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The imidazole catalysed hydrolysis of triacetin: a medium term chemical calibrant for isothermal microcalorimeters

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Abstract

This paper reports an isothermal microcalorimetric study of the imidazole catalysed hydrolysis of triacetin as an example of a solution phase medium term reaction. The results of this study are then discussed in context of its potential as a *quantitative* chemical calibrant for calorimeters, especially isothermal microcalorimeters. It will be also shown that chemical calibrants can be important for the determination of the stability of the calorimetric signal itself and hence to the consideration of location and set-up of the calorimeter. The imidazole catalysed triacetin hydrolysis reaction was studied in the calorimeter over a period of up to 50 days at 298.15 K and at pH 7.09. We have determined that, under these conditions, this reaction is second order with a rate constant of $8.64 \times 10^{-4} \pm 1.5 \times 10^{-5}$ dm^3 mol^{-1} s^{-1} and an associated change in enthalpy of -95.2 ± 1.6 kJ mol^{-1}. The data analysis for this reaction was performed using a new mathematical approach for the calculation of kinetic and thermodynamic parameters from calorimetric data.

Keywords: Isothermal microcalorimetry; Reaction order; Triacetin; Kinetics; Thermodynamics

1. Introduction

There is a wide choice of analytical instruments currently available that can be used to determine the kinetic and thermodynamic parameters necessary for the consideration of the stability of drugs, compounds, industrial materials etc. A suitable analytical instrument, both because of its high sensitivity and that it can record, simultaneously, the kinetic and the thermodynamic parameters of a reaction is an isothermal heat conduction type microcalorimeter (e.g. TAM Thermometric, AB, Jarfalla, Sweden). The isothermal heat conduction microcalorimeter has for some time been proposed as a general and rapid analytical instrument that has a sensitivity to record reactions or physical processes of rates from 1% s^{-1} to rates of 0.06% year^{-1} at ambient temperature and conditions [1]. For example, a reactant that has a rate of 0.06% per year, a Mwt of 500, a reaction enthalpy change of 100 kJ mol^{-1} and a loading mass of 3 g will have a calorimetric signal of 11.4 nW. This is 1.4 nW above the detection limit of the TAM fitted with a nano Watt amplifier [2]. The environmental condition within the reaction ampoule can be precisely controlled in terms of temperature, relative humidity and gas partial pressures. If desired the environment can be made comparable to that the compound is normally subjected to, such as, during storage etc.

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The isothermal microcalorimeter is a versatile instrument in that it allows the measurement of a reaction without the necessity for modification of the compound or intrusive preparation before or during the reaction study. Calorimetric observations can be made under conditions not simply and normally assessable using other analytical techniques [3–5]. For example, HPLC (high pressure liquid chromatography) requires that such reactions are often studied at elevated temperature to accelerate the reaction rate so that there is sufficient degradation product available for the analysis. Under such conditions extrapolation of the reaction kinetics from high temperature to ambient temperature using the Arrhenius equation is not reliable because of possible changes in the reaction mechanism that may occur at elevated temperatures [5].

It has been previously reported [1,6] that it is possible to obtain kinetic, thermodynamic and mechanistic information from the calorimetric data (rate constant, enthalpy change and reaction order). These parameters will then allow access to the determination of activation energy, change in entropy, change in Gibbs energy and heat capacity etc. The calorimetric data can therefore, yield an abundance of information characterising a reaction. It is vital, therefore, that the calorimeter is properly calibrated so that the observed calorimetric signal is a true representation of the reaction studied in the calorimeter. It is also important that the calorimeter is suitably set-up and located so that there is a minimum of external environmental influences e.g. fluctuations in room temperature etc. that could affect the observed signal. Small errors in the calorimetric signal can cause significant errors when calculating the reaction parameters.

The isothermal microcalorimeter possesses an electrical internal calibration device. The calibration device is set to produce a specified heat flow close to the location of the reaction site in the calorimeter. The calorimetric signal is thus adjusted so that it records the same value of heat flow that the calibration device is set to produce. There are several possibilities of error and objections to this type of calibration; firstly it is assumed that the calibration device produces the exact heat flow as that it is set to produce; secondly it is required that a 'base line' (where the calorimetric signal records a heat flow of zero) is defined, using an inert material [7]. It is the result of this separate experiment that is used as the basis for all subsequent experiments. Errors due to differences in heat capacities/thermal conductivities etc. between the calibrant and the subsequent materials can be significant and hence may cause a significant error in subsequent studies.

A more definitive method for calibration is essential if the calorimeter is to be used for high precision quantitative analysis. There have been several proposals [8] for chemical calibrants especially for the isothermal microcalorimeter (TAM). However, there has, as far as we can tell, been no completely satisfactory calibration reaction proposed. A chemical calibrant can be, in principle, any reaction or reaction scheme that can be observed in the calorimeter. The only requirements of a chemical calibrant is that it is characterised in terms of rate constant, order, heat capacity and change in enthalpy for the reaction and that it can be consistently reproduced. An ideal chemical calibrant could therefore, be selected on the grounds that the magnitude and duration of the observed signal would closely match the signal for the subsequent calorimetric study.

The calorimetric data obtained from the chemical calibration is real reaction-based calorimetric data. Therefore, a calibrated response can be defined for many different reaction orders and rates. This can be extended to complex reaction schemes where there may be a combination of reactions. Performing the calibration study under specific reaction conditions will produce an exact and reproducible shape, magnitude and duration for the observed calorimetric signal. The use of a range of chemical calibrants will therefore, allow the evaluation of the performance of the calorimeter, where suitability of the type, set-up and location of the calorimeter can be judged for reactions of similar rates and enthalpy changes. For example a slowly reacting chemical calibrant will give information on the long term stability of the observed calorimetric signal.

The hydrolysis reaction of triacetin was studied in the calorimeter for periods up to 50 days. The data were sequentially analysed using 15 h segments to provide information about the fluctuations in the calorimetric signal over this time period. The calculated reaction parameters (k, ΔH and n) should remain constant for the life time of the reaction (assuming there is no change in reaction mechanism). Errors

between the calculated parameters for each segment of calorimetric data can be ascribed to errors in the calorimetric signal.

2. Experimental materials and methods

The proportions of triacetin, imidazole and acetic acid buffer were used here as previously described [8]. Triacetin and imidazole were obtained from Sigma (purity stated to be better than 99%) and were used without further purification. Ethanoic acid was obtained from BDH (99.9% purity) and the ethanoic acid buffer made, fresh, with de-ionised distilled water before each batch of experiments. Buffer solutions were made by adding 1.6 g ethanoic acid to 2.72 g imidazole (0.4 mol) in 10 ml water. These solutions were kept in stoppered bottles, covered to protect from light and made up freshly before each batch of experiments. The test solutions were made by adding 3 ml imidazole buffer to 0.18 g of triacetin (0.000824 moles) and placed in glass ampoules (supplied by Thermometric, AB, Jarfalla, Sweden). The ampoules were sealed, loaded into the calorimeter and the experiment conducted in the normal way [7]. The reaction was followed in the calorimeter for up to 50 days so that the long term performance of the calorimeter could be determined.

The isothermal microcalorimeter, TAM (Thermometric, AB, Jarfalla, Sweden), was housed in a constant temperature room ($21 \pm 0.1^{\circ}C$) and the experiments were carried out at $25^{\circ}C \pm 1 \times 10^{-4}$. The TAM was set-up and run as described in the manufacturer's manual [7]. For additional operational detail refer to Ref. [9,10]. A 30 min thermal equilibrium period for each sample was allowed before data collection commenced, using the dedicated Digitam software. The collected calorimetric data were then processed using Origin™ [11] graphics package and imported into Mathcad™ [12] for calculation of the reaction parameters.

3. Results

3.1. Parameters obtained from the calorimeter

In a previous communication we detailed a new method for the analysis of calorimetric data that allows the calculation of the kinetic, thermodynamic and mechanistic parameters from calorimetric data. This method of data analysis was designed specifically for reactions considered to be long term in duration [1]. Subsequent to this publication a second method has been developed specifically for calorimetric data that we regard as being fast to medium term in duration [6] (see Table 1 for our assigned definition of reaction duration). It is this method of analysis that has been employed for the characterisation of the hydrolysis of triacetin reaction. The basis of the calorimetric data interpretation is as follows

1. Calculation of the reaction order (n).
2. Calculation of the enthalpy change (ΔH).
3. Calculation of the rate constant (k).

3.1.1. Calculation of the reaction order

For a simple reaction scheme such as $A + B \rightarrow C$ it has been found [6] that the order of the reaction can be

Table 1
Classification of reaction rates, illustrated here using as an example of a first-order reaction, together with the associated half-life and the rate of reaction expressed as percentage degradation as a function of time

First-order rate constant (s^{-1})	Half-life	Percent reaction	
1×10^{-2}	69 s	<1% s^{-1}	Fast
1×10^{-3}	693 s	1% s^{-1}	
1×10^{-4}	1.9 h	30% h^{-1}	
1×10^{-5}	19.25 h	3.5% h^{-1}	Medium
1×10^{-6}	8 days	8% day^{-1}	
1×10^{-7}	11.5 weeks	5.8% $week^{-1}$	
1×10^{-8}	2.2 years	2.4% $month^{-1}$	
1×10^{-9}	22 years	3% $year^{-1}$	Slow
1×10^{-10}	222 years	0.3% $year^{-1}$	
1×10^{-11}	2207 years	0.03% $year^{-1}$	

determined from the ratio of two time points corresponding to two values of dq/dt selected from the power–time calorimetric data. If two values of dq/dt are selected that are defined percentages of the initial power–time signal (i.e. when time = 0) the ratio of the corresponding time values t_1 and t_2 is a constant. This (t_2/t_1) constant is *independent* of the reaction rate constant, change in enthalpy and the initial value of dq/dt chosen. Note, the initial signal can be conveniently chosen near the start of the observed power–time curve. The values for t_2 and t_1 can be then normalised to $t = 0$ by subtraction of the time value that corresponds to this initial power value. The only dependency of the constant is on the *order* of reaction. For example, taking two data points that are say 94% and 4% of the initial signal, the constant for t_2/t_1 will have a value determined solely by n (the reaction order). For a first-order reaction the t_2/t_1 constant will be about 53, for a second-order it will be about 133 and for a 2.5 order reaction it will be about 151 etc. By using a suitable mathematics spread sheet, such as Mathcad™, the experimental t_2/t_1 constant can be compared to computer generated t_2/t_1 values constructed as a function of reaction order (see Appendix A for an example for the construction of a t_2/t_1 versus n table, using Mathcad™). An extension to this Mathcad™ spread sheet will allow the calculation of (n) from pairs of data over the whole of the calorimetric data set. This provides the opportunity for a more statistically precise calculation of (n) as well as a more convenient method to study any changes in the reaction mechanism over the calorimetric observation period [6].

3.1.2. Calculation of enthalpy change (ΔH)

Taking two values of dq/dt from the calorimetric data with the associated values of q (the area under the curve from the initial value of dq/dt at $t = 0$) it is possible to calculate Q, the total enthalpy change for the reaction when it has gone to completion.

Selection of two values of dq/dt at two points on the power–time curve will allow the following two equations to be written Eqs. (1) and (2)

$$\frac{dq}{dt_1} = k\Delta H^{1-n}(Q - q_1)^n \tag{1}$$

and

$$\frac{dq}{dt_2} = k\Delta H^{1-n}(Q - q_2)^n \tag{2}$$

As

$$k\Delta H^{1-n} = \frac{dq/dt_2}{(Q - q_2)^n}$$

a substitution in Eq. (2) can be made. Where $\Phi \equiv dq/dt$ (for simplicity)

$$\left(\frac{\Phi_1}{\Phi_2}\right)^{1/n} = \left[\frac{Q - q_1}{Q - q_2}\right] \tag{3}$$

Therefore,

$$Q = \frac{q_1 - (\Phi_1/\Phi_2)^{1/n} q_2}{1 - (\Phi_1/\Phi_2)^{1/n}} \tag{4}$$

All the values of the term in Eq. (4) are known hence Q can be calculated. ΔH can be thus found from Eq. (5), where, A is the initial quantity of reactant at $t = 0$.

$$\Delta H = \frac{Q}{A} \tag{5}$$

3.1.3. Calculation of the rate constant

For all reaction orders the rate constant can be calculated from Eq. (6)

$$\frac{dq}{dt} = k\Delta H^{1-n}(Q - q)^n \tag{6}$$

where a plot of dq/dt against $(Q - q)^n$ will yield a straight line of slope $k\Delta H^{1-n}$. As ΔH and n have been calculated previously, k can be found.

For the special case of first-order reactions, the rate constant can be determined from a plot of $\ln dq/dt$ against time. A first-order reaction can be described by an equation of the type

$$\frac{dq}{dt} = f\,e^{-kt} \tag{7}$$

where dq/dt is the calorimetric signal, t is time in seconds, k is the first-order rate constant and is the initial signal at time $t = 0$. A plot of $\ln dq/dt$ against time will thus yield a straight line of slope $-k$.

4. Data analysis

The calorimetric data for the imidazole catalysed hydrolysis of triacetin was analysed using the above

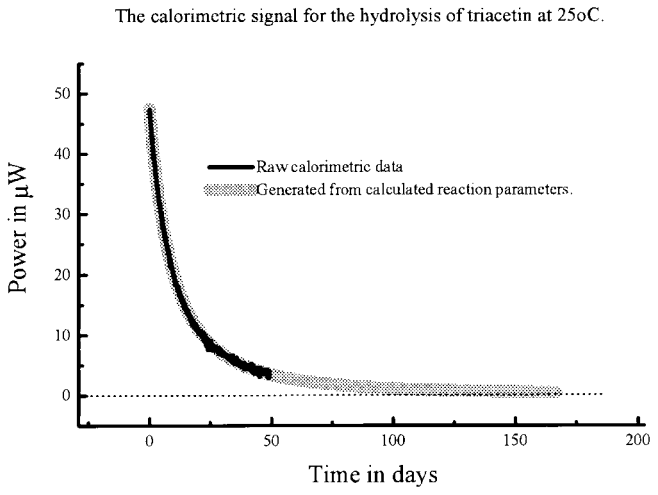


Fig. 1. A comparison of the raw calorimetric data for the hydrolysis of triacetin over a 50-day time period and a computer generated curve constructed using the values of $n = 2$, $k = 8.64 \times 10^{-4}$, $\Delta H = -95.2$ and $Q = 8.14 \times 10^{8}$ in the integral form of Eq. (6); $dq/dt = [kt\Delta H^{1-n}(n-1) + Q^{1-n} + Q^{1-n}]^{n/(1-n)} k\Delta H^{1-n}$ [6].

method. The analysis of the calorimetric data (see Fig. 1) for the hydrolysis of Triacetin showed that the reaction is second order with rate constant of $8.64 \times 10^{-4} \pm 1.5 \times 10^{-5}$ dm^3 mol^{-1} s^{-1} with an associated change in enthalpy of -95.2 ± 1.6 kJ mol^{-1}.

4.1. Determination of the reaction order

Mathcad^{TM} was used to manipulate the calorimetric data to calculate the reaction order. The procedure was to take the ASCII file and divide it half. Using the first data point for the initial value of dq/dt and $t = 0$ pairs of data from the two subsets of data were then taken and solved for (n) using the above procedure. Fig. 2 shows the distribution for the calculated values of (n) for each pair of data points. The value of (n) with the largest number of hits was $n = 2$. This indicates that this is a second-order reaction.

4.2. Calculation of Q (the enthalpy change for the reaction)

This calculation is, again, conveniently performed using Mathcad^{TM}. Inputting an ASCII file containing the dq/dt data with associated enthalpy change data (q), the date set is divided into two and each half is used for values of dq/dt_1, q_1 and dq/dt_2, q_2. The calculation is then performed using the equations described above for each pair of data from the two data sets. The enthalpy change for the reaction (Q) is then determined from the mean value for each pair of data points. The results show the reaction had an enthalpy change of -8.14×10^{8} μJ with a standard deviation of -1.41×10^{6}. The initial quantity of triacetin used in the experiment was 0.1868 g (8.56×10^{-4} mols). Therefore, the molar enthalpy change for the reaction is -95.2 kJ mol$^{-1} \pm 1.6$ kJ mol^{-1}.

4.3. Calculation of the rate constant

The rate constant for the reaction was calculated by plotting dq/dt against $(Q - q)^n$ (see Eq. (6)). The plot should produce a straight line with a slope of $k\Delta H^{1-n}$. For this experiment the slope was found to be $9.0756 \times 10^{-15} \pm 2.7 \times 10^{-18}$ with a linear regression fit (R) of 0.9997 (see Fig. 3). As ΔH and (n) have been previously calculated, (k) can be found by dividing the slope by ΔH^{1-n}. Therefore, k was found to be 8.64×10^{-4} dm^3 mol^{-1} s^{-1}.

4.4. Long-term stability study of the calorimetric signal

The long-term calorimetric signal study was performed by running the imidazole catalysed hydrolysis

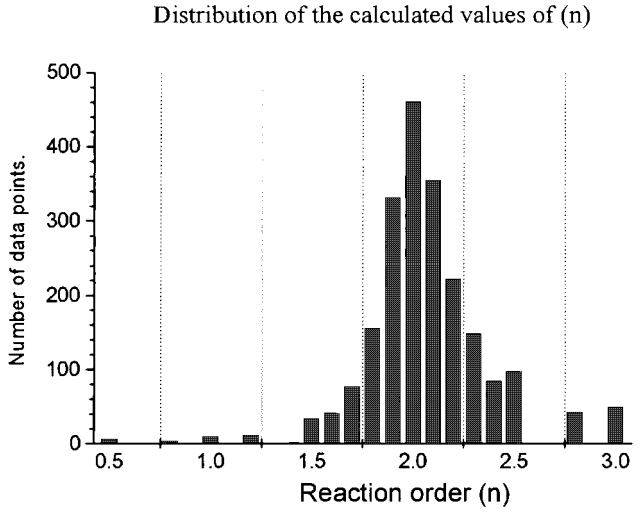


Fig. 2. Shows the distribution of the calculated reaction order for each set of data points. The max. peak indicates a second-order reaction. The distribution around the maximum reflects the error associated with the noise of the calorimetric signal.

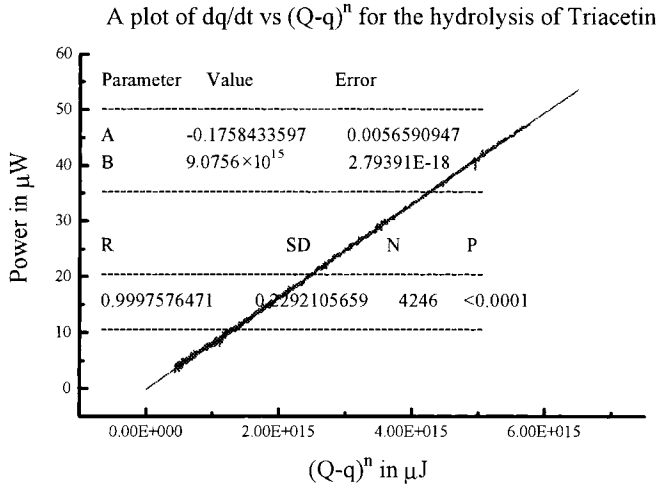


Fig. 3. From Eq. (6) a plot of dq/dt against $(Q-q)^n$ should give a straight line with a slope of $k\Delta H^{1-n}$.

of triacetin reaction over a 50-day time period. The calorimetric data was then divided into six sections. Three sets of data were then constructed by adding section 1 to section 4, section 2 to section 5 and section 4 to section 6. Table 2 shows that, within experimental error, the analysis of these time slices of calorimetric data gave the same values for (n), ΔH and k. This indicates that the calorimetric signal is sufficiently stable over such a time period and is not significantly effected by long-term baseline noise.

5. Conclusion

The hydrolysis of triacetin goes some way towards satisfying the need for a chemical calibrant for reac-

Table 2
Calculated kinetic and thermodynamic parameters for the imidazole catalysed hydrolysis of triacetin

Calorimetric period	Reaction order	% Data conforming to n=2	Rate constant ($dm^3\ mol^{-1}\ s^{-1}$)	Change in enthalpy ($kJ\ mol^{-1}$)
1st division	2	63	8.72×10^{-4}	-95.0 ± 0.87
2nd division	2	45	8.51×10^{-4}	-96.7 ± 1.85
3rd division	2	31	8.86×10^{-4}	-93.6 ± 1.76

The reaction order was determined by the highest number of counts for each value of (n). The distribution spread indicates an increased error as the separation of data is reduced.
Each time period was extracted from the data set and analysed as if it were a separate experiment.

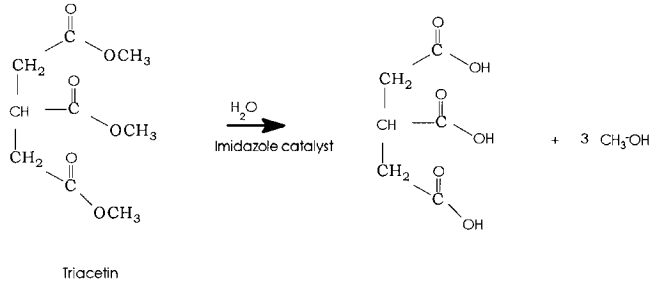
tions that are medium term in duration, i.e. have a half-life of 19 h to 11 weeks (see Table 1). The half-life for the triacetin reaction can be calculated from Eq. (8)

$$t_{1/2} = \frac{2^{n-1} - 1}{(n-1)kA^{n-1}} \tag{8}$$

Using the values of k, n and A from Section 3, half-life for this reaction was found to be 18 days.

The analysis of the calorimetric data using the mathematical procedure previously detailed now provide a powerful method for obtaining reaction kinetics and thermodynamics from relatively short observation times. Combining the principle of the mathematical calculations with a high degree of computing power results in a very flexible and reliable method of analysis. This should lead to a greater understanding of reaction processes as the parameters we now determine have real meaning for the reaction process we observe. They are no longer just constants that best fit the curve.

The hydrolysis of triacetin involves three reaction sites where there is the hydrolysis of a methyl ester group (see Scheme 1). It is possible there would be a difference in reaction mechanism for these ester groups as the central secondary ester group has a different steric environment than the two primary peripheral ester groups. The calorimetric investigation of this reaction does not indicate that there is more than one set of kinetic and thermodynamic parameters for this reaction, as would be expected if the hydrolysis of the ester groups had different reaction mechanisms. Note the calorimetric investigation was carried out over a 50-day time period. Over this period the reaction would be more than 80% completed. If there were significantly different kinetic and thermodynamic parameters associated with the hydrolysis of the primary and secondary ester groups the analysis of the segments of calorimetric data would show changes in the reaction parameters for the different reaction environments (primary and secondary ester hydrolysis). There are two possible explanations for this observation; firstly the kinetic and thermodynamic differences between the three ester groups are beyond the sensitivity of the calorimeter; secondly, the reaction at each ester site may be randomly distributed, i.e. not a sequential ester group reaction sequence. Therefore, the calorimetric data analysis will result in the determination of a change in enthalpy and associated



Triacetin

Scheme 1. The imidazole catalysed hydrolysis of triacetin.

rate constant which is an average value for the three reaction sites.

Appendix A

The construction of a t_2/t_1 versus order table using Mathcad™

Two items of information are required; the t_2/t_1 constant calculated from the experimental data and the associated percentage values of the initial calorimetric signal used to determine the constant.

For example, for a simulated first-order reaction

$$t_2/t_1 = 220, \quad \%1 = 99 \text{ and } \%2 = 10$$

The procedure is to calculate values of t_1 and t_2 from the same percentages of initial calorimetric signal (i.e. 99% and 2%) as a function of reaction order. Using Eq. (8) in Mathcad™ the initial values of calorimetric data can be found as a function of order. Note that t_2/t_1 constant is independent of k, ΔH, A and Q so any values can be used in Eq. (A.1), e.g.

$$k = 1.10^{-2}, \quad Q = 10000, \quad A = 0.1$$
$$\Delta H = Q/A \quad n = 0.1 \text{ to } 3 \text{ in steps of } 0.1$$
$$\frac{\mathrm{d}q}{\mathrm{d}t_{\text{initial}}} = \left[k\Delta H^{1-n}(n-1) + Q^{1-n}\right]^{n/(1-n)} \times k\Delta H^{1-n} \tag{A.1}$$

Hence $\mathrm{d}q/\mathrm{d}t$ (initial) is the calorimetric signal at $t = 0$ as a function of reaction order.

The chosen percentages of the initial signal can be related to Eq. (8) by

$$99\%_{\text{initial}} = \frac{\mathrm{d}q/\mathrm{d}t_{\text{initial}}}{100} \times 99 \quad \text{and}$$
$$10\%_{\text{initial}} = \frac{\mathrm{d}q/\mathrm{d}t_{\text{initial}}}{100} \times 10$$

The time dependence (i.e. time required for the calorimetric signal to reach 99% and 10%, i.e. t_1 and t_2) can thus be found

$$t_1 = \frac{\left(99\%_{\text{initial}}/k\Delta H^{1-n}\right)^{(1-n)/n} - Q^{1-n}}{k\Delta H^{1-n}} \quad \text{and}$$
$$t_2 = \frac{\left(10\%_{\text{initial}}/k\Delta H^{1-n}\right)^{(1-n)/n} - Q^{1-n}}{k\Delta H^{1-n}}$$

Therefore, a table can be constructed where the ratio t_2/t_1 can be displayed as a function of reaction order (n). The experimental value for t_2/t_1 determined by using the same % change in signal (99% and 2%) can be then compared to the Mathcad™ constructed table and the value of (n) read off.

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