**Conclusions** Formulation of solid dispersions for the model drugs investigated showed an increase in the dissolution rate, possibly due to the dispersion of the drug within the carrier as suggested by the DSC scans. Furthermore, investigation of the FTIR spectra suggests the presence of hydrogen bonding between the drug and the carrier in the case of paracetamol.

Sekiguchi, K., Obi, N. (1961) *Chem. Pharm. Bull.* **9**: 866–872

### 57
### Topotecan uptake and elution by drug-eluting embolic beads

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**Objectives** The *in vitro* loading and subsequent elution of topotecan (used to treat a variety of cancers) from drug-eluting beads (DEBs) was assessed, while evaluating the effects of selected physical attributes (size and compressibility).

**Methods** The desired concentration of topotecan (Dabur Pharmaceuticals) was obtained by the dissolution of the drug in pure water. The drug solutions were added to vials containing 1.0 mL of 300–500 $\mu$m DEBs (DC Bead™, Biocompatibles UK; for loading, elution and sizing) and 900–1200 $\mu$m DEBs (for compression), from which the packing solution had been removed by pipette to leave a slurry of beads. A high-performance liquid chromatography method (Xu and Trissel 2003) was used to study the rate of drug uptake by the beads during loading, and to monitor the rate of elution into phosphate-buffered saline (PBS) and a KCl-saturated solution of water and ethanol (0.17 and 0.79 M, respectively). Furthermore, sizing and compressibility of the samples was analysed using an Olympus BX50 microscope and an Instron 4411, as described elsewhere (Lewis et al 2006, Taylor et al 2007).

**Results** The DEBs were found to actively take up the topotecan from solution. The loading was found to be reduced with increased variability when the loading was static compared with when it was dynamic (88% loaded within 3 hours (n = 6, SD ± 8.77%) and > 95% loaded at 5 minutes (n = 6, SD ± 0.58), respectively). Maximum loading for 1.0 mL of DEBs was found to be in the region of 41.0–44.0 mg topotecan. The topotecan was recovered from the DEBs during elution into both PBS and a KCl-saturated solution, demonstrating a reversible interaction. The elution was quicker into the KCl solution (n = 6, 30 minutes) than the PBS (n = 6, 7 hours) due to the ionic strengths of the media. The DEBs were found to significantly reduce in size when loaded with topotecan (n = 200; control, 430 ± 57 $\mu$m; 30.0 mg loaded, 340 ± 47 $\mu$m, $P < 0.01$, by Student's $t$ test), while significantly increasing resistance to compression (n = 5; control, 27.1 ± 0.7 kPa; 30.0 mg loaded, 94.8 ± 10.4 kPa, $P < 0.01$). The observed changes were due to the hydrophobic nature of topotecan displacing the water molecules from the DEBs. These physical changes were found to have no detrimental effects on the handling characteristics of the DEBs.

**Conclusions** Topotecan can be loaded and eluted from the DEBs. The uptake was found to be rapid and more consistent when the DEBs were agitated during loading. All of the topotecan could be recovered during elution, although it took longer when eluting into media of lower ionic strengths. Topotecan loading results in a reduction in the average diameter and an increase in the resistance to compression of the DEBs. Further work is required to demonstrate a correlation between the *in vitro* elution profile and the *in vivo* efficacy of the topotecan-loaded DEBs.

Lewis, A. et al (2006) *J. Vascular Inter. Radiol.* **17**: 335–342
Taylor R. et al (2007) *Eur. J. Pharm. Sci.* **30**: 7–14
Xu, Q., Trissel, L. (2003) *Stability-indicating HPLC methods for drug analysis*, 2nd edn. Pharmaceutical Press

### 58
### Small-angle neutron-scattering studies of the microemulsion component of an aqueous nanosuspomicroemulsion

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**Objectives** To use small-angle neutron scattering (SANS) to examine the stability *in situ* of the microemulsion component of an aqueous nanosuspomicroemulsion containing two water-insoluble drugs. This novel formulation offers the possibility of administering two insoluble drugs at the same time.

**Methods** Drug nanoparticles (≈350 nm, photon-correlation spectroscopy) of the poorly water-soluble drugs griseofulvin and nabumetone were prepared by wet-bead milling (Sepassi et al 2007) in the presence of an aqueous solution of either anionic surfactant (sodium dodecyl sulphate (SDS), griseofulvin) or hydrophilic polymer (hydroxypropyl cellulose, nabumetone). Microemulsions (≈10 nm, photon-correlation spectroscopy, depending upon the precise composition) were made by mixing and heating of oil (trioctanoin), non-ionic surfactant (oleyl(10) polyoxyethylene glycol (Brij 97)) and water and, where appropriate, the drug testosterone propionate (TP) (Malcolmson et al 1998). The nanosuspomicroemulsions were prepared by mixing equal volumes of nanoparticle suspension and microemulsion to give final volume fractions of 0.035 nanoparticles (based on drug) and 0.024 microemulsion (based on surfactant). SANS of the microemulsion part of the nanosuspomicroemulsions was measured every 4–5 hours over a 24 hour period. At the end of the 24 hours, the nanosuspomicroemulsions were centrifuged to precipitate the drug nanoparticles and the SANS of the resultant clear supernatant measured to determine whether any changes observed in the microemulsions were permanent.

**Results** By using the requisite amount of $D_2O$ to $H_2O$ it was possible to prepare the water-continuous nanosuspomicroemulsions and nanosuspensions to make the 'large' nanoparticles invisible to neutrons so that it was possible to 'see' only the microemulsion droplets in the white, opaque nanosuspomicroemulsions. For the nabumetone nanoparticles this necessitated the use of 31.3 vol% $D_2O$ and for the griseofulvin nanoparticles 43.3 vol% $D_2O$. For the griseofulvin nanosuspomicroemulsions, although the microemulsion droplets stabilized by the non-ionic surfactant Brij 97 did not seem to significantly change in size over the 24 hour period, they did acquire a charge, presumably arising from transfer of some negatively charged SDS stabilizing the nanoparticles. Transfer of SDS occurred within 4 hours of preparing the nanosuspomicroemulsions, with no further change occurring over the remaining 20 hours and after centrifugation, suggesting that no griseofulvin dissolved in the microemulsions. In the case of the nabumetone nanosuspomicroemulsions, a gradual but significant change in the shape/size of the microemulsion droplets was observed when the microemulsions contained TP, whereas only very small changes occurred when the microemulsion contained no TP. The changes in the microemulsions may be due to an interaction with the hydroxypropyl cellulose stabilizing the nabumetone nanoparticles. Interestingly, neither hydroxypropyl cellulose nor SDS alone could stabilize trioctanoin microemulsions, nor could Brij 97 stabilize either the griseofulvin or nabumetone nanoparticles.

**Conclusions** SANS can be readily used to monitor *in situ* the stability of microemulsions in contact with drug nanoparticles in cloudy nanosuspomicroemulsion formulations.

Malcolmson, C. et al (1998) *J. Pharm. Sci.* **87**: 109–116
Sepassi, S. et al (2007) *J. Pharm. Sci.* **96**: 2655–2666

## Drug metabolism

### 59
### Trypsin inhibitor may increase the formation of GSH conjugates of potentially toxic reactive intermediates in isolated rat hepatocytes

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**Objectives** To investigate the effect of liver digestion enzymes on the formation of potentially toxic reactive intermediates in suspensions of isolated rat hepatocytes. Isolated hepatocytes are recognized as one of the most relevant and practical models in drug metabolism and toxicity studies. Several modifications of the original two-stage collagenase perfusion technique (Seglen 1972) have been reported for the preparation of hepatocytes. However, there is little information on the effects of the liver digestion enzyme on glutathione (L-γ-glutamyl-L-cysteinyl-glycine; GSH) conjugation of potentially reactive intermediates in isolated rat hepatocytes.

**Methods** Hepatocytes were isolated from male Sprague Dawley rats (180–220 g) using collagenase type II (CII) as described previously (Grant et al 2000). Modifications of this technique using collagenase A/trypsin inhibitor (CA/TI) and collagenase/dispase (C/D) were also investigated. Troglitazone, a known hepatotoxin, was incubated (50 $\mu$M for 120 minutes) with isolated rat hepatocytes to evaluate GSH conjugation of potentially toxic reactive intermediates. Incubations were performed with a cell density of $1\times10^6$ viable cells/mL in Krebs/Hepes buffer, pH 7.4, in rotating round-bottomed flasks at 37°C under an atmosphere of 5% $CO_2$/95% $O_2$. Aliquots (0.25 mL) were removed and centrifuged at 14000 RCF for 5 minutes before liquid chromatography-mass spectrometry (LC-MS) analysis (selective ion monitoring) of the troglitazone ($m/z$ 440) and GSH adduct (troglitazone-SG, $m/z$ 745) anions. The formation of troglitazone-SG was confirmed using LC-MS/MS. In the absence of a suitable standard, the formation of troglitazone-SG was evaluated in relation to the internal standard tolbutamide (anion peak area ratio, PAR). Intracellular GSH levels were measured during hepatocyte incubations. In addition, hepatocyte viability (determined by lactate dehydrogenase leakage from damaged hepatocytes) over 120 minutes was assessed in the presence and absence of troglitazone (50 $\mu$M).

**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

### 60
### 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

### 61
### 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 ($\Delta\varepsilon$, $\tau$, $\varepsilon_\infty$, and $\alpha$), and the Fröhlich parameter $B(T)$ (derived from $\Delta\varepsilon$, $\varepsilon_\infty$) along with values of activation energy $\Delta H$ (from Arrhenius plots of $\tau$ 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

### 62
### 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 > p$K_a$ 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