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A COMPARISON OF THE PERFORMANCE OF CALORIMETERS
Application of a test and reference reaction

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Isothermal calorimetry is finding extensive application in a number of research areas. This popularity is reflected in the number of commercially available instruments which are capable of yielding a variety of thermodynamic and kinetic parameters. Whilst there has been much discussion of ways in which to validate any values returned from these instruments very little has been done quantitatively to compare the relative performances of different instruments. This paper highlights the use of a test and reference reaction quantitatively to compare the performance of three instruments (Thermometric TAM, THT μRC and a Setaram HSDSC III); the specifications of these instruments provide a range from high-sensitivity, long equilibration time to lower-sensitivity, short equilibration time. The comparison is made through a statistical analysis of values returned for the rate constant, enthalpy of reaction and activation energy for the base catalysed hydrolysis of methyl paraben. The statistical analysis from the data set discussed here indicates that there is no significant difference between the returned thermodynamic and kinetic parameters from the TAM and μRC. The analysis revealed however that the HSDSC returns values for the rate constant which are significantly different from both the TAM and μRC, although it is noted that this instrument was not specifically designed to operate in a step-isothermal mode and that it was possible to apply a correction to the data. In all cases the enthalpy data returned from all instruments were statistically similar although the μRC and HSDSC returned values which were, for the rate constant and activation energy, less precise than those obtained from the TAM. As well as highlighting the importance of using test and reference reactions, this study also shows that proper instrument selection is an important factor when designing a calorimetric experimental series.

Keywords: *calibration, isothermal calorimetry, kinetic and thermodynamic analysis, test and reference reaction, validation*

Introduction

Calorimetry is finding increased acceptance for a variety of applications within the pharmaceutical industry and calorimetric methods [1–3] are now becoming more widely used for the investigation and quantification of stability and compatibility of pharmaceutical materials and formulations. Calorimetry confers several advantages over more traditional techniques. In particular it is insensitive to the physical form of the sample (i.e. it can be solid, liquid, gas or any combination of these) and does not require that the sample be altered in any way prior to study. It also has the advantage that the system is monitored indirectly through a change in heat content, i.e. invasive sampling is not required. Calorimetry also offers a level of sensitivity to small changes in the system which is superior to many alternative techniques. This sensitivity means that the sample does not necessarily have to be studied under stressed conditions. Calorimetry does suffer from the limitation that the data offer no molecular information on the system under study and, consequently, no definitive mechanistic information can be derived directly from calorimetric data. It does however reveal kinetic information such as the rate constant and order of reaction which may allow mechanistic information to be inferred.

Calorimeters operate on the principle that nearly all changes (chemical and physical) in a system involve an exchange of heat to, or from, the surroundings. The calorimeter monitors this exchange of heat as a function of time. The returned calorimetric data are of the form power (J s^{-1}) *vs.* time (s). It is therefore possible, using the appropriate equations [4–7], to glean a variety of thermodynamic (e.g. ΔH_R, enthalpy of reaction) and kinetic information (e.g. k, rate constant) from the raw calorimetric data. Moreover, since the calorimeter allows the system to be studied as a function of temperature it becomes possible to derive parameters such as the activation energy, E_a, giving greater insight into the character of the system. Calorimetry is therefore a versatile, sensitive and non-destructive technique, allowing the study of a wide range of materials in a variety of physical forms, making it a potentially very useful tool for the pharmaceutical industry.

There is a range of commercially available calorimeters, supplied by a number of manufacturers, all of which have different capabilities. Some like the

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high sensitivity differential scanning calorimeter (HSDSC, Setaram, France), offer a temperature scanning functionality and rapid thermal equilibration. This functionality requires some compromise on sensitivity. The thermal activity monitor (TAM, Thermometric AB, Sweden) has superior sensitivity (it is now possible to detect heat flow in the nJ s^{-1} range) and baseline stability (permitting long term studies of >100 days) over many instruments but with the concession that rapid temperature changes are not possible (it typically taking ca. 24 h for a TAM to reach equilibrium after a temperature change). Recently a multi-use calorimeter, the micro Reaction Calorimeter (μRC, Thermal Hazard Technology, UK), allowing isothermal, step-isothermal, temperature scanning and titration studies as well as the capacity to stir liquid reaction media, has been developed. This instrument permits rapid temperature changes because its relatively small heat sink reaches thermal equilibrium very rapidly (ca. 20 min). This versatility comes at the cost of long term baseline stability and some compromise on sensitivity (for a comparison of instrument specifications, Table 1). Other instruments are designed for specific applications, such as titration calorimeters, at the cost of some other functionality. Note that most instrument manufacturers have developed removable modules which permit multi-functionality of their instruments. However these accessories can and often do have an impact on the detection limits and sensitivity of the host instrument.

Any instrument design by its very nature must maximise some capability at the cost of other functionality. It is therefore important to choose the instrument carefully to suit the needs of the study to be performed because any limitations of the instrument must be considered when analysing the returned data. In many cases there may be a number of instrument designs available to conduct a study, and proper instrument selection will depend on the nature of the system under investigation.

A number of recent articles [8, 9] have highlighted the importance of validating calorimeters by means of chemical test and reference reactions. Such test and reference reactions have defined values for their kinetic and thermodynamic parameters, allowing assessments to be made of calorimeter performance. These data can be used to ensure the performance of a particular type of instrument falls within its specifications or to allow comparison of the performances of different instrument designs. We have demonstrated the use of a test reaction for the former case previously [10]; here, we report the results of a study to compare the performance of three calorimeters routinely used in our laboratory (those discussed above), through a known hydrolysis reaction; the base catalysed hydrolysis of methyl paraben (BCHMP) [11, 12]. The results highlight the importance of validating instrumental performance under the conditions to be used for any subsequent investigation and show calorimetrists how to ensure that the most appropriate instrument is selected for any particular study.

Experimental

Materials and methods

Methyl 4-hydroxybenzoate (methyl paraben, MP), 99%, was purchased from Fluka. Sodium hydroxide was purchased from VWR. All materials were used as received. Solutions were prepared in distilled, de-ionised water.

Solutions were prepared by dissolving the required amounts of MP (0.38 g) in 50 mL sodium hydroxide solution (0.5 M). Solution pH was measured before and after each experiment and found to be constant at pH 12.3. The time between addition of solute to NaOH (t_0) and commencement of data capture (t_s) was noted and this value added to the time data for each experiment.

Isothermal experiments

Experiments were conducted using a 2277 thermal activity monitor (TAM) at 25, 37 and 40°C. Aliquots of solution (3 mL) were pipetted into standard glass 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 40 min before being lowered into the measurement position. Data capture was then initiated using the dedicated software pack-

Table 1 Manufacturers' specifications for the calorimeters used in this study

Calorimeter	Operating T_{range}/°C	Scanning rate/ K min^{-1}	Resolution/ μW	Noise/ μW	Sample size/ mL	Equilibration time after step *T* change
TAM	12 to 90	*n/a*	0.01	0.008	≤3[b]	Approx. 24 h
μRC	–10 to 200	0.02 to 2	5	5	≤1.5	Approx. 20 min[a]
HSDSC	–20 to 120	0.001 to 1.2	0.03	0.03 (RMS)	≤0.85	Approx. 20 min[a]

[a]These values represent the time taken for equilibration after a step isothermal change of 5°C; greater step changes may require a longer time period for equilibration; [b]The ampoules used for this study were standard 3 mL glass ampoules. 4 and 20 mL ampoules are also available

age Digitam 4.1. Power data (μW) were recorded every 30 s, for approximately 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 validated using the imidazole catalysed hydrolysis of triacetin test and reference reaction (the returned values for the rate constant and enthalpy fell within the accepted limits).

Step-isothermal experiments

For the μRC: Aliquots of solution (1 mL) were pipetted into disposable glass vials; the ampoules were sealed with a screw fit lid. The ampoules were made air-tight thorough the use of a rubber seal situated inside the lid. The reference ampoule was loaded with an aliquot of NaOH solution (0.5 M, 1 mL). The step-isothermal temperature program comprised a 15 min equilibration period at 15°C in order to allow the thermal shock associated with loading to dissipate. Data were then collected for 1 h at 15°C; the temperature was then increased to 25°C and data were collected for a further 1 h. This cycle was repeated for 30 and 35°C. The instrument was calibrated weekly using an electrical substitution method. Samples were run at least in triplicate.

For the HSDSC: aliquots of solution (0.8 mL) were pipetted into hastelloy steel ampoules; the ampoules were sealed with a screw fit lid. The ampoules were made air-tight thorough the use of a rubber o-ring situated around the neck of the ampoule. The reference ampoule was loaded with an aliquot of NaOH (0.5 M, 0.8 mL). The step-isothermal temperature program comprised a 15 min equilibration period at 15°C in order to allow the thermal shock associated with loading to dissipate. Data were then collected for 1 h at 15°C; the temperature was then increased to 25°C and data were collected for a further 1 h. This cycle was repeated for 30 and 35°C. Samples were run at least in triplicate. Calibration of the instrument was achieved through comparison of observed melting points of pure organic compounds, with known reference values, across the temperature range of interest. (The calibrant compounds chosen were biphenyl (*m.p.* 69.7°C), phenyl salicylate (*m.p.* 41.8°C) and phenyl ether (*m.p.* 29.7°C).

Data analysis

Data analysis was performed using Origin (Microcal Software Inc., USA). The difference between t_0 and t_s (in s) was added to the time data to correct for the time-delay between initiation of reaction and the commencement of data capture. All values reported are an average of, at least, 3 repeats with the standard deviation values representing a confidence limit of 68%.

The BCHMP reaction proceeds via first-order kinetics, under the conditions used here, which means that recovery of the rate constant values at each temperature step was easily achieved by plotting ln power *vs.* time; the gradients of the linear sections of data at each temperature give the rate constant values. Arrhenius plots (ln$k$ *vs.* 1/T) were subsequently constructed for each instrument.

It was possible, however, to attain further information from the data sets recorded here by using an iterative procedure (in this case, the non-linear curve fitting tool in origin). Data were fitted to Eq. (1), which describes the power-time response for a single-step reaction following first-order kinetics [13]. This was first published by Bakri [4].

$$\text{power} = \frac{\mathrm{d}q}{\mathrm{d}t} = \Delta H k A_0 \mathrm{e}^{-\mathrm{kt}} \qquad (1)$$

where q is the heat output of the reaction, ΔH is the reaction enthalpy (J mol^{-1}), k is the first-order rate constant (s^{-1}) and A_0 is the initial quantity of reactant (mol). Thus, as well as providing a check on the values of the rate constants, it was possible also to compare the sensitivity of the calorimeters to reaction enthalpy.

Further details of the application of this type of analysis have been given elsewhere [14], but it is important to note that it is imperative that the value of A_o is known. While this is possible for the first temperature used in a step isothermal experiment, it is not possible to calculate how much material reacts during the temperature changes (and the characteristic temperature overshoot). Consequently only the initial temperature step was analysed using this procedure.

Results and discussion

The BCHMP reaction has been proposed [11] as a test and reference reaction for flow-calorimeters and has been the subject of inter- and intra-laboratory trials [12], using previously validated instruments, in order to define a set of reaction parameters; the recommended values for the enthalpy and rate constant (at 298 K) are –50.5±4.0 kJ mol^{-1} and $3.15 \cdot 10^{-4} \pm 1.1 \cdot 10^{-5}$ s^{-1} respectively. It should be noted here that the BCHMP is not a IUPAC (International Union of Pure and Applied Chemistry) recommended test reaction for isothermal calorimetry, the preferred test reaction being the imidazole catalysed hydrolysis of triacetin (ICHT). However, this test reaction has been reported to be unsuitable for use in calorimeters with a low sensitivity [15]. The BCHMP was therefore chosen because of its combination of a reasonably large enthalpy of reaction and high rate of reaction, permitting easy study in calorimeters of lower sensitivity.

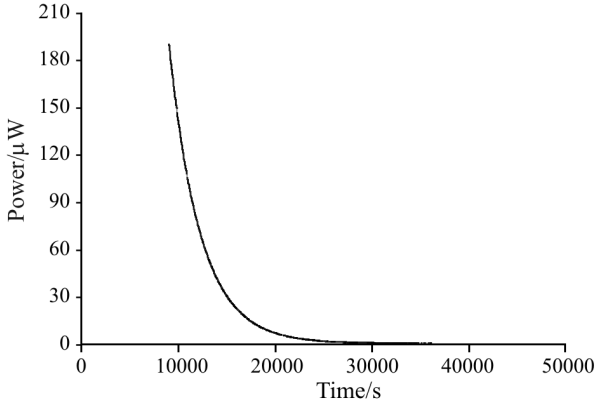

Fig. 1 Power output obtained for the hydrolysis of methyl paraben from the TAM at 25°C

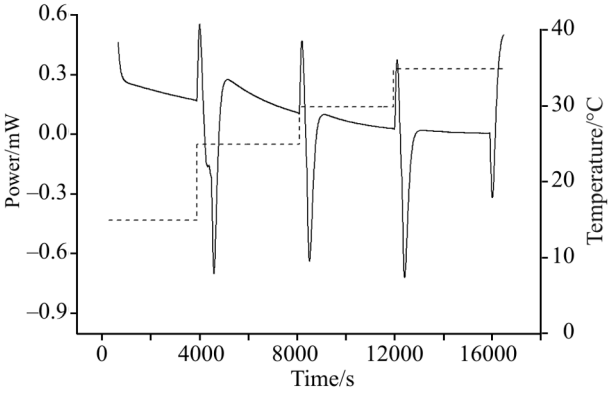

Fig. 3 Power output obtained for the hydrolysis of methyl paraben from the HSDSC running a step-isothermal program. The dotted line represents the temperature program

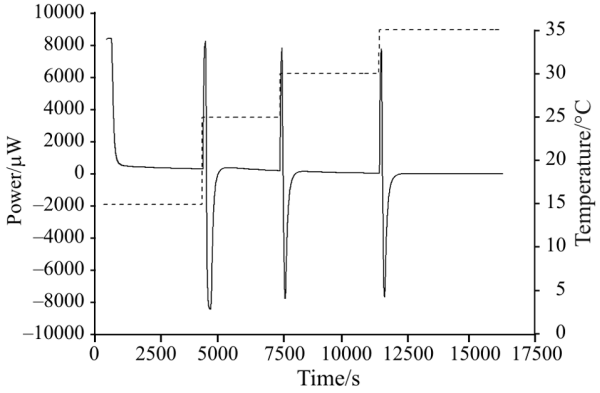

Fig. 2 Power output obtained for the hydrolysis of methyl paraben from the μRC running a step-isothermal program. The dotted line represents the temperature program

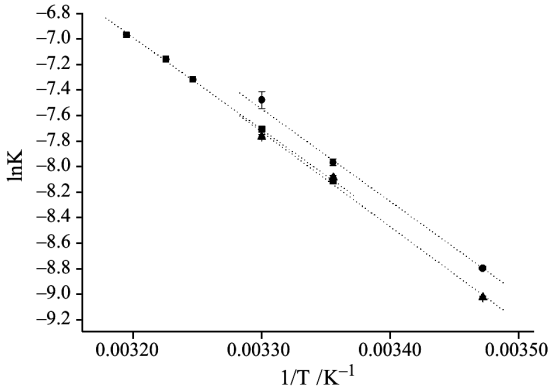

Fig. 4 Arrhenius plots for the ■ – TAM, ● – μRC and ▲ – HSDSC; the dotted lines represent lines of best fit to the data. Data points are the mean of n=3 (minimum) repeats. Error bars represent the standard deviation

Figures 1–3 display representative raw data plots for each instrument. The excellent fits to linear regression analyses of the ln power *vs.* time data shows that for each data set the reaction does conform to first-order kinetics over the lifetime of the reaction at each temperature step. The linearity of each of the Arrhenius plots of the kinetic data obtained from each instrument confirms that the reaction mechanism does not change over the temperature range studied, Fig. 4. Furthermore, the slopes of the Arrhenius plots were used to calculate an observed activation enthalpy, E_a, for the reaction from each instrument. The derived values and their standard deviations are given in Table 2.

From the values reported in Table 2 it is apparent that the values returned from the TAM are statistically indistinguishable from those returned from the μRC. Conversely, the values returned from the HSDSC are statistically different from those returned from both the μRC and the TAM. This observation is further confirmed through analysis using one-way ANOVA and Tukey tests to determine whether the rate constants (Table 3) and enthalpies (Table 4) returned from each calorimeter were statistically similar. The statistical analysis revealed that although the uncertainty in the values for k returned from the μRC are greater than those obtained from the TAM data they are statistically indistinguishable from the TAM values. The rate constants returned from the HSDSC, however, were found to be significantly different from those values obtained from both the TAM and the μRC. Note that only values obtained at 25°C were used for the statistical analyses as this was the only common temperature between all experiments. (TAM data were obtained over a period of time from routine traning and validation exercises whereas the temperatures chosen from the μRC and HSDSC were chosen specifically to alleviate the problem of a short half life for the methyl paraben reaction).

The observation that the rate constants returned from the HSDSC data are significantly different from those obtained from the TAM and μRC is somewhat puzzling. The rate constants obtained from the HSDSC were used to calculate the apparent operating tempera-

Table 2 Average values and the associated standard deviations for the rate constant, enthalpy and activation energy for each calorimeter

Calorimeter	$(k\pm sd)/s^{-1}$	$(\Delta H_R\pm sd)/kJ\ mol^{-1}$	$(E_a\pm sd)/kJ\ mol^{-1}$
TAM	$3.04\cdot10^{-4}\pm3.64\cdot10^{-6}$	50.5±4.3	59.8±0.9
μRC	$3.07\cdot10^{-4}\pm6.00\cdot10^{-6}$	52.1±5.3	60.8±3.5
HSDSC	$3.44\cdot10^{-4}\pm8.72\cdot10^{-6}$	49.0±1.7	60.0±4.4

Table 3 Statistical analysis of rate constant data obtained from the TAM, μRC and HSDSC

Calorimeter (I)	Calorimeter (J)	Mean difference (I–J)	Sig.
TAM	μRC	–0.00001	0.474
	HSDSC	–0.00004*	0.001
μRC	TAM	0.00001	0.474
	HSDSC	–0.00003*	0.023
HSDSC	TAM	0.00004*	0.001
	μRC	0.00003*	0.023

*The mean difference is significant at the 0.05 level

Table 4 Statistical analysis of enthalpy data obtained from the TAM, μRC and HSDSC

Calorimeter (I)	Calorimeter (J)	Mean difference (I–J)	Sig.
TAM	μRC	–1.100	0.849
	HSDSC	1.475	0.747
μRC	TAM	1.100	0.849
	HSDSC	2.575	0.491
HSDSC	TAM	–1.475	0.747
	μRC	–2.575	0.491

In all cases there is no statistical difference at the 0.05 level

Table 5 Statistical analysis of activation energy data obtained from the TAM, μRC and HSDSC

Calorimeter (I)	Calorimeter (J)	Mean difference (I–J)	Sig.
TAM	μRC	–1.000	0.905
	HSDSC	–0.180	0.997
μRC	TAM	1.000	0.905
	HSDSC	0.820	0.943
HSDSC	TAM	0.180	0.997
	μRC	–0.820	0.943

In all cases there is no statistical difference at the 0.05 level

ture of the HSDSC, by interpolation of the Arrhenius plot generated from TAM data. It is found from this analysis that the apparent operating temperature of the HSDSC is approximately 1.8°C higher, in all cases, than the reported temperature. A possible cause for this incongruity is that the system may not have been held for a sufficient time between each temperature step to allow a thermal equilibrium to be established. This may result in biased values for the rate constant being returned. A separate study was conducted to test this hypothesis (data not shown) whereby a series of experiments at a single temperature were conducted (ca. 3 h); however, in all cases the same result was observed. It should be noted here that the HSDSC is designed to operate as a scanning calorimeter or as an isothermal calorimeter it is not specifically designed for step-isothermal experiments. Unsurprisingly therefore its calibration routine does not account for programmed isothermal steps. Once identified however, this discrepancy between actual and operating temperature is not necessarily a cause for concern. The experimental data can be adjusted through appropriate application of reference data [16].

An identical statistical test to that described earlier was performed to establish the significance of any variations in the calculated activation energies. Table 5 reports the results of this statistical test. Here it is seen that, even though the rate constants from the HSDSC are in error, the activation energy is statistically indistinguishable from those obtained from the other instruments. This is not unexpected since it is the temperature dependence of the rate constant, not its absolute value, which influences the value of the apparent activation energy. It should be noted that in the case of the reaction enthalpy, for the data set reported here, the HSDSC returned a value with a substantially smaller standard deviation than those obtained from the TAM or μRC. The reason for this is not known at this time.

Conclusions

The objective of the study reported here was to compare the relative performances of three commercially available calorimeters through the application of a test and reference reaction. It has been shown that, for

the BCHMP reaction system, the TAM and the μRC returned values for the reaction parameters which were statistically identical, although the precision of the μRC was slightly lower than that of the TAM. A potential pitfall in using calorimeters in a mode for which they were not specifically designed has been highlighted in the values obtained, for the rate constants, from the HSDSC. It is possible to correct these values. The values returned for the enthalpy of reaction and the activation energy are, however, statistically indistinguishable from the μRC and TAM.

The data presented here have shown how test and reference reactions can be used to make quantitative comparisons between similar instruments. Such a study allows an operator then to select the most appropriate instrument for a particular sample, taking factors such as experimental run time, precision and cost into consideration.

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