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Review Articles

Purity determinations of drugs with differential scanning calorimetry (DSC) — a critical review

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Summary

This paper reviews the literature on purity determinations with Differential Scanning Calorimetry (DSC). These determinations are based on the van't Hoff's law of melting point depression. The aim of this presentation is to clarify the advantages and pitfalls of the method. Particular emphasis has to be placed on the validity of this law. Since in practice the 'van't Hoff line' generally is not as straight as it should be according to theory, the plot has to be linearized mathematically. The best-known linearization procedures are briefly discussed. The obtained purity values, however, are dependent on the applied operational factors. A discussion is presented of the effects of the instrumental conditions (heating rate) and sample conditions (like sample mass, sample particle size, sample stability in the melting region, sample purity and properties of impurities).

1. Introduction

Differential Scanning Calorimetry (DSC) is defined as a technique in which the difference in energy inputs into a substance and a reference material is measured as a function of temperature whilst the substance and the reference material are subjected to a controlled temperature programme (Lombardi, 1980). Depending on the method of measurement, two types of DSC can be distinguished, namely power-compensation DSC (as used in the Perkin-Elmer type of instrument) and

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heat-flux DSC, which is used in systems with multiple sensors (e.g. the Mettler apparatus) or with thermocouples on a constantan disc (as applied in the DuPont system).

The essential parts of a heat-flux DSC apparatus are given in Fig. 1. The difference in temperature between sample and reference is depicted in the DSC curve. If a transition takes place in the sample during heating or cooling, a peak in the curve is shown. Two energy factors are indicated by the DSC peak: the relative energies required to initiate transitions (indicated by the peak-related temperatures) and the energies involved in the transitions themselves (indicated by the peak areas).

DSC has gained wide-spread popularity in the pharmaceutical field, e.g. in the determination of melting and boiling points, polymorphism, stability and drug–excipient compatibility, kinetic parameters and drug purity (Daly, 1975; Fairbrother, 1978; Müller and Boeke, 1978). In theory, a melting transition of an absolutely pure crystalline compound should occur within an infinitely narrow temperature range. A broadening of the melting range provides a sensitive criterion of impurity. This method of determination is based on van't Hoff's law of melting point depression (Marti, 1972; Burroughs, 1980) which predicts that a plot of absolute sample temperature versus the reciprocal value of the molten fraction should be a straight line. The method has the advantage of speed and simplicity, since it is not necessary to know the nature of impurities. For very pure substances like drugs, the DSC technique of purity determination finds more and more application. The technique is even described in the USP (1980). Once the validity of the method and the purity of a standard material have been established, the characteristics of other batches can be evaluated.

A point to be considered is that the results are expressed as percent of molar impurity. As the molar masses of the impurities are generally not known, it is not possible to convert the results to weight/weight percentages as it is convential in chemical purity analysis. Generally, however, the impurities which are determined

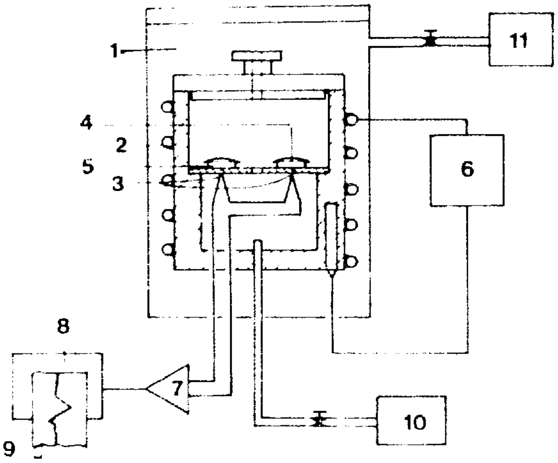

Fig. 1. Essentials of a heat-flux DSC-apparatus. 1 = furnace; 2 = heating coil; 3 = thermocouple junctions; 4 = sample-holder; 5 = reference-holder; 6 = programmable energy supply; 7 and 8 = amplifier with automatic on-off control; 9 = recorder; 10 = gas supply unit; 11 = vacuum pump.

are structurally similar to the major compounds, and their molar masses do not differ much.

2. van't Hoff's law of melting point depression

2.1. Derivation of van't Hoff's law (Martin et al., 1973; Fleming, 1977)

A reversible process is a process that is kept at equilibrium all the time, so that it can be reversed by applying an infinitesimal quantity of work. The work done under this condition is the maximum work possible. The change in free energy $+\Delta G$ is the maximum work that can be obtained from a process at constant pressure and temperature. The free energy change for a reaction, with reactants at arbitrary activities, a_{react}, and products at arbitrary activities, a_{prod}, is:

$$\Delta G = G_{prod} - G_{react} = \Delta G_0 + RT \ln \frac{a_{prod}}{a_{react}} \tag{2.1}$$

where ΔG_0 is the standard free energy change. When a system is in equilibrium, e.g. during melting, ΔG must equal zero. Thus for melting processes, Eqn. 2.1 can be converted to:

$$\Delta G_0 = -RT \ln \frac{a_1^L}{a_1^S} \tag{2.2}$$

where a_1^L and a_1^S are activities of the melting substance 1 in the liquid and the solid phase, respectively. Differentiation of Eqn. 2.2 with respect to temperature gives:

$$\frac{d \ln(a_1^L/a_1^S)}{dT} = -\frac{1}{R}\frac{d(\Delta G_0/T)}{dT} \tag{2.3}$$

We now introduce the Gibbs-Helmholtz equation:

$$\Delta G = \Delta H + \left(\frac{d\Delta G}{dT}\right)_p \tag{2.4}$$

which can also be differentiated towards temperature to give:

$$\frac{d(\Delta G/T)}{dT} = \frac{-\Delta H}{T_m T_0} \tag{2.5}$$

where T_0 is the pure melting point of the main component, T_m is the sample temperature after temperature equilibration. Combination of Eqns. 2.3 and 2.5 gives:

$$\frac{d \ln(a_1^S/a_1^L)}{dT} = \frac{+\Delta H_0}{RT_m T_0} \tag{2.6}$$

Eqn. 2.6 is known as the van't Hoff law. It may be integrated, assuming ΔH_0 to be constant over the temperature range considered:

$$\ln(a_1^S/a_1^L) = \frac{\Delta H_0}{R} \cdot \frac{T_0 - T_m}{T_0 T_m} \tag{2.7}$$

where ΔH_0 is the molar heat of fusion of the main component. Eqn. 2.7 may be rearranged to:

$$T_m = T_0 - \frac{RT_0T_m}{\Delta H_0} \ln(a_1^S/a_1^L) \tag{2.8}$$

We can now introduce the following approximations:
(1) if there is only one impurity present, $a_1 + a_2 = 1$. Then $\ln a_1^L = \ln(1 - a_2^L)$ and if $a_2^L \ll 1$, $\ln(1 - a_2^L) \sim a_2^L$;
(2) $a_2^S \approx 0$, i.e. the activity of the impurity in the solid phase equals zero. Therefore, $\ln a_1^S = \ln(1 - a_2^S) \approx 0$;
(3) for dilute solutions, $a_2^L \sim x_2^L$
(4)$T_0 \sim T_m$.
Furthermore, the concentration of the impurity in the liquid phase x_2^L is given by:

$$x_2^L = x_2 \frac{1}{F} \tag{2.9}$$

where F is the fraction molten. Eqn. 2.9 is only valid above the eutectic temperature. The minimum value that x_2^L can attain is x_2; its maximum value is given by the eutectic composition (Brown, 1979).

Applying the approximations 1–4 and Eqn. 2.9 to Eqn. 2.8 yields the following equation used in purity determinations:

$$T_m = T_0 - \frac{RT_0^2x_2}{\Delta H_0} \cdot \frac{1}{F} \tag{2.10}$$

This equation has the form $y = b - ax$, so the graphic plot of T_m versus $1/F$ yields a straight line with ordinate intersection, T_0, and slope:

$$-\frac{RT_0^2}{\Delta H_0} x_2$$

2.2. *Validity of van't Hoff law*

Essentially, the van't Hoff law describes the molar entropy of mixing and dissolution of the impurity in the melt (Barrall and Diller, 1970). As we have seen, the derivation of the equation is only possible if a number of assumptions is met. This fact limits the applicability of the method to determine purity with DSC, and should always be born in mind.

The most important assumptions are given below (Marti, 1972; Perkin-Elmer, 1967a and b).

(i) The melting point is the triple point.

(ii) ΔG in Eqn. 2.1 must equal zero, so equilibrium should be maintained throughout the fusion process. This limits the method to low heating rates, low sample masses, small temperature gradients within the sample, and a good thermal contact between the sample and its holder.

(iii) The components must form eutectic mixtures and the impurities must give ideal solutions in the molten part of the main component. This means that the equation is inapplicable in cases where the solutions are not ideal because of association or chemical reaction, or when the impurities are insoluble in the liquid phase.

(iv) The system is at constant pressure, which is assumed in the differentiation of Eqn. 2.4. Evaporation before or during melting should not occur and the vapour pressures of the impurities should be very small compared with that of the main component.

(v) ΔH_0, the molar heat of fusion of the main component, is constant over the temperature range and independent of the nature and amount of impurities. This may not be a valid assumption.

(vi) The activity of the impurity in the solid phase equals zero ($a_2^S \sim 0$). This means that no solid solutions may be formed.

(vii) $a_2^L \ll 1$. This approximation is generally very poor at the eutectic composition where the mole fraction of the major and minor component in the liquid phase would be approximately equal. It limits the method to high-purity samples and low $1/F$ values,

(viii) The activity coefficient of the minor component in the liquid phase approximates 1 ($a_2^L \sim x_2^L$). This limits the investigation to high-purity samples and excludes association, H-bridge formation, etc.

(ix) $T_0 \sim T_m$. Except at cryogenic temperatures and very large melting point depressions (i.e. very impure samples), this assumption is valid.

(x) $x_2^L = x_2 \cdot 1/F$. This approximation is found with the lever rule in a simple eutectic phase diagram. It is only applicable if it is assumed that the initial segment of the liquidus curve is linear.

(xi) In a given sample, the mole fraction of impurity must be constant. This again excludes association, but also evaporation of any of the components, and it also excludes determinations of samples which decompose during fusion, which sublimate or give polymorphic changes in the temperature region of interest.

However, the greatest problem encountered arises not from theory, but from a more practical point: the graphic plot of T_m versus $1/F$ is not a straight line. This is most probably because the instrument cannot detect 'early melting' (see Section 3.1.).

Only few authors tried to develop new theories to overcome these limitations. Staub and Perron (1974) introduced a step-heating programming technique, in which the heating rate was in essence zero. The temperature was raised in steps and after each temperature increase the authors waited until equilibrium, that is until the signal on the recorder had returned to the baseline. The sum of the heats of fusion of all peaks between the eutectic temperature and the melting point at completion was equal to the total heat of fusion. A plot of the thus calculated values of T_m and 1/F gave, according to the authors, a straight line. A critical discussion of this technique is given by Palermo and Chiu (1976), Gray (1972), Gray and Fyans (1973) and Ramsland (1980), but due to practical difficulties we do not think that it can replace routine determinations. A new theory was developed by Gustin (1978), who discovered a feature of the melting process which is not affected by heating rate, sample size and thermal conductivity: the point of *maximum melt acceleration*. At this point, the third derivative of the 1/F versus T_m curve equals zero. The tangent at this point can be extrapolated to $1/F = 0$, to find the pure melting point T_0 as the ordinate intercept. Impurities ranging from 1 to 51% gave results by this technique with a coefficient of variation of approximately 5%. Although this method has great potential applicability—for instance, as replacement of chromatographical techniques in stability determinations of drugs—more work is necessary to completely evaluate its merits. Anyway, the method can only be used in power-compensation DSC and not in heat-flux DSC.

3. Linearization

3.1. Introduction

As we already stated in Section 2.3., the van't Hoff plot of T_m versus 1/T is ideally a straight line. Such a straight line can be obtained occasionally with substances with an extremely high purity, but usually this is not the case (Marti, 1972). Many reasons for this discrepancy have been described in the literature, and, indeed, any deviation from ideality in the van't Hoff equation will essentially result in deviation from linearity, for instance:

(a) the condition of thermodynamic equilibrium is not met in a dynamic experiment, not even at low heating rates (Mettler, 1973);

(b) also, thermal equilibrium may not be attained. The resistance to heat transfer between sample-holder and the sample (thermal lag) should therefore be small, which means that the sample size should also be small;

(c) van't Hoff's law only holds in dilute solutions. The molar impurity in the sample should thus be very small;

(d) curvature has also been attributed to solid solution formation. This limitation cannot be controlled for impurities soluble both in the solid and in the melt; and

(e) if thermal equilibrium is maintained and a solid solution is not formed, curvature may result from insensitivity of the instrument to detect 'early melting'. This is the melting in the interval from the eutectic temperature up to the onset temperature found in the DSC curve. The amount of heat for this early melting can range from 0 to 20% of the total enthalpy.

Early melting is generally regarded as the most important reason for nonlinearity (Perkin-Elmer, Instruction Manual (c); Reubke and Mollica, 1967). It is easy to show by calculation from the van't Hoff law that even at small levels of impurity a significant amount of melting will occur over a wide region where the baseline will appear to be horizontal. The neglected portion can be considered negligible with respect to the total area, but it may even be 50–100% of the first few partial areas which are used in the van't Hoff plot. The small amount of lost heat can be retrieved by linearizing the T_m vs $1/F$ plot. An X-correction is introduced, which is simply the amount of melting of the major compound which occurs prior to the onset temperature of the peak. It can be found from:

$$1/F_n = \frac{A+X}{A_n+X} \tag{3.1}$$

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.

Recently, Draguet-Brughmans and Bouché (1981) proposed a polynomial regression procedure to avoid the calculation of the X-correction but more experience is needed to assess the features of this method. Much more attention has been given in the literature to the determination of X, for instance by Ramsland (1980), Sondack (1972), Davis and Porter (1969), Cooksey and Hill (1976), Draguet-Brughmans et al. (1977), and Decker and Young (1978). The most frequently applied methods are the ones of Sondack (1972), and those based on a linear least-squares fitting (Marti, 1972; Barrall and Diller, 1970; Driscoll et al., 1968; Grady et al., 1973; Masse and Chauvet, 1978).

3.2. Sondack's method

Sondack's method (1972) is a three-point correction procedure. Each point n of the plot of T_m versus $1/F$ has the coordinates $(A/A_n, T_n)$. A value of X is sought so that, when the data points n are adjusted to n′, given by the new coordinates $((A+X)/(A_n+X), T_n)$, the points n′ will lie on a straight line. Therefore, any 3 points must satisfy:

$$\frac{T_3 - T_2}{\dfrac{A+X}{A_3+X} - \dfrac{A+X}{A_2+X}} + \frac{T_2 - T_1}{\dfrac{A+X}{A_2+X} - \dfrac{A+X}{A_1+X}} \tag{3.2}$$

By rearranging, simplifying and solving for X, one obtains:

$$X = \frac{\dfrac{T_3 - T_2}{T_2 - T_1} A_3 - \dfrac{A_3 - A_2}{A_2 - A_1} A_1}{\dfrac{A_3 - A_2}{A_2 - A_1} - \dfrac{T_3 - T_2}{T_2 - T_1}} \tag{3.3}$$

When A is expressed in joules, the unit of X is also the joule. The 3 points should be chosen from high, middle and low values of uncorrected $1/F$. In the Mettler procedure (1974), points at 10%, 30% and 50% of maximum peak height are determined. The procedure has the advantage of being fast. However, Sondack (1972) stated that the corrected relation may still take the form of a sigmoidal curve, which could lead to erroneous results. Furthermore, it appears that the choice of the points affects the value of the X-correction, so the change of one point may modify the result considerably (Draguet-Brughmans et al., 1977). A modified procedure, based on Sondack's method, has been described by Brostow et al. (1978).

3.3. *Least-squares fit*

The increment X is frequently determined by manual or automatic iteration. This is done in a trial-and-error procedure, by adding progressively larger area increments and determining the effect on the ($1/F, T_m$) plot. The operation is repeated until a linear plot is obtained. Ten or more points are generally needed (Barrall and Diller, 1970). Such a linearization is only possible within a certain region of the melting curve. This was discussed by Driscoll et al. (1968) who used the 2% molten fraction ($1/F = 50$) as the upper limit, and the point at half-peak height as the lower linerarization limit. This means that the lower limit of $1/F$ is approximately 8 for very pure samples, and about 2.5 for impure samples. Driscoll and co-workers emphasized the importance of the linearization limits, but it should be noted that essentially the choice of these limits is arbitrary. If too little of the curve is used, the purity obtained is too high, and if too much is used, the purity value is too low.

Grady et al. (1973), for instance, noticed that varying the choice of areas in a phenacetin analysis resulted in X-corrections ranging from 1 to 15% of the total peak area and in impurity values ranging from 0.02 to 0.40 mole%. They stated that the T_0 and ΔH_0 values are affected by the random selection of areas, but minor variation in these values has negligible effect on the calculated impurity. However, they found that many drugs give sigmoidal curves, which cannot be even approximately linearized. X-Correction then is entirely arbitrary, which leads to a great variability in the impurity calculation. This was the case with e.g. hexachlorophene and phenacetin. The values they reported for these drugs were found with X-corrections that had the best least-squares fit with the lowest standard error. In cases where the sigmoidal-character is strong, the authors recommended that impurity values be calculated with several X-correction values to assess their confidence. They stated that computer programs calculating a single value for impurity based on a single best fit should be used carefully.

3.4. *Comparison of calculation procedures*

We recently conducted a study to compare the applicability of the Sondack method and the least-squares method (van Dooren and Müller, 1983). The Sondack method tends to underestimate the amount of impurities actually present. Furthermore, greater differences in the $1/F$ values used in the Sondack calculation lead to higher impurity and T_0 values, although all values were less than the theoretical ones. If doped samples are to be assayed with the least-squares technique, the upper linearization limit should be 50% ($1/F = 2$) but if pure samples are to be assayed, linearization limits of 2.5–20% ($1/F = 40–5$) are acceptable.

4. Instrumental conditions

4.1. Introduction

Instrumental factors like sample-holder assembly, calorimetric sensitivity and heating rate may affect purity values obtained with DSC (Marti, 1972). In the Mettler TA 2000, heat transfer occurs mainly through conduction and convection of the furnace atmosphere. This can lead to thermal lags in the sample, which may broaden the curve and thus affect purity values. Therefore, results in other types of instruments may be different. But also in the same instrument, influences of instrumental variables can be superimposed, not only on each other, but also on those of sample variables. As an example, Hunter and Blaine (1982) confirmed in a multi-dimensional critical evaluation of instrumental conditions (mass and impurity level), that errors, caused by deviations from their optimum values, are cumulative.

4.2. Heating rate

Many investigators agree that the heating rate should be low, because the sample is probably not at thermal (and thermodynamic) equilibrium at high heating rates.

Marti (1972) recommends rates of 0.625 or 1.25 °K/min, Palermo and Chiu (1976) prefer rates of less than 1 °K/min. Mettler (1973, 1974) uses rates of 2–5 °K/min. We ourselves found that a heating rate lower than 2.4 °K/min did not have large effects in the purity determination of pure samples (van Dooren and Müller, 1983).

The general requirement that for good data evaluation the peak height should be within 50 and 100% of full recorder scale, forces the heating rate to be low for pure samples and increasingly higher for increasingly impurer samples (Barrall and Diller, 1970). This is to ensure that the start of the peak is detected early enough to give a correct analysis.

Palermo and Chiu (1976) used heating rates up to 10 °K/min for qualitative comparison, unstable compounds, or extremely broad melting peaks.

Barrall and Diller (1970) remarked that a statistical interaction should occur between sample size (total heat uptake) and heating rate. They showed that both high sample sizes and high heating rates caused systematic differences from the known purity value.

5. Sample conditions

5.1. Introduction

The conditions regarding the sample (size, stability, purity, etc.) should be such that thermal and thermodynamic equilibrium are attained as much as possible throughout the determination, to keep van't Hoff's law applicable. All remarks in the following sections are based on this general statement.

5.2. Sample size

For best results, the sample size should be kept small, i.e. a few milligrams. With a sample size greater than 5 mg, thermal equilibrium will not be maintained (Palermo and Chiu, 1976). We also found that sample sizes $\leqslant 2$ mg gave too low purity values at heating rates $\geqslant 2\,^\circ$K/min (van Dooren and Müller, in press). The statistical interaction between sample size and heating rate has already been mentioned. Highly reproducible results are only possible with special care in the weighing and handling procedure.

5.3. Particle size

Only seldom have investigators given attention to the effect of particle size on the purity results. Plato and Glasgow (1969) state that in a larger-than-average crystal, thermal equilibrium cannot be maintained within the main body of the sample. We ourselves (van Dooren and Müller, 1983) recently studied the effects of particle size in purity determinations with 9 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, we often found higher purity values with larger particle sizes, but lower values with still larger particles. At such very high particle sizes the existence of thermal gradients due to lower heat transfer broaden the curve and therefore leads to lower purity values. Very small particles appeared to have relatively low purities. This could be attributed to the following reasons:

(a) smaller particles have relatively more crystal defects, and therefore, a higher energy state (Hüttenrauch, 1978). This would lead to lower peak heights and a broadening of the peak, causing lower purity values. It has been described before (Murphy, 1969) that a further increase in the number of crystal defects, to finally an amorphous state, would lead to complete disappearance of the melting peak; and

(b) it is generally not known how an actually present impurity is distributed over the crystals, but it is not unrealistic to assume that a large amount of the impurity is adsorbed on the crystal surfaces. Smaller particles have the higher surface areas and so the distribution of impurity over a bulk sample of crystals may not be homogeneous, but smaller particles may contain relatively more impurity than the larger ones.

When the particles were aggregated, the purity value was higher than expected. Apparently 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 very rapidly and the melting curve is sharper than for non-aggregated single particles. Therefore the USP (1980) requirement, saying that samples have to be triturated before subjecting them to DSC (to ensure a high thermal conductivity between sample and holder) could lead to too high estimates of purity 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%.

5.4. Sample stability in the melting region

One assumption for the validity of the van't Hoff's law is that in a given sample, the mole fraction of impurity must remain constant. This excludes evaporation of any of the components and to a certain extent it also rules out determinations of samples which decompose during fusion, which sublimate or which give polymorphic changes during the experiment. Reubke and Mollica (1967) reported: samples were selected which would melt without decomposition'. Plato and Glasgow (1969) stated that purity of chemicals that decompose near their melting points cannot be determined by the DSC method. Throughout the literature one can find the statement that the DSC method is unable to assess substances that evaporate or decompose during melting (Marti, 1972); this is, unfortunately, the case with many pharmaceuticals. However, this disadvantage should not be over-emphasized. In the first part of the melting curve the rate of decomposition is often very small and the use of a nitrogen atmosphere instead of air may reduce decomposition by oxidation.

It seems appropriate to carry out the analysis with different heating rates. If the results do not differ too much, decomposition reactions may then be neglected. If decomposition during the experiment causes a higher impurity, the whole peak occurs at a lower temperature level and this leads to a lower T_0 value. If evaporation is the cause of sample instability it is preferable to use hermetically closed holders, even though a slight increase in pressure may be possible then. In pierced sample holders evaporation leads to a decrease in temperatures, especially in the final phase of the melting process, leading to a positive slope of the van't Hoff plot and, thus, to purity values of more than 100%.

5.5. Sample purity

The derivation of van't Hoff's law implies a limitation regarding the maximum concentration of impurities (Marti, 1972). This limit is not properly defined because it would require knowledge of the absolute difference between the theoretical amount of eutectic impurities and the sum of eutectic impurities as determined by DSC. With the lack of such a definition it is not surprising that the maximum concentration for applicability of the van't Hoff law is estimated with considerable differences: Mettler (1973) gives a maximum impurity level of 5%, Blaine (1982) 3%, the USP (1980) 1.5%, de Angelis and Papariello (1968) only 1%.

Only Palermo and Chiu (1976) mention a value of 10 mole%, but this is generally regarded as too optimistic for the normal dynamic technique.

Joy et al. (1971) state that for a thermally stable compound a low DSC purity value is clear evidence that the compound is not of high purity. However, a high purity value is not conclusive evidence that the compound is indeed of high purity. They recommend that in that case confirmatory evidence should be sought from other techniques.

The accuracy range can be extended by the method of de Angelis and Papariello (1968) who mixed in known proportions the sample whose purity is to be determined and a known pure sample (> 99.5%) of the same compound. The amounts were adjusted so that the final purity of the mixture was between 99 and 99.5%. This dilution method is excellent for the solution of special problems. Its practical use is, however, not only limited by the amount of work involved, but also by the difficulty of obtaining known pure substances, and by the problem of getting representative samples of the mixtures.

5.6. Properties of impurities

Molecular structure

The impurities must give ideal solutions in the molten part of the main component. This is one of the conditions for the validity of the van't Hoff's law. de Angelis and Papariello (1968) reported that if the impurities are structurally similar to the main substance, the found purity values were in excellent agreement with the 'true' values. This is important, because these sorts of contaminants (e.g. polymorphs, optical isomers, common synthetic reaction contaminants or degradation products) cannot be easily determined with other methods (like TLC, HPLC, etc.). However, when these investigators made mixtures with structurally dissimilar impurities like formulation excipients, they could not determine their concentration with DSC. It is, therefore, reasonable to expect that when the major and minor component become and more dissimilar, the purity levels found will increasingly become too high.

Vapour pressure

The vapour pressure of the eutectic impurities must not differ much from that of the main component (Marti, 1972). This condition is compulsory during the whole experiment.

Solid solubility

One assumption in the van't Hoff's law is that the solubility of the impurities in the solid phase of the main component should be zero. In other words, no solid solutions must exist. Molecules with a molecular structure similar to that of the major component, can both give ideal solutions in the liquid phase and fit into the solid matrix of the major component without disruption of the lattice, so forming solid solutions. Purity estimates are too high in such a case. This is more common with less ordered crystals as indicated by low heats of fusion (USP, 1980). It has been stated (Marti, 1972; Driscoll et al., 1968) that a proper treatment of systems with solid solution is given by a modification of Eqn. 2.10:

$$T_m = T_0 - \frac{x_2 R T_0^2}{\Delta H_0} \frac{1}{\frac{K}{1-K} + F} \tag{5.1}$$

where K is the distribution ratio of the impurity between the solid and liquid phases:

$$K = \frac{a_2^S}{a_2^l} \tag{5.2}$$

K is zero in the absence of solid solution formation.

5.7. Sample preparation

For routine analysis, samples can be taken from the bulk material, but it must be ensured that they are representative and that the impurities are homogeneously distributed in the bulk. In evaluating a purity programme by DSC, the substance to be tested is often doped with the most probable impurities. In such a case, sampling becomes even more problematic.

De Angelis and Papariello (1968) solved this problem by using a dry-mixing technique, in which both the pure component and the contaminant were separately weighed into a tared sample pan and the weight of each was obtained by subtraction. This procedure is now generally considered as useful (Mettler, 1973).

6. Practical aspects

6.1. Experimental procedure

The experimental procedure consists of the recording and evaluation of the DSC curve of the melting of a substance. It is based on the mathematical principles presented in Section 2 and the instrumental and sample conditions described in Section 4. Of course, the detailed procedure depends on the basic theory chosen (e.g. van't Hoff, Gustin, stepping technique) and if the van't Hoff law is used, it is also dependent on the chosen linearization technique.

An example of a flow chart representing the procedure with the van't Hoff law is given by Brown (1979). A normal evaluation procedure with the aid of a computer consists of:

(i) data entry—in a conversational mode the experimental conditions and known constants are entered by the operator into the terminal;

(ii) data acquisition by any system that functions as an analog–digital interface;

(iii) data processing.

(a) Peak sensing: one possibility is that the operator enters the (known) integration limits. This requires preknowledge of the position of the melting peak. Another possibility is to use an algorithm for peak sensing. This can be accomplished by recording a portion of the baseline preceding the peak, fitting the first data points to a straight line and examining each subsequent point for conformance. As a point has been found to lie outside a predetermined control level (e.g. 2 sigma), just like a number of subsequent points, this point is taken as the peak start. The peak end can be found analogously (Blaine, 1982; Moros and Stewart, 1976).

(b) Baseline construction: once the coordinates of peak start and end have been established, the interpolated baseline can be constructed. In general, a straight baseline is assumed (Marti, 1972; Blaine, 1982), but it must be remembered that the actual course of the baseline may deviate to a large extent from simple interpolation between pre- and post-transition baselines. Hakvoort (1978) described that this was due to a considerable change in heat capacity of the substance during melting. The total heat of fusion is not altered by drawing the correct undulating baseline, but the partial areas corresponding with the molten fractions are larger than when using a straight baseline. This may be another explanation of the X-correction (Section 3.1).

Heuvel and Lind (1970) also give exact mathematical equations for baseline constructions. Another procedure was described by Ramsland (1980).

(c) Peak integration: integration can be done by multiplying the amplifier signal (in J/s) by the data transmission interval, and adding the partial areas. Blaine (1982) describes an interval of 0.2 s, Mettler (1973, 1974) and Perkin-Elmer (1967a and b) use an interval of 1 s.

(d) Determination of partial areas: the ratio F of the area under the DSC curve up to a given temperature is directly proportional to the fraction molten at that temperature. These areas can be calculated using the trapezoidal rule. Marti (1972) divided the whole peak in 30 partial areas; we ourselves found that 10 or 20 partial areas in the linearization region are generally sufficient (van Dooren and Müller, 1983).

(e) Linearization of 1/F with temperature: the linearization in the 1/F versus T_N diagram (the van't Hoff plot) is only possible within a certain region of the melting curve. Driscoll et al. (1968) used for their linearization a constant value of 2% as lower limit and the point at half-peak height as upper value. On this basis Marti (1972) chose 30 segment areas with the greatest density of the points in the expected linearization region. Moros and Stewart (1976) used 1/F fractions from 1 to approximately 75. We found that in the determination of doped samples, 1/F fractions from 2 to 40 are required, but if highly pure samples are to be assayed, 1/F fractions from 5 to 50 are acceptable (van Dooren and Müller, 1983). If the iterative least-squares procedure is used, the X-correction for the partial areas should be such that the plot of 1/F versus T is a satisfactory straight line. To ensure that overcompensation is avoided the linearization should be terminated when the line still has a slight but insignificant upwards curvature (Perkin-Elmer, 1967a). Especially in the case of a very impure sample, the iteration could otherwise produce a better and better, though less and less correct, straight line. Plato and Glasgow (1969) stated that beyond the point where the sample is half-melted (about 2/3 of peak height) melting is too rapid for equilibrium conditions to prevail and the van't Hoff equation does not hold.

(f) Calculation of impurity: the mole fraction of impurity is calculated from the slope of the line that gives the best fit of the corrected data:

$$x_2 = \text{slope} \cdot \frac{\Delta H_0}{R \cdot T_0^2}$$

(g) Data output: together with the calculated (im-) purity and T_0 values, the data output should give all parameters necessary to reconstruct the analysis.

6.2. Accuracy

The important question of accuracy is difficult to handle. All authors agree that the accuracy decreases with increasing content of eutectic impurities. It can be concluded that in normal (high-) purity ranges (> 98%) a relative accuracy with respect to impurity of 25% can generally be reached, together with a coefficient of variation < 1–2%.

Burroughs (1980) and Grady et al. (1973) remark that not all samples are suitable for purity analysis by DSC methods. Estimates range from 20 to 70% of organic compounds being suitable. A first step to a clearer situation regarding problems like decomposition, solid state phase changes, solid solution formation, etc., could be reached by an extensive study on any system which shows great differences in actual and experimental purity values. Elder (1979) studied a ternary system of phenacetin with benzamide and *p*-aminobenzoic acid, and presented evidence that the total DSC-measured impurity is the sum of the individual dopant levels, and independent of their relative proportions.

6.3. *Verification of applicability*

The applicability of a given DSC purity determination programme can be checked by the comparison of data obtained with known values. Such checks may include (Marti, 1972): (1) high-purity substances; (2) mixtures of standard substances; (3) different evaluation procedures; (4) variation of operational variables (sample size, heating rate, etc.); and (5) measurements on substances which decompose in the melting region.

Once the usefulness of the programme has been verified, the exact determination procedures should be developed for each substance. Procedures for such a development have already been described by Marti (1972).

An interesting way of checking the applicability of a purity-determination programme was given by Zynger and Kossoy (1980) who determined molecular weights by using a stepping technique as described before by Zynger (1975).

7. Conclusions

Purity determination by means of melting point depression with Differential Scanning Calorimetry is an interesting and promising technique.

It has many advantages over 'conventional' analytical techniques based on separation of the pure compound and the impurities (like chromatographical techniques). It is a simple and rapid general-purpose means of estimating purity which requires small sample sizes (< 5 mg), and which does not need separation procedures or comparison with known impurity levels. It is ideal for comparison of purities of batches in quality control; especially impurities which are structurally very similar to the pure substance can be determined very well (like polymorphic modifications, intermediates from the manufacturing route and degradation products). Main disadvantages of the method are that the substance should be reasonably stable during melting (no decomposition) and that no solid solutions must exist. Another drawback is the fact that the method only applies to highly pure substances ($\geqslant 98$ mole%). The method appears to be extremely precise; coefficients of variation smaller than 0.1% can easily be reached. This high precision, however, also means that the accuracy is difficult to determine. The operational factors may have significant influences on the measured purity values. For routine procedures, the sample mass should be approximately 2 mg, and the heating rate 0.02–0.04°K/s. Sample-holders should be tightly closed. Particle size affects the results and should be standardized, but powdering of samples should be avoided. As the calculation pocedure, a linear least-squares fit using at least 10 data points is to be preferred.

We believe that further research in this field should in the first instance be aimed at a more in-depth assessment of the effects of particle size. Another important field of interest is a comparison of the effects of different baseline constructions and of the different linearization procedures. Also investigations will be necessary into the possibility to decrease the lower purity limit of samples. A first step toward this goal is the stepping technique or the method proposed by Gustin (1978). If it were possible to assay accurately substances with purities as low as 70–80%, it would be feasible to store samples in DSC holders in furnaces at different temperatures and afterwards to determine the concentrations of the intact ingredients, so that the data could be used for accelerated stability studies.

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