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Thermodynamic and kinetic parameters from isothermal heat conduction microcalorimetry

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Abstract: Recent developments have led to a general procedure that allows the analysis of microcalorimetric data to determine both kinetic and thermodynamic parameters. This makes microcalorimetry a technique with a potential for the accurate prediction of long-term stability, and excipient compatibility, of pharmaceutical compounds. The data presented here show the uses and limitations of this general method when applied to simple solution phase reactions, that are fast to medium term in duration, and suggest how the method may be extended to more complex heterogeneous systems that reflect cases which may be encountered industrially.

INTRODUCTION

The determination of the kinetics of solid-state reactions, specifically, the degradation of drugs, is noted as being a difficult problem (refs. 1-3). At present, it may require many months to attain sufficient data to be confident of knowing the long term stability of both raw and formulated drugs under storage conditions. The maximum extent of degradation allowable for a pharmaceutical product is usually stated to be of the order of 5% in two years (equating to a half-life of 27 years) (ref. 4). The adopted method, at present, for determining solid-state stability is via extrapolation of data obtained at elevated temperatures, since it would be impractical, in most cases, to wait the several years it may require for degradation products to reach detectable levels. In this case, an activation energy is determined at the higher temperature, and the Arrhenius equation employed to calculate the degradation rate at the desired temperature.

This method is valid only if the mechanism of degradation remains constant over the temperature range of both the experiment and the extrapolation. If the Arrhenius plot deviates from linearity outside the experimentally determined region, then there may be a change in mechanism, and the method is invalidated. Although an elevated temperature method is less time consuming than a traditional stability study conducted at room temperature, a full study may take a considerable length of time. It has been shown that, for a compound with a degradation rate of 1%/year at 298K and an activation energy of 75kJ mol^{-1}, the degradation rate at 348K would increase to 1.52%/week (ref. 5). It would, therefore, take about two weeks to obtain any useful data from such an experiment. Repeat experiments over the range of temperatures required to construct an Arrhenius plot would lead to a study time approaching ten weeks.

Since all chemical and physical processes are accompanied by a change in heat content or enthalpy, it is possible, in principle, to study all chemical reactions using microcalorimetry. The sensitivity of a modern, commercially available microcalorimeter (for example, TAM, Thermometric AB, Jarfalla, Sweden) is 0.1μW, which means that a reaction with a first order rate constant of 1×10^{-11} s^{-1} can be detected. This equates to a half-life of 2,200 years - well within the limits for a pharmaceutical product. It can also be shown that the microcalorimeter is 10,000 fold more sensitive than a commercial DSC (for example, Perkin-Elmer DSC 7), a typical instrument on which conventional high temperature studies are performed (ref. 4). Moreover, the technique is non-invasive, non-destructive and not dependent on the physical form of the sample (i.e. the sample may be a solid, liquid or gas, or any combination of these). The reaction environment may also be controlled during the course of an experiment, allowing regulation of parameters such as pH, gas partial pressure, humidity and stirring. The use of microcalorimetry therefore allows the

study of compounds under any particular set of reaction parameters, reproducing storage conditions, for example.

The output from a heat conduction, isothermal microcalorimeter is power versus time, and it follows that analysis of these data allows information to be obtained on both the *thermodynamics* and the *kinetics* of the reaction under investigation. In the absence of information obtained *via* other chemical assay techniques (for example, HPLC), thermodynamic data do not allow molecular interpretation, but kinetic data form the basis of mechanistic investigations. Many systems that are of pharmaceutical interest, including solid-state or heterogeneous mixtures and biological processes, are complex, and analyses of such cases often present considerable difficulties. The general nature of the microcalorimetric method may offer some advantages for the study of these complex reactions, without the need for additional analytical investigation.

Previous work from our group has resulted in a general method that allows the analysis of calorimetric data obtained for reactions that are perceived as being fast to medium term in duration (ref. 6). It is the purpose of this paper to illustrate how this analysis can be applied to simple solution phase reactions, using both simulated and actual calorimetric data, and how it may be extended to more complex, heterogeneous reaction schemes. We present data and analyses for some simulated calorimetric data (to illustrate the application of the general fitting method) and for some real calorimetric data (the imidazole catalysed hydrolysis of triacetin, a reaction which may be of some future use as a chemical calibrant for microcalorimeters).

MATERIALS AND METHODS

Imidazole (1,20-2), acetic acid (32,009-9) and triacetin (24,088-5), were purchased from Aldrich, and were used as received. Solutions were prepared in pH 5.0 deionised water, obtained by passing distilled water through an ion exchange column (Elgastat Micromeg).

The calorimeter used in these studies was an LKB 2277 Thermal Activity Monitor (TAM, Thermometric AB, Jarfalla, Sweden), which was housed in a temperature controlled environment (293 ±0.1K), allowing a baseline stability of ±0.1μW to be attained. The calorimeter was calibrated periodically, using the electrical substitution method. Experiments were performed at 298 K, in glass ampoules. Ampoules were sealed with crimped aluminium caps, the caps being fitted with rubber sealing discs. Loaded ampoules were allowed to equilibrate for 30 min inside the calorimeter before data collection commenced. The heat flow, in or out, of the reaction ampoule was recorded using the dedicated Digitam 2.0 software. Data analysis was performed using the software package ORIGIN™ (Microcal Software Inc., MA, U.S.A.). Simulated data were derived using the mathematical package Mathcad 6.0 (Mathsoft Europe, U.K.).

Buffer solutions for the triacetin experiments were prepared by dissolution of imidazole (2.72g) and acetic acid (1.6g) in deionised water (10mL). Triacetin (0.16g) was added to buffer (3mL) in a sealed glass ampoule, and the mixture was shaken vigourously to ensure complete dissolution. The ampoule was then loaded into the TAM, using buffer (3mL) as a reference. The heat flow, in or out, of the sample ampoule was recorded over a period of four days, and the experiment was repeated seven times.

EXPERIMENTAL

Integration of the heat flow (power, dq/dt (Φ) in watts) versus time (t, in seconds) plot obtained from a microcalorimeter gives the heat output (q, in joules) for a particular reaction. If a suitable kinetic equation can be written that describes the reaction under investigation, then, if heat output is plotted against heat flow, it is possible, using a suitable graphics software package, to determine the constants of the kinetic equation by a process of iteration (ref. 7). Previous work published by our group detailed a general method that allows the analysis of reactions, considered to be long term in duration, in this way (ref. 7). If the reaction is considered to be fast to medium term in duration, i.e. if there is a significant change in the observable calorimetric signal over the observation period, then a second general method for analysis may be applied (ref. 6). It can be shown that, for simple reaction schemes, such as A→B or A+B→C etc.,

equations can be derived that allow the determination of some of the reaction parameters, before the iterative procedure is applied. Knowledge of such parameters simplifies the fitting procedure, because the program is fitting fewer variables. These equations are listed below (ref. 6).

$$\Phi = k.\Delta H^{1-n}.(Q-q)^n \tag{1}$$

$$\frac{t_2}{t_1} = \frac{(\Phi_2)^{\frac{1-n}{n}}}{(\Phi_1)^{\frac{1-n}{n}}} \tag{2}$$

$$Q = \frac{q_1 - R.q_2}{1-R} \tag{3}$$

(where $R = (\Phi_1/\Phi_2)^{1/n}$, Φ_1 and Φ_2 being two power values chosen at convenient intervals from a power-time plot)

$$t_{\frac{1}{2}} = \frac{2^{n-1}-1}{(n-1).k.A^{n-1}} \tag{4}$$

Using the equations outlined above, it should be possible to analyse the power-time signal for any given reaction that can be described by (1). To illustrate how this method may be applied, some power-time data were generated *via* a mathematical software package (Mathcad 6.0), using randomly chosen reaction parameters, fig. 1. These data represent a typical calorimetric trace that one might obtain from a simple solution phase reaction, and were generated using the integrated form of (1). Using a suitable fitting program (Origin™), these data were analysed in a systematic way, using the following procedure. Since the reaction parameters are known in advance, it is possible to cross-check the answers determined at any stage to ensure that the method is valid.

The calculation of reaction order. The reaction order may be determined using (2), since it has been shown that the ratio of two time points, selected at random from any particular power-time plot, will be a constant, for a given order of reaction (ref. 6). Two time points, with associated power values, were selected from the constructed power-time plot (t_1=13600s, Φ_1=59.278μW, t_2=82100s, Φ_2=18.182μW), and the initial power value at t_0 was recorded (Φ_0=84.0μW). The two power values were converted into percentages of the initial power value (70.57% and 21.65% respectively), and a table of t_2/t_1 values was constructed, as a function of the rate constant, fig. 2. Calculating the t_2/t_1 constant for the constructed plot, (82100s/13600s=6.037), allowed the order of reaction to be read off from this table. The closest t_2/t_1 value in the table to the measured t_2/t_1 value is 6.011, giving the correct reaction order of 2.

$n := 2 \quad k := 0.07 \quad H := 30 \cdot 10^9 \quad A := 2 \cdot 10^{-4}$

$Q := A \cdot H \quad t := 0, 100 .. 500000$

$$f(t) := \left[k \cdot H^{1-n} \cdot (n-1) \cdot t + Q^{1-n}\right]^{\frac{1}{1-n}}$$

$$D_t := k \cdot H^{1-n} \cdot f(t)^n$$

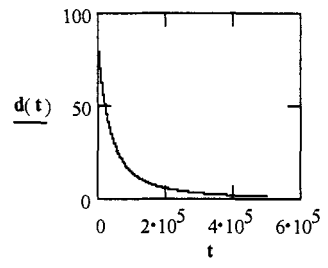

Fig. 1 Simulated power (μW)-time (s) calorimetric data.

The calculation of Q. Once the reaction order has been determined, Q may be calculated. Two power values were chosen from the power-time plot, along with their associated values of q (Φ_1=59.278μW, q_1=959678.21μJ, Φ_2=18.182μW, q_2=3208525.07μJ). Substitution of these values into (3) allows Q to be determined (Q=5999973.72μJ).

The determination of $t_{½}$. Once Q has been determined, $t_{½}$ can be calculated by integrating the power-time plot between t=0 and such a time where the integrated area equals $Q/2$. In this case, $Q/2$=2999986.86μJ, and $t_{½}$ was found to equal 71900s. Note that, because of the nature of the fitting program, this $t_{½}$ value is approximate, since the program can only integrate between actual recorded data points. The value of $t_{½}$ may also be calculated *via* (4), although this requires knowledge of the other reaction parameters. Once these other parameters have been determined, through a process of iteration, the $t_{½}$ value obtained from (4) may be used as a convenient cross-check.

t2(n) / t1(n)	n
5.346	1.5
5.502	1.6
5.646	1.7
5.778	1.8
5.899	1.9
6.011	2
6.114	2.1
6.209	2.2
6.298	2.3
6.381	2.4
6.458	2.5

Fig. 2 Calculated values of t_2/t_1 versus n.

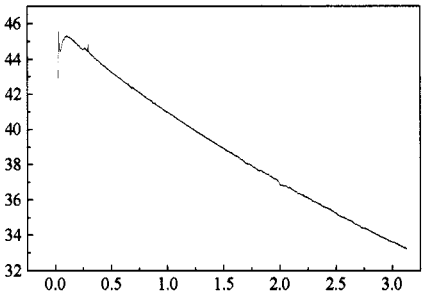

Fig. 3 Power (μW) vs. time (days) for the imidazole catalysed hydrolysis of triacetin.

The calculation of $k.\Delta H^{1-n}$. From (1), a plot of Φ versus $(Q-q)^n$ should give a straight line of slope $k.\Delta H^{1-n}$. This term is a constant, for any given set of reaction parameters, and may be used as a cross-check, after the reaction parameters have been determined. In this case, a plot of Φ versus $(Q-q)^n$ gave a straight line of slope equal 2.33×10^{-12}.

The determination of k, ΔH and A. The simulated data were imported into a graphics fitting program (ORIGIN™), and the iterative fitting procedure was applied, having set the value of n equal 2, using the fitting function of the program and (1). In order to start the fitting process, initial estimates of the constants, (k, ΔH and A), need to be entered into the program. These estimates do not need to be particularly close to the true values, since these would not be known for real data, but, for convenience, should be of the same order of magnitude to that which would be expected. If no initial estimates are entered, the program cannot fit the data, although it was found that a good fit could be obtained, after entering poor estimates, if the iterative procedure was repeated many times. The fitting procedure was continued until there were no more changes in the values of the fitted constants. To further check the validity of the fitted constants, values of $t_{½}$ and $k.\Delta H^{1-n}$ were calculated.

It was found that the fitted values of the constants were very dependent on the quality of the initial estimates, and that a large number of equally valid solutions could be obtained for the fitting equation. These solutions all gave the same value of statistical fit to the simulated data (chi^2 values), and were found to be valid solutions to the cross-checking methods detailed above. These data are represented in table 1. Upon entering the correct value for A into the fitting package, this value being known in this case, and fixing this value constant, the correct values for ΔH and k were obtained. Without prior knowledge of the values of the reaction parameters, there appears to be no way of determining which solution set is correct. It therefore appears that, in order to fit real calorimetric data, some knowledge of the value of at least one of the reaction parameters is required, in order that the fitting procedure can determine the correct parameters. For simple solution phase reactions, the value of A is easy to determine, but a more complicated situation arises for solid-state or heterogeneous reaction systems. It would seem that in these

cases, a more detailed examination of the reaction scheme will be needed before the analysis of calorimetric data can be attempted, such that a reasonable value for A may be determined. In all cases, the maximum value of A will be known, since this will equal the amount of substance added to the reaction cell. Knowledge of one of the other parameters may often be obtained from the literature. It should be noted, however, that the value of n is determinable, *without* prior knowledge of any parameters and, hence, shelf-lives should be calculable for all systems.

TABLE 1. Fitted Reaction Parameters for the Simulated Calorimetric Data

A (mol)	ΔH (kJ mol^{-1})	k	Chi^2	$k.\Delta H^{1-n}$	$t_{½}$ (s)
0.00072	8.33	0.0194	3.5719×10^{-12}	2.33×10^{-12}	71592
0.0004	15.00	0.035	3.5719×10^{-12}	2.33×10^{-12}	71429
0.00013	46.15	0.108	3.5719×10^{-12}	2.34×10^{-12}	71225
0.0001	60.00	0.14	3.5719×10^{-12}	2.33×10^{-12}	71429
0.00009	66.67	0.156	3.5719×10^{-12}	2.34×10^{-12}	71225

The results from the simulated calorimetric data show that it is possible to create a typical calorimetric trace using known reaction parameters, and use the general fitting method to recover those parameters, assuming that one has a good idea of the value of either k, ΔH or A. In order that the method could be applied to real calorimetric data, the imidazole catalysed hydrolysis of triacetin was studied using the microcalorimeter. This reaction was selected because it has been suggested as a possible solution phase calibrant for microcalorimeters (ref. 8), and is accepted as being first order. In the case where a reaction is first order, a plot of $\ln\Phi$ versus time will yield a straight line of slope equal k. Once the value of k has been determined, it should be possible to determine the remaining reaction parameters via the iterative process. The reaction may also be described by (1) and, since it is solution phase, the value of A may be calculated with a reasonable degree of accuracy.

A typical power-time plot obtained for this reaction is given in fig. 3. In order that results from different experiments could be compared directly, the power-time data were analysed between specific time intervals. In this case, the time intervals chosen were 12-24h, 24-48h and 48-72h. The initial data, recorded between 0-12h were disregarded because an initial disturbance necessarily arises from simply loading the calorimeter (ca. 1h). Values of k were determined from plots of $\ln\Phi$ versus time. Although these plots gave a good linear fit (r of the order of -0.99), there was observed to be a distinct curve in the calorimetric data, and the value of k obtained in this way, over the different time intervals, decreased slightly with an increase in the time interval over which the data were analysed (summarised in table 2). Triacetin is a tri-ester, containing reactive esters in two different chemical environments. It may be the case that the least hindered of these esters reacts preferentially and, if the rate of reaction of the esters differ, then the overall rate observed calorimetrically at any particular time may be derived from a combination of several different processes.

TABLE 2. Fitted Reaction Parameters for the Imidazole Catalysed Hydrolysis of Triacetin

Time Interval (h)	ΔH (kJ mol^{-1})	$k\times10^{-6}$ (s^{-1})	$A\times10^{-4}$ (mol)
12-24	-47.93 ± 0.7	1.233 ± 0.03	7.083 ± 0.2
24-48	-50.14 ± 0.7	1.175 ± 0.02	6.750 ± 0.1
48-72	-51.49 ± 0.8	1.135 ± 0.03	6.175 ± 0.2

Each section of data was imported into the fitting program, and the reaction parameters determined, by process of iteration. Initial estimates of k were those determined as above, while, since the value of A necessarily decreases with time, initial estimates of A were determined *via* (5),

$$A = A_0.\exp(-k.t) \tag{5}$$

where A is the amount of material available for reaction at a particular time, t, and A_0 is the amount of material available for reaction at t=0. Entering, and holding constant, these initial estimates for k and A, allowed the fitting program to determine a value for ΔH. Once these values were known, the fitting

process was repeated, allowing all parameters to be varied, until there were no changes in the values of the fitted parameters with repeat iterations. These data are represented in table 2.

The reproducibility of these data is good, and this may facilitate the use of triacetin as a chemical calibrant for solution phase reactions, although repeat experiments using a mono-ester should show if the change in rate constant with increasing time observed for triacetin is a real phenomenon, and may lead to a better choice of calibrant.

SUMMARY

Using the general method of analysis it is possible to analyse calorimetric data from all reaction systems one might choose to study, to determine the reaction order and, hence, calculate Q, with *no* prior knowledge of any reaction parameters or mechanisms of degradation. This allows the fraction of material reacted at any time to be known, and leads to a practical application; the calculation of shelf-lives. However, in order to fit calorimetric data to a suitable model such that the reaction parameters may be determined, it appears that one must have a good initial estimate of at least one of these parameters. Since the value of A must be less than or equal the amount of reactant added to the calorimetric cell, and the value of ΔH might be estimated from mechanistic knowledge or from the literature, this requirement does not seem unreasonable. If the study reaction is known to be first order, then the reaction parameters may be determined without good estimates for the other parameters, since k may be calculated directly.

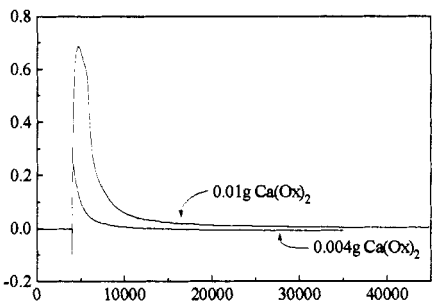

Fig. 4 Power (mW) vs. time (s) for the hydrolysis of calcium oxalate.

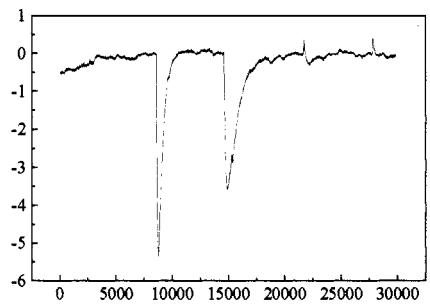

Fig. 5 Power (μW) vs. time (s) for the diffusion of IPM through a (0.005″) Silastic® membrane.

It is possible to obtain calorimetric data for almost any system. More complex examples include the hydration of a metal complex, Fig. 4, and the diffusion of myristic acid isopropyl ester (IPM) through a Silastic® membrane (Sil-Tec Technical products Inc., GA, U.S.A.), Fig. 5. Previously, analyses of such data were considered difficult or impossible, but we have shown that it is now possible to obtain qualitative information from these data, and it is hoped that the general method can be extended to allow full analyses of such systems.

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