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The development of a novel LED photocalorimeter for the assessment of photostability

M. Dhuna[1], S. Gaisford[1], A. E. Beezer[1], J. A. Connor[2] and D. Clapham[3]

[1]The School of Pharmacy, University of London, 29–39 Brunswick Square, London, WC1N 1AX, [2]Medway Sciences, University of Greenwich, Medway Campus, Chatham Maritime, Kent, ME4 4TB and [3]GlaxoSmithKline, New Frontiers Science Park, Harlow, Essex, CM19 5AW, UK. E-mail: meena.dhuna@pharmacy.ac.uk

Objectives To develop a robust and easy to use photocalorimeter, one which allows the quantitative assessment of photostability of pharmaceuticals. The photocalorimeter reported herein was built through a series of iterative prototype designs (Dhuna et al 2007) and can be used for the direct study of liquid, solid or semi-solid systems and, in combination with appropriate data analysis methodologies, the derivation of thermo-kinetic information to monitor photodegradative processes.

Method The photocalorimeter uses a selection of Light-Emitting Diodes (LED) to create an array covering a wavelength spectrum of 350–700 nm in the UV-VIS. The LED array delivers light into the sample and reference cells (stainless steel, 20 mL capacity) of a Thermal Activity Monitor (TAM, Thermometric Ltd) via liquid light guides. The system is powered by a constant current (voltage) input, which is used to balance the power output in the sample and reference cells. The voltage on the sample side is set to a constant level, whilst the voltage on the reference side is adjusted until a zero power signal is obtained prior to sample measurement. Thus, the TAM itself acts as a null-adjuster to ensure a balance in power between the two sides. The light source is mounted on a circuit switch board and each LED can be switched on and off independently. It is, thus, possible to find the causative wavelength range of degradation by selective illumination of LEDs. The instrument's performance was monitored using the photodegradation of a 2-nitrobenzaldehye (2-NB) solution (0.1 M). A single ultra-bright (10 000 mcd) UV LED was used at three different current inputs; 10 V, 15 V and 20 V. All measurements were conducted in triplicate at 298 K and analysed using Origin.

Results The photodegradation of 0.1 M 2-NB showed to follow zero-order kinetics, as observed by a constant calorimetric output. The successive increase in voltage was proportional to the power output of the photodegradation of 2-NB. Power signals of approximately $12.2 \pm 0.3\ \mu$W, $46.9 \pm 1.6\ \mu$W and $85.5 \pm 0.8\ \mu$W were obtained at 10V, 15V and 20V, respectively. This resulted in a linear relationship ($R^2 = 0.99992$) between the power output and voltage. These data are encouraging to the instrument's performance and demonstrate that the novel photocalorimeter is working accordingly.

Conclusions A robust, versatile and rapid photocalorimeter has been successfully developed and is capable of assessing photostability of pharmaceuticals, as demonstrated by the photodegradation of 2-NB. The preliminary data show that LEDs have much potential for application of photostability testing.

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The implementation of FT-Raman spectroscopy to study solid state moisture induced changes in the crystallographic nature of pharmaceutical excipients—a lesson in humidity

P. J. Major[1], J. C. Mitchell[1], V. J. Cornelius[1] and J. Murphy[2]

[1]University of Greenwich at Medway, Medway Campus, Central Avenue, Chatham Maritime, Kent, ME4 4TB and [2]Pfizer Ltd, Sandwich, UK. E-mail: mp68@gre.ac.uk

Objectives To determine the capabilities of FT-Raman in studying the effects of humidity on the storage of pharmaceutical excipients. Increased humidity is known to aid crystallisation by lowering the glass transition temperature (T_g) of an amorphous compound. Sucrose and lactose were chosen as model compounds, as both are used extensively as pharmaceutical excipients. Sucrose exists as either an amorphous or crystalline solid. Lactose exists as either an amorphous solid or in one of three crystalline solid states (α-lactose anhydrous, β-lactose anhydrous and α-lactose monohydrate).

Methods Sucrose and lactose were rendered amorphous by lyophilising a 10%w/w volume of the disaccharide in distilled water, using an Edwards Freeze-Drier, −40°C at 5 mbar for 48 h. The amorphous samples were then stored in one of eight desiccators at 25°C and known humidity, ranging from 0%RH* to 84%RH. Humidity was controlled by using saturated salt solutions and monitored using a Testo 608-H2 Hygrometer (accurate to ±0.1°C and ±2%RH). Each sample was incubated in a desiccator for 1 week, to enable the sample to fully acclimatize to the humidity. Morphological state was then measured by FT-Raman. PXRD was taken of the samples to confirm morphological state. FT-Raman experiments were conducted with a Thermo Nicolet NXR FT-Raman module, with a liquid nitrogen cooled NXR Genie (Germanium) detector. The laser was at 1064 cm^{-1}. 64 scans were taken of each sample over the range 3700–100 cm^{-1} at a resolution factor of 4 (giving ~750 data points per scan). PXRD experiments were performed on a Bruker D8.

Results The sucrose samples stored ≤33%RH remained amorphous after 7 days in the desiccator at 25°C. The sucrose samples stored >33%RH crystallised within 7 days at 25°C. Lactose remained amorphous when stored <43%RH and 25°C. Lactose >57%RH, 25°C had crystallised within 7 days. The sample stored at 43%RH appeared to be in a transitional state, and may have been crystalline. Amorphous lactose crystallised into the α-monohydrate form. These results were shown clearly on the FT-Raman, with diagnostic peaks agreeing with the literature for Raman of α-lactose monohydrate. FT-Raman contains several diagnostic peaks with two chosen for sucrose, and 2 chosen for lactose. These diagnostic peaks have been used to confirm morphological state.

Conclusions FT-Raman has proven to be an effective tool for determining morphological state. % Relative Humidity has been shown to be an important factor in the storage of a pharmaceutical excipient, and by extension the storage of the medicine containing the excipient. Amorphous samples have been shown to remain amorphous at low %RH and to crystallise at higher %RH, where all other factors (e.g. temperature) are equal between samples.

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Application of calorimetry and sub-ambient atomic force microscopy to the study of frozen aqueous trehalose solutions

J. Wu, M. Reading and D. Q. Craig

School of Chemical Sciences & Pharmacy,University of East Anglia, Norwich, NR4 7TJ, UK. E-mail: j.wu@uea.ac.uk

Objective Disaccharides such as trehalose are widely used as cryo-protectants to maintain the activity of proteinaceous drugs during freezing. However, the mechanisms associated with the cryo preservation process are still unclear. One unresolved issue is the double transition, which is observed very commonly in disaccharides solutions in the frozen state; the assignment of these transitions is still controversial. Our objective is to investigate frozen aqueous trehalose solutions by Modulated Temperature Differential Scanning Calorimetry (MTDSC) and Sub-ambient Atomic Force Microscopy (S-AFM) in order to better understand this phenomenon.

Methods MTDSC was used to determine the two sub-ambient transitions and three annealing experiments were performed to assess the relaxation behaviour of frozen trehalose solutions (200 mg/mL). The sample was cooled to the annealing temperatures −40 °C, −34 °C and −25 °C, then held at these temperatures over a time period up to 20 h. The values of the two transitions were re-measured by cooling down the sample to −60 °C then heating up to 0 °C after annealing. We present the first Sub-ambient Atomic Force Microscopy (AFM) results at atmospheric pressure on a frozen aqueous system. AFM can provide spatially resolved physical measurements such as mechanical properties and, therefore, Tg measurements, on the frozen materials. A small volume of solution was loaded on fresh peeled mica and rapidly cooled down to −60 °C then imaged over the relevant temperature range.

Results The Tg values for the unannealed samples were measured as − 36.7°C and −28.8°C for Tg_1 and Tg_2, respectively. The annealing experiment at −40°C only effected the first transition ($T'g_1$; −33.2 °C) but not on the second transition ($T'g_2$). The annealing experiment at −34 °C showed no effect on either transition, while the annealing experiment at −25 °C showed effects only on the second transition ($T'g_2$; −26.8 °C). The topography images from AFM showed the height of crystal-like particles decreasing with the increased stage temperature. At −25 °C, all the crystal-liked particles collapsed, which corresponded to Tg_2 shown by DSC. The force modulated images were able to identify a general softening of the surface in the region of the first transition.

Conclusion The results suggests that Tg_1 is a really glass transition but Tg_2 is not for the following reasons: (1) The unfrozen concentration of amorphous matrix only can be affected by annealing below $T'g_1$ not by annealing below $T'g_2$; $T'g_2$ can only be altered after annealing at −25 °C which is above $T'g_2$. (2) The observed softening indicates the beginning of devitrification of the amorphous phase, the brittle glass changes to a more fluid state, which indicates a real glass transition. The second transition is actually the onset of ice melting as the crystal-like partials collapse and the frozen matrix becomes more mobile after this transition.

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Nano-termal analysis of individual pharmaceutical particles

X. Dai, M. Reading and D. Q. M. Craig

University of East Anglia, Norwich, NR4 7TJ, UK. E-mail: x.dai@uea.ac.uk

Objectives Lactose is a commonly used carrier in dry powder inhaler formulations. Micronisation of crystalline lactose often leads to disruption or activation of the crystalline structure and varying degrees of disorder through the formation of residual amorphous regions. Any amorphous content of micronised lactose will affect the efficiency of dry powder inhaler formulations, hence it is important to characterise the amorphous regions and their distribution within the particles and on their surfaces. Here we describe the novel use of nano-thermal analysis to detect amorphous material on individual particles. The technique involves heating a nano-size probe and measuring the indentation profile as a function of temperature.

Method Amorphous regions on lactose particles were generated by milling lactose anhydrous powder in a simple ceramic ball mill for specified times. The global amorphous content was analysed using MTDSC. After depositing the particles on a glass chip with fast setting araldite, the particle surface is imaged using pulse force mode AFM with nanothermal analysis probes. Selected regions (both crystalline and amorphous region) on the particle surface are characterized using localised thermal analysis.

Results The amorphous content could be detected after 10 min milling using MTDSC. The longer the milling time used, the greater the amorphous content. The pulse force mode images showed two different adhesion regions on the particle surfaces. Local thermal analysis confirmed that the selected two points are at different states (Figure 1), showing the two sets of data observed when assessing the particles. Considered alongside the MTDSC data, we suggest that the lower temperature transition corresponds to the presence of amorphous material, detected at nanoscale resolution.

Conclusion We present evidence that we are able to detect amorphous regions on individual powder particles using nano-thermal analysis. This allows the possibility of both mapping and characterising the amorphous material at a nanoscale resolution.

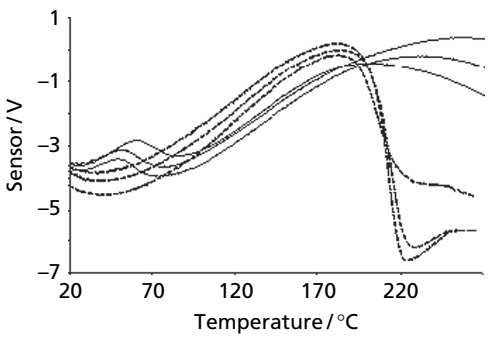

Figure 1 Nanothermal analysis profile of milled lactose showing two populations of response found in different locations on the particle surface.