Poster Session 1 – Material Science

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The evaluation of a wet and dry dispersion particle sizing method

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The FDA recommends that a suitable test method and acceptance criteria should be established for the particle size distribution of a bulk drug if it affects the dissolution, bioavailability, stability, content uniformity, processability or appearance of the drug product. Historically, particle sizing method development at AstraZeneca Macclesfield has routinely involved the use of a laser diffraction instrument (Coulter LS 13 320) with the sample dispersed in a liquid. However, recent problems with the development of liquid dispersion methods resulted in the purchase of a dry powder dispersion instrument (Sympatec Rodos/M). This has allowed a direct comparison of the two dispersion systems to be made on an oncology compound. The particle size results from the dry dispersion method were smaller in comparison with the wet dispersion method, and seemed smaller than the size suggested by light microscopy. Although it is well documented that significant variation in the measured size can be generated by different sizing techniques, it was necessary to understand the causes of the difference and establish which technique was giving a more representative result. The main differences between the dry Sympatec and wet Coulter methods were the instrument itself, the dispersion method (dry versus wet) and the optical model (Fraunhofer versus Mie). To examine the effect of these differences, samples were analysed by the wet dispersion method on a Coulter LS 13 320 or Sympatec Cuvette system using a 10% tween solution buffered to pH 10. Samples were analysed in the dry state using a Sympatec Rodos/M and a dispersion pressure of 3 bar. The particle size distribution of a batch of drug substance analysed using the dry Sympatec method with Fraunhofer (F) and then recalculated using Mie (M) is shown in Table 1. The results demonstrate that although the choice of optical model can have an impact on the particle size data, in this case it is the dispersion method which has a more significant effect. A number of batches were then analysed using the wet and dry dispersion methods on the Sympatec particle size analyser. The data suggests there is a significant difference between the particle size results generated by the wet and dry dispersion method on the same instrument. The distribution for the dry dispersion method has a higher proportion of particles in the 1–5 micron range compared with the wet dispersion method, although at the top end of the distribution the profiles overlap showing no particle comminution has occurred. Scanning Electron Micrographs of the milled batches show there is a significant amount of fines adhered to the surface of the larger particles, not visible in the light microscopy pictures. The difference in particle size results generated by the dry and wet dispersion methods is due to the dry dispersion method removing and then sizing the fine material that is adhered to the surface of the milled batches. This results in a better correlation between the particle size and surface area data for the dry dispersion method, which was also quicker and less complex to develop.

Table 1 Comparison of the particle size results generated using different optical models

Instrument & dispersion technique	Optical model	Particle size (microns)		
		D10	D50	D90
Sympatec-dry	F	1.1	5.2	18.3
Sympatec-dry	M	1.5	6.3	19.5
Coulter-wet	M	2.3	10.0	26.4

The D10 represents the particle size distribution point where 10% of the particles have a volume diameter less than the value stated. The D50 and D90 represent the 50% and 90% distribution points, respectively.

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Influence of particle size on surface energy components of inhalation grade lactose

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Dry Powder Inhaler (DPI) formulations are increasingly used to deliver drugs to the lungs for treatment of pulmonary and systemic diseases. For effective lung deposition the drug particles need to be in the micron range. As the particles in this size range tend to be highly cohesive and have poor flow properties, interactive mixtures are generally used that consist of micronised drug particles adhered to the surface of large carrier particles usually a-lactose monohydrate. Redispersion of the drug particles from the carrier particles is essential for the lung deposition. The interparticulate forces, mainly the van der Waals and polar forces, within the DPI formulations strongly influence the degree of redispersion and therefore extent of the drug delivered to the lungs. It is known that van der Waals forces between the interacting particles depend on their physicochemical properties such as particle size and surface roughness. Accordingly in a number of studies it has been observed that drug deposition in the lungs is primarily influenced by the particle size distribution of carrier and proportion of fine carrier particles. However, to our knowledge, there are no reports characterizing the surface energy components of the lactose with various size fractions of the same grade. It is hypothesized that as the particle size decreases the dispersive surface energy increases and the polar surface energy will also change. The understanding of how dispersive and polar components of surface energy of lactose vary with particle size will shed some light on the otherwise less-understood area of drug-lactose interactions and their impact on drug redispersion. In this study, the surface energy of various sieve (size) fractions of inhalation grade lactose was determined by means of an inverse gas chromatography system (iGC, SMS Ltd, UK) using pulse iGC at infinite dilution at 30°C, 0% relative humidity and a nitrogen carrier flow rate of 10 mL min^{-1}. Decane, nonane, octane, heptane, acetonitrile, acetone, dichloromethane, ethylacetate and ethanol were used as elutants and detected by means of an FID. As can be seen from Table1 the surface energy showed a trend of decreasing dispersive surface energy with increase in the mean volumetric particle diameter (V_{MD}) of lactose. Similarly we have observed changes in their polar surface energy component (i.e. the acid/base parameters). These results are in favour of the above hypothesis and suggest that the surface energy of particles increases with decrease in their particle size probably due to increased surface disorder or changes in the orientation, which influence the interparticle adhesion/separation forces.

Table 1 Surface energy of various fractions of lactose

V_{MD} (μm)	γ (mJ m^{-2})	K_A	K_B
3.3 (0.6)[a]	46.8 (0.4)	0.16 (0.003)	0.0 (0.003)
78.5 (0.8)[b]	45.7 (0.3)	0.15 (0.002)	0.01 (0.002)
58.5 (0.2)	45.0 (0.4)	0.15 (0.001)	0.01 (0.001)
125.4 (0.5)	44.5 (0.5)	0.13 (0.000)	0.04 (0.002)
187.4 (0.4)	42.8 (0.8)	0.13 (0.001)	0.06 (0.005)

[a]Micronised lactose; [b]lactose grade from which other fractions were sieved. V_{MD} = mean volume diameter; γ = dispersive surface energy; K_A = acid parameter; K_B = base parameter.

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The use of inverse phase gas chromatography to study the glass transition temperature of a powder surface

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The amorphous state is commonly characterised through a glass transition temperature (Tg) measured using conventional techniques like differential scanning calorimetry (DSC). Although sorbed water is known to plasticise the amorphous state (lowering the Tg), most of the conventional techniques offer little or no control over the humidity conditions. This issue could be more significant in the case of hydrophobic drugs where water molecules are poorly mobile and are mainly concentrated at the surface. Surface localization of water molecules may also lead to preferential surface plasticization and surface crystallization (Andronis et al 1997). The aim of this work was to measure the glass transition temperature (Tg) at the surface of a hydrophobic particle at different temperatures and humidities. The hypothesis was that the surface may be plasticized to a different extent to the bulk, due to slow water sorption giving a concentration gradient of water through the particles. Amorphous indomethacin was prepared using a standard melt-quench method and then passed through a sieve ($< 350\,\mu$). Amorphous indomethacin was then packed in a pre-silanized glass column and conditioned by dry carrier gas to remove sorbed moisture. It was then exposed to a stepwise increment in the relative humidity (%RH) under isothermal conditions in an inverse gas chromatograph (IGC). At the end of each conditioning step decane injections were made and retention volumes of decane

were calculated using maximum peak height (Vmax) and center of mass (Vcom) methods. These measurements were performed at various temperatures from 25–35°C. The extent of water sorption under the same conditions of %RH and temperature were determined gravimetrically using dynamic vapour sorption (DVS). The Vcom retention volumes were found to deviate from Vmax results at certain critical humidities at each temperature. This was taken as a novel method for determining the Tg of the sample surface at different experimental conditions (on the basis that the change in peak shape was due to a change in the solid allowing probe to be absorbed). Extrapolating the critical RH needed to lower the Tg to experimental temperature to 0%RH yielded a Tg similar to literature values. The amount of water sorbed at this critical RH (from DVS) was lower than the amount of water required to lower the Tg to experimental temperature calculated using the Gordon Taylor equation. This implies a higher concentration of sorbed water at the particle surface and preferential surface plasticization. It is possible to use IGC to determine the Tg of the surface of particles at defined conditions. This overcomes the problems of conventional methods of assessing Tg, relating to disruption of water sorption on heating. This helps in the understanding of the physical form of hydrophobic particle surfaces and how and when these will start to crystallise.

Andronis, V., et al (1997) *J. Pharm. Sci.* **86**: 346–351

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Advances in micro thermal characterisation

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Micro thermal analysis has found a range of applications for a variety of materials (Pollock et al 2001) including a growing number in the field of pharmaceutical materials science (Craig et al 2002). One of the main limitations of the technique has been the spatial resolution. Recently a step change has been achieved both in terms of thermal imaging and local thermal analysis with a new generation of probes. Images with a topographic and thermal resolution of the order of tens of nanometers have been demonstrated. There has also been a corresponding increase in the resolution for local thermal analysis. Another technique that is based on the micro TA instrument is photothermal microspectometry (Hammiche et al 2004). This is a new form of FTIR microscopy, which exploits the photothermal effect in a similar way to photoacoustic spectroscopy but by directly measuring the temperature variations caused by the absorption of IR radiation. Significant improvements in signal to noise of better than an order of magnitude have been made and the limits of the spatial resolution that can be achieved with this method have been defined. In principle this kind of near field measurement can achieve better than diffraction limit resolution. The spatial resolution is governed, not by the wavelength of the incident radiation, but by the thermal diffusion length of the thermal wave created by the interferometer. This parameter can be controlled by the mirror speed. However, increasing mirror speeds decreases signal to noise thus a compromise has to be found. With the new high resolution probes that have a much shorter response time, higher mirrors speeds can be achieved, up to 10 kHz using the Bruker measurement fort this parameter, with reasonable signal to noise. At 280–300 cm^{-1} (C-H stretch) spatial resolution of 3 microns is achieved, which is equivalent to the conventional far field method. It is anticipated that this will be significantly improved upon in the future. Another method is nanosampling. By heating the tip the material adjacent to it can be softened and, consequently, often contaminate the tip when it is withdrawn. This 'nanosample' can then be analysed by a variety of methods including photothermal spectroscopy. It has been shown that spectra can be obtained from samples that are as small as picograms. These methods, used singly and in combination, have advantages for a variety of pharmaceutically relevant analytical applications and even better prospects for the future.

Craig, D. Q. M., et al (2002) *J. Pharm. Sci.* **91**: 1205–1213
Hammiche, A., et al (2004) *Spectroscopy Magazine*, 4 December
Pollock, H. M et al. (2001) *J. Phys.D Appl. Phys.* **34**: R23–R53

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Molecular interactions affecting polymorph behaviour in solution

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Control over conditions affecting polymorph crystallisation and processing characteristics is vital in the development of pharmaceutical and other preparations. To understand fully how and why a particular polymorph is formed or transformed, the kinetic, thermodynamic and molecular recognition considerations associated with particle-particle interactions should be investigated (Bernstein 1993; Davey et al 2001). Various solvents were used to probe the influence of different molecular environments on polymorph formation of the nootropic drug piracetam during molecule-aggregation, nucleation and crystal growth stages of solution crystallisation. For this purpose, a dual-technique approach combining Energy Dispersive *X*-ray Diffraction (ED-XRD) performed with synchrotron radiation at station 16.4 of Daresbury Laboratory CCLRC, UK and UV-VIS, Near IR and FT-Raman Spectroscopy was used to monitor the different crystallisation mechanisms in-situ. Piracetam forms I, II and III and phase-interconversions in each solvent were evident from diffraction data. A typical example, observed when cooling from methanol, showed piracetam form I appearing at around 33°C with characteristic diffraction lines at 3.09 Å, 4.00 Å and 4.73 Å competing with form III which dominates within one minute after cooling to 31°C with strong peaks at 2.41 Å and 3.47 Å. Raman spectra collected at 488 nm, 785 nm and 1064 nm showed significant broadening, shifts and, in some cases, absence of peaks in the solutions spectra when compared with that of the crystalline forms. For example, a characteristic strong peak at 1647.5 cm^{-1} is lost in the saturated solution spectrum at 20°C, the latter peak is clearly observed in the spectrum of form III crystals recovered from methanol at the same temperature. The modifications added to the previously used ED-XRD protocol at station 16.4 (Blagden et al 2002) enabled good control over experimental conditions and generated consistent results from which polymorph inter-relationships were examined. The complimentary Raman data provided key insights into the molecular organisation of the studied environments and how this initial molecular recognition process translates to polymorph crystallisation in solution.

Bernstein, J. (1993) *J. Phys. D-Applied Phys.* **26**: B66–B76
Davey, R. J., et al (2001) *J. Crystal Growth Design* **1**: 59–65
Blagden, N., et al (2002) *J. Crystal Growth Design* **3**: 197–201

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The use of microthermal analysis for the characterisation of compression induced caffeine polymorph interconversion

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This work describes the site-specific characterisation on the tablet surface by the novel use of microthermal analysis (MTA) (Pollock et al 2001). More specifically, we describe the use of the technique as a means of identifying different polymorphic forms of caffeine in a compressed tablet via the use of localized thermal analysis (LTA) (Craig et al 2002). The information obtained is related to the temperature dependent properties such as thermal expansion of the sample or phase transitions. As multiple polymorphic forms may be present within a single compacted sample, there is therefore a potential role for the technique in identifying the presence and distribution of these forms. Compacts of caffeine Form II were prepared by placing approximately 200 mg of pure Form II in an IR press. A mass of 10 tons is applied and the compact was immediately analysed. LTA was performed using a TA instruments mTA 2990 micro thermal analyser calibrated with Nylon 6. LTA data of pure polymorphic forms were obtained by heating the surface of the compact to 80°C (for Form II) and to 170°C (for Form I). The probe was held in-situ for 1 min and retracted; LTA was repeated at the same location at a heating rate of 10°C s^{-1} with a programmed temperature from −50°C to 300°C. LTA studies were then performed over at least seventy site-specific locations covering the whole surface of the tablet. The sensor showed a steady increase in height associated with the sample softening (168.2 ± 6.1°C for Form II and 174.1 ± 2.2°C for Form I) followed by the melting of the sample (232.9 ± 2.5°C for Form II and 238.1 ± 1.8°C for Form I). However, it was also noted that prior to the main transition a discontinuity at circa 140°C was observed for Form II that corresponded well to that associated with the polymorphic transition from Form II to Form I. In contrast, no such step transition was observed for Form I. On this basis it was able to distinguish the LTA sensor responses for Form I and Form II across the tablet surface. LTA data at the edges of the tablet show a lower number of Form II sensor responses to that of the middle section of the tablet, indicating that the conversion of polymorphs induced due to compression is site-specific over the tablet surface. The study has indicated that MTA can distinguish between Form I and Form II caffeine via detection of the transition between the forms. In addition, the technique can distinguish between the two forms in a compressed tablet.

Craig, D. Q. M., et al (2002) *J. Pharm. Sci.* **91**: 1205–1213
Pollock, H. M., et al (2001) *J. Phys. D Appl. Phys.* **34**: R23–R53

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The study of the reaction between xylose and glycine in freeze-dried materials

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The physical state of freeze-dried amorphous materials is one of the factors determining rates of chemical reactions and may affect pharmaceutical amorphous materials quality in process and storage. Recognition that molecules in this state can exhibit significant molecular motion over timescales of pharmaceutical interest, at a temperature both above and below the glass transition temperature (Tg) is key to understand the properties of the amorphous state. The glass transition temperature fixes the temperature range where an amorphous substance goes from a supersaturated, aqueous solution with very high viscosity to a solid. The molecular motion in the form of translational diffusion, essential for any physical or chemical process, can generally be described in terms of temperature, viscosity, and molecular size. Freeze-dried amorphous materials change their physical state at the glass transition temperature range. Below this range, in a highly viscous glassy state, the rate of diffusion, which controls chemical reaction by changing the molecular mobility, is generally regarded as being extremely slow. In dealing with the properties of amorphous pharmaceutical materials, it is inevitable that one must consider the presence of residual water. It has been reported that the residual absorbed water could affect the chemical stability of an amorphous product in at least four ways: water as a reactant; water as a product; water as medium; and water as a plasticizer. The Maillard reaction, which is a non-enzymatic browning reaction between an amino group and a reducing sugar, is a well-studied model of a diffusion-limited chemical reaction. The glass transition temperature, which varies with the water content of system, is an important factor to consider when studying this reaction in the amorphous state. In this study, the xylose-glycine system, which produces a blue pigment in the early stage of a Maillard reaction under mild conditions, was studied. The blue coloured product has a molar extinction coefficient of circa 7×10^4 M^{-1}cm^{-1}, which suggests both a simple and a sensitive spectrophotometric assay. In an attempt to estimate the most important role of water in the xylose-glycine Maillard reaction, this study deals with the effects of some factors, mainly freeze-drying time, glass transition temperature and water content, on the production of blue pigments and the extent of browning reactions.

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Effects of chirality on the salt formation of histidine

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The potential for predicting drug forms and anticipating problems such as hydration and solvation in drug compounds is of great interest to the pharmaceutical industry. This is a possibility resulting from an ever increasing knowledge of the molecular world. A dominant issue in the pharmaceutical industry is polymorphism, as small changes in the crystal structure can greatly affect the physicochemical properties of the drug. This study focuses on the potential packing variations that may arise from a combination of a chiral 'drug-like' molecule and a chiral/achiral counter ion during salt formation. The subtle changes in physicochemical properties as a result of chiral form variation will be probed as a means of characterising each system. Histidine is a particularly suitable probe molecule for this kind of investigation, as it is known to form salts with anionic and cationic counter ions. Some of these salts have hydrated forms. Furthermore, stereochemical effects on salt formation and hydration can be studied by comparing single enantiomer and racemic crystalline forms. Tartaric acid has been chosen as a chiral counter ion owing to the availability of all chiral forms. The 'test-set' of histidine salts created for this study, has been subjected to an array of analytical techniques to classify the solid form characteristics. These include ambient and non-ambient powder X-ray diffraction, TGA, DSC, dynamic vapour sorption, isothermal microcalorimetry, light microscopy and computational calculations. We have combined these analytical approaches with 'data mining' from the Cambridge Structural Database (CSD) (Allen 2002) and focused on the packing arrangements and intermolecular interactions of histidine chiral and achiral salt compounds. We shall present three new histidine tartrate salts structures solved from single crystal X-ray diffraction data: D-histidine D-tartrate, DL-histidine DL-tartrate and D-histidine L-tartrate. All three structures have R-factors less than 6%. Solid form characterisation has begun and isothermal calorimetry results are already showing the variation in lattice energy solvation between the three structures:

D-histidine D-tartrate ΔH_{sol}	32.688 kJ mol^{-1}
DL-histidine DL-tartrate ΔH_{sol}	39.294 kJ mol^{-1}
D-histidine L-tartrate ΔH_{sol}	36.811 kJ mol^{-1}

Allen, F. H. (2002) *Acta Crystallogr. B* **58**: 380–388

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Stabilisation of HFA pMDI formulations through microparticle surface modification: examination of suspension performance using two optical methods

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The required re-formulation of solution and suspension pressurised metered dose inhalers (pMDIs) following the substitution of hydrofluoroalkane (HFA) propellants for conventionally used chlorofluorocarbon (CFC) propellants has prompted a renewed interest in methods for assessing pMDI suspension stability. Homogeneity of pMDI suspension formulations is crucial to ensure dose uniformity (McDonald & Martin 2000). The search for suitable dispersants for use in pMDI reformulation has proved difficult, given that the surfactants used in CFC systems do not dissolve in HFA. In this study, microparticles for inhalation were produced with modified surface properties through the adsorption of a surfactant insoluble in HFA propellants. Suspension formulation stability was then examined using two optical methods. The surface of terbutaline sulphate (TS) was modified through adsorption of the polyoxyethylene-polyoxypropylene block co-polymer surfactant F127. An incubation method was employed, whereby 0.2 g of micronised drug was incubated with 20 mL of a 600 mg L^{-1} surfactant solution in dichloromethane for 3 h in a shaking water bath, maintained at 25°C. Drug-surfactant microparticles were subsequently recovered by filtration under vacuum and drying. Suspensions in both propellants HFA 134a and HFA 227 were prepared at two fill concentrations of 0.15% w/w and 0.3% w/w. Formulations containing TS-F127 microparticles were compared with those containing control micronised drug. Suspension performance was then assessed using the two optical methods: Optical Suspension Characterisation (OSCAR) and Turbiscan. Both methods measure the transmission of infra-red light through a pMDI formulation. In the case of OSCAR, light transmission is measured at the top and bottom of the suspension, whereas Turbiscan measures light transmission over the entire length of the suspension, in addition to measuring light backscattered by the suspension. Formulations were examined over a 3-min period following agitation in an ultrasonic bath. Results from both methods showed an improved suspension stability in formulations containing F127 when compared with control micronised TS. This was observed as a reduced sedimentation rate in HFA 227 and a reducing creaming rate in HFA 134a. The extent of flocculation occurring was also reduced following surfactant adsorption with little fluctuation in light transmission or back scattering observed in formulations containing F127. These improvements appeared more marked in formulations prepared in HFA 134a, and in formulations of 0.15% w/w fill weight. The facility for additional data analysis provided by software accompanying the Turbiscan apparatus allowed direct comparison of formulations in terms of a reduction in mean backscattering over a defined zone of the sample. Table 1 shows the improved suspension properties conferred on formulations following adsorption of F127. By showing a smaller reduction in mean backscattering, formulations containing surfactant can be said to be more stable as a reduction in back scattering over time corresponds to clarification of the suspension due to either sedimentation or creaming. Surfactant adsorption was shown to have improved pMDI suspension stability. Findings suggest that good correlation exists between the two methods. While OSCAR proved a useful and simple tool for assessing stability, the additional information provided by Turbiscan allowing objective comparison between formulations mean that it can be considered the preferred method.

Table 1 Reduction in mean backscattering by the sample cell zone 30–40 mm over 3 min, n = 4

	Reduction in mean backscattering (%)			
	0.3% w/w		0.15% w/w	
	HFA 134a	HFA 227	HFA 134a	HFA 227
TS	12.7 (2.6)	12.5 (2.3)	10.6 (2.2)	11.9 (1.1)
TS-F127	2.7 (1.3)	13.2 (2.6)	1.9 (0.3)	4.3 (3.4)

McDonald, K. J., Martin, G. P. (2000) *Int. J. Pharm.* **201**: 89–107

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The detection of small localized amorphous regions on a compact surface using scanning probe microscopy

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It is recognized that the detection of amorphous material may be a highly useful tool in the quality control of pharmaceutical materials. However, the spatial distribution of such material on compact and particle surfaces remains poorly understood due to the difficulties involved in imaging such substances. The aim of this study is to investigate the generation of such small amounts of amorphous material as well as to detect the amorphous regions on the surface of an otherwise crystalline compact of indomethacin. A compact of the model drug substance, indomethacin (Sigma-Aldrich), was prepared by filling a 13 mm die (Specac, France) with the indomethacin as received, then compressing it for 180 s using a bench press operating under compressing weight of 5 tons. Micro-Thermal Analysis was chosen as the primary means of examining the surface, and was selected due to the ability of the technique to thermally investigate regions approaching tens of microns in size (Murphy et al 2003). A TA Instruments 2990 Micro-Thermal Analyser (TA Instruments, Delaware, USA) was used to analyse multiple regions of the tablet surface within a 50 μm^2 area of the compact surface. The results from this initial analysis showed that the surface was crystalline, with the thermal probe being seen to indent into the sample surface at a temperature of $146 \pm 1°C$. The surface area of interest was then re-scanned to produce an image showing the indentations arising from the thermal analyses. A typical analysis site was identified and selected for re-analysis, the results of which showed an initial partial indentation of the probe into the sample surface at a temperature of $64 \pm 1°C$, before a further indentation at $146 \pm 1°C$, indicating that the regions of the surface which had been subjected to the initial thermal analysis were amorphous in character. For further investigation, a different analysis site within the surface area of interest was identified and subjected to various atomic force microscopy (AFM) techniques such as pulsed force AFM and non-contact AFM, allowing the locally generated small regions of amorphous character to be distinguished from the surrounding surface by their physical properties. It is demonstrated that these combined techniques can differentiate the amorphous from the crystalline material at high resolutions. Furthermore, the study has demonstrated that microthermal analysis may be used as a means of changing the physical integrity of samples on a micron scale.

Murphy, J. R., et al (2003) *Pharm. Res.* **20**: 500–507

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Analysis of parallel processes in pharmaceuticals by isothermal microcalorimetry

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Isothermal microcalorimetry (IM) measures the changes in heat that occur in a sample as it is held at a constant temperature. Nearly all processes occur with a change in heat content and, since IM is both extremely sensitive and indifferent to physical form or sample heterogeneity, it is a tool ideally suited to pharmaceutical stability assessment. However, its use in this area is not widespread, principally because heterogeneous samples can result in complex data that are difficult to interpret and analyse quantitatively. The analytical challenge, therefore, is to derive methodologies that allow successful analysis of complex power-time data. We have shown previously that single-step and consecutive reactions can be successfully interpreted by fitting data to kinetic-based models (Willson et al 1995; Gaisford et al 1999). However, the use of such an approach to the analysis of (the much more likely to be encountered with pharmaceuticals) case of parallel reactions has not been reported in the literature and is the focus of this work. The degradation reactions of binary mixtures of selected parabens (methyl, ethyl and n-propyl) were studied in basic solution at 25°C in a TAM (Thermometric Ltd), these compounds being selected as they are commonly found as preservatives in pharmaceuticals and are often formulated together. Each paraben undergoes hydrolysis, following first-order kinetics, to form an alcohol and p-hydroxybenzoic acid. The power-time data for a binary mixture of parabens thus contains the response of two parallel reactions and can be described by Equation 1:

$$\text{Power} = \frac{dq}{dt} = \Delta H_A \cdot k_A \cdot [A_0] \cdot e^{-k_A \cdot t} + \Delta H_B \cdot k_B \cdot [B_0] \cdot e^{-k_{B2} \cdot t}$$

where ΔH_A and ΔH_B are the enthalpies, k_A and k_B are the degradation rate constants and $[A]_0$ and $[B]_0$ are the initial number of moles of reactants A and B, respectively. Fitting the data to Equation 1 allowed the recovery of the rate constants for both degradation processes (Table 1). Equation 1 also allowed the determination of the reaction enthalpies for the methyl/n-propyl system (data not shown) but it was found that to fit Equation 1 to the other data sets the reaction enthalpies had to be known in advance. This serves to illustrate the practical resolution of the fitting methodology; it appears that to be able to analyse parallel processes with no prior knowledge, one rate constant must be a least twice the magnitude of the other. It was also observed that the rate constants determined for the parabens in the mixed systems were all lower than those expected (cf. methyl, $3.1 \times 10^{-4}\,s^{-1}$; ethyl $-1.5 \times 10^{-4}\,s^{-1}$; n-propyl $-1.1 \times 10^{-4}\,s^{-1}$, determined from calorimetric analysis of individual compounds). This was unexpected and the reasons for this observation are, at present, unclear; however, the fact that the parabens all degrade to a common product (p-hydroxybenzoic acid) may be the causative factor. We intend to study these systems further, but can conclude from this study that it is possible quantitatively to analyse calorimetric data for parallel processes and that the degradation kinetics of materials may alter when formulated in combination, highlighting the need for stability assays capable of analysing heterogeneous systems.

Table 1 Average values for the rate constants for binary mixtures of the parabens determined by fitting data to Equation 1

Ester mix	k_1 (s^{-1})$_{(\pm s.d., n)}$	k_2 (s^{-1})$_{(\pm s.d., n)}$
Methyl/ethyl*	$2.3 \times 10^{-4}{}_{(\pm 0.1,\ n=8)}$	$1.1 \times 10^{-4}{}_{(\pm 0.1,\ n=8)}$
Methyl/n-propyl	$2.2 \times 10^{-4}{}_{(\pm 0.08,\ n=9)}$	$8.0 \times 10^{-5}{}_{(\pm 0.01,\ n=9)}$
Ethyl/n-propyl*	$1.2 \times 10^{-4}{}_{(\pm 0.1,\ n=8)}$	$8.0 \times 10^{-5}{}_{(\pm 0.01,\ n=8)}$

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An investigation into the use of fast heating rates for thermal analysis of pharmaceutical samples

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There has been considerable recent interest in the use of DSC run at fast heating rates (in the region of 100–300°C min^{-1}) (Phil 2002). The aim of the study is to determine the effect of experimental conditions on the melting response of paracetamol, with a view to providing a reference framework for investigators wishing to use fast heating rates. A TA Instruments Q1000 DSC (TA Instruments, Delaware, USA) operating in T4P mode programmed with a standard linear heating rate was used. The instrument was programmed to ramp from 0 to 300°C after an initial isothermal equilibration period of 5 min. The heating rates chosen for the investigation were 10, 50, 100 and 200°C min^{-1}, with the instrument being calibrated for cell constant and temperature at each of these rates using a very small mass of indium (less than 0.1 mg). Nitrogen was used as the purge gas. For the 100 and 200°C min^{-1} heating rates an additional step in the method was used to reprogram the Proportional-band Integral and Derivative parameters of the furnace in the Q1000 instrument, as recommended by the manufacturers when using fast heating rates (Blaine 2003). Samples of paracetamol (Thornton and Ross, B.P. grade) weighing 0.5, 2.5, 7.5 and 15 mg were prepared and sealed into standard TA Instruments aluminium DSC pans before being run in the DSC at the desired heating rate. For systems examined at 200°C min^{-1}, the onset point of melting for 0.5 mg of paracetamol is recorded as 170.7°C (literature value 169°C (DiMartino et al 1996)), whereas the onset of the melting peak for 15 mg of paracetamol is recorded as 172.3°C, a shift of 1.6°C. It is of note that not only had the onset point of melting shifted for the higher sample mass (melting points are first order thermodynamic reactions and therefore should not change with heating rate), but that the peak had also broadened considerably for the 0.5-mg sample. For the 15-mg sample, the peak has lost shape considerably and could no longer be considered reliable. The onset values for the 2.5-mg and 7.5-mg samples run at 200°C min^{-1} were 171.5 and 172.8°C, respectively. For samples examined at 100°C min^{-1}, a shift in observed melting peak onset from 169.6°C for the 0.5-mg sample to 172.3°C for the 15-mg sample is seen. As before the shape of the peak degrades considerably as sample mass is increased, but to a lesser extent than seen at 200°C min^{-1}.

These results suggest that care is required when choosing conditions for high speed DSC runs, particularly in terms of sample mass in relation to scanning speed.

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Stability assessment of pharmaceuticals using isothermal microcalorimetry: the hydrolysis of aspirin

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Isothermal Microcalorimetry (IM) offers the potential to study degradation reactions of pharmaceuticals directly at storage temperatures and it is often the case that 25–50 h of data is sufficient to allow the determination of rate constants and enthalpies (Wilson 1995). Moreover, data analysis using recently developed methodologies allows the determination of the reaction rate constant and enthalpy without the need for the process to go to completion. However, despite these potential advantages, the technique is not widely employed in the pharmaceutical industry, principally because of the perception that IM data are difficult to analyse. In this study we demonstrate the suitability of IM for stability assessment using a model system: the hydrolysis of aspirin. Experiments were conducted using a 2277 Thermal Activity Monitor (TAM) at 25, 40 and 50°C. Aspirin (0.01 M) in HCl solution (0.1 M) was prepared and the power-time data were recorded using Digitam 4.1. Data analysis was performed using the non-linear curve fitting routine in Origin 7. Aspirin degrades in acidic solution following pseudo first-order kinetics. Table 1 shows the average values of rate constants (k) and enthalpies (ΔH) determined by fitting experimental data to a kinetic-based model (Bakri et al 1988) and compare well with those of Angberg & Nyström (1990) (who recorded $9.0 \times 10^{-6}\,s^{-1}$ at 40°C and $22.5 \times 10^{-6}\,s^{-1}$ at 50°C). Extrapolation of the high temperature data using the Arrhenius plot to 25°C resulted in a predicted rate constant of $1.5 \times 10^{-6}\,s^{-1}$, a value which differs considerably from the experimentally measured value of $2.8 \times 10^{-6}\,s^{-1}$. IM is the only analytical technique that measures heat-flow and the model allows the quantification of the reaction enthalpy without the need to allow the reaction to run to completion or for extrapolation. This methodology requires only a limited number of data to be recorded to allow the accurate determination of the rate constant and enthalpy and it was found that as the intensity of the power signal increased the minimum number of data needed to recover the correct reaction parameters reduced. At 25°C it was found that a minimum of 5 h of data (following equilibration) were required; this reduced to 1 h at 40°C and 30 min at 50°C. Given that the first 5 h of data were usually discarded, this means that, even at 25°C, reaction parameters could be determined accurately with just 10 h of data.

Table 1 Average values for the rate constants and reaction enthalpies determined by fitting experimental data to a first order kinetic model

Temperature (°C)	0.1 M HCl k ($\times 10^{-6}\,s^{-1}$)$_{(\pm SD,\ n)}$	ΔH (kJ mol^{-1})$_{(\pm SD,\ n)}$
25	$2.8_{(\pm 0.2,\ n=6)}$	$-23.6_{(\pm 2.4,\ n=6)}$
40	$7.4_{(\pm 0.7,\ n=5)}$	$-34.4_{(\pm 2.9,\ n=5)}$
50	$19.9_{(\pm 3.1,\ n=9)}$	$-30.3_{(\pm 3.9,\ n=9)}$

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Methods to determine the amorphous content of lactose using isothermal microcalorimetry

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Isothermal microcalorimetry (IM) can be used for quantification of amorphous content in pharmaceutical solids by measuring the heat changes that result when a partially crystalline material is exposed to a specific vapour. Lactose crystallisation comprises a number of exo- and endothermic processes and the microcalorimeter records the balance of these events, it being difficult to assign start- and end-points to the event. This has led to discrepancies in the values of the heat of crystallisation (32 and 50 J g^{-1}) reported in the literature (Briggner et al 1994; Sebhatu et al 1994) and makes comparison of data from different sources difficult. It is then necessary to determine the most reproducible method to integrate the crystallisation response of lactose and to assess the use of IM to determine the amorphous content of various lactose samples. Amorphous lactose was prepared by spray-drying (Chidavaenzi et al 1997). Amorphous samples (1–100% w/w) were prepared by mixing known quantities of sieved ($< 425\,\mu m$) amorphous and crystalline lactose. Calorimetric data were recorded using a 2277 Thermal Activity Monitor (Thermometric Ltd) at 25°C. Each sample (~30 mg) was weighed into a 3-mL glass ampoule. Into both the sample and reference ampoules was placed a mini-hygrostat containing a saturated salt solution of magnesium nitrate, which produces an atmosphere of 53% RH at 25°C. Power-Time data were recorded using Digitam 4.1 and analysed using Origin 7 (Microcal Inc.). Differential scanning calorimetry (DSC) data were recorded on a Perkin-Elmer Pyris 1. Samples (1–2 mg) of pure a-lactose monohydrate, recrystallised 100, 85 and 50% w/w amorphous lactose, were placed in non-hermetic aluminium pans and heated from −30°C to 260°C at a scan rate of 200°C min^{-1}. The most reproducible method was the one that integrated the area under the curve between the end of the first exothermic peak and the final baseline (from $y = 0$) of the crystallisation curve of 100% amorphous lactose. This gave a net heat change of 57.3 J g^{-1} for a 100% amorphous sample. The same integration strategy was used to calculate the heat changes of partially amorphous samples. These data were used to calculate the percent amorphous content by using the heat change for the woolly amorphous sample as a reference. Although this methodology proved to be suitable to quantify amorphous content down to 1% w/w, a negative deviation from ideally was observed. As an attempt to explain this DSC experiments were carried out. An H_f of ~169 J g^{-1} for α-lactose and 197 ± 19 J g^{-1} for β-lactose could be estimated from the DSC trace of pure a-lactose monohydrate and crystallised amorphous lactose, which had crystallised to a mixture of α-lactose and β-lactose. The DSC traces for crystallised 85% and 50% amorphous samples showed a change in the ratio of α- to β-lactose formed. In conclusion, a standard methodology to calculate H_c of lactose by IM could be established. Although a non-linear calibration curve was obtained, IM allowed the quantification of amorphous content of lactose samples down to 1% w/w amorphous content.

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075
The preparation and thermal characterisation of ethyl cellulose (EC) films cast from ethanol and acetone

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The choice of solvent is important in influencing the magnitude of internal stresses and performance properties of film coats due to its effect on intermolecular interactions during solvent evaporation (Rowe 1981). Kent & Rowe (1978) demonstrated that poorly hydrogen bonding solvents such as toluene, chloroform and dichloromethane are suitable solvents for ethyl cellulose (EC). However these solvents have toxicity issues and ethanol and acetone are generally more acceptable. Preliminary results showed that cast films made from acetone and ethanol were inhomogeneous in thickness and in some cases cloudy. Hence, the purpose of this study was to investigate the effect of drying conditions, relative humidity (RH) and the addition of triethyl citrate, TEC (as a plasticizer) in preparing clear homogeneous EC cast films from acetone and ethanol. Five-percent (w/v) EC solutions were prepared by dissolving the required amount of EC powder (supplied by Merck Sharp and Dohme) into acetone/ethanol (pharmaceutical grade) using a magnetic stirrer at 45°C. Films were cast onto glass Petri dishes (9 cm). Different drying conditions were conducted by placing EC films in fume cupboard, room condition and glove box (32 cm × 27 cm × 42 cm; area which permits gas exchange was 10.5 cm in diameter), as well as 45°C in a conventional oven for 24 h. Samples were prepared in desiccators with 0% and 60% RH. Different amount of TEC was added into the EC solutions during mixing to yield 5%, 7%, 9% and 11% TEC solutions. T_{zero} DSC (TA Instruments) was used to determine the plasticizing efficiency of TEC. Approximately 5–8 mg of EC films were crimped into non-hermetic aluminum pans (TA Instruments) and scanned at

$2°C\,min^{-1}$ from 10° to 220°C with a modulation of ±0.212°C over 40 s. The amount of residual solvent in the cast films was determined by weight change in thermogravimetric analysis (TGA) from 30° to 120°C. EC films (prepared from acetone and ethanol) dried in the fume cupboard were clear but wrinkly while films dried under room conditions were smooth, although cloudy regions formed within the films. Films prepared under 0% RH were clear and smooth whereas films prepared under 60% RH shrunk and were cloudy. The addition of TEC improved the clarity of the films significantly. The reversing heat flow signal from the MTDSC studies (T_{zero} DSC) clearly showed the T_g of EC was lowered by the addition of TEC and films prepared from ethanol have lower T_g values than films prepared from acetone. For example, 5% TEC decrease the T_g of EC from 128.24°C to 105.87°C and 96.02°C in films prepared from ethanol and acetone, respectively. TGA data indicated that the amount of residual solvent in films prepared from acetone and ethanol was less than 0.8% and 1.2% correspondingly. These results suggest that the choice of solvent and the preparation conditions significantly influences the physical properties of the end product.

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