penetration of drug substances is a key issue for drug delivery and a clear understanding of the mode of action of compounds able to disrupt the lipid structures would greatly improve the further design of better skin-penetration enhancers. However, the lipid bilayer has been extremely difficult to model. Here we report the development of an initial model of the bilayer (using ceramide to create the bilayer as this has been shown to make up to 18% of the lipid within the epidermis and intercellular space), using which we have modelled the mode of action of a number of compounds that have been shown previously to aid drug delivery through the skin.

Methods In the modelling of the lipid bilayer, we considered the use of ceramide (in particular, ω-hydroxyacyl sphingosine) as the lipid mimic. As such, the monolayer was initially created within the CaChe molecular modelling program and each pair of molecule's interactions minimized (using MM3 parameters). As each pair was minimized, the next pair were added and again minimized. Once the monolayer had been created (consisting of some 10×8 molecules), the lower part of the bilayer was then created and minimized (again using MM3 parameters) and the outer surface locked. The penetration enhancers to be studied (e.g. dimethyl sulfoxide, Brij 76T, decylmethyl sulphoxide and *cis*-9-octadecanoic acid) were also produced within CaChe, initially minimized and then inserted into the bilayer, following which the full structure was minimized and the energy of the bilayer with and without the enhancer was determined so as to elucidate the impact of the different enhancers.

Results The minimization of the ceramide molecules resulted in the formation of an interdigitized bilayer with a total span of approximately 80 Å. The incorporation of different penetration enhancers caused different effects, as would be expected; that is, we discovered that the use of Brij 76T resulted in the space between the ceramide molecules increasing. Indeed, the whole molecule was found to fit within the bilayer such that the molecule crossed the span of the bilayer resulting in an overall energy increase of 1631.45 kcal/mol. Our results further support the previous hypotheses that compounds are able to embed themselves within the structure of the bilayer and as a result disrupt the overall structure of the homogenous nature of the bilayer, thereby enhancing the movement of compounds through the creation of 'micro-channels'.

Conclusions We have provided the initial model for the overall structure of the lipid bilayer and using this the mode of action of a range of penetration enhancers may be elucidated.

89
Inhibition of the hydroxysteroid dehydrogenase family of enzymes: an investigation into the specificity of a range of benzyl imidazole-based compounds

I. Shahid, M. S. Olusanjo, S. Dhanani, C. P. Owen and S. Ahmed

Department of Pharmacy, Kingston University, Kingston-upon-Thames, UK. E-mail: s.ahmed@kingston.ac.uk

Objectives The enzyme 17α-hydroxylase/17,20-lyase ($P450_{17\alpha}$) is currently under investigation in the treatment of androgen-dependent prostate cancer. We have previously designed, synthesized and subsequently evaluated a series of compounds based upon benzyl imidazole (Owen et al 2006, Patel et al 2006). Whilst the compounds were equipotent to the standard compound, ketoconazole (KTZ), the inhibitory profile of the compounds against other enzymes was not known. In an effort to determine their specificity, we considered the inhibitory activity of the previously synthesized compounds against two forms of 17β-hydroxysteroid dehydrogenase (17β-HSD); namely, type 1 (17β-HSD1; responsible for the conversion of oestrone to oestradiol) and type 3 (17β-HSD3; responsible for the conversion of androstenedione to testosterone), as well as 3β-hydroxysteroid dehydrogenase (3β-HSD; responsible for the conversion of dehydroepiandrosterone to androstenedione). Here, we report the initial results of our study into the biochemical evaluation (and therefore the determination of the specificity) of the 4-substituted (e.g. F, Cl, Br, I, Me, etc.) benzyl imidazole-based compounds previously reported by us, compared with the three HSD enzymes.

Methods For the current investigation, the target compounds were synthesized using the methodology previously reported by us (Owen et al 2006, Patel et al 2006). The reactions proceeded in good yield (ranging from 40 to 85%) and no major problems were encountered. Biochemical evaluation of the synthesized compounds (final concentration 100 μM) was undertaken using a literature assay procedure using rat testes homogenate and radiolabelled substrates (Lota et al 2006).

Results The results of the biochemical evaluation of the previously reported compounds against the three HSD enzymes show that the compounds were, in general, equipotent to the standard compound, namely KTZ, and which was found to possess 25, 23 and 34% inhibitory activity against 17β-HSD1, 17β-HSD3 and 3β-HSD respectively. However, a small number of compounds were found to possess greater inhibitory activity than KTZ; in particular, the most potent was found to be 4-fluorobenzyl imidazole, which was found to possess 68, 41 and 31% inhibitory activity against 17β-HSD1, 17β-HSD3 and 3β-HSD respectively under similar assay conditions. Further consideration of the inhibitory activity observed against the family of HSD enzymes shows that these compounds possess selectivity between 17β-HSD1 and 17β-HSD3, while possessing extremely poor inhibitory activity against 3β-HSD. For example, 4-bromobenzyl imidazole was found to be a relatively potent inhibitor of the lyase component (possessing an IC50 of 6.8 μM in comparison with 53.4 μM against the 17α-hydroxylase) of $P450_{17\alpha}$ and is found to possess approximately 49% inhibitory activity against 17β-HSD1 and approximately 33% inhibitory activity against 3β-HSD; however, no inhibitory activity was observed against 17β-HSD3.

Conclusions The results suggest that inhibitors of $P450_{17\alpha}$ designed using a benzyl imidazole template possess good selectivity against their target enzyme and are therefore good lead compounds in the design of novel inhibitors of $P450_{17\alpha}$.

Lota, R. et al (2006) *Bioorg. Med. Chem. Lett.* **16**: 4519–4524
Owen, C. P. et al (2006) *Bioorg. Med. Chem. Lett.* **16**: 4011–4015
Patel, C. H. et al (2006) *Bioorg. Med. Chem. Lett.* **16**: 4752–4756

Drug Delivery

90
Design and physico-chemical characterization of a novel drug-delivery system for photodiagnosis and photodynamic therapy of colorectal neoplasias

R. F. Donnelly, D. I. J. Morrow, G. P. Andrews, P. A. McCarron and A. D. Woolfson

School of Pharmacy, Queen's University, Belfast, UK. E-mail: r.donnelly@qub.ac.uk

Objectives Serious cellular abnormalities in the colorectal region are a leading cause of morbidity and mortality in industrialized countries, with an estimated 300000 new cases and 200000 related deaths annually in Europe and the USA. The development of technologies to improve the detection process or enhance treatment would be a welcome addition to current treatment methods. Two such promising procedures are photodynamic therapy and photodiagnosis. The techniques rely on specific accumulation of photosensitizer in a neoplastic lesion with the former therapy used to bring about selective destruction and the latter only making it more conspicuous upon fluorescent emission. Administration of 5-aminolevulinic acid (ALA) leads to selective accumulation of the photosensitizer protoporphyrin IX in neoplastic tissue. However, systemic administration of ALA is associated with significant side effects. In this study, we aimed to design and characterize a novel drug-delivery system that may be of use in photodiagnosis and photodynamic therapy of colorectal neoplasias.

Methods ALA-loaded, poly(ethylene glycol) (PEG) discs were prepared using three PEG molecular weights (1000, 6000 and 10000 Da) and subsequently characterized using friability measurements and differential scanning calorimetry. Drug-release studies were also performed using modified Franz diffusion cells. ALA was quantified by high-performance liquid chromatography (HPLC) employing fluorescence detection, as described previously (Donnelly et al 2006) following derivatization with acetyl acetone and formaldehyde.

Results The disc-shaped delivery system was mechanically robust, as judged by friability measurements. Calorimetric analysis confirmed that low concentrations of ALA (1% w/w) were dispersed completely throughout the PEG matrix, but higher concentrations (5 and 10% w/w) formed crystalline suspensions. The molecular weight of the PEG determined the melting temperature, with PEG 1000 being suitable for melting around body temperature. The drug-release kinetics was shown to be a function of both molecular weight and drug loading. Although the higher-molecular-weight PEG discs were resistant to surface erosion arising from an aqueous receptor phase, this effect was counterbalanced by more rapid and complete release when the ALA loading was increased. The lowest loading used (1% w/w) produced incomplete release, often not exceeding 30% of the total amount of drug.

Conclusions There is little doubt that photodynamic technology could make a significant and important impact in allowing expedient diagnosis or treatment of early-stage lesions without the need for surgery or toxic chemotherapy regimens. In this respect, ALA has many advantages over preformed photosensitizers in both diagnosis and treatment of lesions of the gastrointestinal tract, especially considering its high selectivity for neoplastic cells over normal tissue. The preliminary studies carried out here suggest that dosage forms prepared from poly (ethylene glycol) may be suitable for delivery of ALA to the colorectal region for photodynamic therapy or photodiagnosis of colorectal cancer. Further work is planned to formulate the system in the form of coated tablets to allow for colon-specific delivery after oral administration. Moreover, rectal delivery is to be optimized by shaping the dosage forms as suppositories.

Donnelly, R. F. et al (2006). *J. Photochem. Photobiol. B Biol.* **82**: 59–71

91
Investigating the effect of polymers on film-formation and drug-release properties of monolayered homopolymeric and multipolymeric films with drug and polymers of opposing solubilities

T. Govender[1], V. A. Perumal[1], D. Lutchman[3] and I. Mackraj[2]

[1]School of Pharmacy and Pharmacology, [2]School of Medical Sciences, University of KwaZulu Natal, Durban, South Africa and [3]Abbott South Africa, Gauteng, South Africa. E-mail: govenderth@ukzn.ac.za

Objectives The selection of optimal polymers remains pivotal in the formulation of controlled-release buccal monolayered films. The blending of suitable polymer(s) and drug of opposing solubilities in a monolayered film may be required. The preparation of such a monolayered system presents a challenge since they cannot be conventionally dissolved in a single vehicle and cast. The aim of this study was to investigate the effect of different polymers on film-formation and drug-release characteristics of monolayered homopolymeric and multipolymeric films, comprising a hydrophilic drug (propranolol hydrochloride; PHCl) with polymer(s) of opposing solubilities.

Methods Hydrophilic (chitosan (CHT), hydroxypropyl methylcellulose (HPMC), carboxymethylcellulose (CMC), polyethylene glycol (PEG), poly(vinyl pyrrolidone) (PVP), poly(acrylic acid) (PAA), poly(vinyl alcohol) (PVA), Eudragit® NE30D (EUD NE30D) and sodium alginate) and hydrophobic (ethylcellulose (EC), poly(D,L-lactide-*co*-glycolide) (PLGA) and Eudragit® RS100 (EUD100)) polymers were investigated. Homopolymeric films with PHCl and hydrophilic polymers were prepared by conventional solvent evaporation while homopolymeric/multipolymeric films with hydrophobic polymer(s) were prepared by a modified emulsification method (Perugini et al 2003). Films were cast on to newly designed silicone moulded trays with compartmentalized wells and characterized in terms of drug content (UV-1650PC spectrophotometer), drug release (shaking water bath), mucoadhesion (Texture Analyser XT2*i*) and film thickness (electronic digital micrometer).

Results Anionic polymers such as CMC, PAA, sodium alginate and PLGA could not lead to film formation but rather to complexation due to possible interactions with the cationic PHCl, whereas the cationic and non-ionic polymers could be combined with PHCl to form films. Preparation of homopolymeric PHCl films with hydrophilic polymers (CHT and HPMC) was not able to retard drug release. However, the preparation of monolayered homopolymeric film with PHCl and hydrophobic polymers (EUD100 and EC) was able to provide controlled drug-release kinetics. While unable to retard drug release as a homopolymeric film, HPMC led to a significantly retarded drug release when incorporated into the PHCl:EUD100 film in varying levels. However, the inclusion of CHT at low levels to the PHCl:EUD100 film retarded drug release while higher ratios led to increased drug release. A PHCl:EUD100:CHT (2:20:1) multipolymeric monolayered film was found to display the most suitable controlled release profile over 8 hours. The mucoadhesivity of the homopolymeric (PHCl:EUD100) and PHCl:EUD100:CHT film were 443.40 ± 30.73 and 410.40 ± 30.73 mN respectively.

Conclusions The chemical charge of the polymer and drug dictate homopolymeric film formation. A monolayered homopolymeric film with PHCl and a hydrophobic polymer could be prepared. While hydrophilic homopolymeric films were unable to retard drug release, it could be incorporated with hydrophobic polymers to form monolayered multipolymeric films with controlled drug-release properties and mucoadhesivity. Hence, the modified emulsification/solvent evaporation method/novel casting technique with a polymeric formulation of PHCl:EUD100:CHT (2:20:1) was able to provide a monolayered controlled drug-release film with mucoadhesivity.

Perugini P. et al (2003) *Int. J. Pharm.* **252**: 1–9

92
Determination of release mechanisms for poorly soluble model drugs from PEG8000 solid-dispersion systems

S. Khan, Y. Perrie, H. Batchelor and A. Mohammed

Aston University, Birmingham, UK. E-mail: khanmsa@aston.ac.uk

Objectives Solid dispersions are an attractive means of improving the dissolution rate of poorly soluble drugs but suffer from poor understanding of release mechanisms (Craig 2002). The work presented here investigates the characterization and release mechanisms of two model drugs (indomethacin and phenacetin) formulated as solid dispersions.

Methods Solid dispersions were prepared by melt fusion method. Formulations with 5, 10 and 15% w/w drug loading were investigated. Drug analysis was performed via UV spectrophotometry at 244 and 264 nm for phenacetin and indomethacin respectively. Polymer-release studies were performed using microviscometry (Anton Paar, Austria). Release data was fitted to the Peppas equation to provide information on the mechanism of release (Peppas 1985). Formulations were characterized using differential scanning calorimetry (DSC) and Fourier-transform infrared (FTIR) spectroscopy.

Table 1 Results showing the values of drug-release and polymer-release kinetics from the Peppas model

Formulation	k	n	R^2	k	n	R^2
P 5%	0.0576	0.88	0.94	0.0029	1.68	0.96
P 10%	0.0031	1.66	0.99	0.0039	1.60	0.96
P 15%	0.0085	1.06	0.99	0.0071	1.35	0.91
I 5%	0.0768	0.81	0.97	0.6530	0.11	0.96
I 10%	0.0747	0.84	0.88	0.5296	0.16	0.93
I 15%	0.1245	0.68	0.92	0.4862	0.18	0.92

k, kinetic constant; n, diffusional exponent; *P*, phenacetin; *I*, indomethacin.

Results The formulation of solid dispersions for both the drugs resulted in an enhanced dissolution profile as compared with their physical mix or drug-alone counterparts. The percentage of drug released over time for both the drugs was faster for 5% w/w loading compared with 10 and 15% w/w, suggesting that drug/carrier ratio in a solid dispersion is one of the main influences on the performance of a solid dispersion. Physical characterization of the formulations showed that the drug was present in an amorphous form dispersed within the carrier as suggested by the absence of melting peak and lack of shift in wave numbers studied by DSC and FTIR spectroscopy respectively. Furthermore, the constant calculated from the fitted data (Table 1) suggested that indomethacin release was controlled by the carrier (PEG8000) whereas drug characteristics controlled the release of phenacetin.

Conclusions This study has demonstrated that solid dispersions improved the solubility and dissolution rate of both the drugs studied. The dissolution rate decreases in solid dispersions with increasing concentrations of drug. It is the carrier that controls release of indomethacin and it is drug that controls the release of phenacetin.

Craig, D. M. Q. (2002) *Int. J. Pharm.* **231**: 131–144
Peppas, N. A. (1985) *Pharm. Acta Helv.* **60**: 110–111

93
Nanosizing of hydrocortisone using microfluidic reactors

H. Ali, P. York and N. Blagden

University of Bradford, Bradford, UK. E-mail: hsmali@bradford.ac.uk

Objectives The formulation of poorly water-soluble drugs is a challenging problem within pharmaceutical development. Recently, formulation using nanoparticles was highlighted as showing great potential to improve the dissolution and solubility characteristics of poorly water soluble drugs. This enhancement of solubility is attributed to a massive increase in the overall specific surface area of such particles, which is greater than that possible through micronization (Muller et al 2001). One platform for nanoparticle formation is microfluidic reactors, and this technique involves liquid flows constrained in small-scale channels, with dimensions in the order of micrometres in scale (Whitesides 2006). The unique laminar-flow regime within microfluidic devices (Weibel and Whitesides 2006) presents an opportunity to use liquid–liquid interfaces as templates for crystallization for the production of drug particles below 1 μm with well-defined habit through a solvent drown-out process. The aim of this study is to produce nano-sized particles of hydrocortisone as a model of a hydrophobic drug by controlled precipitation using a microfluidic technique. Experimental parameters for particle formation are multivariable and the critical parameters which are currently being examined include drug concentration, flow rate, reactor channel diameter and inlet angle.

Methods Hydrocortisone solution (12 mg/mL, in ethanol) and water (antisolvent) were pumped at different flow rates (0.5–2.5 mL/minute) through a series of microreactors (Epigem, UK) with different channel diameters (0.1, 0.5 and 1 mm) and inlet angles (10, 25 and 50°). Precipitation (in the form of nanoparticles) was found to occur immediately. The particle size of the produced dispersions was determined by photon correlation spectroscopy (PCS) using a Zetasizer® NanoS (Malvern Instruments, UK). The effects of different drug concentrations, flow rates, internal diameters of the reactors and inlet angles on drug particle size were studied.

Results Nanodispersions of hydrocortisone (≈200 ± 20 nm) with narrow size distribution were obtained. Particle size decreased to less than 100 nm with increasing flow rate, especially the rate of the anti-solvent stream (Table 1). Decreasing drug concentration resulted in larger particles. Sharper inlet angle and smaller internal diameters resulted in smaller drug particle size.

Table 1 Effect of changing the flow rate of anti-solvent (water)/solvent (ethanol) on particle size of hydrocortisone

Anti-solvent/solvent (mL/minute)	Mean particle size (nm)
0.5:0.5	298 ± 15
1:0.5	271 ± 16
1.5:0.5	207 ± 21
2:0.5	152 ± 26
2.5:0.5	80 ± 19

Conclusions Nano-sized dispersions of hydrocortisone with narrow size distribution were successfully synthesized by controlled precipitation in microfluidic channels. Particle size can be influenced by modifying processing conditions and design of microfluidic devices.

Muller, R. H. et al (2001) *Adv. Drug. Del. Rev.* **47**: 3–19
Weibel, D. B., Whitesides G. M. (2006) *Curr. Opin. Chem. Biol.* **10**: 584–591
Whitesides, G. M. (2006) *Nature* **442**: 368–373

94
Cholesterol content in liposomal bilayers versus stability

H. Ali, A. Mohammed and Y. Perrie

Aston University, Birmingham, UK. E-mail: alih4@aston.ac.uk

Objectives In this study our aim was to quantitatively and qualitatively investigate the effect of cholesterol (Chol) concentration on stability of liposomes prepared to deliver solubilized lipophilic drugs, as it is established that the presence of Chol provides a degree of physical bilayer stability, and that liposomes rich in the compound are more stable than low content Chol/Chol-free liposomes. Environmental scanning electron microscopy (ESEM) is a new technique for studying liposomes that can generate images of wet systems without the need for pre-preparation. ESEM was used to follow the dynamic stability of vesicles during dehydration and the effect bilayer composition has on this.

Methods Phosphatidylcholine liposomes were prepared by the well-established hydration method, with the addition of 0–33 total mol% Chol and 1.0 mg of hydrophobic drug (propofol) in all cases. Drug encapsulation and release were measured by spectrophotometric analysis of supernatant (phosphate-buffered saline, pH 7.4) following liposome separation via centrifugation. ESEM analysis (images not shown) of liposomes was performed by gradually reducing operating pressures while maintaining constant temperature (5°C throughout).

Results Liposomes containing various levels of Chol (0–33 total mol%) were subjected to controlled dehydration in the ESEM sample chamber and morphology assessed. At a pressure of 4.5 Torr all preparations exposed fully formed liposomes. Upon gradual reduction of pressure to 3.0 Torr, all samples preserved their spherical morphology. An additional period of controlled dehydration via reduction of the operating pressures from 3.0 to 1.1 Torr revealed no signs of morphological changes of the formulations containing 11 and 20 total mol% Chol. However, this decrease in pressure resulted in the 33 total mol% Chol containing liposomes losing their spherical nature, flattening and spreading to yield lipid patches. Preliminary optimization studies (Table 1) show that increasing Chol content also reduced drug-encapsulation capabilities of liposomes. However, the release profiles of propofol from the various liposome formulations demonstrated that the increase in the molar ratio of Chol progressively decreased the release of propofol from the vesicles (Table 1). These data suggest that there is a competitive interaction between drug loading in the liposomal bilayer and Chol bilayer content.

Table 1 Comparison of formulations containing 0–33 total mol% Chol and 1.00 mg propofol. Results are expressed as the means of at least three experiments ± SD

Parameter	Chol content (total mol%)			
	0	11	20	33
Encapsulation (% mol/mol)	23.9 ± 1.5	20.3 ± 1.8	16.1 ± 2.0	12.1 ± 1.5
Cumulative release after 72 hours (%)	79.4 ± 3.7	52.3 ± 2.5	56.0 ± 5.6	42.5 ± 3.7
Film collapsed under pressure reduction in ESEM chamber	–	No	No	Yes

Conclusions Our results suggest that Chol content in a liposomal bilayer can inhibit drug loading within the liposomal membranes but can improve retention of this loaded drug. Chol content also influenced the stability of liposomes under dehydration conditions.

95
Influence of gelatine bloom strength and concentration on the hardness and disintegration time of rapidly disintegrating solid formulations

F. Alhusban, Y. Perrie and A. Mohammed

Aston University, Birmingham, UK. E-mail: alhusbfa@aston.ac.uk

Objectives Tablets that rapidly dissolve upon contact with saliva in the oral cavity provide a practical solution for buccal, sublingual and oral administration. The current work investigates the effect of gelatine bloom strength and stock-solution concentration on the disintegration time and hardness of lyophilized orally dissolving tablets.

Methods Freeze-drying cycle was optimized for primary as well as secondary drying followed by analysis of moisture content and transition temperature using thermogravimetric analysis and differential scanning calorimetry, respectively. The optimized cycle was applied to produce tablets from 2, 5, 7.5 and 10% w/w of 60 or 225 bloom strength gelatine stock solutions. The disintegration time of the lyophilized tablets was measured according to the US Pharmacopoeia (USP) disintegration test. The hardness of the tablets was evaluated using texture analyser. A penetration probe (5 mm diameter) was used to compress the tablets over a constant distance of 2 mm at a speed of 6 mm/minute. The peak force (N) after 1 mm of penetration was determined.

Results Optimization of freeze-drying regime indicated that primary drying for 48 hours (at a shelf temperature of –40°C and 50 mTorr vacuum) is required to reduce the water content of 2% w/w gelatine (bloom strength 60) tablets to 7.3 ± 1.5% w/w and inclusion of a secondary drying phase for a further 10 hours resulted in the reduction of moisture to 5.0 ± 0.1% w/w (at a shelf temperature of 20°C and 50 mTorr vacuum). A logarithmic relation between moisture content (Mc) and transition temperature (T_g) of the lyophilized tablets was established ($Mc = 485.13*\log(T_g)^{-6.7723}$, $R^2 = 0.99$). Furthermore, the results (Table 1) indicated that the disintegration time of the tablets was dramatically decreased by decreasing the concentration and bloom strength of gelatine in the stock solution, whereas the hardness of the tablets was influenced by the concentration of gelatine rather than the bloom strength.

Conclusions The study suggests that optimization of freeze-drying regime is essential to control the moisture content of lyophilized formulations. Gelatine bloom strength and stock-solution concentration control the disintegration time of lyophilized fast-disintegrating tablets.

Table 1 Measurement of hardness and disintegration time for two types of gelatine (mean ± SD; n = 3)

Gelatine bloom strength	Gelatine concentration % (w/w)	Disintegration time (seconds)	Hardness (N)
60	**2**	3.0 ± 0	1.4 ± 0.4
60	**5**	29.0 ± 2.2	13.5 ± 0.7
60	**7.5**	189.0 ± 13.2	31.7 ± 4.8
60	**10**	360.0 ± 14.2	58.8 ± 2.2
225	**2**	6.0 ± 0.5	2.8 ± 0.2
225	**5**	38.3 ± 1.7	14.2 ± 0.8
225	**7.5**	347.7 ± 23.2	30.2 ± 2.6
225	**10**	808.0 ± 21.9	57.7 ± 3.2

96
Assessment of galactose microneedles for enhanced transdermal drug delivery

D. I. J. Morrow[1], P. A. McCarron[1], M. Gallagher[1], K. Migalska[1], R. Thakur[1], A. Morrissey[2], A. D. Woolfson[1] and R. F. Donnelly[1]

[1]School of Pharmacy, Queen's University, Belfast, UK and [2]Microsystems Team, Tyndall National Institute, Cork, Ireland. E-mail: dmorrow02@qub.ac.uk

Objectives An innovative strategy used to enhance the transdermal delivery of drugs is the use of devices known as microneedle (MN) arrays. To date, the majority of MN-based transdermal studies have employed silicon MNs. However, the use of solid, non-coated MNs generally requires a two-step process, which is undesirable. Furthermore, silicon is not a US Food and Drug Administration (FDA)-approved biomaterial and safety concerns exist in relation to the breaking of

silicon MNs. Recently, sugar MNs have gained attention as possible delivery devices due to their ability to biodegrade in the skin within a short period of time (Kolli and Banga 2008). The aim of this study was to examine the physical characteristics of galactose MNs. In addition, model drugs of both low molecular weight (aminolevulinic acid, ALA) and high molecular weight (bovine serum albumin, BSA) were formulated within the matrix of the MN devices and drug stability and release examined.

Methods Galactose MNs were prepared using inverse silicone moulds of master silicon MN arrays. Briefly, molten galactose (170°C) was poured into the silicone mould and centrifuged at 3500 rpm for 10 minutes. MN stability was assessed by storing galactose MNs at 43% and 75% relative humidity (RH) at either room temperature or at 4°C. MNs were also stored in heat-sealed containers containing silica gel. At pre-defined time intervals the arrays were removed and visualized by light microscopy (GXMGE-5 USB digital microscope). Galactose MNs were tailored to contain a theoretical loading of 1 mg BSA or 19 mg ALA. Actual drug loadings were determined by dissolving the devices in phosphate-buffered saline, pH 7.4, and analysing the solution using high-performance liquid chromatography (HPLC). Drug-release experiments were carried out using the Franz cell model, employing Silescol® as a model membrane.

Results Storage of galactose MNs at 75 and 43% RH resulted in loss of MN shape within 1 and 4 hours, respectively. However, when stored in a heat-sealed, humidity-free environment MNs maintained their shape for up to 96 hours. Analysis of MNs loaded with 1 mg BSA revealed that all of the BSA had degraded during the fabrication process. MNs containing ALA were shown to contain approximately 60% of the theoretical drug content following preparation. However, MNs loaded with ALA were not shown to significantly increase ALA permeation across the model membrane compared with control ($P > 0.126$).

Conclusions This study has highlighted a number of limitations with the use of sugar MNs. Although very sharp needles can be fabricated they are extremely hygroscopic and rapidly change in shape under even ambient conditions. MN preparation requires temperature in excess of 160°C which is catastrophic for peptide stability. In addition, *in vitro* drug-release studies indicated that galactose MNs containing ALA did not enhance delivery compared with control. In conclusion, this study suggests that galactose is not an ideal material for the preparation of rapidly dissolving MNs.

Kolli, C. S., Banga, A. K. (2008) *Pharm. Res.* **25**: 104–113

97
Enhanced *in vitro* release of glibenclamide from drug-in-adhesive silicone layers using solid dispersions of glibenclamide with Solutol-HS15

K. Dodou and A. A. Elkordy

School of Pharmacy, University of Sunderland, Sunderland, UK.
E-mail: kalliopi.dodou@sunderland.ac.uk

Objectives To prepare and compare the properties of solid dispersions of glibenclamide with a non-ionic surfactant (Solutol-HS15) using two different methods and to examine how solid-dispersion properties affect the *in vitro* glibenclamide release from drug-in-adhesive layers.

Methods Solid dispersions of glibenclamide (melting point = 169°C) with Solutol-HS15 (melting point = 30°C) in glibenclamide/Solutol-HS15 weight ratios of 1:9 and 3:7 were prepared using melting and solvent methods. In the melting method (MM), glibenclamide and the surfactant were melted in an oven and the molten products were left to cool at ambient temperature. For the solvent method (SM), glibenclamide and the surfactant were dissolved in ethanol which was evaporated by heating at 40°C (in a controlled-temperature oven) until constant product weight was obtained. Solid dispersions prepared by MM or SM were semi-solid in nature. Solid dispersions were examined for their solubility in phosphate buffer (pH = 5.4), drug content uniformity, microscopic appearance via polarized microscopy, enthalpy changes and melting point using differential scanning calorimetry (DSC) and solid-state interaction using Fourier-transform infrared (FTIR) spectroscopy. Saturation solubility of glibenclamide in a silicone adhesive (BIO-PSA 7-4302) was examined using microscopy. Solid dispersions were then used in the preparation of drug-in-adhesive layers with a target glibenclamide loading of 5 mg and a supersaturated glibenclamide concentration of 2% w/w. Layers containing only glibenclamide were also prepared. Glibenclamide release from each set of layers was tested over 6 hours in a paddle dissolution apparatus using 1 L phosphate buffer (pH = 5.4) under sink conditions at 32°C. The UV spectrophotometric analysis was performed at 224 nm.

Results MM binary mixtures demonstrated better content uniformity compared with SM binary mixtures (intimate contact between glibenclamide and Solutol-HS15, MM): 100.9 ± 3.5, 85.5 ± 10.5, 99.7 ± 2.7 and 91.3 ± 8.9% for 1:9 MM, 1:9 SM, 3:7 MM and 3:7 SM, respectively. Solid dispersion of 1:9 MM exhibited a significant higher solubility (91.2 ± 9.1 μg/mL) in phosphate buffer compared with: (i) 1:9 SM, 79.1 ± 7.9 μg/mL ($P < 0.05$, analysis of variance); (ii) 3:7 SM, 42.2 ± 4.2 μg/mL and (iii) pure drug, 0.95 ± 0.1 μg/mL ($P < 0.001$). Microscopic analysis revealed that all formulations had crystalline structure but crystal morphology was different. DSC data demonstrated that glibenclamide crystals in the 1:9 MM solid dispersion had the lowest melting point (100.8°C) versus 132.5, 137.8, 141.2 and 177.1°C for 1:9 SM, 3:7 MM, 3:7 SM and pure glibenclamide, respectively. There was an additional peak at 207°C for binary mixtures 3:7 MM and SM, suggesting formation of a new crystalline form. This was confirmed by FTIR results. The solubility of glibenclamide in the silicone adhesive was found to be less than 2% w/w. Glibenclamide cumulative percentage release from the drug-in-adhesive layers at 6 hours was 19.5 ± 4.4, 6.9 ± 0.58, 5.1 ± 1.75, 4.9 ± 1.3% and 5.37 ± 0.88% from the 1:9 MM, 1:9 SM, 3:7 MM, 3:7 SM and pure drug, respectively. Glibenclamide release was highest ($P < 0.001$) from the layers containing 1:9 MM and there was insignificant difference in drug release among the rest of the layers.

Conclusions Drug-in-adhesive layers containing the 1:9 MM solid dispersion showed the highest *in vitro* drug release. This indicates that the enhanced dissolution of glibenclamide in the adhesive is a result to the melting point suppression, but it could also be attributed to the higher aqueous solubility of 1:9 MM.

98
Development of liposomes to enhance the delivery of subunit vaccines

M. J. Hussain[1], P. C. Seville[1], T. Rades[2] and Y. Perrie[1]

[1]Aston University, Birmingham, UK and [2]University of Otago, Dunedin, New Zealand.
E-mail: hussaimj@aston.ac.uk

Objectives The aim of this work is to investigate the effect of adding stabilizing lipids to immunostimulatory liposomes and to characterize these particular systems with respect to size and surface charge. Vaccines are an efficient and essential means of health care and their potential impact is evident in the developing world. Millions of fatalities occur from diseases that are often preventable, such as tuberculosis. Thus, the creation of innovative adjuvants that promote targeted immune responses, for example in macrophages, and enhance the efficacy of subunit vaccines are highly favourable. Liposomes composed of dimethyldiocta-decylammonium bromide (DDA) and α,α'-trehalose 6,6′-dibehenate (TDB) are proven to encourage such favourable reactions as this multilamellar vesicle formulation provides a cationic charge ideal for antigen absorption. However, this formulation may have long-term stability issues (Davidsen et al 2005).

Methods DDA/TDB liposomes were prepared via the lipid hydration method at concentrations of 1.25 mg DDA and 0.25 mg TDB per millilitre in Tris buffer (10 mM, pH 7.4) (Davidsen et al 2005). The incorporation of increasing amounts of lipids 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) or 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) at various molar percentage ratios from 0 to 10% were investigated. Average diameter and surface charge of the liposomal formulations were measured using a ZetaPlus (Brookhaven Instrument Corporation) in Tris buffer (1 mM, pH 7.4).

Results DDA/TDB liposomes exhibited an average particle size of approximately 450 nm and an average zeta potential of approximately 50 mV. These values are consistent with previous data from our group (Davidsen et al 2005). Upon addition of 10% DPPC or 10% DSPC, the size of the vesicles increased to approximately 650 and 700 nm respectively. Interestingly, the zeta potential of these vesicles increased by 20–30%. This behaviour was unexpected, as both DPPC and DSPC are neutral, zwitterionic molecules. In contrast to DDA/TDB vesicles (Davidsen et al 2005), upon storage at 4 and 25°C in Tris buffer both DPPC- and DSPC-containing vesicles remain stable in terms of size and zeta potential for 1 week. Given that antigen absorption to DDA-based liposomes is via electrostatic absorption (Davidsen et al 2005), the higher zeta potential of both the DPPC and DSPC systems may further enhance antigen loading and retention. These studies are currently underway.

Conclusions Incorporation of DPPC and DSPC into DDA/TDB vesicles led to an increase in size and zeta potential of the resulting liposomes. These preliminary results form the basis of future studies at various percentage molar ratios of lipid in order to study long-term stability of these formulations and the potential of these systems as adjuvants.

Davidsen, J. et al (2005) *Biochim. Biophys. Acta* **1718**: 22–31

99
Utilization of non-ionic amphiphilic surfactants to enhance dissolution of naproxen from capsule dosage forms

K. W. Wong and A. A. Elkordy

Sunderland School of Pharmacy, University of Sunderland, Sunderland, UK.
E-mail: amal.elkordy@sunderland.ac.uk

Objectives The formulation of hydrophobic drugs as pharmaceuticals is challenging due to poor solubility and poor dissolution of these drugs. The aims

of the study were: (i) to modify dissolution of naproxen, a model hydrophobic drug, by solid dispersions using Solutol-HS15, Cremophor-RH40 (non-ionic amphiphilic surfactants) and Synperonic-PE/F68 (triblock copolymer) and (ii) to assess the effects of these carriers on solubility and integrity of naproxen.

Methods Melting and solvent methods were applied to prepare solid dispersions of naproxen with carriers in 50:50, 30:70, 20:80 and 10:90% w/w drug/carrier ratios. In the melting method, naproxen was dispersed in the molten carrier, whereas in the solvent method acetone was used as a solvent to dissolve naproxen and the carrier. Then, acetone was evaporated at 40°C. All solid dispersions (in quantities equivalent to 12 mg drug) were filled into bodies of hard gelatine size 2 capsules and stored in brown glass bottles at ambient conditions. Solid dispersions were investigated, employing drug-solubility studies, dissolution testing (at 100 rpm and 37°C), drug content uniformity, microscopic examination, differential scanning calorimetry (DSC) to study crystallinity of naproxen formulations and Fourier-transform infrared (FTIR) spectroscopy to study structural changes.

Results Solubility studies showed that formulations containing Solutol-HS15 and Cremophor-RH40 in concentrations of 80 and 90% increased naproxen solubility (4-fold), whereas Synperonic-PE/F68 did not show any increase in drug solubility. Increasing carrier concentrations in solid dispersions showed better drug-content uniformity. Naproxen alone showed a slow release (18% of naproxen released after 10 minutes). Formulations containing non-ionic amphiphilic surfactants in concentrations of 80 and 90% enhanced the drug dissolution rate with immediate pulsed release (about 85% of the drug released after 10 minutes). The solid dispersion of naproxen with Synperonic-PE/F68 has not improved drug dissolution; only 17% of naproxen released after 10 minutes. Solid dispersions prepared by melting method seemed to be more effective in improving dissolution than those prepared by solvent method (e.g. for solid dispersions of 30:70% w/w naproxen/Solutol-HS15, the release rate constant was 2.77 ± 0.55 in the case of the melting method versus 1.86 ± 0.46 in the case of the solvent method) probably due to better drug incorporation into the carrier in the molten state than in the solvent method. The highest ($P < 0.05$, analysis of variance) drug dissolution from 90% non-ionic amphiphilic surfactant formulations could be attributed to: (i) conversion of hydrophobic crystalline drug into amorphous state as confirmed by DSC (in thermograms of these formulations, there was no endotherm for naproxen melting at 160°C as shown in the DSC thermogram of pure naproxen) and polarized microscopy or (ii) solubilization and micelle formation effect of surfactants. Hence, drug crystallinity played an important role in governing drug solubility and dissolution. FTIR analysis demonstrated that solid dispersions of naproxen with 80 and 90% w/w non-ionic amphiphilic surfactants exhibited spectral changes with disappearance of the peak at 1680 cm^{-1}, indicating a drug–carrier interaction that resulted in improvement of drug dissolution. The spectra of all other formulations were similar to that of the pure drug.

Conclusions Solid dispersions of naproxen with Solutol-HS15 and Cremophor-RH40 (non-ionic amphiphilic surfactants) show promise for dissolution enhancement of hydrophobic drugs. Synperonic-PE/F68 proved to be a poor choice of carrier.

Acknowledgements The authors would like to thank IChemE for financial support and Npil Pharmaceuticals UK for allowing access to DSC.

100
Uptake and intracellular trafficking of novel ternary lipoplexes for gene delivery

L. Kudsiova[1], A. Vassa[1], H. C. Hailes[2] and A. B. Tabor[2]

[1]King's College London and [2]University College London, London, UK.
E-mail: laila.kudsiova@kcl.ac.uk

Objectives To date a large number of vesicle-forming cationic lipids and peptides have been synthesized and used for the delivery of DNA with varying degrees of success. To meet therapeutic requirements, however, the DNA-delivering efficiency of those non-viral vectors needs major improvement. One possible strategy is to gain better understanding of their mechanisms of entry in relation to their eventual transfection. Endocytosis is thought to be the major internalization pathway for most non-viral gene-delivery vectors; however, the relative contribution of each distinctive endocytic pathway, including clathrin- and caveolae-mediated endocytosis and/or macropinocytosis, is not yet fully understood. In this study the transfection efficiency and intracellular uptake and trafficking of novel lipid ternary vectors composed of a series of C_{14} glycerol-based analogues of *N*-(1-(2,3-dioleyloxy)propyl)-trimethyl-ammonium chloride (DOTMA) were studied using various endocytotic pathway inhibitors.

Methods The novel C_{14} DOTMA analogues (Writer et al 2006), incorporating *cis*, *trans* and alkyne moieties at C-9 and C-11 positions of the alkyl chain, were synthesized, formulated into vesicles with the neutral lipid dioleoylphosphatidyl-ethanolamine (DOPE) and mixed with pGL-3 plasmid in the presence or absence of an integrin-targeting peptide, Pep6. The novel peptide contains a Lys16 domain at the N-terminus designed to bind and condense the DNA, and a cyclic integrin-targeting recognition site known to bind specifically to the cell-surface protein $\alpha5\beta1$ integrin. Lipid–DNA complexation efficiency was assessed using gel electrophoresis, light scattering and zeta potential. Transfection studies of the lipoplexes in the presence or absence of Pep6 were performed in MDA-MB-231 breast cancer cells in the presence of endocytic inhibitors to determine the route of complex internalization and the relative contributions of each endocytic pathway (Khalil et al 2006). Endocytic inhibitor toxicity studies were also carried out using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay.

Results Zeta potentials, particle-size measurements and gel electrophoresis indicated that complete complexation of DNA occurred between lipid/DNA charge ratios of 2:1 and 4:1, offering partial protection of DNA from DNAseI enzymatic degradation. The presence of Pep6 showed highly improved transfection efficiency compared to the lipoplexes alone, showing up to 3-fold higher transfection efficiencies in some of the lipids when compared with Lipofectamine®. The transfection efficiency of lipoplexes with and without Pep6 was totally inhibited in the presence of chlorpromazine, a clathrin-mediated endocytic inhibitor. No inhibition of transfection was observed in the presence of filipin III or nystatin, both used as inhibitors for caveolae-mediated endocytosis. Some concentration and time-dependent cytotoxicity was observed with chlorpromazine (with T_{50} of 25.2 and 9.2 mg/mL for 30 and 60 minute incubation times respectively); however, any reduction in cell viability was accounted for during the transfection experiments.

Conclusions The proposed ternary lipoplexes are promising candidates for gene delivery due to their high transfection efficiency, although slight structural variations in the lipids also play a major role in their eventual transfection. Clathrin-mediated endocytosis appears to be the major pathway for complex internalization in both the presence and absence of Pep6.

Khalil, I. A. et al (2006) *Pharmacol. Rev.* **58**: 32–45
Writer, M. et al (2006) *J. Liposome Res.* **16**: 373–389

Material Science

101
Amphiphilic copolymers and hydrogels based on 2-hydroxyethylmethacrylate and 2-hydroxyethylacrylate as potential materials for pharmaceutical applications

O. V. Khutoryanskaya[1], Z. A. Mayeva[2], G. A. Mun[2] and V. V. Khutoryanskiy[1]

[1]School of Pharmacy, University of Reading, Reading, UK and [2]Department of Chemical Physics and Macromolecular Chemistry, Kazakh National University, Almaty, Kazakhstan. E-mail: v.khutoryanskiy@reading.ac.uk

Objectives Hydrogels based on poly(2-hydroxyethylmethacrylate) are widely used as components of biomedical devices and drug-delivery systems due to their biocompatibility and excellent physico-chemical properties. The objective of the present work was to synthesize and characterize soluble copolymers and hydrogels by copolymerizing 2-hydroxyethylmethacrylate (HEMA) with 2-hydroxyethyl-acrylate (HEA) and assess the possibility of their application in drug delivery.

Methods A series of soluble copolymers was synthesized by free-radical copolymerization of HEMA and HEA at different monomer ratios (140 minutes at 60°C). These copolymers were purified by dialysis against distilled water. The hydrogels were synthesized using similar reaction mixtures but the copolymerization was conducted for 18.5 hours. The hydrogels were purified by immersing in deionized water, which was changed daily, for 2 weeks to remove any unreacted chemicals. The compositions of soluble copolymers and their molecular weights were determined by ^{1}H-nuclear magnetic resonance spectroscopy and gel-permeation chromatography, respectively. The behaviour of the copolymers in solutions was studied by dynamic light scattering using a Malvern Zetasizer Nano-S (Malvern Instruments, UK). The mechanical properties of the hydrogels were assessed using a TA XT.plus Texture Analyser (Stable Microsystems, UK) in a compression mode at room temperature. The porous structure of the hydrogels was probed by scanning electron microscopy using FEI Quanta FEG 600 environmental scanning electron microscope. The freeze-dried hydrogel samples were sputtered with gold before analysis.

Results HEMA was found to be more reactive in copolymerization compared with HEA, and all the copolymers had higher HEMA content than in the feed mixtures. The copolymers containing up to 47 mol% of HEMA were soluble in water and their solution behaviour was studied by dynamic light scattering at different temperatures. These copolymers exhibited lower critical solution temperature in aqueous solutions; that is, they underwent a phase separation upon increase in temperature. Similar behaviour was previously reported for copolymers of HEA and butyl acrylate (Mun et al 2007). In ethanol solutions HEMA–HEA copolymers showed the presence of an upper critical solution temperature. The analysis of the copolymers by gel-permeation chromatography