Poster Session 2 – Drug Delivery

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Colloidal microgels as potential transdermal delivery systems

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Thermally responsive colloidal microgels of poly(N-isopropylacrylamide) (polyNIPAM) have been reported to act as intelligent materials in controlled drug release (Kikuchi & Okano 2002), in mobilization of enzymes and cells and in separation of aqueous proteins (Jeong *et al* 2002). This paper presents the synthesis of temperature-sensitive microgels based on a copolymer of butyl acrylate (5%) co-polyNIPAM (95%), in the presence of and in the absence of ibuprofen (IBU) and methyl paraben (MP), by a surfactant-free emulsion polymerisation (SFEP) in water. *N*′,*N*′-methylenebisacrylamide was used as a cross-linking agent and potassium persulphate as an initiator. Physico-chemical properties of the microgels were determined using different techniques including dynamic light scattering, Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM) and Differential Scanning Calorimetry (DSC). Absorption of ibuprofen (logP = 3.51) and methyl paraben (logP = 1.92) into the gels, and their subsequent permeation across a model silicone membrane and human skin, were investigated over a range of temperature (292–313 K). The transport rate of IBU from poly(NIPAM) microgel (which at 303 K using silicone membrane was 1.5 $\pm$ 0.2 μg cm^{-2} h^{-1} and using skin was 0.24 $\pm$ 0.1 μg cm^{-2} h^{-1}) is significantly reduced by about two orders of magnitude comparing with the transport rate of saturated solution of IBU (which using silicone membrane was 90 $\pm$ 2.4 μg cm^{-2} $h^{-1}$and using skin was 33.2 $\pm$ 8.4 μg cm^{-2} h^{-1}). A huge reduction in the flux indicates that the microgel retards permeation of the drug across both membranes, and hence the microgel can be considered as a permeation retarder. However, fluxes of MP from poly(NIPAM) microgel (which at 303 K using silicone membrane was 30.9 $\pm$ 2.9 μg cm^{-2} h^{-1} and using skin was 35.6 $\pm$ 4.1 μg cm^{-2} h^{-1}) are of the same order of magnitude as those of saturated solutions of MP (silicone membrane 41.8 $\pm$ 4.0 μg cm^{-2} h^{-1}; skin 66.4 $\pm$ 15.3 μg cm^{-2} h^{-1}). A relationship between the logP value of the drugs and their subsequent release from poly(NIPAM) microgels will be reported.

Jeong, S., *et al*. (2002) *Adv. Drug Deliv. Rev.* **54**: 37
Kikuchi, T. Okano (2002) *Adv. Drug Deliv. Rev.* **54**: 53

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Metronidazole-loaded intravaginal rings for the treatment of bacterial vaginosis

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Bacterial vaginosis (BV) is the most common vaginal infection (Spiegel 1991). The most commonly prescribed treatment is orally or vaginally administered metronidazole. A silicone intravaginal ring containing metronidazole could offer several advantages over current metronidazole regimens including the ability to continuously deliver an effective dose over a number of weeks and thus lower the high rate of recurrence. This study describes the development of a matrix-type metronidazole ring for the treatment of BV, and evaluates in vitro efficacy against *Gardnerella vaginalis*.

Matrix-type intravaginal rings loaded with between 50 and 2000 mg of metronidazole were prepared according to well-established methods already reported in the literature (Malcolm 2003). Fourteen-day in-vitro release studies were performed with daily sampling and replacement of the release medium. Samples were assayed using UV-HPLC. *Gardnerella vaginalis* was grown anaerobicallly and an inoculum of approximately 5.0e5 cfu mL^{-1} was prepared. On-hundred-millilitre volumes of this inoculum were added to metronidazole-loaded vaginal rings. The flasks were then incubated anaerobically and sampled periodically to determine the number of surviving organisms.

In-vitro metronidazole release profiles for the intravaginal rings were typical of matrix devices, showing $t^{1/2}$ kinetics and increasing drug release with drug loading. The influence of the intravaginal ring drug loading on the viability of a culture of *Gardnerella vaginalis* is presented in Table 1. The limited release of metronidazole from the 50-mg-loaded ring did not inhibit the growth of *Gardnerella vaginalis*, as evidenced by the similarity of the profile with that of the placebo ring containing no metronidazole. After 4 h, only the 1000 mg and 2000 mg metronidazole rings had provided a significant decrease (one order of magnitude) in the number of surviving organisms. However, after 24 h, the 125, 250, 500, 1000 and 2000 mg rings provided a concentration-dependent kill, with the 1000 and 2000 mg rings providing complete kill.

Table 1 Effect of metronidazole-loaded vaginal rings on the viability of *Gardnerella vaginalis* ($e^a = \times 10^a$)

Ring type	No. of organisms surviving at time (h)				
	1	2	3	4	24
50 mg matrix	2.9e6	1.9e6	5.0e6	6.2e6	NT
125 mg matrix	1.7e6	1.3e6	3.1e6	4.8e6	5.8e4
250 mg matrix	1.9e6	1.4e6	2.2e6	3.4e6	3.3e3
500 mg matrix	6.2e5	1.3e6	1.6e6	1.3e6	1.3e3
1000 mg matrix	3.1e6	1.1e6	8.5e5	2.0e5	0
2000 mg matrix	1.1e6	1.3e6	1.8e5	4.3e4	0
500mg reserv.	1.9e6	3.1e6	3.7e6	5.6e6	1.3e9
Placebo	1.6e6	2.8e6	3.5e6	7.5e6	1.2e9

The study has demonstrated the potential of developing an intravaginal ring as an alternative treatment for bacterial vaginosis. In particular, a ring-based product would overcome many of the inherent disadvantages associated with the administration, use and acceptability of vaginal gel formulations.

Malcolm, R. K. (2003) The intravaginal ring. In: Rathbone, M. (ed.) *Modified-release drug delivery technology*. Marcel Dekker, New York, pp 775–790
Spiegel, C. A. (1991) *Clin. Microbiol. Rev.* **4**: 485–502

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Design of a silicone reservoir intravaginal ring for the delivery of oxybutynin

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Oxybutynin is the drug of choice in the treatment of urinary incontinence. However, it has a relatively low oral bioavailability (6%) due to an extensive first-past metabolism (Lukkari *et al* 1998) and a high incidence of adverse effects, observed in 30–80% of recipients (Brendler *et al* 1989), resulting in discontinuation in up to 25% of patients, depending on the dosage (Yarker *et al* 1995). This study reports on the design of an oxybutynin intravaginal ring (IVR) to overcome the disadvantages associated with current oral treatments.

Oxybutynin reservoir rings, containing various core lengths and drug loadings (Table 1), were prepared according to published methods (Woolfson *et al* 1999; Russell & Malcolm 2000). Quadruplicate in-vitro release studies under sink conditions were performed with daily sampling and replacement of the release medium. Quantitation of oxybutynin release was measured using UV-HPLC and the release constant D_pC_p was calculated for each ring. The solubilities of oxybutynin in silicone elastomer and fluid were also determined.

Table 1 Design parameters for oxybutynin IVR reservoir devices

IVR	No. of core segments	Core length (mm)	Core OXY loading (mg, % w/w)
OXY 1	1	140.0	100, 5
OXY 2	1	70.0	400, 40
OXY 3	1	35.0	200, 40
OXY 4	1	17.5	100, 40
OXY 5	2	8.75	100, 40
OXY 6	1	11.7	100, 60

Table 2 Release data for oxybutynin IVR reservoir devices

IVR	Day 1 burst (mg)	Mean daily release D3 to D14 (mg)	Day 1/MDR	$D_p C_p$ (mg cm^{-1} day^{-1})
OXY 1	42.74 ± 2.43	—	—	—
OXY 2	34.87 ± 1.86	17.26 ± 1.33	2.02	0.437
OXY 3	20.69 ± 1.61	10.00 ± 1.08	2.07	0.501
OXY 4	11.28 ± 1.10	5.11 ± 0.90	2.21	0.507
OXY 5	12.54 ± 1.19	5.35 ± 1.04	2.34	0.528
OXY 6	7.82 ± 0.82	2.87 ± 0.31	2.72	0.414

An unusually high initial burst release of oxybutynin was observed in-vitro with subsequent non-zero-order drug release (Table 2). Use of fractional cores substantially reduced the burst effect, yielding linear cumulative drug release versus time plots from days 2 to 14. Thus, a 1/8 fractional segment core gave a 24-h burst of 11.28 mg oxybutynin and, thereafter, zero-order release at the target dose of 5 mg daily over 14 days. Two oxybutynin cores, each 1/16 of full length, gave a greater release than a single 1/8 core, due to core segment end effects resulting in an increased surface area for release. The burst release was investigated by determining drug solubilities in the propan-1-ol product of elastomer condensation cure (390 mg mL^{-1}) and in the elastomer itself (13.9–20.21 mg mL^{-1}). These high oxybutynin solubilities were considered the major contributors to the burst effect. It was concluded that use of a fractional segment core would allow development of a suitable oxybutynin reservoir IVR.

Brendler, C. B., *et al*. (1989) *J. Urol.* **141**: 1350–1352
Lukkari, E., *et al*. (1998) *Pharmacol. Toxicol.* **82**: 161–166
Russell, J. A., Malcolm, R. K. (2000) *J. Chromatogr. B*. **744**: 157–163
Woolfson, A. D., *et al*. (1999) J. *Controlled Release* **61**: 319–328
Yarker, Y. E., *et al*. (1995) *Drug Ageing* **6**: 243–262

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In-situ formation of liposomes from proliposomes within a jet nebuliser

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The suitability of nebulisers for delivery of liposomal aerosols is well established. However, liposome instability has led to strategies such as freeze-drying to produce a stable product, deliverable from a nebuliser following rehydration (Bridges & Taylor 2001). In this study, we investigated whether proliposomes (Payne *et al* 1986) could be reconstituted within a nebuliser, to obtain an isotonic formulation of liposomes in-situ.

A pear-shaped flask containing 2 g sucrose carrier particles (300–500 μm) was attached to a modified rotary evaporator under vacuum, and held at 40°C. Egg (EPC) or soya (SPC) phosphatidylcholine (0.4 g) in chloroform (60 mg mL^{-1}) was added in volumes of 0.5–1 mL, through a pipette, and solvent removed under vacuum for 30 min.

Proliposomes (0.56 g) were added to 5 mL deionised water, at room temperature in a Pari LC Plus jet nebuliser, attached to a Pari Master compressor. The reservoir contents were immediately nebulised to dryness. At 1, 2.5, 5 10 and 15 min and at the end of nebulisation (25 min), samples were taken from the reservoir and the size distribution measured by laser diffraction (Malvern Mastersizer). Further samples were studied using light and transmission electron microscopy.

Table 1 Size of liposomes formed from proliposomes during nebulisation

Time (min)	EPC VMD (μm)	Span[a]	SPC VMD (μm)	Span[a]
1	8.38 ± 0.47	2.43 ± 0.47	7.71 ± 0.64	1.85 ± 0.09
2.5	7.33 ± 1.07	2.13 ± 0.49	6.64 ± 0.12	2.09 ± 0.17
5	5.87 ± 0.40	2.15 ± 0.12	4.71 ± 0.15	1.90 ± 0.07
10	3.61 ± 0.15	1.63 ± 0.28	3.42 ± 0.48	2.14 ± 0.56
15	2.97 ± 0.09	1.38 ± 0.08	2.81 ± 0.70	2.31 ± 1.17
25	4.08 ± 0.29	2.39 ± 0.23	3.81 ± 0.55	4.09 ± 2.01

Data are means ± s.d., n = 3.
[a] Span = (90% undersize, 10% undersize)/50% undersize

Microscopy showed multilamellar liposomes in samples at all time intervals, with no evidence of undispersed lipid or undissolved carrier. This was confirmed by laser diffraction which indicated a polydispersed distribution, with evidence of deaggregation and size reduction on nebulisation, up to 15 min. The VMD of liposomes from both lipids initially exceeded 7.5 μm, too large for efficient delivery. However, as for conventional liposomes in this nebuliser, these vesicles were reduced in size by shearing forces to a size appropriate for pulmonary delivery (Bridges & Taylor 2001). At the end of nebulisation, the size and size distribution of liposomes increased, as observed with conventional liposomes (Bridges & Taylor 2001). At all times SPC proliposomes formed liposomes with a smaller median size than EPC (significant ($P < 0.05$) at 5 min), suggesting this may be the lipid of choice for this proliposome formulation and nebuliser.

This study has shown that proliposomes generate liposomes within a nebuliser, without requiring prior hydration, and are a means of producing a stable, isotonic liposome formulation for nebuliser delivery.

Bridges, P. A., Taylor, K. M. G. (2001) *J. Pharm. Pharmacol.* **53**: 393–398
Payne, N. I., *et al*. (1986) *J. Pharm. Sci.* **75**: 325–329

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Effect of formulation variables on the formation and size of liposomes produced from proliposomes

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Proliposomes comprise soluble carrier particles coated with phospholipids, which generate liposomes on adding aqueous phase (Payne *et al* 1986a, b). This study investigated the effect of formulation variable on the formation, morphology and size of liposomes generated from proliposomes.

A pear-shaped flask containing 2 g sorbitol (porous) or lactose monohydrate (non-porous) granules (LMG) was attached to a modified rotary evaporator, held at 40°C under vacuum. Egg (EPC) or soy (SPC) phosphatidylcholine (0.1 or 0.4 g) in chloroform (60 mg mL^{-1}) was pipetted in portions of 0.5–1 mL. After 30 min, free flowing proliposomes resulted, which were stored at −18°C. A sample of proliposomes was hydrated under a light microscope to observe vesicle formation and morphology under static conditions. Proliposomes were also hydrated by addition to deionised water (5% w/v carrier) at room temperature, and particle size determined (Malvern Mastersizer).

Light microscopy showed liposomes instantly budded off from proliposomes on adding 1–3 drops of deionised water. Liposomes from LMG formed in 2 min and dispersed after 5 min aggregation, producing multilamellar liposomes (MLVs) and worm-like multi-vesicular structures. By contrast, MLVs formed from sorbitol proliposomes within 30 s, and dispersed rapidly, with no aggregation or worm-like structures. This may result from the greater aqueous solubility of sorbitol. For each carrier, the size fraction (106–300, 300–500 and 500–710 μm), PC type and PC:carrier ratio had no observed effect on the speed of liposome formation, or vesicle morphology.

Carrier size fraction did not affect liposome size ($P > 0.05$). For each carrier, EPC produced liposomes with larger VMD ($P < 0.05$) than SPC, for equivalent PC:carrier ratios (Table 1).

Table 1 Size of liposomes from proliposomes, 300–500 μm carrier

Proliposome composition (w/w)	VMD (μm)	Span[a]
EPC:sorbitol 1:20	5.87 ± 0.26	1.82 ± 0.02
EPC:sorbitol 1:5	6.83 ± 0.17	1.87 ± 0.03
SPC:sorbitol 1:20	4.81 ± 0.15	1.65 ± 0.04
SPC:sorbitol 1:5	6.09 ± 0.26	1.77 ± 0.05
EPC:LMG 1:20	5.41 ± 0.10	1.34 ± 0.01
EPC:LMG 1:5	6.26 ± 0.06	1.45 ± 0.05
SPC:LMG 1:20	4.95 ± 0.22	1.37 ± 0.10
SPC:LMG 1:5	5.49 ± 0.06	1.38 ± 0.01

Data are means ± s.d., n = 3.
[a] Span = (90% undersize, 10% undersize)/50% undersize

A PC:carrier ratio of 1:5 produced larger liposomes ($P < 0.05$) than 1:20. Sorbitol generally generated liposomes larger than those from LMG. This may be attributed to deposition of thicker PC films on the porous sorbitol surface than are formed on LMG or the larger surface area of LMG carrier, prepared from granulated small particles (40–100 μm).

Thus, liposomes bud off from proliposomes under static conditions, independent of the variables studied. Carrier type determined the rapidity of this process, and morphology of resultant liposomes.

The size of liposomes produced depends on PC type, PC:carrier ratio, and carrier type, though carrier size fraction had no effect.

Payne, N. I., *et al.* (1986a) *J. Pharm. Sci.* **75**: 325–329
Payne, N. I., *et al.* (1986b) *J. Pharm. Sci.* **75**: 330–333

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A solution calorimetry study of the interaction between phospholipids and non-ionic surfactants

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The understanding of phospholipids–surfactant interactions is important for optimising liposomal drug delivery systems, since incorporating surfactants may improve their systemic circulation half-lives (Allen & Chonn 1987), while at high surfactant concentrations liposomal phospholipid is solubilised. Study of surfactant-induced solubilisation also contributes to our understanding of the in-vitro stability of phospholipid bilayers in the presence of amphiphilic additives and degradation products (e.g. lysophospholipids) capable of partitioning between water and the phospholipid membrane.

In this study, the interaction of DMPC with surfactants, $C_{10}(EO)_n$, was investigated by solution calorimetry. Co-films containing DMPC and surfactant were formed in solution calorimetry ampoules. The components were weighed into an ampoule and dissolved in chloroform which was evaporated to deposit a co-film. Co-films were formed with 30 mg DMPC and increasing amounts of $C_{10}(EO)_3$ or $C_{10}(EO)_5$ to give final concentrations of 15, 30, 50, 70 and 90 mol % surfactant. The co-films were mixed with 100 mL deionised water in the solution calorimeter (Thermometric, Sweden) at 37°C. The temperature change associated with the process was recorded, and the heat evolved by the reaction calculated. The response of pure DMPC films or surfactants at 37°C was also measured, providing thermodynamic data on the formation of phospholipid vesicles and surfactant micelles, respectively.

The formation of DMPC vesicles (0 mol % surfactant) produced an endothermic response whereas the formation of surfactant micelles (100 mol % surfactant) was exothermic (Table 1). For DMPC-$C_{10}(EO)_n$ mixtures, the enthalpy values decreased with increasing amounts of surfactant. A reduction in the enthalpy values was related to either a decrease in endothermic responses (e.g. formation of DMPC vesicles) or an increase in exothermic reactions (e.g. formation of micelles), or both.

Table 1 Enthalpies of reaction of DMPC-$C_{10}(EO)_n$ co-films in water at 37°C

Concn (mol % $C_{10}(EO)_n$)	Enthalpy (J g^{-1}) DMPC-$C_{10}(EO)_3$	DMPC-$C_{10}(EO)_5$
0	48.9 ± 6.0	48.9 ± 6.0
15	42.2 ± 4.2	45.7 ± 5.1
30	45.6 ± 3.8	32.9 ± 2.5
50	27.2 ± 6.4	26.3 ± 3.0
70	7.6 ± 0.1	−20.4 ± 1.1
90	−31.8 ± 0.1	−41.1 ± 6.1
100	−50.5 ± 0.3	−68.4 ± 0.7

Data are means ± s.d., n = 3

The solution calorimetry data were consistent with a theoretical three-stage model for the solubilisation of phospholipids by surfactants, proposed by Lichtenberg *et al* (1983). Initially, mixed bilayers were formed up to the ratio where phospholipid bilayers were saturated with surfactant. With increasing amounts of surfactant, surfactant-saturated bilayers and lipid-saturated micelles coexisted, up to the solubilising surfactant to phospholipid ratio (R_e^{sol}). Beyond R_e^{sol}, only mixed micelles were formed. Each phase boundary described was identified as a break point in the enthalpy-concentration curve, with the phospholipid bilayer being most disrupted by $C_{10}(EO)_5$.

Allen, T. M., Chonn, A. (1987) *FEBS Lett.* **223**: 42–46
Lichtenberg, D., *et al.* (1983) *Biochim. Biophys. Acta* **737**: 285–304

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

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Self-curing polymethylmethacrylate (PMMA) based bone cements are extensively used in orthopaedic surgery for the attachment of metal (prosthesis) to living bone. Although joint replacement surgery is often successful there are frequent problems of joint infection, resulting in removal and replacement of the prosthesis, which causes both increased stress for the patient and increased costs to the NHS. In order to aid in the prevention of joint infection it has become common practice to add gentamicin (an antimicrobial drug) to the bone cement mix. However, the addition of gentamicin does not always overcome joint infection as the antibiotic is only released from the surface and superficial layers of the cement resulting in an uncontrolled and erratic release profile (Torrado *et al* 2001). Subsequently, the aim of our research is concerned with sustained and controlled release of antimicrobial agents from bone cements by the synthesis of drug-polymer conjugate systems. A range of drug-conjugate monomers have been designed upon a model whereby they all contain the following groups:

a polymerisable head group (P) for co-polymerisation with MMA;

a functional spacer; an antimicrobial drug (D) — the quinolone antibiotics have been used as they possess carboxylic acid functionality that can be exploited synthetically and are active against typical clinically isolated pathogens;

a neighbouring group (NG) moiety as part of the spacer — by altering the nature, distance and spatial arrangement of this nucleophilic group, the ester hydrolysis of the bond linking drug and the spacer can be controlled, thereby achieving controlled drug release of the antimicrobial drug.
An extensive synthetic scheme has been implemented to produce several nalidixic acid-conjugated monomers. Each of the monomers contains differing neighbouring group participants which affect and control the release of nalidixic acid from the conjugated systems. Essentially, a system has been developed whereby either one monomer, or several different monomers, can be synthetically linked (copolymerised) to the polymeric bone cement to provide a controlled release of nalidixic acid over a required and sustainable time period.

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Torrado, S., *et al.* (2001) *Int. J. Pharm.* **217**: 57–69

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Gentamicin release from bone cement materials

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Bacterial bone infection is a serious complication that has been implicated in 22% of total hip replacement (THR) operations (Tunney *et al* 1998) and in as many as 40% of repeat operations (Lachiewitz *et al* 1996). The incidence of infection requires revision surgery, whereby the prosthesis is removed and replaced, which leads to increased discomfort to the patient and further costs to the NHS. In order to minimise the occurrence of infection it has become common practice to add an antibiotic (usually gentamicin) to the cement formulation. Therefore, as well as providing a support medium for the prosthesis, the polymethylmethacrylate (PMMA) bone cement matrix must also act as drug delivery medium for the incorporated antibiotic. However, despite the widespread clinical use of drug-loaded bone cements, the release profile of gentamicin and the subsequent clinical efficacy of the cement are highly questionable (van de Belt *et al* 2000).
To date there are several contradictory reports regarding the effectiveness of the elution of gentamicin from a variety of bone cements. So, in this study the gentamicin release was examined from bone cement materials that were formulated under controlled conditions, as opposed to other literature studies, which have examined various differing commercial cements. A novel formulation procedure involving MMA, benzoyl peroxide and dimethyl-4-toluidine was used which induced rapid polymerisation and resulted in a uniform dispersion of gentamicin throughout the PMMA film. Gentamicin sulfate was incorporated in the films at concentrations of 1, 2 4 6 and 10% w/w. In conjunction with the release studies, the surface of the bone cement materials were also examined by confocal microscopy both before and after dissolution.
The results of this study demonstrated a poor release profile of gentamicin from the bone cement materials. It was found that 85% of the total amount of gentamicin released was eluted rapidly within the first 24 h of the 7-week period studied, followed by low concentrations released slowly thereafter. Also, it was estimated that no more than 2% of the total amount of gentamicin incorporated was eluted from the cements. Confocal microscopic images of cements that had been in dissolution for the 7-week period revealed a significant quantity of pores, voids and cracks in the surface of the cements, which were found to increase in approximate proportion with gentamicin drug loading. The formation of pores and cracks allows a low release of gentamicin from within their structure long after the initial burst release of gentamicin from the surface of the cement. They could also aid towards further cracking and subsequently mechanical failure of the bone cement if placed under stress.

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Tunney, M. M., *et al.* (1998) *J. Bone Joint Surg.* **80**: 568–572
van de Belt, H., *et al.* (2000) *Biomaterials* **21**: 1981–1987

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Preparation and characterisation of DNA loaded non-ionic surfactant vesicles

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Non-ionic surfactant vesicular delivery systems (niosomes) are formed by the self-assembly of non-ionic surfactant amphiphiles in the presence of aqueous media (Vanlerberghe *et al* 1973) and similar to liposomes these systems have been shown to effectively entrap and deliver DNA vaccines (Perrie *et al* 2002). Here, we further investigate the preparation of niosome-incorporated DNA.
Niosomes composed of 16 μmol 1-monopalmitoyl-rac-glycerol, 8 μmol of cholesterol, 4 μmol of 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine and 2 μmol of cholesteryl 3∃-N-(dimethyl amino ethyl) carbamate were prepared based on dehydration-rehydration method (Perrie & Gregoriadis 2000), which involves freeze-drying preformed small unilamellar vesicles (SUV) in the presence of DNA and rehydrating, under controlled conditions with distilled water. To investigate this method, DNA was added at various alternative stages of the protocol: 1. SUV with no DNA added (Empty SUV); 2. SUV mixed with DNA (SUV+DNA); 3. Dehydration and rehydration of SUV (Empty DRV); 4. Dehydration and rehydration of SUV+DNA (DRV(DNA)); 5. Dehydration of SUV and rehydration with DNA solution (DRV+DNA_1); and 6. Empty DRV mixed with DNA (DRV+DNA_2). Where indicated 200 μg of the plasmid DNA encoding the hepatitis B surface antigen (pRc/CMV HBS) was added. Niosome *z*-average diameter and zeta potential was measured on a ZetaPlus (Brookhaven Instruments) in ddH_2O and 0.001 M PBS respectively and DNA incorporation analysed by gel electrophoresis.
Results showed that of the six niosome preparations, empty SUV have the lowest vesicle size (87 nm) and the highest zeta potential (37 mV), however on addition of DNA (SUV+DNA) the vesicle size increased and the zeta potential reduced (225 nm; 26 mV, respectively) presumably due to complexation of anionic DNA with the cationic SUV. After freeze-drying of SUV and rehydration, larger multilamellar DRV formed, with the presence of DNA, further increasing vesicle size. In the case of DRV the method of preparation influenced the vesicle size of niosomes containing 2 μmol of DC-Chol with a trend of increasing vesicle size in the order empty DRV < DRV(DNA) < DRV+DNA_1 < DRV+DNA_2 (879 nm, 1648 nm, 1855 nm, 2068 nm, respectively). Niosomal DNA encapsulation efficacy was also studied; comparing the intensities of migrated unencapsulated DNA after gel electrophoresis revealed encapsulation efficiency being in the order of DRV(DNA) > DRV+DNA_1 > DRV+DNA_2 > SUV+DNA. In conclusion, these results suggest that similar to DRV liposomes, the dehydration and rehydration of SUV niosomes in the presence of DNA results in the formation of structures with higher DNA incorporation efficiency and in some cases smaller sizes than niosome-DNA complexes. Investigations into the properties of these niosomal DRV(DNA) continue.

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The effect of liposome preparation on the incorporation of DNA within liposomes

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Liposome-mediated DNA vaccination has been shown to enhance both humoral and cell-mediated immune responses, to encoded antigens by the intramuscular, subcutaneous and oral routes (Gregoriadis 1998; Perrie *et al* 2002). Here, we assess several liposomal/DNA systems for their ability to entrap DNA within the

liposome and retain their vaccine payload in the presence of competitive anionic components.

Six liposomal systems, composed of 16 μmol egg phosphatidylcholine, 8 μmol cholesterol and 4 μmol of the cationic lipid cholesterol 3∃-*N*-(dimethyl-aminoethyl)carbamate were prepared (Perrie & Gregoriadis 2000): Small unilamellar vesicles (empty SUV), SUV complexed with DNA, dehydration-rehydration vesicles with no DNA present (empty DRV), preformed empty DRV complexed with plasmid DNA (DRV−DNA), DRV prepared by rehydration with the DNA solution (DRV−[DNA]) and lastly DRV prepared with DNA present during dehydration and rehydration (DRV(DNA)). Where DNA was incorporated, 100 μg of the plasmid encoding the Hepatitis B surface antigen (pRc/CMV HBS) was added. The *z*-average diameter and zeta potential of the each liposomal system was measured on a ZetaPlus (Brookhaven Instruments) in ddH_2O and 0.001 M PBS, respectively.

The addition of DNA to cationic SUV led to the formation of complexes, larger than the original empty SUV (98 nm vs 259 nm, respectively), with a reduced zeta potential (49 mV vs 37 mV, respectively). This decrease in zeta potential reflects neutralisation of the vesicles cationic surface due to adsorption of the DNA. In contrast, empty DRV and DRV dehydration and rehydrated in the presence of DNA (DRV(DNA)) have similar mean z-average diameters (884 nm vs 889 nm, respectively) and zeta potential (54 mV vs 53 mV, respectively), suggesting that in the latter case a high proportion of the DNA is entrapped within the vesicles with little surface neutralisation occurring. Further, the presence of DNA during the dehydration and rehydration steps was shown to be advantageous; rehydration of dehydrated empty SUV with a solution of DNA (DRV-[DNA]) results in the formation of larger vesicle structures (1655 nm) which have a lower zeta potential (47 mV) in comparison with DRV(DNA) (889 nm; 53 mV). The spatial location of the DNA within these systems was further investigated using gel electrophoresis in the presence of sodium dodecyl sulphate that, at the concentrations employed, competes and releases surface bound DNA (Perrie & Gregoriadis 2000). Under these conditions, little DNA was displaced DRV(DNA) compared with the other preparations again suggesting that such DRV are likely to incorporate most of their DNA within closed bilayers. In conclusion, this data suggests that the dehydration and rehydration of liposomes in the presence of DNA produces structures where the DNA is predominantly entrapped within the liposomes and protected against displacement and degradation in biological milieu.

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Investigation of absorption enhancing effects of Acanthophyllum total saponin on intranasal delivery of insulin and blood glucose levels in rat

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Insulin as a protein drug plays a vital role in the treatment of type I diabetes melitus and at present it is administered only parenterally. Due to permanent and daily need of patients to this drug, injection route is not convenient and has many disadvantages (Hinchcliffe & Illum 1999). Therefore it is important to study and investigate other possible routes for insulin delivery. Intranasal route has been studied for several non-peptide and peptide drugs including insulin in recent years and various absorption enhancers have been examined to obtain a proper bioavailability (Illum 2003).

The aim of this research was to investigate the effect of Acanthophyllum total saponin (ATS) on intranasal insulin absorption and to compare it with two other enhancers — Quilaja total saponin (QTS) and sodium cholate (SC) — in rat.

ATS was derived from the root of Acanthophyllum squarrosum by several extraction steps with methanol and n-buthanol, partially purified and finally freeze dried. Blood glucose levels in five fasting rats was determined by a stripe glucometer (Sensorex, Apex Corp.) after instillation of 40 μL solution containing 2 IU regular insulin in the presence of 1% enhancer concentrations into the rat's nostril. The decrease in blood glucose levels due to various enhancers was measured in different time points after instillation and used as an indication of insulin absorption. The decreasing effects of the three enhancers was compared statistically (analysis of variance followed by Tukey-Kramer) with that of control (buffer only) with $P < 0.05$ as significant.

The results showed that ATS has a noticeable effect and can be compared with QTS and SC. Percentage lowering in initial blood glucose concentration against time showed that ATS has a stronger effect than the two other enhancers although the difference was not significant statistically ($P > 0.05$).

130 min after instillation of 20 μL solution containing insulin and different absorption enhancers into the right nostril of rat, the percentage decrease in initial blood glucose were as follows: 72.46% (± 2.39 %) for ATS, 63.22% (± 11.06%) for QTS and 60.06% (± 14.93%) for SC, all significantly different from that of control with either $P < 0.01$ or $P < 0.001$.

It can therefore be concluded that ATS has a considerable absorption enhancing effect and can possibly be used to increase insulin bioavailability via the nasal route.

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Effect of PLA and PLGA blending on drug release from microspheres

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Poly(lactide) and poly(lactide-co-glycolide) polymers have been extensively studied for the preparation of biodegradable microspheres. For wide applications in controlled drug delivery, it is imperative that a range of rates and durations of drug release be achievable. It is possible to obtain a certain copolymer with desired degradation rate and desired drug release profile by careful manipulation of key variables during PLGA copolymer synthesis. The disadvantages of this method are that the the polymerization process is rather difficult and time consuming and needs several analysis steps of the final product.

In this study, attemps were made to control the drug release rate from microspheres by blending different types of PLA and PLGA which are abundant commercially available.

Microspheres containing naltrexone, an opiate antagonist, were prepared by emulsification-solvent evaporation technique. Appropriate amount of naltrexone and different ratios of poly(l-lactide) (MW 579 000 , Resomer L210) (PLLA) and poly(lactide-co-glycolide)50:50 (MW 34 000 , Resomer RG503) (PLGA 50:50) , were dissolved in dichloromethan, emulsified in 0.5%w/w poly(vinylalcohol) solution and stirred up to 4 h until compelete evaporation of dichloromethan and formation of microspheres.

Morphology of microspheres were studied using scanning electron microscopy.

In-vitro drug release studies were conducted in phosphate saline buffer (pH 7.4) in a waterbath at 37°C.

For fabrication of microspheres and control of drug release rate, two different polyesters from lactide–glycolide family were used: semicrystalline poly(l-lactide) which has the most hydrophobic property and slowest degradation rate (about 1.5–2 years) and the opposite one, poly(lactide-co-glycolide) 50:50 which due to the presence of large number of GA units has a high rate of water penetration and degrade within 50–60 days.

Scanning electron micrographs of naltrexone microspheres prepared with PLLA or PLGA 50:50 or blend of both polymers, revealed spherical and nonporous structure.

Investigation of drug release from microspheres composed of PLGA 50:50 with 20%w/w naltrexone loading, showed that these microspheres had a rapid drug

release profile and released about 80% of their drug content in 7 days. On the other hand, microspheres prepared with PLLA and 20%w/w drug loading, released only about 26% of drug content in the same time. By blending these two polymers, microspheres were prepared with PLLA:PLGA 50:50 ratio of 20:80, 40:60, 60:40 and 80:20. In these series, by increasing the PLGA ratio, drug release rate increased which could be contributed to further water penetration into microspheres. Another reason, which was clearly observed during SEM studies, is that due to rapid degradation of PLGA 50:50, numerous pores and channels developed in the microspheres which led to faster drug diffusion through these channels. A simillar effect were observed in formulations with 40%w/w drug content.

By blending of different types of PLA and PLGA with different ratio, it is possible to obtain microspheres which release their drug content in a desired rate and profile.

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Photolytic release of ibuprofen from a 3,5-dimethoxybenzoin ester within a p(HEMA-co-MMA) polymer matrix

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Photochemically-removable protecting groups have several applications in bioorganic chemistry. They provide deprotection that can be accomplished under conditions that leave most other protecting groups untouched; they can also be used in the technique of caging. The design and synthesis of benzoin esters has been investigated in recent years in an attempt to create new photolabile protecting groups in synthesis and combinatorial chemistry (Baldwin *et al* 1990). When a 3,5-dimethoxybenzoin system is employed, maximum quantum yield of a photo-removable carboxylate protecting group is achieved. The process is further advantageous in that the only by-product of the reaction is the potentially biologically benign 2-phenyl-5,7-dimethoxybenzofuran. Previous work investigated the photochemical release of model drugs with carboxylic acid functionality from 3,5-dimethoxybenzoin esters.

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 (Eschbach & Huang 1994). One method of improving the mechanical strength of pHEMA is to employ copolymerisation with methyl methacrylate (MMA). pMMA has been used in medical devices where conditions of rigidity are required, for example in stents (Jones *et al* 1997). In addition to improving the mechanical strength of the polymer, it was anticipated that the presence of the hydrophobic MMA would regulate the release of model drugs such as ibuprofen from the polymer matrix.

This study investigated the incorporation of benzoin esters into novel crosslinked hydrogels comprising 5% 3,5-dimethoxybenzoin ibuprofen ester (w/w) and 93% (w/w) 2-hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) (w/w) in ratios of 90:10 and 70:30. MMA was washed four times with aqueous NaOH (2%) and deionised water in order to remove added inhibitor. The inhibitor-free monomer was dried using anhydrous $MgSO_4$. The photolabile ibuprofen ester was dissolved in the HEMA/MMA solution and polymerised via free radical polymerisation with 1% (w/w) benzoyl peroxide initiator and 1% (w/w) ethylene glycol dimethacrylate (EGDMA) at 90°C for 2 h. The polymers were soaked for 72 h in deionised water in order to remove any unreacted monomer and to hydrate the polymers. Using a cork borer discs were cut from the hydrogel and irradiated in 10 mL of methanol:water (80:20 v/v) 20% methanol solution. Subsequent drug release into the same solvent was characterised using UV-visible spectroscopy. The other major product from drug release experiments was 2-phenyl-5,7-dimethoxy-benzofuran. However, due to the size and hydrophobicity of this molecule it was found to not be released from the hydrogel network. The results also show that increasing the percentage of MMA in the hydrogel prolonged the release time of ibuprofen from the system.

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Release study of rifampicin from polycaprolactone films

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Problems associated with medical devices include bacterial adherence and subsequent biofilm formation, which may ultimately lead to device related infection and removal of device. A possible means of overcoming this problem is to coat the device with a biodegradable polymer, in which surface adherent microorganisms are shed as the polymer degrades hence providing a renewable (biomimetic) surface. Resistance to medical device infection may be enhanced further by the incorporation of an antimicrobial agent within the biomaterial. In this study the antibiotic rifampicin was incorporated into films containing poly-caprolactone (PCL) and polyethylene glycol (PEG 200). This study presents release properties of rifampicin from these potentially novel coatings for medical devices.

Polymeric films were prepared by dissolving PCL (10% w/v), rifampicin (1, 3 and 5% w/w) and PEG (0, 5, 10 and 15% w/w) in dichloromethane with mechanical stirring. Samples (10 mL) of the polymeric solution were then cast onto glass petri dishes and the solvent allowed to fully evaporate at room temperature.

Rifampicin release was studied by placing $1cm^2$ pieces of the films in 10 mL PBS (pH 7.4) in a sealed vial in a shaking water bath (37°C, 100 osc min^{-1}). The film samples were removed and each placed in a vial containing 10 mL of fresh pre-warmed buffer at predetermined times to ensure sink conditions were maintained. The mass of rifampicin released as a function of time was determined using UV spectrophotometry (λ_{max} 475 nm). Table 1 shows the cumulative release of rifampicin after 3 weeks and the percentage of incorporated drug released. The effects of PEG concentration and rifampicin concentration on the mass and % drug release at defined times were statistically evaluated using a two-way analysis of variance ($P < 0.05$ denoted significance).

Table 1 Cumulative (± s.d.) and percentage release of rifampicin over 21 days

% PEG	Cumulative release of Rifampicin ($\mu g/cm^2$)		
	3% RIF	5% RIF	10% RIF
0	225.01 ± 5.2	454.11 ± 4.1	547.36 ± 6.3
	(47.7%)	(57.7%)	(34.8%)
5	211.78 ± 3.2	485.10 ± 4.2	552.79 ± 5.3
	(44.9%)	(61.7%)	(35.2%)
10	231.58 ± 4.2	364.50 ± 7.2	672.86 ± 8.2
	(44.8%)	(46.4%)	(42.8%)
15	185.85 ± 6.1	343.31 ± 5.0	657.94 ± 10.0
	(39.3%)	(43.6%)	(41.8%)

The concentration of PEG was found to significantly affect drug release. Increasing rifampicin concentration in the films significantly increased the mass of drug released. The effect of PEG on rifampicin release was dependent on the concentration of rifampicin present in the films. This dependence of rifampicin release on the concentration of PEG and the lack of correlation between rifampicin concentration and PEG concentration would suggest that the location of the drug in the PCL films differs at lower (3%) and higher concentrations (10%). Accordingly, at higher drug loadings, rifampicin may partition within the PEG domains in the polymer to a greater extent than whenever lower concentrations of this drug are employed. The greater distribution of drug in PEG resulted in greater

drug release. The release of rifampicin coupled with the degradation properties of the PCL indicates that these film formulations have potential as medical device coatings.

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Physicochemical properties of novel degradable coatings for medical devices

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The adherence of bacteria to implanted medical devices can lead to device related infection. A possible means of overcoming this problem is to coat the device with a biodegradable polymer, in which surface adherent microorganisms are shed as the polymer degrades hence providing a renewable surface. Resistance to medical device infection may be enhanced further by the incorporation of an antimicrobial agent within the biomaterial. However, as the mechanical and surface properties of the coating are important considerations in medical device performance, it is important that these properties are not compromised by the presence of therapeutic agents. In this study the effect of rifampicin on the mechanical, surface and biodegradation properties of polycaprolactone, a biodegradable polymer that has attracted attention as a medical device coating biomaterial, are presented.

The films were prepared by dissolving polycaprolactone (PCL 10% w/v) and rifampicin (1, 3 and 5% w/w) in dichloromethane with mechanical stirring. Ten-mL samples of the polymeric solution were then cast onto glass petri dishes and the solvent allowed to fully evaporate at a constant pressure and temperature.

Biodegradation was assessed by placing quadruplicate samples in a shaking water bath (60 osc min^{-1}) containing phosphate buffer (pH 7.4) at 37°C. The samples were removed at defined periods, dried at a reduced pressure for 72 h and the percentage weight loss determined gravimetrically. Surface hydrophobicity was determined using a Cahn Dynamic Contact Angle Analyser using distilled water as the wetting medium. Tensile properties of the films were analysed using a SMS TA-XT^2 Texture Analyser a technique that allows ultimate tensile strength (UTS), % elongation at break and Young's modulus to be calculated (Jones *et al* 2002). Table 1 illustrates the physicochemical properties of the PCL films incorporating increasing concentrations of rifampicin. The effect of rifampicin on the physicochemical properties of PCL were statistically evaluated using a one-way analysis of variance (P 0.05 denoted significance).

Table 1 Physicochemical and degradation properties of candidate PCL/Rif biomaterials

	% Original wt after 20 wks	Advancing contact angle	% Elongation at break	UTS (MPa)	Young's modulus (MPa)
Rif 0%	99.48 ± 3.74	84.44 ± 3.74	15.36 ± 1.74	15.76 ± 4.8	6.48 ± 0.25
Rif 1%	98.97 ± 0.38	86.37 ± 8.63	11.43 ± 1.06	12.04 ± 1.2	6.22 ± 1.80
Rif 3%	95.60 ± 0.93	88.98 ± 4.04	9.39 ± 1.04	11.80 ± 0.9	4.64 ± 2.92
Rif 5%	93.21 ± 1.62	90.38 ± 4.94	7.89 ± 1.21	11.98 ± 1.8	6.14 ± 1.20

Data are means ± s.d., $n \geqslant 4$

Increasing concentrations of rifampicin increased the degradation and surface hydrophobicity of the films. The % elongation of the films decreased with increasing rifampicin concentration whereas the UTS decreased on addition of rifampicin but did not alter significantly with increasing drug concentration. While the addition of the antibiotic rifampicin altered degradation and surface hydrophobicity of PCL, the alterations to the mechanical properties were limited. Therefore, as the inclusion of rifampicin did not compromise the physicochemical properties of PCL, this antimicrobial agent may be useful in the design of antimicrobial biodegradable coatings based on this polymer.

Jones, D. S., *et al.* (2002) *Biomaterials* **23**: 4449–4458

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Physicochemical properties of interpenetrating networks of macroporous hydroxyethylmethacrylate and methacrylic acid

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Polyhydroxyethylmethacrylate (pHEMA) is one of the most widely studied hydrogels for use as a medical device biomaterial due, in part, to the known biocompatibility (Rao *et al* 1994). More recently macroporous pHEMA (macrohema) has been advocated as a novel biomaterial however the poor mechanical properties of this material limit its usefulness. Therefore, this study examined the physicochemical properties of interpenetrating polymer networks (IPNs) of macrohema and methacrylic acid (MA), designed as a means to improve the mechanical properties of macrohema.

Initially macrohema was produced by addition of 0.5% AIBN to a solution containing 0.9% NaCl and HEMA in a ratio 60:40 by weight and polymerised at 60°C for 18 h. IPNs were formed by immersion of sheets of macroporous pHEMA in a 90% MA aqueous solution (containing 0.5% AIBN) for specific times which corresponded to 30, 40, 50, 60 and 70% MA content, following which polymerisation was performed again as described above. The glass transition (Tg), ultimate tensile strength (UTS), % elongation at break (% elong), Young's modulus and dynamic (advancing and receding) contact angles of the IPNs were analysed using dynamic mechanical thermal analysis, tensile and dynamic contact angle analysis, respectively, as previously described (Jones *et al* 1997). Buffer (pH 7.4) uptake was examined using gravimetric analysis. The effect of polymer composition on buffer uptake, mechanical properties and advancing/receding contact angles were evaluated using a one-way analysis of variance ($P < 0.05$ denoting significance).

Table 1 Physicochemical properties of macrohema/MA IPNs

IPN Hema:MA ratio	UTS (MPa)	Tg (°C)	% Elong	Adv. contact angle	Youngs mod (KPa)
Macrohema	0.37	116.5	120.5	70.9	0.553
	± 0.10		± 5.75	± 3.46	± 27.4
IPN 70:30	8.9	190.9	108.5	53.3	77.87
	± 0.15		± 2.44	± 1.09	± 3.5
IPN 60:40	10.2	199.5	96.7	46.9	109.51
	± 0.23		± 5.78	± 0.72	± 3.80
IPN 50:50	8.9	198.6	122.9	42.5	80.57
	± 0.22		± 6.12	± 0.47	± 5.08
IPN 40:60	7.0	206.8	143.8	40.7	29.47
	± 0.15		± 4.17	± 1.81	± 1.12
IPN 30:70	6.3	205.2	169.7	33.8	16.59
	± 0.26		± 5.38	± 0.57	± 3.98

Data are means ± s.d.

IPNs exhibited greater tensile strength, Young's modulus and greater hydrophilicity than macrohema. These properties offer a significant improvement over macrohema as medical device biomaterials. IPNs produced form 40% MA displayed the most favourable properties in terms of tensile strength and elasticity. The glass transition temperature also increased gradually as the % of MA was increased and approached that of pure MA, indicative of network formation. Buffer uptake was dependent on the concentration of MA within the IPNs.

In conclusion, this study highlighted the potential of IPNs composed of macrohema and MA to improve mechanical and surface properties of the parent polymers. These improved properties will enhance the utility of these polymers as medical device biomaterials.

Jones, D. S., *et al.* (1997) *J. Mat. Sci.: Mat. Med.* **8**: 713–717
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Release study of rifampicin from interpenetrating networks of methacrylic acid and macroporous polyhydroxyethylmethacrylate

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Infection is a significant problem associated with indwelling medical devices (Finkelstein *et al* 2000). One approach to overcome this problem involves coating or impregnating the device with an appropriate anti-microbial agent. Hydrogels (e.g., polyhydroxyethylmethacrylate (pHEMA)) are frequently employed as coatings of medical devices. More recently an interest has developed in the use of macroporous pHEMA as a medical device coating. This study examined the release of rifampicin, a model antimicrobial agent with demonstrated efficacy against bacteria associated with device related infection (Monzon *et al* 2002), from interpenetrating polymer networks (IPNs) based on macroporous pHEMA and methacrylic acid (MA).

Initially, macroporous pHEMA was produced by addition of 0.5% AIBN to a solution containing 0.9% NaCl and HEMA in a ratio 60:40 by weight. IPNs were formed by immersion of sheets of macroporous pHEMA in a 90% MA solution (0.5% AIBN) for specific times which corresponded to 30, 40, 50, 60 and 70% MA content. Following this polymerisation of MA within pHEMA was performed again as described above. The drug was loaded into the various materials by immersion of freeze-dried discs of each material in an aqueous solution of rifampicin (50 mg/100 mL) for 24 h. The effect of material composition on the mass of drug released as a function of time was statistically evaluated using a one-way analysis of variance ($P < 0.05$ denoted significance).

Table 1 Cumulative rifampicin (μg) release (± s.d.) from various IPN compositions over 24 h

	1 h	2 h	4 h	8 h	24 h
Hem/MA 70:30	8.17 ± 0.88	8.4 ± 0.01	9.41 ± 0.01	10.4 ± 0.01	11.1 ± 0.18
Hem/MA 60:40	17.4 ± 1.17	22.7 ± 5.3	28.6 ± 5.87	33.7 ± 5.1	36.2 ± 2.5
Hem/MA 50:50	25.3 ± 2.3	29.9 ± 0.4	37.87 ± 2.7	43.2 ± 0.8	50.12 ± 0.8
Hem/MA 40:60	18.6 ± 0.7	28.1 ± 0.7	38.1 ± 0.8	41.9 ± 0.8	43.4 ± 1.1
Hem/MA 30:70	16.1 ± 1.1	22.2 ± 0.4	26.8 ± 1.1	27.3 ± 0.4	28.3 ± 0.7

Drug loading and subsequent release of rifampicin were dependent on the composition of the IPN. There was a correlation between buffer uptake, drug loading and mass of drug released. Maximum drug release and drug loading were observed with the IPN composed of equal ratios of pHEMA and MA. Confocal microscopy of the drug loaded IPNs confirmed that rifampicin was deposited on the surface of the materials and therefore the greater swelling of certain IPNs resulted in a larger surface coverage and not greater depth of drug penetration. Therefore, it is likely that the large molecular weight of rifampicin (823 g mol^{-1}) inhibited diffusion into the polymer network. Whilst these biomaterials offer promise for the release of rifampicin to control microbial biofilm formation, the IPNs may be more suitable for the incorporation (and hence release) of lower molecular weight antimicrobial agents.

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Formulation and characterisation of novel, bioadhesive, binary polymeric gel networks for the treatment of xerostomia

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Xerostomia is a condition characterised by the lowered production or absence of saliva. One method of treatment of xerostomia involves the use of polymeric gel systems (Davis 2000). Bioadhesive/mucoadhesive formulations have received increasing interest since they have the ability to adhere to mucosal surfaces thus increasing their residence at the site of application, a property that would be advantageous for xerostomia. In this investigation, novel bioadhesive formulations containing poly methylvinylether-co-malaeic anhydride (PMVE/MA) or polyvinylpyrrolidone (PVP), with polyethylene oxide (PEO) were examined as potential formulations designed for the improved treatment of xerostomia.

Aqueous neutralised PMVE/MA:PEO formulations (100:0, 80:20, 50:50, 20:80 and 0:100), and PVP: PEO formulations (80:20, 50:50 and 20:80) at two polymer concentrations (10 and 20% w/w) were prepared using mechanical stirring. Dynamic (oscillatory) and continuous shear (flow) rheological analysis of all formulations were performed at 20 ± 0.1°C using a Carri-Med CSL^2–100 rheometer with a 4 or 6 cm diameter parallel plate geometry and a 1mm plate gap. Texture analysis and analysis of the mucoadhesive properties of all formulations were performed as previously described (Jones *et al* 1997, 2001). The effect of polymer concentration and ratio on the oscillatory, textural and mucoadhesive properties were statistically analysed using a two-way analysis of variance ($P < 0.05$ denoting significance). The rheological and mucoadhesive properties of selected formulations are shown in Table 1.

Table 1 Hardness, consistency (Power Law model) and mucoadhesive strength of 20% polymer concentration at range of PVME/MA ratios

PVME/MA	PEO	Hardness (N)[a]	Consist. (Pa s)[a]	Muco strength (N)[a]
20	80	4.62 ± 0.30	3053.8 ± 449.7	11.95 ± 0.11
50	50	1.94 ± 0.15	666.4 ± 36.6	6.85 ± 0.18
80	20	0.43 ± 0.30	10.6 ± 0.1	7.93 ± 0.54

[a] Mean ± s.d. of five replicate measurements

In all formulations viscosity decreased with increasing shear rate, indicative of pseudoplastic behaviour. Increasing the concentration of each polymeric component significantly increased the rheological (storage/loss moduli, dynamic viscosity) and textural parameters (hardness, adhesiveness and compressibility), with the exception of tan δ, which remained constant. These observations may be accredited to increased polymer entanglement. The PMVE/MA:PEO systems possessed greater rheological properties than their PVP:PEO counterparts. With the PMVE/MA:PEO systems, decreasing PEO concentration increased the mucoadhesive properties of the gels. Of the two gel systems studied, the PVME/MA:PEO systems demonstrated the greatest mucoadhesive properties. In conclusion, this study has demonstrated that by appropriate alteration of formulation composition, gels may be prepared that offer ideal textural, rheological and mucoadhesive properties for use as lubricants designed for the treatment of xerostomia

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Characterisation of bioadhesive, polymeric gel systems containing tetracycline, for the treatment of periodontal disease

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Gel based systems have become the focus of research as a treatment strategy for periodontal disease. Formulations containing poly methylvinylether-co-malaeic anhydride (PMVE/MA) and polyvinyl pyrrolidone (PVP) have shown promise as therapeutic drug delivery systems (Jones *et al* 2003). However, there is limited published information concerning the physicochemical properties of these systems and the effect of tetracycline on these properties. Therefore, the aim of this study was to investigate the textural, flow rheological and drug release properties of a range of formulations containing PMVE/MA, PVP and tetracycline, thus providing information, which may allow rational selection of formulations for use as periodontal drug delivery systems.

Neutralised gel formulations were prepared containing PMVE/MA (5 and 15% w/w) and PVP (10 and 15% w/w) by mechanical stirring, following which tetracycline (1 and 10% w/w) was manually dispersed in this system. Flow rheology was performed using a Carri-Med CSL^2–100 rheometer (2 cm parallel plate geometry, 1mm plate gap, 20 $\pm$ 0.1°C). Texture analysis was performed as previously described (Jones *et al* 1997a). In-vitro drug release studies into PBS (pH 7.4) were performed as previously reported by Jones *et al* (1997b). The effect of polymer and tetracycline concentration on the textural, rheological and release properties were statistically evaluated using a three way analysis of variance ($P < 0.05$ denoting significance). The textural and drug release properties of selected formulations are shown in Table 1.

Table 1 Hardness, compressibility and 50% release time for gels composed of 15% PVME/MA

PVP (% w/w)	Tetracycline (%w/w)	Hardness (N)[a]	Compress. (N mm)[a]	50% Release (h)[a]
10	1	0.97 $\pm$ 0.03	6.50 $\pm$ 0.87	3.86 $\pm$ 0.97
10	10	2.59 $\pm$ 0.98	4.60 $\pm$ 0.43	5.48 $\pm$ 0.21
15	1	6.78 $\pm$ 0.22	13.41 $\pm$ 1.62	5.10 $\pm$ 0.88
15	10	9.08 $\pm$ 0.19	14.81 $\pm$ 1.09	5.23 $\pm$ 0.17

[a] Mean $\pm$ s.d. of five replicate measurements

All formulations were found to exhibit pseudoplastic flow with minimal thixotropy. Increases in the concentrations of PMVE/MA and PVP were found to increase all textural (hardness, compressibility and adhesiveness) and rheological (zero-rate viscosity and consistency) properties. This increase was ascribed to a combination of increased polymer chain entanglement with complex formation occurring between PMVE/MA and PVP. All formulations exhibited controlled release of tetracycline with the time required to release 50% of drug ranging from 1.05 $\pm$ 0.08 to 5.97 $\pm$ 0.22 h. The release of tetracycline from these systems was complex, involving a combination of diffusion and polymer dissolution and/or swelling being.

This study has highlighted the potential of PMVE/MA–PVP/tetracycline systems for the management of periodontal disease. It also increases the fundamental understanding of the properties required to optimise the clinical performance of these systems.

Jones, D. S., *et al*. (1997a) *Int. J. Pharm.* **151**: 223–233
Jones, D. S., *et al*. (1997b) *Pharm. Res.* **14**: 450–457
Jones, D. S., *et al*. (2003) *J. Pharm. Sci.* **91**: 2090–2101

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Characterisation of the textural and rheological properties of bioadhesive polymer gels containing nystatin designed for the treatment of oral fungal infections

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Oral fungal infections have traditionally been treated locally with nystatin, azoles or chlorhexidine formulated as gels, lozenges or solutions. However, attention has been focused on the improved delivery of antifungal agents at the site of infection using bioadhesive gel platforms (Ghandi *et al* 1994). These systems offer several advantages over current therapies, including greater retention at the site of application, provision of local and controlled release of antimicrobial agents and decreased systemic side effects. The aim of this study was to texturally and rheologically examine a range of nystatin loaded, bioadhesive gels in order to increase fundamental understanding of their properties and hence optimise their clinical performance as a treatment for oral fungal infections.

Aqueous neutralised gels were prepared containing polymethylvinylether-co-maleic anhydride (PMVE/MA, 5 and 10% w/w) and polyvinylpyrrolidone (PVP 5 and 15% w/w) with the aid of mechanical stirring. Nystatin (0.5, 1 and 5% w/w) was manually dispersed into these systems. All formulations were characterised by continuous shear (flow) and dynamic (oscillatory) rheometry using a Carri-Med CSL^2–100 rheometer at 20 $\pm$ 0.1°C (2 or 4 cm parallel plate geometry, fixed gap of 1000 µm). Texture analysis of all formulations was performed as previously described (Jones *et al* 1997). The rheological and textural properties of selected formulations are shown in Table 1. The effect of polymer and nystatin concentration on these properties were statistically evaluated using a three-way analysis of variance ($P < 0.05$ denoted significance).

Table 1 Hardness, adhesiveness and consistency (Power Law model) for gels composed of 10% PVME/MA

PVP (% w/w)	Nystatin (% w/w)	Hardness (N)[a]	Adhes. (N mm)[a]	Consist (Pa s)[a]
5	1	1.09 $\pm$ 0.10	1.34 $\pm$ 0.09	461.3 $\pm$ 30.3
5	5	2.49 $\pm$ 0.60	2.22 $\pm$ 0.21	817.9 $\pm$ 57.7
15	1	4.19 $\pm$ 0.45	5.54 $\pm$ 0.26	2756.0 $\pm$ 139.2
15	5	7.33 $\pm$ 0.15	9.96 $\pm$ 1.09	10934.00 $\pm$ 667.11

[a] Mean $\pm$ s.d. of five replicate measurements

For all formulations the viscosity decreased with increasing shear rate, indicative of pseudoplastic behaviour. No evidence of thixotropy was observed. Increasing polymer and nystatin concentration resulted in an increase in the rheological (storage/loss moduli, dynamic viscosity, consistency) and textural properties (hardness, adhesiveness and compressibility), however tan δ decreased thereby denoting increased elastic nature. This may be attributed to an increase in interaction and entanglement of the polymer chains. The effect of nystatin on all parameters was dependent upon polymer concentration, and was more significant at higher polymer concentrations. Enhancement of the rheological properties due to the presence of nystatin may be ascribed to the increased semi-solid character of these systems.

Therefore this study has shown that PMVE/MA–PVP gel formulations may offer promise as platforms for the delivery of nystatin to the oral cavity.

Ghandi, R. B., *et al*. (1994) *Adv. Drug Del. Rev.* **13**: 43–74
Jones, D. S., *et al*. (1997) *Int. J. Pharm.* **151**: 223–233

113
Development of a novel hydrogel coating for the endotracheal tube (ET) to prevent ventilator-associated pneumonia (VAP)

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Microbial biofilm on the ET surface has been implicated in the development of VAP (Adair *et al* 2002). The difficulty in eradicating established microbial biofilm using parenteral antibiotics has led to the assessment of nebulised gentamicin as a preventative strategy for VAP with effective results (Adair *et al* 2002). This study describes a hydrogel coating to enhance the residence of gentamicin at the surface of the ET tube post-nebulisation and hence improve the efficacy of this procedure.

A series of hydrogels with varied 2-hydroxyethylmethacrylate:methacrylic acid (HEMA:MAA) ratios were prepared by free radical polymerisation (60°C, 18 h) and loaded with drug by soaking in a buffered gentamicin sulphate solution (Tris pH 7.4, 50 mg mL^{-1}). The mass of incorporated gentamicin was quantified by HPLC following extraction into buffer. The release of gentamicin was performed by immersion of samples in a buffer under shaking conditions at 37°C. At selected

time intervals a sample of the release medium was removed and the mass of gentamicin quantified using HPLC.

To determine resistance of the gentamicin-loaded hydrogels to microbial adherence, stationary phase *Staphylococcus aureus* (approximately 1×10^8cfu mL^{-1}) were added to M^{c}Cartney bottles, each containing three hydrogel discs on a needle and incubated at 37°C (100 oscillations/minute). Following a defined period, adherent bacteria were removed and viable counts determined by serial dilution. The effect of polymer composition on the physical and biological properties of the various materials were statistically evaluated using a one-way analysis of variance ($P < 0.05$ denoted significance).

Table 1 Swelling and gentamicin release properties and resistance to bacterial adherence of various p(HEMA:MAA) copolymers

Polymer (HEMA: MAA)	% wt gain	Drug loading (μg)	% drug release at 24 h	Cumulative drug release at 24 h (μg)
60:40	859.1 ± 21.2	1475.1 ± 2.1	73.7 ± 4.1	1101.2 ± 61.1
70:30	954.3 ± 19.5	2230.3 ± 43.2	98.6 ± 6.9	2500.1 ± 176.2
80:20	734.3 ± 8.4	1283.4 ± 7.1	92.6 ± 8.0	2266.2 ±196.4
90:10	666.2 ± 44.0	1036.0 ± 68.1	100 ± 8.1	858.3 ± 69.4
100:0	55.0 ± 0.4	10.2 ± 3.0	ND	ND

ND, not determined

As the percentage of MAA in the copolymer increased, the degree of swelling increased, peaking with the 70:30 p(HEMA-co-MAA) polymer. Gentamicin loading mirrored the swelling nature of the material. The cumulative release profiles followed the drug-loading trend, with the polymer that exhibited highest drug loading (70:30) releasing the greatest mass of gentamicin, in a diffusion-controlled manner. All co-polymers soaked in gentamicin prevented adherence of *S. aureus* over 4 h except 100% p(HEMA).

The study has illustrated the ability of hydrogel matrices to facilitate the absorption of gentamicin. The use of such coatings will allow entrapment of gentamicin following nebulisation in-situ. This will allow release of drug to occur post-nebulisation, thereby potentially reducing the incidence of VAP.

Adair, C. G., *et al*. (2002) *Intensive Care Med*. **28**: 426–431

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Characterisation of polycation modified microspheres for DNA vaccination

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Ever since the first reports of immune responses following the delivery of plasmid DNA (Tang *et al* 1992), there has been as explosion of research in the field of DNA vaccines. The ability of DNA vaccines to stimulate cellular immune responses, without risk of inadvertent infection, means that DNA vaccines could revolutionize the field of vaccination. However, despite promising results in small animal models, DNA vaccines have proved to be much less immunogenic in primates. A number of approaches have therefore been taken in order to enhance the potency of DNA vaccines. One of the most promising of these has been the incorporation of DNA into biodegradable microparticulate systems such as poly(lactide-co-glycolide) (PLGA) (Jones *et al* 1997). The enhanced immunogenicity can be explained through the protection of DNA from degradation, and passive targeting of the antigen presenting cells (APC) responsible for initiating immune responses. As significant DNA degradation can occur during its encapsulation into microspheres (Capan *et al* 1999), our approach has been to adsorb DNA to preformed PLGA microspheres surface modified with DNA condensing polycations.

This study was conducted to determine the effect of two critical parameters on the properties of microspheres modified with the polycation polyethylenimine (PEI): the effect of PEI molecular weight and the effect of microsphere size. As the PEI molecular weight increased, both the amount of surface incorporated PEI and microsphere surface potential also increased. The DNA binding ability of the microspheres increased markedly as the PEI molecular weight increased from 400 to 2000 Da ($P = 0.0004$), however a further increase to 25 000 Da resulted in little difference in DNA loading ability and some agglomeration of microspheres was observed. It is important to note that no DNA desorption occurred following extensive washing procedures, suggesting that the DNA is tightly bound. DNA presence at the microsphere surface was also confirmed using confocal microscopy. A maximum DNA loading of 3 μg DNA/mg microspheres, which is sufficient for vaccine purposes, was achieved.

The microsphere size was found to have a pronounced effect on both PEI incorporation and subsequent microsphere DNA loading. As microsphere size was reduced from 4.6 to 1.7 μm, the PEI content decreased for both PEI25000 ($P = 0.0004$) and PEI2000 ($P = 0.0044$). In the case of microspheres modified with PEI2000, the DNA loading ability was also found to be higher for the larger microspheres ($P < 0.0001$).

Interestingly, PEI2000 did not adsorb to pre-formed control microspheres. Although some adsorption of PEI25000 was noted, the amount of PEI incorporated was significantly less when compared to microspheres prepared using the usual method ($P = 0.0009$). These results indicate that polycations are predominantly physically entrapped at the microsphere surface, as opposed to simply adsorbed.

In conclusion, PEI surface modified PLGA delivery systems have been developed and extensively characterised and optimised regarding their size and DNA loading for vaccination purposes.

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Jones, D. H., *et al*. (1997) *Vaccine* **15**: 814–817
Tang, A., *et al*. (1992) *Nature* **356**: 152–154

115
Skin electroporation of estradiol in solution and ultradeformable liposomes: the penetration retarding effect of phospholipid

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Many strategies have been developed to improve transdermal drug penetration. Among these techniques are electroporation, creation of pores by using short high-voltage pulses, and use of ultradeformable liposomes (lipid vesicles with special membrane flexibility due to incorporation of an edge activator). Recently, Essa *et al* (2001) successively improved estradiol human skin penetration by the combination of iontophoresis with ultradeformable liposomes.

The aim of this work was to investigate electroporation (5 pulses, 100 V, 100 ms and 1 min spacing) for enhancing transdermal penetration of estradiol from saturated aqueous solution and ultradeformable liposomes (phosphatidylcholine (PC):sodium cholate; 86:14 w/w), all at maximum thermodynamic activity. The work was extended to probe the effect of PC on repairing skin damage after electroporation.

Negatively charged ultradeformable liposomes containing estradiol were prepared by bath sonication and homogenized by extrusion. Skin penetration involved in-vitro occluded passive and electroporation-assisted human epidermal delivery. About 150 μL of liposomal suspension (5% w/v) or estradiol solution was applied in the donor chamber of diffusion cells. Receptor samples were collected and analysed for estradiol by radioactive counting. Total estradiol penetrated over 8 h was compared. The effect of PC on the electrically altered skin was also examined. The same pulsing protocol was applied to a second set of epidermal membranes, with deionized water as donor. Then, half of these membranes were dosed with empty liposome (i.e. without estradiol) suspension (treated-cells), the remainder non-treated epidermis acted as control. After washing the donors, penetration of

saturated estradiol solution through control and treated skin was monitored for three hours (Stage I). To examine further the skin integrity, another set of pulses was applied while estradiol remained in donor (Stage II), and penetration followed for 2 h.

Electroporation of estradiol in solution produced 16-fold increase in drug penetrated compared with passive diffusion ($P < 0.05$, Student's *t*-test). For liposomes, unexpectedly, there was only 1.3-fold increase in estradiol penetration over passive delivery ($P > 0.05$). As liposomes were suspended in saturated (non-entrapped) estradiol solution, a possible role of PC in restoring some of the skin's barrier properties was suggested. In second part of work, the control cells demonstrated improvement in estradiol flux by 33-fold ($P < 0.05$) and 1130-fold ($P < 0.001$) during stages I and II, respectively, compared to passive delivery. For treated-cells, there were only 4- and 22-fold ($P < 0.05$) flux enhancements in Stage I and II, respectively.

Results suggest that during skin electroporation using liposomes, the PC molecules released from liposomes at the sites of damage could improve skin repair. The freed PC monomers would fill up the areas of high skin damage to form liquid crystal microdomains, replacing water molecules. This process overcomes the more usual penetration enhancing effect of phospholipids acting particularly at gel region of the intercellular lipid.

Essa, E. A., Bonner, M. C., Barry, B. W. (2002) *Int. J. Pharm.* **240**: 55–66

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Development of a new in-vitro release model for intravaginal rings

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Silicone intravaginal rings (IVRs) are elastomeric drug delivery devices specifically designed to release substances in a controlled and sustained manner to the vagina for local and systemic effect. Current in-vitro intravaginal models employ 100 mL of aqueous dissolution medium as standard. This volume is replaced on a daily basis to maintain sink conditions. However, this model does not accurately mimic the in-vivo situation, where only 5–10 mL of vaginal fluid is available.

The aims of this study were firstly to observe release from testosterone (TES) loaded matrix IVRs, to measure the solubility of TES in silicone oil and to determine the diffusivity of TES in silicone. The second part of the study was to develop a new in-vitro model accommodating a limited dissolution medium for the purposes of TES release from matrix IVRs.

TES matrix IVRs (50 mg, 125 mg, 250 mg 500 mg and 1000 mg) were placed in stoppered 250-mL conical flasks containing 100 mL 1% benzakonium chloride (BKC). The flasks were placed in an orbital shaking incubator at 37°C and 60 rev min^{-1}. Samples were taken over 8 days with the elution reservoir being replaced each time with freshly prepared 1% BKC. Table 1 summarises daily release of TES into this standard medium.

Table 1 Daily TES release vs drug loading

IVR drug loading	Release using standard in-vitro model (mg)			Release using limited volume model (mg)		
	Day 1	Day 4	Day 8	Day 1	Day 4	Day 8
50 mg	4.8	1.7	1.0	—	—	—
125 mg	7.6	2.7	1.8	—	—	—
250 mg	11.2	3.6	2.6	6.0	4.4	4.4
500 mg	12.6	5.4	3.6	—	—	—
1000 mg	20.8	7.2	4.8	—	—	—

Solubility of TES in silicone oil at 37°C was observed to be 0.065 mg mL^{-1}. TES release results and TES solubility in silicone oil were used together with the Huguchi equation (Malcolm *et al* 2003) to determine the diffusivity of TES in silicone. TES diffusivity in silicone was calculated to be 0.0292 cm^2 day^{-1}.

The new in-vitro release model consisted of 10 mL of simulated vaginal fluid (Owen & Katz 1999) and a matrix TES IVR in a sealed latex balloon, in a 250-mL conical flask containing 200 mL 1% BKC of dissolution medium to represent systemic circulation. The flasks were placed in an orbital shaking incubator at 37°C and 60 rev min^{-1}. Samples were taken over 8 days with the 200 mL 1% BKC elution reservoir being replaced daily. Table 1 summarises daily release for a 250 mg TES matrix IVR.

Malcolm, R. K., Woolfson, A. D., Russell, J. A., *et al*. (2003) Influence of silicone elastomer solubility and diffusivity on the in vitro release of drugs from intravaginal rings. *J. Contolled. Release* In Press

Owen, D. H., Katz, D. F. (1999) *Contraception* **59**: 91–95

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Comparison of plasmid DNA encapsulation methods in biodegradable nanoparticles

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Encapsulation of macromolecules such as DNA in biodegradable nanoparticles affords the possibility of controlled release as well as the avoidance of premature inactivation. The purpose of the work, reported herein, was to encapsulate plasmid DNA using dendrons for the development of a controlled release oral/nasal delivery system for nucleic acids.

Prior to encapsulation, DNA was condensed using cationic poly-lysine based dendrons, forming dendriplexes. Dendrons possessing 16 free amino groups on their outer surface and with varying number of lipid chains in the core (dendron I: $(C_{10})_1Lys_{15}(NH_2)_{16}$ and dendron II: $(C_{18})_3Lys_{15}(NH_2)_{16}$) were tested for their ability to condense DNA (Ramaswamy 2003). The results (Table 1) demonstrate that increasing the dendron:DNA charge ratio progressively generates smaller condensed structures (up to a point), making them amenable for encapsulation.

Table 1 Comparison of naked and polymer-encapsulated dendriplex as assessed by Malvern Zetasizer 4700 and 3000, respectively, n = 3

Charge ratio	Dendron I (nm)		Dendron II (nm)	
	Dendriplex	Encapsulated dendriplex	Dendriplex	Encapsulated dendriplex
2:1	767 ± 9	1340 ± 641	908 ± 167	171 ± 71
5:1	80 ± 2	150 ± 10	64 ± 11	506 ± 313
10:1	165 ± 34	346 ± 62	78	252 ± 134

For polymer encapsulation, two methods were tested to incorporate the dendriplexes in poly(lactide-co-glycolide) PLGA nanoparticles. The nanoprecipitation method (Chorny 2002), involving the dispersion of dendriplex and PLGA in a mixture of volatile solvents in surfactant solution, followed by rotary evaporation, produced large aggregates (< 1 μm), unsuitable for drug delivery applications. The more 'traditional' approach utilising the double emulsion/solvent evaporation technique was then attempted for encapsulation. Briefly, 250 μL of a 1.5% w/v solution of PVA containing the dendriplex was homogenised with 10 mL of PLGA in dichloromethane forming a w/o emulsion. A multiple w/o/w emulsion was then formed with the addition of 100 mL PVA solution (1.25%). Evaporation was effectuated overnight in a fume cupboard, and particles harvested via centrifugation.

The size of the polymer encapsulated dendriplex nanoparticles was again found to be inversely dependent on the molar charge ratio of the dendriplex, with dendron II, that has more lipidic chains, showing a trend in size reduction. Furthermore, upon freeze-drying, the re-dispersibility of the nanoparticles was proportional upon the levels of dendron in the system; thus nanoparticles formulated with dendriplexes at a charge ratio of 5:1 were more easily re-dispersed than those using a charge ratio of 2:1. This appears to indicate that the dendriplexes may be embedded at or near the surface, with the overall positive charge of the dendriplexes providing electrostatic stabilisation. This maybe advantageous if a

rapid release of the DNA is required, for example in genetic vaccination. We are currently evaluating this possible surface localisation of the dendriplexes using surface analysis techniques.

Chorny, M., *et al*. (2002) *J. Controlled Release* **83**: 389–400
Ramaswamy, C., *et al*. (2003) *Int. J. Pharm*. **254**: 17–21

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A bioadhesive patch containing 5-aminolevulinic acid for photodynamic therapy of vulval pathologies

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Over the last 2 decades, the prevalence of vulval pathologies has increased, predominantly in white women younger than 35 years (Hillemanns *et al* 2000). Vulval pathologies include vulval intraepithelial neoplasia (VIN), Paget's disease, vulvodynia and squamous cell carcinoma. Treatment of such conditions presents an especially difficult challenge for dosage form design, given the moist environment and considerable shear forces in patients who remain mobile.

Surgical intervention and laser therapy are the treatments of choice for many vulval conditions. Photodynamic therapy (PDT) uses visible light and a photosensitising drug to destroy abnormal cells. 5-Aminolevulinic acid (ALA) causes rapid accumulation of the photosensitiser protoporphyrin IX (PpIX) in neoplastic cells following topical application. ALA can be used to treat VIN, Paget's disease and other vulval pathologies (Gannon & Brown 1999).

Although the delivery of a light dose is relatively straightforward, delivery of ALA to the vulva using topically applied creams for PDT is problematic. The objective of this work was to design and evaluate a bioadhesive patch which could be used for the treatment of vulval pathologies by PDT.

The patch, composed of a bioadhesive layer based on poly(methylvinylether/maleic anhydride), and containing 38 mg ALA cm^{-2}, and a backing layer based on medical grade poly(vinylchloride) was evaluated in-vitro. Tensile, bioadhesion and swelling analyses were performed. In addition, modified Franz cells were used to investigate the release of ALA from the patch compared to the commercially available cream (Porphin, 20% w/w ALA in Unguentum Merck). The penetration of ALA, released from the patch into excised vaginal tissue was investigated using liquid scintillation spectroscopy and autoradiography employing patches spiked with ^{14}C-ALA. In addition, cytotoxicity to HeLa cells of red-light-activated-PpIX, induced by ALA released from the patch was investigated. Finally the patch was used, in conjunction with red light, to treat patients suffering from vulval pathologies.

Patches exhibited good flexibilities, tensile strengths and bioadhesive properties. Patches released 1.5 times the amount of ALA as Porphin containing the same ALA loading. ALA released from the patch penetrated vaginal tissue to at least 2.5 mm. A concentration of ALA of 0.1 mM was sufficient to reduce the viability of HeLA cells by 95%. Treatment with patches and red light cleared a single patient of Paget's disease. All VIN patients reported reductions in symptoms and histology showed reduction of the severity of VIN in all cases. All vulvodynia patients reported reduction in symptoms. Thus the patch may be a viable alternative to the cream.

Gannon, M. J., Brown, S. B. (1999) *Br. J. Obstet. Gynaecol.* **106**: 1246–1254
Hillemanns, P., Untch, M., Dannecker, C., *et al.* (2000) *Int. J. Cancer* **85**: 649–653

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Nasal absorption of insulin in sheep from a bioadhesive dosage form

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Nasal insulin delivery would avoid the conventional painful injection route currently used, and the use of bioadhesive polymers may overcome the problem of rapid nasal mucociliary clearance. McInnes *et al* (2000) demonstrated that a lyophilised bioadhesive nasal insert can prolong absorption of nicotine in sheep, and more recently (McInnes *et al* 2002) compared a lyophilised nasal insulin formulation with subcutaneous, nasal powder and nasal solution formulations.

Nasal inserts prepared by lyophilisation of 1% and 2% K4MP, and 2% K4MP solutions of pH3 and pH7, were used to assess the effect of HPMC concentration and formulation pH on the absorption and activity of insulin. Eight sheep (50–70 kg) received human insulin (110 IU) delivered to the turbinate site via a naso-pharyngeal tube, either as a simple nasal spray solution, or a lyophilised K4MP nasal insert. Blood samples were collected by venepuncture at intervals up to 480 min, and blood glucose levels were determined at the time of sampling using a PocketScan test meter. Plasma insulin content was determined using an Iodine^{125} radioimmunoassay.

Blood glucose levels were lowered after administration of the nasal spray and the 2% K4MP insert formulations prepared at pH7 and pH 3. The 1% K4MP insert did not decrease blood glucose levels. Plasma insulin pharmacokinetic data are shown in Table 1.

Table 1 Plasma insulin data

Dosage form	C_{max} (μIU mL^{-1})	T_{max} (min)	AUC (μIU h mL^{-1})
Nasal spray 2% K4MP	83.7	10	37.8 ± 20
Nasal insert, pH 7 2% K4MP	33.3	30	16.9 ± 16
Nasal insert pH 3 1% K4MP	18.1	20	52.9 ± 68
Nasal insert pH 7	10.2	5	6.36 ± 8.7

The rapid plasma insulin C_{max} for the nasal spray is observed immediately prior to a corresponding decrease in blood glucose, following which plasma levels tail off swiftly as expected from a simple solution formulation. This is in contrast with a delayed T_{max} and prolonged plasma insulin profile displayed by the 2% K4MP nasal insert formulations. The 1% K4MP formulation did not display such behaviour. Blood glucose AUC values decreased in the order nasal spray > 2% K4MP pH 7 > 2% K4MP pH 3 > 1% K4MP, while plasma insulin AUC values decreased in the order 2% K4MP pH 3 > nasal spray > 2% K4MP pH 7 > 1% K4MP.

The blood glucose levels and plasma insulin profiles offered conflicting information on the effect of the formulation variables. The 2% K4MP lyophilised nasal insert formulation appears to display bioadhesive properties, allowing prolonged absorption of drug from the nasal mucosa.

McInnes, F., Thapa, P., Stevens, H. N. E., *et al.* (2000) *AAPS Pharm. Sci*: **2**: s2121
McInnes, F., Stevens, H. N. E., Nolan, A., *et al.* (2002) *AAPS Pharm. Sci.* **4**: T3176

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Radial ingress of water into lyophilised formulations intended for nasal delivery

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Lyophilised nasal formulations, require re-hydration on the nasal mucosa to become bioadhesive. Therefore initial water ingress is of importance (Hedenus *et al* 2000), since the hydrated surface represents the interface with the mucosa and is involved in the formation of initial adhesive bonds. Hydration rates of such formulations may therefore be considered a potential indicator of bioadhesive performance, and the current study aims to quantify this process.

Disc shaped lyophilisates were prepared from HPMC gels, which were stained with 0.6 g L^{-1} sodium fluoroscein. K4MP lyophilisates (2%) containing 2 mg nicotine hydrogen tartrate (NHT) and 49 IU insulin were also prepared. Lyophilised discs were compressed to ensure consistent depth of sample for microscopy. The compartment of a device containing the sample was flooded with a weak water/rhodamine solution, and radial ingress of water into the sample was analysed using a Bio-Rad MRC 1024ES Laser Scanning Confocal imaging system coupled to a Nikon upright microscope, using the argon laser line at 488 nm. Scans were performed using 10 × 0.25 NA objective, over an area 1400 μm × 1400 μm with a spatial resolution of 1.2 μm. A time series of scans was performed during initial hydration at the edge of the lyophilisate, and subsequently at the progressing water/sample interface.

The initial rate of hydration at the edge of the lyophilisate was rapid compared to a steady rate of hydration, reached after a few minutes. The rate of water ingress decreased in the order 0.5% K4MP >1% K4MP >2% K4MP >3% K4MP (Table 1). Rapid and extensive hydration of the 0.5% K4MP and 2% K100LV formulations within the first minute made it impossible to record a steady rate of hydration. In the presence of NHT and insulin, initial hydration rates were faster in comparison with the 2% K4MP placebo, while in contrast, steady state rates of water ingress were reduced (Table 2).

Table 1 Rate of water ingress

HPMC	Initial rate (mm s^{-1})	Steady rate (mm s^{-1})
0.5% K4MP	80.6 (± 18.2)	–
1% K4MP	52.3 (± 11.7)	3.46 (± 0.53)
2% K4MP	24.2 (± 3.70)	0.98 (± 0.07)
3% K4MP	9.21 (± 5.40)	0.41 (± 0.11)
2% K100LV	55.1 (± 14.2)	–

Means (± s.d.)

Table 2 Effect of drug

Formulation	Initial rate (mm s^{-1})	Steady rate (mm s^{-1})
Placebo	24.2 (± 3.70)	0.98 (± 0.07)
NHT	31.9 (± 10.3)	0.52 (± 0.15)
Insulin	34.5 (± 5.62)	0.70 (± 0.14)

Means (± s.d.)

The rate of water ingress into the lyophilisates was dependant on K4MP concentration, the section of the lyophilisate being studied and addition of drug to the formulation. The technique allowed accurate measurement of rates of water ingress into lyophilised formulations.

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Array-designed small interfering RNA (siRNA) for gene silencing of epidermal growth factor receptor (EGFR) in cancer cells

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Small interfering RNA (siRNA) duplexes represent an advance in recent antisense technology, the duplexes mediate the sequence specific degradation of messenger RNA (mRNA), often referred to as RNA interference (Elbashir *et al* 2001). Chemically synthesised siRNA is now routinely used in gene silencing by transfection (Sharp 2001), although effective design and delivery of siRNA remains an area requiring further investigation. We have previously identified a target region in the epidermal growth factor receptor (EGFR) mRNA (Petch *et al* 1999), which was designed using a scanning oligodeoxynucleotide array technique (Sohail *et al* 1999). EGFR is a receptor tyrosine kinase proto-oncogene that plays a central role in the initiation and development of several human malignancies, notably brain and breast tumours. Here we show the efficacy of an array designed siRNA in knocking down EGFR expression in A431 carcinoma cells when delivered as lipid complexes with Oligofectamine.

The scanning array was used to locate a target sequence of EGFR mRNA as described by Petch *et al* (1999). The constitutively EGFR expressing cell line, A431, was used cultured in DMEM containing 10% FBS, 2 mM L-glutamine, and penicillin/streptomycin. Twenty-four hours post-seeding transfection was carried out using the cationic lipid transfection reagent, Oligofectamine (Invitrogen, UK) following the manufacturer's protocol. This was followed by a second transfection 48 h post-seeding and analysis carried out at 72 h. Optimal siRNA concentration determined over a range of 10–500 nM. In certain studies comparisons were made to the oligodeoxynucleotide recognising the same region. Activity of the siRNA in knocking down in EGFR expression was determined through cell counts (which included the use of Trypan blue viability assessment). Protein expression was examined using Western blotting with an antibody recognising total EGFR. All experiments including a scrambled siRNA duplex, as well as siRNA and transfection reagents alone.

The scanning oligodeoxynucleotide array revealed a hybridisation assessable region (designated HAR-1) within the first 120 nucleotides of the EGFR mRNA sequence. The designed siRNA dose-dependently inhibited cell proliferation with up to 60% decrease at a 100 nM concentration, compared with controls siRNA and transfection reagents alone. Western blotting showed a similar dose-dependent reduction in EGFR protein expression, confirming that potent knockdown of EGFR was observed upon delivery of siRNA as a lipid complex.

In conclusion, an array designed siRNA duplex was capable of suppressing the expression of EGFR in cancer cells. Thus, siRNA represents a pharmaceutically relevant tool with respect to modulation of proliferative cell signaling pathways in EGFR over-expressing cancer cells.

Elbashir, S. M., *et al.* (2001) *Nature* **411**: 494–498
Petch, A. K., *et al.* (1999) *Proc. Int. Symp. Control. Rel. Bioact. Mater. Res.* **26**: 833–834
Sharp, P. A. (2001) *Genes Dev.* **15**: 485–490
Sohail, M., *et al.* (1999) *RNA* **5**: 646–655

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Gene silencing by rationally-designed antisense oligonucleotides, ribozymes and DNA enzymes: a comparative study targeting an array-defined hybridisation accessible region in EGFR mRNA

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The complete sequencing of the human genome has revealed approximately 35 000 genes, many of which have no ascribed function as yet. Rationally designed gene silencing reagents will be useful tools in the annotation of the human genome and may serve as potential therapeutic agents for sequence-specific gene silencing. Gene silencing by antisense oligonucleotoide (ODNs), RNA enzymes (ribozymes) or DNA enzymes (DNAzymes) relies on the fidelity of Watson-Crick base pairing with the complementary mRNA. It is now well known that not all complementary ODNs exhibit antisense activity. Compelling evidence suggests that a major cause of this observation is the intra molecular structure of mRNAs, which renders most of the molecule inaccessible to hybridisation with complementary nucleic acids. In the absence of reliable computational methods for folding mRNAs and also due to the lack of our understanding of their interaction with exogenously administered nucleic acids, empirical methods are often used to discover effective antisense reagents. A recently described empirical technique, scanning oligonucleotide array technology, has been successfully used to provide information on mRNA structure (Sohail *et al* 1999) and to identify antisense sequences targeting hybridisation accessible sites that effectively downregulated target protein expression (Petch *et al* 1999). However, the comparative efficacy of 'array-designed' antisense ODNs, ribozymes or DNAzymes has not been studied. Here, we report on the successful identification of effective antisense ODNs, ribozymes and DNAzymes against human epidermal growth factor receptor (EGFR) mRNA from ODN arrays which inhibited target gene expression in human cancer cells when optimally delivered as cationic lipoplexes.

Hybridization acccessibility profile of the first 120nt in the coding region of the human EGFR mRNA was determined by hybridising a radiolabelled EGFR transcript to a scanning array of 2684 antisense sequences ranging from monomers to 27-mers. An hybridisation accessible region (HAR-1) was identified within the EGFR mRNA and antisense ODNs, hammerhead ribozymes, and DNA enzymes of the 10–23 motif were designed complementary to this site. The ability of the reagents to hydrolyse EGFR mRNA was initially assessed by in vitro cleavage assays and the subsequent knockdown in EGFR protein was assessed in cultured A431 cells following delivery as lipid complexes (polyplexes).

Phosphorothioate (PS)-modified 21-mer antisense oligonucleotide, AS1, targeting the HAR-1 site within EGFR mRNA effectively inhibited the growth of an established human A431 cancer cell line and effectively downregulated EGFR expression by up to 90% when delivered as optimised cationic lipoplex formulations. Although ribozymes and DNAzymes had similar effects on cell growth they were less effective at EGFR protein knockdown (60–70%), than the corresponding antisense oligonucleotide targeting the same hybridisation accessible region in EGFR mRNA. These data suggest that antisense oligonucleotides are somewhat superior gene silencing agents than ribozymes or DNAzymes as used in this study.

Petch, A., *et al.* (1999) *Proc. Int. Symp. Control. Rel. Bioact. Mater. Res.* **26**: 833–834
Sohail, M., *et al.* (1999) *RNA* **5**: 646–655

123
Gene silencing by RNA interference: the biological stability of siRNA containing two 3′-terminal phosphodiester linkages in both strands

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Small interfering siRNA are short RNA duplexes that cause sequence-specific gene silencing by cleaving target mRNA in a putative mechanism involving a multi-protein complex termed RISC. RNA interference, first discovered in *C. elegans* (Fire *et al* 1998), is now widely utilized for gene silencing in mammalian cells. Previous studies with RNA-based therapeutics such as ribozymes have revealed that unmodified RNA is extremely labile and requires chemical modification for enhanced stability in cellular and in-vivo environments (Beigelman *et al* 1995; Hudson *et al* 1996). However, little is known about siRNA biological stability. In this study we evaluated the biological stability of radiolabelled chemically modified siRNA in serum, A431 cell lysates and in DMEM containing 10% serum. Such information will be useful for performing cellular uptake and activity studies with siRNA, and necessary for assessing their suitability as widespread therapeutic use as gene-silencing agents.

A model siRNA duplex (21nt) housing two 3′-terminal phosphodiester linkages in both strands was γ^{32}P-ATP radiolabelled at the 5′-end termini using T4 polynucleotide kinase and purified by Sephadex column chromatography. Samples of radiolabelled siRNA were incubated with the various biological media at 37°C. Samples at different time points were taken and quenched with denaturing (7M Urea) loading buffer and then frozen to halt the degradation reaction. Thawed degradation samples were then analysed by denaturing, Urea/PAGE gels. For comparison, stability studies were also performed with a 21-mer single stranded antisense phosphodiester oligonucleotide.

Analysis of γ^{32}P-ATP radiolabelled siRNA duplexes purified by column chromatography confirmed removal of free γ^{32}P-ATP after two sequential passes through the columns and labelling of intact duplexed siRNA. The 5′end radiolabelling of both strands in duplex siRNA was confirmed by non-denaturing PAGE following siRNA duplex separation into its component strands in 7M urea buffer. In this case, the intact radiolabelled duplex migrated at a higher level than the component single strands.

Degradation of a phosphodiester antisense oligonucleotide in serum was characterized by the appearance of a ladder of degradation products migrating below the intact 21-mer oligonucleotide indicative of exonuclease activity as described by us previously (Akhtar *et al* 1991). The observed half-life of serum degradation of the unmodified phosphodiester oligonucleotide was in the range of 5–10 min. In contrast the terminally-modified duplex siRNA was markedly more stable and had a half-life of degradation in serum in the range of 20–30min. Half-lives for siRNA degradation in A431 cell lysates and in DMEM containing 10% serum were approximately 12 h and 3 h, respectively. In conclusion, these data suggest that duplex siRNA are more stable than unmodified phosphodiester antisense oligonucleotides. The siRNA stability in the different biological milieu suggests that cellular uptake and delivery studies can be performed over conventional time-frames used for antisense oligonucleotide experiments.

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Biegelman, L., *et al.* (1995) *J. Biol. Chem.* **270**: 25701–25708
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Hudson, A. J., *et al.* (1996) *Int. J. Pharm.* **136**: 23–29

124
Silicone intravaginal rings: a possible delivery system for HIV microbicides

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Based on models for predicting silicone elastomer solubility and diffusivity, the potential for the controlled, sustained delivery of antiretrovirals from silicone intravaginal rings (IVRs) for the prevention of heterosexual transmission of HIV has been evaluated.

Intravaginal rings (IVRS) are torus-shaped polymeric drug delivery devices designed to provide controlled sustained release of drugs to the vagina for both local and systemic effect (Woolfson *et al* 2000; Malcolm 2003). Since the first IVR was developed, research has primarily focused on the development of steroid-releasing rings for both contraceptive and hormone replacement therapies (Woolfson *et al* 2000; Malcolm 2003). However, it is now possible that this IVR technology may lend itself to a wider range of applications within the area of women's healthcare including the delivery of microbicides for the prevention of the heterosexual transmission of HIV. In this study, the physicochemical properties of a range of antiretrovirals have been evaluated and the potential for controlled release from an IVR device discussed.

Matrix-type IVRs were manufactured containing various concentrations of estradiol, estradiol-3-acetate, estradiol diacetate, norethisterone, norethisterone acetate, metronidazole, clindamycin and oxybutynin. In-vitro release studies were performed under sink conditions and the $t^{0.5}$ profiles thus obtained evaluated using the Higuchi equation in order to determine the apparent silicone diffusion coefficient for each drug. The octanol/water partition coefficients of all the drugs were calculated using an online demo version of SRC's KowWIN software (http://esc.syrres.com/interkow/kowdemo.htm).

The apparent silicone diffusion coefficients for each of the drugs were plotted as a function of drug molecular weight. The linear relationship allows the diffusion coefficient to be predicted for any other new drug entity. The results suggest that large molecules (> 500 Da) will have limited diffusivity in silicone elastomer and therefore poor IVR permeation characteristics. Based on a study of the physicochemical properties of drugs that have already been evaluated in IVR drug delivery systems, it is apparent that small molecular size and large silicone solubility (as indicated by a large positive value for the octanol/water partition coefficient) are prerequisites for substantive permeation rates (Table 1).

Table 1 Physicochemical characteristics of compounds already evaluated in IVRs

Compounds	RMM (g mol^{-1})	log Ko/w
Norethisterone	298	3.0
Estradiol	272	4.0
Oxybutynin	357	4.0
Metronidazole	171	0.0
Estradiol-3-acetate	314	4.0

Most of the drugs that are known to be effectively released from IVRs have a value of log Ko/w > 3 and a molecular weight less than 400 Da. Thus, similar physicochemical characteristics are to be sought in the antiretroviral compounds being investigated for IVR delivery. These studies have demonstrated that an intravaginal ring device has the potential to release antiretrovirals in sufficient quantities to prevent the heterosexual transmission of HIV.

Malcolm, R. K. (2003) The intravaginal ring. In: Rathbone, M. (ed.) *Modified-release drug delivery technology.* Marcel Dekker, New York, pp 775–790

Woolfson, A. D., Malcolm, R. K., Gallagher, R. (2000) *Crit. Rev. Ther. Drug Carrier Syst.* **17**: 509–555

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Controlled release of dextran sulphate from silicone intravaginal rings

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Intravaginal rings (IVR) containing anti-HIV drugs are being investigated as a woman controlled method that could kill, neutralize or block HIV. The IVR is a flexible, doughnut-shaped drug delivery system. The major advantage of the IVR is its ability and versatility in providing long term, continuous release of drugs at constant predetermined rates. The simplest IVR device contains drug homogeneously dispersed throughout the polymer matrix allowing the whole surface area of the drug to be exposed to the dissolution medium (Malcolm 2003). Dextran sulphate is a viral fusion inhibitor currently being investigated as a vaginal microbicide for the prevention of heterosexual transmission of HIV.

Dextran sulphate proved to be effective in-vitro inhibitors of various enveloped viruses including HIV. It appears to bind to the lymphocyte cells and thereby inhibit the binding of the virus. The evaporative light-scattering detector (ELSD) was mainly used for determination of biological molecules such as triglycerides, fatty acids, esters, steroids and sugars which possess poor chromophores (Li & Fitzloff 2001). Due to the non-chromophoric nature of dextran sulphate the ELSD method was chosen.

Silicone matrix rings were prepared by thoroughly mixing the required concentration of drug and catalyst before being injection moulded (80°C) on a lab scale ring-forming machine. The IVR's were placed in stoppered 250-mL conical flasks containing 100 mL of water. The flasks were placed in a shaking orbital incubator operating at 37°C. Samples were taken daily for HPLC analysis and the water was replaced daily to maintain sink conditions.

The optimum conditions for the ELSD were to inject 100 μL of the samples and run a 500 mg/100 mL standard solution between each sample. Table 1 shows the repeatability studies of 5 injections with 500 mg/100 mL between each sample injection compared to a straight run.

Table 1 Coefficient of variance for 5 injections at each concentration

Concn (mg/100 mL)	500 mg/100 mL	Straight run
5	0.0230	0.0422
10.2	0.0143	0.0286
20	0.0103	0.0233
50.7	0.0080	0.0478
80.1	0.0148	0.0317
103.6	0.0041	0.0225

A standard calibration curve for dextran sulphate based on mean peak areas was constructed over the calibration range 2–100 mg/100 mL. The 10% and 20% release show an initial large release followed by a decrease then further increase. The controlled release of dextran sulphate, a HIV fusion inhibitor, from matrix-type intravaginal rings of varying concentration has been evaluated. A novel chromatographic assay for light scattering detection was developed to quantify release.

Li, W., Fitzloff, J. F. (2001) *J. Pharm. Pharmacol.* **53**: 1637–1643

Malcolm, R. K. (2003) The intravaginal ring. In: Rathbone, M. (ed.) *Modified-release drug delivery technology.* Marcel Dekker, New York, pp 775–790

126
Gelatin adsorbed nanoparticles as adjuvants

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The emergence of "difficult" pathogens (e.g. HIV, malaria and tuberculosis) has intensified the search for immunological adjuvants. Adjuvants are substances used to produce a more "robust immune response than the antigen alone" (O'Hagan *et al* 2001). In this study we investigate the immune response to nanoparticles with surface-adsorbed gelatin A administered by the intramuscular route.

Particles loaded with tetanus toxoid (TT) were prepared using the double emulsion method as described by Eyles *et al* (2003). Samples from this batch were used to produce gelatin A adsorbed particles by adding them to a solution of gelatin A (1% w/v). These suspensions were stirred for 30 min and then collected by centrifugation. The adsorption of gelatin was validated by measuring the zeta potential of the resulting particles.

Female balb/c mice (n = 5) were immunized by intramuscular injection with 10 μg of TT in 50 μL of saline in the formulations listed in Table 1 and boosted with 1 μg of free TT on day 97.

Table 1 Formulations

Group	Formulation
1	TT loaded nanoparticles adsorbed with gelatin A(+)
2	Free TT with free gelatin A(+)
3	TT loaded nanoparticles
4	Free TT

Blood was collected from the tail vein periodically. Serum was analysed using an enzyme linked immunosorbent assay for IgG.

The results showed that the non-modified particles produced the highest IgG titres at all time points other than at 64 days, where particles adsorbed with gelatin A gave the highest response. We postulate that this is a result of gelatin reducing antigen release by shielding the TT. This gives a lower initial response since non-modified particles are unrestricted at releasing antigen to the immune system and so give a higher response. At 64 days we believe the gelatin A particles are still releasing antigen from the surface. This produces a higher response compared to non-modified particles which would have experienced a burst release of antigen and so show lower titres. At day 97 we explain the stronger response displayed by the non-modified particles to be a result of the release of TT from the particles as the polymer begins to degrade and hence releases the encapsulated antigen. Once boosted, all formulations produced higher antibody titres, but still followed the same trends, with non-modified particles producing the highest response.

These results show that gelatin may be able to control antibody levels by retarding the release of antigen and so maybe used to sustain antibody levels over a period of time. Additionally the work provides evidence of the efficacy of nanoparticles as adjuvants.

Further analysis of the sera to look at the IgG subclasses will be performed to look at the type of responses elicited to the formulations. Additionally cytokine profiles will be investigated to provide more information in this regard.

The authors would like to thank Aventis Pasteur, France who provided the tetanus toxoid.

Eyles, J. M., *et al.* (2003) *J. Controlled Release* **86**: 25–32
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Effect of surfactants on the encapsulation of an integrin-binding protein into PLGA microspheres

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Poly-lactide-co-glycolide (PLGA) microspheres manufactured using the double emulsion (water-in-oil-in-water) technique are good candidates for controlled protein delivery and have been tested with tetanus toxoid (Johansen *et al* 1998) and rgp120 (Cleland *et al* 1997). However, low encapsulation efficiencies and protein denaturation during emulsification remain problematic (Van der Weert *et al* 2000). Since polyvinyl alcohol (PVA) is generally used as the surfactant in the emulsification steps, the aim of this study was to explore the effect of alternative surfactants upon the process of encapsulation for a modular protein with integrin-binding activity (FIII9'-10).

Non-ionic surfactants were selected on the basis of their previous use in protein purification/solubilisation techniques: BSA, tween 20, tween 80, triton X-100, IGEPAL CA-630, pluronic F68 and PVA. Surfactant (5 μL) and FIII9'-10 (90 μL, 20 mg mL^{-1}) in PBS were homogenised (15 s, 22 000 rev min^{-1}, IKA-T18) in 950 μL of 5% w/v PLGA in methylene chloride and added to 40 mL 0.5% w/v PVA in distilled water and stirred 2 h at 500 rev min^{-1}. Microspheres were washed with distilled water and dried by lyophilisation. Microsphere morphology, size, encapsulation efficiency and in vitro release, and protein polymerisation were then investigated using scanning electron microscopy (SEM), laser diffraction, Lowry assay and polyacrylamide gel electrophoresis, respectively.

Microsphere diameter (~ 90–140 μm) was little altered by the presence or type of surfactant. The external morphology of microspheres manufactured using either PVA, BSA or no surfactant was extremely porous and perfectly spherical, but the use of tween 80, triton X-100 or pluronic F68 gave microspheres with very small pores. Finally, no pores were seen for microspheres manufactured using tween 20. Compared with microspheres manufactured with no surfactant, FIII9'-10 encapsulation efficiencies were not remarkably altered by the use of BSA, tween 20 or pluronic F68, but PVA increased loading three-fold and triton X-100 and IGEPAL CA-630 increased loading six-fold. On the contrary, tween 80 reduced protein loading by half (Table 1). Although the initial 'burst release' of protein has been attributed to protein diffusion out of the pores, in this study the observed porosity did not reflect the pattern of protein release, since all microspheres showed similar burst release profiles; after 48–72 h little change to the cumulative release was noticed. In summary, it appears that surfactant type greatly affects the encapsulation efficiency and morphology but, surprisingly, not the corresponding in-vitro release profile.

Table 1 Encapsulation efficiency using different surfactants (%)

FIII9'-10 + no surfactant	10
FIII9'-10 + PVA	27.1
FIII9'-10 + BSA	8.5
FIII9'-10 + tween 20	9.4
FIII9'-10 + tween 80	4.5
FIII9'-10 + triton X-100	61.7
FIII9'-10 + IGEPAL CA-630	58.2
FIII9'-10 + pluronic F68	14.7

Cleland, J. L., *et al.* (1997) *J. Controlled Release* **47**: 135–150
Johansen, P., *et al.* (1998) *J. Controlled Release* **56**: 209–217
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Confocal microscopic study on hydration rate of coating formulations for pulsatile delivery

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Previous studies by this group (Leaokittikul *et al* 2001a, b) formulated new press-coated tablet formulations for pulsatile drug delivery. The pulsed release profiles (both lag time and $T_{50\%}$) is controlled either by varying the thickness of a hydrophobic erodible press coating around a core tablet, or the proportional weight ratio between the two components of the coating granules (glyceryl behenate (GB, Gattefossé, France) and low-substituted hydroxypropyl cellulose (L-HPC; LH-21 Grade; Shin-Etsu, Japan)).

In this study, the hydration rates of coating granule formulations for pulsed release press-coated tablets were determined using a Confocal Laser Scanning Microscope (CLSM) (Nikon E600FN microscope, Bio-Rad MRC1024ES scan-head). The disc shaped of 500 mg-compressed coating granules (formulation A-G, see Table 1) were prepared by compression with 5-tons force using the IR Press. The discs were fitted into a specially designed device to create a snug fit for the disc and with a side channel to introduce the fluorescein solution (0.1 mg mL^{-1}). Radial ingression of water into the disc was measured at 30-s intervals over a 30-min period. The experiments were performed in triplicate. The initial hydration rate is calculated from the linear portion of each profile (distance (μm) vs time (s)).

Table 1 Coating granule formulations and hydration rates (n = 3)

Formulation	Ratio of glyceryl behenate and L-HPC	Initial hydration rate (μm s^{-1}) (± s.d.)
A	50:50	0.80 (0.04)
B	60:40	0.65 (0.06)
C	65:35	0.47 (0.19)
D	70:30	0.42 (0.31)
E	75:25	0.39 (0.27)
F	80:20	0.15 (0.04)
G	90:10	0.12 (0.14)

Images captured from CLSM revealed that water ingressed into the coating granules involves the process of absorbing water, swelling and then detachment of LH-21 fragments from the disc by erosion of the coating. The hydration profiles confirmed our hypothesis that the mechanism of water penetration into the coating

formulations consists of many incremental steps illustrating the dispersion of LH-21 particles throughout the compressed disc. The CLSM results (Table 1) demonstrated that the initial hydration rate decreased as the amount of LH-21 decreased, in accordance with predictions. These findings correlate with the dissolution results previously reported in that the lag time and $T_{50\%}$ are also prolonged as the content of LH-21 decreases. This technique provided accurate visualisation and measurement of rates of water ingression into the coating granule formulations, which supported the clarification on the mechanisms taking place for the pulsed release of drug from the press-coated tablets.

Leaokittikul, D., *et al.* (2001a) *BPC Science Proceedings*, p. 54
Leaokittikul, D., *et al.* (2001b) *BPC Science Proceedings*, p. 77

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The effect of precipitation technique on dissolution and particle morphology of hydrochlorothiazide

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The dissolution rate of poorly water-soluble drugs can be enhanced by particle size reduction. Precipitation is a process known to produce particles in the nano- to micron size range and can also change crystal habit (Rasenack & Muller 2002a). In the present study hydrochlorothiazide (HCT) was used in precipitation and granulation studies as a model drug with limited aqueous solubility. Rasenack & Muller (2002b) have reported powder dissolution enhancement by precipitation techniques. However, the effect of downstream processing has largely not been reported.

HCT was dissolved in acetone and precipitated under agitation into an aqueous solution containing either no or 0.5% w/v stabiliser (sodium lauryl sulphate (SLS), Tween 80 and Pluronic F127) by either a direct addition technique (DAT) or a spray technique (ST) at ambient temperature. The slurry was immediately vacuum filtered and the precipitate left to dry at 37°C.

The resultant morphology was studied using scanning electron microscopy (SEM) and polarised light microscopy. HCT samples were analysed by DSC (40 μL crimped aluminium pan; scan rate 10°C min^{-1} from 25–285°C). Wet granulations using HCT precipitates were prepared by a small scale manufacturing technique with glassbeads (106 μm), dicalcium phosphate (DCP) or lactose. Non-precipitated HCT (reference) was granulated in an identical manner. Dissolution was carried out on the granules using a modified USP method II (1000 mL distilled water, paddle speed 50 rev min^{-1}, 37°C).

SEM images indicated plate-like morphology for HCT precipitated using DAT, providing a low surface area to volume (s/v) ratio. ST produced a cuboid-like crystal habit of higher s/v ratio. Polarised light microscopy suggested crystalline material. DSC studies confirmed that all HCT samples were isomorphic (Table 1).

Table 1 Enthalpies of fusion (ΔH_f) and melting point (mp) of precipitated formulations showing no polymorphism

Stabiliser	DAT		ST	
	Mp (°C)	ΔH_f (J g^{-1})	Mp (°C)	ΔH_f (J g^{-1})
None	268	124	270	128
SLS	267	122	264	122
Tween 80	267	119	269	123
F 127	268	125	269	124

Non precipitated HCT: 124 ΔH_f (J g^{-1}); mp 268°C

Dissolution studies confirmed the importance of precipitation technique (HCT dissolution rate for DAT > ST > non-precipitated; analysis of variance, $P < 0.05$) and for excipient selection (HCT dissolution rate for glass beads > lactose >DCP; analysis of variance, $P < 0.05$). HCT precipitated using DAT and granulated with DCP provided a greater than expected dissolution rate than the equivalent ST formulation, despite it having a greater s/v ratio. This may reflect as yet unknown drug-excipient interactions. The results however highlight how dissolution enhancement can be readily lost by inappropriate excipient selection during down stream processing.

Rasenack, N., Muller, B. W. (2002a) *Pharm. Res.* **19**: 1894–1900
Rasenack, N., Muller, B. W. (2002b) *Drug Dev. Ind. Pharm.* **28**: 1071–1089

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Ethylcellulose dipcoating to incorporate time delay in an oil-filled capsule

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Ethylcellulose (EC), a water-insoluble yet water-permeable polymer, is frequently utilised in controlled release systems either as a film coat or a matrix component. We aim to exploit its excellent film-forming properties to incorporate a lag time into our novel delivery system. EC will control the rate of water ingress and constitute a brittle outer coating that will crack and release drug once the pressure generated by the internal swelling agent exceeds the coat's stress limit.

Size 1 hard gelatin capsules were filled with 400 mg of formulations containing 25–45% (w/w) corn oil, 50–70% (w/w) low-substituted hydroxypropylcellulose (grade LH-21, Shin-Etsu) and 5% (w/w) paracetamol. The capsules were suspended on six-armed radial wire frames by strings attached to the heads of the individual capsules with cyanoacrylate-based adhesive. Each six-capsule assembly was dipped into a 3% (w/v) EC (Ethocel Standard 10 grade, Dow Chemical Company) solution in 50:50 acetone/isopropyl alcohol, plasticised with 5%(w/w of EC) triacetin. A drying time of at least one hour was allocated between dipcoats. Capsule sets of differing formulation ratios were dipcoated 5, 7 and 10 times. The coating level was determined by the mean weight gain of the capsules. Automated dissolution testing (USP II) was conducted in a Caleva dissolution apparatus (37°C, 50 rev min^{-1} paddle speed) connected to a Unicam UVII spectrophotometer (249 nm). Six capsules were tested simultaneously in separate dissolution vessels containing 1000 mL distilled, deaerated water each. Dissolution results were interpreted in terms of mean lag times and mean time to 50% drug dissolved ($T_{50\%}$). These values were analysed by one-way analysis of variance.

Dipcoating 5, 7 and 10 times resulted in mean EC coating levels of 2.1 ± 0.5, 2.9 ± 0.3 and 4.0 ± 0.6% (w/w), respectively. In general, the dissolution profile was that of a burst release to 70–90% drug dissolved after an initial lag phase, followed by a gradual release of the remaining drug.

Table 1 Mean lag times and mean $T_{50\%}$ of EC-coated capsules containing varying concentrations of LH-21

LH-21 concn (% w/w)	No. of dipcoats	Mean lag time (± s.d.) (min)	Mean $T_{50\%}$ (± s.d.) (min)
70	0	5 (1)	7 (1)
70	5	31 (2)	40 (7)
70	7	49 (26)	69 (35)
70	10	160 (54)	204 (49)
60	0	10 (2)	13 (1)
60	5	26 (9)	45 (8)
60	7	53 (25)	91 (33)
60	10	259 (216)	331 (206)
50	0	13 (4)	17 (2)
50	5	27 (7)	65 (33)
50	7	131 (74)	173 (46)
50	10	156 (142)	228 (124)

The EC coat built in a lag time prior to capsule rupture ($P < 0.0005$) and consequently increased the mean $T_{50\%}$ ($P < 0.0005$) in a concentration-dependent manner. However, the concentration of the expulsion agent, LH-21, did not significantly affect the two parameters ($P = 0.717$ for lag time and $P = 0.258$ for $T_{50\%}$). The threshold amount of LH-21 required to rupture the coat might therefore be lower than the minimum concentration used in this study.
Dipcoating offered a simple yet effective method to evaluate the role and efficacy of EC in our system. Alternative coating methods are currently being tested.

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An investigation into the effects of particle size and composition on drug release from stearic acid microspheres

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Stearic acid microspheres have been utilised as taste-masking carriers for the delivery of drugs such as antibiotics (Robson *et al* 1999). Earlier studies have indicated a marked pH dependence of the release profile, with higher rates noted under the alkali conditions (Robson *et al* 2000). Investigations into the underlying mechanism are complicated by the fact that BP grade stearic acid contains a range of further fatty acids, including up to 50% palmitic acid. In addition, to date there have been no published studies on the effect of particle size on the release profile. In this investigation spheres containing 10% w/w benzoic acid, used as a model drug, were prepared using a customised spray chilling apparatus. Two materials were used; stearic acid with 99% purity (99SA) and mixes of 99% purity stearic acid and 99% purity palmitic acid 50:50 w/w (50SA). The spheres were sieved into $>180\,\mu m$, 180–106 μm, 106–63 μm, and $<63\,\mu m$ fractions. The systems were exposed to Sorensens modified phosphate buffer (SMPB) pH 8.0 at room temperature, followed by washing and drying. The morphology, particle size, and thermal characteristics were studied using scanning electron microscopy (SEM), laser scattering particle size analyser, and differential scanning calorimetry (DSC). USP II paddle method was used to evaluate drug release from different systems. SEM studies performed prior to exposure to the buffers showed smoother surfaces and a more spherical shape for the 50SA than for the 99SA. However, on exposure to pH 8.0 SMPB, a greater level of imperfection and evidence of fusion were seen on the surfaces of the 50SA spheres. The particle size distributions of the 99SA systems were unchanged after 4 h exposure to the buffer. However, for 50SA spheres a broadening of the distribution and particle size reduction were noted for systems exposed to the alkali medium; for example the mean diameter of the 106–63 μm spheres changed from 52 μm to 28 μm. DSC studies showed single peaks at 67°C for 99SA and 54°C for 50SA respectively prior to exposure to buffer. After 6 h the 99SA spheres showed a single melting peak at circa 72°C a shoulder at 78°C. The 50SA systems, however, showed the main melting peak at 59°C with a shoulder at 52°C, with a third peak seen between 65°C and 69°C that became more marked with the reduction of particle size. Dissolution testing indicated that 40% drug release occurred from 99SA spheres after 30 min and to up to 80% release for 50SA spheres in the same time period for 106–63 μm particles. In both cases, smaller sphere sizes led to faster drug release; for example the release for the 180–106 μm and $<63\,\mu m$ spheres after 30 min for 99SA was 31% and 51%, respectively. The present study has indicated that drug release from stearic acid microspheres is highly dependent on the composition of the stearic acid used and the size of the spheres produced, with the mixed stearic acid/palmitic acid systems and smaller size fractions yielding more rapid release. In addition evidence has been presented for a different level of interaction between the spheres and the medium for the two compositions.

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Polymerised liposomes as adjuvants for nasal delivery

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Particulate delivery systems such as liposomes enhance immune responses to antigen delivery and thus are recognised adjuvants (reviewed by Gregoriadis (1990)). Polymerised liposomes have been projected as sturdier carriers for proteins when compared with conventional liposomes (Okada *et al* 1995; Chen & Langer 1998) and have been proposed for vaccine delivery. The aim of this study is to establish whether polymerised liposomes possess adjuvant activity when administered by the nasal route. We explore the effect of particle size, theoretical loading of antigen, liposomal antigen location (associated and surface adsorbed) using tetanus toxoid (TT) (gift from Aventis Pasteur, France) as a model soluble antigen.
The polymerisable phospholipid 1,2-bis[(2E,4E)-octadecadienoyl]-*sn*-glycero-3-phosphocholin (DODPC) (gift from DOR BioPharma, Inc., IL) was used to prepare liposomes by direct lipid hydration. The vesicles thus formed were modified to the required size by freeze-thaw cycles and passage through an extruder containing either a 1 μm or 100 nm filter. In the case of polymerised liposomes the monomers were polymerised using UV light. TT was associated to polymerised liposomes using a modified Dehydration-Rehydration Vesicle (DRV) method (Gregoriadis *et al* 1999) or simply surface adsorbed and compared to non-polymerised DRVs and free TT. Theoretical loadings of 1% and 5% (mg protein/mg lipid) were compared to assess the effect of the quantity of lipid administered.
Groups of five animals (female BALB/c mice) were immunised nasally with 10 μg TT in solution or associated/adsorbed to liposomes. The mice were boosted on days 34 and 58 with 5 μg of TT in the appropriate formulation and a tail bleed taken on day 68. Serum was analysed for anti-TT IgG using an enzyme linked immunosorbent assay.
Polymerised liposomes produce a significantly greater anti-TT IgG response than non-polymerised liposomes whether the antigen is associated or surface adsorbed. Non-polymerised DRVs did not possess an adjuvant effect when compared to carrier free TT. The mean response is noted to be much greater for surface adsorbed TT polymerised liposomes than the TT associated polymerised liposomes but this is not statistically different. It is also shown that the mixed liposome size formulations give better immune responses than the 100 nm size only. In each case theoretical loadings of 1% gave better results than 5% which is in agreement with our previous studies with polymeric particles (Somavarapu *et al* 1998). The size and theoretical loading differences may be attributable to actual loading and release profile differences. Further work that is being undertaken includes analysis of serum IgA, IgM and IgG subclass responses as well as a cytokine profile.
Preliminary conclusions reveal that polymerised liposomes possess adjuvant activity greater than non-polymerised liposomes and that using a mixed size range (up to 1 μm) elicits an enhanced response.

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PLGA-PCL-co-polymer nanoparticles as a vaccine delivery system

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Poly-ε-caprolactone (PCL) is a biodegradable polymer that has shown potential as a microparticle vaccine delivery system Baras *et al* (1999). Advantages of using

PCL instead of poly-(lactic-co-glycolic acid) (PLGA) for the preparation of drug delivery systems include greater hydrophobicity, less soft, slower degradation and hence generation of a less acidic environment. However, PCL is more difficult to formulate than PLGA into nanoparticles which can be dispersed in water after formulation. It has been shown that vaccine delivery systems containing PCL induce higher immune responses than PLGA or PLA DL) nanoparticles, due to increased hydrophobicity. By using the PLGA-PCL co-polymer as a nanoparticle vaccine delivery system, the properties and the advantages of both polymers are exploited. In this study the two polymers PLGA and PLGA-PCL co-polymer are compared for potential as a vaccine delivery systems

The nanoparticles were prepared using a water-in-oil-in-water solvent evaporation method. The polymer used were PCL-PLGA (75/25 co-polymer) ($\eta = 0.76$) (Lactel) and the PLGA ($\eta = 0.65$) (Boehringer Ingelheim). The size of the nanoparticles was determined using photon correlation spectroscopy. Loading of nanoparticles was determined using a Bicinchoninic Acid Protein Assay. Hydrophobicity of the formulations was determined using Rose Bengal. For the in-vivo evaluation of the different nanoparticle formulations, female BALB/c mice ($n = 3$ per group) were immunised with Diphtheria Toxoid (DT) intramuscularly. The mice received free or encapsulated antigen. Blood was collected after 4, 8 and 12 weeks. Serum was collected and analysed using a Enzyme Linked Immunosorbent Assay.

Characterisation studies (Table 1) show that the nanoparticles prepared were in the range of 249–267 nm in diameter. DT loading efficiencies obtained for the different particles was between 71.38 and 81.00%, with the copolymer having the highest loading. All the nanoparticles obtained were monodisperse and spherical.

Table 1 The effect of polymer on size and loading of polymeric nanoparticles

Polymer	Size (nm)	Loading efficiency (%)	Actual loading (%)
PLGA	249 ± 2	71.38 ± 4.3	1.43 ± 0.09
PLGA-PCL (Co-polymer)	267 ± 2	81.00 ± 1.7	1.62 ± 0.03

Mean ± s.d., n = 3

A positive correlation between IgG antibody titre and hydrophobicity of the nanoparticles was observed. The more hydrophobic nanoparticle formulations induced a higher immune response than the less hydrophobic nanoparticle formulations in the order of PCL-PLGA co-polymer > PLGA > DT. The higher immune response by the more hydrophobic delivery system may be due to more efficient uptake by antigen presenting cells. PCL-PLGA co-polymer and PLGA nanoparticles induced immune responses 8 and 3 times higher than free DT.

The study has shown the potential and advantage of PCL-PLGA co-polymer nanoparticles over PLGA and free antigen solution in obtaining higher immune responses.

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