Poster Session 1 – Drug Delivery

026
Identifying the localisation of drugs in skin using spectroscopic techniques

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Various techniques for quantifying drugs in skin have been published in the literature over the years. In 1998, Touitou *et al* (Touitou *et al* 1998) reviewed such techniques — skin extraction measurements, horizontal stripping and sectioning, removal of hair follicles, quantitative autoradiography and spectroscopic methods — highlighting their merits and disadvantages. Of these techniques only autoradiography enables quantification and visualisation of drugs throughout the entire cross section of skin, but it is only of limited value for screening of drugs and formulation excipients because of the need for use of radiolabelled molecules.

Vibrational spectroscopic techniques such as IR and Raman spectroscopies, have been applied to characterise human and animal skin in fields of research such as medicine, dermatology, cosmetology and pharmacology. The non-invasive characteristic of these vibrational spectroscopic techniques has enabled the composition of individual skin layers on a molecular level, to be determined both in-vivo and in-vitro.

Spectroscopic mapping and imaging techniques allow an area of a sample to be analysed in terms of chemical content on a microscopic scale. Human skin has been examined, to a degree, though not extensively, for the identification of the various layers in whole intact cross sections of skin using IR spectroscopy (Dumas *et al* 2000; Bhargava & Levin 2002).

In this study, Raman spectroscopy and a neoteric mid-IR mapping instrument have been employed to map cross-sections of human skin and to detect the location of drug compounds within skin. Sample preparation for each technique was a key consideration along with the determination of the correct experimental parameters. The spectra obtained for each skin layer have been compared with those published previously and good correlation is seen. Spectral changes due to appendages within the skin have also been identified, which is of great interest with regard to drug localisation within skin.

For comparative purposes, microautoradiography studies have been carried out on an antiviral compound using human split thickness skin. The lateral cross sections of skin showed that the compound located in the upper layers. Cross sections containing this compound were also analysed using Raman mapping and once again the presence of the antiviral agent was visualised in the upper layers of the skin. These results are encouraging, indicating that compounds, which reside in skin in different ways (e.g. within skin appendages, at the dermal/epidermal interface), can be identified by this technique. The methodology is such that it can be implemented within the early stages of discovery and drug development without the need for fluorescence or radiolabelled compounds.

Bhargava, R., Levin, I. W. (2002) *Anal. Chem.* **74**: 1429–1435
Dumas, P., Carr, G. L., Williams, G. P. (2000) *Analysis* **28**: 68–74
Touitou, E., Meidan, V. M., Horwitz, E. (1998) *J. Controlled Release* **56**: 7–21

027
An investigation into polymeric excipient-particle compatibility in solid-liquid formulations

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The current strategy for choosing an appropriate polymer to stabilise a particulate dispersion is somewhat serendipitous. There is no current clear understanding as to why a particular polymer will adsorb and hence confer colloid stability onto a given material. Similarly, it is not understood why the same polymer whilst conferring good stabilisation properties on one compound totally fails when used on a different dispersion.

A number of water-soluble polymers were screened as potential stabilisers for a pharmaceutical dispersion including: methylcellulose, carboxymethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, gum arabic, xantham gum, gum guar, polyvinyl pyrollidone and carrageenan.

Colloidally disperse samples of the particles were found to form upon the addition of the sulphated polysaccharide carrageenan in water at pH 2 and pH 7. Adsorption isotherms revealed adsorption maxima at 80 mg $g-1$ and 90 mg $g-1$, respectively. Dynamic light scattering measurements of the resultant carrageenan-particle complex showed a mean hydrodynamic size of 760 nm, which compared favourably with scanning electron micrographs of the powder 600–800 nm, indicating that the system consisted of disperse unimeric particles. The particle-carrageenan suspension showed excellent dispersibility with the system remaining fully dispersed as monitored by turbidimetric measurements over 3 months.

Adsorption isotherms will be presented for a range of different particulate dispersions where the addition of a polymer clearly confers good stabilisation.

Particles chosen to carry out further polymer compatibility studies will include ionic compounds which are acidic (e.g. ibuprofen in addition to basic compounds such as propanolol).

028
Transdermal delivery of bioactive agents from *Arnica montana*

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Arnica montana (Leopard's bane) is widely used as a topically applied anti-inflammatory herbal medicament. Phytochemical and pharmacological studies on the plant extracts revealed the presence of highly effective bioactive agents (Bisset 1994). However, the efficacy of topically applied *Arnica* preparations is still controversial, hence this study investigates transdermal permeation of bioactive agents from topically applied solutions of *A. montana*.

Permeation experiments over 48 h used human epidermal membranes on Franz type diffusion cells with a methanol-water (1:1 v/v) receptor fluid. Permeants were assayed by a HPLC method adapted from Leven & Willuhn (1987) with a (250 × 4.6 mm) Hypersil ODS 5 μm column and diode array detection at 225 nm. Isocratic analyses at ambient temperature used a methanol–water (1:1 v/v) mobile phase at 1 mL min^{-1}. The injection loop was 100 μL.

A commercially available tincture of *A. montana* derived from dried flowers of Spanish origin was a donor solution. Further donor solutions were prepared from this stock tincture in methanol–water (1:1 v/v), vehicles contained the tincture at 1, 2 and 10-fold concentrations and santonin (pure sesquiterpene lactone compound) was included as an internal standard. A tenfold concentrated sesquiterpene lactone (SL) extract was also tested.

No *A. montana* tincture components were detected in the receptor phase within 12 h of applying the commercial tincture to human epidermal membranes. However, after 12 h, two components were detected in the receptor phase providing peaks at 14.3 ± 0.24 and 23.8 ± 0.16 min (mean ± s.d., n = 3). These components may be ascribed to sesquiterpene lactones (SLs), probably 11, 13-dihydrohelenalin (DH) analogues (6-O- methacryloyl DH and 6-O- tigloyl-DH, respectively). Using the other donor solutions and the concentrated SL extract, these components were also detected as were other permeants providing retention times of 25.4 ± 0.28 and 32.9 ± 0.22 min. These additional peaks are also likely to be SLs, probably 6-O- angelicoyl- DH and 6-O-angelicoyl helenalin, respectively. This is consistent with previous work by Leven & Willuhn (1987) who showed these compounds were present in *Arnica* tinctures and eluted with comparable retention times in relation to the internal standard. Furthermore, Klaas *et al* (2002) showed the anti-inflammatory activities of *A. montana* correlate with SLs content, notably 11,13-dihydrohelenalin (DH) derivatives.

In summary, permeation studies showed that *A. montana* tincture components permeated human skin. These components include the bioactive agents responsible for the anti-inflammatory activity of *A. montana* preparations. These bioactive agents may be isolated and better delivered from new topical formulations.

Bisset, N. G. (ed.) (1994) *Handbook of herbal drugs and phytopharmaceuticals*. CRC Press, London
Klaas, C. A., *et al*. (2002) *Planta Med*. **68**: 385–391
Leven, W., Willuhn, G. (1987) *J. Chromatogr.* **410**: 329–342

029
Surface-enhanced Raman spectroscopy of tetracaine deposition in the *stratum corneum*

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Raman spectroscopy has been employed in the study of various therapeutic and narcotic agents (Hodges & Akhavan 1990) and polymer based drug delivery devices (Bell *et al* 2000), and has proved itself to be an excellent technique for their analysis. Another member of the family of Raman-related techniques is that of surface-enhanced Raman spectroscopy (SERS), which can not only eliminate problems due to sample fluorescence but in addition give rise to markedly enhanced sensitivity for detection of the scattering species. The enhancement of the Raman scattering, by factors of up to 10^6 compared with normal, arises for species on or near the surfaces of noble metals, especially of silver and gold. By far the most popular approach is to use silver and gold colloids as the SERS substrates.
In this study, we have employed the SERS technique in conjunction with 785 nm excitation to characterise the deposition of the local anaesthetic, tetracaine, in the *stratum corneum*. In a pilot clinical study, Ametop gel (Smith & Nephew, UK) was applied to the volar forearm and occluded for 45 min. After this time, the dressing and excess product were removed from the skin surface. The skin surface was gently cleaned and medical grade adhesive tape was used to remove successive layers of the *stratum corneum*. 20 tape strips were collected. Tape strips were also collected from a control site (not exposed to Ametop). Samples were analysed using an Avalon RamanStation R1 instrument (Avalon Instruments, Belfast, UK).
No spectra of tetracaine were observable when individual tape strips were analysed by conventional dispersive Raman spectroscopic methods. Taking successive strips from the same area of skin with a single tape did not noticeably improve the situation. Upon adding colloidal silver to the tape strips, tetracaine could be readily identified in the samples. It was distinct from the components of the tape and the skin spectra, which were characterised separately, both by conventional Raman methods and by SERS. This study has demonstrated that SERS is a viable technique for the rapid analysis of very low levels of tetracaine in clinical samples. These preliminary results provide a further illustration of the potential of SERS for the identification of trace amounts of exogenous chemicals within complex biological matrices. In the case of the skin samples reported on here, several issues remain to be further investigated, particularly the development of optimum procedures for combining tetracaine-treated skin cells with the colloidal SERS media.

Bell, S. E. J., *et al*. (2000) *Analyst* **225**: 541–544
Hodges, C. M., Ahkavan, J. (1990) *Spectro. Acta* **46A**: 303–307

030
Immunogenicity of zein microspheres

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Polymeric microparticles with entrapped antigens are used as controlled-release antigen delivery systems. Most of the polymers are synthetic, however, they may also be extract from natural sources. As previously reported (Hurtado-López & Murdan(2001), we have loaded ovalbumin (OVA), a model antigen, into a novel delivery system: zein microspheres, with a view to determine their adjuvant effect, if any. Zein is the aqueous alcohol-soluble protein of maize endosperm. It is not a homogenous protein; rather it is a mixture of several groups of proteins with similar solubility behaviours, each with a different polypeptide composition (Esen 1987).
We describe here some preliminary observations concerning the use of OVA-loaded zein microspheres as parenteral antigen delivery system.
Five Balb/c female mice were immunised intramuscularly with 30 μL of OVA-loaded zein microsphere suspension containing 150 μg of OVA. Two other groups of five mice were used as controls; each received 30 μL of blank zein microsphere suspension and 150 μg of OVA solution in 30 μL of PBS, respectively. Booster doses were administered 12 weeks after the primary immunisation. Blood samples were collected from the tail veins of the mice at seven weeks after primary immunisation, and at one, four and seven weeks after booster doses. The specific anti-OVA IgG antibody levels of each serum were determined in a standard ELISA assay.
After primary immunisation, non-significant levels of IgG were observed from all three formulations. After boosting, however, high levels of antibodies were measured from both loaded and blank zein microspheres. Secondary response seemed to be due to non-specific anti-zein antibodies. To validate this hypothesis, three approaches were adopted. ELISA microtiter plate wells were coated with diphtheria toxin (DT), phosphate buffer and a solution of zein in 60% ethanol. Reaction of the antisera with both DT and phosphate buffer was randomly detected in the first two coatings. For the latter, booster doses elicited a strong immune response as well, but in this case, IgG levels generated by blank microspheres were higher than those generated by OVA-loaded microspheres.
From the ELISA data, we observe that non-specific anti-zein antibodies present in the sera interfere with the measurement of specific anti-OVA antibodies. On the other hand, high levels of anti-zein IgG appear only after boosting. Since maize is included in the daily diet of mice, some anti-zein antibodies may circulate in the animal sera, and a second injection seems to boost their production, causing an unwanted immune response.
We conclude that zein microspheres are not a good adjuvant for vaccines, when administered intramuscularly.
Oral administration of zein microspheres are underway, with a view of determine adjuvanticity or immunogenicity of the system by mucosal routes; and to investigate oral tolerance.

Esen, A. (1987) *J. Cereal Sci.* **5**: 117–128
Hurtado-López, P., Murdan, S. (2001) BPC Glasgow 2001

031
Ofloxacin release from novel anti-adherent biomaterial coatings

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Major attention has recently been focused upon the treatment of polymeric implant surfaces with a variety of macromolecules to reduce the levels of bacterial attachment, which can ultimately lead to colonization and subsequent implant-

associated infection (Portolés *et al* 1994). Hydrogel polymers are regarded as some of the most biocompatible medical device materials to date however, these are still susceptible to bacterial adhesion and encrustation.

Poloxamers are block copolymers with the ability to form a micellar structure with a hydrophobic inner core and a hydrophilic outer shell in aqueous media as a result of their non-ionic amphiphilic nature. As hydrophobic interactions are utilised effectively for the formation of micellar structures, drug carrier systems for hydrophobic drugs can be constructed using polymeric micelles.

Fluoroquinolones represent a new class of highly potent, broad spectrum agents which have the ability to penetrate bacterial cell walls and inhibit DNA gyrase activity leading to rapid death of the susceptible organism. A novel polymeric coating comprising chemically modified Poloxamer 188 copolymerised with poly(2-hydroxyethyl methacrylate) (pHEMA) has been designed and was previously found to dramatically reduce these phenomena with respect to pHEMA alone which is one of the most commonly used biomaterial coatings (Donnelly *et al* 2002). The incorporation of one of these agents, ofloxacin, which is known to penetrate biofilms reducing the adherence of several bacterial strains including those of *E. coli* in the urinary tract (Multanen *et al* 2000), into the novel materials was carried out and their drug release profiles studied.

1%, 5%, and 10% modified poloxamer:HEMA (w/w) polymers were prepared with the polymerisation reaction initiated using 0.5% 2, 2'-azoisobutyronitrile (w/w). A pHEMA control polymer was also prepared in addition to a pHEMA polymer containing 5% poloxamer (w/w). Ofloxacin loading was achieved via the equilibrium partitioning technique and release was carried out into a 1% benzalkonium chloride solution with samples taken at time periods sufficient to mimic sink conditions. The incorporation of poloxamer 188 into the HEMA hydrogel had no significant effect on the release exponent obtained by the power law model, as shown in Table 1, indicating release is still occurring via a simple diffusion mechanism.

Table 1 Release exponent and fraction ofloxacin released from studied polymers

	Control	1%	5%	10%
Release exponent	0.41 ± 0.01	0.41 ± 0.01	0.41 ± 0.01	0.39 ± 0.02
Fraction Released after 1 h	0.778 ± 0.011	0.309 ± 0.021	0.743 ± 0.015	0.845 ± 0.007

Data are mean ± SD from 3 replicates

Table 1 also shows the fraction of ofloxacin released after one hour. Although the rate of release is similar to the control polymer at the higher poloxamer loadings, at the lower 1% loading the release occurs over a much longer time period providing suitable matrices for a prolonged release application.

Donnelly, L. *et al.* (2002) *J. Pharm. Pharmacol.* **54** (Suppl.): 202
Multanen, M., *et al.* (2000) *Br. J. Urol. Int.* **86**: 966–969
Portolés, M., *et al.* (1994) *J. Biomed. Mater. Res.* **28**: 303–309

032
Physicochemical, binding and cellular uptake of folinate modified macromolecules and gene delivery systems

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Gene therapy will rely on DNA delivery systems, which are effective in-vivo and have good transfection efficiency. Two main requirements for achieving this are: firstly producing small compact particles which can avoid the reticulo-endothelial system in-vivo, secondly enhancing uptake of the particles into the cells.

Most attempts at meeting these two requirements centre on the incorporation of a polyethylene glycol coating onto the particle used, and the use of targeting ligands (Garnett 1999). The difficulty is to incorporate these functionalities without compromising the physicochemical characteristics of the particles. It has previously been reported that it is possible to produce compact self assembling particles with sterically stabilised PEG coating using a mixture of polyamidoamine (PAA) cationic polymer plus PEG-PAA copolymer (Rackstraw *et al* 2002). The transfection activity of these systems was very low; therefore incorporating targeting moieties was one proposed solution to improve the transfection.

Folinic and folic acid as targeting moieties were used to modify the PEGylated polyamidoamine copolymer. Complexes were then made from mixtures of DNA with the modified PEG-PAA and the homopolymer in different proportions. The physicochemical characteristics of the system incorporating the targeting moieties with the DNA were determined using photon correlation spectroscopy (PCS) and transmission electron microscopy (TEM) in both PBS and glucose. These techniques showed well defined small nanoparticles especially at a polymer to DNA ratio of 1.25:1 and 2:1. The particles sizes were less than 150 nm with polydispersity of less than 1.3. The stability of the complexes in PBS and glucose were investigated using a fluorimetric technique. The nanoparticles showed very good stability in presence of both PBS and glucose. The relative fluorescence of the system in presence of ethidium bromide (Et-Br) was less than 40% of that of the DNA at polymer/DNA ratio of 1.25 and 2.

In parallel to the physicochemical studies, binding and uptake studies were carried out. Binding and endocytosis through receptors recognising folic and folinic acid have been investigated using conjugates of these ligands on bovine serum albumin (BSA) with fluorescein (FITC) or tetramethyl rhodamine (TRITC) fluorescent labels. This work was carried out on 791T osteosarcoma and HepG2 liver hepatoma cell lines using fluorescent microscopy. Both cell lines showed binding to both ligands, but with a lower level of folate binding compared to folinate binding. Binding of the folinate ligand was specific, being inhibited by the presence of either folinic acid and or folinic acid-BSA conjugate, but not by folic acid or folic acid–BSA conjugate. In addition, gold particles were formulated by conjugation of folinic acid to BSA then loading the conjugates onto gold spheres (8–12nm). TEM images for the receptors were taken after incubation of these particles with 791T cell line. The images showed clear endocytosic uptake through non-clathrin coated pits with increasing uptake of gold particles with time, using time intervals of 5 min to 4 h. The transfection work to show the effect of incorporating these targeting moieties to the DNA delivery system is ongoing.

Garnett, M. C. (1999) *Crit. Rev. Ther. Drug Deliv. Systems* **16**: 147–207
Rackstraw, B. J., Stalnik, S., Bignotti, F., *et al.* (2002) *Biochim. Biophys. Acta* **1576**: 269–286

033
Chemical imaging of drug delivery systems: applications to pulmonary delivery

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Two common strategies employed to deliver drugs to the lungs include the use of pressurised or dry powder devices. In pressurised devices, the active ingredient and excipients are mixed with a compressed propellant liquid in a canister. On device actuation the propellant expands to produce an aerosol. Alternatively, in dry powder devices the micronised active ingredient is dispersed onto the surface of a carrier particle (usually lactose) to prevent agglomeration. During inhalation the active ingredient detaches from the carrier to allow delivery to the lung. In both of these formulations, knowledge of the distribution and chemical integrity of the components is vital.

Here we demonstrate the use of time-of-flight secondary ion mass spectrometry (TOF-SIMS) and Raman microspectroscopy to characterise the contents expelled from a model combination inhaler device. In such systems factors such as degree of active ingredient mixing, particle size and extent of interaction are important in determining physiological performance.

In addition, TOF-SIMS is used to characterize the coverage of micronised active ingredient on a lactose carrier as compared to scanning electron microscopy (SEM).

TOF-SIMS analysis of the expelled particles from the model combination pressurized device reveal distinct mass fragment signatures for the two active ingredients. This allowed for the distribution of components to be imaged. Results indicated that expelled material consisted both of agglomerates containing both active ingredients, together with individual particles of each component. Knowledge of the proximity of the two components can impact on physiological performance. In addition, the association of excipients such as surfactants with the active ingredients can be studied.

Confocal Raman Microspectroscopy provides a complementary chemical analysis method to TOF-SIMS, capable of resolving individual inhalation microparticles. The ability to extract Raman spectra from selected particles allows an assessment of active ingredient amorphous/crystalline state. This physical state information cannot be obtained using TOF-SIMS alone, highlighting the importance of applying a suite of analytical methods when performing inhalation particle characterisation. Analysis of the Raman spectral data revealed both components in close proximity. However analysis of the Raman spectra does not reveal any indications of interaction between the components.

TOF-SIMS imaging was also used to investigate the distribution of an active ingredient on a lactose carrier particle used in a dry powder device. While SEM revealed fine particles, the active ingredient cannot be distinguished from fine lactose particles. However, using TOF-SIMS imaging, both topographical and chemical information was obtained. A large proportion of the lactose particle surface is covered with active ingredient, however some bare patches of lactose were observed.

The application of high-resolution chemical imaging technologies here has demonstrated considerable potential for solid state analysis of advanced and conventional drug-delivery systems.

034
Lipidic cationic peptide dendrons as a DNA delivery system: influence of lipid chain in stability to disruption by polyanion-heparin

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Dendrimers and their conjugates have potential for DNA complexation and it is recognised that they may be useful as non-viral vectors for gene delivery. Based on initial transfection studies with similar dendrons (Shah *et al* 2000), series of cationic peptide dendrons with or without a lipidic core were synthesised with a view to improve gene transfer. Complexation of these dendrons with DNA to form dendriplexes and physico-chemical properties were characterised (Ramaswamy *et al* 2003). Preliminary studies on DNA transfection by these peptide dendrons showed a relationship between the lipophilicity of the vector and transfection efficiency. Polyvalent delivery systems like PEI and dendrimers are sensitive to the inhibitory effects of glycosaminoglycans (GAGs) on gene transfer (Ruponen *et al* 1999). Extracellular interactions of the dendriplexes with anionic GAGs may affect the stability of the complex and interfere with gene transfer. Interactions of the dendriplex with one of the GAG-heparins were studied using an EtBr intercalation assay. Upon condensation EtBr is expelled from DNA and thus fluorescence decreases. Destabilisation of the complex recovers the fluorescence because of the exposure of EtBr to intercalate with DNA. In brief, calf thymus DNA solutions were prepared in deionised water in presence of ethidium bromide (500 ng mL^{-1}) and the fluorescence was set to 100%. Dendriplexes of 10:1 $\pm$ ratio which showed better transfection were prepared and EtBr was added. Fifty microlitres of 1% heparin solution (well above the 10:1 $\pm$ ratio) was added to the dendriplex containing EtBr. After gentle mixing the solution was left at 37°C for 4 h. The percentage fluorescence recovered was calculated by measuring the fluorescence intensity at an excitation wavelength 260 nm and emission wavelength at 595nm (Table 1).

Table 1 Percentage fluorescence recovered due to the interaction of heparin with dendriplex and transfection activity of dendrons

Dendron nomenclature	MW	Percentage fluorescence	Luciferase activity log (RLU/mg protein)
$(L)_7(NH_2)$*	914	98 $\pm$ 7	4.30 $\pm$ 0.66
$(C10)_3(L)_7(NH_2)$	1422	64 $\pm$ 2	5.76 $\pm$ 0.22
$(C14)_3(L)_7(NH_2)$	1590	58 $\pm$ 3	6.02 $\pm$ 0.29
$(C18)_3(L)_7(NH_2)$	1587	53 $\pm$ 4	7.17 $\pm$ 0.42

* Control with no lipidic core

Dendrons with higher lipophilicity form more stable complexes than non-lipidic or less lipidic dendrons, confirmed by their resistant to disruption following challenge with an anionic species. Non-lipidic dendrons released the DNA completely due to interaction with heparin but release of DNA from the $(C10)_3(L)_7(NH_2)$–DNA complex was 65%, whereas the release was about 60 and 50% for $(C14)_3(L)_7(NH_2)$ and $(C18)_3(L)_7(NH_2)$-dendriplexes, respectively. This indicates the lipid chain present in the dendron core provides the protection to the dendriplexes in the extra-cellular environment. Moreover, the hydrophobicity favours the cellular internalisation of the dendriplexes. Such differences may well influence the biological processing of pDNA molecules and hence contribute to the disparity in cell transfection efficiency observed between different dendrons.

Ramaswamy, C., *et al.* (2003) *Int. J. Pharm.* **254**: 17–21
Ruponen, M., *et al.* (1999) *Biochim. Biophys. Acta* **1415**: 331–341
Shah, D. S., *et al.* (2000) *Int. J. Pharm.* **208**: 41–48

035
Dendrimer-heparin complexation

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Dendrimers are hyperbranched molecules with different surface functionalities (Tomalia *et al* 1990). Interactions between cationic dendrimers and negatively charged DNA is being investigated to improve the generally low penetration rate through lipidic membranes and their short half-life in biological systems. Another anionic macromolecule heparin, a sulfated polysaccharide used as a short-acting anticoagulant, has poor oral absorption due to its hydrophilic, negatively charged nature and the desulfation in the acidic condition of the stomach (Lee & Yamamoto 1990).

The complexation of unfractionated heparin (UFH) (MW 6 000–30 000 Da) with a cationic water soluble polylysine dendrimer (MW 8149 Da) has been studied as a system potentially of use in oral uptake of heparin. The dendrimer studied was synthesized by solid-phase peptide synthesis and purified by dialysis. Spontaneous formation of heparin-dendrimer aggregates occurs upon adding dendrimer solution to an aqueous solution of heparin. Transmission electron microscopy (Philips CM 120 Bio Twin, Einhoven, Netherlands) revealed clearly the formation of these stable nano-aggregates. Z-average diameters and zeta-potentials of the aggregates were studied by photon correlation spectroscopy (PCS) using a Zetasizer 3000 (Malvern Instruments, Malvern, UK; He-Ne laser, the angle of measurement: 90°) (Table 1). The system was found to be stable up to 14 days. Aggregates below a dendrimer/UFH mass ratio 2:1 (4.4:1 molar ratio) showed negative zeta potentials.

Table 1 Z-average diameter and zeta potentials of the nano-aggregates at different mass ratios

Dendrimer/UFH mass ratio	Z-average diameter (nm)	Zeta potential (mV)
0.5:1	99 ± 3.6	−38 ± 1.5
1:1	122 ± 1	−47 ± 2
2:1	121 ± 1.4	+52 ± 1.7
3:1	147 ± 2.6	+56 ± 2.2
4:1	147 ± 1.5	+56 ± 3
5:1	136 ± 2	+60 ± 2

Methylene blue spectroscopy was used to calculate the dendrimer/UFH mass ratio at which complete association of heparin and dendrimer occurs (Leone-Bay *et al* 1998). Upon titration with dendrimer, 100% of the bound methylene blue was displaced from the heparin as shown by a maximum decrease in the absorbance at 568 nm (bound MB maximum) and increase at 664 nm (free MB maximum) at 0.9:1 dendrimer/UFH mass ratio.

From this work, we conclude that an anionic heparin forms spontaneously stable nano-aggregates with a cationic polylysine dendrimer with varied surface charges depending on the component mass ratios. Such phenomena may be utilized in areas such as heparin oral delivery or therapeutically blocking heparin activity and its role in angiogenesis in solid tumours.

Lee, V. H. L, Yamamoto, A. (1990) *Adv. Drug Del. Rev.* **4**: 171–207
Leone-Bay, A., *et al*. (1998) *J. Controlled Release* **50**: 41–49
Tomalia, D. A., *et al*. (1990) *Angew. Chem. Int. Ed. Engl.* **29**: 138–175

036
Positively charged mucoadhesive PLGA microphere for pulmonary delivery of rifampicin

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Nearly one-third of the world's population is infected with tuberculosis (TB), which kills almost 3 million people a year. TB, on a global scale, is the leading cause of death due to an infectious agent. Moreover, TB has seen a steady re-emergence as an increasingly important health problem. Centers for Disease Control suggest that, worldwide, the current high rate of new TB cases in the population is likely to increase over the next 10 years.

The majority of dry powder aerosol research has focused on the aerodynamic properties of drug particles. However, effective drug delivery to the lung following inhalation still depends upon simultaneous, complicated processes (e.g., deposition, dissolution, absorption, metabolism and mucociliary clearance) (Sakagami *et al* 2001). The development of mucoadhesive drug delivery to the lungs as a means of treating TB may be desirable for several reasons. Delivery directly to the site of action may enhance the efficacy of therapeutic agents, enabling reduced doses. Formulation strategies to prolong the retention time of drugs in the lung may be beneficial in order to enhance or retard absorption and, thus also, influence therapeutic effects of agents delivered by this route.

Chitosan has been used for mucoadhesive gene and peptide drug administration via mucosal routes and this has been explored by a number of researchers. We have prepared mucoadhesive positively charged poly lactide-co-glycolide (PLGA) microspheres containing rifampicin. The same system has been evaluated previously by us for enhancement of immune responses to nasally delivered diphtheria toxoid (Somavarapu *et al* 2002).

Microspheres were prepared by double and single emulsion methods. In brief, for single emulsion, polymer and rifampicin was dissolved in dichloromethane (DCM) and added to 2.5% w/v poly vinyl alcohol (PVA) or a 0.75% chitosan solution. This was stirred overnight to evaporate the DCM. For double emulsion, the polymer and rifampicin was dissolved in 5 mL DCM and emulsified with 1.5 mL of 5% PVA or with 0.75% chitosan solution. The microspheres were characterized with reference to size, loading and zeta potential. Microparticles were then harvested by centrifugation and freeze-dried. The microparticles prepared with PVA as an emulsifying agent resulted in nearly 20% loading efficiency using both methods, whereas microparticles prepared with chitosan in both single and double emulsion methods achieved 35% loading efficiency. The size of the microspheres obtained using PVA or chitosan are similar at 2–6 μm. The zeta potential of all rifampicin loaded microparticles was negative except microspheres prepared with chitosan as the emulsifier by the double emulsion method.

The results show that chitosan not only produces positively charged microspheres, but also enhances the loading efficiency of rifampicin. These preparations offer potential for further evaluation both in-vivo and in-vitro.

Sakagami, M., *et al*. (2001) *J. Controlled Release* **77**: 117–129
Somavarapu, S., *et al*. (2002) *J. Pharm. Pharmcol.* **55**S: 549

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The self-assembly of low molecular weight branched polyethylenimine amphiphiles

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Previously we have reported on the self assembly of a number of amphiphilic polymers (Wang *et al* 2001a, b) and have identified structural variables which control the nature of these self assemblies. In the current communication we report on the self-assembly of low molecular weight branched polyethylenimine derivatives (LMBPEI, $M_n = 600$). Seven LMBPEI derivatives were synthesised and structurally characterised, and their self assembly studied by measuring their critical aggregation concentration (CAC) (Uchegbu *et al* 2001) and hydrodynamic radius (Table 1). Self-assembly was also studied in the presence of cholesterol and colloidal aggregates imaged by transmission electron microscopy.

Table 1 LBPEI derivatives synthesised

Sample	Average no. of polyethylene glycol (PEG, Mw ~ 5 kD) chains per molecule	Average no. of cetyl chains per molecule	Average no. of palmitoyl chains per molecule	CAC (g L^{-1})*	Mean particle size (nm) of a 20mg mL^{-1} aqueous dispersion
C1	0	1	0	0.02	15 (P)
C2	0	2	0	0.01	16 (P)
C4	0	4	0	0.025	41 (M)
PP31	3	0	1	2.7	78
PP13	1	0	3	1.3	110
PC11	1	1	0	0.2	174
PC12	1	2	0	0.08	125

P = polydisperse (more than one peak seen in size distribution plot),
M = monodisperse (only 1 peak seen in size distribution plot),
* obtained from two separate experiments (replicate curves showed complete overlap).

For C1, C2 and C4, CAC values mirrored the hydrophobicity of the polymer for C1 and C2 but not for C4, which had a higher CAC than would be predicted from it's structure. By examining the particle size data, it was concluded that C1 and C2 form micellar aggregates (Table 1), while the aggregation of C4 results in a larger molecular arrangement — a nanoparticle. Thus micelles may be formed from LBPEI molecules bearing a maximum of 2 hydrophobic, in this case cetyl, groups per molecule. The hydrophobicity of these cetylated LMBPEI aggregates followed the trend C4 > C2 > C1. The inclusion of hydrophilic PEG chains to erstwhile micelle forming amphiphiles prevents micelle formation (Table 1) as larger aggregates are formed. This indicates that PEG chains also reside within the core of

the aggregates and do not reside exclusively on the aggregate surface. Additionally with the PEGylated amphiphiles the inclusion of palmitoyl chains as opposed to cetyl chains had a drastic effect on the CAC of the aggregates leading to the production of more polar aggregates as the hydrophobicity of PEGylated aggregates followed the trend PC12 > PC11 > PP13 > PP31. Both PC12 and PC11 formed bilayer vesicles in the presence of cholesterol (10–50% w/w). We conclude amphiphilic LMBPEI forms various self-assemblies, the nature of which is controlled by polymer architecture.

Uchegbu, I. F., *et al.* (2001) *Int. J. Pharm.* **224**: 185–199
Wang, W., *et al.* (2001a) *Langmuir* **17**: 631–636
Wang, W., *et al.* (2001b) *J. Coll. Interf. Sci.* **237**: 200–207