Poster Session 1 – Drug Delivery

002
Spray-dried powders for pulmonary drug delivery: comparison with marketed products

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Pulmonary drug delivery offers an attractive route of administration for locally-acting drugs, such as bronchodilators and corticosteroids. Significant attention has been focused on the development of dry powder inhaler (DPI) systems, as such systems could potentially confer many advantages, including increased shelf-life, reduced drug loss on administration, ease of patient use and more efficient delivery to the pulmonary target region. The majority of DPI systems comprise micronised drug, blended with larger carrier particles to improve powder flow and homogeneity during manufacture and to enhance powder aerosolisation properties; however, this traditional approach requires additional steps in the formulation process, including sieving and blending. It has recently been shown that spray-drying lactose-based formulations incorporating amino acids as dispersibility enhancers can successfully generate dry powders with reasonably high fine particle fractions, without the need for large carrier particles (Li et al 2003, 2005; Rabbani & Seville 2004). The aims of this study were to prepare spray-dried powders containing salbutamol (model hydrophilic drug) or beclometasone dipropionate (BDP: model lipophilic drug) and to compare the in vitro aerosolisation performance of these powders with currently marketed single-dose DPI systems. Aqueous ethanol (30% v/v) formulations containing model drug (salbutamol or BDP) and lactose in the presence of leucine were prepared, with a total powder mass of 2% w/v. All formulations were spray-dried (Büchi B-290 mini spray-dryer) using standard operating conditions of inlet temperature, spray flow rate, aspirator and pump rate to generate spray-dried lactose-based powders containing either 4% w/w model drug/6% w/w leucine or 8% w/w model drug/12% w/w leucine. Powder samples ($n = 3$) were loaded into gelatin capsules and aerosolised using a Spinhaler DPI attached to an Andersen-type cascade impactor (ACI; flow rate of $60\,L\,min^{-1}$). The emitted dose (ED) was determined gravimetrically, and UV analysis was used to quantify the fraction of drug deposited at each stage of the ACI, with the fine particle fraction (FPF) determined as the fraction of deposited material less than $5\,\mu m$ aerodynamic diameter. These results were then compared with those obtained using commercially available pre-loaded capsules for inhalation (salbutamol $400\,\mu g$ Cyclocaps and BDP $400\,\mu g$ Cyclocaps; Approved Prescription Services), aerosolised as above using a Cyclohaler DPI. The 8% salbutamol/12% leucine spray-dried powder (FPF $63.2 \pm 2.0\%$) performed statistically (one-way analysis of variance, $P < 0.05$) better than both the 4% salbutamol/6% leucine powder (FPF $47.4 \pm 8.4\%$) and salbutamol Cyclocaps (FPF of $49.4 \pm 1.0\%$). Likewise, the 8% BDP/12% leucine spray-dried powder (FPF $32.2 \pm 1.3\%$) performed statistically better than both the 4% BDP/6% leucine powder (FPF $19.1 \pm 3.8\%$) and BDP Cyclocaps (FPF of $11.3 \pm 1.3\%$). In addition, aerosolisation of the Cyclocaps powders resulted in substantially higher deposition in the upper stages of the ACI; this would be associated with higher oropharyngeal deposition in vivo, with potentially increased local side effects (e.g. oral candidiasis with BDP). This study suggests that the aerosolisation performance of novel spray-dried powders containing salbutamol or BDP can be favourably compared with that of commercially available single-dose products, with the potential for reduced side effects.

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003
The effect of different bases on the release of paracetamol from suppositories

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Paracetamol suppositories provide an alternative drug delivery route for patients who may be unable to take oral tablets. However, the bioavailability of drugs administered via the rectal route is often lower than the same drug administered via alternative routes such as oral (Montgomerry et al 1995; Coulthard et al 1998), intravenous and intramuscular (Moolenaar et al 1980). This project examined the effect of suppository bases (Witepsol H15, Witepsol H32, PEG 600, PEG 1300 and PEG 2000), suppository size (1 g, 3 g), paracetamol concentration (250 mg, 500 mg, and 1000 mg) and an emulsifying agent (Tween 80) on in vitro drug release. Suppositories were formulated using the moulding method and the drug release rate was determined using the USP II dissolution method. All formulated suppositories complied with the uniformity of mass test specified in the European Pharmacopoeia. The paracetamol release rate from suppositories with a polyethylene glycol (PEG) base was more rapid than from suppositories formulated with Witepsol. After 30 min, PEG 600 and 1300 had released the total drug dose, whereas Witepsol H15 had only released 48.1% of the total dose. Variations were also observed in the drug release rate between the different PEGs and between the different Witepsols. Witepsol H32 released the total dose after 105 min, which was more rapid than the release from Witepsol H15 where only 64.8% of the paracetamol dose was released. This observed difference is most likely due to the lower melting point of Witepsol H32. As the molecular weight of the PEG base increased, the rate of drug release decreases. Paracetamol was released most rapidly from suppositories formulated with PEG 600 (87.9% at 15 min and 100% at 30 min), followed by PEG 1300 (43.7% at 15 min and 100% at 30 min) and lastly PEG 2000 (59.7% at 15 min and 74.3% at 30 min). This difference is probably due to the hydroxyl value and viscosity of the compound. The release from 3-g suppositories containing 250, 500 or 1000 mg of paracetamol was statistically significantly different ($P < 0.05$). However, inconsistent results were observed with suppositories of different mould sizes. The incorporation of 2% w/w Tween 80 in paracetamol suppositories produced a significant increase in the release of paracetamol from all of the suppository bases tested (e.g. Tween 80 produced a 50% increase in the amount of paracetamol released from Witepsol H15 base after 120 min). By selecting bases with different physicochemical properties, one can manipulate the release of drug from suppositories. The in vitro drug release from suppositories can be improved by incorporating an emulsifying agent. Further work is required to verify the effect of drug concentration and suppository size on drug release.

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004
Formulating biodegradable microspheres and enhancing their adjuvanticity for use as sub-unit TB vaccine delivery systems

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Biodegradable poly(DL-lactide-co-glycolide) (PLGA) microspheres appear to be an ideal candidate for the delivery of sub-unit vaccines, due to their relative biocompatibility, prolonged drug release profile, and their efficient targeting of professional antigen presenting cells (APC) (Audran et al 2003). The aim of this research is to formulate and characterise effective microsphere delivery systems for a sub-unit (Ag85B-ESAT-6) TB vaccine. To this end, the cationic adjuvant dimethyl dioctadecylammonium bromide (DDA) and the immunomodulating agent trehalose 6,6′-dibehenate (TDB) were added to the formulations, due to their proven ability to initiate a specific immune response (Holten-Andersen et al 2004). Microspheres were prepared by the w/o/w double emulsion-solvent evaporation method previously described (Ogawa et al 1988). Initially, key various parameters of this method were optimised: notably, polymer concentrations of 3% (w/v) in the organic phase, and poly(vinyl alcohol) concentrations of 10% (w/v) in the external aqueous phase, yielded microspheres well within the desired sub-10 μm range ($1.50 \pm 0.13\,\mu m$), while exhibiting a high protein entrapment efficiency ($95 \pm 1.2\%$). Addition of DDA (20% w/w of polymer) to the organic phase led to a marked increase in mean particle size ($20.3 \pm 1.85\,\mu m$) and a reduction in entrapment efficiency ($57 \pm 0.8\%$). In contrast, addition of TDB (2% w/w of polymer) to the organic phase resulted in no significant change in mean particle size ($2.4 \pm 0.20\,\mu m$), but resulted in a 15% reduction in antigen entrapment efficiency. Interestingly, the addition of both DDA and TDB led to only an approximate two-fold increase in mean particle size ($3.16 \pm 0.47\,\mu m$), which is significantly smaller than DDA-microspheres, although this combination resulted in a significant reduction in antigen entrapment, down to $55.3 \pm 2.3\%$. All formulations showed prolonged release profiles, with no initial burst release over the first 24 h, characteristic of most microsphere formulations. Indeed, the addition of DDA, either alone or in combination with TDB, appears to result in a more sustained protein release. From these results, such formulations offer excellent potential for the combination of immunomodulating agents in the design of sub-unit vaccine delivery systems. The addition of TDB to the formulations

appears to have a stabilising effect, while the addition of DDA seems to have a positive effect in terms of prolonging the release of protein antigens, potentially due to partial adsorption of the protein to the DDA cationic head group.

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005
Using yeast to enhance the delivery of insulin through an epithelial monolayer

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Transport of hydrophilic drugs occurs mainly through the paracellular pathway, which is controlled by the presence of tight junctions between neighbouring epithelial cells. Tight junctions form a highly effective impenetrable barrier to paracellular transport; however penetration enhancers work by reversibly opening this barrier (Foraker et al 2003). The aim of this work was to investigate the enhancement of insulin penetration through an epithelial monolayer in vitro by heat-killed yeast cells. To test the ability of yeast cells (*Saccharomyces* spp.) to enhance paracellular permeability, a Caco-2 cell monolayer culture system was used. Caco-2 cells were grown to confluence on a polycarbonate membrane support, effectively imitating the small intestinal epithelium with the formation of brush borders and tight junctions, thus forming an impervious barrier. Yeast suspensions of 2–0.05% (w/v) were applied to cell monolayers for 1 h or 24 h. Permeability enhancement was then assessed by measuring the transepithelial electrical resistance (TEER) across the monolayer and by assessing permeability to both horseradish peroxidase (HRP), a marker protein, and to fluorescently-labelled insulin. Recovery of the permeability of the cell monolayers after removal of yeast was also assessed by TEER and by HRP permeability measurements, and toxicity was assessed through trypan blue dye exclusion. The molecular mode of yeast action was investigated with confocal scanning microscopy using phalloidin to stain actin filaments and an anti ZO-1 (Zonula Occludens-1) antibody to stain one of the scaffolding tight junction proteins. In Caco-2 cells, application of yeast caused a dose-dependent decrease in TEER over 1 h. Opening of tight junctions was confirmed in a monolayer culture after application of a 2% yeast suspension through a 3.8- and 7.4-fold increase in permeability to HRP compared to control, 1 h and 24 h after application respectively. After removal of the yeast, Caco-2 cell monolayers recovered fully as shown by restoration of the TEER and virtual impermeability to HRP after 24 h. The application of yeast did not seem to be toxic to the Caco-2 cells. Following incubation with yeast, the actin cytoskeleton and ZO-1 tight junction proteins were shown to be involved in tight junction opening based on a decrease in fluorescent intensity and a change in their localisation, as observed by confocal microscopy. Heat-killed yeast cells were shown to successfully enhance the penetration of insulin, acting in a reversible, time and dose-dependent manner. Data on the penetration of fluorescently-labelled insulin through Caco-2 cell monolayers will be presented and the potential for using yeast cells to aid drug delivery will be discussed.

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006
Design and assessment of dendrimer–drug conjugates for enhanced drug bioavailability

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Dendrimers represent a relatively new class of highly branched polymers with a well-defined structure that allows precise control of size, shape and terminal group functionality (Tomalia et al 1985). Dendrimers have several pharmaceutical applications (D'Emanuele et al 2003), including the enhancement of drug solubility and permeability. Polyamidoamine (PAMAM) dendrimers show potential for the enhancement of bioavailability via the oral route (D'Emanuele et al 2004). The aim of this study is to design a linker between the drug and carrier (dendrimer) that is stable in the gastrointestinal tract and during transit across epithelial cells, but labile once absorbed. Naproxen, a poorly water-soluble drug, was conjugated to G0 PAMAM dendrimers either directly (amide bond) or via a linker: either L-lactic acid or diethylene glycol (ester bonds). The in vitro chemical and enzymatic hydrolyses of the dendrimer–naproxen conjugates were examined. Conjugates were synthesized using an equimolar ratio of dendrimer and naproxen. The carboxylic acid groups of both naproxen (NAP) and naproxen-lactic acid (NAP-lact) were conjugated to the amine groups of G0 dendimer using N,N'-carbonyldiimidazole (CDI) as a coupling agent. Naproxen-diethylene glycol (NAP-deg) was attached to G0 using 4-nitrophenyl chloroformate. The resulting water-soluble conjugates were characterized by ^{1}H and ^{13}C NMR, mass spectrometry, FTIR and RP-HPLC. Hydrolyses of the prodrugs were studied at 37°C in hydrochloric acid buffer (pH 1.2), phosphate buffer (pH 7.4), borate buffer (pH 8.5), 80% human plasma and 50% rat liver homogenate. It was found that the amide-linked conjugate (NAP-G0) was stable towards hydrolysis over a period of ten days in all media. Similarly, both ester conjugates (NAP-lact-G0 and NAP-deg-G0) were chemically stable in buffer solutions at all pHs over 48 h of incubation. Naproxen was enzymatically released from the L-lactic and diethylene glycol ester conjugates at different rates in plasma and liver homogenate. NAP-lact-G0 was slowly hydrolysed in 80% human plasma (25% of naproxen released in 24 h) and in 50% liver homogenate (pseudo-first order kinetics, $t_{1/2} = 180$ min). In contrast, despite the high stability of the diethylene glycol ester conjugates (NAP-deg-G0) in buffers, this ester was cleaved rapidly with pseudo-first order kinetics in both 80% human plasma ($t_{1/2} = 51$ min) and 50% liver homogenate ($t_{1/2} = 4.7$ min). Dendrimer–drug carriers based on appropriate linkers with ester bonds have potential as carriers for low solubility drugs such as naproxen. The permeation of the conjugates through Caco-2 monolayers is currently under investigation.

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007
Do QPSR models accurately reflect percutaneous absorption?

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Mathematical modelling studies (e.g. Moss & Cronin 2002) based on the Flynn dataset have determined that the main predictors of permeability coefficient (kp) are the size and lipophilicity of the drug molecule. These may be modelled as MW and octanol/water partition coefficient (P), and for absorption from aqueous vehicle $\log kp\ (cm/s) = 0.74 \log P - 0.0091\ MW - 2.39$ (Moss & Cronin 2002). Magnusson et al (2004) suggested that maximal flux (J_m) was more important than k_p when modelling percutaneous absorption and found that MW was its only significant predictor: $\log J_m\ (mole/cm^2/h) = -4.52 - 0.0141\ MW$. The major issue with QSPR models for skin absorption is that they are limited by their original datasets, with particular regard to the range of data and the lack of formulation involved – all the QSPRs are derived from datasets that measure J_m or k_p from saturated aqueous solutions. Recently, the synthesis and percutaneous absorption of a range of captopril prodrugs have been reported (Moss 2004). These prodrugs were developed by using QSPR models to predict ideal prodrug candidates with optimised permeability profiles. QSPR analysis suggests that esters and thiol derivatives of captopril would have lower J_m values than the parent, with J_m decreasing with ester chain length. In practice the esters had flux values up to 0.8 μmole/cm^2/h for the ethyl ester (≈100 times that predicted), peaking for the intermediate chain lengths. These large differences could not be explained by the higher lipohilicity of the esters resulting in higher concentration in the outermost layers, or discrepancies between experimental and calculated solubilities. No rationale can be offered for the lack of applicability of the skin QSPRs. The results of this analysis would suggest that current QSPR models provided, at best, limited estimates of percutaneous transport. It is widely recognised that they cannot account for formulation effects, but the current study would indicate that other factors, such as metabolism and aqueous solubility play important roles. This, combined with the limitations of the dataset (particularly with regard to the range log P), indicate that QSPRs should be applied to "real-life" situations with caution, and the datasets need to be expanded considerably to widen the applicability of these techniques. The use of non-linear modelling may allow a better understanding, and prediction, of percutaneous absorption.

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008
Characterisation of protein antigen loaded surfactant vesicles as subunit vaccine adjuvant system: ESEM analysis of stability

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Previous studies have shown that vesicular adjuvant, dimethyldioctadecyl ammonium (DDA), promotes both cell-mediated and humoral responses in mice when tested for a TB subunit vaccine (Lindblad et al 1997). Unfortunately, these DDA liposomes are physically unstable under ambient storage conditions, forming aggregates over time. Here, an attempt was made to produce a stable formulation by incorporating additional surfactants into this system, altering the method of preparation and evaluate their physico-chemical characteristics. Ag85B-ESAT-6, a recombinant antigen to induce immunity against TB infection (20 μg) was mixed with preformed MLV composed of DDA, with or without α,α'-trehalose 6,6′-dibehenate (TDB) (1:1 mole ratio) prepared by film hydration method or SUV composed of 1-monopalmitoyl-rac-glycerol (MP), cholesterol, DDA with or without TDB (4:4:1:1 mole ratio) which was freeze-dried, followed by controlled rehydration obtaining dehydration-rehydration vesicles (DRV). The vesicle z-average diameter and zeta potential were measured on *Zeta Plus* (Brookhaven Instruments). Encapsulation efficiency was assayed based on the activity of incorporated ^{125}I-labelled protein. Morphological analysis and vesicle stability was examined by environmental scanning electron microscopy (ESEM). Results showed that DDA vesicle sizes after 28 days had increased to 4639.6 ± 1256.8 nm from an initial size of 488.1 ± 123.6 nm at 25°C, presumably due to aggregation as seen from decreased zeta potential (46.2 ± 1.6 mV to 25.7 ± 1.3 mV). Inclusion of TDB slightly improved the vesicle size (3106.6 ± 164.4 nm) with a zeta potential of 35.5 ± 1.5 mV. The efficiency of MP-based adjuvant systems in the presence of cholesterol was previously reported (Brewer & Alexander 1992) and further demonstrated that merely mixing antigen with empty vesicles is not sufficient for adjuvant activity. When MP and cholesterol were incorporated into the DDA system prepared by DRV method, the initial vesicle size (1346.8 ± 140.0 nm) was relatively higher than its DDA counterparts; this is explained mainly because of the high surface free energy induced by the low hydrophobicity of the MP molecule (Wan & Lee 1974) whose size remained at 1490.8 ± 300.3 nm after 28 days without any variation in zeta potential (51.0 ± 4.3 mV). Encapsulation of protein in these preparations was found to be 87.8 ± 0.5% of the initial amount used. Addition of TDB did not affect the system's physical characteristics. Spatial location of protein was also studied by incubating empty DRV with protein and it was found that adsorption of protein on the vesicle surface was minimal (about 10%), suggesting that most of the protein is actually encapsulated within the vesicle. This was further reinforced by ESEM analysis, which revealed that protein incorporated vesicles resisted the destabilisation caused by reduction in operating pressure in the sample chamber up to 2.4 torr unlike the empty vesicles, which collapsed at a pressure of 3.5 torr, suggesting that protein might actually be present within the bilayers increasing the rigidity. In conclusion, MP-based DRV preparations, apart from having inherent characteristics of adjuvant activity, also facilitate encapsulation of protein which in turn make vesicles more stable and possibly possess greater capacity to target antigen presenting cells or ability to form short-lived depots in vivo.

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009
Poly(styrene-co-maleic anhydride)-sulfanilamide conjugate: synthesis and antibacterial activity

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The pendent type of drug delivery systems, where the drug is attached as a side chain to the polymer matrix via biodegradable bond, is the most popular. This bond may be hydrolysable (ester, anhydride) or enzymatically biodegradable (amide). They usually exhibit their microbial activity by slowly releasing the drug fraction via hydrolysis. Such systems mainly aid in increasing the half-life of drug as well the residence at the affected site (Lee et al 2002). They also have some advantages over low molecular weight agents because they are more stable against volatilization, dissolution and diffusion to the surface of material to be protected. In this work, poly(styrene-co-maleic anhydride), PSMA, was used as an intermediate in preparing functional polymer since the active agent containing the amino group can be linked to it via a ring opening reaction. PSMA was prepared by precipitation polymerization (Raval et al 1997). A solution of previously prepared PSMA was added to a solution of sulfanilamide (SA) in anhydrous dimethylformamide (DMF). The coupling reaction was carried out at 40°C for 4 h and monitored with change in acid value. The mixture was precipitated into methanol (80%). The glass transition temperature of PSMA-SA (210°C) conjugate was higher than that PSMA (195°C) due to intermolecular hydrogen bonding. The antimicrobial activity of PSMA-SA was investigated with the shake flask test method towards *Bacillus subtilis*. PSMA-SA, as well as SA, showed strong bactericidal activity against the bacteria (Table 1). The inhibition zone test of PSMA-SA against the bacterial species was also performed. However, an inhibition zone was not observed from the test specimens. This result suggests that the hydrolysis of PSMA-SA may be too slow to generate a sufficient amount of free SA molecules that lead to formation of any inhibition zone under the experimental condition. Therefore it was concluded that PSMA-SA may be bactericidal itself and its bactericidal activity may last for a fairly long period of time under neutral conditions.

Table 1 Antibacterial efficacy against *Bacillus subtilis*

Sample	Reduction (%)	Tg°
Blank	—	—
PSMA	24	195
SA	100	185 (m.p)
PSMA-SA	93.4	210

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010
Improving the intracellular delivery of aminoglycosides using anionic PAMAM dendrimers

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The treatment of intracellular infection poses an ever-increasing challenge. With the incidence of diseases such as tuberculosis and malaria escalating, the demand for antibiotics that can target the site of infection is greater than ever. Aminoglycosides, including gentamicin and streptomycin, are invaluable antibiotics capable of eradicating both Gram-positive bacilli and some Gram-negative bacteria. They do, however, have disadvantages, such as their potential nephrotoxicity and poor physicochemical properties. Aminoglycosides are polycationic, water soluble, and accumulate slowly inside cells because of their limited penetration across lipophilic cell membranes (Lambert & O'Grady 1992). In this work dendrimers were studied as a novel drug delivery system for aminoglycosides. Half generation PAMAM dendrimers terminating in carboxylate groups, which are non-toxic (Malik et al 2000), were used. Dendrimers are taken efficiently into cells by endocytosis (Jevprasesphant et al 2003). This work investigates, firstly, the potential of anionic PAMAM dendrimers to bind with an aminoglycoside and, secondly, the release of the antibiotic and its subsequent activity. The antimicrobial activity of the antibiotics and the antibiotic complexes was evaluated. Two methods of associating aminoglycosides with G3.5 PAMAM dendrimers were employed: electrostatic interaction and covalent conjugation via an acid-labile hydrazone linker. Simple mixing and dialysis of gentamicin and dendrimer yielded an electrostatic complex. ^{1}H NMR and a fluorescamine assay confirmed the presence of the aminoglycoside and indicated that a weak electrostatic interaction was taking place between the two compounds. A conjugate of streptomycin to dendrimer was synthesised using a hyrazone-glycine linker; ^{1}H NMR and the Sackaguchi Method confirmed the presence of the antibiotic. A standard microbial agar assay against *Salmonella* ACTC 23564 proved there was no antimicrobial activity of free dendrimer but when electrostatically combined with either antibiotic, antimicrobial activity was the same as or greater than aminoglycoside alone. Antimicrobial activity was detected in solution after hydrolysis of the streptomycin-dendrimer conjugate at pH4 (endosomal pH). The movement of FITC-labelled antibiotic-dendrimer conjugate into cells was followed by fluorescent microscopy. Electrostatic and covalent dendrimer-aminoglycoside have

been synthesised and characterised. Their activity and uptake into cells have been evaluated. These systems have the potential to enhance delivery of antibiotics to sites of infection traditionally difficult to treat.

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011
Design of Eudragit coated chitosan beads for colon-specific drug delivery

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The purpose of the study was to design and evaluate a multiparticulate colon-specific drug delivery system, combining the pH sensitive property of enteric polymers, Eudragit L100 and Eudragit S100, and biodegradability of chitosan polymer (Yang et al 2002) for efficient targeting of the model drug, meloxicam. Meloxicam is indicated in rheumatoid arthritis and also found to be beneficial in colon cancer therapy (Goldman et al 1998). Chitosan beads were prepared by ionotropic gelation using sodium tripolyphosphate (TPP) as the multivalent counterion (Sezer & Akbuga 1995). The drug solution was dispersed in a 2% (w/v) solution of chitosan in dilute lactic acid and dropped into a gently agitated solution of TPP from an optimum height for crosslinking. After 45 min, the chitosan beads formed were washed and air-dried for 24 h. The beads so formed were coated with Eudragit L100, Eudragit S100 and a 1:1 ratio of both the polymers by solvent evaporation technique. Triethyl citrate (TEC) and talc were used as plasticizer and glidant, respectively, to the coating solution formulation. Various process parameters were optimized to ensure maximum drug loading. In vitro drug release studies were carried out by sequential pH change method to simulate the pH change across the gastro-intestinal tract. SEM studies and optical microscopy was done to characterize the formulation. The drug loading percent of chitosan beads was 9.60 ± 0.06 ($n = 3$). Drug release in the first 3 h was $< 6\%$ for all the batches. The release was fastest for the batch coated with Eudragit L100 and slowest for that coated with Eudragit S100. The best release profile was observed for the batch coated with Eudragit L100 and Eudragit S100 mixture (1:1) where 88.85% drug release occurred in 10 h, with 58.79% drug release occurring in colonic pH conditions. A novel system comprising of crosslinked chitosan beads coated with Eudragit polymers was thus designed for successful delivery of meloxicam to the colon. This route of delivery is particularly advantageous over conventional dosage formulations because delayed and sustained release can be achieved, a reality that is likely to be attained by the designed formulation in treating diseases like rheumatoid arthritis, which is affected by circadian rhythm.

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012
Drug targeting for pulmonary delivery

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The aim of this study is to use surface modification of colloidal particles and investigate the effect of such a modification on the particles interaction with, and uptake by, the BEAS-2B cell line, a model for small airway epithelial cells. This work is a part of our project that aims to examine whether the modified microparticles confer steric stabilisation and thus have reduced macrophage uptake, hence allowing the possibility of improved targeting of epithelial and dendritic cells in the deep lung. Poly ethylene oxide (PEO) modified Poly (D,L lactide-co-glycolide) (PLGA) microparticles in the size range of 1–4 μm, appropriate for inhalation, were produced using an oil in water emulsion technique with certain combinations of Pluronic (P) and Tetronic (T) surfactants. Coumarin-6 labelled PLGA-T908, PLGA-PF127, PLGA-T908&T904 formulations were assessed for cellular association using confocal microscopy and flow cytometry. A PLGA-surfactant free formulation was used as a control. Confocal studies with microparticles and the BEAS-2B cells revealed a reduced association at 4°C compared with 37°C. This is in agreement with flow cytometry findings where less association was seen with BEAS-2B cells at 4°C (i.e. PLGA-surfactant free $8.0\% \pm 1.4$ fluorescence associated with cells as percentage of added microparticle fluorescency) relative to 37°C ($46.0\% \pm 6.7$ fluorescence associated with cells as percentage of added microparticle fluorescency, $P < 0.05$, $n = 4$). The confocal images at 4°C confirmed that microparticles were associated to the outer surface of cells only. However, the association observed at 37°C was from a combination of particles associated to cells outer surface, as well as particles internalised by the cells, suggesting that some particles are taken up at 37°C by an active process. Initial inhibitor studies using cytochalasin D with BEAS-2B cells and PLGA-T908 or PLGA-surfactant free particles reveal that a different mechanism of uptake may be involved for PLGA-T908 particles than PLGA-surfactant free after 1 h at 37°C. There was no evidence of PLGA-T908 particles taken up into BEAS-2B cells, although some PLGA-surfactant free particles were observed to be inside BEAS-2B cells. The inhibitor cytochalasin D interacts with cell cytoskeletal filaments and inhibits the filament polymerization reaction, resulting in changes in cell shape as well as inhibition of some types of cell movements. As no PLGA-T908 particles were internalized in the presence of cytochalasin D and some PLGA-surfactant particles were, initial findings suggest that perhaps the PLGA-T908 particles are taken up into the cell by a process dependent on actin filament polymerization. Further work using different cell uptake inhibitors will be carried out to elucidate how the PLGA-surfactant free particles are internalised.

013
Nanoparticles for pulmonary drug delivery

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Currently, most pressurised metered dose inhalers (pMDI's) are formulated as suspensions in which micronised drug (~2–3 μm diameter) is suspended in the propellant. These suspension formulations are prone to particle sedimentation in the device, which leads to poor dose reproducibility and inefficient lung deposition in the patient. We wish to investigate a novel nanotechnology comprising a low-energy process to prepare surfactant-coated nanoparticles. The selection of an appropriate surfactant system provides the resulting nanoparticles with surface properties that allow for their redispersion in hydrofluoroalkane (HFA) propellant within the chamber of a pMDI. The nano-sized dimensions of the particles and their ability to efficiently disperse in HFA produces stable aerosols capable of efficient pulmonary delivery. A water-in-oil microemulsion system was formed (Dickinson et al 2001), with 15% w/v sulbutamol sulphate solution as water phase, lecithin:iso-propanol 1:3 w/w as surfactant system, and iso-octane as oil phase. A pseudo-ternary phase diagram of this microemulsion system was constructed, and the subsequent selected microemulsion comprising water phase:surfactant:oil phase (22.5:45:32.5 w/w/w) was dried and the waxy mixture of sulbutamol sulphate nanoparticles and lecithin collected in iso-octane. Column chromatography (Strata-X-C) was used to remove surfeit lecithin, with 60% recovery of sulbutamol sulphate. Transmission electron microscopy (TEM, Philips 208) showed that the nanoparticles were spherical in shape. Nanoparticles were subsequently suspended in HFA134A to form a homogeneous dispersion for pulmonary administration. The in vitro deposition of nanoparticles was investigated using an Andersen-type cascade impactor (ACI) (Feddah 2001), at flow rate of 28.3 L min^{-1}. Each stage of the impactor was washed with water:methanol (60:40 v/v, bamethane 7 μg mL^{-1}) and the deposited sulbutamol sulphate was analysed using high-performance liquid chromagtography (HPLC), with a mobile phase (1 mL min^{-1}) of water:methanol (60:40 v/v, heptane sulphuric acid 1.1013 g L^{-1}, pH3.0). Salbutamol sulphate nanoparticles were successfully prepared to a diameter of less than 80 nm, with an average particle size of 11.2 ± 5.3 nm. These particles appeared spherical in shape with TEM confirming that the majority of particles were approximately 10 nm in diameter. The formulation of nanoparticles dispersed in HFA was stable and homogeneous being either transparent or opaque depending on concentration. In vitro deposition demonstrated that the purified nanoparticles are effectively delivered to the pulmonary region, especially the alveolar and lower bronchiolar area, with a fine particle fraction (particle size < 5.8 microns) of 40%. Nanoparticles of sulbutamol sulphate can be produced by a novel low energy process. These nanoparticles can be efficiently delivered to the lower respiratory tract via pMDI.

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014
The importance of co-lipids and phospholipids headgroup type within liposomes for the successful delivery of DNA

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For liposome-mediated DNA delivery, each lipid component used to formulate liposomes is said to determine and influence liposome structure and function when applied in vitro and in vivo. Therefore, the liposomes' primary function can be optimised by utilising the most appropriate lipids for the delivery of plasmid DNA. Here we assess the importance of associating a helper lipid with the cationic lipid to improve transfection efficiency and evaluate the importance of the type of helper lipid used. Small unilamellar vesicles (SUV), consisting of L-a-dioleoyl phosphatidylethanolamine (DOPE) in combination with various cationic lipids (*N*-[1-(2–3-dioleyloxy)propyl]-*N,N,N*-trimethylammonium chloride (DOTMA), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), dipalmitoyl trimethylammonium propane (DPTAP), cholesterol 3β-*N*-(dimethylaminoethyl)carbamate (DC-Chol) and dimethyldioctadecylammonium (DDA), were prepared at a 1:1 molar ratio. Alternative co-lipids (e.g. dioleoyl phosphatidylcholine (DOPC), phosphatidylethanolamine (PE), phosphatidylcholine (PC), dimyristoyl phosphatidylethanolamine (DMPE) or dimyristoyl phosphatidylcholine (DMPC)) were also tested. In vitro transfection studies were performed as per standard protocol for Lipofectin transfection reagent. Lipoplexes were characterised by measuring the *z*-average diameter (nm) and zeta potential (mV) on a ZetaPlus (Brookhaven Instruments) in ddH_2O and 0.001 M PBS, respectively. Lipoplexes containing the cationic lipid DC-Chol, produced the highest level of transfection compared with the other cationic lipids tested and was therefore adopted for all subsequent transfection formulations. Results show that both the co-lipid head group and the alkyl-chain influence transfection efficiency (Table 1), DOPE formulations resulting in the highest transfection levels despite the vesicle size and surface charge of all samples tested being relatively similar, with sizes in the range of 300–500 nm and surface charges of -30 to -50 mV. The effectiveness of DOPE is in line with other previous investigations, which suggest that the architectures of dioleoyl alkyl lipids can facilitate fusion and disruption of the lipoplexes with cellular and endosomal membranes (Farhood et al 1995) through their ability to invert into hexagonal lipid arrangements. However, it is apparent that this can only occur in combination with the PE head-group, which may facilitate the dissociation of the lipoplexes after internalisation. This has been reported (Pedroso de Lima 2001) to be due to a weakening of the otherwise strong cationic lipid–DNA electrostatic binding as a result of the PE amine group interacting with the DNA phosphate groups. This combination of alkyl chain and head-group within the lipid may promote successful release of the DNA into the cytosol. These systems will be further evaluated and correlated in vivo.

Table 1 Effect of liposome formulation on transfection activity

Liposome formulation	Luciferase activity (% lipofectin)
PE:DC-Chol	85.27 ± 19.99
PC:DC-Chol	22.59 ± 0.49
DOPE:DC-Chol	580.70 ± 33.95
DOPC:DC-Chol	11.40 ± 0.61
DMPE:DC-Chol	45.87 ± 14.90
DMPC:DC-Chol	2.41 ± 1.21

Results are means $\pm$ s.d., n = 3.

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015
The effects of excipient surface interactions on aerosol perfomance of pressurised meter dose inhalers

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The incorporation of larger particles of L-isoleucine (L-ile) as the excipient/carrier in interactive drug mixtures has been shown to improve the flowability of the powder blend and the quality of the aerosol generated from pressurised meter dose inhaler (pMDI) formulations (Tran et al 2004). It is believed that yet further improvements in aerosol characteristics may be achieved if the excipients' physical and chemical characteristics are modified. A smoother excipient/carrier is believed to improve the surface interaction with the active drug particle, thereby improving drug dispersion in the suspension and de-aggregation upon aerosolisation. The aim of this particular study was to extend the investigation to an examination of the contribution of excipient surface characteristics to drug surface interactions with different physical/chemical properties of excipient particles to determine the effects on aerosol performance. Solid phase extraction columns contain stationary phase materials that have narrow particle size distributions and size is an important factor in the selection of the aerosol carrier/excipient as it affects the dispersion of the aerosol's cloud (Louey et al 2004). Fortunately, wide ranges of stationary phases are available with different physical/chemical properties that interact with analytes differently. Four of these materials were chosen for their different properties, C18-E, NH_2, Si and WCX (weak cation exchanger), and L-ile (38–63 μm) was used for comparison. Particle size distributions were determined via dry dispersion through laser diffraction using the Malvern Mastersizer 2000. Excipients were blended with salbutamol sulphate in a ratio of 10:1 (w/w). Drug content uniformity was determined by coefficient of variation (CV) and formulations with CVs of 5% or less being accepted. Each formulation was dispensed into a canister, sealed with a 50-μL BK357 valve and filled with 6 mL of HFA 134a (n = 3). In vitro aerosol performance was assessed using an Andersen Cascade Impactor (ACI) coupled with HPLC analysis of salbutamol. Aerosol performance was assessed using the following parameters: fine particle fraction (FPF), fine particle dose (FPD), mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD). Particle size distributions of the four stationary phases were narrower than that of L-ile sieved particles. The formulations demonstrated a wide range of FPFs: C18-E $63.3 \pm 1.24\%$, NH_2 $28.35 \pm 3.83\%$, Si $13.13 \pm 2.61\%$, and WCX $30.64 \pm 6.39\%$, while the L-ile formulation demonstrated FPF of $41.33 \pm 2.00\%$. The C18-E formulation was significantly better than the others, while the Si formulation was significantly worse. Corresponding differences were noted for FPD. The results support the hypothesis that the silanol groups of the Si material offer a highly adsorptive environment for the drug, in contrast to the hydrophobic C18-E surface. The NH2 and WCX materials offer more potential for electronic interactions than the C18-E, thus reducing drug–excipient dispersion during aerosolisation. It is concluded that physicochemical properties of excipient surfaces in these novel pMDI formulations are important in determining aerosol performance and that high FPFs can be achieved by choosing an appropriate excipient.

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016
Drug incorporation and release from functionalized poly glycerol adipate nanoparticles

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Most drug delivery systems suffer from a major drawback of limited drug delivery to target organs and tissues. The drug can be either metabolized or they may exert side effects on non-target tissues. Nanoparticles made by poly-L-lactic-co-glycolic acid (PLGA) and poly-lactic acid (PLA) polymers have been found to be limited in their use as they suffer from poor drug incorporation and rapid drug release (burst release) (Govender et al 1999). Also the drug incorporation depends on both the drug and the polymer properties. Considering this, a system of novel polymers, poly (glycerol adipate) (molar ratio of glycerol/adipic acid: 1/1) was synthesized. This polyester was functionalized by attaching acyl groups of various lengths (C_8, C_{18}) and in different percentages (0%, 20, 40, 60, 80, 100%) to the pendant hydroxyl groups of glycerol on the backbone, to modify the hydrophobic characteristics of the polymer. All the polymer series formed spherical particles of a good homogeneity. To assess the potential use of the polymers as a drug delivery system, we are incorporating anti-inflammatory and anti-cancer drugs. Here we report work on the incorporation and release of dexamethasone phosphate (DXM-P) and cytosine arabinoside (CYT-ARA). Two preparative methods, the interfacial deposition method (ID) (Fessi et al 1989) and the emulsification technique (EM) (Murakami et al 1999), were used to make both empty and drug loaded nanoparticles. So far, results have been obtained related to the incorporation and drug release of DXM-P in particles made with the 12 kDa polymer substituted with the C_8 acyl chain at 0%, 20 and 100% and prepared

using both methods mentioned above. Preliminary studies show that CYT-ARA encapsulation was lower than that of DXM-P in particles made with the same polymers. The particles made using the ID method were approximately 200 nm, while the ζ-potential was approximately – 30mV. The incorporation of DXM-P increased as the acylation of the C_8 polymer increased. The drug loading was 4.1% in 0% substituted particles, 7.8% in 20% C_8-PGA particles and 10.4% in 100% C_8-PGA particles. A 10-fold decrease of the DXM-P loading was obtained in the particles made with the EM but the same trend of drug incorporation was observed. This could be due to the displacement of the drug molecules in the emulsion droplets due to the presence of the surfactant, polysorbate-80, which is necessary for particle production by this method. The method of preparation also affected the size of the particles and the surface charge. Particles prepared by the EM were smaller in size (120–170 nm) and had a surface charge of −45 to −50 mV. Drug release studies were carried out in water. Although the burst release phenomenon was not seen, 96% of DXM-P was released from the 20%C_8-12 kDa and 100% C_8-12 kDa particles after 33 days. The emulsified particles released up to 80% drug after 33 days. In summary, the polymers assembled into well formed spherical nanoparticles of a relatively small size using a very simple technique (ID) in the absence of surfactant with relatively high drug encapsulation and slow drug release making them a promising drug delivery system.

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017
Investigating the use of biologically active polymers as a means of transiently permeabilising the intestinal barrier

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Numerous linear, water-soluble polymers (particularly polyanions) are biologically active and can induce cytokines, such as tumour necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ) and interleukin-2 (IL-2), from cells depending on their molecular weight (Mw), composition or salt form. The proinflammatory cytokines TNF-α and IFN-γ are able to permeabilise epithelial barriers by affecting the protein composition of the tight junction (Walsh et al 2000). The aim of this work is to identify polymers that will promote enhanced drug delivery due to their inherent ability to stimulate TNF-α, IFN-γ and IL-2 release, and therefore transiently permeabilise epithelial barriers. Several linear polyanions already used as excipients, including alginic acid, hyaluronic acid, sodium hyaluronate and polyacrylic acid (Mw 30 kDa, 100 kDa, or 450 kDa), were chosen as the first library to investigate. Branched polymers, including polyamidoamine (PAMAM) generation 3.5 and the polycation polyethylenimine, PEI (Mw 750 kDa), were also studied. Finally, isolated rat intestine (the half cell model) was used to investigate the ability of polymers or cytokines to modulate the transport of the paracellular marker, FITC-dextran (Mw 4 kDa). Initially, polymer cytotoxicity was investigated in B16F10 murine melanoma and ECV304 human endothelial-like cells using the MTT assay (Mosmann 1983) after 1 h or 72 h incubation times. Non-toxic concentrations of polymers (0.1–100 μg mL^{-1}), were then added to DU937 human macrophage-like cells for 24 h, or to B16F10 (0.1 and 1 mg mL^{-1}), or to 246.7 (1 mg mL^{-1}) murine raw monocyte/macrophage cells, for 1 h, to test their ability to induce TNF-α, IFN-γ and IL-2 release. Cytokine release was assayed using human and murine sandwich-ELISA assays. Effects of IFN-γ, TNF-α, IL-2 or polymers on apical to basal or basal to apical FITC-dextran transport, across rat jejunal tissue (with or without Peyer's patches), was measured with the vertical diffusion system (Grass & Sweetana 1988) over 1 h. Viability of the tissue was assessed during these experiments by measuring lactate dehydrogenase (LDH) release. Polyanions showed little cytotoxicity towards B16F10 cells and ECV304 cells at 72 h except polyacrylic acid of Mw 100 kDa in both cell lines, and 450 kDa in B16F10. PEI was cytotoxic even at low concentrations. Decreasing the incubation time to 1 h reduced polymer cytotoxicity (IC_{50} > 1 mg mL^{-1}) with the exception of PEI (IC_{50} 0.04 mg mL^{-1}). Highest amounts of TNF-α, IL-2 and IFN-γ release from B16F10 and 246.7 cells were seen with PEI (1 mg mL^{-1}). Sodium hyaluronate induced TNF-α release (1675–2500 pg mL^{-1}) from DU937 cells in a concentration-dependent manner. In transport studies, FITC-dextran transfer was greatest in the apical to basal direction, and in the absence of Peyer's patches. Apically applied TNF-α (30, 45, 60 pg mL^{-1}) tended to enhance FITC-dextran transport in a concentration-dependent manner. The polyacrylic acids (Mw 100 kDa and 450 kDa) and alginates (1 mg mL^{-1}) showed no increase in apical to basal FITC-dextran transport across non-Peyer's patch tissue. Polyacrylic acid sodium salt (Mw 30 kDa), appeared to induce an increase in transport.

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018
Optimising transfection efficiency of liposome-DNA complexes: effect of co-lipid, lipid:DNA ratio and complex size

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Cationic liposome have been widely used as an efficient means of delivering plasmid DNA and to elicit the appropriate immune responses to DNA encoded antigens (Gregoriadis 1995). Delivery of genes to mammalian cells is influenced by numerous factors, including cell type, type a cationic lipid, lipid to DNA ratio and lipid composition. Here we assess the effect of lipid composition and the lipid to DNA weight ratio on transfection efficiency in vitro. Small unilamellar vesicles (SUV) consisting of a helper lipid, either L-alpha-dioleoyl phosphatidyl ethanolamine (DOPE) or cholesterol (Chol) and cationic lipid cholesterol 3β-*N* (dimethylaminoethyl)carbamate (DC-Chol), were formulated where the total lipid ratio was increased but the molar ratio between the helper lipid and DC-Chol was kept at a constant 1:1 molar ratio. In vitro transfection studies were performed as per standard protocol for Lipofectin transfection reagent. Lipoplexes were characterised by measuring the *z*-average diameter (nm) and zeta potential (mV) on a ZetaPlus (Brookhaven Instruments) in ddH_2O and 0.001 M PBS, respectively. At low lipid to DNA weight ratios (1:1), DOPE:DC-Chol liposomes exhibit minimal transfection levels (23% of Lipofectin control). In comparison, Chol:DC-Chol lipoplexes formulated at the same lipid:DNA weight ratio gave significantly higher transfection (121% of control). As the lipid:DNA ratio increased to 1.6:1, transfection activity increased to 170% and 370% (of control) for the DOPE and Chol liposomes, respectively, again showing the enhanced ability of the cholesterol liposomes to promote transfection at these ratios. Optimum transfection for both systems was achieved at a DNA weight ratio of 3.1:1, although a reversal in terms of efficiency was noted with levels of 739% and 481% for DOPE and Chol liposomes, respectively, with optimum transfection being promoted by DOPE containing vesicles. As expected, the zeta potential for both DOPE and Chol formulations increased with increasing cationic lipid content with optimum transfection occurring at around neutral zeta potential, suggesting that the difference in transfection levels between DOPE and Chol lipoplexes was not related to this factor. However, vesicle size appears to play a more predominant role – DOPE and Chol lipoplexes transfection efficiency is optimum when vesicles are large (754 nm and 104 8 nm, respectively). At low lipid to DNA ratios, Chol lipoplex size was 805 nm and only increased slightly as the ratio increased (1089 nm at highest ratio of 4.2:1), which also reflects the gradual increase in transfection levels. However at the low lipid:DNA weight ratios (1:1), DOPE lipoplexes were less efficient at transfection compared with Chol, which may be due to the significantly smaller vesicle sizes of DOPE lipoplexes (357 nm) at this ratio. Variations in optimum lipid composition at a given weight ratio has been contributed to a range of factors, including: vesicle size, level of DNA condensation, lipoplex stability (Bhattacharya & Haldar 2000), but in this instance zeta potential appears not to be a contributing factor; however, in addition to the presence of DOPE within the lipoplex, the structural size of these systems appear to have a significant role in promoting transfection. To further investigate this, the morphology of these various systems will also be presented.

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019
Combining surface tension measurements, electron paramagnetic resonance (EPR) and small-angle neutron scattering (SANS) to better understand pH-responsive polymer-surfactant interaction

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Polyamidoamines (PAAs) were first developed in the biomedical field for their ability to form complexes with heparin (reviewed by Ferruti et al 2002). Subsequently, these polymers have been developed as drug carriers and as pH-responsive polymers for protein and gene delivery (Richardson et al

2001). The latter, called endosomolytic PAAs, disrupt membranes at low pH as shown by red blood cell and lysosomal membrane models. However, their precise mode of action is still poorly understood. The aim of this study is to further investigate polymer/membrane interactions using techniques normally applied to the study of polymer/synthetic surfactant complexes. Surface tension measurements can be used to study interaction of PAAs with surfactant species (Khayat et al 2004), electron paramagnetic resonance (EPR) to measure PAA-induced change in localised fluidity, and small-angle neutron scattering (SANS) to define the effect of PAA on the micelle shape and size. To date, this sort of concerted experimental approach has not been applied to the study of such biological interfaces. Two systems have been examined: firstly, as a model surface, the anionic surfactant sodium dodecyl sulfate (SDS, 25 mM) to validate the methods and, secondly, 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (L-PC, 10 mM) again as a model surface but one that is a better representation of the true biological membrane. In both cases, the polymer used was ISA23Cl (M_w 65 000 g mol^{-1}, 10 mg mL^{-1}). Previously, surface tension was used to study the interaction between SDS and ISA23Cl polymer. The data have demonstrated a pH-dependent interaction with three distinct responses; "strong"-phase separation of the SDS/ISA23Cl complex; "weak" a pronounced change in the aggregation properties but no phase separation and "no interaction" (Khayat et al 2004). Here, these conclusions were supported by EPR experiments, which showed that the fluidity of the SDS micelles was significantly reduced in the presence of ISA23Cl at $4.5 < pH < 6.5$, but it was unaffected at $pH > 7$. SANS observation reinforce this conclusion. The observed scattering from the ISA23Cl/SDS at $pH = 7.4$ solution is a simple addition of the individual scattering, indicating no change in size or shape of either species. At pH 5.5 and 4.0, the polymer appears to "wrap" around the surface of the SDS micelle, causing a slight change in micelle morphology. The surface tension of L-PC is not affected following the addition of ISA23Cl, between pH 3 and 8, thus indicating no interaction occurs between ISA23Cl and L-PC. Concomitantly, the fluidity (as measured by EPR) of the L-PC micelle was not affected by the presence of ISA23Cl over $3 < pH < 10$. The scattering of L-PC also remains the same following the addition of ISA23Cl at pH 7.4 and 5.5. It can be concluded that ISA23Cl does not interact with the PC surface. These measurements demonstrate that the relative charges on the polymer and the surface play a crucial role in determining PAA-surfactant interaction. It is clear that a minimal or critical charge is necessary to promote interaction. The PAA/L-PC interaction may be understood in the same light given its zwitterionic nature. Over the pH range studied, the L-PC does not bear a significant negative charge, resulting in no measurable interaction.

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020
Biopolymer complexes for in situ formation of delivery systems

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Polyelectrolyte complexes between bio-polyanions, including hyalyronic acid, chondroitin, heparin, cellulose and polymeric cations, are by the nature dynamic structures that undergo assembly/disassembly process in specific conditions. A particularly important aspect is the sensitivity of the assemblies to the nature of the medium, concentration and temperature. This opens a possibility to design polymeric systems sensitive to the changes in the biological environments in the body/cell compartments. In this research, complexation of hyaluronic acid (HA) to form an in situ crosslinkable polymer complex has been investigated. HA is a natural component of the extracellular matrix, making it a highly suitable material for drug delivery formulations. Two different polycations were explored – polyethyleneimine (PEI) (MW 750 000 Da), a branched polymer with a very high charge density arising from every third atom of the polymer being a nitrogen that can be protonated, and chitosan (MW 330 000 Da), a linear copolymer of glucosamine and N-acetylglucosamine, with a low charge density due to the presence of an amino group per two sugar residues. Both the viscosity measurements (Schott Gerate, Hofheim, Germany) and potentiometric titration studies (Mutek Analytic, Germany) on the supernatants from polycation (PEI or chitosan)-HA mixtures, demonstrated an almost complete complexation of the materials, with both species, PEI and HA, and chitosan and HA present in their respective complexes; the phenomenon is called associative phase separation and is characteristic for formation of polyelectrolyte complexes. However, the complexation occurred at different stoichiometries, with PEI interacting with HA in an almost 4:1 and chitosan with HA in a 1:1 stoichiometry; the later result being in a close agreement with previously published research (Nakajima & Shinoda 1975). Therefore, HA complexation can be optimised at specific stoichiometries, with no excess of polycation present, a potential toxic species. To assess the polycation-HA complexes' susceptibility to the changes in the environment, these were subjected to increasing ionic strength buffers, using sodium chloride, and the dissociation of the complexes was assessed by turbidity measurements (Hanna Instruments). Complexes formed with chitosan showed very low dissociation in high ionic strength environment, up to 0.43 M NaCl, in contrast to those formed with PEI, which dissociated at the physiological NaCl concentration (0.15 M), clearly indicating the differences in the properties of the complexes formed by two different polycationic species. Cryo-scanning electron microscope images reveal further differences in morphology of the complexes formed. Highly porous, with pores of several micrometers in size, and 'structured' internal morphology of chitosan-HA complexes can be seen. PEI-chitosan complexes appear with less 'structured' morphology. The visual observations of the gels also indicate 'harder' HA-chitosan and 'softer/gel like' structure of PEI-HA complexes. The results confirm formation of polyelectrolyte complexes with biopolymer, HA, and polymeric cations, whereby the stoichiometry of that interaction, the morphology formed and susceptibility to changes in the environment are all dependent on the nature of the polycation used.

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021
Drug penetration in 3-D cell model

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Many promising new drug therapies, such as gene therapy, are not effective because the large complex drug molecules fail to penetrate into the diseased target tissue. A number of novel cancer therapies combine ionizing radiation with gene therapy (Harari & Huang 2001). Ionizing radiation increases delivery and expression of reporter genes in tumour cell monolayers (Stevens et al 2001). The extracellular matrix may be modified and remodelled in response to radiation. This could affect the penetration of gene vectors. This project will study how changes in tissue structure caused by radiation for cancer can affect the penetration of therapeutic biomolecules, and possibly be exploited for more effective therapy. Multicellular assemblies provide 3-dimensional (3-D) cell–cell interactions, which alter cell properties, and they provide a penetration barrier to large particles, similar to the barrier posed by tissue. Cells grown in 3-D culture are more sensitive to damage by the radiation "radiological bystander effect" than the same cell type grown in monolayer (Boyd et al 2002). We will use multi-cellular spheroids or multilayers to simulate in vivo conditions. In this work, we measure the effects of ionizing radiation on the delivery and expression of reporter genes in tumour cell spheroids and co-culture spheroids and, specifically, detect differences in total reporter expression, duration and spatial distribution. The spheroids were co-cultured with tumour cells and PKH26 stained normal tissue cells. N1 cells were centrifuged. Then diluent C (PKH 26 Red Fluorescent Cell Linker Mini Kit, MINI-26, Sigma) was added and mixed gently then PKH26 dye was added for 2 min. The reaction was stopped by adding FCS for 1 min and diluting with complete medium. The cells were washed 3 times with PBS. The N1 fibroblast cell suspension (12 000 cells per well) were seeded in a 96-well agarose-coated plate. After 3 days incubation, HT1080-GFP or HCT116-GFP cell suspension with different cell number was added. Spheroids were checked consecutively up to 7 days after adding the tumour cells under the confocal microscope (Zeiss LSM 510) on glass cover slips. A slight infiltration of co-culture spheroids could be detected by confocal microscopy. The tumour cells had the inclination of infiltrating the highly compact fibroblast. By the methods mentioned above, we successfully prepared the co-culture spheroids of normal tissue cells and cancer cells, which improved techniques for investigating drug penetration in tissue models to allow more precise measurements without using live animals. The latest quantitative confocal microscopy techniques detected a good image of penetration. It can be used as a reliable method of detecting gene delivery, giving a better understanding of how effective cancer therapies can be designed by maximizing penetration of therapeutic biomolecules.

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022
Dissolved oxygen assessment of a micronutrient minibag base system for parenteral nutrition (PN) patients: effects of light and temperature

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In a previously presented study, the stability of a water-soluble multivitamin injection in 0.9% sodium chloride stored under different storage conditions was examined (Said & Cosslett 2004). One of the parameters that affect micronutrients stability and in particular the vitamins stability in PN formulations is the presence of air or oxygen in solution. Therefore, this study aims to assess the dissolved oxygen content of the base system, 0.9% sodium chloride, stored in its presenting container under varying storage conditions, as well as to assess the container's permeability to oxygen. Air (10 mL) was added to 100 mL 0.9% NaCl in either Freeflex (Fresenius Kabi) plastic bags or glass bottles. The solutions were then stored in a pharmaceutical stability chamber (Sanyo Gallenkamp PSC062) for one week at 4, 25, 30 and 40°C, at a relative humidity of 60%. Additionally, experimental solutions were also tested, by exposing them to artificial light of 0.7 klux intensity, of equivalent office and hospital ward lighting that have specifications of around 500 lux (Baker et al 1993). Solutions without addition of air acted as controls and the dissolved oxygen contents of the solutions were measured separately at 0, 48 and 168 h. The dissolved oxygen content of the solutions were measured by a polarographic system, with a calibrated dissolved oxygen meter (Jenway 9500). It was found that the dissolved oxygen content in both glass and plastic containers, with and without added air, were highest when stored at 4°C and least when stored at 40°C. This is comparable with the dissolved oxygen content of the base solution being dependent on temperature, with the oxygen solubility decreasing with higher temperatures. Also, it was found that samples with added air in Freeflex bags and stored at higher temperatures showed a decreased dissolved oxygen concentration and reaching a plateau upon time, signifying permeability of air through the plastic bags, while those in glass containers showed increased dissolved oxygen concentration from its baseline value. Exposing the samples to light did not have significant effect on the dissolved oxygen content. The results suggest that the dissolved oxygen content for the micronutrient base system is least at higher temperatures and that air is permeable through the plastic packaging bag.

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023
The evaluation of novel cationic peptides as delivery systems for antisense oligonucleotide and siRNA gene silencing nucleic acids

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Antisense oligonucleotides and siRNA can specifically degrade messenger RNA (mRNA) post-transcriptionally to silence the targeted gene. Antisense oligonucleotides act by hybridising to accessible regions of the target mRNA. Gene silencing occurs by recruitment of RNaseH to degrade the mRNA or by blocking ribosomal translation thereby preventing protein synthesis. In contrast, siRNA interacts with the RNA induced silencing complex (RISC) leading to the specific degradation of the target mRNA. Unfortunately, both siRNA and antisense oligonucleotides do not enter cells freely due to their large molecular weights and polyanionic nature. Therefore, to increase the intracellular concentration of the nucleic acids, cationic lipid delivery systems (e.g. Oligofectamine, RNAifect) are often used to interact with the cell membrane and to promote endosomal escape (Gilmore et al 2004). However, these delivery systems can be toxic and can induce gene expression changes (Omidi et al 2003). Therefore, two novel cationic peptides were designed and evaluated for their ability to deliver siRNA, oligonucleotides and plasmid DNA, with the intention of creating a more effective delivery system than commercially available cationic lipids. The two peptides, PIK (9 a.a long) and DSRB (15 a.a long), were synthesised using automated peptide synthesis and complexed at different molar ratios with fluorescent or radiolabelled siRNA and oligonucleotides for 30 min at room temperature. For gene delivery, enhanced green fluorescent protein plasmid was complexed as above. A431 or ECV-304 cells were incubated with peptide/nucleic acid complexes at 37°C for 4 h before nucleic acid uptake and trafficking was measured using flow cytometry, fluorescent microscopy or liquid scintillation counting. Expression of EGFP was measured 24 h post transfection using flow cytometry. Peptide binding to siRNA and plasmid DNA was assessed using electrophoresis mobility shift assays and were performed in 1% agarose gels with ethidium bromide staining. The uptake of a phosporothioate antisense oligonucleotide was significantly increased with a transfection efficiency of 33% and 25% (for PIK and DSRB, respectively) when complexed with high molar ratios of peptide (1000:1 peptide:nucleic acid) ($P = 0.05$). However, despite both peptides binding to siRNA and plasmid DNA, as shown by electrophoresis mobility shift assay, the two peptides when complexed with siRNA and plasmid DNA did not increase the delivery of the nucleic acids. These data suggest that duplex structure of nucleic acids is an important factor in the ability of cationic peptides to act as a delivery system for nucleic acids when used in complexation. Although the peptides were able to form complexes with all the different nucleic acids, they did not increase the cellular uptake of siRNA and plasmid DNA. Work is currently being undertaken to determine the mechanism underlying the disparity between the different nucleic acids. As an alternative strategy we are also examining whether direct covalent linkage between the peptides and nucleic acid is required for effective delivery.

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024
Differential sensitivity of tamoxifen resistant and wild type breast cancer cells to the DNA double strand break-causing drugs, etoposide, bleomycin and doxorubicin

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Tamoxifen is currently the drug of choice for the treatment of hormone responsive breast cancer. However, tumours can develop resistance to tamoxifen within 2–5 years. Epidermal growth factor receptor (EGFR) is an important contributing factor in allowing formerly estrogen sensitive tumours to grow in the presence of tamoxifen (El-Zarruk & van den Berg 1999). High levels of EGFR in tumours generally correlate with a poor prognosis and an increased resistance to cytotoxic drugs, whereas inhibition of EGFR results in an increased sensitivity to cytotoxic drugs. The mechanisms by which EGFR can impart resistance to cells are thought to include modulation of several DNA repair pathways, either by direct interaction between EGFR and DNA repair proteins (Bandyopadhyay et al 1998), or by EGFR-mediated regulation of DNA repair proteins (Yacoub et al 2003). It is the aim of this study to ascertain whether the increased EGFR signalling associated with tamoxifen resistance results in a phenotype more resistant to cytotoxic drugs, and to study the underlying mechanisims. The effect of bleomycin, cisplatin, doxorubicin, etoposide and 5-flourouracil on the growth of epithelial breast cancer cell lines (MCF-7 and T47D cells) and their tamoxifen resistant derivatives (TamR and T47D(Tam) cells, respectively) was observed by cell counting over seven days. Levels of EGFR and phosphorylated EGFR were also examined by western blot. DNA damage in MCF-7 and TamR cells was then examined using comet assay and by observing the presence of γ-H2AX foci by immunocytochemistry. The effect of direct EGFR inhibition on the sensitivity of both tamoxifen sensitive and resistant cell lines to cytotoxic drugs was also examined using the known EGFR inhibitor AG1478. Cisplatin, and 5-flourouracil exhibited a similar effect on growth of MCF-7 and TamR cells. However, contrary to expectations the EGFR rich TamR cells displayed greatly increased sensitivity to etoposide, doxorubicin and bleomycin, with a difference in IC_{50} values of up to 40 fold. This effect was also observed in T47D cells, indicating that it is not a peculiarity of the MCF-7 model. Immunocytochemistry confirmed the presence of γ-H2AX foci in bleomycin treated cells, indicating that bleomycin is eliciting a cytotoxic effect through the formation of double stranded breaks in DNA. γ-H2AX has been detected in both cell lines by western blot, with the extent of phosphorylation in TamR cells much greater than in MCF-7 cells treated with the same amount of bleomycin. These results suggest that despite greatly increased EGFR signalling, TamR cells are much more susceptible to killing with double-strand breakers than their parent MCF-7 cell line. Data from T47D cells demonstrate that this phenomenon is not unique to a particular cell culture model and may represent a general trend. The observed

increase in H2AX phosphorylation would indicate that a greater degree of damage is being caused to TamR cells. While one explanation may be that the different effect might be caused by a difference in uptake/efflux of drugs, the fact that this effect is peculiar to bleomycin, etoposide and doxorubicin and not other cytotoxics would suggest that the difference between these two cell lines may lie in their ability to detect and repair DSBs and not in their ability to efflux or metabolise them. Further work will establish the DNA repair capacity of these cell lines, as well as their tendency to commit to apoptosis when subjected to certain stresses in order to better understand the underlying differences that cause this phenomenon of double strand break sensitivity.

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Poster Session 1 – Materials Science

025
Nanoparticle based ciprofloxacin coating formulation for biomedical application

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One of the solutions to overcome medical device related infection is the use of biodegradable coating incorporated with antimicrobial agent. As most drugs have poor solubility in water, coating formulations are derived by using appropriate solvents. However, these solvents have toxicity issues and the use of ethanol or acetone as solvents is generally more acceptable. Preliminary results with these solvents showed that coating solutions with the selected drug candidate, ciprofloxacin, was inhomogeneous and cloudy. An interesting alternative to this situation is formation of a nano suspension of drug particle in ethanol, acetone, or a mixture of the two. In this work, the preparation of ciprofloxacin nano suspension in acetone is reported. This mixture was used to cast film by evaporation. Coating composition with varying concentration of ciprofloxacin (1, 2 and 3% w/w of copolymer concentration) was prepared by dissolving styrene-maleic anhydride (S-MA) copolymer in acetone. The nano suspension (100 nm) of ciprofloxacin was obtained by simultaneously adding an appropriate amount of non-ionic surfactant, Hyoxide AAO (1% w/v). The films were prepared by applying the resulting mixture on a Teflon panel and drying at room temperature for 24 h. Table 1 shows the coating composition and film properties. Film degradation and ciprofloxacin release were studied by placing 1 square inch of film in 100 mL of simulated gastric fluid (pH 1.2) in a stoppered flask in a rotary shaker (37°C, 100 osc. min^{-1}). The amount of ciprofloxacin released as a function of time was determined using a spectrophotometer at 430 nm. Table 1 shows the degradation characteristics and ciprofloxacin released after 10 days. Increasing the amount of ciprofloxacin in the film significantly increased the degradation and thereby the percentage of drug released. Thus, the release of ciprofloxacin coupled with degradation properties of the film indicated its potential as a medical device coating.

Table 1 Coating composition and film properties

No.	S-MA (% w/v)	Drug (% w/w)	% Elongation	% Weight loss	% Release
SMA0	10	0.0	2.23	35	—
SMA1	10	0.1	2.12	11	43
SMA2	10	0.2	2.04	15	58
SMA3	10	0.3	1.97	21	69

Note: Suspension based on total of 100 mL acetone and 1% w/v of non-ionic surfactant.

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026
In-line techniques for end-point determination in large scale high shear wet granulation

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High shear wet granulation is a size enlargement process whereby powders are converted to larger granules through addition of liquid and under mechanical agitation. Despite its widespread use in the pharmaceutical industry, wet granulation has traditionally been considered an empirical art, although in recent years there have been rapid advances in understanding the fundamental processes involved (Lister 2003). In this work three in-line techniques – Power Consumption, Focused Beam Reflectance Measurement (FBRM) and Acoustic Emission – were evaluated for their ability to monitor the wet granulation of a calcium-phosphate-based formulation, particularly focussing on granulation end-point determination. An experimental matrix was designed, varying each of three processing parameters (water added, impeller speed, granulation duration) at three levels to ascertain the extent to which these techniques could differentiate between granulations producing material of different physical properties. All granulations were performed at 50 kg scale in a high shear bottom-driven granulator (PMA200, GEA). Power consumption, a measure of the torque exerted on the impeller by the granulating mass, was found capable of differentiating between different impeller speeds and quantity of water added. However, it was also found that the power curves tended to reach a maximum and then plateau, despite the continued densification of the granules over time. To improve the correlation with granulation behaviour, power consumption data were integrated to give a measure of the total work done during granulation, and this was found to provide an improved relationship with the extent of granulation. FBRM (Lasentec, Mettler-Toledo Autochem) relies on the emission of a laser from a stainless steel probe placed within the granulator to determine particle size during granulation. It was found that upon addition of water to the dry powder, the fine particle counts ($< 100\,\mu$m) were seen to decrease, accompanied by a sharp increase in the 100–1000 μm range and the median particle size. Particle size changes were also observed with the activation of the chopper, showing a decrease in the coarse particle counts. Furthermore, in-line real time FBRM data could follow median granule size growth with increased water added, impeller speed and granulating time, thus offering a potential to aid process control. Probe fouling was not found to be a significant issue with this formulation due to it largely comprising an inorganic filler. Acoustic Emission was used to monitor the intensity of the acoustic profile in the range of 20 kHz to 1 MHz during granulation. The data collected showed a relatively flat profile during the dry mixing phase, followed by a large peak corresponding with water addition, before returning to baseline. This technique offers the potential to develop a fundamental understanding of the interaction of liquid and the dry mass in the early stages of granulation. Further analysis of specific frequency ranges may yield trends in the data that can help with the identification of granulation end-point (Whitaker et al 2000). This work has outlined three in-line techniques that offer the potential to monitor real time physical changes in the granulation process and thereby improve process understanding.

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027
Study of hydrated sugar lyophiles by low-frequency dielectric spectroscopy

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Lyophilisation is commonly used to prepare stable parenteral formulations from compounds with poor inherent solution stability. The stability of these lyophilised systems depends, in part, on their residual moisture following lyophilisation and their propensity to absorb water during storage. Sugars, such as sucrose, are often used as pharmaceutical excipients in such lyophilised systems. The formation of a nearly dry amorphous sugar 'matrix' can help protect the drug from both chemical and physical degradation during storage. Freeze-dried sugars have a porous structure with a very large surface area. Previous studies of non-pharmaceutical porous materials (porous glasses) have shown that the absorbed water molecules in such systems (Gutina et al 2003) form different populations. However, no such studies have been performed for pharmaceutical excipients, and yet new information about the behaviour of water molecules in amorphous sugar matrices could help in understanding drug stability in such systems, and thus facilitate more rationale formulation design. The aim of this work was to undertake a dielectric relaxation study of molecular dynamics in a model freeze-