

PERFORMANCE VALIDATION OF STEP-ISOTHERMAL CALORIMETERS

Application of a test and reference reaction

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Isothermal calorimetry is becoming indispensable as a tool for the study of a wide variety of systems. As with all scientific instruments it is essential that robust calibration routines be developed in order to validate the data obtained. Chemical test reactions offer many advantages over (the traditionally used) joule effect heating methods, not least because they have the potential to validate instrument performance (i.e. they can be used to assess all aspects of calorimeter operation). In this work the results of a validation exercise, conducted by Thermal Hazard Technology as part of an installation routine, using the base catalysed hydrolysis of methyl paraben are discussed. In the case described, a systematic misreporting of the reported temperature of a calorimeter was identified, caused by an upgrade to the calorimeter's firmware, a discrepancy which may not have been noted using traditional electrical calibration methods and one which highlights the importance of both manufacturers and end-users adopting chemical test reactions into their test and validation routines.

Keywords: calibration, isothermal calorimetry, methyl paraben, rate constants, validation

Introduction

Calorimetric techniques offer several advantages (which have been well documented elsewhere [1–4]) over more traditional analytical techniques, such as HPLC, and are becoming more widespread in their application for the study of a broad variety of systems [5–7]. One particular area in which calorimetric techniques have found many applications is the study of pharmaceutical systems, where as well as being used for the detection of polymorphism and amorphicity [8], they are increasingly being used for stability assessment and compatibility studies [9].

However, with the increasing study of pharmaceutical systems, and the requirement to submit data into regulatory documents, comes the need for instrument and data validation. As with any scientific instrument the accuracy of the returned data are only as good as the accuracy of the calibration routine. There are a number of IUPAC recommended chemical standards for validating differential scanning calorimeters (such as indium, for example) but the majority of isothermal calorimeters are usually calibrated by means of an electrical substitution (Joule effect heating) method. The use of this method has caused some concern, however, because it is unlikely that the processes of heat generation and dissipation from a resistance heater accurately mimic those that occur during

a chemical reaction (for instance, with a resistance heater all the heat is supplied externally from the base (usually) of the ampoule, which can result in temperature gradients being formed, while in a homogeneous solution phase reaction heat is generated uniformly inside the ampoule). This has led to much discussion and attempts to define standard chemical test reactions for isothermal calorimeters, a debate that has recently been summarised in a IUPAC technical report [10].

A further concern of the use of electrical calibration is that it does not provide information on the overall performance of the instrument. Although Joule effect heating can be used to validate power outputs and time constants, its use invokes a number of assumptions; firstly, that the resistance of the heater is known (such that the amount of power supplied is accurate); and secondly that the effects of heat generation from a point source do not generate a temperature gradient in the sample (as noted above, it is likely that temperature gradients form; if so, the application of the equations used to calculate the time constants is compromised). Furthermore, Joule effect heating does not validate the operating temperature of the instrument, which could be different from that indicated by the calorimeter. Clearly this is unsatisfactory and could lead to systematic errors being introduced to experimental data.

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Validation of isothermal instruments through the use of appropriate chemical test reactions avoids these drawbacks because it resolves a kinetic term, the rate constant (k), as well as a thermodynamic term, the reaction enthalpy (ΔH), and can therefore be used for troubleshooting instrumental performance by comparing the actual experimental reaction parameters determined with those expected, a topic recently discussed by Hills *et al.* [11]. Several test reactions have been suggested as suitable for isothermal calorimeters, including the imidazole catalysed hydrolysis of triacetin (ICHT) [12] and the base catalysed hydrolysis of methyl paraben (BCHMP) [13]. ICHT, because of its slow rate constant and smaller power output, is more suited to the validation of medium-term (weeks) stability. BCHMP is ideally suited for experiments run on a shorter time scale (hours or days), or for instruments with lower sensitivities, because its faster rate constant produces a larger power signal.

We have recently been collaborating with an instrument manufacturer (Thermal Hazard Technology) who were very keen to assess the potential role of chemical test reactions in their commissioning and installation routines. In the case discussed here, we show how BCHMP was used to assess the performance of a newly developed instrument, the micro-reaction calorimeter (μRC^{TM}) during installation; the use of the BCHMP test reaction correctly and quantitatively identified a misreporting of the operating temperature of the instrument, a discrepancy that is thought to have occurred following an upgrade to the instrument's firmware and one that would not have been detected through conventional electrical calibration.

Materials and methods

Methyl 4-hydroxybenzoate (methyl paraben, >99%) and sodium hydroxide (ACS) were purchased from Sigma Aldrich. Both materials were used without further purification. Solutions were prepared in purified water (Elga DV25 water purifier).

Experiments were performed using the μRC^{TM} operating in isothermal mode. Methyl paraben (0.38 g) was added to NaOH solution (0.5 mol dm^{-3} , 50 mL) with vigorous stirring. An aliquot of this solution (1.5 mL) was sealed inside a glass HPLC ampoule and loaded into the instrument. Data were recorded vs. an equivalent reference ampoule containing purified water (1.5 mL) using the dedicated instrument software. The instrument was set to an indicated 298 K. Data analysis was performed using the non-linear fitting function in Origin 7.0 (Microcal Software Inc).

Results and discussion

A typical calorimetric output for the BCHMP reaction, recorded by the μRC^{TM} , is shown in Fig. 1. Under the conditions used here, the degradation of methyl paraben has been shown to be first-order [13]. Power-time data for a first-order process are described by Eq. (1) [14–15].

$$\text{Power} = \frac{dq}{dt} = \Delta H \nu k [A_0] e^{-k \cdot t} \quad (1)$$

where q is the heat output of the reaction, ΔH is the reaction enthalpy per mole of product formed, ν is the volume of solution in the ampoule, k is the first-order rate constant and $[A_0]$ is the initial concentration of reactant. Data fitting requires initial estimates for all parameters to be entered into the software. Values for ν (0.0015 dm^3) and $[A_0]$ (0.05 M) were known and therefore kept constant. The initial values entered for ΔH ($1 \cdot 10^{10} \text{ } \mu\text{J mol}^{-1}$) and k ($1 \cdot 10^{-5} \text{ s}^{-1}$), 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. It should be noted that this method of analysis requires that the reaction progresses to completion and that no equilibrium state is reached. We have shown previously [9] that this requirement is met for the methyl paraben system. An alternative method of analysis has been proposed for systems where this is not the case [16].

Under the experimental conditions used here it has been shown that the rate constant and reaction enthalpy for BCHMP are $3.15 \cdot 10^{-4} \pm 0.11 \text{ s}^{-1}$ and $-50.5 \pm 4.0 \text{ kJ mol}^{-1}$ respectively [13]. The experimental data recorded in the μRC^{TM} were fitted to Eq. (1) to derive values for k and ΔH (summarised in Table 1) in the manner described above. It can be seen from the data in Table 1 that when the calorimeter was set at an indicated 298 K

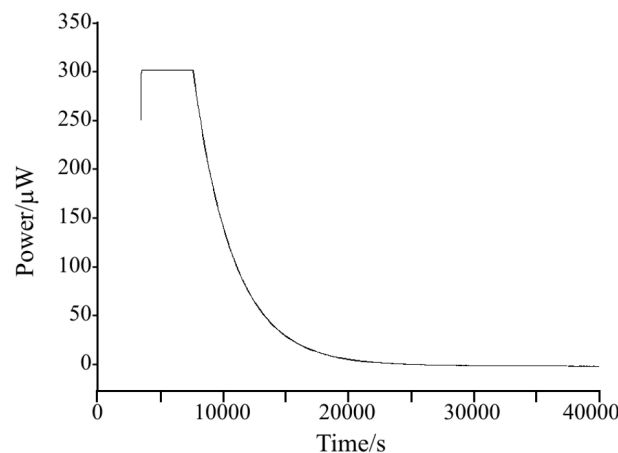


Fig. 1 A typical calorimetric output for the base catalysed hydrolysis of methyl paraben at an indicated 298 K

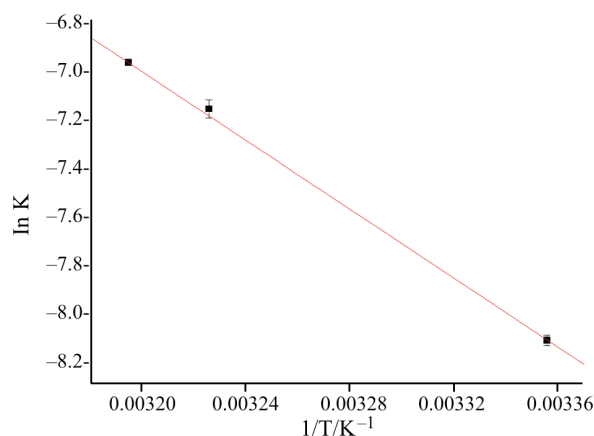
Table 1 A comparison of the reported and predicted (from the Arrhenius plot) temperatures for the μRC^{TM} experiments conducted, as well as the rate constants and enthalpies determined by fitting data to Eq. (1)

Experiment number	Reported temp./K	Predicted temp./K	k/s^{-1}	$\Delta H/\text{kJ mol}^{-1}$
1	298	300.8	$3.75 \cdot 10^{-4}$	-56.8
2	298	300.6	$3.70 \cdot 10^{-4}$	-55.2
3	298	300.8	$3.74 \cdot 10^{-4}$	-54.3
		Mean	$3.73 \pm 0.03 \cdot 10^{-4}$	-55.4 ± 1.3

the returned mean value for the enthalpy was $-55.4 \pm 1.3 \text{ kJ mol}^{-1}$, a value within the range noted above. However, the mean value for the rate constant, $3.73 \pm 0.03 \cdot 10^{-4} \text{ s}^{-1}$, was outside of the accepted range.

As a check of the integrity of the test system used, the methyl paraben solution was placed in a different isothermal calorimeter (TAM, Thermometric AB), set at 298 K. The data returned (not shown) were then fitted to Eq. (1) in the manner described above. The values returned for k ($3.13 \cdot 10^{-4} \pm 0.06 \text{ s}^{-1}$) and ΔH ($-53.5 \pm 1.8 \text{ kJ mol}^{-1}$) were in much better agreement with the expected values and confirmed that the source of the discrepancy was the instrument and not the reaction system.

As noted above, Hills *et al.* [11] have discussed the use of chemical test reactions for troubleshooting calorimeters. They note that one potential cause of outcome seen here (correct reaction enthalpy, incorrect rate constant), once operator or sample errors are ruled out, is an incorrect temperature of the calorimetric block. Since we had previously studied this system over a range of temperatures in a different isothermal calorimeter (TAM, [9, 13]) it was possible to construct an Arrhenius plot ($\ln k$ vs. $1/T$) for methyl paraben degradation, Fig. 2. The actual operating temperature of the μRC^{TM} could then be predicted from the rate constant values derived from the experimental data. The predicted values are shown in Table 1 (mean, 300.7 K). In all cases the predicted tem-

**Fig. 2** An Arrhenius plot for the base catalysed hydrolysis of methyl paraben constructed from TAM data

peratures were several Kelvin higher than the reported temperature, which agrees with the hypothesis suggested from analysis of the test reaction.

Following analysis, the instrument was checked by the engineer and was found to misreporting temperature; the temperature at an indicated 298 K was actually 300.8 K, a value in excellent agreement with the predicted value. The performance of the calorimeter had been verified at the factory following manufacture and it is believed that the discrepancy noted above was caused by an upgrade to the instrument's firmware during installation. Once rectified, the performance of the calorimeter was re-validated using the BCHMP test reaction and the returned values for k and ΔH then fell within the accepted range ($k=3.2 \pm 0.16 \cdot 10^{-4}$; $\Delta H=-56.7 \pm 3.1 \text{ kJ mol}^{-1}$).

Conclusions

The use of isothermal calorimetry for the study of pharmaceuticals is becoming prevalent and with this comes a real need for robust methods for instrument validation. Chemical systems offer many advantages over the electrical substitution method, not least that they can be used to validate temperature as well as power scales, and, if they are easy to perform and use readily available materials, should become more widely adopted.

It became clear from the data obtained by Thermal Hazard Technology that, in one isolated case, data from an instrument during installation returned the correct reaction enthalpy but an incorrect rate constant; one cause for this can be a misreporting of operating temperature. This was found to be the case by the engineer. Estimation of the discrepancy in temperature using an Arrhenius plot matched almost exactly that actually found. Had the calorimeter been calibrated solely by electrical substitution the misreporting of the temperature may not have been identified. Although the usefulness of chemical test reactions is still the subject of much debate, the results shown here indicate that chemical calibration is extremely useful and should be used routinely by both instrument manufacturers and end-users to verify the performance of isothermal calorimeters. Following these

findings, Thermal Hazard Technology has investigated a programme of chemical calibrations during instrument commissioning which is to be repeated during installation.

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