NaOH required to produce full hydrolysis of the majority of the compounds; the time required so as to observe full hydrolysis. As such, the compounds were exposed to the basic conditions and 'assay' run until the complete hydrolysis of the compounds, and the half-life of each compound determined from the plot of absorbance against time.

Results In the development of the assay, we considered the hydrolysis reaction and, in particular, the half-life of the compounds under basic conditions. That is, we concluded that the mimicking of the gem-diol within the active site of ES is best undertaken through the use of HO^- (e.g. through the use of alcoholic NaOH). As such, we considered the time taken for the full hydrolysis of the sulphonate moiety and therefore monitored the production of the phenolic product. From our results, we discovered that the previously reported acetate- and formate-based inhibitors possess a greatly reduced half-life in comparison with the methanesulphonate derivatives (for example, the half-life for the methanesulphonate derivative of 4-hydroxyacetophenone was approximately 77 minutes whereas for the acetate derivative it was approximately 5 minutes; it should be noted that the time for the aminosulphonates was found to be less than 1 minute). As such, this would appear to suggest that the mechanism of action of these two ranges of compounds is similar; that is, hydrolysis of the sulphonate/carboxylate moieties.

Conclusions We have therefore provided a novel non-enzymatic methodology that has been utilized successfully in the study of the mechanism of different ranges of inhibitors of ES.

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Drug Delivery

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An investigation into the use of clathrates in metered-dose inhaler formulations

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Objectives Clathrates are crystalline compounds consisting of a lattice of one type of molecule which hosts a second type of guest molecule within its structure. Guest molecules in clathrates are packed (in channels and cages) in coordination compound frameworks. Clathrates are thermodynamically unstable and tend to dissociate rapidly when removed from the stabilizing medium, due to the presence of large empty cavities at the core of their structure. The guest molecules prevent the collapse of the open framework structure and render the structure more thermodynamically stable. The aim of this study is to investigate and characterize the physico-chemical properties of beclomethasone dipropionate (BDP) crystallized from trichloromonofluoromethane (CFC-11). Although CFC-11 will shortly be phased out of use, the BDP–CFC-11 clathrate is a stable entity and thus easy to use for our initial investigations (Vervaet et al 1999).

Methods BDP is a widely used corticosteroid for the treatment of asthma. It is formulated in metered-dose inhalers (MDIs) in the presence of propellant and other formulation ingredients. Since the solid-state chemistry can significantly alter the therapeutic effect of the formulation, it is crucial to determine the most stable crystalline form in the presence of the propellant. Crystal growth of anhydrous BDP in CFC-11 was examined. The crystal investigated in this study was grown in 0.1 and 0.5% w/w BDP in CFC-11 at ambient room temperature. Under these conditions, BDP crystallizes with a channel structure that allows the insertion of CFC-11 molecules (Kuehl et al 2003). The structure is held together through hydrogen bonding. Anhydrous BDP suspended in CFC-11 resulted in spontaneous crystal growth. The structure of the BDP–CFC-11 clathrate was determined using direct methods such as X-ray photoelectron spectroscopy and X-ray powder diffraction. Atomic force microscopy was used for the determination of surface energy (primarily the dispersive component). In addition, 3M Drug Delivery Systems also supplied an isopropyl alcohol (IPA) clathrate of BDP for investigation.

Results The dispersive surface energy of anhydrous BDP was 47.5 ± 4.9 mJ m^{-2} whereas the dispersive surface energy of the BDP–IPA clathrate was 66.5 ± 6.7 mJ m^{-2}.

Conclusions The higher dispersive surface energy observed for BDP–IPA clathrate is most likely attributable to its non-anhydrous nature, since the IPA associated with the BDP–IPA solvate can promote hydrogen bonding. Thus the surface of BDP will be more 'active' compared with anhydrous BDP. This has implications when considering interparticulate interactions, since a higher surface energy typically results in greater cohesive and adhesive properties. However, the greater dispersive surface energy may not be detrimental to the overall shelf life of the formulation. The surface energy and adhesive interactions of the BDP–CFC-11 clathrate are currently still under investigation and will be reported.

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Physico-mechanical characterization of multipolymeric monolayered films for buccal delivery of propranolol hydrochloride

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Objectives For optimal controlled release and mucoadhesivity of a buccal delivery system, the blending of polymers and drug of opposing solubilities may be required for the formation of monolayered films. The preparation technique of such a system comprising polymers and drug of opposing solubilities presents a challenge since the drug and polymers cannot be dissolved in a single vehicle to form a solution to be cast as a monolayered film. Further, conventional casting onto teflon-coated trays to be cut into films of desired sizes has been shown to suffer from poor drug-content uniformity (Perumal et al 2008). The aim of this study was therefore to prepare and characterize monolayered multipolymeric films comprising a hydrophilic drug (propranolol hydrochloride; PHCl) and polymers of opposing solubilities using a specially designed silicone moulded compartmentalized tray for film casting.

Methods Multipolymeric monolayered films (MMFs) containing PHCl and the hydrophilic polymer chitosan (CHT) and the hydrophobic polymer Eudragit® RS100 (EUD100) with plasticizer (30% w/w) were prepared in various ratios, by a modified emulsification/solvent evaporation method (Perugini et al 2003) and then cast by a new approach using a silicone moulded tray with individual wells. Films were characterized in terms of drug content (UV-1650PC spectrophotometer; Shimadzu), drug release (shaking water bath; Memmert), mucoadhesion and textural analysis (Texture Analyser XT2*i*; Stable Microsystems). Film thickness (electronic digital micrometer), film-surface morphology (Nikon Coolpix 5.1 digital camera and Jeol JSM scanning electron microscope), swelling, erosion and surface pH were also evaluated.

Results MMFs with a hydrophilic drug and polymers of opposing solubilities, i.e. comprising PHCl with EUD100 and CHT (PHCl/EUD100/CHT, 2:20:1), could be successfully prepared. Reproducibility studies demonstrated uniform drug content ($100.71 \pm 2.66\%$), thickness (0.442 ± 0.030 mm), mucoadhesivity (401.40 ± 30.73 mN) and no significant differences between the batches ($P > 0.05$). The films also showed controlled drug-release profiles that were reproducible ($f_2 > 50$). Drug release followed Higuchi's square-root model ($r^2 = 0.9426$). Maximum swelling of the films occurred after 1 hour and 28.26% of the films eroded during the 8 hour test period. Mechanical testing in terms of tensile strength, elongation, elasticity and toughness revealed that the MMFs displayed a greater abrasion resistance, were more elastic and also required more energy to break, rendering them tougher and more suitable for buccal delivery when compared with the monopolymeric PHCl/EUD100 film. The more porous surface morphology of the MMF was attributed to the inclusion of CHT into the PHCl/EUD100 film. The surface pH of the films remained constant at neutral pH throughout the test period.

Conclusions Monolayered multipolymeric films with drug and polymer of opposing solubilities, i.e. PHCl/EUD100/CHT (2:20:1), could be reproducibly prepared. The characterization data obtained in this study confirmed the potential of this MMF system as a promising candidate for the controlled buccal delivery of PHCl.

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Co-delivery of 5-aminolevulinic acid and the novel hydroxypyridinone iron chelator CP-94 from bioadhesive patches for enhanced topical photodynamic therapy

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Objectives In photodynamic therapy (PDT) a combination of a photosensitizing drug and visible light causes selective destruction of neoplastic cells. Administration

of excess exogenous 5-aminolevulinic acid (ALA) yields accumulation of the potent photosensitizer protoporphyrin IX (PpIX) in neoplastic cells. We have previously described (McCarron et al 2004) a bioadhesive patch system containing ALA that allows prolonged delivery to otherwise inaccessible areas of the body, such as the vulva. ALA-induced PpIX accumulation can be enhanced in neoplastic lesions by co-delivery of an iron chelator that, in binding intracellular iron, prevents conversion of PpIX to haem. In so doing, the potency of the photodynamic effect upon irradiation can be enhanced. In this study, the aim was to formulate a bioadhesive patch-based system capable of co-delivery of ALA and the novel iron chelator 1,2-diethyl-3-hydroxypyridin-4-one hydrochloride (CP-94).

Methods Bioadhesive patches were prepared using a 20% w/w poly(methyl vinyl ether/maleic acid)/10% w/w tripropylene glycol monomethyl ether gel. ALA loadings of 19, 38 and 50 mg cm^{-2} were used. For each ALA loading, three CP-94 loadings were used; 1, 5 and 10 mg cm^{-2}. Modified Franz diffusion cells and a model membrane (surface area 0.7 cm^2) used to mimic the disordered stratum corneum overlying neoplastic skin lesions were used to study the release of ALA and CP-94 from the formulated systems. The receiver compartment (10 mL) contained 0.1 M borate buffer pH 5, to maintain ALA stability throughout the 6 hour experiments. ALA released from patches was quantified using high-performance liquid chromatography, employing pre-column derivatization with acetyl acetone and formaldehyde and fluorescence detection. Detection was by fluorescence with excitation at 370 nm and emission at 460 nm (Donnelly et al 2006). Determination of release was by UV spectroscopy at 280 nm using a multiwell plate-reading spectrophotometer.

Results ALA and CP-94 release were found to be mutually independent. Patches containing 19, 38 and 50 mg cm^{-2} ALA yielded ALA receiver compartment concentrations of approximately 1906, 3832 and 5043 $\mu g\ mL^{-1}$, respectively, after 6 hours. Patches containing 1, 5 and 10 mg cm^{-2} CP-94 yielded CP-94 receiver compartment concentrations of approximately 39, 47 and 55 $\mu g\ mL^{-1}$, respectively, after 6 hours.

Conclusions In this study, we have shown that a bioadhesive patch containing ALA and CP-94 is capable of releasing both drugs across a model membrane. Neither drug interfered with release of the other. Importantly, the ALA concentrations achievable after 6 hours' release were significantly greater than those previously shown to produce greater than 90% kill of tumour cells upon irradiation with red light (635 nm) *in vitro* (McCarron et al 2004). Work is currently ongoing to determine the effect of CP-94 on ALA-induced PpIX accumulation in tumour cells *in vitro* with a view to conducting *in vivo* studies on subcutaneously implanted animal tumours using the novel ALA- and CP-94-containing patch described here.

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The effect of temperature on the diffusion of lidocaine base

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Objectives The inherent barrier properties of the skin present a major problem for the transdermal delivery of drugs. Due to the selectively permeable nature of the skin compounds need to be relatively lipophilic with a molecular weight below 500 Da for passive permeation (Finnin et al 1999). Temporary application of heat is a non-invasive method that has been shown to increase systemic drug delivery (Ashburn et al 2003). A rise in skin temperature causes an increase in drug diffusivity throughout the vehicle and temporarily alters the barrier properties of the skin, resulting in enhanced diffusion (Akomeah et al 2004). However, little work has been conducted on the effect of membrane temperature on diffusion of a compound with an inverse relationship between solubility and temperature, for example lidocaine. Therefore, the aim of this study was to show that an increased membrane temperature can enhance the diffusion of lidocaine.

Methods The diffusion of lidocaine base was measured using large unjacketed upright Franz-type diffusion cells (MedPharm, UK) with a diffusion area of 2.0 cm^2 and a volume of 10 mL. Regenerated cellulose membrane pre-soaked in deionized water for 1 hour at 70°C and then rinsed in deionized water was used as the barrier. Phosphate-buffered saline (PBS; 0.15 M, pH 7.3) was employed as the receiver fluid. The donor system was a saturated solution of lidocaine base in PBS. The Franz cells were placed in a thermostatically controlled water bath. Samples of receiver fluid were removed at predetermined time points throughout a 4 hour period and were analysed using a previously developed high-performance liquid chromatography method which was shown to be fit for purpose in terms of precision (coefficient of variation <2%) and accuracy (100.2 ± 0.8%), with a limit of detection of 0.55 μg/mL. The diffusion studies were performed at a range of membrane temperatures between 27 and 45°C.

Results A linear relationship ($R^2 = 0.99$) between the mean increase in temperature and rate of diffusion of lidocaine from a saturated donor solution was observed. The diffusion rate was found to increase by 10.3 $\mu g/cm^2$ per hour for every 1°C rise in temperature. The flux of lidocaine through the membrane increased from 349.1 ± 25.7 $\mu g/cm^2$ per hour at a membrane temperature of 27°C to 534.6 ± 36.9 $\mu g/cm^2$ per hour when the membrane temperature was 45°C. The increase in membrane temperature from 27 to 32°C showed no significant increase in diffusion rate ($P \geq 0.05$, analysis of variance). However, membrane temperatures of 32, 37 and 45°C all had significantly different rates of diffusion ($P \leq 0.05$).

Conclusions An increase in membrane temperature can increase the rate of diffusion irrespective of the exothermic nature of lidocaine dissolution. Applying temporary heat would maximize the efficiency of delivery of lidocaine, therefore providing a non-invasive solution to combat breakthrough pain.

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Aerosolization of protein-loaded poly(D,L-lactide) microsphere freeze-dried powders by nebulization and dry-powder inhalation

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Objectives The aim of this work was to investigate the use of ultrasonic (eFlow, Pari) and air-jet (LC, Pari) nebulizers to enhance the delivery of freeze-dried poly(D,L-lactide) (PLA) microspheres in comparison with dry-powder inhalation.

Methods Bovine serum albumin (BSA)-loaded PLA (50000 Da) microspheres, prepared by the double-emulsion solvent-evaporation technique, were suspended in a 6% w/v trehalose and 1% w/v L-leucine aqueous solution and freeze-dried. Finally the particle size of the freeze-dried powder was reduced by grinding using a pestle and mortar for 10 minutes. Aliquots (150 mg) of this powder were then resuspended in double-distilled water and nebulized, using fill volumes of 4 mL (Pari eFlow and Pari LC) or 8 mL (Pari LC only). Other aliquots (150 mg) of the powder were also filled into eight gelatin capsules (size 2) for dry-powder inhalation using a Rotahaler. The particle size and size distribution in both wet and dry mode were measured by laser diffraction (HELOS particle, Sympatec). The aerodynamic size distribution of the powder was determined using a Multi-Stage Liquid Impinger (MSLI, Westech) at an air-flow rate of 60 L/min. The cut-off diameters of stages 1–4 are 13, 6.8, 3.1 and 1.7 μm respectively. A piece of filter paper was placed into stage 5 to capture the remaining fraction of particles less than 1.7 μm. The mass of BSA deposited on each stage was determined by bicinchoninic acid (BCA) assay.

Results The mean diameter of freeze-dried microsphere-loaded powder after manual grinding was 5.10 ± 0.54 μm. Resuspension of the powder in double-distilled water indicated that the PLA microspheres within the freeze-dried powder had a mean size of 1.13 ± 0.01 μm; this was not significantly different from the freshly prepared microspheres prior to freeze-drying ($P > 0.05$). The larger particle size of powders indicates aggregation of the trehalose and L-leucine with the microspheres; trehalose and L-leucine dissolved after re-suspension to release the entrapped microspheres. As a direct result of their increased size, the freeze-dried PLA microsphere powders showed an extremely poor aerodynamic distribution following administration by dry-powder inhaler, with nearly 40% of the aerosolized dose depositing in the MSLI throat and no material detected further than Stage 2. These powders would be predicted to exhibit high oropharyngeal deposition following inhalation, with a fine-particle fraction (FPF; the fraction being < 5 μm) of only 1.1%. Delivery of these microspheres to MSLI stages corresponding to the central and peripheral regions of the lung was significantly increased by using either the Pari eFlow or the Pari LC (FPF 33.8 and 23.4%, respectively).

Conclusions Nebulization of the rehydrated powder using the Pari eFlow nebulizer resulted in statistically improved aerosolization performance compared with the Pari LC nebulizer. Inclusion of 6% w/v trehalose and 1% w/v leucine when freeze-drying protein-loaded PLA microspheres results in maintenance of protein loading and microsphere size (Song et al 2007); however, the freeze-dried powder exhibits poor aerodynamic properties due to a relatively large particle size and interparticulate cohesion.

Song, X. et al (2007) *Proc. Drug Deliv. Lungs* **18**: 165–168

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Influence of chemical permeation enhancers on topical delivery of aminolevulinic acid from a novel bioadhesive patch

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Objectives Photodynamic therapy based upon topical administration of aminolevulinic acid (ALA) is a novel treatment for premalignant and malignant skin lesions. Application of exogenous ALA stimulates the over-production of protoporphyrin IX (PpIX), an effective endogenous photosensitizer. However, ALA is a small hydrophilic (167.6 Da) molecule and a zwitterion at physiological pH. Consequently, permeation across intact stratum corneum is poor (Casas et al 2000). One of the most common approaches to modifying the horny layer is to employ formulation-based chemical permeation enhancers (PEs). The aim of this study was to incorporate several PEs at various levels of loading into a bioadhesive patch. ALA penetration into porcine tissue *in vitro* from bioadhesive patches containing enhancers was examined. In addition, fluorescence spectrometry was used to determine ALA-induced PpIX fluorescence in the murine model *in vivo*.

Methods Bioadhesive patches were cast from aqueous blends containing 20% w/w poly(methyl vinyl ether/maleic anhydride) and 10% w/w tripropylene glycol methyl ether. Patches were tailored to deliver 19 mg cm^{-2} ALA and contained 2, 5 or 10% w/w of the enhancers dimethylsulphoxide, Labrafac® CC, Labrafac® PG and Labrafil® M1944CS. *In vitro* tissue penetration was quantified by spiking formulations with ^{14}C-radiolabelled drug, and applying to excised neonatal pig skin. The concentration of drug per unit area of tissue was determined by liquid scintillation spectroscopy (Tri-Carb® 2300 liquid scintillation analyser). *In vivo* studies were performed by placing formulations on the dorsal skin of anaesthetized female nude mice for defined time periods (1 and 4 hours), and measuring subsequent PpIX-induced fluorescence using a fibre-optic probe coupled to a Perkin-Elmer LS50B luminescence spectrometer (excitation 407 nm, emission 635 nm).

Results *In vitro* penetration studies showed that formulations containing chemical PE increased the percentage of drug delivered into porcine skin compared with control. However, statistical analysis revealed that these increases were not significant (P values > 0.51). The accumulation kinetics of PpIX followed a similar profile for all patch formulations. Following removal of the vehicle, PpIX fluorescence at the application site peaked at 6–7 hours and reduced to baseline levels at 24 hours. The only exceptions were the standard patch (no PE) and Labrafac CC 10% w/w, which exhibited peak fluorescence at 8 hours. The inclusion of permeation enhancers generally did not significantly enhance PpIX production at the site of application compared with control. The only exception was patches containing 10% w/w Labrafil M1944CS®, which were associated with significantly higher peak fluorescence values than standard patches ($P < 0.05$).

Conclusions For the first time, the influence of PE loading on the delivery of ALA from a bioadhesive patch has been assessed. Chemical enhancers were not shown to significantly increase ALA delivery into porcine skin *in vitro*. In addition, ALA-induced PpIX production *in vivo* was generally not significantly enhanced using PE. In conclusion, these animal studies suggest that there is no value in incorporating PEs into the bioadhesive patch system.

Casas, A. et al (2000) *Br. J. Dermatol.* **143**: 564–572

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Formulation of novel vaccine adjuvants containing cationic lipids and synthetic cord factor

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Objectives To study the efficacy of liposomes as adjuvants in vaccine delivery formed from various combinations of cationic lipids coupled with trehalose 6,6′-dibehenate (TDB). TDB is known to be an important immunostimulant based on previous findings (Vangala et al 2007). Cationic lipids have also been shown to act as immunostimulants in addition to their intrinsic property of self-forming bilayered vesicles when placed in an aqueous environment, thereby providing a suitable vector for drug delivery.

Methods The lipid-film method (Davidsen et al 2005) was used to form liposomes composed of a cationic lipid (dimethyl ammonium bromide (DDA), (*N*-(*N*′,*N*′-dimethylaminoethane)-carbonyl) cholesterol (DC-Chol) or *N*-(1-(2,3-dioleoyloxy)propyl)-*N*,*N*,*N*-trimethyl ammonium chloride (DOTAP)) and TDB at a molar ratio of 8:1. Each formulation was rehydrated using phosphate-buffered saline (PBS; 10 nM) or Tris buffer (10 mM, pH 7.4). The average diameter and zeta potential of the liposomes was studied as an indication of stability over a period of 28 days at both 4 and 25°C.

Results Results show that liposomes rehydrated with PBS produce larger vesicles than equivalent formulations rehydrated with Tris buffer (between 5- and 10-fold larger). The liposomes formed in PBS also displayed a higher zeta potential than their Tris counterparts (generally ≈10 mV higher). Comparison between liposome formulations revealed that those composed of DC-Chol/TDB were the smallest in size (≈250 nm when suspended in Tris) and had the lowest zeta potential (≈20 mV) of those tested. Liposomes composed of DDA/TDB were the largest with the corresponding largest zeta potential (≈600 nm; ≈33 mV). All formulations tested showed no significant change in size or zeta potential over the 28 day period when stored at either 4 or 25°C.

Conclusions Our results show that the buffer employed to re-suspend cationic liposomes for use as vaccine adjuvants makes a significant difference to the liposomal characteristics. The significant differences in vesicle size resulting from different buffers used could result in differences in both physico-chemical and biological activity and therefore due consideration must be given to this in the early stages of developing such formulations. Interestingly, whereas liposomes suspended in Tris buffer showed small vesicle size and potentially longer-term stability as a result, there are concerns regarding potential toxicity when Tris is employed. We are currently investigating the effect of these differences in physico-chemical attributes using cell-culture models to evaluate the various formulations for their cellular toxicity, ability to stimulate macrophages and phagocytic uptake when containing a model antigen. *In vivo* bio-distribution studies will also be reported.

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Physico-chemical characterization of solid dispersions of phenylbutazone and paracetamol with polyethylene glycol 8000

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Objectives The enhancement of bioavailability of poorly water-soluble drugs remains one of the most challenging aspects of drug development. Solid dispersions have been shown to improve the solubility, dissolution and bioavailability of poorly water-soluble drugs (Sekiguchi and Obi 1961). The aim of the present study was to evaluate the physico-chemical properties of solid dispersions of phenylbutazone and paracetamol formulated in polyethylene glycol (PEG) 8000.

Methods Solid dispersions were prepared by the melt fusion method. Characterization was carried out using differential-scanning calorimetry (hyper DSC), Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). Dissolution studies were carried out using a US Pharmacopoeia (USP) method. All the tests were carried out in triplicate.

Results The drugs (phenylbutazone and paracetamol) within the solid dispersions showed an enhanced dissolution rate when compared with their physical mix or drug alone (Table 1). The lack of a melting peak in the DSC scans of a solid dispersion (phenylbutazone and paracetamol) indicated that the drug was present in an amorphous rather than a crystalline form and soluble within the carrier (Table 1). Additionally, SEM analysis of the formulation clearly showed that the drug was dispersed within the carrier. Comparison of the FTIR spectra of the solid dispersion of phenylbutazone and PEG 8000 and the physical mixture showed no differences in the position of the absorption bands, suggesting the absence of any chemical interaction between the two components. However, the absorption bands, which could be assigned to the free hydroxyl group, involved in intermolecular hydrogen bonding disappeared in case of the paracetamol solid dispersion, suggesting the formation of hydrogen bonds between the drug and the carrier.

Table 1 Results from dissolution and DSC studies

Formulation	% Released in 60 min	Melting point (°C)
PEG 8000 only	–	59.1
Phenylbutazone only	5.6 ± 0.2	107.7
Physical mix: phenylbutazone/PEG 8000	20.6 ± 1.2	–
Phenylbutazone/PEG 8000 solid dispersion	39.6 ± 2.3	60.5
Paracetamol only	34.7 ± 2.03	166.7
Physical mix: paracetamol/PEG 8000	38.2 ± 5.0	–
Paracetamol/PEG 8000 solid dispersion	78.9 ± 1.5	59.9

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

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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 μm DEBs (DC Bead™, Biocompatibles UK; for loading, elution and sizing) and 900–1200 μm 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 μm; 30.0 mg loaded, 340 ± 47 μm, $P < 0.01$, 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.

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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

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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 μM for 120 minutes) with isolated rat hepatocytes to evaluate GSH conjugation of potentially toxic reactive intermediates. Incubations were performed with a cell density of 1×10^6 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 μM).