Short Talks on Pharmaceutical Materials Science

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Calculation of the fragility index of indometacin using Thermally Stimulated Current Spectroscopy

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The fragility index of glass forming materials is an important empirical parameter that reflects the stability of a structure to thermally induced transformations and describes the rapidity at which properties of liquids change as the glassy state is approached. It has been reported that values for the fragility index are in the range of 20–214, where these extremes represent "strong" and "fragile" behaviour, respectively. Strong liquids show a steady change in viscosity and no significant change in entropy of the system as it undergoes the glass transition. In this work, the ability to calculate the fragility index from the global Thermally Stimulated Depolarisation Current (TSDC) spectrum and Thermally Stimulated Polarisation Current (TSPC) experimental results is tested. Indometacin exists in four monotropic polymorphic forms and an amorphous form. Amorphous indometacin is prepared from the stable crystalline γ-form by heating above the melting point and then quench cooling. TSC experiments were carried out using a TSC/RMA 9000 spectrometer (Setaram, France). The two TSC techniques used here, TSDC and TSPC, vary in the timing of the application of the electrical field to the sample. In TSDC, the sample is first polarised (electrical pre-treatment), then cooled and a "depolarisation" current measured as the sample is heated and dipoles relax back to their original positions. In contrast, in TSDC, the sample is cooled without electrical pre-treatment, but an electric field is applied as the sample is heated, hence generating a "polarisation" current as the dipoles move. Using TSDC, a glass transition was detected at 314.4 K, with the TSPC results show a lowering of 2 degrees in the recorded glass transition temperature (Tg). The obtained spectra presented show an overlapping distribution of relaxation processes, which complicates measurements of activation energy of the glass transition process. In contrast, a TW-TSDC spectrum shows a narrow distribution of relaxation times and deconvolutes the complex TSDC spectrum, hence an activation energy for each of the separated processes can be easily calculated. From the derived activation energies and the measured glass transition temperature, the value for the fragility index may be calulcated using the following equation, where m = the kinetic fragility, τ = relaxation time of the dipoles and T = temperature:

$$m = \lim_{T \to T_g} \frac{d\log(\tau)}{d(T_g/T)}$$

Fragility indexes of 61.5 and 55 were obtained from the TSDC and TSPC results, respectively. The difference in the values obtained by the two techniques is expected and can be connected to the experimental conditions at which the results were recorded. The fragility values agree with the known structural information about indometacin, where dimers, trimers and tetramers exist due to formation of H-bond structures. The values obtained in this study are in the range of published results (51–81) with the lower values being obtained by the TSC and DSC techniques and higher values by DSC and Dielectrics. However, the advantages of the TSC technique is that it requires fewer experimental runs to generate sufficient data to reliably interpret and that it is directly measuring dipolar relaxation.

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De novo design of novel emulsifiers based on a protein scaffold

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Fibronectin type III domains 9 and 10 (FIII 9–10) was used as a protein scaffold for the design of novel emulsifiers as the wild type protein is a poor emulsifier and the structure of the protein has been solved (Leahy et al 1996) allowing the rational design of mutants. Mutagenesis was carried out using PCR based site directed mutagenesis and the mutations were designed to increase the hydrophobic moment and amphilicity of the protein while maintaining the scaffold's conformation. The recombinant proteins were expressed in BLR(DE3)pLysS with the addition of a six histidine tag to aid purification and 8-L cultures purified using a nickel-NTA sepharose column (26 × 125 mm), washing with 54.5 mM imidazole, before eluting with 250 mM imidazole. The protein was diafiltered into 10 mM potassium phosphate pH 7.0 and 50 mM sodium chloride before characterisation. Emulsion droplet size was analysed over time with a 0.1% (w/v) protein solution and peanut oil that had been homogenised at 10 000 rev min^{-1} for 1 min before loading into a Malvern Mastersizer 2000 (Table 1). Stability studies of the protein emulsion were carried out using a variety of protein concentrations and the emulsion index monitored over the course of two weeks at room temperature. Preliminary Mastersizer data show a general trend in all samples of an initial increase in droplet size before a reduction in the size of the emulsion droplet. It is thought this may be due to a conformational change in the protein. Stability studies indicate that mutations that increase the conformational stability of FIII 9–10 also improve the emulsion stability, whereas mutations in non surface seeking β-strands that increase the mean hydrophobic moment and mean hydrophobicity decrease the emulsion stability. The stability data and droplet size data after 30 min are in close correlation with each other in that the proteins that form the most stable emulsions have the smaller droplet size, while the least stable proteins have the highest droplet size.

Table 1 Variation of emulsion droplet size with time

Time (min)	Droplet size (mM)						
	FIII9' 10	FIII9-10 AV2 CC	FIII9-10	FIII9-10 IDLE	FIII9-10 IEI	FIII9-10 AV2 CC 5 mM DTT	FIII9-10 AV2 CC 20 mM DTT
0	124	60	88	53	74	65	56
1	150	74	120	89	146	74	80
2	131	83	111	113	144	75	108
3	118	86	103	113	134	74	123
4	109	85	97	108	129	73	128
5	101	85	95	107	131	73	128
10	73	85	82	101	182	74	111
15	57	88	87	103	216	79	109
20	54	92	90	110	225	85	108
25	56	95	95	120	225	90	109
30	60	96	106	130	230	95	111

Leahy, D. J. et al (1996) *Cell* **84**: 155–164

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Development and validation of a novel photocalorimeter

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The aim of this project is to develop an analytical method that allows a quantitative assessment of the photostability of pharmaceuticals. Early work resulted in the design, construction and early phase development of a novel photocalorimeter (Morris 2004). The instrument allows the direct study of liquid, solid or semi-solid materials and, in combination with appropriate data analysis methodologies, the recovery of kinetic and thermodynamic parameters as the sample is irradiated with a high-intensity light. However, a major problem encountered during the early work was that of achieving a stable, and zero, baseline; this arose because it is challenging to combine the sensitivity of modern isothermal calorimeter (Thermal Activity Monitor, TAM, Thermometric AB) with a large mass of stainless steel light source equipment. Further problems occur when light is introduced to the calorimetric cells, principally because of the difficulties inherent in trying to ensure an equal amount of light in each cell. We have since redesigned the instrument significantly; here we describe these changes. The principal aims of the redesign were two-fold: firstly, to achieve a zero baseline with no light input and, secondly, to achieve a consistent (ideally zero) baseline with the light on. The performance of the instrument could then be validated using a photochemical standard reaction (in this case the degradation of 2-nitrobenzaldehyde, 2-NB). The photocalorimeter is equipped with a 300 W power xenon arc lamp from which light irradiates through a monochromator and a trifurcated cable of optical fibres. Two of the cables lead into the sample and

reference cells of a TAM and the third can be connected to a spectroradiometer. The irreproducible nature of the baseline with the original design implied that the system was subject to air conduction within the chamber of the TAM. Various design elements of the column were revised. Specially-made plastic supporting lids (with o-rings) were attached on both ends of the column. This allowed air-tight interfaces between the cable and the ampoule and between the apparatus and the TAM, consequently minimising heat losses. This led to a dramatic improvement, reducing the signal by 90% with an average offset of $0.5 \pm 0.3\,\mu$W (cf. an offset of $67.9 \pm 5.1\,\mu$W with the original design). The next stage was to investigate the system's response to light input. This affected the baseline dramatically, deflecting it by $\sim 214.0 \pm 2.6\,\mu$W, suggesting an imbalance in the light delivery via each cable. We have not been able as yet to achieve a zero baseline with the light on, but have implemented further design changes to overcome this; these include the use of a beam-splitter, rather than a trifurcated cable, and the use of liquid-filled light guides rather than fibre-optic cables.

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Impact of protein concentration on the glass transition behaviour of protein-sugar formulations

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Due to the significant increase of protein-based therapeutic applications in recent years the formulation of proteins has become an area of high interest. A common method is the freeze-drying of proteins embedded in amorphous structures formed by sugars. Although these sugar matrices provide some stabilisation of the protein, environmental conditions, such as temperature and humidity, have a strong impact on product performance and storage. For example, an increase in moisture content or temperature may cause the sugar to go through a glass transition or even crystallise, resulting in a loss of thermal stability of the protein (Imamura et al 2000). For this reason, knowledge of physico-chemical properties, such as glass transition and water sorption behaviour, is important. In this study the impact of bovine serum albumin (BSA) on the water sorption and glass transition behaviour of trehalose is studied. Water sorption experiments have been carried out by Dynamic Vapour Sorption (DVS) at 25°C. DVS is a well-established method for the gravimetric determination of vapour sorption isotherms using a Cahn D200 recording ultramicrobalance. The vapour partial pressure around the sample was controlled by mixing saturated and dry carrier gas streams using electronic mass flow controllers. Four different samples were obtained from the same freeze-drying process: trehalose with 30% BSA, with 3% BSA and trehalose without BSA as well as pure BSA. Each sample was initially dried at 0% relative humidity (RH) at the desired temperature. Then, the sample was exposed to a particular RH while monitoring the change in mass. Measurements were carried out both in a step ("isotherm") and ramp mode. All samples containing trehalose showed a significant mass loss between 60 and 70% RH when humidity was increased in steps at 25°C. This decrease in mass is due to a glass transition followed by crystallisation of the trehalose. No mass loss was observed for the pure BSA sample at these humidities, suggesting that the mass loss is due to the collapse of the amorphous sugar matrix. The glass transition at 25°C occurred around 60% RH for the pure trehalose sample. This was shifted to 70% RH for the 30% protein loading. Three-percent protein did not cause any major shift in the glass transition. This was confirmed by ramping experiments. When the humidity is increased continuously the mass will increase until crystallisation occurs. The resulting maximum corresponds to the crystallisation humidity (Burnett & Thielmann 2004). While the sample with 3% loading and the sample without protein showed almost the same crystallisation event at ~55% RH, there was a significant shift for the 30% loading to 72% RH. High contents of BSA (30%) in an amorphous trehalose matrix cause an increase in the humidity at which the glass transition of trehalose occurs at 25°C (anti-plasticization). Small amounts (3%) of BSA do not show any significant impact on the glass transition or water sorption behaviour of trehalose.

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Protein crystal engineering for drug delivery

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Numerous proteins have been identified as potential therapeutic agents and the commercial returns from these agents are growing; global sales of the top ten recombinant protein therapeutics grew by nearly 20% between 2002 and 2003 to around £18 billion (Pavlou & Reichert 2004). However, most therapeutic proteins are highly sensitive to environmental conditions and also degrade or denature rapidly under physiological conditions. One approach to improve protein stability and to control delivery is to engineer the crystal habit of these materials. Here, we have used hen egg white lysozyme, a relatively small protein (around 129 residues, 14 000 Daltons) as a model to investigate additive influences on crystal habit (Oki et al 1999). Protein crystals were grown by sitting-drop vapour diffusion after optimising conditions; lysozyme concentration ranged from 25 to 75 mg mL^{-1}, sodium acetate buffer at pH 3.6, 4.0, 4.4, 4.8, 5.2 and 5.6 was evaluated, sodium chloride concentration was varied and polyethylene glycol (PEG) concentrations screened. Reproducible tetragonal protein crystals resulted using crystallisation conditions of 10% sodium acetate, pH 3.6, 25% PEG and 65% sodium chloride. Single crystal X-ray diffractometry (Rigaku FR-D) characterised the crystals, and visualisation of the crystal packing (Web Lab Viewer Lite) showed the amino acids at the crystal faces and that arginine dominated. Three main types of amino acids were selected to include hydrophobic (methionine, tryptophan), polar (histidine, glutamine) and charged (lysine, arginine, glutamic acid, aspartic acid) examples. These were added to the buffer solution at 0.01–0.2 M. Orthorhombic crystals grew in the presence of L-lysine, L-arginine and L-glutamic acid. Two data sets from the protein crystals (high and low resolution) were collected using the EMBL Synchrotron facility in Hamburg. Examples of the cell dimensions for the untreated and habit modified lysozyme crystals are in Table 1. It can be concluded that while some charged amino acids modified the crystal habit, others such as aspartic acid did not. Thus, while we have shown that including amino acids into the crystallisation media can modify protein habit, the molecular basis for this modification requires further investigation.

Table 1 Cell dimensions of crystalline lysozyme and lysozyme crystals modified by some amino acids

Additive	Type	Shape	Space group	Cell dimensions (Å)		
				a	b	c
None		Tetragonal	$P4_32_12$	78.24	78.24	37.20
L-Lysine	Charged	Orthorhombic	$P2_12_12_1$	30.59	58.00	68.39
L-Arginine	Charged	Orthorhombic	$P2_12_12_1$	31.85	60.60	71.28
L-Aspartic acid	Charged	Tetragonal	$P4_22_12$	78.78	78.78	37.23

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Pavlou, A. K., Reichert, J. M. (2004) *Nat. Biotech.* **22**: 1513–1519

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Relationship between the mechanical and molecular properties of HPMC solutions during the thermally modified sol:gel transition

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Hydroxypropyl methylcellulose (HPMC), a non-ionic cellulose ether, is frequently used as a rate control polymer in extended release hydrophilic matrices. An unusual property of HPMC is its ability to undergo a reversible sol:gel transition at elevated temperatures in aqueous solutions. Modifications in the dynamic viscoelastic functions of HPMC solutions during the phase transition have been reported (Haque et al 1993), although detailed information relating to changes occurring at a molecular level are lacking. As a result

we use Attenuated Total Reflectance–Fourier Transformed Infrared Spectroscopy (ATR-FTIR) to probe molecular changes during the phase transition. This study was undertaken to investigate the relationship between the molecular and mechanical changes occurring in HPMC solutions during the thermally modified sol:gel transition, employing ATR-FTIR and oscillatory rheology experiments, respectively. All spectra of 2% w/w HPMC solutions (Methocel E4M; Colorcon Ltd, Dartford, UK) were collected using a single reflection heated diamond ATR cell (Graseby Specac, UK) coupled to a ThermoNicolet Magna FTIR spectrometer. The dynamic viscoelastic functions (storage modulus G' and loss modulus G'') were determined using a Bohlin C-VOR (Bohlin Instruments Ltd, UK) fitted with acrylic parallel plates (40 mm). A continuous temperature sweep (10–85°C) at a rate of 1°C/min was undertaken. Measurements were carried out at an angular frequency of 0.5 Hz and at 5% strain to ensure the linearity of viscoelasticity. From the ATR-FTIR data, the υ(CO) band that is associated with the C-O stretches of methoxyl, hydroxyl and hydroxypropyl functional groups of HPMC, as well as the glycoside link, showed a marked increase in intensity at approximately 56°C; this marked the onset of gelation. From the rheology data, the crossover of G' and G'' (i.e. tan $\delta = 1$), which has been traditionally used as an indication of the sol:gel transition point, also occurred at 56°C. Before the crossover (G' < G''), the system demonstrated common viscoelastic behaviour of a liquid, whereas following the crossover (G' > G'') a weak but elastic structure was displayed. A large drop in G'' was also observed at approximately 56°C. Upon cooling, both the υ(CO) band intensity and dynamic viscoelastic functions illustrated a deviation from the heating curve; a hysteresis loop was observed during the thermal cycle. This has been attributed to the lifetime of certain polymer interactions such as hydrophobic bonding. A change in the shape of the υ(CO) band was identified as a function of temperature. The methoxyl band (1197 cm^{-1}) component displayed a change in relative intensity during the sol:gel transition with respect to other components of the υ(CO) band, therefore illustrating the prominent role of the hydrophobic group during the phase transition. The ATR-FTIR data was in good agreement with rheological measurements conducted on the same system, molecular and mechanical changes were detected during the sol: gel transition. Changes in the shape of the υ(CO) band indicated hydrophobic polymer chain interactions were involved during the phase transition.

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Single particle surface energy and hardness screening using Atomic Force Microscopy

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There is considerable interest in the potential application of AFM to quantify physical properties of pharmaceutical ingredients. A key advantage of AFM methods is the capability to analyse small amounts of material, allowing application at early stages of formulation development. In the light of this goal, methods for the determination of surface energy and hardness from individual particles are described together with examples of their application to pharmaceutical materials. Surface energy is an intrinsic property of a material used to predict adhesive and cohesive interaction magnitudes. If surface energies are screened at an early stage of active ingredient development it may be possible to avoid pursuing highly adhesive forms. In addition, because surface energies are intrinsic, it is possible to build a library of values that can aid ongoing research. Macroscopic methods to determine surface energy include contact angle measurement and Inverse Gas Chromatography (IGC); however, these require significant amounts of material. Various strategies can be used to extract surface energy values from Atomic Force Microscopy data (Drelich et al 2004). All rely on recording force–distance curves to measure the interaction between the AFM probe and a substrate as a function of distance. A simple approach is to challenge a clean probe to a particle of interest, and calculate the adhesive interaction. Estimation of the contact area and knowledge of the surface energy of the probe material allows the surface energy of the unknown particle to be calculated. Here we demonstrate this approach for crystalline and amorphous lactose, and show consistent differences between these materials. Surface energy values obtained in this way are compared with IGC data. A second example where AFM could serve as a screening tool is the measurement of nanomechanical properties. Knowledge of the hardness and deformation mechanism of ingredients may aid choice of appropriate formulation methods. For example, it is known that materials exhibiting plastic deformation are more suitable for direct compression than those with elastic properties. Here, we investigate this capability to analyse single crystals of alpha-lactose, and spray dried amorphous lactose. As for surface energy measurements, hardness data is obtained using force-distance curves. In this case it is the contact region of the curve that is important. By comparing curves recorded on a particle of interest to those recorded against a hard substrate it is possible to determine the indentation of the probe as a function of applied force. This data can be modelled to generate quantitative values of Young's modulus. In this paper a range of forms of crystalline and amorphous lactose are analysed in this way, and modulus values in the range 2–9 GPa. In addition to quantitative data, the form of force–distance curves provides information about the mechanism by which the probe deforms the surface. For example, in our study, amorphous lactose displayed a dynamic change during the initial contact cycles. This may be indicative of plastic behaviour, with further evidence provided by AFM images recorded after the contacts, which revealed a permanent indentation at the lactose surface.

Drelich, J. et al (2004) *J. Colloid Interface Sci.* **280**: 484–497

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Development of tests to measure the impact and abrasion properties of film-coated tablets

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Failure of tablet coatings during processing and subsequent handling can result in significant financial losses. There is therefore a need to develop tests to assess the potential behaviour of coatings to ensure that they are sufficiently robust to withstand all the forces they will experience in situ. The aim of this project was to develop reliable laboratory scale test methods to measure coating performance with a strong emphasis on assessing mechanical damage. The implications of scale-up, from development batches to full-scale manufacturing capacity, can be unpredictable due to large differences in processing conditions, potentially resulting in inferior quality coatings. The development of new testing methods for coated tablets to better predict the effects of scale-up would lead to increased coating robustness and quality minimising commercial losses. This study has involved the development of novel impact and abrasion testing methods that can be applied to tablet coatings. The impact technique determines the energy required to cause given levels of damage at the face centre and face edge of the tablets. The abrasion technique determines the relative wear rates of the coatings. Tests were carried out on tablet batches with a variety of placebo core formulations (standard, as well as those exhibiting brittle and plastic like properties) and HPMC based film coatings (a conventional coating, A, applied to all cores; and a spray dried coating, B, applied to the standard formulation only) to encompass a range of tablet properties in terms of core hardness and coating quality. Scanning electron microscopy (SEM), compression testing and surface profilometry were used to further characterise the range of tablets. Tables 1 and 2 show typical results from impact and abrasion testing, respectively. The results identify differences between tablet types. The coated brittle cores proved to be the weakest in terms of both impact and abrasion testing, and based on results from Tables 1 and 2, were up to approximately 77% weaker during impact, and wore through after up to 11% less distance (per unit coating thickness). The coated plastic core formulation exhibited significantly better resistance on impact testing, but was slightly inferior during abrasion. The standard core formulation with coating A showed higher impact resistance than coating B, however differences during abrasion were of little significance. Results from compression testing showed that while coating tablets improved their strength, the properties of the core predominate. SEM analysis did not reveal any major differences between the tablet types and in some cases the theoretically better quality coating appeared more porous than the spray dried one. In conclusion, the results indicate that, in coated tablets, the resistance to damage is affected by the core formulation and to some extent the presence of a coating (mainly due to its thickness). The techniques will enable coated tablet performance to be evaluated more thoroughly at an early stage of the development process. This will increase the understanding of the coatings' performance under impact and abrasion conditions and will potentially optimise their performance. The methods developed show potential for future research and development, to generate useful data modelled on real life situations that can be used to predict coated tablet behaviour.

Table 1 Typical impact testing results with 1 mm diameter indenter at tablet face edge – damage threshold energies

Tablet type		Minimum energy required (mJ/μm)	
Placebo core	Coating quality and mean thickness	Coating fracture	Total tablet fracture
Brittle	A, 24.9 μm	0.31	2.39
Plastic	A, 28.1 μm	0.56	6.22
Standard	A, 33.7 μm	1.32	3.71
Standard	B, 26.3 μm	1.25	4.60

Table 2 Typical abrasion testing results (standard placebo cores)

Tablet type		Mean abrasion distance (m/μm)	s.d.
Placebo core	Coating quality and mean thickness		
Brittle	A, 24.9 μm	2.11	0.14
Plastic	A, 28.1 μm	2.36	0.13
Standard	A, 33.7 μm	2.64	0.23
Standard	B, 26.3 μm	2.83	0.21

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The development of powder pocket dynamic mechanical analysis for the detection of amorphous content in salbutamol sulphate: a feasibility study

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Dry powder inhalers are a commonly prescribed dosage form for the treatment of asthma. In essence they consist of an active compound in particulate form (1–5 μm diameter) adhered loosely on the surface of a larger inert particle, usually lactose (25–60 μm diameter). Processing of the pharmaceutical active powder to produce the stated particle size range requires high-energy milling or micronisation. However, this process also breaks down the surface crystalline structure of the particles, introducing an unstable amorphous phase to the powder. This conversion of the crystalline to the amorphous state can be of great pharmaceutical significance. It can influence the bioavailability, stability, formulation and manufacturing of the medicinal product. The milling-induced amorphism must be detected and quantified to prevent stability and formulation consequences. The objective of this study was to evaluate the feasibility of using the novel technique of powder pocket dynamic mechanical analysis (DMA) to detect and quantify amorphous content in pharmaceutical powders. Mixtures of amorphous and crystalline salbutamol sulphate were used as test materials. A wholly amorphous salbutamol sulphate powder system was produced by spray-drying using literature parameters (Chawla et al 1994). Mixing crystalline material with this spray-dried system produced a set of spray-dried, crystalline salbutamol sulphate mixes of known amorphous content. The samples were tested by solution calorimetry to confirm the amorphous content of the mixes and by powder pocket DMA. The sample powders were loaded into the DMA using a metal pocket fabricated from a sheet of stainless steal. This pocket was run in the DMA with a single cantilever bending geometry. Enthalpy of solution varies in a linear manner with amorphous content (Hogan & Buckton 2000), and this rule was observed for the salbutamol sulphate mixes, with an R^2 value of 0.9996 and a limit of detection of 1.1% w/w. The enthalpy of solution of a wholly amorphous spray-dried salbutamol sulphate system was measured as $-54.3\,J\,g^{-1}$ ($n = 3$, s.d. $= 0.5$). The same samples were tested by DMA to investigate the effect on viscoelastic properties with changing amorphous content. Linear correlations were found between the measured tan delta and loss modulus signals; however, the tan delta gave the best regression coefficient of 0.9992. Using the approach developed by Millar & Millar (2000), it was possible to determine a limit of detection of 3.6% w/w for mixtures amorphous salbutamol sulphate in otherwise crystalline powders of salbutamol sulphate. The novel application of powder pocket DMA to analyse small amounts of amorphous content in non-self supporting pharmacuetical powders has been proven to be feasible.

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The development of smart fabrics

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A smart material can be defined as any material that can respond to subtle changes in environmental conditions (e.g., a change in temperature, pH, ionic strength, solvency, light intensity or the influence of an applied electric field). Stimuli-responsive microgels are crosslinked hydrophilic polymeric networks that exhibit various swelling properties, depending on environmental variables such as pH, temperature, ionic strength and electric current (Murray & Snowden 1995). The most widely studied temperature-sensitive microgel is poly N-isopropylacrylamide (poly-NIPAM), which undergoes a conformational transition at 34°C in water, from a swollen state to compact sphere-like structure (Pelton 1995). Another well-studied stimuli-responsive microgel comprises of NIPAM copolymerized with acrylic acid. The presence of carboxylic acid groups results in pH-sensitive behaviour. A high degree of swelling is obtained in basic solutions (pH > pKa) owing to both the osmotic pressure and electrostatic repulsion between the polymer backbones, while the polymer network collapses in acidic solutions (pH < pKa). A number of applications have been found for colloidal microgels in the field of drug delivery (Lowe et al 1999). These include the solvent directed controlled release of a variety of drugs including anti-inflammatory, antibiotics and larger protein based compounds (e.g. insulin). We propose to take colloidal microgels and attach them to the surface of a textile fibre with a view to developing a smart textile that will allow the controlled release of drugs (e.g. in response to the specific environmental conditions of a wound such as an ulcer). A number of strategies exist to facilitate the attachment. These materials should have occlusive properties to maintain a moist environment and to prevent secondary contamination. They should be painlessly and easily removed, and should also contain, for example, an antibacterial drug to protect infection, should permit gas exchange and retain the ability to absorb exudates. Franz cells will be used to quantitatively determine the rate of release of the active drug molecules from the smart dressing. This will allow a kinetic profile for the release of the actives to be undertaken in a variety of solvent conditions. These can be manipulated to mimic a given wound or skin condition. Experiments with poly dimethyl siloxane (PDMS) membrane, which is used as a model for human skin, and an antimicrobial drug methylparaben, were carried out. An experimental flux ($J_{exp} = 88.04\,\mu g\,cm^{-2}\,h^{-1}$) and permeability coefficient ($Kp_{exp} = 0.033\,cm\,h^{-1}$) of methylparaben will be compared with the flux and permeability coefficient obtained from the smart dressing on the membrane.

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The impact of drug substance ageing on the dissolution of an immediate release tablet

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The surface properties of pharmaceutical powders are critical in determining their processing behaviour as they affect their interactions with other phases (e.g. the wetting of a powder by a binder fluid) (Planinsek et al 2000). During the development of a wet granulation process for an AstraZeneca development compound, two batches of an immediate release (IR) tablet failed the proposed dissolution specification. The subsequent investigation found a linear correlation ($r^2 = 0.85$) between the age of the drug substance batch (time delay between micronisation and the manufacture of the drug product batch) and the dissolution result. To investigate this ageing effect, the surface properties of the drug substance batches were investigated using gravimetric vapour sorption (GVS) and inverse gas chromatography (IGC). The GVS studies were undertaken using a Dynamic Vapour Sorption apparatus (DVS; Surface Measurement Systems, UK) at 25°C. Aqueous DVS was used to determine

the moisture uptake characteristics, whereas acetone was used to detect the presence of amorphous material (Mackin et al 2002). The moisture sorption profiles exhibited a high degree of variability, with some batches sorbing more than 1% w/w moisture above 80% relative humidity. This unusually high moisture uptake for a crystalline compound has been correlated with the presence of variable levels of sodium chloride (100–500 ppm) on the surface of the drug substance particles. The acetone sorption profiles for freshly micronised batches exhibit a sharp decrease in weight after the samples sorb 0.2% w/w acetone. This weight loss is due to the re-crystallisation of small amounts of amorphous material (< 5% w/w) generated during micronisation. To further examine the impact of this amorphous material, the dispersive and polar components of the surface energy were determined using pulse IGC (Surface Measurement Systems Ltd, UK) at infinite dilution. Experiments were performed at 30°C and 0% relative humidity using a helium carrier flow rate of $10\,mL\,min^{-1}$. The dispersive surface energy was determined by eluting 3% v/v injections of a homologous series of alkanes (heptane, octane, nonane and decane). The polar component of the surface energy was determined using acetone, ethanol, acetonitrile and ethyl acetate as the elutants. The IGC data for a typical batch of micronised drug substance are shown in Table 1 and indicate that micronisation significantly increases the dispersive surface energy in addition to changing the acidic/basic nature of the highest energy sites. The data also show a significant reduction in the dispersive and polar surface energy over time, which is probably due to the re-crystallisation of the amorphous material on the surface of the particles. The reduction in surface energy occurs over a period of 1–2 years and the rate of change is affected by the presence of sodium chloride on the surface of the particles. To improve the granulation process and reduce the risk of dissolution failures, the granulation processing conditions have been modified and subsequently no further dissolution failures have occurred. Further studies to investigate the relationship between the surface properties of the drug substance and the granulation behaviour of the formulation are ongoing.

Table 1 Change in the dispersive and polar surface energy of micronised drug substance batches

Drug substance age (weeks)	Dispersive surface energy ($mJ\,m^{-2}$)	Ka	Kb
Unmilled	34.7 (0.8)	0.10 (0.00)	0.09 (0.00)
0	87.3 (0.8)	0.18 (0.01)	0.00 (0.00)
11	78.6 (1.7)	0.16 (0.00)	0.01 (0.01)
21	71.2 (1.0)	0.15 (0.00)	0.00 (0.00)
30	68.6 (3.3)	0.15 (0.00)	0.00 (0.00)

Ka and Kb are dimensionless numbers referring to the acidic and basic nature of the surface, respectively.
The values in brackets represent the standard deviation for the two columns

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Use of functional polymers for treatment of pulmonary cystic fibrosis

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Cystic fibrosis (CF) is a hereditary disorder caused by mutations in the Cystic Fibrosis Transmembrane Regulator (CFTR) coding gene. Prognosis and treatment of CF has improved significantly over the past few decades, increasing the life expectancy of the patients (FitzSimmons 1993), although the development of novel therapeutics is required to overcome the present problem of antimicrobial resistance and currently available expensive and time consuming treatments. The issue with current treatment is mainly the mechanisms of delivery. The highly viscous mucus in the lungs of CF patients somewhat compromises the activity of the drugs. The pathogenic microorganisms infecting the CF patients form a safe microenvironment within the inspissated mucus barrier, protecting them from any antibiotics/drugs being administered. As a consequence of this, the microorganisms are becoming resistant to the available antibiotics. To solve this problem, this project aims to develop a drug delivery system that will overcome the mucus barrier, hence aiding the delivery of the accompanying drug. The utilisation of biodegradable polymers for therapeutic uses has been investigated since the early 1970s. To overcome the lack of chemical functionality and limited range of variable physico-chemical properties of polymers, such as PLGA, biodegradable polyesters with backbone functionality have recently been developed. This allows the attachment of chemical moieties or drugs, as well as controlled encapsulation and release of desired molecules (drugs, peptides, proteins). In addition, the physical characteristics (hydrophilicity/hydrophobicity) of the polymer can easily be manipulated by varying the backbone chemistry. We have prepared a family of novel, functionalised polyesters with different backbone chemistry and of various molecular weights via the enzyme-catalysed transesterification of a combination of activated di-acids, glycerol and lactone monomers (Kobayashi 1999). Lipase region-selectivity for primary hydroxyl groups produces linear polyesters with pendant hydroxyl groups that were used for the attachment of drugs or the modification of the polymer properties. From these polymers, submicron sized colloidal particles were prepared via emulsion-solvent evaporation (Song et al 1997). This process was optimised to achieve the best particle formation. The effect of polymer chemistry on the ability to form discrete particles was analysed using Scanning Electron Microscopy. Addition of enzyme or drug to the aqueous or organic phase enabled encapsulation of these species into the particles. Release profiles of both the chosen model enzyme (chymotrypsin) and the drug (ibuprofen) were obtained by adding the particles to a buffered system and taking timely samples. The chymotrypsin released was estimated spectrophotometrically and its proteolytic activity determined using the azocasein proteolytic assay. In addition, the enzyme's hydrolysing activity toward mucin as substrate was measured on the developed mucinolytic assay. The released ibuprofen from the samples was estimated using HPLC. These particles will be further developed as a novel pulmonary drug delivery system designed to incorporate novel active mucinolytic enzymes to aid the delivery of an appropriate drug through the mucus barrier of patients with cystic fibrosis.

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Short Talks on Pharmacognosy

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Studies on the anti-psoriatic activity of gossypol and its derivatives followed by pre-formulation and formulation studies of gossypol into a topical dosage form

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Gossypol, a natural anti-inflammatory compound, has been studied extensively since the discovery of its in vivo male antifertility activity in the late 1960s and has since shown anti-viral, anti-parasitic and anti-tumour activity. Psoriasis is a multifactorial skin condition characterised by benign keratinocyte hyper-proliferation, skin inflammation, defective keratinisation, altered dermal vasculature and insufficient anti-oxidant activity. In this study the in vitro anti-psoriatic activity of gossypol and its derivatives was evaluated using an anti-proliferative assay and an anti-oxidant assay (Dodou et al 2005). In the anti-proliferative study, the sensitivity of an HPV-16 keratinocyte cell line to each compound was determined using an MTT viability assay. The compounds that showed increased inhibition against keratinocyte proliferation were subsequently tested for their anti-oxidant effect against iron/ascorbate dependent lipid peroxidation, using the thiobarbituric acid (TBA) test. Racemic gossypol ($GI_{50} = 5.4 \pm 0.03\,\mu M$) and its enantiomers were the most potent compounds against the proliferation of HPV-16 keratinocytes, followed by the half-Schiff's bases ($GI_{50} = 15–50\,\mu M$), racemic gossypolone ($GI_{50} = 47.3\,\mu M$) and the bis-Schiff's bases ($GI_{50} > 100\,\mu M$). A comparison was made with the data from the MTT assays on HPV-16 keratinocyte cell lines using methotrexate ($GI_{50} = 148\,\mu M$) and dithranol ($GI_{50} = 0.58\,\mu M$). All tested compounds showed similar anti-oxidant activity ($IC_{50} \approx 17\,\mu M$) and were more potent than the positive control propyl gallate ($IC_{70} = 100\,\mu M$). Pre-formulation and formulation studies were then conducted on racemic gossypol, which was the most active compound according to the biological assays. The pre-formulation studies included saturation solubility in hydrophilic and lipophilic vehicles, compatibility with excipients, partition co-efficient over pH range 2–8, and physicochemical stability in solution under extreme light, heat, acidic, basic