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Applications of Differential Scanning Calorimetry in Pharmaceutical Analysis

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The application of differential scanning calorimetry to several aspects of pharmaceutical analysis is presented. These include the utilization of this technique in the quantitative estimation of purity, in the relative determination of purity for a control procedure, and in the detection of polymorphism. The purities and heats of fusion of diallylbarbituric acid, naphthalene, glutethimide, dibucaine, and anthraquinone are given. A comparative analysis of several samples of methyl reserpate is presented and the detection of polymorphism in tripelennamine citrate is discussed.

THE AVAILABILITY of commercial instruments capable of performing various types of thermal analysis has led to an increasing number of publications of their application to organic compounds (1–4). One area of application which is of particular interest to a pharmaceutical analyst, and one which has not been extensively investigated, is the quantitative estimation of purity through thermal analysis. Phase solubility analysis is presently one of the most widely used techniques for purity determination (5, 6), since the equilibrium solubility of a pure compound is as characteristic a property as is the melting point. This method, however, suffers from certain disadvantages. Perhaps the most important of these is that the time required for equilibration may be several weeks. It was decided therefore to see if differential scanning calorimetry could overcome some of these disadvantages in determining the "absolute" purity of a compound. This determination is based on the other fundamental property of a compound, its melting point. This method would overcome some of the disadvantages of phase solubility in that the analysis could be completed in 1 day and since one determines the sum of the impurities, it would be possible to estimate purity to within $\pm 0.1\%$ when dealing with samples at the 99%+ level, even if errors of the order to 20% were introduced.

Two other areas of application were also investigated—namely, the utilization of this technique in the determination of relative purity and in the detection of polymorphism. It provides a rapid method for control procedures since one can compare the endotherm of the sample to endotherms of known purity without the necessity of a complete analysis. The presence of polymorphism in several samples of tripelennamine citrate is easily detected by comparing thermograms of different samples and this substantiated evidence that was obtained by spectroscopic methods.

Received January 31, 1967, from the Research Department, Ciba Pharmaceutical Co., Summit, NJ 07901
Accepted for publication April 10, 1967.
The authors are grateful to Miss Janet Conover for technical assistance.
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EXPERIMENTAL

A Perkin-Elmer model DSC-1B differential scanning calorimeter was used in this study (7). Aluminum sample pans and pan lids which fit the DSC-1B were used for all samples except the anthraquinone. Anthraquinone samples were sealed in a volatile sample sealer. Care was taken in sample handling and in covering the sample holders with the covers in the same relative position in order to minimize base line drift, which, as pointed out by Rogers and Morris (8), can be caused by differences and variations in thermal emissivity. All samples were run in a nitrogen atmosphere. The materials used were of the quality specified in the text.

A semimicro balance with a sensitivity of 0.01 mg. was used to weigh all samples. Areas were generally determined by planimeter, although in some cases areas were determined by cutting and weighing. In either case, the reproducibility of an area determination was to within 3%.

THEORY

Calorimetric methods for the determination of purity are well known (9, 10); hence, only a brief review of the theory will be given. The equation for the lowering of the freezing point in dilute solution is that developed by Van't Hoff:

$$\frac{dT}{dX_2} = \frac{RT^2}{\Delta H_f}(k/k' - 1) \qquad \text{(Eq. 1)}$$

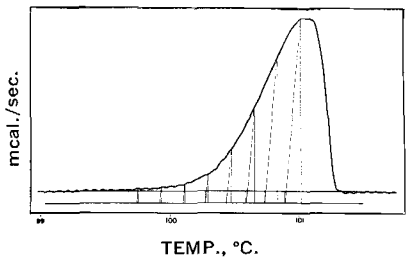

Fig. 1—Thermogram of p-*chlorophenoxyaniline; 1.74 mg.; 1 mcal./sec., full scale; scan rate, 0.625°/min.; chart speed, 4 in./min.*

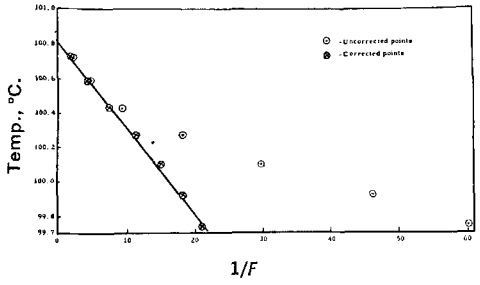

Fig. 2—Plot of T vs. *1/*F *for* p-*chlorophenoxyaniline from thermogram in Fig. 1.*

where T = temperature in °K.,
X_2 = mole fraction of minor component (solute; impurity),
ΔH_f = molar heat of fusion of the major component,
R = gas constant,
k/k' = the distribution of the solute between the solid and liquid phases.

If it is assumed that no solid solutions are formed, *i.e.*, $k/k' = 0$, then upon integration of Eq. 1, one obtains the most commonly used expression for relating mole fraction of impurity to the melting point depression:

$$X_2 = \frac{(T_0 - T_m)\ \Delta H_f}{RT_0^2} \qquad \text{(Eq. 2)}$$

where T_0 = melting point of the pure compound in °K., T_m = melting point of the sample in °K.

If there are no solid solutions formed, then the concentration of impurity in the liquid phase at any temperature during melting is given by:

$$X_2' = X_2/F \qquad \text{(Eq. 3)}$$

where X_2' = mole fraction of impurity in the liquid phase,
X_2 = mole fraction of impurity in the total sample,
F = fraction melted at temperature, T.

Since the melting point depression is directly proportional to the mole fraction of impurity, one obtains:

$$T_0 - T = 1/F\,(T_0 - T_m) \qquad \text{(Eq. 4)}$$

which predicts that a plot of the sample temperature *versus* the reciprocal of the fraction melted at temperature, T, should yield a straight line with a slope equal to $-(T_0 - T_m)$, the melting point depression and the intercept at $1/F = 0$ gives T_0, the melting point of the pure compound.

The value for ΔH_f, the molar heat of fusion, is obtained by determining the area under the melting curve which is proportional to the heat absorbed by the sample during fusion.

These three values, T_0, $T_0 - T_m$, and ΔH_f, may then be introduced into Eq. 2 to calculate the mole fraction of impurity.

RESULTS AND DISCUSSION

Although Eq. 4 predicts a linear relationship in a plot of T *versus* $1/F$, in practice, linear plots were not obtained. Neglecting deviation caused by experimental error, the curvature could result from two sources. [The use of slow scanning rates and small samples of relatively high purity makes errors negligible due to thermal resistance and deviations from "ideality" (11).] If the impurity forms a solid solution with the major component, then the concentration in the liquid phase will be less than that predicted by Eq. 3, and the corresponding equilibrium temperature will be too high; hence, the plot will be concave upward (12). Another factor, which will produce the same type curvature, is the sensitivity limit of the instrument.[1]

With respect to Fig. 1, the melting point depression, $T_0 - T_m$, is equal to 0.048° and T_0 is 100.8° C. If Eq. 4 is rearranged to:

$$F = \frac{T_0 - T_m}{T_0 - T}$$

it is readily seen that at 2° below the melting point, the sample is 2.4% melted, yet the signal at this point is indistinguishable from the base line. If the point at which the signal departs from the base line ($\approx$99.3°) is taken as the starting point for the area measurement, 3–4% of the total area will have been neglected. If the total measured area is designated A, and the partial measured areas are designated a_i, one then has $1/F_i = A/a_i$. The neglected portion could be considered negligible with respect to the total area, but it is significant with respect to the first few partial areas measured as they may be in error by as much as 50–100%. One may compensate for this by adding an adjustable parameter x to linearize the plot, which compensates for the neglected portion which was undetectable due to the sensitivity of the instrument. One then defines:

$$1/F_i = \frac{A + x}{A_i + x}$$

Figure 2 shows a plot of the data before and after correction. The correction in this case amounted to 3.4%. The value x is determined by successive approximation, and if the theory is correct, there should be but one value of x which will yield a linear plot.

However, since no *a priori* knowledge exists as to whether a solid solution is formed, it cannot be stated that the curvature is due entirely to instru-

[1] A complete discussion of the application of the theory to the detailed analysis of a melting curve obtained by a continuously scanning calorimeter was presented by Gray, A. P., in "Determination of Purity by Differential Scanning Calorimetry," presented to Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 1966. He has also treated this subject in the "Thermal Analysis Newsletter," No. 5 and 6, published by the manufacturers of this instrument.

TABLE I—PURITY AND HEAT OF FUSION OF SEVERAL COMPOUNDS DETERMINED BY DIFFERENTIAL SCANNING CALORIMETRY

Sample	Wt., mg.	ΔH, cal./Gm.	T_0, °C.	Purity, mole %
Diallylbarbituric acid[a]	4.04	31.9 ± 1	173.3	99.7_6
	1.14		173.7	99.8_4
Naphthalene[b]	3.60	35.1 (lit. 35.6)[c]	80.3	99.6_4
Glutethimide[d]	4.05	28.7 ± 1	86.8	99.1_1
	4.55		87.0	99.1_1
p-Chlorophenoxyaniline[e]	1.74	32.0	100.8	99.8_8
Dibucaine: 1[f]	2.38	20.2 ± 1	63.6	99.5_5
2[g]			63.1	98.4
Anthraquinone: 1[h]	1.19	38.8 ± 2	285.8	99.9_9
2[i]	1.55	(lit. 37.5)[c]	285.3	99.9_7
2	0.89		285.1	99.9_4
3[j]	1.71		285.6	99.9_1
4[k]	2.18		285.1	99.6_9

[a] Reference standard which analyzed 100.0% by phase solubility. [b] Recrystallized from MeOH and dried *in vacuo*. [c] "Handbook of Chemistry and Physics," 43rd ed., Chemical Rubber Publishing Co., Cleveland, Ohio. [d] Reference standard. [e] Material purified by sublimation. [f] Reference standard. [g] Production sample. [h] Zone-refined material. [i] Synthetic sample for temperature calibration. [j] Chromatographically purified. [k] Production sample—minimum 99.5% by GC.

mental insensitivity. But, because the corrected value $A + x$ gave good results for heat of fusion in those cases where there was a literature value for comparison and the calculated purities were reasonable, it was assumed that this was the main cause of the curvature.

Samples were selected which would melt without decomposition, and in most cases if there was some independent determination of their purity. The first criterion, that the compound fuse without decomposition, eliminates many compounds of pharmaceutical interest, particularly salts which either melt with decomposition or do not behave ideally.

The anthraquinone samples presented an interesting problem. The attempt was made to distinguish among four samples, all of which were at least 99.5% pure. The calculated purities and the heat of fusion are given in Table I. The results agree with what one would predict semiquantitatively, *i.e.*, the zone-refined material should be the purest and the production sample the least pure. A difficulty encountered with the anthraquinone was that it started to sublime well below its melting point, which led to sloping base lines and endotherms which were not resolvable. This was remedied when the samples were enclosed in the volatile sample sealer. Figure 3 shows two of the endotherms, sample 1 (99.9_9%) and sample 3 (99.9_1%). One can detect a visual difference in these two endotherms, yet they differed in purity by approximately 0.1%.

As a control procedure, methyl reserpate is analyzed by the solubility temperature method. The method involves the determination of the temperature at which the material goes into solution and then a comparison with a standard curve of sample purity *versus* solubility temperature. The sample purity for the working curve is determined by phase solubility. The method is tedious and subject to error. It was decided to investigate whether one could differentiate among these samples by DSC. The purity of the samples listed is that obtained by the solubility temperature method. Figure 4 shows the results of these studies at two different scanning rates. It is seen that even at the relatively fast scanning rate of 10°/min. one can

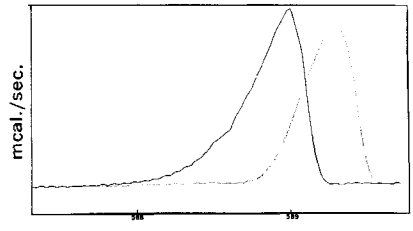

Fig. 3—Thermogram of two samples of anthraquinone; 2 mcal./sec., full scale; scan rate, 0.625°/min.; chart speed, 4 in./min. Key: ·····, zone refined; ———, chromatographically pure.

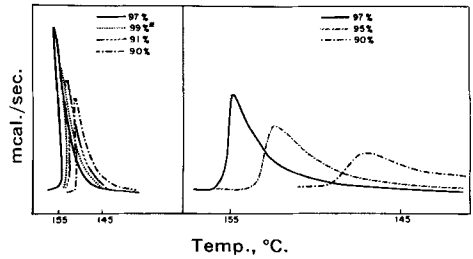

*Fig. 4—Thermograms of several samples of methyl reserpate at scan rates of 10°/min. and 2.5 min.; 8 mcal./sec., full scale; sample sizes were all about 2.5 mg. *Sample which analyzed 99% by temperature solubility method, but was later shown to have a high salt content.*

resolve the sample and is able to estimate the purity to ±1%, although it was found necessary to run at least two compounds of known purity with the unknown in addition to comparing the result to a previously determined working curve in order to compensate for any day-to-day instrumental variation. The method is therefore advantageous in those cases where an existing analytical method is particularly tedious or insensitive. To achieve reproducible results, however, in the authors'

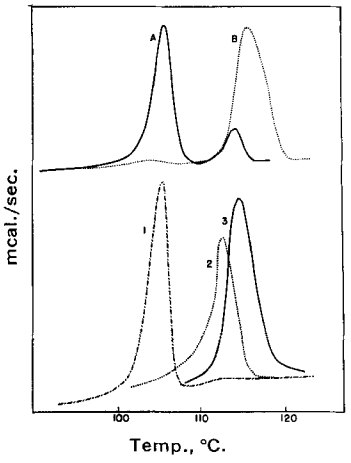

Fig. 5—Thermograms of two samples of tripelennamine citrate (A *and* B). *Curve* 1 *is form* B, *which had been recrystallized and seeded with form* A; *curve* 3 *is form A, which had been recrystallized and seeded with form* B; *curve* 2 *is form* A *after heating. Sample weights were all about 5.3 mg.; scan rate 5°/min.; 8 mcal./sec., full scale.*

opinion, a greater amount of care is necessary than one would have to utilize, for example, in making a spectrophotometric measurement.

During the investigation of various compounds by DSC, it was observed that tripelennamine citrate displayed some anomalous behavior. Two different sources of the material differed in their melting behavior, although chemical analysis showed no significant difference. The thermograms of these two samples are shown in the top half of Fig. 5. It was thought that the tripelennamine citrate existed in two different crystalline forms. The X-ray diffraction patterns and infrared spectra of the two substances also indicated this. If the behavior was caused by different crystalline modifications, then one should be able to transform one into the other. Some preliminary results of these attempts are shown in the lower half of Fig. 5. Form A was carefully recrystallized from acetone and seeded with crystals of form B. Form B was recrystallized from acetone and seeded with crystals of form A. The thermograms of the recrystallized material indicate transformation to forms B and A, respectively. It was also possible to transform A by heating it overnight at a temperature just below its melting point. The data presented above are some preliminary observations. Further investigation on the application of DSC in combination with X-ray analysis on this problem is under way.

SUMMARY

Differential scanning calorimetry provides a rapid and accurate method for purity determination. However, it is limited to those compounds which do not decompose on melting, and unfortunately, therefore, eliminates many compounds of interest. As such, it does not have as wide a range of applicability as does phase solubility analysis. There is also the possibility that the impurity may form a solid solution and thus invalidate the analysis. However, in this respect there are analogous interferences which may invalidate phase solubility analysis (6).

With a reasonable amount of care, the technique can be used to rapidly estimate the purity of a sample by comparison with samples of known purity as evidenced by the differentiation of several samples of methyl reserpate. Also, the technique can be utilized to provide evidence of polymorphic behavior which can complement or supplement spectroscopic analysis.

Differential scanning calorimetry is not the answer to all analytical problems, but it can offer evidence in many areas which is not obtainable by other methods, or it can provide evidence to support that obtained by other techniques.

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