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Isothermal microcalorimetry as a tool to study solid–vapour interactions: design and testing of a novel hydration apparatus

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Abstract

This paper details the development and application of a novel hydration apparatus, used to initiate water-mediated reactions in an isothermal microcalorimeter. Conventionally, the relative humidity (RH) in a sample ampoule is maintained using one of two methods; an amount of a saturated salt solution is held in a small glass tube (Durham tube or mini-hydrostat) sealed within the ampoule or a carrier gas of known RH is flowed over the sample at a constant rate. The Durham tube method is often used on the basis of simplicity and cost, but has several disadvantages; the system may be rate-limited by the small surface area available for water evaporation, the internal surfaces of the ampoule are wetted and the system cannot reach equilibrium before the reaction is initiated. In this work, we show how by switching the placement of the sample and water reservoir (sample in a sealed hydrostat and water reservoir in the ampoule) many of these drawbacks are overcome. The use of the system is demonstrated by studying the hydration of anhydrous ceftazidime.
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1. Introduction

Changes in the physical properties of solids are often induced by the presence of water vapour. For instance, water may initiate a hydrolysis reaction, result in the hydration or deliquescence of an anhydrous solid, mediate a reaction between two solid components or cause recrystallisation of an amorphous material by acting as a plasticiser, lowering its glass transition (T_g) temperature. Any changes resulting in a solid state sample from such interactions may significantly affect its physical and chemical properties. The study and quantification of these effects is therefore of some considerable importance, especially in the field of physical pharmacy since many pharmaceuticals are amorphous or partially crystalline solids or exhibit hydrate formation.

Heat-conduction isothermal microcalorimetry is ideally suited to the study of water vapour–solid interactions because of its inherent characteristic; the ability to monitor, non-invasively, the heat changes in complex heterogeneous systems with high sensitivity. The experimental methodology is simple. The relative

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humidity (RH) in an ampoule containing the relevant sample is controlled and the heat-flow changes occurring as a function of time are measured. The RH is conventionally maintained using one of two techniques. The simplest method involves the placement of a small glass tube (a Durham tube, or hydrostat) holding water or a saturated salt solution directly within an air-tight ampoule containing the sample [1]. Saturated salt solutions will maintain a constant RH within a confined space at equilibrium. The specific RH attained is dependent on the ambient temperature and salt used. An alternative arrangement is to use RH perfusion [2]. In this case the RH of a carrier gas, flowing at a constant rate, is controlled as it passes through the sample ampoule. One such piece of apparatus is available from Thermometric AB (Järfälla, Sweden). In this system, two gas lines are routed into the sample ampoule; one contains dry gas and one contains gas saturated with water vapour—the desired RH in the sample ampoule is achieved by correct mixing of the two gas lines using a flow switch.

Processing of solid state crystalline pharmaceuticals, such as milling, drying or grinding, often induces the formation of small regions of amorphous (disordered) material [3]. Since, relative to the crystalline form, amorphous material is metastable, changes in morphology are likely to occur over time, leading to problems with dosage form development and stability. Whilst the percentage of amorphous material formed may be relatively low (a few percent) the effects of its presence can be significant. This is because the amorphous material will be located primarily on the surface of the particles with the consequence that almost the entire surface area of the processed material will be amorphous [4]. Such changes will inevitably alter the interfacial interactions between the particles. Inducing the recrystallisation of amorphous material by the addition of water vapour results in a quantitative change in heat content that can be measured in a microcalorimeter [5]. Examples of pharmaceuticals that have been investigated in this way include nifedipine [6], griseofulvin [7], albuterol sulfate [8] and acadesine [9].

Buckton and Darcy [10] reviewed the use of the Durham tube method to study the response of amorphous lactose, a widely used pharmaceutical excipient, to changes in RH. In these experiments, the amorphous lactose was sealed in a glass ampoule with a Durham tube containing a small quantity of saturated NaCl solution (which maintains an RH of 75% at 25 °C). A typical power–time response, shown in Fig. 1 [11], shows two distinct regions; a small initial response followed by a large exotherm. The large exotherm reflects crystallisation of the sample and comprises

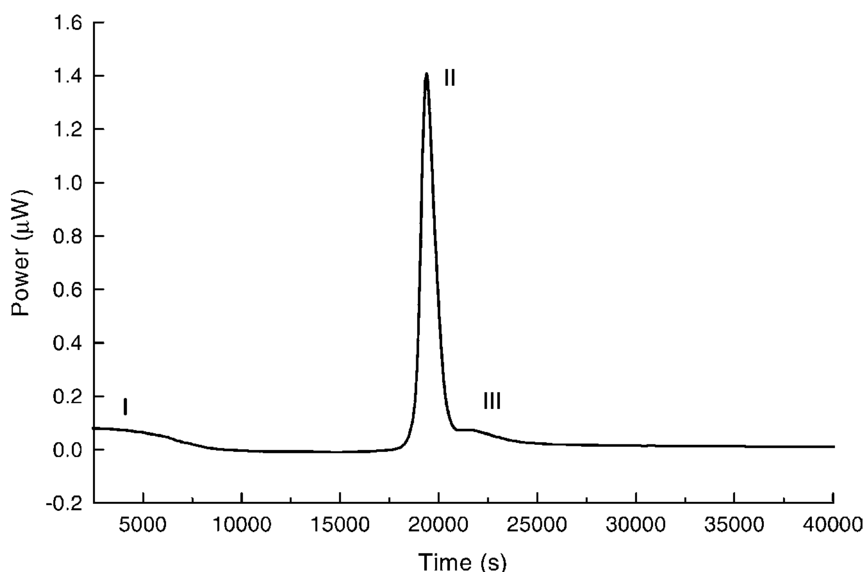

Fig. 1. The power–time signal for amorphous lactose exposed to 75% RH using the hydrostat method [11].

contributions from crystallisation of the sample, expulsion of absorbed water and condensation of that water back into the salt solution. The smaller initial signal reflects heats of friction caused by loading of the sample ampoule, evaporation of water from the reservoir, wetting of the internal surfaces of the ampoule and absorption of water by the sample.

A principal disadvantage of the Durham tube method is that it allows the effect of only one RH upon a sample to be investigated with each experiment. Furthermore, the initial response from such an experiment is never observed as the open Durham tube must be sealed in the ampoule before being loaded into the calorimeter. Discounting the effects of friction and thermal equilibration from a conventional calorimetric experiment means that, typically, the first 30 min of data are lost, during which time a reaction may have progressed to a significant extent. RH perfusion, conversely, can maintain the atmosphere over a sample at zero RH until thermal equilibration has been achieved before being programmed to ramp the RH in the sample cell at either a constant rate or in a series of discreet steps. In this way it is possible to record the response of a sample from time zero (t_0). The uses of such a system have been discussed by, for example, Angberg et al. [1] and Jakobsen et al. [12].

Both the systems have advantages and disadvantages and care must be taken to ensure that unexpected or erroneous heat-flow signals are not simply a result of poor experimental design. To analyse the data quantitatively the effects of water evaporation and wetting of the ampoule need to be removed. For Durham tube experiments this is most conveniently achieved by using an equivalent salt solution in a reference cell, connected in opposition to the sample cell. RH perfusion experiments are usually conducted using a stainless steel ampoule containing a suitable quantity of an inert material as a reference and hence this correction is not possible. Perhaps the most important consideration is to ensure that the supply of water vapour is sufficient such that the measured sample response is not rate-limited. Rate limitation may occur if, for instance, the rate of evaporation of water from the salt reservoir is slower than the rate of water uptake by the sample (in the case of the Durham tube) or if the flow of carrier gas is too slow (in the case of RH perfusion).

In this work, we show how a modification to the standard Durham tube experiment can improve the quality of the power–time data obtained by overcoming most of the disadvantages discussed above. In this case the sample is contained in the Durham tube and the water or salt solution is placed in the sample cell. This affords the largest surface area possible for water evaporation, although it is noted that this does not preclude the possibility that the sample response may be rate limited. A further advantage is that the sample can be sealed into the Durham tube (in this case by a thin sheet of foil and wax). Before data collection is initiated the system can be allowed to reach thermal equilibrium and the internal surfaces of the ampoule, and external surfaces of the Durham tube, can be wetted. Hence, data may be collected from time zero. Once equilibrium has been achieved, the foil seal is broken (by means of a rod that is accessible from outside the instrument) and the sample and water vapour can interact. It should be noted that, of course, the internal surfaces of the Durham tube will be wetted, but this area is significantly smaller than that of the ampoule.

The use of the methodology is demonstrated by studying the hydration of anhydrous ceftazidime, a compound that is known to form a mono-hydrate upon exposure to humidity. Ceftazidime is a third generation cephalosporin, with extended activity against gram negative bacteria. It is usually given by injection in the treatment of pseudomonal infections [13]. As such, it provides a model (and pharmaceutically relevant) system for demonstrating the application of the technique to the pharmaceutical sciences.

2. Materials

Ceftazidime was provided for study, with appropriate background information [14], by GlaxoWellcome and was used as received. The sample was stored in a vacuum desiccator over P_2O_5.

3. Experimental

Experiments were conducted using a 2277 Thermal Activity Monitor (TAM, Thermometric AB) at 25 °C. The apparatus consists of an insert that fits inside one TAM channel, and is shown diagrammatically in Fig. 2. A sample is placed in the standard 5 ml stainless steel ampoule and the ampoule is screwed to the

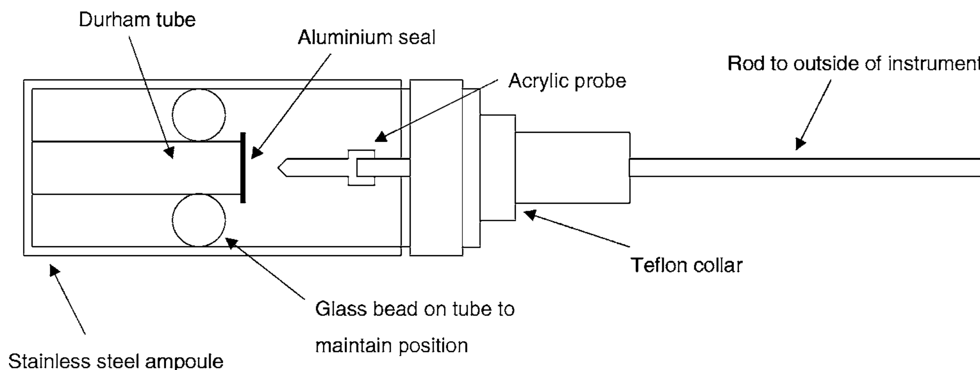

Fig. 2. A schematic diagram of the hydration apparatus.

bottom of the insert. An air-tight seal is ensured by the use of a soft rubber O-ring in the lid. The insert comprises a hollow stainless steel tube through the centre of which runs a metal pole. A teflon shaft is screwed onto the end of the pole nearest to the sample. The shaft enters the ampoule through a teflon collar in the lid and has a sharp acrylic point on its end. The gap between the collar and shaft was adjusted such that the shaft could move with minimal friction but an essentially air-tight seal was maintained. The upper end of the pole extends above the level of the calorimeter so that it can be raised or lowered between two fixed points by an operator.

The Durham tube was approximately 1 cm tall and had an internal diameter of approximately 3 mm. Three evenly spaced glass lugs were placed on the top edge of the tube. This ensured the Durham tube remained vertically aligned and was positioned correctly with respect to the acrylic point after sealing in the apparatus.

Anhydrous ceftazidime was placed within the Durham tube. An air-tight seal was ensured using a small piece of aluminium foil secured to the rim of the Durham tube by dentist's wax. The tube was placed vertically within the ampoule. Water (or a saturated salt solution) was placed at the bottom of the ampoule and the apparatus was loaded into the calorimeter to reach equilibrium. An empty stainless steel cell was used as a reference and the baseline was adjusted to zero before commencement of data capture. Experiments were initiated by lowering the probe from outside the instrument, breaking the foil seal and returning the probe to its initial position. Data were collected using the dedicated software Digitam 4.1 (Thermometric AB) and analysed using Origin™ (Microcal Software Inc.).

4. Results

The inherent thermal response characteristic of the new apparatus was determined by running a number of control experiments. To maximise the power–time signal an empty cell was used as a reference. Fig. 3 shows the power–time trace obtained for control experiment A (no sample in the Durham tube, water in the ampoule) when the foil seal was punctured. The trace gives a small exotherm and returns to a baseline within 1 h. By contrast, the power–time trace for control experiment B (water in Durham tube, empty ampoule), Fig. 3, shows a much larger response. In this case an endothermic signal is obtained which does not return to the baseline for several days. Both the traces reflect the puncturing of the seal, friction from lowering and raising the pole of the insert and adsorption of water to the previously unexposed surfaces of the ampoule or Durham tube. The differences between these traces reflect both the different surface areas that are wetted in each experiment and the different quantities of water that evaporate. In control experiment A, only the inner faces of the Durham tube are being wetted while in control experiment B the outside faces of the Durham tube and the inner faces of the ampoule are being wetted.

Fig. 4 shows the power–time trace obtained when the apparatus was used to study the hydration of

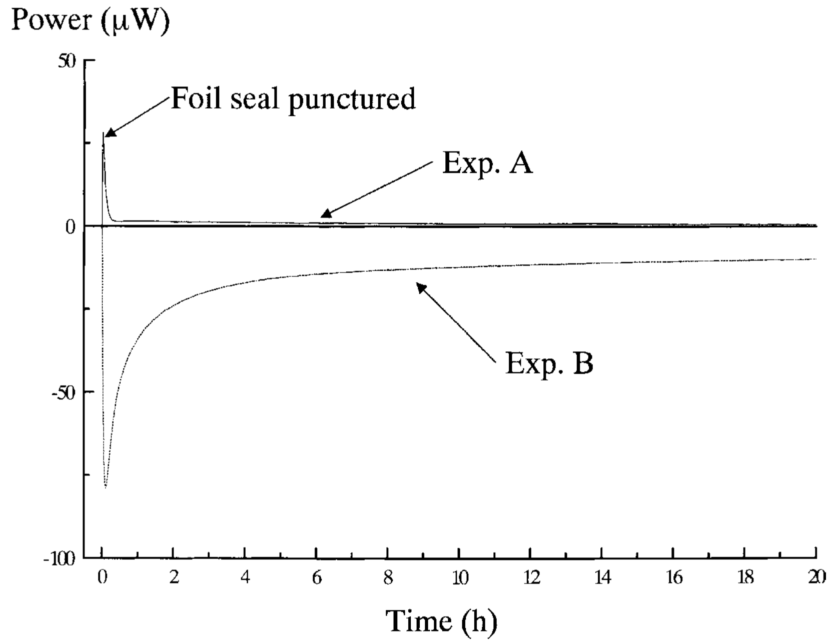

Fig. 3. Power–time traces for two control experiments ((A) water in the ampoule; (B) water in the Durham tube).

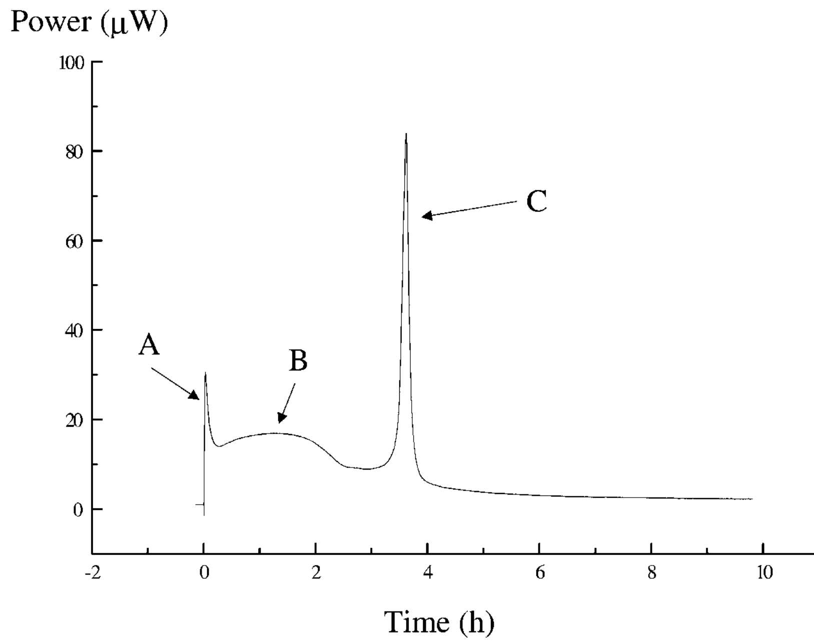

Fig. 4. A typical power–time trace for anhydrous ceftazidime exposed to 100% RH in the new hydration apparatus.

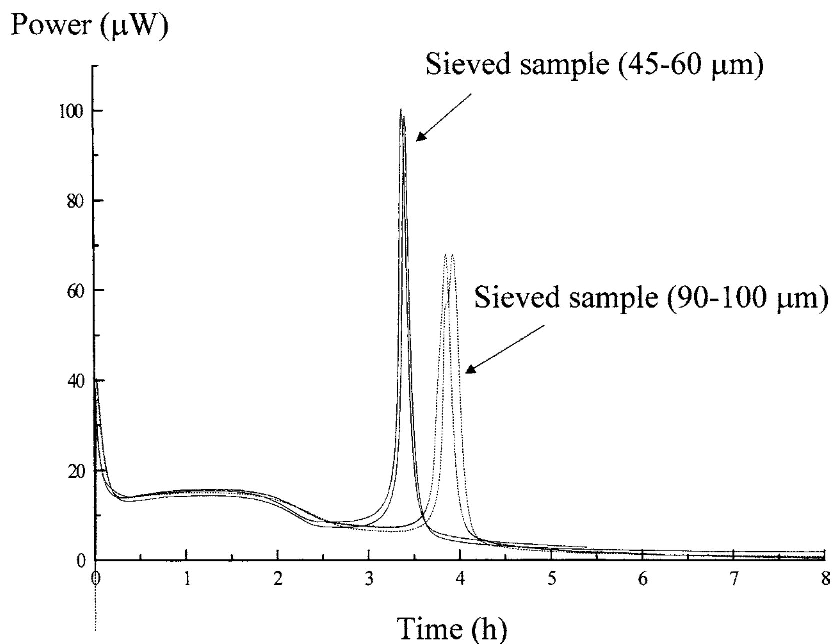

Fig. 5. Power–time traces for the hydration of two different particle size fractions of anhydrous ceftazidime in the new hydration apparatus.

anhydrous ceftazidime. The drug was sealed in the Durham tube and water was placed in the ampoule. After equilibration had been attained the foil was pierced and data collection started. The trace comprises three main regions, termed A, B and C. Region A represents the mechanical effects of breaking the seal and wetting of the cell and sample as discussed above and remains constant for each sample. Region B is

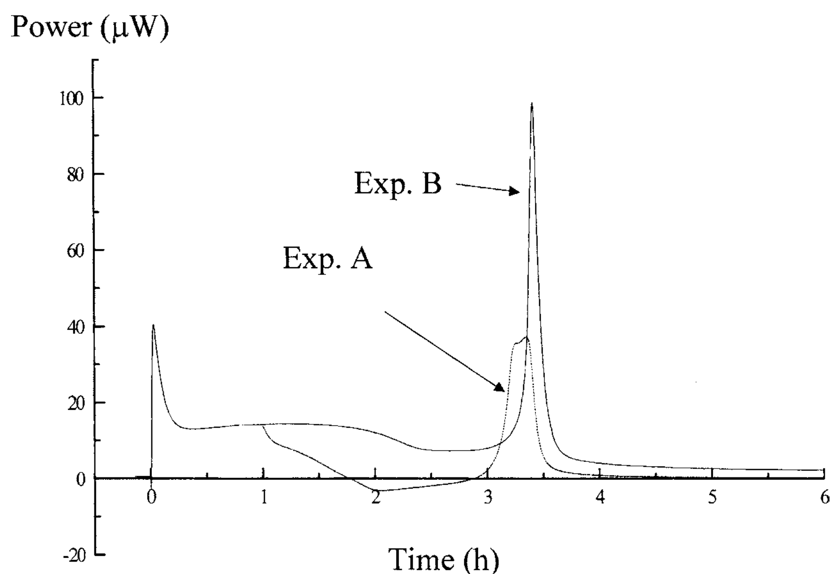

Fig. 6. Power–time data for the hydration of anhydrous ceftazidime using the conventional hydrostat method (experiment A) and the new hydration apparatus (experiment B).

seen to alter as the average particle size distribution of the sample is varied. Fig. 5 shows the power–time traces for two particle size fractions of drug (45–60 and 90–100 μm prepared by sieving). The time over which region B extends is observed to reduce from 3.95 h for the larger particle size fraction to 3.4 h for the smaller particle size fraction. Since the only factor that varies in the two cases is the surface area of the sample, it seems likely that this region is attributable to the absorption of water by the drug. The large exotherm, region C, appears to be slightly sharper with the lower particle size fraction, but the areas under each curve are the same, within experimental error limits, irrespective of the particle size used (45–60 μm, 42.3 J g^{-1}; 90–100 μm, 40.5 J g^{-1}). Powder X-ray studies (data not shown) show that the sample undergoes a change in crystal habit during this phase. The data suggest that this exotherm corresponds to a change in the crystal structure of the solid drug as it forms the mono-hydrate.

Comparison of data recorded using the new apparatus with those recorded using a conventional hydrostat method reveal, as might be expected, some differences, Fig. 6. In the conventional hydrostat experiment (experiment A) the initial data are lost, for the reasons highlighted in the introduction, region B appears to give a smaller signal and region C is broad and shallow. The new apparatus (experiment B) shows all the data from time zero, and the large exotherm is better defined.

5. Summary

The study of interactions between vapours and solids using isothermal microcalorimetry is of importance, especially to pharmaceuticals, but current experimental methodologies have several disadvantages. The mini-hydrostat method is quick and convenient but care must be taken during experimental design. Firstly, it must be ensured that the thermal response of the sample is not limited by the rate of evaporation of water from the reservoir. Secondly, the reference used must be carefully selected such that heat-flows arising from, for instance, wetting of the internal surfaces of the ampoule and evaporation of water from the reservoir are minimised. It is also not possible to record data from time zero, nor study the effects of different RH's using the hydrostat method. RH perfusion allows both the RH to vary during a particular experiment and data collection from time zero. However, there is a large surface area to wet inside the ampoule that is (usually) not compensated for by the use of a suitable reference.

In the work described above, we show how sealing a sample in a Durham tube and placing the hydrating reservoir in the ampoule allows better data to be attained. Data collection can be initiated at time zero and, at that point, the largest possible surface area of the ampoule has been wetted. Care must still be taken to ensure the evaporation rate is not a rate-limiting factor but the hydrating reservoir has the largest possible surface area. In this case, we chose to study the hydration of a drug, ceftazidime, since it provided a convenient, and relevant, model system and we have shown that, in practice, our new experimental methodology has several advantages over existing techniques. We have also used the system to study the hydration of copper(II) chloride [15].

A direct consequence of being able to measure the complete heat response from the sample is that it should, in principle, be possible to analyse the data using the methodology we have described elsewhere [16] to obtain thermodynamic and kinetic reaction parameters. It is not possible to alter the specific RH in the cell during an experiment, which may make the technique unsuitable for some systems where RH perfusion may be a better alternative.

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