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Stability assessment of pharmaceuticals by isothermal calorimetry: two component systems

Cyrus V. Skaria, Simon Gaisford*, Michael A.A. O'Neill,
Graham Buckton, Anthony E. Beezer

Department of Pharmaceutics, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, UK

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Abstract

Isothermal calorimetry offers the potential to determine rapidly the stability of formulated pharmaceuticals because it is indifferent to physical form and sensitive enough to detect extremely small powers; ca. 50 nW at 25 °C. However, its use in this area is not widespread, principally because the power–time data obtained often comprise contributions from more than one process and are thus difficult to analyse quantitatively. In this work, we demonstrate how power–time data recorded for systems in which two components are degrading in parallel (in this case, binary mixtures of selected parabens) can be analysed using a kinetic-based model; the methodology allows the determination of the first-order rate constant and reaction enthalpy for each process, so long as one rate constant is at least twice the magnitude of the other. It was found that the reactions did not need to run to completion in order for the analysis to be successful; a minimum of 15 min of data were required for samples with one degrading component and a minimum of 4 h of data were required for samples with two degrading components. It was observed that the rate constants for paraben degradation in binary systems were significantly lower than expected. This was ascribed to the fact that the parabens degrade to a common product and is an important factor that should be accounted for when the two or more parabens are formulated together.

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Keywords: Isothermal calorimetry; Microcalorimetry; Degradation kinetics; Stability assessment; Methyl paraben; Ethyl paraben; Propyl paraben

1. Introduction

An assessment of the long-term stability of an active pharmaceutical ingredient (API), or the likelihood of any active-excipient incompatibility, is an important part of the formulation process of a medicine. Isothermal calorimetry (IC, the measurement of power as a sample is maintained at constant temperature) is ideally suited for stability assessment of pharmaceuticals for a number of reasons; it is indifferent to the physical form, or heterogeneity, of a sample; the data contain both kinetic and thermodynamic information on

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* Corresponding author. Tel.: +44 20 7753 5863;
fax: +44 20 7754 5942.
E-mail address: simon.gaisford@ulsop.ac.uk (S. Gaisford).

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the process(es) under investigation; samples are studied non-destructively (that is, the calorimeter causes no additional degradation other than that which would have occurred anyway upon storage); and the sensitivity of modern instruments is such that, in principle, reactions with a first-order rate constant of $1 \times 10^{-11}\,s^{-1}$ (corresponding to 0.03% degradation per year) can be detected directly at 25 °C (Willson, 1995).

Pikal (1983) first showed the correlation between the exothermic power output of some pharmaceutical systems and their known degradation rates, showing that degradation rates of the order of 2% per year were easily quantified. Subsequently, IC has been used to investigate the stability of a number of APIs alone (Oliyai and Lindenbaum, 1991; Angberg et al., 1993; Willson et al., 1995a; Hansen et al., 1990; Pikal and Dellerman, 1989) and a number of approaches have been suggested that allow qualitative screens of API-excipient compatibility (Phipps et al., 1998; Schmitt et al., 2001).

However, since heat is a universal indicator of chemical change the data obtained from IC, especially for multi-component systems, are often complex, because the calorimeter records the power changes from all the processes occurring in the sample simultaneously (indeed, care must be taken to ensure that erroneous or unexpected powers do not arise simply as a consequence of poor experimental design or sample handling) and a lack of data analysis routines has limited its more widespread application. The analytical challenge, therefore, is to be able to analyse such complex data, with no prior knowledge of the number or type of reaction processes, and to deconvolute them into their constituent parts.

One promising approach to meet this challenge is to fit IC data to models based on reaction kinetics using an iterative procedure (Willson et al., 1995a,b, 1996). This returns rate constants (k) and reaction enthalpies (ΔH) for each individual reaction step and, in theory, does not require any prior knowledge of reaction mechanism (a range of models is used to fit the data and that which gives the best statistical fit, with the fewest variables, is selected. However, if, as in this case, the reaction mechanism is known (or suspected) then the specific model can be used; see below for further discussion of this point). Importantly, this method does not require the reaction under study to progress to completion (depending upon the reaction parameters, approximately 24 h of power–time data are usually sufficient to allow analysis, although this may need to be extended for reactions that progress slowly or with a small change in enthalpy). We have shown previously how this approach can be used to study: (i) a degradation reaction with three consecutive, first-order steps (Gaisford et al., 1999) and (ii) a second-order hydrolysis (that is now recommended as a chemical test reaction for calorimeters; Beezer et al., 2001a). However, these studies had only one degrading component. A typical formulated pharmaceutical may well have several independently degrading components and, although the degradation kinetics of the individual components of a medicine may be known, their behaviour in combination may be significantly different.

The applicability of IC to the quantitative analysis of systems containing more than one degrading material has not been reported and is the focus of this work. Of particular interest is the ability of the models to determine the number of degrading components in a complex system and the minimum number of data needed to effect an accurate analysis. Kinetic models have been used to analyse power–time data for two-component systems; aqueous solutions containing binary mixtures of selected parabens. The parabens were selected for study because their degradation kinetics are known (Kamada et al., 1973) (more recently, the base-catalysed hydrolysis of methyl paraben (MP) has been suggested as a test reaction for flow microcalorimeters; O'Neill et al., 2003); they can degrade through consecutive steps dependent upon solution pH (Sunderland and Watts, 1984); they provide a model example of parallel degradation; and they have found widespread application in pharmaceuticals, foods and cosmetics as preservatives (where it is usually the case that at least two parabens are in any particular formulation).

2. Materials and methods

2.1. Materials

Methyl 4-hydroxybenzoate (methyl paraben), ethyl 4-hydroxybenzoate (ethyl paraben, EP), *n*-propyl 4-hydroxybenzoate (*n*-propyl paraben, PP) and *p*-hydroxybenzoic acid (pHBA), all >99%, were purchased from Fluka. Sodium hydroxide was purchased from VWR. All materials were used as received. Solutions were prepared in distilled, de-ionised water.

2.2. Isothermal microcalorimetry

Experiments were conducted using a 2277 Thermal Activity Monitor (TAM, Thermometric AB, Järfälla, Sweden) at 25 °C. Solutions were prepared by dissolving the required amounts of MP, EP and/or PP (0.05 M with respect to each component) in sodium hydroxide solution (0.5 M). Solution pHs were measured before and after each experiment and were found to be constant at pH 12.3. The time at which the addition of solute to water was made was noted and designated t_0. Aliquots of solution (3 ml) were pipetted into standard glass TAM ampoules; the ampoules were then sealed with a crimped metal lid. An air-tight enclosure was ensured with the use of a rubber seal on the inside of the lid. Sample ampoules were placed in the thermal equilibration position of the TAM for approximately 20 min before being lowered into the measurement position. Data capture was then initiated using the dedicated software package Digitam 4.1. The time at which data capture started was noted and designated t_s. Power data (μW) were recorded every 30 s, for a minimum of 24 h, with an amplifier setting of 300 μW, against a reference ampoule containing sodium hydroxide solution (0.5 M, 3 ml). The instrument was calibrated weekly using an electrical substitution method, and was zeroed before each experiment using buffer against buffer. Samples were run at least in triplicate.

2.3. Data analysis

Data analysis was performed using Origin (Microcal Software Inc., USA). The difference between t_0 and t_s (in seconds) was added to the x-axis data to correct for the time-delay in initiating recording in the TAM (note that this does not affect the magnitude of the power signal, as the calorimeter records the instantaneous power-output as a function of time). Data were analysed using an iterative procedure (in this case, the non-linear curve fitting tool in Origin 7.0, Microcal Software Inc.). Data were fitted to Eq. (1), which describes the power–time response for a single-step reaction following first-order kinetics (Bakri et al., 1988),

$$\text{Power} = \frac{\mathrm{d}q}{\mathrm{d}t} = \Delta H \upsilon k [A_0]\,\mathrm{e}^{-kt} \qquad (1)$$

where q is the heat output of the reaction, ΔH the reaction enthalpy per mole of product formed, υ the volume of solution in the ampoule, k the first-order rate constant and $[A_0]$ is the initial concentration of reactant. The procedure requires initial estimates for all parameters to be entered into the software. Values for υ (0.003 dm^3) and $[A_0]$ (0.05 M) were known and therefore kept constant. The initial values entered for ΔH (1×10^{10} μJ mol^{-1}) and k (1×10^{-5} s^{-1}), values which are entirely reasonable for chemical degradation, were the same for each data set; the software then altered these values until a good fit to the data (as indicated by a low χ^2-value) was obtained. Were the reaction mechanism not known, then the data would have been fitted to a range of models (such as those in Gaisford, 1997) and that giving the best fit would have been selected.

3. Results and discussion

The degradation kinetics of the parabens in aqueous solution are well discussed in the literature; Kamada et al. (1973) recorded degradation of methyl and *n*-propyl paraben in acidic solutions between 40 and 100 °C, Blaug and Grant (1974) reported base-catalysed degradation of methyl, ethyl and *n*-propyl paraben above 70 °C and Raval and Parrott (1967) reported degradation of methyl paraben at pHs 6–9 between 70 and 85 °C. In the most complete kinetic study to date, Sunderland and Watts (1984) studied the hydrolysis of methyl, ethyl and *n*-propyl paraben between pH 1.26 and 10.59 and noted that degradation was fastest at low pH (acid catalysis predominates) or high pH (base catalysis predominates). They determined rate constants of 3.03, 1.26 and 0.93×10^{-2} s^{-1} for methyl, ethyl and *n*-propyl paraben, respectively, at pH 10.59, although their data were recorded over a range of elevated experimental temperatures and extrapolated to 130.5 °C rendering a direct comparison with the data reported here impossible. O'Neill et al. (2003) reported the rate constant for the hydrolysis of methyl paraben in excess base to be 3.15×10^{-4} s^{-1} at 25 °C and 7.94×10^{-4} s^{-1} at 37 °C.

The degradation of methyl paraben is represented schematically in Fig. 1 (the processes for ethyl and *n*-propyl paraben are analogous, the only difference being the alcohol formed). The initial hydrolysis step follows pseudo first-order kinetics and is pH dependent (Kamada et al., 1973; Sunderland and Watts, 1984), although no literature data are available at pHs above

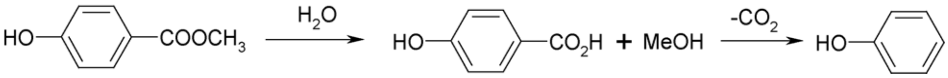

Fig. 1. Reaction pathway for the degradation of methyl paraben.

10.59. Depending upon solution pH, *p*-hydroxybenzoic acid can decarboxylate to form phenol, a reaction first reported by Cazeneuve (1896) whose data showed the acid to be stable in alkaline but unstable in acidic conditions. Quantitative kinetic data are only available for this reaction between pH 1.26 and 10.59, where it has been shown that the rate falls significantly at the higher pHs and the reaction follows first-order kinetics over four to five half-lives (Sunderland and Watts, 1984).

In this study, paraben degradation was studied in NaOH solution (pH 12.3), to ensure that degradation stopped once *p*-hydroxybenzoic acid had formed. The power–time traces obtained for the degradation of the three individual parabens are shown in Fig. 2. The data were fitted to Eq. (1); the fit of each data set to the model is represented by the open circles in Fig. 2. Eq. (1) can be used to derive values for ΔH and k from power–time data, as long as v and $[A_0]$ are known and that the reaction goes to completion. For this system, measurements of paraben degradation performed at 25 and 37 °C resulted in the same total heat output (data not shown) indicating no equilibrium state is reached and, hence, the analysis is appropriate in this case. An alternative analysis has been proposed for systems where this is not the case (Beezer, 2001; Beezer et al., 2001b).

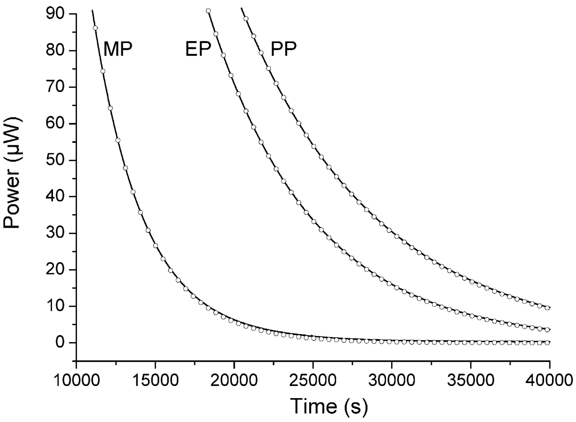

Fig. 2. Power–time data for MP (0.05 M), EP (0.05 M) and PP (0.05 M) in NaOH aqueous solution (0.5 M) at 25 °C and the fit lines (○) generated by application of Eq. (1).

Table 1
Average values for the rate constants and reaction enthalpies for the individual parabens determined by fitting experimental data to Eq. (1)

Ester	k (s^{-1}) (±S.D., n)	ΔH (kJ mol^{-1}) (±S.D., n)
Methyl	3.1×10^{-4} (±0.01, 3)	−59.2 (±0.4, 3)
Ethyl	1.5×10^{-4} (±0.01, 3)	−64.4 (±1.3, 3)
n-Propyl	1.2×10^{-4} (±0.01, 3)	−60.1 (±0.3, 3)

All three parabens were found to degrade following first-order kinetics (as a further check, ln(power) versus time plots were found to be linear, data not shown), allowing rate constant values and reaction enthalpies to be obtained (summarised in Table 1). It is apparent that the degradation rate decreases as the hydrocarbon moiety increases in length, which would be expected on steric grounds, while the reaction enthalpies are roughly equivalent.

It is appropriate to note here the benefits of using this method of analysis compared with other analysis methodologies. Conventionally, the reaction order and the rate constant are determined by plotting some function of power versus some function of time; the plot, which is linear determines the reaction order and the slope gives the rate constant. In the case of a first-order system, a plot of ln(power) versus time, or for a second-order process a plot of $\text{power}^{-0.5}$ versus time, results in a linear relationship. Similarly, enthalpy is conventionally determined by letting the reaction progress to completion and measuring the total heat released. Fitting a section of data to Eq. (1) allows the determination of both the rate constant and enthalpy without needing the reaction to run to completion (often time-consuming) or invoking an extrapolation of data. The approach presented here also allows the determination of reaction order if the general form of Eq. (1) is used (as derived by Willson et al., 1995b; not shown here), and also allows the analysis of non-integral order systems. It was found that the correct reaction parameters were returned even if only the first 15 min of data were used for analysis (Table 2). Fitting greater numbers of data did not alter the fitting values.

Table 2
The reaction parameters obtained by fitting varying time periods of power–time data for MP to Eq. (1)

Time	k (s^{-1})	ΔH (kJ mol^{-1})
15 min	3.1×10^{-4}	−59.1
30 min	3.1×10^{-4}	−59.1
1 h	3.1×10^{-4}	−59.1

Initial experiments on binary systems were conducted on mixtures of MP and PP, since these components had the largest difference in rate constants. Power–time data obtained for MP–PP mixtures could not, as expected, be fitted by Eq. (1) (which, in the absence of any prior knowledge of the system, would have immediately indicated the likelihood of there being more than one event occurring in the sample); the data were, however, described very well by Eq. (2) (which describes two simultaneous first-order decay processes), Fig. 3, allowing the recovery of rate constant and enthalpy values, Table 3.

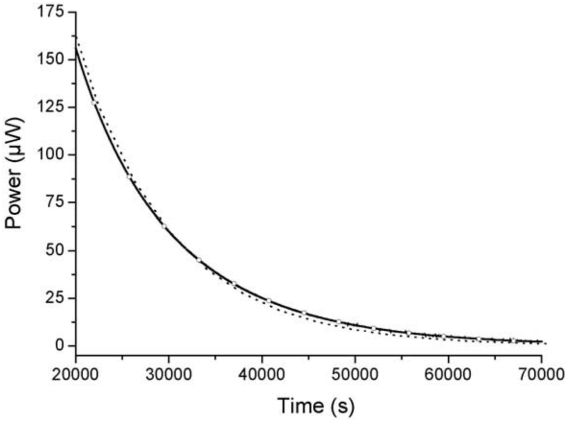

Fig. 3. Power–time data for a binary mixture of MP (0.05 M) and PP (0.05 M) in NaOH aqueous solution (0.5 M) at 25 °C and the fit lines generated by application of Eq. (1) (dotted line) and Eq. (2) (represented by open circles – the fit line to Eq. (2) overlays the raw data).

$$\text{Power} = \frac{dq}{dt} = \Delta H_1 \upsilon k_1 [A_0]\,e^{-k_1 t} + \Delta H_2 \upsilon k_2 [B_0]\,e^{-k_2 t} \qquad (2)$$

where the subscripts 1 and 2 refer to the individual reaction pathways and $[A_0]$ and $[B_0]$ refer to the initial concentrations of reactants A and B, respectively.

Interestingly, while the enthalpy values determined from the mixed system data are the same (within error) as those determined when the species are studied individually, both rate constants are lower than expected. The most likely explanation for this observation is that the two reactants present both degrade to a common product, *p*-hydroxybenzoic acid. The rate of degradation for the degradation of an individual paraben (methyl paraben in this case) is given by,

$$-\frac{d[\text{MP}]}{dt} = \frac{d[\text{pHBA}]}{dt}$$

where [MP] is the concentration of methyl paraben and [pHBA] is the concentration of *p*-hydroxybenzoic acid as a function of time (the rate laws are analogous for the other parabens). In the case of a binary mixture, it is clear that two sources contribute to [pHBA]; this must inevitably cause the rates of disappearance of the two reactants to change.

If MP degradation is measured in a solution of base containing 0.05 M *p*-hydroxybenzoic acid (i.e. a solution that already contains one of the degradation products), the measured rate constant is $2.2 \times 10^{-4}\ \text{s}^{-1}$, a value that is identical to that recorded for MP in the mixed MP:PP system. Furthermore, the total amount of heat released from the binary mixture (16.7 ± 0.6 J) was the same, within error, of that of the sum of heats released by the individual components (17.9 ± 0.6 J), indicating the same extent of reaction taking place in

Table 3
Average values for the rate constants and reaction enthalpies for binary mixtures of the parabens determined by fitting experimental data to Eq. (2)

Ester mix	k_1 (s^{-1}) (±S.D., n)	k_2 (s^{-1}) (±S.D., n)	ΔH_1 (kJ mol^{-1}) (±S.D., n)	ΔH_2 (kJ mol^{-1}) (±S.D., n)
Methyl/ethyl[a]	2.3×10^{-4} (±0.1, 8)	1.1×10^{-4} (±0.1, 8)	n/a	n/a
Methyl/*n*-propyl	2.2×10^{-4} (±0.08, 9)	8.0×10^{-5} (±0.01, 9)	−58.2 (±2.1, 9)	−54.4 (±1.9, 9)
Ethyl/*n*-propyl[a]	1.2×10^{-4} (±0.1, 8)	8.0×10^{-5} (±0.01, 8)	n/a	n/a

[a] The rate constants for these systems were obtained by fixing the enthalpy values constant.

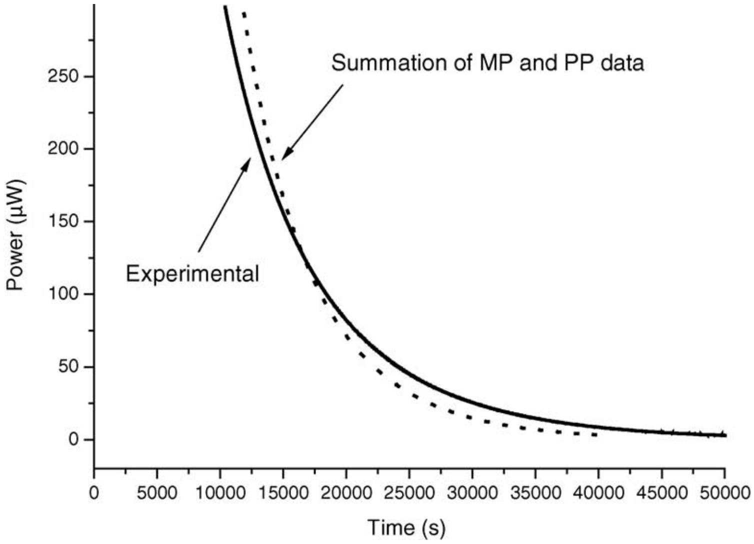

Fig. 4. Power–time data for a binary mixture of MP (0.05 M) and PP (0.05 M) in NaOH aqueous solution (0.5 M) at 25 °C (solid line) and the power–time trace obtained by summation of the data recorded for MP alone and PP alone (dotted line).

all cases. If the power–time data recorded for individual samples of MP and PP are summed, it is clear that the resulting trace differs significantly from that recorded experimentally for the actual binary system (Fig. 4). Fitting these summed data to Eq. (2) resulted in rate constants of 2.9×10^{-4} (±0.1) and $1.1 \times 10^{-4}\ \mathrm{s}^{-1}$ (±0.1) and enthalpies of −60.9 (±2.0) and −53.7 (±2.0) for the methyl and *n*-propyl esters, respectively, values that are in much better agreement with those presented in Table 1.

The observation that degradation rate constants may differ significantly from those expected when materials are formulated in combination is important; in this case, a shelf-life could have been predicted based on the stability data obtained for the individual materials, but the actual shelf-life of the product would have been longer. Other properties of the parabens have been observed to alter when they are formulated in combination (for instance, mixtures of parabens are more effective as preservatives than the individual parabens, Littlejohn and Husa, 1955; Schimmel and Husa, 1956) and this highlights the importance of developing analytical techniques that allow the direct study of heterogeneous samples.

The data for the other two binary mixtures (MP:EP and EP:PP) were found not to be fitted by either Eq. (1) or Eq. (2), when all the parameters were allowed to vary, but were successfully fitted to Eq. (2) when the enthalpy values (as determined for the individual components and shown in Table 1) were fixed. As for the MP:PP binary system, the fitting again returned lower than expected rate constants, Table 3, but these systems also have a common degradation product.

These data serve to show the practical limits of the resolution of the model fitting technique to real data and suggest that one rate constant needs to be at least twice the magnitude of the other to enable a successful analysis, assuming approximately equal enthalpies. If ‘ideal’ data for either the MP:EP or EP:PP system are generated (for instance, using Mathcad) using the data in Table 1 then the model fitting successfully recovers the rate constants using Eq. (2). This suggests that it is the inherent noise in the data that prevents successful analysis, rather than the parameters being too similar. Analysis using simulated data also showed that if one rate constant is more than three orders of magnitude larger than the other, the model fitting is similarly unable to recover both values, because one process dominates the observed signal.

As for the individual components, different sections of data were analysed in order to determine the minimum observation period required to allow the recovery of the correct reaction parameters. Unsurprisingly, the binary mixtures required a greater number of data to be

Table 4
The reaction parameters obtained by fitting varying time periods of power–time data for a binary mixture of MP and PP to Eq. (2)

Time	k_1 (s^{-1})	k_2 (s^{-1})	ΔH_1 (kJ mol^{-1})	ΔH_2 (kJ mol^{-1})
30 min	1.2×10^{-4}	1.2×10^{-4}	−39.1	−50.4
1 h	1.5×10^{-4}	2.0×10^{-5}	−85.2	−24.9
2 h	1.6×10^{-4}	5.0×10^{-5}	−77.3	−29.5
3 h	2.1×10^{-4}	8.0×10^{-5}	−60.1	−52.3
4 h	2.2×10^{-4}	8.0×10^{-5}	−59.1	−56.0

analysed for the fitting method to recover the correct values, Table 4. However, the analysis required just 4 h of data to return the correct parameters; a significant improvement over the methodologies used in existing published work, most of which contain data recorded at a minimum of 70 °C.

As noted above, under certain conditions paraben degradation can proceed via two consecutive steps. If, as expected, *p*-hydroxybenzoic acid is stable under alkaline conditions then paraben degradation should be described by Eq. (1). However, if *p*-hydroxybenzoic acid subsequently degrades to phenol, then the data would be better described by a two-step consecutive model. We have shown previously that a consecutive reaction with two first-order steps is described by Eq. (3) (Gaisford et al., 1999);

$$\text{Power} = \frac{dq}{dt} = \Delta H_1 v k_1 [A_0]\, e^{-k_1 t} + k_1 k_2 H_2 v [A_0] \left(\frac{e^{-k_1 t} - e^{-k_2 t}}{k_2 - k_1} \right) \tag{3}$$

where k_1 and k_2 are the rate constants and ΔH_1 and ΔH_2 are the enthalpies for the two reaction steps, respectively. Power–time data for the degradation of MP were fitted to Eq. (3) and showed a better statistical measure of fit than that obtained when the data were fitted to Eq. (1) ($\chi^2 = 0.008$ for fit to Eq. (3) compared with $\chi^2 = 0.125$ for fit to Eq. (1)). The microcalorimeter provides no direct molecular information and, in the absence of any other supporting data, we have stated that the best approach to determining reaction mechanisms from calorimetric data is to fit the data to a range of models and select that which gives the best fit with the fewest variables (Gaisford et al., 1999). As such, these results suggest that *p*-hydroxybenzoic acid is itself degrading and is not, as suggested by Cazeneuve (1896) stable under alkaline conditions. However, the enthalpy value returned by the fitting process is very small (ca. 0.3 kJ mol^{-1}); if the reaction parameters are used to construct the power–time traces for the two steps, it is clear that the degradation to phenol contributes very little to the observed heat-flow, Fig. 5. An alkaline solution of *p*-hydroxybenzoate gave no detectable heat-flow in the calorimeter over a period of 4 days (data not shown); the same solution also showed no detectable change when analysed by UV spectroscopy over the same time period (*p*-hydroxybenzoic acid λ_{max} 280, phenol λ_{max} 287; data not shown). It may therefore be the case that Eq. (3), having more variables, simply gives a better fit by generating artifacts that are not related to the reaction mechanism and, as stated in the introduction, should be disregarded. Further work will be required using other analytical methods to verify the exact reaction mechanism for the parabens under these conditions; however, since the aim of this study was simply to assess the use of IM to study parallel pro-

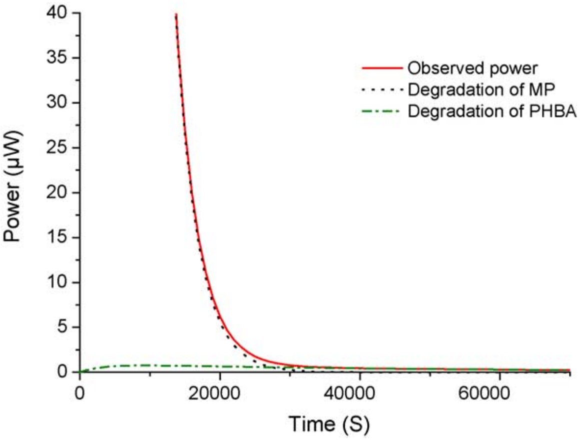

Fig. 5. Power–time data for MP (0.05 M) in NaOH aqueous solution (0.5 M) at 25 °C and the theoretical contributions to the observed signal from the individual steps as determined using Eq. (3); degradation of MP and degradation of *p*-hydroxybenzoic acid.

cesses, rather than to result in a comprehensive study of paraben degradation, these studies were not conducted here. We simply note that the analysis suggests a secondary degradation step could be occurring and we will return to the issue in a future publication. We also note that in order to effect a successful analysis for two parallel processes it appears that one rate constant needs to be twice the magnitude of the other.

4. Conclusion

It has been shown that IM can be used to study samples containing more than one degrading component and that analysis of the power–time data obtained allows the recovery of the rate constant and enthalpy for each species, so long as one rate constant is at least twice the magnitude of the other. Analysis of simulated data showed that the inability of the model to separate rate constants more similar than this was a result of the inherent noise of the signal, although results from fitting simulated data also suggested that a complete analysis would not be possible if the two rate constants were more than three orders of magnitude different.

The data allowed the determination of the rate constants and enthalpies for degradation of methyl, ethyl and *n*-propyl paraben directly at 25 °C, without the need for reaction to progress to completion. Indeed, it was found that as little as 15 min of experimental data were needed to effect a complete analysis when only one degrading component was present. Analysis of data for mixed paraben systems showed that the models were able to detect the presence of two parallel reaction processes and that the degradation rate constants for parabens in combination were considerably lower than expected. This was ascribed to the presence of a common degradation product. A minimum of 4 h of experimental data were needed to ensure an accurate analysis.

Although the subsequent degradation of *p*-hydroxybenzoic acid to phenol should be arrested under our experimental conditions, the fitting methodology suggested that this reaction may be occurring to some extent, because the data were better fitted to a consecutive model. It is unclear at this stage whether this is observation is an artifact of the fitting procedure (the consecutive model having more variables) and we intend to conduct further studies into this effect.

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