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The influence of reflection and transmission geometries on powder X-ray diffraction (pXRD) patterns of an API

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Preferred orientation can be a significant problem in the analysis of APIs by powder X-ray diffraction (pXRD). Early batches of material may have undesirable habits for flat-plate sample presentation methods. In some cases, habit modification of the candidate is not possible and APIs progressed to late development may continue to have an undesirable habit for pXRD analysis. Crystals should be randomly orientated so that all lattice planes are observed in the diffraction pattern. Preferred orientation occurs when the crystals align to present only particular planes to the incident beam. Compound X is an analgesic that is currently entering phase 2 studies in the drug development process. Form 2 of this compound is the desired polymorph for progression but has an acicular habit, which presents significant handling problems for both formulators and physical properties scientists. Diffraction patterns obtained using flat-plate presentations often show significant orientation effects, which lead to interpretation difficulties. The purpose of this study was to identify all of the reflections characteristic for forms 1 and 2 of compound X, and to evaluate the influence of reflection and transmission geometries on the powder pattern of form 2 while also considering the resource demands on instrument usage and operator involvement. The powder patterns from the different geometries were assessed in terms of pattern completeness and resolution. A Phillips X'pert Pro (Phillips Analytical, Holland) with a Cu anode and an X'celerator detector was used. In reflection mode, the diffractometer was fitted with a variable divergence slit on the incident beam side. In transmission mode, a parabolic mirror or an elliptical mirror was used on the incident beam side. In reflection mode, samples were mounted on a zero background silicon wafer while in transmission mode, samples were presented in a 1-mm diameter glass capillary or between X-ray transparent foils. High purity batches of form 1 and 2 were used. The samples were analysed over the range of 3–40°2θ and experiments were performed in duplicate. Samples presented in the capillaries showed additional reflections to those obtained from samples mounted on a silicon wafer. Despite shorter data acquisition times for capillaries analysed using the elliptical mirror, data collected showed better signal-to-noise ratios and better resolution than that collected using the parabolic mirror. Data collected using the transparent foils showed additional reflections to those obtained from samples mounted in a silicon wafer. However, a few of the reflections observed in the capillary datasets were not apparent. In comparison with the transparent foils presentation system, capillary presentation was time consuming due to sample alignment requirements and filling issues: the undesirable habit and electrical properties produced poor flow. Capillary presentation of compound X with the elliptical mirror appears to produce a complete pattern while requiring less instrument time than when using the parabolic mirror. While transparent foils presentation of compound X reduces some preferred orientation, it requires considerably less resource in terms of instrument usage and sample preparation operations.

Poster Session 3 – Drug Delivery

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Enhanced release of acyclovir from intravaginal rings using common vaginal excipients

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The present scale of the HIV pandemic and the failure to develop an effective vaccine have forced scientists to evaluate alternative preventative strategies for reducing the rate of HIV transmission. Since over 90% of new infections result from heterosexual intercourse, the most viable, short-term strategy being pursued is the development of vaginal HIV microbicides. In particular, vaginal rings providing long-term, controlled-release of antiretrovirals over days/weeks/months are actively being investigated for this purpose. Several studies have demonstrated that prior infection with herpes simplex virus type 2 (HSV-2) is an important risk factor for HIV acquisition (Holmberg et al 1988), attributed to the presence of vaginal lesions and subsequent infiltration of CD4 cells to the lesion sites. Therefore, preventing and treating HSV-2 vaginal infections may be a useful strategy in reducing the incidence of female HIV infection. In this study, we evaluate the potential for controlled release of the anti-HSV-2 drug acyclovir from silicone vaginal rings. Silicone, matrix-type vaginal rings containing 10% w/w acyclovir and optionally 15% w/w of various pharmaceutically-acceptable, hydrophilic, vaginal excipients (cross-carmellose, polyacrylic acid, ascorbic acid, octyl dodocanol, povidone iodine, undecylenic acid) were manufactured according to standard methodologies (Malcolm et al 2003). In vitro release studies (37°C, 100 mL aqueous release medium, orbital incubation) were performed to assess the potential of each excipient for providing enhanced release of acyclovir from the silicone devices. Briefly, samples of the release medium were taken daily over a 14-day period, the release medium replaced to ensure sink conditions, and the samples subsequently analysed by UV-HPLC for determination of drug concentrations. For six of the seven excipients, acyclovir release was enhanced over the 14 days compared with the ring formulation containing no excipient (crosscarmellose 152%, povidone iodine 23%, ascorbic acid 18.2%, polyacrylic acid (MW 2100) 11.2%, polyacrylic acid (MW 5100) 10.8% and octyl dodecanol 3.4%). The results demonstrate that hydrophilic excipients provide greatest release enhancement and have the potential to extend the utility of the rings to the release of substances that might otherwise be difficult to release from the hydrophobic silicone elastomer. The enhanced release may be attributed to a number of factors, including water ingress and subsequent dissolution of the acyclovir. Further studies are required to determine the vaginal concentration of acyclovir required to reduce HIV transmission and to optimise the release profile from these modified ring formulations.

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Formulation of antigens in particulate delivery systems for in vitro cell stimulation assays

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Protein antigen entrapped in poly(lactide-co-glycolide) (PLGA) nanospheres has been shown to facilitate increased antigen specific in vitro cell proliferation in conjunction with increased secretion of IFN-γ and IL-6 in primary splenocyte cultures from pre-immunised mice when compared with free antigen (Eyles et al 2003). This mechanism is likely to be distinct from non-specific effects caused by components of the delivery vehicle itself. In addition to offering potential for increased and more sensitive biological readout of immunological parameters, formulation in particulate delivery systems may facilitate analysis of hydrophobic proteins or lipid antigens in aqueous environments normally problematic for such moieties. Additionally, such formulations may alter the in vitro toxicity of some antigens that can limit analysis using immunological assays (Bramwell et al 2002). To address these problems, we have designed systems that are suitable for the delivery of hydrophobic or lipid moieties for in vitro analysis of immunological cell stimulation assays. To minimise the impact of the carrier, biocompatible delivery systems based on biodegradable polymers and liposomes were thought to be promising candidates for this purpose. We used formulations based on PLGA (50:50), poly(methyl methacrylate) (PMMA), phosphatidylcholine (PC) and dioleoyl phosphatidyl ethanolamine (DOPE). Polymer carriers were prepared using a single emulsion and excipients were minimised in order to facilitate rehydration following freeze drying with the cryo/lyoprotectant sucrose. Liposome carriers were formed using thin film hydration (handshaking) in the presence of sucrose and homogenised to obtain a more desirable size range. Initial characterisation of polymer systems in 1.5% PVA is summarised in Table 1. Full characterisation and initial biological evaluation will be presented. The use of methanol (in addition to chloroform) in the polymer formulations as a co-solvent invariably led to an increase in size. The use of PVA facilitated smaller liposomes following homogenisation and did not affect the observed cell viability (in COS-7 cells) at the concentrations used for any of the formulations. Hydrophobic protein and lipid antigens are often problematic to produce and purify and are therefore generally expensive. While entrapment of such moieties in liposomes if often high, definitive analysis of entrapped material can be difficult due to the lack of specific assays, difficulties in extraction of antigen and the small amount of material available. Therefore, systems that incorporate all of the antigen, such as those outlined here, may comprise highly useful and comparatively simple tools for routine analytical purposes, especially when coupled with the versatility offered by an easily rehydrated freeze dried product that retains the characteristics of the initial formulation.

Table 1 Initial characterisation of polymer formulations

Polymer	100% Chloroform		85% Chloroform:15% Methanol	
	1 min*	3 min*	3 min*	After freeze drying
PLGA (50:50)	3.03 ± 0.05	2.56 ± 0.05	3.04 ± 0.11	3.01 ± 0.13
PMMA	3.53 ± 0.12	2.76 ± 0.08	2.91 ± 0.03	2.85 ± 0.03

*Homogenisation time; all results are average ± s.d. of 9 measurements; 3 separate (aliquoted) measurements of each sample.

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An investigation into protein stability following low-frequency ultrasound application

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Low-frequency ultrasound (US) has been shown to be effective in enhancing protein and DNA permeation (Boucaud et al 2002; Tezel et al 2004). US can be applied to the skin as a pre-treatment (i.e. before drug application) or concurrently with the drug. The possible advantage of the latter is potentially higher protein permeation into the skin while a possible disadvantage is protein degradation due to heat and other processes generated by US (e.g. cavitation). This study was conducted to determine the stability of a model protein when exposed to US. A solution of bovine serum albumin (MW 66 kDa) in a thermally insulated container was exposed to US (20 kHz) 70% amplitude for a total sonication time of 5 min using a calibrated sonicator (VCX500) in 3 different modes of US application: continuous mode, 5 s on 5 s off pulse mode (50% duty cycle) and 0.1 s on 0.9 s off pulse mode (10% duty cycle). Temperature changes in the coupling medium were measured throughout the experiments. At the end of the experiments, gel electrophoresis was performed and band intensities were analysed using Scion Image software (Maryland, USA) to assess changes in protein structure. A control experiment where a temperature change of 50% duty cycle US was mimicked was conducted to determine the contribution of heat changes in protein stability, if any. It was found that continuous mode produced the greatest rate of increase in temperature (~50°C) in 5 min. 50% Duty cycle (5 s on, 5 s off) pulse mode caused a similar temperature rise, but over a duration of 10 min. In contrast for the 10% duty cycle (0.1 s on, 0.9 s off) the maximum temperature rise was ~15°C. Analysis of band intensities following SDS-PAGE revealed that continuous mode caused the most severe BSA degradation, followed by 50% duty cycle, then by 10% duty cycle (Table 1). Interestingly the control experiment, which mimicked the temperature rise of 50% duty cycle, showed only a small percentage of degradation. This shows that BSA degradation upon sonication was not wholly due to the heat generated during US application but was related to other effects of US such as cavitation. We conclude that US treatment causes degradation of protein and damage is more likely when low frequency US is applied continuously compared with application in pulses. Thermal effects contribute to protein degradation but are not solely responsible for the degradation. Severe protein degradation during simultaneous sonophoresis may result in loss of activity. Therefore, ultrasound pre-treatment will be considered for our future experiments.

Table 1 Percentage of BSA left after sonication

Ultrasound protocol	Percentage of BSA (n = 4)
Continuous mode	48.4 ± 8.6
5 s on 5 s off pulse mode (50% duty cycle)	71.9 ± 10.5
0.1 s on 0.9 s off pulse mode (10% duty cycle)	87.2 ± 15.5
Control	91.5 ± 7.2

Boucaud, A. et al (2002) *J. Control. Release* **81**: 113–119
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Polymer-based gene delivery system with a novel polymer-multilayer encapsulating structure

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Polymer-based gene delivery systems have been studied as promising formulations for gene therapy. The studies in this area mainly focus on the systems formed by DNA and merely one polycation, which can be denoted as DNA/polycation delivery system. A variety of natural or synthetic polycations, such as polyethyleneimine, polylysine, chitosan, etc., have been used. Although considerable progress has been made in this area, the design of "artificial virus" system that would contain different functional components has not yet been achieved. The objective of this study is to apply a layer-by-layer self-assembly approach (Sukhorukov et al 1998) to build a novel polymer-based gene delivery system, which has an "onion-like" multilayer structure. We apply this approach on gene delivery system in the way of forming a core by condensing DNA with a polycation, and then depositing a polyanion on the formed cores to build an outer layer. The alternative deposition of oppositely charged polymers can be repeated until the desired number of layers is achieved. It is hoped that by formulating such a multilayer structure, the drawbacks of DNA/polycation system can be overcome; moreover, multifunctionality of the delivery system can be further developed based on such a structure. Our study has been carried out using two species of DNA (calf thymus DNA and salmon sperm DNA), the polycations of polyethyleneimine, chitosan and poly (2-(diethylamino)ethyl methacrylate), and the polyanions of poly(styrene sulfonate), poly(aspartic acid) and hyaluronan (both undegraded and degraded). The medium is 10 mM Tris-HCl buffer, at pH 7.4 and pH 4.0, respectively. Ethidium bromide displacement assay, size measurement by PCS, zeta-potential measurement and gel electrophoresis were used to study formation and characterize the system. The results show that the polycations used condense DNA into particles to different degrees, whereby polyethyleneimine appears the most efficient. After the polyanion addition to the DNA/polycation particles, "free" DNA was detected in the case of poly(styrene sulfonate) and poly(aspartic acid), indicating polyelectrolyte competition and DNA displacement from the complexes (Danielsen et al 2005); hence the polymers were not appropriate for our aim. However, when hyaluronan was used, DNA seemed not be released from the system: no "free" DNA was observed in the medium. Moreover, in optimized conditions, particles of smaller size and improved colloidal stability could be achieved. Therefore, we conclude that combination of polyethyleneimine and hyaluronan may be a potential polyelectrolyte system to take part in the building of multilayers. Moreover, as a natural polymer of non-toxic, non-immunogenic and with potentially targeting ability, hyaluronan is advantageous to be used in gene delivery systems.

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Hydrogels based on PLGA-PEG-PLGA triblock co-polymers as sustained release reservoirs for the delivery of pDNA to microneedle treated human skin

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The ability to deliver to and express plasmid DNA (pDNA) within the viable epidermis of skin holds significant therapeutic potential, most notably in genetic vaccination. In this respect the skin is an attractive target due to its considerable immune surveillance capabilities, a consequence of its proximity to the physical environment. However, there are a number of formidable barriers that restrict cutaneous gene delivery; these include the stratum corneum (SC) and the rapid elimination of the vector by the host cells (Hengge et al 1995). To address these issues, microfabricated microneedles were utilized to compromise the SC barrier, creating channels primarily in the epidermis, permitting direct access to the viable cells in a precise and potentially pain free manner. Hydrogel formulations, based on Carbopol-940 and PLGA-PEG-PLGA, were used to provide a sustained release environment for pDNA, replacing that previously eliminated by the

host cells. Microfabricated microneedle arrays, first described by Prausnitz in 1998 (Henry et al 1998), were manufactured using both wet- and dry-etch methodologies and subsequently characterized via scanning electron microscopy (SEM). SC disruption was determined using heat separated human epidermal membranes and human split thickness skin treated with the microneedle device. Conformation of channel formation was made both by SEM and transmission electron microscopy (TEM) and determining the trans-epithelial water loss (TEWL) of skin. Carbopol-940 gels were prepared at varying strengths, each loaded with 100 μg of the pCMVß reporter plasmid, and neutralized with TEA. Polymers of PLGA-PEG-PLGA were synthesized by ring-opening-polymerization (ROP) using stannous octoate as the catalyst (Chen et al (2004) and structurally characterized by ^{1}H NMR, GPC and HPLC. Hydrogels were formed from the polymer at strengths (20–30% w/v) and loaded with 100 μg of the pCMVß reporter plasmid. Transfection studies were performed on fresh, split thickness human skin obtained immediately after surgery. Following microneedle disruption and hydrogel application the tissue was maintained in organ culture for 24 h followed by X-gal staining to give a visual indication of positive transgene expression. Other samples were subject to RNA isolation followed by entry into RT-PCR reactions containing primers specific for the reporter gene messenger RNA (mRNA) transcript. Microneedle treatment resulted in comprehensive disruption of the SC and the formation of channels of 150–200 μm length (i.e. to the basement of the viable epidermis). Additionally, a significant increase in TEWL, through skin treated with both wet- and dry-etched microneedles, was also observed, with the highest levels observed by the dry-etched microneedle array. Analysis of the PLGA-PEG-PLGA polymers showed that its chemical identity conformed to that predicted before synthesis. Hydrogels based on both Carbopol-940 and PLGA-PEG-PLGA could harbour and release functional pDNA, as determined by electrophoresis, which was subsequently shown, by RT-PCR and X-gal staining, to successfully transfect microneedle treated human skin in organ culture. Initial studies suggest that transfection is enhanced when pDNA is released from PLGA-PEG-PLGA based hydrogels compared with those based on Carbopol-940, though more studies are required to verify this and elucidate the reason why. Using an innovative combination of microfabricated microneedles and pDNA-loaded hydrogels it is possible to successfully deliver and express transgenes in human skin maintained in organ culture. These delivery platforms warrant further investigations for their potential in the cutaneous administration of genetic vaccines.

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The influence of alkyl chain symmetry on liposomal encapsulation of poorly water soluble midazolam

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The biphasic nature of the liposomal systems enables efficient solubilisation of both water soluble and insoluble drug moieties. However, careful bilayer characteristics need to be studied to enhance the entrapment of poorly water soluble drugs. This work investigates the role of structural geometry of phosphatidylcholine (PC) analogues on the encapsulation of midazolam, a poorly water soluble model drug candidate. Multi-lamellar vesicles (MLV) were formulated via the hand shaking method, with the addition of 1.0 mg of poorly water soluble midazolam (experimental log**P** = 3.88; Monzon & Yudi 2001). The lipids investigated include symmetrical (1,2-distearoyl-sn-glycero-3-phosphocholine, DSPC; 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, DPPC; 1,2-dimyristoyl-sn-glycero-3-phosphocholine, DMPC) and asymmetrical (1-myristoyl-2-stearoyl-sn-glycero-3-phosphocoline, MSPC; 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, POPC) types, along with the addition of 11% cholesterol (Chol) in all cases. Liposome encapsulation was measured by spectrophotometric analysis of the supernatant following MLV separation by centrifugation, and mean volume diameter noted using a Malvern Mastersizer X. Initial optimisation studies showed that of those tested, 11% cholesterol content encapsulated the highest quantity of midazolam and yielded the largest liposomal diameter size (6.24 μm), therefore suggesting this to be the most efficient and to be investigated further with the various PC analogues and cholesterol. Incorporation studies using PC and its derivates suggest that symmetrical PC derivatives (i.e. DMPC, DPPC and DSPC) provide more efficient encapsulation of midazolam when compared with that of their asymmetrical counterparts and a trend of increasing midazolam incorporation with increasing lipid alkyl chain length in the order of DSPC > DPPC > DMPC > PC (Table 1). This trend correlates with previous work investigating the solubilisation of ibuprofen in liposomes (Mohammed et al 2004) and micellar solubilisation of barbiturates (Arnarson et al 1980) and may be attributed to the increased lipophilic area within these longer lipid bilayers. The two asymmetric lipid (MPSC, POPC) formulations yielded the lowest encapsulation (~4% mol/mol) and a lower MLV size than DSPC, DPPC, and DMPC liposomes, therefore, suggesting geometry to play a key role in drug bilayer loading. Statistical variance analysis showed that the encapsulation values were significantly different for all the formulations ($P < 0.05$) except when comparing DMPC:Chol with MSPC:Chol. The decrease in encapsulation could be as a result of bilayer formation via a mismatch interaction between asymmetric lipids, therefore reducing the overall hydrophobic region when compared with that of symmetric lipid bilayers. In conclusion, these results suggest that alkyl chain symmetry plays a significant role in the incorporation of the poorly water soluble drug midazolam. Investigations continue into combinations of both symmetrical and asymmetrical lipids.

Table 1 The influence of long-chain symmetric and asymmetric lipids on midazolam encapsulation and mean volume diameter

Lipid category	Formulation	Encapsulation (% mol/mol)	Size (μm)
Symmetric	DSPC:Chol	7.09 ± 0.33	8.49 ± 0.19
	DPPC:Chol	6.46 ± 0.12	7.42 ± 0.10
	DMPC:Chol	5.13 ± 0.36	8.32 ± 0.67
	PC:Chol	4.79 ± 0.30	6.24 ± 0.35
Asymmetric	POPC:Chol	4.06 ± 0.48	7.23 ± 0.20
	MSPC:Chol	4.01 ± 0.91	7.14 ± 0.18

Result represent mean ± s.e., n = 3

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Amphiphilic lower generation polypropylenimine dendrimer as a gene delivery agent

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An ideal gene medicine would be orally active, transforming only the cells within the target tissue. Polypropylenimine or DAB (diaminobutamine) dendrimers contain 100% protonable nitrogens and have been used for gene delivery. DAB-16-Am (generation 3, 1687 Da, 16 terminal amine groups) is able to completely condense DNA (Zinselmeyer et al 2002). Administration of DAB-16-Am-DNA complexes via the tail vein to mice was found to produce preferential liver expression of the reporter gene, in contrast with a commercial linear polyethylenimine formulation that resulted in high levels of gene expression in the lung tissue (Schätzlein et al 2005). Intravenous injection of DAB-16-Am-DNA complexes to CD1 nude mice bearing A431 xenografts has also been shown to result in a localisation of gene expression in the tumours (Dufes et al 2005). Lung avoidance is therefore advantageous and possibly occurs because the dendrimer can resist aggregation in the lung capillary bed, an effect which may be influenced by amphiphilicity, molecular size and overall charge of complexes. To test this hypothesis, a cetylated derivative of DAB-16-Am has been synthesised. DAB-16-Am and cetyl DAB-16 dendrimers were complexed with DNA at a 5:1 weight ratio (nitrogen:phosphate molar ratio 30:1) for comparison (Table 1). Visualisation in liquid using tapping mode AFM reveals flower-like strands of DNA protruding from DAB-16-Am-DNA complexes whereas DNA complexed with cetyl DAB-16 appears to be completely 'coated' by the dendrimer. The mean IC_{50} of the DAB-16-Am-DNA formulation determined by MTT assay of treated A431 cells (92.7 μg mL^{-1}) is reduced by one order of magnitude after modification. Cetyl DAB-16 self assembles into bilayer vesicles (of approximately 50 nm diameter) in aqueous media in the presence of cholesterol. This vesicle structure does not alter the mean size and

charge of the cetyl DAB-16-DNA complex but there is a modest improvement in the biocompatibility of the formulation. At the ratio tested, cetyl DAB-16 complexed with DNA transfects A431 cells with the β-galactosidase reporter gene at least as efficiently as the parent dendrimer and is comparable with DOTAP (a commercial in vitro lipid transfection reagent). This amphiphilic dendrimer has potential as an in vivo carrier of therapeutic genes and as a tool to identify key determinants of the biodistribution profile of complexes and factors influencing complex uptake by solid tumours.

Table 1 Key features of the dendrimers and their complexes

Dendrimer (complex with DNA N:P = 30)	Molar % cetylation (chains per molecule)	Mean % DNA binding (n = 3)	Mean hydrodynamic diameter of complex (nm) (n = 3)	Mean zeta potential (mV) (n = 3)
DAB-16-Am Cetyl	0 (0)	80.5 ± 1.0	310.2 ± 0.4	+11.4 ± 0.8
DAB-16	2.4 (0.72)	97.2 ± 1.0	244.7 ± 0.9	+32.8 ± 0.9

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pH-temperature sensitive microgel particles as (trans)dermal drug delivery systems

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Microgels are dispersions of particles in the size range of 1 nm to 1 μm. They have a high degree of sensitivity to changes in environmental conditions (i.e. a change in temperature, pH, ionic strength and solvency). As a function of these changing conditions, microgels undergo rapid changes in their physical properties, including changes in particle size (behaving like "micro-sponges") and surface charge density; taking these properties into account, the term "smart materials" is often applied to microgel particles. In this study, microgels will be used as novel drug carriers (i.e. a novel controlled drug delivery system) for either dermal or transdermal delivery. The steps followed in this project were, firstly, to investigate the uptake and release of model compounds with different physico-chemical properties (i.e. solubility and $\log K_{oct/w}$) to and from a colloidal gel system. Secondly, the study of the permeation of the model compounds across human epidermis. The first part of the project was to co-synthesise pH- and temperature-sensitive colloidal microgels particles based on a co-polymer system of poly(N-isopropylacrylamide) (85%) -co-butyl acrylate (10%) -co-methacrylic acid (5%) (NIPAM/BA/MAA) (85/10/5) (% w/w), in the presence and in the absence of methyl paraben (MP), propyl paraben (PP) and salicylamide (SA), by a surfactant-free emulsion polymerisation (SFEP) in water. Physico-chemical properties of the microgels were determined using different techniques, including Photon Correlation Spectroscopy (PCS) and Nuclear Magnetic Resonance (^{1}H and ^{13}C spectroscopy). The uptake and release of the model compounds to and from colloidal microgel particles was investigated by partitioning (i.e. centrifugation) and in vitro drug release experiments (i.e. dialysis). Results showed that there is a relationship between the amount of compound entrapped and the solubility and $\log K_{oct/w}$ of the compounds. Diffusion studies, across human skin, were performed at 305 K in the range of pH 3–7 for saturated solutions of SA, MP and PP, and for microgel particles incorporating the three compounds. The transport rate for the microgels incorporating MP was reduced by 2–3 fold compared with the saturated solution, by one order of magnitude for PP, meanwhile the transport rate for the microgels incorporating SA is the same order of magnitude as that for the corresponding saturated solutions. (Trans)dermal release studies of the saturated colloidal dispersions indicated that pH control of the drug release was marginal. The incorporation of compounds into the pH/temperature sensitive co-polymer (NIPAM/BA/MAA) (85/10/5) and the subsequent release depends on the $\log K_{oct/w}$ and solubility of the respective compound.

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Drug delivery system induced gene expression changes in cells in vitro and in vivo

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Gene therapy confers enormous potential for the successful treatment of genome-based diseases. To date, a number of gene therapy strategies have reached the stage of clinical trials. The success of gene therapy mainly depends upon the development of suitable delivery systems for in vivo gene transfer. Many clinical trials have been performed with viral vectors, but the potential risks of undesired immune and toxic side reactions have raised concern (Somia & Verma 2000). Furthermore, the potential of viral recombination to replicate competent wild-type virus and insertional mutagenesis needs to be more fully evaluated (Whitehouse 2003). Non-viral vectors are a promising alternative to viral vectors since they offer a higher degree of safety, as well as ease of manufacture. Polycations of lipid and polymers provide great potential for non-viral gene delivery, in which DNA or RNA form lipoplexes and polymers polyplexes with lipid and polymer formulations, respectively. However, little is known about the impact of non-viral vectors at the subcellular level. Here we investigated the toxicogenomics of the cationic polymers, linear and branched poly(ethyleneimine) (PEI), using microarray analysis of both cultured cells and in vivo tumour samples. The carcinoma cell line, A431 was the culture model utilised, both in vitro and in vivo as the basis of the tumour model. Microarray was carried out using 10 k and 20 k gene human arrays (MWG-Biotech), following the protocol described by Omidi et al (2003). The quality of mRNA isolates was confirmed before Cy-dye labelling and microarray hybridisation. Images were then analysed using Imagene 5 and Genesight packages (BioDiscovery, Inc.); further annotation and gene ontology analysis was undertaken using DAVID EASE software. Verification of microarray data was performed using semi-quantitative rt-PCR. Western blot analysis of specific protein expression was used to further verify the microarray findings. Cells in culture were treated with PEI and harvested for analysis 24 or 48 h later. An increased number of gene expression changes was observed with increasing concentration of branched PEI whereby 2.5 μg of PEI effected 875 gene expression changes by greater than 2-fold while at 10 μg PEI, 3468 genes were altered in their expression after 24 h. In a study of the effect of PEI exposure time on toxicogenomics, gene expression changes at 48 h post-transfection were reduced by 45% compared with 4 h post-transfection for branched PEI. In vivo administration of PEI by intra-tumoural injection into A431 xenografts showed that greater than 4 times more gene expression changes in a wide range of gene ontologies were induced by branched PEI compared to linear PEI, suggesting that toxicogenomics are influenced by polymer architecture. These studies suggest that drug delivery systems can markedly alter target cell genomics. They also show that polymer architecture may be an important determinant of the final number and type of genes that are affected in target cells. Thus, a detailed understanding of delivery system genomics will help guide their appropriate use in vivo.

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Liposomal solutions to problematic drugs: the influence of addition of α-tocopherol

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Liposomal encapsulation can be exploited for the enhancement of solubility of poorly soluble drugs. However, careful consideration of both the liposome and drug characteristics are required to achieve optimal solubilisation. This work investigates the role of the anti-oxidant α-tocopherol in liposomal solubilisation systems. Liposomes were prepared by the hand shaking method using phosphatidylcholine (PC), cholesterol (Chol) (16:4 μmoles) and up to 20 mole% of α-tocopherol with 1.25 mg of a range of poorly soluble drugs – ibuprofen ($\log P$ 3.6), flurbiprofen ($\log P$ 4.1), sulindac ($\log P$ 3.4) and progesterone($\log P$ 3.9). Liposome drug loading was assessed by spectrophotometric analysis of the supernatant after liposome separation by centrifugation. Our investigations suggest that in addition to providing oxidation protection, α-tocopherol can influence drug encapsulation; however, this is dependent on

the drug investigated. Initial investigations using low levels (5 mole%) of α-tocopherol had no significant effect on the bilayer loading of the four drugs tested. However, increasing the α-tocopherol concentration to 15 mol% resulted in an increased drug loading of ibuprofen, flurbiprofen and progesterone by approximately 10%. In contrast, 15 mol% of α-tocopherol had no significant effect on sulindac bilayer loading, which remained around 11.5 mol%. Further increases in the α-tocopherol concentration to 20% have a variety of effects depending on the drug: liposome incorporation of flurbiprofen and progesterone again increased to a minor extent (approximately 5%), while incorporation of ibuprofen significantly reduced to below that of formulations without α-tocopherol present (10.7 vs 9.2 mol% respectively). Again sulindac loading was not influenced by the presence of α-tocopherol even at 20 mol%. These results suggest that the effect of tocopherol on drug bilayer solubilisation is dependent on both its concentration and the drug properties. Indeed, previous research investigating micellar drug solubilisation have suggested that the effect of α-tocopherol on drug solublisation within micelles was dependent on the log*P* of the drug candidates (Nielsen et al 2001) with the addition of tocopherol enhancing griseofulvin (log P 2.2) solublisation, whereas drugs with higher log*P* (e.g. Lu28–179 (log*P* 8) and felodipine (log*P* 4.8)) saw a decrease in encapsulation with the addition of α-tocopherol. These variations in encapsulation may be explained by the surfactant nature of α-tocopherol; like cholesterol α-tocopherol is an insoluble, non-swelling amphiphile. Our previous studies (Mohammed et al 2004) have already shown that, similar to α-tocopherol, the cholesterol bilayer content can influence drug solubilisation within liposome bilayers, with low concentrations (20%) enhancing ibuprofen loading while higher concentrations (> 33%) inhibit solublisation. The results suggest that the addition of α-tocopherol to liposomal systems can benefit encapsulation in some cases but this is possibly dependent on the log*P* of the drug candidate.

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An investigation into the compatibility of three water-soluble plasticizers with ethyl cellulose and their effects on drug release

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Plasticizers are widely included in ethyl cellulose (EC) film coats to reduce brittleness, impart flexibility and increase toughness, strength and the impact resistance of the film. A basic requirement of any plasticizer is compatibility with the polymer with which it is formulated. To be considered compatible, the plasticizer must be miscible with the polymer as well as soluble in the solvent system employed to cast the film. In this study, the properties of EC cast films with three water-soluble plasticizers, triethyl citrate (TEC), polyvinylpyrolidone (PVP) and hydroxylpropyl methylcellulose (HPMC), were studied using thermal and imaging methods and the effect of plasticizer compatibility on the subsequent drug release was investigated. Cast films containing 20% w/w plasticizer were prepared by dissolving the appropriate amount of EC and plasticizer in the solvent and mixing for at least 15 min at 45°C. Ethanol was used for EC/TEC and EC/PVP system, whereas a mixture of 75% dichloromethane and 25% 2-propanol was required to fully dissolve the EC/HPMC system. The compatibility of these systems was assessed by scanning electron microscope (Philips XL30 ESEM-TMP), micro-thermal analysis (μ -TA 2990 Micro-Thermal Analyser) and differential scanning calorimetry (Q1000 DSC from TA Instruments). Drug release characteristics of these films were investigated by intrinsic dissolution using Copley Dissolution Tester DIS 8000 with Copley Intrinsic Dissolution Kit. Ibuprofen (30 mg, model drug) was compressed into the die cavity of the intrinsic dissolution holder with a 5 mm diameter exposed surface. Films were then cast directly onto to the exposed surface without removing the punch from the holder. The films were dried overnight and the entire holder was screwed into the shaft of the dissolution tester and immersed into phosphate buffer (pH 7.2 ± 0.1) for dissolution testing. Under the SEM, the cross-sectional appearance of the EC/TEC film was very homogeneous, whereas the EC/PVP film consisted of spheroid structures of approximately 5 μm diameters and the EC/HPMC film revealed two distinctive features. When the films were analysed by micro-thermal analysis, only the thermal conductivity image of the EC/HPMC film demonstrated two unique thermal properties, which may indicate phase separation. DSC results showed that the glass transition temperature (Tg) of EC in EC/HPMC and EC/PVP film was almost unaltered, while the Tg in the EC/TEC film was significantly lowered. These results indicate that only the EC/TEC film is miscible; such compatibility has been previously suggested by Sakellariou et al (1986). Dissolution results showed that at the end of a 24 h period, the amount of ibuprofen released was 36%, 30% and 25% for the EC/TEC, EC/PVP and EC/HPMC film, respectively. This indicates that the inclusion of a non-compatible water-soluble plasticizer actually slows release, despite the expectation that such a system would rapidly form pores on contact with water. It can be concluded that the EC/TEC film is miscible, whereas HPMC and PVP are incompatible with the polymer. However, the EC/plasticizer compatibility strongly affects the drug release characteristics, with compatibility appearing to be associated with faster release.

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Influence of pH on protein aggregation during emulsification

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The biotechnological advances of recent decades have provided a number of therapeutic proteins for human disease management. The rapid dosage form development of such drugs has been challenged by protein instability during manufacturing processes due their fragile nature. During the preparation of micro and nanospheres by the double emulsion (w/o/w) technique, the majority of therapeutic proteins show a reduction in their activity through the creation of protein aggregates. The first step in this process, namely, the emulsification step, has been identified by a number of researchers as the most detrimental (Perez et al 2002). Stresses such as shearing force, cavitation, heating and adsorption of protein at water/organic interface were mentioned as causes of protein denaturation, the latter being the most predominant (Sah 1999). Different approaches have been used in an attempt to prevent protein denaturation and enhance protein stability. These strategies comprise increasing protein concentration, adding another protein, using amphiphilic additives or adding cosolvents (Perez et al 2002). In this study, we explore the pH-dependent integrity of proteins after exposure to the stresses associated with emulsification and relate it to structural properties. Beta-galactosidase was selected as a model protein with an isoelectric point (p*I*) of 4.5. Protein recovery was determined after 2 mL of protein solution (5 mg mL^{-1}) was homogenised with 10 mL of methylene chloride (CH_2Cl_2) using a homogeniser speed of 20 000 rev min^{-1}. The pH of protein solution was varied between 3 and 8.2 using phosphate buffer. For beta-galactosidase a linear relationship between the percentage protein recovered and pH was obtained between pH 4.5 and 8.2. The least square equation form from straight line was: Y (percentage recovered) = 8.08 X (pH) + 13.93. Other points (at pH < 4.5) deviated significantly from this line and indicated that as the protein deviates from p*I* on either side the protein undergoes less aggregation at the interface. This equation was used to extrapolate that when using protein solution at pH 10.94 the percentage of protein recovery after homogenisation would be 93%. The experimentally determined value at this pH showed good agreement (96.6 ± 4.3%). Lysozyme showed similar p*I* dependent aggregation behaviour during emulsification although its p*I* is 10.7. The decreasing electrostatic repulsion between protein molecules at pHs close to p*I* would explain this behaviour (Lefevre & Subirade 2000).

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Use of corrugated gelatin particles in dry powder inhalation aerosols

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Porous particles have been shown to improve aerosol performance of dry powder inhalers at low inspiratory flow rates. Phosphatidylecholine has been employed to produce porous particles, and the formulation has optimum aerosol performance at an inspiratory flow rate of 60 L min^{-1} (Dunbar et al 2002). However, patients such as children, the elderly and those with severe respiratory distress may not be able to generate such a high inhalation flow rate. Hence only a small amount of the aerosol may reach the lower airways. In this study aerosol performance of gelatin particles to be employed in dry powder inhalers and operated at 30 L min^{-1} inspiratory flow rate was investigated. Gelatin solutions with concentrations of 5, 7.5, and 10% w/v were spray

dried using an inlet-temperature of 100°C and an outlet-temperature of 40°C. The resulting particles were kept in sealed containers at 5°C. The particles were viewed using a scanning electron microscope (SEM). Size 2 gelatin capsules were filled manually with 25 mg of the particles and inserted into a Spinhaler. The aerosol device was connected to a biological throat model, which was obtained from a previous study (Ehtezazi et al 2004). Then the model was connected to a filter housing (containing glass wool) to collect the particles that pass through the model, and the filter housing was connected to a vacuum pump, which operated at 30 L min^{-1}. The aerosol performance was assessed before and after one-week storage in a desiccator under vacuum at room temperature. The amount of gelatin deposited in the filter was determined using an UV spectrophotometer at wavelength of 218 nm. SEM study revealed that the spray-dried gelatin particles were porous. However, the particles became corrugated with increasing gelatin concentration while remaining porous. The amount of particles on the filter (respirable fraction) increased with gelatin concentration (Table 1). Thus, corrugation of the particles may have contributed to improving the aerodynamic properties of the particles. Drying the particles under vacuum further improved their aerosol performances. A significant improvement was observed for 10% gelatin particles and this was mirrored by the increase in the respirable fraction from $18.8 \pm 2.1\%$ to $35.5 \pm 7.7\%$. This increase in respirable fraction could be attributed to loss of water from the particles, which not only reduced the adhesive forces among the particles, but also reduced the particle size and increased the corrugation of the particles. In conclusion, gelatin particles with improved respirable fraction at a low inhalation flow rate were prepared. Two factors may have accounted for such a performance, namely the presence of pores and corrugation. Corrugation can be enhanced by increasing the concentration of gelatin, or dehydrating by applying desiccation. Dehydrating may also decrease the geometric size of the particles. The latter observation suggests that heat, or a combination of heat and desiccation may be applied to improve corrugation of gelatin particles and possibly reduce the particle geometric size.

Table 1 Effect of drying gelatine particles under vacuum on respirable fraction of the nominal dose

Gelatin concn	Before desiccating	After desiccating
5%	$11.9 \pm 3.3\%$	$12.8 \pm 2.2\%$
7.5%	$8.8 \pm 3.3\%$	$12.2 \pm 2.5\%$
10%	$18.8 \pm 2.1\%$	$35.5 \pm 7.7\%$

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Development of a micronutrient minibag formulation for parenteral nutrition (PN) patients – effects of light and temperature on stability

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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) and it was found that the vitamins pyridoxine, nicotinamide, thiamine, folic acid (FA) and riboflavin sodium phosphate (RSP) were most stable when kept refrigerated and stored protected from light. This study aims to investigate the stability of the formulations under conditions simulating the clinical environment encountered by PN patients. Solutions in 100 mL Freeflex (Fresenius Kabi) bags were stored in a pharmaceutical refrigerator for 7 or 30 days. The solutions were then exposed to delivery conditions of 25°C or 40°C (to simulate the duration of administration at room temperature or at higher climatic conditions respectively). One set of solutions were protected from light and another set exposed to artificial fluorescent light of 1.2 klux intensity, simulating extreme indoor ward lighting (Baker et al 1993). In addition, the light-exposed samples were further irradiated with ultraviolet A (UVA) light to about 1.2 W h m^{-2} total exposure, this simulating 8 h filtered daylight at approximately one metre away from a window (Tonnesen 2004). Samples were taken at 0 h, on day 7 or day 30, and after exposure to the simulation conditions, and analysed using a validated stability-indicating reversed phase HPLC method. It was found that the vitamins were stable, with less than 10% degradation, when the solutions were stored for both 7 and 30 days in the refrigerator. The vitamins remained stable when subjected to temperatures of either 25°C or 40°C following the storage durations, and if protected from light. When exposed to visible light, nicotinamide, thiamine and FA were stable but RSP was unstable at both temperatures, while pyridoxine was stable when subjected to 40°C following 30 days storage. For simulation of solutions in Freeflex bags to filtered daylight, nicotinamide remained stable, although RSP and pyridoxine were unstable, while FA was only stable at 25°C; as for thiamine, it was unstable when subjected to 40°C following 30 days storage. In conclusion, all vitamins were stable when stored refrigerated up to 30 days. When subjected to different simulation conditions following the next 24 h, the vitamins remained stable if protected from light.

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Topical delivery of aminolevulinic acid and its esters: influence of drug hydrophobicity and vehicle type on in vivo production of protoporphyrin IX

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Photodynamic therapy based upon topical administration of aminolevulinic acid (ALA) is a novel treatment for premalignant and malignant skin lesions. Application of exogenous ALA stimulates the over-production of protoporphyrin IX (PpIX), an effective endogenous photosensitiser. This effect is prevalent in rapidly proliferating neoplastic cells and explains selective lethality of the therapy. However, ALA is a small hydrophilic (167.6 dalton) molecule and zwitterion at physiological pH. Consequently, permeation across intact stratum corneum is poor (Malik et al 1995; Casas et al 2000). ALA esterification is a possible means of enhancing penetration. Attaching alkyl groups to the parent ALA molecule increases hydrophobicity and enhances partitioning into the stratum corneum. Subsequently, cutaneous PpIX is expected to be enhanced. The aim of this study was to test this supposition and examine the effect of ALA esterification on in vivo PpIX production. In addition, the effect of vehicle type, namely a hydrophilic patch and a more lipophilic cream, was compared and the influence of possible hydrophobic interaction between drug and vehicle investigated. ALA and ALA ester-loaded, bioadhesive films were cast from drug containing aqueous blends of poly(methylvinylether. maleic anhyhydride) (PMVE/MA), suitably plasticised using tripropylene glycol methyl ether (TPM). Creams were prepared by mixing ALA and molar equivalent amounts of ALA methylester (ALA-Me) and ALA hexylester (ALA-Hex) into a lipid-rich hydrophilic base (Unguentum Merck). Both formulations were loaded in such a way so as to deliver 5, 19 or 38 mg cm^{-2} of drug to the dorsal skin of anaesthetised female nude mice for defined time periods (1 and 4 h). Upon removal of the vehicle, fluorescence in vivo was measured using a fibre-optic probe coupled to a Perkin-Elmer LS50B luminescence spectrometer (excitation 407 nm, emission 635 nm). The accumulation kinetics of PpIX followed a similar profile for all drug formulations. Following removal of the vehicle, PpIX fluorescence peaked at 3–6 h, and reduced to baseline levels at 24 h. Using the cream formulation, the highest PpIX fluorescence was generated using ALA-Hex. Patch formulations generally resulted in less PpIX production than the corresponding cream. However, at the highest drug loading (38 mg cm^{-2}) there was no significant difference ($P < 0.05$) between cream and patch formulations. PpIX production at distant sites was significantly reduced ($P < 0.05$) when ALA and its esters were formulated in the bioadhesive patch. Topical application of ALA esters was shown to induce equivalent levels of fluorescence as the parent ALA molecule. This indicated that although they are efficient pro-drugs for PDT, they do not improve PpIX production. Fluorescence from patch formulations was generally lower than that from cream, indicating possible differences of flux from both formulations. Importantly, this study demonstrated that PpIX production was localised to underlying tissues when the patch was used. This helps prevent the generalised photosensitivity that sometimes afflicts PDT and is an excellent method of targeting PpIX formation to the site of application.

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Evaluation of poly(lactide-co-glycolide) nanoparticles prepared using a novel procedure comprising controllable and simultaneous diffusion and emulsification steps

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Polymeric, drug-loaded nanoparticles are colloidal systems, typically 10–1000 nm in diameter, with a therapeutic payload entrapped, adsorbed or chemically coupled to a polymer matrix. Their potential as controlled release systems with site-specific delivery capabilities has ensured that interest has been sustained over the years. Nanoparticles can be prepared by methods involving either polymerization of dispersed monomers or a dispersion of preformed polymer. The latter procedure is used extensively with poly(esters) and especially the copolymer of lactide and glycolide (PLGA). Three established techniques have been used for the preparation of PLGA nanoparticles, namely, nanoprecipitation, salting out and emulsion–evaporation. Several difficulties are still encountered when using these techniques, such as low yield, poor entrapment efficiency of drug and high polydispersity index (PI). Therefore, procedural modifications are to be welcomed. The aim of this work is to describe a novel modification of the salting-out procedure by combining a controllable diffusion step with an emulsification step as a way to improve polydispersity and particulate yield. PLGA nanoparticles were prepared by modifying the procedure of Konan et al (2002). PLGA 50:50 (Resomer RG 505S) was dissolved in an organic phase comprising different ratios of acetone and dichloromethane. This was added to an aqueous solution of PVA (Murakami et al 1997) containing 60% $MgCl_2.6H_2O$. The emulsion was sonicated for 3 min (50–55 W, pulse mode in ice bath). Additional aqueous PVA was then added under moderate stirring to initiate selective acetone diffusion. An acetone-only organic phase resulted in a low yield of nanoparticles with substantial aggregation. A dichloromethane-only phase resulted in high yield of nanoparticles (250 nm) but the size distribution was wide ($PI = 0.1 \pm 0.1$). A combination of the two solvents (75% acetone, 25% DCM) produced nanoparticles of 160 nm with a tight monomodal particle size distribution ($PI = 0.04 \pm 0.6$) and in high yield. Other ratios (50% acetone, 50% DCM and 25% acetone, 75% DCM) produced similar sized nanoparticles, but size distribution was found to increase ($PI = 0.06 \pm 0.8$). Varying the concentration of $MgCl_2.6H_2O$, a procedure for altering the acetone diffusion, was found to affect the size distribution, but not the mean particle diameter. Among all the concentrations studied, 45% $MgCl_2.6H_2O$ showed the best polydispersity ($PI = 0.02 \pm 0.5$). Energy input, a way to alter the emulsification step, was studied by varying the sonication time in the primary emulsion stage, which was thought to alter the nascent polymeric droplet size. Sonication for various time intervals was evaluated. Sonication for 3 and 6 min showed the lowest polydispersity ($PI = 0.02 \pm 0.5$) with 250-nm and 150-nm particles produced, respectively. This work showed that PLGA nanoparticles can be prepared in a reproducible process with high yield and of monomodal particle size distribution. Particle size can be controlled by the use of a combination of water miscible (acetone) and water immiscible solvents (dichloromethane) with controlled acetone diffusion by optimizing the concentration of the salting out agent ($MgCl_2.6H_2O$) and use of appropriate sonication to control the emulsification procedure.

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Attenuation of the initial burst release of celecoxib from loaded poly(lactide-co-glycolide) nanoparticles prepared using a novel modification of the salting out procedure

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The burst effect observable during many drug release experiments on nanoparticulate systems is a frustrating drawback. It is readily identifiable as an initial surge in drug release upon placement in the dissolution medium before the release rate reaches its longer-term equilibrium profile (Huang & Brazel 2001). Reasons for the occurrence of a burst release range from processing conditions, nanoparticle surface morphology (porosity, drug adsorption), drug loading and the physicochemical properties of drug and polymer. The large surface area found in colloidal systems exacerbates the problem. The objectives of this work are to describe the formulation of celecoxib-loaded poly(lactide-co-glycolide) nanoparticles, to evaluate the burst release from these nanoparticles and to use formulation modifications to reduce it. Celecoxib-loaded nanoparticles were prepared by modifying the salting out procedure of Konan et al (2002). Celecoxib (10 mg) was dissolved in dichloromethane; poly(lactide-co-glycolide) (Resomer RG 505 S) was dissolved in acetone. The two solutions were mixed to give an organic phase comprising different ratios of the two solvents. This was added to an aqueous PVA solution (2.5% w/v) containing 45% MgCl2.6H2O and sonicated for 3 min in an ice bath using 50–55 W in pulsed mode. Additional aqueous PVA was then added under moderate stirring to initiate selective acetone diffusion. Nanoparticles were recovered using centrifugation (56 000 *g*), washed and freeze-dried. Celecoxib release studies were conducted using 20-nm pore size membrane filters separating both donor and receiver phases. Both phases were methanol/water mixes (50:50) to insure sink conditions across the membrane. Drug release profiles were dependent on changing the ratio of acetone to dichloromethane in the initial organic phase (Table 1). Although all formulations displayed a sustained release profile for celecoxib, the initial burst release effect was seen to vary significantly. As seen in Table 1, an initial organic phase comprising 75% acetone produced a release profile where 22% of the total celecoxib loading was released after 60 min, thereby indicating that the burst was reduced. Celecoxib, being hydrophobic with low aqueous solubility, displays high variability in absorption after oral administration (Subramaian et al 2004). It is envisaged that these nanoparticles will make an ideal alternative, acting as a means of localised release after injection into compartmental spaces. As such, it may be a platform for sustained COX-2 inhibition in synovial fluid. This being the intention, it is, therefore, clear that a burst effect is undesirable and the formulation changes described go some way to achieving this aim.

Table 1 Celecoxib release (%) profiles with different acetone concentrations

Time (min)	Acetone concn			
	75%	50%	25%	0%
15	0 + 0	4 + 3	7 + 3	12 + 1
60	22 + 3	36 + 2	36 + 1	38 + 2
180	54 + 3	69 + 2	69 + 1	57 + 1
300	68 + 1	81 + 2	81 + 1	71 + 2

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Subramaian, N. et al (2004) *Biol. Pharm. Bull.* **27**: 1993–1999

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Temperature responsive lipid-based microparticles as a topical drug delivery systems for photodynamic therapy

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Aminolevulinic acid (ALA)-mediated photodynamic therapy (PDT) of neoplastic disease is a rapidly developing field in cancer therapeutics. Topical application of ALA leads to selective production and accumulation of the potent endogenous photosensitiser, protoporphyrin IX (PpIX), in neoplastic cells. Irradiation of such cells with red light (635 nm) leads to selective cellular destruction via singlet oxygen. ALA is a hydrophilic molecule and, therefore, shows only limited permeation through stratum corneum. To enhance lipophilicity and improve dermal bioavailability, alkyl ALA esters have been used. Other approaches using penetration enhancers, such as 20% dimethylsulphoxide, have demonstrated an improved ALA permeation. Different enhancer types, such as oleic acid, have resulted in similar improvements, thought to be due primarily to an interaction with the intercellular lipids in the skin. The most commonly used formulation clinically is a 20% ALA O/W cream. It has a limited shelf life of two weeks due to ALA instability. A formulation that can maintain ALA in the crystalline phase but undergo a triggered release upon application to skin would be one novel method of circumventing poor stability. Skin temperature is one potential trigger. Thus, the aim of this study was to incorporate ALA into microparticles composed of a low melting point lipid (Witepsol H15) that melts at skin temperature (33°C). In addition, the molten lipid base may fluidise skin lipids and enhance the stratum corneum permeability. The drug loading efficiency of this formulation, drug release and

production of PpIX in cutaneous murine models was evaluated. ALA-loaded microparticles were prepared using a novel spray congealing method with Witepsol H15 used as the lipid matrix of the particle. ALA was encapsulated in poly(caprolactone) microparticles using an emulsification-solvent evaporation method to act as a comparator formulation. The morphology was studied using scanning electron microscopy. Particle size was analysed by laser diffraction. The effect of using different ALA-lipid ratios and temperatures on the ALA release were evaluated. ALA concentration was determined using acetyl acetone and formaldehyde derivatisation and analysed fluorometrically. ALA-loaded microparticles were applied to a 1-cm^2 area on the dorsal side of nude mice and the induced PpIX measured by a fiber-optic probe coupled to a fluorometer (CL50B, Perkin-Elmer, Norwalk, CT, USA). The ALA encapsulation efficiency was low for the emulsification-solvent evaporation methods (0.8%), while the spray congealing method gave 100% drug encapsulation. In vitro release studies showed that the release was increased by increasing the ALA concentration and by increasing the temperature of the release medium. In vivo studies showed that ALA microparticles generated cutaneous PpIX in mice that was localised under the formulation. It can be concluded that ALA release was triggered by skin temperature and that PpIX production was successful. Further studies are underway to evaluate long-term stability of ALA within this novel formulation.

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Antiadherent and antifungal nanoparticles

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Candida albicans is a dimorphic organism present as part of the normal body flora of man. It is frequently found in the oral cavity, gastrointestinal tract and female genital tract. *C. albicans* rarely causes infection in healthy individuals, but tends to become pathogenic in immunocompromised patients, when host defences are impaired. Deep-seated candidal infections are potentially lethal and patients suffering from AIDS, those having undergone several cycles of antibacterial therapy and those having received transplants are particularly susceptible. Candidosis of the oral cavity can manifest in a variety of forms but tends to be non-life threatening. The most common forms seen are oral thrush (characteristic soft white plaques) and denture stomatitis. Oral thrush can develop in up to 5% of neonates and 10% of elderly debilitated patients. Denture stomatitis occurs in up to 50% of denture wearers, possibly due to the adherence of *C. albicans* to denture surfaces (Hugo & Russell 1998). Continuous dosing with antifungal agents may lead to resistance and antibacterial agents can impair local healing of lesions. Consequently, new strategies for the treatment and prevention of oral candidosis are required. We have previously shown that non-drug loaded poly(propylcyanoacrylate) nanoparticles can reduce the adherence of *C. albicans* blastospores to human buccal epithelial cells (BECs) in vitro (McCarron et al 2004). The primary aim of this study was to produce nanoparticles with both anti-adherent and antifungal activity by incorporation of the antifungal drug miconazole into the particles. The first objective was to evaluate the ability of both drug-free and miconazole-loaded particles to prevent adherence of *C. albicans* blastospores to BECs in vitro. The second objective was to determine the ability of the miconazole-loaded nanoparticles to kill *C. albicans* blastospores in vitro. Nanoparticles were produced by the standard emulsion polymerisation method described previously (McCarron et al 1999). Different classes of surfactants were added to the polymerisation medium to alter the surface properties of the particles formed. Miconazole was also incorporated by addition to the polymerisation medium. Physiochemical properties of formed particles were investigated using photon correlation spectroscopy (mean size) and electrophoretic mobility (zeta potential). The incorporation rate of miconazole into the particles and its subsequent release was also determined using UV spectroscopy. Following local ethical committee approval and fully informed consent, BECs were harvested from healthy subjects and suspended to defined optical densities ($OD_{550} = 0.4$) in sterile phosphate buffered saline pH 7.4. BECs were then treated with nanoparticle suspensions ($OD_{550} = 0.5$) for 5 min and exposed to *C. albicans* suspensions ($OD_{550} = 1.0$) for 2 h at 37°C. After 2 h, samples were removed using a bacteriological loop and placed on microscope slides. Cells were flame-fixed and stained with crystal violet. The number of *C. albicans* blastospores adhering to 100 BECs was counted for each type of nanoparticle. The mean number of blastospores adhering to each BEC for each particle type was calculated. The kill rate obtained by incubating *C. albicans* with nanoparticles was also determined in each case. Overall, there was no obvious correlation between physiochemical properties of nanoparticles and their antiadherent or antifungal activity. Pre-treatment with nanoparticles caused significant reductions in numbers of adherent *C. albicans* blastospores to BECS in vitro regardless of the surfactant type or drug loading. For example, particles prepared using cetrimide as surfactant and containing miconazole produced a 55.2% reduction in the number of adhering blastospores and 24% of BECs were free from *C. albicans*. Drug loaded particles caused 100% kill of *C. albicans* following a 24-h incubation, regardless of surfactant type.

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Evaluation of topical patch-based delivery of aminolaevulinic acid through normal skin and nodular basal cell carcinomas

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Topical ALA-photodynamic therapy (PDT) relies on effective penetration of this small, charged drug through the structures of stratum corneum (SC) of skin. Application stimulates the over-production of protoporphyrin IX (PpIX), an effective endogenous photosensitiser. This effect is prevalent in rapidly proliferating neoplastic cells and explains the selective lethality of the therapy. However, in pre-malignant and malignant lesions, the SC is often disordered and has a reduced barrier function allowing increased ALA penetration into these lesions. This explains the good clinical outcome seen with ALA-PDT. However, nodular basal cell carcinomas (BCC) have remained more recalcitrant and shown disappointing results. This was believed to be due to a thicker and more ordered SC overlying these lesions. Consequently, ALA permeation through their full thickness is limited. The distribution of PpIX, the active metabolite of ALA, through these lesions has been studied before, mostly in a qualitative way, but little data on ALA distribution has been elucidated. The aim of this work was to evaluate the clinical potential of a novel bioadhesive patch for delivery of ALA to both normal skin and nodular BCC. In particular, it examines the penetration profile of ALA through both cutaneous structures. The effects of different ALA concentrations and application times on ALA penetration are examined. ALA-loaded, bioadhesive films were cast from drug-containing, aqueous blends of poly(methylvinylether. maleic anhyhydride) (PMVE/MA), suitably plasticised using tripropylene glycol methyl ether (TPM), as detailed by McCarron et al (2005). Punch biopsies (5 mm) of normal skin and nodular BCCs were obtained under appropriate local research ethics committee approval. Each biopsy was mounted in a thermostatically controlled holder on a modified Franz cell apparatus. A bioadhesive ALA patch with a known amount of ^{14}C-ALA was applied. ALA patch concentrations of 19, 38 and 50 mg ALA cm^{-2} were each tested for 2–8 h. Biopsies were subsequently flash frozen and microtomed cryostatically, taking longitudinal 0.25 mm slices into the biopsy, according to the method of Ahmadi et al (2004). The slices were then solubilised, the radioactivity evaluated in each using scintillation spectroscopy and related to the amount of ALA present. The results from this study showed that increasing ALA concentration and application times resulted in improved ALA penetration into normal skin. ALA penetration into nodular BCC lesions was highly variable across all concentrations and application times. Distance-resolved profiles of ALA distribution through these lesions showed an inconsistent pattern. This has important implications for PDT of nodular BCC in that variation in the SC of these lesions, both in terms of integrity and thickness, is more likely to influence the clinical outcome. This will override any improvements that are possible by increasing the dose in a topical system.

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Characterisation of moisture uptake effects on the glass transitional behaviour of polylactide films using modulated temperature DSC

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Polylactide microspheres, films, and implants have been widely used for controlled drug delivery purposes. However, it is perhaps surprising that little is known regarding the effects of moisture on the Tg of these systems, despite

evidence that water sorption may lower the Tg to below body temperature (Passerini & Craig 2001), thereby raising the possibility of polylactides being in the rubbery state within the body. The purpose of this study was to investigate the depression of the glass transition temperature (Tg) on dried polylactide films as a function of the quantity of sorbed water by the application of modulated temperature differential scanning calorimetry (MTDSC). Samples of polylactide-co-glycolide (PLGA) 50:50 were used as received and as films. Solutions of the polymer in acetone was prepared at room temperature, then placed in aluminium hermetic pans without lids, dried on a hot plate at 100°C for 1 h and pretreated under various humidity conditions. The quantity of sorbed water was measured by thermogravimetric analysis (performed using a TGA Hi-Res 2950, a dry nitrogen purge and temp. ramp $10°C\,min^{-1}$). MTDSC runs were performed using a TA 2920 DSC, calibrated at $1°C\,min^{-1}$ with indium and n-octadecane for temperature and enthalpy and alumina for heat capacity calibration. Hermetically sealed pans were used throughout. The following thermal procedure was used: isotherm at 10°C for 1 min, ramp at $1°C\,min^{-1}$ from 10°C to 70°C, amplitude ±0.5°C, period 40 s, isotherm at 70°C for 1 min, ramp at $1°C\,min^{-1}$ from 70°C to 10°C. The glass transition temperature was seen in the reversing signal; the change in heat capacity, width of the transition and magnitude of the endothermic relaxation (seen in the non-reversing signal) were also noted. Sorbed water significantly lowered the glass transition temperature of amorphous PLGA 50:50; for example the Tg for PLGA 50:50 films, stored at 85% RH for 1 h, run in hermetic pans, was determined to be $34.83 \pm 4.7°C$ compared with a measured value of $43.57 \pm 0.32°C$ obtained using the film dried without storage at high RH (the corresponding heat capacity changes through Tg are $0.45 \pm 0.04\,J\,g^{-1}°C^{-1}$). Interestingly, the Tg of the films was found to be higher than that corresponding to the powder as received ($35.6 \pm 0.75°C$), an observation that is not explained by differing solvent levels. The relationship between moisture uptake and Tg is discussed in terms of the Gordon-Taylor equation. In addition, we consider the fragility of the system (itself related to the heat capacity change through the transition) as a function of water content, an issue that will have implications for the change in mechanical properties of the film as a function of water content and temperature. Overall, the study not only demonstrates that PLGA films may, in a humid environment, sorb sufficient water to lower the Tg to below body temperature but also presents more fundamental information on the changes in the film's molecular mobility caused by the plasticisation process.

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Drug product analysis through multidisciplinary solid state characterisation

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While challenges still remain for the solid state characterisation of drug substance (e.g., reliable surface amorphous quantification), there are numerous techniques that can be applied to determine physicochemical properties. In comparison, the number of solid state characterisation methodologies applicable to the study of a formulated drug product is greatly reduced. With increasing regulatory guidance and escalating challenges faced for the incorporation of the API into a viable drug delivery system, more sophisticated characterisation technologies are required for evaluation of resultant physicochemical properties. No one technique is capable of elucidating all properties and here we explore, for the first time, the application of high-resolution solid state characterisation methodologies to study the mechanisms of action of drug delivery systems in vitro. In addition to the conventional requirements of identifying and quantifying the composition of components, resolving spatial distribution is a key element in optimising and understanding the mechanism of action in relation to drug release profiles. Here we have combined the use of the chemical mapping techniques of Confocal Raman microscopy and time-of-flight secondary ion mass spectrometry (TOF-SIMS) with the high resolution imaging capability of X-ray microtomography. Two different but equally complex systems have been studied – a multilayered controlled release pellet and a tableted formulation. Non-destructive imaging by X-ray microtomography has been used to probe the 3-dimensional physical structures of both systems in dry and solution immersed conditions. In the dry state, this has provided 3-dimensional quantified information on particle size (in the range 10–55 μm) and layer thickness (range 15–100 μm) as well as physical properties such as cracks and inherent porosity to be determined. Dynamic studies have for the first time revealed 3-dimensional information about the mechanism of action in solution. X-ray microtomography is, however, limited to a spatial resolution of ~5 μm and does not provide direct chemical identification. TOF-SIMS analysis of cross-sectioned regions was therefore used to correlate the microtomography data to the spatial distribution of chemically identified components within the two systems. Through the application of Raman microscopy the chemical form and interactions between components was also correlated to microtomography data under dry and solution conditions. Application of solid state analytical technologies can be used for the in-depth study of drug product analysis. Through careful combination of relevant techniques, issues relating to the final drug form, particle size, chemical distribution and mechanisms of action can start to be resolved aiding formulation problem solving and optimisation early in drug delivery design.

Poster Session 3 – Materials Science

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Characterisation of two multicomponent adenine complexes

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Many solid forms of active pharmaceutical ingredients (APIs) can be prepared, including polymorphs, solvates, hydrates, salts and amorphous solids. In addition, crystal engineering can be used to design crystals containing multiple component phases involving an API and other molecules. It is possible for an API and a second substance to crystallise together without a reaction taking place. If both substances can exist separately as solids at room temperature then the multicomponent crystal is known as a cocrystal. Multicomponent crystals may form not only from recrystallisation but also by grinding. Desirable or undesirable properties may then result. Recently, several co-crystals of caffeine have been prepared with a view to enhancing physical stability at various humidities (Trask et al 2005). The aim of this study was to prepare multicomponent crystals of adenine. As adenine is a base, it should be possible to combine it with an acid and adipic acid and salicylic acid were selected for complex formation. The optimum characterisation technique used to analyse the products obtained by recrystallisation was X-ray crystallography. Before X-ray investigations, identification methods used on the samples included simple melting points, microscopic examination, FTIR, DSC and density determinations. Single crystal X-ray data, collected on a Bruker-Nonius KappaCCD diffractometer, were used to determine the previously unknown crystal structures. In both cases the solvent used, methanol, became trapped within the crystal lattice. Multicomponent crystals of adenine:adipic acid: methanol (2:1:2) are triclinic with a = 8.9781 (5), b = 9.9334 (5), c = 14.281 (7) Å, $\alpha = 74.908$ (3), $\beta = 85.357$ (3), $\gamma = 66.786$ (3)°. The five molecules in the asymmetric unit of the crystal are linked together by extensive hydrogen bonding. An attempt to cocrystallise adenine and salicylic acid resulted in a methanolated proton transfer complex where the hydrogen from the carboxylic acid group in salicylic acid transferred to a nitrogen in the pyrimidine ring of adenine. The ortho hydroxy group of salicylic acid is also disordered over both possible ortho positions. The cell dimensions of the resulting monoclinic crystals, space group $P2_1/n$, are a = 7.1636 (2), b = 7.9486 (3), c = 24.7336 (9), $\beta = 91.248$ (2). The ratio of molecules in the crystal is 1:1:1. Again, extensive hydrogen bonding is present and for both multicomponent crystals the molecular geometries were characterised using the PLATON (Spek 1998) computer program. It can be concluded that multicomponent crystals of adenine can be formed by recrystallisation with suitable acids and that the architecture of the products can be characterised by single crystal diffraction.

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An approach to normalise inverse gas chromatography data measured with a range of dispersive probes

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Inverse gas chromatography is a precise and reproducible technique to calculate the surface energetics of pharmaceutical solids by measuring the