Results Total troglitazone-SG formation was significantly ($P < 0.05$) greater with CA/TI-isolated hepatocytes (2.45 ± 0.29 PAR) in comparison with CII-isolated hepatocytes (1.21 ± 0.26 PAR). Moreover, significant differences were evident after 60 minutes (2.34 ± 0.18 and 1.22 ± 0.35 PAR for CA/TI and CII respectively). However, liver digestion enzymes did not significantly affect intracellular GSH levels. For example, after 120 minutes intracellular GSH levels were 10.41 ± 3.06 (CII), 10.77 ± 2.42 (CA/TI) and 13.32 ± 2.14 nmol/mg protein (C/D). There were no significant differences in the overall metabolism of troglitazone measured by substrate depletion. In addition, hepatocyte viability (in the presence and absence of troglitazone) was unaffected by the choice of liver digestion enzyme.

Conclusions Results indicate that the presence of trypsin inhibitor in the preparation of isolated rat hepatocytes may increase the formation of GSH conjugates of potentially toxic reactive intermediates. This could have significant implications for the interpretation of metabolism data derived from hepatocytes in suspension.

Grant, M. H. et al (2000) *Hum. Exp. Toxicol.* **19**: 309–317
Seglen, P. (1972) *Biochem. Biophys. Acta* **264**: 398–410

Material Science

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Quality control of powdered pharmaceutical ingredients: developing a caking test for industry

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Objectives Powdered and granulated particulate materials make up most of the ingredients of pharmaceuticals and are often at risk of undergoing unwanted agglomeration, or *caking*, during transport or storage. This is particularly acute when bulk powders are exposed to extreme swings in temperature and relative humidity, which is now common as drugs are produced and administered in increasingly hostile climates and are stored for longer periods of time prior to use. This study explores the possibility of using a uniaxial unconfined compression test to compare the strength of caked agglomerates exposed to different temperatures and relative humidities. This is part of a longer-term study to construct a protocol to predict the caking tendency of a new bulk material from individual particle properties. The main challenge is to develop techniques that provide repeatable results yet are presented simply enough to be useful to a wide range of industries.

Methods Powdered sucrose, a major pharmaceutical ingredient, was poured into a split die and exposed to high and low relative humidity cycles at room temperature. The typical ranges were 20–30% for the lower value and 70–80% for the higher value. The outer die casing was then removed and the resultant agglomerate was subjected to an unconfined compression test using a plunger fitted to a Zwick compression tester. The force against displacement was logged so that the dynamics of failure as well as the failure load of the sample could be recorded. The experimental matrix included varying the number of cycles, the amount between the maximum and minimum relative humidity, the height and diameters of the samples, the number of cycles and the particle size.

Results Trends showed that the tensile strength of the agglomerates increased with the number of cycles and also with the more extreme swings in relative humidity. This agrees with previous work on alternative methods of measuring the tensile strength of sugar agglomerates formed from humidity cycling (Leaper et al 2003).

Conclusions The results show that at the very least the uniaxial tester is a good comparative tester to examine the caking tendency of powdered materials, with a simple arrangement and operation that are compatible with the requirements of industry. However, further work is required to continue to optimize the height/diameter ratio during tests.

Leaper, M. C. et al (2003) *Proc. Inst. Mech. Eng. Part E* **217**: 41–47

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Dielectric relaxation in freeze-dried disaccharides and the potential significance for moisture buffering of freeze-dried products

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Objectives A variety of excipients (including various disaccharides) are used routinely in lyophilized product formulation, to provide for a moisture-buffering environment and thereby sustain shelf life. Our previous work (Ermolina et al 2007) on lactose, sucrose, trehalose and maltose provided insight into the mechanisms and moisture sensitivity of two principal sub-T_g relaxations. Here we examine the potential significance of the relaxation behaviour for the potential moisture-buffering effects of these disaccharides.

Methods Lactose, trehalose, sucrose and maltose were individually freeze-dried to moisture contents in the region of 1–4%, and measured using a Solartron 1296/1255 dielectric analyser, in the frequency and temperature ranges 0.1 Hz to 1 MHz and −100 to 0°C, respectively.

Results All four disaccharides revealed two sub-T_g processes. The faster of the two relaxation processes was singled out for further analysis by fitting the Cole–Cole function to each spectrum. The temperature dependency of the fit parameters ($\Delta\varepsilon$, τ, ε_∞, and α), and the Fröhlich parameter $B(T)$ (derived from $\Delta\varepsilon$, ε_∞) along with values of activation energy ΔH (from Arrhenius plots of τ against 1000/T) were used to speculate as to the potential moisture-buffering effects of the four disaccharides. As before, the mechanism of the *faster relaxation process* was ascribed to the rotation of the pendant hydroxymethyl group on each sugar ring. The magnitude of $B(T)$ for the fast relaxation process follows the trend: trehalose > sucrose > maltose > lactose. Charge screening of dipole–dipole interactions, associated with a tendency for dipoles to orientate anti-parallel to each another, was used to explain the observed trend in $B(T)$. For trehalose (at one end of the rank order) the extent of charge screening is much less than that for lactose (at the other end of the rank order), which must result from extended degrees of molecular freedom of the hydroxymethyl group in trehalose compared with lactose. The moisture sensitivity of $B(T)$ was also considered, and found to increase to a greater extent for trehalose (following a step change in moisture of 2%) than for maltose or sucrose, and finally with lactose displaying the least sensitivity. These trends correlate with earlier findings that moisture-sensitive drugs (similar in structure to ATP) are more stable when freeze-dried with pure lactose, followed by sucrose and finally trehalose (El Moznine et al 2003).

Conclusions It is proposed that the degree of polarization of these sugars, as reflected in the magnitude of $B(T)$, and the temperature dependency of $B(T)$ are key parameters which reflect the degree of molecular mobility, which in turn underpins the diffusion rate of the trace amounts of water within a freeze-dried product. The diffusion coefficient of water impacts the extent of hydrolysis of a moisture-sensitive additive (e.g. drug) and so dielectric measurements may provide a useful means of scoring the relative stabilizing effects of a variety of excipients which are assumed to act as moisture buffers.

El Moznine, R. et al (2003) *J. Phys. D* **36***:* 330–335
Ermolina, I. et al (2007) *J. Pharm. Pharmacol.* **59**: Suppl A42

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Development of anti-infective intraocular lens coatings for prevention of infectious endophthalmitis following cataract surgery

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Objectives Cataract extraction is now the most commonly performed surgery in adults in most developed nations. Endophthalmitis can be defined as inflammation of the ocular cavities due to infection at the time of cataract surgery. The intraocular lens (IOL) implanted during surgery is acknowledged to constitute a risk factor for infection. In order to prevent bacterial attachment to the IOL and hence infectious endophthalmitis, a novel antibiotic-impregnated coating based on 2-hydroxyethyl methacrylate (HEMA) and methacrylic acid (MAA) was proposed which would produce high antibiotic concentrations, specifically gentamicin, at the IOL surface during and immediately following surgery.

Methods A wide variety of methods can be used to quantify aminoglycoside antibiotics. Although radioenzymatic methods and radioimmunoassays are reliable they are very expensive. A high-performance liquid chromatography (HPLC) method would be ideally suited to gentamicin-release studies. The absence of a chromophore on the gentamicin component molecules means that an indirect method is required for its spectrophotometric assay. Modification of two published methods using 2,4-dinitrofluorobenzene as a derivatizing agent (Isoherranen and Soback 2000, Arcelloni et al 2001) resulted in a suitable assay utilizing UV detection. A series of polymers were formulated, ranging from 100% HEMA films to 60% HEMA/40% MAA, with or without additional crosslinker, 1% ethyleneglycol dimethacrylate. Hydration of the materials was determined by drying samples to constant weight at 40°C followed by swelling over 48 hours and re-weighing the hydrated discs. Gentamicin was incorporated into the hydrogel systems by swelling discs cut from the material in buffered gentamicin solution (pH 7.4). Gentamicin release from the formulations was carried out in Tris buffer (pH 7.4, 37°C) over a period of 5 hours.

Results Discs placed into an aqueous environment of pH > pK_a of MAA (Tris buffer, pH 7.4) resulted in ionization of the carboxylic groups, producing an increasingly hydrophilic polymer network as buffer was drawn in. As the percentage

of the MAA was increased the degree of swelling increased, with maximum swelling occurring with films containing 70% HEMA/30% MAA. The gentamicin loading capacity followed a similar trend. 100% HEMA formulations displayed minimal swelling and consequently a low gentamicin loading and release was achieved.

Conclusions Following development of a quick and reliable assay method, this study has shown that, through copolymerizing MAA into poly(HEMA) networks, gentamicin can be successfully incorporated into and released from these hydrogels. Gentamicin levels achieved were in excess of known bactericidal concentrations of obtained clinical endophthalmitis isolates. Increasing crosslink density within the copolymers had a significant effect on decreasing the rate of diffusion of gentamicin from the hydrogel matrix, thus improving the controlled-release nature of the delivery system.

Arcelloni, C. et al (2001) *J. Chrom. B.* **753**: 151–156
Isoherranen, N., Soback, S. (2000) *Clin. Chem.* **46**: 837–842

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Modification of poly(vinyl chloride) biomaterials to yield surface-immobilized hydrogel layers

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Objectives Poly(vinyl chloride) (PVC) is one of the most common polymeric biomaterials currently in use in healthcare devices such as endotracheal tubes and catheters (Pritchard 2002). As is often the case, bacterial colonization of the PVC surface represents a major issue, contributing to poor patient outcomes, increased hospital stays, and increased costs. A prime example of this is the implication of the endotracheal tube in the development of ventilator-associated pneumonia (Vincent 2003). Adherent bacteria readily colonize such surfaces, surrounding themselves with thick exopolysaccharide matrix to form biofilms, which are difficult to treat. In the biofilm form, bacteria become much more resistant to conventional antibiotic therapies, making treatment of resultant ventilator-associated pneumonia difficult (Adair et al 1999). This work will use a surface modification approach to graft a hydrogel layer on to PVC which can be used for local antibiotic delivery to treat infection, while improving the durability of the hydrogel over current coating methods and retaining the bulk mechanical properties required for device performance.

Methods Thiol-mediated chemical functionalization was used to covalently attach allyl mercaptan to PVC. PVC (3 cm × 3 cm × 200 μm) was immersed in a mixture of dimethyl formamide/water (5:1 v/v) with allyl mercaptan (0.01 mol) and stirred at 60°C for 6 hours. The PVC sample was removed, washed and dried before further use. Further modification was achieved by polymerization of 2-hydroxyethyl methacrylate (HEMA) across the vinyl-functionalized PVC surface, using azoisobutyronitrile (AIBN) as an initiator. The surface of these materials was examined throughout the process using attenuated total reflectance (ATR)-Fourier-transform infrared (FTIR) spectroscopy and Raman spectroscopy. Initial drug-loading and -release studies have been performed.

Results Both ATR-FTIR and Raman spectroscopies confirm the initial modification with allyl mercaptan and the subsequent attachment of the hydrogel layer on to PVC. Raman mapping shows complete surface coverage of PVC with poly(HEMA). Initial study of the drug-delivery potential of the material shows promise, indicating successful drug uptake and release from the hydrogel layer.

Conclusions An efficient method for functionalization of PVC surfaces with a hydrogel layer has been demonstrated. Such modification has been shown to represent a potential method for the creation of a permanent drug capture-release coating for delivery of antibiotics directly to the site of the bacterial biofilm.

Adair, C. G. et al (1999) *Intens. Care Med.* **25**: 1072–1076
Pritchard, G. (2002) *PVC World Markets Prospects* **16**: 109–114
Vincent, J. L. (2003) *Lancet* **361**: 2068–2077

Pharmaceutical Technology

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Development and validation of a near-infrared method to monitor the loss on drying within commercial single-pot processors

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Objectives Development and validation of two non-invasive near-infrared (NIR) chemometric calibrations for rapid monitoring of the online percentage loss on drying (% limit of detection, LOD) of a potent product to allow reduced operator exposure was required. Successful calibration models must target a percentage LOD value of 4.6% w/w, with sufficient accuracy to ensure that the predicted NIR result was within the specification limits of 3.8–5.4% w/w.

Methods An ABB Bomem Fourier-transform NIR spectrometer (ABB Bomem, FTPA2000–260-NetworkIR) was installed with two multiplexed diffuse reflectance probes. These probes were fitted to two separate single-pot processors (SPPs, Ultima 300, Collette, N.V, Wommelgem, Belgium). Development and validation spectra were collected external to the SPPs, due to the difficulties of safe operator access within the SPPs with a sample interface designed to mimic the probe's optical configuration within the body of the SPP. An external relative reflectance standard (99% relative reflectance standard) was used for all spectral background measurements. Samples throughout the drying run from five different development batches were removed directly from the SPP, their spectra collected and the registered reference analysis method performed. These spectra were then distributed between a calibration and internal validation set. Each sample's spectra were collected in triplicate over the wavelength range 10000–4000 cm^{-1} and the sample was stirred between replicate spectra. A resolution of 16 cm^{-1} and 64 co-averaged spectra were selected. Correct operation of spectrometer was verified before use with AIRS software (ABB Bomem, AIRS version 3.1 QA/QC data-acquisition and reporting package) and spectral collection was accomplished with GRAMS/AI software (Thermo Galactic, GRAMS-AI7 data-acquisition and spectroscopic package).

Results Spectra and reference results from the calibration sets were used to develop two separate quantitative calibration models for each SPP within the quantitative PLS/IQ software of GRAMS. This enabled accurate prediction of samples with a percentage LOD content of between 2.5 and 8.0% w/w. Predicted spectra that resulted in a F ratio of more than 4.04 (95% probability of being spectrally dissimilar from the calibration model) due to incorrect sample presentation during spectral collection or reference analysis error were rejected (Haaland and Thomas 1998, Mark 1986). The average error of the reference method was determined as 0.4% w/w and was equivalent to the standard error of calibration from the two NIR calibration models. The standard error of prediction was calculated as 0.4 and 0.3% w/w for the two separate SPPs' internal accuracy prediction and 0.7 and 0.3% w/w for an independent sample lot. Spectra from two validation lots on each SPP were predicted with the calibration models and successfully met acceptance criteria for accuracy, linearity, precision, intermediate precision and robustness.

Conclusions The calibration models were successfully validated with a high degree of confidence that at the target value the result would meet the specification and offer reduced exposure, rapid quantification and equivalent accuracy to the registered method.

Haaland, D. M., Thomas, E. V. (1988) *Anal. Chem.* **60**: 1202–1208
Mark, H. (1986) *Anal. Chem.* **58**: 379–384

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Process optimization of aqueous ethylcellulose (Surelease®) film-coated paracetamol pellets with and without a hypromellose pore-forming agent (Opadry®) assessed using accelerated stability testing

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Objectives Sustained release can be achieved by coating with a water-insoluble polymer (ethylcellulose). However, for poorly soluble active agents insufficient control of dissolution is achieved with ethylcellulose. Typically, stability of the films is assured by curing, although this is not essential. This study investigated the effect of coating spray rate on the stability of aqueous ethylcellulose film-coated paracetamol pellets, applied using a MPMicro fluid-bed drier with a Wurster column. Pellets coated with Surelease® combined with a hypromellose pore-former (Opadry®) were also investigated. Each sample was stored at accelerated stability conditions (40°C/75% relative humidity (RH)) for 28 days to assess the physical stability of the film, which was measured by dissolution rate before and after storage. Some samples were cured at 50°C for 12 hours, and the effect of curing was assessed by dissolution rate.

Methods Samples (25 g) of paracetamol (BN9930166) pellets, formed by extrusion spheronization, were prepared by the method described by Chopra et al (2001). They were fluidized and warmed to 37°C in a MPMicro chamber fitted with a Wurster column. The inlet temperature was kept at 65°C for all coating runs. The column height was adjusted so that the pellets 'bubbled' and were fluidized throughout the coating column. Spray rates of 0.55, 0.70, 0.80 and 1.1 g/minute were used to apply the coating solution to the core pellets. Samples coated with 10% w/w ethylcellulose at spray rates of 0.55 and 1.1 g/minute were cured at 50°C for 2 hours. The purpose of the curing process is to thermally seal the film coat and make it physically stable. Dissolution testing was performed before and after