Materials Science

128
Photocalorimetry: a rapid method for assessment of photostability

M. Dhuna, S. Gaisford, M. A. A. O'Neill, C. V. Skaria, A. E. Beezer, J. A. Connor[1] and D. Clapham[2]

School of Pharmacy, University of London, 29–39 Brunswick Square, London. WC1N 1AX, [1]Medway Sciences, University of Greenwich, Medway Campus, Chatham Maritime, Kent. ME4 4TB and [2]GlaxoSmithKline, New Frontiers Science Park, Harlow, Essex. CM19 5AW, UK. simon.gaisford@pharmacy.ac.uk

We have reported previously early development work of our photocalorimeter system (Dhuna et al 2005) the ultimate aim of the project being to produce a robust, quantitative and easy to use system for the assessment of photostability. The original calorimetric system, while useful for providing proof-of-concept data, was not ideal because it proved impossible to get a zero power signal with light irradiating the sample cell, rendering quantitative analysis of data challenging. We thus redesigned the instrument to ameliorate this issue and report the initial results of our trials on liquid and solid samples here. The new system uses an optical beamsplitter and liquid light guides to direct light from a Xe arc lamp into sample and reference ampoules (20 mL volume). The light entering the two cells is adjustable using a number of focusing and shuttering assemblies; the amount of light entering the sample cell is then set to a predefined level (using an actinometer), while the light entering the reference cell is adjusted until a zero baseline signal is achieved. Essentially, the calorimeter itself is used as a null-adjuster to ensure parity between the sample and reference sides. Using the new system we studied a number of liquid and solid samples to evaluate instrumental performance characteristics. Liquid systems: the zero-order photodegradation of 2-nitrobenzaldehyde was selected as a model system, with a view to assessing the suitability of its use as a chemical actinometer. A zero-order reaction should give a constant deflection in the photocalorimeter. It was observed that the calorimetric data did indeed settle to a zero-order deflection ca. 5 h after the light was switched on. However, 5 h is a considerable period of time for the system to attain equilibrium, so we followed the reaction using a pH titration method. It became clear from these data that an additional oxidation event was occurring in addition to photodegradation; when EDTA was added to the system true zero-order degradation was observed, giving a rate constant of ca. 2.5×10^{-7} mol dm^{-3} s^{-1}. The oxidation event would clearly impact upon the measured response in the photocalorimeter and was the likely cause of the non zero-order behaviour seen during the first 5 h of measurement. Solid systems: although at an early stage, we have studied the response of formulations containing retinoic acid (RA). It was found that RA alone gave a zero-order power response, although the magnitude of the deflection was not proportional to the sample mass. Binary mixtures of RA and microcrystalline cellulose (MCC) also gave a zero-order deflection although again power values did not vary linearly with sample masses. The reason for these observations is not yet clear; assuming that sample mixing was uniform, it is likely that one or more instrumental factors are implicated. These may include the depth of the sample, the proportion of sample being irradiated by light and the fact that different powders absorb/reflect light differently. Further work is required and we simply note here that sample effects appear to be important for solid systems.

Dhuna, M. et al (2005) *J. Pharm. Pharmacol*. **57** (Suppl.): S58

129
Influence of protein solid-state form on moisture sorption behaviour

W. L. Hulse, M. A. Mohammad, I. M. Grimsey and R. T. Forbes

School of Pharmacy, University of Bradford, Bradford, BD7 1DP, UK. R.T.Forbes@Bradford.ac.uk

The inherent instability of proteins when isolated from their native conditions creates the necessity of suitable stabilisation techniques. To overcome the lack of long term stability in solution protein pharmaceuticals are often prepared as solid products. The most common method is freeze drying (Arakawa et al 2001). Other methods include spray drying and crystallisation of the protein. Crystallisation of lysozyme has been shown to maintain the conformational integrity better than the spray dried form (Elkordy et al 2002). Water molecules interact with protein molecules, these interactions are one of the major contributors to the conformational stability of proteins. It was accordingly decided to determine the moisture sorption behaviour of crystalline, spray dried and freeze dried Lysozyme. An aqueous solution of lysozyme was dialysed (5% w/w) and was spray dried using a Büchi 190 spray drier. Samples were atomised using a two fluid nozzle (0.5 mm). The solutions were fed to the nozzle using 1mm silicone tubing and a peristaltic pump with a flow rate of 12–13 ml/min and was dried with inlet temperatures of 135–145°C to produce an outlet temperature of 60 ± 2°C. Batch crystallization was used to prepare lysozyme crystals. A solution containing 2% lysozyme and 5% NaCl was sealed and stored for ten days at 20°C before crystal harvesting. A freeze dried sample was used as received. Moisture sorption experiments were carried out using an IGAsorp moisture sorption analyser incorporating a real time processor (Hiden Isochemica, UK). Samples (50–60 mg) were placed in stainless steel sample baskets. The sequence of isothermal steps incorporated drying the samples to 0% RH until constant weight was achieved. The RH was then increased in increments of 10% to a maximum of 95% RH. The results show that the crystals are more hygroscopic than both the spray dried and freeze dried samples. Moisture sorption profiles of the crystals indicated a gradual moisture increase to approximately 60% RH where a significant increase in moisture uptake then occurred. The spray dried and freeze dried samples both showed gradual moisture increase prior to approximately 80% RH where moisture uptake then significantly increased. These results may have implications for the production and storage of lysozyme crystalline samples. It is generally anticipated that crystalline materials are less hygroscopic than their less crystalline counterparts but the complex conformation of lysozyme compared to a small molecule probably contributes to the behaviour. Table 1 shows the total moisture uptake of the protein samples.

Table 1 Water uptake (%) in samples

Lysozyme sample	% Water uptake (25°C)
Freeze dried	25.39 ± 0.3
Spray dried	12.14 ± 9.6
Crystalline	30.95 ± 7.2

Data are means ± s.d.

Arakawa, T. et al (2001) *Adv. Drug Del. Rev*. **46**: 307–326
Elkordy, A. A. et al (2002) *Int. J. Pharm*. **247**: 79–90

130
The use of quasi-isothermal modulated temperature DSC as a novel means of detecting and characterising polymorphic transitions

M. Misici Falzi and D. Q. M. Craig[1]

Departement of Chemical Sciences, University of Camerino, Via S. Agostino, 62032 Camerino, Italy and [1]School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich NR4 7TJ, UK. E-mail: d.craig@uea.ac.uk.

Differential scanning calorimetry (DSC) is one of the most common approaches for characterising drug polymorphic forms. This may be performed in terms of simple detection, whereby the difference in melting point between forms is measured, or alternatively more sophisticated analysis may be performed whereby thermodynamic phase diagrams may be derived from heat of fusion and melting data. However, a difficulty that is intrinsic to all such measurements is that the melting of polymorphs is a highly energetic event, leading to masking of more subtle transitions. Furthermore, as DSC is a dynamic technique it is often very difficult to differentiate between thermodynamic (equilibrium) and kinetic events. In this study we describe the use of quasi-isothermal modulated temperature DSC (QI-MTDSC) as a means of studying the simultaneous melt-crystallisation process of two model systems, polyethylene glycol (PEG) 4000 and indometacin. In brief, the sample is modulated at a series of temperatures and ramped between those temperatures so as to obtain a series of effectively isothermal data sets. In this way the effects of kinetic events may either be effectively eradicated if they are rapid in terms of the timescale of the measurement and may be followed in real time if they are not, thereby offering the possibility of both equilibrium and kinetic measurements. The metastable form of PEG 4000 was prepared by heating samples to 70°C and then cooling to 20°C. Conventional DSC showed melting points corresponding to the coexistence of the metastable once folded (57.8°C) and stable extended (61.8°C) chain forms. Quasi-isothermal experiments allowed the heat capacity to be measured through the transitions in isolation from the kinetic events associated with melting and recrystallisation. It was noted that there was a reversible component to the melting of the stable (extended) form; this has been previously attributed to locally reversible surface melting and crystallization processes in polymers (Cebe 2005). However, the (quasi-) equilibrium melting and recrystallisation of the metastable form could also be identified in isolation and evidence is presented for the approach being able to detect and quantify low temperature, time-dependent changes in structure that may not be observed using conventional DSC, particularly using Lissajous analysis whereby the modulated heat flow is plotted against the modulated temperature. The approach was also used to study the transformation between the α- and γ-forms of indometacin. The melting points of these two forms were measured as 154.4°C and 161.7°C, respectively. However, using the quasi-isothermal technique we have been able to show a melt/recrystallisation process that takes place at 150–151°C, followed by melting of the α-form; this implies the

presence of further metastable form of the drug. The approach has also furnished evidence that after the melting of the α-form, simultaneous recrystallisation/melt of the γ-form takes place over the temperature range between the melting points of the two forms even though no full, macroscopic recrystallisation takes place, a phenomenon which may not be detected using conventional DSC due to the energetic subtlety of the process. We suggest that this may form the basis for a technique whereby one may establish whether the most stable polymorph is present or not.

Cebe, P. (2005) *J. Polym. Sci. Part B: Polym. Phys*. **43**: 629–636

131

An investigation into the recrystallisation behaviour of amorphous paracetamol: spectroscopic studies for the identification of exothermic thermal events

S. Qi and D. Q. M. Craig

School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich, NR4 7TJ, UK. d.craig@uea.ac.uk

Despite the extensive use of paracetamol, the polymorphic conversion behaviour of this drug is still a matter of some controversy. Our focus within this field has been to study the recrystallisation behaviour of amorphous paracetamol using a range of thermal techniques (Qi et al 2005). In our previous study, glassy paracetamol produced by slow cooling exhibited two exothermic transitions at 60–85°C and 120–130°C followed by the melting of the orthorhombic form II (158°C). The purpose of the current study is to identify these exothermic transitions using spectroscopic means. In the first instance, paracetamol form III was prepared in order to provide a standard thermal and spectroscopic reference. DSC results using pinhole pan of the standard form III exhibited an exothermic peak at 127°C followed by melting at 158°C. Variable temperature XRD (VTXRD) confirmed that the spectrum of the material in the temperature region below 120°C is same as reported XRD pattern of paracetamol form III (Rossi et al 2005). Taken together, these data indicate that the exothermic transition at 127°C represents the polymorphic transition of form III to form II. This was confirmed by temperature controlled Raman microscopy results. The behaviour of the amorphous paracetamol was then considered in the context of these results. It was hypothesised that the transition at 60–85°C should relate to the recrystallisation of glassy paracetamol into (at least predominantly) form III. However, the VTXRD and Raman spectrums of amorphous paracetamol heated above 60–85°C were dominated by the XRD patterns of form II. Examination of the ratios of the relative enthalpy values of the exothermic peaks at 120–130°C to the peaks at 60–85°C are in the range of 9.9–2.5%, which indicated that at 60–85°C the majority of glassy paracetamol recrystallise into form II mixed with small fraction of form III, which subsequently also recrystallises to form II. Previous studies also noted that the relative enthalpy of the exothermic transition at 120–130°C could be significantly increased by using pinhole pans as opposed to hermetic pans, indicating that the yield of form III is not only dependent on the thermal history of the glassy sample but the type of sample pan used. Under a high-pressure environment (in this case use of a hermetic pan) the formation of form II is favoured (Boldyreva et al 2003). This explains the almost complete absence of the 120–130°C transition in the samples prepared using hermetic pans under the ambient atmosphere. The study has identified the exothermic transitions seen in the DSC traces of amorphous paracetamol and has also allowed insights into the effects and mechanisms underpinning parameter-related conversions between paracetamol polymorphs following recrystallisation from the amorphous state.

Boldyreva, E. V. (2003) *Cryst. Eng*. **6**: 235–254
Rossi, A. et al (2005) *J. Pharm & Pharmacol.* **57**: S93–S94