SESSION 3

Drug Delivery

167
Optimisation and characterisation of biocompatible, microparticulate, modified-release formulations, for the pulmonary delivery of immunoglobulins

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Poly(lactide-co-glycolide) (PLGA) microspheres, encapsulating a model antibody for pulmonary delivery, have been produced by the spray-drying of double-emulsion formulations. A biocompatible, microparticulate system has been developed using the GRAS excipients dipalmitoylphophatidylcholine (DPPC) and lactose, for emulsion stability and thermal protection, respectively, as well as the biodegradable PLGA, to afford the antibody the required stability, modified-release and aerodynamics for inhalation delivery. In this work, the proportions of each excipient in the formulation have been optimised by factorial experimental design, and an optimal formulation further characterised in terms of release-profile and antibody stability. A 2^4 (+ 3 centre points) factorial experiment was used to investigate the effects of varying the amounts of 7 kDa PLGA (50–350 mg), IgG (formulated 1:1 with sorbitol) (10–50 mg), DPPC (10–40 mg) and lactose (100–500 mg). All batches were produced using identical, previously optimised, emulsification and spray-drying conditions. The measured responses were yield, encapsulation efficiency (EE) and burst-release, determined by Bradford assay, median volume diameter in both cyclohexane and water, determined by laser diffractometry, and morphology categorised on a quantitative scale, from assessment of scanning electron micrographs (SEMs). Response data were analysed by a factorial ANOVA to find main effects and interactions, significant at 95% confidence intervals. The optimal batch was produced in triplicate, and a full release-profile was measured in pH 7.4 and pH 2.5 buffers (Jiang et al 2002). Integrity of the antibody in burst-release samples was measured by capture ELISA, non-reducing SDS-PAGE and field flow fractionation (FFF), with refractive-index and light scattering detectors. Yield increased with PLGA and lactose content, whereas increasing DPPC to 40 mg reduced the yield by approximately 10%. However, large amounts of lactose reduced the EE from 85 to 75%, although this was dependent on the amount of antibody. Increasing the amount of antibody significantly increased the burst-release from less than 15% to almost 50%. Optimal batches were found to have a median geometric volume diameter of 3–5 μm in cyclohexane, and of approximately 500 nm in water, presumably owing to PLGA nanospheres that freely disperse as the lactose shell of the microspheres dissolve. It is expected this mechanism will allow microspheres of inhalable diameter to part-dissolve in pulmonary fluid into nanospheres small enough to avoid phogocytosis. SEMs identified that only optimal excipient combinations produced spherical, non-aggregated microspheres. Table 1 summarises the optimal choice of each excipient amount to produce the desired particle characteristics. The optimised formulations gave different release profiles in the two media. At pH 7.4 very little antibody was released after 7 days, whereas in agreement with Jiang et al (2002), a late phase of release continued in pH 2.5. Analysis of burst-release samples identified the antibody as having full conformational activity (ELISA), and to consist of one molecular weight band of approximately 150 kDa (SDS-PAGE). FFF data agreed with this, and appeared to indicate less dimerisation in the formulated antibody relative to controls. The findings suggest that the optimised formulation meets the aims of providing antibody stability, modified-release and inhalable particle size.

Table 1 Selecting optimal excipient amounts from response data

	PLGA (mg)	Lactose (mg)	DPPC (mg)	IgG (mg)
Yield (max)	200/350	300/500	10 or 25	—
EE (max)	—	100/300	—	10/30
Burst-release (min)	350	—	—	10
Dry D50% (2–5μm)	50/200	300/500	25/40	—
Wet D50% (min)	200/350	—	25/40	30
SEM (max)	50/200	300/500	25/40	—
Optimal	200	300	25	20

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168
Formulation and delivery of p-EGFP DNA condensed by a synthetic lipospermine

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Cationic lipids are non-viral DNA-delivery vectors. These vectors have the ability to condense DNA into particles that can be readily endocytosed in tissue-cultured cell lines. Ultimately, the DNA pay-load is delivered to the nucleus. The aim of this study is DNA formulation with a lipospermine non-viral vector capable of efficiently delivering the desired plasmid to the target nuclei in a variety of cell lines (e.g. primary skin and cancer cells) (Ahmed et al 2005, 2006). We have synthesized and characterized a lipopolyamine in which the tetra-amine spermine, the cationic moiety, and two oleoyl chains as the lipophilic moiety are linked by amide bonds at the secondary amino groups of spermine to form N^4,N^9-dioleoyl spermine. Among the prerequisites for delivery of DNA across intact cytoplasmic membranes are masking the negative charge of the phosphate backbone leading to DNA condensation, we have therefore studied the ability of N^4,N^9-dioleoyl spermine to condense linear calf thymus DNA and circular plasmid DNA (β-galactosidase) using an ethidium bromide (EthBr, $\lambda_{ex} = 260$ nm, $\lambda_{em} = 600$ nm) fluorescence-quenching assay (Geall & Blagbrough 2000) and compared the results with those obtained using poly-L-lysine (PLL, average molecular weights 9.6 and 27 kDa) and polyethylenimine (PEI, average molecular weights 2 and 60 kDa) as model DNA condensing agents. Our results show that N^4,N^9-dioleoyl spermine is able to condense DNA with > 90% fluorescence quenching at ammonium/phosphate N/P charge ratio 2 more efficiently than PLL9.6 and the commercially available formulations Lipofectin and Lipofectamine. The DNA binding constants of these lipopolyamines were calculated, and particles of condensed DNA were detected using a UV light scattering assay at $\lambda = 320$ nm. N^4,N^9-Dioleoyl spermine is able to condense DNA, shown by the significant decrease in (intercalated) EthBr fluorescence intensity and the related increase in apparent absorption in the light scattering experiments. The transfection efficiency and toxicity of non-liposomal N^4,N^9-dioleoyl spermine were also studied in comparison with commercially available liposomal cationic lipid formulations Lipofectin and Lipofectamine. Transfection was performed using plasmid DNA encoding for enhanced green fluorescent protein (pEGFP) as the reporter macromolecule, with its fluorescent imidazolidinone moiety analysed by FACS. We found higher transfection efficiency in both primary skin cells (FEK4, 75%) and in an immortalized cancer cell line in culture (HtTA HeLa, 70%) than Lipofectin that showed 18% and 58% transfection efficiency, respectively. Our synthetic lipospermine showed comparable results with Lipofectamine (66% and 70%) in FEK4 and HtTA cells, respectively. Using the cationic lipid N^4,N^9-dioleoyl spermine, both DNA condensation and gene delivery were achieved at a small N/P charge ratio (2.5 +/–) minimising the toxic effects seen at higher charge ratios (MTT assay). From our results, we conclude that N^4,N^9-dioleoyl spermine is efficient in both lipoplex formation and lipofection.

We acknowledge the financial support of the Egyptian Government (studentship to O.A.A.A.). We are grateful to Prof. R. M. Tyrrell (University of Bath) for the HtTA and FEK4 cell lines and to Dr C. Pourzand (University of Bath) for help in the cell biology studies and for useful discussions.

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169
The development of liposomal subunit TB vaccines: from bench to clinic

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The successful development of a parenteral subunit protein based liposomal vaccine from the bench into the clinic requires the fabrication of a stable, sterile and freeze dried product. The development of a liposomal freeze dried formulation enables to overcome the process of aggregation in solution together with the added advantage of reduction in transition temperature thereby controlling the thermal behaviour of the end product. The aim of this work was to establish the optimum concentration of the cryoprotectant together with terminal sterilization of the freeze dried formulation after exposure to 25KGy dose of gamma radiation. Liposomal formulations consisting of DDA and TDB (1.25 mg/ml and 250 μg/ml, respectively) were prepared by the lipid hydration method. Freeze drying was carried out with the addition of a range of cryoprotectants (sucrose, maltose, trehalose and lysine: 2–10mole/mole, respectively) (pre freezing: –70°C for 30 min, drying phase: –40°C for 48 h). The freeze-dried formulations were exposed to gamma radiation to a dose of 25 KGy using a Schering Healthcare IBL 437C irradiator. The radiation source was ^{137}Cs at a dose rate of 2.8 Gy/min. The liposomes were sized on a Zetaplus (Brookhaven Instruments, UK). Differential Scanning Calorimetry (Pyris Diamond DSC, PerkinElmer, UK) was used to measure the change in transition temperature. Thermogravimetric studies were carried out to measure the moisture content of the freeze dried liposomes using a Perkin Elmer Pyris 1 TGA. The optimization of the cryoprotection was based on a balance between the moisture content, size retention/consistency of the vesicles upon rehydration and transition temperature. The results suggest that the cryoprotectants stabilize the liposomes in a concentration dependent effect (lysine and maltose 4mole/mole; sucrose 8 mole/mole; trehalose 10 mole/mole). Previous studies have shown that cryoprotection was biphasic in nature with both the lower as well as the higher concentrations of the cryoprotectants showing poor lyophilisation when compared with the intermediate concentration (Suzuki & Komatsu 1996; Miyajima 1997). Measurement of the moisture content revealed that all the cryoprotected formulations had low moisture content (1–4% w/w) ensuring long term stabilisation of the freeze dried product. The addition of the cryoprotectant also showed a lowering in the transition temperature of the lyophilized formulation. Lysine lowered the transition temperature to 28.8°C when compared with the transition temperature of the non protected formulation (47.2°C). The optimized formulations were then sterilized by exposure to high energy gamma radiation and no significant difference ($P > 0.05$) in all the parameters measured (size, surface charge, viscosity, chemical integrity) was shown pre and post sterilisation. These results suggest that exposure of freeze dried liposomes to high energy radiation hinders the generation of any free radicals which have been explained as the major source for chemical degradation and hence alters the bilayer characteristics previously reported (Zuidam et al 1995). While minor levels of moisture content were measured (1–4%w/w), it can be concluded that higher levels of moisture than those recorded are needed to generate any free radicals to destabilize the formulation. It is clearly evident that terminal sterilization of freeze dried liposomal formulations by gamma radiations can be an effective approach to produce sterile liposome products.

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170
A novel method to determine the interaction of media and compact using texture analyzer

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Previously this group have studied the single surface erosion characteristics of compacts comprising varying concentrations of glyceryl behenate (GB) and low-substituted hydroxypropylcellulose (L-HPC) as shown in Table 1 and found that a linear relationship existed between the ratio of GB to L-HPC and erosion rate constant (K_{ER}) (Ghimire et al 2006). However erosion characterisation required dissolution studies and gravimetric analysis of compacts at different time intervals making the process laborious and time consuming. Therefore the aim of this study was to develop an alternative more rapid method to determine the interaction of compact with dissolution media using a Texture Analyser (TA). Initially the compact was encased in a holder and retained in a beaker under thermostatic control. The TA was set to "Measure force in compression-hold until time" mode. A sharp increase in the force was recorded as the needle-shaped probe penetrated 1 mm into compact. As the probe reached 1mm into compact, 400 ml of media was poured into a beaker. TA was allowed to run until the force exerted in the probe was less than 12gm. The force felt in the probe decreased in 3 different phases namely, exponential Phase I (stress relaxation within compact in response to external applied force), linear Phase II (interaction of media and compact) and non-linear Phase III (complete erosion of compact). Rate of force change (dF/dt) was obtained from the slope of Phase II and are given in Table 1. A linear relationship was obtained between dF/dt and quantity of L-HPC in compact ($R^2 = 0.98$) but not with the ratio of GB to L-HPC. Therefore, dF/dt does not linearly correlate with K_{ER} from erosion studies but reflects the interaction of L-HPC with dissolution media thus proving that this method is capable of distinguishing between compact compositions. Further to this, we studied the effect of media composition using TA on compact-C by replacing distilled water with milk (skimmed, semi-skimmed, whole). Surprisingly dF/dt was similar in skimmed (1.38 ± 0.21 gmmin^{-1}), semi-skimmed (1.41 ± 0.15 gmmin^{-1}) and whole (1.42 ± 0.23 gmmin^{-1}) milk, however dF/dt was significantly lower than that of distilled water (2.35 ± 0.20 gmmin^{-1}). To validate the result from TA studies, erosion studies were carried out in milk media and the K_{ER} values obtained were 0.012 mgmm^{-2} min^{-1} for skimmed and whole milk which is considerably lower than distilled water. In conclusion, TA studies can be used as an alternative to erosion studies to determine interaction of compact and media.

Acknowledgement: M. Ghimire is partially supported by Overseas Research Student Award Scheme (Universities UK, London). Authors would also like to thank Shin-Etsu for financial support and Gattefossé for the samples.

Table 1 Formulation composition, erosion rate constant (K_{ER}) and rate of force change (dF/dt) of compacts

Compact	Formulation composition (%)		K_{ER} ($mgmm^{-2}$ min^{-1}) (n = 6)	dF/dt ($gmin^{-1}$) mean ± s.d., (n = 3)
	GB	L-HPC		
A	50	50	0.024	4.68 ± 0.51
B	60	40	0.019	2.80 ± 0.34
C	65	35	0.017	2.35 ± 0.20
D	70	30	0.013	1.55 ± 0.32
E	75	25	0.010	1.11 ± 0.11

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171
Investigation of eutectic interactions as a strategy to enhance drug release from silicone vaginal rings

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Eutectic systems have been shown to be useful in enhancing the release of drugs from a number of commercialized drug delivery products, including EMLA topical cream and Nuvaring contraceptive vaginal ring. From a fundamental perspective, the formation of a eutectic mixture produces a decrease in the melting temperature of the components of the system, which leads to enhanced solubility in either the delivery matrix and/or the tissue through which the drug must penetrate (Chein et al 1976). In this study, the formation of a eutectic mixture between the antibacterials agents metronidazole and tinidazole, and their subsequent release from silicone reservoir-type vaginal rings was evaluated. The phase properties of the metronidazole and tinidazole system were investigated using differential scanning calorimetry (DSC). Sample mixtures at 10% concentration intervals were prepared and subjected to a heating programme (10°C/min), and the resulting trend in onset of melting versus concentration plotted to produce a two-component phase diagram. The data confirmed the formation of a eutectic mixture (115.47°C (25% metronidaole, 75% tinidazole)), which was prepared and loaded into reservoir-type silicone elastomer vaginal rings, according to a standard reaction injection moulding process. Each reservoir device (n = 4) was placed in a conical flask containing 100 mL of a 0.3% sodium lauryl sulfate aqueous solution, selected so as to maintain sink conditions. Release studies were performed at a constant temperature (37°C) and constant shaking (60 rpm, 32 mm throw diameter) in an orbital incubator. Samples were taken daily over a period of 14 days with complete replacement of the dissolution medium. An aliquot (1 mL) of each sample was analysed by reverse phase HPLC/UV. Release data showed that the eutectic formulations released both substances at a significantly faster rate than control rings containing metronidazole of tinidazole only. This was attributed to the enhanced solubility (and thus permeation) of the both agents through the silicone elastomer network. The eutectic formulation increased total metronidazole release after 14 days by 23.86% and tinidazole by 36.3% compared with the single drug vaginal rings. The study provides proof of concept for eutectic interactions as a strategy to enhance drug release from reservoir-type silicone elastomer vaginal rings. Importantly, this may be effectively used to deliver multiple drugs from vaginal rings. The study will be expanded to include other eutectic mixtures to investigate whether there are factors other than melting point depression affecting these particular systems.

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172
Use of the MTT assay to evaluate the biocompatibility of β-cyclodextrin derivatives with respiratory epithelial cells

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Cyclodextrins are cyclic oligosaccharides derived from starch, possess a hollow cavity and are able to form water-soluble complexes with hydrophobic molecules. They are mainly used in pharmaceutical formulations to enhance the aqueous solubility and the dissolution rate of drugs with poor aqueous solubility (Loftsson et al 1996). Furthermore, it has recently been shown that cyclodextrin solutions can be aerosolized into droplets with a size optimal for delivery to the lower airways (Evrard et al 2004). They have also been successfully utilised as absorption enhancers for the delivery of macromolecules to the lungs (Yang et al 2004). These promising properties of cyclodextrins as excipients for pulmonary delivery, however, may be overshadowed by concerns about their toxicity. The aim of this study was to investigate any cytotoxic effects of two β-cyclodextrin derivatives, 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) and randomly-methylated-β-cyclodextrin (RAMEB) on the human bronchial epithelial cell line, Calu-3. Two methyl thiazol tetrazolium (MTT) assay protocols were evaluated for measurement of cell viability. Calu-3 cells (passage 27–35) were seeded on 96-well plates at 10 000 cells/well and incubated for 20–24 h. After removing the culture medium, the cells were exposed to 0–100 mM (n = 12 at each concentration) of HP-β-CD or RAMEB in culture medium (100 μl). After 4 h, 500 μg/ml MTT in PBS was presented to the cells using Protocol A (spiking 50 μl of MTT concentrate into the test solution), or Protocol B (replacing the test solution with cyclodextrin-free MTT solution). Cells were then incubated for 2 h, after which the cells and formazan crystals were solubilised by sodium dodecylsulfate 10% in 50:50 dimethylformamide:water at pH 4.7 and absorbance was measured at 570 nm. For RAMEB, cell viability was reduced at concentrations > 10 mM and an LD_{50} of ~25 mM was found using both MTT assay protocols. No effect of HP-β-CD on the cells was observed at concentrations < 100 mM under MTT protocol B. However, a reduction in cell viability occurred at concentrations > 10 mM and an LD_{50} of ~37 mM was obtained using MTT protocol A. This apparent cytotoxicity may be an artefact due to a reduction in MTT availability to the cells caused by complexation of the MTT substrate by HP-β-CD. Cytotoxicity assays are useful indicators of the concentrations at which the ß-cyclodextrin derivatives could be used as safe adjuvants in inhalation therapy. HP-β-CD was shown to be considerably more biocompatible than RAMEB, with no cytotoxicity observed up to a concentration of 100 mM. The potential for cyclodextrins to complex with test substrates and interfere with in vitro assays is highlighted by the comparison of the MTT assay protocols.

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173
An orally dissolving film containing midazolam for paediatric use

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The aim of the study was to develop an orally dissolving film containing midazolam, a short-acting benzodiazepine, presently administered mainly by the parenteral route. Midazolam solution is administered buccally as an unlicensed therapy in children using slow depression of an oral syringe. An orally dissolving formulation would be of benefit, particularly for paediatric administration. Formulations comprised hydroxypropyl methylcellulose (HPMC-15 cps) alone or blended with hydroxypropyl cellulose (HPC-150–700 kcps) to add strength, propylene glycol (plasticizer) and midazolam BP. Midazolam was dissolved in the propylene glycol prior to incorporation into the polymer solutions. Loadings varied from 8.3% to 5.5% w/w with respect to the polymer. The polymer solutions were cast on perspex sheets using a casting knife fixed at 7 mm and dried in an oven at 80°C for 3 h. A film containing 6% w/v HPMC formed the and two combinations of HPMC and HPC (2:1 and 5:1) were successfully formulated. Midazolam release was quantified using a UV assay in relevant media. Release studies for each of the film types were carried out using franz diffusion cells at 37°C (n = 3). The donor chamber contained 1 cm^2 film sections in 1 mL artificial saliva while the receiver solution was 10% v/v aqueous propylene glycol solution to maintain sink conditions. Dialysis membrane was used to separate the chambers and release was measured over 60 min. The following were calculated for each film: cumulative mass of midazolam released, percentage of midazolam released and rate of drug release over the linear portion of each profile. Analysis of variance (ANOVA) was performed to compare percentage of midazolam released and the rate of drug release for the three types of films. Overall, there was no significant difference in the percentage of midazolam released from the three film types ($P > 0.05$) after 60 min. However, there was a significant difference in rate of drug released over 30 min for the three types of films ($P < 0.001$). The HPMC film had the highest rate of drug release, followed by 5:1 (HPMC:HPC) film and lastly the 2:1 (HPMC:HPC) film had the lowest rate of drug release, however, even after 60 min only 50% of midazolam was released. The drug loading could be increased, as could film thickness and the size of the dosage form. Release rates may also be enhanced by processing methods or addition of excipients

to the formulation. Midazolam can be successfully incorporated into and released from films suitable for intra-oral delivery. Studies to increase the drug loading and release rate are ongoing.

174
Microneedle facilitated delivery of pDNA encoding HBsAg to human skin: potential for genetic vaccination

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The skin is an attractive target for the delivery of therapeutic agents for local and systemic activities, including nucleic acid based therapies (e.g. plasmid DNA (pDNA)). The skin is the interface between our bodies and the physical environment and has a protective role and sophisticated immune surveillance system — skin associated lymphoid tissue (SALT). The epidermally resident Langerhans cells, a component of SALT, generate a proficient immune response therefore the skin is an ideal site for genetic vaccination strategies. However, in order to exploit SALT we need to overcome the outermost layer of the skin, the stratum corneum (SC), a considerable barrier to the delivery of macromolecules, such as pDNA. To address this problem we employ arrays of silicon microneedles to create micron sized channels through the SC and into the epidermis. Microneedles were first demonstrated as a mean of compromising the SC for the delivery of therapeutics by Praunitz in 1998 (Henry et al 1998); they are suitable for this purpose as they penetrate the SC and reach only into the epidermis allowing targeting of the delivery formulation to this region without stimulating pain receptors located within the dermis. Microneedle arrays were manufactured from silicon wafers by controlled etching with potassium hydroxide, with morphological characterization by scanning electron microscopy. Microneedle application to human epidermal sheets and trans-epithelial water loss measurements were used to assess disruption to the SC. Microneedles were used to deliver fluorescently labeled albumin to ex vivo human skin and subsequently processed for fluorescent microscopy. The pCMVHB-S2.S vector encoding the small and middle forms of the HBsAg under the control of a CMV promoter (Michel et al 1995) was a gift from Aldevron (North Dakota, USA). Liposome-Protamine-pDNA (LPD) complexes were formed with pCMVHB-S2.S and delivered to A459 cells in vitro, with gene expression determined by immunocytochemisrty (ICC). Microneedle-facilitated delivery of purified HBsAg was demonstrated in ex vivo human skin by immunohistochemistry (IHC). An optimized organ culture system (Birchall et al 2005) was used to demonstrate microneedle facilitated delivery of pCMVHB-S2.S to viable human skin, again visualized by IHC. Microneedle disruption of SC was comprehensive and observed as puncture marks approximately 90 μm in diameter. Microneedle treatment to ex vivo skin results in ~2–3 fold increase in water flux across the skin surface compared to untreated (10.5 g/hm^2 compared with 24.9 g/hm^2). Macromolecular delivery of fluorescent albumin and HBsAg, facilitated by microneedles, to ex vivo skin was demonstrated in both 12 μm cryosection and 6 μm paraffin embedded sections detected by fluorescent microscopy and IHC respectively, both were observed in proximity of SC disruption and associated with micro-channels of ~150 μm depth. Positive transfection of pCMVHB-S2.S in A459 cells was detected by ICC confirming functionality of the plasmid. Positive expression of pCMVHB-S2.S in viable ex vivo skin was determined by creating 6μm paraffin embedded sections and visualization by IHC; expression was confined to the epidermis and clearly associated with micro-channels of ~150 μm depth. The strategy presented offers a potentially more efficient and cost effective method of achieving vaccination than current conventional injection protocols.

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175
A comparison of the in-vitro deposition profiles of drugs from a combination dry powder inhaler (DPI) using the Next Generation Impactor (NGI)

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Patients with asthma and chronic obstructive pulmonary disease (COPD) may need to inhale more than one active ingredient and, therefore, formulations combining two drugs have been developed. Seretide Accuhaler, a DPI, combines fluticasone propionate (FP) with salmeterol xinafoate (SX). Particle size is known to be an important factor in determining the deposition of inhaled particles in the respiratory tract (Visser 1995). Aerodynamic particle size distribution is widely assessed in-vitro using inertial impactors. The objective of this study was to compare the deposition of two drugs from a single DPI at two flow rates using the recently introduced NGI.Ten doses from a Seretide 100 (S100) or Seretide 500 (S500) Accuhaler (GSK, UK) were actuated into the NGI at one of two flow rates; 30 or 66 (± 0.5) L min^{-1}. Each nominal dose contained a combination of 50 μg of SX, and either 100 μg (S100) or 500 μg (S500) of FP. Impactor cut-off diameters were calculated according to the method described in the European Pharmacopoeia (2002). Impactor "collection plates" were coated to minimise particle bounce. Samples were recovered and quantified using a validated HPLC method. Fine particle fractions (FPF) were calculated as the percentage of the recovered dose having an aerodynamic diameter < 3 μm (FPF$_{<3\ \mu m}$) or < 5 μm (FPF$_{<5\ \mu m}$) (Table 1). The mass-median aerodynamic diameter (MMAD) and FPF values determined for both SX and FP showed formulation and flow rate dependency ($P < 0.005$, ANOVA). Table 1 MMAD and FPF values for two Seretide formulations containing a combination of SX and FP obtained using the NGI at different flow rates. The MMAD of FP from each formulation was smaller and the FPF was larger than SX at both flow rates ($P < 0.01$, t-test). Increase in flow rate from 30 to 66 L min^{-1} led to expected reductions in MMAD and increases in FPF values for both drugs. Increase in flow is expected to produce greater turbulence and higher fluidisation energy leading to increased dispersion. The FPF values of FP were higher from the S500. However, when the same concentration of SX was present in both formulations, a lower FPF was obtained with the S100. The increase in active to lactose ratio in the S500 leads to a relative increase in the density on the surface of lactose particles possibly saturating 'active' sites. When drug-drug and/or drug-carrier ratios are varied in combined DPIs, then the pulmonary deposition and bioavailabilities of the individual components can be affected.

Table 1 Mass-median aerodynamic diameter (MMAD) and FPF values for SX and FP

Flow rate (L min^{-1}) (%)	Strength	Active	Mean (RSD)		FPF$_{<5\ \mu m}$
			MMAD (μm)	FPF$_{<3\ \mu m}$ (%)	
30	S100	SX	5.5 (1.3)	3.0 (7.6)	9.1 (9.0)
		FP	5.3 (2.5)	4.6 (11.7)	11.3 (8.4)
	S500	SX	4.3 (1.8)	6.4 (10.8)	13.4 (3.0)
		FP	4.2 (0.6)	7.4 (6.8)	16.4 (3.0)
66	S100	SX	3.8 (7.7)	8.1 (2.6)	14.2 (2.8)
		FP	3.4 (2.0)	11.6 (5.1)	18.2 (9.7)
	S500	SX	3.4 (2.9)	11.2 (9.0)	18.4 (7.9)
		FP	3.3 (2.8)	13.9 (9.4)	23.1 (9.3)

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176
An investigation into using spray chilled Gelucire 50/13 microspheres as potential drug carriers for poorly soluble drugs

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Gelucire 50/13 is a semisolid excipient composed of a mixture of mono-, di- and triglycerides with PEG esters of fatty acids. This material is typically used as a sustained release matrix for oral delivery and exhibits a combination of erosion and diffusion drug release mechanisms (Sutananta et al 1994). Recent studies have indicated that Gelucire 50/13 solid dispersions may improve the bioavailability of some poorly water-soluble drugs in-vivo (Choy et al 2005). This study used a Class II drug, piroxicam, as the model drug to evaluate the potential of Gelucire 50/13 microspheres as a means of improving the solubility, and indirectly, the bioavailability of poorly water soluble drugs. Piroxicam was encapsulated in the Gelucire 50/13 matrix with up to 25% loading using a customized spray-chilling apparatus. The microspheres were determined to have a spherical shape with smooth surfaces and to be 20–200 μm in diameter. Differential scanning calorimetry (DSC) results of the piroxicam loaded microspheres displayed a

single melting transition of Gelucire 50/13 which indicated that there is no interaction between the drug and the excipient. Hot stage microscopy (HSM) experiments in polarized mode proved that the drug particles exist as separate crystals in the microspheres. The dissolution results of the piroxicam loaded microspheres showed significant improvement compared with the drug alone. The microspheres (25% drug loading) with size below 63 μm in diameter showed 78% drug release in the first 30 min, while microspheres with increased particle size exhibited a decreased drug release rate. For example, microspheres (25% drug loading) with a size range of 125–180 μm in diameter exhibited only 44% drug release in the first 30 min. Therefore, it is clear that particle size can significantly influence the release rate of the drug from the microspheres. In the case of large (125–180 μm) and medium (63–125μm) size microspheres, aging studies revealed that the longer the aging period, the faster the drug release rate. For the same groups of microspheres, drug loading displayed a significant effect on the drug release: the lower the loading, the faster the drug release rate. However, both aging and drug loading studies demonstrated that the drug release from Gelucire 50/13 microspheres with a small particle size range (< 63 μm) are less influenced by formulation variables and storage than are systems with large and medium particle sizes. In conclusion, Gelucire 50/13 has been successfully used in a spray chilling process for preparing drug loaded microspheres. More importantly, the study has demonstrated that there is considerable potential in using spray chilled Gelucire 50/13 microspheres as a means of improving the dissolution, and hence by implication bioavailability, of poorly water soluble drugs.

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177
Formulation of a terpene-based HPC gel drug reservoir system for optimizing the in-vitro transdermal permeation of ondansetron hydrochloride

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Ondansetron hydrochloride is a selective 5-HT3 receptor antagonist used in the treatment of chemotherapy-induced nausea and vomiting (Markham & Sorkin 1993). Due to its short half-life and low bioavailability (Bozigian et al 1994), it is administered orally 3 or 4 times a day wherein the patient compliance is low. Even with oral controlled release dosage forms, fluctuation in plasma concentration of drug is high. The broad objective of the study was to design a membrane-moderated transdermal therapeutic system (TTS) for ondansetron hydrochloride. This investigation was carried out to formulate a terpene-based hydroxypropyl cellulose (HPC) gel drug reservoir system for its optimal transdermal permeation. The effect of ethanol-water cosolvent system and ethanolic solution of nerodilol, carvone and limonene on the in-vitro permeation of ondansetron hydrochloride was studied across the rat epidermis in order to select a suitable solvent system and optimal concentration of the terpene enhancer for the development of a membrane-moderated TTS. The solubility of ondansetron hydrochloride in ethanol, water and ethanol-water cosolvent system (20:80v/v, 40:60v/v or 60:40v/v) was determined. The in-vitro transdermal permeation of ondansetron hydrochloride was studied across the rat epidermis, mounted in modified Keshary-Chien diffusion cell. The in-vitro transdermal permeation parameters such as flux of the drug, permeability coefficient and enhancement ratio (ER) were calculated as per the procedure described elsewhere (Krishnaiah et al 2004). The highest transdermal permeation was observed from 60:40v/v of ethanol-water solvent system, which showed highest solubilty, and hence chosen as a vehicle for formulating 2% w/w HPC gel drug reservoir. The HPC gel formulations containing ondansetron hydrochloride (3% w/w) and selected concentrations of carvone (0, 2, 4, 8 and 10% w/w), limonene (0, 2, 3 and 4% w/w) or nerodilol (0, 1, 2, 3 and 4% w/w) were prepared, and evaluated for in-vitro permeation of the drug across rat epidermis. All the three chosen terpene enhancers enhanced the transdermal permeation of ondansetron hydrochloride on adding to HPC gel drug reservoir, but to a varying degree. The optimal transdermal permeation was observed with 8% w/w of carvone (87.4 ± 1.6 μg/cm^2·h), 3% w/w of limonene (181.9 ± 0.9 μg/cm^2·h) or 3% w/w of nerodilol (175.3 ± 3.1 μg/cm^2·h) in HPC gel. The enhancement ratio (ER) in drug flux with 8% w/w carvone, 3% w/w limonene and 3% w/w nerodilol were found to be 10.8, 22.5 and 21.6, respectively, when compared to that obtained without a terpene enhancer (control) in HPC gel drug reservoir. However, the optimal drug flux obtained with 8% w/w carvone was only 1.04-times that of desired drug flux (84 μg/cm^2·h) whereas the drug flux with 3% w/w limonene and 3% w/w was 2.17- and 2.09-times respectively. Due to the possible resistance of TTS components (rate-controlling membrane and adhesive-coat), and due to the difference in permeability of skin model (rat epidermis) and human skin, only 3% w/w limonene and 3% w/w nerodilol are most likely to produce the desired transdermal permeation of the drug from the HPC gel drug reservoir. It is concluded from the results that HPC gel drug reservoir containing either 3% w/w limonene or 3% w/w nerodilol could be used in the design of membrane-moderated TTS of ondansetron hydrochloride.

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178
Modulation of two stable W/O/W multiple emulsions to control delivery of a hydrophilic drug through the skin

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Different strategies have been used in order to improve the diffusion of molecules through the skin. Vehicles can affect both drug delivery and skin permeability properties (Bonina et al 1993). Multiple emulsions are of interest because of their ability to provide sustained release (Raynal et al 1993). By enhancing drug accumulation at the site of administration, multiple emulsions may improve its activity and reduce serious side effects due to undesirable systemic absorption (Youenang Piemi et al 1998). Two water-in-oil-in-water (w/o/w) multiple emulsions (CME1–01 and CME1–02) were obtained for dermato-cosmetic purpose. Caffeine (CAF) was used as a drug model placed in the inner hydrophilic phase of both multiple emulsions (ME). The aims of this study were: to show the ability of CME1–01 and CME1–02 to control the delivery of caffeine (CAF) at the target site, when they were compared with the release profile of CAF from an O/W emulsion of similar composition; and to show the possibility to adjust the release of drugs through the skin by only changing the composition of the Lipophilic emulsifier system (LES). Lipophilic and hydrophilic silicones copolyoles were used as emulsifiers to formulate two multiple emulsions at room temperature using caffeine in the inner phase as a hydrophilic drug model and a mix of reological additives in the external phase. A two-step process was used for the preparations of both ME (Raynal et al 1993). The only difference between both of them was the composition of LES, even though; the total percentage of the lipophilic emulsifier system remained stable. CME1–02 was made by using a mix of PEG/PPG-18/18 Dimethicone and Cethyl PEG/PPG-10/1 Dimethicone (1:5) while CME1–01 had only Cethyl PEG/PPG-10/1 Dimethicone. The delivery behaviour of CAF from both ME were investigated and compared through in vitro skin distributions and permeation studies using Franz cells (Youenang Piemi et al 1998). The cumulative amount of CAF permeated after 3 h (express per ml and cm^2) of CEM1–01, CEM1–02 and CA1–00 were 0.52 ± 0.04; 0.15 ± 0.02; 1.02 ± 0.08, respectively. The cumulative amount of CAF permeated after 6 h (express per ml and cm^2) of CEM1–01, CEM1–02 and CA1–00 were 1.10 ± 0.08; 0.50 ± 0.06; 2.62 ± 0.13, respectively. Both ME studied controlled the release of CAF when they were compared with an O/W emulsion (CA1–00) of similar composition ($P < 0.05$, n = 5). When both formulations (CEM1–01 and CEM2–02) were compared each other, they showed significant different release profiles ($P < 0.05$, n = 5). There is a possibility to adjust the release of drugs from a ME through the skin by changing the composition of the LES.

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179
Combination of inulin as a bacterially degradable polysaccharide and Eudragit RS to achieve colon delivery of indomethacin pellets

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To achieve successful colonic delivery, a drug needs to be protected from absorption and/or degradation in the environment of the upper gastrointestinal tract and then be released into the proximal colon. Various approaches have been used for oral delivery of drugs to colon which includes covalent linkage of a drug with a carrier, coating with pH-sensitive polymers, formulation of time dependent systems and exploitation of carriers that are degraded specifically by colonic bacteria. The method by which the drug release will be triggered by colonic flora appears to be more interesting with regard to the selectivity (Sinha et al 2003).

Our previous studies showed that the free films containing sustained release polymethacrylates such as Eudragit RS in combination with inulin have potential as a coating system for specific colon delivery (Akhgari et al 2006). The aim of this work is to assess the suitability of such an approach for achieving specific delivery of indomethacin to colon using pellets coated with formulations containing Eudragit RS and inulin. Indomethacin was selected as a model drug because it has good indications for colonic delivery. The drug loaded pellets were produced by layering of indomethacin onto the non-pareils using a fluidized bed coating apparatus. Then these pellets were coated with formulations containing Eudragit RS and different percent of inulin (20% and 30%) at the level of coating 5, 10, 15 and 20%. The indomethacin release was evaluated at different pH (1.2, 6.4 and 7.2). The drug release studies were also performed at pH 6.4 in the presence of inulinase. It was shown that in absence of inulinase, drug release was low at different pH, indicating that the integrity of coating remained unchanged, but in presence of inulinase, the enzymatic degradation of inulin provided aqueous pores in the coating layer and the drug release markedly increased. However even after 12 h only 40% of indomethacin was released which may be attributed to an interaction between ionic groups of Eudragit RS and indomethacin. The results of this study also showed that increasing the percentage of inulin from 20% to 30% in the coating formulation has no significant effect on the indomethacin release. The results of this study revealed that incorporation of inulin to Eudragit RS could modulate drug release and make it suitable for colonic delivery of indomethacin.

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180
An investigation into piperine and tetrahydropiperine as chemical enhancers for transdermal drug delivery

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Transdermal drug delivery has many advantages, such as, high patient compliance. However, due to the low permeability of the skin, enhancers are needed to increase drug permeation into the skin and much research is currently conducted on identification and evaluation of transdermal enhancers. Piperine, an alkaloid found naturally in black pepper and long pepper, is a small (MW 285 Da), lipophilic molecule that is used in traditional Ayurvedic and Chinese medicine, and is also available commercially as a nutritional supplement. Interestingly, it has been found to enhance the oral bioavailability of several pharmaceutical compounds (Bano et al 1991). Its potential action on mucosal membrane begged the question of whether piperine could also enhance drug penetration into the skin. Hence, we investigated the potential of piperine as a chemical transdermal enhancer. Tetrahydropiperine (Cosmoperine, obtained from Sabinsa Corp.) was also investigated as preliminary experiments by Sabinsa Corp. (USA) indicated its potential to increase transdermal drug delivery. Permeation experiments were conducted using Franz diffusion cells and excised rat skin. Lidocaine, the model drug, was dissolved in amphiphilogels (gels made solely of surfactant that have been investigated in our laboratories as oral and transdermal delivery vehicles; Jibry & Murdan 2004; Murdan et al 2005). The amphiphilogels were found to be suitable vehicles for the lipophilic, water-insoluble, piperine and its derivative. The effects of piperine (1.5% w/w) and tetrahydropiperine (1.5 and 3.0% w/w) inclusion on drug permeation were investigated. Solid Phase Extraction and HPLC were used to measure drug levels in the receptor phase and in the skin. Each experiment was replicated 4 times. It was found that both piperine and tetrahydropiperine increased the drug flux, to similar extents (1.5–2 ×), compared with the control (absence of piperine/tetrahydropiperine). Increasing the concentration of tetrahydropiperine (from 1.5 to 3.0% w/w) led to a further small increase in drug flux. Calculation of the diffusion and partition coefficients suggest that the enhancing effects of piperine and of tetrahydropiperine was primarily due to changes in the drug's partitioning into the skin. To conclude, we can say that piperine and tetrahydropiperine showed promise as transdermal enhancers. However, the increase in drug permeation, compared with the control (absence of the 2 chemicals), was not substantial at the low concentrations (≤3%w/w) used. Further work must be conducted to establish the optimal levels of piperine and of tetrahydropiperine, differences between the transdermal enhancing ability of the 2 chemicals, their skin irritation potential, their own transdermal absorption and any subsequent pharmacological effect(s), as well their mechanism(s) of action.

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181
Encapsulation of mucinolytic enzymes for treatment of cystic fibrosis

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The basis of pulmonary cystic fibrosis (CF) lies in the imbalance of electrolyte transport, either directly or indirectly, across the epithelial cells. However, this is not the fatal determinant of the disorder. It is the consequences of such an abnormality that render CF the most common lethal genetic disease in Caucasian populations (Quinton 1999), with development of chronic pulmonary disease being the main cause of mortality (over 90% of cases). Prognosis and treatment of CF has improved significantly over the past few decades, increasing the life expectancy of the patients (FitzSimmons 1993). The development of novel therapeutics is required to overcome the present problem of antimicrobial resistance as well as expensive and time consuming treatments. The main problems associated with current treatment concern the mechanisms of delivery to deep within the lung. This project aims to develop a drug delivery system that will penetrate and overcome the thick mucus barrier that develops in pulmonary CF patients. The incorporation of mucinolytic enzymes into the delivery vehicle should improve the efficacy of the accompanying drug by increasing the proportion of drug reaching the target site. Biodegradable polyesters with backbone functionality were synthesised via the enzyme-catalysed transesterification of divinyl adipate, glycerol and ω-pentadecalactone monomers. These were used to form colloidal particles via a multiple emulsion (w/o/w)-solvent evaporation technique. Varying the energy input into the emulsion system corresponded to variations in the sizes of the particles being formed, as well as altering the yields of protein encapsulated within the polymer particles. This system allowed for encapsulation of both a lipophilic drug and a hydrophilic enzyme. Release profiles of both the chosen model enzyme (chymotrypsin) and the drug (ibuprofen) were obtained by adding the particles to a buffered system and sampling regularly. Sustained release of model enzyme was achieved over 2–3 h. The protein was detected spectrophotometrically and the activity of the released enzyme confirmed via the azocasein assay and the active site titration assay. Loss of activity was observed upon maximal release of the enzyme. The released ibuprofen was quantified via HPLC. A burst release of ibuprofen was observed. Future work will involve linking the drug to the polymeric backbone in order to achieve a more controlled release. In addition, enzymes that degrade mucin were investigated. Microorganisms belonging to the streptomycete and related genera that are able to degrade this complex substrate, were isolated from soil samples and characterised. Studies have now shown that these microbially produced enzymes display the concerted effect of both proteolytic and glycosidic enzymes on degrading mucins. Further characterisation and purification of these enzymes will facilitate the more specific targeting of mucin, and so further optimise the delivery system.

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182
Sustained drug release from respirable powders prepared by spray-drying w/o/w double emulsions containing PLGA, chitosan and leucine

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This study investigates the viability of spray-drying water-in-oil-in-water (w/o/w) emulsions containing biocompatible polymers poly-lactide co-glycolide (PLGA 50:50; Chaw et al 2003) and chitosan (Filipovic-Grcic et al 2003) as drug release modifiers to generate highly respirable dry powders that exhibit sustained drug release characteristics, using the amino acid leucine as an aerosolisation enhancer (Rabbani & Seville 2005). Primary emulsions were prepared by vortex-mixing 1 mL water (± 80 mg salbutamol sulphate) with 0.02 mL Span 80 in a solution of 200 mg PLGA (50:50) in 3 mL chloroform (± 80 mg beclometasone dipropionate: BDP). This primary emulsion was subsequently homogenised with 25 mL low molecular weight chitosan gel (4% w/v chitosan in 3% v/v glacial acetic acid) containing 520–680 mg leucine and optionally 160 mg salbutamol, diluted to 100 mL with aqueous ethanol (30% v/v) to form w/o/w emulsions containing salbutamol in the inner aqueous phase and/or BDP in the oil phase and/or salbutamol in the outer aqueous phase. The emulsions were spray-dried using a Buchi B-290 mini spray-drier (Buchi, Switzerland) with high performance cyclone (spray flow rate 600 L/h, inlet temperature 180°C, aspirator 85%, pump 3.2 mL/min). The resultant powders were assessed for thermal and physical characteristics. Aerodynamic diameters, distributions and fractions of the

powders was determined using HPMC capsules (n = 3) loaded with 25 mg spray-dried powder aerosolised using a Spinhaler (Fisons) DPI into a Multi Stage Liquid Impinger (MSLI; Copley Scientific) at a flow rate of 60 L/min. The emitted dose (ED) and powder deposition at each stage of the MSLI was determined by HPLC (salbutamol: 278 nm; BDP: 250 nm). Finally, the tapped density of the powders was assessed and theoretical estimates of primary aerodynamic diameter calculated. In vitro powder dissolution studies were performed using USP 2 dissolution apparatus in phosphate buffer (pH 6.8, 37°C) with quantification of drug release by HPLC. The spray-dried powders had an off-white appearance and good flow properties (Carr's Index 6–35%). Using a high performance cyclone, yields of up to 90% were achieved. SEM indicated that the powders comprised microspheres with a diameter of 1–2 μm. Laser diffraction data supported SEM results, with mean diameters of approximately 2–3 μm and theoretical aerodynamic diameters of 1–2 μm. The aerosolisation testing of the spray-dried powders resulted in an ED of at least 92% of capsule contents and an FPF (fraction < 5 μm) of up to 60% total loaded dose. In vitro dissolution studies indicated that inclusion of PLGA 50:50 and LMW chitosan within the formulation resulted in a sustained release profile of over seven days for salbutamol incorporated in the inner aqueous phase and BDP incorporated in the oil phase. Salbutamol incorporated in the outer aqueous phase did not undergo sustained release, offering the potential for a dual release profile. In conclusion, w/o/w double emulsions containing both PLGA 50:50 and LMW chitosan as drug release modifiers and leucine as an aerosolisation enhancer may be successfully spray-dried to generate respirable powders demonstrating good in vitro dispersibility and sustained drug release.

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183
Using a solvatochromic dye as an indicator of polarity in self-emulsifying drug delivery systems (SEDDS)

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Although SEDDS have been investigated for a number of years as potential drug delivery systems, little is known about the effect of polarity of the constituents on the formation and stability of SEDDS emulsions. In this study, Reichardt's dye and the $E_T(30)$ parameter have been used to probe the polarity at SEDDS interfaces. In solution, intramolecular charge transfer ($\pi \Rightarrow \pi^*$) in Reichardt's dye gives rise to a UV-VIS absorption pattern. The wavelength of maximum absorbance (λ_{max}) is dependent on solvent polarity, ranging from 810 nm in diphenyl ether to 453 nm in water, as a result of differential stabilisation of the ground versus the excited state of the dye. A solvent polarity parameter, the $E_T(30)$ parameter, can be derived from the UV-VIS data, where E_T is an abbreviation for the molar transition energy (usually expressed in kcal/mol) and 30 is the serial number of Reichardt's dye used in the initial investigations. $E_T(30)$ is defined as:

$$E_T(30) = h \,.\, c \,.\, N_A / \lambda_{max}$$

where h, c and N_A are, respectively, Planck's constant, the speed of light and Avogadro's constant. $E_T(30)$ values can range from 63.1 kcal/mol for water to 30.7 kcal/mol for tetramethylsilane. $E_T(30)$ values normalised to a scale of 1.000 (water) to 0.000 (tetramethylsilane) are referred to as $E_T^N(30)$ values and allow a direct comparison of the polarity of a test system to these standard materials. All materials were supplied by Sigma, MO. The following placebo formulations were prepared by vortex mixing: A (65/30/5), B (65/17.5/17.5), and C (65/5/30) (% w/w Soybean Oil/Tween 80/Span 80, respectively. Ibuprofen was added at 300 mg/5 g (6% w/w). In all cases, the drug dissolved completely in the SEDDS. 150 μM of Reichardt's dye and 5 μL of SEDDS were then dispersed in 5 mL of Milli Q water before UV-VIS spectra (n = 3) being collected on a Lambda 35 (Perkin-Elmer, UK). Calculated $E_T^N(30)$ values were as follows: Placebo A (HLB 13.5) 0.721 ± 0.0001, Placebo B (HLB 9.7) 0.7789 ± 0.0002, Drug-loaded A 0.760 ± 0.0014, Drug-loaded B 0.8143 ± 0.0064 and drug-loaded C (HLB 5.8) 0.8740 ± 0.0130. The results indicate that there is a relationship between the HLB of the surfactant system and the polarity as indicated by the $E_T^N(30)$ calculation. However, as the dye probes the interface between the various components of the SEDDS formulation, it may be that the polarity indicator is actually a more precise indicator of the micro-polarity that exists in these systems than the relatively crude HLB calculation, and will be able to allow more precise determination of the effect of varying the SEDDS formulation. The effect that the drug has on the system is highly interesting, as this is usually assumed to be negligible, and reflects the partial surfactant-like behaviour of some drug molecules. This study has shown that it is possible to use Reichardt's dye and the $E_T^N(30)$ parameter to probe the polarity of SEDDS formulation. The effect of inclusion of drug on the behaviour of the formulation as a whole can not be negelcted.

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184
A study of the stability of liposomes to air-jet and vibrating-mesh nebulisation

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Pulmonary delivery of liposomes is commonly achieved using air-jet nebulisers (Farr et al 1985). However, jet-nebulisation may disrupt liposomes, resulting in loss of the entrapped hydrophilic material (Taylor et al 1990). Recently, vibrating-mesh nebulisers have been commercialised, and shown to be suitable for delivering liposomes (Elhissi & Taylor 2005). In this study, using salbutamol sulphate as the model hydrophilic material, the stability of liposomes to nebulisation was evaluated using Pari LC Plus (air-jet), Aeroneb Pro (4 μm-Mesh), and Aeroneb Pro (8 μm-Mesh) nebulisers. A chloroformic solution (60 mg/mL) of soya phosphatidylcholine and cholesterol (1:1, 70 mg) was prepared. A thin lipid film was formed by evaporating chloroform using a rotary evaporator. The film was hydrated with NaCl (0.9%) solution containing salbutamol sulphate (35 mg/mL), and hand shaken for 10 min to form liposomes (35 mg/mL). Drug-free NaCl solution was added to produce dilute liposomes (10 mg/mL) followed by extrusion through 1 μm polycarbonate membrane filters and centrifugation at 40 000g and 4°C to separate the unentrapped drug (supernatant) from the entrapped fraction (Pellet). The pellets were re-dispersed in NaCl solution for laser diffraction size analysis (Malvern Mastersizer S) of liposomes or for nebulisation (5 mL) to 'dryness' into a twin impinger (TI, 60 L/min). Nebulisers and TI stages were washed separately with NaCl solution and contents centrifuged for quantification of entrapment efficiency (EE) using HPLC. EE = (amount of entrapped drug/ total amount of the drug) × 100%. The median size was 5.95 ± 0.88 μm and 1.13 ± 0.02 μm for non-extruded and extruded liposomes, respectively. For all devices and formulations, marked losses of the originally entrapped drug occurred, indicating liposome instability to nebulisation (Table 1). However, drug losses were less for the vibrating-mesh nebulisers (Table 1). The employment of a device with large mesh apertures (8 μm), and liposome extrusion to 1 μm before nebulisation both increased the EE in all compartments (Table 1). Unlike, the jet nebuliser, vibrating-mesh devices showed that EE for liposomes delivered were less than that for vesicles remained in the nebulisers. Moreover, except for the Pari nebuliser, liposomes delivered to the lower stage of the TI had a lower EE than those delivered to the upper stage (Table 1), suggesting that smaller liposomes were delivered in smaller aerosol droplets to the lower stage, and these entrapped a smaller proportion of the drug. In conclusion, this study shows that vibrating-mesh nebulisation are less destructive to liposomes than air-jet nebulisation. However, further work is required to increase the proportion of entrapped to unentrapped drug.

Table 1 Entrapment efficiency of salbutamol sulphate in various compartments using air-jet and vibrating-mesh nebulisers

Nebuliser/Liposome formulation	Entrapment efficiency (%)		
	Residual	Upper stage	Lower stage
Pari (air-jet)/non-extruded	13.66 ± 1.70	10.40 ± 1.33	11.87 ± 2.74
Pari (air-jet)/extruded	33.05 ± 9.08	11.96 ± 0.79	17.94 ± 1.47
Aeroneb Pro (4 μm)/ non-extruded	46.93 ± 17.17	22.85 ± 9.71	12.19 ± 3.48
Aeroneb Pro (4μm)/ extruded	45.07 ± 7.05	35.31 ± 7.38	31.01 ± 1.23
Aeroneb Pro (8μm)/ non-extruded	52.35 ± 6.66	45.40 ± 4.10	24.64 ± 2.41
Aeroneb Pro (8μm)/ extruded	71.78 ± 3.77	63.35 ± 1.98	37.99 ± 1.65

Data are means ± s.d., n = 3.

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185
Supercritical fluid encapsulation of human growth hormone maintains protein integrity during processing and release

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Human growth hormone (hGH) is a 22 kDa protein currently administered via daily to thrice weekly subcutaneous injection for a variety of disorders including growth hormone deficiency, Turners' syndrome and HARS (HIV associated adipose redistribution syndrome). Since long-term treatment is usually required, the development of a controlled release formulation is highly desirable to reduce the frequency of injections and improve patient convenience and compliance. However, hGH is susceptible to aggregation, oxidation and deamidation during storage, processing and encapsulation using traditional formulation techniques (Pearlman & Bewley 1993; Cleland et al 1997). In this paper hGH was encapsulated into poly(D, L-lactic acid) microparticles suitable for subcutaneous injection using supercritical fluid (SCF) processing. The SCF process is a low temperature, solvent free method which utilizes supercritical carbon dioxide ($scCO_2$) to encapsulate drugs in a gentle one step process into a range of product morphologies for controlled release applications (Howdle et al 2001). It is therefore an attractive methodology for formulating labile drugs, such as proteins, into polymeric controlled release delivery systems. Lyophilised hGH powder and poly(D, L-lactic acid) were placed in a high pressure vessel and exposed to $scCO_2$ for 1 h at 40°C. A stirrer was used to promote mixing of the protein into the polymer matrix. Release of the pressure through a nozzle produced hGH loaded microparticles. Protein aggregation and degradation was assessed on processing and release in vitro. The European Pharmacopoeial SEC method was used to monitor hGH aggregation and to quantitate the protein during the release study. The E.P. RP-HPLC method was used to examine the generation of deamidated and oxidized species. Native-PAGE, and SDS-PAGE (reducing and non-reducing conditions) were also used to investigate aggregation and degradation. Results showed that hGH was not significantly affected by $scCO_2$ exposure as determined by SEC. Even without any process optimization and formulation development the protein remained within the European Pharmacopoeial limits for aggregates and related substances. In vitro release studies in preliminary formulations revealed an initial burst release of 21.4% (± 2.6%, n = 3) within the first day and continued hGH release from the microparticles throughout the 28-day study period. In conclusion, these experiments have demonstrated that without process optimization or formulation development hGH encapsulated by this SCF process remains within European Pharmacopoeial limits for aggregates and related compounds. Monomeric hGH was shown to be released in vitro over a 28 day period, with a burst release of around 20% of the loaded protein in the first day. Optimisation of the release profile and in vivo PK/PD studies are underway. Furthermore, studies are being extended to other proteins such as Granulocyte Macrophage Colony Stimulating Factor (GM-CSF), erythropoietin and beta interferon to demonstrate the versatility of this SCF technique.

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186
Size analysis of aerosol droplets generated using vibrating-mesh nebulisers: the effect of fluid physicochemical properties

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Many factors affect the deposition of particles in the respiratory tract. Among these, particle size of the inhaled material may be considered the most essential. Principally, particles smaller than 5 μm are considered necessary for deposition in the peripheral airways (O'Callaghan & Barry 1997). Using air-jet and ultrasonic nebulisers, aerosol droplet size has been found to be dependent on fluid viscosity and to a lesser extent surface tension (McCallion et al 1995). In this study, using the newly marketed type of nebulisers, namely vibrating-mesh nebulisers, the influence of ions and fluid viscosity on the size of aerosol droplets generated was studied. Solutions of fluids having a range of viscosity and constant surface tension were prepared using deionised water as the solvent (Table 1). An Omron NE-U22 or Aeroneb Pro vibrating-mesh nebulisers were filled with 5 ml solution and operated to 'dryness'. The volume median diameter (VMD) of aerosol droplets was measured at midway nebulisation using laser diffraction (Malvern 2600c). All experiments were performed in triplicate. The viscosity was measured using a U-tube viscometer, whilst surface tension measurements were as reported by McCallion et al (1995). Compared with deionised water, the employment of glycerol solution to increase fluid viscosity resulted in a decrease in the VMD of the aerosol droplets for both nebulisers (Table 1). At the highest glycerol concentration (i.e. 30%), no aerosols were generated from the Omron nebuliser (Table 1), indicating that an increase in fluid viscosity may be desirable up to a limit beyond which no aerosols are generated. Moreover, compared with deionised water, NaCl (0.9%) solution produced droplets having smaller and more consistent VMD measurements (Table 1). This indicates that the presence of ions in such small concentration was desirable in generating aerosol droplets with smaller median size. This might be attributed to the reported localisation of sodium ions in the bulk liquid whilst large halides such as Cl anions tend to be close to the liquid-air interfaces (Garrett 2004). In conclusion, this study has shown that the increase in fluid viscosity resulted in a decrease in the size of aerosol droplets generated using vibrating-mesh nebulisers. Moreover, the presence of small salt concentrations may be desirable to reduce the droplet size.

Table 1 The VMD of aerosol droplets generated from nebulisers using a range of fluids

Fluid	Physicochemical-Parameters		VMD (μm) of aerosol droplets	
	Viscosity (cp)	Surface tension (dyne/cm)	Aeroneb Pro	Omron NE-U22
Water	1.00	72.80	6.60 ± 1.95	6.43 ± 0.87
Glycerol (10% v/v)	1.31	72.90	4.61 ± 0.04	5.83 ± 0.08
Glycerol (20% v/v)	1.92	72.54	4.17 ± 0.08	4.69 ± 0.22
Glycerol (30% v/v)	2.74	71.73	4.09 ± 0.04	No aerosols
NaCl (0.9% w/v)	1.02	72.09	4.55 ± 0.04	5.04 ± 0.18

Mean ± s.d., n = 3.

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187
Microwave, a novel percutaneous absorption enhancement method

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Microwaves are a form of electromagnetic energy, like light or radiowaves. Microwaves ranging in frequency from 0.3 to 300 GHz, increasingly present in our daily environment, for example, in microwave ovens, satellite, cellular phone and etc. Exposure to high density microwaves can cause detrimental effects and induce significant biological changes involving the CNS, cardiovascular system, hematopoetic system, uteroplacental function, cutaneous perception, and behavior through thermal action (Nakamura et al 2003). It is well known that percutaneous absorption of drugs can be increased thermally. However, the influence of non-thermal effects of microwave on percutaneous absorption of drug, the subject of the present investigation, has not been studied yet. To perform this investigation, nitrofurazone (MW = 198.14) was chosen as the model penetrant and the effects of microwave on its permeation through excised rat skin was studied for 6.45 h at well controlled temperature of 30°C using Franz-type static diffusion cells. Microwave power densities of 3, 15, 30, 60, 120 W cm^{-2} at frequency of 2450 MHz in a pulsed manner with 45 min exposure intervals were applied to skin. No electromagnetic field was used in control (passive) experiments. Saturated solution of nitrofurazone in distilled water

Table 1 Effect of microwaves on permeation of nitrofurazone through rat skin

Power (W cm^{-2})	Flux ($\mu g\ cm^{-2}\ h^{-1}$)	Enhancement ratio	*P*-value
0 (Control)	1.91 ± 0.31	1.0	—
3	2.09 ± 0.22	1.1	0.991
15	2.38 ± 0.55	1.2	0.008
30	3.26 ± 0.88	1.7	<0.001
60	4.27 ± 0.74	2.2	<0.001
120	5.05 ± 0.32	2.6	<0.001

Data are mean ± s.d., n = 4–5, and are compared statistically by two-tailed t-test analysis.

was used as the donor phase and a mixture of distilled water, acetonitrile and triethanolamine buffer in a ratio of 790:200:10 was used as the receptor phase. Samples were collected each 45 min and drug determination was by UV spectrophotometry at 385 nm. Control studies in the presence and absence of microwave power showed that the material released from skin do not interfere with the assay method. Nitrofurazone flux through rat skin was $1.9 \pm 0.3\ \mu g\ cm^{-2}\ h^{-1}$ in passive condition (control). Application of microwave power at $3\ W\ cm^{-2}$ was not able to significantly increase the nitrofurazone flux ($P = 0.991$, Table 1). The enhancement effect increased with exposure power augmentation and the increase in the flux was significant at microwave intensities of 15–120 $W\ cm^{-2}$ (Table 1). The flux of nitrofurazone reached $5.05 \pm 0.32\ \mu g\ cm^{-2}\ h^{-1}$ at the highest applied power of $120\ W\ cm^{-2}$, which is by about 3 times more than that of control ($P < 0.001$, Table 1). The results clearly show that microwaves increase percutaneous absorption of nitrofurazone, and might be considered as a physical enhancement method for transdermal drug delivery. More investigations are in progress in our laboratories in this regard.

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188
Pluronic and gantrez binary mixes as novel thermosetting, bioadhesive polymer platforms for drug delivery within the periodontal pocket

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A fundamental understanding of polymer gel rheology is paramount to the development of semi-solid drug delivery platforms that are easily administered and retained at the site of application. Moreover, the rheological properties of polymer gels have been shown to significantly influence the erosion and swelling of gel matrices and hence the drug release properties. While the rheological properties of conventional gel systems are often a compromise between the elastic and viscous character of the polymer in aqueous solution, in-situ gelling systems are an extremely exciting alternative that possess a temperature triggered change in gel structure ensuring ease of application while maximizing retention, which has significant promise for ocular, buccal, and vaginal drug delivery. While in-situ gelling systems are extremely promising, one inherent disadvantage is their poor bioadhesion. Should this be improved, these systems could provide extremely efficacious drug delivery platforms. This study aimed to address this current deficit and describes the properties of unique combination of a highly bioadhesive (Gantrez) and a temperature triggered (Pluronic) polymer. Combinations of these two biocompatible polymers and their unique physiochemical properties may be beneficial for in-situ gelling, drug delivery applications, negating premature leakage at the applied site. Pluronic F127 gels (15, 20, 25 and 30% w/w) were formed via the slow addition of polymer to deionised water at 8°C. Pluronic 20 and 25% w/w were chosen as candidates for further analysis through the introduction of 5 and 10% w/w Gantrez. Rheological analysis was carried out at 32°C using a TA AR2000 rheometer with a 2, 4 or 6cm diameter parallel plate geometry and a 1mm plate gap, whereas the mucoadhesive strength was assessed using a published force detachment method (Jones et al 1999, 2001). Increasing the concentration of Pluronic or Gantrez within mono-polymeric gels significantly increased gel elasticity and viscosity (dynamic and zero rate). Combinations of Gantrez and Pluronic yielded gels that had improved flowability and hence a decreased viscosity. Moreover, the combination of the two polymers yielded gel structures with a highly disrupted network and hence decreased elasticity and connectivity. Increasing the ratio of Gantrez within the blend significantly reduced the gel structure, which was observed as a decrease in dynamic viscosity and increased loss tangent values. Gantrez disrupts the interaction between Pluronic chains and inhibits hydrophobic interactions, thus reducing the viscosity and elasticity. Moreover, the interaction between these polymers significantly altered the gelling temperature of Pluronic.

Table 1 Showing Power law viscosity of binary mixes of Gantrez and Pluronic

Polymer (% w/w)	Power law viscosity (Pa.s)	Shear stress (Pa)
20% Pl & 5% Gant	215.5 ± 16.48	500–1200
20% Pl & 10% Gant	110.68 ± 23.49	400–700
25% Pl & 5% Gant	888.77 ± 17.51	500–1000
25% Pl & 10% Gant	222.6 ± 29.9	500–1000

Pl, pluronic; Gant, Gantrez.

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189
Thermoresponsive rheologically structured vehicles for HIV vaccine delivery

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Vaginal mucosal HIV vaccines are gaining increasing attention to combat the spread of sexually transmitted diseases through immunization at the principal site of entry. Rheologically structured vehicles (RSV's) are being developed containing a mucoadhesive component to allow enhanced retention within the vaginal vault to prolong the duration of antigen delivery so as to induce and sustain protective immune responses against HIV pathogens. Upon application, the formulations may experience some change in temperature during the initial spreading process. This change from room to body temperature takes place over a 3–5 min period (Owens et al 2003) and the rheological properties of the formulation can be greatly influenced depending on its components. The inclusion of a temperature sensitive component can exploit the change in temperature that takes place. Pluronics (non-ionic amphihilic block copolymers) form micellar structures in aqueous media which at higher concentrations form aggregates but more interestingly at higher temperatures can undergo gelation leading to increased solution viscosity. By tailoring formulations so as to induce gelation between ambient and body temperature, the formulation can be inserted with ease and spread over the mucosal surfaces before undergoing gelation to form a more structured matrix capable of enhanced retention. In this study, pluronic F127, F108 and F68 were investigated and RSV's were prepared by dissolving sorbic acid (0.1% w/w) and mucoadhesive component (Gantrez SBF97 or Noveon AA1, 3% w/w) in the required amount of H_2O and NaOH. Pluronic (10% w/w) was added followed by hydroxyethylcellulose (5%) and subsequently poly(vinylpyrollidone) (4%w/w). Oscillatory temperature sweeps between 10–38°C on an AR2000 rheometer (T.A. Instruments, Surrey, UK) with a 2 cm diameter parallel plate geometry and a plate gap of 1000μm at 1Hz (n = 5). Table 1 illustrates the storage modulus (G'), a measure of the elastic component of the samples, at 10, 25 and 37°C. Formulation F108 + Gantrez was found to separate out on storage leading to large deviations upon rheological evaluation with the onset of gelation occurring at 25°C. Similarly, its Noveon counterpart underwent gelation at the same temperature. Formulations containing pluronic F127 and either Gantrez or Noveon demonstrated an increase in G' upon reaching 25°C although a slight decrease was then experienced before reaching 37°C which can be attributed to melting of the pluronic. These systems underwent gelation at 15 and 16°C respectively. Conversely, pluronic F68 formulations showed a decrease in G' with increasing temperature with a more marked decrease being experienced in the case of the Gantrez formulation. The thermo-gelation of the RSV's is therefore

Table 1 Values of G' at a range of temperatures for various formulations

Formulation	Thermal response temp.	G' at 10°C	at 25°C	at 37°C
F127 + Gantrez	15°C	1139 ± 82	14970 ± 85	14345 ± 21
F68 + Gantrez	16°C	8951 ± 723	7949 ± 900	3194 ± 860
F108 + Gantrez	25°C	544 ± 472	957 ± 1109	9135 ± 2526
F127 + Noveon	16°C	5590 ± 895	17423 ± 2866	15157 ± 2438
F68 + Noveon	12°C	6846 ± 557	6307 ± 512	5654 ± 451
F108 + Noveon	25°C	5078 ± 256	5684 ± 291	8795 ± 456

Means ± s.d., n = 5.

dependent upon the type of pluronic and mucoadhesive component included, with formulations containing pluronic F127 appearing to be most suitable for the delivery of vaginal vaccines.

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190
Development of rheologically structured vehicles for HIV vaccine delivery

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With the natural transmission of the HIV virus occurring through the mucosal surfaces and 80% of new cases resulting from heterosexual intercourse (Crotty & Andino 2004), vaginal mucosal HIV vaccine strategies are being investigated as the most promising means of combating HIV through immunization at the principal site of entry. Due to poor retention of conventional delivery systems, optimal delivery of an antigen to the vagina represents a significant challenge. In this study, rheologically-structured vehicles (RSV's) have been designed to allow enhanced retention within the vagina to prolong the duration of antigen delivery so as to induce and sustain protective immune responses against HIV pathogens. When applied initially the formulations must have the ability to spread and coat the vaginal epithelial, and the inclusion of a mucoadhesive component should allow intimate contact of the polymer with the mucosal surfaces providing additional persistence. RSV's were designed to include a hydrophilic matrix former (Pluronic F127 and/or hydroxyethylcellulose-HEC), mucoadhesive (polyacrylic acid-Noveon AA1, poly(methyl vinyl ether/maleic anhydride)-Gantrez SBF97), vaginal fluid absorber (polyvinylpyrrolidone-PVP) and preservative system. The RSV's were evaluated by oscillatory rheology and mucoadhesiveness to assess which formulations were suitable for clinical investigation. RSV's were prepared by initially dissolving sorbic acid (0.1% w/w) and mucoadhesive component (3% w/w) in the required amount of sterile H_2O and NaOH required for adjustment to pH6. Pluronic (0–10% w/w) was added, followed by the main matrix component HEC (3–10% w/w) and subsequently PVP (4% w/w). Oscillatory rheological analyses were performed on an AR2000 rheometer (T.A. Instruments) at 25°C with a 2 cm diameter parallel plate geometry and a plate gap of 1000 μm, over a frequency range of 0.1–10Hz. Mucoadhesion studies were performed using a Stable Micro Systems Texture Analyser in adhesion mode, involving the lowering of a hydrated crude porcine disc attached to a 10-mm probe onto the surface of the formulations. A force of 1 N was applied for 30 s before removal at 1 mm/s. The force required to detach the disc from the sample was determined from the peak value of the resultant force-time plot (n = 5 for all experiments). From rheological analyses, the storage modulus (G'), which is a measure of the elastic component of the samples, was determined and for all formulations was found to increase with increasing oscillatory frequency. The presence of pluronic was found to increase G' at 25°C, increasing HEC concentrations also increased G' as did the inclusion of Noveon in place of Gantrez. Statistical analysis (Fischer's PLSD, significance 5%) also showed that while both HEC and pluronic content had no effect on the mucoadhesiveness of the formulations, the mucoadhesive component had a significant effect on the results obtained. In conclusion, the overall structure of the formulations can be modified through alteration of HEC and pluronic levels, while the mucoadhesive component included determines their mucoadhesiveness.

Table 1 Rheological and mucoadhesive properties

Pluronic:HEC: Noveon:Gantrez	G'(Pa)	Mucoadhesion (N)
10:3:3:0	7449 ± 495	0.449 ± 0.014
10:3:0:3	7461 ± 471	0.442 ± 0.034
10:5:3:0	14463 ± 1860	0.378 ± 0.074
10:5:0:3	9394 ± 447	0.446 ± 0.128
10:10:3:0	25200 ± 2155	0.458 ± 0.032
10:10:0:3	20973 ± 1386	0.548 ± 0.043
0:3:3:0	3940 ± 285	0.467 ± 0.072
0:3:0:3	1857 ± 61	0.537 ± 0.041
0:5:3:0	6149 ± 130	0.37 ± 0.041
0:5:0:3	2895 ± 11	0.605 ± 0.076
0:10:3:0	16443 ± 797	0.52 ± 0.078
0:10:0:3	12673 ± 72	0.481 ± 0.081

Mean ± s.d., n = 5.

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191
Microencapsulated calcium-phosphate and chitosan nanoparticles for pulmonary delivery of DNA

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Airway delivery of therapeutic genes represents an attractive modality for systemic and localized treatment of a variety of diseases (Pison et al 2006). Recently non-viral, inorganic calcium-phosphate nanoparticles (Ca-P-NP) have been developed that are suitable for DNA delivery due to their ease of preparation and unique nanomaterial properties; the formation of Ca-P-NP occurs by ionic complexation between divalent calcium ions and DNA phosphate backbone, producing particles < 100 nm (Roy et al 2003). Spray drying is a ubiquitous technique in the pharmaceutical industry, producing dry powder formulations suitable for lung delivery. These experiments examined spray drying of three different systems (1. chitosan-DNA-complexes (CDC), 2. Ca-P-NP encapsulating DNA, and 3. DNA-adsorbed Ca-P-NP) to produce micronised, inorganic DNA carriers for pulmonary delivery. Chitosan was chosen for its proven use in mucosal macromolecule delivery and excellent muco- and bioadhesive properties (Takeuchi et al 2005). Briefly, 25 ml sodium-bis(ethylhexyl)sulphosuccinate (AOT, 0.1 M) in hexane was mixed with 50 μl aqueous calcium chloride ($CaCl_2$, 20% w/v), 400 μl double-distilled water (DDH_2O) and 50 μl plasmid DNA (pDNA, gWIZ luciferase (Aldevron), 6732 bps, 1000 μg/ml) and stirred continuously for 48 h to form microemulsion-1. A second 25 ml aliquot of AOT, 50 μl aqueous disodium hydrogen phosphate (Na_2HPO_4, 5% w/v), 350 μl DDH_2O, 50 μl 0.2 M Tris-HCl buffer (pH 6) and 10 μl pDNA (400 μg/ml) was dissolved by continuous stirring for 48 h to form microemulsion-2. Microemulsion-2 was added to microemulsion-1 (at 5 ml/h) while stirring continuously at < 10°C; stirring was continued for a further 6 h before a 30 min centrifugation. The pellet was washed 3× with absolute ethanol and dispersed in DDH_2O by vortexing to give a clear dispersion. The size of CDC and Ca-P-NP was assessed by photon correlation spectroscopy (Malvern Instruments, UK) and varied between 150–200 nm for the complexes and 80–100nm for both empty and loaded Ca-P-NP, respectively. Aqueous chitosan–pDNA, complexes were prepared by admixing plasmid DNA (100 μg/ml) and chitosan (300 μg/ml) in 2 mL of purified water containing 50 mg of lactose and left for 30 min at room temperature. Dry powder forms of CDC, Ca-P-NP (encapsulating DNA) and empty Ca-P-NP were obtained by laboratory-scale spray-drying (Büchi Mini-spray dryer, Switzerland). The spray-drying parameters used were as follows: T_{Inlet} 145 ± 2°C, T_{outlet} 90 ± 3°C, feed rate 10 ml/min, airflow rate 800 Nl/h and aspirator 80%, respectively. Each formulation was mixed with 2% (w/v) lactose before spray drying (Sigma, UK) and yield varied between 30–35%. After spray drying, particle size was assessed by laser diffraction (MasterSizer, Malvern Instruments, UK) and revealed that CDC had increased to 5–6 μm, whereas for both empty and loaded Ca-P-NP diameter < 5 μm. The integrity (i.e. DNA structure) and entrapment efficiency (EE) of pDNA was examined by agarose gel electrophoresis; in all cases the DNA remained static in the gel, indicating the formation of stable complexes with no evidence of degradation of the DNA structure and EE 60–70%. Results show that inorganic nanoparticles and ionic-complexes of a suitable size for pulmonary delivery can be produced by spray drying, with high EE and excellent stability of encapsulated DNA. Further work will characterize optimum formulation and process parameters and investigate in vitro/in vivo uptake dynamics for Ca-P-NP systems.

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192
Prevention of percutaneous absorption of nicotine by an interacting polymer

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Human skin is one of the most protective barriers of body against the external chemical and physical hazards. However, some toxic material can penetrate the skin well enough to cause poisoning and this is a particularly serious risk in the work environment. Therefore, it is necessary to prevent or decrease the percutaneous absorption of such material. Here, the possibility of preventing the percutaneous absorption of hazardous chemicals by low and high molecular weight interacting polymers has been investigated. To per-

form this study, nicotine was chosen as the model toxic substance and the effect of an interacting polymer in two different molecular weights of low (LMWP) and high (HMWP) on permeation of nicotine through excised rat skin was investigated at 32°C for 10 h, using static diffusion cells. Aqueous solution of nicotine (500 μg ml^{-1}) was used as the donor phase and distilled water as the receptor phase. Polymers were applied to the skin as chloroformic solutions followed by evaporation of the solvent and formation of thin film at the surface. The same procedure was carried out using chloroform (without polymer) in control experiments. Permeated nicotine was measured by UV spectrophotometry at 260 nm. There was no interaction between the material released from the skin and the used measurement method. The flux of nicotine in control experiment (in the absence of the polymer) was measured to be 40.0 ± 6.97 μg cm^{-2} h^{-1}; data are mean ± s.d., n = 3–6. In the presence of 2% (w/v) LMWP film, the flux of nicotine decreased by about 1.5 times, which was marginally significant ($P = 0.097$). The films of 2 and 3% (w/v) HMWP decreased the flux by about 2.6 ($P = 0.001$) and 4.3 times ($P = 0.001$), respectively. The consequence of applied 4% (w/v) film of HMWP was complete inhibition of permeation of nicotine; nicotine was not detectable in the receptor phase by the UV method. These data show that percutaneous absorption of nicotine, and possibly other drugs, can be decreased by the polymer in a molecular weight- and concentration-dependent manner. Further investigations are in progress in our laboratories to determine the mechanism of the observed retardation. The polymer is interacting and our preliminary data shows that both physical and chemical barriers play role in retardation of nicotine by the polymer. The method might be tailored for application in retardation of hazardous chemicals at work.

193
Formulation of hyaluronidase as a temperature-stable dry powder using protein-coated micro-crystals (PCMC)

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Hyaluronidase is well known as a spreading factor to improve the absorption of drugs. Numerous cancers, both of epithelial and mesenchymal origins, have been shown to exhibit elevated levels of hyaluronan relative to normal tissues and so hyaluronidase has attracted interest as an adjuvant for chemeotherapies. The purpose of this study was to produce a hyaluronidase formulation in the form of a free-flowing dry powder based on protein coated micro-crystal (PCMC) technology. Particles of this type could be used to develop alternate topical or inhalation drug-delivery strategies or form the basis for preparing sustained release formulations. Hyaluronidase formulations were made via a one-step process, which rapidly dehydrates then immobilises proteins onto the surface of pharmaceutically acceptable crystalline carriers, such as amino acids, producing PCMCs. Several hyaluronidase PCMC formulations were produced using a 5-factor full-factorial Design of Experiment protocol. These PCMCs were stored under four different storage conditions ranging from room temperature to 40°C, and analysed periodically over 6 months. The stability of formulations were analysed by SDS-PAGE and by measuring the hyaluronidase-PCMC activity using hyaluronic acid. The assay used is based on a turbidity, whereby hyaluronidase breaks down hyaluronic acid. The amount of hyaluronic acid left can be quantified by assessing the turbidity produced when blended with an acid albumin solution, measured at 600 nm. SEM was also used to analyse the size and shape of the crystals produced. Most of the hyaluronidase PCMC formulations were found to produce crystals that were greater than 50% active after 6 months under stressed conditions. Several formulations appeared to have increased activity compared with the supplied hyaluronidase, thought to be due to protein refolding due to a "solvent annealing" process. Seven of the formulations produced were also found to have greater than 100% activity after 6 months. The SDS-PAGE analysis showed no aggregation/dissociation/modification or cleavage in either the formulation stage or in the storage period, however four of the samples became insoluble during the experiment. SEM showed normal size and shape distributions for PCMC crystals. In conclusion optimal hyaluronidase PCMCs formulations exhibited attractive properties with respect to alternate drug-delivery applications. They were produced as free-flowing powders made up of micron-dimension particles resistant to temperature and humidity. The immobilised enzyme remained fully soluble and catalytically active after six months storage under accelerated stress conditions.

194
Novel antiadherent polyurethane for medical device coatings

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Urinary catheters are widely used medical devices whose use is limited by problems associated with microbial biofilm formation (Jones et al 2005). Biofilm formation is of considerable importance because a biofilm can protect bacteria from concentrations of antimicrobial agents that would otherwise be active against planktonic bacteria. One method by which biofilm formation may be reduced involves the controlled release of antimicrobial agents from the catheter surface. However, the diffusion of antimicrobial agents from currently available medical device biomaterials is frequently inappropriate and therefore alternative strategies are required to ensure the optimum drug release rate/duration. Drug diffusion may be controlled is by the use of ion-pairing agents as this offers a means to modify the physicochemical properties of the diffusant. In this study, the effect of ion-pairing the antimicrobial agent chlorhexidine (chx) with myristic acid (ma) on the release profile of chlorhexidine from polyurethane films and on the antiadherant properties of the polyurethane after different periods of exposure to drug release medium are examined. Polymer samples were produced by dissolving polyurethane and additives in tetrahydrofuran, pouring onto glass plates and evaporating the solvent. Films containing: chx 2.5%; chx 2.5% + ma 2.26%; ma 2.26%; polymer alone, were produced. The samples were exposed to a release medium consisting of pH 7 citrate buffer for various time periods. The samples were exposed to an *S. epidermidis* culture for 4 h and 24 h time periods and the adherent bacteria enumerated by serial dilution. In samples not exposed to the buffer (time 0), the level of bacterial adherence was significantly lower in the samples containing chx and the samples containing chx + ma than the blank. As the time of exposure to the buffer increased, the adherence on the chx – only film increased until at 28 days, there was no significant reduction in adherence compared with the blank. In contrast, the film containing chx + ma showed a significant reduction in adhered bacteria after 28 says and 56 days exposure to the buffer. This study demonstrates that ion-pairing chlorhexidine with myristic acid results in an improved duration of the antiadherant properties of a polyurethane biomaterial exposed to a biological fluid.

Table 1 Adherence of *S. epidermidis* to polyurethane samples, pre-exposed to pH 7.2 buffer for differing periods of time, after 4 h exposure to a bacterial suspension

Time of Exposure to buffer (days)	Adherence of bacteria to films containing chlorhexidine only, expressed as a % of blank	Adherence of bacteria to films containing chlorhexidine + myristic acid expressed as % of blank
0	2.43 ± 0.79	1.79 ± 0.82
1	30.83 ± 3.42	4.84 ± 0.27
3	8.62 ± 0.34	6.76 ± 0.45
7	83.62 ± 12.76	2.55 ± 0.12
14	38.23 ± 4.95	22.67 ± 3.67
28	85.42 ± 16.67	15.24 ± 4.97
56	109.69 ± 8.54	6.64 ± 0.77

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195
Preparation and characterization of polymer-lipid composite nanoparticles as DNA carriers

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Nanoparticles formulated from biodegradable polymers such as poly (lactic acid) and poly (lactide-co-glycolide) are being investigated as non-viral delivery systems due to their slow release characteristics and biocompatibility. However, encapsulation of DNA into these carriers suffers from poor loading efficiency and degradation of the encapsulated DNA due to exposure to shear stress and organic solvents during preparation. Furthermore, during the release process, the encapsulated DNA suffers massive degradation due to the acidic microenvironment created inside the particles (Walter et al 1999). The use of poly (lactic acid)-poly(ethyleneglycol) (PLA-PEG) copolymers has been proposed to reduce the latter problem (Perez et al 2001). The objective of the present work is to formulate cationically modified PLA-PEG nanoparticles with defined size and shape to ensure efficient binding of DNA by simple mixing with the nanoparticles. PLA(10k)-b-PEG(1k) copolymer was chosen for this study. In particular, we attempt to design DNA carrier system that combines the reported high transfection efficency of cationic lipids with the stability of biodegradable polymer nanoparticles. With this view, two types of cationic lipids were investigated as possible charge modifiers; 3[N-(N', N'-dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol) and the novel cationic lipidic lysine-

based dendron $(C14)_3Lys_7(NH_2)_8$ (LLD). Particles were prepared using the nanoprecipitation method. Briefly solution of the polymer and the charge modifier in an organic solvent was added drop wise onto water or water/ethanol mixture. Solvents were allowed to evaporate at room temperature. The effect of formulation parameters namely; polymer concentration, solvent composition, antisolvent type, and the cationic component concentration on the particle characteristics such as size, zeta potential, morphology have been studied, and morphology have been studied. DNA loading capacity was optimized by changing the concentration of the cationic lipid, nanoparticles and/or DNA as well as DNA/nanoparticles ratio, and the adsorption medium. Particles less than 200 nm in diameter were obtained using optimal formulation parameters. Conditions to obtain highest loading capacity and a minimal increase in the particle size and polydispersity were identified. The results show that the zeta potential of the particles increased as the cationic lipid concentration increased and reached a plateau, probably due to saturation of the nanoparticle surface. Gel retardation assays showed that the DNA binding capacity of the particles depends upon the type and concentration of the cationic lipid used. For example nanoparticles containing 5% m/m LLD (zeta potential + 30 mV) were able to bind up to 3.5% m/m DNA while those containing the same concentration of DC-Chol, although having a much higher zeta potential (+ 52 mV), bind a maximum of 2% m/m DNA. These results suggest the possible exposure of the cationic lipids on the nanoparticle surface, and hence the involvement of different types of DNA/cationic lipid interactions at the particle interface. LLD containing nanoparticles appear to form much stronger complexes with DNA for all the concentrations tested as shown by anion exchange assay using heparin sulphate. In conclusion, the method described represents a simple and a highly reproducible technique for the preparation of cationic lipid modified nanoparticles with high DNA binding capacity. Further work is currently being undertaken in order to investigate the transfection capacity of mammalian cells and to learn more about their stability in vitro.

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196
Effect of ionising radiation on gene delivery in 3-D cell models

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Many promising new drug therapies such as gene therapy are not effective because the large complex drug molecules fail to penetrate into the diseased target tissue. A number of novel cancer therapies combine ionizing radiation with gene therapy (Harari & Huang 2001). Ionizing radiation increases delivery and expression of reporter genes in tumor cell monolayers (Stevens et al 2001). The extracellular matrix may be modified and remodelled in response to radiation. This could affect the penetration of gene vectors. This project will study how changes in tissue structure caused by radiation for cancer can affect the penetration of therapeutic biomolecules and possibly be exploited for more effective therapy. Multicellular assemblies provide 3-dimensional cell-cell interactions, which alter cell properties, and they provide a penetration barrier to large particles, similar to the barrier posed by tissue. Cells grown in 3-D culture are more sensitive to damage by the radiation "radiological bystander effect" than the same cell type grown in monolayer (Boyd et al 2002). We will use spheroids to simulate in vivo conditions. Measure the effects of ionizing radiation on the delivery and expression of reporter genes in tumor cell monolayers and spheroids. Effect of radiation on gene expression in monolayers was assessed with flow cytometry. Cells were seeded on petri dishes and irradiated at different dose (nonirradiated cells as control). After 36 h infected with optimized amounts of adenovirus cells were then with PBS, fixed and assessed with flow cytometry (Table 1). Effect of radiation on gene expression in HT29 spheroids was carried by disaggregating spheroids into single-cell suspension after the same treatment with monolayers and assessing with flow cytometry. Spheroids growth delay was carried by identifying individual spheroids of their cross-sectional area over 3 weeks with Confocal Microscopy (Zeiss LSM510) using wide-field DIC (interference contrast) and laser scanning confocal fluorescence, exciting fluorescence with blue (488nm) laser lines. In monolayers, control intensities were near zero and not affected by radiation; GFP expression in HCT116 was the greatest and had the strongest enhancement by radiation. HT29 also showed an apparent increase around 7% with radiation dose. Others also showed effect, probably underestimated due to the cell toxicity. HT29 spheroids showed strong enhancement in GFP expression at about 100% increase with radiation. The diameter of spheroids after radiation was 100um smaller than the ones without radiation. Radiation can enhance the expression of GFP in both monolayers and spheroids. The increase is dramatically higher in spheroids than in monolayer. Gene delivery and expression were evenly distributed throughout the spheroids. Radiation also has the growth delay effect on spheroids.

Table 1 Effect of radiation on adenovirus infection efficiency

Cell lines	MOI	Radiation dose (Gy)
HCT116	2,10	0,2,4,6
HT1080	20	0,2,4,6
HT29	20	0,2,4,6
MDA-MB-231	20,50	0,2,4,6

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