International Journal of Pharmaceutics, 72 (1991) 181–191

ADONIS 0378517391002245

IJP 02428

Invited Review

The applications of microcalorimetry in the field of physical pharmacy

Graham Buckton [1] and Anthony E. Beezer [2]

[1] *Department of Pharmaceutics, The School of Pharmacy, University of London, 29–39 Brunswick Square, London WC1N 1AX (U.K.)* and [2] *Chemical Laboratory, The University, Canterbury, Kent CT2 7NH (U.K.)*

(Received 21 January 1991)
(Accepted 19 February 1991)

Key words: Microcalorimetry; DSC; Stability; Wetting; Immersion; Adsorption; Thermodynamics; Crystal properties; Dissolution; Drug targeting

Summary

The technique of microcalorimetry is introduced, the instrumental output is shown to relate to thermodynamics, kinetics and the concentration of the reactants (analysis). The detection sensitivity of the instrument is discussed, with particular reference to possible application to isothermal stability testing of solid-state reactions in pharmaceuticals, at ambient conditions; this particular aspect is compared directly to the current practice of use of differential scanning calorimetry (DSC) to screen for excipient incompatibilities. It is necessary to raise the temperature of a reaction significantly to observe a response in a DSC that is detectable at ambient conditions in a microcalorimeter, thus the DSC experiment may give false conclusions if the reaction which occurs at elevated temperatures is not chemically identical to the reaction that proceeds under ambient conditions. Microcalorimetry detects all processes that occur in the reaction cell, this can have advantages in, for example, studies of mechanism, but can cause problems with regard to quantification, the experiment must often be designed to limit investigation to a specific process that is of interest. A selective literature review of applications is presented, which covers stability testing, studies of powder wettability (by immersion and adsorption), crystal properties, dissolution of tablets in artificial foodstuffs and aspects of drug targeting. These examples do not cover the full list of applications, but demonstrate that microcalorimetry can be used to investigate any stage of the development, production and use of a dosage form, e.g. powder properties, excipient compatibility, product stability, tablet dissolution, direct in vitro studies of biological response etc.

Introduction

All chemical and physical processes are accompanied by changes in heat content, or enthalpy. Thus, in principle, all processes, from the metabolic activity of living species (Beezer, 1980; James, 1989) to physical processes (e.g. the wetting of drug powders; Buckton and Beezer, 1988) may be investigated by this technique. The microcalorimetric system is non-invasive and non-destructive towards the sample and, importantly, is not dependent upon the sample form; the sample can be solid, liquid, gaseous, or any combination thereof,

Correspondence: G. Buckton, Dept. of Pharmaceutics, The School of Pharmacy, University of London, 29–39 Brunswick Square, London WC1N 1AX, U.K.

and unlike other analytical tools (e.g. UV spectroscopy) neither colour, optical transparency nor absence of suspended matter are requirements.

This review will deal predominantly with the possibilities that exist for the use of microcalorimetry in the field of physical pharmacy, or perhaps more accurately, those possibilities which have been explored to date; the total list of potential applications of microcalorimetry is extensive and limits are currently a matter of speculation. Attention should be drawn to the wide range of microcalorimetric biologically based studies, which cover, for example, rapid bioassay procedures, drug/cell (including tissue cell) interaction mechanisms, evaluation of synergy and investigations of antagonism; these subjects will not be discussed here, but detailed reviews on these topics have recently been published (Beezer, 1980; James, 1989; Beezer et al., 1991).

Instrumentation

The principle of operation of a modern microcalorimeter is very simple. A sample cell (typically 3–25 cm^3 for batch cells, and 0.5 cm^3 for flow cells) is surrounded by a semi-conductor thermopile, which in turn is embedded in a high heat capacity, large mass heat sink. The heat sink is maintained, via water thermostation, at a defined temperature (within the range +5 to 95°C) to ± 0.0001°C. Any process that occurs in the sample cell results in a change in enthalpy and thus generates a rapid heat flow across the thermopiles. The output voltage of the sample thermopile is connected in opposition to the thermopile of a reference cell, to eliminate any concurrent environmental change effects. The resultant voltage signal is amplified and recorded (on a microcomputer and/or chart recorder). The signal is the rate of change of heat with time (power) (dq/dt), which is recorded as a function of time (a power-time (p-t) curve). The stability of the signal is such that a baseline fluctuation over a period of 24 h would be no more than 0.1 μW.

A detailed description of the theoretical aspects of, for example, flow microcalorimetry (Beezer and Tyrrell, 1972) shows that, for a first-order process, the output of the microcalorimeter can be described by the following equation (other orders of reaction can be treated in similar fashion),

$$dq/dt = -\Delta HCR(1 - \exp - k_1\tau) - \exp - k_1 t \quad (1)$$

where ΔH is the enthalpy change for the reaction under investigation, C the concentration of the reactant, R the flow rate, k_1 the first-order rate constant, τ the residence time of the flowing solution in the calorimetric flow cell and t the time from initiation of the reaction.

Using this example, it is apparent that the microcalorimeter can be used: (i) analytically, dq/dt is proportional to C; (ii) to determine thermodynamic parameters, via electrical calibration, as dq/dt is proportional to ΔH; and (iii) to investigate reaction mechanisms, as dq/dt is proportional to a function of the rate constant.

Detection sensitivity

The detection sensitivity of a modern microcalorimeter is 0.1 μW, thus 1 μW (i.e. 1 μJ s^{-1}) can be detected with ease, therefore, assuming a modest ΔH of 40 kJ mol^{-1} it is possible to detect a reaction rate of 2.5×10^{-11} mol s^{-1}, which is equivalent to 1.5×10^{-9} mol min^{-1}, or 6.05×10^{-6} mol $month^{-1}$.

It should be noted that, given the sensitivity of the instrument and its temperature stability, an ampoule based (batch) microcalorimetric experiment requires a period of approx. 90 min equilibration, prior to recording the signal. Thus to record a signal for 90 min involves a total time of 3 h from reaction initiation.

To elaborate on the sensitivity of the instrument, it is possible to consider a solid-state reaction (e.g. a powder mix of a drug and an excipient), assuming the conditions described above, with a molecular weight of 400 for the drug, and a sample size of 1 g, then a degradation reaction with a half life of approx. 17 years could be detected within 3 h under ambient conditions. If, however, these conditions are maintained, but ΔH

were 100 kJ mol^{-1} (which is not unreasonable), then a response could be detected in 3 h, under ambient conditions, for a reaction that would take over 200 years to run to completion.

For a pharmaceutical product, the maximum extent of degradation that would usually be acceptable is 5% in 2 years, this would equate to a half-life of approx. 27 years (2% degradation in 2 years having a half-life of approx. 68 years). Thus, at present detection sensitivity limits, certain reactions that will result in unacceptable degradation may not be detected under ambient conditions by microcalorimetry. However, major reactions will be detected, thus allowing rapid screens for excipient compatibility, and minor increases in temperature would allow more accurate prediction of shelf life.

Isoperibol Calorimeters

The instruments that have been described above as microcalorimeters have a detection sensitivity that is orders of magnitude greater than that of isoperibol calorimeters. The principle of operation is different, as isoperibol instruments are not truly isothermal, but rather they are isothermal shield calorimeters, i.e. the temperature of the reaction system approaches that of the shield. The versatility that is such an advantage in microcalorimetry can also be restricted with certain isoperibol instruments, for which it is much more common to offer only titration and breaking ampoule modes, into a vessel which is routinely in the order of 200 ml capacity (N.B. other options exist, e.g. small volume, but most instruments that are currently used are of the type indicated here). Titration allows the study of liquid/liquid interactions. The breaking ampoule principle is where one phase (often a solid) is sealed into a thin-walled glass ampoule (approx. 1 ml), and secured in the filled calorimetric vessel (liquid); after thermal equilibrium is achieved, the ampoule is broken, by a remote operation, and the two phases are allowed to mix. The resultant thermal event is then measured, usually via a thermistor which is housed in the reaction vessel. Events such as a drug being wetted (enthalpy of immersion) or dissolving (enthalpy of solution) are sufficiently large to be measured with ease (e.g. Storey 1985).

Characterising Reactions

Microcalorimetry is general in application, it can be used to investigate chemical reactions, and physical processes that occur within a system consisting of any combination of physical states of matter. This wide ranging generality of application is an obvious advantage of the instrument, however, specificity (i.e. the ability to investigate only one process) must be a consequence of careful experimental design.

The experiment must always be designed and managed carefully. Design involves selection of an appropriate blanking experiment for the reference cell. For a solid-state stability test this reference system may involve something as simple as an identical quantity of a dry stable solid which is of similar heat capacity to the reactants in the sample cell. Management of the experiment involves an appreciation of the sensitivity of the instrument, and the elimination of all other mechanical or artefactual reactions (since, as noted above, all chemical and physical processes contribute to the observed signal); for example, a semi-solid (e.g. a cream) flowing from the side to fill the bottom of a cell will result in a thermal event, as will the adsorption of atmospheric humidity onto previously dry powders which have carelessly been lodged in the screw thread of the lid, or on the outside of a cell; a further problem may be stress relaxation of an over-crimped seal on a rubber-stoppered glass cell. Any moisture that is present on the outside of a cell will tend to evaporate and, for example, water evaporation at the low level of 10 $\mu g\ day^{-1}$ will absorb 10 μW (Hansen et al., 1989); other losses of volatile material from, for example, fingerprints, labels or any other material on the outside of the cell must be avoided. The cell should be airtight to avoid exchange with the atmospheric gases. The cell should not react with or sorb the experimental materials; for this reason glass is preferred, however, sealing of glass usually involves problems of spurious thermal activity from stressed rubber/elastomer seals, or the in-

convenience (and potential problems, e.g. sample melting, stability, etc.) of fusion seals. Clean non-reactive metals can, therefore, be an advantage, thus stainless-steel cells are regularly employed.

As described above, the microcalorimetric output can be used to obtain kinetic, thermodynamic and analytical data concerning a specific reaction, but cannot be used to identify the reaction which is occurring. The interpretation of the molecular detail of a reaction is only possible by chemical investigation (e.g. spectrophotometry, HPLC, etc.) in order to identify the species involved. Whilst the microcalorimeter cannot be used to identify the reaction that is occurring, its capacity to record all events in a non-discriminating manner can be an advantage. Most analytical techniques currently used are selective in that they observe change in only one reaction property, e.g. chromophoric change in spectrophotometry, pH change in potentiometry, etc. Thus, microcalorimetry recording, as it does, a universal property may reveal more detail about the progress, and process, of an overall reaction than may be available from classical analytical techniques. It is possible, for example, to distinguish between certain physical processes (e.g. wetting) which are comparatively rapid events, and for example, degradation processes which are larger (in terms of enthalpy change) and frequently exist as more protracted thermal events.

Solid-state Stability Testing: A Comparison with Differential Scanning Calorimetry

Solid state drug/excipient reactions are often investigated via studies at elevated temperatures, for example in a differential scanning calorimeter (DSC). The Arrhenius equation is then used to predict a reaction rate under ambient conditions. There is, of course, an assumption that the reaction which occurs at the elevated temperature is chemically identical to the reaction that proceeds at 20°C. If this assumption is not valid, then the use of the Arrhenius equation to predict low-temperature stability is inappropriate. A method for establishing stability by direct observation under ambient conditions would be useful, as those analytical methods which are used currently are not sufficiently sensitive to allow slow reactions to be studied; hence, the need for temperature rises to accelerate reaction rates. As described above, isothermal microcalorimeters have potential for application in this field of study.

TABLE 1

Comparison of differential scanning calorimetry and isothermal microcalorimetry

	DSC	Microcalorimeter
Sensitivity (μW)	10	0.1
Sample mass (mg)	10	1000
Specific sensitivity (μW/g)	1000	0.1

To illustrate the application of the isothermal microcalorimeter to excipient compatibility testing, it is possible to compare its performance with that of a DSC. Table 1 shows typical operational parameters for the two types of instruments, and reveals that the microcalorimeter is 10000 times more sensitive than the DSC. Table 2 shows the temperature rise that is required to accelerate reactions with activation energies of 50, 75 and 100 kJ mol^{-1} (reasonable estimates of activation energies), by a factor of anything between 1 and 100000. As a microcalorimeter will be 10000 times more sensitive than a typical DSC, then the temperature of the DSC experiment will have to be raised by 239 (to 259)°C to observe a reaction with an activation energy of 50 kJ mol^{-1}, which could be observed at 20°C in a microcalorimeter.

TABLE 2

The temperature increase, over 20°C, that is required to accelerate a reaction which has an activation energy of 50, 75 or 100 kJ/mol

Factor of rate increase required	50 (kJ/mol)	75 (kJ/mol)	100 (kJ/mol)
×1	0	0	0
×10	37	24	17
×100	85	52	37
×1000	149	85	59
×10000	239	125	85
×100000	375	175	114

Literature Review of Current Applications

Product Interaction and Stability Studies

As was reported above, calorimetry is often used for studies of reaction and decomposition kinetics, and excipient compatibility studies; however, most workers use differential scanning, rather than isothermal, calorimetry for such experiments (such DSC experiments have been reviewed by Ford and Timmins, 1989). Considering the comparison that has been presented above, it is surprising that very little published work exists relating to the use of microcalorimetry for interaction and/or stability studies. Gibson et al. (1988) have reported on the use of immersion calorimetry to study interactions between additives (e.g. pigments, opacifiers and talc) of hydroxypropylmethylcellulose (HPMC) films. These workers (Gibson et al., 1988) concluded that immersional calorimetry "can provide a direct, rapid and simple method of assessing additive-polymer interactions".

Recently, a few reports have been published to demonstrate that microcalorimetry can provide rapid and reliable information on the mechanisms and rates of drug decomposition (e.g. Angberg et al., 1988; Hansen et al., 1989; Oliyai and Lindenbaum, 1989; Pikal and Dellerman, 1989). Angberg et al. (1988) used a standard decomposition reaction as a test system with which to investigate the applicability of microcalorimetry to stability studies. Microcalorimetry was used to monitor the hydrolysis of aspirin in aqueous solution, experiments were undertaken isothermally at each of five temperatures (range 30–50°C) and the accuracy of the determinations was quoted as being acceptable, with rate constants and activation energy values being similar to those obtained by conventional isothermal and non-isothermal analytical methods (Angberg et al., 1988). Pikal and Dellerman (1989) have investigated amorphous, crystalline and solution forms of cephalosporins and found good correlation between microcalorimetric predictions of stability and the long term chemical assay. Pikal and Dellerman (1989) describe a number of different microcalorimetric experiments, ranging from studies of decomposition in solution (where an accurate quantity of a freshly prepared solution was added to a 3 ml rubber-stoppered glass vial, using an identical quantity of water in the reference cell), dry solids (filled into 4 ml stainless-steel cells in an environment of dry air, with glycine used as the reference), and solids which had been stored at different humidities (these were left in the filled calorimeter cell for 18 h prior to measurement, to ensure that the environment had stabilised, i.e. the moisture had equilibrated from the powder to the enclosed air space). Solution loading was between 1 and 2 ml (lower quantities were needed if the experiment was run at slightly elevated temperatures) and for solids 1 g was used. Pikal and Dellerman (1989) used stainless-steel cells when the instrument was used at its highest sensitivity, as thermal events from the rubber stoppers of glass cells were found to add an error of ± 0.5 μW (see Introduction).

Hansen et al. (1989) have used three different isothermal microcalorimeters to investigate (principally) the oxidation of Lovastatin, this being proved by the absence of signal under anaerobic conditions. There was general good agreement between instruments, with overall reproducibility being to ± 0.2 μW at 25°C.

The use of microcalorimetry for stability studies, excipient compatibility test, product/container interaction studies, and material source variation are likely to be the major growth area for this technique in the pharmaceutical field (Anon., 1990).

Wetting

It has always been acknowledged that wettability is important in the preparation, storage and use of pharmaceuticals. Typical examples of the importance of wetting include the dispersion of powders to form suspensions, the addition of binders during the process of wet granulation, the adhesion of polymer films to tablets and the dissolution rate of solid dosage forms in the gastrointestinal tract. Recently, the role of surface energies and polarities has been investigated as a method of predicting interactions between components of a formulation, thus aiding formulation optimisation (for example, Rowe, 1989).

Wettability is usually assessed by means of a contact angle, but for powdered systems this can be problematic (Buckton, 1990), thus alternatives are required. An obvious alternative to contact angle measurements is the use of microcalorimetry to quantify the interaction between the wetting agent and the powder. Thus, wetting and surface energies can be obtained by considering contact angle, vapour adsorption or immersion into liquids.

The Relationships between Adsorption, Immersion and Contact Angle

As wettability can be measured by either contact angle (θ), immersion or adsorption (and often combinations of these) it is necessary to consider the inter-relationships between these different parameters. Schroder (1984) reported the relation of Harkins (1952) which links the enthalpies (H) of adsorption (subscript ads) and immersion (subscript imm):

$$\Delta_{\text{imm}}H_{(x=0)} - \Delta_{\text{imm}}H_{(x=n)} = n\left(\Delta_{\text{ads}}H_{(x=n)} - \Delta_{\text{cond}}H_{\text{L}}\right) \quad (2)$$

where the amount adsorbed (x)varies from 0 to n (mol m^{-2}), and $\Delta_{cond}H_{L}$ is the enthalpy of condensation of the liquid. Immersion can then be related to contact angle:

$$\Delta_{\text{imm}}G_{(x=n)} = -\gamma_{\text{LV}} \cos\theta \quad (3)$$

$$\Delta G = \Delta H - T\Delta S \quad (4)$$

$$\Delta_{\text{imm}}H_{(x=n)} = T\Delta_{\text{imm}}S_{(x=n)} - \gamma_{\text{LV}} \cos\theta \quad (5)$$

where ΔG is the free energy change and ΔS is the entropy change; γ_{LV} is the surface tension of the liquid.

Measuring Enthalpy of Immersion

Most workers have attempted the use of breaking ampoule calorimetry in order to determine the enthalpy of immersion of the solid in the liquid (for example, Hollenbeck et al., 1978; Hansford et al., 1980; Storey, 1985). Generally, the enthalpy is obtained calorimetrically, and the Gibbs function is derived from contact angle data (Hansford et al., 1980; Storey, 1985), the difficulties here are associated with problems of measuring contact angles for powders (see Buckton, 1990). Immersional approaches can be successful, for example in the papers quoted above (Hollenbeck et al., 1978; Hansford et al., 1980; Storey, 1985), Hansford et al. (1980) were able to demonstrate a change in the thermodynamic functions of immersion for different samples of a hydrophobic drug (griseofulvin), which had been milled by different techniques; Storey (1985) considered the effect of various substituents at different positions on an imidazole ring structure and Hollenbeck et al. (1978) measured enthalpies of immersion of samples of microcrystalline cellulose which had been equilibrated with different water contents. Hollenbeck et al. (1978) noted that immersional techniques can be suitable for hydrophilic powders, which are particularly difficult to study by contact angle methods, however, the difficulties associated with the use of immersional calorimetry were not addressed.

Many drug powders are hydrophobic, and incomplete immersion (in water) can result (Buckton, 1985, 1988); although this issue has been addressed (e.g. Everett et al., 1984), uncertainties about the surface coverage of the powder prior to immersion (i.e. the extent of water sorption), and the degree of flotation lead to the need for alternative approaches.

Flow Microcalorimetry

Flow microcalorimetry has been used, where vapours of different humidities have been passed over a powder (Bhatt and Rubinstein, 1983), however, this approach has not been given serious critical examination in the pharmaceutical field. In related subject areas this approach is both used and accepted, for example, carbon interactions with non-aqueous liquids in coal research (Jones, 1989), in the study of surface acidity and basicity (Gervasini and Auroux, 1989) and to measure the enthalpies of adsorption and desorption on

powdered solids (Groszek and Templer, 1989). Indeed, Groszek and Templer (1989) describe an instrument which has been developed for application to adsorption and desorption experiments on powdered solids (Microscal Ltd, London). In flow experiments, there is a displacement of one phase from a surface, by another, e.g. air replaced by a liquid, or one immiscible liquid replaced by another, or one gas replaced by another, etc. These systems are often complicated to interpret, as the response is a summation of the desorption and adsorption processes, and thus is related to competition and interaction between the probe substances. The use of vapour sorption onto a vacuum cleaned surface offers some unique advantages to this field of study.

Adsorption

Although it is acknowledged that immersion is often the process of interest (e.g. for a product to dissolve in the gastrointestinal tract), on a fundamental basis, it is the adsorption of the first layers of water molecules onto a powder surface that is most indicative of the interaction between that powder and the liquid (in line with the BET approach). Further adsorption after these initial molecular layers, will tend to be equivalent to condensation, and immersion of this vapour laden surface will result in a reordering of the molecules of condensed liquid (usually water) to be accommodated in the structure of the bulk liquid. The work of Hollenbeck et al. (1978) demonstrates this point, as the enthalpy of immersion falls exponentially as a function of the moisture content of the powder. The practical difficulty with the approach of Hollenbeck et al. (1978) is that the powder is equilibrated in humidity-controlled chambers, then generally removed into ambient conditions before sealing; this leads to at least some uncertainty about the exact water content. A more disturbing situation is the insistence of some workers to use the powder in the 'as received' form, which will mean that the water content will vary from day to day (depending upon the shape of the adsorption isotherm(s), and on the humidity/temperature profiles in the laboratory), and results should show similar variability. The argument offered to attempt to justify the use of undefined powder surfaces is a need to model the 'practical' situation of product use.

To gain a more fundamental understanding of powder/vapour (and thus liquid) interactions, it is appropriate to consider experiments based on vacuum cleaned surfaces. Therefore, calorimetric vapour sorption studies have been linked with parallel experiments in a vacuum microbalance (Buckton and Beezer, 1988). It is possible to obtain the Gibbs function from an equilibrium constant using the vacuum microbalance, and then to combine the calorimetric power output with the knowledge of the mass adsorbed to obtain the enthalpy of adsorption. This approach has been used to study hydrophobic drugs (Buckton and Beezer, 1988), small differences in surface properties of the same drug (induced by different milling techniques) (Buckton et al., 1988), and the complex sorption processes that make up the interaction between microcrystalline celluloses, starches, and water (Blair et al., 1990). The use of the combination of a vacuum microbalance and the sorption microcalorimeter offers advantages over other systems, especially for hydrophobic powders. Firstly, it is possible to obtain the thermodynamic functions without the need to assess the contact angle. Secondly, the thermodynamic parameters, and the kinetics, can allow a molecular mechanism of the interaction between the vapour and the powder to be postulated; for example, Buckton and Beezer (1988) noted that there was a difference in rate of adsorption of water vapour onto various powder surfaces (hydrophilic powders had adsorption rates similar to that of instrumental response, whilst water vapour adsorbed to hydrophobic powders more slowly); and Blair et al. (1990) reported that the sorption to cellulosic materials followed three distinct kinetic processes (the first being rapid, and being the binding of one water molecule between two anhydroglucose molecules, the second process was slow and corresponded to the development of a 1 : 1 stoichiometry between water and anhydroglucose units, finally there was a further rapid process which was believed to be the sorption of loosely bound water into the amorphous region). Thirdly, the use of the

calorimeter is more accurate and more rapid than the isosteric approaches to this problem.

Crystal Properties

Changes in the crystalline nature of a solid (e.g. polymorphic form, crystal habit and crystal lattice imperfections) result in different physical properties. The changes in properties can alter the processability of the powder (e.g. flow, compression, etc.), and the behaviour (e.g. dissolution rate), and thus the bioavailability of the product. The prevalence of lattice imperfections is influenced by many processes including crystallisation technique (solvent, stirring rate, crystallisation rate, etc.; see York (1983)), drying, milling, and compression (Huttenrauch, 1968).

Solution calorimetry has successfully been employed to investigate differences in crystal form and structure. These differences in crystal properties often correlate with an aspect of the behaviour of the drug/product, e.g. correlation of heats of solution with stability (Pikal et al., 1978).

In the specific case where crystal imperfections are considered, it is possible to engineer the extent of the defects by addition of various amounts of different impurities. To quantify such systems, York and Grant (1985) defined a disruption index. The disruption index relates to entropy changes in the system, and is defined as the rate of change of the difference between the entropy of the solid and that of the liquid, with respect to the ideal entropy of mixing of the components of the solid. Disruption indices have been calculated from heats of fusion, using differential scanning calorimetry data (York and Grant, 1985), and from solution calorimetry (Grant and York, 1986); the solution calorimetry data can be assumed to be more reliable, on the basis that molecules will tend to realign during the heating process that is required during a DSC experiment.

Dissolution

The most popular method of administering medication is by a solid oral dosage form. Generally, before the drug can be systemically effective it is necessary for the drug to be dissolved and absorbed. There are official pharmacopoeial in vitro dissolution tests which are used to assess the rate of drug release in buffered fluid, under constant conditions. The assay from such experiments is generally simply ultraviolet spectroscopy. These simple tests are reproducible and are essential, however, they are not readily adaptable to studies of complex problems such as the influence of food on dissolution. Food is known to influence dissolution profiles, for example, certain sustained release theophylline products have different rates of absorption and bioavailabilities if taken before or after meals (e.g. Jonkman, 1989). It should be expected that many products show significantly different release profiles if the fluid is changed from aqueous buffer to a fatty emulsion. It has been shown (e.g. Maturu, 1986) that the in vivo variation in absorption due to food intake can be paralleled by in vitro tests. To undertake in vitro tests that mimic fed states involves the use of complex media, the major problem being that these fluids are normally coloured and/or contain suspended material, thus they interfere with the standard UV drug assay procedures. Macheras et al. (1987, 1989) have attempted to use a dialysis technique to extract drug from the dissolution fluid, prior to analysis; this is obviously a troublesome technique, and is open to error if all the drug is not extracted during dialysis.

Microcalorimetry has been used to monitor drug dissolution in buffer and model foodstuffs (Ashby et al., 1989; Buckton et al., 1989). In these experiments, a slow release tablet was placed in the cell of the calorimeter and equilibrated to 37°C. The dissolution fluid (from an external thermostated reservoir) was then allowed to flow over the tablet, and solid matter was retained in the cell by use of a mesh positioned over the outlet (the effluent was then returned to the thermostated reservoir). The advantage of microcalorimetry for dissolution experiments is that the presence of model liquid foodstuffs does not interfere with the assay; the disadvantage is that the calorimetric output is not just due to the drug, but also due to the interaction of the fluid with, and/or the dissolution of, the other components of the dosage form. Ashby et al. (1989) have studied, calorimetrically, the

dissolution of an active and placebo slow release theophylline product; the active compound had a significantly higher power output during the process, demonstrating that in this instance the drug contributed significantly to the response. The dissolution of the active was monitored calorimetrically using buffer, a fat emulsion (Intralipid diluted in buffer) and a commercial liquid feed (Ensure diluted in buffer) as dissolution fluids (Ashby et al., 1989). The results (Ashby et al., 1989) demonstrated that the different fluids produced different power/time curves, suggesting that the composition of the fluids affected drug release from these dosage forms. The product that was investigated by Ashby et al. (1989) was Phyllocontin Continus, the different Continus dosage forms are now known to interact with different foods to different extents, such that the various Continus products (e.g. Uniphyllin, Uniphyl, Phyllocontin) should not be regarded as equivalent in their performance (see Jonkman, 1989). It is possible that microcalorimetric experiments could provide an explanation for the range of product/food interactions that exist for the Continus, and other sustained release dosage forms. Further work is necessary to validate the use of calorimetry to probe drug/food interactions, but at present the approach seems to offer considerable promise. A limitation of microcalorimetry for dissolution studies is the requirement for an equilibration stage (see above), for these experiments, the onset of flow will cause a disruption which may require 30–60 min to equilibrate, thus rapidly dissolving tablets could not be studied. However, the above experiment was carried out in a flow system; if it had been undertaken in an ampoule breaking calorimeter (see above) then the initial period would not have been lost; the potential difficulty of an ampoule breaking system is that the liquid volume is often approx. 200 ml, thus it may be difficult to obtain sink conditions throughout the experiment for some drugs.

Microcalorimetry has also been used to investigate interactions between a product and model gastro-intestinal content (Buckton et al., 1989). It is well known that tetracycline interacts with (amongst others) calcium ions, thus calorimetric dissolution experiments were performed using 0.1 N hydrochloric acid, a fat emulsion, milk in buffer and Ensure in buffer; the first two products did not interact with the product and the calorimetric output was due to dissolution only, whilst the (interacting components of) milk and Ensure both chelate the tetracycline, the interaction was indicated by a much larger power output (Buckton et al., 1989).

Drug Targeting

As was mentioned in the Introduction, microcalorimetry has been used extensively to study biological systems; drug targeting falls at the interface of many disciplines, and although it is essentially biological it also fits into the realm of physical pharmacy. It is, therefore, appropriate to give a brief account of possible applications.

One area that has received considerable attention for targeted systems is the interaction between plasma proteins and model drug/carrier complexes. It is acknowledged that surface energy and charge will influence the binding of proteins to such particles (see, for example, Van Oss et al., 1975; Illum and Davis, 1982; Davis and Illum, 1986). Hydrophobic particles are normally removed to the liver rapidly, following a process known as 'opsonisation', during which plasma proteins adhere to the particles making them recognised by phagocytic cells of the mononuclear phagocytic system; this process can be changed (i.e. alter rate, extent and place of deposition) by changes in the surface characteristics of the particle (e.g. Illum et al., 1987). These changes in surface characteristics alter the initial process of adsorption of 'opsonising' proteins. It is possible to study adsorption onto solids from solution, using microcalorimetry. Norde and Lyklema (1972) investigated the adsorption of human serum albumin onto polystyrene latices (these being models of opsonising proteins and hydrophobic carriers respectively). Manzini et al. (1979) investigated the interactions between model drugs and albumins, and was able to identify either primary binding sites or cooperative binding mechanisms.

Illum et al. (1987) demonstrated changes in phagocytic uptake as a result of adsorbing differ-

ent block co-polymers onto polystyrene latices (poloxamer series). Mitchard (1990) has recently undertaken a microcalorimetric investigation of interaction between polystyrene and poloxamers, and the formed complexes and plasma proteins.

The investigation of the interactions between drug/carrier complexes and biological binding sites is obviously well suited as a microcalorimetric experiment as specificity is already built into biological processes. Biological microcalorimetry has been reviewed elsewhere (Beezer et al., 1991).

Conclusion

Microcalorimetry is a versatile technique, by which reactions can be followed. The experimental yield is informative, providing thermodynamic, kinetic and analytical information.

Any phases or combinations thereof can be studied, making the list of potential applications limited almost only by the imagination of the investigator, and the ability to build in the required specificity. Pharmaceutical systems are particularly suitable for investigation by microcalorimetry, as biologically based experiments generally have built in specificity (e.g. an enzyme/substrate interaction), and in physical pharmacy, there is an advantage in that most of the chemicals that are used are of high purity (compared to many other fields of study), thus, once again, allowing the possibility for developing reaction specificity. Potential applications of the instrument are manifold, and cover every stage in development, from the properties of the pure drug (crystal form, wettability, stability, etc.) to interactions with excipients (compatibility testing of binary and then more complicated powder mixtures), to the properties of the final dosage form (e.g. stress relaxation in tablets (N.B. this is an application that has not been reported, but which clearly is possible to investigate), dissolution etc.). Stability testing can be carried out at any stage (from drug powder, to mixes, to final form) often at ambient, or at worst near ambient, conditions, with the possibility of controlling atmospheric conditions (R.H., oxygen, etc.; even light conditions can be altered using fibre optics).

Microcalorimetry seems to be underused in physical pharmacy.

References

Angberg, M., Nyström, C. and Castensson, S., Evaluation of heat conduction microcalorimetry in pharmaceutical stability studies. 1. Precision and accuracy for static experiments in glass vials. *Acta Pharm. Suec.*, 25 (1988) 307–320.

Anon., Use of thermal methods for the characterisation of pharmaceuticals. *Pharm. J.*, 245 (1990) 183–184.

Ashby, L.J., Beezer, A.E. and Buckton, G., In vitro dissolution testing of oral controlled release preparations in the presence of artificial foodstuffs. I. Exploration of alternative methodology: microcalorimetry. *Int. J. Pharm.*, 51 (1989) 245–251.

Beezer, A.E., *Biological Microcalorimetry*, Academic Press, London, 1980.

Beezer, A.E. and Tyrrell, H.J.V., Application of flow microcalorimetry to biological problems. Part 1, Theoretical aspects. *Sci. Tools*, 19 (1972) 13–17.

Beezer, A.E., Russell, S. and Buckton, G., Pharmaceutical microcalorimetry: a selective review. *Thermochim. Acta*, (1991) submitted.

Bhatt, B. and Rubinstein, M.H., The use of low microcalorimetry to characterise powder surfaces. *Drug Dev. Ind. Pharm.*, 9 (1983) 215–222.

Blair, T.C., Buckton, G., Beezer, A.E. and Bloomfield, S.F., The interaction of various types of microcrystalline cellulose and starch with water. *Int. J. Pharm.*, 63 (1990) 251–257.

Buckton, G., Assessment of the wettability of powders. PhD Thesis, University of London (1985).

Buckton, G., The assessment, and pharmaceutical importance, of the solid/liquid and the solid/vapour interface: a review with respect to powders. *Int. J. Pharm.*, 44 (1988) 1–8.

Buckton, G., Contact angle, adsorption and wettability – a review with respect to powders. *Powder Technol.*, 61 (1990) 237–249.

Buckton, G. and Beezer, A.E., A microcalorimetric study of powder surface energetics. *Int. J. Pharm.*, 41 (1988) 139–145.

Buckton, G., Choularton, A., Beezer, A.E. and Chatham, S.M., The effect of comminution technique on the surface energy of a powder. *Int. J. Pharm.*, 47 (1988) 121–128.

Buckton, G., Beezer, A.E., Chatham, S.M. and Patel, K.K., In vitro dissolution testing of oral controlled release preparations in the presence of artificial foodstuffs. II. Probing drug/food interactions using microcalorimetry. *Int. J. Pharm.*, 56 (1989) 151–157.

Davis, S.S. and Illum, L., Colloidal delivery systems – opportunities and challenges. In Tomlinson, E. and Davis, S.S. (Eds), *Site-Specific Drug Delivery*, Wiley, Chichester, 1986, pp. 93–110.

Everett, D.H., Langdon, A.G. and Maher, P., Developments in

immersion calorimetry: design and testing of improved seal-breaking technique. *J. Chem. Thermodyn.*, 16 (1984) 981–992.

Ford, J.L. and Timmins, P., *Pharmaceutical Thermal Analysis: Techniques and Applications*, Ellis Horwood, Chichester, 1989, pp. 85–107; 238–247.

Gervasini, A. and Auroux, A., Microcalorimetric study of the surface acidity and basicity of metal oxide catalysts. *44th Calorimetry Conference*, Oak Ridge, TN, 1989, No. 106.

Gibson, S.H.M., Rowe, R.C. and White, E.F.T., Quantitative assessment of additive-polymer interaction in pigmented hydroxypropyl methyl cellulose formulations using immersion calorimetry. *Int. J. Pharm.*, 48 (1988) 113–117.

Grant, D.J.W. and York, P., A disruption index for quantifying the solid state disorder induced by additives or impurities. II. Evaluation from heat of solution. *Int. J. Pharm.*, 28 (1986) 103–112.

Groszek, A.J. and Templer, M.J., Rapid measurement of the heats of adsorption and desorption of gases on powdered solids by flow and adsorption microcalorimetry. *44th Calorimetry Conference*, Oak Ridge, TN, 1989, No. 31.

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

Hansford, D.T., Grant, D.J.W. and Newton, J.M., Surface energetics of the wetting of a hydrophobic powder. *J. Chem. Soc. Faraday I*, 76 (1980) 2417–2431.

Harkins, W.D., *The Physical Chemistry of Surface Films*, Reinhold, New York, 1952.

Hollenbeck, R.G., Peck, G.E. and Kildsig, D.O., Application of immersional calorimetry to investigation of solid-liquid interactions: Microcrystalline cellulose-water system. *J. Pharm. Sci.*, 67 (1978) 1599–1606.

Huttenrauch, R., Molecular pharmaceutics as a basis of modern pharmaceutical technology. *Acta Pharm. Technol.*, APV Informationsdienst Suppl., 6 (1968) 55–127.

Illum, L. and Davis, S.S., The targeting of drugs parenterally by use of microspheres. *J. Parent. Sci. Technol.*, 36 (1982) 242–248.

Illum, L., Jacobsen, L.O., Muller, R.H., Mak, E. and Davis, S.S., Surface characteristics and the interaction of colloid particles with mouse macrophages. *Biomaterials*, 8 (1987) 113–117.

James, A.M., *Thermal and Energetic Studies of Biological Cellular Systems*, Wright, Bristol, 1989.

Jones, K.L., Time-slicing flow calorimetry data-rationale and advantages in coal research. *44th Calorimetry Conference*, Oak Ridge, TN, 1989, No. 106.

Jonkman, J.H.G., Food interactions with sustained release theophylline preparations. *Clin. Pharmacokinet.*, 16 (1989) 162–179.

Macheras, P., Koupparis, M. and Apostolelli, E., Dissolution of four controlled release theophylline preparations in milk. *Int. J. Pharm.*, 36 (1987) 73–79.

Macheras, P., Koupparis, M. and Antimisiaris, S., An in vitro model of exploring CR theophylline-milk fat interactions. *Int. J. Pharm.*, 54 (1989) 123–130.

Manzini, G., Ciana, A. and Crescenzi, V., The interaction of serum albumins with various drugs in aqueous solution. Gel permeation, calorimetric, and fluorescence data. *Biophys. Chem.*, 10 (1979) 389–396.

Maturu, P.K., Prasad, V.K., Worsley, W.N., Shiu, G.K. and Skelley, J.P., Influence of high fat breakfast on the bioavailability of theophylline controlled release formulations: an in vitro demonstration of an in vivo observation. *J. Pharm. Sci.*, 75 (1986) 1205–1206.

Mitchard, N., A microcalorimetric study of polymer, latex and protein interactions and their relevance in drug delivery. PhD thesis, University of London (1990).

Norde, W. and Lyklema, J., Analytical and microcalorimetric investigation of the adsorption of human serum albumin on well defined polystyrene latices. *Protides Biol. Fluids*, 20 (1972) 467–470.

Oliyai, R. and Lindenbaum, S. Stability testing of pharmaceuticals by isothermal heat conduction calorimetry: ampicillin in aqueous solution. *44th Calorimetry Conference*, Oak Ridge, TN, 1989, No. 30.

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

Pikal, M.J., Lukes, A.L., Lang, J.E. and Gaines, K., Quantitative crystallinity determinations for beta-lactam antibiotics by solution calorimetry: correlations with stability. *J. Pharm. Sci.*, 67 (1978) 767–773.

Rowe, R.C., Polar/non-polar interactions in the granulation of organic substrates with polymer binding agents. *Int. J. Pharm.*, 56 (1989) 117–124.

Schroder, J., On the thermodynamic functions of gas-solid adsorption and liquid-solid immersion and their relation to adhesion, contact angle and surface tension. *Prog. Org. Coatings*, 12 (1984) 159–180.

Storey, D.E., Thermodynamics of the aqueous immersion of a series of imidazoles. *J. Pharm. Pharmacol.*, 37 (1985) 25P.

Van Oss, C.F., Gillman, C.F. and Neuman, A.W., *Phagocytic Engulfment and Cell Adhesiveness*, Dekker, New York, 1975.

York, P., Solid-state properties of powders in the formulation and processing of solid dosage forms. *Int. J. Pharm.*, 14 (1983) 1–28.

York, P. and Grant, D.J.W., A disruption index for quantifying the solid state disorder induced by additives or impurities. I. Definition and evaluation from heat of fusion. *Int. J. Pharm.*, 25 (1985) 57–72.