Application of isothermal microcalorimetry in solid state drug development

Mark A. Phipps and Lesley A. Mackin

Microcalorimetry is an analytical technique that has found numerous applications within the pharmaceutical environment. In the realm of pharmaceutics, especially solid state pharmaceutics, the technique has proved to be an invaluable tool. This review addresses the solid state applications of microcalorimetry within the pharmaceutical industry, with a specific focus on stability, compatibility and amor- phicity determinations.

Stability

Stability can refer to both chemical and physical stability of a raw drug product or formulation. This area also encompasses compatibility of active components with excipients. Microcalorimetry can address chemical and physical stability as it is a direct measurement of reaction processes occurring within a sample. It is equally suited to compatibility studies.

Conventional methods

At present, the standard method used for stability analysis of a solid state pharmaceutical product is HPLC. In summary, the concentration of parent compound and/or the concentration of any daughter compounds produced are determined as a function of storage time. The method has certain drawbacks in that it is often not very sensitive to small changes in concentration, it requires a certain degree of method development to establish a sample preparation and analysis protocol and it relies on the dissolution of the solid product. This last drawback can cause distortions in an assay as a result of rapid acceleration of decomposition when a compound is in a solvated state.

Because of the poor sensitivity of the technique, it is necessary to perform the experiment...
over an extended time frame to allow sufficient reaction to take place. Hence, samples are stored under elevated temperature and humidity after preparation to accelerate the potential decomposition. The samples are then assayed over a period of time that can range from a few weeks to many months to give reaction snapshots along the decomposition profile. For each storage condition, a rate constant, \( k \), is calculated. By plotting \( \ln k \) against \( 1/T \) using the Arrhenius relationship, it is possible to extrapolate back to ambient temperature and hence determine the rate constant at that temperature.

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

where \( k \) is the rate constant, \( A \) is the Arrhenius factor or pre-exponential constant, \( E_a \) is the activation energy, \( R \) is the gas constant, and \( T \) is the temperature. This technique for the determination of stability has been accepted as normal practice for many years. It does, however, rely on some assumptions that are not necessarily true in all cases. It is assumed that the Arrhenius plot gives a linear relationship. This may not be true for many reasons. If there are two competing reactions occurring simultaneously, then they will both have an associated activation energy leading to an incorrect extrapolation. This effect can be dramatic if a second decomposition mechanism only ‘kicks in’ at higher temperatures. The extrapolation would then lead to a major error in calculating the ambient rate constant. Finally, if the reaction does not go by a first order reaction, it is necessary to determine a different rate equation that gives an improved understanding of the system under study. This is not always straightforward, and, for solid state reactions, can be very complex.

Microcalorimetry

An alternative to the standard HPLC methodology is to use isothermal microcalorimetry. Many laboratories have found this technique very useful. Microcalorimetry has been shown, in some instances, to give more information as to the mechanism, kinetics and thermodynamics of a degradation reaction than either the literature or conventional techniques can provide. It has also been demonstrated to be more sensitive than HPLC analysis, enabling less valuable time to be spent on stability or compatibility testing.

Some early work used flow microcalorimetry to calculate reaction rates. Here the reaction to be monitored had to be dissolved into the solution state and the two components were combined in a mixing chamber within the calorimeter. It was demonstrated that it is possible to monitor and quantify both first and second order reactions using this method. Through the use of flow microcalorimetry it has been demonstrated that it is also possible to study dissolution processes in a similar fashion.

When considering the stability of a pharmaceutical product, it is important to consider both the chemical and physical stability of the product. In the field of emulsion technology it has been demonstrated how microcalorimetry can be used to investigate the physical stability of emulsions with respect to component concentration, and, more specifically, surfactant concentration.

Pharmaceutical stability was addressed by Angberg and Nystrom in the first of a series of papers dealing with pharmaceutical stability using isothermal microcalorimetry. Although the reaction in question was studied in solution, the experiment was performed in an ampoule, which is far more convenient than a flow method. The acid hydrolysis of acetylsalicylic acid was investigated at various temperatures and rate constants determined. Using the Arrhenius relationship, an activation energy was also calculated. By using microcalorimetry in combination with other complementary techniques it was possible to determine rate and stability data. Stability data has been generated using HPLC and microcalorimetry in tandem. By using pH determinations to accompany microcalorimetric data it has been shown that it is possible to correlate pH with stability.

Pikal and Dellerman studied the solution and solid state stability of several cephalosporins. Within the solid state, the stability of various physical forms of the cephalosporins was investigated. The reaction enthalpy was also correlated with the concentration of water present. This study gave clear evidence that reaction rates as low as 1% per year are readily observed using isothermal microcalorimetry. This brings the sensitivity of the technique in line with conventional stability analysis techniques, but in a much shorter time frame. With the microcalorimetry experiment, no elevated or stressed sample conditions were used and the analysis was performed in a few hours. This has dramatic advantages over conventional techniques. Similar experiments were performed by Hansen et al. using Lovastatin. In this case, microcalorimetry and HPLC were used as complementary techniques.

The goal of any stability experiment is to obtain the maximum information possible from each analysis. It has been demonstrated how both kinetic and thermodynamic information can be derived from a microcalorimetric experiment alone. These methods do not rely on any assumptions about reaction mechanism and do not require any prior knowledge of the starting concentration of the reactant(s). Experiments were performed on a variety of systems and the results compared to published literature data derived from different techniques. Good correlation was observed with the published data as well as more detail as to the reaction mechanism. For example, the oxidation of ascorbic acid was studied and a mechanistic change after only 1.5 hours of analysis was observed.
detected. Although the mechanism was shown to still be first order, the rate constant changed by almost two orders of magnitude. This rate change in such a short time frame would almost certainly be missed using conventional methods, and only the rate constant for the last part of the reaction would be observed. The oxidation of ascorbic acid had previously been investigated using microcalorimetry by Angberg et al. The ability to observe mechanistic changes and also quantitate the kinetics is an invaluable tool at the disposal of a microcalorimetrist. A theoretical calculation from Willson et al. as to the sensitivity of microcalorimetry in stability investigations was reported. Making assumptions of the enthalpy of reaction, mechanism and molecular weight, the author stated that it is possible to investigate reactions of only 0.68% degradation per year.

Using this new kinetic approach to data analysis, it has been proposed that a standard reaction, analysed in this way, can be used as a chemical calibrant. This is the first example of determining kinetic and thermodynamic parameters from a standard reaction for the purposes of calibration. Another kinetic and thermodynamic analysis approach has been developed for the study of complex, multistep reactions.

As stated previously, to investigate stability it is no longer necessary to have a prior knowledge of the reaction mechanism. For every rate equation it is possible to apply an appropriate heat flow equation to determine the thermodynamic and kinetic parameters. In solution this is relatively simple as there are well defined and well understood rate equations that can apply. In the solid state, the problems become more difficult as solid state reactions are, in general, not well documented or understood, and general kinetic equations to describe general solid state reactions are uncommon. There are, however, many kinetic expressions that can be used to describe specific solid state reactions. These are well documented (Table 1).

There is a general solid state rate equation that refers to all two phase, solid state mechanisms.

where $dx/dt$ is the reaction rate, $A$ is the number of moles of starting material, $k$ is the rate constant, $x$ is the number of moles of starting material, $t$ is time, and $n$ is the order of the reaction.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Rate equation</th>
<th>Calorimetric equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A \rightarrow B$</td>
<td>$\frac{dx}{dt} = k(A - x)^n$</td>
<td>$\frac{dq}{dt} = \Delta H \left[ A - \left( \frac{q}{\Delta H} \right) \right]^n$</td>
</tr>
<tr>
<td>$A + B \rightarrow C$</td>
<td>$\frac{dx}{dt} = k(A - x)^n(B - x)^m$</td>
<td>$\frac{dq}{dt} = \Delta H \left[ A - \left( \frac{q}{\Delta H} \right) \right]^n \left[ B - \left( \frac{q}{\Delta H} \right) \right]^m$</td>
</tr>
<tr>
<td>$A \rightarrow C$</td>
<td>$\frac{dx}{dt} = k(A - x)(B - x)$</td>
<td>$\frac{dq}{dt} = k_0 \left[ (A - x) + (B - x) \right]$</td>
</tr>
<tr>
<td>$A + B \rightarrow C$</td>
<td>$\frac{dx}{dt} = k_0(A - x)(B - x)$</td>
<td>$\frac{dq}{dt} = k_0(A - x) \left[ (A - x) + (B - x) \right]$</td>
</tr>
<tr>
<td>Autocatalytic</td>
<td>$\frac{dx}{dt} = k_0(A - x)(B - x)$</td>
<td>$\frac{dq}{dt} = k_0 \left[ (A - x) + (B - x) \right]$</td>
</tr>
<tr>
<td>Coagulation</td>
<td>$\frac{dx}{dt} = k_0(A - x)^2$</td>
<td>$\frac{dq}{dt} = k_0 \left[ (A - x)^2 \right]$</td>
</tr>
<tr>
<td>Michaelis–Menten</td>
<td>$\frac{dx}{dt} = k_0 \left( \frac{x}{x_k} \right)$</td>
<td>$\frac{dq}{dt} = k_0 \left( \frac{x}{x_k} \right)$</td>
</tr>
</tbody>
</table>

For a more detailed description of these equations, refer to the work of R.J. Willson et al. 17
moles reacted at time $t$, and $m$ and $n$ are fitting constants. From the values of the fitting constants it is possible to determine the type of solid state reaction mechanism in progress. For instance, when $m = 0$ and $n = 1$, the mechanism is unimolecular decay, or when $m = 1$ and $n = 1$ Prout–Tompkins kinetics are valid.

There is a corresponding equation that can be related to microcalorimetric data:

$$\frac{dq}{dt} = -\Delta H \left( \frac{q}{\Delta H} \right)^m \left( 1 - \frac{q}{\Delta H} \right)^n$$

where $dq/dt$ is the heat flow measured by the calorimeter, $q$ is the heat output measured by the calorimeter, and $H$ is the enthalpy of reaction. The fitting parameters, $m$ and $n$, are defined depending on the solid state reaction mechanism. By plotting $dq/dt$ against $q$ it is then possible to determine the other constants by iteration.

Compatibility is an important area in the drug development pipeline. Conventional compatibility testing methods require both multiple sample preparation and long storage times in order to obtain meaningful results. It has been reported that a standard method for compatibility testing of binary mixtures has been developed using isothermal microcalorimetry. The method involves preparing a binary mixture, followed by examination in a microcalorimeter after a period of equilibration.

Figure 1 shows a typical response for an excipient, an active component and a mixture of the two. This combination is clearly incompatible as the mixture profile is very different from the two individual components.

The conditions for analysis are 50°C and 75% RH. The standard method can cope with a majority of active compounds and most common excipients. This means that there is no method development required for every active compound examined, as with HPLC analysis. The samples are run for 15 hours in the calorimeter, which means that a considerable number of compatibility experiments can be performed in a short period of time. This eliminates the lengthy storage conditions required by conventional techniques. The method is only designed as a screen and does not give a quantification of the amount of degraded active. Instead it looks at the amount of heat flow released from the sample and indicates whether this is a compatible or incompatible mixture. This gives the formulator valuable information as to which excipients are likely to be compatible, cutting down the number of conventional compatibility samples to prepare and saving valuable time. Similar methods have been employed by Schmitt using water slurries instead of humidified samples.

Compatibility testing using isothermal microcalorimetry has been extensively used in other application areas. The main exponents of this are those working in the munitions and rocket propellant field. They experience the same sort of problems as we in the pharmaceutical area encounter, but with much more energetic systems.

As mentioned earlier, the physical stability of a product can be just as important as chemical stability. Physical stability becomes critical when the dosage form is involved in respiratory administration. The physical form of the active component will heavily influence the speed with which the drug will be absorbed. The drug is normally prepared as a suspension in a suitable propellant. Other compounds are added to these systems to help stabilize or dilute the drug product. Work has been performed to look at the compatibility of these additives in the propellant as a solvent. These experiments are normally very difficult to perform because of the low boiling point of the propellant. This problem was overcome by these workers and very important results were obtained.

The crystallinity of the solid product suspended in the propellant is also a very important parameter. Different adsorption rates and indeed different product stability profiles will result from varying crystallinity levels in the formulation.

Solid state properties

During the development of a dosage form the solid state properties of the drug substance and excipients must be defined and controlled. This is to ensure that they comply with the regulatory authorities and that the manufacture and performance of the solid dosage form is consistent. York identified...
Table 2. Solid-state properties and potential processing stresses during identified procedures used in solid dosage manufacture

<table>
<thead>
<tr>
<th>Solid state properties</th>
<th>Processing stresses</th>
<th>Manufacturing procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystal structure</td>
<td>Temperature</td>
<td>Crystallization</td>
</tr>
<tr>
<td>Crystal hardness</td>
<td>Pressure</td>
<td>Precipitation</td>
</tr>
<tr>
<td>Crystal habit</td>
<td>Mechanical</td>
<td>Milling</td>
</tr>
<tr>
<td>Polymorphism and</td>
<td>Radiation</td>
<td>Mixing</td>
</tr>
<tr>
<td>solvate forms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wettability, surface</td>
<td>Exposure to liquids</td>
<td>Drying</td>
</tr>
<tr>
<td>polarity and</td>
<td></td>
<td>Granulation</td>
</tr>
<tr>
<td>moisture sorption</td>
<td>Exposure to gases</td>
<td>Compression</td>
</tr>
<tr>
<td></td>
<td>and liquid vapours</td>
<td>Storage</td>
</tr>
</tbody>
</table>

several solid state properties of particular importance to formulation and processing, and these are listed in Table 2. Variations in solid state properties, for example between batches of the same material or resulting from processing, such as through milling or compaction, can modify formulation requirements as well as product performance.  

Over the last ten years, isothermal microcalorimetry has been used to characterize and identify changes in the solid state properties of solid dosage forms that have the potential to cause problems during the formulation and processing of drug product batches. This section of the review will concentrate on the role of isothermal microcalorimetry in the detection of low levels of amorphous material, and the characterization of the surface properties of powders, such as wetting, adsorption and surface energetics.  

Amorphicity

The ability to detect and quantify the amount of amorphous material within a highly crystalline drug is of great importance during the development and subsequent manufacture of a solid dosage form. During the processing of pharmaceutical solids, operations such as milling, spray drying, tablet compaction, mixing, and lyophilization can cause disruption or activation of the crystal structure, leading to various degrees of disorder. If this disorder is more extensive than the occasional molecular dislocation, it can be viewed as an amorphous region of disorder. If this disorder is more extensive than the occasional molecular dislocation, it can be viewed as an amorphous region of disorder. If this disorder is more extensive than the occasional molecular dislocation, it can be viewed as an amorphous region of disorder. If this disorder is more extensive than the occasional molecular dislocation, it can be viewed as an amorphous region within the crystal structure. Because the amorphous state is metastable with respect to the crystalline form, morphology changes are likely to occur, leading to significant physical stability problems during dosage form development and subsequent manufacture. In addition to issues with the physical stability of a product, amorphous material behaves in a different manner to crystalline material, resulting in problems with the processing and quality of the finished product. Many well known excipients, such as microcrystalline cellulose and spray-dried lactose, are semi-crystalline, with regions of amorphous content within their structure that control their physical properties. Amorphous character in highly crystalline solids can be difficult to detect using traditional analytical techniques, such as Powder X-Ray Diffraction (PXRD) and Differential Scanning Calorimetry (DSC), as the limit of detection is 5–10%.

In recent years, several papers have been published that detail the use of isothermal microcalorimetry for the quantification of low levels of amorphous content (<5%). One of the first published studies demonstrated the ability of isothermal microcalorimetry to detect levels of amorphous content that is well outside of the resolution of traditional techniques. Briggner et al. used isothermal microcalorimetry to study changes in the crystallinity of spray dried and micronized lactose monohydrate. By relating the output from the microcalorimeter for the micronized samples to that obtained from an amorphous standard, it was possible to quantify the amorphous material in a micronized sample to a resolution of 1% or less. This study used the miniature humidity chamber technique, where a sample under investigation is placed in a sealed ampoule under conditions that allow the transition to the thermodynamically stable crystalline state to occur. In this study, saturated salt solutions were used to generate humidities between 53% and 85% RH. The absorbed water acts as a plasticizer to lower the glass transition temperature of the amorphous lactose to below the experimental temperature (25°C), at which point recrystallization occurs. A typical response for spray-dried lactose is shown in Fig. 2. The output shown in Fig. 2 is a typical response for the crystallization of an amorphous material on the microcalorimeter. The first part of the output represents a small wetting response, which is thought to be caused by a slight imbalance in generation of water vapour (endothermic) within the vial and the sorption of the water vapour onto the powder (exoenermic response). However, more recently it has been suggested that this initial response, in part, may be because of the amorphous material undergoing structural collapse following absorption of the water. After this initial response there is a large, sharp response from the recrystallization process. The area under this exothermic response represents the total heat of the recrystallization process. A further advantage of isothermal microcalorimetry over other techniques is that at the end of the experiment the sample can be removed and analyzed using more traditional techniques, such as PXRD and DSC to confirm that the material has recrystallized. Buckton and Darcy investigated the influence of additives, such as glass beads, magnesium stearate or microcrystalline cellulose, on the recrystallization of spray-dried lactose, and were able to present a
poor aqueous solubility. The study concluded that isothermal microcalorimetry can be used to assess the crystallinity of both hydrophobic and hydrophilic drugs provided a vapour phase can be produced that can cause the required transition.

In addition to using the miniature humidity chamber technique, solution microcalorimetry has been used to detect small differences in crystallinity between micronized and unmicronized salbutamol sulphate. Through a comparison of $\Delta H_{\text{cryst}}$ values, it was possible to distinguish between unmicronized and micronized material that had been stored at elevated temperature and humidity; that is, recrystallized amorphous material.

Over the last few years, isothermal microcalorimetry has been used to both quantify the amount of amorphous material and to study the crystallization process itself. Larsen used isothermal microcalorimetry to study water-catalysed crystallization of amorphous acadesine. The study showed that above 50% RH the amorphous acadesine crystallized within 1.5 hours, and the initiation time for crystallization was not correlated with the partial pressure of water vapour. From a consideration of the possible mechanisms of water-catalysed crystallization, the author concluded that crystallization proceeded through a metastable hydrate that immediately decomposed to the anhydrous crystal.

Bystrom investigated the relationship between chemical degradation in a micronized powder and low levels of amorphous content using isothermal microcalorimetry. The study found that after storage of micronized lactose samples for one month under controlled temperature and humidity conditions, the amount of degradation products correlated to the heat of crystallization; that is, the amorphous content, determined by isothermal microcalorimetry. The author was therefore able to conclude that the crystalline phase was chemically stable, whereas the reactivity of the amorphous phase was much higher.

Several articles have been published that compare the ability of isothermal microcalorimetry to detect amorphous material with a number of the more traditional techniques. Sebhatu prepared five samples of lactose with varying degrees of disorder, and compared them using isothermal microcalorimetry, Modulated Differential Scanning Calorimetry (MDSC) and PXRD. It was concluded that isothermal microcalorimetry had advantages over the other techniques when estimating the degree of disorder for highly crystalline samples (less than 10% amorphous content).

Most of the investigations reviewed so far have studied hydrophilic compounds, such as lactose, which can be plasticized and recrystallized by water vapour. In the pharmaceutical industry, many drugs have to be micronized to improve their bioavailability because of their low aqueous solubility. Ahmed investigated the use of gas flow isothermal microcalorimetry with non-aqueous vapours to recrystallize amorphous contents of powders with
atmospheres. However, in comparison with the miniature humidity or solvent chamber, very little work on the use of the RH perfusion unit has been published, although considerable work has been performed on an industrial level. One of these investigated the recrystallization of a micronized drug using an isothermal microcalorimeter equipped with an RH perfusion unit53 (Fig. 3).

The heat flow curve could be divided into three distinct phases; adsorption or absorption of moisture, crystallization of the amorphous regions within the sample, and evaporation of excess moisture from the solid following structural collapse and the subsequent crystallization. The area under the crystallization curve was related to the amount of amorphous material present and, as such, the technique can be used to quantify the amount of amorphous material present in the sample. The difference between the RH perfusion unit and the vapour chamber technique is that with the perfusion unit, the wetting and adsorption or absorption response is detected as the solvent vapour is generated outside the sample chamber, whereas with the vapour chamber technique little or no wetting, and adsorption or absorption response is recorded as the vapour phase is generated within the sample chamber.

In recent years, isothermal microcalorimetry has increasingly been used to probe the surface properties of powder materials. Zografi54 stated that subtle variations in surface composition, in terms of the chemical nature and location of chemical groups, is often what causes batch-to-batch variations in raw materials encountered within pharmaceutical systems. In gas flow microcalorimetry the adsorption of different vapour states can be measured over a range of partial pressures. The assumption can be made that the extent of adsorption of a particular gas is related to the interaction energy, which, in turn, reflects the energetics of the solid surface groups.

Microcalorimetry vapour sorption and vacuum microbalance studies have been combined to investigate the effect of different milling processes on the nature of the surface of a model drug56. The wetting mechanism for a range of hydrophobic drugs57 and the interaction between microcrystalline celluloses, starches and water58 have also been investigated. Through the use of this approach, it is possible to obtain the Gibbs function and enthalpy of adsorption of water.

The development of microcalorimetric gas flow cells, where the vapour pressure of water (or organic vapour) is controlled by mixing two different air streams (0% and 100% relative humidity, respectively), offered a very sensitive and extremely reproducible method for determining the surface energetics of powders. This technique has been used to probe the powder surface energetics59 of three different suppliers of α-lactose monohydrate. The three different suppliers could not be distinguished from each other using contact angle measurements, the traditional technique for measuring surface energetics, although the calorimetric adsorption isotherms showed that one of the supplier’s samples had different surface energetics, which correlated to its behaviour in the solid dosage form performance.

In addition to using moisture as a probe to determine powder surface energetics, the microcalorimetric gas flow cell has been evaluated for the determination of the critical RH for three drug substances with different hygroscopic properties60. The isothermal microcalorimetry results were shown to be comparable with those obtained from various weighing methods. The author suggested that the speed, sensitivity and the small amount of sample required would make isothermal microcalorimetry a valuable screening technique for new drug candidates.

The same technique has been used to generate a calorimetric adsorption isotherm of water vapour on sodium benzoate61. The study found that microcalorimetry is a very rapid, sensitive and convenient method for obtaining adsorption isotherms. An equation was presented that allowed the calculation of the water-vapour sorption surface area from the calorimetric data.
Reviews

The technique to the pharmaceutical scientist continues to both these areas and, in the view of the authors, the utility of the development pipeline. Isothermal microcalorimetry may be applied in to avoid drug substance problems further down the development of any drug. The isothermal technique to determine stability and compatibility will be valuable to a pharmaceutical company, the use of screening gated through the use of this technique. As time becomes more amorphicity and physical form characterization can be investigated using microcalorimetry.

Conclusion

This review has attempted to demonstrate how isothermal microcalorimetry has been used in the area of solid state pharmacology. It has been shown how stability, compatibility, amorphous and physical form characterization can be investigated through the use of this technique. As time becomes more valuable to a pharmaceutical company, the use of screening techniques to determine stability and compatibility will become increasingly important. Also, the quality of solid state characterization data needs to be constantly improved in order to avoid drug substance problems further down the development pipeline. Isothermal microcalorimetry may be applied in both these areas and, in the view of the authors, the utility of the technique to the pharmaceutical scientist continues to grow.

References
