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Characterisation of the surface physico-mechanical properties of an active pharmaceutical ingredient by atomic force microscopy

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For inhalable dosage forms, drug actives and/or excipients usually undergo high energy mechanical processing to reduce particle size (e.g. micronisation). Such materials often experience considerable damage to the crystal lattice, resulting in the generation of amorphous regions. The bulk properties of these materials in terms of stability, flowability and others are significantly impacted during the process. This is very likely to be linked to the occurrence of these regions. Recently, Phase-Imaging (an advanced feature of atomic force microscopy (AFM)) has been employed to detect changes in the physico-mechanical properties of crystalline materials that have undergone high-energy processing (Begat et al 2003; Price & Young 2005). Phase-Imaging involves the oscillation of a micro-fabricated AFM imaging probe over a sample. By measuring the phase lag it becomes possible to concurrently measure variations in the physico-mechanical properties of a surface (e.g. viscoelastic response). In this study, selected particles of an active were imaged by AFM before and after mechanical activation. In addition to phase lag data, force-volume measurements of the same areas were conducted. Particles on the AFM stub were mechanically activated using a mechanical press to apply stress to the sample on the AFM stub. The surface morphology of drug particles was investigated using an AFM (Multimode AFM with a Nanoscope 3a controller and extender electronics module (all DI, Cambridge, UK). Topographical data was collected in Tapping mode, at a scan rate of 1.0 Hz using a high aspect ratio AFM probe oscillating 350 kHZ. Height, amplitude and phase data were collected simultaneously. Force volume data were obtained in contact mode, measuring the force between crystalline and activated areas of the drug particle and the AFM tip. AFM topographical studies of crystalline drug particles showed relatively smooth surfaces, with a root mean square roughness (R_{RMS}) of 0.742 nm. Phase imaging of the crystalline drug particles indicated little variation in the physico-mechanical property across the sample surface. However, exposure of the same surface to stress, induced a large effect on the surface characteristics of the crystalline drug particle surface. An increase in surface roughness was observed (R_{RMS} to 18.869 nm), coupled with large variations in the physico-mechanical properties, with the presence of discrete regions across the surface of the sample, as confirmed *via* phase imaging analysis (phase shift > 50 °). Force-volume analysis of the crystalline drug particles pre and post mechanical activation is shown in Table 1. The data suggest that on mechanical activation the surface of the drug particles become significantly ($P < 0.05$) more adhesive than its native crystalline form. This may attribute to the mechanically activated areas contributing to increase the overall adhesive properties of the drug surface, which, in turn, might explain the significant increase in agglomeration of micronised drug particles.

Table 1 Force volume data analysis showing the average adhesion force (nN) of a drug crystal pre and post mechanical activation

	Mean (nN)	StDev	Median (nN)
Crystalline drug	5.76	1.62	5.66
Mechanically activated drug	16.83	2.87	16.54

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A novel combined approach to in-situ analysis of frozen trehalose solution systems

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Sugars and polyols are widely used as a means of stabilizing pharmaceutical preparations during processes such as freeze drying which may otherwise result in extensive drug degradation. In particular, trehalose has been found to be highly effective amongst the disaccharides as a means of preserving and maintaining the activity of complex biomolecules such as proteinaceous drugs during freezing and subsequent water removal. However, the mechanisms associated with the cryopreservation process are still unclear, arguably because of the paucity of techniques that are capable of effectively characterising complex subambient systems. This study describes a combination of calorimetry, dielectric analysis, sub-ambient atomic force microscopy and micro thermal analysis to better understand the glass transition, relaxation behaviour and spatial characteristics of frozen trehalose systems, with a view to developing a novel multifaceted understanding of the structure of the material. Modulated temperature DSC was used to study the freeze concentration process and maximally concentrated T'_g values as follows; four concentration trehalose solutions ranging from 100 mg/ml to 400 mg/ml were studied by cooling them to the onset of lower-temperature transition, $T_1'_g$, then holding at this temperature. The complex heat capacity was measured over a time period up to 20 h, allowing the freeze concentration to be followed in real time via the reversing signal; this represents a novel means of measuring the kinetics of the freeze concentration process. On cooling the sample to –60°C then heating up to 0°C two transitions were observed at -33.1 ± 0.1°C and -29.1 ± 0.1°C. The same annealing conditions result in the same composition for the amorphous phase regardless of the starting concentration of the solution. Dielectric analysis was performed over a frequency range every 10°C from –70°C to 0°C. A clear relaxation peak was observed in 10^6–10^{-2}Hz range from which the relaxation time could be calculated as a function of temperature; this ranged from 0.7 ms to 2.4 ms as the temperature was lowered from –20°C to –70°C. Correlation of these data with the MTDSC findings indicated that the relaxation process was dominated by the unfrozen water component of the system, suggesting this method to be a useful means of characterising the molecular mobility of this component of the system. Atomic force microscopy and micro thermal analysis provide spatially resolved physical measurements such as mechanical properties and T_g measurements on the frozen materials. Subambient AFM images enable the visualisation of the spatial distribution of crystalline and amorphous components. We also describe the combination of thermomechanical measurements with macroscopic dynamic mechanical analysis, thereby allowing mechanical characterisation at a range of scales of scrutiny. Overall, the study has indicated that this unique combination of methodologies allows us to characterise the structure of frozen systems in terms of temperature-dependent behaviour, kinetics of freeze concentration, molecular mobility, mechanical properties and spatial distribution of components. This in turn will allow us to explore for the first time the interrelationship between these parameters.

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Thermoresponsive hydrogel nanoparticles

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Injectable, thermoresponsive materials have potential for use as 'in situ forming' drug delivery systems. A novel class of thermoresponsive hydrogel nanoparticles are investigated here. Previous work has produced such particles using an inverse emulsion photopolymerization process (Missirlis et al 2005), which exhibited temperature-dependent properties (e.g. size, hardness) and in certain conditions of concentration lead to a colloidal gelation (based on the formation of a colloidal glass). Precursors are dissolved in a water solution, whose emulsified viscous droplets are transformed into elastic cross-linked nanoparticles by means of photoactivated curing. A weak point of this process is the poor dimensional control of the emulsion, whose broad distribution in droplet size corresponds to a fairly ill-defined distribution in the produced nanoparticles. The current work describes a further development in the preparative process of these materials, aiming to provide narrow dispersity in size to the nanoparticles by utilising an alternative polymerization method (free-radical polymerization) performed in more stable inverse microemulsion and mini-emulsion systems. As in our former investigations, we employed thermosensitive Pluronic F127 diacrylate (PLUDA, $M_n \approx 13{,}000$, synthesized by double acrylation of Pluronic F127 with acryloyl chloride) in mixture with poly(ethylene glycol) diacrylate (PEGDA, $M_n = 570$). The presence of PEG is necessary for formation of a gel structure in the water phase, since Pluronic diacrylate alone can provide only core-cross-linked micelles. Using a free radical initiator system (ammonium persulphate/N, N, N′, N′-tetramethyl ethylenediamine (APS/TEMED)), we performed the polymerization first in aqueous solutions of PLUDA and PEGDA (9.45% (w/w) and 3.15% (w/w), respectively), to make macroscopic hydrogels, before utilising emulsions. Order of addition of the initiator system components was an unexpectedly important factor. The basicity of TEMED, when added before APS, did not lead to gelation, but to the hydrolysis of acrylate esters which hindered the formation of a cross-linked polymer. However, this was overcome by premixing APS and TEMED, delivering a final pH of 6 (instead of 12). To prepare emulsions for polymerization, we then studied three-component systems comprising a hexane phase, an aqueous phase (both with and without the polymeric polymerization components) and a low HLB emulsifier (Aerosol-OT (AOT) or Span-65), and constructed ternary phase diagrams. To produce a dispersed aqueous phase, we employed high organic/water phase ratios, ranging from 50:50 to 94:6. Surfactant concentrations ranged between 5–20% (w/w). Systems were examined visually, rheologically and by dynamic light scattering to determine those of suitable stability and droplet size for polymerizations. As a result of this, an organic:aqueous phase ratio of 90:10 was selected and AOT chosen as surfactant. Size analysis of polymerized particles, produced within our three component

emulsified systems, showed some size dependence upon surfactant concentration. At 5% (w/w) AOT there was an average particle size of 320 nm, at 10% (w/w) it was 240 nm, and at 20% (w/w) it was 260nm. In conclusion, we have successfully produced smaller particles with narrower size dispersity than in previous work, by utilising this alternative polymerization method. However, further refinements to reduce particle size are required. Future work will address this, with particular focus on temperature-dependence of size and aggregation properties of nanoparticles.

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The influence of PVP incorporation on crystallization rate of amorphous spray-dried lactose powders measured on single particles

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Assessments of the stability of amorphous powders are usually performed with methods that require a sample size of at least several milligrams in weight. In such setup, the crystallization rate for the amorphous powder may not only be governed by nucleation and crystal growth rate but also by the transport of heat and water vapour to and from the powder bulk (Buckton & Darcy 1999). In a recently published study (Mahlin et al 2004), we presented a method for determine the crystallization rate of single amorphous particles using an atomic force microscope (AFM). The particles under study could be kept at well defined relative humidity (RH) and temperature and without influence of neighboring particles as it would have been in a powder bulk. The objective of this study was to further evaluate the possibility to derive rate constants for moisture provoked crystallization of composite particles using the AFM approach reported earlier, as a means to improve our understanding of the role of a stabilizer in a composite. The idea was that a rate constant obtained in this way to a higher degree would reflect the nucleation and crystal growth rate of the amorphous particles than the more traditional "time to crystallization" and crystallization temperature (Berggren & Alderborn 2003). Amorphous particles were prepared by spray drying of water solutions of lactose and PVP. The PVP content of the dried particles was 0, 5 or 25 wt % of either PVP K17 or PVP K90. The particles were confirmed amorphous by x-ray and characterised by AFM and electron spectroscopy for chemical analysis (ESCA), and their response on increased RH was investigated. An enrichment of PVP at the surface of the particles could bee seen from the ESCA measurements. The incorporation of PVP in the particles influenced the way the particles responded to an increase in RH. The crystallization kinetics of single particles was analysed by determining the crystallinity from rugosity of AFM topography images and, subsequently, utilising the JMAK equation. The rate constant for this transformation increased in an exponential manner with increasing RH. Furthermore, above the RH needed for the crystallization to be detected, the exponential increase in the crystallization rate was larger for particles with higher polymer content. This indicates that the stabilising effect, in terms of inhibited nucleation and crystal growth, decreases as the water content in the particles becomes higher.

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Analysis of Fickian and non-Fickian drug release: chlorhexidine ion-pairs diffusion through methacrylate biomaterials

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Matrix devices are probably the most common of the medical devices for controlling the release of drugs. In such a device the drug or bioactive agent is present as a dispersion within the polymers matrix, and they can be constructed by dissolution or melt mixing of the polymer and drug. The drug release properties of matrix devices may be dependent upon the solubility of the drug in the polymer matrix or, in the case of porous matrixes, the solubility in the sink solution within the particle's pore network (Singh et al 1968). It is common practice to add a plasticiser or surfactant, to increase the effectiveness of matrix devices by enhancing the permeability of the drug (Nakagami et al 1991). One method by which drug diffusion may be controlled is by the use of ion-pairing agents as this offers a means to modify the physicochemical properties of the diffusant. Therefore, the purpose of this study was to examine the effect of acidic ion-pairing agents (fatty acids) on the release of chlorhexidine (CHX) from poly(methymethacrylate) (PMMA). Poly(methylmethacrylate) films were prepared by initially dissolving/dispersing a defined mass of CHX in warmed methylmethacrylate monomer (containing benzoyl peroxide 4% w/w, as an initiator), either in the presence or absence of either capric acid, (CA), or oleic acid, (OA). The samples set into a glass mould and free radical polymerisation performed by heating the samples at 60°C for 3 h. To examine drug release, samples (10 mm × 10 mm) were cut from each film and affixed to glass slides using a silicone adhesive. The slides were placed in beakers and immersed in the citric acid release medium (chosen to ensure sink conditions). The beakers were incubated at 37°C and agitated at 30 rpm. Samples were removed from the buffered solution at regular intervals and the mass of CHX determined using reverse phase HPLC (UV detection, amine deactivated C18 column and mobile phase acetonitrile:water 40:60). The release of CHX from the various samples was modelled using a power law relationship (Peppas 1985). The release exponents (derived from the power law model) were significantly affected by the loading of chlorhexidine, the type of fatty acid and the ratio of drug to fatty acid in the PMMA biomaterial (Table 1). In the absence of fatty acids, the solubility of chlorhexidine within the monomer was limited; however, when formulated with fatty acids, CHX was freely soluble in the monomer. Therefore, it may be suggested that increased non-aqueous solubility of CHX was mediated by ion-pairing with the fatty acids under examination. This is reflected by alterations in the FT-IR and NMR spectra of the various materials. Interestingly, the release of chlorhexidine that was devoid of fatty acids was diffusion controlled; the release exponent was ca. 1.0 anomalous release. Conversely, the mechanism of drug release in the presence of the various fatty acids was anomalous for OA (0.8 and 0.5, respectively), and fickian for CA (0.2 and 0.4, respectively). The rate of release, K, increased with the higher molar ratio of the fatty acid.

Table 1 Diffusional release mechanisms from drug release data

Molar ratio of fatty acid	K	n	R^2
CHX No fatty acid	0.1688	0.978	0.985
CA:CHX (0.5:1.0)	0.0061	0.201	0.967
CA:CHX (1.0:1.0)	0.0186	0.390	0.968
OA:CHX (0.5:1.0)	0.0068	0.754	0.979
OA:CHX (1.0:1.0)	0.0347	0.519	0.975

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Production of highly selective binding sites in synthetic polymers via bio-molecular imprinting

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The purpose of this study was to overcome some of the key difficulties known to be associated with preparing molecularly imprinted polymers using proteins. The strategy used was to immobilize the protein in the form of protein coated microcrystals (PCMC) and to use these water-soluble particles as the template. The advantage of using PCMC for this application is that it enables the protein to be immobilized in the dry state in a near native conformation and allows it to be introduced into the same aprotic solvents successfully used for small molecule imprinting of polymers. PCMCs of haemoglobin and myoglobin coated on K_2SO_4, were prepared by coprecipitatation into IPA. The PCMCs were re-suspended with methacrylic acid, 4-vinyl pyridine, cross-linker and AIBN initiator in dry acetonitrile and polymerisation initiated by heating to 60°C for 48 h. Following polymerisation, the polymer was recovered, washed with water, dried overnight in a vacuum oven at 70°C and ground in a steel ball mill for 15 min. Polymer 'imprinted' with pure K_2SO_4 crystals was used as a control. Rebinding was measured by passing a binary protein mixture comprising the target and a control enzyme through polymer particles packed in standard glass Pasteur pipettes. 150 mg of polymer was weighed and added to the columns in each case. Eluted fractions were collected and analysed for protein content. Polymers imprinted for haemoglobin (Hb) and myoglobin (Mb) were found to exhibit highly selective binding with > 98% of the target protein being extracted from the mixture and less than 2% of the control enzyme – either a lipase or protease. In comparison, control polymer imprinted with K_2SO_4 showed no discrimination. Importantly the bound target protein could be easily eluted from the polymer by a change in pH and essentially identical discrimination was obtained in a further rebinding study using the same column. Studies showed that Hb and Mb columns exhibited cross-reactivity indicating protein size was not a major determinant of selectivity. Protein-imprinted polymers can be straightforwardly prepared using PCMC as the templating agent. After removal of water-soluble crystals, the robust polymer can selectively extract the imprinted protein from a mixture. This novel technology is expected to find applications in areas such as protein purification, determination of biomarkers, proteomics and drug-delivery.