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Results of Evaluation of the LKB 2277 Calorimeter for stability testing of Pharmaceuticals

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Instrument LKB 2277 Calorimeter

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Background

At the preformulation stage of product development, estimates of chemical and/or physical stability are normally derived from accelerated stability tests and occasional use of thermal analysis (DSC, DTA, TGA). Although thermal analysis is ideally suited for study of first and second order phase transitions, kinetic studies of either chemical reactions or physical changes are normally limited to extremely high temperatures where the data obtained are of dubious utility in predicting kinetics at temperatures of interest (25° and 40°). Accelerated stability tests are subject to the same criticism if the temperatures are high enough to complete the stability test in a reasonable time (i.e. approximately one week). Further, accelerated stability tests are quite labor intensive due to the need for assay work.

Clearly, a rapid method for estimating rates of chemical and physical changes at temperatures near ambient would be of great utility. LKB-Produkter AB has recently introduced a versatile calorimeter system, the Bioactivity Monitor, which has a unique combination of high sensitivity and high sample capacity. Although the instrument is currently viewed as a tool for microbiological and biochemical research, for example, studies of the effect of cephalosporins and other antibiotics on various microorganisms, it has also found application in shelf-life stability estimation for explosives, a problem similar to that faced by the pharmaceutical industry in estimating the stability of preliminary pharmaceutical formulations.

The utility of the calorimetric technique in kinetic studies depends on the sensitivity of the calorimeter and on the heat of reaction for the change under study. For energetic reactions such as amorphous to crystalline physical changes, acid-base reactions and oxidation reactions, it appears that reaction rates as low as 5% per year could be measured. However, for reactions giving essentially zero heat of reaction, calorimetry would be useless. For many reaction types of potential interest to pharmaceutical research, such as cephalosporin decompositions, the magnitude of the heat of reaction is unknown.

An assessment of the utility of the LKB 2277 Bioactivity Monitor in studying the stability of pharmaceutical systems was performed in the LKB Application Laboratories in Stockholm in co-operation with Dr. Jaak Suurkuusk. The major objective was to determine whether the heat produced during decomposition of representative pharmaceutical systems was sufficient to be quantitatively determined by the calorimeter system at temperatures between 25° and 40°

Results

The samples investigated were chosen mostly from the cephalosporin class but several other materials and two classic pharmaceutical decompositions, aspirin hydrolysis and p-amino salicylic acid decarboxylation, were also studied. In general, about 2 grams of solid were used, and total thermal output was easily quantified for most samples, even at 25°C.

The calorimeter is a differential system and performs best when the reference used is of similar heat capacity as the sample. Obviously the reference must have essentially zero thermal output or the net reading must be corrected for the thermal output of the reference. Crystalline cephalothin sodium, known to be very stable, was used as the reference for all solid samples studied, while pure water was used as reference for the aqueous solution studies. We were able to verify that cephalothin sodium gave essentially zero thermal output at 25° and slightly endothermic (but still negligible) output at 37°

All hygroscopic samples were loaded into sample ampoules in an atmosphere of dry nitrogen using a dry bag. Since facilities for Karl Fisher assay were not available, we were not able to verify water contents after sample loading. Previous experience with this procedure indicates that some moisture increase may occur with this procedure but the increase is usually less than ca 0.2%.

For samples in internal physical equilibrium, reliable data may be obtained within about 1 hour after samples are introduced into the calorimeter. However, for many of the samples studied the power output of the sample was time dependent for about 10 hours after loading. We believe this phenomenon reflects re-equilibration of moisture within the sample. During the loading procedure in the dry bag, some moisture absorption resulted in a system slightly heterogeneous in water content which then required a substantial time to reach equilibrium. Allowing the samples to pre-equilibrate overnight before placing into the calorimeter greatly reduced the period of time-dependent power output.

The results obtained for the solid samples are summarized in Table 1. The reaction rates tabulated are estimated either from published data or Lilly unpublished data. Since the reactivity of solids is well known to be dependent on sample history and often exhibits complex time-dependence in the rate, these values should be regarded only as estimates for the samples investigated at the time the colorimetric data were obtained. It should be emphasized that the calorimeter measures the rate of energy production by the sample. The data recorded in the last column (heat output) are given in units of power per gram of sample or, in the case of solutions, power per ml of sample. The power is given in microwatts. We assume that once the power generated by the sample is essentially independent of time, physical equilibrium is attained, and the power output is then due to chemical reactions alone. The data recorded represent such time invariant power outputs. Here, we use the convention that heat produced (exothermic) is positive.

It should be noted that in nearly every system studied, the heat output is well above the sensitivity of the instrument (~.05 μW/g), even at a sample temperature of 25°C. Moreover, with the exception of Amorphous A samples there is a good correlation between estimated reaction rate and thermal output. For example, as one increases the water content or temperature of amorphous cephalothin sodium, the thermal output increases roughly in proportion to the increase in estimated reaction rate. Also, the solid crystalline A samples (Exp. 15, 17, 18) at 25° show the order in thermal output expected from the estimated reaction rates. The Amorphous A samples are, however, an apparent anomaly. Although both the 25° and 37° data show the expected increase in heat output as the moisture level is increased from 0.5% to 2.0% (Exp. 4 vs. Exp. 6; Exp. 5 vs. Exp. 7), and while the effect of temperature on heat output for the sample containing 2% H_2O (Exp. 6 vs. Exp. 7) correlates with reaction rates, the essentially zero heat output for the 0.5% H_2O samples (Exp. 4,5) requires some interpretation.

Amorphous A decomposes by two mechanisms, conventional rupture of the β-lactam and also by decarboxylation. At low water content, both reactions proceed at roughly the same rate and coincidently have, within experimental error, the same activation energy. While the rupture of the β-lactam would likely proceed with a heat of reaction of the same sign and magnitude as the corresponding reaction in amorphous cephalothin sodium, giving a heat output of roughly 4 μW/g at 25°C (exothermic), the heat of the decarboxylation reaction must also be considered. Note that the decarboxylation of p-amino salicylic acid is endothermic. If we postulate that the heat of decarboxylation of Amorphous A is endothermic and equal in magnitude to the heat of β-lactam rupture, the data (Table 1) are consistent with the chemistry of Amorphous A decomposition. For example, as the water content increases, the rate of β-lactam rupture increases much more than does the rate of decarboxylation and one would expect to observe a net exothermic heat output at 2.0% H_2O, as observed. The net exothermic heat output of Amorphous A formulated with dextran is probably partly due to the higher moisture level in these samples, but may also be due, in part, to a greater suppression of the decarboxylation rate with dextran than obtained with mannitol. The latter speculation may be tested experimentally, and such studies are in progress.

The heat output of a sample is directly proportional to the product of the net heat of reaction and the reaction rate. The heat of rupture of the β-lactam, and subsequent rapid secondary reactions, appear to be strongly exothermic. Estimates using the data in Table 1 suggest exothermic values of the magnitude: 300 Kcal/mole for "dry" amorphous material, 100 Kcal/mole for high water content amorphous material, and 60 Kcal/mole in aqueous solution. Similarly, decomposition of crystalline A is strongly exothermic (roughly 200 Kcal/mole). More accurate values must await simultaneous determination of heat output and rate of product decomposition by chemical means on identical samples.

The most obvious problem in the "blind" use of calorimetry to measure stability is the measurement of zero heat output due to several simultaneous reactions cancelling in heat output. In general, the energy of activation will differ and measurements at several temperatures should therefore prevent the erroneous conclusion of chemical stability. For example, observation of a higher magnitude of heat output as the temperature is lowered would suggest simultaneous reactions cancelling in heat output. However, even in the case of equal activation energy for two simultaneous reactions, calorimetry would be useful in certain stability studies. For example, in studies of the effect of excipients (i.e. mannitol, dextran, etc.) on the rate of decarboxylation of Amorphous A one would expect to see an increase in exothermic heat output as the rate of decarboxylation is reduced by the excipient.

While the data presently available suggest the calorimetric approach will be useful in stability studies, the ultimate power and reliability of the method will depend on how accurately one is able to estimate the heat of reaction for a sample of interest from previously acquired data and chemical intuition.

With the acquisition of the LKB 2277 system, we hope to generate the data base and experience needed to define the role of calorimetry in preformulation stability testing.

The LKB 2277 system is quite versatile and may be configured for use in more conventional calorimetric studies, such as evaluation of the thermodynamics of binding between proteins and small molecules. The sensitivity of the instrument for such studies is roughly a factor of 10 greater than previous LKB instruments and therefore has the sensitivity for work in very dilute solutions. The use of the calorimeter for this type of application was demonstrated for the case of m-cresol binding to Pork Insulin. The data obtained are summarized in Table 2. It should be noted that the heat output is far in excess of the sensitivity of the instrument (ca 0.2 μW/g) and therefore solutions considerably more dilute than those studied could be investigated. The accuracy of the results in Table 2 are limited by the accuracy of the pumping speed calibrations. With greater care in this calibration, the limiting factor in precision and accuracy is normally the noise and drift originating in the pumping speed variability.

Conclusion:

In summary, the calorimeter performed very well during our tests. The calorimetric method for stability studies shows great promise, and the instrument is well designed for the thermodynamic characterization of interactions in dilute solutions.

Even for very slow rates of decomposition (on the order of 2% per year) thermal outputs were easily quantified, and in general, the thermal output was consistent with the chemistry of the sample being studied. Although additional data and experience are needed to precisely define the role of calorimetry in stability testing, two types of applications appear particularly promising:

1) Preliminary screening of formulations for a potential product to select the most stable formulations for further study by conventional means;
2) Preliminary evaluation of the relative stability of raw material as a function of polymorphic form and/or method of manufacture.

For both classes of applications, the sensitivity, precision, and speed of operation characteristic of the calorimetric method would generally allow stability information to be obtained within days whereas conventional methods would require months or years. Moreover calorimetry requires an order of magnitude less labor input to generate the information than chemical-assay-based information requires.

TABLE 1:
HEAT OUTPUT AND ESTIMATED REACTION RATES FOR SELECTED PHARMACEUTICAL SYSTEMS

Exp.=	System	+°C	Estimated Reaction Rate	Heat Output (exothermic > 0)
1	10% aspirin in $NaHCO_3$	25°	3% mo^{-1}	−7.2 μW/g
2	p-amino salicylic acid (ground, PH_2O = 14 mm)	37°	3% mo^{-1}	−3.8 μW/g
3	Amorphous A (12% mannitol) 20% Aqueous Solution	25°	3% d^{-1}	32 μW/ml
4	Amorphous A (12% mannitol) Solid 0.5% H_2O	25°	0.5% mo^{-1}	−.09 μW/g
5	Amorphous A (12% mannitol) Solid 0.5% H_2O	37°	2.0% mo^{-1}	0.04 μW/g
6	Amorphous A (12% mannitol) Solid 2.0% H_2O	25°	1.0% mo^{-1}	2.5 μW/g
7	Amorphous A (12% mannitol) Solid 2.0% H_2O	37°	4.3% mo^{-1}	7.6 μW/g
8	Amorphous A (12% dextran 40) Amorphous Solid 1.0% H_2O	25°	—	2.29 μW/g
9	Amorphous A (12% dextran 70) Amorphous Solid 1.4% H_2O	25°	—	1.72 μW/g
10	Cephalothin Sodium 20% Aqueous Solution	25°	2% d^{-1}	33.5 μW/ml
11	Cephalothin Sodium Amorphous Solid, 0.3% H_2O	25°	0.2% mo^{-1}	3.5 μW/g
12	Cephalothin Sodium Amorphous Solid, 0.3% H_2O	37°	0.9% mo^{-1}	9.2 μW/g
13	Cephalothin Sodium Amorphous Solid, 2.0% H_2O	25°	1.4% mo^{-1}	5.7 μW/g
14	Cephalothin Sodium Amorphous Solid, 2.0% H_2O	37°	6.7% mo^{-1}	20.5 μW/g
15	Crystalline A, stable salt	25°	"stable"	0.74 μW/g
16	Crystalline A, stable salt	37°	"stable"	3.1 μW/g
17	Crystalline A crystallized from methanol	25°	0.5% mo^{-1}	1.2 μW/g
18	Crystalline A crystallized from acetone	25°	3% mo^{-1}	12.7 μW/g

TABLE 2:
HEAT OUTPUT FOR INTERACTION OF M-CRESOL WITH INSULIN 37°C, FLOW, 10.4 ML/HR

Solutions Studied:

I. 7.2 mg/ml (U-200) Purified Pork Zn-Insulin in solvent
II. 20 mg/ml m-cresol in solvent
III. Solvent (16 mg/ml glycerol, pH 7.2)

Exp.	Mixing	Heat Output (exothermic)
A	I with II	118.2 μW
B	II with III	50.1 μW
C	I with III	37.2 μW

Power due to Interaction (Exp. A corrected for heats of dilution B and C): 30.9 μW exothermic
Enthalpy of Interaction: 2.03 Kcal/mole Insulin (exothermic)

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The samples investigated were chosen mostly from the cephalosporin class but several other materials and two classic pharmaceutical decompositions, aspirin hydrolysis and p-amino salicylic acid decarboxylation, were also studied. In general, about 2 grams of solid were used, and total thermal output was easily quantified for most samples, even at 25°C.

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