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Formulation of a stable, sterilised vaccine against *M. tuberculosis* – preservation of effective immune responses

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Objectives The combination of dimethyldioctadecyl ammonium bromide (DDA) and synthetic cord factor, trehalose dibehenate (TDB), can engender protective immune responses against *M. tuberculosis* infection (Holten-Andersen et al 2004). However, problems such as lack of stability and production of an economical sterile product have motivated investigation into formulation aspects such as freeze drying and γ-irradiation sterilisation (Mohammed et al 2006). This study evaluates the immunogenicity of these optimised formulations.

Methods DDA/TDB formulations were prepared by lipid hydration. BALB/c mice (n = 5) were immunised with vaccines incorporating a range of cryoprotectants, or vaccines exposed to gamma radiation (25 KGy) as approved by the local ethical review committee. Immunological analysis was as previously outlined (Vangala et al 2007). Briefly; antibody responses were assessed using ELISA; spleen cell proliferation was quantified using uptake of ^{3}H thymidine following restimulation; and cytokine production was evaluated using DuoSet capture ELISA (R & D Systems).

Results Almost all of the antibody mediated immune responses were comparable with no adverse effect on immunogenicity. However, antigen specific cell proliferation (Figure 1) showed that the inclusion of lysine plus sterilisation facilitated superior cell proliferation to any of the other groups except lysine without sterilisation. The inclusion of lysine has not previously been evaluated for immunogenicity and clearly warrants further investigation. IL-2 levels indicated that the increased cell proliferation was probably associated with enhanced T cell expansion. IL-2 and IFN-γ production mirrored the cell proliferation results.

Conclusions In all tests, the various modifications facilitating production of a sterile and more stable product, do not adversely affect immunogenicity. This study supports the use of these optimisations in the formulation of this vaccine.

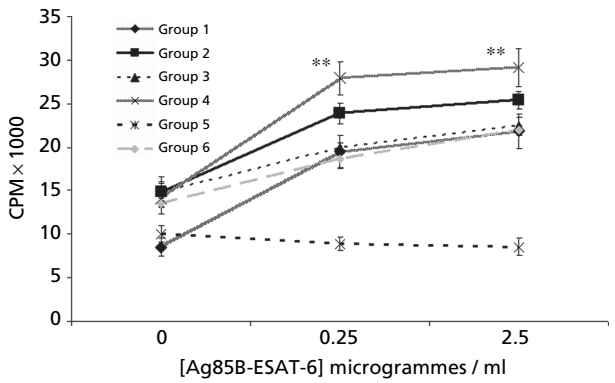

Figure 1 Spleen cell proliferation following stimulation with antigen. 1. DDA/TDB (Freshly Prepared); 2. DDA/TDB (Freeze Dried, Lysine); 3. DDA/TDB (Freeze Dried, Sucrose); 4. DDA/TDB Sterilised (Freeze Dried, Lysine); 5. Naïve Control; 6. DDA/TDB (Freshly Prepared). **denotes significantly increased in comparison to all other groups except group 2 (n = 5, $P < 0.05$).

Holten-Andersen, et al (2004) *Infect. Immun*. **72**: 1608–1617
Mohammed, et al (2006) *Methods* **40**: 30–38
Vangala, et al (2007) *J. Controlled Release* In press

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Modelling the release of drugs from PEG 8000 solid dispersion systems to determine the mechanism of release

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Objectives Solid dispersions are an attractive means of improving the dissolution rate of poorly soluble drugs. However the development of these systems has been hampered due to the poor understanding of the mechanisms of release from these systems. The work presented here investigates the mechanism of release of two drugs from the same carrier to note the effects of drug or carrier and drug loading on the mechanism of drug release. Poorly soluble drugs paracetamol and sulfamethoxazole were investigated within polyethylene glycol (PEG 8000) as a carrier.

Methods Solid dispersions were prepared by melt fusion method. Formulations with 5, 10 and 15% w/w drug loadings were investigated. Dissolution studies were carried out using a dissolution bath at 37 °C in 1000 mL of PBS. Drug analysis was performed via UV spectrophotometry at wavelengths of 252 nm and 240 nm for sulphamathoxazole and paracetamol, respectively. Release data was fitted to both the Peppas equation and the Peppas and Sahlin equation to provide information on the mechanism of release. The Peppas equation provides values for k, a kinetic constant and n, the diffusional exponent. The Peppas and Shalin equation provides values for k_1 and k_2 the kinetic constants associated with diffusional and relaxational release, respectively.

Results Table 1 shows the constant calculated from the fitted data. These results show a tendency towards diffusional rather than relaxational release as k_1 is greater than k_2 in all cases. The similarity of all values indicates that for these two drugs it is the carrier that controls drug release, this is particularly evident for the 5 % w/w loading as the k, k_1 and k_2 values are similar in all cases. As expected there was a general trend of a decrease in the diffusional release rate constant, K_1 as the drug loading increased.

Conclusions This study has demonstrated that for the systems investigated it is the carrier that controls release of these poorly soluble drugs. The drug release data fitted to the Peppas and Peppas and Sahlin equations has allowed comparisons of the theoretical mechanism of release to be compared.

Table 1 Results from the statistical analysis of data using Peppas and Peppas and Sahlin models

Formulation	Peppas Model			Peppas and Sahlin		
	k	n	R^2	K_1	K_2	R^2
P 5%	0.2095	0.43	0.90	0.1963	<0.001	0.89
P 10%	0.2075	0.43	0.92	0.1940	<0.001	0.91
P 15%	0.1462	0.50	0.92	0.1627	0.001	0.92
S 5%	0.2054	0.43	0.88	0.1924	<0.001	0.88
S 10%	0.1205	0.55	0.85	0.1385	0.006	0.84
S 15%	0.1348	0.52	0.90	0.1532	0.004	0.89

(P = Paractamol; S = Sulphamethaoxazole).

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The creation of a vitreous humour replacement: hydrogel formulations and freeze drying of the porcine vitreous humour

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Objectives To create a vitreous humour (VH) replacement using two methods. 1) A completely artificial hydrogel/liquid formulation, 2) Freeze-drying and

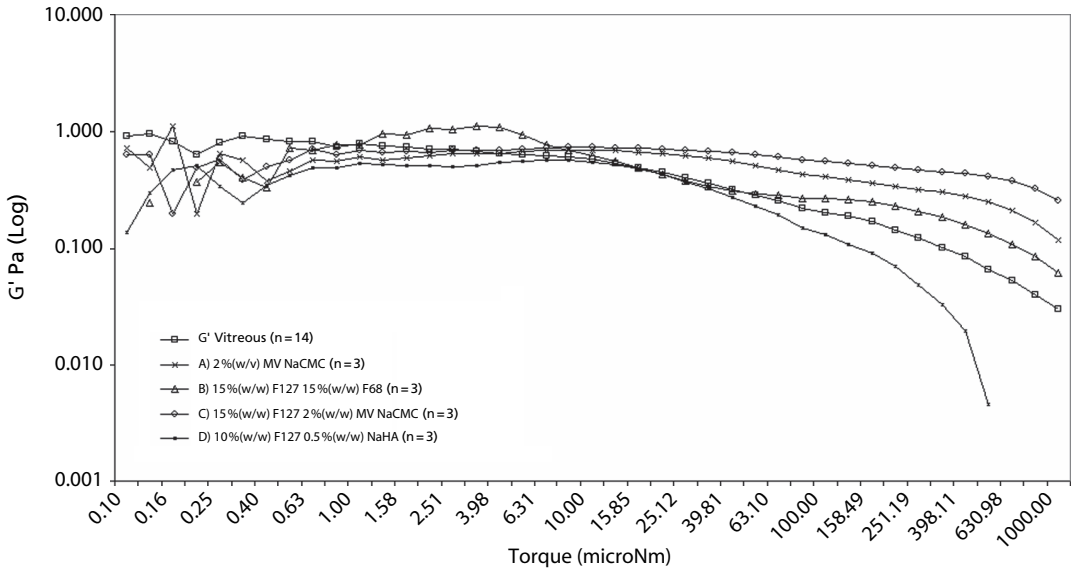

Figure 1 G' behaviour match to fresh (< 4 h post mortem) porcine vitreous humour.

reconstituting the natural vitreous humour. Porcine vitreous humour was used as a model.

Methods 1) Various concentrations of assorted hydrogel powders were dissolved in balanced physiological liquids. The gels produced were tested for clarity, cytotoxicity, pH, density and their capability to performance match natural porcine vitreous humour in rheological terms. 2) Fresh vitreous humour (<4 h post mortem) taken from porcine globes was freeze-dried. Water loss was measured using Thermogravimetric analysis. The freeze-dried samples were reconstituted with an ocular specific physiological saline solution and their integrity measured using texture analysis (TA) (n = 6).

Results 1) Rheological analysis showed that four formulations (A-D) show similar G' values as the natural vitreous humour during a torque sweep (Figure 1). These formulations are completely transparent and show minimal light scatter. Densities (g/cm^3) and pH compared to the natural vitreous (0.97, 8.44) are respectively; A) 0.98, 7.73; B) 1.10, 7.11; C) 1.07,7.19; D) 1.11, 7.24. *In-vitro* cytotoxicity studies (human ARPE-19 cells) of the individual polymer components have shown minimal toxicity issues. 2) Successful VH freeze drying was achieved. Samples taken from the centre of the VH showed an average liquid content of 2.21 ± 0.64%. Samples reconstituted with the whole volume of liquid they had lost gave similar TA values (grams force in compression) to those that had been reconstituted with half the amount when tested *in situ* (0.80 ± 0.02, 0.74 ± 0.02 n = 4). This is in contrast with the fresh porcine vitreous result of 34.32 ± 11.84 (n = 25).

Conclusions 1) Rheological studies on several apparently bio-compatible gel formulations suggest that the physical behaviour can be matched thus the development of a VH replacement is feasible. 2) The VH freeze-dried successfully, producing a nice cake with low moisture levels in all cases. However, on reconstitution only a minor gel component was produced even when the volume of reconstituting medium was decreased. TA data was significantly different to the unprocessed material. Further work is ongoing examining the effect of a cryoprotectant on the structural integrity of the material during freeze-drying.

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Preparation, evaluation and characterization of ciprofloxacin dry powder for inhalation (DPI)

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Objectives Pulmonary administration of antibiotics is a mean of providing a more effective treatment for lung associated diseases by targeting the drug to the infected site as well as decreasing the systemic toxicity of these drugs. In patients with pulmonary infections, particularly those with cystic fibrosis, the pharmacokinetics of the administered antibiotics are usually altered, thus necessitating the prolonged administration of excessive doses, which can lead to the development of adverse effects and antibiotic resistance. This study investigates the possibility of preparing respirable ciprofloxacin (CFX) microparticles in order to improve the drug safety profile. Currently, there are no available dosage forms suitable for pulmonary administration of this antibiotic (Leticia et al 2007).

Methods Accordingly, CFX (100, 250 or 500 mg) was dissolved in a solution of chitosan in acetic, hydrochloric, glutamic or aspartic acids with or without 50 mg of either polyethylene glycol 8000 or dipalmitophosphatidyl choline. Leucine (60, 120 or 180 mg) was finally added as a dispersibility enhancer (Naumana & Peter 2005). The solution was spray dried on a Buchi spray dryer using an inlet temperature of 110 °C, aspiration 85%, pump rate of 4 mL/min and the outlet temperature was 70–72 °C. The particle size and morphology of the resultant powders were evaluated. X-ray diffraction (XRD) and Differential scanning calorimetry (DSC) were used to evaluate the surface crystallinity and thermal properties of the powders. The *in vitro* aerosolization and deposition of the powders were determined using gelatin capsules loaded with 20 mg of the powder and aerosolised with a DPI device (aerolizer) into a twin stage impinger at a flow rate of 60 L/min.

Results The volume median diameter of the particles varied from 2.3 to 6.5 μm. SEM confirmed the results and showed that the morphology of the particles varied with variation of the acid used as well as the drug concentration. X-ray diffraction revealed an amorphous surface. The crystallization of the drug on the surface of the particles with previously used polymers was not seen here. DSC thermograms confirmed the results at the two low drug concentrations. However, with the high drug concentrations some drug crystals might be present inside the particles. More than 95% of the dose was efficiently emitted from the inhaler and a fine particle fraction of more than 60 % was achieved.

Conclusion By using this simple mild aqueous procedure, a high loading of CFX into a mucoadhesive biocompatible polymer was achieved. The spray drying conditions were suitable to produce respirable CFX powders. Lung targeting and a better safety profile are therefore expected. Further studies on the uptake of the particles by the macrophages are being undertaken.

Leticia, E., et al (2007) *Eur. J. Pharm. Biopharm*. **65**: 346–353
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Preparation, evaluation and characterisation of deoxyribonuclease 1 dry powder for inhalation

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Objectives Development of chronic pulmonary diseases is the main cause of mortality in cystic fibrosis (CF) patients. Currently, deoxyribonuclease I (DNase I) (MW 33 KDa) is delivered as a nebulization solution. This mucolytic agent acts locally to cleave undesirable neutrophils-derived DNA. These extracellular DNA, together with bundles of F-actin, are responsible for the presence of viscoelastic

sputum in CF patients. However, current treatment options are limited as certain types of nebulizers may compromise enzyme activity (Gonda 1996). Moreover, the high dosage frequency (2–3 times daily) is a concern with regards to the potential cost of treatment. Anionic polyamino acids and negatively charged polysaccharides have been shown to enhance DNase I activity and reduce the viscosity of CF sputum (Feng et al (1999). Accordingly, we investigated the possibility of combining DNase I with these enhancers in a single formulation that can be delivered as dry powder by inhalation (DPI). This combined therapy can offer the potential of better penetration through the thick mucus as well as decreasing the dose of DNase I and hence the cost of treatment.

Methods DNase I solutions containing hydroxy-propyl–β-cyclodextrin (HP-β-CD), ovalbumin, Ca^{2+}, Mg^{2+} and Na^{+}, leucine, with or without the addition of adjuvants (L-polyglutamic acid, L-polyaspartic acid or dextran sulphate) were prepared. These solutions were then spray dried. The particle size was determined and the morphology of the resultant powders were evaluated by SEM. The *in vitro* aerosolization and deposition of the powders were determined using gelatin capsules loaded with the powder and aerosolised with a DPI device (aerolizer) into a Sympatec inhale system or a twin stage impinger at a flow rate of 60 L/min. To investigate effect of manufacturing parameters on enzyme integrity, the recovered enzyme was subjected to SDS-PAGE. Agarose gel electrophoresis and a single radial enzyme diffusion (SRED) method were used to detect the activity of the recovered enzyme before and after delivery from the inhaler.

Results The process yield was high and reached 90% in presence of the adjuvants. The volume median diameter of the particles was found to be 4–6 μm. More than 96% of the dose was efficiently emitted from the inhaler. A fine particle fraction of about 97% was achieved with the Sympatec inhale system but a corresponding 75% was found when using the twin impinger method. SDS-PAGE confirmed the integrity of the protein. The recovered enzyme retained full activity as detected by the SRED method after calculating the diameters of the zone of inhibition.

Conclusion In conclusion, novel spray dried microparticles containing DNase I were successfully prepared for delivery by dry powder inhalation. The mild manufacturing conditions, together with the stabilizers selected were able to protect the enzyme activity. The effects of the adjuvants used has to be investigated on the mucus of CF patients in order to prove the efficacy of this suggested combined treatment.

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Innovative topical microemulsion gel of babchi oil (*Psoralea corylifolia*) as antipsoriatic agent

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Objective Psoralen, a main constituet of babchi oil (*Psoralea corylifolia*), is one of the naturally occurring furocoumarins, which have been exploited for their photosensitizing properties since ancient medicine, and is used for treatment of psoriasis disorders. The aim of this study was to develop and evaluate a microemulsion gel based system of babchi oil (*Psoralea corylifolia)* for the treatment for psoriasis, which could provide improved permeation of the drug through the skin, reduce the dosing frequency and increase patient compliance.

Method Simultaneous determination of standard psoralen (purchased from Sigma-Aldrich) and eight different brands of babchi oil were quantified at 250 nm by HPTCL method. By HPTLC analysis 1.5 μg/μl psoralen was determined in best brands of babchi oil supplied by Vyas Pharmaceutical (Batch no. 0023). Therefore, this brand was selected for microemulsion gel formulation. The solubility of babchi oil was determined in various oils (oleic acid, castor oil & olive oil), surfactants and cosurfactants. Various O/W microemulsions were prepared by spontaneous emulsification method. Micremulsion area was identified by construcing pseudoternary phase diagrams. Thermodynamic stability studies like centrifugation, heating & cooling cycles and freeze thaw cycles were carried out on selected microemulsion formulations. The formulations that survived thermodynamic stability tests were selected for further skin permeation studies with the skin of albino rat by Franz diffusion cell. Optimized formulation was characterized for particle size, viscosity, pH and refractive index. The microemulsion formulation showing the best *in vitro* skin permeation profile was converted into a gel using 1% carbopol 940. Immunomodulator activity was determined by foot pad reaction test for microemulsion gel formulation.

Result The solubility of babchi oil was found to be highest in oleic acid as compared to other oils. Thus oleic acid was selected as the oil phase for the development of optimal formulation. Highest solubility of drug was seen in Tween 80 and Transcutol-P. Therefore, Tween-80 and Transcutol-P were selected as surfactant and cosurfactant respectively for the study. Microemulsions were successfully prepared by spontaneous emulsification method. Pseudoternary phase diagrams were constructed for selection of microemulsion formulations. Maximum microemulsion region was found in the 1:1 ratio of surfactant to cosurfactant. Thermodynamic stability tests were performed on selected microemulsion formulations to remove metastable formulations. The mean droplet size of all microemulsion formulations was < 100 nm. Significant increase in permeability parameters was observed in optimized microemulsion formulation F1 which consisted of 6.7% w/w of babchi oil, 10% w/w of Oleic acid, 25% w/w of Tween-80, 25% w/w of Transcutol-P and 40% w/w of distilled water. Immunomodulator activity was found to be significant ($P < 0.05$) in micremulsion gel when compared with conventional marketed oil.

Conclusion A safe and effective microemulsion gel formulation of babchi oil consisting of oleic acid, Tween-80, Transcutol-P and distilled water for psoriasis was developed, which provided for enhanced permeation of the drug, and reduced dosing frequency.

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Characterisation of poly(ethylene glycol) plasticized poly(methyl vinyl ether-co-maleic anhydride) films

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Objectives Plasticizers are usually added to improve the mechanical and thermomechanical properties of films. Different molecular weights (MWs) (10 000, 1000 and 200 Daltons) and concentrations of poly(ethylene glycols) (PEGs) were incorporated as plasticizers in poly(methyl vinyl ether-*co*-maleic anhydride) (PMVE/MA) films. Physical, mechanical and thermal properties of cast films were investigated.

Methods A total of 52 films were prepared comprising PMVE/MA at 5, 10, 15 and 20% w/w alone and at 4:1, 2:1, 4:3 and 1:1 w/w ratios with individual PEGs. Various physical-mechanical properties, such as thickness (T), tensile strength (TS), percentage elongation (%E), young's modulus (YM), and work of failure (WF) were evaluated. In addition to this, the films were also investigated for their T_g and water content using DSC and TGA, respectively.

Results The results, showed that either an increase in PMVE/MA or PEG concentration or decrease in MW of PEG, or both, caused decrease in TS, YM, WF and T_g and increase in the %E. Table 1 shows the results of 1:1 ratio of PMVE/MA at 5% w/w and PEG films. However, PMVE/MA cast films at 15% and 20% w/w with PEG 200 in 1:1 ratio were too elastic to be tested for mechanical analysis. On the other hand, films without any plasticizer and PMVE/MA (at 5, 10 and 15% w/w) with different MW PEGs in 4:1 ratio could not be tested due to excessive stress on the equipment. Furthermore, thickness of films was observed to increase with increase in polymer concentration. However, water content of films was not statistically significant ($P > 0.05$) different.

Conclusion In conclusion, PEG 200 showed the highest plasticizing effect at higher concentrations. It is due to the fact that the number of hydroxyl groups per unit mass of low MW PEG is higher than that of high MW PEG, indicating that the low MW PEG is more hydrophilic than the high MW PEGs. Furthermore, increase in the PMVE/MA content also caused greater plasticizing effect, while maintaining similar ratios of PMVE/MA to PEG. Therefore the plasticization effect was related to the concentrations and molecular weights of plasticizers and polymer concentrations.

Table 1 Mechanical and thermal properties of 1:1 ratio of 5% PMVE/MA and PEG (10 000, 1000 and 200 Da) films

	PMVE/MA:PEG, 1:1		
	10 000	1000	200
TS, N/mm^2	31.02	27.29	2.21
YM, N/mm^2	139.82	12.87	1.29
WF, mm^{-1}.s^{-1}	9146.10	2528.32	1500.11
T_g, °C	9.19	8.62	7.04
%E	478.43	603.76	1238.32

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Formulation and characterisation of deformable liposomes of methotrexate using statistical experimental designs

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Objectives To formulate deformable liposomes of MTX using thin film hydration for enhancing topical delivery of methotrexate (MTX) in the treatment of psoriasis.

To apply statistical experimental designs in identifying the significant formulation and process variables affecting percent drug entrapment. Characterisation of optimal formulations for percent drug entrapment (PDE), particle size, polydispersity index, zeta potential, microscopy and in-vitro drug release.

Methods Formulation: liposomes were formulated using thin film hydration method with sodium cholate as the bio-surfactant which imparts deformability. Screening: three formulation and seven formulation variables varied at a high and low levels were screened to identify the important factors using Plackett Burman Screening Design (PBSD). Optimization: drug: phospholipid (X_1) and drug: sodium cholate (X_2) were identified as the significant factors. These formulation variables and their ranges chosen from the knowledge obtained from the PBSD were used to construct a central composite design (CCD) using the software Design Expert V 6.05. Characterisation: check point analysis of the optimal solutions was performed to confirm the utility of the statistical design. PDE was determined by ultracentrifugation at 20,000 X g. Particle size, polydispersity index and zeta potential were determined using laser diffraction (Zetasizer, Malvern Instrument). Morphology of the vesicles was studied by optical microscopy and in-vitro release was carried out using dialysis membrane. Chromatographic conditions: MTX concentration was quantified by HPLC using C18 column, mobile phase of acetonitrile:buffer (pH 6) at a flow rate of 1 ml/min at ambient temperature and 303 nm.

Results Results showed that the PDE ranged from 19.35% to 43.62 %. Analysis of variance indicated a quadratic model of CCD to be best fit model. The correlation co-efficient was found to be 0.8461. Response surface results indicated a positive effect of phospholipid concentration (X_1) i.e., increase in concentration of phospholipid increased entrapment efficiency whereas a negative effect was observed in case of surfactant concentration (X_2) i.e., increase in surfactant concentration decreased PDE. The average diameter of the deformable liposomes after extrusion ranged between 100 and 150 nm with uniform size distribution. Polydispersity index indicated the liposomal formulation was a homogenous dispersion as the values ranged from 0.121 to 0.214. Zeta potential being the overall charge a particle acquires in a particular medium was found to be between −20.8 to −21.9. In-vitro release of MTX from liposomal formulation was $0.81\pm 0.28\times10^{-2}$ and that of pure MTX drug solution was $3.71\pm0.97\times10^{-2}$ which attributes to the entrapment of MTX in liposomes sustaining the release of the drug as only the drug molecules present in the external aqueous phase were able to permeate across the semi-permeable membrane.

Conclusions Results obtained demonstrated the feasibility of applying statistical design models in the formulation of deformable liposomes of methotrexate. Further, this optimized formulation will be taken up for in vivo studies to show the suitability of such delivery systems for prolonged action of the topically applied anti-psoriatic agent, with lesser side effects than the conventional formulations.

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An assessment of pain, penetration efficiency and trans-epidermal water loss in human subjects following application of microfabricated microneedle arrays

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Objectives Microneedles provide a new method for transdermally delivering medicaments and vaccines (Birchall et al 2005). Although it is purported that microneedles can facilitate drug delivery without stimulating underlying pain receptors or blood vessels (Kaushik et al 2001), previous studies did not attempt to demonstrate both microneedle functionality and pain response. In this study we aim to investigate for the first time whether microneedles elicit pain after breaching the stratum corneum (SC).

Methods Following NHS ethics approval participants (n = 12) were randomised to receive on their buttock two types of microneedle array (36 pyramidal microneedles of either 180 μm or 280 μm height) and a 25-gauge subcutaneous hypodermic needle (3 devices). A visual analogue scale (VAS) measured (double-blind) pain where "no pain" = 0 cm and "worst pain imaginable" = 10 cm following application of each device. Formation, and retention, of microchannels was visualised for 24 h by external staining (methylene blue). Trans-epidermal water loss (TEWL) study measurements were taken pre- and up to 24 h post-application.

Results (n = 10 subjects (cf 12)) VAS scores were 1.03 cm for the hypodermic, 0.21 cm for 180 μm microneedle array and 0.005 cm for 280 μm array. The hypodermic causes significantly more pain than the 180 μm ($z = 1.97$, $P = 0.05$) and 280 μm ($z = 2.75$, $P = 0.006$); there was no significant difference between the arrays ($z = 1.61$, $P = 0.11$) (Wilcoxon matched pairs). Methylene blue staining showed microconduit formation with skin repair appearing between 8–24 h. TEWL measurements further demonstrated perbation of the SC barrier with water loss increasing post application of each device. Median TEWL increased immediately post-application for the hypodermic from 3.4 (range 13.6) to 8.7(range 17.4) gm H_2O/sq.m/hour; from 4.85 (range 13.9)–8.7 (range 8.8) for 180 μm; and from 4.1 (range 11.1)–6.1 (range 49.1) for the 280 μm array. Each recovered to baseline value by 24 h.

Conclusions This novel pilot study demonstrates that microneedles represent a less painful method for disrupting skin barrier properties than the hypodermic. Microchannel re-sealing appears to occur within 8 hours though some channels remained open at 24 hours. Studies of administration of a range of therapeutics via microneedles are warranted.

Acknowledgements: The British Skin Foundation for funding.

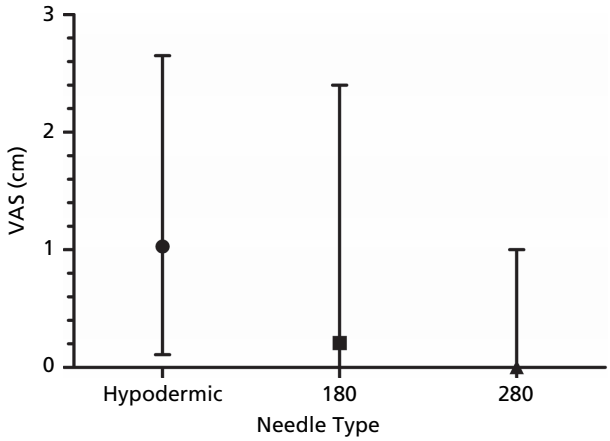

Figure 1 Median and range VAS scores for each device.

Birchall, et al (2005) *J. Drug Targeting* **13**: 415–421
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Drug delivery to the posterior segment of the eye using sub-conjunctival injection of thermo-setting gel formulations

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Objectives Age-related retinal diseases are increasingly common. Current available treatment is through direct intravitreal injection which is highly invasive and may cause further complications, necessitating development of alternative safe and effective ways of delivering drugs to the retina. Here, we have investigated the use of thermo-setting gel formulations, applied to the external scleral surface via sub-conjunctival injection (analogous to sub-cutaneous injection), as a means of targetting the retina in a relatively non-invasive manner.

Methods Fresh porcine eyes were obtained from a local abattoir. Four thermally responsive formulations based on aqueous solutions of poloxamer F127 (P) and alginate (A) were developed and physically characterised using oscillatory rheology and texture analysis. The formulations were: T1 (14% P/0.1% A), T2 (17% P/0.1% A), T3 (14% P/1.0% A) and T4 (14% P and 0.2% polycarbophil), with distilled water as control. Following sub-conjunctival injection into excised porcine eyes (n = 6 per timepoint) and incubation at pig body temperature, the distribution of the tracer sodium fluorescein through the ocular tissues was monitored over 18 h.

Results Texture analysis indicated that T2 showed much greater adhesion to porcine sclera (14.70 ± 4.43 g.s) than the other formulations, eg 0.18 ± 0.02 g.s for T1. Oscillatory rheological measurements showed that all formulations were liquid at room temperature but gelled rapidly (< 1 min) when exposed to body temperature and subsequently behaved in a visco-elastic fashion. Analysis of tracer location after sub-conjunctival injection indicated there was movement of tracer from the injection site through the sclera towards the choroid and the retina, eg T3 showed 8.01 ± 2.22 % retinal tracer accumulation after 18 h. Drug release from all gel formulations was statistically slower than from the control; indicating a sustained drug release. Suprisingly, all gel formulations behaved similarly *ex vivo*, indicating some interaction with the biological system.

Conclusions Sub-conjunctival injection of thermo-setting formulations may provide a novel and relatively simple method of drug delivery to the posterior segments of the eye.

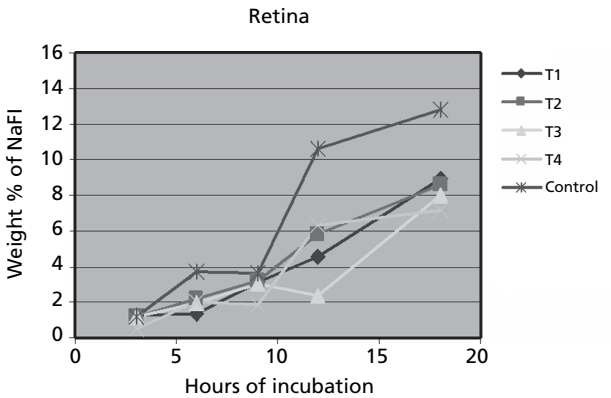

Figure 1 Percentage of tracer in the retina over 18 h following sub-conjunctival injection (error bars removed for clarity).

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Screening of vesicle based delivery systems for adjuvanticity in hepatitis B vaccination

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Objectives To assess the potential of hepatitis B surface antigen (HBsAg) loaded surfactant vesicle systems with two cationic agents, [*N*-(*N'*,*N'*-dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol) or dimethyldioctadecylammonium bromide (DDA). Previous reports showed that whilst most adults recover completely from hepatitis B infection, a percentage of infected individuals become chronic carriers. Thus emphasising a clinical need for effective management of chronic hepatitis B virus infection, vaccination is likely to be the cheapest and the most beneficial treatment (Michel & Mancini-Bourgine 2005). Vesicle based delivery systems such as liposomes and niosomes have been shown to be versatile carriers for various antigens.

Methods Vaccines were prepared, incorporating HBsAg into four compositions of vesicle systems; 1) DDA:trehalose 6,6'-dibehenate (TDB) (4:0.5 μmol); 2) 1-monopalmitoyl glycerol (MP) (16 μmol):cholesterol (Chol) (16 μmol) added to preparation 1; 3) MP:dioleoyl phosphoethanolamine (DOPE):Chol:DC-Chol (16:8:4:4 μmol); 4) MP replaced with phosphatidylcholine (PC) in preparation 3. Mice were immunised subcutaneously (1 μg HBsAg in 0.2 ml/dose) at days 0, 14 and 28 and blood samples were collected at designated time intervals. All *in vivo* protocols have been subject to stringent ethical review. Cytokine production was assessed using DuoSet capture enzyme-linked immunosorbent assay (ELISA) kits. Antigen specific proliferative responses were assessed measuring [3H] thymidine incorporation in cultured splenocytes. Post immunisation sera samples were analysed for the anti-HBsAg IgG1, IgG2a, IgG antibodies by ELISA.

Results The free HBsAg was able to initiate better HBsAg-specific IgG1 and IgG antibody immune responses compared to the surfactant vesicle vaccines. However, IgG2a response was markedly reduced by day 68, while DDA–TDB system showed the most enhanced IgG2a responses compared to the free HBsAg system from day 35 onwards. Thus a trend towards a Th2 response is suggested by the unadjuvanted HBsAg. Considering IgG2a isotype as an indicator for Th1 type response (Bramwell et al 2002), all of MP-containing formulations showed an improved Th1 component. Replacement of PC for MP appears to limit overall IgG and IgG2a responses, whilst having a little effect on IgG1 titers. The DDA–TDB system elicited high titers of all three isotypes. Splenocyte proliferation of DDA–TDB system and DDA–TDB incorporating MP elicited increased clonal expansion in response to HBsAg restimulation shown by a mean of 20 000 and 16 000 CPM, respectively (compared to 2000 CPM, naïve group). An enhanced cytokine production was seen for both of these DDA–TDB based vaccines in terms of antigen specific IFN-γ (5999 and 4709 pg/ml); IL-2 (1593 and 1014 pg/ml); IL-6 (640 and 724 pg/ml) production respectively which are significantly higher than other groups ($P < 0.05$). The PC-based system (PC:DOPE:Chol:DC-Chol) showed significantly high IL-5 levels (202 pg/ml) ($P < 0.05$), indicating a Th2 bias, further corroborating the trend in antibody isotype outlined above which may be interpreted as Th2 for this formulation. MP:Chol:DDA:TDB system elicited IL-6 production comparable to the DDA-TDB group.

Conclusions Overall, DDA-TDB based systems showed markedly increased antigen specific splenocyte proliferation and elicited cytokine production concomitant with a strong T cell driven response, delineating formulations that may be useful for further evaluation of their clinical potential.

Bramwell, V. W., et al (2002) *Immunology* **106**: 412–418
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Alginate beads as potential oral vaccine delivery platforms: effect of coating

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Objectives Alginate beads were used to entrap fluorescent microspheres as a model for Bacillus Calmette-Guerin (BCG) vaccine. Release characteristics of microspheres from the alginate beads both uncoated and coated with poly-L-lysine (PLL) or chitosan were evaluated. The use of alginate beads to entrap and protect bioactive molecules for delivery to the intestine has been widely investigated and provides a potential route to deliver live BCG vaccine orally to animals.

Methods Alginate beads containing microspheres were prepared by mixing a 2%w/w alginate solution (ProtanalSF200 MW387000 Pronova) with fluorescent microspheres (3 μm) at a ratio of 10:1. The mixture was then cross-linked in a 0.2 M $CaCl_2$ solution using the syringe droplet technique producing beads ~2.2 mm in diameter. The beads were removed after 15 min and washed with ddH_2O. Coating was achieved by submerging the prepared beads in 0.5%w/w chitosan (medium MW, Sigma) or a 0.02%w/w poly-L-lysine (high MW, Sigma) solution for 45 mins. 1 g samples of the beads were then submerged using dissolution baskets (rotation 100 pm) in 200 ml 0.1 M HCl pH1.2 at 37 °C for 2 h. The HCl was then replaced with phosphate buffered saline (PBS) pH 6.8 at 37 °C. Samples of HCl and PBS were taken at regular intervals and measured for fluorescence intensity.

Results Release profiles shown in Figure 1 demonstrate the effect coating has on release of 3 μm microspheres from alginate beads. All formulations show no measurable release of fluorescent microspheres in 0.1 M HCl. Following 2 h submerged in 0.1 M HCl pH 1.2 the beads were transferred to PBS pH6.8 where the alginate released 95% of the fluorescent microspheres within 40 min. The PLL coated alginate beads released significantly less with only ~45% released following 1 h; this was reduced further when coated with 0.5% chitosan, with only 11% of the entrapped microspheres released following 1 h in PBS pH6.8.

Conclusions This study demonstrated that 3 μm microspheres can be entrapped in alginate beads via a mild procedure suitable for a live vaccine such as BCG. The release from the alginate formulations was prevented in simulated gastric conditions yet was rapid once in simulated intestinal conditions. However, the release could be modified by coating the beads in poly-L-lysine or chitosan.

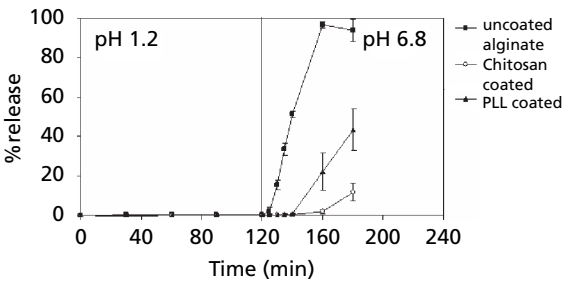

Figure 1 Release profile of 3 μm fluorescent microspheres from beads prepared from 2% alginate: Uncoated beads (filled squares), PLL coated beads (filled triangles) and chitosan coated beads (open circles); vertical error bars indicate standard deviation from n = 3 samples.

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Aminosalicylic acid conjugates of EDTA as potential anti-inflammatory prodrugs: synthesis, copper(II) chelation and superoxide dismutase-like activities

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Objectives Chronic inflammatory diseases are characterised by oxidative stress resulting from an imbalance between fluxes of reactive oxygen and nitrogen species (RONS) and anti-oxidant defences such as superoxide dismutase (SOD). Low-molecular-mass redox-active transition metal ions (mainly Cu(II) and Fe(III)) are credited with enhancing oxidative stress, by formation and/or activation of RONS. The aim of this project is to develop aminosalicylic acid (ASA)–chelator conjugates as a prodrug to: i) deliver ASA to the inflamed GI tract with release of the ASA by microbial enzymes, ii) deliver a chelator to bind deleterious 'labile' redox-active metal ions, and iii) to generate a catalytic antioxidant mimetic (upon metal ion complexation) to reduce oxidative stress in the GI tract.

Methods The methyl esters of 4-ASA and 5-ASA were synthesised as reported previously (Bailey et al 2004) prior to conjugation to the dianhydride of EDTA, using standard amide preparation techniques. Purification involved shaking a 1% w/v suspension of the product in H_2O at 80°C for 3 h, hot-filtering, acetone-washing and drying. Chelation studies were carried out using Job's method and spectroscopic titrations to determine the copper to chelator ratio. The superoxide ($O_2^{\bullet -}$) suppressing (SOD-like) activity was assessed using a modified NBT assay as previously described. All recordings were performed four times and the average taken, where the confidence interval for each average is worked out at 95%.

Results Using Job's method (Huang 1982), both ASA-EDTA conjugates (4-EBAME and 5-EBAME) showed an ideal complexation ratio of 1:1 with Cu(II). This was further verified by spectroscopic titrations conducted over the pH ranges involved in these studies. Spectroscopic studies of the EBAME conjugates with Cu(II) ions showed optimal complexation occurred for the 4-EBAME, with rapid complex formation in both buffer and non-buffered aqueous solutions. The Cu(II) bound conjugates had superior SOD activity in comparison to the metal-free conjugates.

Conclusions The transition metal chelates of the EBAME conjugates showed good SOD mimetic activity. The unbound chelators 4- and 5-EBAME showed very little SOD-like activity, whereas their synthons 4-ASA, 5-ASA and the corresponding methyl esters showed varying degrees of SOD-like activity consistent with previous studies by Gionchetti et al (1991*)*. These conjugates are potential dual-function prodrugs for delivering the active 4- and 5-ASA molecules, with the advantage of providing the SOD-like activity of the released ASA moieties, the conjugate upon Cu(II) complexation and the remnant EDTA moiety. Further work is underway to evaluate these conjugates in biological systems.

Bailey, M. A., et al (2004) *Biochem. Biophy. Res. Commun*. **31**: 1155–1158
Gionchetti, P., et al (1991) *Dig. Dis. Sci*. **36**: 174–178
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Formulation and evaluation of an implantable polymeric configuration for application in AIDS dementia complex utilising nanotechnology

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Objectives To synthesise polymeric nanoparticles to develope a Zidovudine (AZT)-loaded device intended for implantation into the intracranial region of the brain to manage AIDS Dementia Complex (ADC). ADC is a significant CNS complication of late HIV-1 infection. Manifestations of the condition are extremely debilitating, warranting early therapeutic intervention. Oral AZT therapy is effective in managing the condition; however the poor bioavailability of AZT in the brain requires large doses to be administered. The need for localised drug delivery is evident as this will lower the dose administered, eliminating systemic side effects. Particle size determination and surface morphology of the nanoparticles will enable optimisation of the nanoparticles formulated.

Methods AZT-loaded and drug-free nanoparticles were prepared using controlled gelification of alginate. Sodium alginate and pectin were dissolved in deionised water and crosslinked with $CaCl_2$. This was then centrifuged to precipitate nanoparticles and thereafter lyophilised to obtain a free flowing powder. A polymeric dispersion comprising sodium carboxymethyl cellulose (NaCMC), polyethylene oxide (PEO) and epsilon caprolactone (ECL) incorporating the nanoparticles was prepared. A crosslinking solution of $CaCl_2$, $AlCl_3$ and $Na_2S_2O_3$ in deionised water was prepared to formulate the NaCMC-PEO-ECL scaffold. The polymer solution was incorporated into the crosslinking solution and was allowed to cure for 30 min. Crosslinked particles were removed and dried overnight under standard conditions.

Fourier Transform Infrared (FTIR) spectroscopy was performed on the nanoparticles and the scaffold to elucidate changes in the molecular assemblage of the native polymers during formulation. Particle size and surface morphology of the nanoparticles were elucidated using a Zeta Sizer Nano ZS and Transmission Electron Microscopy (TEM). Zeta potential was employed to determine overall surface charge distribution and stability of the nanoparticles. Drug release studies were performed on the nanoparticles and the scaffold in phosphate buffer pH7.4 using an orbital shaker incubator set to rotate at 20 rpm at 37 °C. Samples were taken at predetermined time intervals and analysed using UV spectroscopy.

Results FTIR indicated a change in the molecular assemblage of both the nanoparticles and the scaffold due to surface interactions occurring during the preparation process. However, the basic polymeric structure of the parent compounds was maintained. Particle size analysis in conjunction with TEM indicated the presence of particles in the range 200–700 nm as well as distinct nanotubes having lengths in the range 500–900 nm. Within the nanotubes, particles ranging between 50 and 200 nm were entrapped. Particle size distribution studies revealed an average size distribution of 576.1 nm for AZT-loaded nanoparticles, and 602.4 nm for drug-free nanoparticles. The average zeta potential of AZT-loaded nanoparticles was –0.174 and that of drug-free nanoparticles was –6.39. Drug release studies indicated first-order kinetics from the nanoparticles with 100% being released within 4 h. Incorporation of nanoparticles into the polymeric scaffold significantly retarded drug release (3.43% after 4 h) while inducing a mixture of upcurving and zero-order kinetics.

Conclusion Nanoparticle incorporation into a multipolymeric scaffold restricts AZT release, allowing for the development of a controlled release drug delivery system for site-specific administration of AZT to manage ADC.

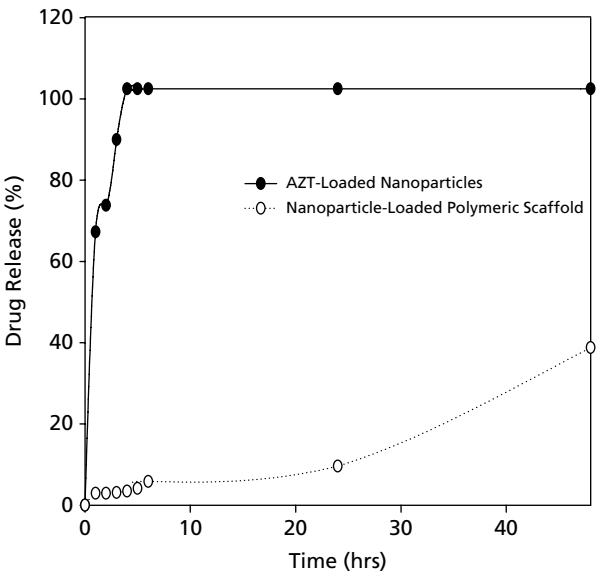

Figure 1 Drug Release profiles of AZT-loaded nanoparticles and polymeric scafflod under cerebrospinal fluid simulated conditions (20rpm, 37ºC, 1M PBS, pH7.4).