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The base catalysed hydrolysis of methyl paraben: a test reaction for flow microcalorimeters used for determination of both kinetic and thermodynamic parameters

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Abstract

The results of an inter/intra-laboratory study into a test and reference reaction for isothermal microcalorimeters, the imidazole catalysed hydrolysis of triacetin, have been reported in a recent paper [Thermochim. Acta 380 (2001) 13]. The results and conclusions drawn from this study have been extended to a consideration of the need for a similar test and reference reaction for isothermal microcalorimeters operating in flow mode. This paper reports the findings of a preliminary inter/intra-laboratory study of the base catalysed hydrolysis of methyl 4-hydroxy benzoate (methyl paraben) and its suitability as a test and reference reaction. The derived values for the hydrolysis reaction were $(3.15 \pm 0.11) \times 10^{-4}\,\mathrm{s}^{-1}$ and $-50.5 \pm 4.3\,\mathrm{kJ\,mol}^{-1}$ for the rate constant and enthalpy, respectively. It is also reported how such a test and reference reaction can be used to validate the thermal output from a LKB 10-700-1 and Thermometric Thermal Activity Monitor (TAM) 2277-202 flow microcalorimeters.

Keywords: Test and reference reaction; Flow microcalorimetry; Methyl paraben; Triacetin

1. Introduction

Recently, the results have been reported of an international inter/intra-laboratory investigation into the application of the imidazole catalysed hydrolysis of triacetin as a potential test and reference reaction for commercially available, static, batch-type, isothermal microcalorimeters [1]. The purpose of this investigation was to determine accurate values for the rate constant, *k*, and the enthalpy, ΔH (subsequently expressed as *H* for simplicity), for the hydrolysis reaction. Such a test reaction provides the means to trace and validate results for new experimental systems. The existence of such a chemical test reaction also allows troubleshooting for potential sources of error in the calorimetric signal [2,3] and comparison of results from different sources and for training of new personnel. It also allows the experimental design of calorimetric experiments to be scrutinised [4]. The hydrolysis reaction was found to be second-order in nature with a rate constant of $(2.8 \pm 0.1) \times 10^{-6}\,\mathrm{dm}^3\,\mathrm{mol}^{-1}\,\mathrm{s}^{-1}$ and an enthalpy of $-91 \pm 3\,\mathrm{kJ\,mol}^{-1}$.

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The work reported in the present paper is concerned with the analysis of calorimetric data obtained from *flow* microcalorimeters. These generally have two modes of operation, flow mix and flow through. For flow-mix systems where instantaneous (that is reactions which are rapid relative to the time constant of the instrument: in this paper, time dependent means those reactions in which this condition does not hold) reactions are initiated actually in the flow-measuring vessel the thermodynamic parameters for the reaction are calculated through the use of the calibration constant determined at the experimental flow rate. In contrast to this simple calibration, a flow-through calorimetric experiment involves a reaction in which the rate of reaction changes continuously with time (lead times from the external reaction reservoir to the calorimetric vessel are of the order of 3–15 min). Furthermore, the simple calibration is not appropriate for a flow-mix vessel in which a time dependent reaction takes place. This is because the observed calorimetric signal describes the mean extent of reaction that occurs in the flowing reacting system over the vessel residence time. The extent of reaction is the integral of the reaction rate over this residence time. It is important to underscore the fact that the results reported here refer *only* to those in which the reaction rate varies continuously with time. They have been derived from flow-through experiments but a similar analysis can be performed for flow-mix experiments in which there is evidence for time dependent reactions.

Previously, kinetic equations were described by Beezer and coworkers [5,6] that express the thermal output for first-order, Eq. (1) and zero-order Eq. (2), reaction systems:

$$\Phi = -FCH(1 - e^{-k_1\tau})e^{-k_1 t} \quad (1)$$

where Φ is the thermal power (W), F the flow rate ($\mathrm{dm^3\,s^{-1}}$), C the concentration ($\mathrm{mol\,dm^{-3}}$), H the enthalpy ($\mathrm{J\,mol^{-1}}$), k_1 the first-order rate constant ($\mathrm{s^{-1}}$), t the time (s) and τ the residence time (s).

The calorimetric output for a zero-order system is:

$$\Phi = -kV_cH \quad (2)$$

where V_c is the thermal volume ($\mathrm{dm^3}$), k the rate constant and H the enthalpy ($\mathrm{J\,mol^{-1}}$).

It is seen in both Eqs. (1) and (2) that the calorimetric analysis for elucidating the rate constant using them hinges on prior knowledge of the residence time, τ (time the reacting solution spends in the calorimetric vessel) and hence the thermal volume, V_c. The residence time is described by Eq. (3).

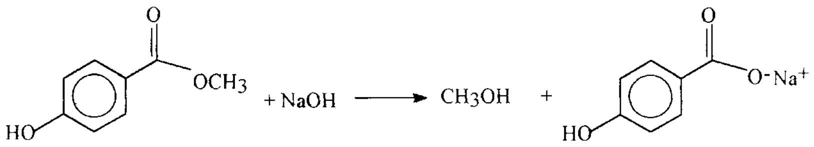
$$\tau = \frac{V_c}{F} \quad (3)$$

Determination of reliable values for rate constants and enthalpy changes from experimental data (power, time data) for reacting systems studied by flow microcalorimetry obviously requires accurate and precise values for τ; which is determined from Eq. (3) through the knowledge of F and V_c.

Until recently, and in the analysis presented earlier [5,6], it was necessary to make the assumption that the thermal volume of the vessel, at *any* flow rate, was the same as its physical volume. Standard flowing reaction systems necessarily result in transport of heat from the detection area. Thus, whilst the physical volume of the vessel can be known, this may not be the effective or the thermal volume, nor indeed the zero-flow-rate volume of the vessel. The determination of an effective or thermal volume is a generic problem associated with all flow microcalorimeters. The thermal volume, V_c is the volume that the calorimeter detects as the solution flows through the vessel. It is affected by several different factors but the most important is the flow rate of the solution since, as the reacting solution flows through the calorimetric vessel, a proportion of the heat will be carried out of it retained in the solution, undetected. As the flow rate is increased, more heat will be carried out by the flowing solution and hence a smaller thermal volume will be observed. Conversely, as the flow rate is decreased, less heat will be lost such that as the flow rate approaches zero, the apparent thermal volume will approach a limiting value. To examine the issue of thermal volume it is necessary to make use of a test reaction, i.e. a reaction for which accurate and precise values of the rate constant and enthalpy are known.

The way forward is to determine the rate constant, k, and the enthalpy, H, for the selected reaction using a batch-type calorimeter equipped with a glass ampoule. Then, these data can be regarded as constants to be inserted into Eq. (1) (for a first-order reaction) and hence, from the flow microcalorimetric data (Φ, t), allow the calculation of τ. Because τ and F are now known, the thermal volume can be calculated at the selected flow rate.

The triacetin test and reference reaction has already been described [1] for static, batch-type calorimeters such as the heat conduction Thermal Activity Monitor (TAM), model 2277-201 (Thermometric AB, Järfälla, Sweden). However, studies by us (data not reported here) of this test reaction have shown it to be unsuitable for use in flow calorimeters of the heat conduction type. Although the enthalpy of the triacetin reaction is relatively large, the rate constant is small, as is the *physical* (as engineered) volume (nominally 0.47 cm^3) of the standard gold flow vessel of the LKB flow calorimeter used in this work. It is reasoned, therefore, the rate of change of signal is insufficient to be reliably followed by the flow calorimeter. It is then necessary to employ a secondary test reaction, i.e. one for which the derived parameters can be traced back to, and validated by, the test reaction in the batch version of the TAM (model 2277-201). This secondary test reaction must also fulfil the requirements set out in [1]. Namely it should be robust, cheap, easy to obtain pure reagents, etc. The results are reported in this paper of an investigation into the base catalysed hydrolysis of methyl 4-hydroxy benzoate (methyl paraben) and its potential use as a secondary test reaction for flow microcalorimeters. This reaction is pseudo first-order in nature, meaning that it is first-order in the presence of excess base. The reaction can be written as:



To validate the results obtained for the hydrolysis of methyl paraben, it was necessary to determine accurate and traceable values for the rate constant and enthalpy for the hydrolysis reaction by an ampoule calorimetric study with a batch version of the TAM (model 2277-201). A temperature dependence study was made using a micro DSC III (Setaram, France). A separate study was carried out by some of us, based at the University of Wales, Aberystwyth, using a purpose built [7] flow module for the TAM and the results are also reported here.

2. Experimental

All calorimeters were operated according to the manufacturer's guidelines as set out in the operator manuals.

2.1. TAM (Thermometric AB, Järfälla, Sweden)

The initial study in the batch module of the TAM, at 298 K, was to provide validated, traceable, accurate values for the rate constant and for the enthalpy for the base catalysed hydrolysis. The TAM had been validated using triacetin in the manner described in [1]. The instrument was housed in a temperature-controlled room held at 23 °C.

0.3808 g of methyl paraben (Sigma, purity 99%) was added to 50 cm^3 of 0.5 mol dm^{-3} NaOH (Sigma, volumetric standard) with vigorous stirring. 3 cm^3 of this solution was run against a 3 cm^3 de-ionised water reference using a protocol similar to that described in [1]. Ampoules were lowered into the equilibrium position and left for 40 min, in order to reach thermal equilibrium with the TAM. The ampoules were then lowered into the measuring position and data collected using the dedicated software package.

2.2. Micro DSC III (Setaram, France)

The reaction solution was prepared as described above. 0.8 g of reaction solution was run against the reference of 0.8 g de-ionised water. The experiment was carried out at 20, 25, 30, 40 and 52 °C.

2.3. LKB 10-700-1 flow calorimeter (extinct LKB AB, Bromma, Sweden)

This instrument is the precursor to the TAM and has a smaller sized, gold flow vessel with nominal (engineered) volume of 0.47 cm^3. The experiments were conducted using the same experimental procedures as described in [8] using methyl paraben made as in

Section 2.1. The reaction solution was prepared as described above. At this instance, the whole solution was run in a continuous loop through the calorimeter at 298 K, meaning that the reaction was initiated in an external temperature-controlled vessel the contents of which were pumped into the calorimeter and the outflow from the calorimeter was returned to the external reaction vessel. The time for the reacting solution to reach the calorimetric cell was approximately 15 min. The calorimeter was housed within a temperature-controlled environment held at 23 °C. The experiment was repeated for flow rates ranging from 6 to 34 $cm^3\,h^{-1}$.

2.4. Thermometric TAM 2277 with customised flow module

TAM has a standard flow module with a nominal (engineered) volume of 0.6 cm^3 that is, of course, a candidate for calibration with methyl paraben. For this study, however, we used a flow module specially designed by Thermometric AB for studying the heat produced by mammalian cells in dilute suspension [7]. For this reason, all the tubing is stainless steel of 1.5 mm (i.d.). This tubing also forms the coiled flow vessel, which has a nominal (engineered) volume of 1 cm^3. In the normal use, the peristaltic pump draws cell suspensions downwards through the flow vessel at 100 $cm^3\,h^{-1}$. Experiments conducted with this calorimeter followed the above protocol for methyl paraben (Section 2.3), but the temperature was 310 K and the flow rates ranged from 30 to 180 $cm^3\,h^{-1}$.

2.5. Data logging and analysis

Data for the LKB 10-700-1 flow, TAM 2277-201 batch, micro DSC and TAM 2277 flow were collected using the dedicated software packages Picolog, Digitam, Setsoft and BioXpert, respectively. All data analysis was conducting using Microcal Origin V 5.0 and Microsoft Excel.

Values for the enthalpy and rate constant derived from TAM data were calculated using the equations and methods outlined in [1,13,14].

Fig. 1 shows the calorimetric output from a typical flow experiment. The hydrolysis of methyl paraben is first-order in nature. Consequently, a log plot of the calorimetric output versus t should yield a straight line

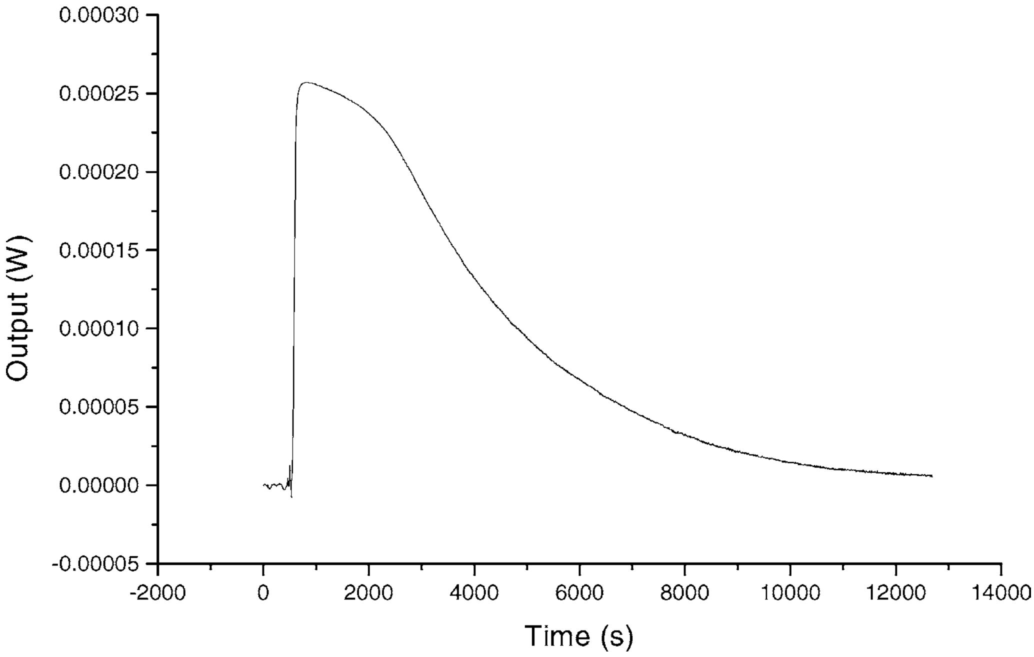


Fig. 1. Calorimetric output for methyl paraben.

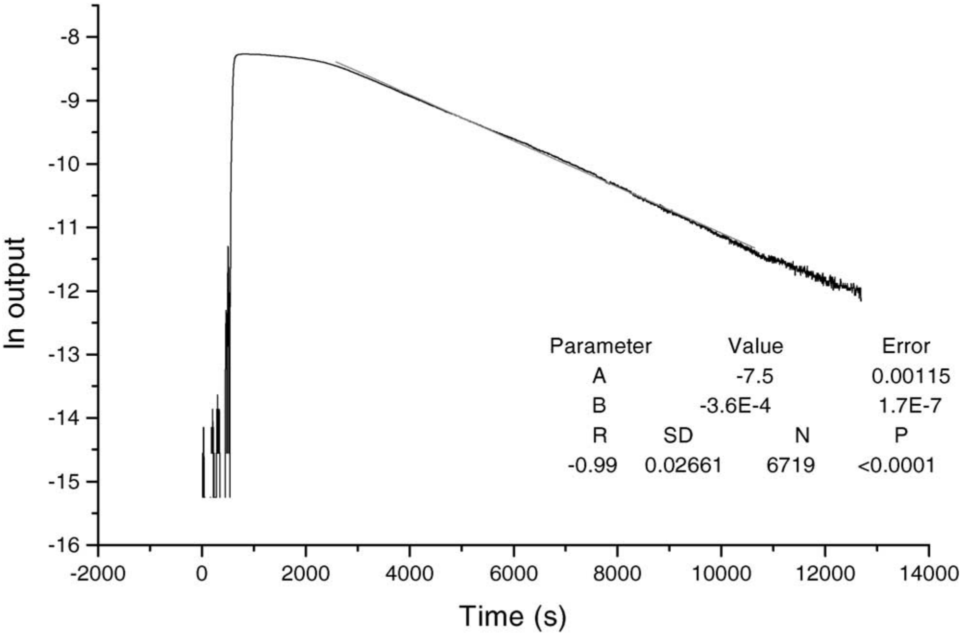


Fig. 2. Methyl paraben flow experiment ln output vs. time.

with a (negative) slope equal to the rate constant (this provides a useful internal check with the TAM data) and a y intercept equal to $\ln \Phi_0$, Fig. 2.

If the value for Φ at $t = 0$ (Φ_0) is used in Eq. (1), then the (e^{-kt}) term becomes equal to 1, leaving Eq. (4),

$$\Phi_0 = -FCH(1 - e^{-k\tau}) \tag{4}$$

Eq. (4) is readily re-arranged to give Eq. (5),

$$\tau = \frac{\ln(1 - (\Phi_0/FCH))}{-k} \tag{5}$$

All parameters, except τ, are now known and hence it is simple to calculate τ for a given flow rate. This was done using a simple Excel spreadsheet.

3. Results and discussion

The derived results for the rate constant and enthalpy, at 298 K, obtained from the ampoule based experiments in the batch TAM are reported in Table 1. The mean values for the enthalpy and rate constant were calculated as $-50.5 \pm 4.3\,\mathrm{kJ\,mol^{-1}}$ and $3.2 \times 10^{-4} \pm 1.1 \times 10^{-5}\,\mathrm{s^{-1}}$ respectively ($t_{1/2}$ for this reaction is approximately 36 min).

The values obtained from the Setaram for the rate constants at various temperatures are given in Table 2. The characteristic temperature dependence of the rate constant is clearly displayed in Fig. 3.

The derived values for rate constant, residence time and thermal volume for the LKB flow calorimeter are

Table 1
Rate constants and enthalpies determined from an ampoule study in the Thermal Activity Monitor 2277 (Thermometric AB, Järfälla, Sweden) 298 K

Experiment	Rate constant ($\mathrm{s^{-1}}$)	Enthalpy ($\mathrm{kJ\,mol^{-1}}$)
1	2.88E−04	45.4
2	3.06E−04	47.9
3	3.26E−04	58.4
4	3.16E−04	47.0
5	3.12E−04	47.2
6	3.03E−04	51.1
7	3.31E−04	51.4
Mean	3.15E−04	50.5
S.D.	1.10E−05	4.3

Table 2
Rate constants determined as a function of temperature using a DSC III (Setaram, France)

Temperature (K)	Rate constant (s^{-1})
293	2.42×10^{-4}
298	3.58×10^{-4}
303	7.27×10^{-4}
313	1.25×10^{-3}
325	3.06×10^{-3}

reported in Table 3. The crucial relationships are dramatically illustrated in Figs. 4 and 5.

The values derived for the rate constant, residence time and thermal volume for the customised TAM 2277 high-flow calorimeter are reported in Table 4.

The results obtained from the Setaram based study were analysed using the Arrhenius equation (Eq. (6)),

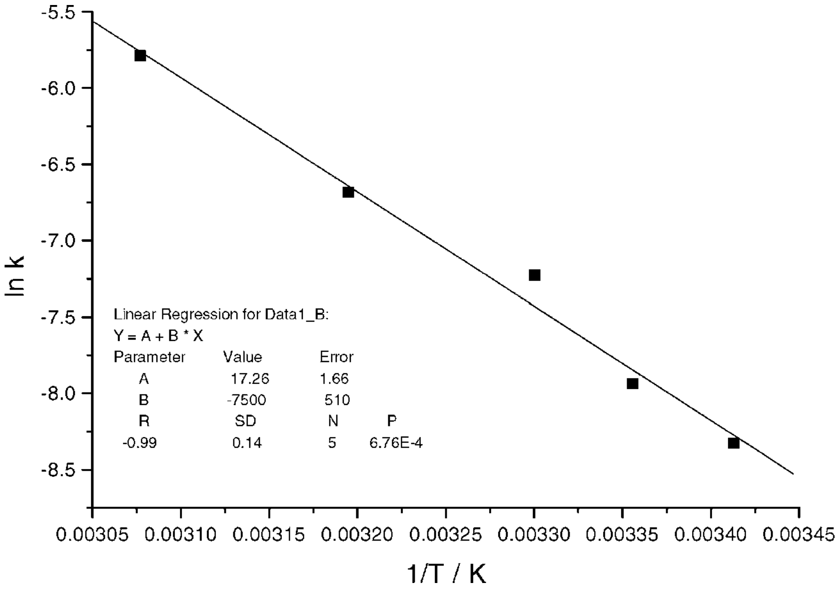
$$k = A e^{-E_a/RT} \tag{6}$$

where k is the rate constant (s^{-1}), A the pre-exponential factor, E_a the activation energy ($J\,mol^{-1}$), R the gas constant and T is the temperature (K).

The Arrhenius equation appears to be obeyed by this system and a plot of $\ln k$ versus $1/T$ yields a straight line with slope $-E_a/R$ (Fig. 1). The activation energy was calculated to be $+63\,kJ\,mol^{-1}$. The good linear fit obtained from this plot also confirmed that the reaction mechanism does not change over the temperature range studied.

Fig. 4 shows a plot of τ versus $1/F$ and, as would be expected from Eq. (3), a linear plot was obtained. As noted in Section 1, the thermal volume should vary with flow rate such that, at high flow rates, the thermal volume approaches zero and at low flow rates the thermal volume approaches the zero-flow-rate volume of the vessel. This reasoning explains the flow rate dependency seen in Fig. 5. It is possible therefore to calculate the zero-flow-rate volume of the cell, from this plot, by extrapolating the curve back to zero flow rate and taking the intercept value The zero-flow-rate value was determined as 0.73 cm^3. This is 65% greater than the nominal (engineered) volume.

The results obtained for the high-flow TAM 2277 instrument are in some respects different from the trend shown by the LKB 10-700-1. It appears that there is no significant dependence of the thermal volume over the flow rates studied for this particular calorimeter. It should be noted that it is of significantly different proportions from that of the flow LKB calorimeter. The insert was designed to sustain a dilute homogenous suspension of cultured Chinese hamster ovary cells [9]. The flow characteristics of such cellular suspensions were known to be significantly different from



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Linear Regression for Data1_B:
Y = A + B * X
Parameter     Value        Error
   A           17.26        1.66
   B          -7500          510
   R            SD           N        P
 -0.99         0.14          5     6.76E-4
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Fig. 3. Arrhenius plot for the base catalysed hydrolysis of methyl paraben.

Table 3

Flow rates, derived rate constants, thermal volume and residence times for the LKB flow microcalorimeter 10-700-1 298 K

Flow rate ($dm^3\,s^{-1}$)	Rate constant (s^{-1})	Thermal volume (cm^3)	τ (s)
1.73×10^{-6}	$3.19 \times 10^{-4} \pm 3.64 \times 10^{-5}$	0.71 ± 0.08	412
4.76×10^{-6}	$3.65 \times 10^{-4} \pm 3.64 \times 10^{-5}$	0.64 ± 0.05	135
5.91×10^{-6}	$3.67 \times 10^{-4} \pm 4.81 \times 10^{-5}$	0.62 ± 0.06	107
8.17×10^{-6}	$3.59 \times 10^{-4} \pm 2.50 \times 10^{-5}$	0.61 ± 0.05	76
9.50×10^{-6}	$3.44 \times 10^{-4} \pm 4.03 \times 10^{-5}$	0.61 ± 0.07	64

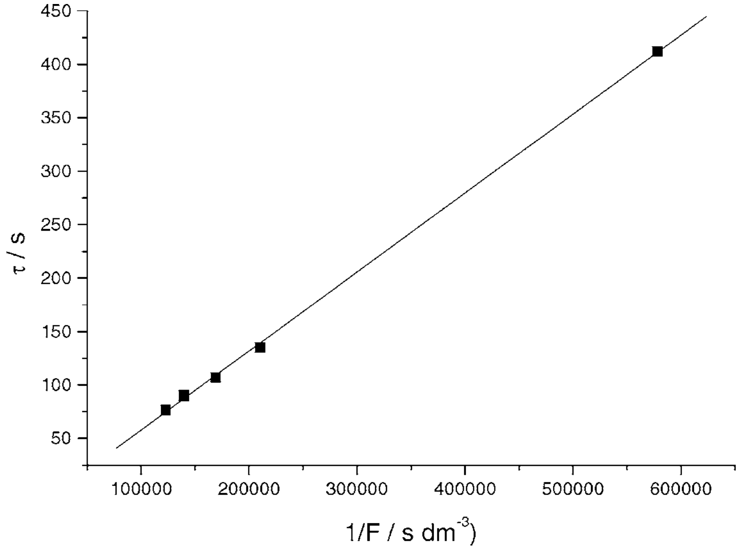

Fig. 4. Residence time vs. reciprocal flow rate.

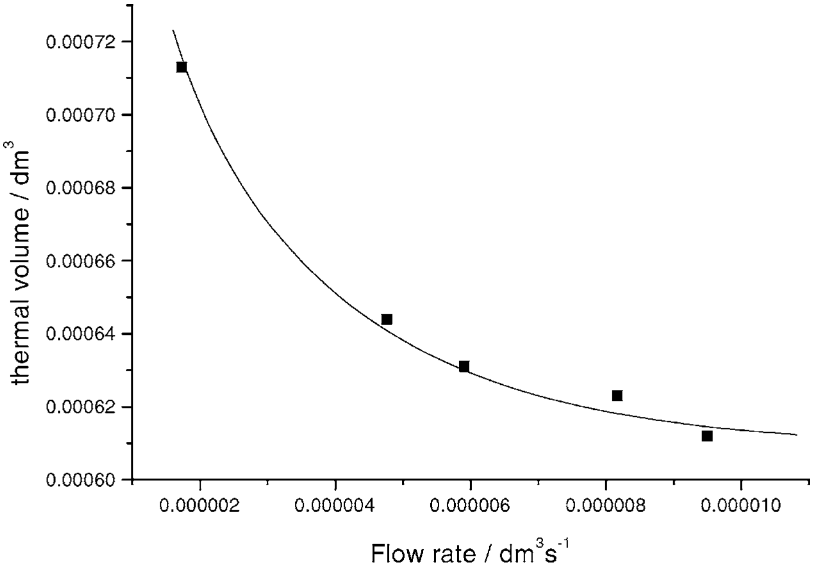

Fig. 5. Thermal volume vs. flow rate.

Table 4
Experimental determination of the thermal volume of the TAM 2277 customised, high-flow calorimeter (Thermometric AB, Järfälla, Sweden) over a range of working flow rates at 310 K

Experiment	Rate constant (s^{-1})	τ (s)	Thermal volume (cm^3)	Flow rate ($dm^3\,s^{-1}$)
1	7.75×10^{-4}	25.74	1.29	5.00×10^{-5}
2	7.58×10^{-4}	25.19	1.26	5.00×10^{-5}
3	7.79×10^{-4}	39.72	1.29	3.33×10^{-5}
4	7.79×10^{-4}	38.73	1.29	3.33×10^{-5}
5	8.01×10^{-4}	47.80	1.33	2.78×10^{-5}
6	8.31×10^{-4}	45.66	1.27	2.78×10^{-5}
7	8.09×10^{-4}	75.03	1.25	1.67×10^{-5}
8	8.03×10^{-4}	75.76	1.26	1.67×10^{-5}
9	8.08×10^{-4}	157.39	1.31	8.33×10^{-6}
10	7.97×10^{-4}	155.37	1.29	8.33×10^{-6}

homogenous solution phase systems [7]. To overcome these differences, wide-bore tubing and relatively fast, downward flow-through rates are employed along with a calorimetric vessel of 1 cm^3 nominal (engineered) volume. Equally important is the fact that, 35 years after the design of the LKB instrument [10], more attention was paid by Thermometric AB to the geometry and hydrodynamics of the flow vessel, the chamber housing it and the positioning of the Peltier heat detector assembly [7] to minimise the flow dependency of the heat detection system. To this end, the measuring chamber housing the coiled flow vessel was relatively large and also contained considerable lengths of transmission tubing to and from the engineered vessel. Heat from this tubing would have contributed to the total heat flow from the measuring chamber to the sink through the thermal detector. Nevertheless, it is anticipated that there will be a limit to the minimisation of such flow consequences that can be achieved by design modifications. It must be emphasised that the test reaction has allowed determination of the zero-flow-rate thermal volume (average 1.28 cm^3 over the range of flow rates) and that this is markedly different from the nominal (engineered) volume (1 cm^3) and from that calculated previously (at 100 $cm^3\,h^{-1}$) using the less satisfactory triacetin reaction as the test [11].

4. Conclusions

Recent work has highlighted the importance of having a test and reference reaction in order to report and reliably compare the results obtained from isothermal microcalorimeters. Such a test reaction must be robust, easy to perform and provide consistent, reproducible results. The imidazole catalysed hydrolysis of triacetin was shown [1] to be ideal for such a test reaction in static, batch-type calorimeters. However, it is not suitable for the validation of flow calorimeters used to determine kinetic parameters. This incongruity means that a secondary test reaction is required for the validation of the flow calorimeter, i.e. one that can be performed in a batch calorimeter such that the values obtained for *k* and *H* can be validated and traced back through the triacetin reaction. This paper has shown that the base catalysed hydrolysis of methyl paraben is a suitable candidate for such a test reaction.

It has been shown that the reaction can be reliably followed using isothermal flow microcalorimetry and the results obtained thus far conform to mathematical and theoretical concepts. There is approximately a 15% variation in thermal volume, for the LKB 10-700-1 instrument, across the range of flow rates studied. Note that, importantly, the zero-flow-rate volume is substantially different (65% greater) from the nominal (engineered) volume (Fig. 1). This indicates that other parts of the flow system contribute to both to the zero-flow-rate volume and to those thermal volumes determined at appropriate flow rates. This conclusion is illustrated in a simple, non-literal fashion in Fig. 6. The results obtained for the high-flow TAM 2277, however, do not follow the expected trend of significant thermal volume dependence on flow rate, possibly because of strenuous efforts by the

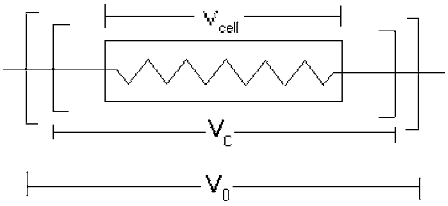

Fig. 6. Schematic for the flow calorimetric cell.

designer to minimise it. It should be noted, though, that the thermal volume of this instrument, is, again, significantly different from its nominal (engineered) volume. This will markedly affect the enthalpy balance equations of the type carried out for living material [12]. It is evident that variation in thermal volume is not predictable. Results reported in this paper show that thermal volume must be determined for all flow calorimeters used for the determination of thermo-kinetic data and that necessarily, determination of thermal volume will incorporate a study of its dependence on flow rate. Hence, the methyl paraben test reaction must be conducted for all flow-type calorimeters used for such thermo-kinetic studies.

This paper has shown the theoretical reasons for the requirement of a secondary test and reference reaction for isothermal flow microcalorimeters. It has also shown that the predicted effects are real and significant for the conditions studied thus far. It is important to note however that for this to become an accepted reference reaction it must be subject to an inter/intra-laboratory trial similar to that described [1] for the triacetin reaction.

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