Solid-State Stability Testing of Drugs by Isothermal Calorimetry

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A new technique has been developed to calculate rapidly the solid-state room-temperature degradation rate of drugs and drug candidates. The technique utilizes measurements of the initial rate of heat output at several elevated temperatures by isothermal calorimetry and the degradation rate of the compound determined at a single elevated temperature by chromatography. The activation energies and degradation rates at 25°C calculated by conventional methods and by isothermal calorimetry are compared and discussed. The compounds studied were phenytoin, triamterene, digoxin, tetracycline, theophylline, diltiazem, and several proprietary ICI compounds.

KEY WORDS: calorimetry; microcalorimetry; stability; kinetics, solid state; degradation.

INTRODUCTION

The solid-state degradation rate of a drug candidate during the initial stages of its development is an important consideration in determining if it can be successfully developed. Its stability needs to be determined as quickly and accurately as possible. Degradation rates as slow as 0.5%/year at 25°C may have an impact upon the development of the drug candidate. Accurately determining the degradation rate of a relatively stable drug candidate at 25°C would be too costly and time-consuming, since it may take several years for enough degradation to occur to be accurately quantified by a stability indicating assay. The conventional technique for determining the degradation rate of drug candidates typically consists of storing a sample at several elevated temperatures (to accelerate the degradation rate) and determining the undegraded fraction at selected intervals. The degradation rate at 25°C is then calculated by extrapolation of the high-temperature data using the Arrhenius equation.

There are several limitations with the conventional technique. In order for high-temperature data to predict a degradation rate accurately at 25°C, the mechanism(s) must be similar over the temperature range of the study and at 25°C. A nonlinear Arrhenius plot may be an indication of a change in mechanism(s), making the extrapolation to a degradation rate at 25°C inaccurate. Drugs that are hydrates or that have unbound water are prone to losing their water at elevated temperatures, thus the degradation mechanism at 25°C and elevated temperatures may be different.

Other limitations with the conventional technique include the amount of time necessary to complete a study and errors associated with the assay. If a compound has a degradation rate of 1.0%/year at 25°C and has an activation energy (E_a) of 18 kcal/mol, then the degradation rate at 85, 75, and 65°C would be approximately 3.14, 1.52, and 0.70%/week, respectively. It would take at least 2 weeks before one would begin to obtain useful data from the 75°C samples and at least 3 weeks for the 65°C samples. The entire study may then take up to 10 weeks. The upper limit of the degradation rate at an elevated temperature may be estimated, but without a reliable estimate of the activation energy an accurate extrapolation to a degradation rate at 25°C is impossible.

Another possible source of error is in the determination of the rate constant at each temperature from the percentage assay vs time plot. Frequently this plot has significant scatter or curvature, making the data difficult to fit. There may also be considerable scatter in the Arrhenius plot. With only three or four points (temperatures), determining the best linear fit through the data can be difficult. The further the extrapolation to 25°C, the greater the error in the estimate of the degradation rate.

Previous workers have used isothermal DSC rate–time curves to determine Arrhenius parameters and kinetics of chemical reactions (1,2). But this requires the rate-time data to be collected at several temperatures and over several reaction half-lives to determine the kinetic model accurately. Hansen *et al.* used isothermal calorimetry to determine the decomposition mechanisms of drugs (3). Their technique also required the rate of heat output to be measured over a substantial portion of the reaction and at several temperatures. Pikal has applied isothermal calorimetry to correlate the decomposition rates of cephalosporins in the solid and aqueous solution states (4).

In our work the initial rate of heat output, measured by isothermal calorimetry at several elevated temperatures, was used to calculate the activation energy of decomposition reactions of several relatively stable pharmaceutical compounds in the solid state. The activation energy and the degradation rate, determined at a single elevated temperature by HPLC, were used to calculate the degradation rate at 25°C. The activation energies and degradation rates at 25°C determined by HPLC and isothermal calorimetry are compared and discussed.

MATERIALS AND METHODS

Materials

Phenytoin, digoxin, tetracycline, theophylline, and diltiazem were obtained from Sigma Chemical Co. (St. Louis, MO). The triamterene used was obtained from Smith, Kline, and Beckman (Swedeland, PA). The purity of all the drugs used was greater than 98%. The reagents used for the preparation of the mobile phases were of HPLC grade and were used without further purification.

Isothermal Calorimetry

The calorimeter used in these studies was an LKB 2277

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Thermal Activity Monitor (TAM) which has been described previously (5). It was equipped with four channels, allowing for the rate of heat output (q) of four drugs to be measured simultaneously. The rate of heat output, which was displayed digitally, was recorded on a strip-chart recorder. Each channel of the calorimeter was independently electronically calibrated with a precision of ±0.2%. The short-term (1-hr) baseline noise of the system was ±0.05 μW at 85°C. Samples were placed in a stainless-steel ampoule with a screw cap and a Teflon seal or in a glass ampoule with a crimp top. Superior results were generally obtained with the stainless-steel ampoules. The sample and reference ampoules were sealed 18–24 hr prior to measurement, since studies performed with empty ampoules showed that steady baselines were obtained and all mechanical stress (which could give a measurable heat output) was released when the ampoules were sealed 18–24 hr prior to measurement. The reference ampoules were left empty since the sample weights were only 0.5–1.0 g. The same sample was used throughout each calorimetric study. Gloves were used for the handling of all ampoules since fingerprints can cause fallacious heat outputs. Compound 1 was known to react with O_2, thus between measurements the ampoule was opened to replenish the supply of O_2. Without the opening of the ampoule between measurements, the O_2 would become depleted in a relatively short period of time (3).

Samples were inserted into the equilibration position of the calorimeter for 1–3 hr prior to measuring the rate of heat output. Although the samples approached thermal equilibrium after about 30 min, it was necessary for the samples to spend additional time in the equilibration position for measurements at the highest sensitivity level (i.e., <0.1 μW) (5). When samples with a high moisture content were equilibrated for shorter periods of time (<2 hr), the rate of heat output fluctuated. This may be attributed to redistribution of moisture in the samples. After 1–3 hr in the equilibration position the samples were lowered into the measurement position. This caused the recorder to go off-scale for about 1 hr. Since q decreased with time, it was necessary to extrapolate back to determine its "initial" value. The initial q was chosen to be 30 min after inserting the sample into the equilibration position of the calorimeter, and all initial measurements of q were linearly extrapolated to this time. The time of 30 min was chosen since this is approximately the length of time necessary for a sample to reach the elevated temperature of the calorimeter, prior to which the sample has not started to degrade at a rate corresponding to the elevated temperature of the calorimeter (5).

The temperature range utilized (usually 40–85°C) for each study was selected such that the value of q was of a measurable magnitude. The q, which could be a positive (exothermic) or negative (endothermic) quantity, was first measured at 25°C. The actual time necessary after equilibration for the measurement of q at each temperature was only about 2 hr. The temperature of the calorimeter was then increased 5–10°C and another measurement taken, up to 85°C or until it became difficult to extrapolate linearly back to the initial value of q.

This procedure enabled one or two measurements to be made per day and an Arrhenius plot could be constructed in about 1 week. After the ampoules were loaded with sample, the actual amount of labor required is very small, since the majority of time the calorimeter is unattended while the samples are reaching thermal equilibrium.

HPLC

The drugs studied were also assayed by HPLC after storage in controlled temperature ovens. Typically, loosely covered amber glass vials containing 200–250 mg of each drug were stored at 70, 80, 90, and 100°C for 4–8 weeks. A vial was removed from each temperature station at 1- or 2-week intervals for the duration of the study and stored at 4°C. At the conclusion of the study a weighed portion of the sample in each vial was assayed by HPLC against a sample that had been stored at 4°C during the course of the study. For those samples having a significant amount of water, Karl Fischer moisture analyses were performed at the beginning and end of the study to determine if there was any change in the moisture content of the samples. Any changes in the moisture content were taken into account in the final calculations. Some studies used preweighed quantities of drug (10–20 mg) in 25-ml volumetric flasks at each temperature station, thus it was not necessary to perform Karl Fischer moisture analyses.

All HPLC assays used in this study were stability indicating. Typically, the HPLC columns used were 25 cm × 4.6-mm I.D. packed with 5-μm ODS. The mobile phases used were a mixture methanol or acetonitrile with an appropriate aqueous buffer. The tetracycline samples were analyzed by an HPLC method adapted from Dihuidi *et al.* (6).

THEORY

An estimate of the degradation rate at 25°C can be obtained by plotting the initial rate constant, k, obtained at each temperature in Arrhenius fashion [Eq. (1)] and extrapolating back to 25°C.

$$k = Ae^{-E_a/RT} \tag{1}$$

In Eq. (1), A is the preexponential factor, E_a is the activation energy, R is the gas constant, and T is the absolute temperature. It has been shown previously that for a zero-order rate law,

$$q/D_0 = -\Delta H\beta k = C = \text{constant} \tag{2}$$

where q is the rate of heat output, D_0 is the initial amount of drug present, ΔH is the enthalpy change for the reaction, and β is the reactive portion of the sample (3). If we assume that $-\Delta H$ and β are not a function of temperature over the range of the experiment, then the rate constant, k, is directly proportional to the initial rate of heat output, q_0. Thus,

$$q_0 = Ck \tag{3}$$

Substituting Eq. (3) into Eq. (1) and expressing the result in logarithmic form results in Eq. (4).

$$\ln q_0 = \ln C - E_a/(RT) \tag{4}$$

If q_0 is measured at several temperatures the slope of a plot of $\ln q_0$ vs $1/T$ will be equal to $-E_a/R$ (3); assuming that a zero-order rate law adequately describes the kinetics during the initial portion of the reaction. Putting Eq. (1) in its log-

arithmic form, differentiating with respect to temperature, integrating between limits, and solving for k_1 result in Eq. (5).

$$k_1 = k_2/\left(\exp\{E_a(T_2 - T_1)/[(T_2T_1)R]\}\right) \quad (5)$$

The activation energy, determined by isothermal calorimetry, and the degradation rate (k_2), determined by HPLC of a sample that has been stored at temperature T_2, can be used to estimate the degradation rate at 25°C (k_1) using Eq. (5). It has been our observation that the estimation of the value of E_a is the most critical parameter when extrapolating to the degradation rate at 25°C from elevated temperature data. The E_a determined from elevated-temperature data may actually be the weighted mean of several activation energies from simultaneously occurring reactions in the solid state.

RESULTS

Arrhenius plots of the calorimetric and HPLC data for Compounds 1 (mesylate and HCl salts), 2, and 3 and digoxin are shown in Figs. 1–5. The degradation rates at 25°C and activation energies for the compounds studied are given in Table I. The k_1 values were calculated using Eq. (5) and values for E_a and k_2, which were determined by calorimetry and a single HPLC assay of a sample after storage at an elevated temperature, respectively. These rates can be compared with those calculated from the HPLC data only (Table I).

The mesylate and hydrochloride salts of Compound 1 were studied by calorimetry and HPLC to determine which had greater stability at 25°C. Prior to the determination of the degradation rate by HPLC, it was known that the drug decomposed via reaction with O_2. The amount of surface area exposed to air was uncontrolled. This resulted in significant scatter in the Arrhenius plots of the HPLC data. The calorimetric technique probably more accurately reflects the conditions seen during bulk storage in which the surface area exposed to air is relatively small.

Two separate studies with Compound 4 (a trihydrate) determined the effect of recrystallization solvent and formulation with sodium carbonate on the activation energy using calorimetry. In the first study Compound 4 was recrystallized in either acetone or tetrahydrofuran (THF), and its initial rate of heat output measured as a function of temperature. The second study looked at the effect of formulating the acetone recrystallized compound with sodium carbonate at a molar ratio of 1.00:0.75 (drug:sodium carbonate). The results are given in Table I. The compound did not degrade at a sufficient rate to allow its E_a to determined by HPLC.

The degradation rate for tetracycline was too slow to be measured at temperatures <100°C over a period of 4 months by monitoring the disappearance of parent using HPLC. Thus, the rate of appearance of degradation products was monitored by HPLC and was assumed to equal the rate of disappearance of parent. After storing the sample for 4 weeks at 100°C the sum of the areas of parent and degradation peaks equaled the initial area to the tetracycline peak, lending validity to the above assumption.

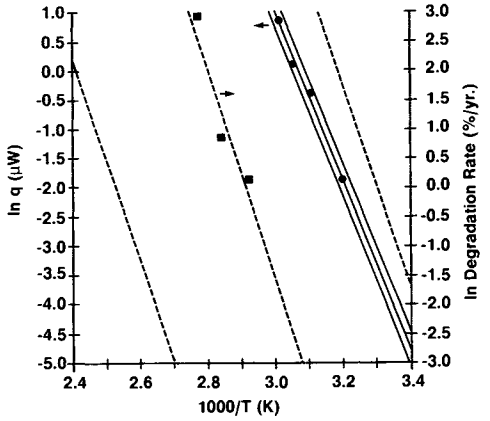

Fig. 2. An Arrhenius plot of the HPLC and microcalorimetry data for Compound 3 and the associated 95% confidence bands. (●) Microcal data, (——) $-14.13X + 43.2$; (■) HPLC data, (– – – –) $-17.29X + 50.3$.

DISCUSSION

The aim of this work was to develop a technique to

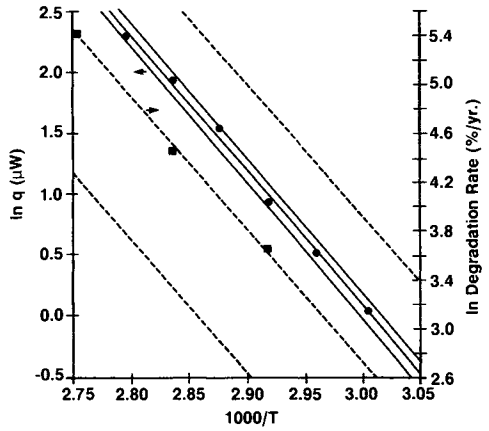

Fig. 1. An Arrhenius plot of the HPLC and isothermal calorimetry data for Compound 2 and the associated 95% confidence bands. (●) Microcal data, (——) $-11.0X + 33.2$; (■) HPLC data, (– – – –) $-10.8X + 35.1$.

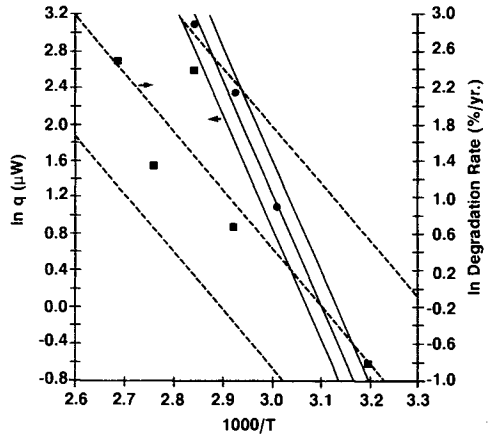

Fig. 3. An Arrhenius plot of the HPLC and isothermal calorimetry data for Compound 1 mesylate salt and the associated 95% confidence bands. (●) Microcal data, (——) $-11.9X + 36.8$; (■) HPLC data, (– – – –) $-6.25X + 19.2$.

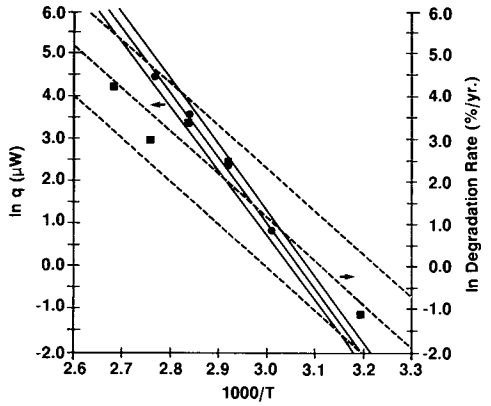

Fig. 4. An Arrhenius plot of the HPLC and isothermal calorimetry data for Compound 1 HCl salt and the associated 95% confidence bands. (●) Microcal data, (——) $-15.1X + 46.3$; (■) HPLC data, (– – – –) $-10.0X + 31.3$.

estimate quickly and accurately the solid-state room-temperature (25°C) degradation rate of drug candidates. For some compounds studied the degradation rate could not be accurately estimated. In these cases the upper limit to the degradation rate was estimated. Determination of the degradation mechanism and the associated rate law of drug candidates can be very-time consuming and is frequently unnecessary during the initial stages of a drugs development. An in-depth interpretation of the degradation mechanisms of the compounds used in this study was not attempted.

A zero-order rate law was assumed during the initial portion of the degradation reaction for all the compounds studied. This assumption was necessary, since for most of the compounds studied, only a very small portion of the q–t curve was acquired. The distinction between a zero- and a first-order rate law is difficult during the initial portion (first 15%) of a degradation reaction, since in general, when $x < 0.15$

$$\ln (1 - x) \sim -x \qquad (6)$$

where x is the reacted fraction (1). The assumption of an incorrect rate law may lead to an inaccurate calculation of the activation energy and the degradation rate at 25°C. The magnitudes of the Arrhenius parameters for a particular reaction are not very sensitive to the kinetic model used in the derivation of the rate coefficients (7). Thus, the assumption of an incorrect rate law would not necessarily invalidate the results.

Another inherent danger with using only initial q values to calculate the activation energy is the possibility of measuring the wrong thermal event. This may be indicated when there is significant curvature in the Arrhenius plot.

The entire q–t curve was determined only for Compound 2. Initially, the curve linearly approached zero, but as the reaction progressed the curve approached zero in an asymptotic fashion.

The main inconsistency in this study was the correlation between the activation energies obtained by calorimetry and those obtained by HPLC. The poor correlation was especially apparent in the studies with Compound 1 (mesylate and HCl salts) and digoxin. It is not surprising that the results of the studies with Compound 1 do not agree, since the compound is known to react with O_2 and the samples for the HPLC and calorimetric studies were handled so differently. For the mesylate and HCl salts of Compound 1 the calculated value for the degradation rate at 25°C was an upper-limit estimate based on the degradation rate determined by HPLC at 80°C. The reason for the poor correlation of the digoxin results is not presently known, but it may be because only three data points were used in determining the E_a from the Arrhenius plot of the HPLC data. A small error in one of the points can significantly affect the estimate of the activation energy.

Several of the compounds studied degraded too slowly to allow their degradation rate to be determined by HPLC, thus the activation energy and a degradation rate at 25°C could not be calculated from the HPLC data alone. In these cases, an upper-limit estimate of the degradation rate (26%/year at 100°C) and a lower estimate of the activation energy (16.9 kcal/mol) were used to calculate the degradation rate at 25°C. Ideally, sufficient degradation should occur to allow Arrhenius plots to be constructed from both the HPLC and the calorimetric data. Agreement in the slopes of the two Arrhenius plots would increase the possibility that the HPLC and calorimeter data were measuring the same degradation reaction.

The degradation rate for tetracycline determined by Dihuidi *et al.* was 3.8%/year at 70°C (6). This is significantly greater than the rate measured in our laboratory and may be due to a higher level of impurities present in their sample of tetracycline (6%, vs <1% for our sample). The greater level of impurities in their sample may have altered the crystal structure of the tetracycline making it more susceptible to thermal degradation.

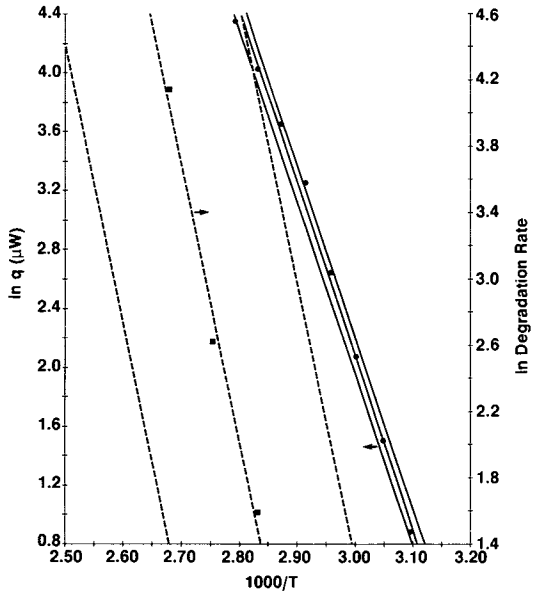

Fig. 5. An Arrhenius plot of the HPLC and isothermal calorimetry data for digoxin and the associated 95% confidence bands. (●) Microcal data, (——) $-11.7X + 37.1$; (■) HPLC data, (– – – –) $-16.8X + 49.0$.

Table I. Activation Energies and Degradation Rates Determined by Isothermal Calorimetry and HPLC for the Compounds Used in this Study

Compound technique	Activation energy, E_a (kcal/mol)		Degradation rate (k_1) at 25°C (%/year)	
	Calorimetry	HPLC	Calorimetry[a]	HPLC
Compound 1, HCl	30.0 ± 1.6[b]	19.9 ± 2.9	0.012	0.088
Compound 1, Mesylate	23.6 ± 3.0	12.4 ± 3.3	0.022	0.17
Compound 2	21.9 ± 0.7	21.4 ± 1.7	0.21	0.20[c]
Compound 3	28.1 ± 1.2	34.4 ± 10.0	0.0022	0.00044
Compound 4				
Acetone recrystallized	18.2 ± 1.2	ND[d]	0.0006	ND
THF recrystallized	23.6 ± 1.2	ND	0.0006	ND
Formulated with Na_2CO_3	29.5 ± 0.7	ND	0.0006	ND
Tetracycline	29.4 ± 2.7	25.7 ± 2.8	0.0011	0.0024
Digoxin	23.2 ± 0.8	33.3 ± 4.2	0.040	0.00068
Phenytoin	19.0 ± 1.4	ND	0.0006	ND
Triamterene	20.6 ± 1.3	ND	0.0006	ND
Diltiazem	24.8 ± 0.8	ND	0.0006	ND
Theophylline	17.9 ± 1.0	ND	0.0006	ND

[a] Calculated using Eq. (5).
[b] The ± values represent 1 SD.
[c] The determination of the HPLC degradation rate at 25°C for Compound 2 utilized samples stored at 25°C and assayed over a 6-month period.
[d] Not determined. The degradation rate was too slow to measure by HPLC.

Phenytoin triamterene, diltiazem, and theophylline were studied by both HPLC and isothermal calorimetry. Insufficient degradation was observed after several weeks at elevated temperatures (90°C) to determine a degradation rate by HPLC alone. The upper limit for the degradation rate at 25°C for these drugs was estimated to be ≤0.0006%/year, using values of 16.9 kcal/mol for the E_a (a lower limit estimate) and 26%/year at 100°C for k_2 (an upper-limit estimate). Arrhenius plots for phenytoin and triamterene are shown in Fig. 6.

Two studies with Compound 4 determined the effect of recrystallization solvent (acetone or THF) and formulation with sodium carbonate after recrystallization in acetone on the activation energy. The activation energy of the degradation reaction of the THF recrystallized compound was slightly greater than that for the acetone recrystallized compound (Table I). The significance of this difference is being studied.

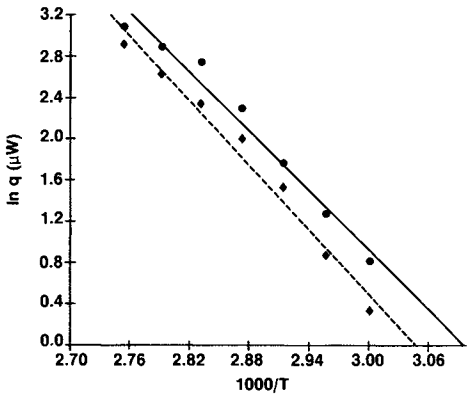

Fig. 6. Arrhenius plot of the isothermal calorimetry data for phenytoin and triamterene. (●) Phenytoin, (——) $-9.95X + 29.6$; (◆) triamterene, (– – – –) $-10.4X + 31.7$.

CONCLUSION

The applicability of isothermal calorimetry to the determination of room-temperature degradation rates and activation energies of several pharmaceutical compounds has been demonstrated. The technique utilizes measurements of the rate of heat output at several elevated temperatures by isothermal calorimetry and the degradation rate of the compound determined at a single temperature by HPLC. In cases where the degradation rate at an elevated temperature cannot be determined by HPLC, an upper-limit estimate of the degradation rate at an elevated temperature can be used, with the activation energy determined by calorimetry to estimate the degradation rate at 25°C.

For all the compounds studied, the activation energies determined by isothermal calorimetry were more precise than those determined by conventional HPLC assay techniques. Isothermal calorimetry can provide a useful check on the results of conventional techniques used to determine solid-state degradation rates. Isothermal calorimetry has also been shown to be applicable to the determination of the activation energies of the degradation reactions of pharmaceutical compounds recrystallized from different solvents and for excipient compatibility studies.

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REFERENCES

1. J. T. Carstensen. Stability of solids and dosage forms. *J. Pharm. Sci.* **63**:1–14 (1974).
2. D. N. Waters and J. L. Paddy. Equations for isothermal differential scanning calorimetric curves. *Anal. Chem.* **60**:53–57 (1988).
3. L. D. Hansen, E. A. Lewis, D. J. Eatough, R. G. Bergstrom, and D. DeGraft-Johnson. Kinetics of drug decomposition by heat conduction calorimetry. *Pharm. Res.* **6**:20–27 (1989).
4. M. J. Pikal and K. M. Dellerman. Stability testing of pharmaceuticals by high-sensitivity isothermal calorimetry at 25°C: Cephalosporins in the solid and aqueous solution states. *Int. J. Pharm.* **50**:233–252 (1989).
5. J. Suurkuusk and I. Wadso. A multichannel microcalorimetry system. *Chem. Scripta* **20**:155–163 (1982).
6. D. Dihuidi, E. Roots, J. Hoogmartens, and H. Vanderhaeghe. Influence of temperature on the stability of solid tetracycline hydrochloride, measured by high-performance liquid chromatography. *J. Chromatogr.* **246**:350–355 (1982).
7. M. E. Brown and A. K. Galwey. Arrhenius parameters for solid-state reactions from isothermal rate-time curves. *Anal. Chem.* **61**:1136–1139 (1989).