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Stability testing of pharmaceuticals by high-sensitivity isothermal calorimetry at 25 °C: cephalosporins in the solid and aqueous solution states *

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Summary

Present methodology for preliminary stability testing requires high temperatures and therefore suffers from the uncertainty of extrapolation of results to the temperature of interest. Clearly, a rapid method for estimating chemical decomposition rates at the temperature of interest would be of great utility. The recent introduction of a high-sensitivity isothermal calorimeter, the LKB 2277 thermal activity monitor (TAM), allows measurement of the rate of heat production, or thermal activity, from a sample (i.e., arising from a chemical reaction) with a sensitivity of about 10^4 greater than is possible with a conventional differential scanning calorimeter. Since the power is equal to the product of the reaction rate and the heat of reaction, ΔH_r, the sensitivity of a calorimetric method depends on the magnitude of ΔH_r. From TAM and chemical assay data on the same samples, ΔH_r data were evaluated for a number of cephalosporins in aqueous solution and in various solid forms (i.e., crystalline forms and the amorphous form at selected water contents). Heats of reaction are exothermic and large in magnitude, several hundred kJ/mol for the solids, yielding a sensitivity to detect decomposition rates as low as $\approx 1\%$/year with an overnight experiment. For crystalline solids and amorphous samples of low to moderate moisture content, ΔH_r is roughly independent of temperature, water content, and polymorphic form. High-moisture amorphous solids and aqueous solutions of all concentrations have a heat of reaction about a factor of 5 less than the corresponding "dry" samples. However, variations in ΔH_r for a series of samples, including solutions, is less than the variation in chemical stability. Consequently, decomposition rate correlates well with thermal activity for a given compound, and TAM data are generally a valid measure of chemical stability. Exceptions to this generalization may arise when thermal activity from physical changes dominate, and if systems which undergo parallel endothermic and exothermic reactions are studied. The stability studies suggest two generalizations of particular interest. (1) The decomposition rates of amorphous cephalosporins increase with increasing water content in highly non-linear fashion, the rates increasing sharply as the water content increases beyond "intermediate" levels. (2) Stability in a series of crystalline pseudo-polymorphs is found to be quantitatively related to the heat of crystallization, and a theoretical model is proposed to interpret this observation.

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Introduction

High temperature (accelerated) stability studies of drugs are normally carried out to guide optimization of the formulation and the manufacturing process. However, pseudo rate constants for pharmaceutical solids often show non-Arrhenius temperature dependence, resulting in significant error in the estimated rate constant at the temperature of practical interest. Important causes of non-Arrhenius behavior for solids are: phase transitions, moisture redistribution between drug and excipients (Zografi et al., 1988), and operational definition of rate constants which are actually combinations of fundamental rate constants (Perlmutter-Hayman, 1976). Moreover, since the dominant reaction at high temperatures may not be the dominant reaction at low temperature, studies of the effect of formulation and process variables at high temperature may not extrapolate even qualitatively to the temperature of interest.

Methodology for accelerated stability testing normally involves chemical assay of samples stored at high temperatures for appropriate time periods. Motivated largely by the desire to increase sample throughput, thermal analysis methods, particularly differential scanning calorimetry (DSC), have been applied in studies of the decomposition kinetics of explosives (Kishore, 1978; Beckmann et al., 1977) and in stability studies of pharmaceutical solids (Po, 1986; Chrzanowski et al., 1986; Signoretti et al., 1986; Hardy, 1982; Smith, 1982). However, sensitivity limitations demand high temperatures in both scanning mode and isothermal mode. In principle, the isothermal mode has the potential to provide data at more realistic temperatures. In isothermal operation, deviation of the sample signal (W) from baseline is the rate of heat production by the sample (dQ/dt) and is proportional to the reaction rate at that temperature (dn/dt), where n is number of moles of parent compound, with the constant of proportionality being the heat of reaction (ΔH_r),

$$dQ/dt = \Delta H_r \, dn/dt \qquad (1)$$

The heat of reaction is not normally known and may be evaluated by integration of dQ/dt over the course of the experiment, provided the sample decomposes completely during the experiment. Thus, extremely high temperatures are required. In principle, one could run at a more moderate temperature, without decomposing the sample greatly, and be content to compare the thermal activities (dQ/dt) for a series of samples. Assuming that the heats of reaction do not vary greatly among the samples studied, this procedure would yield a comparison of reaction rates (Eqn. 1). However, reproducibility of the baseline limits the sensitivity of the measurement to roughly ± 50 μW for a common DSC unit [1]. With this sensitivity and the small sample size ($\leqslant 30$ mg), high temperatures are required to generate reproducible data.

The recent availability of commercial high sensitivity isothermal calorimeters [2] has dramatically increased the potential of calorimetric stability studies. With a sensitivity of ≈ 0.1 μW and a sample capacity of several grams, such units have more than 4 orders of magnitude greater effective sensitivity than a conventional DSC [1]. Thus, assuming a heat of reaction in the tens of kJ/mol, such instrumentation is capable, in principle, of comparative stability studies on relatively stable materials at room temperature. High sensitivity isothermal calorimetry has found application in shelf-life stability estimation for explosives (Elmqvist et al., 1983), and a brief report from this laboratory (Pikal, 1983) suggests that this calorimetric approach would be useful in pharmaceutical stability studies.

The general objective of this study is to provide a more definitive test of the utility of high sensitivity isothermal calorimetry in the estimation of the stability of pharmaceutical systems, particularly solids. Three questions assume particular importance. (1) Does the calorimeter have sufficient sensitivity to quantify the thermal activity arising from decomposition of relatively stable pharmaceuticals at 25 °C? Alternately, what is the effective heat of reaction for decomposition reac-

[1] Taken from specifications for base line reproducibility for the Perkin-Elmer DSC-2.

[2] Specification for the LKB-2277 "Thermal Activity Monitor".

tions of pharmaceutical interest? (2) To what extent does thermal activity arising from physical changes interfere with estimation of chemical stability? And (3) is the effective heat of reaction constant for a series of samples being studied? If not, is the heat of reaction at least "well behaved" in the sense that variations in heat of reaction are significantly less than variations in chemical stability, thus allowing isothermal calorimetry to provide a useful measure of stability?

For this study, cephalosporins in the amorphous state, in the crystalline state, and in aqueous solution were studied as a function of water content at 25 °C and 40 °C. Effective heats of reaction were determined by monitoring both the chemical purity (chemical assay), and the thermal activity as a function of time for the same batch of material. This procedure circumvents the uncertainty caused by batch-to-batch variation in chemical stability often found with solids. The correlation between initial decomposition rate and initial thermal activity was examined, and variations in the effective heat of reaction were studied.

Materials and Methods

Materials

The cephalosporins were obtained as chemical intermediates (Eli Lilly and Co.). The crystalline samples were used as received while the amorphous samples were prepared by freeze-drying aqueous solutions. Moxalactam was obtained as the free acid, neutralized with sodium bicarbonate, and then freeze-dried from about a 25% aqueous solution as the disodium salt. Amorphous ceftazidime was prepared by freeze drying a nearly saturated aqueous solution of the free acid. Cefaclor was freeze dried from a nearly saturated aqueous solution. Cephalothin sodium and cefamandole sodium were freeze-dried from about 25% aqueous solutions. Primary drying, or ice sublimation, was carried out well below the respective collapse temperatures, and secondary drying was carried out at temperatures in the range 40–50 °C. Freeze-dried samples directly from the freeze dryer usually contained about 0.5% water. Crystalline ceftazidime was provided as the pentahydrate. The non-stoichiometric hydrate was prepared from the pentahydrate by vacuum drying, followed by exposure to ambient humidity. Amorphous samples of all the cephalosporins and the non-stoichiometric hydrate of ceftazidime were adjusted to selected moisture levels by either equilibration in a desiccator containing an appropriate saturated salt solution or by vacuum equilibration in a freeze dryer at elevated condenser temperature and subambient shelf temperature (to provide a controlled relative humidity in the drying chamber). Water contents were determined by Karl Fischer titration. All solids were stored at −20 °C until use. Initial purity was greater than 90% except for amorphous ceftazidime (> 80%) and high moisture samples of amorphous cefaclor (> 80%). The methanol used in the HPLC assays was Mallinckrodt HPLC grade, and all other materials were reagent grade.

Assays

Moxalactam and ceftazidime were assayed by the Lilly Analytical Development Division using HPLC methodology while the other cephalosporins were assayed by HPLC procedures in this laboratory using a Dupont Zorbax ODS C18column (4.6 mm × 25 cm.; 5 μm). The mobile phase was 30% methanol and 70% aqueous acetate buffer at pH 3.8. Retention times were in the 3–8 min range for flow rates of ca. 1 ml/min. For each of the above cephalosporins, the reference standard was crystalline material (from Eli Lilly and Co) at the onset of each study. The standards were stored at −20 °C for the duration of the study. The reference material was assigned a purity of 100%. Thus, the purities determined by this procedure are relative to the purity of the standards, which have absolute purities in the high 90% range. As relative purity is sufficient for our use, no attempt was made to place the assay results on an absolute basis. The assay precision is normally better than ±1%. All stability samples were run in duplicate.

Calorimetry

High sensitivity isothermal calorimetry

The system used in these studies was the LKB-277 Thermal Activity Monitor (TAM). The unit

consists of 4 independent differential heat flow microcalorimeters. Calibration was performed electrically with a precision of about ±0.2% and an absolute accuracy of about ±5% in the power (μW).

The use of the instrument in this research used the "ampoule mode" where the sample was loaded into either a stainless-steel cylinder (4 ml) or a rubber-stoppered glass vial (3 ml). The sample and reference containers were lowered to a thermal equilibrium position in the calorimeter and allowed to equilibrate with the calorimeter for about 15 min. The sample and reference were then slowly lowered into the measuring zone of the calorimeter. The thermal excursions dissipated within 30–60 min, leaving the signal representing the thermal activity of the sample. Stopper effects may cause spurious thermal activity on the order of ±0.5 μW, so the stainless-steel containers should be used whenever the highest sensitivity (±0.1 μW) is required. Our practice was to use the stainless-steel containers for solid samples and the glass vials for solutions or materials that might corrode the steel (i.e., cefaclor, as HCl is evolved during decomposition). Solution loading was 2 ml for studies at 25 °C and 1 ml for studies at 40 °C. Loading of solids was variable depending on powder density but was normally about 1 g. Pure water of the same volume as the sample was used as reference in the solution studies while glycine of roughly the same mass as the sample was used as reference for the solid samples. The reference must, of course be thermally inert. In addition, to minimize noise, the heat capacity should approximately match that of the sample. In practice, the heat capacity match is not critical.

Output of the thermal activity was both digital and via strip chart recorders. Recorder output allows one an easy visual record of the thermal equilibration between sample and calorimeter. Although thermal equilibrium was established within 30–60 min, hygroscopic solids commonly showed a moderate (i.e., 1–2 μW/g) decrease in thermal activity for 5–10 h before the output became "steady". In this context, "steady" means a thermal activity that is essentially independent of time over a period of several hours. Preliminary experiments suggested this time depenedence was due to redistribution of traces of moisture sorbed by the sample during loading.

For studies of solution stability, an aliquot of the solution was loaded into the calorimeter immediately after solution preparation. The solution samples were left in the calorimeter, with output being recorded, for the duration of the study. Assays were run immediately to generate the initial value and at selected times (normally 6) during the study. For solid stability studies, aliquots of the solid were loaded into the sample containers, usually stainless steel, and lowered into the calorimeter. To circumvent the moisture redistribution effect, solid samples normally were allowed to remain in the calorimeter for about 18 h (i.e., until the next morning). The solid was assayed for purity on the same day. This procedure was repeated at selected times to generate thermal activity and corresponding purity data at discrete time points (normally 6) during the course of the stability study. All studies, solution and solid, were done in duplicate. Agreement in thermal output per gram of solid was normally within ±10% or ±0.2 μW/g, whichever is larger.

For hygroscopic samples, the loading operation was carried out in a dry bag continuously purged with dry air to minimize moisture sorption during loading Powder must be kept off the threads of the stainless-steel cap of the sample container because interaction with ambient humidity in the calorimeter may produce spurious thermal effects.

Early runs with dilute solutions (≈ 1% solute) at 40 °C showed a sharp increase in thermal activity during the first few hours in the calorimeter. This effect was never observed at 25 °C or with concentrated solutions (i.e., those which produce high thermal activities). The effect was traced to our practice of using the same references sample with a "well aged" stopper for a series of measurements, and of course, using a fresh stopper for the sample each time a new sample was run. The anomalous effect was essentially eliminated if fresh stoppers were used for both sample and reference. Early runs were then corrected for the stopper effect using data from blank runs as long as the magnitude of the correction was moderate. A blank run employed vials filled with water, using a fresh stopper on the sample vial and a well aged stopper

on the reference vial. The blank showed a strong endothermic response which dissipated with time over 24 h. In cases where the blank correction was large, the systems were re-run using fresh stoppers for both sample and reference, and all subsequent experiments with solutions used fresh stoppers for both sample and reference vials. Since it is unlikely that even fresh stoppers are perfectly matched, early time data for dilute solutions may be subject to small residual "stopper effect" artifacts.

Solutions frequently exhibited some time dependence in thermal activity early in the experiment which was clearly not related to the "stopper effect" described above. The thermal activity either increased or decreased, depending on the compound and temperature, and occurred in concentrated solutions as well as in dilute solutions. The origin of the phenomenon remains obscure. However, even when early time drift in thermal activity was observed, the effect dissipated within 3 h (40 °C) or 6 h (25 °C).

Heat of solution measurements

Heat of solution measurements were conducted with a simple batch calorimeter as described previously (Pikal, et al., 1978).

Results

Thermal activity and chemical stability data for cephalothin sodium (Table 1), cefamandole sodium (Table 2), cefaclor (Table 3), ceftazidime (Table 4), and aqueous solutions of cefazolin sodium and cephalexin (Table 5) are summarized with the

TABLE 1

Thermal activity and chemical reactivity of cephalothin sodium

Substance and temperature	% H_2O	A_0 (μW/g)	R_0 (%/mol) [a]	$\langle A \rangle$ (μW/g)	ΔP (%) [τ]	ΔH_r (kJ/mol)
Amorphous solid at 25 °C	0.2	31.9	5.0 ± 2.0	13.3	12.3 [6 mo]	700
	1.2	17.0	5.1 ± 1.8	9.3	11.5 [6 mo]	520
	2.8	16.4	3.6 ± 1.1	6.1	10.6 [6 mo]	370
	3.8	25.4	3.7 ± 1.1	5.9	10.2 [6 mo]	380
	7.4	4.5	– [b]	3.7	8.2 [1 mo]	49
	18.6	11.8	– [b]	4.0	11.7 [1 mo]	37
Amorphous solid at 40 °C	0.2	(87.6) [c]	22.5 ± 5.2	20.9	29.0 [6 mo]	470
	0.8	(75.6) [c]	21.9 ± 5.3	18.7	28.6 [6 mo]	430
	1.4	65.2	6.3 ± 0.9	13.4	37.9 [8 mo]	310
	3.2	52.3	12.4 ± 0.5	15.6	52.3 [6 mo]	190
	4.3	84.8	35.1 ± 3.0	19.2	76.6 [6 mo]	160
Anhydrous crystalline solid at 25 °C	0.1	−0.1	0.0	0.04	0.0 [18 mo]	–
Aqueous solutions at 25 °C	79.6	144	340 ± 9	147	28.8 [3 d]	55
	86.6	156	290 ±12	162	25.5 [3 d]	69
	97.9	219	380 ±15	195	31.9 [3 d]	66
Aqueous solutions at 40 °C	78.5	815	2420 ±33	916	55.3 [1 d]	60
	89.2	1055	2760 ±45	1022	60.2 [1 d]	61
	99.1	1242	2790 ±15	1137	60.5 [1 d]	68

[a] Aqueous solutions and some amorphous solids studied at 40 °C (% H_2O = 1.4%, 3.2%, and 4.3%) showed first-order decomposition kinetics, R_0 data for all other samples were evaluated from the 3-parameter kinetic equation (Eqn. 2, $k \neq 0$).

[b] Samples crystallized during the study, and because of this complication the decomposition kinetics could not be represented by Eqn. 2. ΔP was obtained by graphical smoothing.

[c] Not "steady" values. See text for details.

TABLE 2

Thermal activity and chemical reactivity of cefamandole sodium

Substance and temperature	% H_2O	A_0 (μW/g)	R_0 (%/mo) [a]	$\langle A \rangle$ (μW/g)	ΔP (%) [τ]	ΔH_r (kJ/mol)
Amorphous solids	0.4	1.3	0.6 ± 0.1	1.2	3.4 [6 mo]	270
at 25 °C	0.8	2.2	0.6 ± 0.1	2.1	3.5 [6 mo]	450
	1.2	3.3	2.2 ± 0.8	1.9	3.7 [6 mo]	390
	2.9	4.2	2.1 ± 0.2	1.6	5.2 [6 mo]	230
	8.0	16.3	43 ± 9	11.6	18.6 [1 mo]	78
	13.0	14.7	41 ±19	10.2	23.7 [1 mo]	54
Amorphous solids	0.4	(10.4) [b]	1.1 ± 0.1	2.4	6.7 [6 mo]	270
at 40 °C	2.1	(20.8) [b]	2.3 ± 0.1	3.8	12.6 [6 mo]	230
	10.0	106.3	126 ±13	70.7	46.5 [0.5 mo]	95
	11.8	107.2	116 ±10	76.3	43.6 [0.5 mo]	110
Aqueous solutions	36.9	36.9	78 ±12	34.3	7.6 [3 d]	57
at 25 °C	75.3	44.6	48 ± 3	38.8	4.7 [3 d]	104
	88.0	34.2	45 ± 6	28.7	5.8 [4 d]	83
	99.1	42.6	90 ± 6	38.8	11.2 [4 d]	58
Aqueous solutions	43.2	242	390 ±36	224	12.2 [1 d]	77
at 40 °C	80.3	268	370 ±48	234	11.7 [1 d]	84
	89.7	236	380 ±15	222	11.9 [1 d]	78

[a] At 25 °C, the solid samples having a water content of 1.2% or above required the 3-parameter kinetic equation (Eqn. 2, $k \neq 0$) to represent the purity vs time data. All other stability runs obeyed first order kinetics.
[b] Not "steady" values. See text for details.

TABLE 3

Thermal activity and chemical reactivity of cefaclor

Substance and temperature	% H_2O	A_0 (μW/g)	R_0 (%/mo) [a]	$\langle A \rangle$ (μW/g)	ΔP (%) [τ]	ΔH_r (kJ/mol)
Amorphous solids	0.4	17.9	1.0 ± 0.1	8.1	5.7 [6 mo]	810
at 25 °C	1.1	17.4	1.1 ± 0.1	8.4	6.5 [6 mo]	740
	1.8	23.5	1.4 ± 0.2	8.4	8.2 [6 mo]	580
	5.5	30.9	2.4 ± 0.1	7.8	13.6 [6 mo]	330
	9.0	36.4	3.2 ± 0.8	17.0	3.1 [1 mo]	520
	12.0	80.2	19.0 ± 2.3	36.3	11.4 [1 mo]	300
Amorphous solids	0.5	43.0	– [b]	13.3	14.4 [6 mo]	530
at 40 °C	1.4	48.5	– [b]	18.7	12.7 [6 mo]	840
	8.2	139.8	58 ±12	78.7	15.3 [0.46 mo]	230
	9.1	179.3	85 ±15	129.3	17.7 [0.46 mo]	320
Crystalline pseudo-monohydrate at 25 °C	5.4	0.8	0.14 ± 0.1	0.7	2.4 [18 mo]	500
Aqueous solution at 25 °C	98.8	89.0	66 ± 6	119	6.4 [3 d]	180
Aqueous solution at 40 °C	98.8	857	360 ±90	927	11.3 [1 d]	260

[a] The amorphous sample with 12% water and all solid samples run at 40 °C required the 3-parameter kinetic equation (Eqn. 2, $k \neq 0$) to represent the purity vs time data. All other stability runs obeyed first order kinetics.
[b] Due to a sharp drop in purity between time zero and the first time point, the initial rate could not be obtained with acceptable accuracy.

TABLE 4

Thermal activity and chemical reactivity of ceftazidime

Substance and temperature	% H_2O	A_0 (μW/g)	R_0 (%/mo) [a]	$\langle A \rangle$ (μW/g)	ΔP (%) [τ]	ΔH_r (kJ/mol)
Amorphous solids	1.3	19.4	53	20	18.2 [3 mo]	460
at 25 °C	6.7	58.0	51	13.1 [b]	37.3 [3 mo]	150
	12.2	73.1	100	16.5 [b]	66.1 [3 mo]	110
Amorphous solids	1.3	25.4	61	21.8	38.0 [1.5 mo]	120
at 40 °C	6.7	93.5	175	50.8 [b]	58.1 [1.5 mo]	180
	12.2	194	484	– [c]	79.4 [1.5 mo]	–
Crystalline non-stoichiometric	0.8	2.6	1.2	1.4	3.4 [3 mo]	170
	2.9	4.8	4.4	2.9	5.8 [2 mo]	140
hydrate at 25 °C	5.4	5.9	3.7	2.6	5.8 [3 mo]	190
Crystalline non-stoichiometric hydrate at 40 °C	0.8	9.2	12.0	3.9	11.7 [3 mo]	140
Crystalline penta-hydrate at 25 °C	14.2	0.47	0.3	0.3 [b]	0.8 [3 mo]	– [d]
Crystalline penta-hydrate at 40 °C	14.2	1.8	0.0	−0.4 [b]	0.0 [3 mo]	– [d]

[a] The decomposition kinetics at 25 °C for the non-stoichiometric hydrate with 0.8% water is consistent with first order kinetics. First order kinetics were assumed for the minimal decomposition shown by the pentahydrate. All other samples required the three parameter kinetic equation (Eqn. 2, $k \neq 0$) to represent the purity vs time data (available only at 3 time points). The S.E.M. in the initial rates is estimated to be roughly ±20%.

[b] Value of A is negative or near zero at the end of the study.

[c] Thermal activity determined only at time zero.

[d] Not evaluated due to insignificant decomposition.

TABLE 5

Thermal activity and chemical reactivity of aqueous cefazolin sodium and aqueous cephalexin

Substance and temperature	% H_2O	A_0 (μW/g)	R_0 (%/mo) [a]	$\langle A \rangle$ (μW/g)	ΔP (%) [τ]	ΔH_r (kJ/mol)
Aqueous cefazolin	78.5	33.2	48 ± 12	40.5	4.6 [3 d]	109
at 25 °C	90.0	27.8	36 ± 3	37.0	4.9 [4 d]	120
	98.8	43.9	30 ± 3	38.3	4.0 [4 d]	160
Aqueous cefazolin	68.7	126	450 ± 90	130	14.5 [1 d]	37
at 40 °C	88.4	120	330 ± 60	139	10.0 [1 d]	57
	98.3	107	420 ± 60	132	13.5 [1 d]	40
Aqueous cephalexin at 25 °C	99.0	62.6	45 ± 6	80.7	4.6 [4 d]	210
Aqueous cephalexin at 40 °C	98.8	430	300 ± 150	594	9.0 [1 d]	200

[a] All purity vs time data are consistent with first-order kinetics.

same format. The water content (%H_2O) refers to weight percent. Thermal activity normalized to unit mass of anhydrous material is denoted by the symbol A, and is given in units of $\mu W/g$. Thermal activities for solids decrease significantly as a function of time during the stability study. Only the initial thermal activities, A_0, and the mean thermal activities over the duration of the experiment, denoted $\langle A \rangle$, are reported. For solids, the initial thermal activities actually correspond to a time 18 h after time 0 and with the exception of the values enclosed by parentheses, represent "steady" values. Initial thermal activities for solutions are "steady" values and correspond to times of 6 h (25 °C) and 3 h (40 °C).

The initial chemical reaction rate in %/month (mo), denoted R_0, is the rate calculated at 18 h, $(dP/dt)_{18h}$, where P is the % purity at time t. The rate is evaluated by differentiating the generalized kinetic equation,

$$\ln(P/P^0) = [k_0/k][\exp(-kt) - 1], \qquad (2)$$

where P^0 is the initial purity. Both k_0 and k are kinetic constants. Regression analysis of the raw purity data determines P^0, k_0, and k. In the context of this research, Eqn. 2 is simply an empirical expression which is justified solely because it normally represents the observed kinetics. In the limit as k approaches zero, Eqn. 2 reduces to a simple first order expression. With k significantly greater than zero, Eqn. 2 reproduces the often observed sharp decrease in reaction rate as time increases. The uncertainty given with an initial rate value is the estimated standard error based on the relative standard error in k_0. Since the ceftazidime raw data only included 3 time points, a "statistical" based error estimate could not be made. Here, as a rough guess, the standard error in R_0 is estimated to be about $\pm 20\%$.

The time average thermal output, denoted $\langle A \rangle$, is calculated from the raw data by the relationship

$$\langle A \rangle = (1/\tau) \int_0^\tau A \, dt, \qquad (3)$$

where A is the thermal activity at time t and τ is the duration of the stability run. The integration in Eqn. 3 is carried out numerically using the trapezoidal rule.

The decrease in % purity over the duration of the stability run, denoted ΔP, is calculated from Eqn. 2 ($\Delta P = P^0 - P$) when this equation provides a good fit. For the few cases where Eqn. 2 does not represent the data, ΔP is evaluated by graphical smoothing. In general, the uncertainty in ΔP is estimated at less than $\pm 1\%$.

The effective mean heat of reaction over the duration of the stability study, denoted ΔH_r, is calculated from the decrease in purity, the duration of the stability run in months (τ), mean thermal activity, and the molecular weight, M [3],

$$\Delta H_r(\text{kJ/mol}) = 0.2592\{M\tau(\langle A \rangle)/\Delta P\}. \qquad (4)$$

In essence, Eqn. 4 is a result of the integration of Eqn. 1 over time and substitutions of A for $(dQ/dt)/\text{mass}$ and mass/M for n. The numerical factor is a result of units conversions. In short, with M in g/mol, τ in months, A in $\mu W/g$, and ΔP in %, the mismatch in units requires a conversion factor to yield a result for ΔH_r in units of kJ/mol. The experimental error in ΔH_r increases as the magnitude of ΔP decreases and is estimated to vary between about $\pm 8\%$ (large ΔP) and $\pm 25\%$ ($\Delta P = 5\%$). Note that we use the convention that exothermic corresponds to a positive value for ΔH_r.

Two samples of amorphous cephalothin sodium (Table 1) and two samples of cefamandole sodium (Table 2), identified by the parenthesis around the numerical values, displayed an anomalous time dependence during the first 18 h of the calorimeter run which was reproducible and well in excess of that expected for moisture redistribution. Representative of this effect, two initial samples which showed this effect are compared with more normal samples (samples of the same composition run at the two week time point) in Fig. 1. Thermal activities, A, for initial samples (left axis) show a sharp drop in thermal activity during the 18 h run and,

[3] Molecular weights for the compounds studied are: cephalothin sodium, 418; cefamandole sodium, 484; anhydrous ceftazidime, 544; anhydrous cefaclor, 367; moxalactam, 544; anhydrous cefazolin, 476; anhydrous cephalexin, 347.

even at the end of the experiment, show significant time dependence. By contrast, the 2 week samples (right axis) show, at most, a modest decrease in thermal activity over the course of the experiment, consistent with moisture redistribution.

The time dependence of the heat of reaction can be evaluated from the real time thermal activity and the corresponding derivative, dP/dt. Normally, a combination of random assay error and non-optimum spacing of time points results in excessive error in dP/dt, thus limiting the significance of such a calculation. The 25 °C run for amorphous cefaclor containing 12% water is an exception to this generalization. Spacing of time points was nearly optimum, and the assays were unusually precise (ca. $\pm 0.3\%$). Heats of reaction were calculated from the raw purity data according to the derivative analog of Eqn. 4, where the thermal activity at a given time replaces the mean thermal activity and the derivative, dP/dt, replaces the ratio $\Delta P/\tau$. To minimize systematic errors, derivatives were evaluated by numerical differentiation of the raw purity data. Standard errors in the calculated heats of reaction were estimated assuming a standard error of 0.4 in a purity difference and a relative standard error of 10% in the thermal activity. The calculated heats of reaction (Fig. 2) are approximately independent of time. The slight increase with time is within the estimated uncertainty in the data. Use of derivatives evaluated from differentiation of Eqn. 2 give essentially the same results, differing only in that

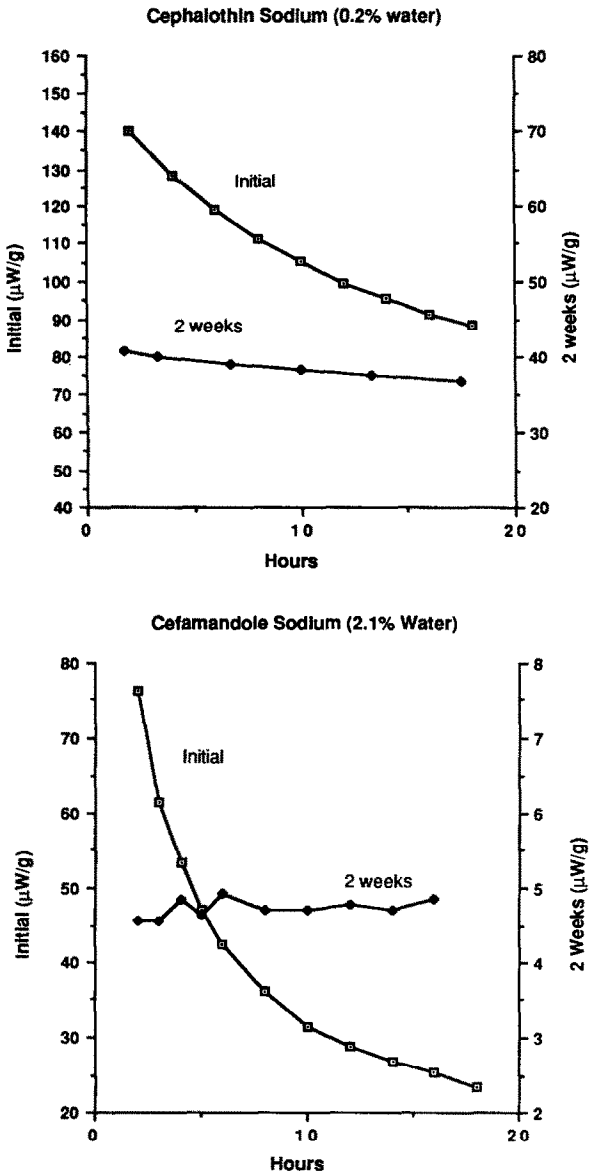

Fig. 1. Time dependence of thermal activity at 40 °C for amorphous samples.

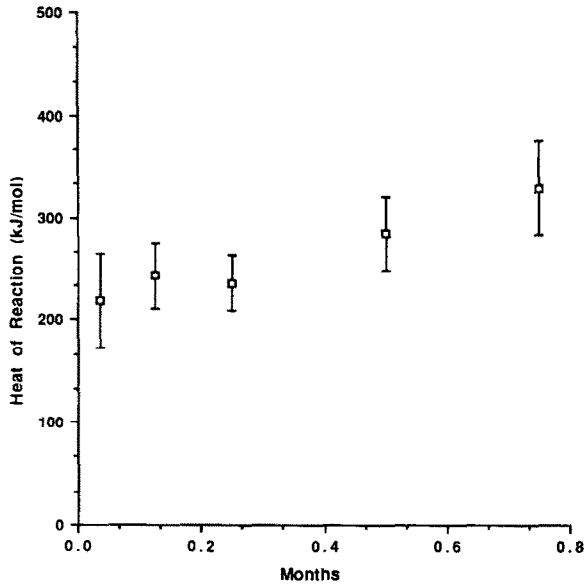

Fig. 2. Time dependence of the heat of reaction for cefaclor (12% H_2O) at 25 °C.

TABLE 6

Thermal activity and chemical stability of amorphous moxalactam formulated with 12% (w/w) mannitol

Lot	t(°C)	% H_2O	A_0 (μW/g solid) [a]		R_0 (%/mo) [b]	
			Exp.	Calc. [c]	Decarboxylation	β-Lactam rupture
S	25	0.5	−0.09	0.3	0.19	0.28
L	25	0.85	0.09	0.7	0.22	0.36
S	25	2.0	2.6	1.4	0.35	0.61
L	25	2.8	2.0	2.0	0.43	0.78
L	25	3.7	3.9	2.6	0.52	0.97
S	37	0.5	0.04	1.2	0.76	1.10
S	37	2.0	7.8	5.3	1.4	2.4
L	40	0.85	3.2	3.3	1.2	1.9
L	40	3.5	11.6	13.1	2.8	5.1

[a] Values are initial thermal activities with essentially no short-term time dependence.

[b] Interpolated from historical data on other lots. Initial purity averages 86.8% (note that 12% of the solid is mannitol).

[c] Calculated from a fit of the experimental data to the rates of decarboxylation and β-lactam rupture with heats of reaction determined by the regression analysis (−1500 kJ/mol for decarboxylation (endothermic) and 1200 kJ/mol for β-lactam rupture (exothermic)). See text for more details.

the curve shows a shallow minimum around 0.5 mo.

Due to the different protocol used in the study of moxalactam, the table format (Table 6) differs from that used for the previous tables. The column headed "Exp." represents the experimentally determined thermal activities. The column headed "Calc." represents corresponding thermal activities calculated from a theoretical model for the decomposition which will be discussed later. All thermal activity data represent initial "steady" values, achieved after 18–48 h in the calorimeter. The decomposition rates represent the average behavior of a number of other lots studied several years earlier (Pikal and Bornstein, 1982). Moxalactam decomposes by two parallel reaction pathways, one involving decarboxylation of a carboxyl group on the *p*-hydroxyphenylmalonyl side chain (7 substituent) and the other involving rupture of the β-lactam with expulsion of the methyltetrazolethio side chain (3′ substituent). Assays for the decarboxylated cephalosporin and the free methyltetrazolethio side chain are used to quantify both reaction rates. Loss of parent moxalactam is consistent with these two pathways being essentially the only decomposition pathways of significance. The initial reaction rates, R_0, for β-lactam rupture and decarboxylation were obtained by a fit of the corresponding assay data to the differential equation analogs of Eqn. 2. Coincidently, the

TABLE 7

Time-dependent energy of an amorphous solid: heats of solution of amorphous cephalothin sodium (0.2% H_2O) in water at 25°C

Lot	Aging time/ 25°C	Thermal activity (μW/g)	Heat of solution (kJ/mol) [a]
A	0	22.8	−18.3±0.1
	2 mo	13.2	−15.0±0.2
B	0	31.8	−17.1±0.3
	2 mo	12.1	−15.1±0.1

[a] Heat of solution to infinite dilution. Uncertainties are S.E.M. based on 3 replicates.

TABLE 8

Heats of solution of cefaclor at infinite dilution in water at 25°C

Substance	% H_2O	Heat of solution (kJ/mol) [a]	Heat of crystallization (kJ/mol)
Amorphous	0.0	−24.2	0
	4.6	−10.2	0
	6.0	−8.4	0
Crystalline			
pseudo-mono-	0.0	−20.5	3.7
hydrate	4.5	−7.1	3.4

[a] Uncertainty ca. ±0.6 kJ/mol.

activation energies for both decarboxylation and β-lactam rupture are essentially equal, ≈ 88 kJ/mol. Since individual lots can vary significantly in measured initial decomposition rates, we cannot be sure that the rates given in Table 6 are quantitative for the lots used to obtain thermal activity data. Thus, our analysis of the moxalactam data can only be qualitative.

Heats of solution of some cephalosporin solids were determined to aid in the interpretation of the stability data. The effect of aging time at 25 °C on the heat of solution of cephalothin sodium is summarized in Table 7. Heats of solution of amorphous and crystalline forms of cefaclor (Table 8) and ceftazidime (Table 9) were determined as a function of water content to allow evaluation of the respective heats of crystallization. Heats of solution for ceftazidime were measured in a mixed solvent system (DMSO : water) because of solubility limitations in pure water. Thus, the heats of solution contain a contribution from the "mixing" of hydrate water with the mixed solvent system as well as the obvious solvation energies arising from ceftazidime interaction with both components of the solvent system. However, since these contributions are cancelled in the calculation of the heat of crystallization, this apparent complication is of no consequence. We define the heat of crystallization, ΔH_{cryst}, as the enthalpy change for the process, crystalline (n_0 H_2O) → amorphous (n_0 H_2O), where n_0 is the number of moles of water per mole of cephalosporin. The heat of crystallization can be evaluated from the heat of solution of amorphous and crystalline solids of the same water content,

$$\Delta H_{cryst} = \Delta H_s(\text{crystal}, n_0\ H_2O) - \Delta H_s(\text{amorph}, n_0\ H_2O), \quad (5)$$

where ΔH_s is a heat of solution to infinite dilution, "crystal" refers to the crystalline phase, and "amorph" refers to the amorphous phase. Eqn. 5 was used to evaluate heats of crystallization (Table 8 & 9) from the corresponding heat of solution data. Since the amorphous phase is a high-energy solid, relative to a crystalline phase, the heat of crystallization as defined here is a positive number whose magnitude is the difference in energy between the amorphous and crystalline phases of the same water content. The heat of crystallization for cefaclor pseudomonohydrate is independent of water content, but for ceftazidime non-stoichiometric hydrate, the heat of crystallization increases as the water content decreases. The pentahydrate of ceftazidime, though having a higher water content than the non-stoichimetric hydrate, has a much higher heat of crystallization.

Comparison of chemical decomposition rates for cephalothin sodium from Table 1 with corresponding data in the literature (Pikal et al., 1977) shows that while agreement is excellent at high water content, the rates from Table 1 are much higher at low water content, particularly for the 25 °C data. The lack of agreement noted here is likely the result of the difference in assay methodology [4]. The chemical stability data for cefamandole sodium given in Table 2 are in good agreement with the corresponding data in the literature (Pikal, et al., 1977) where comparisons are possible.

[4] The iodometric assay used to assay cephalothin sodium in the early study will fail to measure decomposition unless the β-lactam is destroyed or the decomposition product containing an intact β-lactam precipitates from solution during the assay. Desacetylcephalothin and desacetylcephalothin lactone are two decomposition products which have intact beta-lactams (Pikal, et al., 1977). While the lactone does precipitate from aqueous solution when present in sufficient quantity, a small amount of lactone may well have been counted as undecomposed in the iodometric assay. HPLC separates both the lactone (after main peak) and desacetyl species (well before main peak) from cephalothin. In addition, a significant unidentified decomposition peak appears slightly before the main peak. Since the UV detection system is set at the maximum for the β-lactam absorption, it is likely that this peak also represents intact β-lactam. Thus, we tentatively conclude that the lack of agreement between the two data sets is mostly a result of the failure of the iodometric assay to measure decomposition not involving rupture of the β-lactam. The major effect on the conclusions from the early study (Pikal et al., 1977) is that the stability of amorphous cephalothin sodium is relatively insensitive to water content at low to moderate water content.

TABLE 9

Heats of solution of ceftazidime at infinite dilution in DMSO : 0.01 N aqueous HCl(80 : 20) at 25 °C

Substance	% Relative humidity	% H_2O	Heat of solution (kJ/mol)	Heat of crystallization (kJ/mol)
Amorphous	Dried	1.5	−50.7±2.9	0
	11	5.5	−36.7±2.1	0
	30	6.7	−35.0±1.9	0
	58	10.3	−27.1±0.6	0
	76	12.2	−26.3±0.4	0
	93	17.5	−25.3±0.9	0
Crystalline non-stoichiometric hydrate	Dried	0.4	−40.2±1.9	14.6
	30	2.9	−35.4±2.0	10.0
	76	6.1	−27.4±0.8	8.4
Crystalline pentahydrate	30	14.8	−0.3±0.1	24.2
	76	14.6	0.0±0.1	24.5

Discussion

Trends in stability

Kinetics of solid state decomposition and measures of chemical stability

Consistent with previous studies of amorphous cephalosporins (Pikal et al., 1977), the observed kinetics are complex. The solids studied frequently exhibit a sharp decay in decomposition rate with time which is equivalent to high reaction order, but the same material may give apparent first-order kinetics under different conditions of water content and temperature. Except in several unusual cases, such as high moisture cephalothin sodium which crystallized during the stability study, the kinetics can be represented by Eqn. 2, which contains two kinetic constants, k_0 and k. Eqn. 2 can be "derived" assuming a pseudo-first-order decomposition where the rate constant itself, k_1, is time dependent according to the expression, $k_1 = k_0 \exp(-kt)$. The rate constant, k_0, represents the first-order rate constant at time zero, and k is a rate constant which phenomenologically describes the decrease in first order rate constant with time. While several possible causes for "higher order" kinetics have been discussed, a complete understanding of the origin of this phenomenon, remains obscure (Pikal et al., 1977). Of major importance to this study is the fact that two kinetic constants are frequently needed to completely describe the stability of a sample. We use the initial decomposition rate, R_0, for stability comparison since R_0 is essentially a measure of the same kinetic constants, k_0, for all samples, regardless of the value of k. Thus, a series of samples having variable kinetics may be compared on the same basis. The disadvantage of this approach is the R_0 data are frequently subject to large experimental error when $k \neq 0$. Generally, trends in R_0 data are similar to trends in average decomposition rate ($\Delta P/\tau$). One exception, probably reflective of experimental error in R_0, occurs for amorphous ceftazidime at 25 °C. Initial rates are nearly identical for both 1.3% and 6.7% water samples yet the average rate for the 6.7% water sample is significantly greater than the average rate for the lower moisture sample.

Effect of water content

While the stability of aqueous solutions is essentially independent of water content (concentration), all amorphous compounds and the crystalline non-stoichiometric hydrate of ceftazidime show decreased stability at high water content. The relationship between R_0 and stability is not linear. For amorphous samples, the decomposition rate either remains constant (cephalothin sodium) or increases slightly (cefamandole sodium, cefaclor) as water content increases from low to intermediate water. As the water content increases from intermediate levels to high levels, the decomposition rates sharply increase. The definition of "intermediate" water content varies with the compound studied and is ≈ 5% for cephalothin sodium and cefamandole sodium and ≈ 10% for cefaclor. The stability variation with water content is illustrated by the data for amorphous cefaclor at 25 °C (Fig. 3), where the initial rates and initial thermal activities (divided by 5 for purposes of plotting) are given as a function of water content. Both measures of reactivity (or instability) show a linear increase at low to moderate water content followed by a sharp increase between 9% and 12% water.

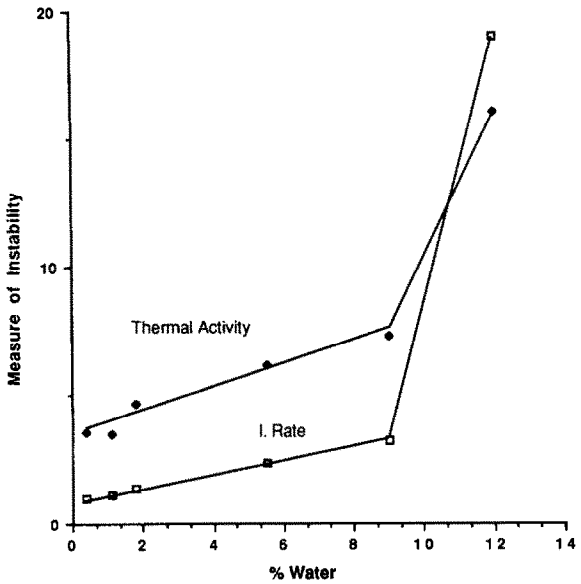

Fig. 3. Correlation between stability and water content for amorphous cefaclor at 25 °C. Comparison of initial reaction rate in %/mo (□) with thermal activity (μW/g) divided by 5 (◆).

Because water can be a reactant in the decomposition of cephalosporins (Pikal et al., 1977), it is not surprising that the decomposition rate increases with water content. Indeed, if the amorphous system behaves as solution undergoing bimolecular kinetics, the initial decomposition rate would be directly proportional to the water concentration. The non-linear relationship between decomposition rate and water content is not consistent with the simple "solution model" but requires a more complex interpretation. The non-linearity may be a result of multiple sorption sites for water. That is, the "intermediate" level of water corresponds to saturation of the relatively non-reactive water binding sites, and further increases in water content result primarily in binding to the reactive sites on the cephalosporin molecule. Alternatively, the non-linearity could be a result of plasticization of the amorphous phase by water, thereby lowering the glass transition temperature of the solid. Here, "intermediate" water content is the water content which is required to lower the glass transition temperature to the temperature of the stability study. Samples with "intermediate" to "high" water content would then be above the glass transition temperature, and the much higher molecular mobility for a system above the glass transition temperature results in greatly increased chemical reactivity. Note that this interpretation demands that the "intermediate" level of water is lower for a stability study at 40 °C than for the corresponding study at 25 °C, a condition which appears to be consistent with the data. While glass transition temperature data are not available for the systems of interest to this research, we note that cephalothin sodium becomes "tacky" at some water content between 4% and 7%, suggesting a glass transition temperature of 25 °C in the vicinity of "intermediate" water content.

The correlation of stability with heat of crystallization

The chemical decomposition data for cephalothin sodium, ceftazidime and cefaclor demonstrate, as expected (Pikal, et al., 1977), that a crystalline phase is much more stable than the corresponding amorphous phase. The corresponding thermal activity data lead to the same conclusion. However, not all crystalline forms of a given compound have the same chemical stability. Rather, at least for ceftazidime, the stability appears to be directly related to the heat of crystallization.

Chemical stability and heat of crystallization are compared (Fig. 4) by calculating a relative reaction rate from the 25 °C data presented earlier (Tables 3 and 4). The relative rate is defined as the initial decomposition rate of the crystalline sample divided by the initial rate of an amorphous sample of the same water content, the latter being determined by interpolation of the data. The relative rate for ceftazidime pentahydrate is not quantitative due to the extreme stability of the pentahydrate. However, the point representing the pentahydrate and the corresponding error bar do set an upper limit for the relative rate. Within experimental error, the ceftazidime relative rates are exponential in the heat of crystallization (i.e., linearity on a semi-log plot). Although the pentahydrate point was not included in the regression analysis used to generate the straight line, this point is consistent with the trend established by

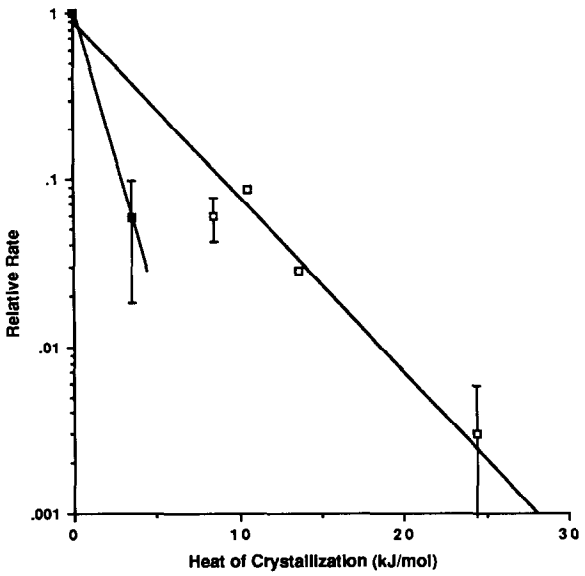

Fig. 4. Correlation of chemical stability at 25 °C with heat of crystallization. The relative rate is defined as the initial rate of the sample divided by the initial rate of the Amorphous solid of the same water content. Data are given for ceftazidime (□) and cefaclor (■).

the 4 other points. The two points available for cefaclor apparently define a different curve with a slope larger in magnitude (-0.35 ± 0.1) than the slope for ceftazidime (-0.11 ± 0.02) [5].

One interpretation of the trends shown in Fig. 4 may be given in terms of transition state theory. We develop our arguments from the theory for a unimolecular reaction, but it should be noted that the results are independent of molecularity. The transition state theory expression for the rate constant of a unimolecular reaction, k_1, may be written (Benson, 1960),

$$k_1 = (k_B T/h) \exp(-\Delta G^*/RT) \tag{6}$$

where k_B is Boltzmann's constant, h is Planck's constant, T is absolute temperature, R is the gas constant, and ΔG^* is the Gibbs free energy change on transition from the normal state to the state where the system is suitably "activated" for the chemical reaction to proceed. We assume the same activated state for all solid forms of a given compound undergoing a particular reaction. Thus, for solid form i, we may write,

$$\Delta G_i^* = G^* - G_i, \tag{7}$$

where ΔG_i^* is the free energy of activation for solid form i, G^* is the free energy in the activated state, and G_i is the free energy of solid form "i" in its normal state. Using Eqn. 6, the ratio of crystalline to amorphous rate constant, denoted k_{rel}, is given by,

$$k_{rel} = \exp(-\Delta G_{cryst}/RT), \tag{8}$$

where ΔG_{cryst} is the free energy of crystallization, $G_{amorphous} - G_{crystalline}$. Since the amorphous state is both a high energy (more exothermic heat of solution) and a high free energy (higher solubility) state relative to a crystalline state, ΔG_{cryst} is positive, and therefore k_{rel} is less than unity. Given the relationship, $\Delta G = \Delta H - T\Delta S$, and assumption of the entropy/enthalpy compensation principle (Grant and York, 1986), $\Delta H = \beta \Delta S$, where β is a constant of the same dimensions as absolute temperature, the free energy of crystallization may be expressed as,

$$\Delta G_{cryst} = \Delta H_{cryst}(1 - T/\beta). \tag{9}$$

Since both the enthalpy and free energy of crystallization are positive, T/β must be less than unity. Combination of Eqn. 8 and 9 then give,

$$\log k_{rel} = -[(1 - T/\beta)/2.303\ RT]\,\Delta H_{cryst}. \tag{10}$$

From Eqn. 10 a plot of log k_{rel} as a function of ΔH_{cryst} should be linear (as observed) with a slope of: $-0.18\ (1 - T/\beta)$. The observed slope for ceftazidime (-0.11) is consistent with Eqn. 10, but the magnitude of the observed slope for cefaclor is somewhat larger than Eqn. 10 would predict. The deviation noted for cefaclor may not be significant, however, given the sizable uncertainty in the slope.

[5] The uncertainty given for ceftazidime is the standard error in the slope as calculated from the results of the regression analysis. For cefaclor, the error limit is a crude estimate of standard error based on the uncertainty in the relative reaction rate.

Time dependence in thermal activity

Physical changes: annealing of amorphous solids

In principle, the decrease in energy of an amorphous solid as a function of storage time, or "annealing" (Pikal et al., 1978) could contribute to the thermal activity of amorphous solids. We propose that rapid annealing at 40 °C is responsible for the time dependence shown in Fig. 1. Since the reaction rate calculated from Eqn. 2 decreases only slightly over the time period relevant to Fig. 1, it does not seem likely that the time dependence of thermal activity is due to decreasing reaction rate. Of course, there remains a slight possibility that the kinetics deviate from that represented by Eqn. 2 for the first few days at 40 °C. Several orders of magnitude more precision in the assay methodology would be needed to verify such an effect. Physical changes in the solid present a more attractive interpretation. Decreases in energy of the solid as a function time, or "annealing", has been reported (Pikal et al., 1978) for cefamandole sodium. Cephalothin sodium also shows an apparent annealing effect (Table 7). For both lots of cephalothin sodium investigated, the heat of solution of the aged sample (2 mo at 25 °C) is significantly more endothermic (lower-energy solid). No information on the kinetics of annealing is available from the limited heat of solution data given here or in the literature (Pikal et al., 1978). A linear decrease over the 2 month aging period (Table 7) would give an annealing contribution of 1.5 μW/g at 25 °C, which is likely a lower bound for the effect. At 40 °C, the thermal activity of annealing would presumably be much greater initially, but as the sample approaches equilibrium, the contribution of annealing to thermal activity would vanish. Thus, if the rate of annealing is fast compared to the rate of chemical decomposition, the observed thermal activity would decrease sharply with time as observed in Fig. 1. We speculate that annealing also contributes to some of reported "steady" thermal activities. In particular, the irregularities in A_0 data for amorphous cephalothin sodium at 25 °C (Table 1) may be a result of variable annealing rates, and some of the decrease in "steady" thermal activity values during the stability study could be a result of slow annealing. Exposing a series of samples to a higher temperature for a brief period (i.e., 40 °C for 2 weeks) to anneal the samples before calorimetric stability measurements are initiated may circumvent problems caused by variations in annealing. Finally, while physical changes may be an interference in calorimetric studies of chemical reactivity, high-sensitivity isothermal calorimetry could be used to monitor the progress of physical changes in systems where chemical changes are negligible or separable.

Decreasing chemical reaction rate

At least for solids, the mean thermal activity, $\langle A \rangle$, is almost always significantly less than the initial thermal activity, A_0, reflecting the general trend of decreasing thermal activity as a function of time. While this trend could reflect a decrease in the effective heat of reaction due to annealing effects or interference from decompositon products, the reaction rate also normally decreases significantly as a function of time, thereby decreasing the thermal activity. At least for amorphous cefaclor (12% water), the heat of reaction *is* independent of time (Fig. 2), and the sharp decrease in thermal activity with time (factor of 3 over 1 mo) is simply a reflection of the decomposition kinetics. In general, most of the time dependence in A for other systems is probably due to decreasing reaction rate. However, we do not mean to imply that the heat of reaction is always independent of time. For example, the ceftazidime data that show a change in sign for the thermal activity (Table 4, footnote 6) are clearly not consistent with a time-invariant heat of reaction. Presumably, the decomposition products have an endothermic thermal activity. Moreover, in those cases where annealing phenomena contribute significantly to the thermal activity (likely for cephalothin sodium), the apparent heat of reaction will decrease with time.

Trends in mean heats of reaction

In general, mean effective heats of reaction for the decomposition of cephalosporins are large and exothermic. Heats of reaction on the order of several hundred kJ/mol are observed for solids,

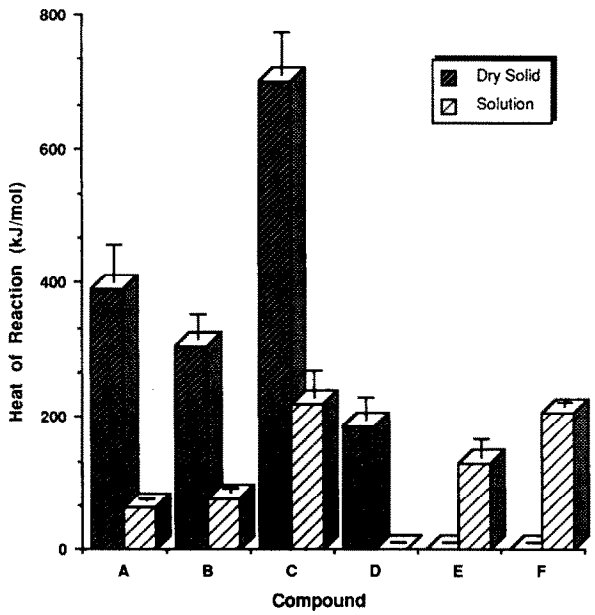

Fig. 5. Comparison of heats of reaction for cephalosporins. Dry solids have dark shading and solutions have light shading. Compounds are cephalothin sodium (A); cefamandole sodium (B); cefaclor (C); ceftazidime (D); cefazolin at 25 °C (E); and cephalexin (F).

which is of the same magnitude as found for decomposition of explosives (Tall and Zeman, 1977). Therefore, sensitivity of the calorimeter is not a limitation of the calorimetric procedure for stability estimation. A summary of the heat of reaction data is provided by Fig. 5, where mean values for the "dry" solids and aqueous solutions studied are compared. The term "dry" refers to water contents in the "low range", where the heat of reaction is relatively insensitive to water content. An error bar reflects the standard error. The heat of reaction given for cefazolin sodium is the mean of the 25 °C data. Significant differences exist between the different compounds, and the heats of reaction for the dry solids are roughly a factor of 5 higher than the values for the corresponding solutions. Given the complexity and individuality of cephalosporin decompositions in solution (Indelicato et al., 1974; Bundgaard, 1975; Indelicato et al., 1977) and the differences between solution and solid state decompositions (Pikal et al., 1977), the observed variations in heats of reaction are not surprising.

One measure of the correlation of chemical stability with thermal activity is provided by an examination of variations in the heats of reaction. If the correlation between time average thermal activity and time average reaction rate were perfect, the heat of reaction would be constant within experimental error (generally ca. $\pm 15\%$ in ΔH_r).

The average heat of reaction for ceftazidime is 184 kJ/mol with the only significant trend being the decrease in ΔH_r as the water content increases for the amorphous phase at 25 °C. Even this trend may not be real. Indeed, if the apparently anomalous high ΔH_r for the 1.3% water sample at 25 °C is rejected, the remaining heats of reaction are constant (150 kJ/mol) within the expected experimental error.

Heats of reaction for solutions show no significant variation with water content (i.e., concentration), and with the exception of cefazolin sodium (Table 5) are also independent of temperature. For example, the heats of reaction for aqueous cephalothin sodium average 63 kJ/mol with a relative S.D. of $\pm 9\%$, indicating a constant heat of reaction within the expected experimental error. Amorphous materials of very high water content have heats of solution comparable in magnitude to the corresponding solutions, while amorphous samples of lower water content show much higher effective heats of reaction (Fig. 5) with a trend toward lower heats of reaction as the moisture content increases. This trend is illustrated in Fig. 6 where the heat of reaction is plotted as a function of water content for all the cephalothin sodium and cefaclor systems studied. The corresponding data for cefamandole sodium shows a trend intermediate between the trends noted in Fig. 6. No significant trends with temperature are observable in the cefamandole sodium or cefaclor data. In the case of cephalothin sodium, the heats of reaction for solids at 40 °C are systematically slightly less than the corresponding data at 25 °C. However, for all 3 compounds, the major variable is water content. While the smooth lines drawn (Fig. 6) are somewhat arbitrary given the scatter, the data suggest either a modest decrease in heat of reaction from low to intermediate water content (cephalothin sodium and cefamadole sodium) or no significant change over this range of water

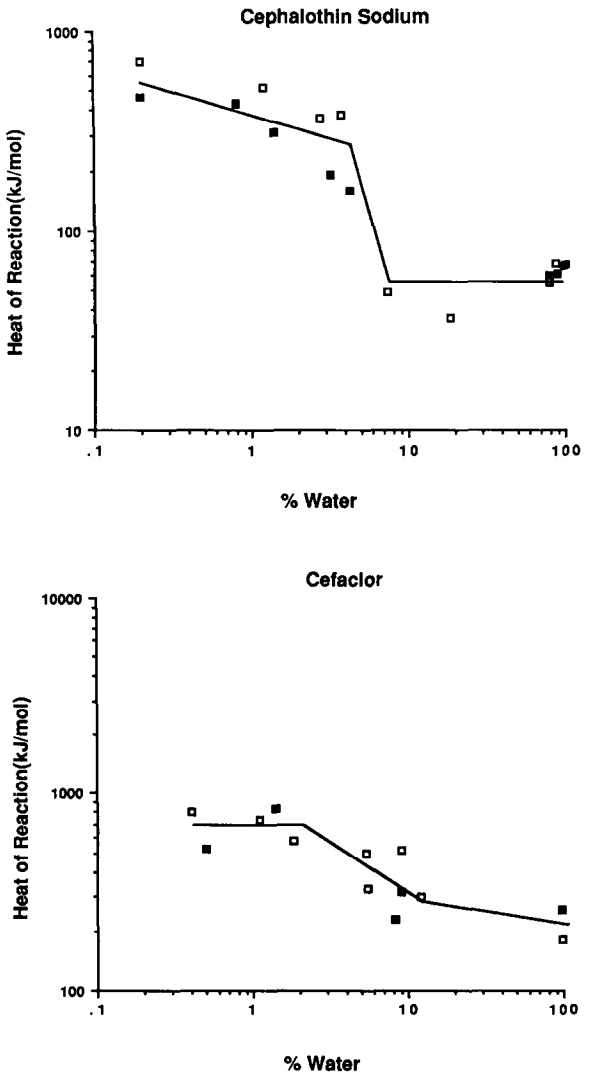

Fig. 6. Correlation of heats of reaction with water content for cephalotin sodium and cefaclor systems. Open symbols are 25 °C and filled symbols are 40 °C.

content (cefaclor), followed by a sharp decrease in heat of reaction around 5% water, particularly for cephalothin sodium. Since the major reaction products formed in high water content systems are different from those formed in low moisture systems (Pikal et al., 1977), differences in heat of reaction might also be expected. The sharp decrease in heat of reaction occurs in roughly the same water content region as the sharp decrease in stability, suggesting that the two phenomena have a common cause. If this cause is a glass transition, as discussed earlier, the relative contribution of annealing effects should decrease sharply in the intermediate water content range; i.e., an annealing effect for an amorphous system above the glass transition temperature is unlikely, and an aqueous solution cannot anneal. Thus, at least for cephalothin sodium, the trends noted in Fig. 6 could reflect, in part, a loss of the thermal activity of annealing as increasing water content lowers the glass transition temperature to the temperature of the stability study.

Correlation of thermal activity and stability

Moxalactam is atypical and will be discussed first. At low water content (0.5% and 0.85%), the experimental thermal activities at 25 °C are nearly zero (Table 6), although the decomposition rates are clearly not zero. A similar observation can be made for the 0.5% water content sample at 37 °C. At higher water content, a sizable thermal activity is measured. It would appear that, at low moisture, the effective heat of reaction is nearly zero, but becomes significantly different from zero as the water content increases. We postulate that the near-zero effective heat of reaction is a result of cancellation of the exothermic heat of β-lactam rupture by the endothermic heat of decarboxylation. At low water content, the cancellation is essentially complete. At high water content, the rate of β-lactam rupture increases more than does the rate of decarboxylation. Consequently, the rupture of the β-lactam dominates the heat of reaction, and the net effect is a strong exothermic heat of reaction. While we have no direct evidence that decarboxylation of moxalactam is endothermic, we have measured the thermal activity for crystalline *p*-aminosalicylic acid (which undergoes decarboxylation), and have found it to be endothermic.

As a check on the plausibility of this interpretation, we fit the thermal activity data to a model based on Eqn. 4 but modified to include two reactions having different heats of reaction,

$$A_0 = (0.2592\ M)^{-1}\left(\Delta H_D (R_0)_D + \Delta H_\beta (R_0)_\beta\right) \quad (11)$$

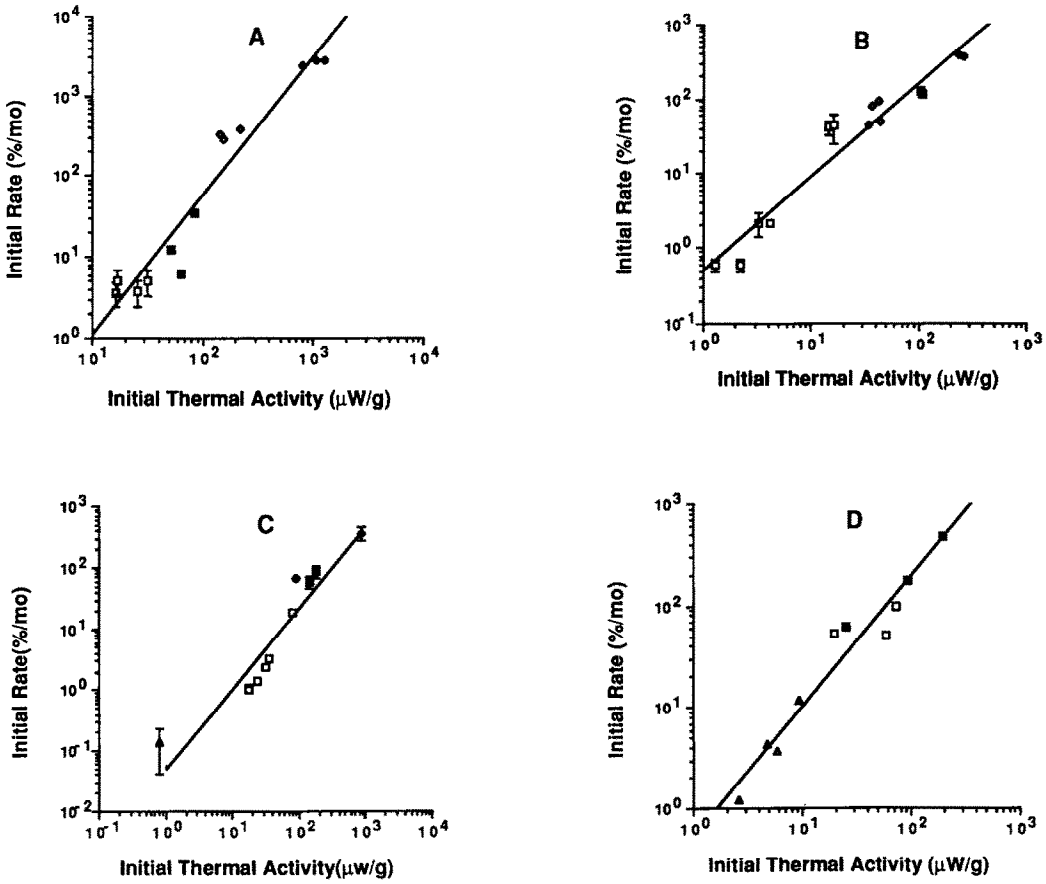

Fig. 7. Correlation of initial decomposition rate with initial thermal activity for cephalosporin systems. A: cephalothin sodium. B: cefamandole sodium. C: cefaclor. D: ceftazidime. Open symbols are 25 °C, and filled symbols are 40 °C. Amorphous solids are squares, crystalline solids are triangles, and aqueous solutions are diamonds.

where the subscript D refers to the decarboxylation reaction and β refers to β-lactam rupture. Comparing Eqn. 11 with Eqn. 4, A_0 corresponds to the mean thermal activity and R_0 corresponds to the ratio $\Delta P/\tau$. The best fit is obtained with the heat of decarboxylation, ΔH_D, being endothermic (-1500 ± 800 kJ/mol) and the heat of β-lactam rupture, ΔH_β, being exothermic (1200 ± 500 kJ/mol). Thermal activities calculated from Eqn. 11, using the above heats of reaction and the R_0 data (Table 6), are compared with the experimental thermal activity data in the column headed Calc. (Table 6). While some differences between experimental and calculated values are outside normal experimental error in the thermal activity measurements, the agreement is satisfactory in view of the fact that different lots are being compared.

For cephalothin sodium, cefamandole sodium, cefaclor, and ceftazidime, thermal activity and chemical stability are well correlated. To illustrate this correlation, we plot the initial reaction rate against the initial thermal activity (Fig. 7). This format is used because applications of isothermal calorimetry to stability problems would likely involve comparisons of initial thermal activities (to allow rapid accumulation of data). Systems where the initial reaction rate could not be measured with acceptable accuracy are excluded from this analysis. Likewise, systems which did not give "steady" initial thermal activity are excluded. Error bars reflect the standard error in the initial

rate but are used only when the estimated uncertainty is significantly larger than the size of the plotting symbol. The straight line is the result of regression analysis:

Cephalothin sodium,

$$R_0 = 0.020(A_0)^{1.728},\ R^* = 0.966, \quad (12)$$

Cefamandole sodium,

$$R_0 = 0.51(A_0)^{1.250},\ R^* = 0.972, \quad (13)$$

Cefaclor,

$$R_0 = 0.056(A_0)^{1.296},\ R^* = 0.943, \quad (14)$$

Ceftazidime,

$$R_0 = 0.534(A_0)^{1.286},\ R^* = 0.970, \quad (15)$$

where R* denotes the correlation coefficient. With few exceptions the data fall as close to the correlation lines as one could expect given the sizable uncertainty in R_0 and A_0. Variations in solid form, or polymorph, do not appear to affect the correlation. The data set for ceftazidime (Fig. 7D) are particularly impressive since it includes two crystalline forms as well as the amorphous form. Since the pentahydrate does not decompose at a measurable rate, the pentahydrate data could not be included on a log-log plot. However, the small values of R_0 calculated (Eqn. 15) from the observed values of A_0 (Table 4) are consistent with the observed stability of the pentahydrate.Thus, there is an excellent correlation between thermal activity and decomposition rate for all compounds studied except moxalactam [6].

[6] While the correlations are excellent, it should be noted that if the initial rate were directly proportional to the initial thermal activity (i.e., the initial heat of reaction is constant), the exponent would be unity. Except for ceftazidime, the deviation of the exponent from unity would be predicted from the dependence of the average heat of reaction on water content. That is, the more reactive systems are high water content systems which have lower average heats of reaction. This trend would have the effect of increasing the exponent. With ceftazidime, the time average heat of reaction is nearly constant, and only the initial heat of reaction is variable.

In summary, thermal activity is generally a valid measure of the decomposition rate. While small variations in stability of solids may not be predicted correctly via calorimetry, small variations are also frequently buried in the experimental error of decomposition rates. Either isothermal calorimetry at the temperature of interest or storage/assay methods of stability testing at high temperature may fail to give the correct answer. However, since they will generally fail for different reasons, it is unlikely that both methods will fail in the same study, and the probabilty of misleading information would be greatly reduced if both techniques were used on the same problem. Calorimetry should be particularly useful in examinations of relative stability for different solid forms and in studies of the effect of process changes on stability. Though not explicitly studied in this research, calorimetry should also find application in studies of drug-excipient interactions.

Conclusions

Stability trends

(1) The degree of stability enhancement provided by the crystalline state appears to be quantitatively related to the heat of crystallization.

(2) The decomposition rate of amorphous cephalosporins increases with increasing water content in highly non-linear fashion, the rate increasing sharply as the water content increases beyond an "intermediate" level.

Stability studies via calorimetry

(1) Sensitivity is not a problem in this application of calorimetry to the study of chemical stability because heats of reactions in the systems studied are large.

(2) While physical changes (annealing of amorphous material) can contribute significantly to the measured thermal activity, particularly for "initial" samples, such effects normally do not dominate the thermal activity.

(3) High-moisture amorphous solids and aqueous solutions usually have significantly lower heats of reaction than the corresponding lower-moisture amorphous solids and crystalline forms.

(4) For most systems studied, the heat of reaction may be considered "well behaved" in the sense that variations in heat of reaction for a series of samples is less than the variation in chemical stability. Thus, a good correlation between thermal activity and chemical stability normally is observed, and calorimetry provides a useful measure of stability. The ability to obtain a stability estimate at room temperature with an overnight experiment makes high sensitivity isothermal calorimetry an attractive technique for use in screening the relative stability of pharmaceutical systems.

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