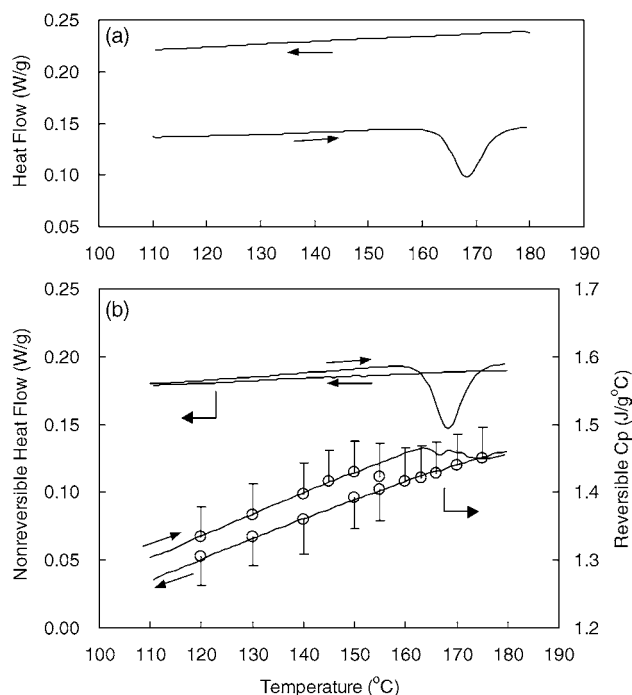
Fig. 7. Non-isothermal measurement of SLM: (a) total heat flow and (b) separated heat flows. The modulation condition was (2, 60, 0.75). After reaching 180 °C, the temperature was held for 3 min and then decreased to 110 °C. Open circles are the heat capacity values obtained in the quasi-isothermal experiments (see text). The standard deviations of three runs were indicated by error bars.

higher activation energy of TLB for the transition than that of FRS. This case could also be attributed to the second-order transition.

3.3. Kinetically irreversible transition

Fig. 7 shows the thermogram of SLM, which showed enantiotropic transition at 168 °C. This transition can be attributed to the transition from form II to form I. As will be discussed later, this transition temperature was greatly affected by the ramp rate. (For example, 180.0 °C for 10 °C/min.) The transition enthalpy was 10.9 J/g. Those values agreed with the reported values [16]. Unlike the cases of FRS and TLB, this endothermic peak was reproduced in the nonreversible heat flow. The reversible heat capacity was nearly consistent with that from the quasi-isothermal experiment. A small inconsistency was observed after the transition peak. This may be inevitable because of the irreversible nature of this transition. The transition temperature of the two experiments should be different, because we applied 30 min long modulation for the quasi-isothermal experiments at one temperature, which seemed to be long enough to alter the crystal form even at temperatures lower than the observed transition in the non-isothermal experiment. After reaching 180 °C, the sample was cooled to observe the heat capacity of form I, which was lower than that of form II. The enthalpy jump was not

observed as expected in this process. This result can be explained by Fig. 1(A), which is accepted as the most typical phase diagram of enantiotropic transition. This example of the transition can be interpreted as of the first-order.

3.4. Summary of the variations of enantiotropic transition

After the review of Burger and Ramberger [1,2], the endothermic transition observed by DSC analysis has been regarded as evidence of enantiotropic transition. The origin of the peak has been believed to be an enthalpy jump as shown in Fig. 1(A). However, we have shown that this may not be true in some cases, because the endothermic transition peaks of FRS and TLB were explained only by the reversible heat capacity. Based on the assumption that the transition is of the first-order and ΔH is positive, the enthalpy-temperature curves of polymorphs do not cross. However, this occurs if the transition is of the second-order. This finding explains why there are two types of enantiotropic transitions: kinetically reversible and irreversible ones. This difference is most likely to originate from the difference in the order of the transition. If the transition accompanies the enthalpy jump, the transition may be irreversible.

Fig. 8 shows the dependence of the reduced transition temperature on the ramp rate. The reduced transition temperature was defined as $\Delta T_t / T_{t0}$, where T_{t0} is the transition temperature observed in the non-isothermal MTDSC measurements described above and ΔT_t is the increment of the transition temperature compared to T_{t0} at higher ramp rates. As can be clearly seen, the dependence of the transition temperature of SLM on the ramp rate was significantly larger than those of other compounds. This difference may also be due to the difference in the order of the transition. If Kissinger's
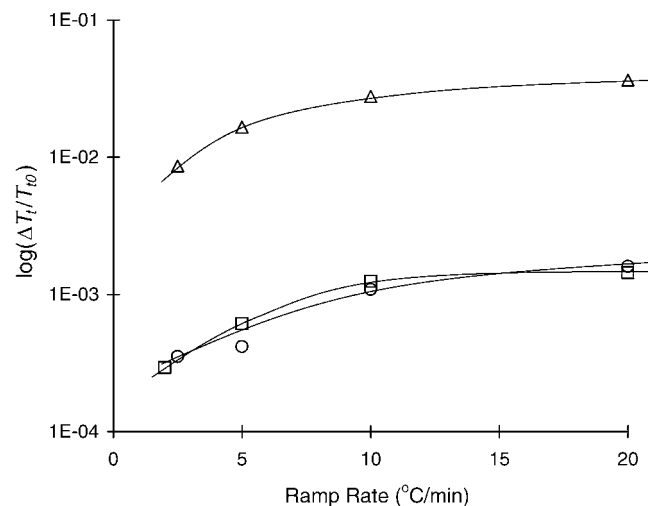

Fig. 8. Dependence of the reduced transition temperature of FRS (squares), TLB (circles), and SLM (triangles) on the ramp rate. The error bars (standard deviations) were omitted, because they are smaller than the symbols. Observations were done in the standard mode (i.e., no modulation was applied).

analysis was applied to calculate the activation energy, although Kissinger's derivation did not suppose this kind of transition [20], it would be 266 kJ/mol for SLM. However, this analysis provides 3700 kJ/mol for TLB and 6400 kJ/mol FRS. These values are obviously unreasonable, suggesting a difference in the transition mechanism between SLM and the other compounds.

4. Conclusions

We have shown some examples of MTDSC analysis of polymorphic transitions. The transitions of FRS and TLB were attributed to of the second-order, because the transition peak could not be explained by the enthalpy jump but could be by the reversible heat capacity change. These transitions could not be explained by the widely accepted phase diagram (Fig. 1(A)) but interpreted by the modified one (Fig. 6). In the case of SLM, the transition peak could be explained mainly by the enthalpy jump, and thus this case was elucidated as of the first-order transition. This transition behavior followed the typical phase diagram (Fig. 1(A)). Some enantiotropically related polymorphs are known to be kinetically reversible but others are not. This difference was most likely to be explained by the difference in the order of the transition. Also discussed in this paper was the applicability of the fractal analysis for quantifying the disorder of the Lissajous figure. It may be too soon to set the criteria of the fractal dimensions for the acceptable figures. Further analysis of various thermal events should develop this analysis into a powerful method for validating the modulation conditions.

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