Drug Delivery

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Determination of solubility of naproxen in a wax based matrix using thermal, visible microscopic and Higuchi release approach

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Objective Determination of the solubility of drugs in semi-solids is often a problem, especially for transdermal formulations. In this study, the solubility of naproxen in Witepsol H15, a semi-solid wax matrix, was determined using microscopy, Higuchi release rates and HyperDSC (high speed differential scanning calorimetry).

Methods For microscopy studies, granules were formed using witepsol and naproxen at the following concentrations of naproxen; 1, 2, 5, 7.5, 10, 15, 20, 25, 30, 35, 40 and 50% w/w drug loading by employing a method described by Oladiran & Batchelor (2007). Then, a thin film of the prepared granules was smeared over a microscope slide for microscopic analysis using a microscope with a 4× objective lens and images were captured using Axiovision camera. From the granules (above), tablets were made via a method described by Oladiran & Batchelor (2007) and Higuchi release was carried out by directly putting a tablet in the USP II basket adapted for small volumes using PBS, pH 7.4 maintained at 33°C as the release media. In the case of hyper-DSC, the approach described by Gramaglia et al (2005), naproxen loaded wax based matrices were prepared by combining the drug with Witepsol H15 (via melting) at concentrations of 0.5, 1, 2, 5, 10, 20, 30, 35, 40 and 45% w/w. Samples (1.90 ± 0.05 mg) were sealed in air tight aluminium pans (Perkin-Elmer) and analysed at 500°C/min over a temperature range of −30°C to 200°C with helium as a purge gas using a Diamond DSC, Perkin Elmer. The resulting thermogram was analysed using Perkin-Elmer Pyris series 5.0 software. Five repeats were performed and an empty pan was used as a reference in all cases. The drug solubility in the wax via this method was determined using an approach first described by Theeuwes et al (1974) and recently applied by Oladiran & Batchelor (2007). For each of the approaches, three repeats of each analysis were carried out.

Results Solubility values of about 20% w/w, 20.5% w/w and 18.4% w/w resulted from microscopy, Higuchi release rates and HyperDSC techniques, respectively. Microscopy was useful in additionally examining crystal size, shape and homogeneity. Release rate experiments showed that release from these formulations followed Higuchi kinetics with an inflection in release rate constant at the drug loading corresponding to drug solubility. HyperDSC not only measured solubility but also determined the melting point of the formulation.

Conclusions The results from these three techniques correlated well, suggesting that the simpler techniques of microscopy or HyperDSC are appropriate to determine the solubility in semi-solid pharmaceutical formulations.

Gramaglia, D., et al (2005) *Int. J. Pharm*. **301**: 1–5
Oladiran, G. S., Batchelor, H. K. (2007) *Eur. J. Pharm. Biopharm*. In press.
Theeuwes, T., et al (1974) *J. Pharm. Sci*. **63**: 427–429

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Novel nanoparticles for pulmonary gene delivery

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Objectives A number of respiratory diseases could potentially benefit from gene transfer to the lung, including cystic fibrosis, asthma and emphysema. Jet nebulisation, the current standard for introducing gene therapy formulations into the lung, is highly inefficient. While newer nebuliser technologies are under development, pressurised metered dose inhalers (pMDIs) offer an alternative with potential advantages of more efficacious and rapid administration. As both solution and suspension pMDI formulations have drawbacks relating to stability and dose reproducibility,

we aim to explore the potential of modifying a novel low-energy nanotechnology process (Dickinson et al 2001) to prepare surfactant-coated plasmid DNA (pDNA) nanoparticles for pulmonary gene delivery via a dispersion pMDI.

Methods Water-in-oil microemulsions containing pEGFP-N1 reporter plasmid were prepared from sucrose solution (as cryoprotectant and aqueous phase), lecithin:propan-2-ol (as stabilising surfactant) and iso-octane (as organic phase). Resultant microemulsions were snap frozen in liquid nitrogen and lyophilised. Excess surfactant was removed by repeated washes with iso-octane and centrifugation. Scanning electron microscopy (SEM) and gel electrophoresis were used to characterise surface morphology and deduce pDNA integrity, respectively.

Results A ternary phase diagram was constructed to identify optimised microemulsion compositions (Figure 1). Microemulsions with a surfactant to water ratio of 1.5 and above formed stable water in oil isotropic systems. Unstable biphasic systems were formed when the surfactant to water ratio fell below 1.5. Optimised formulations resulted in effective incorporation of pDNA into the aqueous pool of reverse micelles. Controlled lyophilisation enabled the formation of novel surfactant-coated pDNA nanoparticles. A qualitative analysis performed using gel electrophoresis showed that the freeze-dried particles retained pDNA structural integrity. SEM images conferred aggregates of DNA-cryoprotectant particles.

Conclusions Freeze-drying pDNA microemulsions produced surfactant coated pDNA particles whilst successfully maintaining the integrity of the pDNA. The nanotechnology process used offers the potential for the incorporation of pDNA nanoparticles into pMDI systems for pulmonary delivery of gene vectors.

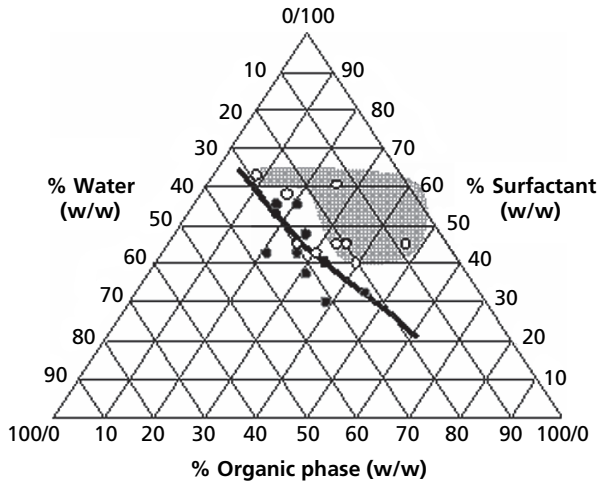

Figure 1 Ternary phase diagram of lecithin:propan-2-ol/iso-octane:water. (○) indicates formation of a stable microemulsion. (●) indicates phase separation. Black line (—) indicates phase boundary. (▦) indicates stable w/o microemulsion

Dickinson, P. A., et al (2001) *J Drug Target*. **9**: 295–302.

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The use of amino acids as cryoprotectants during lyophilisation of microspheres

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Objectives To investigate the potential of amino acids as an alternative to traditional sugar-based cryoprotectants, in terms of maintaining physico-chemical stability during the freeze-drying of immunomodulatory microspheres.

Methods Poly(DL-lactide-co-glycolide) (75:25) microspheres, incorporating the immunologically active adjuvant dimethyldioctadecyl ammonium (DDA), were prepared by double emulsion solvent evaporation (w/o/w), harvested by centrifugation and then freeze-dried in the presence of a range of cryoprotectants. Six different amino acids were chosen: lysine, arginine, histidine, glycine, leucine and valine. Following lyophilisation, microspheres were characterised in terms of volume mean diameter and zeta potential.

Results Initially, investigations were based around lysine due to previous reports of effective stabilisation of liposomal preparations upon lyophilisation (Mohammed et al 2007). Results suggest that lysine may not be as effective for the microsphere systems as it has shown to be for liposomes, although there is some cryoprotective effect as compared with no cryoprotectant, with an optimum concentration around 3% (w/v) (an increase in mean diameter after freeze-drying by a factor of 3.66 compared with 10.02 with no cryoprotectant), beyond which the protective effect begins to diminish. Subsequently, a further two positively charged amino acids, arginine and histidine, were investigated, with positive results emerging for high concentrations of arginine (10% w/v) and relatively low concentrations of histidine (2% w/v) (increase in mean diameter by a factor of 2.05 and 2.16, respectively). Additionally, amino acids bearing an inactive hydrophobic side chain were studied, all of which showed good cryoprotective ability at relatively low concentrations (2% w/v) (increase in mean diameter by a factor of 1.36, 1.01 and 1.79 for glycine, leucine and valine, respectively). Furthermore, there is a definite trend showing concentration dependence on cryoprotective ability, indicating that these hydrophobic compounds associate with the microspheres, forming a protective layer, which consequently prevents aggregation of the particles. Indeed, freeze-drying in the presence of the hydrophobic amino acids visually improves cake formation compared with freeze-drying in the presence of either sugar or lysine, for example, which may suggest better ability to replace bound water during lyophilisation. Interestingly, the presence of the positively charged amino acids profoundly affects the zeta potential exhibited by the systems, suggesting some interaction with the surface of the microspheres, possibly through hydrogen bonding with the polymer. Nevertheless the microspheres retain a cationic charge when the inactive hydrophobic moieties are employed, further supporting the hypothesis of the protective layer postulated above. However, further investigations would be required to fully elucidate the mechanism of action of these two groups of compounds with regards to their cryoprotective function.

Conclusions Amino acids, particularly those possessing an inactive hydrophobic side chain, have been shown to offer potential for cryoprotection of microspheres at relatively low concentrations as compared to traditional sugar-based cryoprotectants.

Mohammed, A. R., et al (2007) *Eur. J. Pharm. Sci*. **30**: 406–413

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Optimization, characterization and in vitro release study of poly (D,L-lactide) microspheres for pulmonary delivery

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Objectives Polymeric microspheres have been shown to be a potential candidate for drug delivery. Local delivery via the lung can be more efficient in lung disease therapy. The initial objectives of this work were to optimise the formulation of poly (D,L-lactide) (PLA) microspheres to obtain particles of a suitable size for inhalation (0.5–3 μm), determine the physicochemical characteristics of the microspheres and investigate the in vitro drug release properties.

Methods Bovine serum albumin (BSA) loaded PLA (MW 50 000) microspheres were prepared using the double emulsion solvent evaporation technique. Different concentrations of PLA in the organic phase (0.5–10% w/v) were investigated to optimize the particle size distribution. The particle size and size distribution of the resultant microspheres were measured using laser diffraction (Helos/BF system, Sympatec, Ltd). Microspheres were then dispersed in 7% w/v trehalose aqueous solution and freeze-dried to generate a dry powder. The residual moisture content of the powder after freeze drying was determined by thermogravimetric analysis (Pyris 1 TGA; Perkin Elmer Instruments) with a dry nitrogen purge, an open pan and a heating rate of 10 °C/min over the temperature range 40–150 °C. Differential scanning calorimetry (Pyris Diamond DSC and Intracooler 2P; Perkin Elmer Instruments) was used to identify the crystalline nature of the freeze-dried powders, with samples sealed in hermetic pans and a heating rate of 50 °C/min over the temperature range 40–300 °C. The drug entrapment efficiency and rate of drug release was quantified using a BCA assay and a microplate reader (BioRad).

Results The mean particle diameter decreased with increasing PLA concentration over the concentration range 0.5–3% w/v, whilst a reversed trend was observed over the concentration range 3–10% w/v. At the optimal PLA concentration (3% w/v), a mean particle size of 1.13 ± 0.01 μm (n = 3) was obtained. The residual moisture content of the BSA-loaded freeze-dried powder was comparable with that of the BSA-free freeze-dried powder ($4.23 \pm 0.20\%$ vs $3.98 \pm 0.12\%$, respectively, n = 3). Differential scanning calorimetry indicated that the powders were more amorphous rather than crystalline. Following the initial formulation optimisation studies, investigations into microsphere loading using BSA as a model protein demonstrated an entrapment efficiency of over 60%. The in vitro release profiles suggested an initial burst release within the first 24 h, followed by a sustained release with over 70% of BSA released over seven days and 100% BSA released after three weeks.

Conclusions Using appropriate concentrations of polymer in organic phase, the double emulsion solvent evaporation technique can be successfully used to prepare high entrapment efficiency microspheres with of an appropriate size for pulmonary drug delivery.

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Pressurised metered dose inhaler formulations for pulmonary delivery of proteins

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Objectives The objective of this study was to develop pressurised metered dose inhaler (pMDI) protein formulations for pulmonary delivery. Spray-drying, a verified approach to fabricate respirable-sized particles, was employed to prepare dry particles of bovine serum albumin (BSA) with alpha-cyclodextrin (α-CD) included as a thermoprotectant (Li 2005). The influence of the inclusion of surfactants on the stability and the aerosolisation characteristics of the pMDI formulations was investigated.

Methods The solubility of Tween 20 and Brij 30 in propellant was determined by mixing 100 mg of the surfactants with 10 mL HFA 134a in the presence or absence of 0.5 mL ethanol as a cosolvent. A series of aqueous solutions containing 1% w/v BSA and 0.5–10% w/v α-CD were spray-dried using standard operation parameters (Mini-spray dryer B-290; Buchi, Switzerland: inlet temperature 180 °C, flow rate 600 L/h, aspiration 85%, outlet temperature 60 °C) to prepare dry powders. Surfactant-modified powders were prepared by adding Tween 20 or Brij 30 into the solutions (surfactant:BSA ratio 1:1, 10:1, 50:1) before spray-drying. pMDI formulations were prepared by dispersing sufficient spray-dried powder in 5 mL HFA 134a (± 0.25 mL ethanol) to give a theoretical dose of 50 μg BSA per 50 μL actuation. In-vitro deposition was investigated using a twin-stage impinger (TSI), operated at a flow rate of 60 L/min. The mass of BSA deposited within the TSI was determined by Bicinchoninic Acid (BCA) protein assay.

Results The solubility study indicated that Brij 30 was soluble in HFA-134a, whereas Tween 20 was soluble in HFA-134a/ethanol. The spray-dried BSA powders were successfully prepared with yields up to 62%. Analysis of the BSA content of the powders indicated that the optimal spray-drying formulation comprised 1% w/v BSA and 3% w/v α-CD; this powder contained 98% of the expected BSA content. The inclusion of surfactant in the spray-drying solution reduced the yield of the spray-dried powder, but did not adversely affect BSA loading. The surfactant-modified pMDI formulations demonstrated better dispersion and greater stability than the unmodified pMDI formulations, and in-vitro deposition studies indicated enhanced aerosolisation characteristics for the modified spray-dried powders compared to the unmodified powders. The spray-dried powders containing surfactant:BSA 10:1 exhibited the best TSI deposition profile, with a respirable fraction (particles <6.8 μm) of 32.2% and 25.9% for Tween 20 and Brij 30 modified powders, respectively, compared with only 12.8% for the unmodified (surfactant-free) spray-dried powders.

Conclusions These preliminary investigations into the development of protein pMDI formulations demonstrate that spray-drying can be used to generate spray-dried powders incorporating α-CD as a thermoprotectant and Tween 20/Brij 30 to improve pMDI suspension stability. These surfactant-modified spray-dried powders demonstrate high protein loading and enhanced aerosolisation properties compared to unmodified powders.

Li, H. Y. (2005) *J. Gene Med.* **7**: 1035–1043

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Comparison of three techniques to determine the solubility of drugs in wax

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Objectives The solubility of ibuprofen in Witepsol H15 was determined via three methods: microscopy was used to visualise the loading at which drug crystals were first observed; theoretical calculations based on the ideal solution theory were used based on chemical structures; the final technique investigated the authors' theory that the density of ibuprofen dissolved in wax would be different to the density of a system where ibuprofen is dispersed due to greater disruption of the three dimensional crystal structure in a dispersed system. Pre-calibrated suppository moulds were used to maintain a constant volume so that density differences could be measured by changes in mass.

Methods Suppositories of ibuprofen in wax were prepared at drug loadings of 1, 5, 10, 15, 20 and 25% w/w and weighed on an analytical balance. Samples from each drug loading were examined under a microscope with a 4× objective lens for the presence of ibuprofen crystals. The structures of ibuprofen and Witepsol were used to calculate the theoretical solubility according to the ideal solubility equation. The structure of Witepsol H15 was assumed to have three lauric acid groups providing a MW of 638.

Results The microscopy study suggested that the solubility of ibuprofen in Witepsol was 15–20% w/w as crystals were first observed in the 20%w/w loaded formulation. The theoretical solubility prediction was 5.04% w/w. The results of the drug loading versus mass of the suppositories can be seen in Figure 1. The results from this were inconclusive.

Conclusions This study demonstrated that the solubility of ibuprofen in Witepsol can be measured using microscopy, providing a value in agreement with reported values (Oladiran & Batchelor 2007). The theoretical prediction of solubility was lower than expected; this may be due to assumptions about the ideal solution and the chemical structure of Witepsol H15. The theory that the density of a drug in wax blend alters when the drug is no longer soluble but suspended within the wax cannot be answered by the data presented. This may be due to the loadings selected or the lack of sensitivity in the apparatus. This study demonstrates the complexity of measuring solubility in semi-solids and suggests that microscopy is the simplest and most reliable technique.

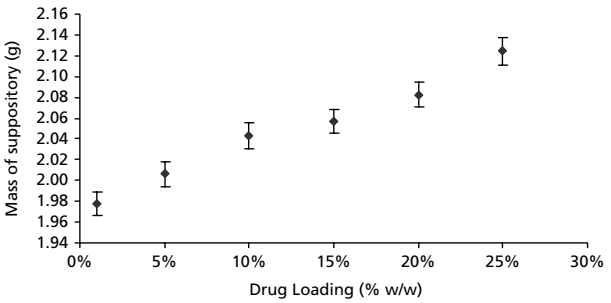

Figure 1 Graph of drug loading against mass of suppository for ibuprofen loaded Witepsol formulations.

Oladiran, G., Batchelor, H. (2007) *Eur. J. Pharm. Biopharm.* Available online 27-01-07

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Effect of the liposomal morphological phase and liposome-DNA complex size on in vitro DNA transfection

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Objectives Morphology of liposome-DNA complexes vary depending on the type of neutral lipid included within the formulation. Two different morphological phases exist; multilamellar structure (L_α) with the DNA confined between two lipid bilayers, as usually shown for phosphatidylcholines (PC's) and columnar inverted hexagonal liquid-crystalline structure (H_{II}) where the lipid surrounds the DNA and arranges itself into an unstable hexagonal lattice, usually associated with phosphatidylethanolamines (PE's). The transfection efficiency of liposomes will vary, depending on the lipids included within the bilayers. Here we assess the affect of lipid composition, structure and size on transfection efficiency *in vitro*.

Methods Small unilamellar vesicles (SUV) consisting of helper lipid, either phosphatidylcholine (PC), L-alpha-dioleoylphosphatidylethanolamine (DOPE) or cholesterol (Chol) and cholesterol 3β-*N* (dimethylaminoethyl)carbamate (DC-Chol), were formulated at various molar ratios. *In vitro* transfection studies were performed as per standard protocol for Lipofectin® transfection reagent. Lipoplexes were characterised by measuring *z*-average diameter (nm) and zeta potential (mV) on a ZetaPlus (Brookhaven Instruments) in ddH_2O and 0.001 M PBS, respectively.

Results Transfection efficiency of PC:DC-Chol (1:1 molar ratio) SUV-DNA complexes is enhanced with the addition of DOPE, from 38% to 123% (of lipofectin control), respectively, for PC:DOPE:DC-Chol (1:1:1). The addition of DOPE could cause a slight destabilisation of lipid bilayers causing the once stable bilayers to 'soften' (May et al 2000). However, liposomes composed of DOPE:DC-Chol (1:1) in the absence of PC exhibited extremely higher levels of activity (485% of lipofectin control). Therefore, secondly, the liposome-DNA complex suspension composed of PC:DOPE:DC-Chol (1:1:1) could contain a heterogeneous population of vesicles with a coexistence of complexes differing in structure and composition, where there is a coexistence of both stable L_α and fusogenic H_{II} structures. By reducing DOPE content within PC:DOPE:DC-Chol (0.5:0.5:1), transfection efficiency is significantly reduced to 85% (of lipofectin control), which is intermediate between PC:DC-Chol omitting DOPE and PC:DOPE:DC-Chol (1:1:1). Vesicle size is unaltered for each formulation, with and without DOPE, demonstrating that in this instance size is not the contributing factor and must be associated with the bilayers mechanics as described. With regard to Chol:DC-Chol (1:1) liposomes, addition of PC also significantly reduces transfection efficiency from 384% to 12% (of lipofectin control) respectively. However, this coincides and is associated with the considerable reduction in complex size, from 945 nm for Chol:DC-Chol to 391 nm for

PC:Chol:DC-Chol, as complexes with diameters in the range 700–1000 nm produce elevated levels of activity when compared with smaller complexes of 300–350 nm (Felgner et al 1995; Kawaura et al 1998). Further characterisation studies were carried out (i.e. gel electrophoresis and transmission electron microscopy).

Conclusions The fusogenicity of DOPE plays a predominant role in the high transfection efficiency of DOPE. The inclusion of increasing amounts of DOPE within PC-based formulations enhances transfection efficiency accordingly. Complex structure and size both play an essential role in transfection efficiency.

Felgner, et al (1995) *Ann. NY Acad. Sci*. **772**: 126–139
Kawaura, et al (1998) *FEBS Lett*. **421**: 69–72
May, et al (2000) *Biophys. J*. **78**: 1681–1697

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Experimental determination of the solubility of a model drug in drug-in-glue transdermal patches to validate a theoretical solubility calculator

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Objectives The solubility of drugs in semi-solid transdermal preparations is paramount in predicting their in vivo performance. Experimental measurement of solubility in semi-solids is difficult, however an on-line solubility calculator based on linear free-energy relationships is available for Duro-Tak adhesives, (http://www.nationaladh.com). The Log10 of the partition coefficient and the drug's solubility in water are the required inputs for the solubility calculator. This tool has previously only been validated via microscopy, used to observe the drug loading at which solid material was first visible, which provided only semi-quantitative data (Foreman et al 2005). This study validates the theoretical solubility calculator by comparing the solubility of sodium fluorescein, a model hydrophilic drug, in a range of Duro-Tak glues using Higuchi kinetics to calculate the experimental solubility.

Methods Drug-in-glue patches were prepared at six different fluorescein loading concentrations with Duro-Tak 87–900A, 87–9301, (3)87–2525 and 87–2852 adhesives. At least three drug loadings either side of the theoretical solubility were prepared. The release of drug from each formulation was measured using dissolution apparatus over an 8-h period in PBS as dissolution media and quantified using spectrofluorimetry. The release profiles were fitted to Higuchi kinetics and a plot of the release rate constant versus drug loading for each glue was plotted, where the inflection predicted the solubility.

Results All release profiles followed Higuchi kinetics. All graphs demonstrated an inflection in the drug loading graph and this value was reported as the experimental solubility value. Table 1 shows the results of the predicted solubility and the solubility values measured via Higuchi kinetics for all glues examined (mean value ± standard deviations are shown, n = 4). As noted there was good correlation in the experimental and theoretical values suggesting that the theoretical predictor is a valuable tool in product development.

Conclusions This study demonstrated that the online tool provided a good estimation of solubility as validated using Higuchi analysis. The results obtained from this study demonstrated better substantiation compared to the initial validation where microscopy was used and differences of up to 100% between experimental and theoretical values were reported.

Table 1 Predicted and experimental solubility values

Durotak adhesive	Predicted solubility (% w/w)	Experimental solubility (% w/w)	Difference (%)
87–900A	2.579	1.820 ± 0.010	+29.4
87–9301	3.161	3.356 ± 0.188	−6.2
(3)87–2525	2.001	2.370 ± 0.320	−18.4
87–2852	1.686	2.345 ± 0.266	−39.1

Foreman, P., et al (2005) *Drug Deliv. Technol*. **5**: 1–6

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Formulation of stabilised micellar based systems for the delivery of poorly water-soluble chemotherapeutics

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Objectives The poor water solubility of some anti-cancer analogues limits their use as therapeutic agents. For example, it has been reported that poor delivery of paclitaxel has been associated with its poor aqueous solubility (<1 μg/mL). Currently, paclitaxel is formulated in a mixture of dehydrated alcohol and Cremophor EL. This formulation has caused adverse effects upon administration such as hypersensitivity and nausea. To overcome such issues other research groups have shown that micellar systems constructed using poloxamers can solubilise and enhance the action of compounds like paclitaxel due to the P-glycoprotein (P-gp) inhibitory activity of the surfactant. Poloxamers form micelles, however they rapidly dissociate upon injection into a large volume; this is due to lowering the critical micelle concentration (cmc). It is possible to prevent micelle dissociation by means of internal cross-linking or coating. Currently, these two approaches are being investigated. One involves the creation of an *in situ* generation of an interpenetrating polymeric (poly N,N-diethylacrylamide) network within, and around, the poloxamer (P-105) micelles using a method adapted from Husseini et al (2002). The other method concerns the coating of micelles, which is the focus of the abstract.

Methods Micelles were constructed using poloxamer P105 in an aqueous medium, after which a hydrophobic dye Oil Red O (MW 408; aqueous solubility of ~ 0.1 mg/mL) was encapsulated into the micelle core as a proxy for hazardous chemotherapeutic compounds. The unincorporated dye was removed via filtration and polyacrylamide was added to the micelle stock solution at room temperature. Unbound polyacrylamide was re-moved via dialysis in formulations with excess poly-acrylamide.

Results Monodisperse micelles (polydispersity index (P.D.I) 0.1) were constructed with a particle size of 18 ± 2 nm, as determined by dynamic light scattering. Coating the micelles led to an increase in particle size to 22 ± 1 nm, again with a low P.D.I. Subsequently, dye loading also showed an increase in particle size of 20 ± 3 nm without coating. It was noted that after coating the formulation the average size of the micelle with the sequestered dye increased to 28 ± 2 nm in diameter with respect to the amount of polyacrylamide added. Release studies were carried out for both non-coated micelles (control) and stabilised coated micelles via dialysis. The exterior dialysis medium was analysed by a simple spectrophotometric assay. Over a period of 8 h the approximate dye release from the control system was 68%. In contrast, the stabilised coated system showed no dye release at the same time point. DSC thermal analysis has revealed that a blend composed of the poloxamer and polyacrylamide show significant binding interactions.

Conclusions In conclusion our data has shown that we have been able to quantify dye release from the two stabilised systems with a higher degree of stability expressed by the coated micelle system in comparison to the *in situ* polymer generation. On-going studies are testing the coated formulations with methotrexate and paclitaxel on breast cancer cell lines MCF7 and also MCF7-ADR, which over expresses the drug efflux pump called P-gp.

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The use of polycaprolactone (PCL)-polyethylene glycol (PEG) nanoparticles as targeted vaccine delivery systems

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Objectives To use biocompatible and biodegradable PCL polymeric nanoparticles with entrapped tetanus toxoid (TT) for the ultimate aim of developing a controlled release vaccine that requires only a single mucosal administration of nanoparticles. The attachment of mannose or galactose residues on nanoparticles could be utilised for targeting lectins on antigen presenting cells which in turn could improve specific antigen presentation by dendritic cells (DC) and the overall vaccine immunogenicity (Higashi et al 2002).

Methods PEG-galactose and PEG-mannose conjugates were synthesised by the reaction between m-PEG-NH_2 (5 kDa) and galactose or mannose isothiocyanate. This was confirmed by using both Fourier Transform (FT) ^{1}H NMR spectroscopy and mass spectrometry (MS). PCL (80 kDa) nanoparticles were prepared using a modified w/o/w solvent evaporation technique. 10% m/m of PEG-sugar conjugates was blended with PCL. PCL, PCL-PEG-galactose and PCL-PEG-mannose nanoparticles were administered through the intranasal (I.N.) route in mice. Serum samples were collected after 4 and 8 and 12 weeks and the immune response was detected using ELISA technique. Animal work was conducted with appropriate ethical approval.

Results Following I.N. administration, PCL-PEG-mannose and PCL-PEG-galactose induced TT-specific serum IgG antibody responses significantly higher than PCL-PEG and PCL nanoparticles (Figure 1). This could be, in part, due to the role of PEG in stabilising nanoencapsulated TT in the nasal mucosal fluids. Also, PEG improves the hydrophilicity and the mucoadhesive properties of the nanoparticles, and hence, their ability to cross the nasal epithelium with improved uptake by M-cells of the NALT, thus eliciting a high and long lasting immune response (Vila et al 2004).

Conclusions The use of PEG-sugar conjugates would provide an approach for targeting biodegradable nanoparticles to the macrophage mannose receptor and the macrophage lectin specific for galactose (MGL), which were found only in immature DC, thereby improving specific antigen presentation and the overall vaccine immunogenicity.

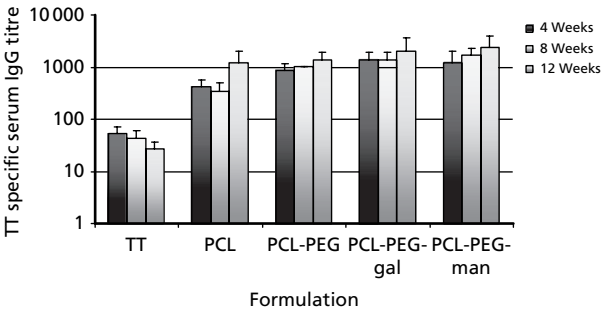

Figure 1 TT specific serum IgG antibody titres after 4, 8 and 12 weeks following I.N. administration of 5LF TT in polymeric PCL, PCL-PEG, PCL-PEG-gal, and PCL-PEG-man nanoparticles or as free TT (n = 3, mean ± s.d.).

Higashi, N., et al (2002) *J. Biol. Chem.* **277**: 20686–20693
Vila, A., et al (2004) *J. Controlled Release* **98**: 231–244

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Cellular immune response to surface-modified chitosan nanoparticles as DNA vaccine delivery systems

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Objectives Since the mucosal surfaces are the main entry site for hepatitis B virus (HBV), it is crucial to develop mucosal vaccination against HBV as the vaccines currently available produce only humoral immunity. The development of a DNA vaccine against HBV will provide the advantage of stimulating cellular immunity as the antigenic protein is produced intracellularly, with the advantage of fighting the intracellular virus and protecting the liver from its harmful effect (Jaganathan & Vyas 2006).

Methods Chitosan/DNA nanoparticles containing pCMV-S plasmid encoding the hepatitis B surface antigen (HBSA) were prepared by an ionic gelation procedure (Li et al 2004). To introduce the multivalent hydrophilic polymer coating, poly-N-(2-hydroxypropyl) methacrylamide (pHPMA) (35 kDa) (20 mg/mL in water) was added to the particles in 10 mM HEPES, pH 6.8, applying different pHPMA/chitosan molar ratios. Naked DNA, pHPMA-coated and uncoated chitosan/DNA nanoparticles were administered to mice via both S.C. and I.N. routes. Serum samples were collected after 2 and 6 and 10 weeks and the immune response was detected using ELISA technique. All animal work was undertaken with the appropriate approval.

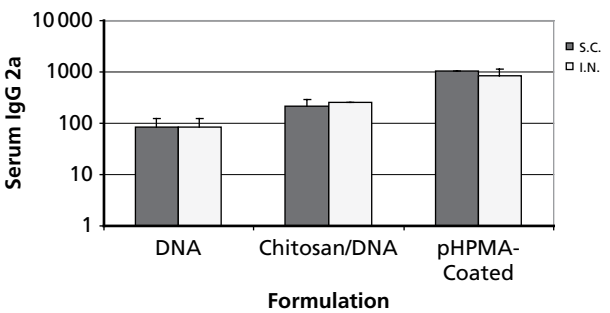

Figure 1 Hepatitis B surface antigen specific serum IgG2a antibody titres 2 weeks (week 10) following I.N. and S.C. administration of the final dose of 50 μg of pCMV-S plasmid encoding the HBSA.

Results The more hydrophilic pHPMA-coated particles were successful in inducing the production of T-dependant antibody response to the encoded HBSA following both S.C. and I.N. administration (Figure 1). The stealth like property gave the coated nanoparticles the advantage of their wide distribution after S.C. administration, so there would be a great chance for them to be encountered by dendritic cells. Following I.N. administration, this hydrophilic coat also provided the particles with both steric and lateral stabilisation, and hence, an improved stability in the nasal mucosal fluids, with improved overall uptake by the immune system.

Conclusions The findings of this study demonstrate that the hydrophilic polymer pHPMA can be efficiently used to stabilize chitosan particles for improved *in vivo* vaccine administration.

Jaganathan, K. S., Vyas S. P. (2006) *Vaccine* **24**: 4201–4211
Li, X. W., et al (2004) *Biochim. Biophys. Acta* **1630**: 7–18

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Enhanced transdermal delivery of aceclofenac using nanoemulsion technique

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Objectives Aceclofenac, a nonsteroidal anti-inflammatory drug (NSAID), has been recommended orally for the treatment of rheumatoid arthritis and osteoarthritis. The oral administration of aceclofenac causes gastrointestinal ulcers and gastrointestinal bleeding upon chronic use. Therefore the objective of the present study was to investigate the potential of nanoemulsion formulation for transdermal delivery of aceclofenac.

Methods The solubility of aceclofenac in various oils, surfactants and cosurfactants was determined by dissolving excess amount of aceclofenac in 2 mL of each of the selected oils, surfactants and cosurfactants in 5 mL capacity stoppered vials separately. Various o/w nanoemulsions were prepared by spontaneous emulsification method. Nanoemulsion area was identified by constructing pseudoternary phase diagrams. The prepared nanoemulsions were subjected to different thermodynamic stability tests like centrifugation, heating and cooling cycles and freeze thaw cycles. The nanoemulsion formulations which passed thermodynamic stability tests were characterized for viscosity, droplet size and transmission electron microscopy (TEM). Transdermal permeation of aceclofenac nanoemulsions through rat abdominal skin was determined by Franz diffusion cell. The optimized nanoemulsion was converted into nanoemulsion gel using 1% w/w Carbopol-940. The *in vitro* skin permeation profile of optimized nanoemulsion formulation was compared with conventional aceclofenac gel and nanoemulsion gel. The in-vivo anti-inflammatory studies were also performed on optimized nanoemulsion formulation and compared with conventional aceclofenac gel and nanoemulsion gel.

Results The higher solubility of the drug in the oil phase is important for the nanoemulsion to maintain the drug in solubilized form. The solubility of aceclofenac was found to be highest in 2:1 combination of Labrafil and Triacetin (48.95 ± 2.22 mg/mL) as compared with other oils, and combination of oils. Thus this combination was selected as the oil phase for the development of optimal formulation. Highest solubility of drug was seen in Tween 80 (398.21 ± 2.89 mg/mL) and Transcutol-P (292.42 ± 2.80 mg/mL). Therefore, Tween-80 and Transcutol-P were selected as surfactant and cosurfactant respectively for the phase study. Nanoemulsions were successfully prepared by spontaneous emulsification method. Pseudoternary phase diagrams were constructed for selection of nanoemulsion formulations. Maximum nanoemulsion region was found in the 2:1 ratio of surfactant to cosurfactant. Thermodynamic stability tests were performed on selected nanoemulsion formulations to remove metastable formulations. The droplets of nanoemulsion were found spherical using TEM. The mean droplet size of all nanoemulsion formulations was < 100 nm. The viscosity of nanoemulsion formulations was also lower. Significant increase in permeability parameters like steady state flux (J_{ss}), permeability coefficient (K_p) and enhancement ratio (E_r) was observed in optimized nanoemulsion formulation F1, which consisted of 2% w/w of aceclofenac, 10% w/w of Labrafil, 5% w/w of Triacetin, 35.33% w/w of Tween-80, 17.66% w/w of Transcutol-P and 32% w/w of distilled water. The anti-inflammatory effects of formulation F1 showed significant increase ($P < 0.05$) in % inhibition value after 24 h when compared with conventional aceclofenac gel and nanoemulsion gel on carrageenan-induced paw edema in rats.

Conclusion These results suggested that nanoemulsions are potential vehicles for improved transdermal delivery of aceclofenac.

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A high sensitivity calorimetric approach for the determination of steroid entrapment in nebulised liposomes generated from proliposomes

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Objectives Proliposomes are carbohydrate carrier particles coated with phospholipid (Payne et al 1986). The aerosolised liposomes were generated from proliposomes by hydration within a jet nebuliser (Elhissi & Taylor 2005) and a novel approach was developed for the detection of the entrapment of beclometasone dipropionate (BDP) in nebulised dimyristoylphosphatidylcholine (DMPC) liposomes using high sensitivity differential scanning calorimetry (DSC).

Methods Proliposomes having 1:5 w/w DMPC to carrier ratio were manufactured in a modified rotary evaporator using sucrose as carrier particles. Proliposomes (1.25 g) were placed in a Pari LC Plus air-jet nebuliser. Deionised water (5 mL, 40 °C) was added and nebulisation commenced to "dryness" in front of a twin impinger (TI). Aerosol output was determined gravimetrically and phospholipid output estimated colorimetrically. Size distribution of liposomes was analysed using laser diffraction. The contents in the TI and nebuliser were collected separately and centrifuged at 40 000 *g* and 4 °C. The supernatants were decanted and the liposome pellets redispersed in deionised water to comprise a DMPC concentration of 50 mg/mL for DSC. The onset temperature of the pretransition (T_{pre}) and main transition (T_m), and the enthalpy of the pretransition ($\triangle H_{pre}$) and main transition ($\triangle H_m$) of DMPC molecules were investigated using the Setaram Micro DSCIII.

Results Compared with the delivered liposomes, vesicles remaining in the nebuliser had a larger volume median diameter (VMD) (Table 1). High aerosol outputs were achieved for formulations with 0% and 5% BDP, being 79.58% and 75.34%, respectively. Phospholipid outputs were lower than aerosol output, being 61.62% and 49.41% for the 0% and 5% BDP formulations, respectively, indicating solvent evaporation and subsequent liposome concentration in the nebuliser. Steroid inclusion resulted in a slight reduction in the T_{pre} and T_m, a marked depression of the $\triangle H_{pre}$ and an elevation in the $\triangle H_{m.}$ (Table 1), indicating BDP entrapment in the liposome bilayers. For each formulation, no apparent difference was observed in the thermotropic properties between delivered and undelivered liposomes (Table 1). Using DSC, maximum incorporation of a material in liposome bilayers may be determined as the material concentration above which phase separation of the main transition occurs (Fildes & Oliver 1978). Inclusion of BDP did not completely remove the pretransition (Table 1) or cause phase separation of the main transition. This suggests that the maximum entrapment of this steroid has not been reached using 5% BDP.

Conclusions This study has demonstrated that high sensitivity DSC can be employed in the detection of steroid entrapment in the bilayers of nebulised liposomes.

Table 1 Thermal properties and size of liposomes (n = 2)

BDP Mol%	Pretransition		Main transition		VMD (μM)
	T_{pre} (°C)	$\triangle H_{pre}$ (KJ/mol)	T_m (°C)	$\triangle H_m$ (KJ/mol)	
0% (Nebuliser)	13.02	1.03	23.96	20.79	7.88
0% (TI)	12.99	0.91	23.99	20.54	5.12
5% (Nebuliser)	10.96	0.38	23.74	25.65	6.95
5% (TI)	10.78	0.59	23.65	25.07	4.30

Elhissi, A. M. A., Taylor, K. M. G. (2005) *J. Drug Deliv. Sci. Technol*. **15**: 261–265
Fildes, F. J. T., Oliver, J. E. (1978) *J. Pharm. Pharmacol*. **30**: 337–342
Payne, N. I., et al (1986) *J. Pharm. Sci*. **75**: 325–329

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Chain length modulation in symmetrical lipopolyamines and the effect on nanoparticle formulations for gene delivery

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Objectives Poly-nucleic acid delivery has many potential applications in medicine, especially for gene (DNA) therapy and also for small interfering RNA (siRNA) delivery. Our non-viral gene delivery systems are centred upon formulations of DNA complexed with synthetic cationic lipids, lipopolyamines. These vectors have the advantages of simplicity of use and ease of large-scale production.

Methods A first step in gene formulation is DNA condensation into nanoparticles, through masking the negative charges of the phosphate backbone by titration. Particle sizes were in the range 150–190 nm determined on a Nanosight Nanoscope. We are studying how lipopolyamines interact with circular plasmid DNA in order to produce nanometre-sized particles suitable for transfecting cells with high efficiency and low toxicity. These bio-nanoparticles are assessed by physico- and biochemical techniques (fluorescence quenching, gel shift, and DNase assays) target cell transfection (with and without serum) and toxicity (MTT assay).

Results and Conclusions The results for both the transfection efficiency and cytotoxicity show that for symmetric lipospermines as lipid chain length is reduced from C18 (N^4,N^9-dioleoyl spermine, Lipogen) to C10 (N^4,N^9-didecanoyl spermine) through C14 (N^4,N^9-dimyristoleoyl spermine and N^4,N^9-dimyristoyl spermine) and C12 (N^4,N^9-dilauroyl spermine) while transfection of HeLa derived cancer cell line HtTA (~67%), and the primary skin cell line FEK4 (~80%) remains constant, the formulations become toxic (cell viability drops from ~70% to ~10%) (Figure 1). The results based on both the DNase protection assay and transfection (pEGFP) efficiency for both primary and cancer cell lines show no significant difference between the presence and absence of serum in the medium indicating that our lipospermine compounds protect DNA from DNase enzyme.

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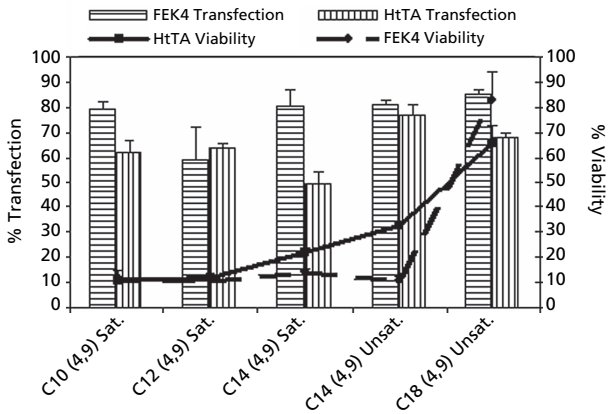

Figure 1 Chain length in lipospermine formulations versus transfection with pEGFP and viability of FEK4 and HtTA cells.

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Formulation and delivery of fluorescent siRNA by lipospermine nanoparticle complex formation

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Objectives RNA interference (RNAi), although only recently discovered, has already gained world-wide prominence as an important gene-silencing tool. The silencing "triggers" are long double-stranded RNA (dsRNA) molecules complementary to specific mRNA sequences. RNAi is now being exploited for a variety of laboratory applications in biology and as a promising therapeutic product. Any clinical use of small interfering RNA (siRNA) is hindered by significant problems, e.g. rapid enzymatic degradation, poor cellular uptake. These problems can be partly overcome by mixing siRNAs with lipopolyamines (synthetic cationic lipids) to form complexes (cf lipoplexes) (McLaggan et al 2006; Zhou et al 2006), which can also be surface-modified with specific ligands allowing tissue targeting. These vectors not only circumvent the drawbacks of viral vectors, but also they have the advantages of simplicity of use and ease of large-scale production. The first key step in siRNA formulation is RNA condensation into a nanoparticle form, through masking the negative charges of the dsRNA phosphate backbone. The formation of these bio-nanoparticles is important for high delivery efficiency. We are studying how lipopolyamines interact with RNA by using fluorescein tagged siRNA (Mirus Delivery Control).

Methods In this study, we synthesized and formulated novel non-liposomal lipospermines in which the tetra-amine spermine (the cationic moiety) and two fatty-acid chains (the lipophilic moiety) are linked by amide bonds at the secondary amino groups of spermine to form N^4,N^9-difatty acid spermine. The particle size determined on a NanoSight LM10 nanoparticle detection and analysis system. We typically measured 1–2 μg of RNA in a small sample volume (200 μL). The system gave video of the particle motion and particle sizing for each individual nanoparticle. 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 fluorescent siRNA analysed by Fluorescent Activated Cell Sorter (FACS) and cell viability by the MTT assay.

Results Our results show that the particle size for the formed nanoparticles is in the range 50–190 nm. FACS analysis showed that most of our lipospermines are effective transfecting agents (75–90%) in a tissue cultured skin fibroblast primary cell line (FEK4 cells) and in HtTA cancer cells, but with high toxicity except for N^4-oleoyl,N^9-stearoyl spermine which displayed both high cell viability ~80% and high transfection ~82%.

Conclusions N^4-oleoyl, N^9-stearoyl spermine shows promising results as a siRNA transfecting agent with high cell viability.

Acknowledgements We acknowledge the financial support of the Egyptian Government (fully-funded studentship to H.M.G.), the generous loan of a NanoSight LM10 nanoparticle detection and analysis system, and helpful discussions with Dr Alastair Hood, NanoSight. We are grateful to the EPSRC National Mass Spectrometry Service Centre, Swansea for rapid and efficient HRMS provision. We are also grateful to Prof. R. M. Tyrrell (University of Bath) for the FEK4 and HtTA cell lines.

McLaggan, D., et al (2006) *BMC Biotechnol*. **6**: 6 doi: 10.1186/1472–6750–6–6.
Zhou, J., et al (2006) *Chem. Commun*. 2362–2364

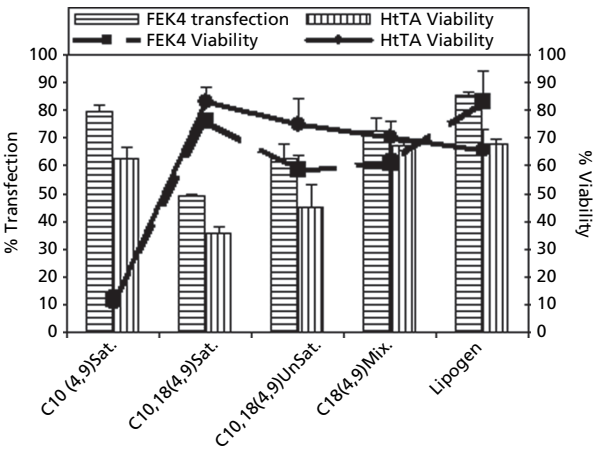

Figure 1 Lipofection and viability of FEK4 and HtTA cells transfected with pEGFP formulated with different C10 and C18 series

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Efficient novel unsymmetrical lipopolyamine formulations for gene delivery

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Objectives We have designed a series of novel unsymmetrical lipopolyamine formulations for non-viral gene delivery. These cationic lipids are based upon consideration of mammalian diglyceride esters where the two long lipid chains are not the same, incorporating different chain lengths and/or different oxidation states.

Methods Binding such acyl lipids to spermine affords novel unsymmetrical lipospermines as vectors that are designed to circumvent the problems associated with viral gene delivery systems, together with simplicity of use based upon DNA condensation by titration. These are not liposomal formulations, rather they form lipoplexes that efficiently transfect target cells.

Results and Conclusions Unsymmetrical lipopolyamines (N^4-decanoyl, N^9-oleoyl-), (N^4-decanoyl, N^9-stearoyl-), (N^4-oleoyl, N^9-stearoyl-), and (N^4-myristoleoyl, N^9-myristoyl-spermine) show promising transfection results in HeLa derived HtTA cancer cells and in skin fibroblast primary FEK4 cells with excellent cell survival ratios. There was no loss of potency on transfection in the presence of serum in either cell line; we conclude that the pEFGP DNA is effectively protected from DNase. In detail, transfection efficiency results and cytotoxicity results, for both unsymmetrical C10,C18 (N^4-decanoyl, N^9-oleoyl spermine) and its saturated (N^4-decanoyl, N^9-stearoyl spermine) analogue, show that while transfection of the HtTA cells (~40%) and the primary FEK4 skin cells (~60%) are both lower than symmetrical diC10 (N^4,N^9-didecanoyl spermine) (62% and 80%, respectively), the formulations are significantly less toxic (cell viability up from ~10% to ~75%). Unsymmetrical diC18 (N^4-oleoyl, N^9-stearoyl spermine) HRMS m/z, FAB$^+$ found 733.7283 Da, ($M^+ + 1$), $C_{46}H_{93}N_4O_2$ requires $M^+ + 1$ 733.7293), is much better in both transfection (~75%) and cell viability (~70%). Unsymmetrical diC14 (N^4-myristoleoyl, N^9-myristoyl spermine) also shows promising results in both transfection (~60%) and viability (~70%).

Acknowledgements We acknowledge the financial support of the Egyptian Government (fully-funded studentship to H.M.G.), the generous loan of a NanoSight LM10 nanoparticle detection and analysis system, and helpful discussions with Dr Alastair Hood, NanoSight. We are grateful to the EPSRC National Mass Spectrometry Service Centre, Swansea for rapid and efficient HRMS provision. We are also grateful to Prof. R. M. Tyrrell (University of Bath) for the FEK4 and HtTA cell lines.