Stability Assessment Of
Pharmaceuticals Using Isothermal
Calorimetry

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DECLARATION

This thesis describes research conducted in the School of Pharmacy, University of London between 2003 and 2006 under the supervision of Dr. Simon Gaisford and Professor Graham Buckton. I certify that the research described is original and that any parts of the work that have been conducted by collaboration are clearly indicated. I also certify that I have written all the text herein and have clearly indicated by suitable citation any part of this dissertation that has already appeared in publication.

Signature  23-10-2007

Date
Abstract

Pharmaceuticals are formulated to be stable products and studying their stability using conventional techniques can be difficult and time consuming. Isothermal calorimetry has the potential to study the stability of pharmaceuticals, as it is sensitive enough to detect extremely small heat flows associated with change (chemical or physical). Its use in the pharmaceutical arena has not been widespread because of the difficulties associated with analysing data recorded from complex processes, since heat is ubiquitous. The work presented in this thesis demonstrates the applicability of isothermal calorimetry in the stability assessment of pharmaceutical model systems. Calorimetric data recorded for complex processes were analysed based on kinetic modelling.

Initial studies involved the degradation of simple single-step processes (hydrolysis of aspirin at 25 °C returned rate constants of $2.8 \times 10^{-6}$ and $5.3 \times 10^{-6}$ s$^{-1}$ in 0.1 M HCl and citrate buffer respectively), followed by model systems with increasing complexity. Degradation involving parallel processes - binary mixtures of parabens were studied. Using kinetic models, rate constants and reaction enthalpies were determined for individual processes. Rate constants of $2.2 \times 10^{-4}$ and $0.8 \times 10^{-4}$ s$^{-1}$ for methyl and propyl paraben present in a binary mixture were lower than when recorded individually ($3.1 \times 10^{-4}$ and $1.2 \times 10^{-4}$ s$^{-1}$). Data recorded for consecutive reactions, commonly encountered in pharmaceuticals can be difficult to analyse if exact mechanisms are unknown. The use of chemometric techniques to analyse calorimetric data recorded for complex processes offers great potential when reaction mechanisms are unknown. The two-step consecutive reaction of potassium hydroxy lamine disulfonate was successfully analysed for reaction parameters using kinetic-based models and chemometric analysis.

Significant proportions of degradation in pharmaceuticals proceed at a slow rate and can pose difficulty in their assessment. Minimum desirable reaction parameters required to successfully analyse calorimetric data for slow reactions were generated and its use in designing calorimetric experiments was demonstrated. Finally the use of IC in the preformulation stages of drug development is emphasised, particularly its role in purity determination of impure aspirin and drug excipient compatibility. Compatibility between aspirin and magnesium stearate was investigated using IC and reaction parameters obtained were compared with those obtained using conventional chromatographic techniques.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Φ</td>
<td>Calorimetric power</td>
</tr>
<tr>
<td>α</td>
<td>Fraction of material decomposed at time ( t )</td>
</tr>
<tr>
<td>( \chi^2 )</td>
<td>Statistical measure of fit</td>
</tr>
<tr>
<td>( \Delta G )</td>
<td>Change in Gibbs free energy</td>
</tr>
<tr>
<td>( \Delta H )</td>
<td>Change in Calorimetric enthalpy</td>
</tr>
<tr>
<td>( \Delta H_f )</td>
<td>Enthalpy of fusion</td>
</tr>
<tr>
<td>( \Delta H_i )</td>
<td>Enthalpy change for impure drug</td>
</tr>
<tr>
<td>( \Delta H_p )</td>
<td>Enthalpy change for pure drug</td>
</tr>
<tr>
<td>( \Delta S )</td>
<td>Change in Entropy</td>
</tr>
<tr>
<td>([A_0])</td>
<td>Initial concentration of reactant</td>
</tr>
<tr>
<td>( \mu W )</td>
<td>micro Watt</td>
</tr>
<tr>
<td>1D</td>
<td>First derivative spectroscopy</td>
</tr>
<tr>
<td>AMP</td>
<td>1-amino-4-methyl-piperazine</td>
</tr>
<tr>
<td>API</td>
<td>Active Pharmaceutical Ingredient</td>
</tr>
<tr>
<td>CHN</td>
<td>Carbon Hydrogen Nitrogen combustion analysis</td>
</tr>
<tr>
<td>( dq/dt )</td>
<td>Rate of change of heat flow with time (power)</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential Scanning Calorimeter</td>
</tr>
<tr>
<td>( E_a )</td>
<td>Activation energy</td>
</tr>
<tr>
<td>( F )</td>
<td>Fraction of total material melted</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>HSDSC</td>
<td>High Sensitivity Differential Scanning Calorimeter</td>
</tr>
<tr>
<td>IC</td>
<td>Isothermal Calorimetry</td>
</tr>
<tr>
<td>K</td>
<td>Kelvin</td>
</tr>
<tr>
<td>( k )</td>
<td>Rate constant</td>
</tr>
<tr>
<td>M</td>
<td>Molar</td>
</tr>
<tr>
<td>MF</td>
<td>Meclofenoxate hydrochloride</td>
</tr>
<tr>
<td>ml</td>
<td>millilitre</td>
</tr>
<tr>
<td>( n )</td>
<td>Reaction order</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>( q )</td>
<td>Heat evolved or absorbed at time ( t )</td>
</tr>
<tr>
<td>( Q )</td>
<td>Total heat evolved or produced by a reaction</td>
</tr>
</tbody>
</table>
\( Q_i \)  
\( Q \) of impure substance

\( Q_p \)  
\( Q \) of pure substance

\( R \)  
Gas constant (8.314 \( \text{J K}^{-1} \text{ mol}^{-1} \))

\( \text{RH} \)  
Relative Humidity

\( \text{RIF} \)  
Rifampicin

\( \text{RSV} \)  
3-formylrifamycin SV

\( T \)  
Temperature

\( t \)  
Time

\( \text{TAM} \)  
Thermal Activity Monitor

\( \text{TLC} \)  
Thin Layer Chromatography

\( \text{TM} \)  
Trimebutine maleate

\( \text{UV} \)  
Ultra Violet Spectroscopy

\( v \)  
Volume of solution in TAM ampoule

\( \text{w/v} \)  
weight/volume

\( x \)  
Number of moles of product formed at time \( t \)

\( x(t) \)  
Function describing rate of change of power with time for reaction \( x \rightarrow \) product

\( X_2 \)  
Mole fraction of impurity
Chapter 1

INTRODUCTION
Chapter 1 – Introduction

1. INTRODUCTION

Drug substances can be fragile entities and any type of stress (environmental or process related; turning an Active Pharmaceutical Ingredient into a finished product for instance) has the potential to cause changes that can compromise the quality of a medication. The quality of the drug product changes with time and it is usually the case that the initial state is the one with the desired properties. It is therefore imperative that manufacturers state a time frame during which the product is considered therapeutically efficacious. This is usually stated as the period when the potency of the drug is at least 90 % as when formulated originally.

The challenge therefore is to be able to quantify these inevitable changes as rapidly and economically as feasible. A number of analytical tools are utilised to meet this challenge, but some prerequisite factors are required to be fulfilled. For spectroscopic analysis, the molecule must possess a suitable chromophore, while it should have some affinity for the column material in case of chromatographic analysis. Pharmaceuticals are however formulated to be stable systems (within pharmacopoeial limits) for the duration of their shelf lives. Hence there is no appreciable loss in drug activity under normal conditions that can be measured effectively using current techniques. This is ameliorated by studying the degradations under stressed conditions, which involve an increase in either temperature and/or relative humidity (RH). The increased degradation rates allow for rate constants to be determined at each of the temperatures studied. Plotting of an Arrhenius relationship (In $k$ versus 1/T) followed by an extrapolation, returns rate constants under storage conditions. This procedure however requires the assumption that the analysis results in a linear relationship and that the reaction processes occurring under stress conditions are the same as those that would occur under storage conditions.

Calorimetry offers an alternative approach to stability assessment of pharmaceuticals. It measures the heat change that is invariably associated with any process (physical or chemical). Modern day calorimeters are sensitive enough to detect very small heat changes (0.05 μW) directly at storage temperatures. Moreover, it allows the sample to be studied non-destructively. Since heat is ubiquitous, calorimetric data recorded can arise from a number of processes and hence careful experimental design and technique is essential. However, its use in the pharmaceutical industry is not widespread, due to lack of data analysis techniques and the complex nature of pharmaceutical degradations. It is the aim of this thesis to study various pharmaceutical model degradation systems using

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calorimetry and analyse the data recorded to obtain kinetic (rate constants) and thermodynamic (reaction enthalpies) parameters.

1.1 Effects Of Instability In Pharmaceuticals

The stability of drugs and drug products has been an important area of research in the development of pharmaceuticals. Changes in the drug (such as caused during dissolution, comminution or addition of other materials) or its environment can alter its stability (Conners et al, 1979). They are therefore susceptible to some form of chemical degradation or physical change when formulated. Such changes can be brought about by a number of factors and include hydrolysis, oxidation, photolysis, isomerization, change in crystal structure or interaction with other material.

Instability in pharmaceutical products can be complex and lead to a wide variety of undesirable adverse effects. Degradations can lead to loss of potency of the drug thereby affecting the therapeutic activity of the drug (Yoshioka and Stella, 2000). This can have a detrimental effect on the quality and safety of the product. Identifying degradation products and pathways is vital in order to minimise the deleterious effects. The degradation rates should also be determined, so that shelf-life predictions can be made. Commercial pharmaceutical products should have a shelf-life of three years, where the potency should not fall below 95 % under the recommended storage conditions (Wells, 2002). Increased API (Active Pharmaceutical Ingredient) concentration can occur if there is a loss of vehicle, which can lead to deleterious effects. In such cases, an upper potency value (110 %) should be defined (Carstensen, 2000).

Degradation in some cases can lead to the formation of toxic compounds or may cause a change in the physical appearance of the product making it aesthetically unacceptable. Discoloration or change in odour may occur because of oxidation or photolysis. Epinephrine is oxidised to adrenochrome producing a pink tinge (Yoshioka and Stella, 2000).
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1.2 Modes Of Degradation

Pharmaceutical degradations can be broadly classified as chemical or physical degradations depending on the mechanism. Substances used as API's have great structural variety and pharmaceutical formulations tend to be complex blends. Hence, a number of degradation reactions are possible and it is essential to identify potential reactions and determine their rates.

1.2.1 Chemical degradations

The reduction in potency of an API, due to loss through chemical reaction is the most widely and easily understood form of drug instability. The API's can chemically degrade through several pathways and include hydrolysis, oxidation, dehydration, isomerization and racemization, elimination, photolysis and complex interactions with excipients and other drugs. The most commonly encountered mechanisms in pharmaceutical degradations are hydrolysis or oxidation.

Degradations that involve reaction with water are called hydrolysis and are one of the most common degradation processes. It is often the main reaction pathway for drugs that have an ester or amide functional group. The rate of hydrolysis is influenced by temperature, pH, buffer salts, solvent, ionic strength, presence of complexing agents, surfactants and excipients (Stewart and Tucker, 1985; Waterman et al., 2002). Hydrolysis reactions are typically catalysed by hydrogen ions (specific acid-catalysed) or hydroxyl ions (specific base-catalysed). The hydrolytic susceptibility of an API is tested by exposure to acidic, neutral and basic conditions (pH range 1 – 13), preferably at 100% aqueous conditions. This is important when ionisable functional groups are present and exist in different ionization states under different aqueous conditions.

Oxidation is a well-known chemical pathway for degradation in pharmaceuticals and is divided into two types: reaction with molecular oxygen and reaction with other oxidising agents present in the formulation. Oxygen is abundant in the environment and invariably pharmaceuticals are exposed, either during processing or long-term storage. Moreover, even small traces of oxygen or metal ions are sufficient to cause significant stability problems. Some drugs tend to form coloured products due to oxidation and although does not lead to loss of potency, cannot be used. Oxidation reactions involve a series of
reactions and the initial reaction is usually catalysed by metal ions, which is the rate-limiting step (Waterman et al., 2002a). A large proportion of oxidative degradations in pharmaceuticals is via autoxidation and involves the generation of free radicals.

Isomerisation is the process of conversion of a drug from one molecular structure into another (isomer) whose component atoms are the same but arranged in a different geometrical structure. Isomerisation can lead to loss of therapeutic activity and some of the drugs that have been reported to degrade via isomerization include, all-trans retinoic acid to cis-retinoic acid (Lehman et al., 1960), cephalosporin (Richter et al., 1990) and tetracycline (Remmers et al., 1963).

Photodegradation occurs in a large number of pharmaceutical compounds resulting from the absorption of radiant energy in the form of light. Common degradative routes include oxidation, ring rearrangement, polymerisation and dehydrogenation, which generally yield numerous products through complex pathways. Some of the drugs that photo degrade are Chlorpromazine (Merkle and Discher, 1964), hydrocortisone (Hamlin et al., 1960), chlorodiazepoxide (Reisch et al., 1992) and nifedipine (Matsuda et al., 1989).

Excipient compatibility forms an important step during the pre-formulation stage of new drug development. Some excipients when formulated along with an API are known to enhance the degradation rates of the drug. Excipient compatibility testing is usually performed at elevated stress, such as high temperature and/or increased humidity. Some of the excipient compatibility studies recorded in the literature include compatibility of excipients with cefaclor (Terada et al., 2006), amino-n-caproic acid (Schmitt et al., 2001), phosphomycin (Vecchio et al., 2001), aspirin (Al-Gohary and Al-Kassas, 2000; Ahlineck et al., 1987; Wissing, et al., 2000;), nefazodone (Balestrieri et al., 1996) and niclosamide (Malan et al., 1997).

1.2.2 Physical changes

Determination of physical stability of pharmaceutical formulations is important especially over long storage periods. Physical state of a drug determines its physical properties such as solubility, which in turn affects the efficacy and safety of drug substances. Drug substances and excipients exist in various microscopic physical states differing in the degrees of order and can include amorphous, crystalline, hydrated or solvated states. They
usually change from an unstable or metastable state to a more thermodynamically stable state over a period depending on the free-energy difference between the states and the energy barrier. Examples of physical changes that are most commonly encountered include crystal growth, change in crystal form and change in dissolution time or colour formation.

Poorly water-soluble drugs are often formulated in their amorphous state because of the higher solubility compared to the crystalline state. However, amorphous substances tend to crystallise over time because of the lower free energy of the crystalline state and may have a disastrous effect on the efficacy of the formulated product. The crystalline form has a slower dissolution and consequently lower solubility, resulting in reduced bioavailability of the drug. Polymorphic transitions (change in the crystalline form) can occur during storage and affects the solubility and dissolution rate of the drug substances. Crystals within a formulation can grow or decrease in size provided there is sufficient mobility. In solid dosage forms such as tablets and granules, drugs and excipients may recrystallise or sublime onto the surface of the dosage form during storage (Yoshioka and Stella, 2000). Absorption of moisture by solid pharmaceuticals during storage may cause changes in appearance and dissolution rates.

1.3 Requirements For A Reaction

If one considers a reaction in its simplest form, where reactant A forms product B, the reaction proceeds only if there is an available reaction pathway. In some cases only one pathway exists, but for many reactions a number of different pathways may be available and the reaction mechanism becomes complex. Irrespective of the number of pathways or complexity of the reaction, three criteria need to be fulfilled for the reaction to occur – the reaction must be mechanistically, thermodynamically and kinetically feasible. For a reaction to occur, all three factors must be satisfied, failing which the system is stable and no change occurs. Changing reaction conditions will result in an unstable system and favour the reaction.
1.3.1 Mechanistic factors

The first requirement for a feasible reaction is that the reactants possess the ability to react. This is related to the physical properties of the reactants and should have the correct functional groups, properly oriented in order to maximize interaction with the reactive groups of other compounds.

1.3.2 Thermodynamic factors

The second requirement for a reaction to occur is that it should be thermodynamically feasible. A thermodynamic parameter which serves as a good indicator for the potential of a reaction to occur is the Gibbs free energy ($\Delta G$), also known as Gibbs function. It is given by equation 1.1 and is comprised of two terms, the enthalpy change ($\Delta H$) and the change in entropy ($\Delta S$).

$$\Delta G = \Delta H - T\Delta S$$  \hspace{1cm} \text{Equation 1.1}

Enthalpy change is equal to the heat gained or lost during a process at constant pressure and almost all processes are accompanied by a change in enthalpy. When the change occurs with the evolution of heat to the surroundings (at constant pressure), it is referred to as an exothermic process and the enthalpy of the system decreases ($\Delta H$ is negative), while if the change takes heat from the surroundings it is endothermic ($\Delta H$ is positive). Entropy is a measure of the disorderliness of a system. A change in enthalpy is always accompanied by a change in the entropy and vice versa. A reaction is favoured if the value of $\Delta G$ is negative, which is the case when $\Delta H$ is negative (exothermic process). For an endothermic process, where $\Delta H$ is positive, a negative value for $\Delta S$ can result in a net negative $\Delta G$ thereby favouring a reaction (the reaction is entropy driven). However, no information regarding the rate of reaction can be obtained from the mechanism or the thermodynamic term.

1.3.3 Kinetic factors

The third criterion essential for a reaction to occur is that it should be kinetically feasible. Reaction rates vary from very fast to very slow, but only a range in-between this is measurable. Three main parameters that govern the rate of reaction are the quantity of
reactant available for reaction, the fraction of reactant that possesses sufficient energy to overcome the activation energy barrier and the order of reaction. This is given by:

\[
\frac{dx}{dt} = k(A_0 - x)^n
\]

Equation 1.2

where \(\frac{dx}{dt}\) is the rate of reaction, \(k\) is the rate constant, \(A_0\) is the initial quantity of material that can react, \(x\) is the amount reacted in time \(t\) and \(n\) is the order of reaction. The rate constant, \(k\) is determined from the Arrhenius equation and is proportional to the quantity of reactant \((A_0 - x)\) that possess sufficient energy to overcome the activation energy barrier and is given by,

\[
k = Ae^{-\frac{E_a}{RT}}
\]

Equation 1.3

where \(A\) is the frequency factor or pre-exponential factor, \(E_a\) is the activation energy, \(R\) is the universal gas constant and \(T\) is the temperature (in Kelvin). The logarithmic form is given by,

\[
\ln k = \ln A - \frac{E_a}{RT}
\]

Equation 1.4

The Arrhenius equation is valid only on the assumption that the activation energy remains constant over the temperature range studied, indicating no change in reaction mechanism. A plot of \(\ln k\) versus \(1/T\) will be linear with a slope of \(-E_a/R\).

1.4 Reaction Kinetics

Reaction kinetics is the study of reaction rates of a chemical change and factors that affect the rate. Kinetic studies give an insight into the mechanisms involved in the change and allow a prediction of the rate of change that will occur. During a chemical reaction, the amount of reactants decreases with time and that of the product increases with time. In some cases, the reaction may proceed via the formation of an intermediate, whereby the concentration increases with time, reaches a maximum and then decreases to zero as the reaction goes to completion. For a reaction that proceeds through several steps, the overall rate is determined by the slowest step. The average rate of reaction is characterised by the
rate of change in the concentration of the reactants or products over a given time period and is represented as,

\[
\text{Rate} = -\frac{d[\text{reactant}]}{dt} = \frac{d[\text{product}]}{dt}
\]

Equation 1.5

For a hypothetical reaction of stoichiometry given by,

\[aA + bB \rightarrow cC + dD\]

Equation 1.6

the rate of reaction is expressed as:

\[
\text{Rate} = \frac{1}{a} \frac{d[A]}{dt} = \frac{1}{b} \frac{d[B]}{dt} = \frac{1}{c} \frac{d[C]}{dt} = \frac{1}{d} \frac{d[D]}{dt}
\]

Equation 1.7

where \([A], [B], [C] \text{ and } [D]\) are the concentrations terms. The rate is proportional to the product of powers of concentration and can be expressed as,

\[
\text{Rate} = k[A]^a[B]^b
\]

Equation 1.8

where, \(k\) is the proportionality constant known as rate constant and \(a\) and \(b\) are the orders of reaction with respect to \(A\) and \(B\) respectively. The overall reaction order, \(n\) is defined as the sum of the individual orders.

The order of a reaction is the power to which the concentration of a species (reactant or product) is raised in the rate equation. It is different from the molecularity of the reaction, which provides information about the number of molecules that take part in a single reaction step and is obtained from a balanced equation. For a single step reaction, the order is often determined by the stoichiometry of the reactants, but for complex reaction schemes the observed order can be different from the stoichiometry of the reaction. It is determined experimentally and is used to describe the dependence of reaction rate on the concentration of reactants. Degradation of pharmaceuticals can be treated as zero-order, first-order or pseudo-first order, although many of the pharmaceutical compounds degrade by complicated mechanisms (Lachman et al., 1991)
In zero-order reactions, the degradation proceeds at a constant rate and are independent of the concentration of the reactants. Processes occurring at phase boundaries usually follow zero-order kinetics, where the concentration at the surface remains constant (reaction sites are saturated or constantly replenished by diffusion). Hydrolysis of API’s in suspensions or degradation of solids in limited moisture follows zero-order kinetics. A rate expression for zero-order processes is given in Table 1.1. For a first-order reaction, the rate depends on the concentration of a single reactant and is most common in pharmaceuticals. An API directly degrades to one or more products and the rate is proportional to the concentration of the reactant.

The rate of reaction at any time \( t \) can be calculated from the slope of a concentration versus time plot. However, it is inaccurate and a laborious process, especially for slow reactions. The use of specific rate equations, for the system under investigation is more appropriate and rate equations for many types of reactions have been derived. Such equations relate the rate of reaction at any time \( t \) to the concentration of reactant or product (differential rate equation) or as a function of time (integrated rate equation). Reactions occurring in solutions are generally found to obey specific kinetic schemes and are either zero-order, first-order, second-order or third-order. The differential and integrated rate equations commonly used to describe zero-, first- and second-order reactions are shown in Table 1.1.

<table>
<thead>
<tr>
<th>Overall reaction order</th>
<th>Differential rate form</th>
<th>Integrated rate form</th>
<th>Linear plot to determine ( k )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero ( \frac{-d[A]}{dt} = k )</td>
<td>([A] - [A_0] = -kt)</td>
<td>([A] ) vs ( t )</td>
<td></td>
</tr>
<tr>
<td>First ( \frac{-d[A]}{dt} = k[A] )</td>
<td>(\ln \frac{[A]}{[A_0]} = -kt)</td>
<td>(\ln[A] ) vs ( t )</td>
<td></td>
</tr>
<tr>
<td>Second ( \frac{-d[A]}{dt} = k[A]^2 )</td>
<td>(\frac{1}{[A]} - \frac{1}{[A_0]} = kt)</td>
<td>(\frac{1}{[A]} ) vs ( t )</td>
<td></td>
</tr>
</tbody>
</table>

Table 1.1: Differential and integrated rate expressions for zero-, first- and second-order reaction schemes.

1.5 Accelerated Stability Testing

Pharmaceuticals are formulated to be stable systems (within pharmacopoeial limits) for the duration of the shelf-life. Hence, instabilities under normal conditions are often detectable only after considerable storage periods. To reduce the time required for testing,
pharmaceuticals are exposed to conditions of high stress thereby increasing the rates of any degradation process. This enables a greater amount of data to be obtained in a short time span and also allows screening of unstable formulations during early stages of the study. However, extrapolation of data obtained for accelerated stability studies to normal storage conditions, should be carried out with caution as degradation mechanisms could change under increased stress. Therefore long-term stability assessments (real time data) must also be done under normal conditions to back up such accelerated stability data.

The three main objectives of an accelerated stability study are firstly to detect rapidly any degradation in different initial formulations of the same drug, thereby enabling a proper selection of a formulation with the best stability characteristics. Secondly, to predict the shelf-life of a formulated product when stored under expected or directed storage conditions and finally to provide a rapid quality control method that ensures no unexpected changes in the product. The main variables to be considered for accelerated stability testing are temperature, moisture and light.

1.5.1 Effect of temperature

An increase in temperature causes an increase in the degradation rate and generally, a ten-degree rise in temperature doubles the reaction rate (Atkins, 1998). Samples are stored at temperatures high enough to induce degradation and analysed at various time intervals to determine the extent of degradation. Studies are carried out at several elevated temperatures and an activation energy is determined (Equation 1.3). Application of an Arrhenius relationship allows for the determination of degradation rate at any desired temperature from which shelf-lives can be calculated.

The effects caused by high temperatures may sometimes be confused with those that arise from the effects of low relative humidity (RH). The RH inside a high temperature storage cabinet will be lower than that in surrounding room and may cause some loss of moisture from the sample. This may lead to an apparent increase in reactant concentration and if not accounted for in subsequent calculations, degradation may be unsuspected.

Although accelerated stability testing using the Arrhenius relationship has proved invaluable in the development of stable formulations, there are some limitations. The order of reaction for the degradation needs to be known and is a time consuming process.
Extrapolation of high temperature data must be done with caution, as these extrapolations are valid only on the assumption that reactions mechanisms are independent of temperature. Moreover, accelerated stability studies of pharmaceuticals are often conducted over narrow temperature ranges (typically 35 – 70 °C) and it is difficult to observe non-Arrhenius behaviour in experimental data even though non-linearity may be expected from the reaction mechanism (Fung, 2002).

The Arrhenius equation applies to simple (single-step) degradation mechanisms as it involves only one rate constant and cannot be used for complex reactions (parallel, consecutive) or heterogeneous processes involving phase boundaries (Pugh, 2002). Additional factors such as rate of dissolution, diffusion from within a matrix and melting are important and measured to determine degradation. The procedure may also be invalidated by number of factors such as; reduction of hydrolysis rate caused by reduced moisture content at higher temperatures, melting or softening of gelatine or splitting of tablet coatings. The effects of temperature on photochemical and microbiological degradation are unpredictable.

1.5.2 Effect of humidity

Stability testing should always include samples that have been artificially stressed by the addition of moisture (Carstensen, 1974). Accelerated degradation rates are observed when products susceptible to hydrolysis are stored in high RH atmospheres. Addition of moisture has been shown to decrease the lag time and increase the zero-order rate constant for the degradation of aminosalicylic acid (Kornblum and Sciarrone, 1964). Mechanistic information on the degradation processes occurring in the sample can be deduced. Such tests give an indication of the minimum level of RH that can be tolerated by the product and are useful in determining the degree of protection that is required to afford suitable protection over the course of its shelf-life.

1.5.3 Shelf-life prediction from accelerated stability testing data

In pharmaceuticals, shelf life is the length of time that elapses before the drug content no longer exceeds 90 %, when stored in accordance to the label instructions. Shelf-life predictions are based on the application of the Arrhenius equation (Equation 1.3), which
indicates the effect of temperature on the rate constant $k$ of a chemical reaction. A plot of $\ln k$ versus the reciprocal of temperature $1/T$ yields a straight line (provided reaction mechanisms do not change) and from an extrapolation, the rate constant at any desired temperature can be determined. The extent of degradation at any given time can be calculated by substituting value of $k$ into the appropriate rate equation that depicts the particular degradation. This however requires knowledge of the order of reaction.

Some of the limitations are that the application of high stress may cause reactions that would not possibly take place under lower stress associated with normal storage conditions. Furthermore, it is difficult to predict the exact shelf-life of a product because of variations in the conditions likely to be encountered under normal storage conditions. This normally involves accepting the shortest shelf-life for the range of conditions likely to be encountered.

Formulated products are complex systems and often degrade via complex reaction schemes and may involve parallel, consecutive or chain reactions. Determining the extent of degradation is a challenging task and requires prolonged tests under normal conditions.

### 1.6 Conventional Stability Study Assay Techniques

The progression of a reaction from reactants to products is possible, only if there is a suitable reaction pathway and is thermodynamically and kinetically feasible. However, in order to successfully investigate the reaction, it must proceed at an observable rate. The instrument used for chemical assays should ideally give information on both, the kinetics and thermodynamics of the process and be capable of studying reactions that proceed at different rates. It should be sensitive to determine reaction parameters under ambient conditions in the shortest experimental time. Moreover, it should be versatile enough to study a range of products in different phases.

A number of analytical techniques have been conventionally used for pharmaceutical stability assessment, but the most widely used are those based on spectroscopic and chromatographic principles. High performance liquid chromatography (HPLC) is the most prevalent technique used in the pharmaceutical industry to assess drug stability (Heinanen et al., 2001; Bempong et al, 2005; Rao and Nagaraju, 2003; Shao et al., 2004; Papadoyannis et al., 2000). This technique involves separation of reaction components as
the mixture is passed through a column at high pressure. This is achieved by exploiting physical characteristics like size, charge or affinity. Components eluted from the column are then measured with the help of a detector, which requires that it have a suitable functionality (chromophore). The reaction kinetics is analysed by quantifying the concentrations of the parent compound and/or any degradation products formed as a function of storage time.

The use of HPLC in stability assessment is however, limited by two factors. First is its relative insensitivity to small changes in concentration. This is a problem with all samples that have slow degradation rates and consequently have to be studied under accelerated stress conditions or requires the reaction to proceed for considerable period of time in order to generate detectable limits of products. The second is the requirement that samples are dissolved in a suitable solvent prior to analysis. This is not a problem for compounds normally formulated in solutions, but can cause accelerated decomposition and increased rates for some compounds especially solid pharmaceuticals. Moreover, HPLC analysis cannot detect if a solid drug has changed polymorph because dissolution of the sample before analysis removes any solid-state history. It is also incapable of continuously monitoring the sample and requires that aliquots be taken from a stock at regular time intervals and analysed.

Spectroscopic techniques may also be used to study reaction kinetics and include infrared spectroscopy (Chen et al., 2005), ultra-violet spectroscopy (Seridi et al., 2006) and nuclear magnetic resonance spectroscopy (Naidong et al., 1993; Muangsiri et al., 2005). Spectroscopic techniques are capable of monitoring a sample continuously as it is non-invasive and in many cases provides excellent kinetic information.

Most analytical techniques are capable of producing quantifiable kinetic data but are incapable of producing thermodynamic data. This requires additional studies to be performed using different techniques. Calorimetry offers an alternative approach to conventional stability techniques as it has the potential to obtain both kinetic and thermodynamic data.
1.7 Calorimetry

A calorimeter is essentially made up of a vessel (in which the thermal phenomena under investigation is carried out and studied) placed within a cavity (Calvet and Prat, 1963). The use of one of the first calorimeters is usually regarded to be that by Lavoisier and Laplace (1780), to measure the heat produced as a result of respiration in a guinea pig. The construction of the calorimeter was simple with an outer shell packed with snow, which melted and maintained a constant temperature of 0 °C around an inner shell that was filled with ice (Figure 1.1). A guinea pig was placed in the centre of the chamber and the heat generated as a result of its metabolism, melted the ice. From the amount of water collected and the latent enthalpy of fusion of water, it was possible to determine the total heat output for the process $Q$. If the body-mass of the guinea pig was known, then the enthalpy change (in kJ Kg$^{-1}$) for metabolism can be determined.

Figure 1.1: Guinea pig calorimeter designed by Lavoisier and Laplace.
1.7.1 Types of calorimeters

Modern calorimeters are based on similar design principle and measure power, but with greater accuracy. They are capable of measuring both exothermic as well as endothermic events. Both thermodynamic (heat output from a reaction, $q$) and kinetic (heat output as a function of time, power, $dq/dt$) information can be obtained. Based on the method by which heat is measured experimentally, calorimeters are categorised as, adiabatic calorimeters, power compensation calorimeters and heat conduction calorimeters (Wadsö and Goldberg, 2001).

In an ideal adiabatic calorimeter, there is no heat exchange between the calorimetric vessel and its surroundings. Hence, the temperature of the calorimeter would rise (exothermic process) or fall (endothermic process) as the heat content of the sample changes during the course of the reaction. The change in heat is equal to the product of the temperature change and the heat capacity of the system. In practice, true adiabatic conditions are difficult to obtain and some heat leak to the surroundings do occur (semi-adiabatic or isoperibol).

In a power compensation calorimeter, heat is added or removed from the calorimetric vessel by an electrical element, so as to maintain the sample and vessel at a given temperature. The power supplied by the element is thus the inverse of that generated by the sample and is based on the Peltier principle.

In a heat conduction calorimeter, heat released or absorbed is allowed to flow between the reaction vessel and a heat sink. A thermopile is present between the vessel and the heat sink, which generates a voltage signal that is proportional to the heat flow across it. The temperature of the heat sink is kept constant.

1.7.2 The principles of Isothermal Calorimetry

Isothermal calorimeters, sometimes referred to as heat conduction or heat flow calorimeters are surrounded by a heat sink and maintain the reaction system at a constant temperature. Any thermal changes occurring in the sample causes a small temperature difference relative to the sink, thus causing a heat-flow either to or from the heat sink. The magnitude of the temperature difference is directly proportional to the heat-flow. Heat that
is absorbed or released by the reacting system is quantitatively exchanged with the heat sink via an array of thermopile. The potential generated by the thermopiles is then amplified and recorded as the heat-flow (\(dq/dt\), power) as a function of time. Various designs of heat sinks have been used and include thermostatted water/oil bath (TAM and Calorimetry Sciences Corporation series of instruments), thermostatted air box (LKB batch calorimeters) and temperature-regulated Peltier units (Setaram).

Calorimetric studies reported in this thesis were conducted using a 2277 Thermal Analysis Monitor (TAM, Thermometric AB, Jarfalla, Sweden).

1.7.3 **Thermal Activity Monitor**

The TAM is a multi-channel calorimeter comprising of four independently operated heat-conduction microcalorimetric channels, which are seated in a 25 litre thermostatted water bath (Figure 1.2). The temperature of the water bath can be finely set from 5 – 90 °C with the help of thermostats and heaters and has a stability of about 1 \(\times\) 10^{-4} °C. The water thermostat is of the overflow type, where water is circulated with the help of a circulating pump positioned under the bath. Water flows through the pump outlet and into the inner vessel of the bath, through a grid, which ensures uniform upward flow, which then overflows between the inner and outer vessel before returning to the pump. The volume between the two vessels acts as a buffer volume, thereby maintaining the volume in the inner vessel, while accommodating any changes in the number of calorimeters in the bath. Both the vessels are cylindrical and made from stainless steel, the inner vessel holding about 22 litres with a water flow rate of 30 litres min\(^{-1}\) (Suurkuusk and Wadsö, 1982).

A schematic design of a twin calorimetric channel is shown in Figure 1.3 and is made up of a cylindrical stainless steel vessel comprising of an aluminium block assembly; which has two main cylindrical blocks and four small nearly cubical blocks in contact with the thermopile and serves as the calorimetric heat sinks. A twin calorimetric unit consists of two ampoule holders each surrounded by two Peltier effect plates which form thermal bridges to aluminium blocks. The Peltier effect plates are electrically connected in series, while the two thermopiles thus formed are connected in opposition, thereby giving a differential voltage signal. Calibration heaters are placed in vertical bores in the middle section of the ampoule holders.
Figure 1.2: Schematic of the 2277 TAM (Thermometric AB): a, console for electronic modules; b, ampoule lifter; c, inner lid; d, outer lid; e, heat exchanger; f, calorimetric unit; g, water bath; h, pump outlet tube; i, polyurethane foam insulation; j, water bath circulating pump; k, water bath temperature control unit; l, digital display and control panel unit (Adapted from Thermometric).

Figure 1.3: Twin calorimetric channel: a, g, Ryton connecting tubes; b, lid; c, 16 mm steel tubes; d, 6 mm steel tubes; e, steel lid; f, steel vessel; h, j, aluminium block; i, small aluminium block; k, steel spring; l, ampoule holder; m, peltier effect plate; n, calibration heater (Suurkuusk and Wadsø, 1982).
1.7.3.1 Calibration of a calorimetric unit

It is important that calorimeters are calibrated regularly and after any modification to the system (change of temperature, amplifier setting). The accuracy of a calorimetric measurement is determined by the reliability of the calibration routine of the instrument. Modern calorimeters are calibrated by means of an integral internal electrical calibration heater and work by generating a known quantity of heat.

Two identical ampoules are lowered simultaneously into the sample and reference chamber to the thermal equilibration position. This is usually for about 30 min to allow for thermal equilibration, but depends on the heat capacity of the ampoules and their contents. The ampoules are then lowered to the measurement position (in between the thermopiles) (Figure 1.4). The lowering of the ampoules will cause frictional heat to be generated and must be left (approximately 30 min) to allow for the thermal fluctuations to dissipate. The thermal power is then set to zero (zero baseline) with the help of the potentiometer. A predefined thermal power is then generated through the use of the electric calibration heaters located at the base of the calorimetric chamber (for an amplifier set to a range of 100 μW, the electrical heater delivers a thermal power equal to 99.7 μW). After a stable signal is observed the upper limit is adjusted such that the output recorded by the calorimeter is the same as that delivered by the calibration heaters. The calibration heaters are then turned off and the power signal returns to zero. The recorded output is checked and the procedure is repeated if necessary.

Figure 1.4: The heat-sink/thermopile arrangement and heat flow through thermopile blankets (Peltier element) arrangement in the TAM.
Electrical calibration does have some limitations. The heat generated during the electrical calibration must be entirely that from the heater and no other source and all heat generated must be transferred to the calorimeter. There is inevitably some loss due to electrical resistance during transfer through wires. Moreover, the behaviour of the internal calibration heaters may vary over a period of time thereby introducing systemic errors in the recorded data.

### 1.7.3.2 Sample preparation

Sample preparation is an important step in an ampoule calorimetric experiment. Since heat is ubiquitous, errors can easily arise from poor sample preparation and corrupt experimental data. Care must be taken to avoid contamination of samples and ensuring that ampoules are clean and properly sealed. Experiments reported in this thesis were conducted using 3 ml glass ampoules. Details about sample preparation methods are discussed in relevant experimental sections, but a general idea about sample preparation is given here.

Experiments conducted were either solution phase or solid phase systems. For solution phase studies, the relevant material (API) was accurately weighed and dissolved in appropriate amounts of solvent/buffer. The time at which the solute was added to the solvent was noted and designated $t_0$. Aliquots of solution (3 ml) were pipetted into standard glass TAM ampoules. For solid-phase reactions, samples were accurately weighed directly into the ampoules. Micro hydrostat tubes containing appropriate saturated salt solutions (to obtain desired RH) were inserted with care in order to avoid spillage into the ampoule or the sides of the micro hydrostat. The ampoules were sealed with a crimped metal lid and an air-tight enclosure was ensured with the use of a rubber seal on the inside of the lid. Reference ampoules were prepared with the same proportion of solvent and sealed. The ampoules were then placed in the TAM in the thermal equilibration position for approximately 30 min before being lowered into the measurement position. Data capture was initiated using the dedicated software package Digitam 4.1. The time at which data capture was initiated was noted ($t_i$). The method was set to record power data (μW) either every 30 or 60 s depending on the duration of the experiment. Solution phase reactions were studied for a minimum of 24 h, while the duration of solid-state experiments were considerably longer.
1.7.3.3 Calorimetric Data

All physical and chemical processes result in a heat change and therefore can be studied calorimetrically. The heat flow to or from the heat sink depends on whether the process being studied is exothermic or endothermic. The heat flow for a process is recorded by the TAM as $\frac{dq}{dt}$ or $\Phi$ and is the rate of change of heat flow or thermal power (Watts, J s$^{-1}$) as a function of time (s). Data obtained from the TAM is effectively a plot of thermal power versus time, the power-time curve. Integrating the area under the power-time cure, gives the time dependent heat output $q$ or enthalpy change for the reaction at any given time $t$. Therefore two types of data can be obtained from the recorded IC data, the heat flow (kinetic term) and the time dependent enthalpy change for a process (thermodynamic process).

For a reaction that is allowed to go to completion ($t = \infty$), the total heat output (evolved or absorbed) for the reaction can be denoted by $Q$ and the heat change at any particular time $t$ can be denoted by $q$. Both $Q$ and $q$ are derived by integrating the area under the power-time curve ($\Phi$ versus $t$) for $t = 0$ to $t = \infty$ and $t = 0$ to $t = t$ respectively. The magnitude of $Q$, the total heat output for a process is dependent on the energetics of the process and the amount of material available for the process. For a process that has gone to completion, it is equal to the product of the enthalpy change ($\Delta H$) and the amount of material available ($A_0$, in moles) and is given by,

$$Q = A_0 \Delta H$$  \hspace{1cm} \text{Equation 1.9}

The amount of material reacted at time $t$ equals $x$, hence

$$q = x \Delta H$$  \hspace{1cm} \text{Equation 1.10}

For a simple, solution phase first-order reaction $A \rightarrow P$, the general rate expression for the appearance of product is given by,

$$\frac{dx}{dt} = k v (A_0 - x)$$  \hspace{1cm} \text{Equation 1.11}
where \( \frac{dx}{dt} \) is the rate of appearance of product, \( k \) is the first-order rate constant, \( v \) is the volume of solution and \( A_0 \) is the number of moles of reactant available for the reaction. Substituting the value of \( x = \frac{q}{\Delta H} \) (Equation 1.10) in Equation 1.11 gives,

\[
\frac{dq}{dt} = k \Delta H v \left( A_0 - \frac{q}{\Delta H} \right)
\]

Equation 1.12

Calorimetric kinetic equations that describe a range of commonly encountered mechanisms have been written by Willson et al., (1995) and given in Table 1.2. Gaisford et al., (1997, 1999) have derived calorimetric equations that describe more complex reaction schemes such as consecutive and parallel reactions. Calorimetric equations that describe solid-state reactions are discussed in Chapter 5. The calorimetric equation for a two-step consecutive reaction following first-order kinetics as depicted by Equation 1.13 is given by Equation 1.14.

\[
A \xrightarrow{k_1} B \xrightarrow{k_2} P
\]

Equation 1.13

\[
\frac{dq}{dt} = k_1 \Delta H_1 A_0 e^{-k_1 t} + k_1 k_2 \Delta H_2 A_0 \frac{e^{-k_1 t} - e^{-k_2 t}}{k_2 - k_1}
\]

Equation 1.14

Calorimetric equation that describes a three-step consecutive reaction of the type \( A \rightarrow B \rightarrow C \rightarrow P \) where all individual steps follow first order is given by,

\[
\frac{dq}{dt} = k_1 \Delta H_1 A_0 e^{-k_1 t} + k_1 k_2 \Delta H_2 A_0 \frac{e^{-k_1 t} - e^{-k_2 t}}{k_2 - k_1} + \Delta H_3 A_0 k_1 k_2 k_3 \left( \frac{e^{-k_1 t}}{(k_2 - k_3)(k_3 - k_1)} + \frac{e^{-k_2 t}}{(k_1 - k_2)(k_3 - k_2)} + \frac{e^{-k_3 t}}{(k_1 - k_3)(k_2 - k_3)} \right)
\]

Equation 1.15

For a parallel reaction scheme (\( A \rightarrow P \) and \( B \rightarrow P \) that both follow first-order kinetics the calorimetric equation is described as,

\[
\frac{dq}{dt} = \Delta H_1 v k_1 [A_0] e^{-k_1 t} + \Delta H_2 v k_2 [B_0] e^{-k_2 t}
\]

Equation 1.16
### Table 1.2: Transformed calorimetric equations for some commonly encountered reaction mechanisms (Willson et al., 1995).

<table>
<thead>
<tr>
<th>Reaction scheme</th>
<th>Kinetic expression</th>
<th>Transformed expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>A $\rightarrow$ B</td>
<td>$\frac{dx}{dt} = k (A-x)^n$</td>
<td>$\frac{dq}{dt} = \Delta H k (A - \frac{q}{\Delta H})^n$</td>
</tr>
<tr>
<td>A + B $\rightarrow$ C</td>
<td>$\frac{dx}{dt} = k (A-x)^n (B-x)^n$</td>
<td>$\frac{dq}{dt} = \Delta H k (A - \frac{q}{\Delta H})^n (B - \frac{q}{\Delta H})^n$</td>
</tr>
<tr>
<td>A $\leftrightarrow$ B</td>
<td>$\frac{dx}{dt} = k \left( \frac{A}{x_e} \right) (x_e - x)$</td>
<td>$\frac{dq}{dt} = k A (\Delta H - \frac{q}{x_e})$</td>
</tr>
<tr>
<td>A + B $\leftrightarrow$ C</td>
<td>$\frac{dx}{dt} = k_1 (A-x)(B-x)$</td>
<td>$\frac{dq}{dt} = k_1 (A \Delta H - q)(B \Delta H - q) - (k_1, q)$</td>
</tr>
<tr>
<td>Ng equation</td>
<td>$\frac{dx}{dt} = Ax \left( \frac{x}{A} \right)^n (1 - \frac{x}{A})^n$</td>
<td>$\frac{dq}{dt} = Ak \Delta H \left( \frac{q}{A \Delta H} \right)^n (1 - \frac{q}{A \Delta H})$</td>
</tr>
<tr>
<td>Auto-catalytic</td>
<td>$\frac{dx}{dt} = k(A-x)(x_e + x)$</td>
<td>$\frac{dq}{dt} = k(A \Delta H - q)(x_e \Delta H + q)$</td>
</tr>
</tbody>
</table>

**1.7.3.4 Data analysis**

Calorimetric data analysis was performed using Origin™ 7.0 (Microcal Software Inc., USA). The difference in time from $t_0$ to $t_e$ was added to the time axis to correct for the time delay in initiating recording in TAM. Calorimetric data analysis reported in this thesis involved kinetic modelling to various equations and are discussed in relevant sections. Analysis involved an iterative procedure (non-linear curve fitting tool in Origin 7.0).

For a solution phase single-step first-order process calorimetric data were fitted to Equation 1.12 which can also be written as,

$$\text{Power} = \frac{dq}{dt} = k \Delta H v [A_q] e^{-kt}$$  \hspace{1cm} \text{Equation 1.17}$$

where, $q$ is the heat output of the reaction, $k$ is the first-order rate constant, $\Delta H$ is the reaction enthalpy per mole of product formed, $v$ is the volume of solution in the ampoule.
and \([A_0]\) is the initial concentration of reactant. Equation 2.8 can be used to derive values for \(\Delta H\) and \(k\) from the power-time data, as long as \(v\) and \([A_0]\) are known and the reaction proceeds to completion. The iteration procedure requires initial estimates for all parameters to be entered into the software. Values for \(v\) and \([A_0]\) are known for degradation reactions in solutions and are therefore kept constant. Values for \(k\) and \(\Delta H\) are usually unknown and an estimated value is entered and allowed to vary during the iteration procedure until a good fit to the data (indicated by a low \(\chi^2\) value, the program's statistical measure to the fit) is obtained. If the reaction mechanism is not known then the data are fitted to a range of models and that which gives the best fit with least variables is selected. This can be complicated and time consuming if complex reaction pathways are involved and are discussed in Chapter 3.

### 1.7.4 Isothermal Calorimetry in stability testing

Calorimetry (Latin *calor* means heat; Greek *metry* means measurement) is defined as the measurement of heat. A calorimeter is a device capable of measuring the heat flow either to or from the surrounding. Isothermal calorimetry (IC) is the measurement of heat absorbed or released during a process at a constant temperature, with time. All processes, chemical or physical are accompanied by a change in heat content or enthalpy and it is possible therefore, in principle, to study such changes calorimetrically. Calorimetry is non-specific and since heat is ubiquitous, it monitors and records the heat flow changes of all processes occurring in a system.

IC offers a number of advantages over many other analytical techniques. The samples are studied non-destructively (does not cause any extra degradation other than that which would have occurred upon storage) directly under storage conditions. This enables further analytical tests to be carried out on the sample following calorimetric observations. Studies can be carried out on a wide range of materials including solid, liquid, gas and heterogeneous mixtures. Thus, it is possible to study most pharmaceutical preparations directly. It requires no prior knowledge of reaction mechanism or structural information as compared to other analytical techniques like HPLC.

Modern calorimeters such as the Thermal Activity Monitor (TAM, Thermometric AB, Järfälla, Sweden) are versatile and highly sensitive compared to other techniques. It has been shown that the TAM is 10,000 fold more sensitive than a commercial Differential
Scanning Calorimeter (DSC 7, Perkin Elmer) (Buckton and Beezer, 1991). The high sensitivity allows for compounds to be studied directly under ambient conditions and various reaction parameters like pH, RH or temperature can be precisely controlled. The TAM can detect heat flow signals as low as 0.1 μW and it has been reported that the instrument can discriminate between reactions that have a first-order rate constant of $1 \times 10^{-11}$ and $2 \times 10^{-11}$ s\(^{-1}\) with just 50 h of calorimetric data (Willson, 1995). A reaction that progresses at a rate of $1 \times 10^{-11}$ s\(^{-1}\) has a half-life of approximately 2200 years. The design, principle and working of the TAM has been discussed earlier in the chapter.

The high sensitivity of IC offers a huge potential for the study of pharmaceuticals, but its use has so far been limited by the lack of methods for analysing calorimetric data quantitatively. In the past decade or so, a number of approaches to analyse calorimetric data have been reported (Angberg et al., 1988; Willson et al., 1995; Gaisford, 1997; Beezer et al., 1998; Beezer et al., 2001). The different methods of analysing calorimetric power-time data are empirical fitting, kinetic modelling, direct calculation and chemometric approach.

Empirical fitting is the simplest approach to modelling calorimetric data and involves fitting calorimetric data to generic equations. The equation fits the data but does not describe the reaction processes. In most cases, the data are fitted to exponential decay model equations by least-squares minimization. From the determined equation parameters, the data are extrapolated to time at which the power signal falls to zero. The area under the extrapolated power-time curve is calculated and represents the total heat ($Q$) that would have been generated had the reaction gone to completion. Percentages of reaction at any time ($t$) can be determined by analysing fractional areas.

Kinetic modelling involves fitting equations that are constructed based on solution or solid phase kinetics, by a process of iteration. Ideally, it requires the knowledge of reaction mechanisms and is used to determine reaction parameters. For a simple solution phase reaction $A \rightarrow P$, the kinetic expression that describes the rate of disappearance of reactant $A$ is given by Equation 1.2,

$$\frac{dx}{dt} = k(A_0 - x)^n$$

Equation 1.2

where $dx/dt$ is the rate of reaction, $k$ is the rate constant, $A_0$ is the initial quantity of reactant $A$ that is available for the reaction, $x$ is the amount of reactant at time $t$ and $n$ is the order of reaction (integral or non integral). The total heat evolved $Q$ for a reaction that
has proceeded to completion is equal to the product of the enthalpy of reaction $\Delta H$ and the number of moles of material reacted $A_0$ and is given by,

$$ Q = A_0 \Delta H $$  \hspace{1cm} \text{Equation 1.9}

where $q$ is the heat evolved at time $t$.

Substituting values of $x = q/\Delta H$ and $A_0 = Q/\Delta H$ in Equation 1.9, gives

$$ \frac{dq}{dt} = \Phi = k \Delta H^{1-x}(Q - q)^x $$  \hspace{1cm} \text{Equation 1.18}

where $\Phi$ is the calorimetric power (in Watts). The integrated form of Equation 1.18 is given by,

$$ (\Phi - q) = [kt \Delta H^{1-x}(n-1) + Q^{1-x}]^{1-x} $$  \hspace{1cm} \text{Equation 1.19}

Equation 1.19 is then substituted into Equation 1.18 to give Equation 1.20 which describes the calorimetric power-time data,

$$ \Phi = k \Delta H^{1-x}[kt \Delta H^{1-x}(n-1) + Q^{1-x}]^{1-x} $$  \hspace{1cm} \text{Equation 1.20}

The above equation describes the calorimetric data that is derived from reactions which follow the general rate expression (Equation 1.7) for any single-step reaction. Calorimetric data obtained can be fitted to Equation 1.18 by using suitable mathematical software (Origin™ 7.0 (Microcal Software Inc., USA) by a process of iteration and is known as the Willson method (Willson, 1995; Willson et al., 1995). The process was first described by Bakri et al., (1988).

The Willson method has been extended to allow the analysis of various solution phase reactions of varying complexity. Gaisford, (1997) derived calorimetric equations that describe various parallel reaction schemes, consecutive reaction schemes and those that involve both parallel and consecutive steps. Analysing such data that arises from many processes is a challenging task. This thesis is concerned with the stability assessment of complex pharmaceutical systems. An attempt is made to analyse calorimetric data recorded for the degradation of pharmaceutical systems using available data analysis techniques.
Chapter 1 – Introduction

The Specific Aims And Objectives Are:

The aim of this thesis was to study various pharmaceutical model degradation systems using calorimetry and analyse the data recorded to obtain kinetic (rate constants) and thermodynamic (reaction enthalpies) parameters.

- To study degradations of API's involving single component system, analyse calorimetric data to obtain reaction parameters and to assess the applicability of using an Arrhenius relationship in determining rate constants at lower temperatures.
- To assess the applicability of IC in the quantitative analysis of degradation systems containing more than one degrading material – parallel processes.
- To study a two-step consecutive reaction using IC and analyse calorimetric data recorded for complex reactions using kinetic modelling and chemometric analysis.
- Determine minimum desired values for reaction parameters and initial concentration of reactant that are required in order to successfully study slow reaction with small reaction enthalpy using IC.
- Assess the use of IC during pharmaceutical preformulation stage - purity determination of API's and excipient compatibility.
Chapter 2

SOLUTION PHASE KINETICS
2.1 Introduction

Chapter 1 discussed the problems encountered when interpreting calorimetric data and current methodologies involving the use of kinetic models to obtain reaction parameters. In this chapter an attempt is made to analyse the calorimetric data recorded when either a single or a multi-component pharmaceutical model system undergoes degradation in known conditions. It is divided into two parts; the first deals with a simple system while the second deals with a binary system that is likely to be encountered in pharmaceuticals.

In the first part, a study of the degradation of a common pharmaceutical drug, aspirin, was carried out since its degradation kinetics have been widely studied in different buffer solutions and at a range of temperatures. Moreover, its degradation kinetics have been investigated using calorimetry. Studying its degradation in the calorimeter and comparing the reaction parameters derived by using current methods of data analysis enabled a comparison with those in the literature (reaction parameters obtained using different method of data analysis).

Having familiarised the data analysing method and having evaluated its ability to generate accurate reaction parameters for the degradation of single component systems, its applicability to complex pharmaceutical systems was tested. Degradation involving a multi-component system (typically encountered in pharmaceutical formulations) was investigated. In this work, the applicability of analysing calorimetric data obtained when two different components were degrading simultaneously in a system is demonstrated. With the application of kinetic modelling, it was hoped to determine the reaction parameters and deconvolute them into their constituent parts.

2.2 Aspirin In Solution

2.2.1 Introduction

Aspirin (acetyl salicylic acid), a substituted phenyl ester, is relatively unstable towards hydrolysis and forms salicylic acid and acetic acid by the cleavage of the ester group (Figure 2.1).
The degradation rate for the hydrolysis of aspirin exhibits pH dependence, being fastest below pH 2 (acid catalysis predominates) and above pH 9 (base catalysis predominates) and is pH independent between pH 5 and 9 (approximately) (Edwards, 1950; Garrett, 1957). At pH 7.0 and 25 °C, the hydrolysis has a rate constant of $3.7 \times 10^6$ s$^{-1}$, which corresponds to a half-life of about 52 h (Garrett, 1957). A pH-rate profile for aspirin hydrolysis is shown in Figure 2.2, with maximum stability occurring at pH 2.5 (Garrett, 1957; Connors et al., 1979). The rate and mechanism of hydrolysis are affected by the ionization of the carboxylic acid group of aspirin ($pK_a = 3.6$). Several degradation pathways are available and the hydrolysis of aspirin could occur via any of the six simultaneous reaction schemes shown below, the rate constants for each of them being second-order. One or more pathways dominate at various pH values, but the overall reaction follows first-order kinetics and is a function of the six second-order constants (Edwards, 1950; Kelly, 1970).

\[
\begin{align*}
\text{CH}_3\text{COOC}_6\text{H}_4\text{COOH} + \text{H}_3\text{O}^+ & \rightarrow k_1 \rightarrow \text{HOC}_6\text{H}_4\text{COOH} + \text{CH}_3\text{COOH} + \text{H}^+ \\
\text{CH}_3\text{COOC}_6\text{H}_4\text{COOH} + \text{H}_2\text{O} & \rightarrow k_2 \rightarrow \text{HOC}_6\text{H}_4\text{COOH} + \text{CH}_3\text{COOH} \\
\text{CH}_3\text{COOC}_6\text{H}_4\text{COOH} + \text{OH}^- & \rightarrow k_3 \rightarrow \text{HOC}_6\text{H}_4\text{COOH} + \text{CH}_3\text{COO}^- \\
& \quad \text{or} \quad \text{HOC}_6\text{H}_4\text{COO}^- + \text{CH}_3\text{COOH} \\
\text{CH}_3\text{COOC}_6\text{H}_4\text{COOH} + \text{H}_3\text{O}^+ & \rightarrow k_4 \rightarrow \text{HOC}_6\text{H}_4\text{COOH} + \text{CH}_3\text{COOH} \\
\text{CH}_3\text{COOC}_6\text{H}_4\text{COOH} + \text{H}_2\text{O} & \rightarrow k_5 \rightarrow \text{HOC}_6\text{H}_4\text{COOH} + \text{CH}_3\text{COO}^- \\
& \quad \text{or} \quad \text{HOC}_6\text{H}_4\text{COO}^- + \text{CH}_3\text{COOH} \\
\text{CH}_3\text{COOC}_6\text{H}_4\text{COOH} + \text{OH}^- & \rightarrow k_6 \rightarrow \text{HOC}_6\text{H}_4\text{COO}^- + \text{CH}_3\text{COO}^- 
\end{align*}
\]
The overall hydrolysis of aspirin may be expressed by

\[
\frac{d([C_4H_4(OOCCH_3)COOH] + [C_6H_4(OOCCH_3)COO'])}{dt} = -k_1[H^+][C_4H_4(OOCCH_3)COOH] \\
- (k_4[H^+] + k_5[C_2H_5O] + k_6[OH^-])[C_6H_4(OOCCH_3)COO']
\]

\[= -k([C_4H_4(OOCCH_3)COOH] + [C_6H_4(OOCCH_3)COO'])\]

where,

\[
k = \frac{k_1[H^+]}{1 + K_a/[H^+]} + \frac{(k_4[H^+] + k_5[C_2H_5O] + k_6[OH^-])}{1 + [H^+]/K_a}
\]

where \(K_a\) is the dissociation constant of aspirin and \(k\) is the first-order rate constant at a constant pH.

![Figure 2.2: pH-rate profile for hydrolysis of aspirin at 25 °C (Garrett, 1957).](image)

Aspirin was chosen as a model drug because its degradation kinetics in solutions have been widely studied by a variety of techniques including isothermal microcalorimetry
and a direct comparison of the kinetic and thermodynamic data obtained can be made with those in the literature.

Hydrolysis of aspirin at various pHs has been widely studied mainly at higher temperatures. The rate constants at room temperatures were determined from the calculated Arrhenius activation energy. Angberg and Nystrom, (1988) studied the acid catalysed degradation of aspirin in an aqueous solution of HCl (0.1 M) at a temperature range between 30 and 50 °C using isothermal microcalorimetry. From the gradient of the ln(power) versus time plot, the rate constants at higher temperatures were determined and from an Arrhenius relationship the rate constant at 25 °C was determined (Table 2.1). In another microcalorimetric study, Angberg, et al., (1990), studied the degradation of aspirin as a function of pH at two different temperatures of 40 and 50 °C. In acetate buffer pH 4.8, rate constants of $14.0 \times 10^{-6}$ and $34.2 \times 10^{-6}$ were obtained at 40 and 50 °C respectively (Table 2.2).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Rate constants (s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>$2.3 \times 10^{-6}$</td>
</tr>
<tr>
<td>30</td>
<td>$3.9 \times 10^{-6}$</td>
</tr>
<tr>
<td>35</td>
<td>$5.9 \times 10^{-6}$</td>
</tr>
<tr>
<td>40</td>
<td>$9.0 \times 10^{-6}$</td>
</tr>
<tr>
<td>45</td>
<td>$14.5 \times 10^{-6}$</td>
</tr>
<tr>
<td>50</td>
<td>$22.5 \times 10^{-6}$</td>
</tr>
</tbody>
</table>

**Table 2.1:** Rate constants for the degradation of aspirin in 0.1 M HCl at different temperatures as determined by Angberg and Nyström (1988); † extrapolated value.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>pH</th>
<th>Rate constant (s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>4.8</td>
<td>$14.0 \times 10^{-6}$</td>
</tr>
<tr>
<td></td>
<td>5.4</td>
<td>$13.4 \times 10^{-6}$</td>
</tr>
<tr>
<td>50</td>
<td>4.8</td>
<td>$34.2 \times 10^{-6}$</td>
</tr>
<tr>
<td></td>
<td>5.4</td>
<td>$35.0 \times 10^{-6}$</td>
</tr>
</tbody>
</table>

**Table 2.2:** Rate constants for the degradation of aspirin at 40 and 50 °C in acetate buffer pH 4.8 and 5.4 (Angberg et al., 1990).
Chapter 2 – Solution phase kinetics

The objectives of this work were to analyse the power-time data obtained for the degradation of aspirin, using kinetic based models. The applicability of using an Arrhenius plot to high temperature stability data in determining rate constants at room temperature was evaluated. Finally, to determine the minimum observation time required to obtain sufficient data for analysis, in order to obtain the correct values of rate constant and reaction enthalpy.

2.2.2 Materials and methods

2.2.2.1 Materials

Aspirin (99%, lot 119H0175) was purchased from Sigma. Citric acid monohydrate (99%, lot 24203BB) was purchased from Aldrich. Di-sodium hydrogen orthophosphate dodecahydrate (lot K91569674306) and 2 M hydrochloric acid (lot 0C309162) were from BDH. All materials were used as received and solutions were prepared using distilled de-ionized water.

Buffer Preparation

Citrate-phosphate (McIlvaine) buffer pH 5.0 was prepared by dissolving 3.69 g of disodium hydrogen phosphate (dodecahydrate) and 1.02 g of citric acid (monohydrate) per 100 ml of distilled de-ionised water. The pH was adjusted by adding hydrochloric acid and sodium hydroxide solutions.

Buffers were freshly prepared every week and the pH was measured before each experiment using a HANNA HI 9024 pH meter.

2.2.2.2 Isothermal microcalorimetry

Experiments were conducted using a 2277 Thermal Activity Monitor (TAM, Thermometric AB, Järfälla, Sweden) at 25, 40 and 50 °C. Aspirin solutions (0.01 M) were prepared by dissolving 180 mg in 100 ml of hydrochloric acid solution (0.1 M) or citrate buffer (pH 5.0). These solutions were sonicated for 10 min to aid dissolution and equilibrated in a water bath, maintained at the same temperature as the TAM while the drug dissolved (~ 1 h). The time at which the aspirin was added to the buffer was noted.
and designated \( t_0 \). Sample and reference ampoules were prepared using 3ml aliquots of drug solutions or buffer (Section 1.7.3.2) and were placed in the thermal equilibration position of the TAM for at least 20 min before being lowered into the measurement position. Data capture was initiated using the dedicated software package Digitam 4.1 and the time at which data capture started was noted and designated \( t_0 \). Power data (\( \mu \text{W} \)) were recorded every 30 s, for a minimum of 48 h, with an amplifier setting of 30 \( \mu \text{W} \). The instrument was calibrated weekly using an electrical substitution method as mentioned in Section 1.7.3.1. Samples were run at least in quadruplicate.

### 2.2.2.3 Data analysis

Data analysis was performed using Origin™ 7.0 (Microcal Software Inc., USA). The difference between \( t_s \) and \( t_0 \) (in seconds) was added to the x-axis data to correct for the time-delay in initiating recording in the TAM and the onset of degradation. Data were analysed using an iterative procedure, which in Origin 7.0 is the non-linear curve fitting tool. The power-time profile for a reaction that follows first-order kinetics has been shown to be described by Equation 2.1 (Bakri et al., 1988).

\[
\text{Power} = \frac{dq}{dt} = \Delta H . v . k . [A_0] . e^{-kt} \quad \text{Equation 2.1}
\]

where, \( q \) is the heat output of the reaction, \( \Delta H \) is the reaction enthalpy per mole of product formed, \( v \) is the volume of solution in the ampoule, \( k \) is the first-order rate constant and \([A_0] \) is the initial concentration of reactant. Equation 2.1 can be used to derive values for \( \Delta H \) and \( k \) from the power-time data, as long as \( v \) and \([A_0] \) are known and the reaction proceeds to completion. A UV assay conducted on a sample of aspirin removed from the TAM after the heat-flow had reduced to zero, did not show the presence of any active and hence the analysis was appropriate in this study (data not shown).

The procedure requires initial estimates for all parameters to be entered into the software. Values for \( v \) (0.003 dm\(^3\)) and \([A_0] \) (0.01 M) were known and therefore kept constant, while initial values entered for \( \Delta H \) (1 x 10\(^{10} \) \( \mu \text{J} \text{mol}^{-1} \)) and \( k \) (1 x 10\(^{-5} \) s\(^{-1} \)) were entirely reasonable for a chemical degradation and were the same for each data set. The software
then altered these values until a good fit to the data was obtained, as indicated by a low $\chi^2$-value.

### 2.2.3 Results and discussion

The representative power-time curves obtained for the degradation of aspirin in 0.1 M HCl and citrate buffer (pH 5.0) at 25, 40 and 50 °C are shown in Figures 2.3 and 2.4 respectively. Plots of ln(power) versus time (Figures 2.5 and 2.6) for all data sets were linear and gave excellent fits to linear regression analysis for the duration of the experiment, confirming that the degradation followed first-order kinetics at the different temperatures. The raw power-time data were then fitted to Equation 2.1, which describes the power-time response for a single step reaction following first-order kinetics. The fit to each data set is represented by open circles (o) (in Figures 2.3 and 2.4). The power signal for aspirin degradation at 25 °C was very small in comparison to those at higher temperatures, but the data were sufficiently resolved to allow a complete analysis.

![Figure 2.3: Power-time data for the degradation of aspirin (0.01 M) in HCl aqueous solution (0.1 M) at 25, 40 & 50 °C and the fit lines (°) generated by application of Equation 2.1.](image)
Figure 2.4: Power-time data for degradation of aspirin (0.01 M) in Citrate buffer (pH 5.0) at 25, 40 & 50 °C and the fit lines (—) generated by application of Equation 2.1.

Figure 2.5: ln(power)-time data for the degradation of aspirin (0.01 M) in HCl aqueous solution (0.1 M) at 25, 40 and 50 °C and fit lines (—) generated by application of linear regression analysis.
Figure 2.6: ln(power)-time data for the degradation of aspirin (0.01 M) in citrate buffer (pH 5.0) at 25, 40 and 50 °C and fit lines (—) generated by application of linear regression analysis.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>0.1 M HCl</th>
<th>Citrate buffer pH 5.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k$ (s$^{-1}$) (S.D., n)</td>
<td>$\Delta H$ (kJ mol$^{-1}$) (S.D., n)</td>
</tr>
<tr>
<td>25</td>
<td>$2.8 \times 10^6$ (±0.2, 6)</td>
<td>-23.6 (±2.4, 6)</td>
</tr>
<tr>
<td>40</td>
<td>$7.4 \times 10^6$ (±0.7, 5)</td>
<td>-34.4 (±2.9, 5)</td>
</tr>
<tr>
<td>50</td>
<td>$19.9 \times 10^6$ (±3.1, 9)</td>
<td>-30.3 (±3.9, 9)</td>
</tr>
</tbody>
</table>

Table 2.3: Average values for the rate constants and reaction enthalpies for the degradation of aspirin determined by fitting experimental data to Equation 2.1.

Rate constants for the degradation of aspirin as a function of temperature and pH were obtained from the slopes of the ln(power) versus time plots and the average values are summarised in Table 2.3. Rate constants for the degradation of aspirin at a series of temperatures (30 – 50 °C) in an aqueous solution of 0.1 M HCl (pH 1.0) were recorded by Angberg and Nystrom, (1988) from the gradient of ln(power) versus time plots. In 0.1 M HCl they obtained a rate constant of $9.0 \times 10^6$ s$^{-1}$ at 40 °C which increased to $22.5 \times 10^6$ s$^{-1}$ at 50 °C, while in an acetic acid buffer (pH 4.8) rate constant of $14 \times 10^6$ s$^{-1}$ at 40 °C and $34.1 \times 10^6$ s$^{-1}$ at 50 °C were determined (Angberg et al., 1990).
Values obtained in this study were slightly lower than those recorded by Angberg, but nevertheless compare well.

Enthalpy is an important thermodynamic parameter and calorimetry is the only analytical technique that measures heat-flow from which reaction enthalpies can be quantified. The reaction enthalpy ($\Delta H$) is conventionally determined by dividing the total heat output ($Q$) of a reaction by the number of moles or mass of the reactant, provided the reaction progresses to completion during the course of the experiment. This might be time consuming if the reaction is slow and occurs over a prolonged period (longer than the calorimetric experimental measurement period). Another method used in determining $\Delta H$ is by measuring the initial heat output of the sample (i.e., power at $t = 0$), as mentioned in Section 1.7.3.3, and Equation 1.17 can be re-written for a first-order process as:

$$\Phi_0 = \Delta H k[A_0]$$  \hspace{1cm} \text{Equation 2.2}

where, $\Phi_0$ is the power signal at $t = 0$, $k$ is the rate constant and $[A_0]$ is the initial concentration of the sample. Angberg (1992) determined the enthalpy of hydrolysis of aspirin to be approximately -29 kJ mol$^{-1}$. However, in an IC ampoule experiment the initial power signal is always lost and hence it is usually deduced through extrapolation of the experimental power signal which can inherently introduce errors. By fitting the experimental data to Equation 2.1, value of $\Delta H$ can be directly determined without the need to either determine the initial power signal or having to let the reaction run to completion (to determine the value of $Q$). Table 2.3 summarises the enthalpy values determined for the hydrolysis of aspirin as a function of temperature and pH and agrees well with those stated by Angberg (1992).

The enthalpy values determined for the hydrolysis of aspirin in the higher pH were lower than those in 0.1 M HCl. These on the contrary should have been identical, as the start and end points in both the media were the same and enthalpy change is independent of the reaction pathway. The calorimeter records the power changes from all the processes occurring in the sample system simultaneously. As the $pK_a$'s of both the acids produced are below 5 (salicylic acid ~ 3.0 and acetic acid ~ 4.8) both the acids will deprotonate in the higher pH medium, which is accompanied by an endothermic heat response, hence lowering the observed net enthalpy. However, in the lower pH medium, significant
deprotonation will not occur and hence these values are in better agreement with those determined by Angberg (1992).

Advancements in amplifier technology have led to improvements in detection sensitivities and the development of highly sensitive microcalorimeters, which can detect extremely small powers; ca. 50 nW at 25 °C. Calorimetric data were directly recorded at 25 °C, and rate constants of $2.8 \times 10^{-6}$ s$^{-1}$ and $5.3 \times 10^{-6}$ s$^{-1}$ for the degradation of aspirin in 0.1 M HCl and citrate buffer pH (5.0) respectively were obtained. Angberg and Nystrom (1988), using the Arrhenius relationship (Equation 1.3), predicted a rate constant of $2.3 \times 10^{-6}$ s$^{-1}$ for aspirin degradation in 0.1 M HCl at 25 °C. This in fact was lower than the rate constant obtained by direct measurement at 25 °C. Moreover, application of an Arrhenius plot to current data (Figures 2.7 and 2.8) did not result in a linear fit, thereby suggesting that there might be a change in reaction mechanism with the change in temperature, which can be expected since aspirin can hydrolyse through one of six pathways depending on the ionization of the carboxylic acid group of aspirin, and it is pH dependent.

Extrapolation from the high temperature data gave a predicted rate constant of $1.6 \times 10^{-6}$ s$^{-1}$ and $2.9 \times 10^{-6}$ s$^{-1}$ in the 0.1 M HCl and citrate buffer (pH 5.0) respectively at 25 °C (Rate constant of $6.27 \times 10^{-6}$ s$^{-1}$ for the degradation of aspirin in 0.1 M HCl at 37 °C was obtained in a later study (Chapter 5) and was used in the Arrhenius plot). However, in the Arrhenius plot for aspirin degrading in citrate buffer, only two higher temperature data points were used for the extrapolation. These predicted rate constants were significantly different from those determined experimentally (Table 2.3) and should be treated with caution especially when predicting shelf-life from elevated temperature stability data.
Figure 2.7: An Arrhenius plot for aspirin rate constants determined in HCl aqueous solution (0.1 M) at 25, 37, 40 & 50 °C.

Figure 2.8: An Arrhenius plot for aspirin rate constants determined in citrate buffer pH 5.0 at 25, 40 & 50 °C.
2.2.3.1 Minimum experimental data

Different sections of the power-time data were analysed to determine the minimum observation period required to obtain accurate values of reaction parameters. Calorimetric data at fixed time intervals of 0-1, 0-2, 0-3, 0-4 to 0-7 h (following equilibration) were fitted to Equation 2.1, to evaluate the minimum data required to return correct reaction parameters. It was found that the minimum number of data points needed to accurately recover the correct reaction parameters, reduced with an increase in the intensity of the power signal. It was found that at 25 °C, a minimum of 5 h of data (following equilibration) were needed (Table 2.4), which reduced to 1 h at 40 °C and 30 min at 50 °C. Therefore, there was no need to run the reaction to completion and just 5 h of data (following equilibration) were sufficient to determine the reaction parameters accurately at 25 °C.

<table>
<thead>
<tr>
<th>Time interval (h)</th>
<th>$k$ (s$^{-1}$)</th>
<th>$\Delta H$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 1</td>
<td>$3.7 \times 10^{-6}$</td>
<td>14.3</td>
</tr>
<tr>
<td>0 – 2</td>
<td>$4.5 \times 10^{-6}$</td>
<td>8.6</td>
</tr>
<tr>
<td>0 – 3</td>
<td>$3.3 \times 10^{-6}$</td>
<td>20.4</td>
</tr>
<tr>
<td>0 – 4</td>
<td>$3.2 \times 10^{-6}$</td>
<td>19.2</td>
</tr>
<tr>
<td>0 – 5</td>
<td>$2.9 \times 10^{-6}$</td>
<td>22.9</td>
</tr>
<tr>
<td>0 – 6</td>
<td>$2.7 \times 10^{-6}$</td>
<td>23.1</td>
</tr>
<tr>
<td>0 – 7</td>
<td>$2.8 \times 10^{-6}$</td>
<td>22.5</td>
</tr>
</tbody>
</table>

Table 2.4: Reaction parameters obtained by fitting varying time periods of power-time data for the hydrolysis of aspirin in 0.1 M HCl at 25 °C.

2.2.4 Conclusions

The degradation of aspirin in solution was studied directly at 25 °C and fitting the data to a first-order kinetic model allowed for the recovery of rate constants and reaction enthalpy. The rate constants obtained at 40 and 50 °C were in good agreement with those obtained by Angberg. However, the high sensitivity of IC at room temperature eliminates the need of performing high temperature studies and the subsequent application of an Arrhenius plot. Extrapolation of the high temperature data to 25 °C resulted in a predicted rate constant ($1.6 \times 10^{-6}$ and $2.9 \times 10^{-6}$ s$^{-1}$ in 0.1 M HCl and citrate buffer pH...
which differed considerably from the measured values \(2.8 \times 10^{-6}\) and \(5.3 \times 10^{-6}\ \text{s}^{-1}\) in 0.1 M HCl and citrate buffer pH 5.0, indicating that the reaction mechanisms varied with temperature. The rate of degradation of aspirin varied as a function of pH as stated in the literature and the net enthalpy at the higher pH was lower, possibly due to the deprotonation effect.

The data allowed the determination of rate constants and enthalpy change for the degradation of aspirin even at 25 °C, without having to let the reaction proceed to completion or determination of the initial power signal. Correct reaction parameters were returned with ca. 5 h of data. At higher temperatures, shorter observation times were required to determine the correct values for the reaction parameters (1 h and 30 min at 40 and 50 °C respectively).

2.3 Parabens

2.3.1 Introduction

The alkyl \(p\)-hydroxybenzoate esters, commonly known as parabens are one of the most important group of chemical agents used as pharmaceutical preservatives (Gottfried, 1962). Parabens are widely used as antimicrobial preservatives in cosmetics (Nikitakis, 1988; Blaug, 1984; Gottfried, 1962; Sunderland and Watts, 1984), food products (Smolinske, 1992; Daniel, 1986) and pharmaceutical formulations (Boehm and Maddox, 1972; Sabalitschka, 1930). The most commonly used are methyl, ethyl, \(n\)-propyl and butyl esters and used either alone or in combination.

Paraben esters are chemically stable in most pharmaceutical systems and hydrolyse only under the most drastic of conditions. At near neutral or slightly acidic pH, an insignificant amount of hydrolysis was observed after boiling for several hours (Aalto et al., 1953; Gottfried, 1962). In an aqueous alkaline solution, they hydrolyse to \(p\)-hydroxybenzoic acid and the corresponding alcohol following pseudo first-order kinetics and are pH dependent (Kamadaa et al., 1973; Sunderland and Watts, 1984). Cazeneuve (1896) first reported the decarboxylation of \(p\)-hydroxybenzoic acid to phenol in acidic medium (but is relatively stable in the alkaline medium). The overall degradation pathway for the paraben ester can be depicted as:
Parabens can undergo either an acid-catalysed or a base-catalysed ester hydrolysis, but are most stable in the pH range of 4-5. The pH-rate profile for the hydrolysis of methyl paraben at 70 °C is shown in Figure 2.10. The hydrolysis at very high pH is complicated by the ionization of the phenolic group (Connors et al., 1979).
The kinetics of the parabens degrading in an aqueous solution has been well discussed in the literature. Raval and Parrott (1967) studied the degradation of methyl paraben in aqueous solution (pH 6-9) at 70, 80 and 85 °C, using a spectrophotometric method and recorded rate constants of 0.51, 1.36 and 1.89 x 10⁻¹ s⁻¹ at the respective temperatures (pH 9.0). Degradation of methyl and n-propyl paraben in acidic solution was recorded by Kamada et al., (1973) between 40 and 100 °C. Blaug and Grant (1974) reported the base-catalysed degradation of methyl, ethyl and n-propyl paraben above 70 °C. Sunderland and Watts (1984) recorded the degradation kinetics of methyl, ethyl and n-propyl paraben between pH 1.26 and 10.59 at the sterilization temperature of 130.5 °C and noted that the degradation was fastest, either at low pH (acid catalysed) or at high pH (base catalysed). They determined the rate constants for methyl, ethyl and n-propyl paraben to be 3.03, 1.26 and 0.93 x 10⁻² s⁻¹ respectively, at pH 10.59.

O'Neill, et al., (2003) studied the base catalysed hydrolysis of methyl paraben as a test reaction for the flow microcalorimeter. They performed a comparative study on the hydrolysis of methyl paraben using the TAM (2277-201 model, Thermometric), micro DSC (Setaram) and the LKB 10-700 flow calorimeter (Bromma) and recorded a value of 3.15 x 10⁻⁴ ± 1.1 x 10⁻⁵ s⁻¹ for the rate constant and an enthalpy value of -50.5 ± 4.3 kJ mol⁻¹ in the TAM at 25 °C.

The parabens were used as model systems since their degradation have been comprehensively studied under various conditions. Moreover, the parabens have been
widely used in numerous marketed formulations either separately or in combination as preservatives. Hence, it is an ideal model system for studying the degradation involving a combination of substances.

The overall aims were to quantify the applicability of using kinetic models to analyse the calorimetric data obtained, when more than one degrading component was present in the system and to assess if the reaction parameters could be obtained for the individual components. The initial study involved the degradation of the parabens on their own, followed by a binary mixture of the parabens (MP:EP, EP:PP, MP:PP).

2.3.2 Materials and methods

2.3.2.1 Materials

Methyl 4-hydroxybenzoate (methyl paraben, MP, lot 449396/1), ethyl 4-hydroxybenzoate (ethyl paraben, EP, lot 440453/1), n-propyl 4-hydroxybenzoate (n-propyl paraben, PP, lot 438404/1) (all 99+ %) and methyl 4-aminobenzoate (≥98%, lot 010351/1) were purchased from Fluka. Methyl 3-hydroxybenzoate (99%, lot S15081-063), 4-hydroxybenzoic acid (p-HBA, 99+%, lot 15203TA) and methyl 2,6-dihydroxy-4-methylbenzoate (98%, lot 30831S03308LK) were purchased from Aldrich. Sodium hydroxide was purchased from VWR. All materials were used as received and solutions were prepared in distilled, de-ionised water.

2.3.2.2 Isothermal microcalorimetry

Experiments were conducted using a 2277 Thermal Activity Monitor (TAM, Thermometric AB, Järfälla, Sweden) at 25 °C. Solutions were prepared by dissolving 0.38 g MP, 0.415 g EP and/or 0.45 g PP (0.05M with respect to each component) in 50 ml of sodium hydroxide solution (0.5 M) and equilibrated in a water-bath maintained at 25 °C. For the binary systems, required amounts of MP:EP, MP:PP and EP:PP were dissolved in sodium hydroxide solution (0.5 M) to obtain the desired concentration. Solution pHs were measured before and after each experiment and were found to be constant at 12.3. Experiments were set up as mentioned in section 1.7.3.2. Power data
(μW) were recorded every 30 s, for a minimum of 24 h, with an amplifier setting of 300 μW. Samples were run at least in triplicate.

2.3.2.3 Data analysis

Data analysis was performed using Origin 7.0 (Microcal Software Inc., USA). For the single component system, the power-time data were analysed by fitting to a first order kinetic model equation (Equation 2.1). The initial values entered for the iterative procedure were constant for ν (0.003 dm³) and [A₀] (0.05 M), while initial values entered for ΔH (1 x 10¹⁰ μJ mol⁻¹) and k (1 x 10⁻⁵ s⁻¹), were those entirely reasonable for a chemical degradation and were the same for each data set. The software then altered these values until a good fit to the data were obtained, as indicated by a low χ² value.

2.3.3 Results and discussions

2.3.3.1 Single component system

The representative power-time traces obtained for the degradation of the three individual parabens in 0.5 M NaOH at 25 °C are shown in Figure 2.11. The data fitted well to Equation 2.1, which describes the power-time response for a single step reaction that follows first order kinetics. The fit lines to the curve are represented by open circles (o). Plots of ln(power) versus time for each data set resulted in a linear relationship, confirming that degradation of all three parabens followed first-order kinetics at 25 °C. From the slope of the line, the first-order rate constants were obtained (Table 2.5).

Fitting the power-time data to Equation 2.1 allowed for the recovery of reaction enthalpies (Table 2.5). The degradation rate constants decreased as the hydrocarbon chain increased in length and could be accounted for due to the sterric hindrance, while the reaction enthalpies were roughly similar. The rate constant obtained for methyl paraben compares well with that obtained by O'Neill et al., (2003).
Table 2.5: Average values for the rate constants and reaction enthalpies for the individual parabens at 25 °C determined by fitting experimental data to Equation 2.1.

<table>
<thead>
<tr>
<th>Paraben ester</th>
<th>$k$ (s$^{-1}$) (±S.D., n)</th>
<th>$\Delta H$ (kJ mol$^{-1}$) (±S.D., n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl</td>
<td>$3.1 \times 10^{-3}$ (±0.01, 3)</td>
<td>-59.2 (±0.4, 3)</td>
</tr>
<tr>
<td>Ethyl</td>
<td>$1.5 \times 10^{-3}$ (±0.01, 3)</td>
<td>-64.4 (±1.3, 3)</td>
</tr>
<tr>
<td>n-Propyl</td>
<td>$1.2 \times 10^{-3}$ (±0.01, 3)</td>
<td>-60.1 (±0.3, 3)</td>
</tr>
</tbody>
</table>

Different sections of the power-time data were analysed to determine the minimum observation period required to deduce accurate values of reaction parameters. Correct values were returned with only the first 15 min (following equilibration) of data used for analysis (Table 2.6). Fitting greater number of data did not result in any significant difference in fitting values.

Table 2.6: Reaction parameters obtained by fitting varying time periods of power-time data for MP to Equation 2.1.
2.3.3.2 Binary mixtures

Binary system experiments conducted were initially on mixtures of MP and PP, since this ester combination is most frequently used as an antimicrobial preservative in cosmetics, food and pharmaceutical formulations. Besides, it has the largest difference in rate constants among the three combinations. The power-time data obtained for the degradation of the MP-PP mixture is shown in Figure 2.12. The power-time data however, could not be fitted by Equation 2.1 (Figure 2.12) (as expected), and in the lack of any prior knowledge of the system, would have immediately implied the probability of multiple events occurring in the system. Since the number of degrading components was known, the data were fitted to an equation that describes the occurrence of two parallel first-order reactions and is derived below.

The contribution from each first-order process is as follows

\[ \begin{align*}
A & \xrightarrow{k_1} P \\
B & \xrightarrow{k_2} P
\end{align*} \]

Equation 2.3

Equation 2.4

The rate of disappearance of the reactants \(A\) and \(B\) are given by,

\[ \frac{d[A]}{dt} = -k_1.[A] \]

Equation 2.5

\[ \frac{d[B]}{dt} = -k_2.[B] \]

Equation 2.6

Integration of the Equations 2.5 and 2.6 yields,

\[ [A] = A_o.e^{-k_1.\tau} \]

Equation 2.7

\[ [B] = B_o.e^{-k_2.\tau} \]

Equation 2.8

Inserting values of \([A]\) and \([B]\) into Equation 2.5 and 2.6 gives,

\[ \frac{d[A]}{dt} = -k_1.A_o.e^{-k_1.\tau} \]

Equation 2.9

68
\[ \frac{d[B]}{dt} = -k_2.B_o.e^{-k_1.t} \]  
Equation 2.10

Since,
\[ q_A = [A].\Delta H_1 \]
\[ q_B = [B].\Delta H_2 \]

Equation 2.9 and 2.10 become,
\[ \frac{dq_A}{dt} = \Delta H_1.k_1.A_o.e^{-k_1.t} \]  
Equation 2.11
\[ \frac{dq_B}{dt} = \Delta H_2.k_2.B_o.e^{-k_1.t} \]  
Equation 2.12

Since the power signal is contributed from both the individual reactions, the total power is the sum of the individual processes and is given by,
\[ \frac{dq}{dt} = \frac{dq_A}{dt} + \frac{dq_B}{dt} \]  
Equation 2.13

\[ \text{Power} = \frac{dq}{dt} = \Delta H_1.v.k_1.[A_o].e^{-k_1.t} + \Delta H_2.v.k_2.[B_o].e^{-k_1.t} \]  
Equation 2.14

where the subscripts 1 and 2 refer to the individual reaction pathways and \([A_o]\) and \([B_o]\) refer to the initial concentrations of reactant A and B (MP and PP in this case), respectively.

The power-time data for the MP-PP binary mixtures were well depicted by Equation 2.14, which describes the power-time response for two simultaneous first-order decay processes and is shown in Figure 2.13.
Figure 2.12: Power-time data for a binary mixture of MP (0.05 M) and PP (0.05 M) in NaOH aqueous solution (0.5 M) at 25 °C and the fit line (•) generated by application of Equation 2.1.

Figure 2.13: Power-time data for a binary mixture of MP (0.05 M) and PP (0.05 M) in NaOH aqueous solution (0.5 M) at 25 °C and the fit line (o) generated by application of Equation 2.14.
Fitting the power-time data to Equation 2.14 allowed for the recovery of rate constant and enthalpy values, for both the individual degradation reactions in the binary mixture (Table 2.7). Enthalpy values determined from the mixed system data are, within error limits same as those determined from the degradation of the individual species, while the rate constants are lower than expected. This interesting observation can be attributed to the fact that the two reactants present degrade to a common product, \( p \)-hydroxybenzoic acid. For the degradation of an individual paraben the rate of degradation may be defined as the rate of change of concentration of the reactant or product and is given by,

\[
\frac{d[\text{Paraben}]}{dt} = \frac{d[p\text{HBA}]}{dt}
\]

where \([\text{Paraben}]\) is the concentration of either paraben and \([p\text{HBA}]\) is the concentration of \( p \)-hydroxybenzoic acid as a function of time. In the binary mixture, it is evident that \([p\text{HBA}]\) is contributed from two sources, thereby causing the rate of disappearance of the two reactants to inevitably reduce to some extent.

<table>
<thead>
<tr>
<th>Ester mix</th>
<th>( k_1 ) (s(^{-1})) (S.D., ( n ))</th>
<th>( k_2 ) (s(^{-1})) (S.D., ( n ))</th>
<th>( \Delta H_1 ) (kJ mol(^{-1})) (S.D., ( n ))</th>
<th>( \Delta H_2 ) (kJ mol(^{-1})) (S.D., ( n ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl/ethyl</td>
<td>( 2.3 \times 10^{-4} ) (±0.10, 8)</td>
<td>( 1.1 \times 10^{-4} ) (±0.10, 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl/( n )-propyl</td>
<td>( 2.2 \times 10^{-4} ) (±0.08, 9)</td>
<td>( 8.0 \times 10^{-5} ) (±0.01, 9)</td>
<td>(-58.2) (±2.1, 9)</td>
<td>(-54.4) (±1.9, 9)</td>
</tr>
<tr>
<td>Ethyl/( n )-propyl</td>
<td>( 1.2 \times 10^{-4} ) (±0.10, 8)</td>
<td>( 8.0 \times 10^{-5} ) (±0.01, 8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.7**: Average values for the rate constants and reaction enthalpies for binary mixtures of the parabens determined by fitting experimental data to Equation 2.14.

The influence of \( p \)-hydroxybenzoic acid on the degradation rate of individual parabens in the binary mixtures was studied by measuring the degradation of paraben in a solution of base containing 0.05 M \( p \)-hydroxybenzoic acid (concentration equivalent to one degradation product). Fitting the power-time data (Figure 2.14) to a first-order kinetic model, returned rate constants of 2.2, 1.0 and \( 8.0 \times 10^{-4} \) s\(^{-1}\) for MP, EP and PP respectively (Table 2.8) which were identical to those recorded from the binary mixtures. This suggests that formation of a common product (\( p \)-hydroxybenzoic acid) from two
different starting materials in the binary system does influence the degradation rates of the individual components.

![Figure 2.14: Power time data for MP (0.05 M) EP (0.05 M) and PP (0.05 M) in NaOH aqueous solution (0.05 M) containing 0.05 M p-hydroxybenzoic acid at 25 °C and the fit lines (o) generated by application of Equation 2.1.](image)

<table>
<thead>
<tr>
<th>Paraben ester</th>
<th>$k$ (s$^{-1}$)</th>
<th>$k$ (s$^{-1}$) in PHBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl</td>
<td>$3.1 \times 10^{-4}$</td>
<td>$2.2 \times 10^{-4}$</td>
</tr>
<tr>
<td>Ethyl</td>
<td>$1.5 \times 10^{-4}$</td>
<td>$1.0 \times 10^{-4}$</td>
</tr>
<tr>
<td>n-Propyl</td>
<td>$1.2 \times 10^{-4}$</td>
<td>$0.8 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

Table 2.8: Comparison of average values of rate constants for the individual parabens in NaOH and in p-hydroxybenzoic acid at 25 °C determined by fitting experimental data to Equation 2.1.

Total amount of heat released during the degradation process was measured by integrating the area under the power-time curve. Heat released from the binary mixture ($16.7 \pm 0.6$ J) was calculated to be the same (within error) as the sum of the heats released by the individual components ($17.9 \pm 0.6$ J), thus indicating the same extent to which the reaction proceeded in all cases.
Power-time data plots for the other two binary mixtures of MP:EP and EP:PP are shown in Figure 2.15, and could not be fitted by either Equation 2.1 or Equation 2.14, when all the unknown parameters were allowed to vary. However, fitting the data to Equation 2.14 and fixing the enthalpy values equal to those obtained from the individual components (Table 2.5) resulted in a successful fit (Figure 2.15). Rate constants returned (Table 2.7) were again lower than that expected from the individual parabens, but are in accordance with those recorded in Table 2.8 as these systems also have a common degradation product.

The data from the binary system shows the practical limits of the resolution of the model fitting technique to real data. Assuming approximately equal enthalpies, the data analysis implied that the rate constant of one component had to be at least twice the magnitude of the other to enable a successful analysis. When 'ideal' data (generated using values in Table 2.5, in Mathcad) for the MP:EP and EP:PP systems were fitted to Equation 2.14 (Figure 2.16), correct values of rate constants were recovered, suggesting that it was the
inherent noise in the data that prevented a successful fit rather than the parameters being too similar.

![Graph showing power-time data for binary mixture of MP:EP and EP:PP](image)

**Figure 2.16:** Simulated power-time data for binary mixture of MP:EP and EP:PP generated using values from Table 2.5 and the fit lines (o) generated by application of Equation 2.14.

The observation that degradation rate constants may vary significantly than expected when materials are formulated together is of great significance, especially if shelf-life were predicted based on stability data obtained for the individual components. Mixtures of parabens have been used in various cosmetic, foods, and pharmaceutical formulations because of improved antimicrobial properties compared to individual parabens. In this case, the shelf-life would have been longer, and hence the need for developing analytical techniques that would allow the study of heterogeneous samples.

Determination of the minimum observation period required to allow the recovery of the correct reaction parameters, were carried out by analysing different sections of the power-time data. Greater amount of binary system data were needed for the model fitting to recover the correct values compared to a system having a single degrading component. Correct parameters were returned with just 4 h of data (Table 2.9) and are a significant improvement in comparison to other methodologies used in existing published work.
Degradation of parabens under certain conditions can proceed to the formation of phenol and can then be described by a two-step consecutive reaction. However, if the p-hydroxybenzoic acid is stable as expected under alkaline conditions, then the degradation should be described by Equation 2.1. Had the degradation proceeded to phenol the data would be better described by a two-step consecutive model, which is represented by Equation 2.15 (Gaisford et al., 1999).

$$ \text{Power} = \frac{dq}{dt} = \Delta H_1 \cdot v \cdot k_1 [A_0] \cdot e^{-k_1 \cdot t} + k_1 \cdot k_2 \cdot \Delta H_2 \cdot v \cdot [A_0] \cdot \left( \frac{e^{-k_1 \cdot t} - e^{-k_2 \cdot t}}{k_2 - k_1} \right) \quad \textbf{Equation 2.15} $$

where $k_1$ and $k_2$ are the rate constants and $\Delta H_1$ and $\Delta H_2$ are the enthalpies for the two reaction steps respectively. Fitting the power-time data for the degradation of MP to Equation 2.15 (Figure 2.17) resulted in a better statistical measure of fit (indicated by a lower chi squared value; $\chi^2 = 0.008$) than that obtained by a fit to Equation 2.1 ($\chi^2 = 0.125$). It has been suggested earlier (Gaisford et al., 1999) that in the absence of any supporting data of the reacting systems, the best approach to determine reaction mechanisms from calorimetric data is to fit the data to a range of kinetic models and select that one which gives the best fit with least variables. This would suggest that the p-hydroxybenzoic acid is not stable under alkaline conditions and undergoes further decarboxylation (contrary to that suggested by Cazeneuve (1896)) and returned very small value for the enthalpy change (ca. 0.3 kJ mol$^{-1}$).
Figure 2.17: Power-time data for MP (0.05 M) in NaOH aqueous solution (0.05 M) at 25 °C and the fit lines (○ and *) generated by application of Equation 2.1 and 2.15 respectively.

Power-time traces were generated (Mathcad) for the two steps based on the reaction parameters obtained from the fit of the data to Equation 2.15 and is shown in Figure 2.18. The degradation of p-hydroxybenzoic acid to phenol contributed very little to the observed heat flow and an alkaline solution of p-hydroxybenzoic acid (0.05 M) gave no detectable heat-flow in the calorimeter over a period of eight days (Figure 2.19). A better fit of the data to Equation 2.15 can be ascribed to the greater number of variables and not related to the reaction mechanisms and should be disregarded. The aim of this study was to assess the use of IC to study parallel processes and a comprehensive study of the paraben degradation is beyond the scope of this project.
Figure 2.18: Power-time data for MP, EP and PP (0.05M) in NaOH aqueous solution (0.5M) at 25 °C (—) and the theoretical contributions to the observed signal from the individual steps as determined using Equation 2.14; degradation of the individual parabens (—) and degradation of $p$-hydroxybenzoic acid (—).

Figure 2.19: Power-time data for $p$-hydroxybenzoic acid (0.05 M) in NaOH aqueous solution (0.5 M) at 25 °C.
2.3.4 Conclusions

IM data allowed for the determination of rate constants and enthalpies for the degradation of the individual methyl, ethyl and n-propyl parabens directly at 25 °C, without the need for the reaction to reach completion. The rate constants obtained for the degradation of methyl paraben were in good comparison to the literature and it was found that when the system had only one degrading component, a minimum of 15 min of experimental data (following equilibration) was sufficient to effect a complete analysis.

Analysis of calorimetric data for the binary mixtures showed that the kinetic models were able to detect the presence of two parallel reaction processes. The degradation rate constants for the parabens in combination were considerably lower than expected and were ascribed to the common degradation product being formed. This observation is of great importance especially if substances behave differently when used in combinations, but their degradation properties were studied individually. The recovery of rate constants and reaction enthalpies of each species in a mixed system were possible, as long as one rate constant was at least twice the magnitude of the other. Analysis of simulated data successfully recovered the rate constants, which showed that the inherent noise in the data prevented successful analysis rather than the parameters being too similar, although results from simulated data also suggested that a complete analysis would not be possible if the magnitude of the rate constants were more than three times the other. A minimum of 4 h of experimental data (following equilibration) was sufficient to recover correct reaction parameters.

2.4 Summary

This chapter showed that IM can be used to study the degradation of API in solutions. In the absence of any prior knowledge on the reaction mechanism, the best approach is to fit calorimetric data to various kinetic models and select that which gives the best fit with fewest variables.

The first part of this chapter demonstrated the capability of the model fitting process to recover correct reaction parameters, by comparing experimental data with those in the literature. Rate constants and enthalpy values were determined without having to let the reaction run to completion and in fact, 5 h of data (following equilibration) were
sufficient to accurately determine the reaction parameters at 25 °C. Moreover, as calorimetric data can be measured directly at 25 °C the determination of rate constants without invoking the assumptions inherent in application of the Arrhenius relationship should be avoided. Reaction enthalpies were easily quantified for the power-time data obtained from IM and did not require an extrapolation of data to \( t = 0 \) or for the reaction to run to completion.

The advantages of fitting calorimetric data to kinetic models were clearly evident in the second part of this chapter, where complex reactions were occurring in a binary system. Fitting the data to a range of models helped deduce the likely reaction kinetics occurring in the system. It was also possible to recover rate constants for individual species in a system that was undergoing parallel degradation. Although it is possible to recover rate constants having small magnitude of differences, through model fitting, the inherent noise in calorimetric data prevents its greater application.

This has shown that it is possible to successfully study the degradation kinetics of API’s that have a fast rate of reaction (order of \( 10^4 \) s\(^{-1}\)) and those that have a medium rate of reaction (order of \( 10^6 \) s\(^{-1}\)) directly at 25 °C in the calorimeter.
Chapter 3

CONSECUTIVE REACTION
3.1 Introduction

Pharmaceutical formulations are complex and most often multi-component systems. Instabilities in these heterogeneous systems are complex and difficult to analyze using standard analytical tools. Isothermal calorimetry offers an alternative approach to conventional stability assessment techniques for analyzing complex degradation reactions. Degradation reaction mechanisms in pharmaceuticals are numerous and among them include parallel, consecutive and/or a combination of such processes. Chapter 2 demonstrated the applicability of using kinetic models to analyse calorimetric data when two parallel first-order processes occurred simultaneously. This chapter aims to study a more complex reaction associated with pharmaceutical degradations: the consecutive reaction.

Consecutive reactions are complex, and the most commonly encountered in pharmaceuticals are either a two-step, three-step or four-step process. Using conventional techniques such as High Performance Liquid Chromatography (HPLC) and spectrophotometry to study the kinetics of the various steps is complicated and involves careful planning and design. IC offers the advantage where in the individual steps of a consecutive reaction do not have to be isolated in order to be studied, as it measures the thermal changes occurring as the reactants turn into products.

Since heat is ubiquitous, IC records thermal changes occurring over the entire reaction and hence cannot be differentiated for each individual steps. However, it has been shown that with the aid of kinetic modelling, calorimetric data can be iterated to obtain kinetic and thermodynamic information about the individual steps. For complex reactions, prior knowledge of reaction mechanisms is essential to enable a good fit. The alternative approach would be to systematically fit the data to increasingly complex kinetic models until a satisfactory fit is obtained (Gaisford et al., 1999). This is time consuming and requires the derivation of many complex equations.

A more recent approach to complexity in multivariate data sets is the application of chemometrics. Chemometrics is the application of mathematical or statistical methods to chemical data to quantitatively model and produce visual representations of information. The procedure involves application of techniques such as principal component analysis (PCA), to determine and deconvolute the individual processes that contribute to the overall process. The use of chemometrics as a data analysis tool is becoming increasingly
popular especially in multivariate calibrations (Martens, 1999; Kowalski and Seasholtz, 1991); quantitative structure-activity modelling (Wold et al., 1993), pattern recognition (Albano et al., 1978) and multivariate statistical process monitoring and control (Workman et al., 1999). In this study, chemometric techniques involving pattern searching from a data matrix in a model-free approach was used.

In this chapter the degradation of potassium hydroxylamine disulfonate in solution was studied which proceeds by a two-step consecutive reaction scheme. The disulfonate hydrolysis to hydroxylamine monosulfonate and ultimately to hydroxylamine, both steps being first-order. The degradation reactions were studied in the calorimeter and the data obtained were subjected to kinetic modelling to obtain the reaction parameters for the individual steps. The calorimetric data were further subjected to chemometric analysis that involved pattern searching (multivariate analysis) in order to obtain information of the individual reaction steps. This essentially is a model-free approach and does not require the need of imposing kinetic models on the system. A comparison of reaction parameters obtained from both techniques was thus possible.

3.2 Consecutive Reaction

Degradations through consecutive reaction are common in pharmaceuticals and pose considerable difficulty in elucidating their kinetics. The various steps can have different orders of reaction. Calorimetry is an ideal analytical tool for studying complex degradations involving consecutive reactions. Kinetic equations can be written which could best describe the processes under consideration and be used to fit calorimetric data, provided the mechanisms are known. Calorimetric equations that describe a two-step, three-step and four-step consecutive reaction schemes had been derived (Gaisford, 1997).

3.2.1 Hydrolysis of potassium hydroxylamine-NO-disulfonate

The degradation kinetics of potassium hydroxylamine-NO-disulfonate had been studied earlier based on titration using $^{35}$S labelled compounds (Candlin and Wilkins, 1961). It is an intermediate product formed when potassium hydroxylamine trisulfonate is subjected to acid catalysed hydrolysis. Potassium hydroxylamine trisulfonate hydrolysis by a three-step consecutive reaction mechanism to first form potassium hydroxylamine disulfonate,
which further hydrolysis to the monosulfonate and finally to hydroxylamine. All three steps follow first-order kinetics and hydrogen sulphate is formed at each step. A reaction scheme that represents the hydrolysis of potassium hydroxylamine disulfonate to the monosulfonate ion and finally to hydroxylamine is shown below.

\[
\text{SO}_3\text{NH}_2\text{O}_3\text{SO}_3^{2-} + \text{H}_2\text{O} \xrightarrow{k_1} \text{NH}_2\text{O}_3\text{SO}_3^- + \text{HSO}_4^- \\
\text{NH}_2\text{O}_3\text{SO}_3^- + \text{H}_2\text{O} \xrightarrow{k_2} \text{NH}_2\text{OH} + \text{HSO}_4^-
\]

**Figure 3.1:** Reaction pathway for the hydrolysis of hydroxylamine disulfonate to hydroxylamine monosulfonate and finally to hydroxylamine by a two-step consecutive reaction. Hydrogen sulphate is formed at each step.

The three-step acid catalysed hydrolysis of potassium hydroxylamine trisulfonate had been studied in the TAM as a function of temperature and perchloric acid concentration (Gaisford, 1997). The recorded calorimetric power-time data was subjected to kinetic model fitting and showed an excellent fit to a three-step first-order consecutive reaction scheme. For each of the three individual steps, rate constants and reaction enthalpies were recorded (Table 3.1). The first step had the fastest rate constant while the second and third steps had slower and almost similar rates. The reaction enthalpies recorded for each step varied with temperature and although a number of values were returned, only those with the lowest \( \chi^2 \) (statistical measure of fit) value were considered. The values recorded for \( \Delta H_1 \) were positive and \( \Delta H_2 \) were negative while those for \( \Delta H_2 \) varied with temperature (Gaisford et al., 1999).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>( k_1 ) (s(^{-1}))</th>
<th>( \Delta H_1 ) (kJ mol(^{-1}))</th>
<th>( k_2 ) (s(^{-1}))</th>
<th>( \Delta H_2 ) (kJ mol(^{-1}))</th>
<th>( k_3 ) (s(^{-1}))</th>
<th>( \Delta H_3 ) (kJ mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>4.2 x 10(^4)</td>
<td>28.3</td>
<td>4.6 x 10(^{-6})</td>
<td>-82.2</td>
<td>6.7 x 10(^{-6})</td>
<td>-47.4</td>
</tr>
<tr>
<td>30</td>
<td>4.4 x 10(^4)</td>
<td>±0.1 x 10(^{-9})</td>
<td>95.8</td>
<td>±55.8(^\dagger)</td>
<td>2.8 x 10(^{-5})</td>
<td>±33.5</td>
</tr>
<tr>
<td>35</td>
<td>5.1 x 10(^4)</td>
<td>±0.6 x 10(^{-4})</td>
<td>140.5</td>
<td>±9.1(^\dagger)</td>
<td>4.8 x 10(^{-5})</td>
<td>±22.9</td>
</tr>
</tbody>
</table>

**Table 3.1:** Rate constants and reaction enthalpies for the hydrolysis of potassium hydroxylamine trisulfonate (0.001 M) in perchloric acid solution (0.0005 M), determined by fitting power-time data to a three-step first-order consecutive reaction model (Gaisford, 1997). \( \dagger \) represents the standard deviation with \( n = 2 \).
3.2.2 Two-step first-order consecutive reaction

Calorimetric equations that describe a two-step consecutive reaction scheme following first-order kinetics had been derived by Gaisford (1997). A typical two-step consecutive reaction is shown in Equation 3.1;

\[ A \xrightarrow{k_1} B \xrightarrow{k_2} P \]  

Equation 3.1

where \( k_1 \) and \( k_2 \) are the first-order rate constants and \( \Delta H_1 \) and \( \Delta H_2 \) are the reaction enthalpies for the first and second steps respectively. The rate equations that describe each reaction step are given below:

\[
\frac{d[A]}{dt} = -k_1[A]  
\]

Equation 3.2

\[
\frac{d[B]}{dt} = k_1[A] - k_2[B]  
\]

Equation 3.3

\[
\frac{d[P]}{dt} = k_2[B]  
\]

Equation 3.4

Integration of equation 3.2 yields:

\[ [A] = A_0 e^{-k_1t} \]

Equation 3.5

The reaction \( A \rightarrow B \) may be described by:

\[
\frac{d[A]}{dt} = -k_1 A_0 e^{-k_1t}  
\]

Equation 3.6

and hence the power associated with reaction \( A \rightarrow B \), \( dq_{A}/dt \) is given by:

\[
\frac{dq_A}{dt} = -k_1 \Delta H_1 A_0 e^{-k_1t}  
\]

Equation 3.7
Equation 3.7 also describes the power-time response for any single-step reaction following solution phase first-order kinetics.

Substituting value of \([A]\) into equation 3.3 gives:

\[
\frac{d[B]}{dt} = k_1 \cdot A_0 \cdot e^{-k_1 \cdot t} - k_2 \cdot [B] \quad \text{Equation 3.8}
\]

Integrating equation 3.8 using an integrating factor, if \(B_0 = 0\), would give:

\[
[B] = \frac{A_0 \cdot k_1}{k_2 - k_1} \cdot (e^{-k_1 \cdot t} - e^{-k_2 \cdot t}) \quad \text{Equation 3.9}
\]

In the absence of \(B\) and \(P\) (initially at \(t = 0\)) the total reactant concentration must equal \(A_0\), then:

\[
[P] = A_0 - [A] - [B] \quad \text{Equation 3.10}
\]

So:

\[
[P] = A_0 \left[1 + \frac{1}{k_1 - k_2} \cdot (k_2 \cdot e^{-k_1 \cdot t} - k_1 \cdot e^{-k_2 \cdot t}) \right] \quad \text{Equation 3.11}
\]

Differentiation of Equation 3.11 gives:

\[
\frac{d[P]}{dt} = k_1 \cdot k_2 \cdot A_0 \cdot \left(\frac{e^{-k_1 \cdot t} - e^{-k_2 \cdot t}}{k_2 - k_1}\right) \quad \text{Equation 3.12}
\]

and hence the power associated with the reaction \(B \rightarrow P\), \(dq/dt\) is given by:

\[
\frac{dq_p}{dt} = k_1 \cdot k_2 \cdot \Delta H_2 \cdot A_0 \cdot \left(\frac{e^{-k_1 \cdot t} - e^{-k_2 \cdot t}}{k_2 - k_1}\right) \quad \text{Equation 3.13}
\]

The overall power-time data that would be observed for a reaction following a consecutive, two-step, first-order reaction scheme can be obtained by combining Equations 3.7 and 3.13:
Equation 3.14 is used to fit calorimetric power-time data recorded for a two-step consecutive reaction, when both the steps follow first-order kinetics.

### 3.3 Chemometrics

Analysing calorimetric data recorded for complex reactions such as the consecutive reactions (two-step or three-step) can be complicated and very time consuming, especially if exact mechanisms are not known. Fitting such complex data to increasingly complex kinetic models may also give rise to problems associated with many perfect fits, but returning different reaction parameters. However, only those fits which result in a low \( \chi^2 \) value (the program’s statistical measure of fit) and having the least number of variables should be considered.

Calorimetric power time data recorded from complex reactions are a sum of the powers of all individual processes occurring in the system. A newer approach to analyse such complex multivariate data sets is the application of chemometrics. Chemometrics is a data analytical methodology based on multivariate mathematical (or statistical) modelling and analysis of all data. Chemometric techniques are widespread and have various applications: collecting good data (optimisation of experimental parameters, calibration, signal processing) or extracting information from data (pattern recognition, modelling, statistics). Techniques such as principal component analysis (PCA), partial least squares (PLS) and multiple linear regression (MLR) are well established for spectroscopic data (Roggo et al., 2007).

Extracting information from data using chemometric techniques, involves pattern searching from a matrix of data (requires at least three variables) in a model-free way. These approaches have been used for the analysis of non-isothermal (scanning) calorimetric data, where changes in temperature during the course of the experiment bring about complexity in the data (Roduit, 2000; Brown et al., 2000; Maciejewski, 2000; Vyazovkin, 2000). These data matrix contains three variables (power, time and temperature) which can facilitate multivariate analysis. IC data has however not been

\[
\frac{dq_{obs}}{dt} = k_1 \cdot \Delta H_1 \cdot A_o \cdot e^{-k_1 \cdot t} + k_1 \cdot k_2 \cdot \Delta H_2 \cdot A_o \cdot \frac{e^{-k_1 \cdot t} - e^{-k_2 \cdot t}}{k_2 - k_1}
\]  

Equation 3.14
subjected to extensive chemometric analysis (model-free), because only recently has IC been actively used as a quantitative tool. The increasing use of isothermal calorimetric instruments in studying complex systems, commonly encountered in pharmaceuticals, has resulted in an urge to develop new techniques to analyse complex data sets.

### 3.3.1 Chemometric techniques in analysing isothermal calorimetric data

Chemometric techniques have been successfully applied to spectroscopic systems, mainly because of the generation of copious quantities of data (intensity and wave number) as a function of other variables such as pH, time and temperature. Since IC data consist of only two components (power and time), there is a need to generate a third variable component. The power-time data recorded by the calorimeter is a sum of all power outputs of each component reaction of the system, at any defined time. At any given temperature, each component of the complex system will exhibit a unique maximum, thereby suggesting that the data could be analysed via multivariate method provided a suitable matrix can be generated. Generating a third component with available calorimetric instruments is difficult and it is also not clear what form this third variable should be. Small differences in replicate runs of a system, caused by small differences in loaded sample mass is thought to cause appreciable variations in the data to permit a successful chemometric analysis (Gaisford and O'Neill, 2006). Data of this type has x, y and z property which describe row, column and intensity information respectively and is shown in Figure 3.2.

![Figure 3.2: Schematic data format suitable for deconvolution. P, power; S, sample mass; T, time (Gaisford and O'Neill, 2006).](image)
Calorimetric power-time data are univariate in nature resulting in $t$ time points and $P$ powers. The third variable, intensity $S$ can be engineered by obtaining replicate runs of the same sample and can be designed such that each varies from the other in the total power output. Variations in sample weighing or loading for repeated runs results in $S$ sets of runs. However, to create a suitable matrix $[P]$ that can be subjected to chemometric analysis would require $2X + 2$ replicate runs, where $X$ is the number of species that evolve after time $t$ for a system (Gaisford and O'Neill, 2006). This can be a problem when limited quantities of material are available as in a discovery laboratory.

Deconvoluted calorimetric data following chemometric analysis should represent the individual patterns of the processes occurring in the system. Hence, they should confirm to single-step kinetic equations depending on the number of reaction steps and in principle can be analysed for kinetic and thermodynamic reaction parameters through an iterative procedure. However, the deconvoluted data returned is in the form of intensity versus time and the relationship between intensity and power is not well understood. Hence, accurate thermodynamic information cannot be determined from the deconvoluted data. The shape of the data is however not altered and hence kinetic information can be obtained through analysis.

The aim of this study was to analyse a two-step consecutive reaction using isothermal calorimetry. Potassium hydroxylamine disulfonate that undergoes a two-step consecutive reaction would be synthesised and its degradation recorded in the calorimeter. The data obtained would be subjected to kinetic modelling using an iterative procedure in order to obtain kinetic and thermodynamic information for the individual step. Chemometric analysis of the recorded calorimetric data would help determine the number of steps involved in the reaction and the deconvoluted data should be representative of each reaction step. Fitting the deconvoluted data to single-step kinetic equations should return reaction parameters for the individual reaction enabling a comparison to be made with those obtained earlier.
3.4 Materials And Methods

3.4.1 Materials

Hydroxylamine-O-sulfonic acid (97%, lot S20376-244), glacial acetic acid (≥99.7%, lot 17628MB-144), potassium hydrogen carbonate (≥99.7%, lot 16422MB) and potassium nitrite (≥96%, lot 11721DC) were from Aldrich. Potassium hydroxide (85+, lot 0551602) was from Fisher Scientific. Barium chloride (≥99.9%, lot 1060345), perchloric acid and sulphur dioxide (≥99.9%, lot 1119688) were from Fluka. Hydrochloric acid (5 M, lot 0C534447) was from BDH.

All materials were used as received and all solutions were prepared using distilled deionised water.

3.4.2 Methods

3.4.2.1 Preparation of potassium hydroxylamine-NO-disulfonate

Potassium bicarbonate (22 g) was dissolved in an acidic solution of glacial acetic acid (15 ml glacial acetic acid in 40 ml deionised water). The resulting temperature of the solution rapidly dropped to approximately 0 °C, to which potassium nitrate (17 g) was dissolved, followed by addition of crushed ice (300 g) to the reaction mixture. A rapid stream of sulphur dioxide was passed through the solution and with constant and vigorous stirring, the low temperature (approximately 0 °C) was maintained. After all the ice had melted (20-30 min), the solution smelt faintly of sulphur dioxide and the white precipitate of potassium hydroxylamine-NO-disulfonate that formed was filtered off. The crystals were washed with water and methanol and then vacuum dried for 24 h.

3.4.2.2 Isothermal calorimetry

Experiments were conducted using a 2277 Thermal Activity Monitor at 40 °C. Sample solutions were prepared by dissolving either 0.0113 g (0.001 M) of hydroxylamine-O-sulfonic acid or 0.0216 g (0.001) of potassium hydroxylamine-NO-disulfonate in 100 ml of perchloric acid solution (0.0005 M). The time of mixing the solutions were noted. 3 ml
aliquots of the solution were filled into TAM ampoules, sealed and placed in the thermal equilibration position of the TAM (Section 1.7.3.3). All samples were repeated for a minimum of six times.

3.4.2.2.1 Data analysis

Data analysis was performed using Origin 7.0 (Microcal Software Inc., USA). The power-time data obtained for the hydrolysis of hydroxylamine-O-sulfonic acid were fitted to a single-step first-order kinetic model (Equation 3.7) while the hydrolysis of hydroxylamine-NO-disulfonate was fitted to a two-step consecutive reaction kinetic model (Equation 3.14) until a good fit was obtained. Value entered for \([A_0]\) 3 x 10^{-6} was known and therefore kept constant. Initial value entered for \(k\) was 1 x 10^{-4} s^{-1} and for \(\Delta H\) was 1 x 10^{10} \mu J mol^{-1} and were altered by the fitting process until a good fit to the data (indicated by a low \(\chi^2\) value) were obtained.

3.4.2.2 Chemometric analysis

To enable a chemometric analysis, 2X +2 calometric data sets would be required in order to generate a suitable matrix. The hydrolysis of potassium hydroxylamine disulfonate by a two-step consecutive reaction involves the generation of two species and hence six calorimetric data sets are required. The power-time data were used to create a suitable matrix from which factor analysis could be used to deduce the data matrix. This would allow for the determination of the number of reaction steps that contribute to the overall mechanism. The returned component heat outputs for each individual reaction steps were analysed for reaction parameters by fitting these single-step heat output data, to a single-step kinetic equations that describes each step of the consecutive reaction. This would enable a comparison of the reaction parameters obtained to those returned from an iterative fit of calorimetric data. Chemometric analysis was done by Dr. John Tetteh (University of Greenwich).
3.5 Results And Discussion

3.5.1 Hydrolysis of hydroxylamine-O-sulfonic acid

Initial calorimetric experiments were carried out on the hydrolysis of hydroxylamine-O-sulfonic acid. A typical power-time trace for the hydrolysis of 0.001 M hydroxylamine-O-sulfonic acid in 0.0005 M perchloric acid at 40 °C is shown in Figure 3.3. From the power-time plot, it is apparent that the hydrolytic reaction is an endothermic process as the magnitude of the power signal is negative. For a first-order process the ln(power)-time curve is linear and from the slope, the first-order rate constant is obtained. Since the power recorded by the calorimeter for an endothermic process is negative, the ln(power) value cannot be calculated. Inversing the power value (additive inverse) would enable a ln((inverse)power)-time curve to be plotted. For this discussion, additive inverse (calculated by multiplying by -1) is referred to simply as inverse and should not be confused with a multiplicative inverse (x^-1).

A plot of ln((inverse)power) versus time for the degradation of hydroxylamine-O-sulfonic acid was a straight line (Figure 3.4) which indicated that the reaction was first-order. The slope of the line would give the first-order rate constant, but the sign was reversed to correct for the inversed power value. Plots for the remaining data sets were linear and from the slopes an average first-order rate constant of 4.09 x 10^{-5} s^{-1} (S.D. ± 0.8 x 10^{-5}, n = 6) was obtained. There is no literature value for the degradation rates of hydroxylamine-O-sulfonic acid at 40 °C, however, Gaisford (1997) studied the degradation of potassium hydroxylamine trisulfonate in 0.0005 M perchloric acid at 35 °C; which degrades via a three-step consecutive reaction, the third step being the hydrolysis of hydroxylamine-O-sulfonate ion to hydroxylamine and hydrogen sulphate. Using ^{35}S-labelled compounds Candlin and Wilkins (1961) showed that the second and third hydrolytic reactions proceeded at similar rates. Gaisford, (1997) determined first-order rate constants of 4.8 x 10^{-5} and 2.3 x 10^{-4} s^{-1} for the second and third steps respectively. Results obtained from the slope of the ln((inverse)power)-time curve were slightly slower but compares well with those in the literature.
Figure 3.3: Power-time data for the hydrolysis of 0.001 M hydroxylamine-O-sulfonic acid in perchloric acid solution (0.0005 M) at 40 °C.

Figure 3.4: ln((inverse)power)-time data for the hydrolysis of 0.001 M hydroxylamine-O-sulfonic acid in perchloric acid solution (0.0005 M) at 40 °C and the fit line (—) generated by application of linear regression analysis.
Power-time data were fitted to Equation 3.7 using an iterative procedure (non-linear curve fitting tool in Origin 7.0) and the initial estimates for the reaction enthalpy and rate constants used were $1 \times 10^{10}$ $\mu$J mol$^{-1}$ and $1 \times 10^{-4}$ s$^{-1}$ respectively, while $[A_0]$ was set as constant at $3 \times 10^6$. The power-time data fitted well to a single step first-order kinetic model (Figure 3.5) and the average value for reaction enthalpy returned by the fitting process was 23.03 kJ mol$^{-1}$ (S.D. ± 3.87, n = 6). There is no literature value to enable a comparison for the enthalpy change at this temperature. Reaction enthalpies recorded by Gaisford (1997) indicated that the hydrolysis of hydroxylamine monosulfonate was exothermic when it was part of a three-step consecutive reaction. However, when the hydrolysis of hydroxylamine sulfonic acid was studied as a separate reaction it exhibited an endothermic property that indicates that they might behave differently when studied as part of a three-step consecutive reaction.

**Figure 3.5:** Power-time data for the hydrolysis of 0.001 M hydroxylamine-O-sulfonic acid in perchloric acid solution (0.0005 M) at 40 $^\circ$C and the fit line (°) generated by application of equation 3.7.
3.5.2 Hydrolysis of potassium hydroxylamine-NO-disulfonate

3.5.2.1 Yield and purity of potassium hydroxylamine-NO-disulfonate

After vacuum drying for 24 h, 9.3 g of white crystals of potassium hydroxylamine-NO-disulfonate was obtained. The purity of the sample was determined by nitrogen and sulphur analysis. The nitrogen content determined by CHN combustion analysis was 2.76% (expected N, 2.62%), while the sulphur content was determined by volumetric analysis. The disulfonate was allowed to hydrolyse completely in the presence of concentrated hydrochloric acid in a boiling water bath for 2 h, which resulted in the formation of the sulphate. This was measured by the addition of sufficient barium chloride to form a precipitate of barium sulphate. The sulphur content was determined to be 23.7% (expected S, 23.8%).

3.5.2.2 Calorimetric study of the hydrolysis of potassium hydroxylamine-NO-disulfonate

A typical power-time trace for the hydrolysis of potassium hydroxylamine disulfonate (0.001 M) in perchloric acid (0.0005 M) at 40 °C is shown in Figure 3.6. The data did not fit a single-step first-order kinetic model (Figure 3.6), thus indicating the likelihood of more that one event occurring in the system. However, since the mechanism of degradation was known, the power-time data were fitted to a two-step consecutive reaction model (Equation 3.14), which resulted in an excellent fit to the model (Figure 3.7). This shows the sensitivity of model fitting in distinguishing mechanistic information. If no prior knowledge of the reaction mechanism were available, then the ideal approach would be to systematically fit the data to a range of increasingly complex kinetic models until a satisfactory fit was obtained and select that which gives the best fit with the fewest variables (Gaisford et al., 1999). When fitting data to increasingly complex kinetic models, a large number of data sets may be obtained, each of which may appear to be equally valid; but data sets which give the lowest \( \chi^2 \) (the program’s statistical measure of fit) values should be chosen to determine reaction parameters. From the curve fitting procedure, reaction parameters were recovered for both the individual steps and are summarised in Table 3.2.
Figure 3.6: Power-time data for the hydrolysis of 0.001 M potassium hydroxylamine-NO-disulfonate in perchloric acid solution (0.0005 M) at 40 °C and the fit line (•) generated by application of single-step first-order kinetic model (Equation 3.7).

Figure 3.7: Power-time data for the hydrolysis of 0.001 M potassium hydroxylamine-NO-disulfonate in perchloric acid solution (0.0005 M) at 40 °C and the fit line (■) generated by application of a two-step first-order consecutive model (Equation 3.14).
Table 3.2: Average values for the rate constants and reaction enthalpies for the acid catalysed hydrolysis of potassium hydroxylamine disulfonate (0.001 M) at 40 °C, determined by fitting experimental data to Equation 3.14.

<table>
<thead>
<tr>
<th>$k_1$ (s$^{-1}$)</th>
<th>$\Delta H_1$ (kJ mol$^{-1}$)</th>
<th>$k_2$ (s$^{-1}$)</th>
<th>$\Delta H_2$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>1.16 x 10$^{-4}$</td>
<td>$-95.06$</td>
<td>2.01 x 10$^{-4}$</td>
</tr>
<tr>
<td>± S.D.</td>
<td>$\pm 0.47 x 10^{-4}$</td>
<td>$\pm 14.19$</td>
<td>$\pm 0.64 x 10^{-4}$</td>
</tr>
</tbody>
</table>

The power-time data fitted well to a two-step first-order reaction scheme ($\chi^2 = 0.0008$; fit to Equation 3.7, $\chi^2 = 0.006$) and returned rate constants of $1.16 \times 10^{-4}$ and $2.01 \times 10^{-4}$ s$^{-1}$ for the first and second hydrolytic steps respectively. The rate constant for the second step of the consecutive reaction (hydrolysis of hydroxylamine monosulfonate ion) was slightly faster than when studied as a single step first-order process ($4.09 \times 10^{-5}$ s$^{-1}$). Gaisford (1997) determined the rate constants to be $4.8 \times 10^{-5}$ and $2.3 \times 10^{-4}$ s$^{-1}$ at 35 °C for the two steps, being step two and three of a three-step consecutive reaction (hydrolysis of potassium hydroxylamine trisulfonate). Although the value recorded were those at a lower temperature, they compare well. This indicates that reactions behave differently when it is part of a wider reaction scheme (in this case a two-step consecutive scheme) than when studied as an individual reaction.

Fitting calorimetric data to a two-step first-order consecutive kinetic model also allowed for the recovery of reaction enthalpies for both individual steps. The signs for the reaction enthalpy values returned from the fitting process were inverted, to correct for the way the calorimeter records data. The reaction enthalpies obtained immediately suggest that both the reaction steps proceed with different heat changes. The first step was exothermic and has a negative reaction enthalpy ($-95.06$ kJ mol$^{-1}$) while the second step is endothermic (28.96 kJ mol$^{-1}$). The reaction enthalpy for the second step was consistent with the endothermic nature of the hydrolysis of hydroxylamine sulfonic acid ($\Delta H = 23.03$ kJ mol$^{-1}$) obtained earlier. Values of reaction enthalpy obtained for the first step (Gaisford, 1997) were -82.2, -33.5 and 122.9 kJ mol$^{-1}$ at 25, 30 and 35 °C respectively and were stated to vary with temperature.

3.5.2.3 Chemometric analysis of calorimetric data

A minimum of six calorimetric power-time data sets for the hydrolysis of potassium hydroxylamine disulfonate were recorded. This enabled the generation of a suitable data
matrix, which was subjected to factor analysis to deconvolute the data. Factor analysis was used on the assumption that the power signal at any time $t$ represents the total power of all species present at that moment and is proportional to the sample load in the calorimeter (Gaisford and O’Neill, 2006). Chemometric analysis of the raw power data showed the presence of two individual reaction steps. Figure 3.8 shows the deconvoluted heat outputs for each reaction step. It is evident that Factor 1 had a much higher positive heat output signal and represents an exothermic process. The initial heat output for Factor 2 was negative which increased as the reaction progressed, indicating the likelihood of an endothermic event.

![Figure 3.8: Deconvoluted power-time trends for the two individual steps in the hydrolysis of potassium hydroxylamine disulfonate obtained from chemometric analysis. Factor 1 and 2 represent steps A→B and B→P respectively in the two-step consecutive reaction.](image)

Chemometric analysis is a ‘model-free’ approach of data analysis and the only information presented to the software is the overall calorimetric power-time data for each of the repeats. Following chemometric analysis, the individual ‘patterns’ recovered, should be representative of each step of the overall reaction. They should then confirm to the single-step kinetic equations from which kinetic information can be obtained. Factors 1 and 2 (Figure 3.8) represent the first (A→B) and second (B→P) steps respectively of the two-step consecutive reaction, the hydrolysis of potassium hydroxylamine disulfonate.
Chapter 3 - Consecutive reaction

Factor 1 (hydrolysis of hydroxylamine disulfonate to hydroxylamine monosulfonate) should confirm to single-step first-order kinetics (Equation 3.7), while factor 2 which represents the hydrolysis of hydroxylamine monosulfonate to hydroxylamine should be better fitted by Equation 3.13.

Factor 1 represents the hydrolysis of hydroxylamine disulfonate to hydroxylamine monosulfonate. A log plot of Factor 1 (Figure 3.9) yielded a straight line, the slope of which gave a first-order rate constant of $1.63 \times 10^{4} \text{ s}^{-1}$. This was similar (within error limits) to that calculated from calorimetric power-time data analysis ($1.16 \times 10^{4} \text{ s}^{-1}$) through model-fitting to a two-step consecutive reaction scheme. Factor 1 data was fitted to Equation 3.7 with the initial estimates of $k$ ($1 \times 10^{4} \text{ s}^{-1}$) and $\Delta H$ ($1 \times 10^{10} \text{ J mol}^{-1}$) allowed to vary, while $[A_0]$ ($3 \times 10^{6}$) was kept constant. The iteration resulted in a good fit (Figure 3.9), but value for reaction enthalpy (-266.1 kJ mol$^{-1}$) returned, was higher than those obtained earlier (-95.06 kJ mol$^{-1}$). This is in accordance to that stated earlier that accurate thermodynamic information cannot be obtained from deconvoluted data because of the lack in clarity of the relation between intensity and power.

![Figure 3.9: Deconvoluted data representing step one (Factor 1) of the hydrolysis of hydroxylamine disulfonate following chemometric analysis and the fit line (- -) generated by application of Equation 3.7. L represents the ln(Intensity) data and the fit line (- -) obtained by application of linear regression analysis.](image-url)
Factor 2 in Figure 3.8 represents the hydrolysis of hydroxylamine monosulfonate to hydroxylamine. The data were therefore fitted to Equation 3.13, which describes the power-time data for the second step of a two-step consecutive reaction (Figure 3.10). The initial estimate for $[A_0] \times 10^6$ was kept constant while initial values for the rate constants and $\Delta H_2$ were set as $1 \times 10^{-4}$ s$^{-1}$ and $1 \times 10^{10}$ μJ mol$^{-1}$ respectively and were allowed to vary until a good fit was obtained. The first-order rate constant for the second step, returned by the fitting process was $8 \times 10^5$ s$^{-1}$ with an enthalpy change value of 16.74 kJ mol$^{-1}$. Although a comparison of the enthalpy values obtained cannot be made due to lack of knowledge of the relation between power and intensity, the rate constants obtained were slower than those obtained when calorimetric power-time data were subjected to model fitting. Although the difference in the rate constants obtained was small, the fact that the power contributed by the second step of the reaction was small and significant contributions of baseline noise could influence the chemometric analysis. The values of $k_2$ returned were the same even if $k_1$ was kept constant during the iterative procedure.

**Figure 3.10:** Deconvoluted data representing step two of the hydrolysis of hydroxylamine disulfonate following chemometric analysis and the fit line (°) generated by application of equation 3.13.
Simulated power-time data that represents the hydrolysis of hydroxylamine disulfonate were generated using a mathematical worksheet (MathCad® 2001) and it is possible to deconvolute the power-time curves for such complex reactions into the individual reactions. Figure 3.11 shows the curves representing the two individual hydrolytic steps constructed by entering values of reaction parameters obtained from the fitting process (Table 3.2). The overall power signals, represents the sum of the powers for each individual reaction and was lower than the power signal for the first hydrolytic step.

The power value for the first step was significantly high while that for the second step was comparatively lower. Rate constants for both steps are fast and hence the duration of the hydrolysis is relatively shorter than a reaction with a comparatively slower rate (Refer Chapter 4). From the simulated power-time curve for the second step, it is evident that the magnitude of power signal reaches below 1 μW within 6 h, at which point the instrumental noise could contribute significantly to the net observed power. Hence, at such low magnitudes of power the recorded values are not entirely contributed by the reaction under consideration, which could explain the deviation in the rate constants recovered from fitting deconvoluted chemometric data.
3.6 Conclusions

It has been shown in this chapter that IC can be used to study complex degradation reactions occurring in solutions. The analysis of complex calorimetric data, as obtained from a two-step consecutive reaction can be successfully analysed through model fitting, provided suitable kinetic equations can be written. This invariably requires knowledge of reaction mechanisms and the need to impose a model to the system. In the absence of any such information, the ideal approach is to fit calorimetric data to increasingly complex kinetic models and select that which gives the best fit with least variables.

Analysis of the calorimetric data obtained for the hydrolysis of potassium hydroxylamine disulfonate through model fitting, allowed for the recovery of reaction parameters for each individual reaction step. Kinetic model fitting was sensitive enough to distinguish between a single-step and two-step consecutive reactions. Although the rate constants obtained for the two steps were almost identical in magnitude, the model successfully resolved the processes. This was due to the large difference in reaction enthalpies for the two hydrolytic steps.

Model fitting can be time-consuming especially when more complex reaction schemes are involved. A newer model-free approach towards complex data of this nature is the application of chemometric analysis. Chemometric analysis of IC data is relatively new and still in its infancy. The need to generate large number of repeats to substitute for the third variable (to generate a suitable matrix) can be challenging especially in research involving new drugs with limited quantity. Moreover, the relation between the recovered intensity and original power components needs to be understood. However, the use of chemometric techniques to determine the number of reaction steps occurring in a reaction scheme is greatly advantageous. Successful deconvolution produces individual patterns that contributed towards the overall process. From these individual patterns, reaction kinetics were obtained, which were similar to those obtained through model fitting.

Calorimetry does not provide any molecular information about the processes being investigated and additional studies involving other techniques are essential to obtain such information. Although possible mechanistic information can be deduced from model fitting (provided suitable model equations can be written), in case of complex reactions it is time consuming and requires additional confirmation using ancillary studies. Application of chemometric techniques to complex calorimetric data is clearly
advantageous especially when reaction mechanisms are unknown. It can successfully deconvolute complex calorimetric data (recorded when multiple reactions occur at a given time) into the individual contributions in a model-free way, hence throwing light on the number of processes occurring. Hence, it aids the process of model fitting of calorimetric data when reaction mechanisms are unknown and negates the need to fit many kinetic models.

The use of IC in studying simple to increasingly complex (parallel and consecutive) reactions was demonstrated. The reactions involved were relatively fast ($1 \times 10^{-4}$ to $1 \times 10^{-6}$ s$^{-1}$) and had a high reaction enthalpy, which generated power signals that were relatively high and easily analysable. However, studying slower reactions with small enthalpy changes and consequently low power signals can be a tricky situation and needs to be scrutinized. The next chapter deals with the issues encountered when studying slow reactions in the calorimeter.
Chapter 4

THEORETICAL LIMITS
4.1 Introduction

In chapters 2 and 3 the degradation of API’s in solutions that followed simple to increasing complex kinetics had been studied. The reaction kinetics were first-order and systems studied were single-step degradations followed by parallel and later two-step consecutive reaction. The reactions had a medium rate and a moderate enthalpy change and the degradations were nearly completed in a day. Degradations commonly occurring in pharmaceuticals are however much slower and considerably difficult to study. This chapter aims to deal with such slow reactions, common in pharmaceuticals and emphasises the issues encountered when studying slow reactions with small enthalpy change using IC. Fast and medium rate reactions generally have a relatively higher calorimetric power signal compared to degradation reactions having slower kinetics.

Pharmaceuticals are formulated to be stable products for the duration of their shelf life, typically five years, during which the potency of the API should not fall below 90% at the recommended storage conditions and the product should possess the same therapeutic effect it had when originally manufactured (Carstensen, 2000). Stability in the final product is enhanced by a category of excipients called preservatives that ensures the integrity of the drug and the product. In spite of this, many drugs are susceptible to some form of chemical degradation. The rate at which degradation occurs is of considerable importance in determining whether such reactions can be successfully studied in the calorimeter.

Almost all reactions are accompanied with a change in heat content or enthalpy and can in principle be studied using IC. The sensitivity of modern calorimeters is such that, in principle, measurements for a range of reaction rates (from 1% s\(^{-1}\) to 0.03 % year\(^{-1}\)) can be studied. A slow reaction with a first-order rate constant of \(1 \times 10^{-11} \text{ s}^{-1}\) (corresponding to 0.03 % degradation per year) can in principle be detected directly at 25 °C (Willson, 1995). The rate of a chemical reaction has been classified as fast, medium or slow depending on the percentage of reaction that has occurred over a specific time interval. A slow reaction is considered to have a reaction rate between 3% and 0.03 % reaction per year, corresponding to a first-order rate constant of \(1 \times 10^{-9}\) and \(1 \times 10^{-11} \text{ s}^{-1}\). A medium rate reaction has a reaction rate between 3.5% per hour and 2.4% per month and corresponds to a first-order rate constant of \(1 \times 10^{-5}\) and \(1 \times 10^{-9} \text{ s}^{-1}\). While a fast reaction has a reaction rate between less that 1% per second to 30% an hour (Willson, 1995) and are summarised in Table 4.1.
Table 4.1: Comparison of rate of chemical reaction to the first-order rate constants (Willson, 1995).

<table>
<thead>
<tr>
<th>Rate</th>
<th>% Reaction rate</th>
<th>Half life</th>
<th>First order rate constant (s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td>&lt;1% s⁻¹</td>
<td>69 s</td>
<td>1 x 10²</td>
</tr>
<tr>
<td></td>
<td>1% s⁻¹</td>
<td>693 s</td>
<td>1 x 10³</td>
</tr>
<tr>
<td></td>
<td>30% hour⁻¹</td>
<td>1.9 h</td>
<td>1 x 10⁴</td>
</tr>
<tr>
<td></td>
<td>3.5% hour⁻¹</td>
<td>19.25 h</td>
<td>1 x 10⁵</td>
</tr>
<tr>
<td>Medium</td>
<td>8% day⁻¹</td>
<td>8 days</td>
<td>1 x 10⁶</td>
</tr>
<tr>
<td></td>
<td>5.8% week⁻¹</td>
<td>11.5 weeks</td>
<td>1 x 10⁷</td>
</tr>
<tr>
<td></td>
<td>2.4% month⁻¹</td>
<td>2.2 years</td>
<td>1 x 10⁸</td>
</tr>
<tr>
<td></td>
<td>3% year⁻¹</td>
<td>22 years</td>
<td>1 x 10⁹</td>
</tr>
<tr>
<td>Slow</td>
<td>0.3% year⁻¹</td>
<td>222 years</td>
<td>1 x 10¹⁰</td>
</tr>
<tr>
<td></td>
<td>0.03% year⁻¹</td>
<td>2207 years</td>
<td>1 x 10¹¹</td>
</tr>
</tbody>
</table>

Theoretically, reactions having a first-order rate constant of $1 \times 10^{11}$ s⁻¹ corresponding to a half life of 2207 years should be easily detected directly at 25 °C in the TAM with just 50 h of data (Willson, 1995; Beezer et al., 1998). The dearth in current literature of any experimental data of this magnitude, could suggest that similar slow reactions have not been studied calorimetrically or else the calorimeter is not sensitive enough under certain reaction conditions.

Modern calorimeters despite their high sensitivity, should have limits of detectivity beyond which measurements are either not possible or the data obtained could be flawed. For a heat conduction calorimeter these boundaries of detection are limited to the sensitivity of the instrument to record very minute changes in thermal events that occur when a system undergoes change (physical or chemical). In case of a chemical change (such as degradations in API's), the detection limits are pertinent to the reaction parameters (rate, enthalpy change, order and amount of reactant that can react) for that reaction.

Calorimetric data recorded in the TAM is in the form of power (μW) versus time (s). When the degradation is first-order the calorimetric power signal recorded for a reaction
under study, is a function of the enthalpy change ($\Delta H$), the rate constant ($k$) and the initial amount of material that can react ($A_0$). For a system undergoing solution phase single-step first-order degradation kinetics, the power-time response recorded by the TAM can be described by Equation 4.1 (Bakri et al., 1988),

$$\text{Power} = \frac{dq}{dt} = \Delta H \cdot v \cdot k \cdot [A_0] \cdot e^{kt}$$  \hspace{1cm} \text{Equation 4.1}

The magnitude of the power signal recorded by the calorimeter is a function of the change in reaction enthalpy, rate constant, initial quantity of reactant that can react and the order of the reaction. Variations among these parameters determine the duration and size of the calorimetric power signal. The rate constant for a chemical degradation is temperature dependent while the enthalpy change is independent. Theoretical limits can be set for the reaction parameters based on an acceptable magnitude of power signal (at a particular time) below which, instrumental parameters such as baseline noise contributes significantly to the calorimetric power signal. For the following discussions solution phase degradation following first-order kinetics is considered.

In solution phase degradations following first-order kinetics, the reaction enthalpy and rate constants are specific to the system, whereas the initial concentration of reactant can be varied up to its maximum solubility in a given solvent system. The reaction enthalpy for the system undergoing degradation is constant while the rate constant is temperature dependant. Degradation reactions are accompanied with a net change in enthalpy which can be small or large. Similarly, rate constants can be slow or fast. The power signal generated during degradation can be scaled depending on the magnitude of the reaction parameters and are summarised in Table 4.2.

<table>
<thead>
<tr>
<th>Reaction rate ($k$ s$^{-1}$)</th>
<th>Enthalpy change ($\Delta H$ kJ mol$^{-1}$)</th>
<th>Thermal power signal (P $\mu$W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow</td>
<td>Small</td>
<td>Small</td>
</tr>
<tr>
<td>Slow</td>
<td>Large</td>
<td>Moderate</td>
</tr>
<tr>
<td>Fast</td>
<td>Small</td>
<td>Moderate</td>
</tr>
<tr>
<td>Fast</td>
<td>Large</td>
<td>Large</td>
</tr>
</tbody>
</table>

Table 4.2: Interdependence of power signal with reaction rate and reaction enthalpy.
In the above table, a slow reaction is considered to have a rate constant between $1 \times 10^9$ and $1 \times 10^{11}$ s$^{-1}$ while a fast reaction between $1 \times 10^2$ and $1 \times 10^4$ s$^{-1}$. Lower enthalpy change values can be attributed to hydrolytic bond cleavage while higher values are indicative of oxidative reactions. The power signal can be classified as small, moderate or large depending on the value observed (after initial thermal equilibration) and its deviation, relative to the baseline.

4.1.1 TAM detection limits

The principle, working and importance of calibration of the TAM have been discussed in detail in Sections 1.7.2 and 1.7.3.1. The sensitivity and performance of the TAM is greatly influenced by its immediate surroundings, being very sensitive to fluctuations in the external room temperature. The manufacturer's (Thermometric AB, Järfälla, Sweden) optimal conditions for operating the TAM at temperatures above 12 °C, requires that it be housed in a temperature-controlled environment (20 – 30 °C) and maintained constant (within ±1.0 °C) for best performance. It should be placed away from room radiators or air conditioning ducts and from excessive light and shade conditions such as near windows that would reduce its optimum performance.

For a TAM fitted with a 4 ml, Twin Ampoule Calorimetric Unit (2277-201) and the Standard Amplifier Module (2277-101), the manufacturers (TAM Thermometric AB, Järfälla, Sweden) stated technical performance specifications are given in Table 4.3.

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<th>Performance specifications</th>
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</thead>
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<tr>
<td>Limit of detectability</td>
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<td>Precision</td>
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<td>300 μW$^\dagger$ 0.1 %</td>
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<td>Baseline stability$^\S$</td>
<td>±0.2 μW</td>
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Table 4.3: Technical specifications for a 4 ml twin ampoule calorimetric unit (TAM 2277-201) (Appendix 2, 2277 TAM Instruction Manuel).

$^\dagger$ Based on the standard deviation obtained with 5 repeated electrical calibrations.

$^\S$ Refers to the mean value over 8 hours.
Chapter 4 – Theoretical limits

The detection limit for the 2277 TAM as stated by Thermometric is 0.15 μW, however when housed in a temperature-controlled environment (21 °C ± 0.1 °C) an improved reproducible detection limit of 0.05 μW was recorded (Willson, 1995). The short-term baseline noise is less than ± 0.2 μW while the drift (long-term baseline stability) of the instrument measured over 24 hours should also be less than ± 0.2 μW/24 h when equipped with the standard amplifier and less than ± 0.1 μW/24 h when equipped with a nano amplifier (Thermometric, Experimental and technical note EN 016). The precision when using a 30 μW amplifier setting is 0.5 % which corresponds to 0.15 μW whereas if a 300 μW amplifier setting was used, the precision is 0.1 % (0.3 μW). Summing up the values of the short-term and long-term baseline stability noise and the precision data, a value of 0.55 μW and 0.7 μW for the 30 and 300 μW amplifier settings respectively were calculated. These values are representative of a worst-case scenario and significantly lower deviations would be observed if a temperature-controlled environment is maintained (constant to ± 0.1 °C) as shown by Willson (1995). The total noise would be significantly lower if a nano amplifier is used instead of the standard amplifier. In the following discussions a power signal below 0.5 μW is considered to be contributed mainly by instrumental noise and is therefore used as a baseline value. Moreover ln(power) values at this magnitude is inherently noisy and is shown in the experimental section later in the chapter.

In any stability study, time and accuracy is of the essence and while performance of the calorimeter can be tremendously improved by rigidly controlled conditions of temperature and humidity fluctuations, short experimental time is desirable. Calorimetric observation times needed for slower degradation are much longer compared to a faster degradation (typically 50 h to distinguish between a first-order rate constant of $1 \times 10^{-10} \text{s}^{-1}$ and $2 \times 10^{-10} \text{s}^{-1}$ Willson, (1995)). In case of pharmaceuticals, it is essential to predict long-term stability, and measuring small changes (chemical and physical) over short experimental time is desirable. It is possible to study a fast or medium rate first-order degradation reaction (Table 4.1) for the entire life of the reaction, provided majority of the reaction does not occur during the time required for sample preparation and thermal equilibration in the TAM. Using reaction rates (Table 4.1), an enthalpy change of 50 kJ mol$^{-1}$ (reasonable for a first-order chemical degradation) and an initial concentration of 0.01 M, power-time data that depicts first-order degradation (Equation 4.1) were generated (Mathcad® 2001) and are shown in Figures 4.1 to 4.3.
Figure 4.1: Simulated power-time data generated for a solution phase first-order reaction (fast) using Equation 4.1, with fixed enthalpy change and initial concentration and different rate constant.

Figure 4.2: Simulated power-time data generated for a solution phase first-order reaction (medium rate) using Equation 4.1, with fixed enthalpy change and initial concentration and different rate constant.
From the simulated power-time graph in Figure 4.1, reactions having a first-order rate constant of $1 \times 10^{-2}$ or $1 \times 10^{-3}$ s$^{-1}$ have high initial power values that rapidly reduce to zero. Since the reaction would be completed within a couple of hours, which is the time when no meaningful data is recorded (sample preparation and thermal equilibrium); reactions with similar rates are potentially difficult to study using IC. Reactions with first-order rate constants between $1 \times 10^{-4}$ to $1 \times 10^{-6}$ s$^{-1}$ (Figure 4.1 and 4.2) have power values significantly higher than the detection limit of the calorimeter and can be studied using the calorimeter. As the reaction rate gets slower the magnitude of the power signal reduces significantly and remains flat. The simulated power-time curves for slow reactions ($1 \times 10^{-9}$ to $1 \times 10^{-11}$ s$^{-1}$, Figure 4.3) are below the detection limit of the calorimeter and can be difficult to study using IC. Reaction parameters used for generating power-time data are entirely reasonable for a chemical degradation.

The enthalpy change for a reaction is unique for a particular system while the rate of reaction is temperature dependent, but the initial amount of reactant used can be predetermined depending on its solubility properties. Therefore, there arises the need for a database of reaction parameters, from which the likelihood of successfully studying a reaction with similar parameters in the TAM can be made. Creating such a table requires
the generation of large number of data plots with a range of reaction parameters that
would give a power signal above the detectable limit of the TAM.

The aims of this chapter were, first to generate a matrix with various combinations of
reaction parameters (rate constant, enthalpy change and amount of material) that would
teoretically generate a power signal value greater than the detection limit of the TAM.
The second was to test the practical applicability of such matrix tables in designing
calorimetric experiments, by studying degradation reactions using reaction parameters
from these tables. The degradation kinetics of Trimebutine maleate and Rifampicin in
aqueous solution was carried out with the help of reaction parameters in Tables 4.4 – 4.6.

4.2 Determination Of Minimum Reaction Parameters Required For Feasible TAM Study.

Power-time data were generated using Mathcad® 2001 with various values for reaction
parameters. The power-time data generated depicted a first-order solution phase reaction,
described by Equation 4.1. Values for enthalpy change ($\Delta H$, kJ mol$^{-1}$), initial
concentration ($[A_0]$ (expressed as $M$, mol/L) and volume of solution in the ampoule
($v$, 3 ml) were kept constant while the rate constant ($k$, s$^{-1}$) was altered until a power signal
($dQ/dt$) of 0.5 $\mu$W at $t = 24$ h was obtained. It should be noted that for each combination of
reaction enthalpy and initial concentration there are two values of rate constants that can
be generated, both giving a power signal value of 0.5 $\mu$W at $t = 24$ h. The reaction with
the higher rate constant had a large initial power signal which reduced rapidly whereas the
slower reaction had a power signal that was flat. Figure 4.4 depicts a plot for the two
possible first-order rate constants both having a power signal value of 0.5 $\mu$W at $t = 24$ h
for a fixed combination of enthalpy change (50 kJ mol$^{-1}$) and initial concentration
(0.05 M).
Figure 4.4: Simulated power-time data for a solution phase first-order reaction having different magnitudes of reaction rates but same enthalpy change (50 kJ mol\(^{-1}\)) and initial concentration of reactant (0.05 M) both reaching a power signal value of 0.5 \(\mu\text{W}\) (at \(t = 24\) h).

The lower value of rate constant was used to predict the theoretical minimum rate that is desired in order to obtain a detectable power signal value of 0.5 \(\mu\text{W}\) (at \(t = 24\) h) for a particular combination of reaction parameters. A matrix and three-dimensional plots of the theoretical minimum value of rate constants desired for a given combination of reaction enthalpy and initial concentration of reactant are shown in Tables 4.4 to 4.6 and Figures 4.5 to 4.7.
Table 4.4: Matrix for the theoretical minimum value of first-order rate constants (s⁻¹) for a solution phase reaction for a given combination of enthalpy change ($\Delta H$) and initial concentration of reactant that can react ($[A_0]$ = 0.001 – 0.01 M) that is required in order to obtain a theoretical calorimetric power signal of 0.5 pW at $t = 24$ h.
Figure 4.5: A three-dimensional representation of the minimum value of rate constant desired to obtain an analysable calorimetric power signal, for a given combination of enthalpy change (10 - 200 kJ mol⁻¹) and initial concentration of reactant (0.001 - 0.01 M). Refer Table 4.4 for numeric values for corresponding combination.
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Table 4.5: Matrix for the theoretical minimum value of first-order rate constants (s$^{-1}$) for a solution phase reaction for a given combination of enthalpy change ($\Delta H$) and initial concentration of reactant that can react ([$A_o$] = 0.01 – 0.1 M) that is required in order to obtain a theoretical calorimetric power signal of 0.5 µW at $t = 24$ h.
Figure 4.6: A three-dimensional representation of the minimum value of rate constant desired to obtain an analysable calorimetric power signal, for a given combination of enthalpy change (10 – 200 kJ mol⁻¹) and initial concentration of reactant (0.01 – 0.1 M). Refer Table 4.5 for numeric values for corresponding combination.
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<td>3.32E-09</td>
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<td>1.99E-09</td>
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<td>1.06E-09</td>
<td>9.41E-10</td>
<td>8.48E-10</td>
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</table>

Table 4.6: Matrix for the theoretical minimum value of first-order rate constants (s⁻¹) for a solution phase reaction for a given combination of enthalpy change (ΔH) and initial concentration of reactant that can react ([Aₕ] = 0.1 – 1.0 M) that is required in order to obtain a theoretical calorimetric power signal of 0.5 μW at t = 24 h.
Figure 4.7: A three-dimensional representation of the minimum value of rate constant desired to obtain an analyzable calorimetric power signal, for a given combination of enthalpy change ($10^{-200}$ kJ mol$^{-1}$) and initial concentration of reactant ($0.1$ – $1.0$ M). Refer Table 4.6 for numeric values for corresponding combination.
Tables 4.4 – 4.6 lists the theoretical minimum values for the desired first-order rate constants for a slow reaction to be successfully studied in the TAM with corresponding enthalpy change values. These values are applicable for both exothermic and endothermic reactions. The rate constants should be used as a guide to determine whether the system degrading with first-order kinetics and known enthalpy change can be studied in the TAM. It has been stated that in principle, 50 h of calorimetric power-time data is sufficient to study slow reactions and discriminate between the first-order rate constants of $1 \times 10^{-11}$ and $2 \times 10^{-11}$ s$^{-1}$ (Willson, 1995). It is however desirable to obtain reaction parameters for pharmaceutical degradations in the shortest possible time, in order to predict long-term stability. For reactions having a medium rate of reaction, 24 h of calorimetric data is sufficient in order to determine reaction parameters. In Chapter 2 it was shown that much lesser time was required to successfully obtain reaction parameters through kinetic model fitting.

The reaction parameter values in Tables 4.4 – 4.6 can be used as an aid in designing an IC experiment. If the rate constant for a degrading system is known and the theoretical enthalpy change for the process calculated (from gas phase bond energies), then the desired initial concentration of reactant required to obtain an analysable calorimetric signal can be determined from these tables. This is helpful when limited sample amounts are available thereby reducing the number of experiments needed, to determine the optimum concentration. The added advantage is that if degradation rate constants of a reaction at higher temperatures are available, then the feasibility of successfully studying the reaction at a different temperature in the TAM can be made (determining rate constant at experimental temperature using an Arrhenius relationship).

The following section is a discussion of some of the solution-phase reaction systems, which degrade by first-order kinetics that have been successfully studied in the TAM. That is followed by reactions that have been studied using other analytical methods and a comparison is made as to whether they can be effectively studied in the TAM. The rate constants obtained from other techniques were used as basis to design the calorimetric experiment thus showing the advantage of having such tables with rate constants, enthalpy change values and initial concentration.
4.3 Reactions Studied In The TAM

Modern calorimeters are highly sensitive and when housed in a temperature-controlled environment it has been stated that, in principle, reactions with a first-order rate constant of $1 \times 10^{11}$ s$^{-1}$ can be studied calorimetrically at 25 °C. Many API's have been subjected to calorimetric investigation, but majority of systems studied in solution phase have a first-order rate constant in the magnitude between $10^{-4}$ to $10^{-7}$ s$^{-1}$. This section details some of the solution phase reactions studied in the TAM which follow first-order kinetics and are recorded in the literature.

4.3.1 Ampicillin in aqueous solution

The degradation of ampicillin in an aqueous solution as a function of pH and temperature has been studied in the TAM (Oliyai and Lindenbaum, 1991). The pseudo first-order rate constants were calculated from slope of the ln(power) versus time curves and are summarised in Table 4.7.

<table>
<thead>
<tr>
<th>Temp/pH</th>
<th>$k$ (s$^{-1}$)</th>
<th>$\Delta H$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>$6.39 \times 10^{-6}$ (± 0.2)</td>
<td>103 (± 4)</td>
</tr>
<tr>
<td>3.0</td>
<td>$5.00 \times 10^{-6}$ (± 0.08)</td>
<td>84 (± 3)</td>
</tr>
<tr>
<td>7.0</td>
<td>$2.06 \times 10^{-6}$ (± 0.08)</td>
<td>97 (± 5)</td>
</tr>
<tr>
<td>8.0</td>
<td>$3.33 \times 10^{-6}$ (± 0.05)</td>
<td>108 (± 2)</td>
</tr>
<tr>
<td>37 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>$1.81 \times 10^{-5}$ (± 0.02)</td>
<td>85 (± 4)</td>
</tr>
<tr>
<td>3.0</td>
<td>$1.42 \times 10^{-5}$ (± 0.08)</td>
<td>101 (± 2)</td>
</tr>
<tr>
<td>7.0</td>
<td>$7.78 \times 10^{-6}$ (± 0.1)</td>
<td>96 (± 1)</td>
</tr>
<tr>
<td>8.0</td>
<td>$1.06 \times 10^{-5}$ (± 0.08)</td>
<td>104 (± 4)</td>
</tr>
<tr>
<td>50 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>$6.06 \times 10^{-5}$ (± 0.2)</td>
<td>97 (± 6)</td>
</tr>
<tr>
<td>3.0</td>
<td>$4.08 \times 10^{-5}$ (± 0.1)</td>
<td>73 (± 4)</td>
</tr>
<tr>
<td>7.0</td>
<td>$1.39 \times 10^{-5}$ (± 0.02)</td>
<td>88 (± 2)</td>
</tr>
<tr>
<td>8.0</td>
<td>$3.25 \times 10^{-5}$ (± 0.08)</td>
<td>63 (± 2)</td>
</tr>
</tbody>
</table>

Table 4.7: Observed rate constants and reaction enthalpies for the degradation of ampicillin in aqueous solution as a function of temperature and pH (Oliyai and Lindenbaum, 1991).
At 25 °C and pH 7.0, a first-order rate constant of $2.06 \times 10^{-6}$ s$^{-1}$ and reaction enthalpy of 97 kJ mol$^{-1}$ is recorded with an initial concentration of 0.0053 M ampicillin. The rate constant is above the minimum desirable value corresponding to an enthalpy change of 100 kJ mol$^{-1}$ and initial concentration of 0.005 M (Table 4.4) and hence would generate a power signal above the theoretical analysable limit of 0.05 μW. Similarly, the first-order rate constants for the hydrolytic degradation of ampicillin in solution at other temperature and pH values, (Oliyai and Lindenbaum, 1991) were higher than the theoretical analysable limit.

### 4.3.2 Meclofenoxate

The degradation of meclofenoxate hydrochloride (MF) in pH 2.9 and 6.4 buffers at 25 °C was studied using the TAM and HPLC, which enabled a comparison of the derived rate constants to be made (Otsuka et al., 1994). MF is hydrolysed at its ester bond in aqueous solution and follows first-order kinetics. The first-order rate constants calculated from the TAM data were $1.14 \times 10^{-4}$ and $9.7 \times 10^{-7}$ s$^{-1}$ for hydrolysis in pH buffers 6.4 and 2.9 respectively. An enthalpy change value of -7.4 kJ mol$^{-1}$ was recorded from the TAM study carried out at 45 °C.

In pH 6.4 buffer, the initial amount of MF used to study its hydrolysis in the TAM at 25 °C was 1.0 mg/ml, corresponding to a concentration of 0.0034 M. There is no value for the theoretical minimum rate constant corresponding to these values of enthalpy change and concentration in Table 4.4. This is because the reaction was fast (first-order rate constant of $1.14 \times 10^{-4}$ s$^{-1}$) and the power signal value dropped to the baseline before 24 h. For fast reactions of this magnitude, the power signal is considerably high and a successful analysis can only be done if sufficient calorimetric data for the reaction can be recorded (equivalent to at least three half-lives). Furthermore, rate constants with similar magnitude have been reported earlier (Section 2.3.3.1) and only 15 min of calorimetric data (following equilibration) was sufficient to recover accurate reaction parameters. Figure 4.8 shows a simulated power-time plot for the hydrolysis of MF in buffer pH 6.4 using the reaction parameters obtained by Otsuka et al. (1994).
Higher initial amount of MF (20 mg/ml = 0.068 M) was used in the hydrolysis in pH 2.9 buffer at 25 °C using the TAM. The calculated rate constant of $9.7 \times 10^7$ s$^{-1}$ was higher than the minimum required for the similar combination of reaction parameters (Table 4.5).

### 4.3.3 Ascorbic acid in aqueous solution

Ascorbic acid is oxidised in aqueous solution to dehydroascorbic acid by molecular oxygen. Willson, (1995) carried out the kinetic study of the oxidation of L-ascorbic acid as a function of pH, oxygen concentration, temperature, concentration of ascorbic acid and addition of radical scavengers in the TAM. The enthalpy change for the oxidation step was determined to be -130.9 kJ mol$^{-1}$, while the first-order rate constant was recorded as $1.82 \times 10^4$ s$^{-1}$. These values would result in a considerably high power signal and hence would be easily analysable. Oxidation reactions are accompanied with a large enthalpy change which generates large power signal values.
4.4 Potential Reaction Systems That Can Be Studied Calorimetrically

Solution phase degradation reactions that follow first-order kinetics, having a slow rate of reaction and a small enthalpy change, results in a low power signal value causing considerable difficulty in analysis. The inherent baseline noise of the calorimeter contributes significantly to the recorded power signal, especially when the magnitude of the power value is close to the baseline. Housing the TAM in a temperature-controlled environment reduces the baseline noise and improves the performance of the TAM. However, there is a limit at which current instruments can record data which can be successfully analysed using available techniques.

In the previous sections, these theoretical limits were arrived at by generating copious amounts of data and finding out the minimum value of rate constant that is required for a fixed set of enthalpy change and initial concentration, in order to allow a successful analysis. In this section, the practical application of these generated minimum reaction parameters is tested by studying degradations of API's in the TAM. Experimental conditions were based on reaction parameters available in the literature and with the help of Tables 4.4 – 4.6, the ideal experimental parameters were selected to affect a successful analysis of calorimetric data.

Rate constants for the degradation of Trimebutine maleate and Rifampicin were available in the literature. From these values and calculated theoretical enthalpy change, the minimum amount of initial material required to react, to produce a calorimetric signal that could be successfully analysed was determined (Tables 4.4 – 4.6). The next section deals with the degradation studies of Trimebutine maleate and rifampicin in aqueous solutions.

4.4.1 Hydrolysis of Trimebutine maleate

4.4.1.1 Introduction

Trimebutine maleate (TM), 2-dimethylamino-2-phenylbutyl-3,4,5-trimethoxybenzoate hydrogen maleate is an antispasmodic and is clinically used for treatment of various gastrointestinal disorders including irritable bowel syndrome. TM (I) degrades by hydrolysis of the ester bond producing the acid 3,4,5-trimethoxy benzoic acid (II) and the corresponding alcohol 2-(dimethyl amino)-2-phenylbutanol (III) (Figure 4.9). Hydrolysis
is either acid or base catalysed and TM is most stable at pH 3.3. The pH-rate profile for the degradation in Britton-Robinson buffer at 80 °C is shown in Figure 5.10 (El-Gindy et al., 2003).

Figure 4.9: Reaction pathway for the degradation of trimebutine maleate (I).

Figure 4.10: pH-rate profile for the degradation of TM in Britton-Robinson buffer at 80 °C (El-Gindy et al., 2003).
The degradation kinetics of TM is documented in the literature. El-Gindy et al., (2003) carried out a comprehensive study of the acid and base catalysed hydrolysis of TM at a range of temperatures between 30 to 90 °C. The degradation studies were performed using HPLC and first derivative spectrophotometry (\(^1\)D). The acid catalysed ester hydrolysis of TM was studied in 1 M HCl from 60 to 90 °C. The degradation process followed pseudo first-order kinetics and from the slope of the log concentration versus time plots, first-order rate constants were recorded for each temperature (Table 4.8).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>(k) (s(^{-1}))</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>HPLC</td>
</tr>
<tr>
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</tr>
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<td>80</td>
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</tr>
<tr>
<td>85</td>
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</tr>
<tr>
<td>90</td>
<td>5.17 x 10(^{-5})</td>
</tr>
<tr>
<td>25</td>
<td>4.34 x 10(^{-8})†</td>
</tr>
<tr>
<td>50</td>
<td>9.34 x 10(^{-7})†</td>
</tr>
</tbody>
</table>

Table 4.8: Degradation rate constant (\(k\)) for TM in 1 M hydrochloric acid determined by HPLC and \(^1\)D methods (El-Gindy et al., (2003)). † denotes rate constants predicted using an Arrhenius plot.

An Arrhenius plot of log(rate constant) versus 1/T was linear for the degradation of TM in 1 M HCl at the temperature range studied. From an extrapolation of the Arrhenius relationship, rate constants of 4.34 x 10\(^{-8}\) and 9.34 x 10\(^{-7}\) s\(^{-1}\) were obtained for 25 and 50 °C respectively.

There is a lack of literature data for enthalpy change values for the degradation of TM in aqueous solutions. However, calculation of the theoretical enthalpy change for the degradation of TM in aqueous acid solution from gas phase bond energies (not an accurate method) resulted in a null value, as the sum of the standard enthalpies of formation of products and reactants were equal.

From the values of the first-order rate constants (Table 4.8) and enthalpy change, a prediction can be made whether the degradation reaction would produce a theoretically
analysable power signal (0.5 μW at \( t = 24 \text{ h} \)) (Tables 4.4 – 4.6). From Tables 4.4 – 4.6 it is evident that assuming an enthalpy change of approximately 10 kJ mol\(^{-1}\), the degradation of TM in 1 M HCl at 80, 85 and 90 °C would theoretically produce a power signal of at least 0.5 μW (\( t = 24 \text{ h} \)) if the initial concentration of TM was approximately 0.004 M. While at 60 and 70 °C an initial concentration of approximately 0.008 and 0.005 M respectively would be required to obtain a calorimetric signal of 0.5 μW (\( t = 24 \text{ h} \)). Similarly, to study the degradation of TM at 25 and 50 °C the initial amount of TM required to produce analysable power data would increase. The minimum initial concentration of TM required would be 0.4 and 0.02 M to obtain a power value of 0.5 μW (\( t = 24 \text{ h} \)) at 25 and 50 °C respectively.

However, reactions having a first-order rate constant in the magnitude of \( 10^{-3} \) to \( 10^{-6} \) s\(^{-1}\) would require less than 24 h of calorimetric data to recover accurate reaction parameters. It has been shown earlier that reactions having a first-order rate constant in the magnitude of \( 10^{-6} \) s\(^{-1}\) (\( \Delta H \approx 30 \text{ kJ mol}^{-1} \)) would require only 10 h of calorimetric data (section 3.2.3), while those with a magnitude of \( 10^{-4} \) s\(^{-1}\) (\( \Delta H \approx 60 \text{ kJ mol}^{-1} \)) would require 15 min of calorimetric data (section 4.3.3.1) to successfully recover reaction parameters. As the magnitude of the reaction rate decreases, greater calorimetric observation times are required, typically 50 h of calorimetric data for a first-order rate constant of \( 1 \times 10^{-11} \) s\(^{-1}\) (Willson, 1995).

The aim of this study therefore was to determine whether the degradation of TM could be studied successfully in the TAM at a lower temperature that those in the literature. Furthermore the usefulness of Tables 4.4 – 4.6 in determining the minimum initial concentration of TM required, in order to observe analysable calorimetric power-time data, when reaction parameters are know were studied. The degradation of TM in 1 M HCl at 25 and 50 °C was studied in the TAM and data obtained was analysed to determine reaction parameters. The availability of published literature values for the degradation rate constants formed the basis of designing a TAM experiment.
4.4.1.2. Materials and methods

4.4.1.2.1 Materials

Trimebutine maleate (98 %, lot 080H0755) was purchased from Sigma and refrigerated (2 – 4 °C) once opened. 5 M hydrochloric acid (lot 0C534447) was from BDH. All solutions were prepared using distilled de-ionized water.

4.4.1.2.2 Methods

Experiments were conducted using a 2277 TAM at 25 and 50 °C. TM solutions were prepared by dissolving the appropriate quantities in 1 M HCl to produce a final TM concentration of 0.2 M. The solutions were prepared and 3 ml aliquots were filled into TAM ampoules, sealed and placed in thermal equilibration position of the TAM (Section 1.7.3.3). Data capture and analysis were carried out as mentioned in Section 1.7.3.4.

4.4.1.3 Results and discussion

Initial experiments were carried out on the acid catalysed hydrolysis of TM (0.2 M) in 1 M HCl at 50 °C. The ln(power)-time plots were linear which confirmed that the reaction was first-order at this temperature and from the slope the first-order rate constants were obtained by linear regression analysis (Figure 4.11 and Table 4.9). A typical power-time plot obtained for the degradation of TM is shown in Figure 4.12 and the data were fitted to Equation 4.1. The fit of the data set to the model is represented by open circles (○). Reaction parameters obtained from the fit of the data to Equation 4.1 are given in Table 4.9.
Figure 4.11: ln(power)-time data for the degradation of TM (0.2 M) in HCl aqueous solution (1.0 M) at 50 °C and the fit line (—) generated by application of linear regression analysis.

Figure 4.12: Power-time data for the degradation of TM (0.2 M) in HCl aqueous solution (1.0 M) at 50 °C and the fit line (•) generated by application of Equation 4.1.
An average rate constant of $6.96 \times 10^{-7}$ s$^{-1}$ was obtained from the gradient of the ln(power)-time plot for the degradation of TM at 50 °C. This compares well with that obtained by application of an Arrhenius relationship to TM degradation data (El-Gindy et al., 2003) and extrapolating to 50 °C ($9.34 \times 10^{-7}$ s$^{-1}$). The rate constant determined was greater than the minimum desirable value of rate constant required to generate a power signal of 0.5 µW ($t = 24$ h), corresponding to an enthalpy change of 10 kJ mol$^{-1}$ and an initial reactant concentration of 0.2 M (Table 4.5).

Fitting the power-time data to Equation 4.1 allowed for the recovery of reaction enthalpy. The enthalpy change for the degradation of TM was determined to equal -5.25 kJ mol$^{-1}$ and since no literature value is available, a comparison cannot be made. However the enthalpy change calculated from the standard enthalpy of formation of reaction and products (gas-phase bond energies), gave a zero value and hence the value returned from fitting are assumed to be reasonably correct.

As mentioned earlier, the degradation of TM in 1 M HCl at 25 °C required an initial TM concentration of at least 0.4 M (assuming $\Delta H \approx 10$ kJ mol$^{-1}$) in order to obtain an analysable power signal of 0.5 µW ($t = 24$ h). However, the maximum aqueous solubility of TM as stated by the manufacturer (0.2 M) was lower and hence would produce a power signal lower than 0.5 µW. The sensitivity of the TAM would play a crucial roll under such circumstances and housing the TAM in a temperature-controlled environment (< 0.1 °C variation) would greatly improve the sensitivity, thereby reducing the fluctuations in the power signal and reducing the limit of 0.5 µW to 0.1 µW.

Degradation of TM in 1 M HCl with an initial concentration of 0.2 M was studied in the TAM at 25 °C. Two sets of experiment were done, first in a TAM that was housed in a

<table>
<thead>
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<th>Temperature (°C)</th>
<th>$k$ (s$^{-1}$)</th>
<th>$\Delta H$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
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<td>50</td>
<td>$7.74 \times 10^{-7}$</td>
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</tr>
<tr>
<td>50</td>
<td>$6.17 \times 10^{-7}$</td>
<td>-5.34</td>
</tr>
<tr>
<td>Average</td>
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<td>-5.25</td>
</tr>
<tr>
<td>±1.11 x 10$^{-7}$</td>
<td>±0.13</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.9: Reaction parameters determined for the degradation of TM (0.2 M) in HCl aqueous solution (1.0 M) at 50 °C.
temperature-controlled room and the second where there was no control on the temperature in the TAM housing room. Typical plots of the power-time data for the degradation of TM (0.2 M) in 1 M HCl at 25 °C are shown in Figure 4.19.

![Power-time and ln(power)-time plots](image)

**Figure 4.13:** Power-time (A and B) and ln(power)-time (C and D) data for degradation of TM (0.2 M) in 1 M HCl at 25 °C in the TAM; housed in a temperature-controlled room (A, C) and where temperature-controlled conditions were not maintained (B, D).

From the power-time curves, it is evident that, there were random changes in the power signal recorded in the TAM where there was no control on the temperature in the housing room. The sporadic changes in the power signal were also observed with data recorded in other channels (data not shown). These small fluctuations in the power signals are critical when slow reactions are studied in the TAM. The fluctuations in the power-time curve could be attributed to sudden electrical surges/spikes, which would be characterised by sudden sharp peaks. This emphasizes the need for very strict calorimetric operational parameters.

Although both power-time traces for the degradation of TM follow the same pattern, the ln(power)-time plots of only one set of data are analysable (Figure 4.13). The random fluctuations recorded in the power-time curve causes significant deflections in the ln(power)-time plots, making them difficult to analyse. Moreover, the ln(power)-time curve showed significant noise when the power signal was below 0.2 μW, even when the
TAM was housed in a temperature-controlled environment. The power-time data recorded were therefore entirely contributed by the degradation process, until the power signal reached 0.2 µW, below which, instrumental noise contributed significantly. Modern calorimeters equipped with nanowatt amplifiers, housed in a temperature-controlled environment and protected from electrical fluctuation have a sensitivity of 0.05 µW, thereby enhancing the reliability of the power signal recorded for slow reactions.

Fitting the power-time data to Equation 4.1 did not return correct reaction parameters. However, fitting simulated power-time data generated using Mathcad® 2001 (reaction parameters from Tables 4.8 and 4.9) to Equation 4.1 returned correct reaction parameters (data not shown), thereby suggesting that the inherent noise in the recorded calorimetric data prevented successful analysis.

4.4.2 Degradation of Rifampicin

4.4.2.1 Introduction

Rifampicin (rifampin, 3-(4-methyl-1-piperazinyliminomethyl)rifamycin SV; Figure 4.14) is a semi-synthetic antibiotic used clinically as an anti-tubercular drug. Rifampicin contains an azomethine bond (-N=CH-) and the degradation kinetics of such compounds are complex. The degradation of API's containing an azomethine bond have been studied, hydrolysis of diazepam (Han et al., 1977; Nakano et al., 1981; Connors et al; 1986a), nitrofurantoin (Inotsume and Nakano, 1981; Connors et al; 1986b) and ebifuramin (Prankerd and Stella, 1989) and is proposed to involve reversible azomethine bond cleavage.
The solubility of rifampicin (RIF) in aqueous solutions is temperature and pH dependent. The solubility being approximately 10 g/100 ml (pH 2.0), 0.4 g/100 ml (pH 5.3) and 0.28 g/100 ml (pH 7.5) at 25 °C (Kenny and Strates, 1981). Aqueous solutions of rifampicin are relatively unstable and the degradation process is pH dependent (Gallo and Radaelli, 1976). At low pH, rifampicin (I) undergoes specific acid catalysed hydrolysis with the formation of 3-formylrifamycin SV (RSV, II) and 1-amino-4-methyl-piperazine (AMP, III). At alkaline pH, oxidation of rifampicin occurs in the presence of atmospheric oxygen. The degraded product formed being rifampin-quinone. Degradation studies of RIF in aqueous solutions (pH 2.3 and 8.0) showed that oxidation to quinone occurred at the higher pH whereas hydrolysis occurred at acidic pH at initial concentrations of 200 µg/ml (Maggi et al., 1966).

Seydel, (1970) carried out a comprehensive study on the degradation kinetics of rifampicin in 0.1 M HCl as a function of temperature and different initial concentrations using UV-spectrophotometry and thin layer chromatography. Activation energy of 79.5 kJ mol⁻¹ was obtained after application of an Arrhenius relationship to the pseudo first-order rate constants. A plot of the first-order rate constants (obtained for different initial concentrations of RIF, Table 4.10) versus concentration is exponential (Figure 4.15) indicating that the rate constant decreased with increasing initial concentration of RIF.
<table>
<thead>
<tr>
<th>Concentration (M)</th>
<th>Rate constant (s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.40 x 10⁻⁵</td>
<td>6.05 x 10⁻⁴</td>
</tr>
<tr>
<td>6.07 x 10⁻⁵</td>
<td>3.60 x 10⁻⁴</td>
</tr>
<tr>
<td>1.23 x 10⁻⁴</td>
<td>2.36 x 10⁻⁴</td>
</tr>
<tr>
<td>2.40 x 10⁻⁴</td>
<td>2.10 x 10⁻⁴</td>
</tr>
<tr>
<td>4.25 x 10⁻⁴</td>
<td>1.44 x 10⁻⁴</td>
</tr>
<tr>
<td>6.07 x 10⁻⁴</td>
<td>1.36 x 10⁻⁴</td>
</tr>
<tr>
<td>1.23 x 10⁻³</td>
<td>0.97 x 10⁻⁴</td>
</tr>
</tbody>
</table>

Table 4.10: Apparent first-order rate constants for the hydrolysis of rifampicin in 0.1 M HCl at 50 °C, for a range of different initial concentrations (Seydel, 1970).

Prankerd et al., (1992), studied the kinetics of RIF hydrolysis in acidic solutions at a range of pH values from pH 1 to 5 at 37 °C. Equation 4.2 describes the proposed hydrolysis of RIF involving the azomethine bond, where the forward reaction is pseudo-first order \( (k_f) \), whereas the reverse reaction is pseudo-second order \( (k_r) \).
However their plots of log(concentration) versus time were bi-exponential, with the initial phase being linear for about three half-lives, followed by a slower linear terminal phase. The two linear portions were interpreted in terms of a fast initial conversion of RIF to the degraded aldehyde (RSV) and piperazine (AMP) leading to a dynamic equilibrium between RIF and RSV; followed by a slower reaction(s) corresponding to loss of either RIF or its degradation products by parallel pseudo-first order reactions (Prankerd et al., 1992). Hence, the degradation of RIF in aqueous acidic solution can be studied in the TAM for the duration of the initial rapid hydrolysis.

There is no published value of enthalpy change for the degradation of RIF in aqueous solutions. Therefore, an estimate of the theoretical enthalpy change for the process from the gas phase bond energies was calculated to be -38 kJ mol⁻¹.

The first-order rate constants for the hydrolysis of RIF in 0.1 M HCl at 50 °C as a function of initial concentrations are given in Table 4.10. Corresponding to an enthalpy change of approximately -40 kJ mol⁻¹ (Table 4.4), even with an initial concentration of 0.001 M, the first-order rate constant of 9.7 x 10⁻⁵ s⁻¹ (0.00123 M initial concentration) for the degradation of RIF at 50 °C is above the minimum detectable limit to obtain a power of 0.5 μW (t = 24 h). Hence the degradation of RIF in 0.1 M HCl at 50 °C can be successfully studied in the TAM with a concentration as low as 0.001 M.

The aim of this work was to study the acid catalysed hydrolysis of RIF in 0.1 M HCl at 50 °C for duration of at least three half-lives. The recorded power-time data was then analysed to obtain reaction parameters that would enable a comparison to be made with those in the literature. Moreover it would justify the use of Tables 4.4 – 4.6 in determining initial concentrations of materials required in order to successfully undertake a TAM experiment when possible reaction parameters are known.
4.4.2.2 Materials and Methods

4.4.2.2.1 Materials

Rifampicin (≥97.0%, lot 1178137) was purchased from Fluka and kept refrigerated (2 – 4 °C) once opened. Hydrochloric acid (0.2 M, lot 00109KD) was from Aldrich. All solutions were prepared using distilled de-ionised water.

4.4.2.2.2 Methods

Experiments were conducted using a 2277 TAM at 50 °C. RIF solutions were prepared by dissolving the required amount in 0.1 M HCl to produce a final concentration of 0.0015 M. Aliquots of the solution (3 ml) were pipetted into standard glass TAM ampoules and sealed. The ampoules were placed in the thermal equilibration position of the TAM for approximately 20 min after which they were lowered and data capture was initiated (Section 1.7.3.3). Samples were run in triplicate.

4.4.2.3 Results and discussions

Degradation of RIF in 0.1 M HCl was observed in the TAM for a minimum duration of three half-lives. At acidic pH the degradation of RIF occurs by the hydrolysis of azomethine bond. The inherent design of a TAM ampoule experiment is such that the ampoule forms an airtight seal and if filled prevents any possible interaction with air in the headspace of the ampoule. Hence, there is no oxidation to the quinine derivative. A ln(power)-time plot (Figure 4.16) was linear for the first three half lives and hence confirms that the reaction followed first-order kinetics. This initial reaction corresponds to the hydrolysis of RIF to RSV and AMP. From the slope of the ln(power)-time plot the first-order rate constant was obtained (Table 4.11).
Figure 4.16: ln(power)-time data for the degradation of RIF (0.0015 M) in HCl aqueous solution (0.1 M) at 50 °C and fit line (—) generated by application of linear regression analysis.

<table>
<thead>
<tr>
<th>$k$ (s$^{-1}$)</th>
<th>$\Delta H$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.05 x 10$^{-5}$</td>
<td>-34.86</td>
</tr>
<tr>
<td>1.89 x 10$^{-5}$</td>
<td>-36.75</td>
</tr>
<tr>
<td>1.71 x 10$^{-5}$</td>
<td>-36.91</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>1.88 x 10$^{-5}$ ± 0.17</strong></td>
</tr>
<tr>
<td></td>
<td><strong>-36.17 ± 1.14</strong></td>
</tr>
</tbody>
</table>

Table 4.11: Values for the rate constants and reaction enthalpies for the initial phase of degradation of RIF in 0.1 M HCl at 50 °C determined by fitting experimental data to Equation 4.1.

The average rate constant (1.88 x 10$^{-5}$ s$^{-1}$) for the initial phase of degradation of RIF, recorded from the calorimetric data was lower than that in the literature (9.7 x 10$^{-5}$ s$^{-1}$; Seydel, 1970). This could be because the degradation rate is dependent on the initial concentration of RIF and has an exponential relationship (Figure 4.15). Initial concentration of RIF (0.0015 M) used in the TAM experiment was fractionally higher than reported by Seydel, (1970), which could explain the lower rate.
The hydrolysis of RIF is an exothermic process and the reaction enthalpy was determined by fitting a portion (equivalent to three half-lives) of the power-time curve to Equation 4.1 (Figure 4.17). Values entered for $v$ (0.003 dm$^3$) and $[A_0]$ (0.0015 M) were kept constant, while those for $\Delta H$ (1 x 10$^{10}$ $\mu$J mol$^{-1}$) and $k$ (1 x 10$^{-5}$ s$^{-1}$) were allowed to vary until a good fit was obtained. The fitting procedure returned an enthalpy change value of -36.14 kJ mol$^{-1}$ (Table 4.12). Although there is no literature value to compare with, it is within error limits to that calculated from the gas-phase bond energies (-38 kJ mol$^{-1}$).

**Figure 4.17:** Power-time curve for the degradation of RIF (0.0015 M) in HCl aqueous solution (0.1 M) at 50 °C and the fit line ($\sigma$) generated by application of Equation 4.1 to a portion of the data.

The hydrolysis of RIF is an exothermic process and the total enthalpy change of the reaction is the sum of all enthalpy changes. Hydrolysis of RIF is a reversible process with the forward reaction being pseudo-first order for about three half-lives after which a dynamic equilibrium between RIF and RSV is reached. This is followed by a slower reaction which corresponds to loss of either RIF or its degradation products by parallel pseudo-first order reactions (Pranker et al., 1992). The forward reaction is exothermic whereas the reverse reaction is endothermic. The net enthalpy change for the reaction would approach zero if the extent of forward and reverse reactions were similar, but would shift to either exothermic or endothermic when the forward or reverse phases predominate at equilibrium.
4.5 Conclusion

In this chapter, the problems associated when studying solution-phase degradation reactions having slow reaction rates and small enthalpy change was discussed. These reactions result in a small power signal when studied in the TAM and instrumental noise has a significant contribution to the overall recorded calorimetric data. This is the reason for the difficulty in analysing the recorded data. Housing the TAM in a temperature-controlled environment greatly improves the calorimetric data that is recorded.

Calorimetric power-time data recorded for solution phase first-order reactions are a function of the reaction rate, enthalpy change and amount of material that can react. For slow reactions accompanied with small enthalpy change there is a theoretical minimum value of rate constant, below which data recorded cannot be successfully analysed using current analysing techniques. Tables were generated for various combinations of reaction parameters that would result in a theoretical power value above the minimum detection value. These tables would help in deciding whether a reaction with know set of reaction parameters would generate calorimetric data that could be successfully analysed. It can also be used to determine the minimum concentration of material required to successfully study a degradation reaction when rate constants and enthalpy change for the process are known. The use of these reaction parameters would considerably save time and are greatly useful when amount of material available is limited.

The degradation of Trimebutine maleate in aqueous solution was studied using the TAM. Rate constants recorded in the literature in conjunction with the tables, were used in designing a TAM experiment. The minimum amount of material needed, in order to observe a power value greater than the minimum detectable was determined with the help of tables generated earlier. A first-order rate constant of $6.96 \times 10^{-7}$ s$^{-1}$ and a reaction enthalpy of $-5.25$ kJ mol$^{-1}$ were obtained for the degradation of TM in 1 M HCl solution at 50 °C and compares well with those in the literature. To observe a calorimetric power signal of $0.5 \mu W (t = 24 \text{ h})$ at 25 °C would require an initial concentration of 0.8 M which was greater than the solubility limit of TM (0.2 M). Moreover, at such low observed power values, instrumental noise contributed significantly, which prevented successful analysis for reaction parameters.

The hydrolysis of RIF was studied in the TAM at 50 °C for the duration of the forward reaction (three half-lives). The initial amount of RIF that was required in order to observe
an analysable power signal was determined using the tables generated earlier. Analysis of the recorded power-time data returned a first-order rate constant of $1.88 \times 10^5 \text{ s}^{-1}$ and a reaction enthalpy of $-36.14 \text{ kJ mol}^{-1}$.

In this chapter, minimum reaction parameters desired, in order to observe a calorimetric power value above the detection limit of the TAM were generated and the application of such tables in designing calorimetric experiments were demonstrated. Having tables that provide minimum values for the reactions parameters is clearly advantageous (when some reaction parameters are known), as it saves time in method development and reduces the need for unnecessary experiments to determine optimum initial concentrations to observe analysable power signal. Although the purpose of many TAM experiments are to obtain reaction parameters for the process under study, the greater applicability of these tables in studying other applications such as in purity testing (discussed in next chapter) is paramount. The next chapter deals with the application of IC in various pre-formulation stages of pharmaceutical product development.
Chapter 5

IC IN PREFORMULATION
5.1 Introduction

The system studied so far in this thesis involved degradations occurring in solution, with different kinetic profiles. In Chapter 2 degradations involving single component first-order kinetics were studied, followed by investigation of parallel degradation systems. A degradation reaction involving a two-step consecutive reaction pathway was studied in Chapter 3 while Chapter 4 discussed issues encountered when slow reactions in solutions are studied calorimetrically. All degradation reactions studied so far, involved stability assessment of API's that potentially formed the active part of a finished product. This chapter aims to demonstrate the role of IC at the preformulation stage of drug development.

Preformulation is a stage of drug development, during which biopharmaceutical principles are utilised to determine physicochemical properties of the new drug substance. A thorough understanding of these properties is essential for the development of an efficacious dosage form. A list of properties essential in preformulation evaluation of a new drug is listed in Table 5.1. During the initial stages, when only limited amounts of new drug is available, only the most essential properties should be characterised that are mandatory.

A large number of analytical techniques are available during preformulation characterization and among the most widely used is HPLC. These techniques do provide valuable information about the system being studied but have distinct disadvantages. IC in some cases may offer an alternative to conventional methods for quantification of some physicochemical parameters (Buckton et al., 1991). IC data are currently, not accepted by pharmaceutical regulatory authorities, but their use as supporting evidence for data obtained from accepted techniques opens a new dimension. In this chapter, two important drug characterization parameters essential for new product development – drug purity and excipient compatibility are investigated using IC.
Table 5.1: Essential information in preformulation drug characterization (Wells and Aulton, 2000).

<table>
<thead>
<tr>
<th>Test</th>
<th>Method/function/characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Spectroscopy</td>
<td>UV assay</td>
</tr>
<tr>
<td>2 Solubility</td>
<td>DSC/IC – Phase solubility, purity</td>
</tr>
<tr>
<td></td>
<td>aqueous</td>
</tr>
<tr>
<td></td>
<td>pK&lt;sub&gt;a&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>salts</td>
</tr>
<tr>
<td></td>
<td>solvents</td>
</tr>
<tr>
<td></td>
<td>partition coefficient</td>
</tr>
<tr>
<td></td>
<td>dissolution</td>
</tr>
<tr>
<td>3 Melting point</td>
<td>DSC – polymorphism, hydrates, solvates</td>
</tr>
<tr>
<td>4 Assay development</td>
<td>HPLC, UV, TLC</td>
</tr>
<tr>
<td>5 Stability (in solution and solid state)</td>
<td>Thermal, hydrolysis, oxidation, photolysis, metal ions, pH</td>
</tr>
<tr>
<td>6 Microscopy</td>
<td>Morphology, particle size</td>
</tr>
<tr>
<td>7 Powder flow (bulk density, angle of repose)</td>
<td>Tablet and capsule formulation</td>
</tr>
<tr>
<td>8 Compression properties</td>
<td>Aids excipient choice</td>
</tr>
<tr>
<td>9 Excipient compatibility</td>
<td>Preliminary screening by DSC, IC</td>
</tr>
</tbody>
</table>

5.2 Purity Determination

5.2.1 Introduction

All pharmaceutical substances unavoidably contain some form of impurity and its control in the final product is a challenging task in drug development. Impurities in drug products are more difficult to analyse than those in drug substances but needs to be evaluated both qualitatively and quantitatively. A number of factors contribute to the nature and quality of impurities present and includes quality of starting material, reaction conditions, synthetic route, residual solvents, and purification steps.

Impurities in drug substances can be classified as organic impurities, inorganic impurities and residual solvents (ICH 1996). Organic impurities can result from the manufacturing
process and/or storage of the drug substance and consists of the starting material, by-products, intermediates, degradation products and/or reagents. Inorganic impurities can result from the manufacturing process and consists of inorganic salts, reagents and heavy metals. Solvents can be inorganic or organic liquids used as vehicles in the synthesis of drug substances.

Whatever the source, an impurity profile for the drug substance must be defined and a number of analytical tools including chromatography (Nageswara Rao and Nagaraju, 2003; Sandra et al., 2002; Ferenczi-Fodor et al., 2006), mass spectrometry (Govearts et al., 2001) and Differential Scanning Calorimetry (Khattab, 1983; Giron, 1986) have been used to characterise the impurities. Determining the amount of impurities present is also of vital importance. Chromatographic techniques are widely employed, but the need for method development can be time consuming. Differential Scanning Calorimetry (DSC) has been used to determine the purity of organic materials (van Dooren and Muller, 1984) and is being widely used in purity determination of pharmaceuticals.

### 5.2.1.1 DSC in purity determination

The use of DSC in determining the absolute purity of organic materials has been an accepted technique in the pharmaceutical industry (Giron and Goldbronn, 1995). Purity determination by DSC is based on the fact that minute amounts of impurity in a material broadens its melting range and lowers the final melting point of the material from $T_m$, the melting temperature of an infinitely pure material, to a lesser temperature $T'_m$. As the impurity content increases, the melting point decreases and the range of melting (melting endotherm) broadens.

DSC data can be analysed qualitatively and quantitatively. In qualitative analysis, melting points and peak shapes of an unknown material can be compared with known standards, while the percent purity can be determined by a quantitative approach. This is based on the van’t Hoff isochore and was first described by Gray and Fyans, (1973) and reviewed by Brennan et al., (1984):

$$T'_m = T_m - \frac{RT_0^2 X \frac{1}{\Delta H_f}}{F}$$  \hspace{1cm} \text{Equation 5.1}
where $T_s$ is the sample temperature (K), $T_0$ the melting temperature of pure material (K), $R$ the molar gas constant (8.314 J mol$^{-1}$ K$^{-1}$), $X_s$ the mol fraction of the impurity, $\Delta H_f$ the enthalpy of fusion (J mol$^{-1}$) and $F$ the fraction of total material melted at $T_s$. A plot of $1/F$ versus $T_s$ should yield a straight line with an intercept temperature ($T_0$) and a slope of $-RT_0^2X_s/\Delta H_f$. The value of $F$ at a given $T_s$ is directly proportional to the area under the DSC curve, up to the sample temperature and is determined from the calorimetric data. The impurity level is then easily determined from the slope.

Purity determination using DSC is advantageous as it can be done quickly (often in less than an hour) and easily with small amounts (milligram quantity) and it requires neither the identification of the impurities nor a comparison to standard materials. However as with any analytical technique there are some limitations – only samples of high purity (>97 mol %) (Hunter and Blaine, 1984) can be accurately measured and neither should the impurities form a solid solution with the main component nor should the sample decompose on melting.

5.2.1.2 IC in purity determination

IC can be used in purity determinations of drug substances provided the substance can undergo a chemical change with a measurable heat flow. Moreover, the recorded calorimetric signal should arise solely from the chemical change in the drug substance. O’Neill (2002) studied the purity of triacetin using IC. The purity is calculated from the observed calorimetric data and requires knowledge of the reaction enthalpy for the pure substance.

Purity was calculated by taking the ratio of $Q$ (area under the power-time curve when all the reactant is consumed) for the impure substance ($Q_i$) to that of the pure drug ($Q_p$).

$$\% \text{ purity} = \frac{Q_i}{Q_p} \times 100$$ \hspace{1cm} \text{Equation 5.2}

The value of $Q$ can be easily determined by integrating the area under the calorimetric curve from $t = 0$ to $t_{\text{end}}$, provided the reaction rate is rapid enough to permit a complete reaction within a measurable period of time. However, reactions commonly encountered in pharmaceutical are slow and may take months or years to complete. Hence, it is
necessary to determine \( Q \) from experimental data. From chapter 1, the general equation that describes the calorimetric power (\( \Phi \)) for a simple solution phase reaction \( A \rightarrow P \) is given by:

\[
\Phi = k \Delta H^{1-n} (Q - q)^n
\]

Equation 5.3

where \( k \) is the rate constant, \( \Delta H \) is the reaction enthalpy, \( q \) is the time dependent enthalpy change and \( n \) is the order of reaction. Taking two values of \( \Phi \) at different points on the calorimetric curve gives:

\[
\Phi_1 = -\Delta H^{1-n} k (Q - q_1)^n
\]

Equation 5.4

\[
\Phi_2 = -\Delta H^{1-n} k (Q - q_2)^n
\]

Equation 5.5

So:

\[
\frac{\Phi_1}{\Phi_2} = \left( \frac{Q - q_1}{Q - q_2} \right)^n
\]

Equation 5.6

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

Equation 5.7

Setting:

\[
\left( \frac{\Phi_1}{\Phi_2} \right)^\frac{1}{n} = R
\]

Equation 5.8

The value for \( Q \) is then derived by:

\[
Q = \frac{(q_1 - Rq_2)}{1 - R}
\]

Equation 5.9

If however the mechanism of the chemical change in the impure pharmaceutical substance is known, then an alternative approach to determine purity is employed. For a substance that undergoes chemical change through first-order kinetics the ratio of enthalpy change for impure drug (\( \Delta H_i \)) to that of pure drug (\( \Delta H_p \)) gives the percent purity.
For a chemical change that follows a solution phase single-step first-order kinetics the measured power signal is a function of the first-order rate constant, the reaction enthalpy and the amount of material present and is given by Equation 5.11 (Bakri et al., 1988):

\[
\text{Power} = \frac{dq}{dt} = \Delta H \cdot v \cdot k \cdot [A_0] \cdot e^{-k \cdot t}
\]  

where \( q \) is the heat output of the reaction, \( \Delta H \) is the reaction enthalpy per mole of product formed, \( v \) the volume of solution, \( k \) the first-order rate constant and \( [A_0] \) is the initial concentration of substance. Fitting the power-time data obtained for pure substance (\( v \) and \( [A_0] \) are known and hence fixed while \( k \) and \( \Delta H \) are allowed to vary) will return values of rate constant and reaction enthalpy. The rate constant is unique for a substance at a particular temperature. The purity of aspirin was studied using IC where the ratio of enthalpy change for impure aspirin to that of pure aspirin gave the percent purity.

The aim of this study was to determine the purity of various aspirin samples containing different amounts of salicylic acid as an added impurity. The power-time data obtained would then be fitted to obtain reaction enthalpies from which the purity would be determined.

### 5.2.2 Materials and Methods

#### 5.2.2.1 Materials

Aspirin (99%, lot 045K0101) was purchased from Sigma. Salicylic acid (≥99.0 %) was from Fluka. Hydrochloric acid (0.2 M, lot 00109KD) was from Aldrich. All materials were used as received. All solutions were prepared using distilled de-ionised water.
5.2.2.2 Methods

5.2.2.2.1 Isothermal microcalorimetry

Experiments were conducted using a 2277 thermal activity monitor (TAM, Thermometric AB, Järfälla, Sweden) at 37 °C. Aspirin solution (100%) was prepared by dissolving 180 mg of aspirin in 100 ml of hydrochloric acid solution (0.1 M). Impure aspirin solutions (representing different percentage purities: 90, 95, 97, 98 and 99% w/v) were prepared with the addition of an appropriate quantity of salicylic acid (10, 5, 3, 2 and 1% w/v) to aspirin (Table 5.2). 3 ml aliquots of solution were pipetted into standard glass TAM ampoules; the ampoules were then sealed with a crimped metal lid and placed in the thermal equilibration position of the TAM for approximately 20 min before being lowered into the measurement position. Refer Section 1.7.3 for detailed discussion. Data capture was then initiated and the power data (μW) were recorded every 30 s for approximately 24 h with an amplifier setting of 100 μW, against a reference ampoule containing hydrochloric acid solution (0.1 M, 3 ml). The time between addition of solute to HCl (t₀) and the commencement of data capture (tₛ) by the software package was noted and added to the time data for each experiment.

<table>
<thead>
<tr>
<th>% purity</th>
<th>Aspirin (mg)</th>
<th>Salicylic acid (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>162.0</td>
<td>13.81</td>
</tr>
<tr>
<td>95</td>
<td>171.0</td>
<td>6.91</td>
</tr>
<tr>
<td>97</td>
<td>174.6</td>
<td>4.14</td>
</tr>
<tr>
<td>98</td>
<td>176.4</td>
<td>2.76</td>
</tr>
<tr>
<td>99</td>
<td>178.2</td>
<td>1.38</td>
</tr>
<tr>
<td>100</td>
<td>180.0</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 5.2: Quantity of aspirin and salicylic acid dissolved in 100 ml HCl (0.1M) to obtain required percentage purity.

5.2.2.2.2 Data analysis

Data analysis was performed using Origin 7.0 (Microcal Software Inc., USA). The difference between t₀ and tₛ (in s) was added to the time data to correct for the time-delay between initiation of reaction and commencement of data capture. Power-time data were
analysed using an iterative procedure (the non-linear curve fitting tool in Origin). Data were fitted to Equation 5.11, which describes the power-time response for a single-step reaction following first order kinetics (Bakri et al., 1988).

\[
\text{Power} = \frac{dq}{dt} = \Delta H \cdot v \cdot k \cdot [A_0] \cdot e^{-kt}
\]

Equation 5.11

where \( q \) is the heat output of the reaction, \( \Delta H \) is the reaction enthalpy per mole of product formed, \( v \) the volume of solution in the ampoule, \( k \) the first-order rate constant and \( [A_0] \) is the initial concentration of reactant. Value for \( v \) (0.003 dm\(^3\)) and \( [A_0] \) (0.01 M) were known and therefore kept constant. For the pure aspirin solution (100%) the initial values entered for \( \Delta H \) (1 x 10\(^{10}\) µJ mol\(^{-1}\)) and \( k \) (1 x 10\(^{-4}\) s\(^{-1}\)) were the same for each data set; the software then altered these values until a good fit to the data (as indicated by a low \( \chi^2 \) value) was obtained. For the impure aspirin solutions (90, 95, 97, 98 and 99% w/v), the value entered for \( k \) was that obtained from the fit of pure aspirin and was kept constant. \( \Delta H \) (1 x 10\(^{10}\) µJ mol\(^{-1}\)) was altered by the software until a good fit to the data was obtained.

5.2.3 Results and discussion

The power-time trace obtained for the degradation of pure aspirin (100%) in 0.1 M HCl at 37 °C is represented schematically in Figure 5.1. Plots of ln(power)-time (Figure 5.2) resulted in a linear relationship, confirming that the reaction followed first-order kinetics. From the slope, an average first-order rate constant of 6.27 x 10\(^{-4}\) s\(^{-1}\) was obtained. The power-time data were fitted to Equation 5.11, the fit of the data set to the model is represented by the open circles (Figure 5.1). Fitting the data resulted in a good fit (low \( \chi^2 \) value) and the average value for \( \Delta H \) returned was 37.31 kJ mol\(^{-1}\) (Table 6.3).
Chapter 5 – IC in Preformulation

Figure 5.1: Power-time data for pure aspirin (100%) in HCl aqueous solution (0.1 M) at 37 °C and the fit lines (o) generated by application of Equation 5.11.

Figure 5.2: ln(power)-time data for aspirin (100%) in HCl aqueous solution (0.1 M) at 37 °C and the fit line (—) obtained by application of linear regression analysis.
Table 5.3: Values for the rate constant and reaction enthalpy for pure aspirin (100%) determined by fitting experimental data to Equation 5.11.

<table>
<thead>
<tr>
<th>100 %</th>
<th>( k \text{ (s}^{-1}\text{)} )</th>
<th>( \Delta H \text{ (kJ mol}^{-1}\text{)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.12 (\times 10^{-6})</td>
<td>36.77</td>
<td></td>
</tr>
<tr>
<td>6.23 (\times 10^{-6})</td>
<td>37.36</td>
<td></td>
</tr>
<tr>
<td>6.51 (\times 10^{-6})</td>
<td>34.78</td>
<td></td>
</tr>
<tr>
<td>6.18 (\times 10^{-6})</td>
<td>37.02</td>
<td></td>
</tr>
<tr>
<td>6.29 (\times 10^{-6})</td>
<td>39.05</td>
<td></td>
</tr>
<tr>
<td>6.23 (\times 10^{-6})</td>
<td>37.94</td>
<td></td>
</tr>
<tr>
<td>6.37 (\times 10^{-6})</td>
<td>38.24</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>6.27 (\times 10^{-6})</td>
<td>37.31 ± 0.13 (\times 10^{-6}) ± 1.35</td>
</tr>
</tbody>
</table>

The power-time data obtained for the degradation of impure aspirin (different initial concentration of aspirin; 90, 95, 97, 98 and 99 % w/v) are represented schematically in Figure 5.3. Plots of ln(power)-time for each data set were linear, confirming that the degradation of impure aspirin solutions followed first-order kinetics. Power-time data were fitted to the Equation 5.11 by keeping values for \( v \) (0.003 dm\(^3\)) \(, [A_0] \) (0.01 M) and \( k \) (6.27 \(\times 10^{-6}\) s\(^{-1}\), average value derived from pure aspirin data) constant, and varying \( \Delta H \) (1 \(\times 10^{10} \mu J \text{ mol}^{-1}\)) until a good fit to the data was obtained (indicated by a low chi\(^2\) value). Values of enthalpy change returned from the fitting process are summarized in Table 5.4.

The values of enthalpy change returned from the fitting process were used to determine the purity of the impure aspirin. The percentage purity of the impure aspirin was calculated from the ratio of the enthalpy change of the impure aspirin (\(\Delta H_i\)) to that of pure aspirin (\(\Delta H_p\)) (Equation 5.12; Table 5.4).

\[
\text{Percentage purity (\%) = \frac{\Delta H_i}{\Delta H_p} \times 100}
\]  

Equation 5.12
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Figure 5.3: Power-time data for impure and pure aspirin (90, 95, 97, 98, 99 and 100%) in HCl aqueous solution (0.1 M) at 37 °C.

<table>
<thead>
<tr>
<th>% Aspirin</th>
<th>ΔH (kJ mol⁻¹)</th>
<th>% Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>33.71 ± 1.46</td>
<td>90.34</td>
</tr>
<tr>
<td>95</td>
<td>35.55 ± 0.82</td>
<td>95.27</td>
</tr>
<tr>
<td>97</td>
<td>36.17 ± 1.19</td>
<td>96.92</td>
</tr>
<tr>
<td>98</td>
<td>36.37 ± 0.86</td>
<td>97.46</td>
</tr>
<tr>
<td>99</td>
<td>36.40 ± 0.82</td>
<td>97.55</td>
</tr>
</tbody>
</table>

Table 5.4: Average values for the reaction enthalpies of the impure aspirin determined by fitting experimental data to Equation 5.11 and the calculated percentage purities.

The percentage purity obtained for 90, 95 and 97% correlate well with the theoretical value, whereas those obtained for 98 and 99% differ from those expected. From the percentage purity data in Table 5.4, it is evident that for the 98% aspirin sample the error in the calculated percentage purity was 0.6%, which increased to 1.5% for the 99% aspirin sample. The errors for the other impure aspirin samples were 0.4, 0.3 and 0.1 for 90, 95 and 97% respectively. The failure to distinguish small differences in purity may be
attributed to either the inability of the iteration process to distinguish small differences or the insensitivity of the TAM to record small differences.

‘Ideal’ power-time data that represents degradation of impure and pure aspirin (90, 95, 97, 98, 99 and 100 % w/v) were generated in Mathcad® 2001 using Equation 5.11. The value for $v$ (0.003 dm$^3$), $k$ ($6.27 \times 10^5$ s$^{-1}$) and $\Delta H$ (37.31 kJ mol$^{-1}$) were kept constant, while that for $[A_0]$ (0.0090, 0.0095, 0.0097, 0.0098, 0.0099 and 0.01 M) were changed accordingly, to generate power-time data to represent the various percentage purities. Representative power-time data generated for the different initial percentages of aspirin are shown in Figure 5.4. The simulated data were imported into Origin 7.0 and analysed using the iterative procedure (non-linear cure fitting tool). The data were fitted to Equation 5.11; the initial values for $v$ (0.003 dm$^3$), $[A_0]$ (0.01 M) and $k$ ($6.3 \times 10^5$ s$^{-1}$) were kept constant while $\Delta H$ ($1 \times 10^{10}$ µJ mol$^{-1}$) was altered by the software until a good fit was obtained. Enthalpy values determined by fitting the simulated data are summarised in Table 5.5.

![Figure 5.4: Simulated power-time data for pure and impure aspirin in HCl (0.1 M) at 37 °C generated in Mathcad® 2001f.](image)
Table 5.5: Values for the enthalpy change returned by fitting simulated power-time data for impure and pure aspirin data to Equation 5.11 and the calculated percentage purity.

<table>
<thead>
<tr>
<th>% aspirin</th>
<th>$\Delta H$ (kJ mol$^{-1}$)</th>
<th>% purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>33.6</td>
<td>90.1</td>
</tr>
<tr>
<td>95</td>
<td>35.4</td>
<td>94.9</td>
</tr>
<tr>
<td>97</td>
<td>36.2</td>
<td>97.1</td>
</tr>
<tr>
<td>98</td>
<td>36.6</td>
<td>98.1</td>
</tr>
<tr>
<td>99</td>
<td>36.9</td>
<td>98.9</td>
</tr>
<tr>
<td>100</td>
<td>37.3</td>
<td></td>
</tr>
</tbody>
</table>

From the enthalpy change values (Tables 5.4 and 5.5) it is apparent that the values returned from the experimental data were, within error limits, similar to those returned from fitting ‘ideal’ data, thereby illustrating the robustness of the fitting process. However, the experimental enthalpy change determined for the 98 and 99 % aspirin solutions were identical in magnitude and resulted in a percentage purity value that was indistinguishable. A possible reason could be that both the solutions reached similar equilibrium concentrations when data capture was initiated and therefore cannot be resolved. These data assists in showing the practical limits of a TAM experiment, to clearly distinguish very small differences in initial concentrations of starting material. The percentage purity data for the 90, 95 and 97% aspirin samples however compare well and are within 0.4 % error limits.

5.2.4 Conclusions

It has been shown that IC can be used in purity determination of aspirin and level of impurity can be determined. However since calorimetric data does not provide any molecular information, identifying these impurities requires the application of supplementary techniques such as chromatographic or spectrophotometric methods.

Aspirin samples with varying purities were studied in the TAM and the power-time data recoded were iterated to determine reaction parameters. The values of rate constant obtained for the pure sample were then used in the analysis of impure aspirin samples. This enabled determination of enthalpy change for various impure samples. From the ratio of enthalpy change for impure to pure sample the percentage purity was determined.
Percentage purity obtained for 90, 95 and 97\% were as expected while those for 98 and 99\% differed from the theoretical estimates.

5.3 Solid-State Drug Excipient Compatibility Testing

5.3.1 Introduction

A vast majority of pharmaceuticals are formulated as solid-state systems and assessing their stability can be a challenging task. This is because degradations in solid-state are complex and not well understood. An important area where calorimetry plays a vital role is during the preformulation stage of new drug development, especially the compatibility assessment of various excipients. The use of excipients such as binders, lubricants and fillers in the formulation of solid dosage forms can often impair the stability of an API. The study of possible incompatibilities between an API and different excipients, forms an integral part of the preformulation stage of product development. Conventional stability procedures involve, preparation of samples containing the drug and excipient, storage under stressed conditions and systematically analysing the sample using suitable stability-indicating methods at set time periods. These procedures are time-consuming, very expensive and labour intensive. Newer approaches involve excipient compatibility screening and are an essential part of the development process (Wells, 1988; Serajuddin et al., 1999). Solid-state reactions are complex and much less understood compared to solution phase reactions (Byrn, 2001). A number of factors affect the stability of solid formulations and degradation kinetics (Figure 5.5).

![Factors Affecting Excipient Compatibility](image_url)

**Figure 5.5:** Factors affecting solid formulation stability (Schmitt et al., 2001).
Despite the importance of drug-excipient compatibility testing, no universally accepted protocol is available, although thermal methods such as DSC and High Sensitivity DSC (HSDSC) are being actively used as screening tools. IC is another approach for evaluating stability and excipient compatibility. It can measure the minute heat changes that are accompanied with the degradation of drugs caused by the incompatibility between a drug and an excipient (Selzer et al., 1998; Selzer et al., 1999; Schmitt et al., 2001; Terada et al., 2006).

### 5.3.1.1 Stress conditions

Instabilities in modern formulations maintained at normal conditions, are detectable only after prolonged storage period. To reduce the time required for testing, formulated products are usually exposed to high stress conditions to enhance its deterioration, which enables more data to be collected. Common high stress conditions involve thermal stress, mechanical stress, humidity and exposure to light.

Elevated temperatures are often used to accelerate the degradation rate, with the assumption that the kinetics of the reaction follows linear Arrhenius behaviour. Samples are analysed at various time intervals to determine the extent of degradation. Rate constants are obtained at each of the higher temperatures and from the Arrhenius plot (log rate constant versus reciprocal temperature), rate constant at any temperature and the activation energy is obtained.

Mechanical stress is introduced into systems by grinding, milling or co-milling the drug or blend. This causes amorphous pockets of drug to be formed or induction of crystal defects that cause an increase in the degradation rate. The increased surface area leads to more intimate interactions of drug with excipient.

Storage of products in high humidity atmospheres accelerates decomposition that result from hydrolysis. Most excipients contain significant amounts of free water or can absorb moisture from the immediate environment. Effect of water on the system can be studied by preparation of slurries or suspensions, adding water into a closed system or exposing the system to controlled humidity. Traditional methods of excipient compatibility testing, involves exposure to controlled humidity using saturated salt solutions 25 °C/60% RH, 30 °C/60% RH or 40 °C/75% RH (FDA guidelines).
5.3.1.2 Analysis

Initial assessment for excipient compatibility involves visual inspection of the samples for colour change and deliquescence. These changes can indicate signs of instability of drug and excipient. Instability is detected by measuring the amount of degradation of the API and HPLC is the most widely used technique in determining and quantifying the degradants. However, it requires the preparation of the sample for extracting the degradant and API from the excipient mixture and also needs significant amount of degradant to be formed that is above the detectable limit.

In DSC and HSDSC, a power differential between reference and sample is measured as a function of temperature. Any change in the melting behaviour between the mixture and the individual components indicates an incompatibility.

5.3.1.3 Kinetics of solid-state reactions

Although solid dosage forms, form a great bulk of pharmaceutical formulations, its kinetics in general are not well understood and present considerable problems. Reactions in the solid-state show complex rate behaviours and cannot always be fitted to a simple kinetic model. Solid-state pharmaceuticals have been widely subjected to kinetic studies to determine shelf-lives and expiry dates (Carstensen, 1974; Lachman, 1965; Carstensen, 2000). Kinetic studies of solid-state reactions involve plotting a graph of fraction decomposed, $\alpha$, versus time, from which rate equations that best describe the data are used. Two typical curves of $\alpha$ versus time are shown in Figure 5.6. The curve on the left has a distinct induction period during which the change of $\alpha$ with $t$ is small. This is then followed by an accelerating period until point $\alpha_{1/2}$ when the rate of reaction is at a maximum, after which the reaction rate slows down until completion. The kinetics of solid-state reactions are described by various equations which are used in analysing $\alpha$ versus $t$ plots.
Figure 5.6: Typical fraction of degradation ($\alpha$) versus time curve showing different stages of the reaction, where I is an induction period, A is accelerating period and D is decelerating period. $\alpha_{1/2}$ represents point of maximum rate. Adapted from Ng, (1975) and Byrn, (1999).

The kinetics of solid-state reactions involving nucleation has been described by the Prout-Tompkins or Avrami-Erofe'ev equations. The Prout-Tompkins equation (Equation 5.13) can be derived, if the rate of a solid-state reaction is assumed to be controlled by linearly growing nuclei that branch into chains and are terminated more rapidly with increasing number of nuclei (Prout and Tompkins, 1944).

$$k.t = \ln\left(\frac{1}{1 - \alpha}\right)$$ \hspace{1cm} \text{Equation 5.13}

If however the rate of a solid-state reaction is assumed to be governed by random nuclei growing in three dimensions and ingest other nuclei, then the Avrami-Erofe'ev equation (Equation 5.14) can be derived (Avrami, 1940; Avrami, 1941; Erofe'ev, 1946).

$$k.t = \left[-\ln(1 - \alpha)\right]^n$$ \hspace{1cm} \text{Equation 5.14}

Rates of solid-state reactions controlled by phase boundaries rather than formation of nuclei are described by one, two or three dimensional advancement of a phase boundary, from the outside of the crystal inwards. If the reaction occurs along one direction of the crystal, then the rate is a function of time only and a zero-order rate equation (Equation 5.15) applies,
In case of two-dimensional advancement of phase boundaries, the reaction is assumed to proceed from the surface of a circular disk or cylinder inwards and Equation 5.16 can be derived (Sharp et al., 1966),

\[ k \cdot t = 1 - (1 - \alpha)^{\frac{1}{2}} \quad \text{Equation 5.16} \]

If the reaction is assumed to proceed from the surface of a sphere inwards in three dimensions (three-dimensional advancement of a phase boundary) then Equation 5.17 may be derived (Sharp et al., 1966),

\[ k \cdot t = 1 - (1 - \alpha)^{\frac{1}{3}} \quad \text{Equation 5.17} \]

Rates of solid-state reactions controlled by diffusion rather than nucleation or phase-boundary advancement can similarly be described by one-dimensional, two-dimensional or three-dimensional diffusions and are given by Equation 5.18 (Sharp et al., 1966), Equation 5.19 (Holt et al., 1962) and Equation 5.20 (Carter, 1961) respectively,

\[ k \cdot t = \alpha^2 \quad \text{Equation 5.18} \]
\[ k \cdot t = (1 - \alpha).\ln(1 - \alpha) + \alpha \quad \text{Equation 5.19} \]
\[ k \cdot t = 1 - \frac{2}{3}.\alpha - (1 - \alpha)^{\frac{2}{3}} \quad \text{Equation 5.20} \]

In order to successfully analyse a solid-state reaction, it is therefore necessary to have knowledge of the physical process by which the reaction proceeds, so that correct equations can be used. However, the Ng equation (Ng, 1975) is claimed to describe all solid-state reactions. Reactions in the solid-state commence at points of defect in the structure, particularly dislocations such as flaws in the crystal lattice. At the site of these dislocations, an interface of reactants and products is formed, which causes a localised increase in stress in the crystal lattice of the solid. This causes more dislocations to be
formed thereby accelerating the rate of reaction. The rate accelerates until the fraction degraded ($\alpha$) reaches $a_{1/2}$ when it is maximum after which it declines until $\alpha = 1$ where the reaction has gone to completion (Figure 5.6). The acceleration period has been attributed to a number of possible events: auto catalytic effect of the solid product phase (Erofeev, 1961); partial melting as a result of dissolved products (Bawn, 1955); additional nucleation from stress caused by the growing nuclei (Herley and Levy, 1972).

When the reaction involves nucleation, the maximum number of nucleation sites is created at $a_{1/2}$. Since the rate of reaction is proportional to the number of reaction sites, the rate from $a_{1/2}$ is dependent on the amount of material left at each nucleation site and hence will start to decline. If the number of nucleation sites are doubled, the rate would also be doubled. Hence the rate of reaction from $t = 0$ to the time when $\alpha = a_{1/2}$ is proportional to the quantity of material reacted ($\alpha$). The rate is thus given by Equation 5.21 and describes the accelerating process. For the period after $a_{1/2}$ (deceleration period) the rate is proportional to the fraction of material left un-reacted at each reaction site. Since no additional reaction sites are being produced, the rate of reaction starts to decline and is described by Equation 5.22.

$$\frac{d\alpha}{dt} = k.\alpha^{1-m}$$  \hspace{1cm} \text{Equation 5.21}

$$\frac{d\alpha}{dt} = k.(1-\alpha)^{1-n}$$  \hspace{1cm} \text{Equation 5.22}

Combining Equations 5.21 and 5.22 gives Equation 5.23, which is known as the Ng equation and can be applied to all solid-state degradation reactions.

$$\frac{d\alpha}{dt} = k.\alpha^{1-m}.(1-\alpha)^{1-n}$$  \hspace{1cm} \text{Equation 5.23}

Where $d\alpha/dt$ is the rate of reaction, $k$ is the rate constant for the reaction (units of s$^{-1}$ for all solid-state reactions schemes), $\alpha$ is the fraction reacted and $m$ and $n$ are the fitting constants for the reaction (not representative of reaction order), indicating the mechanism for the degradation path.
The calorimetric form of the Ng equation has been obtained by Willson, (1995) and given below. As \( \alpha = \frac{x}{A} \), where \( x \) is the quantity of starting material \( (A) \) that has reacted over time, \( \frac{x}{A} \) can be substituted for \( \alpha \) in Equation 5.23.

\[
\frac{dx}{dt} = A \cdot k \left( \frac{x}{A} \right)^m \left( 1 - \frac{x}{A} \right)^n \quad \text{Equation 5.24}
\]

As discussed earlier, \( q = \Delta H \cdot x \), where \( q \) is the heat output for the reaction, \( \Delta H \) is the change in enthalpy for the reaction and \( x \) is the amount of starting material reacted over time. Substituting \( \frac{q}{\Delta H} \) for \( x \) in Equation 5.24 gives,

\[
\frac{dq}{dt} = \Phi = A \cdot k \cdot \Delta H \left( \frac{q}{A \cdot \Delta H} \right)^m \left( 1 - \frac{q}{A \cdot \Delta H} \right)^n \quad \text{Equation 5.25}
\]

Equation 5.25 can be used to fit calorimetric data (\( \Phi \) versus \( q \)) recorded from solid-state reactions. Using an iterative procedure (non-linear curve fitting tool in Origin 7.0) the values of \( k, \Delta H, m \) and \( n \) can be determined. From the values of the parameters \( m \) and \( n \) returned from the fitting process, the type of degradation mechanism can be characterised (Table 5.5).

<table>
<thead>
<tr>
<th>( m )</th>
<th>( n )</th>
<th>( \alpha_{1/2} )</th>
<th>Equation</th>
<th>Character</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>-</td>
<td>( \frac{d\alpha}{dt} = k )</td>
<td>Linear</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>1</td>
<td>( \frac{d\alpha}{dt} = k \cdot \alpha )</td>
<td>Exponential</td>
</tr>
<tr>
<td>0.5</td>
<td>0</td>
<td>1</td>
<td>( \frac{d\alpha}{dt} = k \cdot \alpha^{1/2} )</td>
<td>Square</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>0</td>
<td>( \frac{d\alpha}{dt} = k \cdot (1 - \alpha) )</td>
<td>Unimolecular decay</td>
</tr>
<tr>
<td>0</td>
<td>0.5</td>
<td>0</td>
<td>( \frac{d\alpha}{dt} = k \cdot (1 - \alpha)^{1/2} )</td>
<td>Contracting surface</td>
</tr>
<tr>
<td>0</td>
<td>0.66</td>
<td>0</td>
<td>( \frac{d\alpha}{dt} = k \cdot (1 - \alpha)^{2/3} )</td>
<td>Contracting sphere</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>0.5</td>
<td>( \frac{d\alpha}{dt} = k \cdot \alpha \cdot (1 - \alpha) )</td>
<td>Prout-Tompkins</td>
</tr>
<tr>
<td>0.66</td>
<td>0.66</td>
<td>0.5</td>
<td>( \frac{d\alpha}{dt} = k \cdot \alpha^{2/3} \cdot (1 - \alpha)^{2/3} )</td>
<td>Roginskii-Shultz</td>
</tr>
</tbody>
</table>

\textbf{Table 5.6:} Reaction mechanisms described by the Ng equation for certain values of \( m \) and \( n \) (Willson, 1995).
5.3.1.4 Degradation of aspirin in a binary mixture with magnesium stearate

Aspirin hydrolyse in the presence of water to form salicylic acid and acetic acid (refer Chapter 2 for mechanism of degradation). Incompatibility between aspirin and magnesium stearate has been well documented and it degrades to a number of potentially immunogenic products including salicylic acid, salicylsalicylic acid and acetylsalicylcalicylic acid (Bendgaard and DeWeck, 1975; Reepmeyer and Kirchhoefer, 1979; Mroso et al., 1982; Patel et al., 1982; Taguchi et al., 1981). A number of degradation mechanisms for the incompatibility have been proposed. In general, drug-excipient interactions can occur in different ways: the drug and excipient react with each other, change of pH in the microenvironment due to the presence of the excipient or change in the hygroscopicity of the excipient, causing an increase in the amount of water sorbed by the system (Ahlneck and Lundgren, 1985; Ahlneck et al., 1987).

The presence of impurities such as magnesium oxide is thought to form an alkaline environment, thereby facilitating hydration (Jaminet and Louis, 1968). Mroso et al., (1982) suggested that the addition of magnesium stearate resulted in a lower melting point of aspirin, thereby generating a liquid layer on the outside of the magnesium stearate particles, which accelerated the degradation. This liquid decomposition layer present on the surface of the particle diffuses inwards as the reaction proceeds and the reaction rate is diffusion limited (Jander, 1927; Nelson, 1982). The increased degradation rates of aspirin in mixtures with magnesium stearate are thought to be dependant on a pH effect caused by the dissolution of the magnesium stearate (Ahlneck et al., 1987).

In this study, the compatibility between aspirin and magnesium stearate was investigated using IC and HPLC. In order to increase the amount of interaction, both powder fractions were used in equal proportions (1:1) and had similar particle size fraction. Aspirin was subjected to size reduction using the ball mill. The drug and excipients were studied separately in the TAM, followed by the binary mixtures to assess for any incompatibility. To aid degradations, 100% RH conditions were maintained with the aid of a micro hydrostat tube. Calorimetric studies were supplemented by studying the degradation in a bench experiment and assaying for extent of degradations using HPLC techniques.

The aims of this study were to investigate the drug-excipient compatibility of aspirin with lactose and magnesium stearate using the TAM and compare it with an HPLC study. Secondly, to determine whether reaction parameters for the solid-state degradation could
be obtained from the calorimetric data and compare them with those obtained from the bench experiment.

5.3.2 Materials and Methods

5.3.2.1 Materials

Acetylsalicylic acid (≥99.0%, lot 045K0101) and lactose monohydrate (lot 102K0107) was from Sigma. Magnesium stearate (Technical grade, lot U06963) and potassium chloride (99.99%) were from Aldrich. Acetonitril (lot 0736912), HPLC grade water (lot 0600610), propan-2-ol (99.5+%, lot 0552315) and trifluoroacetic acid (99.0+%, lot 0694969) were from Fisher Scientific. All solutions were prepared using HPLC grade water.

Powder samples of aspirin were subjected to milling using a ball mill (Pulpersette 5) for a period of 1 h. Milled samples were stored in an airtight container until further analysis.

5.3.2.2 Methods

5.3.2.2.1 Sample preparation

Aspirin, lactose and magnesium stearate were classified into various particle sizes by means of standard sieves (mesh size 53, 75, 90 and 125 μm). Particle size fraction used in this study were aspirin (75/125 μm), lactose (53/75μm) and magnesium stearate (75/90 μm).

Binary mixtures were prepared by mixing 50 mg (±0.1 mg) of aspirin with an equal weight of either magnesium stearate or lactose. The drug and excipient were weighed directly into a 3ml TAM ampoule and the powders were mixed with a spatula for about 30 s to ensure thorough mixing. Precautions were taken to prevent any loss of material or excess adherence to the walls of the glass ampoule. Micro hydrostat tubes filled with 0.2 ml of either saturated potassium chloride solution or water were inserted into the ampoule to obtain a RH of 82 and 100% respectively. An airtight enclosure was ensured by sealing it with an aluminium lid having a rubber seal on the inside or with parafilm. The ampoules
sealed with parafilm were placed in an oven maintained at 37 °C. Sufficient binary mixtures were prepared to allow daily analysis for 10 days. Bench experiments were run in triplicate.

5.3.2.2.2 HPLC

Binary systems studied were analysed by HPLC after storage in controlled temperature ovens (37 °C). The binary mixtures were placed in 3 ml TAM ampoules that were sealed with parafilm to prevent moisture loss and depict conditions in a TAM ampoule experiment. The ampoules were removed after 24, 48, 72, 96, 120, 144, 168, 192, 216 and 240 h and assayed for aspirin content by HPLC.

Sample analysis

Samples were assayed for aspirin content using a previously validated reverse phase/isocratic method (Fogel et al., 1984). The HPLC assays were carried out using a Hewlett-Packard HP1050 system (Hewlett-Packard, Waldbronn, Germany). The system consisted of an HP 1050 autosampler, an HP 1050 pump and HP 1050 multiple wavelength detector system. The detector was interfaced with a computer having the PC/Chrome software (H & A Scientific Inc. Greenville, NC, USA).

Sample preparation

For HPLC analysis, the samples were prepared by carefully transferring all powder mixtures into a 50 ml volumetric flask and rinsing out the ampoules with extraction solvent [acetonitrile:water:phosphoric acid (50:50:0.5)]. The washings were added to the volumetric flask and volume made up with the extraction solvent. The flask was sonicated for 2 min and then shaken briefly to enable complete dissolution. A portion of the solution mixture was filtered using a Millipore filter (0.22 μm) and transferred into a 2 ml glass vial.

Chromatographic conditions

The mobile phase consisted of water-acetonitrile-phosphoric acid (76:24:0.5). The chromatographic column was X Terra® RP18, 4.6 x 100 mm (Waters). Other details were Injection volume: 10 μL; Flow rate: 1 ml/min; Assay wavelength 295 nm; Pressure: 2 bar and column temperature 40 °C. In these conditions, the retention time of aspirin was 3.3 min.
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**Calibration of HPLC assay method**

A stock solution of aspirin was prepared by dissolving 0.2000 g of pure aspirin (= 2 mg/ml) in 100 ml of the extracting solution. Standard solutions containing 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mg/ml were then prepared from the stock solution by taking appropriate aliquots of stock solution and diluting with the extracting solution. These were repeated five times. The standard solutions were injected using the above conditions and from the peak areas obtained, a five-point calibration curve was plotted.

**Validation of HPLC assay method**

The assay was evaluated for accuracy, detection limit, linearity and stability. Accuracy was expressed as percent recovery of aspirin and it was found to be 99%. Linearity was tested at six concentrations (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mg/ml) and it was found to be \( r^2 = 0.9998 \). Limit of detection (LOD) was found to be 0.05 mg/ml. The aspirin solution in mobile phase exhibited no chromatographic or spectrophotometric changes for 10 h when stored at room temperature. No interference peaks were observed.

### 5.3.2.2.3 TAM

Calorimetric experiments were conducted using a 2277 TAM (Thermometric AB, Järfälla, Sweden) at 37 °C. Samples were prepared in a similar way to those prepared for HPLC analysis. RH of 82 or 100% in the ampoules was obtained by inserting micro hydrostat tubes filled with 0.2 ml of saturated potassium chloride or water respectively. The ampoules were sealed with a crimped metal lid and an air-tight enclosure was ensured with the use of a rubber seal on the inside of the lid. Reference ampoules contained an equivalent weight of talc. Sample and reference ampoules were placed in the thermal equilibrium position of the TAM for approximately 30 min before being lowered into the measurement position. Data capture was initiated using the dedicated software Digitam 4.1. Power data (μW) were recorded every 30 s with an amplifier setting of 100 μW. Samples were run at least in duplicate. At the end of the TAM experiment, the samples were subjected to HPLC analysis to determine the extent of degradation.
5.3.3 Results and discussion

Initial experiments were carried out on pure drug and pure excipient at 0 and 100% RH at 37 °C. This was followed by study on binary mixture (1:1) of aspirin and lactose or magnesium stearate. Bench experiments were carried out followed by observation of possible drug-excipient interaction in the TAM. Samples were assayed using HPLC to determine aspirin content.

5.3.3.1 Aspirin at 0 and 100 % RH

The power time data obtained for the degradation of aspirin at 0 and 100 % RH are shown in Figure 5.7. The power signal settled to a constant value within 5 h. This was the time taken for the sample to attain thermodynamic equilibrium and in case of the sample maintained at 100 % RH, it was the time taken for the headspace to attain vapour saturation. The power signal for the aspirin sample at 0 % RH conditions remained flat and was zero for about six days. The aspirin sample maintained at 100 % RH had a significantly noisier power signal, which was caused by the constant vaporisation and condensation of water from the micro hydrostat tube. Samples removed from the TAM were subjected to HPLC analysis to determine aspirin content. No significant degradations were observed even after 10 days (<2%).

![Figure 5.7: Power-time data for the degradation of 0.0500 g of aspirin at 37 °C under 0 and 100 % RH.](image-url)
5.3.3.2 Magnesium stearate at 0 and 100 % RH

The power-time curve for magnesium stearate at 0 % RH is essentially flat after about 6 h. The initial value could be due to frictional heat from sample loading and thermal equilibration of the sample. However, significant heat flow was observed when the sample was placed at 100 % RH conditions (Figure 5.8). Magnesium stearate is classified as non-hygroscopic (Callahan et al., 1982), however it is known to exist as four different pseudopolymorphs: as an anhydrous form, a monohydrate, a lamellar dihydrate and needle-like trihydrate (Muller, 1977; Miller, 1985; Ertel and Carstensen, 1988; Rowe et al., 2003).

The hydrated forms are relatively stable in the presence of moisture, while the anhydrous form adsorbs moisture at RH up to 50% and is present as adsorbed surface moisture. However, when the RH exceeded 50%, the anhydrate rehydrated to form a trihydrate (Rajala and Laine, 1995). This rehydration in the presence of excess moisture is shown by the exothermic peak in the power-time curve (Figure 5.8). The initial exothermic power lasted for about 24 h, which was due to the rehydration of the upper portion of the powder bed. The movement of moisture from the upper layers to the lower layers is diffusion controlled and depicted by the small but gradual decrease in the power signal that lasted for approximately 40 h.

5.3.3.3 Lactose at 0 and 100% RH

The power-time data for lactose monohydrate (0% RH) was flat and is shown in Figure 5.9. There was slight deflection in the power signal for the lactose maintained at 100% RH conditions, but was stable after 24 h.

5.3.3.4 Aspirin-lactose binary mixture at 100 % RH

Initial TAM studies on a binary mixture of aspirin and lactose (1:1 ratio) in 100% RH gave no significant heat flow signal (Figure 5.10). The power-time curve was flat for about 50 h after the initial heat flow (caused due to friction of loading), which lasted for about 5 h. HPLC assay on the amount of aspirin degraded after the end of the TAM
experiment was negligible (< 1%) and showed that Lactose does not cause any excess aspirin degradation when present in the binary mixture.

**Figure 5.8:** Power-time curve for 0.0500 g magnesium stearate at 0 and 100 % RH and 37 °C.

**Figure 5.9:** Power-time data for 0.0500 g lactose at 0 and 100 % RH and 37 °C.
5.3.3.5 Aspirin-Magnesium stearate (1:1) binary mixture at 0% RH and 37 °C.

Power-time curves for a 1:1 binary mixture of aspirin and magnesium stearate at 0% RH conditions and 37 °C is shown in Figure 5.11. The power signal value was constant after approximately 8 h. The small initial exothermic heat flow was due to the friction of ampoule loading and thermal equilibration. After about 10 h the power signal returned to zero, indicating that there was no significant interaction between aspirin and magnesium stearate in the binary mixture for the duration of the experiment. HPLC analysis for the aspirin content in the mixture after the TAM experiment showed no significant degradation (<5%). Moreover, a bench experiment conducted with the binary mixture under similar conditions showed similar aspirin content after 10 days, when analysed by HPLC (<5%).
5.3.3.6 Aspirin-Magnesium stearate (1:1) binary mixture at 100% RH and 37 °C.

Aspirin-magnesium stearate compatibility studies were carried out on a 1:1 drug:excipient ratio (50% w/w), to maximise degradation in the shortest time. Binary mixtures were prepared by accurately weighing the powders directly into the ampoule and physically mixing with spatula. This minimises the variations that are likely to occur if sampling was done from a bulk mixture.

Aspirin (1.40 g/cm³) has a higher true density than magnesium stearate (1.092 g/cm³) and hence in a binary mixture, a higher percentage of aspirin is likely to occur in the lower portion of a powder bed, leading to non-homogeneity. This invariably causes errors in the proportions of the two powders if the individual binary samples were weighed out from a bulk mixture. Hence, weighing out individual powders and mixing them in the final containers, reduces errors caused by non-uniform distribution of the powders in the mixture.

For this study, the mechanism of aspirin degradation when present in a binary mixture with magnesium stearate and small amounts of water is proposed as follows. After mixing, magnesium stearate forms a powder coat around the aspirin. The binary powder
bed in the ampoule is thought to have a greater proportion of aspirin in the lower part while the upper portion has a higher proportion of magnesium stearate because of different densities. After sealing the ampoule containing the micro hydrostat, the headspace above the mixture gets saturated with vapours evaporated from the micro hydrostat. At saturation point the vapours begin to condense and are actively sorbed by magnesium stearate which gets hydrated (Rajala and Laine, 1995), the rate being diffusion controlled.

The unbound water from the hydrated magnesium stearate can then attach itself to the aspirin molecule (Figure 5.12). The degradation of aspirin begins at the surface active sites such as surface defects (edges, ridges etc). The amount of water available to the mixture increases with time, thereby increasing the reaction rate. Once degraded, the water molecules are replaced by acetic acid molecule (Figure 5.12 C). Thus, after a certain amount of time the amount of acetic acid generated increases, thereby reducing the pH in the microenvironment. The decrease in the pH has a synergistic effect on the reaction rate (seen later in calorimetric data as sharp rise in power signal). The degradation rate increases rapidly until all the water is available in the mixture and has a maximum number of reaction sites. From that point, the amount of aspirin remaining forms the limiting factor and the degradation continues until all the aspirin has degraded.

**Figure 5.12:** Model for aspirin degradation in the presence of magnesium stearate (MGS). A — binary mixture of aspirin and MGS. B — the presence of water that forms a film layer around aspirin. C — the hydrolysis of aspirin to salicylic acid (SA) and replacement of water with a molecule of acetic acid (AA).
Chapter 5 – IC in Preformulation

Binary aspirin-magnesium stearate mixtures (1:1) maintained at 37 °C were analysed for aspirin content at 0, 24, 48, 72, 96, 120, 144, 168, 192, 216 and 240 h using HPLC. Ampoules containing the binary mixture were removed from the oven and assayed immediately. No interference in the peaks was observed and aspirin eluted out after 3.3 min (Figure 5.13). From the peak areas obtained, the amount of aspirin left in the mixture was calculated with the help of a calibration curve. A plot of percentage degraded versus time was sigmoid (Figure 5.14).

A reduced induction period or lag phase was observed under these experimental conditions. Increased surface area of aspirin molecule (by size reduction) leads to the generation a larger number of active sites that aid degradation. Hence, there are a larger number of active reaction sites. Water from the micro hydrostat gains sufficient kinetic energy to vaporise and fill the air space above the aspirin mixture. The vapours are rapidly adsorbed by the anhydrous magnesium stearate present on the surface of the powder bed and are hydrated to form the trihydrate.

![Figure 5.13: Typical HPLC chromatogram of aspirin (1) and magnesium stearate binary mixture at 37 °C/100% RH and the degraded product salicylic acid (3)](image-url)
The degradation rate was limited by the amount of moisture available for the reaction. With the increase in available water, the number of reaction sites increased leading to an increase in the reaction rates. This was seen with the sharp rise in the degradation, from 48 – 96 h when 50% of the drug had degraded. At this point, the number of active sites was at a maximum and the amount of drug remaining was the limiting factor. The degradation then decelerated until it reached completion. The reaction was studied for a period of 10 days where 85 % of degradation had occurred. Figure 5.15 is a plot of concentration of aspirin remaining as a function of time.

From the concentration-time curve, there appears to be a change in the reaction mechanism after 48 h. The solid-state degradation of aspirin in the binary mixture appears to follow zero-order kinetics for the first 48 h, followed by possible first-order kinetics. A plot of concentration versus time for the first three data points (0, 24 and 72 h) was linear, from which a zero-order rate constant of $4.22 \times 10^{-3} \text{ s}^{-1}$ was obtained. A $\ln$(concentration)-time plot (Figure 5.16) was linear from 72 h, which suggests that the degradation of aspirin followed first-order kinetics. Application of linear regression analysis resulted in a first-order rate constant of $2.51 \times 10^{-6} \text{ s}^{-1}$. 
Figure 5.15: Concentration-time curve for the degradation of aspirin (50 mg) in a 1:1 binary mixture containing magnesium stearate at 37 °C and 100% RH.

Figure 5.16: ln(concentration)-time data for the degradation of aspirin (50 mg) in a 1:1 binary mixture with magnesium stearate at 37 °C and 100% RH and the fit line (—) generated by application of linear regression analysis.
The physical nature of the binary mixture was studied on the bench experiment sample maintained at 37 °C and 100% RH. The powder mixture was free flowing and was white in appearance when prepared. The free-flowing nature was seen until day two, after which the powder mixture formed a semisolid mass, which increased in compactness with time. Samples observed on day 10 were light pink in colour and appeared to be a sticky mass which appeared to have partially melted.

Degradation of aspirin in a 1:1 binary mixture with magnesium stearate was studied in the TAM at 37 °C under 100% RH conditions. A representative power-time data obtained from the TAM is shown in Figure 5.17. From the power-time data it is clearly evident that there is a change in reaction mechanism after approximately 90 h.

Heat is ubiquitous and as mentioned in Chapter 1 the heat flow recorded by the calorimeter is a sum of the heat flows for all processes occurring at a given time. For the degradation of aspirin in the binary mixture a number of thermal events occur, all contributing to the overall recorded power signal. Water present in the micro hydrostat tube absorbed heat, which increased its kinetic energy and lead to evaporation (endothermic event). The vapour formed collected in the space above the powder mixture and after reaching saturation point, began to condense, with the release of heat energy. Anhydrous magnesium stearate is hygroscopic under 100% RH conditions and rehydrates to form the trihydrate (Ertel and Carstensen, 1988; Ertel and Carstensen, 1988a). This is an exothermic process contributing to the overall power signal recorded. Studies carried out in the calorimeter on magnesium stearate at 100% RH, showed that the exothermic heat flow lasted for about 20 h.

The observed calorimetric power-time curve is a sum of all the instantaneous heat flows for all the processes. After an initial exothermic peak that lasted for approximately 24 h, there was a gradual increase in the recorded power value until about 55 h. The power value then increased more rapidly until there was a change in reaction mechanism at approximately 90 h, after which there was a decrease in the power.
A sequence of events can be related to the observed thermal heat flow. Initial thermal events were a result of evaporation of water from the micro hydrostat tube. This movement of water from the micro hydrostat to the powder bed was observed on the bench experiment. It was found that the transfer by evaporation and condensation was complete by day 4 (96 h). Evaporation is an endothermic event and should have had a negative power value had the thermal event been recorded exclusively for the process. Condensation occurs after the vapour reaches saturation point and should have had the same magnitude of power (reverse phenomena of evaporation) with the sign reversed, as it is an exothermic process.

The initially observed exothermic peak was mainly contributed by the rehydration of anhydrous magnesium stearate that occurred for approximately 20 h. The increase in the power from 24 to 90 h ('accelerating phase') can be attributed to a number of thermal events, which include initial adsorption of water on the surface of the aspirin molecule followed by dissolution, which is an exothermic process. The rate at which this occurred, increased with the increase in the available water, which could explain the increasing
power value. This happens until the water initially present in the micro hydrostat is available to the powder mixture. At this point, there is the maximum number of active degradation sites on the aspirin molecule and any further degradation is limited to the amount of aspirin available. Thereafter the power signal drops as the reaction rate for the hydrolysis of aspirin is dependent on the quantity of material left at each nucleation site that can react.

The kinetics of both the accelerating and deceleration phases was different, as it involved different amounts of moisture. In the acceleration phase, moisture is limiting while in the deceleration phase the amount of aspirin is the limiting value. Water adsorbed by the powder mixture increases and is at a maximum at approximately 90 h. From this point onwards, water is in excess and the degradation reaction is effectively pseudo first-order.

The calorimetric data up to 90 h represents the acceleration period of the solid-state degradation of aspirin in the binary mixture with magnesium stearate. Calorimetric data (\(\Phi\) versus \(q\)) that represents the acceleration period were fitted to Equation 5.25 using Origin 7.0 (non-linear curve fitting tool), to obtain reaction parameters and fitting parameters. As the constitution of the binary mixture changed with increasing moisture content, data representing 0-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, and 80-90 h were fitted to obtain reaction parameters. Data from 0-10 and 10-20 h could not be fitted and is thought to involve thermodynamic and humidity equilibration. Fitting data from 20-90 h returned reaction parameters summarised in Table 5.7.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Rate constant (s(^{-1}))</th>
<th>Enthalpy change (kJ mol(^{-1}))</th>
<th>(m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-40</td>
<td>2.11 (\times) 10(^{-6})</td>
<td>24.38</td>
<td>0.17</td>
</tr>
<tr>
<td>40-50</td>
<td>7.42 (\times) 10(^{-6})</td>
<td>32.88</td>
<td>0.34</td>
</tr>
<tr>
<td>50-60</td>
<td>9.75 (\times) 10(^{-6})</td>
<td>30.68</td>
<td>0.37</td>
</tr>
<tr>
<td>60-70</td>
<td>1.40 (\times) 10(^{-5})</td>
<td>37.68</td>
<td>0.38</td>
</tr>
<tr>
<td>70-80</td>
<td>2.00 (\times) 10(^{-5})</td>
<td>34.12</td>
<td>0.43</td>
</tr>
<tr>
<td>80-90</td>
<td>5.88 (\times) 10(^{-6})</td>
<td>37.17</td>
<td>0.29</td>
</tr>
</tbody>
</table>

**Table 5.7:** Reaction parameters for the degradation of aspirin present in a binary mixture (1:1) with magnesium stearate at 37 °C and 100% RH by application of Equation 5.25.
Rate constants recovered from the fitting procedure are shown to increase with time. This is because of the increasing number of reaction sites being available and the increasing amount of moisture being available to the aspirin. The reaction enthalpy obtained from the fitting procedure were however similar.

Calorimetric data representing the deceleration period however were not fitted by Equation 5.25. This might suggest that the degradation did not follow solid-state kinetics. A ln(power)-time plot was linear from approximately 90 h, suggesting that the reaction was first-order. Data were fitted to linear regression analysis and the values of rate constants obtained are given in Table 5.8. The power-time data that represents the decelerating phase were fitted to a single-step first-order kinetic equation (Equation 5.11). The rate constant was kept fixed while the enthalpy change and initial concentration were allowed to vary until a good fit was obtained. The data resulted in a good fit and returned an average enthalpy change of 32.94 kJ mol\(^{-1}\). The average first-order rate constant of 2.26 x 10\(^{-5}\) s\(^{-1}\) was similar to those obtained by Angberg et al., (1990) (1.05 x 10\(^{-5}\) s\(^{-1}\) at 40°C, acetate buffer pH 3.8).

![ln(power)-time data](image)
<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Rate constant (s(^{-1}))</th>
<th>Calculated pH(^{c})</th>
</tr>
</thead>
<tbody>
<tr>
<td>95-100</td>
<td>1.59 (\times 10^{-5})</td>
<td>2.46</td>
</tr>
<tr>
<td>100-110</td>
<td>1.59 (\times 10^{-5})</td>
<td>2.46</td>
</tr>
<tr>
<td>110-120</td>
<td>2.00 (\times 10^{-5})</td>
<td>2.45</td>
</tr>
<tr>
<td>120-130</td>
<td>1.76 (\times 10^{-5})</td>
<td>2.44</td>
</tr>
<tr>
<td>130-140</td>
<td>2.13 (\times 10^{-5})</td>
<td>2.42</td>
</tr>
<tr>
<td>140-150</td>
<td>-</td>
<td>2.41</td>
</tr>
<tr>
<td>150-160</td>
<td>-</td>
<td>2.40</td>
</tr>
<tr>
<td>160-170</td>
<td>3.12 (\times 10^{-5})</td>
<td>2.39</td>
</tr>
<tr>
<td>170-180</td>
<td>3.61 (\times 10^{-5})</td>
<td>2.38</td>
</tr>
</tbody>
</table>

**Table 5.8:** First-order rate constants for the degradation of aspirin present in a binary mixture (1:1) with magnesium stearate at 37 °C and 100% RH obtained by application of linear regression analysis.

\(^{d}\) from ln\(\text{inverse(power)}\)-time data. \(^{e}\) calculated based on amount of acetic acid formed during degradation of aspirin.

**Figure 5.19:** Power-time data for the degradation of aspirin (50 mg) present in a 1:1 binary mixture with magnesium stearate at 37 °C and 100% RH and the fit line (--) generated by application of Equation 5.11.
The rate constants were seen to increase with time and can be attributed to the increasing amounts of acetic acid being produced. This invariably would cause a decrease in the pH value and hence increased degradation. The pH of the micro environment cannot be measured but theoretical estimates were calculated. A number of parameters control the pH of the adsorbed water layer; the generation of acetic and salicylic acid which gradually decreases the pH. Rate constants for calorimetric data between 140 – 160 h could not be analysed, as the power values were low and could not be distinguished from instrumental noise.

5.3.4 Conclusions

This study demonstrates the potential of using IC in excipient compatibility studies under certain circumstances. The advantage is that, no special sample preparation is required and the non-invasive nature of the study makes it an ideal asset in studying pharmaceuticals. Chemical degradations in pharmaceuticals are accompanied with heat flow, which can be detected by IC. Since calorimetry is a non-specific technique and heat being ubiquitous, the power signal recorded is a sum of all thermal events occurring and hence other analytical techniques are necessary to confirm calorimetric data. It is however difficult to predict whether any physical processes contributed to the heat flow recorded and the proportions that each process (physical transformation and chemical degradation) contribute to the overall power needs to be investigated.

It was observed that aspirin degrades in the presence of magnesium stearate and humidity. There was no significant degradation observed for aspirin itself (0 and 100% RH) and in a binary mixture with magnesium stearate at 0% RH. This shows that moisture is required to initiate the degradation of aspirin, when present in a binary mixture with magnesium stearate.

Aspirin undergoes solid-state degradation when small amounts of water are present. Fitting calorimetric data to the Ng equation allowed for the recovery of reaction parameters. The reaction rate increased as the amount of water available to the mixture increased. This was seen as a gradual increase in the power signal recorded. Degradation of aspirin produces acetic acid which dissolves in the liquid film around the aspirin molecule. This causes the pH of the microenvironment to get progressively acidic with
time, which in turn increases the degradation rate. This was observed as a sharp rise in the power-time data recorded (approximately 60 h).

There is a change in reaction mechanism at $\alpha_{1/2}$, the point of maximum degradation. During this period the calorimetric data, neither fits solid-state nor solution phase kinetics. Degradation of aspirin in the deceleration period follows solution phase kinetics and fitting power-time data to a first-order kinetic model allowed for the recovery of reaction parameters. The rate constant obtained were similar to those recorded for solution phase reactions.

5.4 Summary

In this chapter, the use of IC as an analytical tool in the preformulation stage of drug development in certain circumstances has been investigated. In conjunction with other techniques, IC is a powerful alternative to conventional techniques of stability testing.

The use of IC in purity determination in certain instances has been demonstrated. No mechanistic information is obtained from calorimetric data and it is necessary to perform ancillary measurements to identify the impurity. IC can be used to accurately determine percent purities below 97%. However, its inability in distinguishing greater percent purities is due to the long sample preparation and TAM equilibration time (typically 30-60 min), during which samples with small initial differences all attain similar equilibrium state.

Stability assessment in solid-state is extremely complicated and are dependent on many factors. The application of IC in screening drug-excipient compatibility is rapidly gaining popularity in the pharmaceutical arena. It was possible to study the incompatibility of magnesium stearate with aspirin. Accelerated stability assessment confirms that the presence of moisture is essential for the increased degradation of aspirin in a binary mixture with magnesium stearate. A change in reaction mechanism was observed when the amount of water increased. At low water concentrations, the reaction followed solid-state kinetics but changed to solution phase when the amount of water increased.
Chapter 6

CONCLUSIONS AND FUTURE WORK
CONCLUSIONS

The work presented in this thesis shows the usefulness of using IC in stability assessment. As heat is a universal indicator of a chemical or physical change, its measurement can throw significant light into studying the change. IC is capable of measuring such small heat changes associated when a drug substance undergoes change. Until recently, IC has not been actively used as an analytical tool mainly because of the inability to successfully analyse calorimetric data recorded for complex reactions. The development of kinetic models and the process of iteration to analyse IC data has been a significant advancement in the field of calorimetry. The usefulness of kinetic modelling of calorimetric data has been tested using simulated data but not been widely used in testing real data.

It was the aim of this thesis to apply actively these models to calorimetric data recorded for real systems. Kinetic modelling has been applied to model degradation systems. Initially simple solution phase systems that involved single step degradations were studied. Calorimetric data for the degradation of aspirin in solution was successfully analysed using kinetic models. Rate constants of $2.8 \times 10^6$ and $5.3 \times 10^6$ s$^{-1}$ were obtained for the degradation in 0.1 M HCl and citrate buffer (pH 5.0) at 25 °C respectively. It was found that considerably shorter durations of calorimetric experimental time was needed to obtain reaction parameters compared to conventional techniques. Even with just 5 h (following equilibration) of calorimetric data, rate constants and reaction enthalpies could be accurately determined for degradations at 25 °C. This minimum observation period reduced to 1 h and 30 m for degradation studied at 40 and 50 °C respectively. This has great potential for its use in stability testing of pharmaceuticals. Moreover the sensitivities of modern calorimeters is such that there is no need for accelerated stability testing as it is sensitive to measure degradations at storage conditions. The use of Arrhenius relationship in predicting rate constants at room temperature is based on the assumption that reaction mechanism remains the same. In reality, this might not be the case, as new degradation pathways are forced, which would normally not occur at storage temperatures. This could cause an increased rate constant to be recorded and a shorter shelf-life to be predicted, when in fact the drug would have had a longer life. Application of an Arrhenius plot to high temperature data and extrapolation to storage temperature showed significant deviation. The extrapolated rate constant of $1.6 \times 10^6$ s$^{-1}$ differed from that actually measured at 25 °C and such data should be treated with caution.
A study of parallel reactions (binary mixture of parabens) in the calorimeter was successfully carried out. The kinetic models were sensitive enough to differentiate between the two reactions. Data analysis using a kinetic model successfully returned rate constants of $2.2 \times 10^{-4}$ and $8.0 \times 10^{-3}$ s$^{-1}$ and reaction enthalpies of -58.2 and -54.4 kJ mol$^{-1}$ for MP and PP present in the binary mixture. However, when reaction parameters were almost similar, the rate constant of one needed to be twice the magnitude of the other to be successfully resolved. This was because of the inherent noise in the data recorded and significant improvements in the baseline stability of the TAM would ameliorate the problem.

The usefulness of kinetic modelling in resolving complex data obtained for a two-step consecutive reactions (the hydrolysis of potassium hydroxylamine disulfonate) has been successfully demonstrated. Rate constants of $1.16 \times 10^{-4}$ and $2.01 \times 10^{-3}$ s$^{-1}$ and enthalpy change of -95.06 and 28.96 kJ mol$^{-1}$ were obtained for the individual steps. In the absence of any knowledge of reaction mechanisms, it has been stated that the ideal approach is to successively fit the data to increasingly complex models until a good fit is obtained with the least variables (Gaisford, 1999). This however can be a time consuming process for complex reactions like consecutive reactions. The use of chemometric methods for analysing calorimetric data is a significant development. Chemometrics is a model free method of data analysis and the only criteria is that there is sufficient variability among the data. Subjecting calorimetric data recorded for a two-step consecutive reaction to chemometric analysis successfully showed the likelihood of two events that were occurring in the system. Moreover, it successfully resolved the data into the individual patterns. This allowed the resolved data to be analysed for reaction parameters. Rate constants of $1.63 \times 10^{-4}$ and $8.0 \times 10^{-4}$ s$^{-1}$ were obtained for the first and second step respectively. Analysing the resolved data returned accurate kinetic data (within error limits) but thermodynamic data could not be obtained. This is because the relationship between the chemometric intensity and calorimetric power needs to be deciphered.

IC has been successfully used to analyse reactions with a moderate rate and significant reaction enthalpy. However, studying slow reactions with small enthalpy change can be a challenging task. The calorimetric power signal is a function of the reaction rate, the enthalpy change and the amount of material available to react. To analyse successfully calorimetric data recorded for slow reactions that are accompanied with a negligible
enthalpy change, there is a minimum amount of material that is required that will produce a power signal greater than the instrumental noise. A table with a range of minimum reaction parameters that are desired in order to observe an analysable power signal was generated. Such a table would instantly determine whether a slow reaction can be successfully analysed calorimetrically and would give an idea as to the minimum amount of material required. For instance, a reaction that has a reaction enthalpy of 10 kJ mol\(^{-1}\) and a maximum solubility profile of 0.05 M would need a minimum rate constant of \(3.47 \times 10^{-7}\) s\(^{-1}\) to be successfully analysed. This can be achieved by studying the reaction at a temperature where comparable kinetic rates might be achievable. Alternatively if the reaction rates and enthalpy change are known then the amount of material required to produce an analysable power signal can be determined.

The use of IC in the pre-formulation stage of new drug development has been demonstrated. IC was used to determine the purity of aspirin containing varying amount of its degradant product, salicylic acid. It was found that the purity of aspirin could be successfully analysed when it was lower than 97 %. Samples of purity greater than 97 % could not be accurately measured and was attributed to the design of a calorimetric experiment where no meaningful data is recorded for the first couple of hours. The fact that samples of marginally different purity (98 – 100 %) would all attain a similar equilibrium in that time frame, renders the analysis ineffective. Another important pre-formulation test, excipient compatibility was demonstrated. The use of IC in studying incompatibility between aspirin and magnesium stearate was compared to HPLC. IC can be used as a fast and accurate analytical tool in determining compatibility. Differences in the power-time data for mixtures of drug and excipient compared to the individual components is an indication of incompatibility. When an incompatibility is identified, the excipient is normally eliminated from the formulation and no further studies are carried out to determine the extent of incompatibility. This is because of the huge amount of time and resources that is needed to generate any meaningful data using existing techniques. However, IC has been used to demonstrate its applicability in studying the kinetics involved in compatibility. The techniques used in this thesis have demonstrated the applicability of using IC in stability testing of some pharmaceutical model systems.

Pharmaceuticals are multi-component systems and degradations involving processes that are more complex needs to be studied. Model systems that depict simultaneous parallel and consecutive reactions must be tested for recovery of rate constants and reaction enthalpies for the individual steps of each process. Analysis of tertiary and quaternary
mixtures and the ability of kinetic modelling to recover reaction parameters can be assessed. Moreover, calorimetric data recorded for these multi process degradation schemes should be subjected to chemometric analysis, which would give an insight into the number of underlying processes. The deconvoluted chemometric output can then be tested to obtain kinetic parameters. The relationship between calorimetric power and chemometric intensity need to be deciphered. In this project, chemometrics was used in determining the number of steps in a consecutive reaction, but has not been tested when there are two or more degradation schemes (parallel and consecutive). The applicability of chemometrics to analyse calorimetric data opens the door to complexity especially in solid-state degradations. Degradations in the solid-state are complex and not well understood and could involve a number of chemical and/or physical processes. Chemometrics could play an important role in understanding these various processes that occur and its use in differentiating chemical degradations from physical transformations needs to be tested.
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Stability assessment of pharmaceuticals by isothermal calorimetry: two component systems


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Abstract

Isothermal calorimetry offers the potential to determine rapidly the stability of formulated pharmaceuticals because it is indifferent to physical form and sensitive enough to detect extremely small powers; ca. 50 nW at 25 °C. However, its use in this area is not widespread, principally because the power–time data obtained often comprise contributions from more than one process and are thus difficult to analyse quantitatively. In this work, we demonstrate how power–time data recorded for systems in which two components are degrading in parallel (in this case, binary mixtures of selected parabens) can be analysed using a kinetic-based model; the methodology allows the determination of the first-order rate constant and reaction enthalpy for each process, so long as one rate constant is at least twice the magnitude of the other. It was found that the reactions did not need to run to completion in order for the analysis to be successful; a minimum of 15 min of data were required for samples with one degrading component and a minimum of 4 h of data were required for samples with two degrading components. It was observed that the rate constants for paraben degradation in binary systems were significantly lower than expected. This was ascribed to the fact that the parabens degrade to a common product and is an important factor that should be accounted for when the two or more parabens are formulated together.

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Keywords: Isothermal calorimetry; Microcalorimetry; Degradation kinetics; Stability assessment; Methyl paraben; Ethyl paraben; Propyl paraben

1. Introduction

An assessment of the long-term stability of an active pharmaceutical ingredient (API), or the likelihood of any active-exciipient incompatibility, is an important part of the formulation process of a medicine. Isothermal calorimetry (IC, the measurement of power as a sample is maintained at constant temperature) is ideally suited for stability assessment of pharmaceuticals for a number of reasons; it is indifferent to the physical form, or heterogeneity, of a sample; the data contain both kinetic and thermodynamic information on
the process(es) under investigation; samples are studied non-destructively (that is, the calorimeter causes no additional degradation other than that which would have occurred anyway upon storage); and the sensitivity of modern instruments is such that, in principle, reactions with a first-order rate constant of \(1 \times 10^{-11} \text{ s}^{-1}\) (corresponding to 0.03% degradation per year) can be detected directly at 25 °C (Willson, 1995).

Pikal (1983) first showed the correlation between the exothermic power output of some pharmaceutical systems and their known degradation rates, showing that degradation rates of the order of 2% per year were easily quantified. Subsequently, IC has been used to investigate the stability of a number of APIs alone (Oliyai and Lindenbaum, 1991; Angberg et al., 1993; Willson et al., 1995a; Hansen et al., 1990; Pikal and Dellerman, 1989) and a number of approaches have been suggested that allow qualitative screens of API-excipient compatibility (Phipps et al., 1998; Schmitt et al., 2001).

However, since heat is a universal indicator of chemical change the data obtained from IC, especially for multi-component systems, are often complex, because the calorimeter records the power changes from all the processes occurring in the sample simultaneously (indeed, care must be taken to ensure that erroneous or unexpected powers do not arise simply as a consequence of poor experimental design or sample handling) and a lack of data analysis routines has limited its more widespread application. The analytical challenge, therefore, is to be able to analyse such complex data, with no prior knowledge of the number or type of reaction processes, and to deconvolute them into their constituent parts.

One promising approach to meet this challenge is to fit IC data to models based on reaction kinetics using an iterative procedure (Willson et al., 1995a,b, 1996). This returns rate constants \((k)\) and reaction enthalpies \((\Delta H)\) for each individual reaction step and, in theory, does not require any prior knowledge of reaction mechanism (a range of models is used to fit the data and that which gives the best statistical fit, with the fewest variables, is selected. However, if, as in this case, the reaction mechanism is known (or suspected) then the specific model can be used; see below for further discussion of this point). Importantly, this method does not require the reaction under study to progress to completion (depending upon the reaction parameters, approximately 24 h of power–time data are usually sufficient to allow analysis, although this may need to be extended for reactions that progress slowly or with a small change in enthalpy). We have shown previously how this approach can be used to study: (i) a degradation reaction with three consecutive, first-order steps (Gaisford et al., 1999) and (ii) a second-order hydrolysis (that is now recommended as a chemical test reaction for calorimeters; Beezer et al., 2001a). However, these studies had only one degrading component. A typical formulated pharmaceutical may well have several independently degrading components and, although the degradation kinetics of the individual components of a medicine may be known, their behaviour in combination may be significantly different.

The applicability of IC to the quantitative analysis of systems containing more than one degrading material has not been reported and is the focus of this work. Of particular interest is the ability of the models to determine the number of degrading components in a complex system and the minimum number of data needed to effect an accurate analysis. Kinetic models have been used to analyse power–time data for two-component systems; aqueous solutions containing binary mixtures of selected parabens. The parabens were selected for study because their degradation kinetics are known (Kamada et al., 1973) (more recently, the base-catalysed hydrolysis of methyl paraben (MP) has been suggested as a test reaction for flow microcalorimeters; O'Neill et al., 2003); they can degrade through consecutive steps dependent upon solution pH (Sunderland and Watts, 1984); they provide a model example of parallel degradation; and they have found widespread application in pharmaceuticals, foods and cosmetics as preservatives (where it is usually the case that at least two parabens are in any particular formulation).

2. Materials and methods

2.1. Materials

Methyl 4-hydroxybenzoate (methyl paraben), ethyl 4-hydroxybenzoate (ethyl paraben, EP), \(n\)-propyl 4-hydroxybenzoate (\(n\)-propyl paraben, PP) and \(p\)-hydroxybenzoic acid (pHBA), all >99%, were purchased from Fluka. Sodium hydroxide was purchased from VWR. All materials were used as received. Solutions were prepared in distilled, de-ionised water.
2.2. Isothermal microcalorimetry

Experiments were conducted using a 2277 Thermal Activity Monitor (TAM, Thermometric AB, Järfälla, Sweden) at 25 °C. Solutions were prepared by dissolving the required amounts of MP, EP and/or PP (0.05 M with respect to each component) in sodium hydroxide solution (0.5 M). Solution pHs were measured before and after each experiment and were found to be constant at pH 12.3. The time at which the addition of solute to water was made was noted and designated \( t_0 \). Aliquots of solution (3 ml) were pipetted into standard glass TAM ampoules; the ampoules were then sealed with a crimped metal lid. An air-tight enclosure was ensured with the use of a rubber seal on the inside of the lid. Sample ampoules were placed in the thermal equilibration position of the TAM for approximately 20 min before being lowered into the measurement position. Data capture was then initiated using the dedicated software package Digitam 4.1. The time at which data capture started was noted and designated \( t^* \).

Power data \((\mu W)\) were recorded every 30 s, for a minimum of 24 h, with an amplifier setting of 300 \( \mu W \), against a reference ampoule containing sodium hydroxide solution (0.5 M, 3 ml). The instrument was calibrated weekly using an electrical substitution method, and was zeroed before each experiment using buffer against buffer. Samples were run at least in triplicate.

2.3. Data analysis

Data analysis was performed using Origin (Microcal Software Inc., USA). The difference between \( t_0 \) and \( t^* \) (in seconds) was added to the x-axis data to correct for the time-delay in initiating recording in the TAM (note that this does not affect the magnitude of the power signal, as the calorimeter records the instantaneous power-output as a function of time). Data were analysed using an iterative procedure (in this case, the non-linear curve fitting tool in Origin 7.0, Microcal Software Inc.). Data were fitted to Eq. (1), which describes the power-time response for a single-step reaction following first-order kinetics (Bakri et al., 1988),

\[
\text{Power} = \frac{dq}{dt} = \Delta H v k [A_0] e^{-kt}
\]  

where \( q \) is the heat output of the reaction, \( \Delta H \) the reaction enthalpy per mole of product formed, \( v \) the volume of solution in the ampoule, \( k \) the first-order rate constant and \( [A_0] \) is the initial concentration of reactant. The procedure requires initial estimates for all parameters to be entered into the software. Values for \( v (0.003 \, \text{dm}^3) \) and \( [A_0] (0.05 \, \text{M}) \) were known and therefore kept constant. The initial values entered for \( \Delta H (1 \times 10^{10} \, \mu J \, \text{mol}^{-1}) \) and \( k (1 \times 10^{-5} \, \text{s}^{-1}) \), values which are entirely reasonable for chemical degradation, were the same for each data set; the software then altered these values until a good fit to the data (as indicated by a low \( \chi^2 \)-value) was obtained. Were the reaction mechanism not known, then the data would have been fitted to a range of models (such as those in Gaisford, 1997) and that giving the best fit would have been selected.

3. Results and discussion

The degradation kinetics of the parabens in aqueous solution are well discussed in the literature; Kamada et al. (1973) recorded degradation of methyl and n-propyl paraben in acidic solutions between 40 and 100 °C, Blaug and Grant (1974) reported base-catalysed degradation of methyl, ethyl and n-propyl paraben above 70 °C and Raval and Parrott (1967) reported degradation of methyl paraben at pHs 6-9 between 70 and 85 °C. In the most complete kinetic study to date, Sunderland and Watts (1984) studied the hydrolysis of methyl, ethyl and n-propyl paraben between pH 1.26 and 10.59 and noted that degradation was fastest at low pH (acid catalysis predominates) or high pH (base catalysis predominates). They determined rate constants of 3.03, 1.26 and 0.93 \( \times 10^{-1} \, \text{s}^{-1} \) for methyl, ethyl and n-propyl paraben, respectively, at pH 10.59, although their data were recorded over a range of elevated experimental temperatures and extrapolated to 130.5 °C rendering a direct comparison with the data reported here impossible. O'Neill et al. (2003) reported the rate constant for the hydrolysis of methyl paraben in excess base to be \( 3.15 \times 10^{-4} \, \text{s}^{-1} \) at 25 °C and \( 7.94 \times 10^{-4} \, \text{s}^{-1} \) at 37 °C.

The degradation of methyl paraben is represented schematically in Fig. 1 (the processes for ethyl and n-propyl paraben are analogous, the only difference being the alcohol formed). The initial hydrolysis step follows pseudo first-order kinetics and is pH dependent (Kamada et al., 1973; Sunderland and Watts, 1984), although no literature data are available at pHs above...
10.59. Depending upon solution pH, p-hydroxybenzoic acid can decarboxylate to form phenol, a reaction first reported by Cazeneuve (1896) whose data showed the acid to be stable in alkaline but unstable in acidic conditions. Quantitative kinetic data are only available for this reaction between pH 1.26 and 10.59, where it has been shown that the rate falls significantly at the higher pHs and the reaction follows first-order kinetics over four to five half-lives (Sunderland and Watts, 1984).

In this study, paraben degradation was studied in NaOH solution (pH 12.3), to ensure that degradation stopped once p-hydroxybenzoic acid had formed. The power–time traces obtained for the degradation of the three individual parabens are shown in Fig. 2. The data were fitted to Eq. (1); the fit of each data set to the model is represented by the open circles in Fig. 2. Eq. (1) can be used to derive values for $\Delta H$ and $k$ from power–time data, as long as $v$ and $[A_0]$ are known and that the reaction goes to completion. For this system, measurements of paraben degradation performed at 25 and 37 °C resulted in the same total heat output (data not shown) indicating no equilibrium state is reached and, hence, the analysis is appropriate in this case. An alternative analysis has been proposed for systems where this is not the case (Beezer, 2001; Beezer et al., 2001b).

<table>
<thead>
<tr>
<th>Ester</th>
<th>$k$ (s$^{-1}$) (±S.D., n)</th>
<th>$\Delta H$ (kJ mol$^{-1}$) (±S.D., n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl</td>
<td>$3.1 \times 10^{-4}$ (±0.01, 3)</td>
<td>-59.2 (±0.4, 3)</td>
</tr>
<tr>
<td>Ethyl</td>
<td>$1.5 \times 10^{-4}$ (±0.01, 3)</td>
<td>-64.4 (±1.3, 3)</td>
</tr>
<tr>
<td>n-Propyl</td>
<td>$1.2 \times 10^{-4}$ (±0.01, 3)</td>
<td>-60.1 (±0.3, 3)</td>
</tr>
</tbody>
</table>

All three parabens were found to degrade following first-order kinetics (as a further check, In(power) versus time plots were found to be linear, data not shown), allowing rate constant values and reaction enthalpies to be obtained (summarised in Table 1). It is apparent that the degradation rate decreases as the hydrocarbon moiety increases in length, which would be expected on steric grounds, while the reaction enthalpies are roughly equivalent.

It is appropriate to note here the benefits of using this method of analysis compared with other analysis methodologies. Conventionally, the reaction order and the rate constant are determined by plotting some function of power versus some function of time; the plot, which is linear determines the reaction order and the slope gives the rate constant. In the case of a first-order system, a plot of ln(power) versus time, or for a second-order process a plot of power$^{-0.5}$ versus time, results in a linear relationship. Similarly, enthalpy is conventionally determined by letting the reaction progress to completion and measuring the total heat released. Fitting a section of data to Eq. (1) allows the determination of both the rate constant and enthalpy without needing the reaction to run to completion (often time-consuming) or invoking an extrapolation of data. The approach presented here also allows the determination of reaction order if the general form of Eq. (1) is used (as derived by Willson et al., 1995b; not shown here), and also allows the analysis of non-integral order systems. It was found that the correct reaction parameters were returned even if only the first 15 min of data were used for analysis (Table 2). Fitting greater numbers of data did not alter the fitting values.
Table 2
The reaction parameters obtained by fitting varying time periods of power-time data for MP to Eq. (1)

<table>
<thead>
<tr>
<th>Time</th>
<th>k (s⁻¹)</th>
<th>ΔH (kJ mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>3.1 × 10⁻⁴</td>
<td>-59.1</td>
</tr>
<tr>
<td>30 min</td>
<td>3.1 × 10⁻⁴</td>
<td>-59.1</td>
</tr>
<tr>
<td>1 h</td>
<td>3.1 × 10⁻⁴</td>
<td>-59.1</td>
</tr>
</tbody>
</table>

Initial experiments on binary systems were conducted on mixtures of MP and PP, since these components had the largest difference in rate constants. Power-time data obtained for MP-PP mixtures could not, as expected, be fitted by Eq. (1) (which, in the absence of any prior knowledge of the system, would have immediately indicated the likelihood of there being more than one event occurring in the sample); the data were, however, described very well by Eq. (2) (which describes two simultaneous first-order decay processes), Fig. 3, allowing the recovery of rate constant and enthalpy values, Table 3.

\[
\text{Power} = \frac{dq}{dt} = \Delta H_1 v k_1 [A_0] e^{-k_1 t} + \Delta H_2 v k_2 [B_0] e^{-k_2 t}
\]

where the subscripts 1 and 2 refer to the individual reaction pathways and \([A_0]\) and \([B_0]\) refer to the initial concentrations of reactants A and B, respectively.

Interestingly, while the enthalpy values determined from the mixed system data are the same (within error) as those determined when the species are studied individually, both rate constants are lower than expected. The most likely explanation for this observation is that the two reactants present both degrade to a common product, \(p\)-hydroxybenzoic acid. The rate of degradation for the degradation of an individual paraben (methyl paraben in this case) is given by,

\[
\frac{d[MP]}{dt} = \frac{d[pHBA]}{dt}
\]

where \([MP]\) is the concentration of methyl paraben and \([pHBA]\) is the concentration of \(p\)-hydroxybenzoic acid as a function of time (the rate laws are analogous for the other parabens). In the case of a binary mixture, it is clear that two sources contribute to \([pHBA]\); this must inevitably cause the rates of disappearance of the two reactants to change.

If MP degradation is measured in a solution of base containing 0.05 M \(p\)-hydroxybenzoic acid (i.e. a solution that already contains one of the degradation products), the measured rate constant is \(2.2 \times 10^{-4}\) s⁻¹, a value that is identical to that recorded for MP in the mixed MP:PP system. Furthermore, the total amount of heat released from the binary mixture (16.7 ± 0.6 J) was the same, within error, of that of the sum of heats released by the individual components (17.9 ± 0.6 J), indicating the same extent of reaction taking place in

Table 3
Average values for the rate constants and reaction enthalpies for binary mixtures of the parabens determined by fitting experimental data to Eq. (2)

<table>
<thead>
<tr>
<th>Ester mix</th>
<th>(k_1) (s⁻¹) (±S.D., n)</th>
<th>(k_2) (s⁻¹) (±S.D., n)</th>
<th>(\Delta H_1) (kJ mol⁻¹) (±S.D., n)</th>
<th>(\Delta H_2) (kJ mol⁻¹) (±S.D., n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl/ethyl</td>
<td>2.3 × 10⁻⁴ (±0.1, 8)</td>
<td>1.1 × 10⁻⁴ (±0.1, 8)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Methyl/n-propyl</td>
<td>2.2 × 10⁻⁴ (±0.08, 9)</td>
<td>8.0 × 10⁻⁵ (±0.01, 9)</td>
<td>-58.2 (±2.1, 9)</td>
<td>-54.4 (±1.9, 9)</td>
</tr>
<tr>
<td>Ethyl/n-propyl</td>
<td>1.2 × 10⁻⁴ (±0.1, 8)</td>
<td>8.0 × 10⁻⁵ (±0.01, 8)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

* The rate constants for these systems were obtained by fixing the enthalpy values constant.
all cases. If the power–time data recorded for individual samples of MP and PP are summed, it is clear that the resulting trace differs significantly from that recorded experimentally for the actual binary system (Fig. 4). Fitting these summed data to Eq. (2) resulted in rate constants of $2.9 \times 10^{-4}$ (±0.1) and $1.1 \times 10^{-4}$ s$^{-1}$ (±0.1) and enthalpies of $-60.9$ (±2.0) and $-53.7$ (±2.0) for the methyl and n-propyl esters, respectively, values that are in much better agreement with those presented in Table 1.

The observation that degradation rate constants may differ significantly from those expected when materials are formulated in combination is important; in this case, a shelf-life could have been predicted based on the stability data obtained for the individual materials, but the actual shelf-life of the product would have been longer. Other properties of the parabens have been observed to alter when they are formulated in combination (for instance, mixtures of parabens are more effective as preservatives than the individual parabens, Littlejohn and Husa, 1955; Schimmel and Husa, 1956) and this highlights the importance of developing analytical techniques that allow the direct study of heterogeneous samples.

The data for the other two binary mixtures (MP:EP and EP:PP) were found not to be fitted by either Eq. (1) or Eq. (2), when all the parameters were allowed to vary, but were successfully fitted to Eq. (2) when the enthalpy values (as determined for the individual components and shown in Table 1) were fixed. As for the MP:PP binary system, the fitting again returned lower than expected rate constants, Table 3, but these systems also have a common degradation product.

These data serve to show the practical limits of the resolution of the model fitting technique to real data and suggest that one rate constant needs to be at least twice the magnitude of the other to enable a successful analysis, assuming approximately equal enthalpies. If ‘ideal’ data for either the MP:EP or EP:PP system are generated (for instance, using Mathcad) using the data in Table 1 then the model fitting successfully recovers the rate constants using Eq. (2). This suggests that it is the inherent noise in the data that prevents successful analysis, rather than the parameters being too similar. Analysis using simulated data also showed that if one rate constant is more than three orders of magnitude larger than the other, the model fitting is similarly unable to recover both values, because one process dominates the observed signal.

As for the individual components, different sections of data were analysed in order to determine the minimum observation period required to allow the recovery of the correct reaction parameters. Unsurprisingly, the binary mixtures required a greater number of data to be...
analysed for the fitting method to recover the correct values, Table 4. However, the analysis required just 4 h of data to return the correct parameters; a significant improvement over the methodologies used in existing published work, most of which contain data recorded at a minimum of 70 °C.

As noted above, under certain conditions paraben degradation can proceed via two consecutive steps. If, as expected, p-hydroxybenzoic acid is stable under alkaline conditions then paraben degradation should be described by Eq. (1). However, if p-hydroxybenzoic acid subsequently degrades to phenol, then the data would be better described by a two-step consecutive model. We have shown previously that a consecutive reaction with two first-order steps is described by Eq. (3) (Gaisford et al., 1999):

\[
\text{Power} = \frac{dq}{dt} = \Delta H_1 v k_1 [A_0] e^{-k_1 t} + k_1 k_2 v [A_0] \left( \frac{e^{-k_1 t} - e^{-k_2 t}}{k_2 - k_1} \right)
\]

where \( k_1 \) and \( k_2 \) are the rate constants and \( \Delta H_1 \) and \( \Delta H_2 \) are the enthalpies for the two reaction steps, respectively. Power–time data for the degradation of MP were fitted to Eq. (3) and showed a better statistical measure of fit than that obtained when the data were fitted to Eq. (1) (\( \chi^2 = 0.008 \) for fit to Eq. (3) compared with \( \chi^2 = 0.125 \) for fit to Eq. (1)). The microcalorimeter provides no direct molecular information and, in the absence of any other supporting data, we have stated that the best approach to determining reaction mechanisms from calorimetric data is to fit the data to a range of models and select that which gives the best fit with the fewest variables (Gaisford et al., 1999). As such, these results suggest that p-hydroxybenzoic acid is itself degrading and is not, as suggested by Cazeneuve (1896) stable under alkaline conditions. However, the enthalpy value returned by the fitting process is very small (ca. 0.3 kJ mol\(^{-1}\)); if the reaction parameters are used to construct the power–time traces for the two steps, it is clear that the degradation to phenol contributes very little to the observed heat-flow, Fig. 5. An alkaline solution of p-hydroxybenzoate gave no detectable heat-flow in the calorimeter over a period of 4 days (data not shown); the same solution also showed no detectable change when analysed by UV spectroscopy over the same time period (p-hydroxybenzoic acid \( \lambda_{\text{max}} \) 280, phenol \( \lambda_{\text{max}} \) 287; data not shown). It may therefore be the case that Eq. (3), having more variables, simply gives a better fit by generating artifacts that are not related to the reaction mechanism and, as stated in the introduction, should be disregarded. Further work will be required using other analytical methods to verify the exact reaction mechanism for the parabens under these conditions; however, since the aim of this study was simply to assess the use of IM to study parallel pro-

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Table 4

<table>
<thead>
<tr>
<th>Time</th>
<th>( k_1 ) (s(^{-1}))</th>
<th>( k_2 ) (s(^{-1}))</th>
<th>( \Delta H_1 ) (kJ mol(^{-1}))</th>
<th>( \Delta H_2 ) (kJ mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min</td>
<td>( 1.2 \times 10^{-4} )</td>
<td>( 1.2 \times 10^{-4} )</td>
<td>(-39.1)</td>
<td>(-50.4)</td>
</tr>
<tr>
<td>1 h</td>
<td>( 1.5 \times 10^{-4} )</td>
<td>( 2.0 \times 10^{-5} )</td>
<td>(-85.2)</td>
<td>(-24.9)</td>
</tr>
<tr>
<td>2 h</td>
<td>( 1.6 \times 10^{-4} )</td>
<td>( 5.0 \times 10^{-5} )</td>
<td>(-77.3)</td>
<td>(-29.5)</td>
</tr>
<tr>
<td>3 h</td>
<td>( 2.1 \times 10^{-4} )</td>
<td>( 8.0 \times 10^{-5} )</td>
<td>(-60.1)</td>
<td>(-52.3)</td>
</tr>
<tr>
<td>4 h</td>
<td>( 2.2 \times 10^{-4} )</td>
<td>( 8.0 \times 10^{-5} )</td>
<td>(-59.1)</td>
<td>(-56.0)</td>
</tr>
</tbody>
</table>

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Fig. 5. Power–time data for MP (0.05 M) in NaOH aqueous solution (0.5 M) at 25 °C and the theoretical contributions to the observed signal from the individual steps as determined using Eq. (3); degradation of MP and degradation of p-hydroxybenzoic acid.
cesses, rather than to result in a comprehensive study of paraben degradation, these studies were not conducted here. We simply note that the analysis suggests a secondary degradation step could be occurring and we will return to the issue in a future publication. We also note that in order to effect a successful analysis for two parallel processes it appears that one rate constant needs to be twice the magnitude of the other.

4. Conclusion

It has been shown that IM can be used to study samples containing more than one degrading component and that analysis of the power–time data obtained allows the recovery of the rate constant and enthalpy for each species, so long as one rate constant is at least twice the magnitude of the other. Analysis of simulated data showed that the inability of the model to separate rate constants more similar than this was a result of the inherent noise of the signal, although results from fitting simulated data also suggested that a complete analysis would not be possible if the two rate constants were more than three orders of magnitude different.

The data allowed the determination of the rate constants and enthalpies for degradation of methyl, ethyl and n-propyl paraben directly at 25 °C, without the need for reaction to progress to completion. Indeed, it was found that as little as 15 min of experimental data were needed to effect a complete analysis when only one degrading component was present. Analysis of data for mixed paraben systems showed that the models were able to detect the presence of two parallel reaction processes and that the degradation rate constants for parabens in combination were considerably lower than expected. This was ascribed to the presence of a common degradation product. A minimum of 4 h of experimental data were needed to ensure an accurate analysis.

Although the subsequent degradation of p-hydroxybenzoic acid to phenol should be arrested under our experimental conditions, the fitting methodology suggested that this reaction may be occurring to some extent, because the data were better fitted to a consecutive model. It is unclear at this stage whether this observation is an artifact of the fitting procedure (the consecutive model having more variables) and we intend to conduct further studies into this effect.

Acknowledgement

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References


A COMPARISON OF THE PERFORMANCE OF CALORIMETERS
Application of a test and reference reaction

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Isothermal calorimetry is finding extensive application in a number of research areas. This popularity is reflected in the number of commercially available instruments which are capable of yielding a variety of thermodynamic and kinetic parameters. Whilst there has been much discussion of ways in which to validate any values returned from these instruments very little has been done quantitatively to compare the relative performances of different instruments. This paper highlights the use of a test and reference reaction quantitatively to compare the performance of three instruments (Thermometric TAM, THT µRC and a Setaram HSDSC III); the specifications of these instruments provide a range from high-sensitivity, long equilibration time to lower-sensitivity, short equilibration time. The comparison is made through a statistical analysis of values returned for the rate constant, enthalpy of reaction and activation energy for the base catalysed hydrolysis of methyl paraben. The statistical analysis from the data set discussed here indicates that there is no significant difference between the returned thermodynamic and kinetic parameters from the TAM and µRC. The analysis revealed however that the HSDSC returns values for the rate constant which are significantly different from both the TAM and µRC, although it is noted that this instrument was not specifically designed to operate in a step-isothermal mode and that it was possible to apply a correction to the data. In all cases the enthalpy data returned from all instruments were statistically similar although the µRC and HSDSC returned values which were, for the rate constant and activation energy, less precise than those obtained from the TAM. As well as highlighting the importance of using test and reference reactions, this study also shows that proper instrument selection is an important factor when designing a calorimetric experimental series.

Keywords: calibration, isothermal calorimetry, kinetic and thermodynamic analysis, test and reference reaction, validation

Introduction

Calorimetry is finding increased acceptance for a variety of applications within the pharmaceutical industry and calorimetric methods [1–3] are now becoming more widely used for the investigation and quantification of stability and compatibility of pharmaceutical materials and formulations. Calorimetry confers several advantages over more traditional techniques. In particular it is insensitive to the physical form of the sample (i.e. it can be solid, liquid, gas or any combination of these) and does not require that the sample be altered in any way prior to study. It also has the advantage that the system is monitored indirectly through a change in heat content, i.e. invasive sampling is not required. Calorimetry also offers a level of sensitivity to small changes in the system which is superior to many alternative techniques. This sensitivity means that the sample does not necessarily have to be studied under stressed conditions. Calorimetry does suffer from the limitation that the data offer no molecular information on the system under study and, consequently, no definitive mechanistic information can be derived directly from calorimetric data. It does however reveal kinetic information such as the rate constant and order of reaction which may allow mechanistic information to be inferred.

Calorimeters operate on the principle that nearly all changes (chemical and physical) in a system involve an exchange of heat to, or from, the surroundings. The calorimeter monitors this exchange of heat as a function of time. The returned calorimetric data are of the form power (J s⁻¹) vs. time (s). It is therefore possible, using the appropriate equations [4–7], to glean a variety of thermodynamic (e.g. \( \Delta H_r \), enthalpy of reaction) and kinetic information (e.g. \( k \), rate constant) from the raw calorimetric data. Moreover, since the calorimeter allows the system to be studied as a function of temperature it becomes possible to derive parameters such as the activation energy, \( E_a \), giving greater insight into the character of the system. Calorimetry is therefore a versatile, sensitive and non-destructive technique, allowing the study of a wide range of materials in a variety of physical forms, making it a potentially very useful tool for the pharmaceutical industry.

There is a range of commercially available calorimeters, supplied by a number of manufacturers, all of which have different capabilities. Some like the
Table 1 Manufacturers’ specifications for the calorimeters used in this study

<table>
<thead>
<tr>
<th>Calorimeter</th>
<th>Operating ( T_{\text{range}} )/°C</th>
<th>Scanning rate/ ( \text{K min}^{-1} )</th>
<th>Resolution/ ( \mu \text{W} )</th>
<th>Noise/ ( \mu \text{W} )</th>
<th>Sample size/ mL</th>
<th>Equilibration time after step ( T ) change</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAM</td>
<td>12 to 90</td>
<td>n/a</td>
<td>0.01</td>
<td>0.008</td>
<td>≤3°</td>
<td>Approx. 24 h</td>
</tr>
<tr>
<td>( \mu \text{RC} )</td>
<td>–10 to 200</td>
<td>0.02 to 2</td>
<td>5</td>
<td>5</td>
<td>≤1.5</td>
<td>Approx. 20 min°</td>
</tr>
<tr>
<td>HSDSC</td>
<td>–20 to 120</td>
<td>0.001 to 1.2</td>
<td>0.03</td>
<td>0.03 (RMS)</td>
<td>≤0.85</td>
<td>Approx. 20 min°</td>
</tr>
</tbody>
</table>

°These values represent the time taken for equilibration after a step isothermal change of 5°C; greater step changes may require a longer time period for equilibration; °The ampoules used for this study were standard 3 mL glass ampoules. 4 and 20 mL ampoules are also available.

Experimental

Materials and methods

Methyl 4-hydroxybenzoate (methyl paraben, MP), 99%, was purchased from Fluka. Sodium hydroxide was purchased from VWR. All materials were used as received. Solutions were prepared in distilled, de-ionised water.

Solutions were prepared by dissolving the required amounts of MP (0.38 g) in 50 mL sodium hydroxide solution (0.5 M). Solution pH was measured before and after each experiment and found to be constant at pH 12.3. The time between addition of solute to NaOH (\( t_0 \)) and commencement of data capture (\( t_s \)) was noted and this value added to the time data for each experiment.

Isothermal experiments

Experiments were conducted using a 2277 thermal activity monitor (TAM) at 25, 37 and 40°C. Aliquots of solution (3 mL) were pipetted into standard glass ampoules; the ampoules were then sealed with a crimped metal lid. An air-tight enclosure was ensured with the use of a rubber seal on the inside of the lid. Sample ampoules were placed in the thermal equilibration position of the TAM for 40 min before being lowered into the measurement position. Data capture was then initiated using the dedicated software pack-
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Data analysis was performed using Origin (Microcal Software Inc., USA). The difference between \( t_0 \) and \( t_e \) (in s) was added to the time data to correct for the time-delay between initiation of reaction and the commencement of data capture. All values reported are an average of, at least, 3 repeats with the standard deviation values representing a confidence limit of 68%.

The BCHMP reaction proceeds via first-order kinetics, under the conditions used here, which means that recovery of the rate constant values at each temperature step was easily achieved by plotting \( \ln k \) vs. \( 1/T \); the gradients of the linear sections of data at each temperature give the rate constant values. Arrhenius plots (\( \ln k \) vs. \( 1/T \)) were subsequently constructed for each instrument.

It was possible, however, to attain further information from the data sets recorded here by using an iterative procedure (in this case, the non-linear curve fitting tool in origin). Data were fitted to Eq. (1), which describes the power-time response for a single-step reaction following first-order kinetics [13]. This was first published by Bakri [4].

\[
power = \frac{dq}{dt} = \Delta H k A_0 e^{-\frac{q}{kt}}
\]

where \( q \) is the heat output of the reaction, \( \Delta H \) is the reaction enthalpy (J mol\(^{-1}\)), \( k \) is the first-order rate constant (s\(^{-1}\)) and \( A_0 \) is the initial quantity of reactant (mol). Thus, as well as providing a check on the values of the rate constants, it was possible also to compare the sensitivity of the calorimeters to reaction enthalpy.

Further details of the application of this type of analysis have been given elsewhere [14], but it is important to note that it is imperative that the value of \( A_0 \) is known. While this is possible for the first temperature used in a step isothermal experiment, it is not possible to calculate how much material reacts during the temperature changes (and the characteristic temperature overshoot). Consequently only the initial temperature step was analysed using this procedure.

Results and discussion

The BCHMP reaction has been proposed [11] as a test and reference reaction for flow-calorimeters and has been the subject of inter- and intra-laboratory trials [12], using previously validated instruments, in order to define a set of reaction parameters; the recommended values for the enthalpy and rate constant (at 298 K) are \(-50.5\pm4.0\) kJ mol\(^{-1}\) and \(3.15\times10^4\pm1.1\times10^5\) s\(^{-1}\) respectively. It should be noted here that the BCHMP is not a IUPAC (International Union of Pure and Applied Chemistry) recommended test reaction for isothermal calorimetry, the preferred test reaction being the imidazole catalysed hydrolysis of triacetin (ICHT). However, this test reaction has been reported to be unsuitable for use in calorimeters with a low sensitivity [15]. The BCHMP was therefore chosen because of its combination of a reasonably large enthalpy of reaction and high rate of reaction, permitting easy study in calorimeters of lower sensitivity.

...
Figure 1: Power output obtained for the hydrolysis of methyl paraben from the TAM at 25°C.

Figure 2: Power output obtained for the hydrolysis of methyl paraben from the μRC running a step-isothermal program. The dotted line represents the temperature program.

Figures 1–3 display representative raw data plots for each instrument. The excellent fits to linear regression analyses of the ln power vs. time data shows that for each data set the reaction does conform to first-order kinetics over the lifetime of the reaction at each temperature step. The linearity of each of the Arrhenius plots of the kinetic data obtained from each instrument confirms that the reaction mechanism does not change over the temperature range studied, Fig. 4. Furthermore, the slopes of the Arrhenius plots were used to calculate an observed activation enthalpy, $E_a$, for the reaction from each instrument. The derived values and their standard deviations are given in Table 2.

From the values reported in Table 2 it is apparent that the values returned from the TAM are statistically indistinguishable from those returned from the μRC. Conversely, the values returned from the HSDSC are statistically different from those returned from both the μRC and the TAM. This observation is further confirmed through analysis using one-way ANOVA and Tukey tests to determine whether the rate constants (Table 3) and enthalpies (Table 4) returned from each calorimeter were statistically similar. The statistical analysis revealed that although the uncertainty in the values for $k$ returned from the μRC are greater than those obtained from the TAM data they are statistically indistinguishable from the TAM values. The rate constants returned from the HSDSC, however, were found to be significantly different from those values obtained from both the TAM and the μRC. Note that only values obtained at 25°C were used for the statistical analyses as this was the only common temperature between all experiments. (TAM data were obtained over a period of time from routine training and validation exercises whereas the temperatures chosen from the μRC and HSDSC were chosen specifically to alleviate the problem of a short half life for the methyl paraben reaction).

The observation that the rate constants returned from the HSDSC data are significantly different from those obtained from the TAM and μRC is somewhat puzzling. The rate constants obtained from the HSDSC were used to calculate the apparent operating tempera-
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Table 2: Average values and the associated standard deviations for the rate constant, enthalpy and activation energy for each calorimeter

<table>
<thead>
<tr>
<th>Calorimeter</th>
<th>( (k_{\text{std}}) ) s (^{-1} )</th>
<th>( (\Delta H_{\text{std}}) ) kJ mol (^{-1} )</th>
<th>( (E_{\text{std}}) ) kJ mol (^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAM</td>
<td>( 3.04 \times 10^{-4} \pm 3.64 \times 10^{-6} )</td>
<td>( 50.5 \pm 4.3 )</td>
<td>( 59.8 \pm 0.9 )</td>
</tr>
<tr>
<td>µRC</td>
<td>( 3.07 \times 10^{-4} \pm 6.00 \times 10^{-6} )</td>
<td>( 52.1 \pm 5.3 )</td>
<td>( 60.8 \pm 3.5 )</td>
</tr>
<tr>
<td>HSDSC</td>
<td>( 3.44 \times 10^{-4} \pm 8.72 \times 10^{-6} )</td>
<td>( 49.0 \pm 1.7 )</td>
<td>( 60.0 \pm 4.4 )</td>
</tr>
</tbody>
</table>

Table 3: Statistical analysis of rate constant data obtained from the TAM, µRC and HSDSC

<table>
<thead>
<tr>
<th>Calorimeter (I)</th>
<th>Calorimeter (J)</th>
<th>Mean difference (I-J)</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAM</td>
<td>µRC</td>
<td>-0.00001</td>
<td>0.474</td>
</tr>
<tr>
<td>TAM</td>
<td>HSDSC</td>
<td>-0.00004*</td>
<td>0.001</td>
</tr>
<tr>
<td>µRC</td>
<td>TAM</td>
<td>0.00001</td>
<td>0.474</td>
</tr>
<tr>
<td>µRC</td>
<td>HSDSC</td>
<td>-0.00003*</td>
<td>0.023</td>
</tr>
<tr>
<td>HSDSC</td>
<td>TAM</td>
<td>0.00004*</td>
<td>0.001</td>
</tr>
<tr>
<td>HSDSC</td>
<td>µRC</td>
<td>0.00003*</td>
<td>0.023</td>
</tr>
</tbody>
</table>

*The mean difference is significant at the 0.05 level

Table 4: Statistical analysis of enthalpy data obtained from the TAM, µRC and HSDSC

<table>
<thead>
<tr>
<th>Calorimeter (I)</th>
<th>Calorimeter (J)</th>
<th>Mean difference (I-J)</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAM</td>
<td>µRC</td>
<td>-1.100</td>
<td>0.849</td>
</tr>
<tr>
<td>TAM</td>
<td>HSDSC</td>
<td>1.475</td>
<td>0.747</td>
</tr>
<tr>
<td>µRC</td>
<td>TAM</td>
<td>1.100</td>
<td>0.849</td>
</tr>
<tr>
<td>µRC</td>
<td>HSDSC</td>
<td>2.575</td>
<td>0.491</td>
</tr>
<tr>
<td>HSDSC</td>
<td>TAM</td>
<td>-1.475</td>
<td>0.747</td>
</tr>
<tr>
<td>HSDSC</td>
<td>µRC</td>
<td>-2.575</td>
<td>0.491</td>
</tr>
</tbody>
</table>

In all cases there is no statistical difference at the 0.05 level

Table 5: Statistical analysis of activation energy data obtained from the TAM, µRC and HSDSC

<table>
<thead>
<tr>
<th>Calorimeter (I)</th>
<th>Calorimeter (J)</th>
<th>Mean difference (I-J)</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAM</td>
<td>µRC</td>
<td>-1.000</td>
<td>0.905</td>
</tr>
<tr>
<td>TAM</td>
<td>HSDSC</td>
<td>-0.180</td>
<td>0.997</td>
</tr>
<tr>
<td>µRC</td>
<td>TAM</td>
<td>1.000</td>
<td>0.905</td>
</tr>
<tr>
<td>µRC</td>
<td>HSDSC</td>
<td>0.820</td>
<td>0.943</td>
</tr>
<tr>
<td>HSDSC</td>
<td>TAM</td>
<td>0.180</td>
<td>0.997</td>
</tr>
<tr>
<td>HSDSC</td>
<td>µRC</td>
<td>-0.820</td>
<td>0.943</td>
</tr>
</tbody>
</table>

In all cases there is no statistical difference at the 0.05 level

An identical statistical test to that described earlier was performed to establish the significance of any variations in the calculated activation energies. Table 5 reports the results of this statistical test. Here it is seen that, even though the rate constants from the HSDSC are in error, the activation energy is statistically indistinguishable from those obtained from the other instruments. This is not unexpected since it is the temperature dependence of the rate constant, not its absolute value, which influences the value of the apparent activation energy. It should be noted that in the case of the reaction enthalpy, for the data set reported here, the HSDSC returned a value with a substantially smaller standard deviation than those obtained from the TAM or µRC. The reason for this is not known at this time.

Conclusions

The objective of the study reported here was to compare the relative performances of three commercially available calorimeters through the application of a test and reference reaction. It has been shown that, for...
the BCHMP reaction system, the TAM and the μRC returned values for the reaction parameters which were statistically identical, although the precision of the μRC was slightly lower than that of the TAM. A potential pitfall in using calorimeters in a mode for which they were not specifically designed has been highlighted in the values obtained, for the rate constants, from the HSDSC. It is possible to correct these values. The values returned for the enthalpy of reaction and the activation energy are, however, statistically indistinguishable from the μRC and TAM.

The data presented here have shown how test and reference reactions can be used to make quantitative comparisons between similar instruments. Such a study allows an operator then to select the most appropriate instrument for a particular sample, taking factors such as experimental run time, precision and cost into consideration.

References


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