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Practical and theoretical consideration of flow-through microcalorimetry: determination of "thermal volume" and its flow rate dependence

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Abstract

The results have recently been reported of a preliminary study into the base catalysed hydrolysis of methyl paraben as a test and reference reaction for flow-through microcalorimeters. The values quoted for the rate constant and the molar enthalpy change are $(3.15\pm0.11)\times10^{-4}\,\mathrm{s}^{-1}$ and $-50.5 \pm 4.3\,\mathrm{kJ\,mol}^{-1}$, respectively. The present paper explains the results of further studies of the methyl paraben reaction and also describes some practical and theoretical considerations of flow microcalorimetric experiments. It is shown that experimental design (sample and reference arrangements) has a significant impact upon the derived data. Particular attention is paid to the theoretical range of values for the molar enthalpy change and the rate constant, for reactions that could be studied successfully by flow calorimetry.

Keywords: Test and reference reaction; Flow microcalorimetry; Methyl paraben; Rate constant

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1. Introduction

The requirement for test and reference reactions to use in the validation of kinetic and thermodynamic data derived from isothermal microcalorimeters have been known for over 20 years [1]. The results of an inter/intra laboratory study into the use of the imidazole catalysed hydrolysis of triacetin as a test and reference reaction for batch-type microcalorimeters have been described in previous publications [2–7]. O'Neill et al. [8] examined the question of the applicability of the triacetin reaction for the validation of data obtained from flow-through calorimetry and concluded for reasons elaborated later in this paper that it was necessary to develop a reaction with different kinetics for this purpose. They then went on to report the results of an investigation into the applicability of the base catalysed hydrolysis of methyl paraben as a test and reference reaction for flow isothermal calorimeters.

Such instruments generally have two modes of operation, flow mix and flow through. In both instances the reagents are pre-equilibrated outside the calorimeter in a flow-mix experiment the reagents are mixed actually inside the cell and the reacting solution is flowed to waste (or retained for further analysis). For a flow-*through* experiment, the reaction is initiated outside the calorimeter and the reacting solution can be recycled (or again retained for further analysis). 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. 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 re-

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action 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.

A classic example of such studies is found in calorimetric investigations of microorganisms which, by their very nature, experience a change in rate of reaction with time. The importance of deriving kinetic and thermodynamic information from such systems was recognised over 25 years ago [9], however at that time the equations required for such analysis had not been derived. This paper will describe the equations that can, and should, be applied to the analysis of calorimetric data obtained from these complex systems.

The test and reference reaction was found to be 1st order with respect to methyl paraben and the derived values for the rate constant and the molar enthalpy change were $(3.15 \pm 0.11) \times 10^{-4}\,\mathrm{s}^{-1}$ and $(-50.5 \pm 4.3)\,\mathrm{kJ\,mol}^{-1}$, respectively [8]. The results of an investigation to explore the theoretical limitations of studies using flow calorimetry are reported in this paper. This is achieved through a consideration of the range of values for the molar enthalpy change ("enthalpy" for shorthand) and the rate constant of hypothetical reactions that would allow successful analysis. In addition, the work extends the analysis of the thermal volume data described previously [8] and highlights the crucial importance of experimental design in determining the thermal volume.

2. Background to the concept of thermal volume and development of the calorimetric equations

Kinetic equations have been previously derived, which describe the calorimetric output from a flowing system [10,11]. These equations can be derived from simple kinetic expressions such as Eq. (1). Integration of this expression to give Eq. (2) allows a general equation which can be applied to all simple $A \rightarrow B$ reactions to derive Eq. (3). A more detailed discussion of these and related equations can be found in Refs. [3,7]:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = k(C - x)^n \tag{1}$$

$$kt + a = \frac{1}{n-1}(C - x)(C - x)^{-n} \tag{2}$$

When $t = 0$, $x = 0$:

$$a = \frac{1}{n-1}C^{1-n}$$

substituting and rearranging gives

$$x = C - (C^{-n}(-ktC^n + kntC^n + C))^{1/(1-n)} \tag{3}$$

where x is the amount of material reacted to time, t; $\mathrm{d}x/\mathrm{d}t$ the rate of change of x with time; k the appropriate rate constant; C the initial concentration of reactant; n the order of reaction; and a the constant of integration.

The calorimetric output for a flowing system is the average signal over the time during which the reacting solution is in the calorimetric vessel, the residence time, τ. Equations can now be written which describe the calorimetric output for any kinetic order. In this paper, only zero, 1st and 2nd order reactions are derived as Eqs. (4)–(6), respectively (3rd order equations have been derived but are not included here):

$$\Phi = k\tau FH \tag{4}$$

$$\Phi = -FCH(1 - \mathrm{e}^{-k\tau})\,\mathrm{e}^{-kt} \tag{5}$$

$$\Phi = \frac{HkC^2}{(1 + ktC)(1 + ktC + k\tau C)} \tag{6}$$

where Φ is the calorimetric signal ($\mathrm{J\,s}^{-1}$), F the flow rate of reacting solution ($\mathrm{dm}^3\,\mathrm{s}^{-1}$), C the initial concentration of reactant ($\mathrm{mol\,dm}^{-3}$), H the enthalpy ($\mathrm{J\,mol}^{-1}$), k the appropriate rate constant, τ the residence time (s) and t the time elapsed from initiation of the reaction. It should be noted that now it is possible to derive equations which describe the thermodynamic and kinetic behaviour of complex reaction schemes, however these equations, thus far, have only been derived for reactions studied by isothermal, batch-type calorimeters [12,13] and so far have not been extended to describe such systems studied using flow calorimeters.

It was stated earlier [7] that, in order to calculate the rate constant and enthalpy using Eqs. (4)–(6) requires prior knowledge of the residence time, τ (Note the 1st order rate constant is always accessible from the slope of the line from the $\ln\Phi$ vs. t plot).

The residence time can be calculated from the following equation which relates the flow rate to the thermal volume of the calorimetric vessel:

$$\tau = \frac{V_\mathrm{c}}{F} \tag{7}$$

where V_c is the effective thermal volume (dm^3). For a more detailed discussion see [6]. In this earlier article it was explained that, from Eq. (7), a plot of τ vs. $1/F$ yields a straight line. Further analysis of the simple equation that describes this line, Eq. (7), suggests a procedure that would allow a better determination of V_c and, moreover, a direct determination of the zero flow rate volume, V_0:

$$V_\mathrm{c} = V_0 - FI \tag{8}$$

where V_0 is the volume at zero flow rate (dm^3) (in principle this should be equal to the physical volume of the calorimetric vessel and I the incremental change in residence time per unit flow rate (the product FI is therefore the incremental volume change per unit flow rate). It is important to realise that a procedure is suggested from Eq. (8) that allows a better determination of V_c than using Eq. (7) and, moreover, a direct determination of the zero flow rate volume, V_0.

If Eq. (8) is divided by the flow rate, then

$$\frac{V_c}{F} = \frac{V_0}{F} - I \qquad (9)$$

From Eq. (7), it is seen that Eq. (9) can be simplified to yield

$$\tau = \frac{V_0}{F} - I \qquad (10)$$

From Eq. (10) it is apparent that a plot of τ vs. $1/F$ yields a straight line with the slope equal to the zero flow rate volume, V_0, and the intercept equal to the incremental change in the residence time per unit flow rate, I. Previously the thermal volume at zero flow rate was obtained by extrapolation from a thermal volume vs. flow rate plot. The relationship outlined above now allows statistically the more accurate value for V_0, to be taken from the average over a series of flow rates.

These developments allow the accurate determination of the volume at zero flow rate, V_0, and the thermal volume, V_c, and consequently the residence time, τ. These parameters have been defined for the first generation LKB 10700-1 flow calorimeter and a recently fabricated, customised flow insert for the TAM, as discussed in [6]. The variation in V_c for the LKB instrument changed considerably with the flow rate whereas the customised flow insert, designed to minimise such effects for the TAM, exhibited no observable flow rate-dependent change in V_c. It was found that the value of V_0, in both cases was significantly different from the nominal (engineered) volume of the vessel (approximately 60 and 30% greater for the LKB and TAM instruments, respectively). For a more detailed discussion see [6].

3. Experimental studies

The initial studies [8] have now been followed by identical ones in principle, conducted by Vine (Greenwich, UK) and Oliveira and Volpe (Campinas, Brazil) using different individual instruments (TAM) of the type used in Kemp's laboratory [14] but, in this instance, using the standard, non-modified, flow module (nominal engineered volume = 0.6 cm^3) produced by Thermometric AB (Järfälla, Sweden). The experiments conducted by Vine were performed at 37 °C and for flow rates ranging from approximately 6–40 cm^3 h^{-1}. The studies performed by Volpe and Olivera were conducted at 25 °C and for flow rates ranging from 10 to 33 cm^3 h^{-1}. All data analysis was conducted to an identical protocol to that in [6].

For the module employed by Vine it was found that the effective thermal volume was dependent on flow rate, increasing from 0.58 to 0.96 cm^3 over the above flow rate range (Fig. 1). Moreover, it was found that the zero flow rate volume is markedly different from that of the nominal engineered volume (Fig. 2). In this instance the calculated zero flow rate volume (1.04 cm^3) is approximately 60% greater than the nominal physical volume (0.6 cm^3). These observations are in contrast to those made for the customised flow module where it was found [8] that whilst effective thermal volume does not vary with flow rate there is a significant contribution to the thermal volume from some other factor.

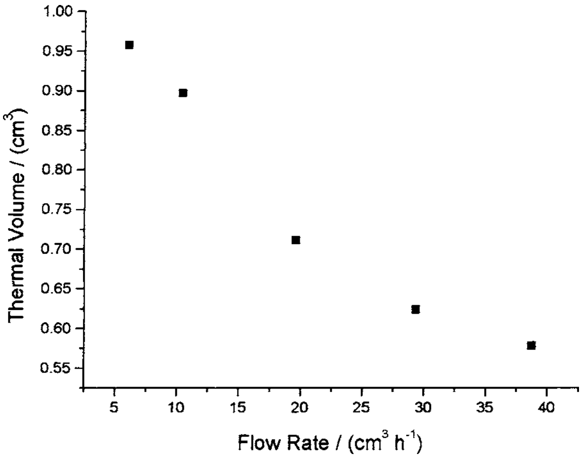

Fig. 1. Thermal volume vs. flow rate for the TAM flow module employed by Vine.

The observations made by Volpe and Olivera are again different from those made previously [8] and those subsequently made by Vine. Volpe and Olivera found that there is no observable effect of flow rate on thermal volume (see Fig. 3) i.e. the value for V_0 is indistinguishable from the nominal physical volume. This does not agree with the observations made for the standard flow module (employed by Vine), the customised flow module and the LKB instrument where it was found that, for both instruments, there is a significant dependence of thermal volume on flow rate. These observations can, in part, be attributed to the sample and reference arrangements of the calorimeters in question. The LKB instrument is a different model having as its reference a sealed flow-mix chamber containing, in our experiments, only air. The flow vessel for the standard TAM flow module is a length of tubing wound round a cup into which can be

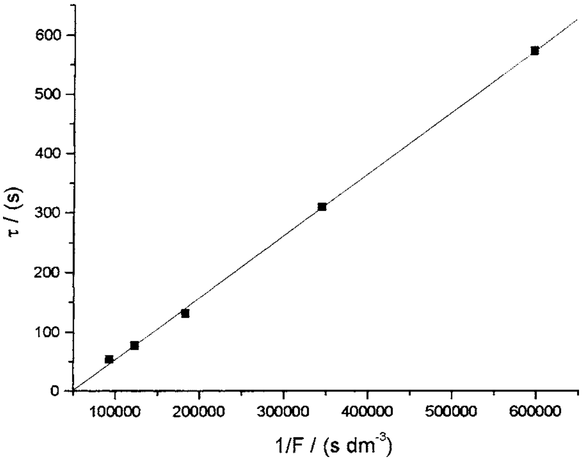

Fig. 2. Residence time vs. reciprocal flow rate for the standard TAM flow module employed by Vine.

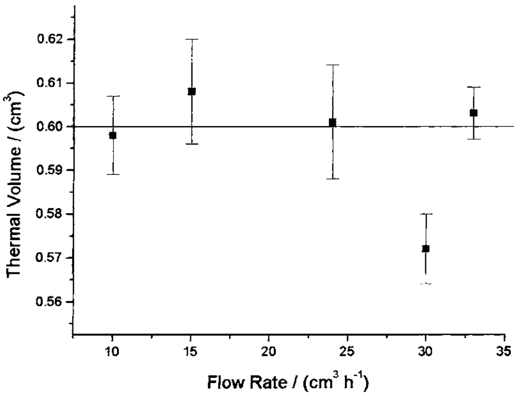

Fig. 3. Dependence of the thermal volume on the flow rate for the standard flow module, used by Volpe and Olivera in the TAM.

inserted an ampoule. An identical configuration is used for the reference side. Because it is very difficult to attain the same flow rates in the sample and reference flow vessels, Vine chose as the reference a glass ampoule containing 0.6 cm^3 distilled water inserted into the cup on the reference side. The modified insert used by Kemp has a twin set of tubing—one used as the sample side and the other is filled with distilled water (stopped flow) and used as the reference. Volpe and Olivera used an empty ampoule in the reference side as their reference. From this information, it can be seen that the instrument set-up has a significant effect on the flow rate/thermal volume relationship (as seen in the comparison between the results obtained by Volpe and Oliveira, and Vine).

To investigate the effect of the instrument set-up a series of single experiments was conducted by Vine using a different arrangement from that described earlier, in this case an empty ampoule was lowered into the sample cup retaining the reference ampoule as described earlier (i.e. containing 0.6 cm^3 water). The methyl paraben experiment was repeated in an identical fashion and with identical flow rates to those described earlier. The results shown in Fig. 4 are remarkably different across the range.

Assuming that the S.D. for these latest experiments is similar to that for the initial ones, it is clear that the different ampoule arrangement does indeed have a significant effect on the flow rate dependence of thermal volume. For the new arrangement the calculated zero flow rate volume, V_0, is 0.43 cm^3 and hence the limiting flow rate is now approximately 44 $cm^3\,h^{-1}$. These are radically different from the values calculated in the main study and further highlight the caution required when performing measurements using flow microcalorimetry. They also highlight the need for the flow characteristics of the calorimeter to be determined by the use of a test and reference reaction prior to any experimental study and whenever the set-up is changed.

There is another possibility which might give rise to the observed differences in behaviour. The inserts and indeed calorimeters are manufactured on a unit basis. It is possible therefore that minor variations introduced during the manufacturing process may also contribute to the observed differences.

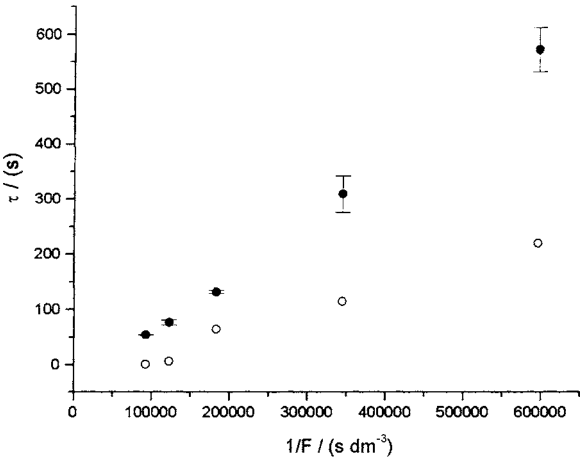

Fig. 4. Effect of the ampoule arrangement on the flow rate dependence of the thermal volume, V_c for the flow module employed by Vine. The closed circle points represent the flow rate dependence seen for the original study with no ampoule in the sample side. The open circle data points represent the flow rate dependence seen when an empty ampoule is present in the sample side of the calorimeter.

4. Theoretical studies

Knowledge of V_0 and flow rate, F, allows the calculation of τ and hence Φ at any flow rate. Eq. (5) describes the calorimetric response, for a 1st order reaction, in terms of k, H and τ as a function of time, t. If the maximum possible signal for a 1st order reaction (the only reaction order explored here) is considered, i.e. at initiation of the reaction when $t = 0$, then Eq. (5) can be re-written as

$$\Phi_0 = FCH(1 - e^{-k\tau}) \qquad (11)$$

It is now possible to explore both separately and in combination, the effects of flow rate, concentration, enthalpy and rate constant on a simulated chemical reaction studied in a flow calorimeter.

It should be noted that control is achievable over the flow rate, the concentration and to a lesser extent over the rate constant, the latter by changing the temperature. However, it is not possible to alter the enthalpy, assuming that it has no temperature dependence (it is possible, in some circumstances, to magnify the enthalpy by careful choice of an appropriate buffer system [15]). The changes in the concentration and the rate constant must also satisfy the requirement that the mechanism of reaction is not affected as a consequence. Changing the flow rate will have no impact on the mechanism since it is purely a physical change to the system.

Figs. 5 and 6 demonstrate how the initial signal Φ_0 varies with the flow rate for a fixed rate constant and varying initial concentration of the reagent. The range of values that Φ_0 can take for a range of selected flow rates and concentrations

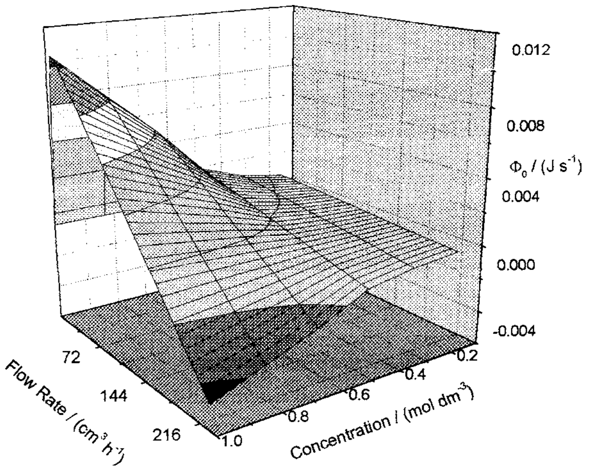

Fig. 5. A 3D representation of the effect of varying the flow rate and the initial concentration, [A_0], on the observed initial output for the fixed rate constant.

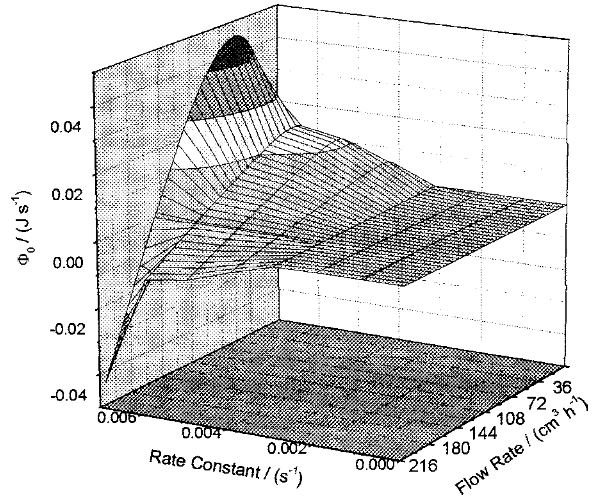

Fig. 7. A 3D representation of the effect of varying the flow rate and the rate constant on the observed initial output for a fixed initial concentration.

is given in Fig. 5. If the upper and lower detection limits for the calorimeter are known then it is possible to choose the combination of values for the concentration and the flow rate that yield an appropriate initial signal. These data are also presented as a series of 2D plots in Fig. 6. It is clear from it that if the flow rate is selected and the initial concentration C is increased then, as expected, Φ_0 increases in direct proportion to C. The calculated values for Φ_0 increase in a linear fashion from 0 to a maximum value governed by the value of C and then decrease in a different linear fashion to zero. If C is now held constant and the flow rate F is varied it becomes apparent from Fig. 5 that the dependence of Φ_0 on flow rate is complex. This is readily explained by a consideration of Eq. (5).

As the flow rate tends to zero τ increases and hence the value of $(e^{-k\tau})$ tends to zero. Hence the term $(1 - e^{-k\tau})$ becomes equal to 1. Therefore at low flow rates, for fixed C and enthalpy, the thermal output is dependent only on the flow rate. As the flow rate increases the term RCH has a greater significance for the value of Φ_0 to increase the initial signal. However, as flow rate increases the residence time, τ, decreases and the term $(1 - e^{-k\tau})$ tends to zero and so the observed output also tends to zero.

A similar study of the effect of rate constant can also be carried out. Figs. 7 and 8 describe the effect of varying the rate constant for a given initial concentration with constant enthalpy. Again Fig. 7 is a 3D representation of these data; which are also expressed as a series of 2D plots in Fig. 8. Consider the situation where the flow rate remains constant but the rate constant is increased. It becomes apparent that Φ_0 also increases. However, in this instance, the relationship is not a direct proportionality. Again this can be readily explained by a consideration of Eq. (5). In this case the rate constant does not have a direct multiplicative effect on Φ_0. In this case the relationship is embedded in an exponential term and hence it will not be linear. If the rate constant

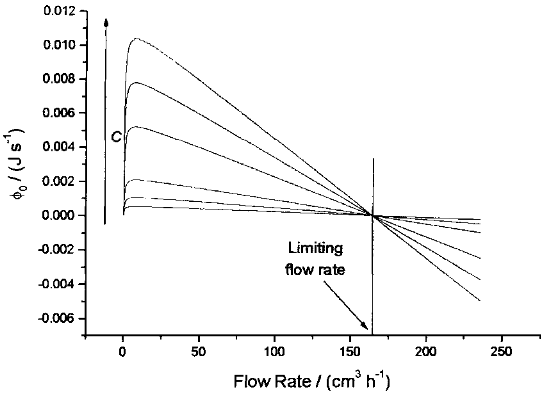

Fig. 6. A plot to show the effect of the increase in the flow rate on the observed initial signal for the fixed initial concentration and the varying rate constant.

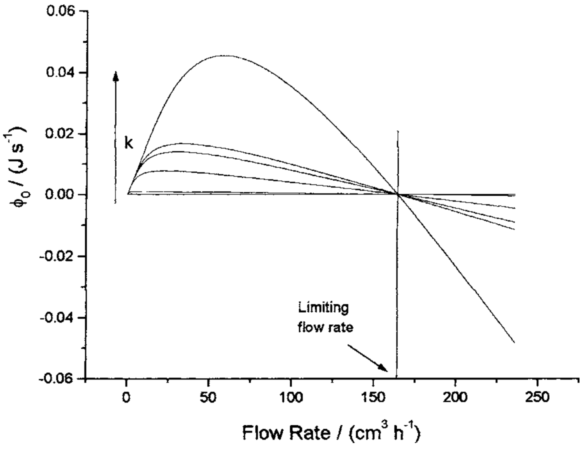

Fig. 8. A plot to show the effect of the increase in the flow rate on the observed initial signal for the fixed initial concentration and the varying rate constant.

is now fixed and the flow rate is increased then the same relationship, as observed above, is evident. Again this flow rate dependence is seen clearly in Fig. 8.

An important observation is that Φ_0 will always go to zero at a limiting flow rate (see Figs. 6 and 8). This is an important result because it says that regardless of the thermo-kinetic parameters of the system all heat generated within the vessel is carried out by the solution when flowing at the limiting flow rate. This rate can be determined from a rearrangement of Eq. (8).

If Φ_0 is equal to zero then the thermal volume, V_c, must also be zero, hence

$$V_0 - FI = 0 \tag{12}$$

$$\frac{V_0}{I} = F \tag{13}$$

Therefore, the limiting flow rate at which Φ_0 is zero (for the LKB 10700-1 calorimeter) is approximately 165 $\mathrm{cm^3\,h^{-1}}$ (this is calculated as approximately 71 $\mathrm{cm^3\,h^{-1}}$ for the standard Thermal Activity Monitor (TAM) 2277 flow module). These observations show the need for caution when investigating unknown reaction systems. The effects can be illustrated through a study of the imidazole catalysed hydrolysis of triacetin. This reaction is well known, as a test and reference reaction [2], and has been characterised in terms of the reaction order ($n = 2$), the molar enthalpy change ($-91.7\,\mathrm{kJ} \pm 3\,\mathrm{kJ\,mol^{-1}}$) and the rate constant ($2.8 \times 10^{-6} \pm 0.097\,\mathrm{dm^3\,mol^{-1}\,s^{-1}}$). This reaction has been investigated using flow calorimetry but has been found to be unsuitable for study in the LKB 10700-1 calorimeter [7] because a known 2nd order reaction appears to yield a small calorimetric signal with apparent zero order kinetics.

It has already been seen from the equations described earlier that the calorimetric signal obtained from flow calorimetry is dependent upon thermal volume (which is related to flow rate) and the value of the product of the rate constant and enthalpy. It is clear that the experimental flow rate has a significant effect on the observed calorimetric output from the calorimeter. With knowledge of this flow rate dependence a study was conducted to determine whether the experimental observation was a consequence of the thermo-kinetic parameters being unsuitable or the flow rate used was inappropriate for the instrument. The range of theoretical values for Φ_0 as a function of flow rate for the hydrolysis of methyl paraben and for triacetin is shown in Fig. 9. It is important to note that the dashed line perpendicular to the x-axis is the experimental flow rate for the triacetin reaction.

It is obvious that for the ideal condition, where Φ_0 is at a maximum, the methyl paraben reaction yields a signal that is approximately 10-fold greater than that for the triacetin reaction. It is also the case that the maximum possible signal for the triacetin reaction is almost at the limit of detection for the used instrument settings. This clearly provides an explanation for the experimental observations described above and previously in [6].

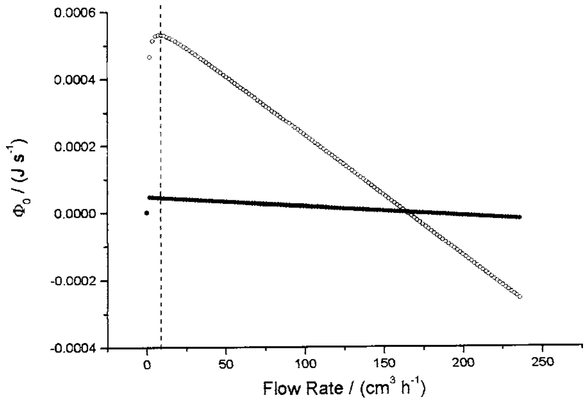

Fig. 9. Range of theoretical values for Φ_0 for the imidazole catalysed hydrolysis of triacetin (closed circles) and the base catalysed hydrolysis of methyl paraben (open circles). The dashed vertical line represents the flow rate employed by O'Neill for the study using the LKB 10700-1 instrument.

5. Conclusions

Our previous article [6] highlighted the requirements and importance of having test and reference reactions, for not only batch-type calorimeters, but also for flow calorimeters. Through the study of this the methyl paraben reaction it was seen that the thermal volume of the flow-through cell does indeed vary with flow rate (although it can be minimised by careful calorimetric vessel design [14]) and, importantly, that the zero flow rate volume is not necessarily the same as the physical volume of the cell. It was seen that the operating volume varies from the nominal (engineered) volume by up to 60%. As a consequence of these observations it was reported, and is emphasised here, that the observation of an apparent zero order signal does not necessarily reflect a zero order reaction mechanism. It may be that the signal is beyond the detection limits of the calorimeter (defined by the flow rate/thermal volume relationship) and hence requires further study to confirm the kinetics of that system.

These observations have been confirmed by the studies conducted by Vine. The study by Volpe and Oliveira has shown however that this effect cannot be predicted from one instrument to the next. Their study revealed that, for the particular flow module configuration employed by them (and which is nominally the same as that used by Vine), the thermal volume is independent of flow rate. Moreover, they found that the effect of flow rate on the thermal volume cannot be predicted in that their insert was found to have a zero flow rate volume identical to that of the physical volume of the calorimetric cell. Factors that may affect the observed value of thermal volume include contributions from the transmission tubing to/from the calorimetric vessels and the arrangement of the ampoules in the sample and reference cups. It was highlighted, through the study by Vine, that the experimental set-up of the calorimeter (sample and reference arrangement) has a significant and greater impact than that of the contribution from the lead-in and lead-out transmission tubing upon the flow rate dependence of the thermal volume. The effect upon V_c from having an empty ampoule within the sample side cup of the flow-through module is complex and is dependent upon the thermal conductivity of the ampoule and the increased heat capacity of the system.

Hence it is essential that a study to determine the flow characteristics of any flow-through vessel is conducted prior to analysis of data obtained from uncharacterised systems. It should be noted that the experiments performed in the LKB instrument and those by Volpe and Olivera in the TAM were conducted at 25 °C and those conducted by Kemp and Olomolayie and by Vine were performed at 37 °C. However, it is not anticipated that this temperature difference would have any significant effect on the observed thermal volume.

Knowledge of the characterising parameters, the zero flow rate volume, V_0, and the incremental change in residence time per unit flow rate, I, for flow-through calorimeters allows an exploration of the theoretical range of rate constants and initial concentrations that can be studied by any particular instrument. An interesting result of this study was the determination of a limiting flow rate at which no calorimetric signal can be observed, regardless of the thermo-kinetic parameters associated with the reaction. For the LKB 10700-1 calorimeter this was determined to be $165\,\mathrm{cm^3\,h^{-1}}$ and approximately $71\,\mathrm{cm^3\,h^{-1}}$ for the flow module (TAM 2277) used by Vine. It was also shown that the arrangement of ampoules within the sample and reference chambers has a significant impact on the flow rate/thermal volume relationship of Thermometric 2277 standard flow calorimeter.

The results reported in this paper highlight the need for caution and careful experimental design when using flow calorimeters. Results previously reported from studies using this technique (where no consideration of the thermal volume has been made) may be in error, especially for those quoting values for the molar enthalpy change. Such errors can now be rectified through the use of the methyl paraben test and reference reaction and the determination of the appropriate thermal volume.

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