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EFFECTS OF EXPERIMENTAL VARIABLES ON PURITY DETERMINATIONS WITH DIFFERENTIAL SCANNING CALORIMETRY

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ABSTRACT

A description is given of the influence of calculation procedure, heating rate, sample mass, particle size and atmosphere on the determination of molar purity by means of melting point depression with differential scanning calorimetry. This determination is the most important pharmaceutical application of DSC. Nine substances, melting in the region 100–250°C were studied and factorial designs were used. A large sample mass leads to high purity values because of thermal insulation within the powder bed. Values even greater than 100 mole% were found. Since at high heating rates thermal and thermodynamic equilibrium cannot be attained, there must be an upper limit on heating rate in purity determinations; this appeared to be 0.05 K sec^{-1}. However, sometimes heating rates below this value influence both the purity value and pure melting point, T_0.

The particle size of the sample also affected its purity value. The effects could be explained in terms of thermal lags, decomposition and evaporation during melting, or sublimation before melting. Decrease of particle size may either lead to low purity values, due to concurrent increase in structural disorder, or to increase in purity values, due to the formation of aggregates by electrostatic forces. It was concluded that if trituration of samples before subjecting them to DSC analysis causes aggregate formation, purity values which are too high may result.

Very large particles give lower purity values because of thermal gradients in the sample.

INTRODUCTION

The determination of molar impurity by means of melting point depression is one of the most important pharmaceutical applications of differential scanning calorimetry [1,2]. This determination is based upon van't Hoff's law

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of melting point depression, which essentially describes the molar entropy of mixing and dissolution of the impurity in the melt [3]. The van't Hoff equation is given by

$$T_{\mathrm{m}} = T_0 - \frac{RT_0^2 x_2}{\Delta H_0} \times \frac{1}{F} \tag{1}$$

where T_{m} is the sample temperature at equilibration, T_0 is the pure melting point of the main component, R is the molar gas constant, x_2 is the concentration of impurity present in the pure substance (in mole %), ΔH_0 is the molar heat of fusion of infinitely pure substance, and F is the fraction which is molten at equilibration.

The derivation of this equation is only possible if a number of assumptions are met, the most important being the proviso that thermal and thermodynamic equilibrium should be maintained throughout the whole fusion process. Moreover, the components must form simple eutectic mixtures and no solid solutions must exist. Another problem is that the "van't Hoff plot" of T_{m} vs. $1/F$ is not a straight line. This is most probably because the instrument cannot detect 'early melting' [1,3,4]. The lost heat due to this early melting can be retrieved by linearizing the plot. An X-correction is introduced, defined as

$$\frac{1}{F_n} = \frac{A + X}{A_n + X} \tag{2}$$

where A is the total area under the curve, A_n is the area at point n, and F_n is the fraction molten at point n.

The most frequently applied methods are the 3-point correction method by Sondack [12] and those based on a linear least-squares fitting [1,3,7,13].

Instrumental factors (like sample holder assembly, calorimetric sensitivity and heating rate [1–4]), as well as sample conditions (like sample size [4], stability in the melting region [5], sample purity [2], and the molecular structure, vapour pressure and solid solubility of impurities [6,7]) may affect the results. As an example, Hunter and Blaine [8] showed that errors caused by changes in instrumental and sample conditions are cumulative.

However, only seldom have investigators given attention to the effect of particle size on the purity results. Plato and Glasgow [9] stated that in a larger-than-average crystal, thermal equilibrium cannot be maintained within the main body of the sample. The United States Pharmacopoeia [2] prescribes the use of finely powdered samples to ensure a high thermal conductivity between the sample and the holder. We have shown previously [10] that particle size can have a large effect on the temperature values, temperature intervals and specific enthalpies of even pure melts and therefore we conducted a series of factorial experiments to study the effects of experimental variables, with particular emphasis on particle size, on the purity results.

EXPERIMENTAL

Materials

We used the following substances (melting temperatures, in °C, in brackets): adipic acid, $C_6H_{10}O_4$ (151); amidopyrine, $C_{13}H_{17}N_3O$ (about 108); benzoic acid, $C_7H_6O_2$ (121.5–123.5); caffeine, $C_8H_{10}N_4O_2$ (234–239); phenacetin, $C_{10}H_{13}NO_2$ (134–137); benzamide, C_7H_7NO (about 130); acetanilide, C_8H_9NO (113–115); dydrogesterone, $C_{21}H_{28}O_2$ (167–171); and naphazoline nitrate, $C_{14}H_{15}N_3O_3$ (about 168). All substances were of a high-purity grade.

The (mean) particle sizes of the different fractions are given in Table 1.

TABLE 1

Mean particle sizes of the substances investigated
Standard deviations (in μm) are given in brackets.

Substance	Mean particle size (μm) of the fractions				
	Comminuted	Small	Medium	Large	Complete size spectrum
Adipic acid	< 10	29(18)	80(32)	230(149)	62 [a]
Amidopyrine	34(6)	94 [b]	256 [c]	920 [d]	146(12)
Benzoic acid	14(9)	[e]	41 [f]	235 [g]	67(57)
Caffeine	10(5)	8(6)	11(10)	20(15)	15(4)
Phenacetin	< 10	24(10)	36(20)	[h]	31(17)
Benzamide	9(6)	36(22)	[i]	[i]	[i]
Acetanilide	10(4)	17(9)	44(6)	91(59)	27(5)
Dydrogesterone	< 10	49 [j]	162(34)	260(48)	[k]
Naphazoline nitrate	< 10	16(11)	72(8)	566(183)	71(8)

[a] Irregular size distribution, $0 < 96\% \leqslant 250$ μm.

[b] Irregular size distribution, $20 < 90\% \leqslant 200$ μm.

[c] Irregular size distribution, $50 < 90\% \leqslant 500$ μm.

[d] Irregular size distribution, $100 < 95\% \leqslant 1100$ μm.

[e] No fraction obtained (needles).

[f] Irregular size distribution, $10 < 90\% \leqslant 80$ μm.

[g] Irregular size distribution, $100 < 95\% \leqslant 800$ μm.

[h] No fraction obtained due to the presence of hard conglomerates which were not disintegrated by sieving.

[i] Mean particle size could not be determined since sample had hard conglomerates which were not homogeneously distributed.

[j] Irregular size distribution, $87\% \leqslant 106$ μm.

[k] Distribution curve with two tops: $d_1 = 25$ μm(5); $d_2 = 260$ μm (67).

Calibration

For calorimetric and temperature calibration we used ultrapure indium (purity > 99.99%).

Apparatus

The equipment used was a Mettler TA 2000A heat-flux DSC system, which was described previously [11]. Weighings were done on an electronic Mettler ME 30 microbalance and were accurate within 0.040 mg.

STUDY DESIGN

All tests were carried out in random order. No reference or diluting agent was used

I. Check of applicability of calculation procedures (factorial, in triplicate)

Substances	phenacetin (complete size spectrum, sample taken from bulk), and phenacetin doped with 0.9741 mole % benzamide
Heating rate	0.02 K $\sec^{-1}$
Sample mass	2.0 mg
Atmosphere	nitrogen 30 ml $\min^{-1}$
Sample holders	pierced
Variables	number of fractions in least-squares linearization: 5, 10, 20 linearization limits (in percent of total peak area): 1–50, 2.5–20, 2.5–50, 5–20

The curves were also evaluated manually to compare the on-line least-squares linearization procedure with the Sondack fit.

II. Check of effect of heating rate (in triplicate)

Substance	phenacetin, complete size spectrum (from bulk)
Sample mass	2.0 mg
Atmosphere	nitrogen 30 ml $\min^{-1}$
Sample holders	pierced
Number of fractions in linearization	20
Linearization limits	2.5–20 (% of total peak area)
Heating rates (K $\sec^{-1}$)	0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08

III. Factorial design with heating rate and sample mass as the variables (in triplicate)

Substances	phenacetin, adipic acid, amidopyrine, dydrogesterone
Atmosphere	nitrogen 30 ml min^{-1}
Sample holders	pierced
Particle size	medium-sized fraction
Number of fractions in linearization	20
Linearization limits	2.5–20% of total peak area
Variables	heating rate (K sec^{-1}): 0.02, 0.04, 0.08 sample mass (mg): 1.0, 2.0, 4.0, 6.0, 8.0

The curves of phenacetin were also evaluated manually and the van't Hoff plots were then linearized using Sondack's procedure.

IV. Factorial design with heating rate and particle size as the variables (in quadruplicate)

Substances	all substances mentioned in the Materials section
Sample mass	2.0 mg
Atmosphere	nitrogen 30 ml min^{-1}
Sample holders	tightly closed
Number of fractions in linearization	20
Linearization limits	2.5–20 % of total peak area
Variables	heating rate (K sec^{-1}): 0.02, 0.04 particle size: comminuted, small, medium, large, complete size spectrum

The curves of naphazoline nitrate were also evaluated manually and the van't Hoff plots were then linearized using Sondack's procedure.

V. Factorial design to study possible decomposition during melting (in triplicate)

Substances	adipic acid, benzoic acid [a], dydrogesterone, naphazoline nitrate
Sample mass	2.0 mg
Sample holders	pierced *
Number of fractions in linearization	20
Variables	heating rate (K sec^{-1}): 0.02, 0.04 particle size: comminuted, medium size fraction atmosphere *: nitrogen, oxygen (30 ml min^{-1}) linearization limits (percent of total peak area): 2.5–20; 2.5–50

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* With benzoic acid, which is known to sublimate, the atmosphere was always nitrogen, but variation was made in the sample holders: pierced or tightly closed.

The curves of naphazoline nitrate were also evaluated manually and the van't Hoff plots were then linearized using Sondack's procedure.

Statistical analysis

Effects of the variables (factors) and their interactions on molar purity and pure melting point, T_0, were investigated by analysis of variance. Significance testing was done using the F-test [14]. Standard deviations of the single determinations were also calculated. In those cases where no or only slight interactions were found, the results were summarized in tables presenting the mean values per factor (averaged over the other factors).

On-line calculation (least-squares fit)

Data acquisition was controlled by a CBM 3016 personal computer, interfaced to the Mettler TA 2000 with an ICS 4880 IEEE 488-40 bits parallel interface. The analog output was digitized by a Keithley 179 multimeter. Data were acquired once a second. The algorithm was such that a linear function was fitted from the first 60 contiguous data points, and the first point of a series of 30 consecutive points falling ouside the limit of two times the standard deviation of the linear function was assumed to be the start of the peak. The peak end was found analogously. The interpolated base line was assumed to be a linear function of time.

The molten fraction, F_n, of the sample at any temperature, T_n, was calculated from the heat entered at that temperature. By means of a linear least-squares function, the data pairs $1/F_n$, T_n were fitted to a straight line, from which the molar purity and pure melting point, T_0, were determined using the van't Hoff law.

Manual calculation (Sondack procedure)

In some instances the peaks were also evaluated manually. In each peak, 3 points (P1, P2, P3) were chosen at 10, 30 and 50% of the peak height [16]. The temperatures (T_n, in K) and partial enthalpy changes (ΔH_n) at these points were calculated, together with the total enthalpy change (ΔH_{tot}) from the area of the complete peak. To linearize the plot of T_n ($n = 1, 2, 3$) against $1/F_n$ ($n = 1, 2, 3$, where $F_n = \Delta H_n/\Delta H_{tot}$), the correction factor, X, was found using Sondack's formula [12] and the purity level (in mole%) and the pure melting point T_0 (°C) were found using the van't Hoff law.

RESULTS AND DISCUSSION

Applicability of calculation procedures

Table 2 gives the results of the investigation of the applicability of the calculation procedures. The purity and T_0 values of the pure sample were not significantly affected by the variables, nor were the T_0 values for the doped sample.

The purity of the doped sample, calculated with the least-squares linearization procedure, was significantly different from the one found using the Sondack method. Both these purities also differed from the theoretical value and were determined with fairly large standard deviations. This suggests that the preparation method for the doped samples (which was by means of a resolidified melt technique) may lead to substantial sampling errors. Doping of a pure substance with an impurity should therefore preferably be done in the sample holder itself (by weighing the compounds on top of each other) [6,15].

The Sondack method tends to underestimate the amount of impurity actually present. It should be noted that Sondack's linearization involves only 3 points of the curve. The points that we used (at 10, 30 and 50% of the peak height) are recommended in the literature [16]. However, the choice of these points considerably affects the results. To check this, 2.293 mg pure phenacetin was weighed in a sample holder and 0.093 mg benzamide was weighed on top of it. In the resulting DSC curve, points were chosen at 10, 20, 30, 40, 50, 62.5 and 75% of the peak height. From the residual peak areas the corresponding $1/F$ values were calculated: these were 55.6, 11.9, 6.3, 4.4,

TABLE 2

Results of phenacetin purity analysis using different calculation procedures (experiment I)

Sample	Linearization procedure			
	Least squares		Sondack	
	Mean value [a]	Standard deviation	Mean value	Standard deviation
Pure				
Purity (mole %)	99.937	0.09	99.986	0.03
T_0(°C)	134.30	0.08	134.23	0.11
Doped (0.9741% benzamide)				
Purity (mole %)	98.824	0.21	99.459	0.13
T_0(°C)	134.43	0.16	134.37	0.11

[a] Averaged over all variables.

3.4, 2.7 and 2.2, respectively. The purity was then determined using all sets of 3 points. Some examples are given in Table 3. In general, it was concluded that greater differences in the $1/F$ values used in the Sondack calculation lead to higher impurity and T_0 values, although all results were less than the theoretical value. Morever, the choice of the data points seems to be arbitrary.

The T_0 values for the pure and doped samples obtained using the Sondack procedure (Table 2) differed significantly from each other ($P \leqslant 0.01$). If calculated using the least-squares technique, they only differed ($P \leqslant 0.05$) if the linearization limits 2.5–20% or 5–20% were used, the values for the doped samples then being 0.2 K higher than those for the pure samples. It is concluded that if doped samples are to be assayed using the least-squares technique, the upper linearization limit should be 50% ($1/F = 2$), but if the effects of experimental variables on pure samples are to be determined, linearization limits of 2.5–20% ($1/F = 40$–5) are acceptable.

Check on effect of heating rate

The results of the check on the effects of heating rate are given in Table 4. A heating rate lower than 0.04 K sec^{-1} appears to have no large effects on the purity determination of pure samples. Rates higher than 0.04 K sec^{-1} lead to too low purity values and too high T_0 values, and also to very high

TABLE 3

Determination of impurity and T_0 using Sondack's procedure with different points (phenacetin with 2.740 mole % benzamide)

Data points (percent of peak height)			Impurity (mole %)	T_0 (°C)
10	20	30	0.993	132.06
10	20	40	1.147	132.44
10	20	50	1.361	132.94
10	20	62.5	1.529	133.31
10	20	75	1.548	133.35
10	30	40	1.437	132.92
10	30	50	1.800	133.55
10	30	62.5	1.999	133.88
10	30	75	1.929	133.76
10	40	50	2.416	134.35
10	40	62.5	2.481	134.43
10	40	75	2.206	134.06
10	50	62.5	2.548	134.51
10	50	75	2.102	133.95
10	62.5	75	1.681	133.50

TABLE 4

Check on effect of heating rate with pure phenacetin (experiment II)

Heating rate (K $\sec^{-1}$)	Purity (mole %)	T_0 (°C)	X-correction (%)
0.02	99.960	134.31	5.01
0.03	99.993	134.24	2.23
0.04	100.001	134.21	2.04
0.05	99.663	134.32	10.62
0.06	98.808	134.58	16.11
0.07	98.248	134.80	17.00
0.08	98.244	135.32	18.05

X-correction factors. The X-correction is generally thought to be caused by the inability of the system to attain thermodynamic and thermal equilibrium and by the insensitivity of the system to detect early melting. The authors have shown previously [10] that an increase in heating rate shifts the melting peak to lower temperatures, because at high rates, temperature gradients exist in the powder bed. These cause particles at the 'hot' side of the bed to melt earlier, which leads to an early deviation from the base line and therefore, to a broadening of the curve [10]. This affects the determination of the onset temperature. Moreover, because the system is more off-equilibrium at the higher heating rates, a larger X-correction factor is required to obtain a straight line, and this leads to steeper van't Hoff plots and hence to higher impurity levels and higher T_0 values.

Effects of heating rate and sample mass

Phenacetin

Table 5 contains the purity values of phenacetin obtained in the factorial study with sample mass and heating rate as the variables. Evidently, the two lower masses give purity values which are too low at the higher heating rates. This effect can be explained in the same way as in the preceding section.

For the other sample masses, an overall mean purity of 100.012 mole % was obtained, and the purity value was affected by the heating rate. It is interesting to note that this effect is highly significant ($P < 0.001$), although the differences in purity levels are very small. This is attributable to the small coefficient of variation (0.01%).

A purity greater than 100 mole % indicates that the slope of the van't Hoff plot is positive. This means that the temperatures in the earlier part of the melting peak (where $1/F$ is high) must be higher than those nearer to the peak top. We believe that this comes from thermal lags in the powder bed, which produce self-cooling effects. These effects have more influence at

TABLE 5

Effects of sample mass and heating rate on purity of phenacetin (experiment III)

Sample mass (mg)	Purity (mole %) for heating rates (K sec^{-1})		
	0.02	0.04	0.08
1	99.935	92.580	60.673
2	99.958	99.932	96.728
4	99.966	100.014	100.041
6	99.967	100.017	100.057
8	99.974	100.026	100.047

higher heating rates, i.e. when thermodynamic and thermal equilibrium are not well achieved.

Using the Sondack method, no significant effects of either sample mass or heating rate were obtained; the C.V. (coefficient of variation) was 0.09 %. Even at the low masses and high rates, the values were the same. It must be concluded that this linearization procedure is much more inaccurate than the least-squares technique but also less sensitive to the setting of the operational variables. Obviously, only 3 data points from the curve give less information than a linear regression with 20 data points.

Adipic acid

Similar results were obtained for adipic acid and phenacetin. Small sample masses and high heating rates produced far too low purity values; with large sample masses we found purities greater than 100 mole %. The mean purity (averaged over masses 2–8 mg) was 100.070 mole %, (C.V. = 0.01%), and the mean T_0 was 151.29°C (C.V. = 0.11 %).

Amidopyrine

For amidopyrine, the C.V. of the purity determination was 0.04 % and the mean value was 99.893 mole %. The 1 mg sample showed a mean purity of 99.631 mole % at 0.08 K sec^{-1}; this value was significantly different from the others ($P < 0.001$).

All purities found were below 100 mole %, but a slight increase with sample mass was again observed.

The T_0 value showed similar behaviour; the mean value was 108.23°C (C.V. = 0.08 %), and at 1 mg and 0.08 K sec^{-1}, its value was 108.84°C.

Dydrogesterone

Table 6 shows that the purity value of dydrogesterone is significantly higher at higher heating rates ($P < 0.001$) and also (although less pro-

TABLE 6

Effects of sample mass and heating rate on purity (mole %) of dydrogesterone (C.V. = 0.08 %) (experiment III)

Sample mass (mg)	Purity (mole %)			
	Heating rate (K $\sec^{-1}$)			Mean
	0.02	0.04	0.08	
1	99.118	99.287	99.542	99.315
2	99.255	99.413	99.624	99.431
4	99.196	99.402	99.700	99.433
6	99.215	99.428	99.762	99.468
8	99.168	99.438	99.777	99.461
Mean	99.190	99.394	99.681	99.422

nounced) at higher sample masses ($P < 0.01$). Dydrogesterone slightly oxidizes during melting, and, as was found earlier [10], high heating rates lead to shorter transition times, so that less time is available for the oxidation and less impurity can be formed. Higher sample masses will also give rise to less oxidation (and, therefore, higher purity values). At lower masses, the concentration of the oxidation product formed will be greater than at the higher masses. Moreover, during the first phase of melting (the phase used in the van't Hoff calculation), thermal lags and self-cooling effects are more pronounced in larger sample masses, so that the temperatures in the powder beds decrease, leading to lower decomposition rates.

For the T_0 values in this experiment (mean value 169.44°C, C.V. = 0.05 %), a significant interaction ($P < 0.05$) was caused by the fact that the value at the highest mass (8 mg) did not increase with increasing heating rate, unlike those at the other masses. Essentially, a higher T_0 value with higher heating rates is in accordance with what has been found with phenacetin, and may be caused by superheating effects: at higher heating rates, the system is more off-equilibrium, which leads to a larger X-correction, to steeper van't Hoff plots and, hence, to higher T_0 values. Apparently, this effect of superheating is counteracted by self-cooling properties of large sample masses.

Effects of heating rate and particle size

Introduction

From the experiments where particle size and heating rate were varied, the mean purity and T_0 values were calculated: the results are presented in Table 7. The coefficients of variation were 0.06 % for both responses. This is well

TABLE 7

Factorial studies with heating rates and particle sizes as variables. Mean values and standard deviations (s) (experiment IV)

Substance	Purity (mole %)		T_0(°C)	
	Mean	s	Mean	s
Adipic acid	99.872	0.039	151.07	0.09
Amidopyrine	99.726	0.020	107.93	0.06
Benzoic acid	99.980	0.022	122.02	0.06
Caffeine	99.875	0.061	235.27	0.08
Phenacetin	99.872	0.063	134.11	0.05
Benzamide	99.243	0.073	125.72	0.13
Acetanilide	99.827	0.079	113.92	0.07
Dydrogesterone	98.977	0.141	169.03	0.15
Naphazoline nitrate	99.718	0.067	168.41	0.13

within the reproducibility given in the literature: the USP [2] states that the measured impurities are reproducible, and probably reliable, within 0.1 %, Blaine [17] estimates the intra-laboratory standard deviation of purity 0.06 %, Reubke and Mollica [4] claim an absolute purity error of 0.1 %, and Joy et al. [18] mention an absolute error of 0.05 %.

Because of the high precision with respect to purity, we found many statistically significant effects and interactions. On the following pages we will present and try to explain these effects, but it must be kept in mind that their importance in normal daily practice may only be small. It should also be emphasized that our explanations, often in terms of thermal lags within the powder bed, apply only to our own apparatus (Mettler TA 2000), where heat transfer is not only by conduction, but also by convection of the furnace atmosphere.

Adipic acid

Table 8 shows that the purities of the comminuted and small-size samples of adipic acid appear to be higher than those of the other size fractions ($P < 0.001$). A higher purity level corresponds with a sharper melting curve in the $1/F$ range of interest. This corresponds with the observation that lower purity levels lead to a broadening of the DSC-curve and to lower peak temperatures [5].

Sharper melting peaks indicate that the melt acceleration is higher [19], or, in other words, that a small temperature difference gives rise to a high increment in the amount of molten material. If the substance was absolutely pure, the complete sample would melt at exactly the same temperature, meaning that the slope of the van't Hoff plot was zero. In practice, however,

TABLE 8

Effects of particle sizes on measured purities of different substances (experiment IV)

Substance	Purity [e] (mole %)				
	Comminuted	Small	Medium	Large	Complete spectrum
Adipic acid [a]	99.940	99.898	99.832	99.855	99.833
Amidopyrine	99.568 [b]	99.753	99.780	99.773	99.757
Benzoic acid	99.988	[d]	99.986	99.965	99.982
Caffeine [a]	99.922	99.847	99.867	99.815	99.922
Phenacetin [a]	99.846	99.933	99.759	[d]	99.949
Benzamide [c]	99.183	99.138	[d]	[d]	99.408
Acetanilide [c]	99.951	99.713	99.884	99.644 [b]	99.943
Dydrogesterone [a]	99.106	99.842	99.105	98.907	98.925
Naphazoline nitrate	99.774	99.219 [b]	99.892	99.796	99.911

[a] Significant difference between levels.

[b] Evidently deviating from other levels.

[c] Significant interaction between particle size and heating rate.

[d] No sieve fraction available.

[e] Averaged over both heating rates.

there will always be thermal lags within the powder bed, leading to melting ranges. Such ranges are therefore not due to the actual impurity present, but only arise from the need to transport heat in the sample.

The DSC system used (the Mettler TA 2000 A, where the sample holder is placed on a thermal insulator, and the energy transport is both by conduction and by convection) is particularly sensitive to these thermal lags.

Evidently, particle size affects these thermal gradients in the sample bed: smaller particles lead to smaller thermal lags, and therefore to sharper peaks and higher purities. It should be noted, however, that a successive decrease in particle size may lead to lower purities as will be seen with benzamide.

The low T_0 value for the large-particle sample (Table 9) corresponds with the low enthalpy of fusion that was found for this sample (248.6 J g^{-1} against 255 J g^{-1} for the other fractions). Such a decrease in enthalpy of fusion for larger particles was also found previously [10] together with a considerably lower onset temperature. It was explained in ref. 10 as being due to greater thermal resistances in larger particles.

T_0 at $\beta = 0.01$ K sec^{-1} was 151.11°C, and at 0.04 K sec^{-1} it was 151.04°C, the difference being significant at the 5 % level. The authors have shown previously [20] that adipic acid may sublimate before melting. It may be expected that at the lower heating rate, the time before the beginning of melting is longer, so that a higher pressure is allowed to build up. Hence,

TABLE 9

Effects of particle sizes on T_0 values of different substances (experiment IV)

Substance	T_0 [e] (°C)				
	Comminuted	Small	Medium	Large	Complete spectrum
Adipic acid [a]	151.18	151.18	151.06	150.87	151.08
Amidopyrine	107.91	107.79	107.89	108.18 [b]	107.86
Benzoic acid	122.00	[d]	122.00	122.07	122.02
Caffeine	235.26	235.34	235.29	235.22	235.27
Phenacetin	134.19 [b]	134.07	134.08	[d]	134.09
Benzamide [c]	125.72	126.04	[d]	[d]	125.40
Acetanilide [c]	113.86	113.99	113.82	114.07	113.86
Dydrogesterone [c]	169.20	168.84	168.93	169.20	168.99
Naphazoline nitrate	168.17	168.09	168.39	169.09 [b]	168.29

[a] Significant difference between levels.

[b] Evidently deviating from other levels.

[c] Significant interaction between particle size and heating rate.

[d] No sieve fraction available.

[e] Averaged over both heating rates.

melting would occur at a higher temperature, so that the van't Hoff plot would lead to a higher T_0 value. This may explain the slight decrease in T_0 with increasing heating rate.

Amidopyrine

The mean purity and T_0 values of amidopyrine are in accordance with those reported by Masse and Chauvet [21]. Tables 8 and 9 show the low purity of the comminuted fraction and the high T_0 for the large-particle samples. For both responses, the differences between the fractions were highly significant ($P < 0.001$).

The broader peak of the milled samples can be attributed to their greater amount of surface defects or to the formation of decomposition products during the comminution. The T_0 values of the large particles are higher because the melting peak of these particles is shifted to a higher temperature level because of their greater thermal resistance.

A slight increase in apparent purity and T_0 with increasing heating rate were also observed. At $\beta = 0.02$ K sec^{-1} the purity was 99.714 mole % and T_0 was 107.89°C, and at $\beta = 0.04$ K sec^{-1}, the purity was 99.738 mole % and T_0 was 107.96°C. These differences were significant at $P < 0.001$, but we do not believe that they have any actual practical importance. It has already been observed that only rates higher than 0.04 K sec^{-1} lead to thermal inequilibrium giving lower purity and higher T_0 values. Indeed, for a number of substances (adipic acid, benzoic acid, caffeine, phenacetin) the T_0 value even decreased, slightly but significantly, if the heating rate was increased from 0.02 to 0.04 K $\sec^{-1}$.

Benzoic acid

The mean purity and T_0 values of benzoic acid were 99.980 mole % and 122.02°C (C.V. = 0.02 and 0.05 %, respectively). No significant main effects or interactions on the purity value were found, and there was only a slight effect of heating rate on T_0: at $\beta = 0.02$ K $\sec^{-1}$, T_0 was 122.05°C and at $\beta = 0.04$ K $\sec^{-1}$, T_0 was 122.00°C ($P < 0.05$). We do not believe that this effect is of practical importance. It is known that benzoic acid may sublimate before melting [22]. To reduce this, tightly closed sample holders were used. A pressure of benzoic acid could therefore have built up in the holders. This slightly higher pressure makes the van't Hoff law theoretically invalid [1]. Apparently, the particle size does not affect the building up of this pressure; the heating rate, however, could affect it in the same way as was discussed for adipic acid.

Caffeine

Table 8 shows that the measured purities of the comminuted and complete-size spectrum samples of caffeine are higher than those of the other fractions. A wet sieve analysis in *n*-heptane (previously saturated with caffeine), which was carried out on all fractions to determine mean particle sizes, showed that in the comminuted and complete-size samples hard lumps were present which disappeared by ultrasonic vibration. These lumps were not present in the other samples, as they had probably already disintegrated during their passage through the sieve. The lumps are thought to consist of smaller single particles, held together by electrostatic forces. It is known that milling may enhance these forces, giving aggregate formation. Apparently, these lumps lead to higher purity values. The authors have shown earlier [10] that aggregates give higher values for transition enthalpy and onset temperature, but a lower onset interval. It costs more energy to start the melting of aggregates, but once the onset of melting has been reached, the temperature level is so high that the melting accelerates rapidly and the melting curve is sharper than for non-aggregated single particles. This sharper peak therefore leads to a higher purity value.

From this observation it is concluded that the USP requirement [2], saying that samples have to be triturated before subjecting them to DSC, could lead to estimates of purity which are too high if the trituration is accompanied by the formation of aggregates due to electrostatic charges.

In our opinion, it would be better to first judge the amount of lumps in the bulk sample. If aggregates are present, one should preferably sieve out a medium-sized fraction without lumps to get 'correct' results. Especially when comparing different batches, this may be the proper procedure because the differences in purity levels may amount to 0.2 mole % (as with benzamide).

The mean T_0 of caffeine found here (235.27°C) corresponds well with the value obtained by Grady et al. [13]. The T_0 values were not significantly affected by particle size, although we had expected that the higher purities of the comminuted and complete-size samples corresponded with a lower T_0 value. The fact that this was not observed again indicates that the temperature levels of melting of these two samples are indeed higher than those of the other samples. The heating rate affected ($P < 0.01$) the T_0 values: 235.34°C at the lower rate and 235.21°C at the higher rate. According to the Merck index [22], caffeine may sublimate before melting, and therefore this effect of heating rate can be explained in the same way as was discussed for adipic acid.

Phenacetin

As with caffeine, the phenacetin bulk sample consisted of many aggregates. Table 8 shows that this again leads to a high purity value. Due to these lumps, the large-particle sample, obtained by collecting the particles that did not pass a 63 μm sieve in the dry state, could not be used in the DSC experiments, because the 'wet' analysis showed that 95% of the single particles were smaller than 63 μm. The medium-sized particles were obtained by dry sieving with 63 and 40 μm sieves, but the 'wet' analysis revealed that these particles had sizes between 7 and 53 μm. Only the fraction with small particles had a narrow distribution, with a mean diameter by weight of 24 μm. Because of better thermal conductivities in such smaller particles their melting peaks were sharper, yielding higher purities than those of the medium-size samples.

The comminuted sample also had a low purity value. This may be because phenacetin slightly decomposes during comminuting, but the greater amount of structural defects in the comminuted sample may also lead to a lower purity value, as was observed for amidopyrine. Unlike amidopyrine, however, the low purity of the milled phenacetin corresponded with a high T_0 value.

Benzamide

For both purity and T_0, we observed significant interactions ($P < 0.001$) between particle size and heating rate (Tables 8 and 9). The bulk sample of benzamide consisted of a fine and a coarse fraction, the latter amounting to about 20% of weight and consisting of aggregates which could not be disintegrated easily and which were not homogeneously distributed. Again, such aggregates led to a higher purity level, the difference with the other fractions even being 0.2 mole %. Sieve analysis was impossible, and therefore we could not test the 'large' and medium sized samples.

To investigate the effects of single particles, we prepared a number of size fractions by subjecting the samples to 10 min of ultrasonic vibration in *n*-heptane (previously saturated with benzamide), followed by 'wet' sieving in a sieve turret. The sieve fractions were then evaporated to complete dryness, and subjected to DSC analysis with $\beta = 0.02$ K sec^{-1}. The purity and T_0 values obtained for these fractions are presented in Table 10 and Fig. 1. Table 10 shows that the purity becomes higher as the particle size increases, reaches a plateau and then decreases if the particles are still larger; the effect on T_0 is reversed. We believe that the lower purity at the very high particle sizes is because of the existence of thermal gradients due to their lower heat-transfer properties, broadening the curve and therefore leading to lower purity values.

The relatively low purity measured at particle sizes < 150 μm could be attributed to the reasons given.

(1) Smaller particles have relatively more crystal defects, and therefore, a higher energy state [23]. This would lead to lower peak heights and a broadening of the peak, causing lower purity values. It has been discussed previously [24] that a further increase in the number of crystal defects, finally to an amorphous state, would lead to complete disappearance of the melting peak.

(2) It is generally not known how an impurity is distributed over the crystals, but it is not unrealistic to assume that a large amount of impurity is adsorbed on the crystal surfaces. Smaller particles have the higher surface

TABLE 10

Purity and T_0 values of different sieve fractions of benzamide (obtained with 'wet' sieve analysis, see text)

Sieve fraction (μm)	Purity (mole %)	T_0 (°C)
10– 20	99.105	126.12
20– 38	98.810	126.15
38– 45	98.964	125.91
45– 53	99.243	125.52
53– 63	99.332	125.33
63– 90	99.463	124.99
90–150	99.485	124.95
150–212	99.415	125.08
212–300	99.279	125.33
300–425	99.302	125.46
425–500	99.304	125.48
500–628	99.256	125.54
628–730	99.193	125.73
730–840	99.085	126.01
⩾ 840	98.962	126.19
Mean	99.213	125.59

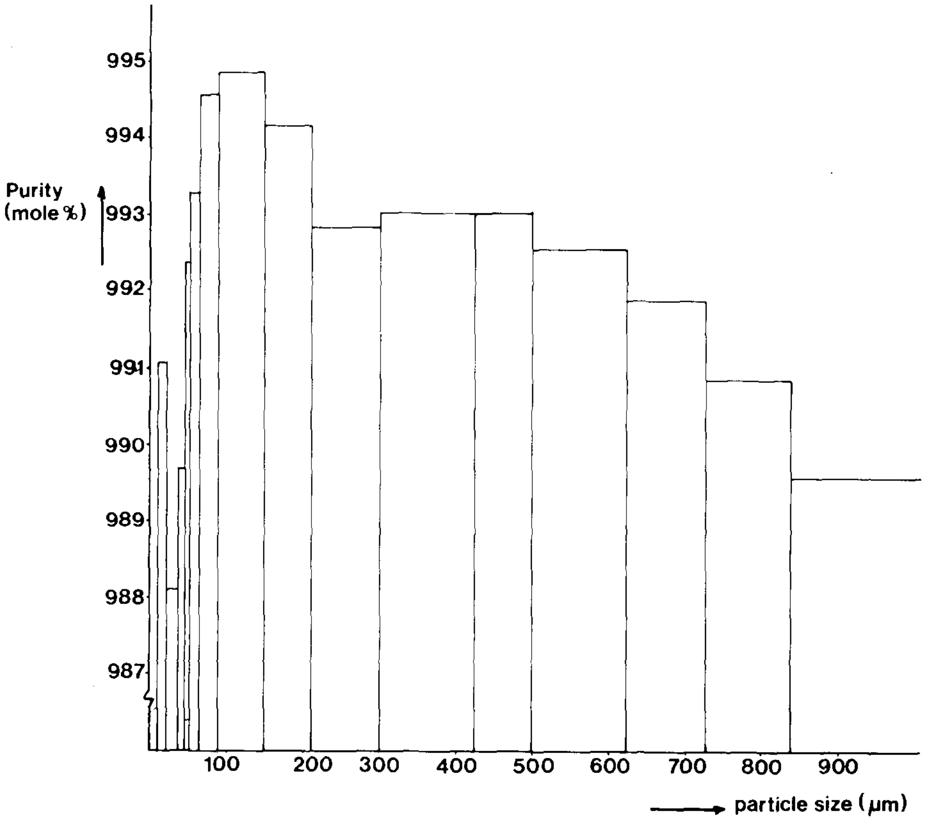

Fig. 1. Purity values obtained for different sieve fractions of benzamide. (Data extracted from Table 10.)

areas and so the distribution of impurity over a bulk sample of crystals may not be homogeneous, but smaller particles my contain relatively more impurity than the larger ones.

It is interesting to note that the smallest particles (< 20 μm) have higher purity values. Processes different from the ones mentioned above should account for this observation. We believe that extremely small particles first melt upon heating, but their contribution to the thermal effect is so slight that the base line is very gradually falling off. Since in the van't Hoff plot the first 2.5% of the peak area is not used, this would mean that the calculation starts at a relatively high temperature and the data points in the calculation are derived from a relatively steep part of the peak, which leads to a higher purity value.

The higher purity levels in Table 10 correspond with lower T_0 values and vice-versa. A higher purity correlates with a more nearly horizontal van't Hoff plot than a lower purity value. A less steep van't Hoff plot leads to a higher T_0 value (which is obtained by extrapolation to $1/F = 0$).

Acetanilide

The purity data for acetanilide are presented in Table 11. It can be seen that the significant interaction that was found ($P < 0.001$) is most probably due to the high decrease in response at the higher heating rate for the large-particle sample. The mean size of these large particles was approximately two times greater than that of the medium-size sample, and these large particles have a greater thermal resistance than smaller particles. Therefore, especially at the higher heating rate, some of these larger particles may reach a high temperature before releasing their energy to surrounding particles. Not only may these high-temperature particles melt earlier, leading to a general peak widening, but they may even evaporate prior to the end of the melting process. It is known that acetanilide has a high vapour pressure [1] so evaporation may occur. The overall result is a very low purity value at the higher heating rate with the large-particles fraction. The significant interaction ($P < 0.01$) that we observed for the T_0 values is probably due to the same reason: at 0.04 K sec^{-1} the T_0 value for the large particles was 114.16°C, whereas at 0.02 K sec^{-1} it was 113.97°C.

Dydrogesterone

At $\beta = 0.02$ K sec^{-1}, the purity of dydrogesterone was 98.891 mole %, at $\beta = 0.04$ K sec^{-1} it was 99.063 mole %. The difference was significant at $P < 0.001$. It is known that dydrogesterone melts with slight decomposition. This decomposition is due to oxidation, and at the lower heating rate, there is more time for the oxidation to occur, so that the purity is then expected to be lower than at the higher rate.

This decomposition may also account for the effects of particle size, as smaller particles are easier to oxidize due to their larger surface area. The lower purity value of the large particles (and of the complete size fraction,

TABLE 11

Purity data of acetanilide, at changes of heating rate and particle size (experiment V)

Particle size (μm)	Purity (mole %)		
	Heating rate (K sec^{-1})		Mean values
	0.02	0.04	
Milled (10)	99.974	99.928	99.951
Small (17)	99.694	99.733	99.713
Medium (44)	99.834	99.935	99.884
Large (91)	99.853	99.436	99.644
Complete size spectrum (27)	99.935	99.950	99.943
Mean values	99.858	99.796	99.827

which has a large proportion of large particles) must come from their greater thermal lags (Table 8). The comminuted fraction consisted of very small particles which were difficult to handle because of electrostatic charges. These charges induce aggregate formation leading to high purity values, and high T_0 values (because the temperatures must be higher to start melting).

Apparently, as the T_0 value of the large particles is also relatively high, the descending line of their melting peak is at an overall higher temperature than that of the other sieved fractions. This supports our statement that the lower purity observed for this fraction is due to its low thermal conductivity, because a lower T_0 value should have been found if the lower purity would have been caused by oxidation during melting.

Naphazoline nitrate

The smaller particles of naphazoline nitrate have a lower purity value than the other sieve fractions. The mean diameters by weight (d) of the small and medium particles are 16 and 72 μm. If they are assumed to be spheres, the surface area (πd^2) of the small particles is more than 20 times larger than that of the medium-sized particles. Naphazoline nitrate melts with oxidation and it is clear that particles with the larger surface area decompose most, so that their purity value (and T_0 value, see Table 9) is low. The high T_0 value for the large-particle sample may again be caused by its lower conductivity, although in this case it does not correspond with a broader peak.

The decomposition also accounts for the increase of T_0 with 0.33 K, which was observed with increasing heating rate.

The naphazoline nitrate curves were also evaluated manually, and then linearization was accomplished using the Sondack method. The results are given in Table 12. It can be seen that the Sondack method gives the same results as the least-squares technique, that is, a lower purity for the small particles, a higher T_0 value for the large particles and an increase in T_0 with increasing heating rate. The standard deviation is, however, slightly higher. Comparison of Tables 8, 9 and 12 shows that the purity values obtained using the Sondack method are higher than those obtained by the least-squares technique, although the extent depends on the particle size. The Sondack T_0 is 0.135 K lower (with 95% confidence limits 0.061–0.209 K) than the least-squares value. This difference is independent of particle size. It can therefore be concluded that the least-squares technique is more reproducible and gives lower purity values, which are expected to be more precise because more points of the curve are used.

Effects of decomposition during melting

Adipic acid

The mean purity value of adipic acid, obtained in experiment V in which possible decomposition was studied in pierced sample holders (where heating

TABLE 12

Effects of particle size and heating rate on purity and T_0 of naphazoline nitrate, calculated with the Sondack method (experiment V)

Size fraction	Heating rate (K Sec^{-1})	Purity (mole %)	T_0 (°C)
Comminuted [c]		99.921	168.10
Small [c]		99.746 [a]	167.89
Medium [c]		99.954	168.23
Large [c]		99.924	169.02 [a]
Complete spectrum [c]		99.940	168.12
	0.02 [d]	99.876	168.12 [b]
	0.04 [d]	99.918	168.43 [b]
Mean value		99.897	168.27
Standard deviation		0.08	0.21

[a] Deviating from other fractions, differences between fractions significant, $P < 0.001$.

[b] Difference between levels at two heating rates significant, $P < 0.001$.

[c] Values averaged over both heating rates.

[d] Values averaged over all size fractions.

rate, particle size, atmosphere and evaluation limits were varied), was 100.031 mole % ($s = 0.02$ mole %) and the mean T_0 value was 151.28°C ($s = 0.06$ K). These values differ from those presented in Tables 8 and 9. The latter data were obtained with samples in tightly closed holders, where no evaporation could take place, although a slight increase in pressure was possible. These closed holders are preferred over pierced ones, where evaporation leads to decrease in temperatures, especially in the final phase of the melting process, leading to a positive slope in the van't Hoff plot, and, thus, to purity values of more than 100%.

The significant difference in purity ($P \leqslant 0.01$) which we observed between the two heating rates (100.014 mole % at $\beta = 0.02$ K sec^{-1} and 100.048 mole % at $\beta = 0.04$ K sec^{-1}) can also be explained by evaporation during the melting process: at the higher rate, the sample is more off equilibrium, so that thermal gradients exist within the sample.

Because of this, some of the sample is at a relatively higher temperature and therefore more subject to evaporation than other parts. Therefore, at the higher heating rate, the van't Hoff plot has a higher positive slope and thus a higher negative impurity value. For the same reason, T_0 at 0.04 K sec^{-1} (151.21°C) is also significantly ($P \leqslant 0.01$) lower than that at 0.02 K sec^{-1} (151.36°C). As the milled particles have a higher surface area, they are more subject to sublimation and give a higher purity value (100.038 mole %) than the medium-size particles (100.024 mole %).

As we did not observe a significant difference in responses between oxygen and nitrogen, we conclude that oxidation does not have a significant effect on the determination of the purity of adipic acid, although small particles of adipic acid may oxidise during melting.

Benzoic acid

Benzoic acid sublimates before melting [25], and as for adipic acid, it could therefore be expected that more sublimation gave higher purity values. However, as the standard deviations of the benzoic acid experiment ($s = 0.04$ mole % for the purity and 0.08 K for the T_0 determinations) were slightly higher than for adipic acid, not all expected effects were observed at a statistically significant level. Only the purity value with pierced sample holders (100.000 mole %) was higher ($P \leqslant 0.01$) than with tightly closed holders (99.951 mole %). The explanation for this in terms of sublimation is made plausible by the different values in specific enthalpy between the pierced and closed holders (136.3 and 146.0 J g^{-1}). Other significant main effects or interactions between experimental variables were not observed.

Dydrogesterone

The mean responses [99.127 mole % purity ($s = 0.12$) and $T_0 = 169.29$°C ($s = 0.11$)] were higher than those found in the study with different particle sizes (Tables 8 and 9). This is clearly due to the atmosphere; in the study with different particle sizes only air was used, but in the study with decomposition, nitrogen and oxygen were used alternatively. The atmosphere has, indeed, a significant effect on the purity value of dydrogesterone: in nitrogen the measured purity was 99.404 mole %; in oxygen it was 98.851 mole %. The effect of heating rate which was observed, is also due to oxidation, because at the lower rate, when the transition time was longer, more substance was oxidised. The purity value then was 99.040 mole %, whereas at 0.04 K sec^{-1} it was 99.214 mole %. Significant interactions were observed between atmosphere and heating rate (in oxygen the increase in purity at the higher heating rate is more pronounced than in nitrogen) and between atmosphere and sieve fraction (the milled fraction with the higher area has a much lower purity in oxygen than the medium-sized fraction).

The authors have previously observed that broader peaks (higher impurities) correspond with higher T_0 values. However, the results for dydrogesterone indicate that if decomposition during the experiment is the reason for the higher impurity, the whole peak occurs at a lower temperature level. This was already shown for naphazoline nitrate [10] and it explains the slightly lower dydrogesterone T_0 value (169.26°C) for $\beta = 0.02$ K sec^{-1}, as compared to the value at 0.04 K sec^{-1} (169.33°C), and also the significant interaction between heating rate and atmosphere (at 0.02 K sec^{-1}, the T_0 value in oxygen is much lower than in nitrogen).

Naphazoline nitrate

The mean purity of naphazoline nitrate was 99.875 mole % ($s = 0.05$ %).

Significant effects of heating rates, particle sizes and evaluation limits, but not of atmosphere, were found. Naphazoline nitrate oxidises during melting and it was expected that in oxygen lower purity values would be obtained than in nitrogen. That oxidation had occurred, was indicated by the lower specific enthalpy and T_0 value in oxygen (92.8 J g^{-1} and 168.47°C) than in nitrogen (100.3 J g^{-1} and 169.04°C). Therefore, it must be concluded that the setting of the other variables in this experiment obscures the effect of atmosphere. This becomes clear when the significant ($P \leqslant 0.01$) interactions in which the atmosphere plays a role are examined. Table 13 shows that the purity value in nitrogen is lower than expected when the upper evaluation limit is 20% of the total peak area and also when comminuted particles are used. A three-factor interaction between atmosphere, heating rate and particle size ($P \leqslant 0.05$) was also observed. It is emphasized that in nitrogen atmosphere, there may still be less than 1% oxygen present [11]. At such a low oxygen concentration, oxidation only takes place in the first section of the melting curve or even before melting, and if a larger part of the peak is used in the calculation, the effect of oxidation is relatively small. This oxidation due to a small concentration of oxygen occurs particularly in comminuted particles which have a large surface area. This explains the significant interaction between particle size and evaluation limits ($P \leqslant 0.01$): if $F = 2.5$—20%, the comminuted sample had a purity of 99.77 mole %, but if $F = 2.5$–50% and also if the medium sized sample was used, the purity was 99.90 mole %.

Many main effects and interactions were observed on the T_0 value of naphazoline nitrate. They can be explained similarly as for dydrogesterone, because decomposition during melting leads to a lower level of peak-related temperatures and so to a lower T_0 value. The mean T_0 value, averaged over all tests in the experiment, was 168.76°C ($s = 0.09$ K).

The purity and T_0 values obtained using the Sondack method were 99.912

TABLE 13

Significant interactions on purity of naphazoline nitrate in the study of decomposition ($P \leqslant 0.01$) (experiment V)

Evaluation limits (%)	Purity (mole %) in	
	N_2	O_2
2.5–20	99.827	99.874
2.5–50	99.929	99.870
Fraction		
Comminuted	99.812	99.864
Medium	99.943	99.880

mole % and 168.62°C, with standard deviations 0.27 mole % and 0.50 K, respectively. No significant effects on purity were observed which was probably due to the relatively large variability. The differences between these values and those obtained with the least-squares linearization were dependent on the setting of the variables. As in the study of particle size, the least-squares method was more reproducible than the Sondack procedure.

SUMMARY AND CONCLUSIONS

With factorial designs, we carried out experiments to determine the influences of calculation procedure, heating rate, sample mass, particle size and atmosphere on the determination (with melting point depression) of molar purity values and pure melting point values, T_0.

Two linearization procedures were used: a 3-point correction (Sondack method) and a linear least-squares fit using 20 data points. The former method appeared to be less accurate than the latter one, but also less sensitive to the setting of the operational variables.

The choice of the 3 points in the Sondack correction method affects the calculated purity value and is somewhat arbitrary. To a smaller extent, the choice of data points in the linear least-squares fit also affected the results. It was concluded that if doped samples are to be assayed with the least-squares technique, an upper linearization limit of 50% is necessary, but if the effects of experimental variables on pure samples are to be determined, limits of 2.5–20% are acceptable.

Doping of samples by a resolidified melt technique leads to substantial sampling errors; weighing the pure substance and the impurity on top of each other is then preferred.

A heating rate lower than 0.04 K sec^{-1} has no substantial effects on the purity determination of pure samples, but higher rates lead to too low purity values and too high T_0 values. This may be evoked by thermal gradients in the sample powder bed.

The same explanation applied to a significant interaction which was found between sample mass and heating rate, viz. the lower masses (1–2 mg), gave much too low purity values at the higher rates. At higher sample masses, the measured purities were not so sensitive to increases in heating rate. Purity values greater than 100 mole % were observed at higher sample masses, which was due to the self-cooling properties of these masses.

The effects of particle size on the purity determination were studied with nine substances (melting in the region 100–250°C). Although generally valid conclusions could seldom be drawn because the influences of particle size were different for different substances, higher purity values were often found with larger particle sizes, but lower values were found with still larger particles. With extremely small particles higher purity values were often found and when the particles were aggregated, the purity value was also higher than expected. This latter effect means that trituration of samples, as required by the USP, is not always preferable. These effects of particle sizes could generally be attributed to heat transfer to and thermal conductivity in the sample powder bed, the crystal ordering of the sample, different distribution of impurities over the particles of the pure substance, and to sublimation, evaporation or oxidation of the sample. When decomposition occurred during melting, the heating rate, particle size and atmosphere could interactively affect the purity values.

In conclusion, the many effects of experimental variables that were observed necessitate a careful selection of these variables to get reliable results in the purity determination with DSC using van't Hoff's law of melting point depression.

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