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Chemical calibration of both flow and insertion calorimetric vessels for biological applications: limitations and solutions

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Abstract

It is frequently stated that calorimetric vessels should be calibrated by a chemical reaction as well as electrically by the Joule effect. For calibrating relatively rapid, purely chemical reactions, there are many well-characterised systems. However, biological reactions and the decomposition of many compounds are slow and so far only the hydrolysis of triacetin has been suggested as a suitable candidate. Measurement of the 4-cm^3 glass ampoule of a Thermometric TAM batch calorimeter gave a small thermal overestimate that was close to the quantity measured by others. In earlier work, it had been found that the thermal volume of the TAM standard and customised flow vessels varied in thermal size that depended on the rate of pumping through them.

The analysis in this paper proved that it is preferable to use rigorous thermal kinetic equations rather than the empirical second-order polynomial expansion often advocated for fitting the experimental data of the type found in the hydrolysis of triacetin. By fitting the data to both the first-order equation and the one that was not first order, it was unequivocally demonstrated that the best fit for this reaction is first order rather than the assumed second order. Attention was drawn to the advantage of obtaining the absolute zero time for the reaction because only then will there be true values for the rate constant and the molar reaction enthalpy. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Heat conduction calorimetry; Chemical calibration; Triacetin hydrolysis; Reaction order; Rate constant

1. Introduction

Heat conduction calorimeters were commercialised over 40 years ago (see reviews in Refs. [1,2]), Ever since then, the designers and manufacturers of the equipment have urged users to calibrate the measuring vessels by a chemical reaction as well as to undertake the regular electrical calibration with a resister of known ohms that produces a proportional amount of heat by the Joule effect. The reason why this is necessary is that, although the resister gives an immutable heat flow rate at source by which to calibrate the electrical signal from the thermopiles, it cannot allow the user to make an adjustment for the physical conditions under which the measurement is made in the vessel. In modern calorimeters, the resister is carefully placed in proximity to the measuring vessel [3,4]. Even so, electrical calibration fails to correct for the systematic errors that are due to the uncertain

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relationship of the set-place resister to the contents of the vessel and/or the contours of the vessel and/or the effects of agitation, stirring or flow. By failing to calibrate chemically and relying solely on the electrical Joule effect, the results become subject to systematic errors that cause deviations from the true values of the heat flow rate. There are at least two reasons for carrying out a further and more objective calibration. The first is to check the correctness of the electrical calibration. Secondly, if there is a systematic error, then an appropriate methodology ought to be applied to correct the data and thus obtain the true heat flow rate.

These days many scientists using calorimetry are not physical chemists. Their major problem is that, while there is a clear protocol for obtaining an electrical calibration that is frequently incorporated into the software of the instrument, there is no clear direction for chemical calibration in terms of the nature and the type of reaction. What biologists need is a reaction with kinetics comparable to Lavoisier's 'slow combustion' that characterises cellular metabolism. Even for physical chemists there is a requirement for reactions that can be used to check the extremely slow decomposition reactions of, for instance, polymers and pharmaceuticals. In recent times, the search for a suitable 'slow' reaction has focussed on the well-established model system of the imidazole-catalysed hydrolysis of triacetin that has been carefully characterised by Chen and Wadsö [5].

Misunderstandings about the nature of the systematic error compound the absence of guidance about the most appropriate system to use for calibration. First, it is often thought that, if it is assumed Φ_{real} is the true heat flow rate of a reaction and Φ_{exp} is the experimentally recorded heat flow rate after an electrical calibration, then providing that $\Phi_{exp}=\Phi_{real}$, the performed electrical calibration is error-free. There is no means to know this equivalence, however, because of the systematic error. So, it has to be assumed that $\Phi_{exp}\neq\Phi_{real}$ and a chemical correction is required for every vessel. Secondly, the deviation of Φ_{exp} from Φ_{real} is regarded intuitively as a linear relationship, i.e. Φ_{exp}/Φ_{exp}=constant for a given reaction system and calorimeter. However, by analysing the kinetics of the triacetin calibration reaction, our recent work shows that there is a clear non-linear relationship between Φ_{exp} and Φ_{real}. In this paper, it is intended to show the importance of adopting a model to express this non-linear relationship and highlight it by exposing the possible errors of using a linear correlation between Φ_{exp} and Φ_{real}.

A further striking issue raised in this paper is that up to now the published chemical calibration data have been focussed on the use of glass insertion ampoules [5,6]. The one exception is the recently presented results [7] for a standard Thermometric 0.6-cm^3 flow-through vessel in which liquid was pumped at the recommended flow rate of 35 cm^3 h^{-1}. In biological calorimetry, the most widely used containers are the various open perfusion/insertion and flow-through vessels, rather than glass ampoules. The reason to avoid the ampoule is that it is completely sealed and so not suitable for maintaining the oxidoreductive process of cell growth [8]. This situation is exacerbated by the sedimentation of the cells causing the so-called 'crowding effect' because there can be no stirring in the glass ampoule (see for instance Ref. [9]). A further disadvantage of the sealed ampoule is that it is obviously not possible to introduce bioactive materials, such as drugs [10], during the measurement. It is necessary, therefore, to extend chemical calibration beyond ampoules to the vessels most suitable for use in recording the heat flow rate of biological material. In summary, the aims of this paper are to calibrate the various types of calorimetric vessel based on the true kinetics for the triacetin hydrolysis reaction, and then to compare the data for the detection of possible systemic errors.

2. Thermal kinetics of chemical calibration

Having recognised the necessity of performing a chemical calibration, the next outstanding issue is to determine which chemical reaction is most suitable for such a purpose in the context of slow biological reactions. First, the reaction must be stable for a long-time run. Once this condition is satisfied, selection is based on two categories of information: These are (1) the range of heat flow rate at certain concentrations; and (2) the change of heat flow rate with time. Except for the starting concentration of the reactant, both the kinetic and the thermodynamic parameters of the reaction determine these two aspects. An investigation of them should help to

clarify the concepts and criteria to be used for selecting suitable chemical reaction systems for the calibration of heat conduction calorimeters and slow decomposition reactions.

With regard to the range of heat flow rate measured by calorimetry, the 4-channel Thermometric calorimeter (TAM) [3] is used as an example. This modular instrument is most suitable, in terms of design, to detect the heat flow rate from slow to moderate chemical or biological reactions. For this reason, many of the TAM applications have been for biologically significant chemical reaction systems and living cells [2,4,8]. For instance, the typical heat flow rates for cultures of animal cells and of microbes are in the range of 10–50 μW [11] and 50–200 μW [12], respectively. To calibrate the vessels used for living cells, it is axiomatic that the chosen chemical reaction system must generate heat flow rates within the required range and have the appropriately thermal kinetics.

The approach adopted in this paper is based on classical chemical reaction kinetics (see, for instance Refs. [13–15]). It is reasonably assumed that the partial molar reaction enthalpy is both independent of the reaction process and constant. Then, it can be derived [16] that the heat flow rate of a chemical reaction is described by the following equations that differ in terms of the order of reaction (n):

$$\frac{dq}{dt} = \Phi = k\Delta H_m[c_0^{1-n} - (1-n)kt]^{n/(1-n)} \text{ (if } n \neq 1) \tag{1}$$

$$\frac{dq}{dt} = \Phi = kc_0\Delta H_m e^{-kt} \text{ (if } n \neq 1) \tag{2}$$

Note that q refers to the amount of heat generated from a unit volume.

From Eqs. (1) and (2) at time zero, there is the following combined form in terms of reaction order:

$$\frac{dq}{dt}\Big|_{t=0} = kc_0^n\Delta H_m \tag{3}$$

Therefore, the heat flow rate at time zero is determined by reaction rate constant, the molar reaction enthalpy, the starting concentration of the reactant (c_0) and the reaction order. In Eq. (3), the only variable for a chosen system is c_0. Triacetin hydrolysis in imidazole buffer [5] is the frequently adopted calibrant, therefore, to provide the different ranges of heat flow rate by simply varying the triacetin concentration, while keeping fixed the amount of imidazole. In Table 1, there is a list of the heat flow rates at time zero and at 48 h for various concentrations of triacetin in the 1-cm^3 flow-through vessel of a specially designed module [17] for the Thermometric TAM. In this case, the thermal volume is very similar to the spatial volume of the flow-through vessel (1 cm^3) [17], meaning that the instrument reading is very close to the real heat flow rate expressed as μW cm^{-3}. An interesting point from Table 1 is that a triacetin concentration of 0.15 M is suitable for calibrating the heat flow rate range 0–30 μ W cm^{-3} while a concentration of 1.5 M looks more appropriate for calibrating the range 0–300 μW cm^{-3}.

The second issue concerns the rate of decline with time of the heat flow rate. Empirically, calorimetric calibration processes require long-lasting, chemical reactions because the instrument is usually capable of measuring long term changes of heat flow rate. As a consequence, the chosen chemical reaction should be suitable for validating the stability test over a long time period. In technical terms, further good reason to have the potential for a long time scale is that it can take some hours for the instrument to achieve thermal equilibration.

Table 1
The effect of the triacetin concentration on the heat flow rate at time zero and at 48 h in a 1-cm^3 flow-through vessel[a] at a flow rate of 100 cm^3 h^{-1} during the triacetin chemical calibration

Heat flow rate (μW cm^{-3})	Triacetin starting concentrations (M)						
	0.10	0.15	0.20	0.50	1.00	1.50	2.00
At initial time	16.2	22.8	32.4	80.9	1.62	243	324
At 48 h	11.8	17.7	23.6	59.0	118	177	236

[a] See Ref. [17] for technical details of this newly designed flow-through vessel.

The slope of the curve for heat flow rate with time may be characterised by the quantity $\mathrm{d}\Phi/\Phi\mathrm{d}t$. From Eqs. (1) and (2) respectively, this quantity is given by,

$$\frac{1}{\Phi}\frac{\mathrm{d}\Phi}{\mathrm{d}t} = k \ (\text{for } n = 1) \tag{4}$$

and

$$\frac{1}{\Phi}\frac{\mathrm{d}\Phi}{\mathrm{d}t} = \frac{k}{c_0^{1-n} + (n-1)kt} \ (\text{for } n \neq 1) \tag{5}$$

It is interesting to note that $\mathrm{d}\Phi/\Phi\mathrm{d}t$ is independent of the molar reaction enthalpy, but is determined by the kinetic parameters, rate constant and reaction order. By comparing Eq. (4) with Eq. (5), it is discovered that, for reactions with the same rate constant, an increase in the reaction order can cause a significant decline in the heat flow rate in a relatively short elapsed time. This analysis indicates that, to obtain a long-lasting chemical reaction, a lower order of reaction would be preferable to a higher one. As will be proved later, triacetin hydrolysis is a first-order reaction and therefore can be regarded as a suitable 'lower order' reaction. Wadsö ([see Refs. [4,5,18]) has advocated its use for calibrating 'slow' biological reactions since the early 1980s. At that time [5], no attempt was made to quantify the kinetics of this reaction. The following second-order polynomial expansion in terms of time was proposed, however, as an approach to the real thermal kinetics of the triacetin hydrolysis [5],

$$\Phi' = \frac{\Phi}{\rho} = a - bt + ct^2 \tag{6}$$

where Φ' is heat flow rate per unit mass of the reaction solution, a, b, c, the corresponding coefficients for this thermal kinetic equation, and ρ the density of the reaction solution. Since then, Wadsö [4] has provided a set of compositions and measured the true heat flow rate histories for specified triacetin solutions.

The major feature of the thermal kinetic equation, Eq. (6), is that it is purely empirical, being based on a second-order polynomial expansion with time. In the 1970s and 1980s, second virial coefficients were widely accepted experimentally in solution chemistry [19,20] as a correction to ideal solutions and this may have influenced the decision to explore this type of second-order expansion for the thermal kinetics of triacetin hydrolysis. Because of the empirical nature of Eq. (6), its applicability is restricted to a fairly short time span. As has been shown, an accurate description of the thermal kinetics of the calibration reaction is necessary for performing accurate and long-time chemical calibration.

As a more general view resulting from the criteria given here for selecting chemical calibration systems, it is highly probable that other chemical reactions exist to meet the requirements for a chemical calibration. It may be that alternative systems have not been validated in the past because the significance of chemical calibration was only realised by a few calorimetrists.

3. Consideration of reaction systems for microcalorimetry

After the *intrinsic* thermal kinetics of the chemical calibrating reaction is established, it is important to consider various reaction systems for measuring their *apparent* thermal kinetics. There are a variety of useful ways to classify chemical reactions in chemical kinetics and chemical reaction engineering [13,14]. For heat conduction calorimetry, it is proposed that the natural way is to characterise a chemical or biological reaction in terms of the type of vessel used to measure it. This type of classification is illustrated below for the Thermometric TAM.

The three predominant types of measuring vessels that are customarily used for biological and cellular systems are the glass ampoule, the perfusion/titration (p/t) insertion vessel and the flow-through vessel [1,2,4,8]. In terms of biochemical engineering, both the glass ampoule and the p/t vessel can be regarded as batch reactors. However, the latter is normally used with an integral stirrer that gives rise to a homogeneous system in terms of both the concentration profiles of any species involved in the reaction and the temperature distribution. In principle, then, the p/t vessel is the same as a continuously stirred tank reactor (CSTR) or a perfectly mixed vessel. In this sense, the reaction in such a vessel can be described by a CSTR iso-thermal system and is reflected by the true kinetics of the calibrating reaction, Eqs. (4) and (5). This is because a CSTR is free of problems such as molecular diffusion and heat transfer inside the reac-

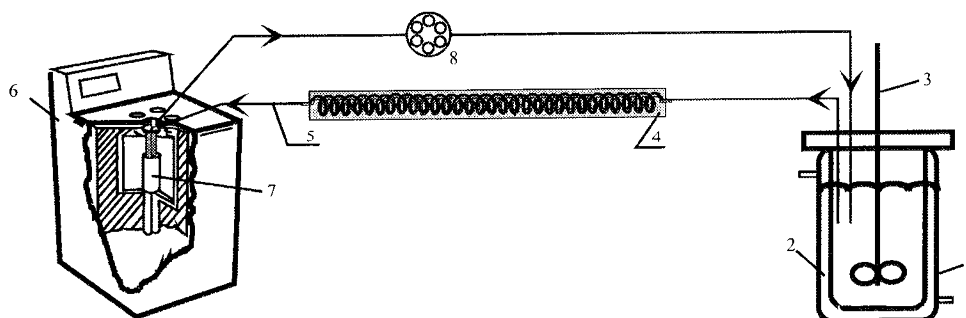

Fig. 1. A schematic drawing of the relationship between the reactor for the bulk triacetin solution and the customised, flow-through measuring vessel of the Thermometric calorimeter. 1, reactor; 2, jacket water for temperature control in the reactor; 3, agitator; 4, Nichrome wire wrapped around the insulated stainless-steel transmission tubing to reduce the heat loss of the cell suspension in transit from the reactor to the calorimeter; 5, Viton tubing; 6, the 4-channel Thermometric microcalorimeter (TAM); 7, the flow-through measuring vessel assembly; 8, the peristaltic pump.

tion vessel. Notwithstanding this, the heat flow rate measured by a p/t vessel is usually quite 'noisy' and prone to fluctuations caused by environmental factors. This is because such a vessel is usually open to the environment whereas the glass ampoule is a true closed system. The stirring shaft creates a conduit for interaction between the reacting species in the vessel and the changing environment. Despite the lack of stirring, in reality it is appropriate to approximate the reaction system in a sealed ampoule containing a small volume (a few cm^3) of solution to a CSTR isothermal system.

In engineering terms, at first glance the flow-through vessel appears to be like a plug-flow reactor rather than a CSTR. However, the following in-depth analysis imposes a completely different view. In order to accurately calibrate the flow vessel used to obtain the heat flow rate of CHO320 cells in a bioreactor (see Ref. [7]), the same experimental set-up is employed for the triacetin chemical calibration (Fig. 1). In this system, the bulk triacetin reaction mixture is kept in a stirred vessel that has a precision temperature control. A very small portion of the reaction solution is pumped in a continuous stream through the flow vessel as a closed loop to the bulk solution. The transmission line from the bulk vessel to the calorimeter is thermally well insulated and temperature-compensated to ensure there is no temperature drop. The part of the transmission line inside the calorimeter involves two heat exchangers to compensate further for any possible heat loss outside the calorimeter [17]. This same overall design involving the bioreactor had been used previously for the standard, commercial flow module [7]. The bulk stirring vessel is obviously best described as a CSTR. On the other hand, the reaction in the transmission line including the flow vessel is better described as a plug-flow reactor. When compared to the 'slow' reaction rate for triacetin hydrolysis, however, the residence time (τ) of the reaction solution in the transmission system is so short that it is justifiable to neglect the compositional difference for the solutions in the bioreactor and in the flow-through vessel of the calorimeter. It was thus reasoned that the entire reaction system should be modelled as a CSTR with a detection loop of which the flow-through vessel is an integral part. Thus, the intrinsic thermal kinetic equations described by Eqs. (4) and (5) can be applied in their original forms for this iso-thermal system.

4. Experimental

The reagents for the triacetin hydrolysis were of the highest commercial grade from Sigma and further purified, where necessary, according to the procedures adopted by Chen and Wadsö [5]. The triacetin solution was prepared according to a composition (solution C) originally formulated by them [5] to contain the following ingredients: 11.96% (w/w) acetic acid, 18.10% (w/w) imidazole and 3.47% (w/w) triacetin. The solution was kept in a freezer at −20°C for not more than 1 month. Before use, the frozen solution

was warmed to near 37°C in a water bath and re-stirred using a magnetic flea. The re-mixing step is important in that sometimes there is a phase separation after this viscous solution is frozen.

It has been shown theoretically that sharp changes in the reaction rate occur immediately after mixing the reactants (c_0 in Eq. (3)). Therefore, it is vital to record the reaction from the absolute zero time of mixing the ingredients [16]. Failure to do so makes the result difficult to be repeated by other operators and invalidates the values for the thermodynamic and kinetic parameters calculated from the experimental result. This is because the triacetin concentration c_0 is strictly applicable only for absolute zero time. In these experiments, the best marker for zero time was when the solution reached the reaction temperature, 37°C. Immediately, the data logging program (Applikon BioXpert v.2.10) was set to zero. In the later data processing stage, these recorded but unstable data prior to thermal equilibration of the insertion vessel were not utilised in the curve correlation for both the empirical and the theoretical data treatments.

The experiments were performed using three different vessels in a 4-channel Thermometric TAM microcalorimeter. The 3-cm^3 glass ampoule contained 2 cm^3 triacetin solution and it was operated according to the previously documented procedure [5]. The flow-through measuring vessel constituted an external loop to the 3-dm^3 Applikon glass bioreactor (see Fig. 1). The stainless-steel tubing for the bioreactor and calorimeter, together with the Viton tubing of the pump were properly tested to ensure that they were chemical inert to the triacetin hydrolysis mixture. Technical details on the use of the flow vessel have been given elsewhere [7,17,21]. The third vessel was the stainless-steel perfusion/titration (p/t) type with stirrer. Precisely 3 cm^3 triacetin solution was added to it. It was then lowered stepwise to the measuring position within 1 h, according to the instructions from the manufacturer [22].

5. Numerical calculations

The numerical calculations are aimed at discovering the best estimations for the parameters in thermal kinetic equations, typically Eqs. (1) and (2). Since these are for different reaction order ranges, a set of experimentally measured heat flow rate data were thus tested for each equation with details given below. If it is assumed that Φ_i^* and Φ_i are the i-th measured and calculated heat flow rates, respectively, at time t_i and m is total number of measurements. The following quantity, Q_E, may be defined for quantifying the degree of fitness of these two equations to the experimental data,

$$Q_E = \frac{\sum_{i=1}^{m}(\Phi_i - \Phi_i^*)^2}{m} \tag{7}$$

Obviously, a smaller value of Q_E means a better fit to the particular thermal kinetic equation. For the fittings given in this paper, the difference between Eqs. (1) and (2) is often obvious without resort to the value for Q_E.

5.1. Fitting to equation (2) when the reaction order n=1

This test involves only two parameters, the molar reaction enthalpy (ΔH_m) and rate constant (k). Eq. (2) can be expressed in the following linear form:

$$\ln \Phi = \ln(kc_0\Delta H_m) - kt \tag{8}$$

If ln Φ is plotted against t and then the least-square method is applied, the best fit linear curve is obtained with slope $-k$ and intercept b. The molar reaction enthalpy can then be calculated by:

$$\Delta H_m = \frac{e^b}{kc_0} \tag{9}$$

5.2. Fitting to equation (1) when reaction order $n \neq 1$

When considering the reaction order other than n=1, target function $F(k,\Delta H_m,n)$ is defined by,

$$F(k,\Delta H_m,n) = \sum_{i=1}^{m}(\Phi_i - \Phi_i^*)^2 = \sum_{i=1}^{m}\left\{k\Delta H_m[c_0^{1-n} - (1-n)kt_i]^{n/(1-n)} - \Phi_i^*\right\}^2 \tag{10}$$

To find a set of values for $(k,\Delta H_m,n)$, the function $F(k,\Delta H_m,n)$ needs to be minimised in terms of the three parameters. Although it is mathematically pos-

sible to determine the molar reaction enthalpy using only Eq. (10) based on the principle shown below, the error involved for ΔH_m by such a calculation is in fact much higher than the value of this quantity obtained from Eq. (9). This is because the first-order thermal kinetic equation gives a much better fit to the experimental data and therefore the first order is in fact a good approximation to the triacetin hydrolysis reaction. In the calculation using Eq. (2), the value of ΔH_m obtained from the first-order assumption was thus chosen for use. As a consequence, only two parameters, the rate constant and the reaction order, need to be decided in Eq. (10) and so the function $F(k,\Delta H_m,n)$ is simplified to $F(k,n)$.

In order to minimise $F(k,n)$, it is required that,

$$\frac{\partial F}{\partial k} = 2\sum_{i=1}^{m}\left[(\Phi_i - \Phi_i^*)\frac{\partial \Phi_i}{\partial k}\right] = 0 \tag{11}$$

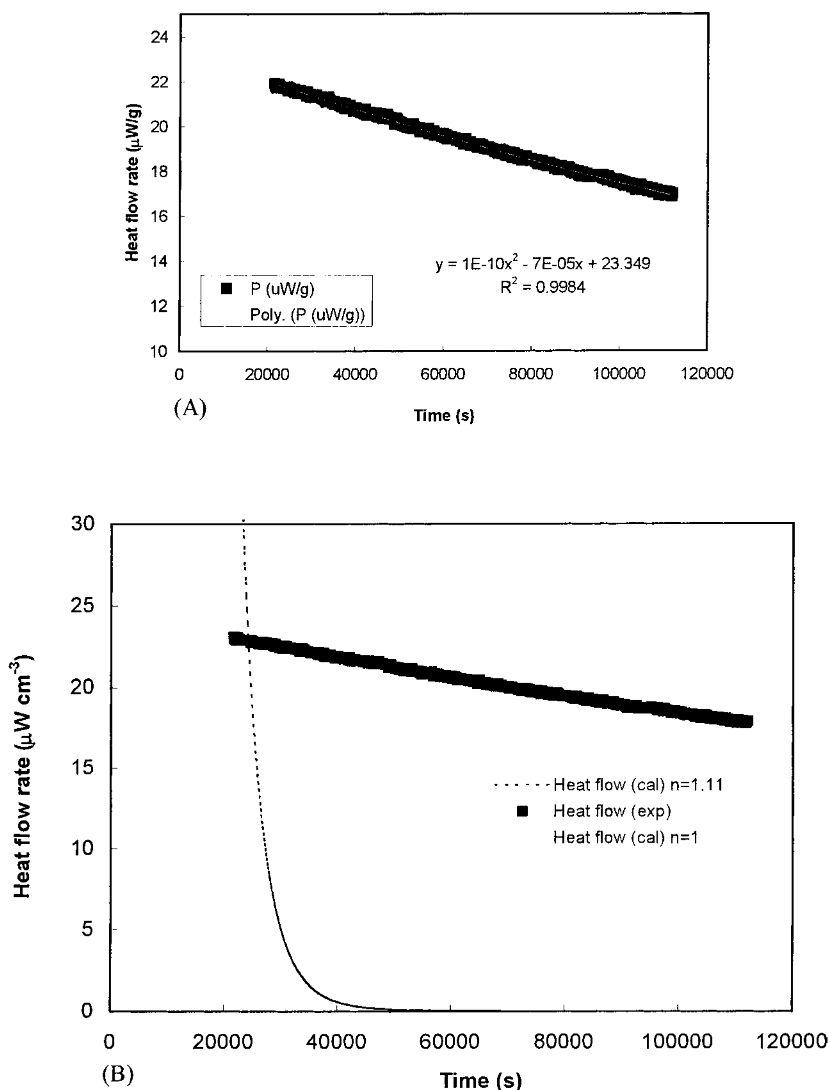

Fig. 2. Triacetin hydrolysis in a 2-cm^3 glass ampoule. (A) The correlation was made using a second-order polynomial expansion $\Phi'=\Phi/\rho=a-bt+ct^2$ (B) The correlation was made using thermal kinetic equations: $dq/dt=\Phi=k\Delta H_m[c_0^{1-n}-(1-n)kt]^{n/(1-n)}$ (if $n\neq1$) (dotted line) and $dq/dt=\Phi=kc_0\Delta H_m e^{-kt}$ (the 'white space').

and

$$\frac{\partial F}{\partial n} = 2\sum_{i=1}^{m}\left[(\Phi_i - \Phi_i^*)\frac{\partial \Phi_i}{\partial n}\right] = 0 \qquad (12)$$

in Eqs. (11) and (12)

$$\frac{\partial \Phi}{\partial k} = \Delta H_{\rm m}\{[c_0^{1-n} - (1-n)kt]^{n/(1-n)} - nkt[c_0^{1-n} - (1-n)kt]^{(2n-1)/(1-n)}\} \qquad (13)$$

$$\frac{\partial \Phi}{\partial n} = k\Delta H_{\rm m}{\rm e}^{\{(n)/(1-n)\ln[c_0^{1-n}-(1-n)kt]\}\{(1)/(1-n)^2\ln[c_0^{1-n}-(1-n)kt]+(n)/(1-n)[c_0^{1-n}-(1-n)kt]^{-1}[kt-c_0^{1-n}\ln c_0]\}} \qquad (14)$$

The calculation was made by a modified Gauss–Newton algorithm [23] and a NAG Fortran library routine (Mark 18), E04HYF, was used.

6. Results and discussion

6.1. Triacetin hydrolysis for chemical calibration is a first-order reaction

As outlined in the above sections, there are three equations for fitting to the experimentally measured heat flow rates for the different measuring vessels.

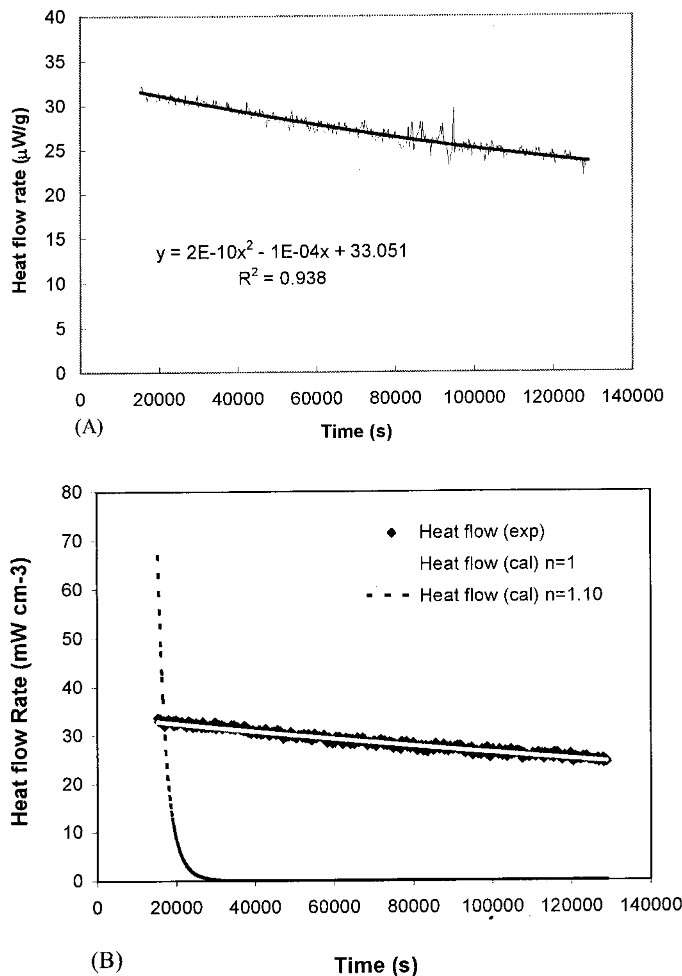

Fig. 3. Triacetin hydrolysis in the 1-cm^3 flow-through vessel of the customised TAM flow module at a flow rate of 50 cm^3 h^{-1}. (A) The correlation was made using a second-order polynomial expansion. (B) The correlation was made using thermal kinetic equations. Details of the corresponding equations are explained in the legend to Fig. 2.

These are the second-order polynomial expansion in terms of time (an empirical equation), i.e. Eq. (6), the equation for the first-order reaction (i.e., Eq. (2)) and the equation for other than first order (i.e. Eq. (1)). The measurements were made at two different flow rates for the flow through vessel and at two different stirring rates for the p/t vessel. Graphical representations of the fits are shown in Figs. 2–6. In all cases, clearly satisfactory fits are seen for Eq. (6) and for Eq. (2). The good fit of the first-order equation (i.e. Eq. (2)) is more remarkable than that for Eq. (6). This is because the former contains only two parameters (the rate constant and the reaction molar enthalpy), each of which has a clear physical meaning, whereas the second-order polynomial expansion in terms of time (i.e. Eq. (6)), contains three parameters (a, b and c). In order to emphasise the point that the triacetin reaction is first order, it should be noted that the best fit to Eq. (1) (i.e. $n \neq 1$) is consistently unsatisfactory, as can be seen in Fig. 2B, Fig. 3B, Fig. 4B, Fig. 5B and Fig. 6B. Quantified results for the fitted parameters and the assessment quantity, Q_E, are given in Table 2. Although these results show that Eq. (1) is inapplicable, it is however very interesting to note that the best

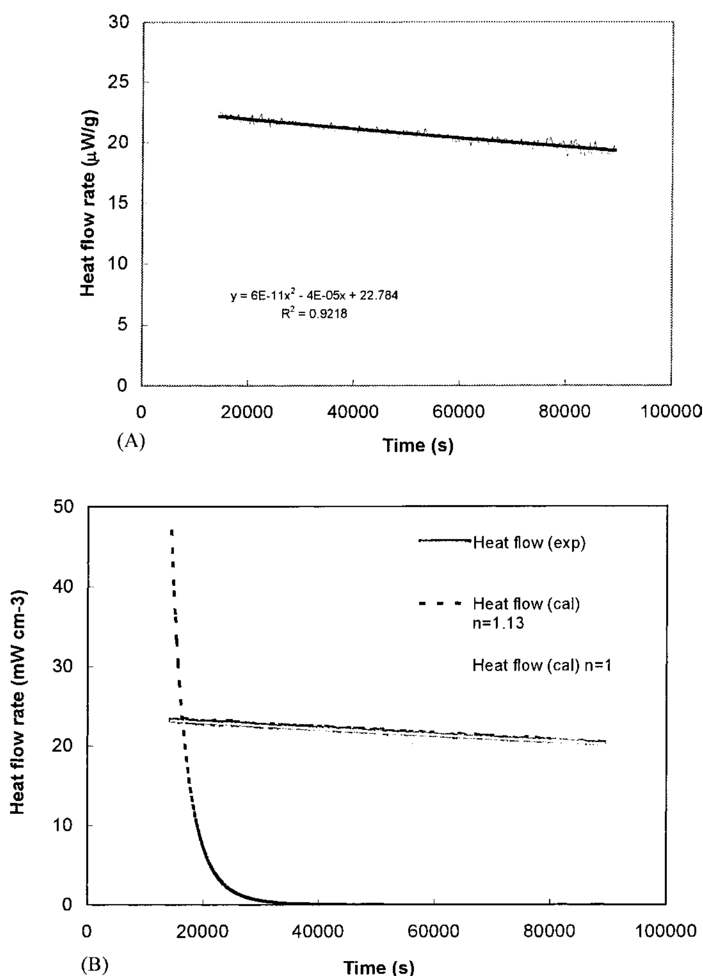

Fig. 4. Triacetin hydrolysis in the 1-cm^3 flow-through vessel run at the higher flow rate than for the data depicted in Fig. 3, 100 cm^3 h^{-1}. (A) The correlation was made using a second-order polynomial expansion. (B) The correlation was made using thermal kinetic equations. Details of the corresponding equations are explained in the legend to Fig. 2.

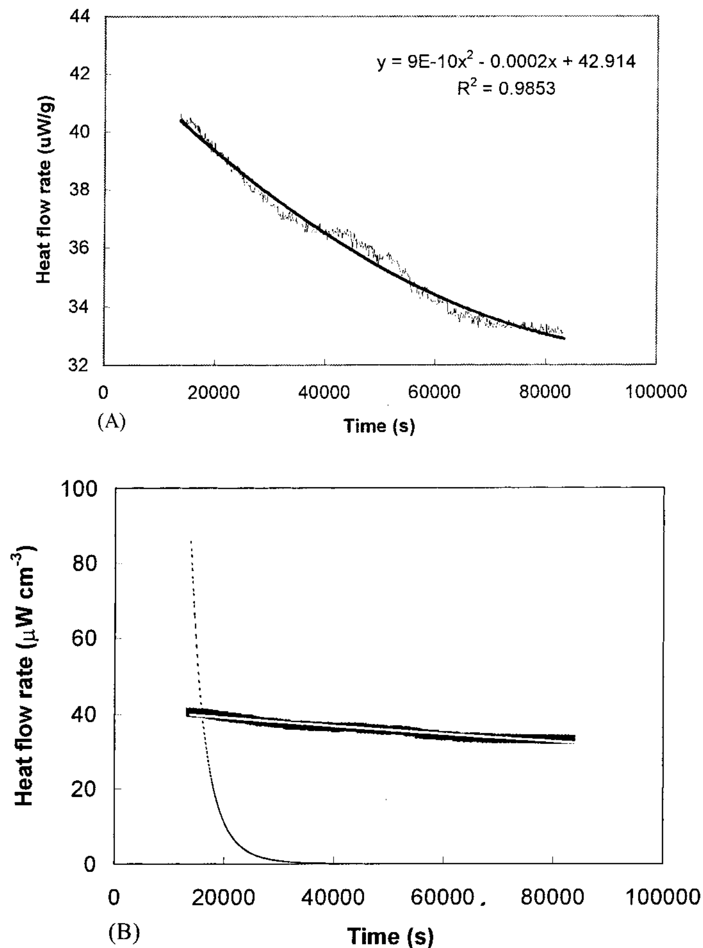

Fig. 5. Chemical calibration using triacetin for the 4-cm^3 perfusion/perfusion insertion vessel of the Thermometric TAM. There was no stirring of the reactants. (A) The correlation was made using a second-order polynomial expansion. (B) The correlation was made using thermal kinetic equations. Details of the corresponding equations are explained in the legend to Fig. 2.

fit to this equation gives rise to a reaction order of 1.1 in a consistent way. Given that mathematically the value of the reaction order, n, in Eq. (1) can not take 1, even this negative result implies that the reaction order for the triacetin hydrolysis should have an order close to 1.

Willson et al. [24] recently confirmed their finding [6] that the triacetin reaction is second order. A noticed difference between their experimental data and the current work is that the former was obtained at 25°C whereas the present results were recorded at 37°C. It seems unlikely, however, that this variance could markedly alter the reaction order. It is possible that an arbitrary zero time was adopted for the earlier work [6,24] and thus a pseudo second-order reaction was detected for certain periods of time. Reasons for the difference are discussed elsewhere [16].

6.2. Significance of the non-linearity

It would be simple if the systematic error involved in the chemical calibration were linear. For the empirical equation, Eq. (6), this means that there is the following relationship for the different sets of parameters a, b and c:

$$\frac{\Phi_1}{\Phi_2} = \frac{a_1}{a_2} = \frac{b_1}{b_2} = \frac{c_1}{c_2} \tag{15}$$

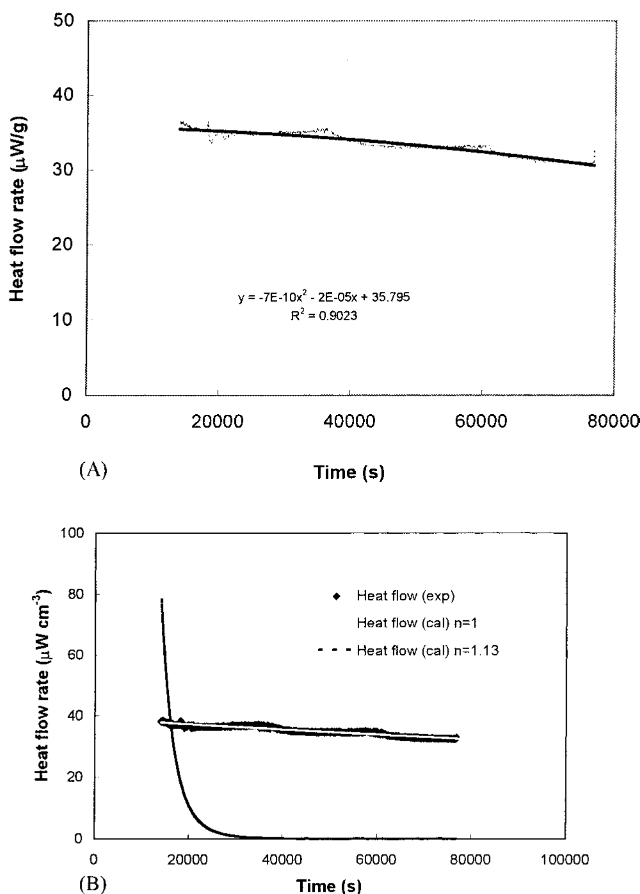

Fig. 6. Triacetin hydrolysis in the same 4-cm^3 vessel used to obtain the data for Fig. 5, but the solution was stirred at a rate of 60 rpm. (A) The correlation was made using a second-order polynomial expansion. (B) The correlation was made using thermal kinetic equations. Details of the corresponding equations are explained in the legend to Fig. 2.

However, the data in Table 3 do not support this relationship and at best they only allow the following approximation:

$$\frac{\Phi_1}{\Phi_2} \approx \frac{a_1}{a_2} \neq \frac{b_1}{b_2} \neq \frac{c_1}{c_2} \tag{16}$$

This situation shows that the correction made by a calibration can contain significant non-linear elements. The fact that there is a non-linear relationship in making the correction to experimentally recorded results was well shown for the triacetin hydrolysis reaction in which the reaction order is 1 [16]. Let

$$A = \frac{k_{\mathrm{real}} c_0 \Delta H_{\mathrm{m,real}}}{(k_{\mathrm{exp}} c_0 \Delta H_{\mathrm{m,exp}})^{k_{\mathrm{real}}/k_{\mathrm{exp}}}} \tag{17}$$

and

$$\gamma = \frac{k_{\mathrm{real}}}{k_{\mathrm{exp}}} \tag{18}$$

Then it follows that [16],

$$\Phi_{\mathrm{real}} = A(\Phi_{\mathrm{exp}})^{\gamma} \tag{19}$$

Eq. (19) is far superior to the criteria given by Eqs. (15) and (16) in that the non-linearity can be reflected by just a single parameter γ in it. As listed in Table 4, the γ value varies from 1.21 to as high as 1.78. None of them is close to unity and this clearly signifies the importance of considering the non-linear correction based on chemical calibration.

Table 2
The results obtained for the kinetic and thermodynamic parameters in the triacetin hydrolysis reaction using the thermal kinetic equation for a first-order reaction (n=1) and for one that is not first order ($n\neq1$)

Measuring vessels	Thermal kinetic equations	
	$q=c_0\Delta H_m(1-e^{-kt})$ for n=1	$q=\Delta H_m\{c_0-[c_0^{1-n}-(1-n)kt]^{1/(1-n)}\}$ for $n\neq1$
1-cm^3 Flow vessel at 100 cm^3 h^{-1}	ΔH_m=88.4 kJ/mol k=1.83×10^{-6} (s^{-1}) Q_E=0.13 (μW g^{-3})	k=3.09×10^{-4} n=1.13 Q_E=19.6 (μW g^{-1})
1-cm^3 Flow vessel at 50 cm^3 h^{-1}	ΔH_m=89.9 kJ/mol k=2.58×10^{-6} (s^{-1}) Q_E=0.46 (μW g^{-3})	k=4.28×10^{-4} n=1.10 Q_E=27.9 (μW g^{-1})
2-cm^3 Glass ampoule	ΔH_m=58.1 kJ/mol k=2.87×10^{-6} (s^{-1}) Q_E=0.075 (μW g^{-3})	k=2.40×10^{-4} n=1.11 Q_E=19.2 (μW g^{-1})
4-cm^3 Insertion vessel without stirring	ΔH_m=94.40 kJ/mol k=2.98×10^{-6} (s^{-1}) Q_E=0.39 (μW g^{-3})	k=2.90×10^{-4} n=1.13 Q_E=33.8 (μW g^{-1})
4-cm^3 Insertion vessel at 60 rpm	ΔH_m=108.2 kJ/mol k=2.33×10^{-6} (s^{-1}) Q_E=0.51 (μW g^{-3})	k=3.00×10^{-4} n=1.13 Q_E=26.7 (μW g^{-1})

Table 3
Parameters for the empirical equation $\Phi'=a-bt+ct^2$ to represent the thermal kinetics of the triacetin hydrolysis in different measuring vessels of the Thermometric microcalorimeter

	Parameters for Chen–Wadsö equation: $\Phi'=a-bt+ct^2$			Correlation coefficient (R)
	a (μW g^{-1})	b (μW g^{-1} s^{-1})	c (μW g^{-1} s^{-2})	
2-cm^3 Glass ampoule [5]	21.81	7.9×10^{-5}	3.5×10^{-10}	high
2-cm^3 Glass ampoule	23.35	7.0×10^{-5}	1.0×10^{-10}	0.998
1-cm^3 Flow-through vessel at 50 cm^3 h^{-1}	33.05	1.0×10^{-4}	2.0×10^{-10}	0.938
1-cm^3 Flow-through vessel at 100 cm^3 h^{-1}	22.78	4.0×10^{-5}	6.0×10^{-11}	0.922
4-cm^3 Insertion vessel without stirring	42.91	2.0×10^{-4}	9.0×10^{-10}	0.985
4-cm^3 Insertion vessel stirred at 60 rpm	35.80	2.0×10^{-5}	−7.0×10^{-10}	0.902

Table 4
The non-linearity parameter γ in terms of 2-cm^3 glass ampoule for flow-through and perfusion/titration calorimetric measuring vessels

Calorimetric measuring vessel	Non-linearity parameter γ of Eq. (18)
1-cm^3 Flow vessel at 100 cm^3 h^{-1}	1.29
1-cm^3 Flow vessel at 50 cm^3 h^{-1}	1.78
4-cm^3 Insertion vessel without stirring	1.21
4-cm^3 Insertion vessel at 60 rpm	1.25

Table 5
Thermal kinetic parameters of the first-order triacetin hydrolysis reaction (Eq. (2)), truncated to second order (Eq. (20))[a]

	Expanded terms as appear in Eq. (20)		
	$kc_0\Delta H_m/\rho$ (μW g^{-1})	$k^2c_0\Delta H_m/\rho$ (μW g^{-1} s^{-1})	$k^3c_0\Delta H_m/\rho$ (μW g^{-1} s^{-2})
2-cm^3 Glass ampoule	23.32	6.7×10^{-5}	9.6×10^{-11}
1-cm^3 Flow-through vessel at 50 cm^3 h^{-1}	32.43	8.4×10^{-5}	1.1×10^{-10}
1-cm^3 Flow-through vessel at 100 cm^3 h^{-1}	22.62	4.1×10^{-5}	3.8×10^{-11}
4-cm^3 Insertion vessel without stirring	39.34	1.2×10^{-4}	1.7×10^{-10}
4-cm^3 Insertion vessel stirred at 60 rpm	35.25	8.2×10^{-5}	9.6×10^{-11}

[a] Thermal kinetic parameters were obtained using previously best-fit results as shown in Table 2 for the first-order kinetic assumption. A purpose of these results is to justify numerically the correctness of Eq. (21). The corresponding values (a, b and c) in the left sides of Eq. (25) can be seen in Table 3.

6.3. Convergence of equation (2) with equation (6)

The theoretically correct thermal kinetic equation for the triacetin hydrolysis has been clearly established in this paper. This is now an appropriate place to compare intensively the theoretically sound equation, Eq. (2) with the very useful empirical equation, Eq. (6).

Expansion of Eq. (2) truncated at the second-order leads to,

$$\Phi = kc_0\Delta H_m\left(1 - kt + \frac{k^2}{2}t^2\right) \tag{20}$$

A comparison of Eq. (20) with Eq. (6) leads to the following approximation:

$$\left.\begin{aligned} a &\cong kc_0\Delta H_m/\rho \\ b &\cong k^2c_0\Delta H_m/\rho \\ c &\cong k^3c_0\Delta H_m/2\rho \end{aligned}\right\} \tag{21}$$

In Table 5, the values on the right side of Eq. (21) were calculated for comparison with the values on the left side of Eq. (21) (already listed in Table 3). The closeness of the corresponding values is very convincing, especially for the first and second terms on the right side of Eq. (20) in order. It confirms that the hydrolysis of triacetin is a first-order reaction. Further, it authenticates the intuition of Chen and Wadsö [5] in formulating Eq. (6).

The condition for the accuracy of Eq. (20) and for the correctness of Eq. (21) is that the value of kt should be fairly small, meaning that the applicability of Eq. (6) is restricted to a relatively short period of time. As a consequence of this, it would be inaccurate to apply Eq. (6) to an experiment lasting for more than 48 h.

7. Conclusions

This paper emphasises the necessity of adopting chemical calibration for slow biological reactions studied by calorimeter. The presently favoured reaction for this purpose is the imidazole-catalysed hydrolysis of triacetin. The various general aspects associated with a chemical calibration were studied, such as the selection of the appropriate reaction solutions and the use of existing thermal kinetic equations. Clear solutions or guidance are given to calorimetric users concerning the accuracy of the measured slow chemical reactions, such as decomposition, and the reactions in biological systems. Particular attention has been paid to the true reaction order of triacetin hydrolysis. Some of the problems involved in chemical calibration are illustrated quantitatively, including the non-linear correction to the calibration. Thus, it has been possible to define conceptually the applicability of and the restrictions to the empirical equation often used in calculating the correction.

8. Nomenclature

A	combined quantity as defined in Eq. (17)
a, b, c	coefficients of the second-order polynomial expansion, see Eq. (6)
c_0	concentration of the reactants at the time of mixing
F	target function defined in Eq. (10)
ΔH_m	molar reaction enthalpy (J mol^{-1})
k	rate constant (unit depending on the reaction order)
m	number of experimental measurement

n reaction order
Q_E quantity defined in Eq. (7) for assessing degree of curve fitness
dq/dt heat flow rate (W cm^3)
t time (s)

Greek letters
β ratio of heat flow rate at time t to that at absolute time zero
γ parameter characterising non-linearity of chemical calibration as defined in Eq. (18)
ρ density of solution (g cm^{-3})
τ residence time
Φ heat flow rate (W cm^{-3})
Φ' heat flow rate (W g^{-1})

Subscripts
E estimate
exp experimental result
i i-th molar
m molar
0 absolute zero time
real true result

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