Poster Session 2 – Drug Delivery

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Use of automated transepithelial resistance to measure cell monolayer integrity

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Caco-2 cell monolayers grown on permeable filter supports are widely used to predict drug absorption. The integrity of these monolayers must be assessed to ensure accuracy of the data and currently radiolabelled mannitol and manual transepithelial resistance measurements (TEER values) are routinely and extensively used. Automation of TEER measurements may offer a faster, cheaper and safer alternative to radiolabelled mannitol and manual TEER measurements.

The Automated Resistance Measurement System (REMS) from World Precision Instruments has been evaluated to establish whether it is a suitable alternative to radiolabelled mannitol for measuring cell monolayer integrity. TEER values have been compared with the permeability of mannitol through Caco-2 cells grown on Falcon 24 well plates. Studies have been undertaken to determine whether there is a correlation between the two techniques and whether a reproducible TEER value is obtained where loss of monolayer integrity is observed. Initially, TEER measurements were carried out at room temperature but subsequently, to minimize variability, a 37°C incubator was used to house the REMS system.

There was a good correlation between mannitol permeability and TEER values in a temperature-controlled environment at 37C. Currently, a mannitol permeability of $< 1 \times 10^{-6}$ cm s^{-1} is regarded as an intact monolayer. Linear regression analysis, with a non-parametric lowess smoothing line fitted, shows that an average resistance reading of approximately 800 ohms corresponds to a 1×10^{-6} cm s^{-1} Papp for mannitol.

Measurement of transepithelial resistance using the automated system is considered to be valuable in assessing Caco-2 monolayer integrity. The system is rapid and robust and has the added advantage of being able to predict cell integrity prior to conducting transport studies.

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Drug solubilisation using block copolymers of ethylene oxide and styrene oxide

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The commercial block copolymers currently available for use as solubilising agents in pharmaceutical formulations are propylene oxide (P)/ethylene oxide (E) triblock copolymers, which suffer the disadvantage of low solubilisation capacity when used for the micellar solubilisation of poorly soluble aromatic drugs. Replacement of the P block with an aromatic, highly hydrophobic styrene oxide (S) block should provide micelles having a more favourable domain for solubilisation of such drugs. In this investigation we examine the potential of styrene oxide/ethylene oxide di- and triblock copolymers to enhance the solubility of the poorly water-soluble hydrophobic drug griseofulvin.

The copolymers were synthesised by sequential anionic copolymerisation of ethylene oxide and styrene oxide using the methods described by Mai (2000). Characterisation by gel permeation chromatography and ^{13}C NMR gave the compositions shown in Table 1. Saturation solubilities of griseofulvin in 1% w/v aqueous solutions at 25°C are presented in Table 1, which shows the influence of the chain architecture, the hydrophobic block length and the copolymer composition on the solubilisation capacity of the block copolymer micelles.

Table 1 Solubilisation of griseofulvin

Diblock copolymers	mg (g coplymer)$^{-1}$
$S_{20}E_{65}$	16.6
$S_{17}E_{65}$	15.3
$S_{15}E_{65}$	15.2
$S_{13}E_{60}$	15.1
$E_{51}S_{6.5}$	5.9
$E_{26}B_{13}$	4.2
Triblock copolymers	
$E_{67}S_{15}E_{67}$	9.8
$E_{66}S_{13}E_{66}$	7.8
$E_{65}S_{11}E_{65}$	5.4

The styrene oxide copolymer $S_{13}E_{60}$ solubilizes four times more griseofulvin by weight than a butylene oxide copolymer of identical hydrophobic block length, $E_{26}B_{13}$, which is a consequence of the better environment for the griseofulvin molecule provided by the more hydrophobic, aromatic styrene oxide block. Table 1 shows that an important consideration is the length of the hydrophobic block in copolymers of similar block architecture: $S_{13}E_{60}$ solubilises 2.5 times more griseofulvin than the shorter hydrophobic block length copolymer $E_{51}S_{6.5}$ because of the significantly larger micelle formed by $S_{13}E_{60}$. The block architecture is also an important factor in the solubilization process as illustrated by the higher solubilisation capacities of the diblock copolymers compared with those of the triblock copolymers of similar composition. The block architecture dictates that the maximum possible diameter of spherical micelles formed from a linear diblock copolymer will be twice (or more) that of micelles formed from a triblock copolymer (Booth & Attwood 2000). The resultant larger core volume is responsible for the higher solubilisation capacity.

This study has highlighted the importance of three structural factors influencing the solubilisation of a hydrophobic drug by block copolymer micelles and provides a basis for the design of block copolymers with enhanced solubilisation capacity.

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Transfection of mammalian cells with chitosan nano particles prepared by an ionic gelation method

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Chitosan has attracted recent interest as a material for gene delivery because it is biodegradable, has a low toxicity and, being positively charged, is able to complex plasmid DNA (MacLaughlin et al 1998; Alonso et al 1999). In this work, chitosan was prepared into nanoparticles of 40–80nm by an ionic gelation technique using sodium tripolyphosphate as a cross-linker (Calvo et al 1997). Ionic gelated chitosan particles complexed DNA completely (determined by gel retardation) at a ratio of chitosan:DNA of 4:1 (w/w) and above. The plasmid pGL3-Control (Promega) encoding firefly luciferase was used to transfect HEK 293 cells. Chitosan particles containing 0.5–3μg of plasmid were added to each well (n = 3). After 48 h, cells were assayed using the Steady-Glo Luciferase Assay System (Promega) and relative light units (RLU) determined using luminescence spectroscopy. The RLU were normalized to protein concentrations in the cell extracts using the Bio-Rad protein assay. SuperFect (Qiagen) was used as a positive control. Chitosan particles complexed to a plasmid encoding β-gal (pCH110, Life Sciences) were used to investigate transfection efficiency in CHO-K1 cells. Following X-gal staining, transfected cell numbers were determined by standard counting procedures.

To evaluate the transfection process, a PCR technique was used. The total DNA of the cells was extracted and purified from the transfected cells. The extracted DNA was then reacted with primers for a fragment sequence in the Amp^r gene using the Ready-to-Go PCR Beads Kit (Amersham Pharmacia Biotech Inc.).

The experimental data indicated that the chitosan particle-pGL3 Control system gave a level of transfection in HEK 293 cells comparable with that of the commercially available transfection reagent (Table 1). The transfection efficiency increased with increasing amounts of DNA until the amount of DNA reached 1.5 μg. Chitosan particles also showed a more sustained expression in comparison to SuperFect with expression levels being maintained for 15 days whereas the expression level was gradually reduced following transfection with SuperFect.

Table 1 Transgene expression in HEK 293 cells as measured by the quantification of luciferase activity

	C	Chitosan nanoparticles					SF
DNA (μg)	0	0.5	1.0	1.5	2.0	2.5	1.0
RLU/mg protein (× 10000)	0.2	95	115	143	154	141	122
St. Dev.	0.01	8	16	74	25	10	87

Cells exposed to DNA alone showed negligible transfection not significantly different from control cells (C). SF = SuperFect

For the β-Gal gene transfection of CHO-K1 cells, chitosan prticles engendered increased transgene expression giving 65% transfection, whilst SuperFect gave a 47% transfection efficiency following transfection with 1 μg plasmid DNA in comparison with < 5% transfection observed in control cells. PCR experiments indicated that the sequence of the Amp^r gene in the reporter plasmid pGL3-Control could be determined only in the cells that showed effective expression.

Alonso, M. J., et al (1999) *EP* 0 860 166 A1
Calvo, P., et al (1997) *J. Appl. Polym. Sci.* **63**: 125–132
MacLaughlin, I. C., et al (1998) *J. Control. Release* **56**: 259–272

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Role of magnesium hydroxide on poly(L-lactide) degradation (ii) microscopy study

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The behaviour of the biodegradable polymers such as lactic/glycolic acid is very important in drug delivery systems (DDS). Biodegradable polymers can be categorized into two groups on the basis of the mechanism or process by which they degrade. These processes are bulk degradation and surface degradation. The lactic/glycolic acid polymers belong to the category of polymers that undergo bulk degradation, which isn't suitable in DDS. However, it is possible to control the degradation mechanism. Maulding et al (1986) mentioned that PLA contains pH sensitive linkage and their hydrolysis rate can be readily controlled by the incorporation of acidic, basic or buffering agent excipients in to the polymer matrix. Our aim is to investigate the effect of various concentrations of $Mg(OH)_2$, on controlling PLA degradation.

High-molecular-weight poly (L-lactide) (PLA) was synthesized via bulk polymerization of lactide using stannous octoate as catalyst. Film samples were prepared by solvent casting from a solution of the polymer in chloroform by adding 1, 3 and 5% $Mg(OH)_2$. Degradation studies were performed in distillated water at 37°C. After 3 and 6 months, surfaces and cross-sections of the films were monitored by SEM.

Table 1 shows that distribution of $Mg(OH)_2$ along the thickness of the films are not homogeneous probably due to bad interaction between the matrix and $Mg(OH)_2$.

Table 1 distribution of $Mg(OH)_2$ in PLA films

	1% Mg	3% Mg	5% Mg
Upper surface	0/67%	0/8%	0/92%
Lower surface	1/44%	3/62%	11/25%

The figures prove that the upper and lower surfaces of the reference (blank) PLA film are identical and the X-section is a homogenous bulky material. After 3 months of storing the blank sample under the degradation conditions, some crystallites have grown identically over the upper and lower surface of the film. One can also see some hollow spaces among the crystallites, probably due to degradation and solubilization of the amorphous section of the film. After 6 months of storage under degradation conditions, the size of both crystallites and the pores have increased. Some crystallites observed in the upper surfaces of PLA films with $Mg(OH)_2$ after 3 months degradation, but the lower surfaces were homogenous. X-Section study shows that $Mg(OH)_2$ controlled number and size of hollow spaces.

$Mg(OH)_2$ acts as buffering agent excipient in PLA polymer matrix, and it controls the pH polymer matrix. This method can be use to stabilize the interior of the PLA-based devices.

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Liposome incorporation of hydrophobic drugs: the effect of charged lipid surfactants

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Due to their unique structural characteristics, liposomes are able to deliver hydrophobic, amphiphilic and hydrophilic drugs (Swarbrick & Boylan 1994). In this respect, liposomes have been extensively exploited for delivery of several poorly soluble drugs (e.g. estradiol) with the advantage of the system being able to provide high entrapment efficiency and improved drug absorption and/or targeting (El Maghraby et al 2000). Despite this large body of work, the effect of lipid bilayer composition on liposomal delivery of hydrophobic drugs has received little attention. Here, we assess the influence of charged surfactant lipids on encapsulation and release of the poorly soluble drug ibuprofen.

Multilamellar vesicles (MLV) incorporating ibuprofen (2.5 mg) were prepared using egg phosphatidylcholine and cholesterol (PC:Chol; 16:4 μmol) by the hand shaking method (Bangham et al 1965). Stearylamine (SA) and dicetyl phosphate (DCP) were used to prepare cationic and anionic formulations, respectively. Drug loading in the liposomes was assessed by spectrophotometric analysis (221 nm) of the supernatant after liposome separation by centrifugation. Similarly, drug release from the various liposomal formulations was monitored over a 24-h period at 37°C in phosphate-buffered saline (pH 7.4; PBS). Liposomes were sized on a Malvern Mastersizer X at 20°C in ddH_2O and the corresponding zeta potential measured on a ZetaPlus (Brookhaven Instruments) in 0.001M PBS.

Ibuprofen incorporation efficiency in liposomes composed of PC:Chol was 39.0 ± 0.3% of the initial amount used. The addition of SA (2 μmol) to the PC:Chol liposome formulation significantly enhanced ($P < 0.001$) the encapsulation efficiency of ibuprofen to 47.2 ± 2.0%. This increased loading may be attributed to the electrostatic attraction between the positively charged head group in stearylamine and the carboxyl group in ibuprofen. Indeed substitution of stearylamine with dicetyl phosphate, an anionic lipid, resulted in reduced ibuprofen entrapment resulting in an incorporation efficiency (34.3 ± 0.3%) below that of the PC:Chol liposomes.

The presence of the charged lipids in the vesicle bilayer also influenced drug release: both the SA- and DCP-containing liposomes released more incorporated drug (71.2 ± 2.8% and 69.0 ± 3.7%, respectively) over 24 h compared with PC:Chol-only liposomes (41.4 ± 0.1%). This increased release rate may be due to repulsive forces between the charged lipids in the bilayer producing a less rigid structure that is less able to retain the incorporated hydrophobic drug.

Investigation of the physical characteristics of the liposomes revealed that the addition of 2 μmol SA to the formulation increased the liposome zeta potential (an indirect measure of surface charge) by 26.0 mV and the size by 0.78 μm when compared with the PC:Chol MLV's (5.34 ± 0.4 μm). In contrast, DCP did not significantly influence liposome size but did reduce the vesicles zeta potential by 18.4 mV, presumably due to the anionic nature of the DCP head-group. The increase in vesicle size noted with the cationic liposomes may be a result of the increased ibuprofen loading creating a more swollen bilayer structure rather than

due to repulsion between the charged SA head-groups since liposome size was unchanged when anionic DCP lipid was included in the formulation.

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El Maghraby, G. M., Williams, A. C., Barry, B. W. (2000) *Int. J. Pharmaceutics* **204**: 159–169
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The preparation of liposomes using supercritical fluid technology

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The vast majority of methods currently employed for producing liposomes utilise toxic organic solvents that are hazardous to health and harmful to the environment. The object of this work has, therefore, been to investigate methods for producing pharmaceutically relevant, drug-loaded liposomes using supercritical carbon dioxide ($scCO_2$) and small quantities of ethanol (5–10 mol %) in place of less desirable solvent systems.

Supercritical fluids are substances existing beyond their critical point, above which a vapour/liquid equilibrium cannot exist. The liquid-like densities of these fluids enable them to act as solvents, while gas-like viscosities and diffusivities facilitate high mass transfer and reaction rates.

The supercritical fluid used in these experiments was CO_2 as it possesses several advantageous characteristics. It has an easily attainable critical point (31.1C and 73.8 bar), enjoys GRAS status (generally regarded as safe), is environmentally acceptable and is relatively cheap.

The experimental rig built for the work was adapted from the design of Frederiksen et al (1997).

The first stage in the method development process was to quantitatively determine the solubility of various natural and synthetic phospholipids in $scCO_2$ at a range of pressures, temperatures and co-solvent (ethanol) concentrations. Methods for producing liposomes were then investigated that used $scCO_2$ as either a solvent, to dissolve liposomal components, or an antisolvent to promote rapid and uniform precipitation of phospholipids from saturated ethanolic solutions. The techniques allowed for the production of aqueous liposomal suspensions and finely divided, solvent free phospholipid powders for reconstitution.

Liposomal preparations loaded with both hydrophilic (ciprofloxacin HCl) and hydrophobic (hydrocortisone) model drugs have been successfully produced using all the investigated methods. Size analysis of the vesicles using photon correlation spectroscopy (PCS) and transmission electron microscopy showed that the liposomes in the aqueous suspensions had mean diameters between 100–200 nm. The polydispersity indices of these formulations, as measured using PCS, ranged from 0.2–0.4.

It has been demonstrated that drug-loaded liposomes with size distributions suitable for intravenous drug delivery can be produced with $scCO_2$ without the need for toxic organic solvents. It is also anticipated that the methods used in this work have greater potential for industrial scale-up than many existing techniques.

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Liposomes as oral drug delivery carriers: the effect of bilayer composition

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The oral route, although the most convenient approach for drug administration is, however, not always applicable for substances that are unstable or poorly absorbed through the gastro-intestinal tract. Liposomes have previously been shown (Gregoriadis 1985) to potentially circumvent these problems by both providing protection (e.g. from gastrointestinal enzymes and pH) and improving oral bioavailability of poorly soluble drugs after oral administration. Here, we assess the effect of liposomal bilayer composition on hydrophobic drug loading and retention in simulated gastro-intestinal fluids.

Methyl hydroxbenzoate (MP; 1 mg) was incorporated into multilamellar vesicles (MLV) (Bangham et al 1965) prepared from egg phosphatidylcholine (PC), or distearoyl phosphatidylcholine (DSPC) (16 μmol) and cholesterol (4 μmol). Liposomal drug release in the presence of simulated gastric fluid (SGF; 0.01 M HCl, made isotonic with NaCl) and fasted intestinal fluid (FaSSIF; 5mM sodium taurocholate, 3mM $CaCl_2$, phospholipase A_2, 5 U mL^{-1}) was monitored over 24 h at 37°C using a modified version of USP Apparatus 2; constant agitation was employed in replacement of paddles. The extent of initial liposomal drug loading and release was estimated based on spectrophotometric analysis (258 nm) of the liposome supernatant after separation via centrifugation.

Incorporation studies revealed no significant difference in MP incorporation between egg PC and DSPC liposomes. In contrast, even after 1 h in the presence of SGF, DSPC liposomes were able to retain significantly more ($p < 0.05$) of the incorporated drug compared with their egg PC counterparts ($99.1 \pm 0.11\%$ vs $96.5 \pm 1.35\%$, respectively). Similarly, in the presence of FaSSIF, $88.1 \pm 0.74\%$ of drug was retained in DSPC liposomes compared with $77.2 \pm 2.79\%$ in PC liposomes after 1 h. Continued monitoring of drug release in these model media for up to 24 h revealed no further increase in drug release suggesting that liposome destabilisation occurring in such media can be rapidly assessed. The use of high gel–liquid crystalline transition temperature lipids (e.g. DSPC) results in liposomes with more rigid bilayers due to the high transition temperature (56°C). The results above show that these rigid bilayers are advantageous in the harsh environment of the gastrointestinal-tract fluids, allowing significantly more drug retention compared to liposomes containing egg PC which has a transition temperature less than 37°C.

In other experiments, cell viability in the presence of the various liposome formulations was determined using the MTT (3[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. All liposomal compositions tested were shown to have no detrimental effect on cell viability (> 85%). Additionally, the effect of simulated gastrointestinal fluids on the cell models was also tested: in agreement with previously reported investigations (Ingels et al 2002), FaSSIF did not influence cell viability (not less than 100%), however, SGF dramatically reduced cell viability to less than 30% under similar conditions.

These results demonstrate the potential of liposomes as oral drug delivery systems for hydrophobic drugs by providing high drug retention and hence protection in the harsh gastrointestinal environment. Work continues, based on the initial toxicity profiles to investigate the effect of these carrier systems on uptake and transport of drugs across a Caco-2 cell monolayer (modelling intestinal absorption).

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Gregoriadis, G. (1985) *TIBS* **3**: 235–241
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A multi-disciplinary approach to the analysis of drug delivery systems

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The current trend to evermore complex drug delivery systems requires increasingly sophisticated analytical methods for comprehensive evaluation. In addition to the

conventional requirements of determining composition of components and their quantification, for drug delivery systems the spatial distribution of the various components is a key element in the final answer. Faced with these technical challenges, it is clear that no one technique is capable of elucidating the complete answer. The solution therefore is to approach the problem holistically, and a structured multidisciplinary approach will generate the most cost-effective answer. In this presentation the capabilities of a variety of techniques will be compared and the relevance to particular problems will be highlighted.

Here, multi-layered controlled release pellet composed of a core containing chloropheniramine encapsulated by three different coating polymers was used as an example to demonstrate the multi-disciplinary approach. Data from time-of-flight secondary ion mass spectroscopy (TOF-SIMS) imaging showed high spatial and chemical resolution required to understand the release profile of a complex multi-layered controlled release pellet. This data was supported by vibrational spectroscopy through the use of a Raman microprobe and infrared microspectroscopy, and the complementary nature of different spectroscopic methods were highlighted. Finally, the role of a range of emergent scanning probe microscopies were demonstrated. The complex microstructure of a controlled release polymer was examined using high resolution imaging via atomic force microscopy (AFM) as a function of ageing. The results showed that the morphology was time dependent and the impact on the polymers capability as a controlled release layer was assessed. Additionally, results from scanning thermal microscopy (SThM) illustrated the important role of localised thermal analysis.

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The use of a buccal hydrogel controlled release delivery system in the treatment of Sjögren's syndrome

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Sjögren's syndrome (SS) is a chronic systemic autoimmune exocrinopathy characterised by the loss of lacrimal and salivary gland function. Common symptoms of the condition include dry mouth (xerostomia) and dry eyes. Xerostomia is associated with difficulty in speaking, eating and swallowing, ulceration or soreness of the mouth and a high incidence of dental caries. It has been estimated that there are 2–4 million sufferers of SS in the USA (NIH Guide 1992). One treatment for SS is pilocarpine, a cholinergic parasympathomimetic drug with a predominantly muscarinic action. It is currently on the market as a 5-mg tablet (Salagen) to be taken three times a day. Pilocarpine stimulates salivary flow, thus reducing the symptoms of xerostomia. However, due to the systemic action of the drug, treatment is associated with unpleasant side effects including stomach cramping, sweating and lachrymation. The aim of this work was to produce a locally acting controlled release delivery system for pilocarpine, to reduce the incidence of side effects.

A hydrogel polymer system suitable for drug delivery to the oral mucosa has been developed. To optimise drug release from the hydrogel, polymer slices of varying thickness (0.6, 0.8 or 1.0 mm) were loaded with 5 mg of pilocarpine HCl by a solution diffusion method. Assessment of drug release characteristics from the slices was carried out by in-vitro dissolution in deionised water at 37°C (average of six slices) (Caleva 8ST apparatus, rotating basket method) and UV spectrophotometry (Philips PU8620 UV/Vis Spectrophotometer) (detection at 215 nm). The results are summarised in Table 1, and a trend is clearly visible whereby drug release is prolonged by increasing polymer slice thickness. Results up to one hour in the dissolution medium are shown; it would be expected that in–vivo dissolution would occur over a longer time period, due to the reduced quantities of saliva present in the oral mucosa.

Table 1 In-vitro and in-vivo drug release from hydrogel slices

Thickness (mm)	Drug release (%)					
	In-vitro (n = 6)			In-vivo (n = 4)		
	10 min	20 min	60 min	60 min	120 min	180 min
0.6	76.0	88.8	94.5	70.0	83.4	94.0
0.8	52.0	84.6	93.6	60.0	73.2	87.0
1.0	38.3	72.2	100.9	48.0	73.2	84.0

A preliminary in-vivo study was carried out in four patients using hydrogel slices of the same dose and thicknesses as used in the in-vitro study. Patients were instructed to keep each thickness of the buccal unit in-situ for 60, 120 or 180 min, and the residual drug content of each unit subsequent to dosing was determined by HPLC (Robertson et al 2001). Again, data are summarised in Table 1. The same trend is observed, whereby drug release is prolonged by increased polymer slice thickness. Preliminary clinical data indicate that buccal adminisatration of 5 mg pilocarpine HCl via the hydrogel is capable of stimulating salivary flow, with no incidence of side effects. Further patient data are being collected.

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Encapsulation of PEI/DNA complexes into PLGA microparticles for mucosal delivery of gene vaccines

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A number of non-viral gene transfection systems such as liposomes, polypeptides, polysaccharides and synthetic cationic polymers has been investigated. The cationic polymer polyethylenimine (PEI, HO-$(CH_2)_2$-$(CH_2$-CH_2-NH) n-$(CH_2)_2$-OH) interacts electrostatically with DNA to form PEI/DNA complexes (polyplexes) ranging from 30 to 100nm. The excess cationic charge permits cell entry via anionic proteoglycans found at cell surfaces (Mislick et al 1996). PEI exhibits endosomolytic properties and consequently gives high transfection efficiencies (Boussif et al 1995). These systems, therefore, show great potential for DNA vaccines (Howard & Alpar 2002). This work describes the development of polyplex-based oral vaccines. A requirement for oral immunisation is the protection of antigen from gastric breakdown. The approach taken in this work is the encapsulation of PEI/DNA complexes into polymeric microparticles composed of poly (lactide-co-glycolide) for DNA protection and delivery to sites responsible for the induction of mucosal immune responses.

PEI/DNA polyplexes were prepared at pH 7.4 at an N/P ratio of 5:1 using linear PEI (25 kDa) and pLG3-control plasmid DNA coding luciferase. Luciferase gene expresion (RLU (mg protein)$^{-1}$) was demonstrated in B16 F10 and A594 cell lines using these polyplexes. Salt-induced polyplex aggregation resulted in higher expression than non-aggregated polyplex measured. The sizes of PEI/DNA complexes were related to electrolyte concentrations in the preparation system. Additionally, particle size was affected by the sequnce of addition of PEI to DNA. The PEI/DNA polyplex was then encapsulated into PLGA (50:50 14 kDa) microparticles using a modified solvent evaporation technique. Studies on extracted polyplex from the PLGA microparticles demonstrated that DNA was still associated with the PEI by the requirement of poly (aspartic acid) (PAA) for DNA displacement from the PEI (Table 1). In-vitro release studies further demonstrated the controlled release of intact polyplex from the microparticulate system.

Table 1 The comparison of DNA amount in different samples Determined by the Picogreen method

Sample	PEI/DNA ($20\,\mu g\,mL^{-1}$)	Extract samples from PEI/DNA encapsulated PLGA particles		Negative controls	
		Without PAA	Added PAA	PAA	H_2O
DNA ($ng\,mL^{-1}$)	—	—	489	—	—

PEI/DNA polyplexes were stabilised during the microencapsulation process by conjugation of varying amounts of poly (ethylene glycol). PEGylation resulted in lower in-vitro transfection levels of the non-encapsulated polyplex when the ratio of PEG/PEI exceeded 5/1. In-vivo experiments, however, showed significant levels of gene expression in Peyer's patches, spleen and liver in mice, orally administrated with microencapsulated polyplex with low and high amounts of PEG (PEG/PEI = 1/1 w/w and 60/1 w/w).

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Howard, K. A., Alpar, H. O. (2002) *J. Drug Targeting* **10**: 143–151
Mislick, K. A., et al (1996) *Proc. Natl Acad. Sci. USA* **93**: 12349–12354

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Incorporation of 1-alkylcarbonyloxymethyl (ACOM) prodrugs of 5-fluorouracil into nanoparticulate drug delivery systems

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In the formulation of any drug delivery system, there are problems which are peculiar to that type of system, with nanoparticulate systems being no exception. Aside from difficulties in the analysis and characterisation of such systems, nanoparticulate drug delivery poses particular problems when dealing with water-soluble drugs. Most methods of nanoparticle manufacture involve the use of organic solvents to dissolve preformed polymers or the use of hydrophobic monomers, each of which lead to poor loading of hydrophilic drugs. One such drug is 5-fluorouracil (5-FU), which is freely soluble in water and hence poorly loaded in nanoparticulate formulations. To increase the loading of 5-FU, ACOM prodrugs, with an increasing lipophilicity as R-group chain length increased, were used. These prodrugs have been subject to investigation (Taylor & Sloan 1998) to enhance transdermal drug delivery of 5-FU.

Briefly, 100 mg of polylactide-co-glycolide (PLGA) was dissolved in 1 mL of acetonitrile and 5 mg of prodrug added. Deionised water (15 mL) was preloaded with 4 mL of acetonitrile and the polymer/prodrug solution added drop-wise with stirring. The suspension was stirred for 12 h to allow evaporation of the solvent and precipitation of the polymer to occur. Particles were then centrifuged at 26 000 rev min^{-1} for 10 min and the pellet and supernatant separated. After treatment with sodium hydroxide, to hydrolyse the prodrugs back to 5-FU, analysis of the supernatant and pellet was performed by HPLC using a 90:10 acetate buffer to methanol ratio. 5-FU was detected using UV absorbance at 266 nm, giving a typical retention time of 5 min. Sizing and zeta potential measurements were performed by taking one drop of the final suspension of particles and diluting with 50 mL deionised water.

Six prodrugs in the series were analysed (ranging from C_1 to C_9) with drug loading efficiency increasing with chain length and hydrophobicity. However, the loading of the C_9 pro-drug was found to be less than that of the C_7 due to a reduced lipophilicity, in spite of the reduced hydrophilicity of the compound. It was therefore postulated that a combination of lipophilicity and hydrophobicity were involved in the loading of the nanoparticles, with the C_7 prodrug having the best profile in this respect.

Drug loading efficiency of the pellet was found to range from 8% with the C_1 prodrug to over 68% with the C_7 prodrug. This compares favourably with other studies performed with 5-FU, in which loading efficiency has been around 4% (McCarron et al 2000). The average size of the particles was found to be 220 nm irrespective of prodrug used. Zeta potentials were approximately –10 mV, again with little inter-batch variation.

In conclusion, the use of ACOM prodrugs of 5-FU significantly increase the loading efficiency of nanoparticulate drug delivery systems when compared to that achieved using 5-FU. Loading of prodrugs is seen to increase with lipophilicity and reduced hydrophilicity, with the C_7 prodrug having the highest incorporation. Hence, these prodrugs show significant advantages over existing approaches employing nanoparticulate systems for the delivery of 5-FU.

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Synthesis of polyamidoamine (pamam) dendrimer conjugates for use in targeted drug delivery

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Dendrimers are well-defined, hyper-branched, globular macromolecules constructed around a core unit (Tomalia 1995). Due to their unique structural characteristics, dendrimers have potential as vehicles for drug delivery, to which targeting and chemotherapeutic moieties may be conjugated (Baker et al 2001). In the system currently under investigation, it is intended to link the single chain antibody fragment (ScFv) MFE-23 (MW ~27kDa) to a polyethylene glycol (PEG) modified G4 PAMAM dendrimer with the aim of targeting the tumour-specific carcinoembryonic antigen (CEA). The present work describes the addition of a linker molecule suitable for subsequent attachment of the antibody fragment; the protection of the amine groups of the dendrimer by the addition of a t-boc protecting group to ensure a single-point antibody attachment; and the addition of PEG chains to enhance the bioavailability of the system as well as to reduce conjugate immunogenicity and toxicity.

The linker molecule selected for attachment to the amine-terminated G4 PAMAM dendrimer (Dendritech) was α-bromo-*p*-toluic acid (BrTA), the carboxyl end of which could be subsequently activated for the attachment of antibody to the dendrimer. Linker addition was achieved by adding BrTA and dendrimer to methanol in a 1:4 ratio, and stirring at room temperature (RT) for 3 h. PEGylation of this modified dendrimer was achieved by adding freshly prepared tresylated monomethoxyPEG (TMPEG) to an aqueous solution of the modified dendrimer in a ratio of 4:1 at pH 8 (adjusted with dilute HCl), and stirring at RT for 4 h. The PEGylated dendrimer was isolated, resuspended in methanol, and di-*tert*-butyl dicarbonate (used to deliver the *t*-boc protecting group) was added in excess and left stirring overnight at RT.

^{1}H NMR analysis showed the presence of an average of 3.5 BrTA linker molecules per dendrimer and the protection of the NH_2 terminals. Both ^{1}H NMR and a trinitrophenylbenzenesulfonic acid assay confirmed that 98% of the amines were protected. The protecting group could be cleaved under relatively mild conditions (10 v-% TFA). ^{1}H NMR analysis indicated the presence of an average of 4 PEG molecules per dendrimer.

Subsequent attachment of the MFE-23 antibody fragment to this modified dendrimer will enable targeting of a drug payload to cancer cells possessing the CEA antigen. Removal of the t-boc protecting groups will confer a positively charged surface with the advantage of increased tumour permeability due to increased interaction with the negatively charged cell walls (Dellian et al 2000).

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Dellian, M., et al (2000) *Br. J. Cancer* **82**: 1513–1518
Tomalia, D. A. (1995) *Sci. Am.* **272**: 42–46

128
Photolabile protecting groups for acidic and basic drug release

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The design and synthesis of benzoin derivatives has been investigated in recent years in an attempt to create new photolabile protecting groups in synthesis and combinatorial chemistry (Baldwin et al 1990). This study extends these applications to provide a novel technique for drug release by photochemically releasing a model drug with either a carboxylic acid functionality from a benzoin ester, or an amine from a benzoin carbamate.

The efficiency of the photocyclisation process of the benzoin derivatives, with concomitant acid/base liberation, is highly dependent on the nature of the substituents on the phenyl rings. When a 3,5-dimethoxybenzoin system is employed, maximum quantum yield of the photoremovable protecting group is achieved (Cameron et al 1997). The process is further advantageous in that the only by-product of the reaction is the potentially biologically benign 2-phenyl-5,7-dimethoxybenzofuran (Pirrung & Shuey 1994).

Esters of candidate model acidic compounds and drugs were prepared by reaction of the parent benzoin (developed from cognate preparations by Corrie & Trentham (1992) and Sheehan et al (1971)) with the appropriate carboxylic acid or acyl chloride. Benzoin carbamates were prepared by treating 3,5-dimethoxybenzoin with *p*-nitrophenyl chloroformate to form an activated mixed carbonate followed by reaction with the free amine. Due to complicated cyclisation reactions upon irradiation when primary amines are employed this strategy was restricted to the use of secondary amines whereby the photolysis is clean (Papageorgiou & Corrie 1997). Several benzoin esters have been synthesised and subsequent photolysis reactions carried out. The identities of the 3,5-dimethoxybenzoin esters, carbamates and photolysis products were confirmed using ^{1}H NMR,^{13}C NMR, infrared spectroscopy, mass spectrometry, and, where possible, single X-ray crystallography.

Complete liberation of drug from the synthesised conjugate was achieved by means of photolysis of the appropriate solution with UV irradiation (15 W, 365 nm, 5 cm). The photolysis products were confirmed by preparative-scale thin layer chromatography and subsequent full characterisation to be only the expected free drug and benzofuran.

The synthesis and characterisation presented here provide an important foundation in the evaluation of the release of model drugs from photoactive drug delivery systems and demonstrate that different drug functional groups can be exploited to give photolabile precursors which can be incorporated into polymer scaffolds for triggered release applications.

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Cameron, J. F., et al (1997) *J. Chem. Soc. Perkin Trans.* **1**: 2429–2442
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Sheehan, J. C., et al (1971) *J. Am. Chem. Soc.* **93**: 7222–7228

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Confocal imaging and drug release from novel sequential interpenetrating networks

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Poly(2-hydroxyethyl methacrylate) (pHEMA) has been used extensively in the synthesis of biomaterials due to its excellent biocompatibility (Eschbach & Huang 1994). A major disadvantage of pHEMA hydrogels, however, is their poor mechanical strength. One approach to overcome this is the formation of interpenetrating networks containing both hydrophilic and hydrophobic components (Peppas et al 1995).

This study involved the synthesis of novel sequential interpenetrating networks (SIPNs) comprising 10% of the poly(ε-caprolactone) (pCL) (w/w) and 88% HEMA, methacrylic acid (MA) (w/w), or HEMA plus MA. They were synthesised via free radical polymerisation with 1% (w/w) benzoyl peroxide initiator and 1% (w/w) ethylene glycol dimethacrylate crosslinker using a modification of the method previously described by Eschbach & Huang (1994). pCL and MA were employed because pCL is a non toxic, hydrophobic, biodegradable, aliphatic polyester which is compatible with various polymer blends and has been shown to enhance the mechanical strength of pHEMA hydrogels (Eschbach & Huang, 1994) and its degradation may be enhanced by the presence of intra-network acidic residues of MA.

The physical nature of the SIPNs was investigated using confocal laser-scanning microscopy (CLSM). The polymers were stained with a dye mixture comprising 0.1% (w/v) hydrophilic acridine orange and 0.02% (w/v) hydrophobic rhodamine B base. Acridine orange exclusively stained the hydrophilic pHEMA/pMA regions in the network whilst the slightly water soluble rhodamine B base stained the pCL regions preferentially. A further advantage of this choice of dual stain is that emission from each dye can be recorded independently. This is possible as each dye can be excited by a laser line at whose wavelength the other dye does not absorb. No significant energy transfer between chromophores was observed and emission was detected selectively for each dye using the spectrophotometric detector of the CLSM as the emission maxima of the dyes are well separated. Using line-by-line sequential scanning, images were obtained which allow the macro domains of both amorphous and crystalline pCL to be visualised along with the interpenetrating channels of the pHEMA/pMA. This gives insight into the growth of the pHEMA/MA copolymer within pCL to give the SIPN and the nature of the final material.

The second part of this study involved incorporating a drug into the polymer matrix during the polymerisation process. A maximum loading of 10% (w/w) metronidazole was achieved and the subsequent drug release into pH 7.4 tris buffer solution was monitored by UV spectroscopy. The results showed that the addition of pCL in the SIPN slowed the drug release rate compared to control materials without pCL while the presence of pMA in the polymer accelerated metronidazole release rate with respect to pHEMA.

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Peppas, N. A., et al (1995) *React. Pol.* **25**: 127–137

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Confocal laser scanning microscopy as a probe of photochromism in responsive hydrogels

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Spiropyrans are molecules that exhibit photochromism, a phenomenon based upon a reversible change between a colourless form (spiropyran) and a coloured form (merocyanine) with the colour change being induced in at least one direction by light of an appropriate wavelength (Feringa et al 1993). Conversion to the merocyanine form is also accompanied by a separation of charges and a large change in dipole moment. If appropriately incorporated into a polymer network, the charge generation which occurs upon merocyanine formation can therefore exert an influence on that network through changes in osmotic potential and this feature can be exploited for the purposes of controlled drug delivery as externally-regulated swelling/deswelling can be effected.

Novel vinyl-functionalized spiropyran molecules were copolymerised with 2-hydroxyethyl methacrylate (HEMA), leading to the formation of a photoresponsive polymer with the properties of the copolymer dependant upon the form in which the photochromic molecule exists.

In addition to being photochromic, these molecules have also been found to be photofluorochromic (i.e. they exhibit photoinduced fluorescence (Barachevsky 2000)). In this study, confocal laser scanning microscopy (CLSM) has been utilized

as an in-situ probe to quantitate conformational changes within a novel spiropyran hydrogel. The photoinduced emissions that accompany photochromic transformations of the spiropyran system allow the simultaneous investigation of the aggregation and photochromism behaviour of these systems to be investigated by CLSM. Equilibrium-swollen copolymer segments of various compositions have been studied in order to visualise spiropyran concentration effects on the appearance of the copolymer.

In one such copolymer, with a concentration of 1% spiropyran:pHEMA (w/w), aggregation does not occur. Upon increasing the spiropyran content to 2%, some indications of aggregation become noticeable with the phenomenon becoming much more pronounced upon increasing the concentration to 4%.

A UV light source focussed through the objective of a CLSM was used to selectively irradiate a small section of each copolymer for controlled periods of time. Following irradiation, the levels of fluorescence intensity from both irradiated and non-irradiated sections of polymer were obtained as a function of wavelength using the spectrophotometric detector of the CLSM, recording fluorescence within a 5-nm window. Data was collected as a series of wavelength-dependent images between 600 and 700 nm, in which range the merocyanine form fluoresces. This enabled ratiometric quantitation of the degree of photoisomerisation that can be correlated with the swelling behaviour of the hydrogel upon irradiation.

From the collected data, plots of maximum fluorescence intensity against time clearly illustrated the increase in fluorescence intensity as a function of duration of UV irradiation within the irradiated zone and conversion of the spiropyran component to the merocyanine form. In contrast, outside the irradiated zone, fluorescence intensity stayed comparatively constant showing that UV light is responsible for the observed effect.

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Feringa, B. L et al (1993) *Tetrahedron* **49**: 8267–8305

131
Positively charged mucoadhesive PLA microspheres as carriers for nasally delivered diphtheria toxoid

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Surface-active agents have previously been used to modify the surface characteristics of, and immune response to, antigen-loaded oil-in-water (o/w) emulsions. The importance of surface characteristics of polymeric colloidal carriers for their adjuvant activity has been highlighted for the nasal route (Alpar et al 1994). The use of chitosan for mucoadhesive, gene and peptide drug administration via mucosal routes has been explored by a number of researchers (Singla & Chawla 2001). In this study, the formation of chitosan-coated microparticles was achieved by a single step procedure that involved the incorporation of the positively charged polysaccharide in the microparticle formulation medium.

A solution of diphtheria toxoid was emulsified with a 5% (w/v) solution of PLA kDa (10 mL) in dichloromethane. The water-in-oil (w/o) emulsion was then emulsified with a 0.5% (w/v) chitosan solution or 1.5% w/v PVA 13–23 kDa producing a water-in-oil-in-water (w/o/w) emulsion. This was stirred overnight to allow the evaporation of the organic solvent. Microparticles were then harvested by centrifugation and freeze-dried. BALB/c mice received two doses of 10 Lf units intranasally and serum DT specific antibody titres were measured using ELISA.

Table 1 Loading, size and surface charge characteristics of DT-loaded microparticles prepared with the use of either PVA or chitosan as the stabilizing agent

Stabilizing agent	DT loading (± s.d.)	Particle size volume (μm) mean ± s.d.	Surface charge (ζ) potential (±s.d.)
Chitosan (0.75% w/v internal, 0.5% w/v external)	9.47 ± 2.2	5.06 ± 2.06	28 ± 1.6
PVA (2.5% w/v internal, 1.5% w/v external)	10.34 ± 1.4	5.18 ± 2.42	−23 ± 1.6

Comparable antigen loading and size characteristics were achieved for both the PVA and chitosan emulsified formulations (Table 1). Microencapsulated antigen was markedly more effective than free antigen in effecting antibody responses. The use of chitosan further increased the observed immune response in comparison with the PVA emulsified formulation. Additionally, the magnitude of the response was increased for the duration of the experiment. Antigen-specific antibody titres following dosing with the chitosan formulation (> 10 000) were on average twice that of the PVA formulation (n = 5), which was in turn an order of magnitude greater than the titres observed following administration of free antigen.

Chitosan may be able to activate components of the non-specific immune system such as macrophages, and may also have absorption enhancing properties, which have also been correlated with enhanced immunogenicity (Alpar et al 2001). While the exact mechanism of action of chitosan warrants further investigation, we conclude that the incorporation of chitosan into microparticulate formulations in the method outlined here offers considerable potential for the intranasal delivery of proteins for the purposes of vaccination.

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Alpar, H. O., et al (2001) *Adv. Drug Deliv. Rev.* **51**: 173–201
Singla, A. K., Chawla, M. (2001) *J. Pharm. Pharmacol.* **53**: 1047–1067

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LEAPT — Lectin-directed enzyme activated prodrug therapy

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The ability to site-selectively deliver pharmaceuticals via a tuneable mechanism is highly desirable on grounds of reduced toxicity, enhanced efficiency and interchangeable-target potential. Carbohydrates are recognised as having profound importance in the body, and many specific carbohydrate-based ligand-receptor mechanisms have been documented. These may be valuably exploited in drug delivery.

Here we describe a novel approach to drug delivery involving the administration of carbohydrate–enzyme conjugates to specific cell-types within the body, followed by a prodrug activated only by that pre-delivered enzyme at the desired site of action.

Galactose and mannose moieties, including a novel dendrimeric glycosylation reagent, have been conjugated to an α-rhamnosidase purified from naringinase (*Penicillium decumbens*), allowing exploitation of the asialoglycoprotein receptor (ASGPR) and mannose binding protein (MBP) in-vivo. Gamma scintigraphy (for selected results see Table 1), microautoradiography and confocal microscopy have been used to determine delivery and drug activation on whole body, organ and cellular levels. Competition experiments using alternative receptor ligands illustrate the specificity of delivery.

Table 1 Qualitative analysis of gamma scintigraphy

Enzyme construct	Target	Blocker?	Δ
MonoGal	ASGPR	$\overset{X}{\surd}$ - AF	35%
diGal	ASGPR	$\overset{X}{\surd}$ - AF	50%

AF = asialofetuin

Novel prodrugs with rhamnose caps and collapsible linkers have been designed and synthesised and the kinetics of release investigated. The structured design approach readily allows the substitution of a wide range of active drug molecules to the systems, further enhancing the range of disease states suitable for treatment by this system.

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Novel biocompatible block copolymers for drug delivery

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Micelle-based drug delivery systems have previously been developed based around the polymers poly(propylene oxide), poly(ethylene oxide), and the multiblock copolymer Poloxomers. However, there is growing evidence that Poloxomer-based systems can elicit an immune response that results in macrophage activation and subsequent scavenging of the Poloxomer micelles (Moghimi & Hunter 2001). Phosphorylcholine (PC)-based copolymers demonstrate biocompatible properties that make them ideal candidates for further development as drug delivery systems (Lewis 2000). Recent developments in atom transfer radical polymerization (ATRP) has provided a viable route for the synthesis of a wide range of well-defined block copolymers with low polydispersities (Lobb et al 2001). The aim of this work has been to investigate the potential for these systems to form polymeric micelles for drug delivery applications.

pH-Sensitive block copolymers of 2-methacryloyloxyethyl phosphorylcholine (MPC) and 2-(diethylamino)ethyl methacrylate (DEA) were prepared by atom transfer radical polymerisation (ATRP) in alcoholic media (Lobb et al 2001). By varying the amounts of MPC and DEA used during synthesis, a number of differing MPC to DEA block ratios were produced (Table 1). The MPC-DEA block copolymers were dissolved in McIlvaines buffer at a concentration of 1 mM and at pH 4. The pH was then adjusted with NaOH to pH 8, and 10.8. These samples were then serially diluted. The hydrophobic dye Orange OT (0.1% w/v in ethanol) was coated into the wells of a 96-well assay plate. The polymer solutions were applied to individual coated wells of the assay plate, and incubated for 18 h at 37°C. The samples were then transferred to fresh uncoated 96-well plates, and the absorbance measured at 492 nm. The readings obtained were converted to percentage solubilisation of Orange OT, as an indication of drug loading potential. Miscellisation was monitored using photon correlation spectroscopy (PCS).

Table 1 Solubilisation of drug analogue

Sample (1 mM)	Mean % (n = 6) solubilisation	± s.e.m.
MPC20-DEA20 (pH 4)	0.40	0.02
MPC20-DEA20 (pH 8)	15.02	0.15
MPC20-DEA20 (pH 10.8)	26.87	1.40
MPC30-DEA30 (pH 4)	0.40	0.01
MPC30-DEA30 (pH 8)	10.46	0.10
MPC30-DEA30 (pH 10.8)	28.17	0.39
MPC30-DEA60 (pH 4)	0.41	0.02
MPC30-DEA60 (pH 8)	33.60	0.34
MPC30-DEA60 (pH 10.8)	62.76	0.99

The MPC-DEA micelle systems were shown to entrap the hydrophobic drug analogue (Table 1). The amount of entrapped drug was shown to be tunable by pH and DEA block length. These initial studies have demonstrated proof of principle and indicate that MPC-DEA polymeric micelles systems have the potential to act as carriers for hydrophobic drugs.

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Moghimi, S. M., Hunter, A. C. (2001) *Crit. Rev. Ther. Drug Carrier Syst.* **18**: 527–550

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Mechanisms involved in drug release from silicone matrix intravaginal rings containing water-soluble excipients

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The release of actives from conventional intravaginal rings (IVRs) is based solely on solubility and diffusivity of the drugs within the silicone elastomer Chien (1982). For more hydrophilic drugs with poor release characteristics, a number of release enhancement strategies are available, the most common of which is the incorporation of hydrophilic excipients into the silicone elastomer.

Two possible mechanisms have been proposed to explain the enhanced release on incorporation of hydrophilic excipients: firstly, the enhanced surface area model — when the IVR comes into contact with the aqueous medium, the water-soluble excipient at the surface swells then dissolves out into the surrounding aqueous medium and increases surface area of the IVR thereby enhancing release; and secondly, the aqueous pathway model — when the IVR comes into contact with the aqueous medium, the excipient at the surface swells and causes pores to form within the silicone elastomer allowing aqueous medium to ingress further into the elastomer, further excipient swelling and pore formation takes place within the matrix thus enhancing release.

In this study a range of analytical techniques, including scanning electron microscopy (SEM), texture analysis and thermal gravitational analysis (TGA), have been employed to elucidate which mechanism predominates in a number of model silicone+excipient systems. D-Glucose and hydroxyethylcellulose were examined at different concentrations and particle size ranges.

TGA was performed on sections taken from D-glucose-loaded dry IVRs and IVRs which had been wetted in aqueous medium for one week. Samples were taken at different distances from the surface of the IVRs and heated up to 150°C at a rate of 10°C per minute and the percentage mass loss measured. A significant percentage mass loss difference was observed between wetted and dry D-glucose loaded IVRs. The dry IVRs showed negligible percentage mass loss and wetted IVRs showed significant percentage mass loss. Percentage mass loss of wetted IVRs was greatest at the surface and decreasing going towards the centre of the IVR. Wetted IVRs containing higher concentrations of excipient demonstrated increased percentage mass loss over IVRs containing less excipient.

SEM was performed on excipient loaded strips and IVRs. Differences in surface appearance of strips occurred after release into aqueous medium. SEM of cross-sections of IVRs revealed differences in water-soluble excipient appearance after release. In D-glucose-loaded rings, the excipient nearest the IVR surface appeared to have dissolved out into the aqueous medium, while in the centre of the IVR D-glucose was still present

The results suggest that the aqueous pathway mechanism of enhanced release predominates.

Chien, Y. K. (1982) *Novel drug delivery systems*. Marcel Dekker, pp 465–573

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Enhancement of drug release from silicone intravaginal rings by incorporation of water-soluble excipients

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Intra-vaginal rings (IVRs) are elastomeric drug delivery devices specifically designed to release substances in a controlled and sustained manner to the vagina, for local or systemic effect. IVRs have been already been commercialised for contraceptive and hormone replacement therapies, with other applications currently being investigated, including treatment of urinary urge incontinence and the

delivery of anti-HIV microbicides (Woolfson et al 2000; Malcolm & Woolfson 2001). The release of drugs from conventional matrix and reservoir-type IVRs is now well understood — the major factors influencing release are the silicone solubility and silicone diffusivity of the permeating molecule (Chien 1982). However, the hydrophobic nature of silicone limits the type of drug molecule that can be released from these systems. One strategy for enhancing release of more hydrophilic molecules is the addition of water-soluble excipients to the silicone IVRs. The aim of this study was to investigate the possibility of enhancing drug release from matrix silicone IVRs by incorporation of the water-soluble excipients hydroxyethylcellulose (HEC) and D-glucose.

The influence of excipient particle size and concentration were investigated in the study. Table 1 summarises the 14-day cumulative release of metronidazole (MET) from MET-loaded (250 mg) IVRs containing various concentrations of HEC. A 75% increase in the amount of MET is observed for the 10% HEC IVR compared with that containing no HEC.

Table 1 Influence of HEC concentration on MET release

HEC concn	Cumulative MET release (day 14)
0%	29.31 ± 0.33 mg
1%	34.83 ± 0.29 mg
5%	40.01 ± 0.32 mg
10%	51.52 ± 0.59 mg

The influence of HEC particle size (constant 5% HEC concn) is presented in Table 2. Clearly, decreased particle size enhances release by up to 30%.

Table 2 Influence of HEC particle size on MET release

HEC particle size	Cumulative MET release (day 14)
No HEC	29.31 ± 0.33 mg
180–500 μm	34.37 ± 0.36 mg
90–180 μm	38.04 ± 1.97 mg
1–90 μm	38.58 ± 2.27 mg

The above results illustrate the significant release enhancement possible with the incorporation of water-soluble excipients.

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Tetraoleyloxysilane: a novel silicone crosslinker for the preparation of biomimetic silicone elastomers

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Due to their excellent versatility and biocompatibility, crosslinked silicone elastomers are widely used in the manufacture of medical devices. (Arkles 1983). The RTV-2 type silicones are manufactured by crosslinking linear, hydroxyl-terminated polydimethylsiloxane chains with a tri- or tetra- functional crosslinking agent, usually tetrapropoxysilane (TPOS). The condensation-type curing reaction produces propanol as a by-product, which diffuses to the surface where it either evaporates or is removed by dissolution. Recently, a novel crosslinker, tetraoleyloxysilane (TOLOS), was synthesised whose oleyl alcohol (OA) condensation product permeates to the surface producing a lubricious surface. This product may lend itself to the development of silicone urinary catheters that are significantly easier to insert and remove than those made of standard silicones.

To evaluate the performance of the resultant elastomer, an assay was required to quantitatively investigate the rate of OA release. Previous reported assays (Sanchez et al 1992) for higher alcohols, including oleyl alcohol, required a GC/MS/FID system which required the extraction of the alcohols into a volatile, combustible solvent with resultant experimental error. A review of the literature suggested that the evaporative light scattering (ELS) detector in conjunction with HPLC could be used to quantify non-chromophoric compounds. The objective of this study were therefore to produce an HPLC assay with online ELS detection for the quantitation of OA and to measure the rate of OA release from a TOLOS crosslinked elastomer.

An HPLC assay was developed that has a linear response over the required concentration range ($r^2 > 0.999$). In repeatability studies of the technique the coefficient of variation (CV) for 10 samples is 8.5×10^{-3} and the reproducibility over a period of 5 days the CV is 11×10^{-3}. The minimum detection limit was found to be 15 ppm.

The resultant assay was used to quantify the release of OA from a silicone elastomer crosslinked with varying concentrations of the TOLOS and TPOS crosslinking reagents keeping the same overall molar concentration of crosslinker.

Table 1 Cumulative release of OA from a silicone elastomer crosslinked with TOLOS

Molar concn	Cumulative release per unit area (mg cm^{-2})					
TOLOS/TPOS	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
100/0	9.54	14.4	17.25	18.82	19.82	20.37
80/20	5.36	8.70	10.83	12.46	13.50	14.13
50/50	3.75	5.56	6.85	7.67	8.20	8.53
20/80	2.10	3.20	3.92	4.36	4.69	4.91

Table 1 demonstrates that the OA condensate exhibits a typical matrix release profile with an immediate burst effect followed by sustained release.

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Time delayed delivery of theophylline

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The ability to deliver drugs after a defined lag period is of increasing interest in the pharmaceutical industry. For example, asthmatics often experience attacks during the night while rheumatoid arthritis sufferers often have exacerbation of symptoms in the early morning. In such diseases it would be beneficial to be able to administer drug formulations at bedtime which only release the drug at the required time. This would minimise sleep disturbance and the distress experienced while waiting for the drug to exert it's actions. A capsule-based delayed-release formulation has been developed (Ross et al 2000). This comprises an insoluble capsule body sealed with an erodible tablet plug prepared from hydroxypropyl-methylcellulose (HPMC). The capsule body contains a swellable expulsion system and a theophylline tablet. After swallowing,the plug slowly erodes at a rate dependent on the HPMC content. When erosion is complete, fluid enters the capsule causing the expulsion system to swell, pushing the theophylline tablet into the lumen of the gastrointestinal tract.

A combined scintigraphy and pharmacokinetic study was conducted to compare the time and site of release and gastrointestinal transit with the plasma concentration time-profiles of theophylline. This was a single centre, randomised, three-way crossover study in eight healthy male subjects. The study was approved by the local ethics committee and ARSAC. A technetium-99m DTPA marker was incorporated into the theophylline tablet during manufacture to allow time and site

of expulsion and complete disintegration to be determined. The capsule body was labelled with indium-111 DTPA to allow transit of the device to be assessed separately.

After an overnight fast, subjects received a light snack 30 min before dosing. The subjects were given one of three formulations with 240 mL water (20%, 24% or 35% HPMC) according to a randomisation schedule. All subjects received lunch (1300 kJ) 4 h post-dose, an afternoon snack (600 kJ) 7 h post-dose and an evening meal (2400 kJ) 10 h post-dose. Anterior and posterior static acquisitions of 30 s duration were collected every 15 min until burst was observed scintigraphically, then hourly to 12 h post-dose. Blood samples were withdrawn at pre-defined intervals. Samples were centrifuged and the plasma fraction removed and stored at –20°C. Plasma theophylline levels were determined using a validated HPLC technique with UV detection.

Gastric emptying times were not significantly different between treatments (range 1–3 h). The formulations were observed to release in the small intestine although 6 of the 24 failed to release. Mean release times for 20%, 24% and 35% HPMC formulations were 2.82 h (± 0.53, n = 7), 3.15 h (± 0.38, n = 5) and 4.17 h (± 0.74, n = 6), respectively. These release times showed good correlation with in-vitro dissolution data. Scintigraphic release was confirmed by plasma theophylline levels.

In conclusion, within the limitations of this pilot study, the feasibility of time delayed delivery of theophylline has been demonstrated in-vivo.

Ross, A. C. et al (2000) *J. Pharm. Pharmacol.* **52**: 903–909

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Formulation of a novel time-controlled explosion system

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Previous work described an HS Capsule that was capable of providing time-delayed release in-vitro and in-vivo. The system comprised an inner size 0 capsule containing drug positioned within a size 000 capsule, with the space between the two capsules filled with lightly consolidated hydroxypropylmethyl cellulose. We now report an extension of that work with polyethylene glycol 8000 (PEG)–glyceryl monostearate (GMS) mixture as the inter-capsule layer.

An inner hard gelatin capsule (size 4) containing 108 mg low-substituted hydroxypropylcellulose (LH 21) and 12 mg paracetamol was centrally located within a size 000 hard gelatin capsule using a specially designed positioning tool. The void between the capsules was then completely filled, incrementally, with 1500 mg of a molten PEG–GMS mixture (0–10% w/w GMS). The dissolution profile from each capsule batch was determined (USP XXIII Apparatus 2) at 37°C, paddle speed 50 rev min^{-1}. The mean T_{50} release time for each formulation in distilled water (n = 6) is shown in Table 1.

Table 1 The influence of GMS matrix composition on mean T_{50} value from dosage form

Matrix composition (% w/w)		T_{50} (min)
PEG 8000	GMS	
100	0	46 ± 2
97.5	2.5	175 ± 21
95.0	5.0	257 ± 48
92.5	7.5	414 ± 56
90.0	10.0	424 ± 64

Data is presented as means ± s.d.

A linear relationship between mean T_{50} value and GMS content was observed (r^2 = 0.96), indicating that time-delayed drug release was directly controlled by the erosion rate of the PEG–GMS matrix which influenced the rate at which water could enter the inner capsule containing the disintegrant-drug mixture. Contact with dissolution fluid then resulted in rapid disintegration of the inner capsule.

The effect of pH on capsule dissolution was tested with phosphate buffer solution (pH 7.4) and hydrochloric acid solution (pH 2). Dissolution testing was done on capsules constructed using a 95% PEG–5% GMS matrix. The onset time of capsule rupture and the resulting T_{50} (n = 6) in assorted dissolution media is shown in Table 2.

Table 2 The influence of dissolution media on capsule performance

Dissolution medium	Capsule rupture (min)	T_{50} (min)
Distilled water	249 ± 31	257 ± 48
Phosphate buffer	290 ± 42	308 ± 35
Hydrochloric acid	322 ± 22*	402 ± 13**

Data are presented as means ± s.d.* $P < 0.009$, **$P < 0.0001$, compared with water and phosphate media

The dissolution studies showed that the onset of capsule bursting and the release profile was influenced by media pH. However, the physiological relevance of these in-vitro observations is yet to be determined.

In conclusion, this time-delayed release device offers a novel and convenient means of releasing drugs within the gastrointestinal tract after a predetermined lag-time by simple manipulation of PEG–GMS matrix composition.

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Towards a QCRS-based biosensor for monitoring interactions of microparticulates with the H376 epithelial cell line

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Quartz crystal resonant sensors (QCRS) are mass-sensitive measuring devices capable of monitoring receptor–ligand interactions in real-time, without the use of markers. In recent years, the technology has been utilised for a variety of sensing applications which incorporate antibodies, antigens, nucleic acids, etc., as part of the sensing system design.

A limited number of studies have utilised QCRS to monitor kinetics of in-situ adhesion and spreading of mammalian and bacterial cells (Wegener et al 1998). More importantly, this has recently been extended to monitoring the response of mammalian cells to external chemical and biological stimuli (Marx et al 2001). To date, the sensitive capabilities of the QCRS technique have not been applied to the monitoring of interactions between cell surfaces and microparticulates. Microparticulates are potentially useful constituents or oral formulations (i.e. mouthwashes), and their surface properties determine residence times within the oral cavity. Increasing the bioadhesiveness of such particles may promote retention within the oral cavity and even be used to target specific sites. This has led to the rationale to develop a cellular biosensor using a suitable oral epithelial cell line to assess the interaction of different microparticles, with respect to optimisation of formulation constituent residence times.

The H376 cell line is an oral squamous cell carcinoma line of sublingual tissue origin. Cells at passage 27 were trypsinised, pelleted by centrifugation and seeded at a density of 6000 cells/cm^2 into 60-mm^2 petri dishes containing 10 MHz polished crystals previously modified with spin-coated polystyrene films. Media was changed on the first day after passage and the cells left to spread on the resonator surface until day 3 after passage, when the cells are known to have formed a sub-confluent monolayer. Cell-coated crystals were removed from culture, installed in a novel flow cell and gassed media (95% O_2/5% CO_2) was passed over the crystal surface at a rate of 4 μL min^{-1}. The crystal was connected to a dual oscillator circuit and frequency response recorded. Once a stable frequency baseline had been established, 0.1-μm diameter FITC-labelled

polystyrene microspheres (diluted 0.1% w/v in media) were introduced to the flow cell via a Rheodyne HPLC injection valve.

Frequency measurements taken at 1-s intervals reveal a tri-phasic response of the resonator to each of three microparticulate injections. These have been postulated to involve an initial binding phase of the microspheres with the cell surface, followed by a possible molecular rearrangement at the cell surface or an endocytic process. Fluorescent microscopy of DAPI-stained H376 cells on the resonator surface at the conclusion of the experiment confirm the interaction of the microspheres with the cells and minimal interaction of microspheres with cell-free regions. Further experiments are required to study these processes and more information should be gleaned with examination of the ADC data (not shown), which considers the energy dissipation of the sensor during these microparticulate interactions.

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Wegener, J., Janshoff, A., Galla, H. J. (1998) *Eur. Biophys. J.* **28**: 26–37

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Influence of processing temperature on dissolution from a time-delayed capsule formulation, based on erosion of a PEG 8000/wax layer

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As an extension to earlier work from this group on time-delayed capsule formulations, we have replaced the loose-fill hydrophilic gel-forming polymer of the HS Capsule described by Ross et al (2000), by an erodible matrix formed by co-melting polyethylene glycol 8000 (PEG) and glyceryl monostearate (GMS), to simplify formulation assembly. One major limitation of PEG noted by Craig & Newton (1991), is that its thermal history may influence the dissolution properties of the product by modifying the degree of crystallinity. In this study the influence of processing and storage temperature on time-delayed drug dissolution was investigated.

A capsule (size 4) containing 108 mg low-substituted hydroxypropylcellulose (LH-21, Shin-Etsu, Japan) and 12 mg paracetamol was centrally positioned within a size 000 gelatin capsule using a specially designed positioning tool. The void between the two capsules was filled, incrementally, with 1500 mg of a PEG–GMS (95:5) molten mixture at 80°C. After the assembly of the capsules, the PEG-GMS mixture was allowed to solidify for 2 h at either 3, 20 or 50°C. Subsequently the capsules were stored for a further 13 h at either 3, 20 or 50°C (Table 1). The dissolution profile from each batch (n = 6) was determined in 1000 mL water (USP XXIII Apparatus 2, 37°C, 50 rev min^{-1}). Dissolution profiles were dramatically influenced by the processing/storage technique as indicated by the mean T_{50} release time for each formulation (Table 1).

Table 1 The influence of manufacture and storage temperature on mean T_{50} value

Formulation solidifying temp. (°C)	Post-manufacture storage temp. (°C)	T_{50} (min)
3	3	67 ± 6
3	50	216 ± 36
20	20	257 ± 48
50	3	190 ± 59
50	50	297 ± 74

Data are presented as means ± s.d.

DSC studies (Model 30, Mettler Toledo, UK) on PEG–GMS (95:5) melts (n = 2) demonstrated that storage temperature during solidification influenced the admixture endotherm (Table 2).

Table 2 Enthalpies of fusion and melting points of PEG–GMS melts solidified at different temperatures

Processing and storage temp. (°C)	Enthalpies of fusion[a] (J g^{-1})	DSC peak[a] (°C)
3	147.8	43.9
20	163.7	59.5
50	18.2	49.2

[a] 7 mg sample analysed in a 40-μL crimped aluminium pan; scan rate 4°C min^{-1} from 20°C to 80°C

Polarised microscopy of samples also confirmed the structural re-arrangement suggested from the DSC studies with different crystals sizes and arrangements being observed.

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Precipitating improved delivery of therapeutic proteins

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When using proteins and peptides in the preparation of pharmaceutical drug delivery formulations or in industrial biocatalysis it is paramount that the final protein conformation after formulation is free from degradation, resulting in loss of function. We have previously described a novel method for producing stable protein-coated microcrystals (PCMCs), free from degradation encountered in conventional production methods, that are very promising candidates for pulmonary and parenteral protein drug delivery (Ross et al 2001). However, in any process it is essential to understand the mechanism underlying the production process and the influence of parameters on it. Here, we examine the influence of processing conditions on PCMC production.

Using an innovative self-assembly method that uses the crystal lattice energy of the core carrier material, we have prepared in one-step, biomolecule-coated microcrystals in the micron-size range that can incorporate a range of biomolecules such as: proteins, peptides, DNA/RNA or sugars. The process is versatile and the PCMCs can be stored in solvent for longer than 1 year at room temperature or air dried to a fine powder. Processing parameters such as droplet delivery and rate of agitation were investigated. The resultant crystal size and morphology were studied using a Mastersizer 2000, a Multi-Stage Liquid Impinger (MSLI) and Scanning Electon Microscopy (SEM).

A simple system where a protein/excipient solution was dispensed into propan-2-ol with and without stirring was used. The PCMC size varied as the conditions were changed as follows: fast addition, no stirring > slow addition, no stirring > fast addition, stirring > slow addition, stirring. Therefore rate of agitation and addition alter PCMC particle size. Using the Mastersizer 2000 to allow greater control over the rate of agitation we dispensed an aqueous excipient solution directly into the mastersizer sample holder containing propan-2-ol and measured the size of the precipitating microcrystals.

Table 1 Effect of stirrer speed on precipitating excipient particle size

Stirrer speed (rev min^{-1})	Relative size
1500	100 (8.3)
2000	64 (0.3)
2500	45 (0.6)
3000	32 (1.6)
3500	26 (1.5)

Results are means of 3 (s.d.); the values show a significant difference ($P < 0.05$)

The greater the stirrer speed, the smaller the microcrystals produced, suggesting that greater dispersion of the aqueous solution in the propan-2-ol resulted in a more rapid dehydration of the particles and hence a smaller particle size (Table 1). Furthermore, delivery of smaller aqueous solution droplets into the organic solvent produced smaller particles. The process can be optimized to produce PCMCs of a desired size range (including sizes suitable for pulmonary drug delivery) with a narrow particle size distribution.

In conclusion, the particle size of PCMCs produced using a novel precipitation technique can be altered due to changes in the aqueous droplet size, rate of addition and rate of agitation.

Ross, A. C., et al (2001) *British Pharmaceutical Conference Science Proceedings*. London: Pharmaceutical Press, p. 70

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Incorporation of a targeting moiety into a multicomponent DNA delivery system

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One of the major problems facing the clinical application of gene therapy is gene delivery. A vehicle is required for gene transfer to enhance DNA uptake into the cell and its translocation to the nucleus (Garnett 1999).

One approach to tackle this problem is to use cationic polymer to condense DNA. In this work cationic polyamidoamines (PAAs) with proven efficiency in DNA delivery, were used. PAA type homopolymer condenses DNA effectively only in the presence of excess polymer. In-vivo, these positively charged complexes will be opsonised and cleared by the reticuloendothelial system (RES). However, PAA–PEG copolymers mixed with DNA produce condensed particles with poor physicochemical characteristics. Multicomponent DNA delivery systems (MCDS) are being investigated to address this problem (Rackstraw et al 2002).

The physicochemical characteristics of PAAs both as homopolymer and copolymer with PEG were evaluated using various techniques (photon correlation spectroscopy, transmission electron microcroscopy (TEM), and gel electrophoresis techniques) to engineer a novel system, which is able to condense DNA with a lower polymer:DNA ratio, with a small particle size, lower polydispersity and with efficient binding to DNA. Different combinations of homopolymer to copolymer ratios (1:1, 2:1, 3:1 and 4:1) with DNA were investigated, the best results were obtained at a 3:1 ratio. The results of the mixed systems showed a considerable improvement in the physicochemical properties of the mixtures in comparison with either the homo or the copolymer alone. A particle size of 145 nm and polydispersity of 0.1 were achieved. Non-aggregating nanoparticles were also produced within the range 1.25–1.75 of mixed polymer to DNA.

MCDS has a condensed core with a PEG corona surrounding it. The presence of the PEG corona in MCDS results in a low transfection efficiency. Therefore, incorporation of a targeting moiety was suggested to potentiate transfection of cells. In this study we have used folinic acid as a targeting moiety for several reasons: firstly, folates are relatively easy to couple to the polymers, folinic acid can be taken up in a variety of tissues and finally it is not removed from the receptor in the presence of folic acid in the culture medium in-vitro or by dietary folic acid in-vivo.

The conjugate of folinic acid and PEG–PAA were synthesised by carbidiimide-mediated coupling of folinic acid to terminal amino group of the PEG–PAA. The ligand produced was characterised using Lowry assay to assess the polymer content and using UV spectrophotometry at λ 280 nm to measure the folinic acid content. The results have shown the formation of folinic acid–PAA ligand with molar ratio 2:1 of folinic acid to polymer. Confirmative studies have shown that the conjugate produced could be used with homopolymers to condense DNA. These ligand-targeted complexes will be assessed for enhancement of transfection efficiency.

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Rackstraw, B. J., et al (2002) *Biochem. Biophys. Acta* In press

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Preparation of water-impermeable capsule bodies for a pulsatile drug delivery system

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A pulsed release drug delivery system has been developed to exploit the possibilities of providing dosage forms attuned to the circadian rhythms of disease states (Lemmer 1991). The delivery device consists of a water-impermeable capsule body, an expulsion excipient, a solid dosage form and an erodible plug (Stevens et al 1995).

Size 0 hard gelatin capsules were coated using an Aeromatic Strea-1 fluidised bed coater. Ethyl cellulose as an aqueous suspension of Surelease plasticized with dibutyl sebacate (DBS) or an organic based solution containing Ethocel and a 50:50 mixture of IPA–acetone plasticized with either dibutyl phthalate (DBS) or phamacopoeial approved triacetin (TRI) were used as coating agents. Plasticizers are used to provide even and malleable coating, (Oh & Luner 1999) by lowering the glass transition temperature (T_g) of coating polymers to allow fluid-flow and coalescence.

Non-contact laser-profilometry was conducted on sections of Surelease and Ethocel (DBP) coated capsules. The average roughness parameter (R_a) was used to assess the extent of coating to a depth of 10 mm from the open end of the capsule body. Texture analysis was used to determine the initial compression strength of the coated capsules. Capsules were exposed to a controlled environment at 95% relative humidity (RH) for 999 minutes at 25°C using dynamic vapour sorption (DVS). After removal further texture analysis was conducted. Comparison of the initial and final compression strengths were used to determine a capsule integrity ratio.

Table 1 Integrity and roughness of water-impermeable capsule bodies (s.d.)

Sample	Initial strength, a (N) (n=6)	Final strength, b (N)	Capsule integrity ratio (b/a)	Average roughness (R_a) (n=10)
Srelease	148.4 (9.6)	27.0 (6.4)	0.18	2.24 (0.49)
Ethocel*	227.4 (8.0)	86.2 (14.5)	0.40	3.58 (2.07)
Ethocel†	156.7 (15.6)	59.4 (7.2)	0.38	—

*Contains DBP; †contains TRI

R_a values (Table 1) indicated the surface of the Surelease capsules were smoother compared with the Ethocel capsules. Highest R_a values were obtained nearest to the open end of the capsule (data not shown). This is due to an increased ability of organic solvents to spread across the gelatin, allowing an internal coating to be applied. Capsule integrity ratio values for the Ethocel-coated capsules are higher than for the Surelease-coated capsules, indicating less permeability to water at 95% RH. In conclusion, an organic-based coating process affords greater structural integrity and an increased internal coating level applied, providing better impermeability. This has a direct effect on the functionality of a pulsed-release capsule device. Ethocel when plasticized with triacetin maintains integrity on exposure to humidity and produces capsules suitable for human use.

Lemmer, B. (1991) *J. Control. Release* **16**: 63–74

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Stevens, H. N. E., et al (1995) *US Patent* 5474784

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Quaternary ammonium polypropylenimine dendrimers for gene delivery

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The clinical use of gene therapy is currently hampered by a number of limiting delivery issues (Brown et al 2001). In an effort to circumvent some of the problems associated with delivering DNA we have been exploring the use of polypropyleninime dendrimers as gene delivery agents and have indeed established that the lower generation (generation 2) polypropylenimine dendrimer is an effective non-toxic gene delivery system (Zinselmeyer et al 2002). Our attention has now turned to ways in which the higher generation materials, which are more effective at DNA binding than their lower-generation counterparts, may be used for gene transfer. A strategy to overcome the toxicity of these higher generation materials is reported here.

Generations 3 (DAB 16) and 4 (DAB 32) polypropylenimine dendrimers were converted to quaternary ammonium derivatives (Q16 and Q32 respectively) by reaction with methyl iodide. Products were isolated by precipitation in ether, ion exchange chromatography and freeze-drying. Quaternary ammonium derivatives were analysed by ^{1}H NMR and elemental analysis and their DNA binding measured using the ethidium bromide exclusion assay. Cell cytotoxicity (MTT) and transfection efficacy (using the β-galactosidase reporter gene) assays on the products and starting materials were performed in the A431 cell line. The cationic liposome formulation *N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium methylsulphate (DOTAP) was used as a positive control and naked DNA as a negative control in transfection experiments.

Table 1 The properties of generation 3 and 4 polypropylenimine dendrimer formulations

Optimum dendrimer, DNA formulations	Particle size (nm)	IC50 ($\mu g\,mL^{-1}$)	%DNA transfection relative to DOTAP at equivalent DNA doses (DNA dose in μg per 96 plate well) DOTAP = 100%
DAB 16, DNA (5:1)	180	36	24% (20)
Q16, DNA (3:1)	259	129	76% (20)
DAB 32, DNA (3:1)	163	6	18% (10)
Q32, DNA (3:1)	300	33	33% (10)

Quaternisation was confirmed by ^{1}H NMR. Elemental analysis revealed data which was consistent with most of the available terminal primary amines being quaternised in the case of Q16 (97%), complete quaternisation of the outer primary amines in the case of Q32 (100%) and additional quaternisation of the inner shell of tertiary amines for Q32 (75%). Complete DNA binding, as measured by the limiting fluorescence of ethidium bromide, was detected at a nitrogen to phosphate ratio of 1.3: 1 for Q16 and 2.5: 1 for Q32 in comparison to a nitrogen to phosphate ratio of 1.5: 1 for the parent molecules. Quaternisation resulted in an increase in particle size of the dendrimer–DNA complexes and an improvement in both cell cytotoxicity and transfection efficacy (Table 1).

In conclusion, we state that a reduction in the cytotoxicity of generation 3 and 4 polypropylenimine dendrimer–DNA complexes may be obtained by quaternisation of the amino groups with resultant improvements in gene transfer.

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Zinselmeyer, B. H., Mackay, S. P., Schätzlein, A. G., Uchegbu, I. F. (2002) *Pharm. Res.* **19**: In press

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Liposomal and niosomal DNA vaccination via the subcutaneous route

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Compared with naked DNA immunisation, entrapment of plasmid-based DNA vaccines into liposomes, by the dehydration-rehydration method (Gregoriadis et al 1999) has been shown to enhance both humoural and cell-mediated immune responses to encoded antigens by a variety of routes including subcutaneous administration (Gregoriadis 1998). Here we compare the ability of liposomes to niosomes to mediate immune responses to plasmid encoded antigens after subcutaneous immunisation.

Plasmid pI.18Sfi/NP containing the nucleoprotein (NP) gene of A/Sichuan/2/87 influenza virus in the pI.18 expression vector (up to 150:g) was entrapped in dehydration-rehydration vesicles (DRV) (Gregoriadis et al 1999; Perrie & Gregoriadis 2000) prepared from 16:mol egg phosphatidylcholine (PC), 8:mol dioleoyl phosphatidylethanolamine (DOPE) and 4:mol of either of the cationic lipids 1,2-dioleoyl-3-(trimethylammonium) propane (DOTAP) or cholesterol 3∃-*N*-(dimethyl-aminoethyl)carbamate (DC-Chol) (liposomes) or 16:mol 1-monopalmitoyl-rac-glycerol (C16:0) (Monopal), 8:mol DOPE, 4:mol cholesterol (CHOL) and 4:mol DC-Chol (niosomes). Female B57 black mice 6–8 weeks old (20–25 g each) were given two, 28 days apart, subcutaneous injections of 10:g (in 0.1 mL PBS) of either naked or DRV-entrapped pI.18Sfi/NP plasmid. Sera samples collected at time intervals, were tested for anti-NP total IgG (IgG_T), IgG_1, and IgG_{2a} by the enzyme-linked immunoadsorbent assay (ELISA).

As previously reported with other plasmids (Gregoriadis et al 1999), pI.18SFi/NP entrapment (% of total used) in both liposomes and niosomes was considerable (79–93%). Such entrapment values, based on the assay of ^{35}S radioactivity have been shown (Perrie & Gregoriadis 2000) to be reproducible and to predominantly reflect actual DNA entrapment within the bilayers as-opposed to complexation of DNA with the vesicle surface.

Analysis of anti-NP IgG_T responses in mice subcutaneously immunised with either naked or DRV-entrapped pI.18Sfi/NP reveal that, at all time points measured up to 114 days after the first injection, responses (log_{10} values) in mice immunised with naked DNA were low (1.8–2.1), similarly to values seen in control, non-immunised mice (2.0). In contrast, by day 49, responses became significantly higher in mice injected with DNA entrapped in niosomes (2.6, $P < 0.03$) or DOTAP liposomes (2.8; $P < 0.02$) and remained so to the end of the experiment (114 days). By day 56, IgG_T responses in mice immunised with DC-Chol liposomes also attained significantly higher values ($P < 0.04$–0.0001) than those mice immunised with naked DNA. Moreover by day 56, comparison of responses between the various DRV formulations revealed no significant difference except at day 114 where the group injected with DC-Chol liposomes declined to significantly lower levels ($P < 0.05$). Similar results were also obtained when the IgG_1 and IgG_{2a} subclass responses were determined.

In other experiments mice were injected subcutaneously with 10:g free or liposome-entrapped (PC:DOPE:DOTAP) plasmid DNA encoding the enhanced fluorescent green protein pCMV.efgp and 48 h later lymph nodes draining the injection site were collected and sections examined microscopically. Results revealed a greater level of gene expression in the lymph nodes of mice dosed with liposomal DNA than that (background autofluorescence) seen in the nodes of mice dosed with naked DNA or in control animals.

Our results suggest that formulation of plasmid vaccines in DRV liposomes or niosomes comparatively enhances humoural immune responses after subcutaneous immunization. This can be attributed to the ability of vesicles to not only protect their DNA content from nuclease attack (Gregoriadis et al 1999) but also deliver it to APC infiltrating the site of injection or in the lymphatics.

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Investigating the effect of carbohydrates on liposomal drug loading during the dehydration–rehydration process

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Recently, a novel method for producing high-yield entrapment of a wide variety of solutes in small liposomes was reported (Zadi & Gregoriadis 2000). The procedure is based on the Dehydration–Rehydration method (Kirby & Gregoriadis 1984) with the modification that during the dehydration–rehydration process sucrose, at appropriate sugar to lipid mass ratios, is present. It is proposed (Zadi & Gregoriadis 2000) that the presence of sucrose allows the destabilization of small unilamellar vesicles (SUV) to an extent to which entry of solute during the rehydration occurs whilst controlling vesicle fusion and hence allowing the SUV to reform either to their original state or to moderately larger sizes. Here we further investigate this novel drug entrapment procedure to elaborate the controlling factors involved in this method.

Dehydration–rehydration vesicles (DRV) in the absence and presence of carbohydrates were prepared as previously described (Kirby & Gregoriadis 1984; Zadi & Gregoriadis 2000). Briefly SUV composed of phosphatidylcholine and cholesterol (16: 16 μmol) were prepared and mixed with water containing 1 mg of riboflavin and in the case of the modified DRV method the appropriate carbohydrate (lactose, glucose, sorbitol, trehalose, sucrose) at a final concentration of 10–300 mM. These mixtures were then freeze-dried and reconstituted as for the standard DRV method. Liposome-entrapped drug was separated from free drug using column chromatography and riboflavin entrapment determined fluorimetrically (excitation 445 nm, emission 520 nm). Effective diameter of the vesicles was determined using a Brookhaven ZetaPlus.

Initially the effect of varying the sucrose to lipid mass ratio was investigated. Results reveal that as the sucrose concentration increased from 10–50 mM the effective diameter of the liposomes decreased from 528 ± 42 nm to 208 ± 21 nm with no significant decrease in drug entrapment measured (18–24% of total amount used). Increasing in the sucrose concentration further to 100 mM resulted in a continued decrease in vesicle size (115 ± 27 nm) but was accompanied by a significant ($P < 0.05$) drop in encapsulation efficiency (8.8 ± 2.3%). A continued increase in the sucrose concentration up to 300 mM produced no further decrease in vesicle diameter however entrapment efficiency continued to drop to as low as 4.7 ± 0.5%. These results coincide with the proposed mechanism of drug entrapment during the modified DRV method: increasing the sucrose concentration allows for less vesicle destabilisation and therefore less solute entry into the liposomes which occurs in conjunction with a decrease in vesicle fusion during destabilisation (highlighted by the reduction in vesicle size).

In another experiment, we attempted to identify if addition of the sucrose after completion of the freeze-drying process (i.e., having the sucrose present in the rehydration solution) would have the same effect as if added before freeze-drying. The entrapment achieved in the former case was significantly lower ($P < 0.005$) than that achieved when the carbohydrate was present during dehydration of the vesicles (11.0 ± 1.7% vs 24.6 ± 2.0%, respectively) suggesting that control destabilisation and fusion of liposomes must occur at the dehydration stage and not the rehydration stage.

Work continues to investigate the efficiency of different carbohydrates when applied to this modified DRV method. Initial studies reveal that sucrose gives significantly higher drug entrapment and lower vesicle size ($P < 0.05$) than all other carbohydrates tested suggesting it to be the most effective carbohydrate to use in this liposome preparation method.

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A peptide-based pro-drug approach for the selective delivery of cytotoxic agents to live tumour cells

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Several membrane translocating peptides, exhibiting highly efficient intracellular import properties, have been reported over the past few years. These have been used in attempts to actively target and deliver cytotoxic agents to neoplastic cells. One such peptide is derived from the HIV-1 TAT (TransActivator of Transcription) protein, an 86-amino-acid residue protein expressed by the human immunodeficiency virus type-1. A highly basic cluster of amino acids extending from residues 47–57 (YGRKKRRQRRR) has been assigned this translocating activity (Vives et al 1997).

The $TAT_{47–57}$-derived peptide has been shown to rapidly and efficiently translocate through the plasma membrane and accumulate in the cell nucleus (Vives et al 1997; Lindgren et al 2001). Chemical coupling of several macromolecules to the $TAT_{47–57}$-derived peptide allowed their efficient internalization into many cell types (Fawell et al 1994; Schwarze et al 2000). However, one major difficulty in using the $TAT_{47–58}$-derived peptide as a cargo system for the import of therapeutically active agents to target cells is the TAT peptides' demonstrated ability to traverse all cell types, thus posing a selectivity problem especially in the delivery of cytotoxic agents to tumour cells.

We are interested in the development of strategies aimed at the selective delivery of pharmacologically active agents to live tumour cell lines using a protease-activated approach. We wish to report on initial results of studies using a masked $TAT_{47–57}$-derived pro-drug which can be unmasked only by the action of tumour-associated proteases

$TAT_{47–57}$-derived peptides with carboxyfluorescein(CF) covalently linked at the N-terminal and CF-$TAT_{47–58}$-derived peptides modified with peptidyl side-chain recognition motifs for Urokinase plasminogen activator (uPA), at lysine residues in positions 51 or 52 were synthesized. HeLa human cervical cancer cells were seeded out at a concentration of 1.5×10^5 mL^{-1} on a 96-well plate. After overnight incubation cells were washed with serum free media and peptide treatments added (100 μL). Peptides were tested at 100 μM for 15, 30, 60 and 120 min. Images of TAT-mediated carboxyfluorescein uptake in HeLa cells were captured and analysed. The results show a general and marked reduction in the amount of fluorescence observed for Hela cells incubated with $TAT_{47–57}$ peptide modified with a side-chain recognition motif for uPA, over each time period, compared to the unmodified CF-$TAT_{47–57}$.

This represents a potential peptidyl based pro-drug approach for the selective delivery of cytotxic agents to tumour cells by exploitation of highly specific tumour-associated proteases as pro-drug activators.

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An investigation into the influence of purity on the interaction of stearic acid microspheres with buffer systems

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Stearic acid has found increasing application as a carrier for drug delivery (Killen & Corrigan 2001; Robson et al 2001). However, commercial stearic acid may contain a range of further fatty acids, including up to 50% palmitic acid, hence it is

essential to have an understanding of how the purity of the material used influences performance. In this investigation we have studied the interaction of stearic acid microspheres prepared by a spray-chilling technique with Sorensens pH 8.0 buffer, both in terms of the morphology and thermal properties of the spheres. Microspheres were prepared using a customised spray chilling apparatus using stearic acid with 99% purity (99SAM), 95% purity (95SAM), mixes of 99% purity stearic acid and 99% purity palmitic acid 50:50 w/w (50SA50PAM) and 30:70 w/w (30SA70PAM). Systems were exposed to Sorensens modified phosphate buffer (SMPB) pH 8.0 at room temperature for 0.5, 1, 2, 4 and 6 h, respectively, followed by washing and drying. Morphological evaluations of original microspheres and systems exposed to buffer were performed using scanning electron microscopy (SEM). Differential scanning calorimetry (DSC) was performed from 20 to 200°C using a heating rate of 10°C min^{-1} and a sample mass range of 1.0–2.0mg. In the cases of two stearic acid microsphere formulations, SEM studies performed before exposure to the buffer showed a greater degree of surface imperfection for the 95SAM than for the 99SAM. Similarly, the 50SA50PAM and 30SA70PAM were observed to have much smoother surfaces and a more spherical shape than the stearic acid alone. On exposure to buffer, imperfections and small scales were found on the surfaces of all systems, with greater changes noted for the 95SAM and 99SAM spheres. DSC indicated that single peaks were seen for the four systems before treatment, indicating melting of the fatty acids. After two hours exposure, further peaks were seen for all four sets of spheres, with the 99SAM showing a main melting peak at circa 70°C and a shoulder at 77–80°C, the 95SAM showing the most pronounced development of the shoulder peak (68°C, 76–79°C) and the 50SA50PAM (57°C, 66–68°C) and 30SA70PAM (56°C, 65–68°C) showing similar behaviour. These data are of significance due to the observation by Robson et al (2001) that there appeared to be a correlation between the appearance of the second peak and the dissolution rate of cefuroxime axetil from stearic acid microspheres, possibly due to acid soap formation. This study has indicated that the formation of such peaks may be dependent on the composition of the stearic acid sample used.

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Hydration effects on lyophilised formulations intended for nasal delivery

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Bioadhesive formulations for nasal administration must be capable of hydrating rapidly, to adhere to the nasal mucosa before being removed by the cilia. Conversely, over-hydration may cause loss of bioadhesion and be detrimental to the required adhesive effect. A novel Dynamic Adhesion technique designed to mimic forces in the nasal cavity for assessment of adhesion of lyophilised formulations was previously reported (McInnes et al 2001). A more detailed characterisation of hydration properties has been carried out.

A simple hydration test assessing extent of water uptake by the inserts (0.5–3% K4MP before lyophilisation) was employed. The inserts were weighed and placed on an agar surface simulating the nasal mucosa with limited availability of water, removed after a defined length of time, re-weighed and water uptake calculated.

Secondly, a test for quantification of adhesive forces in relation to the Dynamic Adhesion test was designed. HPMC gel (1 or 2% K4MP) was lyophilised onto glass sintered discs, ensuring a layer of lyophilised HPMC extended from one surface. The disc was placed on an agar plate, with HPMC in contact with the agar, under a force of 5 g and allowed to hydrate. A Texture Analyser (TA) was used to measure the force required to pull the sintered disc parallel to the surface of the agar at 2 mm s^{-1}. The area under the curve (AUC) was calculated as a measure of work done.

Results for the simple hydration test (n = 6) show that all formulations absorbed the equivalent of over ten times their weight in water in the time period studied (6 h). At this time point, little difference could be seen between the formulations, except the 0.5% K4MP formulation which had begun to show a plateau effect. Each formulation displayed rapid initial hydration, with rate of hydration in the first 45 min decreasing in the order 0.5% K4MP = 1% K4MP > 2% K4MP > 3% K4MP. Results from the TA show that initial adhesion of the formulations is similar, followed by a gradual loss of adhesion over time due to over-hydration. The 2% K4MP formulation displayed greater adhesion at this time than 1% K4MP. Examples of this effect are shown in Table 1 (n = 6). These results confirm findings from the Dynamic Adhesion method in which adhesion was found to be higher in the first 90 min, then gradually decreasing to a plateau effect by approximately 180 min.

Table 1 AUC for TA measurements

Formulation	AUC (N/s)		
	Initial	at 180 min	at 300 min
1% K4MP	2.10 ± 0.27	0.66 ± 0.21	0.57 ± 0.04
2% K4MP	2.88 ± 0.30	1.04 ± 0.15	1.14 ± 0.09

HPMC formulations studied displayed rapid and extensive hydration, ideal properties for a formulation intended for nasal delivery and bioadhesion. The TA appears to be a useful tool for evaluation of loss of adhesion of lyophilised formulations due to over-hydration.

McInnes, F., Stevens, H. N. E., Baillie, A. J. (2001) *BPC Science Proceedings*: 259

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Palmitoyl chitosan polymeric vesicles

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We have previously reported the formation of drug delivery vesicles at neutral pH from palmitoyl glycol chitosan, the amphiphilic derivative of the water-soluble chitosan derivative, glycol chitosan (Uchegbu et al 1998). These vesicles are able to increase the cellular uptake of macromolecules (Dufes et al 2000). Additionally vesicles may be prepared from octadecyl chitosan in the presence of hydrochloric acid (Wakita & Hashimoto 1995) as chitosan is generally insoluble at neutral pH. In this communication we report a method to prepare drug delivery vesicles from hydrophobised chitosan in water as the presence of free hydrochloric acid may not be suitable for the encapsulation of acid labile drugs such as peptides.

Chitosan with a degree of deacetylation of 98 mol% and a molecular weight of 201 kD was degraded in the presence of concentrated hydrochloric acid (4 M). The molecular weight of the degraded material was determined by gel permeation chromatography with multi-angle laser light scattering (GPC-MALLS). The degraded chitosan was palmitoylated by reaction with palmitic acid *N*-hydroxysuccinimide in either a one phase acid medium (pH = 5.2) or a two-phase basic medium with the chitosan present as a slurry (pH = 8.1) to yield PCS2 or PCS1, respectively. The product was isolated by exhaustive dialysis, freeze-drying and an ethereal washing step. The resulting polymer was characterized by ^{1}H NMR and GPC-MALLS. Polymeric vesicular self-assemblies were prepared by probe sonication of PCS1, cholesterol, Solulan C24 (2:1:1 weight ratio) and PCS2, cholesterol (5:1 weight ratio). Polymeric vesicles were imaged by transmission electron microscopy with negative staining and freeze fracture electron microscopy. Polymeric vesicle assemblies were also sized by photon correlation

spectroscopy and loaded with fluorescein isothiocyanate (FITC)-dextran (MW = 4 kD).

Table 1 Palmitoyl chitosan based vesicles

Sample	Palmitoyal level	MW (kD)	Vesicle size (nm)	Encapsulation of FITC-dextran g per g of polymer
PCS1	35	21.9	617 ± 19	0.10 ± 0.024
PCS2	13	13.8	191 ± 1	0.16 ± 0.021

Degraded chitosan had a molecular weight of 10.1 kD but was still insoluble at alkaline pH. The level of palmitoylation obtained by the nucleophilic substitution reaction was predictably higher in PCS1 prepared in an alkaline medium when compared with PCS2, prepared in an acid medium (Table 1). The more hydrophilic PCS2 (hydrochloride salt) produced stable (morphologically stable after 6 months storage at room temperature) unilamellar vesicles in the presence of cholesterol alone and the more hydrophobic material PCS1 (unionized) only produced vesicles on addition of the hydrophilic surfactant Solulan C24 to PCS1, cholesterol formulations. Both vesicles efficiently encapsulated the hydrophilic macromolecule FITC-dextran (Table 1). In conclusion, palmitoyl chitosan vesicles may be formed by probe sonication in water of palmitoyl chitosan hydrochloride with a low level of hydrophobicity and also from unionized palmitoyl chitosan with a higher level of hydrophobicity, provided that a soluble surfactant is added to the latter formulation.

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Nanoparticles formed by the self assembly of a new spermine-based amphiphile

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Bolaamphiles are compounds in which a hydrophobic spacer bridges two hydrophilic moieties and their self assembly has been studied since the 1980s (Fuhrhop & Fritsch 1986) with vesicles and micelles usually reported. The current study describes the self-assembly of a new spermine based bolaamphiphile (DISPERM) to give unusual small 25-nm particles or fused clusters of small particles which are capable of supporting a high hydrophilic load. Such nanoparticles may find use as drug delivery particles.

DISPERM was synthesised by the reaction of spermine with 1,12-dibromododecane. The product was isolated by exhaustive dialysis and the bolaamphiphile structure confirmed by ^{1}H NMR and mass spectrometry. The aggregation of the bolaamphiphiles was studied using a pyrene probe, methyl orange probe and surface tension measurements. DISPERM aggregates were imaged by freeze fracture and transmission electron microscopy with negative staining. The mobility of the aggregate hydrophobic domains in aqueous media was studied using ^{1}H NMR and the nanoviscosity of the hydrophobic domains studied using the dipyrene probe – Dipyme. Tween 20 and Span 60 were used as controls representing a fully hydrated and a clearly hydrophobic aggregate respectively. Finally the presence of hydrophilic domains within the nanoparticles was probed by the encapsulation of fluorescein isothiocyanate (FITC)–dextran (MW = 4 kD).

DISPERM was found to self-assemble at concentrations in excess of 1.0 mg mL^{-1} and produced clear liquids below concentrations of 6 mg mL^{-1} and turbid liquids thereafter. Imaging studies on 1.5 mg mL^{-1} and 20 mg mL^{-1} samples showed small 25–50-nm non-bilayer particles. Particles were easily fractured thus discounting a membrane spanning vesicle structure. Larger fused aggregates consisting of hundreds of smaller particles were seen in the sample imaged at the higher concentration (20 mg mL^{-1}). Fusion is thought to occur by the bridging of two particles by single bolaamphiphiles which prevents fracturing of the fusion points. ^{1}H NMR studies revealed the entire length of the hydrophobic chain to be accessible to water and the nanoviscosity data indicated the presence of comparably viscous hydrophobic domains. Surprisingly nanoparticles could be loaded with a high hydrophilic load when compared to conventional vesicular systems studied at the same level of amphiphile/lipid (Table 1).

Table 1 The loading of FITC–dextran (MW = 4 kD) on to bolaamphiphile nanoparticles

Particulate sample	% w/w FITC-dextran
Span 60, cholesterol (2:1)	0.017 ± 0.0016
Span 60	0.051 ± 0.0018
DISPERM	2.32 ± 0.028

Data are presented as mean ± s.d.

We conclude that the new spermine based bolaamphiphile assembles into small nanoparticles with fusion of the nanoparticles occurring at higher bolaamphiphile concentrations due to the ability of the bolaamphiphile to span 2 particles during formation. The nanoparticles consist of well hydrated but immobile hydrophobic regions and significant hydrophilic domains giving them a high capacity for the entrapment of aqueous soluble drugs.

Fuhrhop, J. H., Fritsch, D. (1986) *Acc. Chem. Res.* **19**: 130–137

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Synthesis and physicochemical analysis of amide crosslinked polyanhydride poly(GluSA:SA) as a potential drug delivery carrier

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In this study, an amide crosslinking unit, sebacyl di-glutamic acid (GluSA), was designed and introduced into the backbone of the polyanhydride p(CPP:SA) (Staubli et al 1989), with the intention of reducing the crystallinity of the polyanhydride. This may improve its mechanical and thermal properties allowing the preparation of high quality microspheres. A series of GluSA crosslinked copolymers, with a portion of GluSA from 5 to 95%, was synthesised using a modified literature method (Domb et al 1987). Briefly, acylation of glutamic dimethyl ester with sebacic acid followed by removal of methyl groups gave GluSA, which was converted to the tetra-acetate anhydride for melt polymerisation.

Molecular weights (MW) were determined using GPC and the identity of the polymers confirmed using IR, NMR and MS spectroscopic techniques. A trend of decreases in melting points ($n = 3$, $P < 5.09 \times 10^{-5}$) was observed when GluSA portion increased from 0 to 30% (Table 1). No melting points were detected using DSC with GluSA content above 30%. The heat of fusion values for the polymers decreases sharply, as GluSA portion increases. These imply a decrease in crystallinity of the polymers and an increase in the amorphous component.

Table 1 Some physicochemical properties of P(GluAS:SA)

Composition P(glySA:SA)	MW	M. pt (°C)	Heat of fusion (J g^{-1})
0:100	39 000	78–80	116.45
5:95	29 650	69–70	56.49
10:90	20 540	66–70	54.71
20:80	16 791	61–64	45.54
30:70	10 250	45–60	9.91
40:60	5000	*	*
50:50	8460	*	*
60:40	6500	*	*
70:30	6430	*	*
80:20	2700	*	*
10:90	1360	*	*
0:100	968	*	*

*none detected

The solubility of the polymers was determined in dichloromethane (DCM) and chloroform (Table 2) and it is seen to decrease with increasing SA content.

Table 2 Solubility and physical appearance of synthesised P(Glu:SA)

Composition P(GluSA:SA)	Physical appearance	Solubility (% w/v)
5:95	Slightly yellow solid	>10
10:90	Yellow solid	>10
20:80	Yellow flexible solid	>10
30:70	Brown rubber	>20
40:60	Brown rubber	>30
50:50	Sticky syrup	>50
60:40	Sticky syrup	>50
70:30	Sticky syrup	>50
80:20	Very sticky syrup	>50
90:10	Very sticky syrup	>80
100:0	Very sticky syrup	>80

Tensile strength of the polymers has been studied through forming films via solvent evaporation. We observed that GluSA increases the tensility of the polymers while SA is crucial for film transparency. This study demonstrated that GluSA is useful for the preparation of crosslinking polyanhydrides. By manipulating the portion of GluSA in the copolymers, a crosslinking polyanhydride with desired properties could be prepared.

Domb, A. J., et al (1987) *J. Polym. Sci.* **25**; 3373
Staubli, A., et al (1989) *J. Am. Chem. Soc.* **112**: 4419