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POSTERS

SESSION 1
Drug Delivery

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Effect of chitosan encapsulation conditions on immunogenicity of diphtheria toxoid as a mucosal carrier for intranasal administration in guinea pig

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Mucosal immunization is an alternative to parenteral immunization as with the appropriate delivery system it is possible to stimulate both humoral and cell-mediated responses and to induce mucosal and systemic immunity simultaneously. Most human pathogens initiate their infection processes at mucosal surfaces; however, most of the conventional vaccines available against these agents are usually given parenterally. Mucosal surfaces are the most common and convenient routes for delivering drugs to the body. Over the last decades significant efforts have been dedicated to explore new routes, alternative to injection, for the administration of macromolecules such as peptides and proteins. Among them, the transmucosal routes such as the nasal, pulmonary and oral routes are those which have received the most important deal of attention (Clement et al 2004). In this study the effect of chitosan microparticles as an immunostimulator in mucosal immunization of diphtheria toxoid (DT) was investigated. Chitosan (CS) microparticles were prepared as described previously (Mortazavi et al 2004). Briefly, chitosan solutions (high, medium and low molecular weight [Sigma-Alderich, USA]) containing different amount of DT (Razi vaccine and serum research institute) were emulsified in castor oil as a medium using a homogenizer set at 20000 rpm for 5 min. at 4°C and then cross-linked by gluteraldehyde (Glu) solution or 5% w/v Na tripolyphosphate (Na Tpp). Microparticles were evaluated morphologically using particle size analyzer and SEM. Loading efficiency was determined by BCA micro kit assay. In-vitro release was carried out in PBS pH 7.2 for a two weeks period. Then DT incorporated in CS microparticles (20 μg) was administered in 40 μl of PBS pH 7.4 on days 0, 7, 14 and 21 to 3 different groups of five guinea pigs. The fourth group was vaccinated with DTP vaccine as I.p. and the last group was received only free toxoid in PBS pH 7.4. The humoral responses to the intranasal vaccination were evaluated by determination of IgG in serum using indirect ELISA method after 7, 14, 21, 48 and 70 days fallow the first dose. Then exudates of GI tract and nasal were collected using Elson method (Elson et al 1984) and sIgA level was determined by ELISA. Evaluation of IgG and sIgA antibody levels after intranasal or I.p. administration of DT in different formulation of CS microparticles and in solution, were shown that in general trend is that CS microparticles generated IgG and sIgA levels significantly were higher than those elicited by the fluid vaccine ($P<0.05$). As well as, microparticles prepared by high Mw CS and cross linked by Glu induce higher level of immune responses in both IgG and sIgA than microparticles prepared by low Mw CS and cross linked by Na Tpp.

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Design of lipospermine non-viral gene delivery vectors for self-assembly DNA nanoparticle formulations

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We are working to design and develop efficient, non-toxic, non-viral vectors for in vitro and possible in vivo applications. These could be achieved using our novel spermine conjugates, based on change to the type, length and position of the hydrophobic anchors. These lipids probably assist in the self-assembling of polycationic scaffolds, as well as facilitating absorptive endocytosis and/or fusion with cell membranes. These lipospermines form spontaneous complexes with negatively charged poly-nucleic acids to condense DNA (using pEGFP as the reporter gene), leading to formation of nanoparticles after removal of small counterions from both cationic carriers and DNA (a thermodynamically favoured step that drives and stabilizes complex formation). Thus, these formed nanoparticles are suitable for gene (or siRNA) delivery. The formed complexes are monitored using ethidium bromide (EthBr) fluorescence quenching, transfection efficiency, and MTT cytotoxicity assays. In this study, we synthesized and formulated a novel non-liposomal lipospermine in which the tetra-amine spermine (the cationic moiety) and two C14 or two C18 saturated and non saturated fatty-acid chains (the lipophilic moiety) are linked by amide bonds at the primary amino groups of spermine to form N^1,N^{12}-dimyristoyl spermine, N^1,N^{12}-dimyristoleoyl spermine, N^1,N^{12}-distearoyl spermine, N^1,N^{12}-dioleoyl spermine and compared them with N^4,N^9-dioleoyl spermine (Lipogen) (Ahmed et al 2005). The binding affinities of these novel non-viral vectors for EGFP DNA were determined using an EthBr fluorescence-quenching assay (λ_{ex} = 260 nm, λ_{em} = 600 nm) (Geall & Blagbrough 2000). The transfection efficiencies of the synthesized lipopolyamines were studied in primary skin cells (FEK4) and in an immortalized (HeLa derived HtTA) cancer cell line using plasmid DNA encoding for enhanced green fluorescent protein (pEGFP, Clontech) as the reporter macromolecule with its fluorescent imidazolidinone moiety analysed by Fluorescent Activated Cell Sorter (FACS). The cytotoxicity of these compounds was studied in both primary skin and immortalised cancer cell lines using an MTT assay (Ahmed et al 2006). The results based on both the transfection efficiency and cytotoxicity showed that two unsaturated C18 chains (N^1,N^{12}-dioleoyl spermine at N/P 3) are better than two C14 chains (N^1,N^{12}-dimyristoyl spermine at N/P 12) and N^1,N^{12}-dimyristoleoyl spermine(N/P 12). Also, C18 unsaturated N^1,N^{12}-dioleoyl spermine is more efficient than C18 saturated N^1,N^{12}-distearoyl spermine (N/P 30), but the N^4,N^9-pattern (Lipogen) (N/P 2.5) is significantly better than the N^1,N^{12}-substitution pattern. In conclusion, the 4,9-substitution pattern on spermine is more effective than the 1,12-pattern. N^4,N^9-Dioleoyl spermine shows promising transfection results in a tissue cultured skin fibroblast primary cell line, it is a candidate medicine worthy of further development.

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Design and synthesis of a series of novel non-viral cationic lipid vectors for plasmid DNA formulation

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Gene therapy is now a popular method for the investigation of genetic diseases and more research with cationic non-viral vectors (Lehn et al 1998; Blagbrough et al 2003) will potentially lead to a practical treatment. The moieties in cationic lipids can each be modified to provide structure-activity relationship data on the medicines. These polyamine conjugates efficiently condense DNA into nanoparticles for cellular uptake by endocytosis, with DNA delivery ultimately to the nucleus. We have designed and synthesized a series of novel lipospermines, conjugates where the tetra-amine spermine (the cationic moiety) and two fatty acid chains (the lipophilic moiety) are linked by amide bonds at the secondary or primary amine groups to form N^4,N^9-diacyl or N^1,N^{12}-diacyl spermine conjugates, respectively. In cell membranes, some glycerophospholipids are built up with unsymmetrical aliphatic chains, some of different lengths, while others are varied in their degree of unsaturation. Therefore, to obtain potentially highly efficient and less toxic non-viral vectors, we have also designed and synthesized some unsymmetrical N^4,N^9-diacyl spermine conjugates by various routes. To synthesize these target medicines, we used selective amine protecting groups, and then added two aliphatic chains with regiochemical control. For N^1,N^{12}-diacyl spermines, the secondary amines were protected with *t*-butyl carbamate (BOC) groups, after trifluoroacetyl (TFA) groups were added to the primary amines (followed by removal of those TFA groups with potassium carbonate in refluxing methanol:water, 10:1, v/v). The

required diamides (e.g. didecanoyl, dimyristoleoyl, dimyristoyl, dioleoyl, distearoyl) were formed by acylation (e.g. acid chloride in pyridine). BOC groups were removed in dichloromethane and trifluoroacetic acid (18:1, v/v). For N^4,N^9-diacyl spermines, di-TFA protected spermine was diacylated and then deprotected to yield the required diamides (e.g. dimyristoleoyl). N^4,N^9-Unsymmetrical spermine conjugates were prepared using TFA as the initial amine protecting group, followed by 0.8 equivalent BOC-reagent to protect only one secondary amine. After conjugation with only one aliphatic chain (e.g. C18 saturated or a shorter chain), the BOC group was removed, allowing us to introduce a different long fatty chain (e.g. C18 un/saturated, C14 unsaturated) on the other secondary amine. The final product was obtained after removal of the two TFA protecting groups. Another route to our unsymmetrical targets starts from N^1,N^4,N^{12}-tri-BOC-spermine (Nagarajan & Ganem 1985). After mono-acylation, all three BOC protecting groups were removed. The two primary amines were protected, acylation gave N^1,N^{12}-di-TFA-N^4,N^9-unsymmetrical diacyl spermine, and TFA deprotection yielded the desired unsymmetrical cationic lipid. We have prepared a designed series of lipid vectors whose efficiency versus toxicity can now be compared. All our synthetic target medicines displayed satisfactory NMR data, required M+1 (FAB +ve) MS data, and accurate MS data within 5 ppm e.g. $C_{38}H_{75}N_4O_2$ N^4,N^9-dimyristoleoyl spermine found 619.5872 (100%) requires 619.5890, accompanied by e.g. $C_{37}{}^{13}CH_{74}N_4O_2$ N^4,N^9-dimyristoleoyl spermine found 618.5775 (7%) requires 618.5767.

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Evaluation of the effect of ibuprofen crystallisation on its release from silicone and acrylic ibuprofen-adhesive layers

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Maximum drug release from transdermal patches is achieved when the formulation is at its maximum thermodynamic state. The attainment of such a state can be problematic when the drug is prone to crystallisation (Kim & Choi 2002). In this work, ibuprofen, a weakly acidic compound ($pK_a = 4.8$) with low aqueous solubility, was used as the model drug together with two silicone (silicone-1, silicone-2) and an acrylic polymer adhesives. The saturation solubility of ibuprofen in each of the adhesives was examined by mixing ibuprofen in increasing amounts with the liquid adhesives and then examining the dried mixtures for undissolved drug and crystal formation over a period of one week, using a polarised light microscope. The saturation concentration of ibuprofen was found to be $6.5 \pm 0.5\%$ w/w in silicone-1 and $5 \pm 0.5\%$ w/w in silicone-2. In the acrylic adhesive there was an initial crystal formation of ibuprofen at concentrations between 13 and 15% w/w. Circular ibuprofen-adhesive layers (mean surface area of 9.5 ± 0.5 cm^2) on a fluoropolymer liner were prepared, containing ibuprofen in a near-saturation concentration ($6.5 \pm 0.7\%$ w/w, $5 \pm 0.4\%$ w/w, $13 \pm 0.5\%$ w/w) and then in a supersaturated concentration ($16.5 \pm 0.5\%$ w/w, $14 \pm 1\%$ w/w, $22.5 \pm 0.5\%$ w/w) with silicone-1, silicone-2 and the acrylic adhesive, respectively. The supersaturated layers with the silicone adhesives showed white ibuprofen crystals under the microscope, in contrast to the acrylic layers, which turned clear on storage. Each set of drug-adhesive layers ($n = 4$) were tested for their drug release profile over 7 h in a paddle dissolution apparatus using citrophosphate buffer ($pH = 5.6$) as the dissolution medium under sink conditions, at 32°C. The UV analysis was carried out at 272 nm. The mean cumulative ibuprofen release (mg/cm^2) at 7 h was 0.62 ± 0.2, 0.5 ± 0.2 and 0.57 ± 0.16 from the near-saturated layers; 0.68 ± 0.07, 0.49 ± 0.1 and 1.7 ± 0.11 from the supersaturated layers, with silicone-1, silicone-2 and acrylic adhesive, respectively. All sets of layers showed biphasic drug release, with a second burst after 2–4 h. The non-significant change of ibuprofen release from the silicone-1 (t-test, $P = 0.879$) and silicone-2 (t-test, $P = 0.227$) between near-saturated and supersaturated formulations agree with previous studies where the presence of ibuprofen crystals suppressed further drug release from supersaturated silicone formulations (Cilurzo et al 2005). The absence of ibuprofen crystals even at high loadings (> 22% w/w) in the acrylic adhesive, without the addition of crystallisation inhibitors, and the significantly higher ($P = 0.001$) ibuprofen release from the clear concentrated ibuprofen-acrylic formulations, render acrylic-based adhesives good candidates for further studies on the formulation of drug-in-adhesive layers with ibuprofen.

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Intranasal immunization: effect of alginate encapsulation parameters

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Nowadays, several mucosal surfaces, such as the nasal, pulmonary and per-oral mucosa, are being extensively explored as alternative routes for the systemic administration of macromolecular drugs and vaccines. Among them, the nasal mucosa is receiving a great deal of attention due to its particularly high permeability and easy access of the drug to the absorption site (Behl et al 1998). The nasal route holds great promise from the perspective of vaccination due to the particular organization of the nasal mucosa (Partidos 2000). The mucosa is the first site of contact with inhaled antigens, and the nasal-associated lymphatic tissue (NALT) at the base of the nasal cavity is important in the defence of the mucosal surface. Based on this information, the aim of this study was to explore further the potential of alginate (Alg) microspheres as a vehicle for the nasal administration of vaccines. Low and high molecular weight sodium alginate (Alg) samples with viscosities (a 2% w/v solution) of 1500 and 14000 cP, respectively, Purified Diphtheria Toxoid (DT, 1500 Lf/ml) in phosphate buffer saline pH 7.4, purified equine DT anti-toxin (100 Lf/ml), Purified guinea pig immunoglobulin (reagent grade), rabbit anti-guinea pig immunoglobulin G (whole molecule) peroxidase conjugate, goat anti-guinea pig immunoglobulin A (α-chain specific) peroxidase conjugate, and all other regents were supplied by Sigma Chemical (USA). Alg microspheres were prepared by water-in-oil (w/o) emulsion-solidification technique (Cho et al 1998). Briefly, aqueous Alg solutions at various concentrations of 0.5, 1, 1.5 and 2% w/v were prepared. 4 ml samples of these solutions were individually emulsified in 20 ml of an oily phase (n-octanol, mineral oil or castor oil) containing a 1:1 ratio of a mixture of Tween80:Span 80 (as surfactant) at a 1% v/v concentration, using a homogenizer set at 20000 rpm in an ice bath at 4°C for 30 s. In addition, 100 ml of a second emulsion containing the oily phases mentioned above was prepared using 3 ml of a cross-linker (1 M aqueous or 3.75% w/v octanolic $CaCl_2$) along with a 1 % v/v mixture (1:1) of Tween 80:Span 80, using a homogenizer set at 20000 rpm for 30 s. Next, the first emulsion was added to the second emulsion under continuous stirring at 1000 rpm for at least 5 h at room temperature. Microspheres loaded with DT were prepared by adding 312–8790 μg of DT to the polymer (Alg) solutions. Initially, the particle size distribution, encapsulation efficiency, SEM microscopy photograph and in-vitro release of microspheres prepared were investigated. Immunogenicity of the Alg formulations was assessed in guinea pig following intranasal immunization. A dose (30 μg) of DT incorporated in 100 μg Alg microspheres were given in 40 μl of PBS pH 7.4 (20 μl into each nostril) on days 0, 7, 14 and 21 to four different groups of five guinea pigs. All animals were conscious during the administration. In this study, two groups treated with DT-loaded Alg microspheres, and the third group with DTP vaccine (Smithkline Beecham, Madrid, Spain) adsorbed on Alum as Ip (30 μg/guinea pig) and the last group received free toxoid (30 μg/ guinea pig) in PBS pH 7.4. As mentioned earlier the aim of this study was to prepare microspheres containing DT for purpose of mucosal immunization. For this purpose Alg microspheres were prepared using a simple technique base on water-in-oil emulsion-solidification technique. The particle size distribution of microsphere was determined using a particle size analyzer. The particle size of all microspheres were below 10 μm with an average 1.09 ± 1.07 and 1.46 ± 0.30 μm for aqueous and octanolic samples, respectively. The loading level and encapsulation efficiency of DT within microspheres prepared using different Alg Mw shows that the actual amount of DT loaded within Alg microspheres, and as a result, their encapsulation efficiencies are significantly (ANOVA, $P < 0.05$) affected by the Mw of the Alg solution and amount of DT added to the first emulsion. The in-vitro release of antigenically active DT from Alg microspheres shows that the all of formulation exhibited two release phases, a rapid release over the first 24 h followed by a slow release for up to 8 days. Evaluation of IgG and IgA antibody levels after intranasal or Ip administration of DT in different formulation of Alg microspheres and in solution, shows that in general trend is that Alg microspheres generated IgG and IgA levels significantly higher than those elicited by the fluid vaccine ($P < 0.05$). As well as, microspheres prepared by high Mw Alg and cross linked by octanolic $CaCl_2$ induce higher level of immune responses in both IgG and IgA than microspheres prepared by low Mw Alg and cross linked by 1 M $CaCl_2$.

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Optimisation of freeze drying conditions for Ice Creams

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Ice cream is a complex food colloid with a typical composition of milk-fat, milk solids-non-fat, sweeteners, stabilizers/emulsifiers, water and a significant amount of air along with ice crystals and fat globules (Goff 1997). Freeze drying of ice cream preserves its original properties by freezing the contained water and then removing the ice by sublimation (Rey & May 1999). The aim of the work presented here is to decipher the optimal freeze drying cycle to obtain a porous, free flowing and stable freeze dried ice cream. Five different ice creams were tested (Formulation 1: SPAR Chocolate, 2: SPAR Vanilla, 3: Marks and Spencer low Calorie, 4: Haagen Dazs Vanilla, and 5: Green and Blacks Organic Vanilla). The freeze drying protocol consisted of various conditions (pre freezing (0.5 h)/primary drying (24 h)/secondary drying (6 h): protocol 1: −70°C/−50°C /−30°C, protocol 2: −50°C/−40°C/−25°C: protocol 3: −30°C/−20°C/−10°C). The various physical parameters tested include cake volume, residual moisture content (Thermal gravimetric analysis), time of rehydration, powder flow properties (modified powder flow analyzer), morphology and viscosity (falling sphere viscometer). Initially the ideal freeze drying protocol was determined by subjecting formulation 2 to three different freeze drying protocols followed by measurement of cake volume, residual moisture content and powder flow characteristics. Protocol 3 showed better powder flow characteristics ($P < 0.05$) in comparison to Protocol 1 and 2 where its end-product turned out to be porous and dry in appearance. The cake volume for all three protocols was not significantly different (0.98 ± 0.08 cm^3 to 1.11 ± 0.15 cm^3). The moisture content for all the cycles was 2.7–3.3% ($P > 0.05$). Further freeze drying was carried out using protocol 3. After carrying out tests on the different ice creams, it was found that not one product has all the best characteristics of an optimum freeze dried product. Both formulation 1 and 2 have the lowest viscosity (around 138 ± 0.2 cm^2 s^{-1}), fairly low residual moisture content (3.42% and 3.34%, respectively) and the best powder flow properties (13.6 and 12.5g/min, respectively). However, they both took the longest time to rehydrate (11 and 9 min, respectively). Alternatively formulation 3 showed the poorest product for freeze drying as it had the largest cake volume, highest residual moisture content (5.4 ± 0.2%) and poor powder flow properties. It was flaky in appearance as opposed to porous and had a shiny surface. However, formulation 4 had the lowest residual moisture content at about 2.35% with good flow properties and morphology, but took a longer time to rehydrate (9.5 ± 0.6 min). Finally, formulation 5 showed good morphological characteristics with the shortest time for rehydration (1.1 ± 0.05 min) but yielded the most viscous product. There was no significant difference in cake volume for all the five ice creams with volumes ranging from 1.05 to 1.29cm^3 ($P > 0.05$). The results suggest that tailor made freeze drying conditions need to be optimized for individual formulation to achieve a balance between the desired characteristics.

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Stabilisation of non-aqueous emulsions

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The delivery of poorly water-soluble drugs has been the subject of much research, as approximately 40% of new chemical entities are hydrophobic in nature. There are numerous approaches that can be used to overcome problems associated with the use of such drugs; however, new methods of drug delivery are required. One area in which published literature is lacking is the field of non-aqueous emulsions and some researchers have used polyethylene glycol (PEG) as a continuous phase for such emulsions (Peterson & Hamill 1968; Amemiya et al 1998). In this study, an attempt has been made to develop oil-in-PEG emulsions for drug loading. The surfactants, Span 60 and Tween 60, were selected based upon previous work by other researchers in non-aqueous emulsions (Peterson & Hamill 1968). Vegetable oils were initially selected as the disperse phase, and from preliminary investigation sesame oil proved the most successful in forming stable emulsions. Surfactants (Span 60 [2%w/w] and Tween 60 [2%w/w]) were heated in PEG 400 [67.2%w/w] for 60 min in a 55°C water bath. The mixture was homogenised (Silverson) and the oil phase (sesame oil [28.8%w/w]) was added slowly. Processing was continued, at room temperature, for a set duration at a certain speed. Emulsions were prepared at a batch size of 6.25 g. The emulsions were visually observed over time to assess short-term stability. In initial experiments to investigate the manufacturing process, emulsions were prepared at different homogenisation speeds (1000–7000 rpm) with duration of 10 min and different homogenisation durations (5–20 min) at a speed of 4500 rpm. The resulting emulsions were assessed for the height of separation over time. It was found that the optimum speed for homogenisation was 4500 rpm – this may reflect the importance of shear in these systems. When the shear is low, collection of surfactant at the droplet interface may be impeded; however, with a high shear the smaller droplets produced may have too high a surface area for sufficient stabilisation by the surfactants at the concentrations used – both resulting in unstable systems. With regard homogenisation duration, the time that produced the most stable emulsion was found to be 20 min. A non-aqueous oil-in-PEG 400 emulsion was prepared, and the manufacturing process was investigated. Future work will involve the use of experimental design to further investigate the formulation and manufacturing parameters critical to the process of these systems prior to drug loading and assessment of dissolution performance.

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Improvements in the release profile of antibiotics from PMMA based bone cements via drug-polymer conjugated systems

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Cemented prostheses have been used in Total Hip Arthroplasty (THA) since 1958, when Charnley used acrylic bone cement to bond femoral head prostheses to the femur. Since then the use of bone cement (BC) has become widespread and gained considerable acceptance. Initially the cementing technique involved mixing methyl methacrylate (MMA) with poly methyl methacrylate (pMMA), N, N-dimethyl toluidine (DMT) and benzoyl peroxide (BPO) in an open bowl (Smith 2005). The resulting dough was packed into the cavity by the surgeon. The most significant change was the incorporation of antibiotics, like gentamicin, into the BC in the 1970's to help prevent infection and sepsis by mixing it with the components prior to polymerising them. There are a number of problems with this approach — PMMA is a hydrophobic polymer and water penetration into the polymer network is minimal, resulting in an initial burst of gentamicin release from the surface of the PMMA but little further sustained release (Baker & Greenham 1988). Also, the incorporation of gentamicin and barium sulphate causes mechanical defects in the polymer network (Kurtz et al 2005), which can lead to premature failure of the joint. This situation is worsened by agglomerates of either antibiotic or radiopacifer acting as stress foci or fatigue initiation sites. The aim of this work was to produce a system capable of delivering antibiotic release over a sustained period. To achieve this a strategy was developed whereby antibiotic was linked directly to the polymer backbone using a drug-polymer conjugate system consisting of nalidixic acid, a quinolone antibiotic joined via an ester bond to a linking group containing a nucleophilic centre and a vinyl group to allow it to co-polymerise with MMA. Conjugates where the neighbouring group was 2-pyridyl (conjugate 1), N-phenol (conjugate 2) and phenyl (conjugate 3) were synthesised and incorporated into PMMA, the subsequent release of nalidixic acid from the system was dependent on the hydrolysis rate of the drug-conjugates which in turn was determined by the nucleophilic properties of the neighbouring groups. 4% w/w of each drug conjugate was mixed with MMA and heated to 60°C for 5 min to allow the conjugate to dissolve. Following that 2% w/w BPO was

Table 1 Nalidixic acid released as a proportion of that originally in the sample

Time (days)	Percentage fraction of drug released		
	Drug conjugate 1	Drug conjugate 2	Drug conjugate 3
1	0.05	0.21	0.10
2	0.05	0.21	0.10
4	0.14	0.33	0.20
7	0.23	1.09	0.31
9	0.33	1.20	0.31
11	0.48	1.27	0.39
14	0.55	1.50	0.51
16	0.82	1.65	0.56
18	1.54	2.23	0.88
21	1.97	2.82	1.10
23	2.11	3.15	1.25
25	2.17	3.61	1.40
32	2.66	3.74	1.48

added. The plates were then incubated at 60°C for 2 h. Upon removal the plates were cut into 1 cm by 1 cm squares. The samples were then placed into 10 ml 0.1 M Phosphate buffer and incubated at 37°C and the release of nalidixic acid was determined using UV spectroscopy at a wavelength of 334.9 nm. This approach has been successful in prolonging the release of nalidixic acid, with the three conjugates releasing significantly different amounts of drug over a one month period (Table 1).

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The role of a fully charged bilayer on liposomal encapsulation of a poorly water soluble drug

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Liposomes (multilamellar vesicles; MLV) are versatile delivery systems for insoluble drugs. However, to effectively deliver solubilised lipophilic drugs certain bilayer characteristics need to be optimized to enhance the entrapment of poorly water soluble drugs. This work investigates the role of a fully charged bilayer on the encapsulation of propofol, a poorly water soluble model drug candidate. Liposomes were prepared by an already well-established hydration method, with the addition of 1.0 mg of poorly water soluble propofol (logP = 3.79). The lipids investigated were the zwitterionic 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and their corresponding charged derivatives, 1,2-distearoyl-3-trimethylammonium-propane (DSTAP), 1,2-dipalmitoyl-3-trimethylammonium-propane (DPTAP) and 1,2-dimyristoyl-3-trimethylammonium-propane (DMTAP), respectively, along with the addition of 11% cholesterol (Chol) in all cases. Liposome encapsulation was measured by spectrophotometric analysis of the supernatant following MLV separation by centrifugation or ultracentrifugation. Initial optimization studies showed that of those tested (0, 11, 20, 33, 50 total molar %), 0 and 11 total molar % Chol content encapsulated the highest quantity of propofol, suggesting this to be the most efficient. Since previous studies (Arnarson et al 1980; Monzon & Yudi 2001) suggest that the presence of Chol provides an enhanced degree of physical bilayer stability as compared to formulations without the Chol, a Chol content of 11% was implemented for all further formulations. Investigations of charged lipids reveal a trend of increasing liposome-entrapment of propofol with an increase in alkyl chain length in the order of DSTAP > DPTAP > DMTAP ($P < 0.05$) (Table 1). This trend appears to correlate with data obtained for their corresponding zwitterionic lipid derivatives where the trend of DSPC > DPPC = DMPC was seen ($P < 0.05$). Interestingly, results reveal a significant ($P < 0.05$) general improvement in propofol-encapsulation by fully charged liposomes compared with their zwitterionic counterparts. DSTAP and DPTAP encapsulated a significantly greater quantity of drug than their zwitterionic analogues, DSPC and DPPC, respectively, with DMTAP revealing no significant difference in propofol-loading when weighed against DMPC. The increase in drug-incorporation by the fully charged liposomes could be due to complete ionisation of propofol during the hydration step of the preparation process. At pH 5.3 (of hydration media), theoretically, enough H^+ species is present to fully ionise the weakly basic propofol drug (pKa = 8.3) with the resulting ionic moiety favouring the charged liposomal environment more than the bilayer possessed by zwitterionic liposomes, resulting in an enhanced bilayer drug-encapsulation. The results appear to correlate with data obtained by Song & Kim (2006) where cationic liposomes incorporated a near three-fold quantity of low-molecular-weight heparin compared to their zwitterionic counterparts.

As expected, the charged liposomes exhibited zeta potential values of around 40 mV (Table 1), therefore confirming the charged nature of the liposomal surface.

Table 1 Propofol encapsulation in cationic and zwitterionic formulations

Formulation (16:2 μmol)	Alkyl chain length (C)	Encapsulation (% mol/mol)	Zeta potential (mV)
DSTAP:Chol	18	27.6 ± 0.9	39.4 ± 3.3
DSPC:Chol	18	24.8 ± 1.5	4.0 ± 0.8
DPTAP:Chol	16	25.0 ± 0.6	40.9 ± 3.8
DPPC:Chol	16	19.8 ± 1.1	1.4 ± 1.0
DMTAP:Chol	14	21.8 ± 1.3	53.0 ± 11.2
DMPC:Chol	14	22.4 ± 1.6	0.2 ± 1.4

C = number of carbons.

In conclusion, results suggest that a fully charged bilayer plays an influential role in the encapsulation of the poorly water soluble model drug candidate, propofol.

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24
A comparison of predicted and experimentally measured descriptors in QSPRs

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Prodrugs of captopril, systematically modified by the use of quantitative structure-permeability relationships, have recently been shown to provide substantially greater percutaneous absorption that the parent drug (Moss et al 2006). In this study, the authors demonstrated that the software available to predict the physicochemical properties of a molecules, such as the melting point [EPI (Estimations Programme Interface) Suite software] gave significantly different results than those measured experimentally. In the case of captopril and its n-carboxyl esters, this difference was particularly striking as the pure captopril prodrugs were oils at room temperature, whereas software packages gave predictions of melting point that increased, not decreased with molecular weight (Moss et al 2006). Such software packages use a range of algorithms based on molecular properties and fragments in order to offer predictions of physicochemical parameters. In the case of melting point, this is clearly an issue and may impact upon other estimations of molecular properties, particularly solubility. Therefore, the aim of this study was to compare experimentally determined log P values with those predicted by a range of widely used software packages. Using SMILES (Simplified Molecular In-Line Entry System) codes, log P (and solubility, log S) were calculated from the IA program, the EPI Suite software and Virtual Computational Chemistry Laboratory. Experimental log P values were determined and validated using the Organisation for Economic Co-operation and Development (OECD) Method 107 "Shake Flask Method" and were analysed via LC-MS. While the trend for theoretical vs experimental values is similar ($r^2 > 0.96$) the theoretical results appear to significantly overestimate experimental results. For example, the range of log P values obtained for captopril and its C_1 – C_6 n-carboxyl esters range from 0.55 to 3.79, whereas the experimental values range from 0.05 to 1.83. The values for permeability coefficient (k_p, the concentrated-corrected flux), differs by at least one order of magnitude, with the predictive software significantly over-estimating flux in every case. This disparity has a significant effect on the interpretation of results and questions the value of using predicted equations to estimate percutaneous absorption, particularly if they are based, wholly or partially, on predicted estimates of physicochemical parameters.

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Formulation and optimization of sustained release tablets of isoxuprine hydrochloride using response surface methodology

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Isoxuprine hydrochloride is used as vasodilator or uterine relaxant and also used in treatment of cerebral and peripheral vascular disease. In high dose it may cause several adverse effects like hypotension, tacycardia, gastrointestinal disturbance, etc. Conventional tablet required being administered 3–4 doses/day (Satoskar 2002). The aim of this study was to formulate and evaluate a sustained release tablet of Isoxuprine HCl using hydrophobic polymer Acrycoat PL-20 as release retardant. Tablets were prepared by wet granulation using isopropyl alcohol (IPA) as a binder. A 3^2 full factorial design was applied to carry out systematic study and for optimization response surface method was used (Bolton 1990). The amount of Dibasic Calcium Phosphate (DCP) (X_1) and Lactose (X_2) were selected as independent variables (Donald 1988). The time required for 90% (t_{90}) drug release was selected as dependant variable. In vitro drug release test was carried out using USP apparatus I in 900ml distilled water at 100 RPM. All the tablet formulation showed acceptable pharmacotechnical properties and complied for weight variation, hardness and friability. Acrycoat PL-20 was found to be very effective in sustained release formulation of isoxuprine hydrochloride at very low concentration. Regression analysis was

carried out on t_{90} value of each batch with transformed factor of X_1 and X_2 to generate polynominal equation. The results of 3^2 full factorial design indicate that the X_1 and X_2 have significant effect on t_{90}. Results showed an increase in concentration of DCP (X_1) leads to an increase in t_{90}, which restricts the entry of solvent, while increase in concentration of lactose leads to decrease in t_{90}, which generates channel for entry of solvent. It shows DCP has positive effect while Lactose has negative effect on t_{90} value. Form the response surface plot of t_{90} Vs X_1 and X_2 a checkpoint batch was prepared. From polynominal equation t_{90} value of checkpoint batch was 10.22 hr, where as practically it was found to be 10.14 h. This approach revealed that the amount of DCP and Lactose significantly affected the dependent variable. So, it was concluded that formulation variables like diluents might affect the formulation properties.

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Incorporation of lipid adjuvants into lipid vesicles: adjuvant action in mice following intramuscular administration of the TB H1 fusion protein

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Tuberculosis (TB) remains one of the leading causes of morbidity and mortality in man. The resurgence of TB has been attributed to the HIV epidemic, immigration, and the emergence of drug resistant variants. An effective vaccine against TB is an invaluable tool in the fight against this disease. The protection afforded by the currently available vaccine, bacillus Calmette–Guérin (BCG) is insufficient and new vaccine strategies are urgently needed (Agger & Andersen 2002). A number of mycobacterial proteins have shown promise as vaccine candidates. One of these is a fusion protein (H1) comprising the 6-kDa early secretory antigenic target (ESAT-6), and antigen 85B, which has shown protective efficacy in the guinea pig model (Olsen et al 2004). Such protein subunit vaccines have the requirement for the use of adjuvants in order to maximise their protective efficacy. Moreover, the adjuvant determines the qualitative nature of the immune response, crucial in the elicitation of protective immune responses. The adjuvant activity of cationic vesicles composed of the quaternary ammonium compound dimethyldioctadecylammonium (DDA) is markedly improved by the incorporation of the glycolipid trehalose 6,6′-dibehenate (TDB) when used for immunisation of mice with the mycobacterial H1 fusion protein (Davidsen et al 2005). Indeed, the DDA/TDB combination represents a potent adjuvant and as such is used as a positive standard for the evaluation of alternative adjuvants for the H1 fusion protein in our studies. We have used thin film hydration in order to incorporate alternative non-polar adjuvant moieties in DDA vesicles for evaluation as adjuvants for the H1 fusion protein when delivered intramuscularly in BALB/c mice. Initial immunogenicity studies in mice presented here have shown that a glycerol based non-polar mycobacterial lipid analogue adjuvant formulated in DDA vesicles can give IgG2a mediated anti H1 antibody titres at least as high as DDA/TDB with 100 fold less of the adjuvant moiety present compared to TDB ($P = 0.867$ after the third dose). This is important because the IgG2a antibody titre delineates a Th1 type response in BALB/c mice, thought to correlate strongly with protective immune responses. While traditionally used experimental and clinical adjuvants, such as Incomplete Freund's (ICF) and alum, respectively, tend to engender Th2 responses, exemplified by the low IgG2a to IgG1 titres elicited by ICF in Table 1, there is a need for adjuvants that can elicit specific Th1 responses. These results also show the potential for DDA vesicles as a platform for the delivery of lipid based co-adjuvants in order to elicit potentially effective Th1 biased immune responses against H1 and other protein subunit vaccines.

Table 1 Serum H1 specific IgG2a and IgG1 titres

	Second dose + 12 days*		**Third dose + 12 days***	
	IgG2a	IgG1	IgG2a	IgG1
DDA/TDB	52	205	533	1109
DDA/glycerol**	290	179	597	811
Incomplete Freund's	2	410	32	853

Shows average positive serum end point dilution × 100 (n = 3). *BALB/c mice received a total of three doses of 2 μg H1 with 250 μg DDA and 50 μg TDB or 0.5 μg lipid adjuvant. **Synthetic glycerol based non-polar mycobacterial lipid analogue.

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Investigation of drug release from a hydrophobic multiparticulate dosage form

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The development of safer, more efficient drugs to improve the quality of patient care has been a topic of much interest and research over the years (Vervaet et al 1995; Hossain et al 2004). Sustained-release formulations are developed to improve oral drug therapies, by increasing patient compliance and reducing the risk of adverse side effects associated with conventional immediate-release dosage forms (Bacon et al 2002). This is achieved by regulating the drug release within a therapeutic range for extended periods of time. The main objective of the study was the production of a sustained-release hydrophobic matrix pellet drug delivery system, incorporating acetaminophen as the active ingredient. Hydrophobic pellet production was achieved utilizing extrusion/spheronisation and the effects of three formulation variables, wax content, spheronisation time and pellet size distribution, were investigated. All granule formulations were prepared from glyceryl monostearate and acetaminophen, utilizing wax fusion. The wax granules produced were incorporated into pellets by the addition of Avicel PH101, Kollidon 30 and a water/ethanol mixture, utilizing extrusion/spheronisation. Production of granules and pellets (pellets produced from the granules) with varying wax contents (5–90% w/w), varying size distributions (0.5–4 mm) and varying spheronisation times (2–14 min) were performed and the release profiles of the resulting granules and pellets were determined utilizing an automated dissolution apparatus (USP XXII Apparatus 2). All dissolution testing was performed at a paddle speed of 50 rpm, using 1000 ml distilled water maintained at a temperature of 37°C. Modified release was achieved by varying the wax content of the granules. Significant differences were observed between the release profiles for 10–90% w/w and acetaminophen powder (f_2 values < 50). However, no significant difference was exhibited between the release profiles for 40–90% w/w granules and pellets (f_2 value > 50). Therefore as the wax content increased (5–40% w/w) the rate of drug release from the pellets decreased. As spheronisation time increased from 2 to 4 min, the rate of drug release from the pellets increased (f_2 value < 50). However, there was no significant difference between spheronisation time and drug release rate between 6–14 min (f_2 value > 50) and pellet agglomeration was observed. A general trend was observed between the release profiles for different pellet size distributions. As the pellet size increases the rate of drug release from the pellets decreases as anticipated by the Noyes Whitney equation. Further work to establish the fundamental relationship between the drug and matrix pellets in realising optimal control and drug from pellet formulations is ongoing.

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28
Liposomes as efficient protein carriers: liposome formulation and the effect of lipid composition

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There are approximately 3 million deaths per year worldwide due to infections of tuberculosis, with approximately a third of the world's population having been infected. Therefore, it is evident that an effective vaccine candidate is developed. Cationic liposome consisting of dimethyldioctadecylammonium bromide (DDA) alone have been shown to enhance immunity, although they are poor at inducing a Th1 response (required for protection against tuberculosis), therefore, by the addition of immunomodulators, the immune response is enhanced even further and essentially high levels of protective immunity against tuberculosis is produced. As the adjuvant forms a depot the possible role of DDA within these liposomal

adjuvant systems could be to assist and enhance in the uptake of antigen and immunomodulator together into antigen presenting cells (Holten-Anderson et al 2004). Here we assess the effect of adding further phospholipids to increase liposome stability and to enhance this depot effect on the immune responses against tuberculosis. Distearoyl phosphotidylcholine (DSPC):cholesterol (Chol) (8:8 μmole/ml), DSPC:Chol:trehalose dibehenate (TDB) (8:8 μmole:12.5 μg/ml), DSPC:Chol:DDA (8:8:2 μmole/ml), DSPC:Chol:DDA:TDB (8:8:2 μmole:12.5 μg/ml) were prepared by the dehydration-rehydration procedure established by Kirby & Gregoriadis (1984), to which 10 μg/ml of the fusion protein, Ag85B-ESAT-6, was added. The z-average diameter and zeta potential was measured in double-distilled water or 0.001 M PBS, respectively, using a Brookhaven ZetaPlus. Protein incorporation efficiency was determined by ^{125}I-labelled protein. For protein release, each formulation was incubated at 37°C maintained within sink conditions. At various time points protein release from each formulation was determined by ^{125}I-labelled protein. C57Bl/6j mice were subcutaneously immunised with liposomes containing 2 μg Ag85B-ESAT-6 fusion protein. Isolated lymphocytes were restimulated with Ag85B-ESAT-6 in vitro, both one week after second and one week after the third immunization and IFN-γ production was measured as an indicator of the Th1 immune response. Analysis of variance (anova) and tukey's post hoc test were performed, with the statistical significance determined to 0.05 confidence intervals ($P < 0.05$). Protein entrapment values significantly increased ($P < 0.05$, anova) with the inclusion of DDA, from 65% to 81% of liposomes composed of DSPC:Chol:TDB. By the addition of DDA, the liposome surface rendered positively charged, as confirmed by an increase in zeta potential values from −14mV to 50mV. The high positive charge apposed on liposome vesicles consequently reduces vesicle size significantly ($P < 0.05$, anova) from 2066 nm to 782 nm, as a result of the charged surfaces repelling each other sufficiently to prevent membrane adhesion and liposome fusion upon rehydration during the DRV procedure. Neutral liposomes are significantly larger due to the absence of DDA and vesicle repulsion, resulting in liposome fusion and the formation of larger vesicles (2066nm). However, the addition of the immunomodulator TDB did not affect the physiochemical characteristics. Liposomes were examined by transmission electron microscopy (TEM) and environmental electron microscopy (ESEM). Protein release from the cationic liposomes was significantly slower than neutral liposomes, as only 19% of its entrapped protein was released after 14 days compared with 45%, respectively. However, these neutral liposomes exhibited a slower rate of protein release as 20% protein still remained entrapped within neutral liposomes compared to 9% for both cationic formulations after 42 days incubation. The permeability of the liposome bilayer increases while bilayer rigidity and stability decreases when imposing a positive surface charge with the addition of a cationic lipid to the formulation (Mohammed et al 2004), therefore the rate protein release is unrestrained. When these liposome formulations were subcutaneously administered in vivo, results show that all four liposome formulations tested induced production and release of IFN-γ, one week and three weeks after the last immunisation. However, results show that the inclusion of TDB within these liposomal formulations enhanced IFN-γ production compared with those formulations omitting TDB.

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Development of microspheres for pulmonary delivery: optimisation of size distribution

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For pulmonary delivery, particle size is one of the most important factors that affect deposition in the lung (Labiris & Dolovich 2003). Solid microspheres with mean size of 0.5–3 μm can reach the alveoli for effective delivery of pulmonary therapeutics (El-Baseir 1997). The initial objective of this work is to develop polymer microsphere carrier systems that may have application for pulmonary delivery by initial manipulation of size characteristics using different concentrations of emulsification agent (PVA) and poly(lactide) (PLA) and through the use of different polymer molecular weights. Microspheres were prepared by the double emulsion solvent evaporation technique. To investigate the influence of PVA concentration on the size distribution of the microspheres, an internal aqueous phase (10 μL) was vortex-mixed for 90 s with a fixed concentration of PLA in chloroform (417 μL, 3% w/v) to form a primary w/o emulsion. This was subsequently emulsified by high speed homogenisation (6000 rpm) with an aqueous solution of PVA (10 mL, 0.5–10% w/v). The solvent was left to evaporate for 18 h with stirring at room temperature. Microspheres were harvested by centrifugation (20 min, 10 000 rpm) and then washed by re-suspension in three 10 mL aliquots of distilled water. To investigate the influence of PLA concentration on the size distribution of the microspheres, further formulations were prepared using a variable concentration of PLA in chloroform (417 μL, 0.5–7% w/v) and a fixed concentration of PVA in the external aqueous phase (10 mL, 10% w/v). In addition, the influence of PLA molecular weight on microsphere size was assessed using PLA of variable MW (50–300 kDa). The size of the resultant microspheres was measured using laser diffraction (Malvern Mastersizer). Increasing PVA concentration in the external aqueous phase at a fixed PLA concentration decreased the size of the microspheres, with a linear relationship observed between PVA concentration and microsphere size (PVA 3–10% w/v, $r^2 = 0.9987$). Similarly, increasing PLA concentration within the organic phase at a fixed PVA concentration generally resulted in a decrease in microsphere diameter. Furthermore, increasing the molecular weight of the PLA resulted in an increase in microsphere size (Table 1). It is proposed that increasing the concentration of emulsifying agent in the formulation facilitates a small particle size by reducing droplet coalescence during emulsion preparation (El-Baseir 1997). This study demonstrates that, by using appropriate concentrations of emulsifying agents when preparing the formulation, the double emulsion solvent evaporation technique can be successfully used to prepare microspheres of an appropriate size for pulmonary drug delivery.

Table 1 Microsphere diameter (μm)

PVA (% w/v) (3% PLA)		PLA (% w/v) (10% PVA)		PLA molecular weight (kDa)	
0.5	2.60 ± 0.07	0.5	4.72 ± 1.00		
1.0	2.56 ± 0.01	1.0	2.90 ± 0.13	50	1.1 ± 0.2
3.0	1.81 ± 0.01	2.0	1.88 ± 0.13	150	1.5 ± 0.2
5.0	1.60 ± 0.02	3.0	1.13 ± 0.01	300	2.5 ± 0.4
7.0	1.43 ± 0.01	5.0	1.43 ± 0.04		
10	1.12 ± 0.01	7.0	1.50 ± 0.09		

Data are mean ± s.d.

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The structural changes of sodium lauryl sulphate/cetostearyl alcohol/water ternary systems induced by ultrasonication and the effect of these on the stabilization of nanoemulsions

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Nanoemulsions are kinetically stable systems in which the nano-sized droplets may be formed by ultrasonication. Such emulsions have potential as drug carriers for dermatological delivery because the small droplet sizes provide a large interfacial area in contact with skin. This work reports preliminary investigations into the structure and stability of liquid paraffin in water nanoemulsions stabilized by the mixed emulsifier sodium lauryl sulphate (SLS) and cetostearyl alcohol (CSA). This mixed emulsifier stabilizes macroemulsions by the formation of a lamellar gel network phase when the mixed emulsifier interacts with water (Eccleston 1997). The aim of this work is to investigate the influence of ultrasonication on ternary system gel networks and on the stability of corresponding nanoemulsions. Ternary SLS/CSA/water systems and liquid paraffin in water emulsions (3 g) containing between 1%, and 6% w/w mixed emulsifier (1:3 and 1:9 ratios of SLS:CSA) were sonicated at 20 KHz, 30% amplitude by ultrasound (Sonics Vibra cell VCX 500). The systems were examined at 25°C before and after ultrasonication by microscopy (Polyvar, UK), rheology (CSL 100 rheometer, TA instruments, UK) and by transmittance readings (%) using UV 1 (Unicam UK). Emulsion droplet sizes were measured by dynamic light scattering (Zetasizer 3000HS; Malvern, UK). For emulsion stability studies, the dye Sudan Red 7B was added to the liquid paraffin phase. Before ultrasonication, the ternary systems and emulsions were white with thicknesses varying from fluid to semisolid, depending both on the ratio of SLS to CSA and the total concentration of mixed emulsifier. In general, systems containing 1:9 ratios had higher apparent viscosities than those containing similar concentrations at a 1:3 ratio (Table 1). Lamellar structures were detected between crossed polars in all systems. The gel network structure was destroyed by ultrasonication. All the systems were mobile immediately after ultrasonication, and no structure was observed microscopically. The nanoemulsions were white (transmittance values < 0.1) with droplet diameters ranging from 200 to 300 nm, whereas the ternary systems varied in clarity with transmittance values varying from 19.2% – 0.23% (Table 1). On storage over 8 weeks, the gel networks partially reformed. The ternary systems became

Table 1 Transmittance and apparent viscosity values for ternary systems before and immediately after ultrasonication (Ult)

Emulsifying wax		Transmittance		Viscosity (Pas)	
SLS: CSA	Conc. (%)	Before Ult.	After Ult.	Before Ult.	After Ult.
1:3	1.0	0.34	19.20	0.03	2.92×10^{-3}
	6.0	<0.1	0.3	0.65	5.41×10^{-3}
1:9	1.0	0.24	15.5	0.04	3.31×10^{-3}
	6.0	<0.1	0.23	0.39	0.03

more opaque with an average decrease in transmittance of 80% and apparent viscosities of both ternary systems and emulsions increased, but were ~ten-fold lower than those before ultrasonication. Emulsions containing 1% and 2% emulsifier were unstable whereas emulsions containing 4 and 6% remained stable, as indicated by Sudan dye. In conclusion, the lamellar gel network structures are destroyed by ultrasonication, but rebuilt with time. Nanoemulsions with excess emulsifier (over 4%) remain stable because sufficient gel network can rebuild in time to prevent coalescence.

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Enhanced release of acyclovir from silicone intravaginal rings using synthesised acyclovir prodrugs

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As the vagina is now an established mode of entry for microbicides it can act as a barrier for all viral infections using treatments with local or systemic effects. A silicone device, loaded with such treatments that can be placed in the vaginal and retained for periods of time is currently being researched as a new method of controlled release of drugs to the human vagina for treatment of various infections within the female population. There is currently much interest in developing a silicone intravaginal rings (IVR's) as a controlled release system for long-term administration of HIV microbicides to the vagina, as a prevention strategy for HIV transmission. Several studies have demonstrated that prior infection with herpes simplex virus (HSV) type 2 is an important risk factor for HIV acquisition (Holmberg et al 1988), thus a treatment that prevents and treats HSV type-2 outbreaks within the vagina may be the first step towards decrease in female HIV infection. Acyclovir, a common treatment for HSV type one and two outbreaks, was prepared and loaded into silicone matrix IVRs, manufactured according to standard methodologies (Malcolm et al 2003). Acyclovir release from such a device was compared with three acyclovir esters prepared by chemical addition of a hydrophobic group according to a method by Shao et al (1993). The three esters synthesised were acetyl-acyclovir (MW 267.25, log P –0.69) butyl-acyclovir (MW 295.3, log P 0.29) and hexyl-acyclovir (MW 323.35, log P 1.27). A controlled release assay of acyclovir and acyclovir esters was carried out in vitro by immersing the drug loaded ring in a known volume of release medium and incubating at 37°C on an orbital shaker. Samples of the release medium were taken every 24 hs over a 14-day period and the release medium was changed daily at this point to ensure sink conditions. The amount active released within each 24-h time period was detected using high performance liquid chromatography coupled with UV detection. There was an average release of 0.54 mg of acyclovir from the IVR over the fourteen-day period, compared with 3.04 mg for acetyl-acyclovir, 5.89 mg for butyl-acyclovir and 46.75 mg for the hexyl-acyclovir. The data obtained illustrated that as hydrophobicity of the ester increased across the three newly synthesised esters, the release from within the hydrophobic silicone matrix was enhanced compared to the original molecule. This may be in part due to an increase in the hydrophobicily of the newly synthesised compounds making permeation through the hydrophobic silicone matrix much better. It is also worth noting that as the hydrophobicity of the three new esters increased the molecular weight increased. However, this increase in molecular weight did not have an overall inhibition on the release characteristics of each ester from the rings.

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Study of processing factors in glyceryl monostearate matrix pellets based on direct warm spheronisation

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This study was designed to develop glyceryl monosterate matrix pellets based on a novel direct warm spheronisation method without prior extrusion. Employing this method eradicated the need for implementation of traditional extrusion-spheronisation techniques that can lead to problems in temperature control of extrusion. This pelletisation technique utilized granulation and warm-spheronisation procedures to prepare matrix pellets which contain paracetamol, dicalcium phosphate (Emcompress) and glyceryl monosterate (GMS). Various factors in formulation, granulation and spheronisation process affected pellet morphology and drug dissolution. Variation of GMS content in pellet formulations provided varied drug release. Increasing GMS content from 40% w/w to 90% w/w in the formulation decreased drug dissolution. A postulated mechanism suggested that the increasing GMS content increased matrix lipophilicity and reduced wettability, resulting in a decreased drug diffusion that provided a sustained drug release profile. In addition, other mixtures of waxes in pellet formulations such as glyceryl dibehenate (GDB or Compritol; HLB 2), polyethylene glycol-8 beeswax (PG8BX or Apifil Pastilles; HLB 9) and glyceryl palmitosterate (Precirol; HLB 2) in the same HLB 3 as GMS provided different dissolution profile and DSC profile. Moreover, milling time in granule preparation influenced granule size range. The optimal milling time was 50 s, which it provided the maximum amount of granules in the size range 1.00–1.18 mm (the desired granule size range). Spheronisation duration and speed affected pellet sphericity and drug release. As spheronisation time and speed increased, drug release rate decreased. The optimal spheronisation time and speed were 4 min and 6 respectively (Spheroniser model 120, Caleva) because they provided suitably spherical pellets with sustained drug release. Furthermore, drug dissolution of GMS pellets stored for 2.5 months decreased significantly upon storage due to polymorphic transformation of the GMS component. Yajima et al (2002) found that the mechanism of transformation of GMS was from the α-form to ß′-form and then to the stable ß-form under ambient conditions. All of these factors must be carefully controlled in achieving and sustaining the desired performance of pellets. Therefore, suitable wax content, milling time, spheronisation time and speed as well as formulation aging provided optimum characteristic of matrix pellets.

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Vitamin E nanoparticles for dermal application

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Vitamin E is a fat-soluble vitamin that has been widely used in topical formulations. It has been reported to quench free radicals, protect the skin from UV damage, prevent skin aging, and reduce inflammation (Packer & Valacchi 2002). D-L-alpha tocopheryl acetate (TA) also known as (R, R, R)-alpha tocopherol acetate is the predominant form of vitamin E derived from natural sources. TA is highly hydrophobic and thus is often formulated as a topical ointment which displays poor aesthetic characteristics. The aim of this study was to develop a TA loaded nanoparticle gel formulation with acceptable aesthetic characteristics. Vitamin E loaded nanoparticles were prepared via the phase-inversion method followed by shock precipitation (Heurtault et al 2002). Lipoid (1.75% w/w), labrafac (17.5% w/w), and solutol (17.5%) were mixed and heated up to 60°C using a magnetic hot plate (Stuart, UK) after which 3% NaCl solution (63% w/w) was added. The mixture was heated up to 85°C at a rate of approximately 4°C/min and then cooled at the same rate to 60°C (repeated three times). The mixture was diluted with de-ionized water (0°C). The physical stability of placebo and loaded samples were evaluated by measuring their size using a Zetasizer 90plus (Brookhaven, USA, fitted with a 488 nm laser beam at a fixed angle (90°) at 25°C) when stored at 4°C, 25°C and 40°C for 7, 14, and 28 days. Nanoparticle purification was performed using the L7 ultracentrifuge (Beckman, USA) for 1 h at 20°C, 40000 rpm. The purified nanoparticles were recovered and analysed by HPLC. The hyaluronan acid used was sodium hyaluronate (KZ 60254, molecular weight 8.5310 Da), was supplied by MedPharm Ltd. Rheological characterisation was performed using a cone and plate rheometer (4.0 cm cone diameter, 1.510 degree), CSL 100 (Carri-med, USA), with 250 μm gap. The phase inversion process was driven to completion during the three phase cycles indicated by the drop in conductivity. The purified nanoparticles generated by this method displayed a mean effective volume diameter of 50.83 ± 1.75 nm

(n = 3) and contained 57.7% ± 4.9 of the original 110 mg of TA added. Upon storage the mean effective volume diameters of the loaded particles increased by 5.1% ± 0.75 at 4°C, 5.0% ± 0.94 at 25°C and 1.81% ± 0.68 at 40°C, respectively. Rheological assessment of the nanoparticles when loaded into the HA gel showed that the G′ and G″ (Pa) cross-over decreased from 8.7 Hz for HA gel (2%w/w) alone compared with 2.75 Hz for 2% HA gel contained 50% purified vitamin E loaded nanoparticles. This decrease in G′ and G″ (Pa) cross-over suggests that the nanoparticles modify the micro-structure of the HA gel network. The manufacturing process described here-in produced a monodisperse, solvent-free, nano-sized carrier system containing TA. The loading of the drug within the nanoparticles was acceptable and the physical stability even at elevated temperatures was good. Incorporation of the nanoparticles within a HA gel produced a cosmetically acceptable homogenous dosage form suitable for topical use.

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Study of polymorphic transformation of glyceryl monostearate

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Glyceryl monostearate (GMS) is composed of not less than 90% monoglycerides that are mixtures of glyceryl esters of fatty acids, mainly glyceryl monostearate($C_{21}H_{42}O_4$) and glyceryl monopalmitate($C_{19}H_{38}O_4$). It was postulated that rapid cooling of melted GMS yields the α-form that is successively transformed to the stable ß-form under ambient conditions. Moreover, the ß-form of GMS has a higher melting point and density than the α-form (Maruyama et al 1971), which may impact on processing and/or its performance in drug delivery systems. GMS samples were melted and cooled at different temperatures (3, ambient, 37 and 50°C) to provide the α-form and untreated GMS samples in the ß-form. FT-IR and DSC were used to monitor the polymorphic form of the wax and differences were found between untreated GMS and re-solidified GMS at t = 0. Differences between the two polymorphs can be identified at the wave numbers 1050 and 1100 cm^{-1} (Alcohol C-O bond) in the FT-IR spectra. Moreover, differences in DSC extrapolated peak temperatures between untreated GMS (61°C) and re-solidified GMS (59°C) were observed at day 1. Only re-solidified GMS stored at 3°C for up to 3 months was still unchanged due to the low temperature preventing polymorphic transformation. The DSC profile of re-solidified GMS stored at room temperature had a noticeable shoulder peak, however samples stored at 37 and 50°C for 1, 2 or 3 months had extrapolated peak temperatures that shifted from 59°C to 64°C and their profiles had a noticeable shoulder peak due to transformation from the unstable to stable polymorphic form. Previous studies suggested that optimum heat treatment temperature for transformation of GMS from the α-form to ß-form is 50°C for 90 min (Yajima et al 2002). Therefore, high temperature (37 and 50°C) and duration of storage might accelerate transformation from an unstable to stable form and hence provided consistent performance in the final product. It has been suggested that slow cooling (cooled at 0.17°C/min) leads to fractionation of wax components into different regions at the microscopic scale, whilst fast cooling (suddenly cooled to –50°C) results in homogeneous chemical structures (Sutananta et al 1994). DSC profiles of slow cooled GMS stored from 1 day to 2 months increase high shoulder peak due to possible segregation of components into different melting fractions. In fast cooled GMS stored for 1 day, 1 week and 2 months, the shoulder peak temperature moved from 47°C to 51°C and then disappeared because of possible recombination of wax components. The dissolution profile of matrix pellets which contained paracetamol, dicalcium phosphate (Emcompress) and GMS decreased significantly upon storage due to polymorphic transformation of the GMS component. Thermal annealing provided an opportunity to rapidly transform GMS to its most stable polymorph. Storage at 46°C for 7 days was found to be the optimum temperature to rapidly convert GMS to the stable form and to allow reproducible dissolution performance.

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Dissolution enhancement of spironolactone by *in situ* lyophilisation

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Spironolactone is a poorly water-soluble drug that presents challenges for oral delivery due to its low bioavailability. Innovation in processing techniques has permitted development of poorly water-soluble candidates as orally available formulations. Micronisation of drug, administration of hydrophobic drugs in oily vehicles and delivery of amorphous drug forms enhance the dissolution of these drugs. Our aims were to develop a novel method of producing amorphous spironolactone formulations (in-situ lyophilisation) in hard gelatin capsules and to assess the effects of mannitol, sodium dodecyl sulphate (SDS) and citric acid on dissolution characteristics and conformational integrity of spironolactone. A co-solvent system of *tert*-butyl alcohol and water in a 60:40 (% v/v) ratio was used to achieve a concentration of 25 mg/mL of spironolactone. Three excipients were used to modify the dissolution rate: mannitol (1 and 1.5% w/v), SDS (5, 10 and 15% w/v) and citric acid (5% w/v). The solutions were filled into the bodies of size 000 hard gelatin capsules and freeze dried. Upon complete drying, capsules were capped and stored in polythene bags over a dessicant. All prepared formulations were investigated employing X-ray powder diffractometry, dissolution testing, FT-IR spectroscopy (spectra were collected at 4 cm^{-1} resolution and spectra of excipients were subtracted from drug-excipients spectra before comparing with spironolactone control spectrum) and differential scanning calorimetry (DSC). It was confirmed by X-ray powder diffraction that all formulations were entirely amorphous. The basic formulation of the drug alone in the co-solvent system showed a linear release profile. Formulations containing SDS and/or mannitol enhanced the dissolution of the drug with an immediate pulsed release observed. After 30 min, formulations with SDS (5% w/v) and mannitol (1.5% w/v) had released 47 and 39% of total spironolactone dose, respectively, compared with 18% from spironolactone control. For formulations containing a mixture of SDS and mannitol, the release was more rapid; after 30 min, about 85% of the total drug was released. Mannitol produced lyophilisates with improved physical structure. Inclusion of SDS in the mannitol (1%w/v)-containing formulations increased the dissolution rates in a concentration-dependent manner. FT-IR analysis demonstrated that formulations containing SDS or mannitol exhibited some physical changes. Formulations containing a mixture of the two excipients revealed no structural differences compared with FT-IR spectrum of spironolactone control, suggesting no drug-excipient interactions. The DSC results confirmed FT-IR data. DSC thermograms revealed that the melting point (T_m) of spironolactone control was about 202°C. Inclusion of mannitol led to shifting of T_m to a lower temperature (199°C). For SDS-containing preparations the calorimetric enthalpy of the endotherm characteristic to spironolactone was smaller than the enthalpy of control drug. Citric acid proved to be a poor choice of excipient. It compromised the structure of the lyophilisate due to its hygroscopicity. Citric acid-containing preparations showed completely perturbed FT-IR spectra and DSC thermograms. This loss of structure correlated with poor dissolution from those formulations. In conclusion, the initial studies for in-situ lyophilisation of spironolactone with mannitol and SDS show promise for dissolution enhancement of the drug. The next step is to protect these moisture-sensitive formulations.

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The use of emulsion stability as a screening tool for microparticle formulation

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Emulsification solvent evaporation (ESE) is one of the methods that has been used for microencapsulation. In this method, the formation process of microparticles involves two steps. The first step is the emulsification of a polymer solution containing the encapsulated substance followed by particle hardening through solvent evaporation and polymer precipitation. The particle formation process is of critical importance and determines the morphology, size distribution and other properties of microparticles (Rafati et al 1997). Studies of the stability of the emulsion system would help to understand the formation process of microparticles and improve drug release and sphericity of the microparticles. The aims of this project are to investigate the stability of the emulsion system of differrent pH sensitive polymers (Eudragit L100, S100, cellulose acetate phthalate (CAP), cellulose acetate trimellitate (CAT) and hydroxypropyl methylcellulose phthalate (HP55), which have threshold pH 6.0,7.0,6.0, 4.8 and 5.5, respectively) and establish the relationship between stability and micoparticle properties. Microparticles were prepared by using the novel process, based on ESE (Kendall et al 2005). The polymer dissolved in an organic solvent (ethanol) and emulsified into a non solvent phase (liquid paraffin) under agitation. Stability was evaluated by the measurement of globule size and size distribution with time using an optical microscope. Microparticles were characterised by Scanning Electron Microscope (SEM) and particle size analysis (Mastersizer). In-vitro dissolution studies were carried out using USP II dissolution apparatus. The emulsion stability of CAP and CAT formulation was very poor, as cracking of the emulsion was found after preparation. Also the globules of these emulsions were unstable and easily broken. The SEM of CAP and CAT formulations revealed irregular shaped particles. Mean globule size of L100 at initial, 1 h, 3 h and 6 h was 36.2, 37.5, 41.2 and 41.9 μm; S100 was 51.7, 61.9, 62.9 and 78.6 μm; HP55 was 55.8, 77.9, 81.6 and 89.3 μm, respectively. The slow rate of globule size increase of L100 formulation indicated that it was more

stable than S100 and HP55. When comparing the size of the microparticles, L100 microparticles were the smallest in size, the largest size of microparticles were produced by HP55, therefore this emulsion was less stable in comparison to L100 and S100 emulsion formulations. The globule size distribution of all the formulations was shown to be polydisperse. Both the globule size and particle size distribution for HP55 was broader than L100 and S100 formulation showing that the globules size distribution is related to the microparticle size distribution. In-vitro dissolution studies revealed that L100, S100 and HP55 microparticles were able to prevent drug release (<10%) at acidic pH while allowing rapid drug release in proximal small intestine pH (L100 and HP55 microparticles) and distal small intestinal pH (S100 microparticles). In contrast the drug release at acidic pH of CAP and CAT microparticles was found to be 23.60 and 71.21%, respectively. This study has shown that it is possible to use emulsion stability as a screening tool for microparticle formulation as stability of the emulsion is of critical importance and predetermines the morphology of the microparticles.

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Preparation and characterisation of microspheres of a novel polyester polymer conjugated to ibuprofen

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A novel semi-crystalline polylactone polymer was produced using an enzymatic polymerization technique (MW 15kDa). Ibuprofen was conjugated to the free hydroxyl groups of this polymer in a pyridine catalysed reaction to 100, 50 or 25% of the free hydroxyl groups. The 100% conjugate was prepared into microspheres using (oil-in-water) emulsion solvent evaporation (ESE) and spray drying (SD) methods. The purpose of this work was to reduce the burst release of ibuprofen. Previous unpublished work that involved manufacture of microspheres with this polymer and non-conjugated ibuprofen produced a burst of at least 33% after 30 min. Morphology was determined by scanning electron microscopy (Leo S430 electron microscope). Thermal properties were determined using a TA instruments Q100 differential scanning calorimeter. Ibuprofen release was carried out in pH7.4 buffer at 37°C; samples were analyzed using both UV and HPLC methods. Drug loading was determined by a UV method with samples dissolved in chloroform. The 100% ESE microspheres were spherical with rough, uneven surfaces. The 100% SD microspheres were smoother, but highly aggregated. The morphology of the conjugated material was rough and mostly non-spherical as all of the polymer/drug conjugated materials had been pulverised using a mortar and pestle prior to characterisation. Thermal scans of the 100, 50 and 25% conjugates produced multiple peaks for melting on the first heating cycle. This may indicate that some of the ibuprofen was not covalently attached as a single melting peak should have been seen if the conjugates existed as homogeneous compounds. In contrast, the 100% ESE and SD microspheres produced single melting peaks (53 to 56°C). All peaks on the first heating cycle were very broad. The T_g of all samples was –33 to –27°C. Drug loading and release for the conjugates can be seen in Table 1. Drug loading for the 100% ESE conjugate was lower than the 100% conjugate as some ibuprofen was lost to the external phase of the emulsion. Release from the 100% conjugate was overall very slow. However burst release was still present with 13% being released within the first 30 min. Release from the 25 and 50% conjugates was faster with greater bursts and faster overall release rates. This seems to confirm that some non-bound ibuprofen was present. HPLC analysis of the samples showed multiple peaks. One large peak could be attributed to non-bound ibuprofen, the multiple smaller peaks were unknowns. These other peaks may be oligomeric conjugates (Oh et al 1999; Ustariz-Peyret et al 2000). Release from the 100% ESE and SD microspheres was initially much slower. However subsequent release was faster than the 100% conjugate. Reduction in burst may have been due to the entrapment of any non-bound ibuprofen during microsphere production. However, when this ibuprofen did eventually diffuse out it may have aided matrix breakdown thereby increasing the total percentage released. Conjugation of ibuprofen to this novel polyester reduced but did not eliminate burst release. Further work would establish whether or not conjugation is completely efficient.

Table 1 Drug loading and release before and after Microencapsulation

Conjugate	Drug loading/%	Drug release at 30 min/%	Drug release at 5 days/%
100%	12.6 ± 0	13 ± 3	22 ± 1
50%	6.8 ± 0	14 ± 1	77 ± 4
25%	3.4 ± 0	18 ± 1	85 ± 2
100% ESE	11.5 ± 1	9 ± 7	83 ± 8
100% SD	12.6 ± 0	6 ± 2	63 ± 4

Data are means ± s.d., n = 3.

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Spray-dried w/o/w double emulsions can generate respirable powders for sustained drug delivery

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This study investigates how the incorporation of leucine as an aerosolisation enhancer (Li et al 2003; Rabbani & Seville 2005) and poly-lactide co-glycolide (PLGA 75:25; Chaw et al 2003) as a drug release modifier in water-in-oil-in-water (w/o/w) double emulsions can generate highly respirable spray-dried powders that exhibit sustained drug release characteristics. W/o/w double emulsions were prepared by vortex-mixing 0.4 mL water (±160 mg salbutamol sulphate) with 0.1 mL Span 80 in a solution of 200 mg PLGA (75:25) in 12 mL chloroform (±160 mg beclometasone dipropionate, BDP), subsequently homogenised with 288 mL 10% w/v PVA aqueous solution containing 720 mg leucine (dispersibility enhancer), 600–920 mg lactose (bulking agent) and optionally 160 mg salbutamol to form w/o/w double emulsions containing salbutamol in the inner aqueous phase and/or BDP in the oil phase and/or salbutamol in the outer aqueous phase. The emulsions were then spray-dried under standard operating conditions using a Buchi B-290 mini spray-drier (Buchi, Switzerland) fitted with a high performance cyclone. The physical and thermal characteristics of the resultant powders were assessed using laser diffraction, SEM, DSC and TGA. The aerodynamic performance of the powders was determined using HPMC capsules (n = 3) loaded with 25 mg spray-dried powder aerosolised using a Spinhaler (Fisons) DPI into an Andersen Cascade Impactor (60 L/min ACI; Copley Scientific) at a flow rate of 60 L/min. The emitted dose (ED) and powder deposition at each stage of the ACI was determined with analysis by UV spectroscopy (salbutamol: 278 nm; BDP: 250 nm). Finally, the tapped density of the powders was assessed and theoretical estimates of primary aerodynamic diameter calculated. In-vitro powder dissolution studies were performed using USP 2 dissolution apparatus in phosphate buffer (pH 6.8, 37°C) with quantification of drug release by UV spectroscopy. The spray-dried powders had an off-white appearance and exhibited Carr's Index values of 21–32%, suggesting fluid powders. Using the high performance cyclone, spray-drying yields of up to 74% could be achieved. SEM indicated that the powders comprised microspheres with a diameter of 0.25–3 μm. Laser diffraction data supported the SEM results, with mean diameters of approximately 3–5 μm. The aerosolisation testing of the spray-dried powders resulted in an ED of at least 90% of capsule contents and a fine particle fraction (FPF: fraction < 5 μm) of up to 40% total loaded dose. In vitro dissolution studies indicated that inclusion of PLGA within the formulation resulted in a sustained release profile over seven days for drugs incorporated in the inner aqueous phase and the oil phase. Salbutamol incorporated in the outer aqueous phase did not undergo sustained release, offering the potential for a dual release profile. Drug release from PLGA microspheres appeared to be erosion mediated, as little difference was observed in the release profiles of hydrophilic and hydrophobic agents. We have shown that highly dispersible spray-dried powders can exhibit an in vitro sustained release profile by spray-drying a w/o/w double emulsion incorporating PLGA (controlled release entity) and leucine (dispersibility enhancer).

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Rabbani, N. R., Seville, P. C. (2005) *J. Controlled Release* **110**: 130–140

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Influence of chitosan molecular weight on drug release from respirable spray-dried powders

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This study investigates how varying the molecular weight of the matrix polymer chitosan (Filipovic-Grcic et al 2003) can modify the drug release profile from highly respirable spray-dried powders incorporating the aerosolisation enhancer leucine (Li et al 2003, Rabbani & Seville 2005). Aqueous ethanol (30% v/v) formulations of

4% w/w terbutaline sulfate (hydrophilic model drug) and/or 4% beclometasone dipropionate (BDP: hydrophobic model drug), 36% w/w leucine, 50% w/w chitosan and 6–10% lactose (bulking agent), with a total powder mass of 2% w/v, were spray dried under standard operating conditions using a Buchi B-290 mini spray-drier (Buchi, Switzerland) fitted with a high performance cyclone. The influence of chitosan molecular weight on the release profile of the resultant spray-dried powders was investigated through the use of low, medium and high MW chitosan, either alone or in combination. The physical and thermal characteristics of the powders were investigated by particle sizing, scanning electron microscopy, PXRD, DSC, TGA and TAM. The aerodynamic performance of the powders was determined using HPMC capsules (n = 3) loaded with 25 mg spray-dried powder aerosolised using a Spinhaler (Fisons, UK) DPI into an Andersen Cascade Impactor (60 L/min ACI: Copley, UK) at 60 L/min. The tapped density of the powders was also assessed and theoretical estimates of primary aerodynamic diameter calculated. In vitro drug release from the powders was assessed using a modified USP 2 dissolution apparatus in phosphate buffer (pH 6.8, 37°C) with analysis by HPLC. Spray-drying yields of up to 90% were obtained using a high performance cyclone; resultant powders were off-white in appearance with Carr's Index values of 17–33%, suggesting fluid powders, as evidenced by more than 95% capsule contents emitted during aerosolisation. SEM revealed rough surfaced microspheres, 1–15 μm in size. PXRD revealed amorphous powders with only a partial crystallisation peak of terbutaline after TAM, suggesting good stability. DSC revealed no degradation of any of the components during spray drying and TGA revealed a water weight of 5–10% in freshly spray dried samples. ACI data revealed that with appropriate selection of chitosan molecular weight (low/medium/high), a fine particle fraction (FPF: fraction <5 μm) of 69% could be achieved, with low MW chitosan powders having higher FPF and high MW chitosan powders demonstrating lower FPF. Terbutaline-only powders typically demonstrated higher FPF than powders containing both terbutaline and BDP, whereas BDP-only powders exhibited lower FPF; this is presumably related to the observation that BDP formulations were suspensions rather than solutions. In vitro dissolution studies revealed that the drug release profile was dependent upon chitosan MW, with release profiles over 12 h attainable. We have shown that the incorporation of low, medium or high MW chitosan and an aerosolisation enhancer (e.g. leucine) into a spray-drying formulation offers the opportunity to generate highly dispersible powders that exhibit an in vitro sustained release drug profile.

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A preliminary investigation into the topical delivery of pre-formed photosensitisers by jet injection

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Photodynamic therapy (PDT) involves administration of a tumour localising photosensitising agent. Subsequent activation of the agent by light of a specific wavelength results in a sequence of photochemical and photobiological processes culminating in irreversible damage to the tumour tissue. Although there are advantages of aminolevulinic acid (ALA) based PDT, there are still drawbacks associated with the treatment. As the photosensitisation is porphyrin mediated, excitation of PpIX occurs at 630 nm, limiting the depth of penetration of the light into the skin. The hydrophilic nature of ALA somehow limits the penetration through the *stratum corneum* (SC) of the skin. Due to this reason a number of methods have been investigated and employed to improve ALA penetration. These include tape stripping, permeation enhancers, ion pairing, iontophoresis, laser SC ablation and formulation development. Needle free jet injector devices employ a high-speed jet to puncture the skin and deliver drugs intradermally without the use of a needle (Baxter & Mitragotri 2005). This novel drug delivery strategy may be one way of localising photosensitiser administration and thus preventing generalised photosensitivity, which is frequently a problem when employing these types of agents. In this study we aimed to enhance topical delivery of both ALA and preformed photosensitisers using needle-free injection technology to by-pass the SC. A proprietary device (SQ-Pen) was used to blast a high pressure jet of liquid containing either ALA or fluorescein into a cross-linked hydrogel (30% poly vinyl alcohol/3% sodium tetraborate), which was used as a transparent skin model, and later, into neonatal porcine skin. By controlling the viscosity of the injected solution it was possible to control the depth of penetration of the drug. To establish the validity of the results, the mechanical strengths of the model gel, and neonatal porcine skin were compared using texture profile analysis. As such, a predictive model was set up, so that the results of these preliminary investigations may be put into context regarding the needle-free injection delivery of photosensitisers across the SC to surface lesions. This investigation has highlighted the potential application of utilising the jet needle device to deliver photosensitisers selectively to surface skin lesions. It was proposed that by adjusting the viscosity of the jet propelled solution that a resultant change in the depth of penetration into a model skin gel would occur. Indeed this was found to be the case, with a linear relationship observed between depth of penetration and jet viscosity. To correlate these results into what may occur when applied to jet delivery to the skin, a predictive model has been set up that will allow estimation of the distance a jet may travel into porcine skin, following observations made in the model gels.

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