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Pharmaceutical microcalorimetry: applications to long-term stability studies

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Abstract

Calorimetry has been a mainstay of stability analyses for some time in the form of differential scanning microcalorimetry (DSC). This technique exploits high (relatively) temperature studies of pure materials and of formulations to accelerate any degradation or interactions. The behaviour of the material at storage or ambient conditions is then estimated via extrapolation from the Arrhenius equation. Recent developments in isothermal microcalorimetry allow the direct determination of both kinetic and thermodynamic parameters for long, slow reactions from studies conducted at appropriate temperatures and under designated environmental control (pH, pO_2, RH etc.). This review introduces the kinetic analysis of microcalorimetric data and, through selected examples, shows applications of the method.

Keywords: Isothermal microcalorimetry; Kinetics; Stability

1. Introduction

Differential scanning calorimetry (DSC) has a long and extensive application in the pharmaceutical industry as a method for the investigation of purity, glass transitions, degradative stability, compatibility etc. (for a review see Ford and Timmins, 1989). The technique is used because the rates of the processes under observation are accelerated at high temperature and hence significant time in evaluating performance under storage conditions is saved. Of course, the data relevant to the storage conditions are deduced by extrapolation through the application of the Arrhenius equation. There is, naturally, the assumption that the process which occurs at the low temperature is the same as that which occurs at the higher temperature. That this is not necessarily true, and that this use of that procedure can introduce difficulty, is illustrated in the example below.

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Table 1
Experimental data for the reaction shown in Scheme A

Temperature (T, °C)	25	35	45	55	60	65
k_{obs} (s^{-1})	0.0047	0.0146	0.054	0.228	0.477	1.00

Consider the mechanism below (Scheme A).

$k_1,\ E_1,\ k_2,\ E_2$

$$A \rightarrow B \rightarrow C \qquad \text{(Scheme A)}$$

Let $E_1 = 62.8$ kJ mol^{-1}, $E_2 = 146$ kJ mol^{-1} and $k_{obs} = k_1 + k_2$. The experimental data are represented in Table 1. Analysis of the last three data points, i.e. using high temperature DSC data shows a linear plot (correlation coefficient 0.999) and a predicted value of k_{obs} at 25°C of 0.0015. This value is wrong by a factor of three. Here, competing processes have been shown to have a significant impact upon the predicted outcome of a reaction at 25°C. Examples of such issues are phase changes, moisture transfer (e.g. excipient–drug) etc. As such, DSC experiments are conducted at high temperature, then it is difficult to control the environmental factors such as RH, pH, oxygen tension etc.

A method for establishing stability by direct observation under controlled ambient conditions would be useful as those analytical methods which are currently used are not sufficiently sensitive to allow slow reactions to be studied: hence the need for temperature rises to accelerate reaction rates. To illustrate the application of isothermal microcalorimetry to excipient compatibility testing, and to stability assessment in general, it is instructive to compare the performance of a DSC with that of a typical isothermal heat conduction microcalorimeter such as the TAM (Thermal Activity Monitor, Thermometric AB, Järfälla, Sweden). Table 2 shows the typical performance parameters for these two types of instrument and reveals that the TAM is 10 000-fold more sensitive than is a standard DSC.

Table 3 shows the temperature rise that is required to accelerate reactions with activation enthalpies of 50, 75 and 100 kJ mol^{-1} by factors of between 1 and 100 000.

This table shows that if a reaction of rate 1 and activation enthalpy of 50 kJ mol^{-1} is observable in a TAM at 20°C then for this reaction to be observed in a DSC the temperature will have to be raised by 239°C. An extrapolation over 239°C would be hazardous. There has not been, as yet, a satisfactory method for the deconvolution of microcalorimetric data to determine rate constants, order of reaction and enthalpy of reaction. Recently Willson has described a general procedure for the determination of both thermodynamic and kinetic parameters from microcalorimetric output data (Willson, 1995; Willson et al., 1995b). The procedure takes a kinetic equation for a particular reaction, and modifies it such that it applies directly to microcalorimetric data. This is achieved by recognition of the fact that the total heat evolved during the course of a reaction (Q) is equal to the total number of moles of material reacted (A_o) multiplied by the change in molar enthalpy for that reaction (ΔH) (Eq. (1)).

$$Q = A_o\ \Delta H \qquad (1)$$

Similarly, the heat evolved at time $t(q)$ is equal to the number of moles of material reacted (x) at time t multiplied by the change in molar enthalpy for that reaction (Eq. (2)).

$$q = x\ \Delta H \qquad (2)$$

Eq. (2) may be substituted into a general rate expression of the form dx/dt to give an expression

Table 2
Comparison of differential calorimetry and isothermal microcalorimetry

	DSC	TAM
Sensitivity (W)	10	0.1
Sample mass (mg)	10	1000
Specific sensitivity (W g^{-1})	1000	0.1

Table 3
The temperature increase, over 20°C, that is required to accelerate a reaction by the specified factors

Factor of rate increase	Activation enthalpies (kJ mol^{-1})		
	50	75	100
1	0	0	0
10	37	24	17
100	85	52	37
1000	149	85	59
10 000	239	125	85
100 000	375	175	114

of the form dq/dt (or power). For example, the general rate expression for a simple, first-order, A → B process is given by Eq. (3).

$$\frac{dx}{dt} = k(A_o - x) \quad (3)$$

Substitution of Eq. (2) into Eq. (3) yields,

$$\frac{dq}{dt} = k\,\Delta H\left(A_o - \frac{q}{\Delta H}\right) \quad (4)$$

This modified rate expression may be used to fit power–time data recorded using the microcalorimeter by a process of iteration. Using this method, Willson showed how it is possible to write calorimetric equations that describe a range of commonly encountered mechanisms (Willson, 1995; Willson et al., 1995b) (Table 4). It is also possible, if the integrated form of the transformed calorimetric equation is known, to simulate calorimetric data using a suitable mathematical worksheet (for example, Mathcad 6.0, Mathsoft Inc.) (Willson, 1995). In this way, it is possible to obtain values for reaction parameter by fitting real calorimetric data and deconvolute complex data into their component parts using the worksheet.

Of these equations, perhaps the most interesting is the Ng equation (Ng, 1975; Table 4), an equation which is stated to describe all two-state solid phase reactions. If the calorimetric form of the Ng equation is used to fit suitable calorimetric data then it is possible, from a knowledge of the values of the constants *m* and *n*, to infer some information on the likely process by which the study reaction occurs. Values of *m* and *n* and the related respective reaction processes are given in Table 5.

Calorimetric forms of the Ng equation were used by Willson et al. (1995a) to analyse the solid-state degradation of L-ascorbic acid. Known amounts (0.5g) of dry L-ascorbic acid were placed in an ampoule along with a known quantity of water and the heat changes in the sample were recorded using an isothermal microcalorimeter.

Table 4
Transformed calorimetric equations for a range of reaction types.

Reaction scheme	Kinetic expression	Transformed expression
A → B	$\frac{dx}{dt} = k(A-x)^m$	$\frac{dq}{dt} = \Delta H k\left(A - \frac{q}{\Delta H}\right)^m$
A + B → C	$\frac{dx}{dt} = k(A-x)^m \times (B-x)^n$	$\frac{dq}{dt} = \Delta H k\left(A - \frac{q}{\Delta H}\right)^m \times \left(B - \frac{q}{\Delta H}\right)^n$
A ⇔ B	$\frac{dx}{dt} = k\left(\frac{A}{x_e}\right)(x_e - x)$	$\frac{dq}{dt} = kA\left(\Delta H - \frac{q}{x_e}\right)$
A + B ⇔ C	$\frac{dx}{dt} = k_1(A-x) \times (B-x)$	$\frac{dq}{dt} = k_1(A\,\Delta H - q) \times (B\,\Delta H - q)(k_{-1}q)$
Ng* equation	$\frac{dx}{dt} = Ak\left(\frac{x}{A}\right)^m \left(1 - \frac{x}{A}\right)^n$	$\frac{dq}{dt} = Ak\,\Delta H\left(\frac{q}{A\,\Delta H}\right)^m \times \left(1 - \frac{q}{A\,\Delta H}\right)$
Auto-catalytic	$\frac{dx}{dt} = k(A-x) \times (x_c + x)$	$\frac{dq}{dt} = k(A\,\Delta H - q) \times (x_c\,\Delta H + q)$
Coagulation	$\frac{dx}{dt} = k(n-x)^2$	$\frac{dq}{dt} = k\,\Delta H\left(n - \frac{q}{\Delta H}\right)^2$
Michaelis–Menten	$\frac{d[ES]}{dt} = k(K_c[E][S])$	$\frac{dq}{dt} = k\,\Delta H\left(k_c[E][S] - \frac{q}{\Delta H}\right)$

Table 5
Reaction mechanisms described by the Ng equation with the particular variables shown

m	n	Equation	Character
0	0	$d\alpha/dt = k$	Linear
1	0	$d\alpha/dt = k\alpha$	Exponential
0.5	0	$d\alpha/dt = k\alpha^{1/2}$	Square
0	1	$d\alpha/dt = k(1-\alpha)$	Unimolecular decay
0	0.5	$d\alpha/dt = k(1-\alpha)^{1-1/2}$	Contracting surface
0	0.66	$d\alpha/dt = k(1-\alpha)^{2/3}$	Contracting sphere
1	1	$d\alpha/dt = k\alpha(1-\alpha)$	Prout–Tompkins
0.66	0.66	$d\alpha/dt = k\alpha^{2/3}(1-\alpha)^{2/3}$	Roginskii–Shultz

The power-time data obtained were analysed using the transformed Ng equation and the parameters obtained are shown in Table 6. It was shown that, at low added quantities of added water, the reaction could be satisfactorily described by solid-state kinetics, but at high added quantities of water (more than 500 μl) the reaction was best described by solution phase kinetics. Indeed, the derived rate constants for 0.5 g ascorbic acid plus water (500 μl or greater) were the same as had been determined from a solution phase study (Willson et al., 1995a). Thus, it is possible to infer mechanistic information from a direct calorimetric measurement and to indicate under what conditions this reaction may be specified as solid-state (water ≤ 200 μl) and as solution state (water ≥ 500 μl).

Willson (1995) also studied the degradation of di-benzoyl peroxide using isothermal microcalorimetry. Literature values for the degradation of di-benzoyl peroxide at 100°C are 3.8×10^{-4} s^{-1} with an activation energy of 125.52 kJ mol^{-1} (Pryor, 1966). Using microcalorimetry, it was possible to study the reaction directly at 25°C. The compound was placed into an ampoule and the heat changes associated with degradation were recorded over a period of 100 h. Since it was assumed that the degradation was mediated by water, and no attempt was made to keep the sample dry, the data were analysed using solution phase kinetics, the results being given in Table 7. Using the Arrhenius equation, the literature data at 100°C were extrapolated to 25°C to give a rate constant of 1.37×10^{-8} s^{-1}, a value which compares favourably with the calorimetrically determined value of 5.205×10^{-8} s^{-1}. The enthalpy change for the reaction was determined to equal -19.2 kJ mol^{-1}. No literature value for this parameter was available, but it compares favourably with the value determined from gas phase bond enthalpies of -20.5 kJ mol^{-1}. These data show that it is possible to analyse relatively complex, solid phase reactions to obtain good estimates of kinetic and thermodynamic parameters. The data presented in Table 7 show that the reproducibility of the technique is excellent.

The interconversion of nitritopentamminecobalt (III) chloride to nitropentamminecobalt (III) chloride was also studied by Willson (1995). This reaction has been the subject of previous studies using IR spectroscopy (Penland et al., 1956) and XRD (Lecompte and Duval, 1945) but no study had determined the kinetic parameter for the reaction. Using isothermal microcalorimetry,

Table 6
Reaction parameters determined for the reaction between ascorbic acid and various quantities of water[a]

Added water (μl)	Rate constant (s^{-1})	ΔH (kJ mol^{-1})	A_o (moles)	m	n
Dry	3.15×10^{-6}	199	4.39×10^{-7}	−0.01	0.9
20	4.10×10^{-6}	199	2.25×10^{-6}	−0.02	0.6
30	4.35×10^{-6}	199	2.39×10^{-6}	−0.01	0.8
50	5.21×10^{-6}	197	3.93×10^{-6}	−0.01	0.8
100	5.62×10^{-6}	180	4.27×10^{-6}	−0.12	0.6
200	4.22×10^{-6}	188	8.05×10^{-6}	−0.09	0.6
Average	4.10×10^{-6}	193		−0.02	0.71

[a] If more than 500 μl of water was added, the reaction followed solution phase kinetics (Willson et al., 1995a).

Table 7
Reaction parameters determined for the degradation of di-benzoyl peroxide at 25°C (Willson, 1995)

	k (s^{-1})	ΔH (kJ mol^{-1})	Quantity of material reacted (moles)	Order
	5.190×10^{-8}	−19.2	1.0×10^{-4}	1
	5.219×10^{-8}	−18.6	1.03×10^{-4}	1
	5.208×10^{-8}	−19.9	8.7×10^{-5}	1
Average	$5.205\times10^{-8}\pm1.46\times10^{-10}$	-19.2 ± 0.65	$9.6\times10^{-5}\pm8.5\times10^{-6}$	1

Willson (1995) obtained an estimate for this parameter, and the thermodynamic reaction parameters, directly at 25°C by fitting the data to the transformed Ng equation (Table 8). The interconversion of one complex to the other is regarded as a physical process whereas the reaction of ascorbic acid, discussed above, is a chemical process. Using the Willson method it is possible to study both types of reaction directly. Note here that the iterative method is successful for a reaction of very low enthalpy change; ΔH equals ≈ 3 kJ mol^{-1}.

More recent work has shown that it is possible to extend the Willson method of analysis to more complex reaction schemes (Gaisford, 1997). For example, it is possible to apply the Willson method to reaction schemes following consecutive, first-order mechanisms. Take, for example, the case shown below;

$$A \overset{k_1}{\rightarrow} B \overset{k_2}{\rightarrow} P$$

(First-order, ΔH_1 and ΔH_2 respectively).

It has been shown (Gaisford, 1997) that the calorimetric equation which describes this reaction is,

$$\frac{dq_{obs}}{dt} k_1 \Delta H_1 A_o e^{-k_1 t} + k_1 k_2 \Delta H_2 A_o \frac{e^{-k_1 t} - e^{-k_2 t}}{k_2 - k_1} \quad (5)$$

Similarly, if the related, three-step mechanism is considered;

$$A \overset{k_1}{\rightarrow} B \overset{k_2}{\rightarrow} C \overset{k_3}{\rightarrow} P$$

(First-order, ΔH_1, ΔH_2 and ΔH_3, respectively).

It has been shown (Gaisford, 1997) that the calorimetric equation that describes this mechanism is,

$$\begin{aligned}\frac{dq_{obs}}{dt} &= k_1 \Delta H_1 A_o e^{-k_1 t} \\ &+ k_1 k_2 \Delta H_2 A_o \frac{e^{-k_1 t} - e^{-k_2 t}}{k_2 - k_1} \\ &+ \Delta H_3 \Bigg(A_o k_1 e^{-k_1 t} \\ &- \frac{A_o k_1}{(k_2 - k_1)} (-k_1 e^{-k_1 t} + k_2 e^{-k_2 t}) \\ &+ \frac{k_2^2 k_1 A_o e^{-k_2 t}}{-k_1 k_2 - k_3 k_2 + k_2^2 + k_3 k_1} + \\ &\frac{k_2 k_1^2 A_o e^{-k_1 t}}{-k_1 k_2 + k_3 k_2 - k_3 k_1 + k_1^2} \\ &- \frac{-k_2 k_1 A_o k_3}{-k_1 k_2 - k_3 k_2 + k_2^2 + k_3 k_1} \\ &- \frac{k_2 k_1 A_o e^{-k_3 t}}{-k_1 k_2 + k_3 k_2 - k_3 k_1 + k_1^2} \Bigg) \end{aligned} \quad (6)$$

The use of these equations to analyse calorimetric data has been demonstrated using a model reaction, the acid catalysed hydrolysis of potassium hydroxylamine trisulfonate (Gaisford, 1997; Gaisford et al., 1998), which reacts according to the following model;

$$(SO_3)_2N.O.SO_3^{3-} + H_2O \overset{k_1,\ \Delta H_1}{\rightarrow} SO_3.NH.O.SO_3^{2-} + HSO_4^-$$

$$NH.O.SO_3^{2-} + H_2O \overset{k_2,\ \Delta H_2}{\rightarrow} NH_2.O.SO_3^- + HSO_4^-$$

$$NH_2.O.SO_3^- + H_2O \overset{k_3,\ \Delta H_3}{\rightarrow} NH_2.OH + HSO_4^-$$

The reaction has been studied previously using a titration method (Candlin and Wilkins, 1960, 1961). The hydrolysis of the trisulfonate ion is the fastest of the three steps, and produces the relatively stable hydroxylamine-*NO*-disulfonate ion. Potassium hydroxylamine-*NO*-disulfonate is sta-

Table 8
Reaction parameters determined for the interconversion of nitritopentamminecobalt (III) chloride to nitropentamminecobalt (III) chloride (Willson, 1995)

	Rate constant	ΔH (kJ mol^{-1})	Quantity of material reacted (moles)	m	n
Analysis 1	3.99×10^{-6} s^{-1}	−1.73	0.0035	1.14	0.45
Analysis 2	3.55×10^{-6} s^{-1}	−2.65	0.0039	1.099	0.45
Analysis 3	1.4×10^{-6} s^{-1}	−4.60	0.0045	1.1	0.3
Average	$2.98\times10^{-6}\pm$ 1.58×10^{-6} s^{-1}	$-2.99\pm$ −1.2	$0.0039\pm$ 0.0004	1.1	0.4

ble enough to be recrystallised from dilute acid solution. The disulfonate is hydrolysed slowly in dilute acid solution forming the hydroxylamine-*O*-sulfonate ion and, eventually, hydroxylamine and hydrogen sulfate. Hydrogen sulfate is formed in each reaction step, and chemical assays for this ion were used to perform the early kinetic studies. It was observed, using ^{35}S-labelled compounds, that the first hydrolytic step proceeded at a much faster rate than the second and third hydrolyses, the final two hydrolyses proceeding at similar, slow, rates. The authors of the original work were unable to study the slower reaction steps at room temperature, and had to use temperatures of up to 70°C. Using the microcalorimeter, it was possible to study the reaction directly at 25, 30 and 35°C.

The power–time data obtained were fitted to Eq. (6) to determine the values of the reaction parameters. A typical fit line is represented in Fig. 1. The data were also fitted to Eq. (5) to see if the models possessed sufficient sensitivity to distinguish mechanistic information (Fig. 2). It is immediately apparent that the simpler model did not fit the experimental data satisfactorily while the more complex model gave an excellent fit, indicating that the power–time data recorded derive from a reaction undergoing a three step mechanism. The two step model gives a reasonable fit over the initial section of data, where the first two hydrolysis reactions predominate, but cannot fit the section of data where all three hydrolyses are occurring. Such an experiment shows that the models are sufficiently sensitive to discriminate between different reaction mechanisms.

Analysis of the data allowed estimates to be made for all the reaction parameters. Those deter-

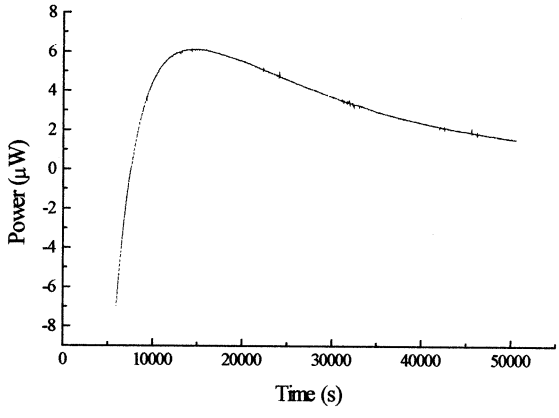

Fig. 1. Power–time data for the acid catalysed hydrolysis of potassium hydroxylamine trisulfonate (0.005 M) at 35°C (0.0005 M acid added), and the fit line obtained after fitting the data to Eq. (6).

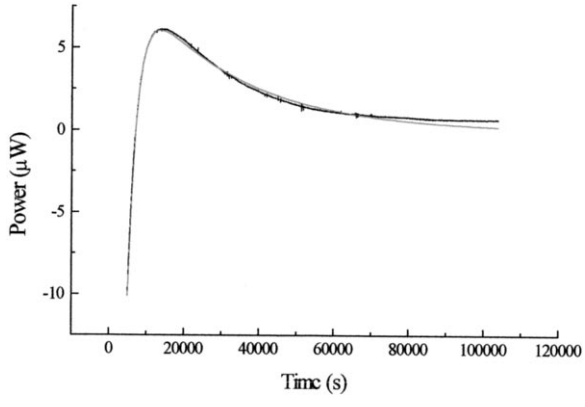

Fig. 2. Power–time data for the acid catalysed hydrolysis of potassium hydroxylamine trisulfonate (0.005 M) at 35°C (0.0005 M acid added), and the fit line obtained after fitting the data to Eq. (5).

Table 9
ΔH values for the acid catalysed hydrolysis of potassium hydroxylamine trisulfonate (0.005M), determined by fitting power–time data to Eq. (6) (Gaisford et al., 1998)

Temperature (°C)	ΔH_1 (kJ mol^{-1})	ΔH_2 (kJ mol^{-1})	ΔH_3 (kJ mol^{-1})
35	134.0	104.8	−167.7
35	147.0	141.0	−82.36
35 (Average)	140.5	122.9	−125.0
30	135.3	−31.75	−37.44
30	56.37	−35.21	−36.23
30 (Average)	95.84	−33.48	−36.84
25	28.30	−82.23	−47.39

Table 10
Rate constant values for the acid catalysed hydrolysis of potassium hydroxylamine trisulfonate (0.005 M), determined by fitting power–time data to Eq. (6) (Gaisford et al., 1998)

Temperature (°C)	k_1 (s^{-1})	k_2 (s^{-1})	k_3 (s^{-1})
35	5.547×10^{-4}	4.393×10^{-5}	2.589×10^{-4}
35	4.705×10^{-4}	5.018×10^{-5}	2.051×10^{-4}
35 (Average)	5.126×10^{-4}	4.751×10^{-5}	2.320×10^{-4}
30	4.378×10^{-4}	2.926×10^{-5}	5.802×10^{-5}
30	4.387×10^{-4}	2.599×10^{-5}	6.648×10^{-5}
30 (Average)	4.381×10^{-4}	2.763×10^{-5}	6.225×10^{-5}
25	4.246×10^{-4}	4.561×10^{-6}	6.712×10^{-6}

mined at an added acid concentration of 0.0005 M, are shown in Tables 9 and 10 (Gaisford et al., 1998). It is apparent that, in most cases, the rate constants determined for the second and third hydrolyses are smaller than those for the first hydrolysis, an observation in accordance with expectation.

2. Summary

The isothermal microcalorimeter is shown to have application to slow reactions. It is also indifferent (in principle) to the physical state of the sample. The examples illustrating this review indicate applications to solid and solution state processes and to processes of low enthalpy. Furthermore, even though the isothermal miycrocalorimeter does not, directly, reveal molecular detail it has been shown that mechanistic information can be deduced from output data.

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