International Journal of Pharmaceutics 417 (2011) 83–93

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Application and use of isothermal calorimetry in pharmaceutical development

Michael A.A. O'Neill[a], Simon Gaisford[b,c,*]

[a] Department of Pharmacy, School of Life and Health Sciences, Aston University, Aston Triangle, Birmingham, B4 7ET, UK
[b] School of Pharmacy, University of London, 29-39 Brunswick Square, London, WC1N 1AX, UK
[c] Kuecept Ltd, Tredomen Business and Technology Centre, Ystrad Mynach, Hengoed, CF82 7FN, UK

ARTICLE INFO

Article history:
Received 22 November 2010
Received in revised form 10 January 2011
Accepted 17 January 2011
Available online 26 January 2011

Keywords:
Isothermal calorimetry
Isothermal titration calorimetry
Solution calorimetry
Gas-perfusion calorimetry
Pharmaceutical

ABSTRACT

There are many steps involved in developing a drug candidate into a formulated medicine and many involve analysis of chemical interaction or physical change. Calorimetry is particularly suited to such analyses as it offers the capacity to observe and quantify both chemical and physical changes in virtually any sample. Differential scanning calorimetry (DSC) is ubiquitous in pharmaceutical development, but the related technique of isothermal calorimetry (IC) is complementary and can be used to investigate a range of processes not amenable to analysis by DSC. Typically, IC is used for longer-term stability indicating or excipient compatibility assays because both the temperature and relative humidity (RH) in the sample ampoule can be controlled. However, instrument design and configuration, such as titration, gas perfusion or ampoule-breaking (solution) calorimetry, allow quantification of more specific values, such as binding enthalpies, heats of solution and quantification of amorphous content. As ever, instrument selection, experiment design and sample preparation are critical to ensuring the relevance of any data recorded. This article reviews the use of isothermal, titration, gas-perfusion and solution calorimetry in the context of pharmaceutical development, with a focus on instrument and experimental design factors, highlighted with examples from the recent literature.

1. Introduction

When not at equilibrium a system is bound by the laws of thermodynamics to change from its current state to a more stable state. A material may change its physical form or chemical structure either through a simple unilateral event or by interaction with another species. The only consideration to be taken into account in determining if such change is of importance is length of time over which it will occur. The pivotal consideration of time is especially important for the pharmaceutical industry particularly in the characterisation of drug substances in both raw and formulated forms since this ultimately informs the safe therapeutic shelf-life of a product.

The measurement and prediction of change is therefore of primary importance and much time and effort is expended in trying to optimize such analyses. Typically a pharmaceutical assay will involve separation of analytes by chromatography (commonly HPLC) and a spectroscopic finish. While of great importance, such measurements can be compromised by the necessity for the analyte to have specific properties (such as a suitable chromophore) and sample preparation requirements. For HPLC the sample is required in a solution form (thus removing information on physical form) and the measurement is not performed in real-time. In the best case this results in a specific assay being developed for any particular sample and in the worst case renders some samples unsuitable for analysis.

A further problem with stability assays is that during development products are formulated to be stable (and hence will not degrade to any considerable extent during the measurement period). In other words, if an assay cannot detect any change in a product, a decision must be made as to whether that is because the product has not actually changed or because any change was below the detection limit (DL) of the measuring technique. This problem is mitigated, to an extent, by conducting stability assays under stress conditions. Usually this would involve an increase in temperature and/or relative humidity (RH).

1.1. Heat as an indicator of reaction process and progress

Calorimetry, the measurement of heat, offers an alternative approach for quantifying the process and progress of change. Its versatility derives from the fact that when change occurs it invariably occurs with a change in heat (ΔH). Thus, *heat is a universal accompaniment to chemical and physical change.* The result is that calorimetry can detect, and potentially quantify, changes in a wide

* Corresponding author at: School of Pharmacy, University of London, 29-39 Brunswick Square, London, WC1N 1AX, UK. Tel.: +44 0 20 7753 5863; fax: +44 0 20 7753 5942.
E-mail address: simon.gaisford@pharmacy.ac.uk (S. Gaisford).

0378-5173/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2011.01.038

range of materials. The only properties required of the sample are that the process being followed results in a detectable quantity of heat and that the sample (or at least a representative part of it) fits within the calorimetric vessel.

Isothermal calorimetry (IC, the measurement of heat change with time, at a constant temperature) is particularly suited to pharmaceutical applications because it is sensitive enough to allow the analysis of samples non-destructively (that is, it does not cause any extra degradation other than that which would have occurred upon storage), directly under storage conditions. This means there is often no need for elevated temperature stability studies (and hence no requirement for extrapolation of data) and samples can be recovered and used in other studies (particularly important in the early stages of the drug discovery process when samples may only exist only in milligram quantities). IC is also invariant to physical form, meaning that complex heterogeneous systems are open to investigation. Furthermore, because both physical and chemical processes occur with a change in heat content, the technique is not limited in its detection ability to chemical degradation, in the way HPLC is.

Careful experimental design allows the investigation of virtually any system and recent advances in data analysis and interpretation methodologies have resulted in the increasing application of the technique to stability testing of pharmaceuticals. Indeed, careful data analysis can result in a description of the reaction process in terms of both thermodynamics and kinetics, the only technique for which such a complete analysis is possible. Controlling the environment to which the sample is exposed also allows much information to be gleaned on molecular interactions or physical form. However, experiment design, instrument selection and sample preparation are critical factors in ensuring calorimetric data are representative of change in the sample.

This review will concentrate on the most popular applications of IC focusing on areas of particular interest to the pharmaceutical industry and will include discussion of good experimental practice and design illustrated with case studies.

2. Applications of isothermal calorimetry

In its most basic form a sample is hermetically sealed in an ampoule before being placed in the calorimeter. Any heat change is then monitored with time. This is known as ampoule calorimetry. Other derivatives of calorimetry essentially change the way the sample is loaded, handled during the experiment or change the local environment around the sample. Typical modes of operation include titration, gas-perfusion, and solution. This paper will briefly explore some of the principles of these modes of operation and their uses with reference to literature examples.

2.1. Ampoule calorimetry

This mode of operation represents perhaps the simplest experimental arrangement. It consists of a hermetically sealed ampoule containing the sample of interest (or a representative portion of it), which is measured against a reference ampoule. The reference material can either be air (i.e. an empty ampoule) or an inert material of similar heat capacity to that of the sample. The ampoules can be disposable (glass) or can be reusable (hastelloy, steel or glass). Glass ampoules should be avoided where studies on proteins or biologicals are conducted. Enzymes, for example, are well known for their affinity for surfaces and the Si–O terminal groups found on glass surfaces are ideal to immobilize enzymes and potentially alter their enzymatic activity (Guisan, 2006).

2.1.1. Calibration

Calibration is usually by means of an electrical heater that generates a known ΔH over a defined period of time allowing the calorimetric output to be appropriately quantified. This method of calibration is not without its problems notably for its lack of relevance to measurements that are made over extended time periods (it does not account for baseline drift for instance) and its inability to flag up instrument problems (for instance, if the operating temperature of the calorimeter is incorrect). Wadsö (2010) provides a detailed review of electrical calibration methods. Finnin et al. (2006) give an example of the problem of relying solely on electrical calibration. The authors report that a simple experiment investigating the known reaction of the base catalysed hydrolysis of methyl paraben (O'Neill et al., 2003), while returning correct values for the enthalpy of reaction, returned values for the rate constant that were not consistent for the reported operational temperature of the calorimeter. Upon further investigation it was discovered that the instrument was operating at a different temperature from that which the software reported. Such an error would not have been apparent if the kinetic parameters for the reaction system under investigation were not known. O'Neill et al. (2006) also use the same test reaction to compare the performance of different calorimeters.

Chen and Wadsö (1982) proposed an alternative calibration strategy whereby electrical calibration was supplemented with a known (in terms of kinetic and thermodynamic parameters) chemical test and reference reaction. The chemical system they chose was the imidazole-catalysed hydrolysis of triacetin, a system that was subsequently the subject of an extensive inter- and intra-laboratory IUPAC (international union of pure and applied chemistry) trial (Beezer et al., 2001; Parmar et al., 2004). When conducted properly, the reaction should return values for the reaction enthalpy ($\Delta_R H$) of $-91.7 \pm 3\,\mathrm{kJ\,mol^{-1}}$ and rate constant (k) of $2.8 \pm 0.1 \times 10^{-6}\,\mathrm{dm^3\,mol^{-1}\,s^{-1}}$. It must be noted that the test and reference reaction noted above is specific to solution phase systems and also that different chemical test and reference reactions are available for the other forms of isothermal calorimetry (discussed below). Although ampoule calorimetry is extensively used for studies on solid state samples (Phipps and Mackin, 2000) no equivalent solid-state test and reference reaction exists to date.

Ampoule experiments are perhaps the most commonly performed experiment since they lend themselves to a wide range of sample materials including solutions, suspensions (Zaman et al., 2001), powders and biologicals and has been applied to variety of drug molecules (Angberg and Nyström, 1988; Pikal and Dellerman, 1989; Hansen et al., 1989). In addition ampoule measurements are useful at all stages of the developmental process of a pharmaceutical from preformulation to interaction with packaging in the final product.

2.1.2. Drug-excipient compatibility screening

A primary application of IC in this configuration is screening for drug-excipient compatibility/stability (Skaria et al., 2005). The basic methodology is very simple. Samples of the active pharmaceutical ingredient (API) and excipient(s) in question are run individually to assess their response under the conditions of interest (temperature, RH, etc.) against an inert reference. The materials are then run in binary mixtures (or tertiary, quaternary as necessary), usually in equi-mass ratios and under 100% RH as this will ensure the maximum possible interaction and increase the probability of observing any interaction in the calorimeter. If no interaction is observed under these conditions then it is usually the case that the materials will not interact under less stressed conditions.

The calorimetric outputs observed for the individual samples are then summed to give a theoretical response (remembering that the calorimetric output is the sum of all the heat changes within

a system), which represents the calorimetric output that would be expected *if* the two materials do not interact. This theoretical response is then compared with the calorimetric output for the real mixture. If the materials interact in some way (i.e. there is some incompatibility) then the observed calorimetric response will differ from the theoretical response. The observed response may reveal an incompatibility whereby any degradation reaction is accelerated and hence the overall shape of the calorimetric curve will be the same (i.e. the mechanism is unchanged) but the rate at which the signal decays will be greater. Alternatively the calorimetric response may reveal that a new mechanistic pathway is now possible in the presence of the excipient and the calorimetric curve is observably different (more complex).

Observation of any deviation from the theoretical output is thus an indicator of interaction and possible incompatibility. At this stage the excipient can be rejected out of hand (if the goal is a preliminary screen) or investigated further if the excipient is necessary for the formulation. Schmitt et al. (2001) have evaluated the capacity of calorimetry to effect a rapid and practical screen for predicting compatibility between binary mixtures of drug and excipient. They showed that it is possible to predict relative stability within a functional class (binders, diluents, etc.). Their study, although unable to provide quantitative information, predicted the worst-case incompatibility for a formulation with known stability problems. However it should be noted that the results reported in their study were obtained from systems under stressed conditions of temperature (50 °C) and by adding water (20%, w/w) which although not unprecedented still represents a significant deviation from normal storage conditions. The authors of this article also make the important point that;

> "The ability to predict reactions in dosage forms depends on the similarity of the binary mixture to the formulation."

This is of course an inherent assumption behind any compatibility study and limits the validity of the calorimetric data if used as a sole indicator of compatibility. Such studies must therefore, be treated with caution and any inferences made from the calorimetric data be backed up with information derived from ancillary sources.

A case in point is provided by Selzer et al. (1998, 1999) who report a study on the stability and excipient compatibility of an unnamed drug. They contend that a long, slow, underlying signal is due to water transfer within the structure of their excipient, microcrystalline cellulose. Given the nature of the analysis this conclusion is not necessarily supported by the data and would, at least, require some ancillary measurement to show that the signal was in fact attributable to water redistribution.

It is worthwhile noting that stability/compatibility studies are often strongly focused on the compounds within the formulation, but the packaging material is also part of the formulation and as such should also be considered when conducting compatibility tests. The primary function of the packaging material is to protect the formulation from its environment and hence it is implicated in the shelf life of the product (i.e. if it does not provide adequate protection from light, moisture, $O_{2(g)}$, etc. then the shelf life of the product may be severely compromised). Moreover the packaging itself is a chemical entity (or a combination of chemical entities) and hence may potentially interact with the formulation. The methodology for this aspect of formulation testing is largely the same as that described above for incompatibility testing albeit that the formulation is now studied (rather than the individual components) in the presence of various packaging materials. If, for instance, the API is known to oxidize then stability trials of the packaged formulation should not show any thermal signature, confirming successful exclusion of oxygen.

2.1.3. Quantitative analysis of processes

If there is only one reacting component then the reaction will be of integral order and it is relatively easy to determine the rate constant by replotting the data.

Thus, for solution-phase reactions;

- If the power signal is constant as a function of time, then the process is zero-order. The rate constant can be determined from the power value if the number of moles of material and reaction enthalpy are known (the deflection is equal to $k \cdot \Delta H \cdot V$ (where V is the volume of sample).
- If a plot of ln(power) versus time is linear, then the process is first-order and the rate constant is given by the negative gradient of the line.
- If a plot of square root power versus time is linear, then the process is second-order and the rate constant is given by the gradient of the line.

If the data do not appear to be zero-, first- or second-order, there may be multiple processes occurring or (unusually) the process may have a non-integral order. In this case a more complex analysis using an approach such as kinetic modeling, direct calculation or chemometrics must be used to discern the reaction order and/or number of reaction steps. O'Neill et al. (2007) describe a methodology for determining, at the very least, the number of processes occurring within a complex reaction system using chemometric analysis. Kong et al. (2009) similarly use chemometric analysis to interpret complex (in their case microbiological) IC data. Sousa et al. (2010) developed a series of methods for quantitative analysis of solid-state IC data once each individual process had been isolated.

Koenigbauer et al. (1992) determined the activation energies for the degradation of several drugs, including phenytoin, triamterene, digoxin, tetracycline, theophylline and diltiazem, using the initial power rates, measured using IC at several elevated temperatures. The results were compared with HPLC data recorded at a single temperature and it was shown that the IC data were more precise. Similarly, Hansen et al. (1990) showed that the shelf-life of a product, degrading via an autocatalytic reaction, was inversely proportional to the rate of heat production during the induction period, using lovastatin as an example.

Many pharmaceuticals are susceptible to hydrolysis and, since water is difficult to remove entirely from a formulation, hydrolysis is a common cause of chemical instability. There are numerous examples in the literature where hydrolysis reactions have been studied calorimetrically. For instance, the degradation rate of meclofenoxate hydrochloride, which hydrolyses in aqueous solution, has been determined using IC (Otsuka et al., 1994). By plotting ln(power) versus time, the degradation rate constants for MF at pH 6.4 and 2.9 were determined to equal $1.14 \times 10^{-4}\,s^{-1}$ and $9.7 \times 10^{-7}\,s^{-1}$ respectively. Comparison of these data with rate constant values determined using HPLC ($1.29 \times 10^{-4}\,s^{-1}$ and $9.0 \times 10^{-7}\,s^{-1}$) revealed the utility of the calorimetric technique. A similar approach has been used to determine the rate constants for ampicillin degradation in aqueous buffers (Oliyai and Lindenbaum, 1991) from pH 2 to 8 and a number of cephalosporins (Pikal and Dellerman, 1989).

Aspirin hydrolysis in aqueous solution as a function of pH was investigated by Angberg and Nyström (1988). In these studies, rate constants were derived from the gradient of ln(power) versus time plots at a series of temperatures (30–50 °C). In 0.1 M HCl at 40 °C, the rate constant was $9.0 \times 10^{-6}\,s^{-1}$, increasing to $22.5 \times 10^{-6}\,s^{-1}$ at 50 °C, while in pH 4.8 acetic acid buffer rate constants of $14 \times 10^{-6}\,s^{-1}$ and $34.1 \times 10^{-6}\,s^{-1}$ were determined at 40 and 50 °C respectively (Angberg et al., 1990). Using the Arrhenius

relationship, a rate constant for aspirin degradation at 25 °C in 0.1 M HCl of 2.3×10^{-6} s^{-1} was predicted.

Otsuka et al. (1994) investigated the oxidation of *dl*-α-tocopherol using IC. Samples of the drug (a slightly viscous liquid) were placed in glass ampoules that were left open to the atmosphere in ovens at 50, 40, 30 and 23 °C for varying lengths of time. Each sample was then capped before being placed in the calorimeter. Equivalent samples were analysed using HPLC. First-order rate constants for the samples were determined at each temperature. An Arrhenius plot of the data revealed a linear correlation and an excellent agreement between the HPLC and calorimetric data.

Tan et al. (1992) studied tretinoin (all-trans-retinoic acid), which degrades upon exposure to heat, light and/or oxygen principally to form one of two geometrical isomers, 13-cis-retinoic acid (isotretinoin) or 9-cis-retinoic acid. While isotretinoin exhibits almost the same clinical activity as tretinoin, and indeed is formulated for systemic administration, 9-cis-retinoic acid is clinically inactive and, hence, the precise quantification of the rates of formation of the breakdown products is of some considerable importance. It was demonstrated that under air the decomposition of isotretinoin was autocatalytic while the decomposition of tretinoin followed zero-order kinetics. In both cases, HPLC analysis showed the appearance of degradation products, although the mechanisms were complex. Under a nitrogen atmosphere both compounds showed a first-order kinetic event, but simultaneous HPLC analysis showed no evidence of chemical degradation, indicating a physical change was occurring.

A similar approach has shown that solid-state lovastatin degrades in the presence of oxygen (Hansen et al., 1989). In this case, the degradation mechanisms were shown to change between 50 and 60 °C.

Simoncic et al. (2008) used IC to quantify the first-order degradation kinetics and thermodynamics of perindopril erbumine in aqueous solution as a function of temperature and pH, reporting that at pH 6.8 only hydrolysis was occurring and that the kinetic data were in agreement with those recorded by HPLC. Simoncic et al. (2007) also investigated the degradation of enalapril maleate in tablet formulations and showed that the degradation rate determined with IC increased with water content and the results were in satisfactory agreement with a supporting HPLC assay.

Gaisford et al. (2006) show how IC data can be used as an aid to reformulating a product to achieve an increase in shelf-life. The authors report the results of a degradation study of Busuflan, a cytotoxic drug used in the treatment of cancer, which undergoes simple hydrolytic degradation. The reaction parameters were determined at a variety of temperatures (25, 30, 37 and 45 °C). The data were plotted in accordance with the Arrhenius relationship and used to establish the shelf life of the reconstituted drug at its storage temperature of 6 °C (approx 15 h). Such a short shelf-life compromised the use of the drug and led to much wastage. The authors then explored the effect on stability of reformulating the drug in a smaller volume, discovering that it was possible to increase the shelf-life to ca. 50 h at 6 °C. The potential power of IC is clearly expressed in the time taken to collect the data that allow this prediction. The data in these experiments were all collected over a period of 24 h and with all repeats considered the data took a little over a week to collect, representing a benefit in terms of the study time which may have been on the order of weeks or months had conventional methods been employed.

It is worth noting that although quantitative information is generally sought IC can also provide powerful insights into complex systems through semi-quantitative and/or qualitative analysis. This is particularly the case in complex biological systems. Kong et al. (2009) used chemometric analysis to interpret the impact of emodin on growth of *Candida albicans*. Bonander et al. (2009) report a method by which IC can be used to demonstrate the up-regulation of recombinant protein production in modified yeast cells. While it is accepted by the authors that the derived data do not yield truly quantitative information IC data permit an examination of the same organism (in its wild type and mutant type) and its rate of protein production under different conditions allowing for optimisation and refinement of experimental technique. It is appropriate at this point to reiterate the dangers of ascribing specific biological, chemical or physical events to calorimetric output without ancillary information particularly for such complex systems as microbiological species. Calorimetric data do not yield sufficient information in isolation. More recently IC has been used in tandem with gas chromatographic/tandem mass spectrometry to quantify the activity of a number of fungal species (Li et al., 2007). IC has been applied to a wide array microbiological species and indeed this was one of the technique's first main areas of application. Of particular interest is the capacity to construct quantitative structure activity relationships for the response of such organisms to drugs. Such studies are outside the scope of this article but excellent reviews of this area are given by Montanari et al. (2004) and Vine and Bishop (2005).

2.2. Isothermal titration calorimetry

In isothermal titration calorimetry (ITC) known aliquots of a titrant are added sequentially (typically 10–30 injections) to a solution within the calorimetric ampoule and the heat change per addition is recorded. Commercial instruments are available that operate on either heat-conduction or power-compensation principles; irrespective of the design, the principles of experimental construction, discussed below, are the same. It is a widespread and powerful technique in the investigation of biological interactions and can be used as a powerful tool to drive rational drug design (Chaires, 2008). A number of experimental advantages explain its popularity; it does not require optically clear solutions; there is no particular assay development step required for each interaction; it is rapid and directly measures the enthalpy of binding ($\Delta_b H$), the equilibrium binding constant (K_b) and the reaction stoicheometry (n) in a matter of hours. If the experiments are performed as a function of temperature then the heat capacity change (ΔC_p) can also be measured.

The experimental set up is very similar to that described for ampoule calorimetry. The system comprises a sample cell containing typically 0.5–1.5 mL of substrate solution into which aliquots of ligand are injected. The reference cell contains an identical amount of water, buffer or solvent to provide a reference of similar heat capacity (at least, before any injections are made). Proper understanding of the origin of the power signal, and its correction to remove experimental artifacts, is essential when undertaking ITC experiments. Critically, it must be ensured that the correct blank experiments are performed and subtracted from the binding data. Usually, as a minimum, three blank experiments are required.

- Dilution of the ligand by the solvent.
- Dilution of the substrate by the solvent.
- Solvent mixing.

The first experiment is performed by injecting ligand solution into solvent, the second by injecting solvent into substrate solution and the third by injecting solvent into solvent. Of the three experiments, the correction for the dilution of the ligand is often most significant, because its initial concentration is high as small aliquots are titrated. If a series of experiments are being performed with the same substrate, then this blank needs only be recorded once, as it is then common to every subsequent experiment. The third blank should be small if the same solvent system is used for both the ligand and substrate solutions. If not, large enthalpies of mixing

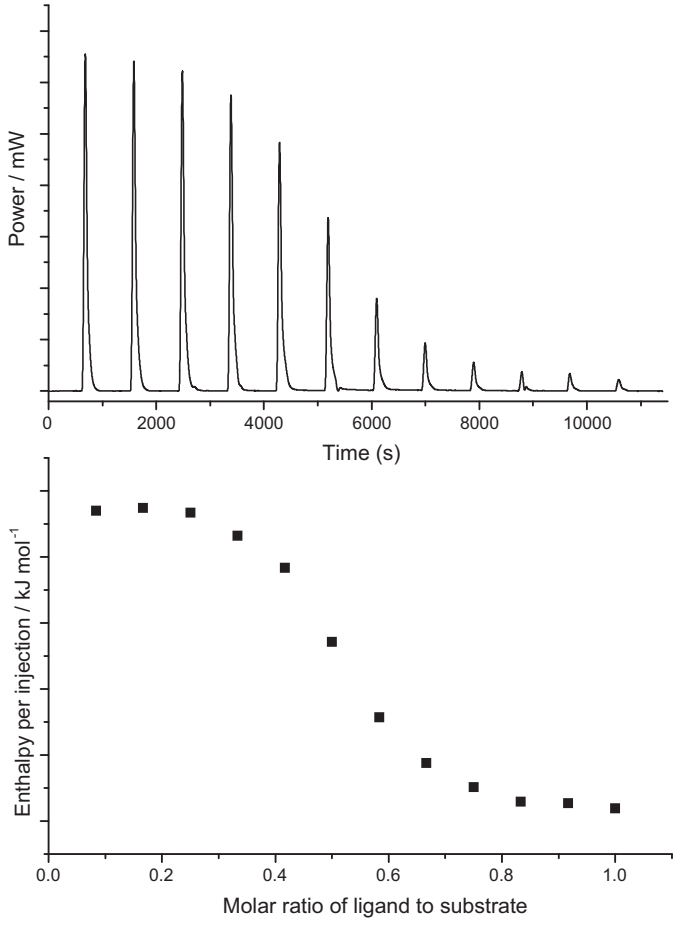

Fig. 1. Typical power–time data for the sequential titration of aliquots of a ligand into a substrate solution (top) and the subsequent binding isotherm (bottom).

(different solvents) or ionization (different buffers, or batches of the same buffer) may be recorded that are likely to be of a magnitude significantly larger than the binding interaction.

The output from a conventional ITC experiment is a plot of power versus time showing a series of peaks corresponding to sequential injections of ligand solution into substrate solution (Fig. 1 top). Integration of each peak results in a binding isotherm of enthalpy (kJ mol^{-1}) versus molar ratio of ligand to substrate (Fig. 1 bottom). The binding isotherm is then fitted to a binding model, by least squares minimization, in order to derive the value of K_b (and, in the process, $\Delta_b H$). It is also possible to derive the value of $\Delta_b H$ by direct experimental measurement. To do so, the experiment must be performed under conditions where the substrate is present at a much higher concentration than the ligand; this then allows the assumption to be made that total binding occurs with each injection of ligand. Thus, the area under each peak for sequential injections should be equal. Knowledge of the number of moles of ligand titrated thus allows the simple determination of the binding enthalpy.

In order to derive meaningful K_b values it is essential that the concentrations of the two species are selected such that the binding passes through its saturation point. An easy way to confirm this is that the titration peaks at the end of the experiment should have settled to a constant, and minimum, value (note here that since these peaks essentially represent the dilution of the ligand and substrate, it is possible to use the value to correct the earlier peaks, thus obviating the need for blank experiments, although this is a approximation).

Given the nature of the measurement there are several cautionary points to note. The first is that the enthalpy of the interaction is temperature dependent (i.e. it derives from the heat capacity change of the system). Therefore a non-detectable signal may not be indicative of no interaction but merely that the enthalpy change at the temperature of study is too low (and in the limit is zero) to be detected by the instrument. The heat capacity dependence is unique to each different system and therefore needs to be considered each time a new experiment is performed. As a consequence it is vital to report the temperature at which the measurement is made.

Method development and optimisation of ITC experiments can potentially be highly material consuming. To lessen some of these issues a novel *in silico* approach to mapping theoretical experiments has been developed (Biswas and Tsodikov, 2010). The nuances of ITC are many and varied and as such caution must be exercised when preparing and performing such experiments. Given the complex nature of these experiments they cannot be adequately dealt with in a review article such as this. For an excellent description of the practical and theoretical aspects of ITC the reader is directed to Ladbury and Doyle (2004).

2.2.1. Calibration

As discussed for ampoule calorimetry there are strong reasons for the use of chemical test and reference reactions in order to assess the performance of an ITC instrument. Two principal systems are indicated. The first is the binding of Ba^{2+} to 18-crown-6 (1,4,7,10,13,26-hexa-oxacyclooctadecane) (Briggner and Wadsö, 1991). The values for the enthalpy and equilibrium constant (at 298.15 K), when run under the conditions specified in the paper are 31.42 ± 0.20 kJ mol^{-1} and $5.90 \pm 0.20 \times 10^3$ mol^{-1} dm^3 respectively. Mizoue and Tellinghuisen (2004) discuss a comparison of the van't Hoff and calorimetric enthalpies of binding for this reaction. The second reaction is the binding of 2′CMP to RnaseA (Wadsö and Goldberg, 2001). The derived thermodynamic properties for this reaction are highly dependent on pH, ionic strength, temperature and RnaseA concentration and hence its use as a test reaction is limited. The IUPAC committee has proposed standard conditions (potassium acetate, 0.2 mol dm^{-3}; potassium chloride, 0.2 mol dm^{-3}; RnaseA, 0.175 mol dm^{-3} at pH 5.5) for which values of the enthalpy and equilibrium constant, respectively, of -50 ± 3 kJ mol^{-1} and $1.20 \pm 0.05 \times 10^5$ mol^{-1} dm^3 should be attained.

ITC is perhaps unique in the fact that over the course of the experiment the volume in the sample cell is either changing as more titrant is added or material is allowed to overflow from the sample ampoule on addition in order to maintain a constant volume for measurement, the latter being the most prevalent. This in itself can lead to systematic errors being introduced to any data derived from the experiment (Tellinghuisen, 2004). Thus care should be taken to map the effects of such an arrangement and to this end a chemical test and reference reaction utilizing the dilution of NaCl(aq) has been reported (Tellinghuisen, 2007). This is an interesting system, utilising the fact that the enthalpy of dilution of NaCl in water varies non-linearly at concentrations below 0.2 M, and the author constructed an algorithm to calibrate the instrument both for enthalpy and dilution.

2.2.2. Ligand–substrate binding

ITC is used widely in the biological and drug discovery context and numerous articles report a vast array of systems that have been characterised using this technique. Specific examples include; ligand–substrate binding (Saboury, 2006); biological macromolecule and metal ion complexation studies (Wilcox, 2008); protein–protein interactions (Liang, 2006). Such is the sensitivity of some modern instruments it is now possible to conduct

experiments at concentrations that previously were only accessible using fluorescence techniques thus opening up the possibilities of studying such systems without fluorescent moieites (Moreno et al., 2010). This universal applicability of ITC to probe interactions has been used to good effect in characterising novel drug formulations and drug delivery systems (Bouchemal, 2008, Holdgate and Ward, 2005). Ikonen et al. (2010) describe how ITC can be used to investigate the binding and concomitant loading of 4 drugs (alprenolol, labetalol, propranolol and tetracaine) into Palmitoyl derived liposomes. In particular they were able to show that the experimentally determined liposome–water partition co-efficients for each drug appeared to be lower than that predicted by the octanol-water partition co-efficient. Such information is crucial for the intelligent design and characterisation of new drug delivery systems.

ITC studies have also been conducted on model membrane systems to predict the efficacy of antibacterials and their potential mechanism of interaction with bacterial cell walls (Al-Kaddah et al., 2010). Here the authors compared two antibacterials, gallidermin and vancomycin, and their mechanisms of membrane interaction using a model liposome. They conclude that although the two drugs both target a key molecule of peptidoglycan biosynthesis, the cell wall precursor lipid II, they exhibit different modes of membrane interaction with gallidermin exhibiting a much higher enthalpy of interaction suggesting stronger, non-targeted membrane interactions with gallidermin compared with vancomycin. This then suggests that the antimicrobial activity of vancomycin is determined by the availability of lipid II in the bacterial membrane and that the activity of gallidermin is mediated by other cell wall characteristics.

One major drawback of ITC is that it is not really amenable to high-throughput analysis, each experiment being individually set up and run (and lasting an hour or more). This fact, to a large degree, means that alternative assays are used for screening compound libraries and ITC is used to verify the results of the top hits. The realisation of the potential of calorimetry in HTS awaits the availability of instrumentation capable of detecting small heat changes in large numbers of samples on a time scale that is both sympathetic to protein stability and conducive to high throughput operation.

2.3. Isothermal gas perfusion calorimetry

One of the major benefits of isothermal calorimetry is the ability to control the atmosphere in the sample ampoule—usually in terms of relative humidity (RH) or, less commonly, relative vapour pressure (RVP). Immediate benefits of such control are the opportunity to replicate storage conditions, study polymorphic conversion, quantify sample hygroscopicity and determine amorphous contents.

2.3.1. Calibration

When an experiment is performed in a closed system using a saturated salt solution, no test for verification of RH is required (although it should be ensured that the salt solution reservoir contains a number of salt crystals, to prevent creation of a supersaturated solution as water evaporates) and there is no indicated test and reference material for water uptake. When the humidity or relative partial pressure are being controlled by proportionally mixing gas lines then using tests to calibrate specific RH values is both possible and important. Where the RH can be increased linearly with time then a deliquescent salt may be employed, since it will deliquesce at a specific RH. An alternative is to place a reservoir of saturated salt solution in the ampoule and perform a linear RH ramp. When the RH set by the instrument is below that which the saturated salt solution will maintain water will evaporate from the reservoir and an endothermic signal will be recorded. Conversely, as the RH is increased above that which the saturated salt solution maintains water will condense to the reservoir and an exothermic signal will be observed. Only when the RH set by the instrument coincides with that maintained by the saturated salt solution will no power be recorded. Baronsky et al. (2009) published an alternative method when relative vapour pressures need to be calibrated.

2.3.2. Amorphous content quantification

To determine amorphous content a partially amorphous sample is exposed to an elevated RH. The material both adsorbs and absorbs moisture and is plasticised; when the glass transition temperature (T_g) reduces to below that of the experimental temperature any amorphous regions will crystallise and a large exothermic signal will be recorded.

In order to achieve control of RH there must be some method of introducing humidity into the sample ampoule. Conventionally two approaches are adopted. The simplest method is to place a small glass hygrostat containing a reservoir of pure water (100% RH) or a saturated salt solution (to maintain a specific, lower RH) directly in the ampoule. After sealing the ampoule, water will either evaporate from, or condense into, the reservoir to maintain a specific RH. The second method is isothermal gas perfusion calorimetry (IGPC) wherein three gas lines are routed through the sample ampoule. Two of the lines allow an inert carrier gas to enter the ampoule and the third allows the gas to exit to the surroundings. One of the inlet lines carries dry gas direct from a cylinder, while the other passes the gas through two water (or other plasticising solvents, typically ethanol) reservoirs prior to the sample ampoule. Hence the two inlet lines can be considered to provide gases at either 0 or 100% RH or RVP. The flow rates of the gas lines are controlled by mass-flow controllers and the exact RH or RVP experienced by the sample can be controlled by adjusting the flow rates proportionately.

In principle, either approach allows for proper construction of experiments, but in practice each system has drawbacks. A detailed discussion of the problems inherent in using mini-hygrostats is available in the excellent review by Khalef et al. (2010). One of the principal assumptions made in the use of mini-hygrostats is that the rates of water evaporation and condensation from the reservoir are equal and opposite, over the experimental timeframe. Thus, when considering crystallisation of an amorphous material it is usually assumed that the following processes contribute to the observed power data;

- Water evaporation from the reservoir.
- Wetting of the ampoule surfaces.
- Water absorption and adsorption by the sample.
- Crystallisation and simultaneous water expulsion.
- Condensation of expelled water into the reservoir.

The net power changes that accompany these processes appear as discrete entities with time and crystallisation is often preceded with a small exotherm, taken to represent wetting of the ampoule and sample. Following this is a sharp crystallisation peak which decays back to baseline concomitantly with condensation of expelled water back to the reservoir. The decision as to which parts of the data to integrate in order to get the heat of crystallisation often reduces to experimenter preference, although Dilworth et al. (2004) provide a quantitative example of different integration strategies for a study of amorphous lactose. What is clear is that whatever part of the data is integrated it is not possible to correct for the heat of wetting of the ampoule surfaces, since this is a one-off, irreversible event. Further, Khalef et al. (2010) discuss several further issues related to the salt solution (dilution of the saturated solution, hydration/ionization of the salt solution following dilution and dissolution of salt crystals in the condensed water) that may contribute to the measured power but that cannot be corrected for. The net result is that use of a mini-hygrostat often means some

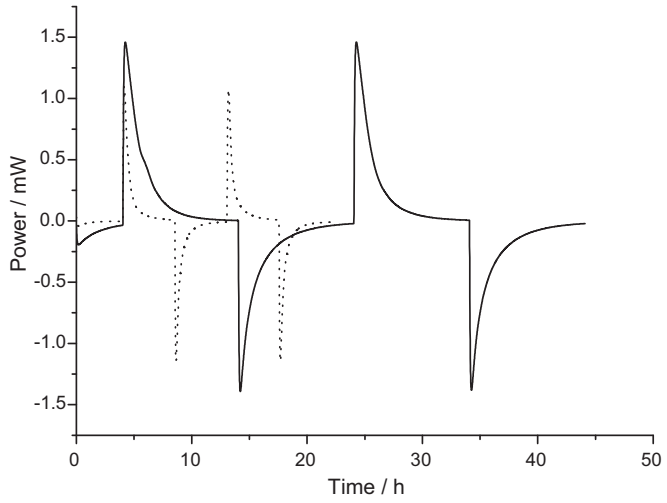

Fig. 2. Power–time data for two samples of a drug, both nominally 5% (w/w) amorphous but with different sample masses (50 mg, dotted line; 250 mg, solid line). The sample was exposed to 5 humidity periods; 0–95–0–95–0. The onset of each peak denotes the change in RH.

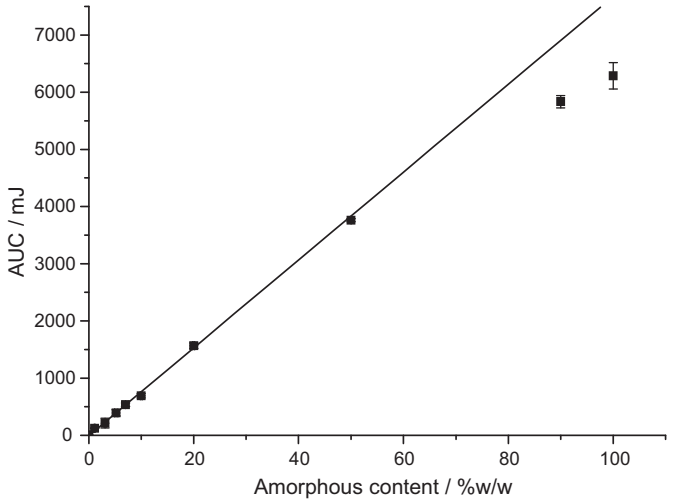

Fig. 3. A calibration line for amorphous content in salbutamol sulphate ($n = 3$) showing a deviation from linearity as the amorphous content approaches 100%.

processes cannot be accounted for which manifest themselves as hysteresis in any derived calibration line for amorphous content.

Use of IGPC ameliorates most of the issues noted above, simply because salt solutions are not involved. In addition, IGPC allows the sample to be held under a dry atmosphere to reach thermal (and moisture) equilibrium prior to experimentation. The RH can be increased linearly or altered in discrete steps at any point during the experiment. From the perspective of amorphous content quantification, if the RH is increased in a single step to above the critical RH value (the RH at which crystallisation will occur) then the measured power will comprise the following processes;

- Wetting of the ampoule surfaces.
- Water absorption and adsorption by the sample.
- Crystallisation and water expulsion into the flowing gas stream.

Again, it must be assumed that water adsorption and expulsion proceed with equal but opposite enthalpies. This being so, the net heat change during the period at elevated RH must then comprise wetting of the sample surface and ampoule and crystallisation. If the sample is subsequently held under a dry atmosphere, the only processes that should occur are drying of the sample and drying of the ampoule surfaces. The sum of areas (elevated RH + dry atmosphere) should then cancel all events except crystallisation.

There are, of course, assumptions with this approach also. One is that the sample that wets (partially amorphous, probably at the surface) is not the same as the sample that dries (crystalline). Another is that no other humidity-induced processes (such as muta-rotation, hydrolysis or change in polymorph) occur. If these are suspected, then one resolution is to wet the sample a second time. In this instance, subtraction of the heats recorded during the two elevated humidity phases should suffice to cancel any heat not arising from crystallisation.

Experimental design also affects amorphous content quantification. Since it is a requirement that all the sample is exposed to the humidity in the ampoule it is not advisable to use large sample masses, since this will create a column of powder and the particles nearer the base will not wet. An example of this effect is provided by the data in Fig. 2, which show the power–time changes for two samples of a drug, nominally 5% (w/w) amorphous, at two mass loadings (50 mg and 250 mg). Although the 250 mg sample gives a larger power, subtraction of the heats recorded during the two wetting periods gives 95 ± 10 mJ for the 50 mg sample and 228 ± 22 mJ for the 250 mg sample. Were the response proportional to sample mass, a heat of 477.5 mJ would have been expected for the larger sample; the reduction in heat can only be ascribed to some of the powder mass not being exposed to the humidity.

A second issue concerns preparation of calibration standards and their physical resemblance to processed samples. The only way to prepare samples of defined amorphous content is to blend nominally 100% amorphous material (typically spray-dried) with crystalline material. Hence, the calibration standards will comprise powder blends where each individual particle is either amorphous or crystalline. Samples to be analysed are frequently micronized powders, wherein each particle will have a crystalline core surrounded by a corona of amorphous material. Not only will micronized powders thus wet differently, the amorphous fraction is always in intimate contact with a crystalline seed, which will result in a faster rate of crystallisation. The issue of seed material also affects the calibration standards. Assuming all particles are the same size and are uniformly blended then at amorphous contents up to 50% (w/w) each amorphous particle should be in contact with a crystalline particle. At higher amorphous contents the number of amorphous particles will always exceed that of the crystalline particles and at 100% no crystal particles are present. This can typically mean that high amorphous content sample either do not crystallise fully, or may crystallise to a different polymorph or hydrate, meaning linearity in the calibration curve is lost. This is demonstrated by the data in Fig. 3, which show a calibration curve for amorphous content in salbutamol sulphate and is also alluded to in a publication by Ramos et al. (2005a).

A final issue concerns applicability of the technique to various samples. It is common to quote a sensitivity of measurement of a particular technique for a given type of analysis. However, the response of the calorimeter is dependent upon the enthalpy of crystallisation of the sample. Fig. 4 shows calibration lines determined with IGPC for two commercial drug candidates, A and B. Drug A had a high enthalpy of crystallisation and thus the detection limit (DL) and quantification limit (QL) were very good (DL, 0.16%, w/w; QL, 0.48%, w/w) while drug B had a low enthalpy of crystallisation and hence the assay was reasonably poor (DL, 1.7%, w/w; QL, 5.2%, w/w).

2.3.3. Polymorph conversion

While differential scanning calorimetry (DSC) is more commonly associated with polymorph characterisation, it is also

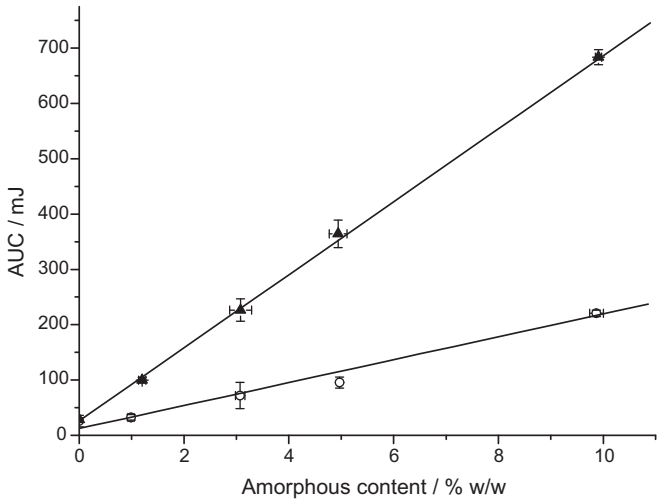

Fig. 4. Calibration lines for amorphous content for two drugs ($n = 3$). Drug A (solid triangle) has a high enthalpy of crystallisation while Drug B (open circles) has a low enthalpy of crystallisation.

possible to determine polymorphic transition behaviour of a system through its isothermal heat output, providing the rate of conversion is reasonably fast (the extent of conversion, α, is >0.15 over the lifetime of the experiment (hours).

Seratrodast (an anti-asthmatic drug) has been studied via an isothermal technique (Urakami and Beezer, 2003). Here the drug was studied under controlled atmospheres of defined RH and the drug allowed to interconvert over a period of approximately 20 h. Elevated temperatures (50–65 °C) were used to ensure that a sufficient fraction of the material had converted in order to perform the analysis. Not only was the enthalpy of the transition ($-5.70 \pm 1.13 \times 10^{-1}$ kJ mol^{-1}) amenable to measurement, quantification of the kinetic nature of the transition was also accessible using the Hancock–Sharp equation (Hancock and Sharp, 1972);

$$\ln(-\ln(1-\alpha)) = \ln B + m \ln t (0.15 \leq \alpha \leq 0.5) \quad (1)$$

where B is a constant and m is a parameter that defines the reaction mechanism. From the values derived ($m = 2.00$, 2.09 and 2.1) the authors concluded that for each of the three sieved fractions the mechanism of transformation of seratrodast is best described by the two dimensional growth of nuclei model. They also showed it possible to calculate the rate constant for the transformation for each sieved fraction and that the rate constant increases as particle size decreases.

2.3.4. Hygroscopicity

The potential for, and the extent to which, a material will take on moisture from its surroundings is described by an equilibrium term; hygroscopicity. The two parameters, potential and extent, are intrinsically linked to the RH of the system and the temperature of the atmosphere surrounding the sample. Because hygroscopicity is an equilibrium term a sample will either lose moisture or gain moisture when placed into an atmosphere at constant RH. This transfer will continue until a new equilibrium is reached, at which point the quantity of water in the sample is called the equilibrium moisture content (EMC). The value of the EMC and the rate at which a sample attains equilibrium is governed by several factors including surface area, temperature and RH.

Nearly every material will exchange moisture with its surrounding atmosphere and hence the understanding and regulation of hygroscopicity is vital for the pre-formulation phase of a pharmaceutical product. Markova et al. (2001) describe a novel isothermal sorption microcalorimeter which permits the measurement of a variety of thermodynamic and kinetic parameters associated with hygroscopicity. It is reported that the instrument can reliably measure the differential enthalpy of sorption, condensation of solvents in capillaries, study the recrystallisation of amorphous materials (lactose in this instance), study lyoptropic phase transitions and monitor solvate formation. Jakobsen et al. (1997) determined the critical RH values for two highly water soluble drugs and one hydrophobic drug and showed that the results (rapidly obtained) were directly comparable to conventional weighing methods.

2.4. Solution calorimetry

The principle of solution (ampoule-breaking) calorimetry is simple; the heat change when a small quantity of a solid or liquid sample is dispersed into a (relatively) large volume of solvent is measured (either directly or by measuring a temperature change which is subsequently converted to a heat change). For a pure material dissolving into a solvent this results in the enthalpy of solution ($\Delta_{sol}H$), an enthalpy change that reflects contributions from the bonds broken when the crystal lattice dissolves ($\Delta_{lattice}H$) and from the bonds formed when the molecules are solvated ($\Delta_{solvation}H$). This can be expressed by Eq. (2);

$$\Delta_{sol}H = \Delta_{lattice}H + \Delta_{solvation}H \quad (2)$$

Depending on the relative magnitudes of $\Delta_{lattice}H$ and $\Delta_{solvation}H$, the heat of solution can have a positive (endothermic) or negative (exothermic) sign. Note here that $\Delta_{lattice}H$ for a completely, and perfectly, amorphous material is zero, which causes a large difference in $\Delta_{sol}H$ for crystalline and amorphous samples of the same materials (this is the basis of the sensitivity of the technique to small amorphous contents); in practice, most amorphous materials will possess some degree of short-range order which may mean that there will be a small heat change associated with dissolution, which will act to reduce the sensitivity of the technique. Furthermore, the value of $\Delta_{lattice}H$, and hence $\Delta_{sol}H$, will vary with each polymorph whereas the $\Delta_{solvation}H$ will remain constant (which is the basis of the sensitivity of the technique to polymorph identification).

Two types of solution calorimeter design are commercially available; instruments that record a temperature change upon reaction (semi-adiabatic) and instruments that record a power change directly upon reaction (heat-conduction). The sensitivities, quantities of solute and solvent required for experiment, operating principles and methods of validation of these instruments vary considerably and it is imperative to ensure proper instrument selection and sample preparation. These issues have been discussed elsewhere (Ramos et al., 2005a).

2.4.1. Calibration

Calibration of solution calorimeters with chemical test reactions is important; an investigation of materials for calibration recently concluded that the dissolution of KCl into water was the most robust test routine for semi-adiabatic instruments (Yff et al., 2004). Ramos et al. (2005b) re-examined these materials for heat-conduction solution calorimeters and concluded that the best reference material was sucrose and not KCl.

2.4.2. Solid form characterisation

Solution calorimetry is often used for polymorphism detection and characterisation. For instance, two polymorphs of cyclopenthiazide (forms I and II) have been shown to have comparable $\Delta_{sol}H$ values (~6 kJ mol^{-1}) while form III has a higher (15 kJ mol^{-1}) enthalpy of solution of (Gerber et al., 1991). Similarly, solution calorimetry has been used to characterise the polymorphs of Abbott-79175 (Li et al., 1996) and MK996, an angiotensin II antagonist agent (Jahansouz et al., 1999).

The correlation of dissolution rates with $\Delta_{sol}H$ for different polymorphs of a drug has also been attempted. For instance, Terada et al. (2000) showed there was a linear correlation between $\Delta_{sol}H$ values of different polymorphs of indomethacin and the logarithms of their initial dissolution rates (determined by the rotating disk method). The same authors showed a similar relationship for a range of samples of terfenadine of varying crystallinity.

2.4.3. Amorphous content quantification

The use of solution calorimetry to quantify the degree of crystallinity assumes that the enthalpy of solution of a mixture varies proportionately with the weight fractions of the crystalline and amorphous states present. The usual methodology involves the preparation of a calibration line of $\Delta_{sol}H$ against degree of crystallinity using a number of known standards (prepared by blending the appropriate mass quantities of entirely amorphous and entirely crystalline material).

There are two potential drawbacks to using this approach. Firstly, the sample may exhibit polymorphism; if this is the case then it must be ensured that the crystalline material used to prepare the standards and the sample to be analysed have the same polymorphic composition. Secondly, it is likely that the interactions in a particle that has a crystalline core and amorphous material on its surface (the likely case for a processed pharmaceutical) differ from those of the calibration standards, which may result in the calibration plot producing spurious results.

Pikal et al. (1978) were the first to use solution calorimetry for the quantitative measurement of the degree of crystallinity of pharmaceuticals by measuring the heat of solution of various β-lactam antibiotics. Subsequent studies have included the analysis of sulphamethoxazole from different sources (Guillory and Erb, 1985), sucrose (Gao and Rytting, 1997) and clathrate warfarin sodium (Gao and Rytting, 1997).

Quantifying large degrees of crystallinity presents little problem for solution calorimetry; however, detection limits are important if the objective is to quantify a small amorphous content in what is a predominantly crystalline sample. The use of solution calorimetry to study small amorphous contents in solid pharmaceuticals was assessed by Hogan and Buckton (2000). They found amorphous contents could be quantified to ±0.5% (w/w) but noted that care needed to be taken when preparing the ampoules, because ingress of even small amounts of humidity caused partial recrystallisation of the sample before measurement.

Usually, in experiments designed to measure degrees of crystallinity of amorphous content a solvent is selected in which the solute is freely soluble. This ensures complete dissolution of the sample within the time frame of the experiment. Harjunen et al. (2004) studied the dissolution of lactose into saturated aqueous lactose solutions, a system where clearly the solute would not completely dissolve. Interestingly, they observed a linear relationship between the amorphous content of the lactose solute and the measured heat of solution in the saturated lactose solution ($\Delta_{sat}H$). Similarly, a linear relationship was found between the amorphous content of lactose and $\Delta_{sat}H$ in methanolic saturated solutions of lactose (Katainen et al., 2003).

2.4.4. Other pharmaceutical applications

Chadha et al. (2002) used solution calorimetry to probe the interactions of diclofenac sodium in cyclodextrin solutions and water/ethanol mixtures. Tong et al. (1991) used solution calorimetry to evaluate the stability constants and enthalpy changes associated with the formation of complexes between 2-hydroxypropyl-β-cyclodextrin and a group of 12 amine compounds which all had a diphenylmethyl functional group. They found that only terfenadine HCl formed a 1:2 complex with the β-cyclodextrin, the other 11 compounds all forming 1:1 complexes.

Solution calorimetry has also been used to measure the enthalpy of solution of diclofenac sodium, paracetamol and their binary mixtures (Chada et al., 2003) and to evaluate the in vitro compatibility of amoxicillin/clavulanic acid and ampicillin/sulbactam with ciprofloxacin (Chada et al., 2004).

Jain et al. (2000) used solution calorimetry to probe the possible interactions between ampicillin and amoxicillin, a combination known to act synergistically. Traditional spectroscopic analysis is not possible because of the proximity of their λ_{max} values. Using solution calorimetry they were able to determine the $\Delta_{sol}H$ values at a variety of pHs as well as the excess molar enthalpies of solution of the binary mixture. From the low values of the excess molar enthalpies they conclude that the two antibiotics do not interact with each other to any great extent.

A similar study (Alves et al., 2005) reports the measurement of $\Delta_{sol}H$ of diclofenac sodium and paracetamol individually and the excess molar enthalpy of binary mixtures in order to determine whether there was any interaction when in solution. It is reported that the $\Delta_{sol}H$ values of diclofenac sodium and paracetamol, at pH 7.0, are $50.24 \pm 0.04\,kJ\,mol^{-1}$ and $24.76 \pm 0.04\,kJ\,mol^{-1}$ respectively. Again the values of the excess molar enthalpy are small and indicate that little or no interaction exists.

Perlovich and Bauer-Brandl (2003) have discussed the use of heat of solution data to predict drug solubility using two model compounds, benzoic acid and aspirin. Their data suggest it may be possible to predict the solubility or solvation of a drug in different media. Similarly, Willson and Sokoloski (2004), as part of a study developing a method to rank the stability of drug polymorphs, correlated solution calorimetry measurements to conventionally determined solubility data. Solution calorimetry has also been employed to study the dissolution and solvation of the model systems, flurbiprofen and diflunisal (Perlovich et al., 2003) and to compare the solvation of (+)-naproxen with three model NSAIDs (benzoic acid, diflunisal and flurbiprofen) (Perlovich et al., 2004). Johari (1995) shows how heats of solution can be used to determine the internal energies of polymorphs.

3. Summary

Isothermal calorimetry has many applications in pharmaceutical development and stands distinct from the more commonly used technique of DSC in that it studies time-dependent phase transitions and chemical processes rather than just temperature-driven phase transitions. This means its applications complement those of DSC and the techniques can rapidly advance development and characterisation of a new pharmaceutical. In its simplest form IC takes samples loaded in hermetically sealed ampoules. This configuration is appropriate for stability-indicating assays, including drug-excipient compatibility assessment, and for determination of reaction kinetics and thermodynamics. Investigation of more specific phenomena requires proper selection of instrument configuration and experimental design. In the pharmaceutical arena the most commonly used configurations are titration, gas-perfusion and solution (ampoule-breaking), since these allow quantification of inter-molecular interactions and determination of physical form characteristics.

References

Al-Kaddah, S., Reder-Christ, K., Klocek, G., Wiedemann, I., Brunschweiger, M., Bendas, G., 2010. Analysis of membrane interactions of antibiotic peptides using ITC and biosensor measurements. Biophys. Chem. 152, 145–152.

Alves, N., Bai, G., Bastos, M., 2005. Enthalpies of solution of paracetamol and sodium diclofenac in phosphate buffer and in DMSO at 298.15K. Thermochim. Acta 441, 16–19.

Angberg, M., Nyström, C., 1988. Evaluation of heat-conduction microcalorimetry in pharmaceutical stability studies. I. Precision and accuracy for static experiments in glass vials. Acta Pharm. Suec. 25, 307–320.

Angberg, M., Nyström, C., Castensson, S., 1990. Evaluation of heat-conduction microcalorimetry in pharmaceutical stability studies. II. Methods to evaluate the microcalorimetric response. Int. J. Pharm. 61, 67–77.

Baronsky, J., Bongaerts, S., Traeubel, M., Urbanetz, N., 2009. Isothermal microcalorimetry perfusion experiments: a method to verify the relative vapour pressure of organic liquids inside the sample vessel. Thermochim. Acta 493, 37–41.

Beezer, A.E., Hills, A.K., O'Neill, M.A.A., Morris, A.C., Kierstan, K.T.E., Deal, R.M., Waters, L.J., Hadgraft, J., Mitchell, J.C., Connor, J.C., Orchard, J.A., Willson, R.J., Hofelich, T.C., Beaudin, J., Wolf, G., Baitalow, F., Gaisford, S., Lane, R.A., Buckton, G., Phipps, M.A., Winneke, R.A., Schmitt, E.A., Hansen, L.D., O'Sullivan, D., Parmar, M.K., 2001. The imidazole catalysed hydrolysis of triacetin: an inter and intra laboratory development of a test reaction for isothermal heat conduction microcalorimeters used for determination of both kinetic and thermodynamic parameters. Thermochim. Acta 380, 13–17.

Biswas, T., Tsodikov, O.V., 2010. An easy-to-use tool for planning and modeling a calorimetric titration. Anal. Biochem. 406, 91–93.

Bonander, N., Darby, R.A.J., Grgic, L., Bora, N., Wen, J., Brogna, S., Poyner, D.R., O'Neill, M.A.A., Bill, R.M., 2009. Altering the ribosomal subunit ratio in yeast maximises recombinant protein yield. Micro. Cell Factories, doi:10.1186/1475-2859-8-10.

Bouchemal, K., 2008. New challenges for pharmaceutical formulations and drug delivery system characterization using isothermal titration calorimetry. Drug Discov. Today 13, 960–972.

Briggner, L.E., Wadsö, I., 1991. Test and calibration processes for microcalorimeters, with special reference to heat-conduction instruments used with aqueous systems. J. Biochem. Biophys. Methods 22, 101–118.

Chadha, R., Kashid, N., Kumar, A., Jain, D.V.S., 2002. Calorimetric studies of diclofenac sodium in aqueous solution of cyclodextrin and water–ethanol mixtures. J. Pharm. Pharmacol. 54, 481–486.

Chada, R., Kashid, N., Jain, D.V.S., 2003. Microcalorimetric studies to determine the enthalpy of solution of diclofenac sodium, paracetamol and their binary mixtures at 310.15 K. J. Pharm. Biomed. Anal. 30, 1515–1522.

Chada, R., Kashid, N., Jain, D.V.S., 2004. Microcalorimetric evaluation of the in vitro compatibility of amoxicillin/clavulanic acid and ampicillin/sulbactam with ciprofloxacin. J. Pharm. Biomed. Anal. 36, 295–307.

Chaires, J.B., 2008. Calorimetry and thermodynamics in drug design. Annu. Rev. Biophys. 37, 135–151.

Chen, A.T., Wadsö, I., 1982. A test and calibration process for micro-calorimeters used as thermal power meters. J. Biochem. Biophys. Methods 6, 297–306.

Dilworth, S.E., Buckton, G., Gaisford, S., Ramos, R., 2004. Approaches to determine the enthalpy of crystallisation, and amorphous content, of lactose from isothermal calorimetric data. Int. J. Pharm. 284, 83–94.

Finnin, B.A., O'Neill, M.A.A., Gaisford, S., Beezer, A.E., Hadgraft, J., Sears, P., 2006. Performance validation of step-isothermal calorimeters: application of a test and reference reaction. J. Therm. Anal. Calorim. 83, 331–334.

Gaisford, S., O'Neill, M.A.A., Thompson, L., Chan, K.-L., 2006. Shelf-life prediction of busulfan I.V. (Busilvex™) by isothermal calorimetry. Hosp. Pharm. 13, 295–298.

Gao, D., Rytting, J.H., 1997. Use of solution calorimetry to determine the extent of crystallinity of drugs and excipients. Int. J. Pharm. 151, 183–192.

Gerber, J.J., vanderWatt, J.G., Lötter, A.P., 1991. Physical characterization of solid forms of cyclopenthiazide. Int. J. Pharm. 73, 137–145.

Guillory, J.K., Erb, D.M., 1985. Using solution calorimetry to quantitate binary mixtures of three crystalline forms of sulfamethoxazole. Pharm. Manuf. (September), 28–33.

Guisan, J.M., 2006. Immobilization of Enzymes and Cells, 2nd ed. Humana Press Inc., New Jersey.

Harjunen, P., Lehto, V.-P., Koivisto, M., Levonen, E., Paronen, P., Järvinen, K., 2004. Determination of amorphous content of lactose samples by solution calorimetry. Drug Dev. Ind. Pharm. 30, 809–815.

Hancock, J.D., Sharp, J.H., 1972. Method of comparing solid-state kinetic data and its application to the decomposition of kaolinite, brucite, and $BaCO_3$. J. Am. Ceram. Soc. 55, 74–77.

Hansen, L.D., Lewis, E.A., Eatough, D.J., Bergstrom, R.G., DeGraft-Johnson, D., 1989. Kinetics of drug decomposition by heat conduction calorimetry. Pharm. Res. 6, 20–27.

Hansen, L.D., Eatough, D.J., Lewis, E.A., Bergstrom, R.G., Degraft-Johnson, D., Cassidy-Thompson, K., 1990. Shelf-life prediction from induction period calorimetric measurements on materials undergoing autocatalytic decomposition. Can. J. Chem. 68, 2111–2114.

Hogan, S., Buckton, G., 2000. The quantification of small degrees of disorder in lactose using solution calorimetry. Int. J. Pharm. 207, 57–64.

Holdgate, G.A., Ward, W.H.J., 2005. Measurements of binding thermodynamics in drug discovery. Drug Discov. Today 10, 1543–1549.

Ikonen, M., Murtomaki, L., Kontturi, K., 2010. Microcalorimetric and zeta potential study on binding of drugs on liposomes. Colloids Surf. B Biointerfaces 78, 275–282.

Jahansouz, H., Thompson, K.C., Brenner, G.S., Kaufman, M.J., 1999. Investigation of the polymorphism of the angiotensin II antagonist agent MK-996. Pharm. Dev. Technol. 4, 181–187.

Jain, D.V.S., Kashid, N., Kapoor, S., Chadha, R., 2000. Enthalpies of solution of ampicillin, amoxycillin and their binary mixtures at 310.15 K. Int. J. Pharm. 201, 1–6.

Jakobsen, D.F., Frokjaer, S., Larsen, C., Niemann, H., Buur, A., 1997. Application of isothermal microcalorimetry in preformulation. I. Hygroscopicity of drug substances. Int. J. Pharm. 156, 67–77.

Johari, G.P., 1995. Obtaining further information from calorimetry. Thermochim. Acta 266, 31–47.

Katainen, E., Niemelä, P., Päällysaho, M., Harjunen, P., Suhonen, J., Järvinen, K., 2003. Evaluation of the amorphous content of lactose by solution calorimetry and Raman spectroscopy. Eur. J. Pharm. Sci. Abs. 19, S36.

Khalef, N., Pinal, R., Bakri, A., 2010. Limitations of amorphous content quantification by isothermal calorimetry using saturated salt solutions to control relative humidity: alternative methods. J. Pharm. Sci. 99, 2080–2089.

Koenigbauer, M.J., Brooks, S.H., Rullo, G., Couch, R.A., 1992. Solid-state stability testing of drugs by isothermal calorimetry. Pharm. Res. 9, 939–944.

Kong, W.J., Wang, J.B., Jin, C., Zhao, Y.L., Dai, C.M., Xiao, X.H., Li, Z.L., 2009. Effect of emodin on *Candida albicans* growth investigated by microcalorimetry combined with chemometric analysis. Appl. Microbiol. Biotechnol. 83, 1183–1190.

Ladbury, J.E., Doyle, M.L., 2004. Biocalorimetry 2: Applications of Calorimetry in the Biological Sciences. John Wiley and Sons, Chichester, England.

Li, R.C.Y., Mayer, P.T., Trivedi, J.S., Fort, J.J., 1996. Polymorphism and crystallization behaviour of Abbott-79175, a second generation 5-lipoxygenase inhibitor. J. Pharm. Sci. 85, 773–780.

Li, Y., Wadsö, L., Larsson, L., Bjurman, J., 2007. Correlating two methods of quantifying fungal activity: heat produciton by isothermal calorimetry and ergosterol amount by gas chromatography-tandem mass spectrometry. Thermochim. Acta 458, 77–83.

Liang, Y., 2006. Applications of isothermal titration calorimetry in protein folding and molecular recognition. J. Iran. Chem. Soc. 3, 209–219.

Markova, N., Sparr, E., Wadsö, L., 2001. On application of an isothermal sorption microcalorimeter. Thermochim. Acta 374, 93–104.

Montanari, M.L.C., Andricopulo, A.D., Montanari, C.A., 2004. Calorimetry and structure–activity relationships for a series of antimicrobial hydrazides. Thermochim. Acta 417, 283–294.

Moreno, M.J., Bastos, M., Velazquez-Campoy, A., 2010. Partition to lipid bilayers by ITC. Anal. Biochem. 399, 44–47.

Mizoue, L.S., Tellinghuisen, J., 2004. Calorimetric vs. van't Hoff binding enthalpies from isothermal titration calorimetry: Ba^{2+} - crown ether complexation. Biophys. Chem. 110, 15–24.

Oliyai, R., Lindenbaum, S., 1991. Stability testing of pharmaceuticals by isothermal heat conduction calorimetry: ampicillin in aqueous solution. Int. J. Pharm. 73, 33–36.

O'Neill, M.A.A., Beezer, A.E., Labetoulle, C., Nicolaides, L., Mitchell, J.C., Orchard, J.A., Connor, J.A., Kemp, R.B., Olomolaiye, D., 2003. The base catalysed hydrolysis of methyl paraben: a test reaction for flow microcalorimeters used for determination of both thermodynamic and kinetic parameters. Thermochim. Acta 399, 63–71.

O'Neill, M.A.A., Gaisford, S., Beezer, A.E., Skaria, C.V., Sears, P., 2006. A comparison of the performance of calorimeters. Application of a test and reference reaction. J. Therm. Anal. Calorim. 84, 301–306.

O'Neill, M.A.A., Beezer, A.E., Tetteh, J., Gaisford, S., Dhuna, M., 2007. Application of chemometric analysis to complexity in isothermal calorimetric data. J. Phys. Chem. B 111, 8145–8149.

Otsuka, T., Yoshioka, S., Aso, Y., Terao, T., 1994. Application of microcalorimetry to stability testing of meclofenoxate hydrochloride and dl-α-tocopherol. Chem. Pharm. Bull. 42, 130–132.

Parmar, M.K., Beezer, A.E., Willson, R.J., 2004. An isothermal microcalorimetric study of the imidazole catalysed hydrolysis of triacetin and the observed rate constant dependence of triacetin concentration. Thermochim. Acta 424, 219–222.

Perlovich, G.L., Bauer-Brandl, A., 2003. Thermodynamics of solutions I. Benzoic acid and acetylsalicylic acid as models for drug substances and the prediction of solubility. Pharm. Res. 20, 471–478.

Perlovich, G.L., Kurkov, S.V., Bauer-Brandl, A., 2003. Thermodynamics of solutions: II. Flurbiprofen and diflunisal as models for studying solvation of drug substances. Eur. J. Pharm. Sci. 19, 423–432.

Perlovich, G.L., Kurkov, S.V., Kinchin, A.N., Bauer-Brandl, A., 2004. Thermodynamics of solutions III. Comparison of the solvation of (+)-naproxen with other NSAIDs. Eur. J. Pharm. Biopharm. 57, 411–420.

Phipps, M.A., Mackin, L.A., 2000. Application of isothermal microcalorimetry in solid state drug development. Pharm. Sci. Technol. Today 3, 9–17.

Pikal, M.J., Dellerman, K.N., 1989. Stability testing of pharmaceuticals by high-sensitivity isothermal calorimetry at 25 °C: cephalosporins in the solid and aqueous solution states. Int. J. Pharm. 50, 233–252.

Pikal, M.J., Lukes, A.L., Lang, J.E., Gaines, K., 1978. Quantitative crystallinity determinations for β-lactam antiobiotics by solution calorimetry: correlations with stability. J. Pharm. Sci. 67, 767–772.

Ramos, R., Gaisford, S., Buckton, G., 2005a. Calorimetric determination of amorphous content in lactose: a note on the preparation of calibration curves. Int. J. Pharm. 300, 13–21.

Ramos, R., Gaisford, S., Buckton, G., Royall, P.G., Yff, B.T.S., O'Neill, M.A.A., 2005b. A comparison of chemical reference materials for solution calorimeters. Int. J. Pharm. 299, 72–83.

Saboury, A.A., 2006. A review on the ligand binding studies by isothermal titration calorimetry. J. Iran. Chem. Soc. 3, 1–21.

Schmitt, E.A., Peck, K., Sun, Y., Deoffroy, J.M., 2001. Rapid, practical and predictive excipient compatibility screeing using isothermal microcalorimetry. Thermochim. Acta 380, 175–183.

Selzer, T., Radau, M., Kreuter, J., 1998. Use of isothermal heat-conduction microcalorimetry to evaluate stability and excipient compatibility of a solid drug. Int. J. Pharm. 171, 227–241.

Selzer, T., Radau, M., Kreuter, J., 1999. The use of isothermal heat conduction microcalorimetry to evaluate drug stability in tablets. Int. J. Pharm. 184, 199–206.

Simoncic, Z., Rokar, R., Gartner, A., Kogej, K., Kmetec, V., 2008. The use of microcalorimetry and HPLC for the determination of degradation kinetics and thermodynamic parameters of perindopril erbumine in aqueous solutions. Int. J. Pharm. 356, 200–2005.

Simoncic, Z., Zupancic, P., Roskar, R., Gartner, A., Kogej, K., Kmetec, V., 2007. Use of microcalorimetry in determination of stability of enalapril maleate and enalapril maleate tablet formulations. Int. J. Pharm. 342, 145–151.

Skaria, C.V., Gaisford, S., O'Neill, M.A.A., Buckton, G., Beezer, A.E., 2005. Stability assessment of pharmaceuticals by isothermal calorimetry: two component systems. Int. J. Pharm. 292, 127–135.

Sousa, L.A., Alem, N., Beezer, A.E., O'Neill, M.A.A., Gaisford, S., 2010. Quantitative analysis of solid-state processes studied with isothermal microcalorimetry. J. Phys. Chem. B 114, 13173–13178.

Tan, X., Meltzer, N., Lindenbaum, S., 1992. Solid-state stability studies of 13-cis-retinoic acid and all-trans-retinoic acid using microcalorimetry and HPLC analysis. Pharm. Res. 9, 1203–1208.

Tellinghuisen, J., 2004. Volume errors in isothermal titration calorimetry. Anal. Biochem. 333, 405–406.

Tellinghuisen, J., 2007. Calibration in isothermal titration calorimetry: heat and cell volume from heat of dilution of NaCl(aq). Anal. Biochem. 360, 47–55.

Terada, K., Kitano, H., Yoshihashi, Y., Yonemochi, E., 2000. Quantitative correlation between initial dissolution rate and heat of solution of drug. Pharm. Res. 17, 920–924.

Tong, W.-Q., Lach, J.L., Chin, T.-F., Guillory, J.K., 1991. Microcalorimetric investigation of the complexation between 2-hydroxypropyl-β-cyclodextrin and amine drugs with the diphenylmethyl functionality. J. Pharm. Biomed. Anal. 9, 1139–1146.

Urakami, K., Beezer, A.E., 2003. A kinetic and thermodynamic study of seratrodast polymorphic transition by isothermal microcalorimetry. Int. J. Pharm. 257, 265–271.

Vine, G.J., Bishop, A.H., 2005. The analysis of microorganisms by microcalorimetry in the pharmaceutical industry. Curr. Pharm. Biotechnol. 6, 223–238.

Wadsö, L., 2010. Operational issues in isothermal calorimetry. Cement Concrete Res. 40, 1129–1137.

Wadsö, I., Goldberg, R.N., 2001. Standards in isothermal microcalorimetry (IUPAC Technical Report). Pure Appl. Chem. 73, 1625–1639.

Wilcox, D.E., 2008. Isothermal titration calorimetry of metal ions binding to proteins: an overview of recent studies. Inorgânica Chimica Acta 361, 857–867.

Willson, R.J., Sokoloski, T.D., 2004. Ranking of polymorph stability for a pharmaceutical drug using the Noyes-Whitney titration template method. Thermochim. Acta 417, 239–243.

Yff, B.T.S., Royall, P.G., Brown, M.B., Martin, G.P., 2004. An investigation of calibration methods for solution calorimetry. Int. J. Pharm. 269, 361–372.

Zaman, F., Beezer, A.E., Mitchell, J.C., Clarkson, J., Elliot, J., Davis, A.F., Willson, R.J., 2001. The stability of benzoyl peroxide by isothermal microcalorimetry. Int. J. Pharm. 227, 133–137.