Drug Delivery

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Gamma radiation: an ally or foe for subunit vaccines?

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Objectives The successful development of a parenteral liposome based vaccine requires the fabrication of a stable, sterile and freeze dried product. One of the major concerns in the process is the design of a pathogen free formulation, which can either be developed using a continuous aseptic process or end sterilized. Of the plethora of the sterilization techniques available, gamma radiation has shown to be a convenient, promising method for freeze dried liposomes (Strensrud et al 1999). The aim of the current work was to establish the influence of exposing a freeze-dried cationic liposome-based vaccine to 25KGy gamma radiation so as to produce a sterile product.

Methods Liposomal formulations consisting of DDA and TDB (1.25 mg/mL and 250 μg/mL, respectively) were prepared by the lipid hydration method. The freeze-dried formulations were exposed to gamma radiation to a dose of 25 KGy. Validation was carried out by exposing strips impregnated with Bacillus pumilus spores (2.5×10^6 CFU) to gamma radiation followed by culturing the strips in trypton soya media. Chemical stability was studied by NMR spectra using a Bruker AC-250 spectrometer at ^{1}H (250.1 MHz). Physico-chemical analysis, including: vesicle size, surface charge, viscosity, pH and antigen release profiles, was also conducted.

Results The results from culturing the sterilized strips indicate that the exposure to gamma radiation was an effective method in completely eradicating the growth of bacterial spores. Positive controls confirmed that the media used supported the spore growth.

NMR scans before and post gamma sterilization were similar, suggesting the absence of any degradation products. The ^{1}H NMR spectrum for the DDA lipid presents a sharp peak (1.2 ppm) representing the methylene groups present in the long alkyl chain. The peak at 0.84 ppm represents the end methyl groups attached to the long carbon chain. The chemical shift observed at 3.37 ppm represents both methyl as well as the methylene groups present surrounding the nitrogen atom. Similarly, no significant changes in the various physico-chemical parameters measured were detected. Previously the damage caused to liposomes by exposure to gamma radiation has been attributed to two factors: direct influence of radiation on the bilayer and an indirect effect caused by the action of the reactive species that are generated by gamma radiation on the bilayer (Zuidam et al 1995); however, the composition of the formulation may also control degradation (Stensrud et al 1999).

Conclusions The physico-chemical stability of the freeze dried DDA liposomes can therefore be due to the presence of cationic nitrogen at the head group which resists any radiation induced damage unlike the negatively charged DSPG liposomes which showed a change in the phospholipids concentration post radiation (Stensrud et al 1999). Additionally the development of a freeze dried product with low levels of moisture content better also adds to the stability. Gamma sterilization therefore provides the opportunity to develop sterile and chemically stable vaccine formulations.

Stensrud, G., et al (1999) *Radiation Physics Chemistry* **56**: 611–622
Zuidam, N. J., et al (1995) *Pharm. Res*. **12**: 1761–1768

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Synthesis and assessment of dendrimer prodrugs to enhance the cellular permeability of P-gp substrates

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Objectives The aim of this study is to evaluate the potential use of first generation (G1) polyamidoamine (PAMAM) dendrimers as drug carriers to enhance the permeability, hence oral absorption, of drugs that are substrates for P-glycoprotein (P-gp) efflux transporters.

Methods G1 PAMAM dendrimer-based prodrugs of the water-insoluble P-gp substrate terfenadine (Ter) were synthesized using succinic acid (suc) or succinyl-diethylene glycol (suc-deg) as a linker/spacer (to yield G1-suc-Ter and G1-suc-deg-Ter, respectively). In addition, two lauroyl chains (L) (permeability enhancer) were attached to G1-suc-deg-Ter to yield L2-G1-suc-deg-Ter. The resulting conjugates were characterized by ^{1}H and ^{13}C NMR spectroscopy and RP-HPLC. The influence of the dendrimer prodrugs on the integrity and viability of human caucasian colon adenocarcinoma cells (Caco-2) was determined by measuring the transepithelial electrical resistance (TEER) and leakage of lactate dehydrogenase (LDH) enzyme, respectively. Permeability coefficient (P_{app}) of terfenadine and conjugates through Caco-2 cell monolayers was measured in both the apical (A)-to-basolateral (B) and B→A directions at 37 °C.

Results The LDH assay indicated that G1 PAMAM dendrimer had no impact on the viability of Caco-2 cells up to a concentration of 1 mM and had a significantly lower toxicity than that of the higher generation G3 PAMAM dendrimer. However, the IC_{50} of the prodrugs was lower than that of G1 PAMAM dendrimer because of the intrinsic toxicity of terfenadine. The presence of G1 PAMAM dendrimer in the transport medium had no impact on the transport profile of terfenadine, but the measurements of the transport of dendrimer prodrugs across monolayers of Caco-2

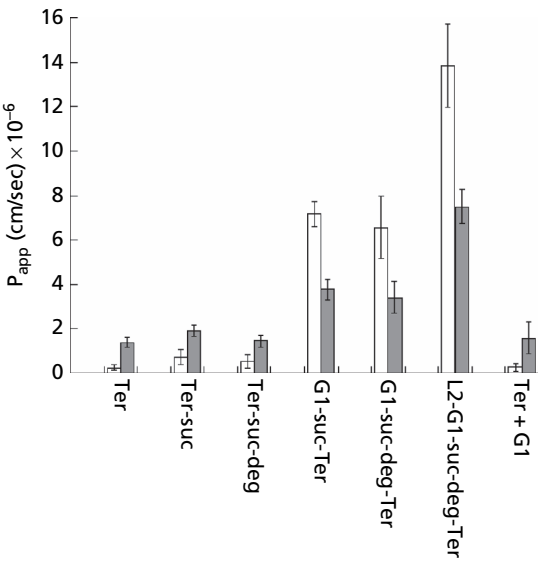

Figure 1 The A→B (□) and B→A (■) permeability of free terfenadine, conjugates, and terfenadine in the presence of G1 PAMAM dendrimer across Caco-2 cell monolayers at 37°C (mean ± S.D., n = 4).

cells showed an increase of the apparent permeability coefficient (P_{app}) of terfenadine in both apical-to-basolateral (A→B) and basolateral-to-apical (B→A) directions after its conjugation to G1 PAMAM dendrimer. The A→B P_{app} of the dendrimer prodrugs was significantly greater than B→A P_{app}. A more pronounced increase of A→B terfenadine permeability was found when lauroyl-modified dendrimer prodrug (L2-G1-suc-deg-Ter) was used as a carrier (Figure 1). The transport mechanism of G1-terfenadine conjugates is thought to involve both transcellular and paracellular pathways.

Conclusion The design, synthesis, characterization and transport studies of terfenadine conjugates of G1 PAMAM dendrimer using biodegradable spacer/linkers are reported. Our results suggest that G1 PAMAM dendrimers demonstrate potential as nanocarriers for the enhancement of oral bioavailability of terfenadine, a model low solubility drug and P-gp substrate.

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Product development of liposome based subunit TB vaccine

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Objectives The successful development of a parenteral subunit protein based liposomal vaccine from bench into the clinic requires careful optimization of the process conditions coupled with the fabrication of a stable and sterile product. The aim of the work presented here is to investigate the parameters controlling the development of freeze dried product together with the analysis of residual solvent content to comply with the regulatory authorities.

Methods Liposomal formulations consisting of DDA and TDB (1.25 mg/mL and 250 μg/mL, respectively) were prepared by the lipid hydration method. Freeze drying was carried out with the addition of a range of cryoprotectants (sucrose, maltose, trehalose and lysine: 2–10 mole/mole respectively) (pre freezing: −70°C for 30 min, drying phase: −40°C for 48 h). Residual solvent analysis was carried using gas liquid chromatography set at the following conditions: column = spiral Pora pak Q column (length: 2 m; diameter: 4 mm); Internal standard = isopropanol; column temperature = 200°C; detector temperature = 250°C; injector temperature = 220°C; nitrogen flow rate = 30 mL/min; detector = flame ionization detector (FID); detector sensitivity = 4 (on a scale of 1–1024); sample volume (injection volume) = 5 μL; run time = approx. 8–10 min.

Results The optimization of the cryoprotection was based on a balance between the moisture content, size of the vesicles upon rehydration and transition temperature. The results suggest that the cryoprotectants stabilize the liposomes in a concentration dependent effect. Lysine and maltose required the smallest concentration (4 mole/mole) with sucrose in the intermediate range (8 mole/mole), while trehalose showed effect at 10 mole/mole. These results are in line with the previously reported studies showing a biphasic nature for cryoprotection effect (Suzuki & Komatsu 1996; Miyajima et al 1997). Both the lower as well as the higher concentrations of the cryoprotectants did not stabilize the liposomes when compared with the intermediate, which effectively lyophilized the formulation. Measurement of residual solvent indicates that no detectable amounts of chloroform were present either in the hydrated or the freeze dried sterilized liposomes. However, low concentrations of methanol (223 ± 9.6 ppm: hydrated liposomes; 235 ± 3.7 ppm: freeze dried) were present at both the stages of formulation. The maximum permissible amounts of chloroform and methanol are 50 and 1000 ppm according to the European Pharmacopeia. The low concentrations of methanol remaining could possibly be explained on the basis of differences in vapour pressure of the two solvents.

Conclusions It can be concluded that careful optimization of process parameters is crucial for the development of a stable, regulatory acceptable product.

Miyajima, K., (1997) *Adv. Drug Del. Rev*. **24**: 151–159
Suzuki, T., Komatsu, H. (1996) *BBA – Biomembranes* **1278**:176–182

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Transdermal patch formulation and diffusion studies for captopril ethyl ester prodrug

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Objectives To use adhesion analysis as a screen for transdermal formulations of captopril ethyl ester prodrug, and to determine optimal flux using diffusion analysis. Analogues of captopril have previously been designed and manufactured (Moss et al 2006). The physicochemical properties of these prodrugs are such that they readily penetrate the skin. In the case of the optimum prodrug (captopril carboxyl ethyl ester), percutaneous absorption is sufficient to exert a systemic therapeutic effect. This suggests that the ethyl ester prodrug is an ideal candidate for further development into clinically viable dosage forms (i.e. transdermal patch development).

Methods Formulations containing a wide range of prodrug concentration and increasing concentrations of pressure-sensitive adhesive (Duro-Tak 387–2054) were prepared. Their adhesion properties were characterised as Force of Adhesion (N) and Work of Adhesion (N.mm) (Woolfson et al 1998). Those displaying similar adhesive properties to the benchmark commercial product (Nicorette patch) were selected for subsequent analysis by *in vitro* diffusion experiments. This was initially achieved using Silastic (polydimethylsiloxane) membranes.

Results The formulations found to display optimal adhesive properties contained 30% w/w adhesive. Diffusion through Silastic membrane of the 30% w/w formulations showed an increase in flux as concentration of prodrug increased in the formulation. This reached a maximum flux at approximately 0.3 mg/cm^2/h obtained from the formulation containing 2% w/w captopril ethyl ester. The maximum flux obtained may be due to saturation of drug in the formulation matrix, membrane saturation, or as a result of binding to crosslinked adhesive (Mehdizadeh et al 2006). The chemical nature of the formulation was further investigated by FT-IR. This indicated that hydrogen-bonding exists between the prodrug and the adhesive, as represented by the shift of the amide I peak from 1640 cm^{-1} to 1650 cm^{-1} and 1617 cm^{-1}. The additional peaks suggest the presence of two different hydrogen bonds in the formulation, which may be due to the hydrogen bonding occurring between the amide and the functional groups in the adhesive.

Conclusions The formulations displaying optimal adhesive properties contained 30% w/w adhesive, and the formulation affording optimal flux through Silastic membrane contained 2% w/w prodrug.

Mehdizadeh, A., et al (2006) *Acta Pharm*. **56**: 219–229
Moss, G. P., et al (2006) *J. Pharm. Pharmacol*. **58**: 167–177
Woolfson, A. D., et al (1998) *Int. J. Pharm*. **169**: 83–94

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Characterization of the decomposition profile of S-nitrosoglutathione and its inhibitory effect on platelet aggregation

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Objective S-nitrosoglutathione (GSNO) is one of the most abundant S-nitrosothiols present in the body, playing an important role in many important physiological functions. Depletion of GSNO in some pathophysiological conditions makes GSNO a potentially interesting therapeutic molecule. The aim of this study was to investigate the decomposition of GSNO under various physicochemical and biological stress conditions and to characterise the inhibitory effect of GSNO on platelet aggregation.

Method GSNO was freshly prepared from equimolar concentrations of glutathione (GSH) and acidic sodium nitrite and quantified using UV/Vis absorption at 334 nm. Samples of GSNO were incubated under different conditions including exposure to UV/Vis irradiation, varying pH values, temperatures, and concentrations of exogenous GSH. The percentage of intact GSNO was determined after 0.5, 1, 3, 24 and 48 h. GSNO decomposition was also examined in the presence of bronchial epithelial cells. The kinetics of enzymatic metabolism of GSNO by GSNO reductase (GSNOR; from *Candida boidinii*) was characterised using fluorescence quantification of the cofactor, NADH. Dose-dependent inhibition of platelet aggregation by GSNO was determined using a Payton 600B aggregometer.

Results The study showed that UV/Vis irradiation is a major factor affecting the stability of GSNO. Increases in temperature reduced GSNO stability, with the highest stability observed at 4°C. An acidic pH protected against GSNO decomposition, while increasing the pH caused a decrease in GSNO stability. Presence of GSH led to GSNO decomposition in a dose-dependent manner and the decomposition of GSNO was also accelerated in the presence of bronchial epithelial cells. GSNOR rapidly metabolised GSNO showing Michaelis-Menten kinetics with non-competitive substrate inhibition (Ksi = 75.22 μM). Platelet aggregation could be inhibited completely at a concentration of 125 μM GSNO. As the concentration decreased, the inhibitory effect on platelet aggregation decreased in a dose-dependent manner.

Conclusions GSNO was shown to be most stable when protected from light, stored at low temperatures and at an acidic pH. Decomposition was accelerated in the presence of bronchial epithelial cells and GSNOR, an enzyme recently discovered to metabolise GSNO. GSNO had a strong, dose-dependent inhibitory effect on platelet aggregation and may therefore have potential as a therapeutic molecule; however, formulation strategies to increase the compound stability may be required prior to its use therapeutically.

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D-optimal designing and optimization of aripiprazole in-situ implant formulation

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Objectives Drug therapy of schizophrenia requires continuous administration of atypical antipsychotic drugs for a long period. In this work a long-acting in-situ implant-based depot injectable formulation of aripiprazole was developed by using D-optimal statistical design. PLGA (different molecular weight) and cholesterol were used as biodegradable carriers for the formation of in-situ implants.

Methods A weighed amount of aripiprazole was dissolved in PLGA/cholesterol solutions and stirred for few minutes. To verify the formation of biodegradable in-situ implants the formed solution/suspension mixture was injected into phosphate buffer solution (pH 7.4). Instant formation of a precipitated gel matrix of drug and carrier conformed the formation of in-situ implant. Design expert software was used for experimental designing and optimization of the implant formulation.

Results In the preliminary studies the application of four polymers, viz., PLGA 50:50, PLGA 75:25, PLGA 85:15 and cholesterol, respectively, was screened for implant formation and drug release characteristics. Based on the better drug release profile PLGA 50:50 and cholesterol were selected for the final implant designing and optimization studies. Fourteen experiments were designed by using D-optimal experimental design technique. Owing to the number of constraints for experimental design, this technique was employed. Fraction of designed space and standard error of the designed graphs confirmed the validity of experimental design. PLGA 50:50, cholesterol, aripiprazole and release at 1,3,7, and 14 day were selected as independent variable and response variable, respectively. Polynomial equations were generated for each response variable and, as per the desired objectives, the optimum formulation was predicted by the software. The results of drug release studies showed that the cholesterol-containing formulation prohibited initial drug release as compared with PLGA-based formulations. The optimization results also supported the same observation. The response surface and contour plots were constructed for the optimization of formulation. The predicted optimized formulation was experimentally validated. The linear and residual plots between observed and predicted formulations were also constructed.

Conclusion This study demonstrates the feasibility of an implant system of aripiprazole using cholesterol as carrier for staged drug release upto 2 weeks. The D-optimal technique of formulation optimization was found to be very useful in the designing and development of the above system.

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Needle-free jet injection for topical delivery of ALA and a model pre-formed photosensitiser

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Objectives Photodynamic therapy is a non-invasive treatment for pre-maligant and malignant skin cancers. Administration of a tumour localising photo-sensitising agent followed by irradiation at the appropriate wavelength leads to a sequence of photochemical and photobiological processes, culminating in irreversible damage to tumour tissue. Systemic administration of photosensitisers can lead to prolonged skin photosensitivity persisting up to 10 weeks (De Rosa & Bentley 2000), and topical penetration tends to be poor due to their relatively high molecular weights (RMM > 1000). An alternative approach is the topical administration of aminolevulinic acid (ALA). Application of exogenous ALA stimulates the over-production of protoporphyrin IX (PpIX), an effective endogenous photosensitiser. However, ALA is a small hydrophilic (167.6 dalton) molecule and zwitterionic at physiological pH. Consequently, permeation across intact stratum corneum is said to be poor (Casas et al 2000). Needle-free injector devices employ a high speed jet to puncture the skin. In this study we aimed to enhance and target topical delivery of both ALA and the photosensitiser (N-methyl-4-pyridyl) porphine tetra tosylate (TMP) into porcine skin using needle-free injection technology.

Methods *In vitro* permeation studies were performed using the Franz cell model, employing excised neonatal pigskin. C^{14} radiolabelled ALA and porphyrin-loaded bioadhesive films were cast from drug containing aqueous blends of poly(methylvinylether.maleic anhyhydride), suitably plasticised using tripropylene glycol methyl ether. Films were applied to excised pigskin for 4 and 6 h for ALA and TMP formulations, respectively. A proprietary device (SQ Pen) was used to deliver a high pressure jet of liquid containing either ALA or TMP into full thickness porcine skin. Tissue penetration was determined by liquid scintillation spectroscopy (ALA) or fluorescence spectroscopy (TMP). Porphyrin delivery was visualised by taking cross-sections of the skin and photographing using a digital camera.

Results A significant increase in drug delivery was determined with the needle-free strategy in comparison with the bioadhesive patch ($P < 0.05$). Indeed an 80-fold increase in the total amount of TMP, and a 2.25-fold increase in the total amount of ALA, delivered into skin was seen with the jet injector device. Furthermore, by altering the viscosity of the injected liquid, the drugs could be targeted to various depths within the skin. Cross-sectioning the skin revealed that porphyrin delivery was restricted to the site of injection.

Conclusions This investigation has demonstrated how needle-free injection can be used to significantly enhance topical delivery of ALA and a model pre-formed photosensitiser. This technology has the potential to increase the efficacy of this treatment modality and enhance the range of photosensitisers that can be employed. Furthermore, targeted delivery by jet injection can negate the effects of prolonged photosensitivity frequently seen with systemic administration of pre-formed photosensitisers.

Casas, A., et al (2000) *Br. J. Dermatol*. **143**: 564–572
De Rosa, F. S., Bentley, M. V. L. B. (2000) *Pharm. Res*. **17**: 1447–1455

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Modulation of stable W/O/W multiple emulsions to target the epidermis and decrease the percutaneous absorption of a hydrophilic drug

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Objectives The aim of this study was to evaluate the influences of two different W/O/W multiple emulsions (CME1-01 and CME1-02) on the penetration of caffeine through the skin when they were compared with an O/W emulsion (CA1-00) of similar composition. By enhancing drug accumulation at the site of administration, multiple emulsions may improve its activity and reduce serious side effects due to undesirable systemic absorption (Youenang Piemi et al 1998).

Methods Lipophilic and hydrophilic silicones copolyoles were used as emulsifiers to formulate two stable W/O/W multiple emulsions (ME) at room temperature using 1% caffeine (CAF) in the inner phase as a hydrophilic drug model and a mix of reological additives in the external phase (Mouriño de Cortese et al 2006). A two-step process was used for the preparations of both ME (Raynal et al 1993). The total percentage of the liphophilic emulsifier system (LES) remained stable; the only difference between both of them was the composition of LES. CME1-02 was made by using a mix of PEG/PPG-18/18 dimeticone and Cethyl PEG/PPG-10/1 dimeticone (1:5), while CME1-01 had only Cethyl PEG/PPG-10/1 dimeticone. To avoid any influence of the ingredients of the formulations and to study only the effect of the emulsion type, the multiple emulsions and the O/W emulsion (CA1-00) were prepared with exactly the same composition. The distribution of CAF within skin tissue was studied by the tape stripping method after 24-h i*n vitro* permeation study using Franz Cells. Caffeine levels were analysed in epidermis and dermis, by liquid chromatography.

Results The topic application of both formulations studied (CME1-01 and CME1-02) significantly reduced the amount of CAF permeated when compared with the O/W emulsion (CA1-01) as discussed in previous works (Mouriño de Cortese et al 2006). In addition, the amount of CAF trapped in the epidermis after a 24-h i*n vitro* permeation study was significantly higher when CME1-01 and CME1-02 were compared with CA1-01 ($P < 0.05$, $n = 5$). The total amount of CAF found in the epidermis after 24 h (expressed as a percentage of the applied dose) of CEM1-01, CEM1-02 and CA1-00 were 8.87 ± 1.15; 8.52 ± 2.53; 3.63 ± 1.90, respectively. When both ME formulations (CEM1-01 and CEM2-02) were compared with each other, they did not show significant different release profiles ($P < 0.05$, $n = 5$).

Conclusions Both formulations (CME1-01 and CME1-02) seemed to be efficient vehicles to control caffeine distribution within the skin despite the liphophilic emulsifier system used.

Mouriño de Cortese, V., Serrao, R. (2006) *J. Pharm. Pharmacol*. Suppl. 1: A66
Raynal, et al (1993) *J. Controlled Release* **26**: 129–140
Youenang Piemi, et al (1998) *Int. J. Pharm*. **171**: 207–215

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ESEM: a possible vehicle for assessing liposome stability?

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Objectives Within this study our aim was to investigate an alternative assay for determining stability of liposomes (multilamellar vesicles; MLV) prepared to deliver solubilised lipophilic drugs. The potential application of liposomes as therapeutic tools is still limited by their innate physical and chemical instabilities, which can lead to an enhanced bilayer permeability and eventual loss of drug, vesicle aggregation/fusion, and precipitation (Mohammed et al 2007). To effectively deliver a formulation, the preparation must display a degree of stability. Using environmental scanning electron microscopy (ESEM) we have followed the dynamic stability of liposomes during dehydration and investigated the effect bilayer composition has on this.

Methods Liposomes were prepared by an already well-established hydration method (Bangham et al 1965), with the addition of 1.0 mg of poorly water-soluble propofol (logP = 3.79). MLV were prepared from 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) with varying concentrations of cholesterol (11, 20 and 33 total molar %). ESEM analysis of liposomes encapsulating propofol was performed by gradually reducing operating pressures whilst maintaining constant temperature (5 °C throughout). Since it is widely established (Manosroi et al 2003) that the presence of cholesterol provides an enhanced degree of physical bilayer stability as compared with formulations without the cholesterol, a sample without the stabiliser was not examined.

Results ESEM investigations were initiated at a pressure of 4.5 Torr and at a constant operating temperature of around 5 °C, at which point all samples revealed fully formed liposomes. Upon gradual decrease of pressure to 3.0 Torr, all preparations maintained their spherical morphology. A further period of controlled dehydration via reduction of the operating pressures from 3.0 Torr to 1.1 Torr showed no signs of morphological alterations of the formulations consisting of 11 and 20 total molar% cholesterol. The vesicles were spherical, intact and resisted any variations in the pressure changes. However, the reduction in pressure resulted in the liposomes containing 33% cholesterol losing their spherical shape as widespread flattening and spreading occurred, to yield lipid patches. The results suggest DSPC formulation prepared with the inclusion of 33% cholesterol and propofol (1.0 mg) is not as stable as the formulations prepared from 11% and 20% cholesterol. The ESEM analysis appear to relate to preliminary optimisation studies that showed that increasing cholesterol content of MLV to 33% results in inferior drug-encapsulation capabilities compared to samples enclosing 11 and 20% stabiliser, therefore supporting the microscopy findings of a less stable MLV formulation at relatively high levels of cholesterol content.

Conclusions Studies confirm the use of ESEM for monitoring the alterations in MLV morphology in real time under varied pressure and humidity concentrations, thereby potentially providing a substitute assay of investigating liposome stability.

Bangham, A. D., et al (1965) *J. Mol. Biol*. **13**: 325–328
Manosroi, A., et al (2003) *Colloids Surf. B* **30**: 129–138
Mohammed, A. R., et al (2007) *Eur. J. Pharm. Sci*. **30**: 406–413

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Liposome formulations containing an alternative to cholesterol

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Objectives The aim of this work was to investigate the role of phospholipid alkyl chain length on encapsulation and drug release from various propofol-encapsulated phosphatidylcholine liposomes (multi-lamellar vesicles; MLV) stabilised using tetradecanol as an alternative to cholesterol. Cholesterol is extensively used in liposome formulation and is generally accepted to enhance bilayer stability, retard permeation of solutes and prevent leakiness. Recently, stable niosomes have successfully been formulated from fatty alcohols (Devaraj et al 2002). Therefore it would be instructive to study the effect of a fatty alcohol (tetradecanol) on liposomal drug-loading and release.

Methods MLV were formulated via the well-established hand shaking method (Bangham et al 1965), with the addition of 1.0 mg hydrophobic propofol. The lipids investigated were 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC, C_{18} alkyl chain length), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC, C_{16} alkyl chain length), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC, C_{14} alkyl chain length) and phosphatidylcholine (PC, C_{12} alkyl chain length) along with addition of 11 total molar% tetradecanol in all cases, as decided by preliminary optimisation studies. Drug encapsulation and release were measured by spectrophotometric analysis of supernatant following MLV separation via centrifugation.

Results Incorporation studies using PC and its derivates reveal a general increase in propofol MLV-encapsulation with an increase in lipid chain length in the order of DSPC > DPPC = DMPC > PC ($P < 0.05$). This correlation between alkyl chain length and drug-loading may be attributed to increased lipophilic area within these longer lipid bilayers. Significant changes in release rates were observed with changes in alkyl chain length of phospholipid. The release rate generally increased with a decrease in the chain length of phospholipids in the order of PC ≥ DMPC > DPPC > DSPC following the 72-h study at 37°C ($P < 0.05$). Both DSPC and DPPC formulations showed sustained release of propofol (45% and 65% of the total entrapped, respectively) after 72 h. The PC and DMPC formulations, while initially (up to 48 h) showing a sustained release profile, rose to about 90% after 72 h. Bilayer drug release is recognised to be influenced by the phase transition temperature (T_C) of the lipid excipients within the vesicles, which in turn is significantly dictated by the alkyl hydrocarbon chain length. As the hydrocarbon chain length is increased, van der Waal's interactions between the alkyl chains become stronger necessitating additional energy to disrupt the ordered packing, thus increasing phase transition temperature. As a consequence, at 37°C, both PC ($T_C < 0$°C) and DMPC ($T_C = 23$°C) vesicles will be in the fluid state, leading to an enhanced drug-leakage when weighed against its higher transition temperature counterparts, DPPC ($T_C = 45$°C) and DSPC ($T_C = 55$°C).

Conclusions Liposomes could successfully be formulated with phosphatidylcholine and its derivatives using tetradecanol as bilayer stabiliser. Generally, encapsulation and retention of propofol was enhanced by employing longer-chain phospholipids.

Bangham, A. D., et al (1965) *J. Mol. Biol*. **13**: 325–328
Devaraj, G. N., et al (2002) *J. Colloid Interface Sci*. **251**: 360–365

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Bisepoxide-crosslinked-PEI nanoparticle for gene delivery: development and characterization

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Objective Cationic polymers (i.e. polyethyleneimine, polyallylamine, poly-L-lysine) having amino groups are poor transfection agents and possess high cytotoxicity index when used without any chemical modification and usually entail specific receptor-mediated endocytosis or lysosomotropic agents to execute efficient gene delivery. In this report, amino groups of polyethyleneimine (PEI, 25 kDa) were reacted with a homobifunctional crosslinker, 1,4-butanediol diglycidyl ether (Bisepoxide reagent), to prepare bPEI-BE nanoparticles. The novel cross-linkers have not only reduced the toxicity of high molecular weight PEI but also improved the transfection efficiency remarkably, which may be due to small size and efficient internalization into the mammalian cells.

Methods The efficacy of bPEI-BE nanoparticles in delivering a plasmid encoding enhanced green fluorescent protein (EGFP) gene was assessed in HEK293 and COS-1cell lines, where their cytotoxicity was also investigated. The PEI was chemically modified by cross-linking with Bisepoxide reagent to synthesize compact nanoparticles. The cross-linking was controlled by adjusting the weight ratio of the bisepoxide reagent and PEI for coupling reaction. The extent of cross-linking was determined by ^{1}H NMR. The bPEI-BE nanoparticles were further characterized by measuring the particle size (dynamic light scattering and atomic force microscopy), surface charge (zeta potential), DNA accessibility experiment and buffering capacity. The cytotoxicity was examined using the MTT method.

Results In vitro transfection efficiency of synthesized nanoparticles is increased up to several folds compared with native polymer, while maintaining the cell viability nearly 100% in the cell lines. Nanoparticles possess positive zeta potential at 25–30 mV and a size range of 85–100 nm (by DLS) in water. As the cross-linking was increased, a concomitant decrease in size of the nanoparticles was observed but an increase in the size of DNA loaded particles was observed compared with unloaded ones. However, in the presence of 10% serum, the hydrodynamic diameter of samples reduced significantly. The surface charge of nanoparticles was found to decrease by increase in percentage of cross-linking. Zeta potential profile showed that the excess surface charge on PEI was effectively masked in case of nanoparticles. The zeta potential of all nanoparticle formulations also showed similar trend after complexing with DNA, while maintaining the overall positive charge. The zeta potential of the nanoparticles decreased on forming a complex with DNA. The zeta potential of nanoparticles/DNA complexes was found to be negative in FCS. The protonation tendency of bPEI-BE nanoparticles changed only marginally by cross-linking with Bisepoxide reagent as compared to native PEI, indicating that the charge is not destroyed on crosslinking with Bisepoxide reagent. The DNA accessibility experiment demonstrated that nanoparticles formed relatively loose complexes with DNA compared with native PEI. An acid-base titration showed marginally reduced buffering capacity in comparision with the unmodified PEI.

Conclusions The bPEI-BE nanoparticles nanoparticles reveal tremendous potential as novel delivery system for achieving improved transfection efficiency, while keeping the cells at ease.

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The diversity of alginates as drug delivery systems

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Objectives Alginates are versatile and have wide use within the pharmaceutical industry. With regard to liquid pharmaceuticals, sodium alginate has been used to produce anti-reflux preparations such as Gaviscon. Experimentally, alginates have been used to produce a gastro-retentive dosage form that has the potential to improve drug bioavailability compared with that from many commercially available immediate and modified release products. The current work objectives were: to investigate the *in vitro* release of a model drug, riboflavin, from calcium alginate beads in media designed to reflect pH differences through the gastrointestinal tract; and to investigate the raft thickness and raft strength of oral alginate preparations using different concentrations of hydrochloric acid, (HCl).

Methods Calcium alginate beads were prepared by extruding sodium alginate solution 2% w/w into calcium chloride 0.02 M solution. Precipitated calcium alginate beads were freeze-dried. The formula was modified to produce calcium alginate beads containing a model drug, and citric acid, an agent known to retard gastric emptying. A modified USP XXII apparatus was used to study the *in vitro* release of riboflavin from the beads. The release profiles were compared using the f_2 equation (US Department of Health and Human Services, FDA 1997). For raft preparations, alginate tablets and suspensions were added to warmed amounts of HCl of different molarities and the raft thickness measured. A modified beam balance measured the force required to pull a horizontal wire probe through the alginate and determined the raft strength. Statistical analysis determined if there were significant differences in raft strength and thickness between formulations at different HCl molarities.

Results The slowest riboflavin release from the beads occurred in acidic media, (t_{50} ~ 150 min); the fastest riboflavin release occurred in near neutral media (t_{50} ~ 40 min). In small intestine conditions, rapid drug release commensurate with calcium alginate solubility was observed. Therefore the current formulation is unsuitable as a gastro-retentive dosage form and requires modification. When placed in selected media, for each formula of beads containing set concentrations of riboflavin and citric acid, similarity within the profiles was demonstrated. Addition of citric acid to the bead formula did not affect riboflavin release from the matrix. Regarding raft formulations, no rafts were formed using tablet or suspension formulations at HCl concentrations of 0.01 or 0.02 M. When tablets were used to form the raft, a decrease in raft thickness with an increase in acid molarity from 0.03 M to 0.15 M was noted. Raft formulations exhibited a maximum requirement for HCl concentrations and the thickness of rafts converged to common values at 0.15 M. Raft strengths decreased with increasing raft thickness. A significant difference was noted between suspensions containing 65–75%, 40–45% and 35% guluronic acid at molarities of 0.03, 0.07, 0.14 and 0.15 M. The differences may have important implications for the treatment of gastric disorders.

Conclusion The versatility of alginates has been confirmed. The dosage forms investigated are contrasting pharmaceuticals that can be used not exclusively for but with particular regard to the treatment of gastric disorders.

US Department of Health and Human Services, FDA, CDER (1997) *Guidance for Industry. Dissolution testing of immediate release solid oral dosage forms*. August 1997.

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Effect of adapalene pretreatment on in vitro skin penetration and distribution of topically applied clindamycin phosphate

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Objectives Adapalene and clindamycin phosphate have demonstrated clinical efficacy in the treatment of acne vulgaris. The objective of this study was to evaluate the effect on skin penetration and distribution of clindamycin phosphate when it is applied concomitantly or after time duration with adapalene.

Methods The in vitro skin penetration and distribution studies were carried out using excised rat skin mounted on modified Franz diffusion cells. To rat skin sections the clindamycin phosphate (1%) gel at a target dose of 5 mg/dose/cm^2 was applied concomitantly and after pretreatment of skin for 3, 5 and 10 min with 10 mg of adapalene (0.1%) gel, clindamycin phosphate gel was applied alone to serve as control. At the end of the application period (12 h), the entire dosing area of skin was collected and clindamycin phosphate was quantified in the skin compartments and in the receptor fluid by using HPLC.

Results Adapalene act as a penetration enhancer and concomitant application of adapalene gel increases the penetration of clindamycin phosphate (8.7%) as compared with control (5.13%). In vitro skin penetration and distribution of clindamycin phosphate seems to be related to the pretreatment time. Pretreatment of skin with adapalene gel for 3 and 5 min before the application of clindamycin gel significantly increases the penetration to 12.75% and 15.5% ($P < 0.05$), respectively, with a higher proportion of clindamycin phosphate (7.5% and 9.4% of the applied dose, respectively) in viable skin. Furthermore, with increase in pretreatment time to 10 min, no statistically significant difference was observed. Modest amounts of clindamycin phosphate were analysed in the receptor phase indicating minimized systemic side effects.

Conclusions As shown in this study, the characteristic property of adapalene, to enhance the penetration of clindamycin phosphate into skin, makes it a good choice as a therapy in combination with clindamycin phosphate for the treatment of acne. Application of clindamycin phosphate gel after pretreatment of skin with adapalene gel for 5 min may contribute significantly to increased efficacy of therapy.

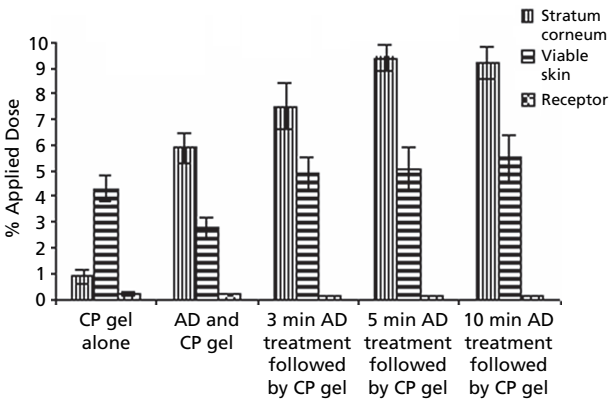

Figure 1 Effect of adapalene (AD) on skin distribution profile of clindamycin phosphate (CP).

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Enhanced release of BSA from silicone elastomers crosslinked with vinyl-terminated PEG

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Objectives Silicone elastomer is widely used in the manufacture of medical and controlled-release drug delivery devices due to its flexibility and biological inertness. Due to the hydrophobic nature of poly(dimethylsiloxane) (PDMS) there is interest in developing silicone elastomer biomaterials that have enhanced hydrophilicity. In this study, vinyl-terminated poly(ethyleneglycol) (AAPEG) and/or vinyl-terminated poly(dimethylsiloxane) were used to crosslink hydride-functionalised PDMS in a platinum-catalysed addition-cure reaction to produce silicone elastomers with inherent hydrophilicity. The objective of this study was to investigate the bovine serum albumin (BSA) release characteristics of the AAPEG-crosslinked silicone elastomers compared with conventional poly(dimethylsiloxane) elastomer systems that contained no AAPEG.

Methods Formulations tested contained hydride-functionalised PDMS crosslinked with vinyl-terminated PDMS:AAPEG (molar ratio) 100:0, 75:25, 50:50, 25:75, 5:95, 1:99 and 0:100. BSA was incorporated into the modified silicone materials at 1%w/w loading. Control samples were silicone crosslinked with vinyl-terminated PDMS only. The silicone elastomer samples prepared were placed into sample flasks containing release medium (distilled water 10 mL) and placed in an incubator at 37 °C and 60 rpm. The ability of the BSA to be released *in vitro* from the silicone matrices was analysed using a Micro BCA Protein Assay Kit. Five replicates, each from a single preparation, were analysed for each formulation.

Table 1 Summary of release of BSA from silicone crosslinked with AAPEG and/or vinyl-terminated PDMS

PDMS:AAPEG (molar ratio)	AAPEG (%w/w)	Day 1 BSA release (μg)	Day 14 BSA release (%)
100:0	0	16.8 ± 5.2	2.3
75:25	0.3	17.3 ± 3.1	6.3
50:50	1	28.3 ± 22.2	8.5
25:75	3	26.5 ± 4.3	12.5
5:95	12	48.5 ± 13.3	29.5
1:99	22	56.9 ± 28.0	32.2
0:100	28	195.6 ± 22.5	44.5

Results Release data demonstrated that the pegylated silicone elastomers facilitated enhanced release of BSA compared with the control that contained no AAPEG. The amount of BSA released increased with increasing AAPEG content in the formulations. Table 1 summarises the release of BSA over 14 days.

Conclusion Silicone modified with AAPEG could potentially be used in medical devices to provide enhanced release of BSA that might not otherwise be effectively released from conventional poly(dimethylsiloxane) elastomer systems.

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Closed loop glucose control of diabetic rats

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Objectives Diabetes is a disease in which continuously good control of glucose would be a major improvement on current treatment regimens, for the prevention of the sequelae of diabetes. Here, a previously described negative feedback response (or closed loop) system (Taylor et al 2003) has been further tested to include several sequential glucose challenges in vivo. The system consists of a polycarbonate insulin reservoir with a glucose-sensitive membrane for peritoneal implantation where mesenteric uptake is fast enough for the variable output to be transmitted to the liver.

Method The glucose-sensitive polymer was produced by UV irradiation of derivatised dextran and concanavalin A (Tanna et al 2006) and the sterilised devices implanted into Sprague-Dawley rats made diabetic later using streptozotocin. The rats were then challenged on several days using standard and triple sized oral glucose tolerance tests and the profiles monitored by blood glucose sampling at convenient time intervals for several hours.

Results For the test systems using the smart material, daily readings showed good control and when challenged with either size of tolerance test, the excursions from normal were minimal compared with control systems (Figure 1). This was repeated over several days until the device was exhausted and, based on the requirements of similar but non-implanted diabetic rats and also on in vitro output rates, implied a mean basal dose rate of 0.5 U/kg/30 mmol/L/h with boosts created by an approximately doubled output until threatened blood glucose rises were in control.

Conclusion This is a design for closed loop insulin delivery that is not based upon biological or electronic principles. The device is simple, needs neither electrical power nor immunosuppression. Self-adjusting insulin delivery in portable form has not yet been achieved for routine clinical use, although implantable electronic pumps are presently being assessed for such a purpose. Assuming the design challenges for safety and refillability can be solved, this method presents a possible alternative that could be cheap and available in large numbers. The appropriate ethical approval was obtained.

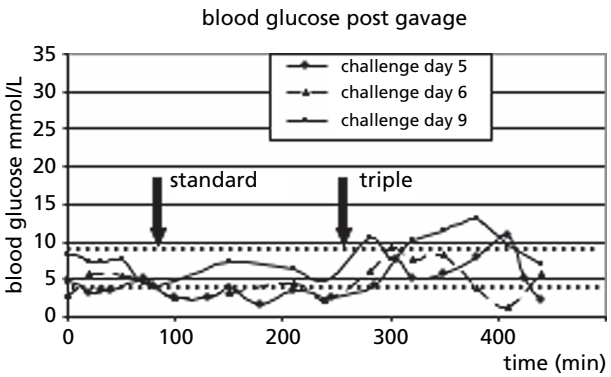

Figure 1 The first challenge (standard size) and the triple size challenge profiles compared with normal tolerance zone (grey), on each of three days.

Tanna, S., et al (2006) *Biomaterials* **27**: 498–507
Taylor, M., et al (2003) *Drug Deliv. Systems Sci*. **3**: 1117