Application of Differential Scanning Calorimetry to the Estimation of Drug Purity: Various Problems and Their Solutions in Purity Analysis

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To establish conditions for differential scanning calorimetry (DSC) purity analysis, problems of instrumental differences, sample size, heating rate and details of the calculation of such analysis were examined. Two kinds of DSC instruments were used to examine instrumental differences, and changing the sample size or heating rate resulted in a difference in the effect of purity value between these two instruments. The analytical region in the linearization of the van't Hoff plot influenced the purity result, while calibration of the heat resistance using indium had little effect. A calculation without a eutectic peak area caused an overestimation of the purity.

According to the above examination, the appropriate conditions for purity determination with our instruments were 1.0±0.1 mg sample size, 2.0 °C/min heating rate and the linearizing region in the van't Hoff plot 10—50% of peak height. Applying this technique to some doped model drugs, acetaminophen, nicotinamide and ethenzamide, impurities of under 2 mol% were accurately estimated except for acetaminophen. Acetaminophen was unsuitable for this method, because overlap of the melting peak and the other phase transitions was detected by modulated DSC.

Key words DSC (differential scanning calorimetry); purity determination; pharmaceutical; van't Hoff equation; eutectic impurity

Differential scanning calorimetry (DSC) can be used to observe the thermal characteristics of drugs such as their melting points, heats of fusion, heat capacities and glass transition temperatures. This technique can also be used to examine drug stability, complex formation, polymorphism, the phase diagram, solid–solid interactions, chemical bond strength, purity and to discriminate between free and bound water.[1–10] Recently, new techniques of DSC analyses, such as temperature modulated DSC (MDSC), have been developed,[11,12] and their applications to drugs are anticipated.

In the United States Pharmacopoeia (USP) XXIII, DSC has been approved to determine transition temperature, eutectic impurity analysis and for characterization of polyethylene containers.[13] In the Pharmacopoeia of Japan (JP) XIII, DSC has not yet been approved as a general test.[14]

Many pharmaceutical methods dealing with purity determination, for example, TLC, HPLC, GC, spectrophotometry, fluorometry and titration, have been approved.[13,14] These methods for purity analysis are occasionally used not for the purpose of detecting unknown impurities but to detect known impurities.[15] In analysis of an unknown impurity using HPLC, the results are expressed in uncertain indices like the ratio of peak areas. In the titration method, it is often difficult to measure expensive or small amounts of samples, like standard reference materials, because several grams of the sample are required. Chromatographic and spectroscopic methods often require pure standard reference materials for quantitative analyses. Further, the HPLC and GC methods require the proper column, detector, mobile phase and a complicated instrumental system.

For calorimetric purity determination, the purity result is based on acquired data and complicated calculations by the van't Hoff equation. The concentration of the impurity in a material is inversely proportional to the melting point, and an increase in the impurity reduces the melting point and broadens the melting range. It is worth noting that this method can accurately estimate total amounts of unknown impurities with a small amount of sample and without pure standard reference materials.[16,17] Furthermore, the instrumental system of DSC is simple, and samples which are suitable for DSC purity analysis can, in many cases, be measured under similar conditions.

Although this method has many merits, in an actual analysis there are several problems: for example, instrumental differences, sample size, heating rate, the region of the data points for calculation, the treatment of the eutectic peak area, and correction of the heat resistance using pure indium. The purity result depends on these parameters.[18] Thus, although this method is mentioned as a US pharmacopoeia general test, there are few examples of its use as a test in a pharmacopoeia monograph. Even this fact, however, cannot deny the merit of DSC purity analysis.

The purpose of this paper is to resolve the calorimetric purity determination for drugs. We used two instruments to resolve the instrumental difference. The optimal conditions for measurements and the calculations of purity analysis were examined with a thermal analysis purity set obtained from the National Institute of Standards and Technology (NIST), and application of the DSC method to purity analysis of some drugs doped with related substances was also examined.

Experimental[19–27]

Instrument The differential scanning calorimeters used were Model DSC220C (SII, Chiba, Japan) and Model DSC-50 (Shimadzu, Kyoto, Japan), which are both heat flux types. The MDSC measurement was carried out using a differential scanning calorimeter Model DSC 2920 (TA Instrument, New Castle, DE). Weighing of the samples was performed using a Mettler M3 microbalance (Greifensee, Switzerland).

Reagents 99.999% pure indium was obtained from Aldrich Chemical Inc. (Milwaukee, WI). A thermal analysis purity set (SRM 1514, control #782705), phenacetin doped with 0—5 mol% *p*-aminobenzoic acid, was from NIST (Gaithersburg, MD). Acetaminophen (control #881),

nicotinamide (#891), ethenzamide (#881) and nicotinic acid (#815), JP reference standards (JP Std.), were supplied by the National Institute of Health Sciences (NIHS, Tokyo, Japan). *p*-Aminophenol was purchased from Sigma Chemical Co., Ltd. (St. Louis, MO), and 2-hydroxybenzamide was from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Preparation of Doped Samples Acetaminophen, nicotinamide and ethenzamide were used as major constituents, and *p*-aminophenol, nicotinic acid and 2-hydroxybenzamide as minor constituents. Both groups were accurately weighed and dissolved in ethanol, the solvent was evaporated and the material dried in a desiccator under vacuum for more than 12 h.

Experimental Conditions The temperature and heat-flow scales of the DSC apparatus were respectively calibrated using the melting point, 156.634 °C, and heat of fusion, 28.59 J/g, of metallic indium.[23] Two kinds of aluminum crimp pans, 5 i.d. × 2.5 mm for DSC220C and 6 i.d. × 1.5 mm for DSC-50, were used as sample containers for the DSC measurements. The former were crimped by hand, and the latter were done using a SII sample sealer to provide good contact throughout the sample. The temperature programs for measurements were set in the range from room temperature to about 5 °C beyond the endset of the melting peak, and the heating rate was typically 2.0 °C/min. Samples were accurately weighed as 1.0±0.1 mg in sample containers unless otherwise specified. Dry nitrogen gas was used as the atmosphere, and the flow rate was controlled at 20 ml/min. The interval of data collection was 2 points/s in both instruments. α-Alumina was used as the reference material in the DSC measurements.

In the MDSC measurements, heating rate was 1 °C/min with the modulation of ±0.2 °C/min.

Purity Calculation The resistance to heat flow between the sample and the detector results in a lag between the temperature of the two. The term 'heat resistance' is used to define this phenomenon. Though indium is often used for calibrating the heat resistance, in this study correction of the heat resistance was typically not carried out; this is considered later.

The basis for the purity determination is the van't Hoff equation, as shown in Eq. 1

$$T = T_0 - \frac{RT_0^2 x}{\Delta H_0} \cdot \frac{1}{F} \tag{1}$$

where T is the sample temperature in K units, T_0 the melting point of the pure sample, R the gas constant, x the mole fraction of impurity, ΔH_0 the heat of fusion of the pure sample, and F the fraction of total sample melted at T.

The partial areas of the melting endotherm should be accurately determined. Figure 1 schematically shows a typical DSC curve with a melting peak. The partial area (A) of the melting endotherm at a temperature T is the sum of $A1$ (the closed area shown as a–d–e in Fig. 1) and $A2$ (the closed area shown by f–g–c in Fig. 1). $A1$ can be detected by actual data, and $A2$ can be calculated using Eq. 2 because the curve b–c was given by Eq. 3, and Eq. 2 was given by the integral of Eq. 3 from t_g to t_c,

$$A2 = \int_{t_g}^{t_c} \alpha \exp(\beta t)\, dt \tag{2}$$

$$\Delta T = \alpha \exp(\beta t) \tag{3}$$

where t_g is the time at g in Fig. 1, t_c is the time at c in the figure and ΔT is the signal of the DSC at t_g or t_c. The parameters α, β are values given by Eq. 3 and two data points on the curve of b–c in the figure. Here, 10 and 40% of the maximum peak height were typically used. Three points, at 10, 30 and 50% of the maximum peak height on curve a–b in the figure, were selected (see top of Fig. 2). F is the fraction of total sample melted at T. According to Eq. 1, plots of the reciprocal area fraction ($1/F$) *vs.* the corresponding temperature (T) ideally show a straight line having an ordinate intercept of T_0 ($1/F=0$) with the slope being $-RT_0^2 x/\Delta H$. However, the plot is usually not a straight line but is upwardly concave (see bottom of Fig. 2). This is explained by the undetected endotherm of the early stage of the melting peak. The correction for linearization of the plot is done using an adjustable parameter δ, which is obtained from Sondack's equation (Eq. 4),[28]

$$\delta = \frac{\left[\frac{T_{50}-T_{30}}{T_{30}-T_{10}}\right]A_{50} - \left[\frac{A_{50}-A_{30}}{A_{30}-A_{10}}\right]A_{10}}{\frac{A_{50}-A_{10}}{A_{30}-A_{10}} - \frac{T_{50}-T_{30}}{T_{30}-T_{10}}} \tag{4}$$

where T_{50}, T_{30} and T_{10} are the temperatures of points at 50, 30 and 10% of peak height and A_{50}, A_{30} and A_{10} are the partial areas of the melting endotherm of those points, respectively. F may be described as follows:

$$F = \frac{A+\delta}{A_{\text{Total}}+\delta} \tag{5}$$

where A and A_{Total} are the uncorrected area under the curve up to T in Fig. 1 and the total area under the curve, respectively.

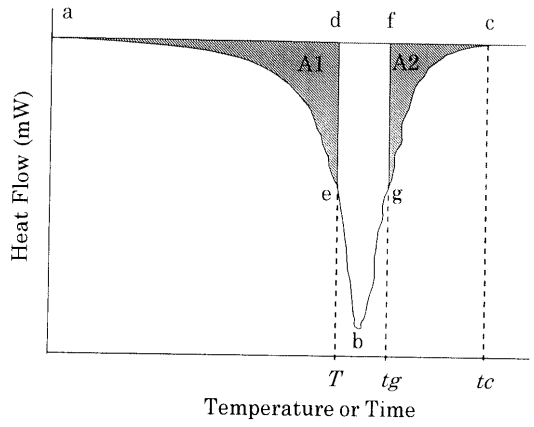

Fig. 1. Diagram of Fraction Melted

a, starting point of melting peak (onset); b, melting peak top; c, ending point of melting peak (endset); d, intersection of line a–c and vertical line drawn from e; e, point on melting peak at temperature T; f, intersection of line a–c and vertical line drawn from g; g, intersection of line b–c and parallel line with line a–c drawn from e; t_g, time at point g; t_c, time at point c.

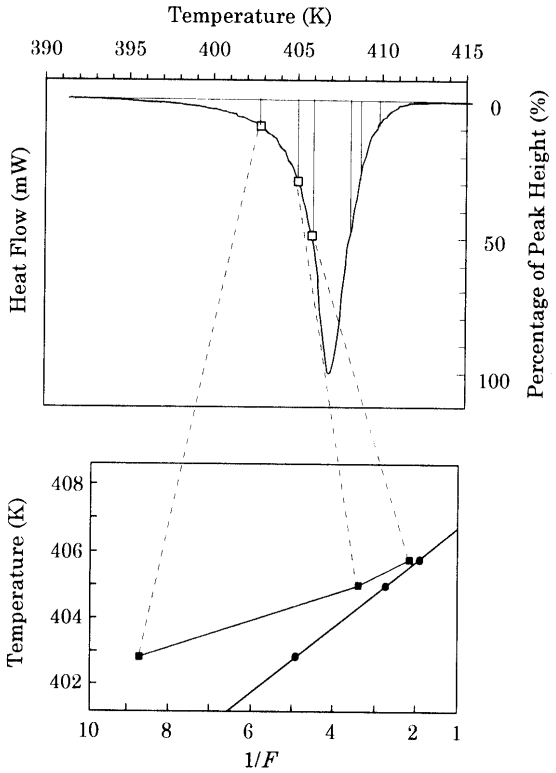

Fig. 2. DSC Curve of 98.09% Phenacetin and the Corresponding van't Hoff Plot Using the Shimadzu DSC-50

Key: □, the point at 10, 30 and 50% of maximum peak height; —■—, uncorrected; —●—, corrected by adjustable parameter δ.

Results and Discussion

Heating Rate and Sample Size Figures 3 and 4 show the influence of heating rate and sample size on the purity result using 98.09 mol% phenacetin.

The slope (denoted by K in Fig. 2) is proportional to x in Eq. 1, meaning that the slope value gives the degree of impurity. There is a difference between the two instruments in these results on effect of sample size and heating rate. The main reason for this is a structural difference in the instruments, no doubt because the purity results use the same calculation methods for the two instruments. For example, although the SII DSC-220C has a relatively basic structure of the sample holder, the Shimadzu DSC-50 has a unique structure called a "pinwheel detector."

Consequently, the appropriate measurement conditions for purity determination for our instruments was 1.0 ± 0.1 mg sample size and 2.0 °C/min heating rate, because, under these conditions, the purity results were most similar to the purity value of 98.09% phenacetin certified by the NIST.

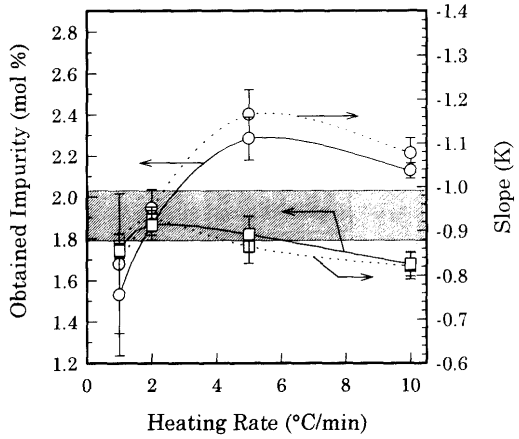

Fig. 3. Influence of Heating Rates on Evaluated Purities and the Corresponding Slopes in the Case of 98.09 mol% Phenacetin

Key: —○—, purity value using the Shimadzu DSC-50; —□—, purity value using the SII DSC220C; - - -○- - -, corresponding slope value using the Shimadzu DSC-50; - - -□- - -, corresponding slope value using the SII DSC220C. The shaded portion indicates purity range certified by NIST. Each value represents the mean ± S.D. of three experiments.

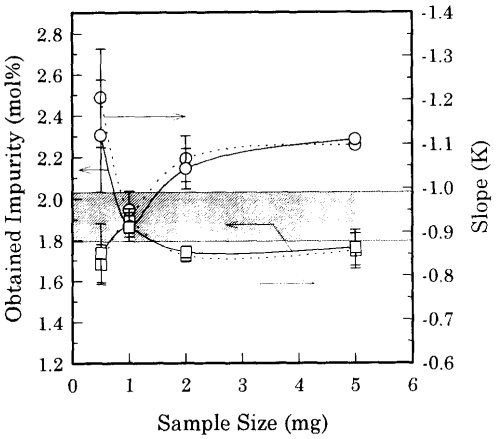

Fig. 4. Influence of Sample Size on Purity Value and the Corresponding Slope Values

Key: —○—, purity value using the Shimadzu DSC-50; —□—, purity value using the SII DSC220C; - - -○- - -, corresponding slope value using the Shimadzu DSC-50; - - -□- - -, corresponding slope value using the SII DSC220C. The shaded portion indicates purity range certified by NIST. Each value represents the mean ± S.D. of three experiments.

Many investigators have suggested that the heating rate should be low and the sample size small, because of the thermal equilibrium. This result, however, does not indicate that the smaller sample size and the slower heating rate are better. If the heating rate is too low, the instruments cannot accurately control heating of the samples, and if sample size is too small, the melting peak will not be large enough to analyze the purity.

Conditions in Purity Calculation Having determined the appropriate measurement conditions, the next consideration is the calculation conditions. Accurate purity results can be achieved with appropriate conditions in the purity calculation, and it is important to consider the region of linearizing of the van't Hoff plot, the eutectic peak area, correction of the heat resistance and area A2 mentioned above.

In linearizing the van't Hoff plot, the analytical region of $1/F$ should be carefully chosen. Table 1 shows the influence of the regions of $1/F$ on the purity analysis using the SII DSC220C. The regions in the linearization were between 10, 30, 50 or 70% of the maximum peak height as the upper limit of $1/F$ (onset) and 30, 50, 70, 80, 90 or 100% of the maximum peak height as the lower limit of $1/F$ (endset). A large endset resulted in a larger impurity value than the value certified by NIST. Linearizing using the peak top such as the regions of 70 to 90 or 100% of the peak height caused the purity results to be greatly different from the value certified by NIST. Consequently, the appropriate region for linearizing the van't Hoff plot was between 10% of the maximum peak height as the onset and 50% of the maximum peak height as the endset, because when this region was used the purity results were within the limit of the values certified by the NIST and the standard deviations were lowest. In other words, it follows that the DSC curve of this region corresponds to the van't Hoff equation.

Figure 5 shows the influence of certain parameters on purity result using the SII DSC 220C. The eutectic peak is the characteristic peak of the eutectic mixture and appears before the melting peak of the main material. Calculation without the eutectic peak area resulted in a decrease in the impurity value obtained; calculation without the area A2 resulted in an underestimation of the impurity result. The calibration of heat resistance using

Table 1. Effect of the Linearizing Region on Obtained Purities of 98.09% Phenacetin Using SII DSC220C

Endset (%)	Obtained impurity (mol%)				
	Onset (%)	10	30	50	70
30		1.97 ± 0.23			
50		1.86 ± 0.07	1.95 ± 0.41		
70		2.03 ± 0.11	2.46 ± 0.12	3.38 ± 1.45	
90		2.28 ± 0.09	2.72 ± 0.26	3.04 ± 0.65	−4.13 ± 7.79
100		2.54 ± 0.20	3.41 ± 0.43	3.99 ± 0.64	10.06 ± 4.49

The underlined data are within the range certified by NIST. For example, if the onset and endset are chosen as the top of Fig. 2, they should be 10% and 50%, and the obtained impurity value is shown as 1.86 ± 0.07%. Each value represents the mean ± S.D. of three experiments.

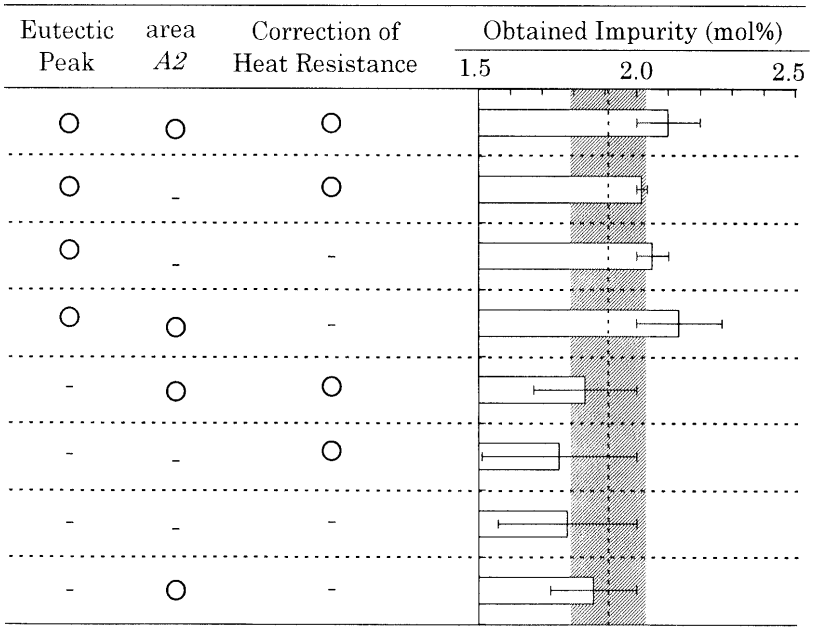

Fig. 5. Influence of Area A2, Calibration of Heat Resistance and Treatment of Eutectic Peak on Purity Value Using the SII DSC220C

Key: (○), calculation with; (-), calculation without. The shaded portion indicates purity range certified by NIST. Each value represents the mean ± S.D. of three experiments.

indium had only a slight influence on the impurity result; the main reason is that the heat conductivity of indium is much greater than the conductivity of an organic compound, and indium has a relatively vertical melting peak. Namely, the calibration of heat resistance using indium caused only a slight change in sample temperature. Even if the DSC purity determination using an organic calibrant for correction of heat resistance is used, it may be difficult to select an appropriate calibrant, no doubt because each sample has peculiar heat conductivity. Furthermore, the particle size of the sample also influences the heat conductivity.

Based on the above, the area A2 theoretically should not be disregarded; thus in practice, this area was basically not disregarded. The eutectic peak was disregarded here except for the case of nicotinamide, and this question will be discussed further later. No correction of the heat resistance was made.

Measurements of Various Drugs Figure 6 shows the DSC curves of pure and various doped drugs. Simply comparing the melting peak of doped drugs with the pure drugs shows that there were obvious differences between them. The lowering of purity caused a broader melting peak, a lowering of the heat of fusion and an increase in the eutectic peak.

Figure 7 shows the comparison between the amounts of doped impurities and the impurity values estimated by DSC. Impurity levels of 2 mol% were precisely estimated with the exception of acetaminophen using the SII DSC220C. In the case of 95.13 mol% nicotinamide, the regular analytical region could not be used for the purity calculation because the main melting peak and the eutectic peak overlapped. Therefore, in the linearization of the van't Hoff plot, the melted fraction F was calculated by adding the eutectic peak area to the total peak area, and the region between 20 and 50% of the peak height was

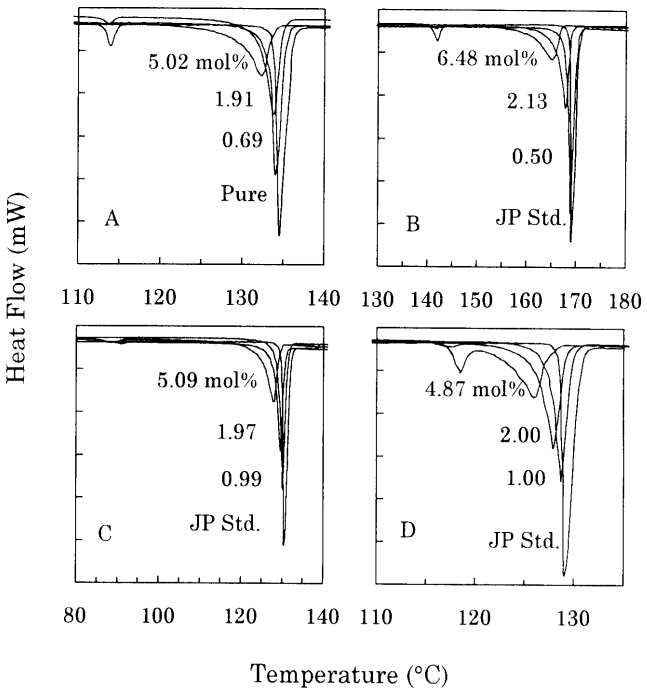

Fig. 6. DSC Curves of Various Drugs Using the Shimadzu DSC-50

A, phenacetin (doped with *p*-aminobenzoic acid); B, acetaminophen (doped with *p*-aminophenol); C, ethenzamide (doped with nicotinic acid); D, nicotinamide (doped with 2-hydroxybenzamide).

used. This case shows that addition of the eutectic peak area to the total peak area can give an accurate purity result. Figure 8 shows a DSC curve of 95.13 mol% nicotinamide. The main melting peak could not be separated from the eutectic peak, and the onset of the main melting peak was apart from the baseline represented by the line A–C. Assuming the line C–B to be the baseline, the purity obtained is inaccurate, because of inaccurate calculation of the fraction melted. It seems reasonable that

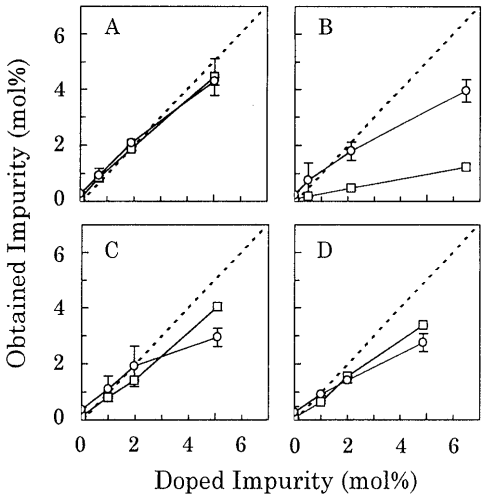

Fig. 7. Comparison between Doped Impurity Amount and Calorimetric Impurity Value

A, phenacetin (doped with *p*-aminobenzoic acid); B, acetaminophen (doped with *p*-aminophenol); C, ethenzamide (doped with nicotinic acid); D, nicotinamide (doped with 2-hydroxybenzamide).

Key: —○—, using the Shimadzu DSC-50; —□—, using the SII DSC220C. The shaded portion indicates purity range certified by NIST. Each value represents the mean ± S.D. of three experiments.

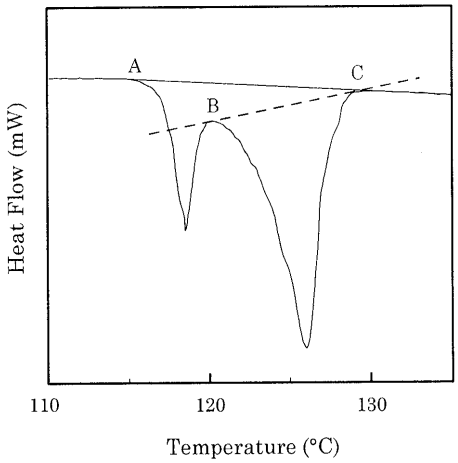

Fig. 8. DSC Curves of 95.13 mol% Nicotinamide Using the Shimadzu DSC-50

Key: (——), accurate base line; (– – –), erroneous base line.

the fraction melted should be calculated by addition of the eutectic peak area when the main melting peak cannot be separated from the eutectic peak, because the increase in the total melting peak area by addition of the eutectic peak area was compensated by the addition of δ.

There is a great difference between the obtained purity values for acetaminophen using the two instruments; there are at least two reasons for this. One is the difference in their instrumental structure, and the other is the thermal characteristic of the JP acetaminophen reference standard. There is also a great difference between the melting points obtained from the first heating and the second heating; therefore, the JP acetaminophen reference standard has polymorphism. Even so, the purity result should be accurate when the melting and the other phase transitions do not overlap. However, the conventional DSC instrument really cannot distinguish the case of the melting peak overlapping with other phase transitions from the

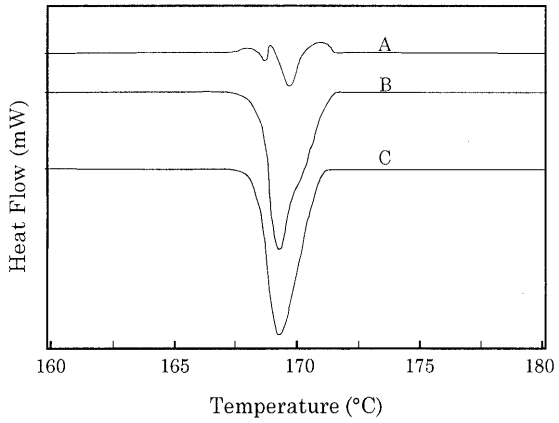

Fig. 9. MDSC Signals of Japan Pharmacopoeia Acetaminophen Standard

A, non-reversing signal; B, reversing signal; C, total signal.

case of the melting peak alone. We used the temperature modulated DSC (MDSC) technique to show the details of thermal behavior near the melting point; this technique can recognize the existence of other phase transitions. In MDSC the heating rate is modulated by a form of perturbation. Mathematical treatment of actual MDSC data can reveal reversing and non-reversing signals as well as the conventional DSC signal. The MDSC total signal is equal to the conventional DSC curve; the reversing signal is based on the heat capacity and the non-reversing signal is calculated by subtracting the reversing signal from the total signal. The MDSC technique is particularly useful for the determination of thermal behavior which has some varieties of phase transitions at approximately the same temperature. Figure 9 shows the reversing, non-reversing and total signals for JP acetaminophen (JP Std.) using MDSC. The melt, which is the reversing signal, and the other phase transition, which is a non-reversing signal, overlapped. For this reason, the purity result using SII DSC220C was far from the doped impurity value, and the purity result using the Shimadzu DSC-50 was obtained as a value near the doped impurity value merely by chance.

Conclusion

We determined here that there was an instrumental difference in calorimetric purity analysis, and appropriate parameters of measurement and calculation for each instrument were required. We did not use the manufacturers' purity analysis software to examine the instrumental differences with the same calculation but used an ordinary spread sheet program. Some manufacturers' purity analysis software, however, have fixed calculative parameters; thus, there may be cases when the software does not have the optimum calculative condition. The software should be able to select an analytical region of $1/F$ at least in linearizing the van't Hoff plot. This work has shown that purity results depend on analytical conditions. Therefore, validation of DSC purity determination is important, and reference standards for the validation are necessary.

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