Drug delivery

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Molecular simulations of skin lipids with DMSO: insights into penetration enhancer activity

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The lipid layers of the stratum corneum (SC) constitute the main barrier to penetration of exogenous substances. Overcoming this barrier is the foremost problem in transdermal delivery. One approach is to use penetration enhancer molecules that interact with the skin lipids to facilitate the transport of the molecule through skin. While there is considerable interest in identifying penetration enhancers, their mechanism of action at the molecular level is not fully understood. A greater understanding of the mechanism of action of these molecules could assist in their rational design. Dimethylsulphoxide (DMSO) at high concentrations enhances the penetration of both hydrophilic and hydrophobic drugs. While it is clear that DMSO interacts with the lipid layers of the SC, its precise mechanism of action remains elusive (Williams & Barry 2004). The lipid layers of the SC comprise mainly ceramides, cholesterol and free fatty acids. In this study we investigate the effects of DMSO on the properties of ceramide bilayers with the aim of gaining insights into the possible mechanism of action of DMSO. We have carried out atomistic molecular dynamics simulations of up to 40ns of bilayers consisting of 128 or 512 ceramides with a range of concentrations (0–60%) of DMSO. In a molecular dynamics simulation one follows the trajectory of the interacting atoms in the system as a function of time. Thus, it is possible to gain a molecular level view of a system, which may not be accessible by experiment. In our earlier work we observed spontaneous pore formation in phospholipid bilayers with high concentrations of DMSO. We have more recently carried out simulations on ceramide bilayers to characterise the free energy barrier to pore formation in the presence and absence of DMSO. Our results show that DMSO acts primarily through interactions at the bilayer/water interface. At low concentrations (up to 30% DMSO) the DMSO accumulates at the headgroup region of the bilayer but has little effect on the properties of the bilayer. At high concentrations (40% and above), DMSO induces a transition in the bilayer from the ordered, tightly-packed gel phase, which is characteristic of the skin lipids, to the disordered, loosely-packed liquid crystalline phase. Alongside this phase transition, we observe a significant expansion of the bilayer (increased area per lipid), a decrease in the bilayer thickness, an increase in the depth of penetration of the solvent into the bilayer, and a decrease in the bilayer elastic modulus. Our preliminary results from the pore formation studies indicate that pore formation in ceramide bilayers is a very difficult process but that it becomes significantly easier in the presence of DMSO. In conclusion, it has been found that DMSO fluidises ceramide bilayers at high concentrations. This fluidisation is likely to result in a significant reduction of the barrier to penetration of substances. DMSO also induces the formation of water pores that may important for enhancing the diffusion of hydrophilic molecules. The results also suggest that DMSO reduces the length of the molecular diffusion pathway.

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Application of gas-filled liposomes as pressure probes for oil extraction: a novel and simple preparation technique

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Although known for delivering drugs, liposomes encapsulating gas are reported to be useful in magnetic resonance imaging (MRI) as pressure probes (Alexander et al 1996). The relationship between MRI signal from this biphasic fluid and permeability of the rock is a measure of the pressure required to obtain a given fluid rate in porous media, a fundamental characteristic of oil reservoirs. This knowledge can be harnessed in recovering greater amount of oil from the porous rock using gas-filled liposomes (GFL) (Perrie 2005). In preliminary characterisation studies, we investigated the effect of lipid composition on the GFL size and stability prepared using a novel homogenisation technique. 50 mg of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) or 1,2-distearoyl-*sn*-glycero-3-phosphocoline (DSPC) was homogenised for 2 minutes below phase transition temperature (T_c) of lipid in 5 ml water with or without cholesterol and stearylamine. 0.2 ml of GFL was dispersed in 1.8 ml of 2%w/v methyl cellulose to give a final GFL concentration of 10%v/v. Vesicle size was measured on Malvern Mastersizer and zeta potential on ZetaPlus (Brookhaven Instruments, USA). Vesicle size was reduced by extruding (Avestin, Canada) GFL through polycarbonate membrane and their morphology was studied on optical microscope. Results showed that, though initial sizes of DPPC and DSPC based GFL were 8.6 ± 1.0 μm and 9.2 ± 0.9 μm, respectively, on day 7 at 25°C, the sizes significantly ($P < 0.05$) increased to 12.3 ± 1.0 μm and 28.3 ± 0.3 μm, respectively, presumably due to vesicle fusion and/or aggregation. Preparation of GFL below T_c was considered to be crucial since, an attempt to produce GFL using 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and egg-phosphatidylcholine (PC) has failed presumably due to low T_c which in turn yielded liposomes with aqueous core. When DPPC was supplemented with cholesterol at 1:0.5 and 1:1 molar ratios, a significant increase ($P < 0.05$) in the vesicle sizes (i.e., 12.9 ± 0.8 μm and 12.4 ± 0.2 μm, respectively) was observed. However, the vesicle sizes remained unchanged over 14 days at both the ratios stored at 25°C and 4°C, indicating a stabilising effect of cholesterol on the vesicles which is known not only to enhance the bilayer rigidity but also improve the resistance against aggregation and fusion of vesicles. To induce surface charge on the GFL, DPPC and cholesterol (1:0.5 molar ratio) was further supplemented with stearylamine (a positively charged lipid) at molar ratios of 0.25 and 0.125. Interestingly, the zeta potential values remained neutral (1.8 ± 0.3 mV) comparable with those in the absence of stearylamine, suggesting that stearylamine did not get accommodated within the bilayer, presumably due to the rigid gel phase of DPPC making it difficult for the lipid to incorporate in the bilayer. Microscopic analysis of GFL showed spherical structures with size distribution between 1 and 8 μm. Extrusion of these preparations through 800 nm polycarbonate membrane lead to a uniform size distribution of 0.8–1 μm. In conclusion, GFL prepared using DPPC in the presence of cholesterol (1:0.5 molar ratio) suspended in 2%w/v methyl cellulose yielded a stable preparation at both 25°C and 4°C over 14 day time period and could serve as potential pressure probes.

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Poly(vinyl alcohol) hydrogels using a multifunctional cross-linker, PD2000

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A novel multifunctional reagent, PD2000, has been used to produce poly(vinyl alcohol) (PVA) chemical hydrogels, which are currently finding application in the biomedical field as matrices for drug release and temporary tissue protection and substitution. The mechanism of action of PD2000 is based on the acid-catalysed formation of cyclic acetals with PVA alcohol groups. However, since PVA exhibits three regiochemically distinct alcohol groupings (the regioregular 1,3 diols are flanked by variable quantities of 1,2 and 1,4 diols arising from polymerisation head-to-head or head-to-tail defects), it is unclear whether PD2000 preferentially or selectively binds to one of these groups. The reaction of PD2000 aldehydes was first studied with low MW compounds that model the different PVA alcohol groupings. Results suggest 1,2 and 1,3 diols react similarly, while isolated alcohols (used as model for 1,4 diols) seem not to show significant reactivity. The reaction was then performed on PVA samples characterised by different MWs, degree of hydrolysis (= amount of residual acetyl groups) and 1,2 diol content and studied by recording gelation kinetics and cross-linking density of the final gels. The results showed a strong influence of the cross-linker concentration (expressed as ratio between reacting groups) and of the degree of hydrolysis. Both factors boost the number of reacting groups. The polymer MW had a moderate influence; shorter "medium MW" (always in the $100–200 \times 10^3$ g/mol) polymers apparently react faster than larger ones; however, "low MW" polymers react slowly. There was a negligible effect of the presence of 1,2 diols, which confirms the results obtained for low MW model compounds. This finding was further confirmed by the fact that gels were obtained also performing the reaction on PVA samples without 1,2 diols (obtained by cleaving PVA diols with periodic acid). PVA was characterised using the Mark Houwink equation to calculate molecular weight, the Periodate test to calculate 1,2 diol content, and IR and NMR to calculate the degree of hydrolysis. Reactions with model compounds were followed by monitoring the aldehyde concentration through IR absorption and fluorescent labelling (Alizarin Red S). Gelation experiments were followed through shear rheometry, monitoring the increase in elastic and viscous modulus with time. The complex modulus was used to calculate the number of cross-links formed. The gelation of PVA with PD2000 has been demonstrated to occur between 1,2 and 1,3 diols with little preference, but not to occur with isolated alcohols or 1,4 diols.

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In-vitro release of the HIV microbicide TMC120 from a silicone intravaginal ring

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The antiretroviral candidate TMC120 (Dapivirine) is currently being evaluated for use as a vaginal HIV microbicide. The potent, hydrophobic nature and small molecular size of this compound make it an ideal candidate for release from a silicone vaginal ring (VR) system. To date, most VR in-vitro release studies have been performed under sink conditions thereby providing an environment whereby all drug released from the VR dissolves in the surrounding dissolution medium (Malcolm et al 2005). Under these conditions the rate of drug release is said to be device-controlled. Although such conditions provide useful information regarding the diffusive release of drugs from VR systems, they may not be truly representative of in-vivo conditions. Therefore, it is necessary to investigate release under conditions that more closely mimic those of the vaginal environment. In this study, the potential for the controlled delivery of TMC120 from silicone VRs under both sink and non-sink conditions have been evaluated. TMC120 reservoir-type VRs incorporating 5% w/w TMC120 (2.0 mm core and 7.6 mm ring cross-sectional diameters) were manufactured on a laboratory-scale, ring-making machine according to standard methodology outlined in the literature (Woolfson et al 2003). A series of in-vitro dissolution studies were preformed using a variety of dissolution media including a 1:1 isopropanol (IPA)/water mixture designed to provide sink conditions, simulated vaginal fluid (SVF) designed to provide non-sink conditions and a micellar phospholipid solution, Lipoid 75, designed to mimic the phospholipid cell membranes of vaginal tissue and to account for the likely occurrence of tissue uptake. Samples were taken on a daily basis with complete replacement of the dissolution media. In-vitro release characteristics were evaluated using HPLC analysis. The cumulative release data (Table 1) demonstrates that TMC120 release from reservoir-type VRs may be achieved under both sink and non-sink conditions. However, the composition of the release media greatly influences release characteristics and rates. As expected, release under sink conditions provided maximum TMC120 release rates. Controlled, sustained release was achieved throughout the four week dissolution study. However, due to the extremely low aqueous solubility of TMC120, release rates into SVF, an essentially aqueous medium that possesses the same general chemical composition, pH and osmolarity of human vaginal fluid (Owen & Katz 1999), were extremely poor with daily release levels remaining below the HPLC limit of detection until day eighteen, after which daily concentrations of less than 1 μg/day were detected. By comparison, release of TMC120 into Lipoid 75 solution provided intermediate levels of release; although significantly less than those achieved under sink conditions there was substantial uptake of TMC120 by the phospholipid micelles. These results suggest that a release model that takes into account the possibility of tissue uptake may be more representative of the in-vivo scenario than release into a completely aqueous dissolution medium.

Table 1 Cumulative release data for TMC120-loaded reservoir-type VRs

Time (days)	Cumulative release (μg) into 100 ml		
	IPA/water	SVF	Lipoid 75
1	110.0	0	16.9
7	425.9	0	99.1
14	835.9	0	171.4
21	1268.8	3.9	239.4

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The use of AFM to investigate how fines improve DPI performance

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The addition of a small amount of fine excipient ("fines") to a carrier-based dry powder inhalation (DPI) system is known to improve formulation performance (Islam et al 2004). Two hypotheses have been proposed to explain this effect – the saturation of strong binding sites on the carrier or the formation of mixed agglomerates of fines and drug particles (Islam et al 2004). The cohesive-adhesive balance (CAB) approach to adhesive force measurement by atomic force microscopy (AFM) (Begat et al 2004a, b) was utilised to investigate these hypotheses. This normalises the variation in the contact area of different AFM colloid probes, giving a quantitative measure of the ratio between the cohesion and adhesion of different materials (Begat et al 2004a). Four drugs (budesonide, formoterol fumarate dihydrate (FFD) and drugs A and B) and four excipients (erythritol, lactose, mannitol and trehalose) were used. The cohesion of three colloid probes of each drug and their adhesion to each fine excipient was measured using force-volume AFM, as previously described (Begat et al 2004a). The cohesion of each colloid probe was plotted against its adhesion to each excipient, in accordance with the CAB method. Linear regression was used to normalise the variation in adhesion caused by variation in the contact area of different probes ($R^2 > 0.83$ in all cases), the gradient of each line giving the ratio between the cohesion of the drug and its adhesion to the excipient in question. This is known as a CAB ratio. Binary and ternary formulations of each drug-fine excipient combination were produced, containing 1:67.5 drug:coarse lactose carrier. Ternary formulations also contained 10% micronised excipient (erythritol, lactose, mannitol or trehalose). Formulations were tested by aerosolisation from a Rotahaler at 60 L.min^{-1} into a NGI and the results summarised in the table. As has been previously described, fine particle dose (FPD) was increased by the addition of fines (Table 1). To allow the performance of different drugs to be compared, ternary formulation FPD was normalised by division by the relevant binary formulation FPD. A plot of normalised FPD vs. CAB ratio demonstrated that when the drug was more adhesive to the fines than cohesive (CAB

Table 1 Mean FPD (mcg ± SD) of each formulation (n = 4)

	Binary	Erythritol fines	Lactose fines	Mannitol fines	Trehalose fines
Budesonide	18.9 ± 3.5	22.1 ± 0.9	34.8 ± 3.7	31.9 ± 2.6	20.7 ± 1.7
FFD	15.6 ± 1.6	16.5 ± 0.6	37.0 ± 1.4	25.9 ± 2.4	22.3 ± 4.2
Drug A	6.8 ± 0.8	12.1 ± 2.0	20.6 ± 2.0	22.9 ± 3.4	18.5 ± 1.8
Drug B	12.6 ± 1.0	27.4 ± 11.7	28.7 ± 3.2	37.3 ± 9.5	29.1 ± 9.9

ratio < 1), stronger drug-fines adhesion was associated with larger FPD. This concurs with the recent hypothesis that DPI performance is optimised when the drug is aerosolised in agglomerates of a critical size, but with internal adhesive forces that are not excessive (Begat et al 2004b). Increased drug-fines adhesion is likely to result in increased agglomerate size, giving better performance due to the larger drag forces that act on larger agglomerates (Begat et al 2004b). When the drug was more cohesive than adhesive to the fines (CAB ratio > 1), formulation performance was not influenced by drug-fines interparticulate interactions. This study suggests that for certain drug-fines combinations, formulation performance is improved via the formation of mixed agglomerates of drug and fines, as has been previously suggested (Islam et al 2004). For other combinations, however, alternative mechanisms might be responsible for improving formulation performance.

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Liposomal adjuvant systems: the effect of surface charge and liposome morphology on protein release and protective immunity

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Liposomes have been extensively recognised as potent stimulators of the immune response (Therien & Shahum 1996). However, the physiochemical characteristics of liposomes and their adjuvanticity is dependent on the manner in which the protein antigen is physically associated with liposomes (i.e. surface-adsorbed or entrapped) and the way in which the antigen is presented may govern the type of the immune response (Leserman 2004). Here we assess the effect of liposome morphology, method of antigen incorporation and lipid composition on liposome physiochemical characteristics, protein release in vitro and essentially the level of protective immunity in vivo. Cationic liposomes composed of 2 μmol dimyritoylphosphotidylcholine, 1 μmol stearylamine and 0.3 μmol trehalosedibehenate were prepared either prepared by the dehydration-rehydration procedure (DRV) entrapping protein antigen established by Kirby & Gregoriadis (1984) or by film technique method to produce multilamellar vesicles (MLV) liposomes with surface adsorbed antigen. Anionic DRV liposomes composed of 1.8 μmol dimyritoylphosphotidylcholine, 0.2 μmol phosphatidylglycerol and 0.3 μmol trehalose dibehenate. The z-average diameter and zeta potential was measured in double-distilled water or 0.001 M PBS, respectively, using a Brookhaven ZetaPlus. P rotein incorporation was determined by ^{125}I-labelled protein. For protein release, each formulation was incubated at 37°C maintained within sink conditions. At various time points protein release from each formulation was determined by ^{125}I-labelled protein. C57Bl/6j mice were subcutaneously immunised with liposomes containing 2 μg Ag85B-ESAT-6 fusion protein. Isolated lymphocytes were restimulated with Ag85B-ESAT-6 in vitro, both one week after second and one week after the third immunization and IFN-γ production was measured as an indicator of the Th1 immune response. Analysis of variance (anova) and tukey's post hoc test were performed, with the statistical significance determined to 0.05 confidence intervals ($P < 0.05$). Cationic DRV liposomes were significantly ($P < 0.05$) larger (924 nm) than anionic DRV or cationic MLV liposomes (479 nm and 595 nm, respectively). High protein entrapment determined for SA-containing DRV liposomes (97%) could be attributed to electrostatic interactions between the cationic charges of these liposomes and the anionic charges of the protein (i.e. Ag85B-ESAT-6), as the antigen is highly negatively charged (Davidsen et al 2005). In addition, the incorporation of protein within anionic liposomes is significantly less ($P < 0.05$) (77%), presumably due to the lack of these electrostatic interactions. In this instance, the protein antigen could be bound to the liposomes inner bilayers by hydrophobic interactions. MLV are preformed structures, in which the internal lamellar layers are inaccessible to antigen, resulting in relatively low passive incorporation efficiencies of 60%, significantly ($P < 0.05$) lower than DRV systems. Anionic DRV liposomes, consisting of PG, released its protein content much more rapidly than either of the cationic liposome preparations, as 86% of total entrapped protein was released after 14 days incubation at 37°C, whereas SA-containing DRV and MLV liposomes exhibited a significantly slower release rate (52% and 24%, respectively) at the same time period. Conversely, after 28 days cationic MLV liposomes exhibited a burst release of 90% protein antigen whereas DRV liposomes maintained its sustained release (77% of protein entrapped). After 42 days incubation all formulations released of 90–94% antigen, however, the rate of release over time varies to some extent. Anionic liposomes show an initial enhancement in IFN-γ release, however, one week after the third immunisation, cationic DRV liposomes produced considerably higher values of IFN-γ production compared to anionic DRV and cationic MLV liposomes. Presumably due to the slow and sustained rate of protein release from these cationic DRV liposomes. Liposome-entrapped protein, prepared by the DRV procedure, generates considerably higher values of protein incorporation than surface adsorption. Therefore, with the protein entrapped within the lipid bilayers, the protein content is released at a slow and sustained rate, therefore maintaining therapeutic levels of antigen for presentation to APC.

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Hyper-DSC as a tool to determine solubility of ibuprofen in a wax based matrix

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Hyper–Differential Scanning Calorimetry is a relatively new approach used to determine solubility of drugs in semi-solids (Gramaglia et al 2005). In this study, we applied this approach to determine the solubility of a model drug; ibuprofen, in wax based system. Ibuprofen loaded wax based matrices were prepared by combining ibuprofen with Witepsol H15 (via melting) at the following concentrations 1, 2, 5, 10, 20, 25, 30 and 50% w/w. Samples (1.80 ± 0.20 mg), cut from the ibuprofen loaded wax based matrix were sealed in air tight aluminium pans (Perkin-Elmer) and analyzed at 500°C/min over a temperature range of −30°C to 200°C with Helium as a purge gas using a Diamond DSC, Perkin Elmer. The resulting thermogram was analyzed using Perkin-Elmer Pyris series 5.0 software. Five repeats were performed and an empty pan was used as a reference in all cases. Theeuwes et al (1974) identified that the fraction of drug solubilised in a matrix does not contribute to the associated endothermic peak observed when the drug is dispersed in the same matrix. Therefore a separate melting endotherm for the drug compared to the wax was used as an indicator that the drug was not soluble in the wax. At ibuprofen concentrations below 25% w/w of the wax matrix, only one well defined endothermic peak in the region of the wax melting point (25–30°C) was observed. At 30% w/w, another smaller endothermic peak (68 ± 1°C) was seen which became more prominent at 50%w/w ibuprofen loading. This second peak is due to dispersed drug within the matrix although this is observed at a lower temperature than the melting point of the pure drug (due to the mixing of this with the wax). Using the highest scan rate possible (500°C/min), ibuprofen solubility was found to be between 25 and 30% w/w. Drug release studies will be performed to determine whether Higuchi kinetics support this finding. The main advantage of this approach is the inhibition of kinetically controlled transitions such as polymorphs inter-conversion and recrystallisation due to the fast heating rates. This study has shown that solubility of ibuprofen in wax based matrix can be determined via hyper –DSC thermal analysis. This may be used as a technique to measure drug solubility within suppositories or other wax-based dosage forms.

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Microfabricated microneedles: a novel strategy for enhancing topical delivery of 5-aminolevulinic acid and preformed photosensitisers

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Photodynamic therapy, based upon topical administration of aminolevulinic acid (ALA), is a novel treatment for premalignant and malignant skin lesions. Application of exogenous ALA stimulates the over-production of protoporphyrin IX (PpIX), an effective endogenous photosensitiser. This effect is prevalent in rapidly proliferating neoplastic cells and explains selective lethality of the therapy. However, ALA is a small hydrophilic (167.6 dalton) molecule and zwitterionic at physiological pH. Consequently, permeation across intact *stratum corneum* is poor (Malik et al 1995; Casas et al 2000). Due to this reason, a number of methods have been investigated and employed to improve ALA penetration. These include tape stripping, permeation enhancers, ion pairing, iontophoresis, laser SC ablation and formulation development. In this study, we aimed to enhance topical delivery of both ALA and preformed photosensitisers using novel microneedle (MN) technology. A silicon MN is a mechanical approach to bypass the outermost layer of the skin, the stratum corneum (SC) and is a physical technique to increase drug delivery through skin, working simply by puncturing the SC. Importantly though, these solid needles do not protrude far enough to reach pain receptors, ensuring that application is without sensation. ALA and porphyrin loaded, bioadhesive films were cast from drug containing aqueous blends of

poly(methylvinylether.maleic anhyhydride) (PMVE/MA), suitably plasticised using tripropylene glycol methyl ether (TPM). Silicone MN arrays were fabricated using wet etch technology to produce needles of approximately 250 μm in length and 240 μm in base diameter with interspacing of approximately 750 μm. In vitro permeation studies were performed using the Franz cell model, employing both silicone and excised mouse skin as model membranes. Animal experiments were approved by the animal department of the Norwegian Radium Hospital. In vivo PpIX accumulation studies were performed by first puncturing the dorsal skin of anaesthetised female nude mice for 15 s using the MN array. Subsequently formulations were applied for 4 h. Upon removal of the vehicle, fluorescence in-vivo was measured using a fiber-optic probe coupled to a Perkin-Elmer LS50B luminescence spectrometer (excitation 407 nm, emission 635 nm). In vitro permeation studies showed a 200-fold increase in ALA penetration across the model silicone membrane and a 3-fold increase across full thickness mouse skin. The penetration of a pre-formed photosensitiser was also shown to be significantly enhanced. The accumulation kinetics of PpIX followed a similar profile for all drug formulations. Following removal of the vehicle, PpIX fluorescence peaked at 3–6 h and reduced to baseline levels at 24 h. Puncturing the skin using MN arrays was shown to enhance photosensitiser production significantly when compared to control ($P = < 0.05$). To date, topical PDT has been restricted due to the relatively poor penetration of ALA and pre-formed photosensitisers into lesions. This study illustrates that MN technology is a novel strategy of overcoming the principle barrier to drug penetration into skin.

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The thermochemical stability of methadone dosed via reefers

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For many years, opiate addicts were treated with injectable heroin although by the late 1970s the amount of heroin prescribed had decreased as it was replaced by the safer alternative, methadone. While the majority of patients receive their methadone replacement therapy in the form of an oral liquid or an intravenous injection, a small proportion are prescribed reefers, usually containing 30 mg or 60 mg of methadone per cigarette. However, moral and legal concerns related to the prescription and supply of tobacco-based reefers has led some physicians to switch addicts over to reefers based on herbal cigarettes. The use of reefers as a delivery system poses a number of important questions; what proportion of the methadone is available to the patient and how much is lost during combustion? What combustion products may be inhaled and could they be considered toxic? What may be the impact of change in the base material from tobacco to some other herb? In a commercial setting, such data would be required before a regulatory agency would grant a license or license variation but as the supply of reefers is essentially extemporaneous dispensing, no such requirements exist. In this study, 30 mg methadone reefers were prepared according to a standard procedure (Anon 1993) and were combusted so as to allow collection of the mainstream (= inhaled) smoke, sidestream smoke, ash, filter and the oily deposit carried by the smoke. These various fractions were extracted and qualitatively and quantitatively analysed by HPLC, LC-MS and GC-MS. A number of reefers were quantitatively analysed by HPLC prior to combustion whilst thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were also performed on the methadone hydrochloride starting material. The data obtained indicates that approximately 44% of the methadone in a reefer was recovered from the various fractions collected. Of this, 54% was found in the filter with only 46% being present in the smoke, that figure comprising both the mainstream smoke (43%) and sidestream smoke (3%). Obviously, the amount actually inhaled by a patient using a reefer would depend on factors such as duration & vigour of inhalation, factors that would modulate the mainstream:sidestream smoke ratio. Notwithstanding that, however, it would appear that only approximately 20% of the dose of methadone in a 30 mg reefer would be inhaled. The actual bioavailability of inhaled drug would depend on the particle size of the inhaled methadone, if it is adsorbed onto smoke particles rather than being a true vapour, as well as the state of health of the patient’s lungs. LC-MS of the extracts of the mainstream smoke revealed the presence of degradants, which appear to be intramolecular ring-closure products. These include the N-desmethyl product 2-ethylidene-1,5-dimethy-3,3-diphenylpyrrolidine (EDDP) which has previously been reported as being a metabolite present in the urine of methadone patients (Ferrari et al 2004).

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An investigation into electroporation to increase drug permeation into the eye

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Drug delivery to the eye, especially to treat intraocular diseases, is a formidable challenge since the eye is exquisitely impervious to foreign substances. For many diseases affecting the inner eye, intravitreal injection is the main route of drug delivery. To avoid the inherent disadvantages of intravitreal injection, we investigated electroporation — the application of brief pulses of large voltage — as a means of enhancing the permeation of topically applied drugs into the eye. Electroporation to the eye is currently being researched for gene delivery (Oshima et al 1998). In-vitro experiments were conducted using a specially designed diffusion cell and fresh cattle eyeballs. The latter were placed in the diffusion cells, with the cornea facing upwards and the back of the eye ball being bathed in a liquid medium. A flanged cylinder was placed on the cornea and a donor lidocaine solution was added. Pulses of high voltage were applied via a cathode to the donor solution which was then left in place for 1 h. The anode was present underneath the eye ball. Four different electric protocols (where pulse number, voltage, duration and interval were varied) were used. The control experiments were conducted in the same way, except that no voltage was applied. At the end of the experiment, the eyeball was dissected and lidocaine levels in the different eye tissues was measured (shown in Table 1). It can be seen that electrical application had a significant effect on drug permeation into the sclera (T test, $P < 0.05$), but not into the cornea or the lens and the vitreous humor. The absence of drug in the deeper tissues and the lack of drug permeation enhancement into the cornea could be due to the relatively mild electrical protocols that were used to avoid damage to the eye. To conclude, we have shown the potential of electroporation to increase drug permeation into the eye. In future, stronger electrical protocols will be investigated to determine effects on drug permeation into the inner eye tissues, bearing in mind the potential for eye irritation.

Table 1 Drug levels in eye tissues

Eye tissues	Drug concn (mcg/g, mcg/ml)				
	Control	Protocol 1	Protocol 2	Protocol 3	Protocol 4
Cornea	4179 (517)	5135 (158)*	3945 (386)	4390 (677)	4908 (255)
Aqueous humour	1139 (537)	1637 (446)	1504 (453)	1657 (302)	1349 (202)
Sclera	149 (54)	227 (46)*	261 (23)*	299 (99)*	263 (49)*
Lens	ND	ND	ND	ND	ND
Vitreous Humour	ND	ND	ND	ND	ND

Mean (and s.d.) are shown, n = 5. ND – not detected;
*$P < 0.05$vs control (Students *t*-test).

Oshima, Y. et al (1998) *Gene Ther*. **5**: 1347–1354

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Formulating cationic microspheres for the delivery of a sub-unit TB vaccine antigen

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Biodegradable poly(DL-lactide-co-glycolide) (PLGA) microspheres have long been exploited for their potential as controlled delivery systems for a wide range of applications due to their relative biocompatibility, as well as their proven ability to efficiently target professional antigen presenting cells (APC) (Audran et al 2003). The aim of this research is to formulate and characterise an effective microsphere delivery system with a positive surface charge to facilitate adsorption of the novel sub-unit tuberculosis vaccine, Ag85B-ESAT-6. The adjuvant dimethyl dioctadecylammonium bromide (DDA) was, therefore, added to the formulations due to its’ cationic nature, and also the proven ability to promote a Th1 immune response (Brandt et al 2000). Microspheres were prepared using a single oil-in-water emulsion solvent evaporation technique, similar to that described elsewhere (Ogawa et al 1988), using emulsion

stabilisers of a varying nature (i.e. Poly(vinyl alcohol) (PVA) (10% w/v and 1.5% w/v), Poly(vinyl pyrrolidone) (PVP) (10% w/v), Chitosan (0.75% w/v) and Protasan (a water soluble Chitosan) (0.75% w/v)). Those microspheres formulated with PVA as emulsion stabiliser at concentrations of both 10% and 1.5%, despite exhibiting mean diameters well within the desired sub-10 μm range ($0.67 \pm 0.07\ \mu$m and $1.85 \pm 0.10\ \mu$m, respectively), were, unfortunately, characterised by a negative surface charge (-5.11 ± 1.53 mV and -8.32 ± 0.97 mV, respectively), possibly as a consequence of internalisation of the DDA molecules or a masking of their cationic charge by the residual PVA on the surface. Nevertheless, all other emulsion stabilisers investigated produced microspheres that were cationic in nature whilst remaining within the desired size range: the PVP and Chitosan stabilised microspheres showed mean diameters of $5.7 \pm 1.1\ \mu$m and $4.7 \pm 1.1\ \mu$m, respectively, with corresponding zeta potentials of 20.4 ± 2.4 mV and 21.8 ± 1.0 mV, with no significant difference in size or zeta potential between the two groups ($P > 0.05$). In comparison, microspheres stabilised by Protasan exhibited significantly decreased mean diameter ($3.1 \pm 0.1\ \mu$m) and increased zeta potential (27.5 ± 2.6 mV) ($P < 0.05$). The three cationic formulations were then studied for their ability to effectively adsorb the Ag85B-ESAT-6 antigen, and although those microspheres stabilised by both PVP and Chitosan showed encouragingly good adsorption efficiencies of $74.3 \pm 1.2\%$ and $77.4 \pm 6.5\%$, respectively, the Protasan stabilised particles showed a significantly lower adsorption efficiency of $27.5 \pm 2.6\%$ ($P < 0.005$). These results show that immunomodulatory polymeric microsphere delivery systems can be formulated to exhibit the desired physico-chemical characteristics, namely size and surface charge, by optimising particular aspects or materials involved in the formulation process, in this case the nature of the emulsion stabiliser. Further studies are ongoing to determine release profiles, stability and the effect of freeze-drying of these systems.

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Needle-free skin immunization using low frequency ultrasound

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Needle-free immunization is desirable due to inherent needle-associated problems and alternative ways of vaccination are sought. One technique is the application of low-frequency ultrasound to the skin to permeabilize the latter before vaccine application. Indeed low-frequency ultrasound has been shown to assist transcutaneous vaccination (Tezel et al 2005). Here, we report our study on the influence of the various experimental parameters on the immune responses in an attempt to optimize the protocol. In-vitro results have shown a marked dependence of the extent of antigen permeation on experimental conditions, such as ultrasound protocol, nature and volume of coupling medium and distance of probe from skin (Dahlan et al 2005). All procedures had been approved by the School's Ethical Review Committee and were conducted in accordance with the Animals (Scientific Procedures) Act 1986. Pulses of ultrasound (20 kHz) were applied to the shaved abdominal skin of anesthetized Balb/c mice via a coupling medium (20 ml; water or sodium dodecyl sulphate (SDS) solution) for a total sonication time of 45 s using a probe at 7.5 mm from the skin. After sonication, vaccine (tetanus toxoid) solution was applied to the treated skin for 1 h. Two booster doses were given on days 15 and 46. Mice were bled on days 14, 45 and 60 and the serum was analyzed for antibody levels using ELISA. Intramuscular (IM) (± Alum) injections and topical applications (± SDS 1% w/v) without US were the controls. The influence of SDS concentration (0, 0.5 & 1% w/v) in the coupling medium and of ultrasound duty cycle (0.1s ON, 0.9s OFF vs 0.2s ON, 0.8s OFF in every second until total 'on' time was 45s) on antibody titres was determined. Statistical tests (Kruskal-Wallis test followed by Nemenyi test) were used. The mean antibody titres (± s.d., n = 4–5) are shown in Table 1. As expected, the negative controls and the positive controls showed low and high immune responses, respectively. Ultrasound (US) treatment alone only resulted in increased antibody titres after the second boost when it was statistically the same as the IM response ($P > 0.05$). Combination of SDS 1% w/v and US resulted in increased immune responses after first dose ($P < 0.05$) and antibody levels for all combinations of US and SDS increased with boosting. After the first dose, no difference was found between 0.5 and 1% w/v SDS concentrations ($P > 0.05$). The lower SDS concentration has advantage that SDS-associated skin irritancy is expected to be less. 10% duty cycle yielded similar immune response as 20% US duty cycle after the first 2 doses, but was surprisingly higher after the third dose. To conclude, we have shown that the extent of the immune response can be modulated by the ultrasound experimental parameters.

Table 1 IgG levels of animal groups

Treatment group	Day 14	Day 45	Day 60
IM injection	3.3 ± 0.9	3.9 ± 0.3	4.5 ± 0
IM injection + Alum	3.6 ± 0.2	3.9 ± 0.1	5.4 ± 0.3
Topical application	1.3 ± 0.1	1.2 ± 0	1.3 ± 0.1
SDS (1% w/v) only	1.2 ± 0	1.2 ± 0	1.2 ± 0
US (20%) alone	1.4 ± 0.1	1.4 ± 0.4	3.7 ± 0.7
US (20%) + SDS (0.5%w/v)	1.6 ± 0.3	2.5 ± 0.6	4.8 ± 0.5
US (20%) + SDS (1% w/v)	2.1 ± 0.2	2.4 ± 0.5	3.7 ± 1.1
US (10%) + SDS (1% w/v)	2.2 ± 0.7	2.8 ± 0.5	4.2 ± 0.5

Numbers represent log serum dilution that gave an OD of 0.2 are shown.

Dahlan, A. et al (2005) *S. J. Pharm. Pharmacol.* **57**: S92
Tezel, A. et al (2005) *Vaccine* **23**: 3800–3807

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Effect of SDS concentration in the coupling medium on ultrasound-induced changes in skin barrier

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Low-frequency ultrasound (US) has been shown to increase drug permeation into the skin. Sodium dodecyl sulphate (SDS) is often included in the coupling medium at 1% w/v. We have shown a highly irregular relationship between the surfactant concentration and protein flux through skin in in vitro experiments (Table 1 Dahlan et al 2005). The in-vitro data suggested that lower SDS concentrations might induce similar protein permeation into the skin; lower SDS levels are expected to cause less skin irritancy. We, therefore, studied the effects of SDS concentration on US-induced changes in skin barrier properties in-vivo, in mice and rats. Pulses of ultrasound (20 kHz) were applied to the shaved abdominal skin of anesthetized animals via a coupling medium containing different concentrations of SDS (0, 0.001, 0.01, 0.1, 0.5 (mice only) and 1 % w/v) for a total sonication time of 60s. US protocol for mice was 7.5 mm probe distance from skin, 20 ml coupling medium, 0.2s ON, 0.8s OFF and 20% amplitude while US protocol for rats was 5 mm probe distance, 20 ml, 0.5s ON, 0.5s OFF and 30% amplitude. Mice received a milder US treatment to avoid damage to their thinner skin. After US application, trans-epidermal water loss (TEWL, an indicator of changes in skin barrier properties;) were measured at 5, 15, 30, 45 and 60 min. High TEWL values are related to high water loss (i.e. reduction in stratum corneum barrier). All procedures had been approved by the School's Ethical Review Committee and were conducted in accordance with the Home Office standards under the Animals (Scientific Procedures) Act, 1986. In mice, the application of US in the presence of SDS at concentration below 0.5% w/v consistently showed low TEWL readings that were statistically the same as baseline (i.e. readings before US application), indicating a good state of the skin barrier at all times. At higher SDS concentrations (0.5 and 1% w/v), TEWL measurements increased significantly with 1% w/v SDS giving the highest TEWL readings (at 60 min post-sonication TEWL was 5.3 times higher than baseline). Higher TEWL values with higher SDS concentration could not necessarily be predicted as the extent of cavitation (i.e. formation and collapse of gaseous bubbles which is expected to damage the skin) is inversely related to surface tension of the coupling medium. Therefore, similar TEWL values for high and low SDS concentration could have been expected. Interestingly in rats, TEWL measurements of all treatment groups were 2.5 times greater than baseline, but were similar to one another at all times. No obvious relationship was seen between presence and absence of SDS and its concentration and TEWL. Surprisingly, lower SDS concentrations caused similar skin damage to higher SDS

Table 1 Protein sonophoresis through rat skin

SDS concn (% w/v) in coupling medium	Radiolabelled protein permeated (cpm)
0	85770 ± 3054
0.001	83250 ± 2085
0.004	83020 ± 1509
0.01	43980 ± 1349
0.04	29111 ± 1579
0.1	41910 ± 3502
1	74841 ± 3502

Data represents mean ± s.d, n = 5.

concentrations. It is possible that the harsher US conditions override any synergistic effects between US and SDS. It is not possible to compare the mice and the rat data as the US protocol was optimized for each species. We can, therefore, conclude that in mice, 0.5% w/v SDS might be sufficient for our subsequent in-vivo experiments, while in rats lower SDS concentrations might be sufficient.

Dahlan, A. et al (2005) *J. Pharm. Pharmacol*. **57**: S92

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A study into the application of ultrasound and liposome combination on skin permeability

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The skin is a good barrier and drug permeation has to be assisted for transdermal delivery. Enhancers include physical techniques such as application of electricity, ultrasound (US), chemical enhancers (e.g. azone) and vehicles (e.g. elastic liposomes). Often, enhancers are used in combination for synergistic activity. Surprisingly, a combination of liposomes and electric pulses was found to decrease permeation of drugs through the skin compared to electricity alone (Essa et al 2003); liposomal lipids were thought to accelerate the repair of electric-induced skin damage by infiltrating into skin lipid bilayers and thereby reduce drug flux. The aim of our study was to determine whether such skin repair ability of liposomes would also apply to damage caused by low-frequency ultrasound. The latter is being investigated in our laboratories for skin vaccination. Thus, the effects of US and liposomes on the in-vitro skin permeation of a model antigen (bovine serum albumin (BSA)) and the in-vivo trans-epidermal water loss (TEWL, an indicator of skin barrier properties) were determined. Permeation studies were conducted using Franz diffusion cells and full thickness rat skin. US waves (30% amplitude, 0.5 s ON, 0.5 s OFF, sonication time 2 min, 5 mm probe distance from skin) were applied to the skin via a coupling medium (PBS or SDS 1% w/v aqueous solution), followed by liposomes (MLVs or SUVs) for 5 or 60 min followed by the application of BSA. All procedures had been approved by the School's Ethical Review Committee and were conducted in accordance with the Animals (Scientific Procedures) Act 1986. In-vivo experiments were conducted in rats, with the same experimental protocols except that no vaccine was applied and TEWL was measured at different times post-liposome application. The effect of liposome application on in vitro antigen flux through skin and on TEWL is shown in Table 1. When coupling medium was PBS, application of liposomes (for either 5 or 60 min) post-sonication decreased BSA flux through the skin compared with the controls (US, but no liposome). This negative effect of liposomes on permeation enhancement correlates with similar negative effect of liposomes on electrically-assisted enhancement. Liposomal lipids seem to repair the skin barrier post-sonication, before protein is applied. Interestingly, a 5-min liposome application was as good as a 60-min application. In-vivo, 5-min liposome application seems to reduce TEWL, with smaller vesicles being more effective than larger ones at skin repair. When SDS was included in the coupling medium, liposomes reduced the protein flux, but did not have any significant effect on TEWL. SDS, a surfactant, is expected to be integrated within skin lipid bilayers and the subsequent addition of lipid (from the liposomes) to the skin does not seem to have any skin repair effect in-vivo. To conclude, liposomes have been shown to be effective in repairing skin that has been disrupted by ultrasound, but not when SDS is also present.

Table 1 TEWL values for animal treatment groups

Time	0 min	5 min	15 min	30 min	45 min	60 min
US	10.6 ± 0.7	24.7 ± 5.9	21.9 ± 4.4	18.6 ± 3.1	18.3 ± 3.3	20.3 ± 3.9
US+MLVs	10.3 ± 0.9	18.2 ± 3.9	14.9 ± 5.0	14.5 ± 3.8	13.9 ± 3.7	13.1 ± 3.5
US+SUVs	10.9 ± 1.3	15.8 ± 1.7	11.2 ± 2.2	10.5 ± 1.5	11.1 ± 1.7	11.1 ± 1.9
US(SDS)	10.0 ± 1.3	33.5 ± 1.8	31.7 ± 1.7	28.6 ± 2.6	27.2 ± 2.3	27.2 ± 3.4
US(SDS) +MLVs	10.9 ± 0.6	31.0 ± 2.1	24.8 ± 3.5	24.8 ± 5.0	23.6 ± 4.3	23.0 ± 3.3
US(SDS) +SUVs	10.2 ± 0.8	33.5 ± 2.5	24.9 ± 4.4	22.0 ± 5.1	20.9 ± 5.0	22.1 ± 4.5
SDS	11.4 ± 1.1	15.8 ± 2.4	11.3 ± 1.2	10.3 ± 0.4	10.5 ± 1.3	10.0 ± 1.2

Data represents mean ± s.d., n = 5.

Essa, E. A. et al (2003) *J. Controlled Release* **92**: 163–172

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The colon as a target for vaccination: quantification of lymphoid tissue in mouse colon prior to vaccination

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Currently, most vaccines are given by injection. However, due to the inherent problems associated with injections, other routes of drug delivery are being researched, among them, the oral route. So far, research into oral vaccination has not differentiated between vaccine uptake by the different parts of the gastro-intestinal tract, such as the small and large intestine. It is likely that following oral vaccine administration, the vaccine is mostly taken up by the lymphoid tissue of the small intestine. We are investigating vaccine delivery targeted to the colon, as the differences between the small and large intestine's immunological environment may mean that colonic vaccines are more appropriate for certain diseases such as Crohn's disease. Before administering vaccines to the colon, we need to establish the presence of, and quantify, the colonic lymphoid follicles, with respect to the small intestine in the mouse (our experimental animals), as the literature is scant. The colonic lymphoid follicles in man have been quantified and shown to exist as patches, similar to those in the small intestine (Langman & Rowland 1986). While Owen et al (1991) have demonstrated microscopically that lymphoid tissue and M cells are present in the mouse colon, no significant quantitative and comparative data exist. The aim of the investigation was therefore to determine the number and location of the lymphoid tissue patches in the colon, in comparison to the small intestine. The caecum was also investigated. Ten female adult Sox-1 and T/O mice were used. All procedures had been approved by the School's Ethical Review Committee and were conducted in accordance with the Home Office standards under the Animals (Scientific Procedures) Act, 1986. Mice were culled by cervical dislocation and dissected. The gastrointestinal tract was removed and sectioned (small intestine, caecum and colon). The sections were flushed with water, opened lengthways, and then incubated in 10% v/v acetic acid aqueous solution overnight at 5°C. The following day the sections were examined visually and the patches of lymphoid tissue and the number of follicles within were counted and photographed. The results are shown in Table 1. Patches were evenly distributed within the small and large intestines. However, they were denser in the large intestine, as seen from the number of patches per cm of intestine (Table 1). The photographic images showed clearly the presence of the lymphoid tissue in the colon, in which the follicular nature of the patch can be seen. Visually, the patches were smaller in the colon, and they contained fewer follicles, which were the same size as those of the small intestine. It was thus concluded that, as for man, patches of lymphoid tissue are present in the mouse colon to a significant degree in comparison to the small intestine, and are similar in appearance to, although smaller than, the Peyer's Patches. Since the target tissue for colonic vaccination is present, further colonic immunisation studies can be undertaken using the mouse as the experimental animal.

Table 1 Average lengths, numbers of lymphoid tissue patches and follicles in the small intestine, colon and caecum of 10 Sox-1 and T/O mice

	Small intestine	Colon	Caecum
Length (cm) (Range)	28.43 (24.7–31.9)	7.57 (6.8–9.7)	—
No. of patches (Range)	8.3 (5–12)	6.9 (3–15)	1.4 (1–3)
Total no. of follicles (Range)	51.1 (32–68)	17.2 (8–35)	7 (5–13)
No. of follicles per patch	6.4	2.5	5
No. of patches per cm of intestine	0.29	0.9	—

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Transdermal formulations of triclosan

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Triclosan was identified as a potential topical antimicrobial agent because of its high lipophilicity with a log P of 4.76 (Moss et al 2000). This enables the drug to diffuse through to the upper layers of the skin more readily. Transdermal pressure sensitive adhesives provide a simple means of achieving the desired drug dosage to the skin. This study aims to compare the rate of drug release and

in vitro permeation from two commercially available acrylic based glues through silastic membrane. Model patches were prepared using Durotak 900a and 2677 acrylic adhesives with 80 mg of triclosan per 3.142 cm^2. The rate of drug release was measured from the patches into a phosphate buffer solution (PBS): ethanol 60:40 composition (pH 7.4) over 9 h. The release rate was calculated as the gradient of the mass released versus the time. The percentage of total dose released and the release rate is shown in Table 1. Both patches showed very similar release rates, the percentage of total dose released was also very similar. Patch 2677 gave a slightly higher release rate than patch 900a and the percentage of total dose released was also slightly higher for patch 2677 than 900a. Permeability of the triclosan patches across silastic membrane was also monitored in static Franz diffusion cells. PBS:ethanol 60:40 was used as the receiver medium and this was stirred continuously at a rate of 550 rpm using a Teflon coated magnetic stirrer. The silastic membrane was mounted between the donor and receiver compartments. A 2 cm^2 patch was used in each experiment; the patches matched the diameter of the Franz cell. A 1 cm^3 sample was drawn and replaced with freshly prepared receiver medium at set time points; 15, 30, 45, 60, 90, 120, 150, 180, 240, 360, 480 and 540 min. All analysis was determined by HPLC. The drug flux was calculated as the gradient of the mass released per cm^2 versus time, this was used to calculate the permeability coefficient constant Kp. Kp and the percentage of dose permeated is shown in Table 2. There was no significant difference between the Kp value and the total % dose permeated. However, both values were slightly higher for patch 900a compared with patch 2677.

Table 1 Comparison of the release rate and % of dose released from transdermal patches formulated using Durotak 2677 and 900a

Matrix (Durotak)	Release rate (ug/h)	% Dose released
2677	5225.41	21.96
900a	4846.41	21.10

Table 2 Comparison of Kp and % of dose permeated from transdermal patches formulated using Durotak® 2677 and 900a

Matrix (Durotak)	Kp (cm/h)	% Dose permeated
2677	0.0036	1.99
900a	0.0039	2.1

Moss, T. et al (2000) *J. Food Chem. Toxicol*. **38**: 361–370

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Determination of the diffusion coefficient of cyanophenol in carbosil membrane using ATR-FTIR spectroscopy

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Model membranes are often used in place of human skin when evaluating dermal drug delivery systems. This is because they offer several advantages compared with human epidermis including their ready availability, uniformity and chemical purity. Carbosil membrane is a polydimethylsiloxane (PDMS) – polycarbonate (PC) block copolymer which has been suggested as a model for skin. Analogous with human skin epidermis it has a heterophasic, heteropolar structure and is thought therefore to be a better model for percutaneous absorption than conventional silicone membrane models. The mechanism of drug permeation across skin involves drug diffusion and partitioning between polar and lipophilic phases of the intercellular channels and so a heterophasic, heteropolar model membrane should mimic the behaviour of skin more closely. This study has been undertaken to determine the diffusion coefficient of a model drug (cyanophenol) in Carbosil membrane to allow comparison with previously determined values in silicone membrane and human stratum corneum. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy was used to monitor the diffusion of cyanophenol through the Carbosil membrane. ATR-FTIR allows the permeation of drugs through membranes to be measured by taking infrared scans at predetermined intervals. Peaks associated with the drug molecule in this case, the cyano peak may then be assayed. A prehydrated membrane was placed on the ATR crystal surface and a saturated solution of cyanophenol in water was applied on top of the membrane. Drug permeation was monitored by taking infrared scans every two minutes. The data were modelled using Scientist (Micromath) software with appropriate boundary conditions applied to Fick's second law which allows calculation of the diffusion coefficient of cyanophenol in the membrane. A value of 5.73 ($\pm$ 0.66) $\times 10^{-6}$ cm^2/h was obtained. This compares with a value of 6.34 $\times 10^{-4}$ cm^2/h (Pellett et al 1997) obtained for cyanophenol in silicone membrane and 6.12 $\times 10^{-7}$ cm^2/h in human stratum corneum (Stinchcomb et al 1999). This indicates that Carbosil membrane has a much lower permeability to cyanophenol than silicone membrane and the diffusion coefficient calculated appears to be closer in magnitude to that of human stratum corneum. Thus these preliminary data suggest that Carbosil may be a better model for dermal permeation of cyanophenol than silicone membrane. This study has also shown the ability to derive the diffusion coefficient of drugs in membranes by modelling data obtained by ATR-FTIR experiments in a novel way using Scientist.

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Physical characterisation of vitreous humour and the development of a vitreous humour substitute

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The gel-like vitreous humour is an integral structure of the mammalian eye. It has evolved to provide mechanical support to the retina and other ocular tissues, thereby protecting the tissues from external damage, permitting the circulation of macromolecules and allowing light to pass through to the retina unhindered (Chirila & Hong 1997). A vitreous substitute is required for two reasons. Firstly, pathologies affecting the ocular tissues such as proliferative diabetic retinopathy may warrant the complete removal of the vitreous humour during a vitrectomy procedure. A replacement is then needed to retain the benefits of the natural vitreous humour. Secondly, if retinal detachment has developed and the natural vitreous is unable to force the retina back into place due to age-related vitreal degeneration, a substitute or supplement may be needed. Replacements currently available such as silicon are not an ideal solution as it is inevitable that cataracts form when silicon is left inside the globe for longer that a couple of months (Hutton & Azen 1994). Research has brought about many other potential replacements such as perfluorocarbon liquids (Velikay et al 1995) but none have been completely successful due to cytotoxicity or rapid biodegradability. As a first step towards development of a replacement vitreous humour, the physical properties of the natural material were characterised. Fresh post-mortem porcine eyes were obtained from the local abattoir. Texture Analysis using a TA-XT2 (Stable Microsystems, UK) involved measuring the force (g) used to push an 8 mm diameter probe into the vitreous humour of the eye in-situ. This was proven to be a destructive technique when repeated on the same sample. Results (mean $\pm$ s.d., n = 25) for individual samples tested when fresh and aged refrigerated for 24, 48 and 72 h were, respectively, 35.94 $\pm$ 11.84 g, 46.49 $\pm$ 14.19 g, 48.65 $\pm$ 21.93 g and 57.97 $\pm$ 18.85 g. Additionally, the results for samples which were frozen and defrosted were 22.69 $\pm$ 13.75 g. These results indicated that the storage condition will have an effect on the measured physical properties of vitreous humour and that the history of any pre-clinical or clinical sample must be known before any conclusions may be drawn on tests performed on them. Rheological tests using a TA Instruments Rheometer AR-1000 indicated that the vitreous humour is a viscoelastic gel that intrinsically has either plastic or pseudoplastic flow. The linear viscoelastic region (LVER) was found to occur at extremely low values of torque, on the limit of the capability of the equipment. In fact, identification of the LVER was possible only by using creep curves. Both techniques showed considerable variation in the results, which is believed to be commensurate with that of a biological system. Iso-osmotic gels produced from sodium hyaluronan in Ringers solution are currently being developed. Such gels have excellent optical clarity and have proven non-cytotoxic in a short term in-vitro cytotoxicity experiment, where the gel is placed in contact with human retinal pigment epithelial cells and the cytotoxicity is measured using a lactose dehydrogenase assay.

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