United Kingdom & Ireland
Controlled Release Society

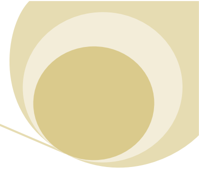
www.ukicrs.org

1st May – Workshop

2nd May – Symposium
Aston University, Birmingham, UK

UKICRS 2012

Drug Delivery: 'through the ages'



WELCOME

PROF Perrie to Write Preface

Workshop

1st May 2012 1pm-6pm

The UKICRS is hosting a free workshop for all attendees at this year's symposium in Aston University. The workshop will comprise of short presentations and hands-on demonstrations of equipment and technology companies.

The UKICRS committee is grateful to the sponsors of the workshop day.

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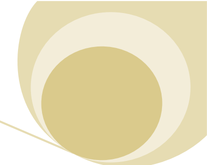


PROGRAMME

2nd May 2012

8.30 am	Registration / Poster Setup
9.30 am	**Prof Yvonne Perrie** (Aston University) Welcome & Opening Remarks
9.40 am	**Prof Terry Allen** (University of Alberta) Controlled release delivery systems: from coated aspirin to personalized nanomedicines
10.25 am	**Lauren Shurety** (Q-Chip Cardiff) Q-Sphera: from proof of principle microfluidic chips to full scale aseptic manufacturing platform
10.40 am	Tea / Coffee
11.05 am	**Dr Randip Kaur** (Aston University) Pegylation of DDA:TDB liposomal adjuvants reduces the vaccine depot effect and alters the Th1/Th2 immune responses
11.20 am	**Dr Viraj Mane** (University of Maryland USA) ICAM-1-mediated targeting and endocytosis in the gastrointestinal tract in mice
11.35 am	**Timothy Doody** (University College Cork) Developing a murine pulmonary model for assessment of nanoparticle delivery *in-vivo*
11.50 am	Poster Session 1
12.40 pm	Lunch
1.40 pm	**Prof Molly Stevens** (Imperial) Biomaterials-based strategies for regenerative medicine and biosensing
2.10 pm	**Nicola Irwin** (Queen's University Belfast) Kinetic and thermodynamic control of antibiotic release from infection and pH-responsive hydrogels
2.55 pm	**Michael Cook** (University of Reading) Dehydrated hydrogel matrices for the oral delivery of probiotic bacteria
2.40 pm	**Amy Judd** (Keele University) Nanomaterials for the prevention of infectious diseases
2.55 pm	Tea / Coffee
3.15 pm	Poster Session 2
3.45 pm	**Wilson Oguejiofor** (Aston University) Spray dried combinations of lactoferrin with antibiotics appear superior to monotherapy for reducing biofilm formation by *Pseudomonas aeruginosa*
4.00 pm	**Rosalind Chong** (Cardiff University) Delivery of siRNA to skin using microneedle devicese: *in-vitro* and *in-vivo* proof of concept
4.15 pm	**Saif Shubber** (University of Nottingham) In-vitro evaluation of the mechanism of action of a novel absorption enhancer criticalsorb®
4.30 pm	Poster Prizes / Close of Meeting

Graduate Network

Dear Delegates,

As the chairperson for the graduate network I would like to invite all graduates to our UKICRS facebook group where we currently have 82 members.

The UKICRS Graduate Network includes postgraduate students and representatives from universities across the UK and Ireland. As the key link between the committee and the universities, the members of the Graduate Network can provide information and help coordinate support for UKICRS various events.

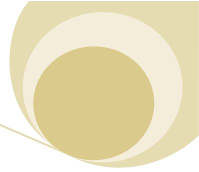
The main aims of the graduate network are to raise awareness of the UKICRS to all researchers across the UK and Ireland and for students to raise their opinions on what they would like to see at future conferences. If you would like to join the graduate network and think you could make an active contribution, please contact us and join the facebook group.

Furthermore, if you would like to become a representative of your university please approach any of the committee members during the symposium where we would be happy to enlist you.

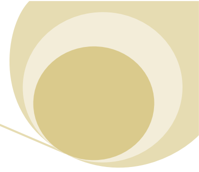
Facebook Group: http://www.facebook.com/groups/UKICRS/
Linkedin Group: United Kingdom and Ireland Controlled Release Society (UKICRS)

Hope you all have fun at the Symposium!

Jitinder Singh Wilkhu
UKICRS Graduate Chairperson



Invited Speakers

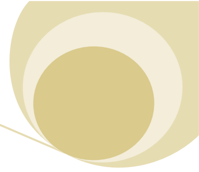


Professor Theresa Allen- University of Alberta

"Controlled release delivery systems: from coated aspirin to personalized nanomedicines"

Theresa M. Allen, PhD, FRSC, is a Professor Emeritus of Pharmacology and Oncology at the University of Alberta. She has been active in the drug delivery field for over 30 years, and has made important contributions to the development of nanomedicines, including the product Doxil®, and of ligand-targeted nanomedicines for anticancer drugs and gene medicines. She has over 200 peer-reviewed publications and several patents.

Dr. Allen is a founding member and Division Chair, Drug Delivery, at the Centre for Drug Research & Development (www.cdrd.ca), which is a novel hybrid organization devoted to advancing promising medical discoveries from academia to a commercially attractive stage. She has received a number of national and international awards, including Fellow of the Royal Society of Canada, and College of Fellows of the Controlled Release Society.

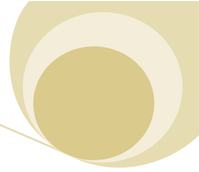


Professor Molly Stevens- Imperial

"Biomaterials-based strategies for regenerative medicine and biosensing "

Molly Stevens is currently Professor of Biomedical Materials and Regenerative Medicine and the Research Director for Biomedical Material Sciences in the Institute of Biomedical Engineering. She joined Imperial in 2004 after a Postdoctoral training in the field of tissue engineering with Professor Robert Langer in the Chemical Engineering Department at the Massachusetts Institute of Technology (MIT). Prior to this she graduated from Bath University with a First Class Honours degree in Pharmaceutical Sciences and was then awarded a PhD in 2000 in biophysical investigations of specific biomolecular interactions and single biomolecule mechanics from the Laboratory of Biophysics and Surface Analysis at the University of Nottingham.

She has a large and extremely multidisciplinary research group of students and postdocs/fellows. Research in regenerative medicine within her group includes the directed differentiation of stem cells, the design of novel bioactive scaffolds and new approaches towards tissue regeneration. She has developed novel approaches to tissue engineering that are likely to prove very powerful in the engineering of large quantities of human mature bone for autologous transplantation as well as other vital organs such as liver and pancreas, which have proven elusive with other approaches. This has led to moves to commercialise the technology (she is the co-founder of RepRegen, formally BioCeramic Therapeutics, and InTiGen) and set-up a clinical trial for bone regeneration in humans. In the field of nanotechnology the group has current research efforts in exploiting specific biomolecular recognition and self-assembly mechanisms to create new dynamic nano-materials, biosensors and drug delivery systems. Recent efforts by the Stevens group in peptide-functionalised nanoparticles for enzyme biosensing have enabled the most sensitive facile enzyme detection to date and have a host of applications across diseases ranging from cancer to global health application



ORAL- ABSTRACTS

Q-SPHERA: FROM PROOF-OF-PRINCIPLE MICROFLUIDIC CHIPS TO FULL SCALE ASEPTIC MANUFACTURING PLATFORM

Lauren Shurety, Bartley, S.J., Seaman, P.F. and Palmer, D.D.

Q Chip Ltd, Oddfellows House, 19 Newport Road, Cardiff, CF24 0AA

Microfluidic lab-scale production of polymer microspheres has long been a reality. Transition to full scale production however can prove difficult due to the complexity of volumetric scale-up. Here we describe the scale up of a microsphere production method to a clinical scale aseptic process, with applications in sustained release parenteral peptide therapeutics. Parenteral drug delivery products account for nearly 30% of total global pharmaceutical sales and were valued at $27 billion in 2011, with figures rising to a predicted $51 billion in 2015.

The original process employed microfluidic chips to create discrete droplets of bioresorbable polymer and active ingredient and used chemical, UV curing or temperature to solidify into microspheres. This method gave microspheres with highly desirable characteristics but proved difficult to adapt to large scale manufacture. To address this we developed a new droplet generation technology which attained a 1000-fold increase in production capability. Additional parallel units can be added to further increase output. The platform is currently undergoing validation for the production of clinical material. During the transition from laboratory to full production scale we have maintained the original desirable product characteristics and in some cases improved them. We present here monodisperse microspheres with a mean diameter of 40µm and a CV below 6%. Drug loadings between 6 and 9% are described, alongside *in vitro* release profiles illustrating low initial release followed by consistent sustained release over a 1 month period.

The semi-continuous process converts liquid starting material into solid microspheres in the absence of harsh solvents, extreme temperatures or shearing forces. The spheres are immediately dried and collected, resulting in a free-flowing powder, presented here as resuspendable and injectable, with a 50mg/ml suspension passing through a 25G needle. In summary, we describe a 1000-fold in increase in the production of microspheres with highly desirable characteristics in a benign process.

PEGYLATION OF DDA:TDB LIPOSOMAL ADJUVANTS REDUCES THE VACCINE DEPOT EFFECT AND ALTERS THE TH1/TH2 IMMUNE RESPONSES

Randip Kaur[1], Vincent W. Bramwell[1], Yvonne Perrie[1]

[1]School of Life and Health Sciences, Aston University, Aston Triangle, Birmingham, B4 7ET.

The adjuvant efficacy of the cationic liposome system composed of dimethyldioctadecylammonium bromide and trehalose dibehenate (DDA:TDB) is well established and one of the proposed mechanisms behind the immunostimulatory effect obtained with DDA:TDB is the 'depot effect' in which the liposomal carrier helps to retain the antigen at the injection site, thereby increasing the time of vaccine exposure to the immune cells. The depot effect has been suggested to be primarily due to their cationic nature which results in electrostatic adsorption of the antigen and aggregation of the vesicles at the site of injection.

The aim of the study was to further test this hypothesis by investigating whether sterically stabilising the DDA:TDB system with polyethylene glycol (PEG) reduces aggregation, and subsequently influences the formation of a depot at the site of injection and the immune response. Liposomes composed of DDA in combination with the synthetic bacterial cell wall glycolipid TDB were produced using the lipid-film method [1] and the dehydration-rehydration method with various concentrations of PEG, 5% and 25 mol%. Ag85B-ESAT-6 was added to a final antigen concentration of 2μg/dose.

Results reported within this study demonstrate that higher levels of PEG i.e. 25 % was able to significantly inhibit the formation of a liposome depot at the injection site and also severely limit the retention of antigen at the site therefore resulting in a faster drainage of the liposomes from the site of injection. This was reflected by the immunisation study, where lower levels of IgG2b antibody, IFN-□ and IL2 was found compared to the DDA:TDB formulation, and higher level of IL-5 cytokine suggesting that the pegylated formulations stimulate a Th_2 type immune response. This study further supports the hypothesis that the depot formation is due to electrostatic forces between the net negatively charged protein and the positively charged liposomes.

References

[1] J. Davidsen, I. Rosenkrands, D. Christensen, A. Vangala, D. Kirby, Y. Perrie, E.M. Agger and P. Andersen. (2005) *Biochimica et Biophysica Acta.* 1718: 22-31.

ICAM-1-MEDIATED TARGETING AND ENDOCYTOSIS IN THE GASTROINTESTINAL TRACT IN MICE

Viraj Mane[1] and Silvia Muro[1,2]

[1]*Institute for Bioscience and Biotechnology Research, University of Maryland, College Park, MD, 20742, USA* [2]*Fischell Department of Bioengineering, University of Maryland, College Park, MD, 20742, USA*

Nanocarriers have several tunable characteristics (size, shape, surface) and can be further functionalized by linking targeting moieties as well as therapeutic or imaging reagents. Our targeting approach was directed at intercellular adhesion molecule 1 (ICAM-1), a surface marker found on gastrointestinal epithelial cells and many other tissues, as a potential method for intraoral drug biodistribution. We first visualized the migration of nontargeting, fluorescent IgG antibody through the gastrointestinal tract following gastric gavage into mice. Radiolabeled IgG was then measured in gastrointestinal compartments, revealing accumulation of over 35% within the stomach and small intestines. To determine the effects of nanocarrier complexation on antibody biodistribution and degradation, we compared free IgG to IgG coupled onto model polymer nanocarriers. Accumulation of IgG-nanocarriers in the stomach was 1.7-fold greater than free IgG; however, gastrointestinal degradation was similar in both formulations. Gastric gavage of IgG-nanocarriers in alkaline buffer accelerated the distal migration of our formulations compared to neutral buffer, but did not protect against degradation, suggesting that degradation is not mediated primarily by pH. We verified this *in vitro* by establishing that degradation depends on digestive enzymes, but not pH. Finally, we determined that retention of ICAM-specific antibodies in proximal gastrointestinal compartments was up to 4-fold greater than IgG, and was further enhanced by coupling anti-ICAM antibodies onto nanocarriers, indicating that ICAM-targeting redistributes orally-delivered formulations within the gastrointestinal tract. Fluorescence imaging of anti-ICAM nanocarriers demonstrated attachment to gastrointestinal epithelium, and transmission electron microscopy further showed the internalization and accumulation of anti-ICAM nanocarriers into endocytic vesicles of epithelium. Taken together, these results indicate that ICAM-1 targeting may find use in directing the retention and epithelial uptake of oral therapies in specific gastrointestinal compartments.

Acknowledgments

Funding: American Heart Association 09BGIA2450014 & National Institutes of Health R01HL098416.

DEVELOPING A MURINE PULMONARY MODEL FOR ASSESMENT OF NANOPARTICLE DELIVERY IN VIVO

Timothy Doody ,[1] Satti A[2], MacLaughlin R[3], Gon'ko YK[2], Volkov Y[4], Ryan K[1]

[1]School of Pharmacy, University College Cork, Cork., [2]School of Chemistry and CRANN, Trinity College, Dublin., [3]Aerogen, Galway Business Park, Dangan, Galway., [4]School of Medicine and CRANN, Trinity College, Dublin.

The pulmonary route has received much attention as a site for non-invasive administration of therapeutic agents for both local and systemic delivery. This is due to factors including a large absorptive surface area and an excellent blood supply. Delivery by nebulisation can target specific areas of the lung depending on the properties of the aerosol, for example to the central conducting airways or to the alveolar region of the lung. We have evaluated an Aerogen® nebulisation device with the aim of utilising it for the delivery of nanoparticle platform for the delivery of drug and diagnostic agents.

Using red fluorescent nanoparticles as a marker we were able to demonstrate that the Aerogen® system was effective for delivering nanoparticles to the murine pulmonary system. IVIS imaging, histology and fluorescent measurements showed the presence of the particles within the lungs of treated animals. Using Evans Blue dye as a model for detection we were able to determine the optimal parameters for nebulisation using the Aerogen® nebulisation device.

We have demonstrated that it is feasible to deliver nanoparticles to the mouse lung using the Aerogen® nebulisation system. In future we hope to deliver a novel drug loaded nanoparticle system using this nebuliser and to demonstrate the bio-compatibility of our nanoparticles.

KINETIC AND THERMODYNAMIC CONTROL OF ANTIBIOTIC RELEASE FROM INFECTION- AND pH-RESPONSIVE HYDROGELS

Nicola Irwin, McCoy C.P., Gorman S.P., Jones D.J

Department of Biomaterials, School of Pharmacy, Queen's University, Belfast.

Urinary catheter infections represent a significant healthcare problem; statistics suggest that indwelling urinary devices are responsible for an estimated 80% nosocomial urinary infections (1). Furthermore, elevations of urinary pH from normal values of approximately pH 6 to levels up to pH 9.1 are frequently reported during infections by urease-producing urinary pathogens, including *Proteus mirabilis,* as a result of urease-catalysed hydrolysis of urea into ammonia (2). This pH elevation, whilst often the cause of increased patient morbidity and mortality through its role in crystalline biofilm formation, is exploited in this report to act as a potential trigger for 'intelligent' drug release in direct response to the onset of infection, without any external intervention.

Nalidixic acid, a model quinolone antibiotic with poor aqueous solubility at normal physiological urine pH, was incorporated into a p(HEMA) hydrogel by a novel, rationally-designed loading technique involving both conventional dispersion of dissolved drug and surface localisation of particulate matter. By exploitation of the rapid dissolution kinetics and significantly increased thermodynamic solubility of nalidixic acid in alkaline media, a paradigm for pH-responsive drug delivery is presented. The drug itself, by responding to the pH trigger, is inherently responsible for its own release, rates of which are enhanced by factors of 50 and 10 in alkaline media compared with release at pH 5 and 7 respectively, and not dependent on the polymer carrier. This represents a novel approach towards achieving rapid, infection-responsive drug delivery.

References

1. Jacobsen S, Stickler D, Mobley H, Shirtliff M. Clin. Microbiol. Rev. 2008; 21: 26-59.
2. Stickler DJ. J. Med. Microbiol. 2006; 55: 489-494.

DEHYDRATED HYDROGEL MATRICES FOR THE ORAL DELIVERY OF PROBIOTIC BACTERIA

Michael. T. Cook[1], G. Tzortzis[2], V. Khutoryanskiy[1] and D. Charalampopoulos[1]

[1] *University of Reading, Reading, Berkshire, RG6 6AD, U.K.;* [2]*,Clasado Ltd, Reading, Berkshire, RG6 6AD, U.K.*

Probiotic bacteria have gained worldwide popularity as a safe method of alleviating the ill-effects of poor gut health. However, many strains of probiotic bacteria are acid sensitive, so do not survive passage through the low pH juices of the stomach after oral administration. It has been demonstrated that the microencapsulation of probiotic cells can improve the survival of these acid-sensitive species during exposure to low pH. Hydrogel matrices may offer a desirable environment for entrapment of cells and can be produced in ways which are 'gentle' enough to not harm the cells so are of interest in this field of research. The presented work focuses on the encapsulation of the acid-sensitive probiotic *Bifidobacterium Breve* into ionic alginate gels which have been coated by chitosan as a means of improving the efficacy of orally administered probiotics as well as targeting the delivery of the probiotic to the intestine. In addition, the dehydration of these capsules by a variety of techniques was investigated and fluid-bed drying chosen as the most favourable in terms of speed of drying and quality of product. Dehydration is essential for a nutraceutical of this type to ensure the stability of the probiotic cells during storage. The described research includes an investigation into several facets of the encapsulation system using techniques including gravimetric analysis, turbidometry and confocal microscopy. Once physically characterised, these systems were tested for both their ability to protect *Bifidobacterium breve* from a simulated gastric solution and to control the release of the cells during a simulated gastrointestinal passage. The ability of the matrices to protect the cells from acid after dehydration was also determined.

NANOMATERIALS FOR THE PREVENTION OF INFECTIOUS DISEASES

Amy Judd, Jon Heylings[2], Ka-Wai-Wan[3] and Gary Moss[1].

[1]School of Pharmacy, Keele University, Staffordshire, UK, [2]Dermal Technology Laboratory Ltd, Keele, Staffordshire, UK, [3]School of Pharmacy, University of Central Lancashire, Preston, UK.

The Department of Health stated that each infection acquired in hospital costs between £4,000 and £10,000 totaling £1 billion pounds each year for the NHS[1]. To reduce morbidity and the financial cost, innovation is required within the infection control arena to provide novel antimicrobial compounds and drug delivery platforms.

This project has further investigated the antimicrobial properties of poly(amidoamine) (PAMAM) dendrimers. The inhibitory concentration (IC_{50}) has been determined for generation (G=) 2, 3, 4 and 5 of amine terminated PAMAM dendrimers and also for generation 3.5 which are carboxyl terminated PAMAM dendrimers. The IC_{50} for PAMAM dendrimer G=2 was 26.90 µg/mL, G=3; 15.25 µg/mL, G=4; 7.43 µg/mL and G=5; 2.89 µg/mL. Calculated IC_{50} values plotted against amine terminated PAMAM generation to demonstrate linear relationship. To further elucidate the biocidal effect and the mechanism of the PAMAM dendrimers a cell membrane integrity study, an inner membrane permeabilization assay were conducted and SEM micrographs was taken to visualize the membrane disruption.

PAMAM dendrimers are not only antimicrobial in their own right but also enhance the permeation of conventional antiseptics into the non viable upper skin strata. A topical pre-dose formulation of increasing concentrations of PAMAM dendrimer generation 3 followed by a clinical dose of chlorhexidine digluconate (2 % w/v) has been investigated *in vitro* using a Franz diffusion cell model. An *in vitro* porcine epidermal permeation study revealed that with the lowest concentration PAMAM pre-dose formulation tested (1 mM, generation 3) resulted in a ten-fold increase of absorbed chlorhexidine digluconate after 24 hours, compared to the control pre-dose formulation. Dendrimers have the capacity to be versatile polyvalent biocides/drug delivery platforms that have many biomedical applications.

References

[1] Department of Health, (2006) Going further faster: Implementing the saving lives delivery programme sustainable change for cleaner safer care. London. http://www.dh.gov.uk/en/Publicationsandstatistics.

SPRAY DRIED COMBINATIONS OF LACTOFERRIN WITH ANTIBIOTICS APPEAR SUPERIOR TO MONOTHERAPY FOR REDUCING BIOFILM FORMATION BY *Pseudomonas aeruginosa*

Wilson Oguejiofor, Andrew Ingham, Lindsay Marshall

School of Life & Health Sciences, Aston University, Birmingham, B4 7ET.
Email: oguejiow@aston.ac.uk

Chronic lung infections with the opportunistic pathogen *Pseudomonas aeruginosa* are a major contributor to the high morbidity and mortality amongst people with cystic fibrosis (CF). In this study, we report on the production and application of a novel, spray dried combination therapy; employing an aminoglycoside antibiotic (tobramycin or gentamicin) with an antimicrobial protein (lactoferrin or apo-lactoferrin). Biofilms were prepared in 96 well polystyrene plates. Combinations of the antibiotics and various lactoferrin preparations were spray dried. The bacterial cell viability of the various spray dried combinations were determined. Iron-free lactoferrin (apo lactoferrin) induced a 3 log reduction in the killing of planktonic cell by the aminoglycoside antibiotics ($p < 0.01$) and also reduced both the initiation and persistence of *P. aeruginosa* biofilms ($p < 0.01$). These combinations may provide a superior treatment strategy to those currently being employed to combat *P. aeruginosa* biofilms in the airways.

DELIVERY OF siRNA TO SKIN USING MICRONEEDLE DEVICES: IN VITRO AND IN VIVO PROOF-OF-CONCEPT

Rosalind. Chong[1,2], E. Gonzalez-Gonzalez[3], M.F. Lara[4], T.J. Speaker[4], R.L. Kaspar[4], S.A. Coulman[1], R. Hargest[2] and J.C. Birchall[1]

[1] *School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, CF10 3NB, UK.* [2] *Academic Department of Surgery, University Hospital of Wales, Cardiff University, Heath Park, Cardiff, CF14 4XN, UK.* [3] *Department of Pediatrics, Stanford School of Medicine, Stanford, California, USA.* [4] *Transderm Inc., Santa Cruz, California, USA.*
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One of the greatest challenges in gene therapy is to deliver the nucleic acid effectively to the intracellular target. This challenge is further complicated in skin by the stratum corneum barrier. We aim to optimise methods for microneedle-assisted delivery of small interfering ribonucleic acid (siRNA) to skin, with a future view of modifying gene expression in dermatopathogenic conditions.

The ability to dry coat siRNA formulation onto microneedles was investigated using lamin A/C as a model gene for RNA interference studies. Following optimisation of the coating procedure, naked siRNA was efficiently (30g per device) dry-coated onto the surface of microneedles. The coating process did not reduce the biological functionality of the siRNA, as demonstrated by a significant reduction in gene expression/protein synthesis of lamin A/C in keratinocyte culture models.
In vivo functional delivery of siRNA using microneedles was investigated using a transgenic mouse model expressing the hMGFP gene, which produced a phenotype that resulted in GFP expression in the upper layers of epidermis1. The middle region of one mouse paw was microneedle-treated with Accell CBL3 siRNA targeted against the hMGFP gene and the other paw with Accell TD101 siRNA (control). The mouse paws were imaged using the Maestro fluorescent imaging system prior to sacrifice for qPCR analysis and histology sectioning.
Results from the *in vivo* study were equivocal. Whilst microneedle delivery of siRNA led to an apparent reduction in protein expression in certain microneedle treatment groups, this reduction was not always reflected in qPCR data. Despite some evidence of gene expression modification at the protein level, there are significant practical challenges in determining the molecular effects of siRNA delivery to skin. Current studies are therefore characterising microneedle delivery of fluorescently labelled siRNA to human skin explants, alongside keratinocyte cultures, to determine correlation between siRNA delivery, uptake and subsequent gene expression reduction.

References

1. E Gonzalez-Gonzalez, *et. al.*, siRNA silencing of keratinocyte-specific GFP expression in a transgenic mouse skin model, *Gene Therapy*, **16**, 963–972 (2009).

Acknowledgements

R.P. Hickerson (*Transderm Inc., Santa Cruz, California, USA*) for siRNA, M.A. Flores (*Transderm Inc., Santa Cruz, California, USA*) for technical support, C.H. Contag (*Department of Pediatrics, Stanford School of Medicine, Stanford, California, USA*) for access to imaging facilities. NIH GO Delivery Grant for funding.

IN VITRO EVALUATION OF THE MECHANISM OF ACTION OF A NOVEL ABSORPTION ENHANCER - CRITICALSORB®

Saif Shubber[1], Faron Jordan[2], Andy Lewis[2], Snjezana Stolnik[1]

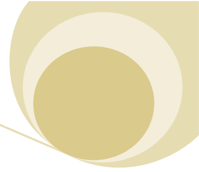
[1]Division of Drug Delivery and Tissue Engineering, School of Pharmacy, Boots Science Building, University of Nottingham, University Park, Nottingham, NG7 2RD [2]Critical Pharmaceuticals Limited, BioCity Nottingham, Pennyfoot Street, Nottingham, United Kingdom, NG1 1GF

The therapeutic benefit mucosal drug delivery offers over parenteral has attracted a considerable research interest and investment particularly when attempting to deliver peptides, proteins and the greater spectrum of biotechnological based therapeutics. Absorption of these therapeutics across mucosal membranes is often low resulting in low bioavailability and therefore different approaches, such as manipulation of the formulation, the API and/or the incorporation of absorption enhancing agents, have been adopted to increase the absorption and ultimately improve bioavailability.

CriticalSorb® is a novel mucosal absorption enhancer developed by Critical Pharmaceuticals which is currently in clinical development as nasal spray formulation for human growth hormone. As its absorption enhancing mechanism of is currently unknown, we have probed physicochemical and biological properties of CriticalSorb® formulations. In aqueous solution CriticalSorb® formed micellar structures with a mean particle size of 12-14 nm, and relatively narrow particle size distribution, with the critical micellar concentration (CMC) at 0.005-0.01 %w/v, as judged from pyrene 1:3 normalisation studies.

When applied to Calu-3 monolayers, *in vitro* model of respiratory mucosa, the range of CriticalSorb® concentrations below and above the critical micelle concentration caused no substantial change in transepithelial electrical resistance (TEER) suggesting minimal tight junction regulation. Cell viability studies, measuring cell metabolic activity (MTS) and cell membrane integrity (LDH) indicate low toxicity of CriticalSorb® up to concentrations of 0.5 %w/v for a 3 hour experiment.

Transport studies, using FITC-insulin as permeant, showed significant difference in insulin transport across the Calu-3 cell monolayer in the presence and absence of CriticalSorb® at 37 ^{o}C, indicating its permeability enhancing properties. Studies conducted at 4 ^{o}C showed no significant differences in the transport of FITC-insulin in the presence of CriticalSorb®. This data may indicate that mechanism of enhancement operates through a transcellular route, possibly affecting the membrane fluid bilayer although further studies would be needed to confirm this.



POSTER- ABSTRACTS

AQUAPARTICULATES FOR PROTIEN DELIVERY

Fadi A. Abdulrazzaq, Yvonne Perrie, and Deborah Lowry

Aston University, Birmingham, B4 7ET, United Kingdom

Aquasomes are recent delivery systems for protein/peptide-based pharmaceuticals. Aquasomes retain conformational integrity of loaded molecules, which enable them to overcome some of the problems, such as poor bioavailability. They are self-assembled systems consists from an inner solid core, a middle polyhydroxy carbohydrate coating and an outer drug layer. The purpose of this study is to show whether aquasomes are able to prolong the release of BSA. Solid cores were manufactured by reacting monobasic sodium phosphate and calcium chloride. The solid cores were coated by mixing with lactose solution for 2.5 hrs and freeze-dried. The coated cores were loaded by mixing with BSA solution 1 mg/mL for 2.5 hrs and freeze-dried. Different manufacturing conditions were carried out 4°C, 25°C and 50°C. An *in vitro* release study was performed on the freeze-dried samples. The samples were redistributed in 10 mL of water and then placed in a shaking water bath at 37°C and 100 rpm. A quantity of 0.5 mL was taken for HPLC analysis at a number of time points. The aquasomes were calculated to have a BSA loading of between 40-50%. Solid cores where manufactured at 25°C, as when manufactured at 4°C and 50°C the cores were rod shaped rather than oval. The BSA release studies showed an initial burst effect over the first 2 hrs followed by a constant release over 24 hrs. Formulation A (coated and loaded at 4°C) showed higher release rates than formulation B (coated and loaded at 25°C) due to the manufacturing conditions. The lower temperatures allowed a higher rate of physical adsorption for the coating and drug loading. Aquasomes loaded with BSA were successfully manufactured. The *in vitro* release studies of BSA show constant release over 24 hrs. Currently, manufacturing conditions are being explored to optimize aquasomes release properties.

DESIGN OF LECTIN CONJUGATED MICROSPHERES IN THE ERADICATION OF H-PYLORI INFECTIONS FOR THE TREATMENT OF PEPTIC ULCER

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Helicobacter pylori has been implicated in the aetiology of diseases including acute chronic gastritis, gastric lymphomas and peptic ulcers [1]. Obstacles to the successful eradication of these infections include: antibiotic-resistant bacteria; complicated dosing schedules and bacterial residence in environments where high drug concentrations are difficult to achieve [2]. Conventional oral formulations have a short gastric residence time, thus limiting the duration of exposure to the bacteria. Gastro-retentive formulations may prolong gastric residence and also maintain controlled release of drug. Lectins have the potential to target drugs to different parts of the GI tract or even to different cells and are resistant to digestion within that environment. The purpose of this study is to characterize Concanavalin A-conjugated ethylcellulose microspheres loaded with clarithromycin and to evaluate *in vitro* floating, mucoadhesive and drug release properties.

SEM showed microspheres that were spherical with a smooth surface. Drug loading efficiencies were above 80% in all formulations and particle sizes were between 65 and 200□m. About 65% of the microparticles remained floating over SGF after 12 hours. Lectin conjugation efficiency was 60.1 ± 9.4%. DSC and FTIR scans showed no interactions between the clarithromycin and the polymers. Also, FTIR confirmed the conjugation of the lectins to the microsphere surface. Mucoadhesion of conjugated microspheres to porcine gastric mucosa was about 78% compared with 35% of the unconjugated microspheres. Zeta potentials of unconjugated and lectin conjugated microspheres were -10.8 ± 0.6 mV and +49.4 ± 1.07 mV respectively. *In vitro* release was biphasic with an initial burst release followed by slow release which continued beyond 12 hours.

The conjugation of Concanavalin A to ethylcellulose microspheres improved the mucoadhesion of the formulation and could provide a means for targeted delivery of antibiotics in the eradication of *H-pylori*.

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INVESTIGATION AND COMPARISON OF VARIOUS DRUG-LOADING PROCESSES ONTO MESOPOROUS SILICA (SBA-15)

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Many of the drugs developed today exhibit poor water solubility, slow dissolution rate and limited oral bioavailability. If the dissolution rate of poorly-water soluble drugs can be improved, it would allow their development in pharmaceutical products. This can be achieved by increasing their surface area by loading onto a high surface area carrier like mesoporous silica (SBA-15). SBA-15 is an excellent carrier for poorly-water soluble drugs because it has a large surface area, pore volume with a narrow pore size distribution, which allows for homogonous and reproducible drug adsorption and release (Manzano et al., 2009).

The aim of this study was to investigate the methods used to load the poorly-water soluble drug fenofibrate onto SBA-15. A number of methods including physical mixing, solvent impregnation, near-critical (liquid) CO_2 and supercritical CO_2 (SC-CO_2) were used. The impact of the loading method was quantified in terms of the drug's physicochemical properties. Techniques used included powder x-ray diffraction (pXRD) and differential scanning calorimetry (DSC) to study drug crystalline form; BET surface area and pore volume analysis to determine changes in SBA-15 surface and pore properties and *in-vitro* release studies to quantify drug dissolution rate.

The fenofibrate loaded onto SBA-15 using the physical mixing method was crystalline to some degree. For all other samples, it was in the amorphous form. There was a large increase in effective surface area of drug in contact with the dissolution medium, which resulted in enhanced drug release rate after the drug was loaded onto SBA-15. The physically mixed sample showed a modest increase in drug release rate whereas for all other samples, there was a large increase in drug release rate.

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THE POTENTIAL USE OF TRITIATED WATER IN ASSESSING THE OCULAR TISSUE INTEGRITY OF AN IN-VITRO EYE MODEL

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Posterior eye disorders are on the rise due to an aging population, an increase in the number of diabetic and obese patients and are the major cause of blindness in the developed world. Novel delivery systems such as nanoparticles, liposomes and dendrimers have been explored to enhance the delivery of therapeutic agents to the posterior eye segment. These novel delivery systems have in general been assessed using *in-vivo* animal models, despite ethical concerns for animal wellbeing and criticism of the validity and the precision of the animal models. The development of an *in-vitro* eye model to test potential formulations and to minimise the number of animals used could be an ideal alternative. Commonly the barrier integrity of tissues used in *in-vitro* eye models is assessed by monitoring the permeation of a marker compound, either concurrently, before or after the permeation of the drug. The limitations of this technique are that it is time consuming, costly and might alter the permeation profile of the drug. In this study, tritiated water (^3H_2O) was used as a rapid and inexpensive method to determine the tissue barrier integrity of porcine ocular tissues. 100µl of ^3H_2O (14.8 KBq) was applied to porcine ocular tissue (3 layers consisting of sclera, chroid and retina) mounted on a Franz diffusion cell. ^3H_2O permeation was measured over 15, 30, 60, 120, 180 and 240 min before it was removed and 1 ml of Triamcinolone Acetonide (TA) was applied as a model drug. The permeation of TA over a 28 hour period was measured and the results correlated with tritium permeation at each time point. The best correlation was found after 15 min exposure to ^3H_2O when R^2= 0.91(n=12). Tissue exposure to ^3H_2O for more than 15 min was not discriminative between compromised and non-compromised tissue, probably due to the high permeability of ^3H_2O through the ocular tissue. In conclusion, ^3H_2O permeation over 15 minutes can be potentially exploited as a rapid screening test to determine ocular tissue integrity prior to time consuming drug permeation studies. It may also be used as a method to normalise drug flux based on inherent variability between tissue samples thus increasing accuracy of the *in-vitro* model.

DIRECTLY COMPRESSED ORALLY DISINTEGRATING TABLETS FOR PAEDIATRIC DRUG DELIVERY: A NOVEL HEAT-COOL PROCESS STRATEGY

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Orally disintegrating tablets (ODTs) are gaining an increasing attention from healthcare professionals as a drug delivery system with a potential to overcome swallowing difficulties in specific target patient populations such as paediatrics. ODTs offer many advantages including ease of handling (as they exist as tablets), dose uniformity and their conversion into liquid upon contact with saliva. This study aims to develop an ODT base using a novel heat-cool blending process and consequent tableting by direct compression. An investigation was carried out to assess the suitability of α-tocopheryl polyethylene glycol succinate (TPGS) as a binder (due to cohesiveness) and disintegrant (melts at body temperature 37°C) for ODTs.

Powders comprising different concentrations of D-mannitol (89% - 97%) and TPGS (2% - 10%) were blended for 5 min before blending with magnesium stearate (1%) for further 1 min. A heating step was introduced during blending of powders using the same concentrations and mixing procedure. Tablets (500 mg, 13 mm in diameter) were prepared from each blend at 15 KN followed by assessment of hardness, disintegration time and friability. D-mannitol was sieved to two fractions of 63 µm and < 53 µm in particle size followed by blending each fraction with TPGS and magnesium stearate then pressed into tablets to investigate the influence of particle size on tablet properties. Although hardness was not influenced, results showed a positive impact on tablet friability upon introducing a heating step during blending possibly due to improved blending homogeneity and uniform distribution of TPGS. Friability decreased from 2.73% and 3.16% to 1.74% and 1.77% for blends made from < 53 µm and 63 µm respectively. Disintegration time was dependant on particle size of D-mannitol when tablets were produced by heat approach in contrast to cool approach where disintegration depended both on particle size of D-mannitol and on TPGS.

EXTENDED RELEASE MEDICATED MUCOADHESIVE TOOTHPASTE: AN ATTEMPT TO TREAT PERIDONTAL INFECTION

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Periodontal disease refers to a group of problems that arise in the sulcus, the crevice between the gum and the tooth. Present work, it was planned to prepare therapeutic medicated dental pastes for treatment of periodontitis (dental inflammation) and pain which provide effect for extended period of time. Stress was given for local action of the drug and improve mucoadhesive characteristics of the prepared formulations by adding required amount of mucoadhesive polymers. Diclofenac sodium and nimesulide was chosen as an analgesic and anti-inflammatory drug due to safety, low toxicity and high efficacy. Pastes were comprising of stabilizer, mucoretention/ muco-adhesive polymers such as methyl cellulose, hydroxy ethyl cellulose and carboxy methyl cellulose sodium and subjected for various physicochemical parameters like pH, spreadability, extrudability, drug content, viscosity, mucoadhesive study and IR studies. *In vitro* drug release studies was carried out in phosphate buffer pH 6.4. Stability studies were also conducted at temperature 45±2ºC and 5ºC. During physicochemical studies significant results were obtained and the *in vitro* drug release in pastes were found to exhibit extended release with good adhesion to the oral mucosa revealing more retention time in mouth. The present study envisage that the prepared mucoadhesive dental paste formulations with prolonged retention time in oral cavity will be useful than mouth rinses, gels and tooth pastes which have very short retention time.

PROLIPOSOME FORMULATIONS FOR GENERATION OF IMMUNOGLOBULIN G LIPOSOMES

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Liposomes are well-established biodegradable drug carriers and a range of anticancer and antimicrobial liposome formulations have been approved worldwide for clinical use. Unfortunately, the number of clinically approved liposome formulations is limited because of the instability of liposome phospholipids in aqueous media. Various approaches have been employed to stabilize liposomes; one of these is freeze drying of liposomes (1). Alternatively, proliposomes are stable phospholipid formulations that generate liposomes upon addition of aqueous phase (2,3). Liposomes entrapping Immunoglobulin G (IgG) were prepared using thin film hydration, ethanol-based proliposomes and particulate-based proliposomes. Different concentrations of IgG were incorporated into the formulations and the effect of IgG concentration on the entrapment efficiency was studied.

Soya phosphatidylcholine: cholesterol (2:1) multilamellar vesicles (MLVs) were prepared using the thin film method. Other liposomes were generated from ethanol-based proliposomes and particulate based proliposomes as adapted from Perrett et al. (2) and Payne et al. (3) respectively. The IgG used was a human Immune Globulin Intravenous (IVIG) solution provided by Instituto Grifols (Spain). The Entrapment efficiency of IgG was determined via high performance liquid chromatography (HPLC) using a size exclusion chromatography (SEC) column.

Results have shown an inverse relationship between IgG concentration and its entrapment efficiency for the three different methods. Moreover, the trend of encapsulation efficiency was the same for the different concentrations of IgG, and was as follows: ethanol-based proliposomes > particulate-based proliposomes > thin film method.

Overall, this study has demonstrated the relationship between protein concentration and its entrapment efficiency using different liposome preparation methods. Moreover, these results have confirmed the validity of the different liposomal methods in entrapping IgG, and the superiority of the proliposome methods over the conventional thin film method in entrapping IgG especially when the ethanol-based proliposome approach was selected.

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THE ART OF MINIATURISATION: A MICROFLUIDICS APPROACH TO THE DESIGN AND DELIVERY OF NANOPARTICLE DRUG FORMULATIONS

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The formulation of nanoparticulate drug delivery systems requires complex post-processing steps to ensure that the appropriate size formulations are produced. Whilst there have been many successes in this approach, the rapid and fast screening of novel drug formulations requires a high throughput production systems. We describe a rapid and reproducible method of preparing liposomal formulation and monitoring release through novel microfluidics and microdialysis based methodologies.

Egg phosphatidylcholine-cholesterol (EggPC/chol) liposomes were prepared using hydrodynamic flow focussing (HFF) on a glass Y-channel microfluidic chip under laminar flow conditions. EggPC/chol (6:1) in an ethanolic solution and rhoadmine-123 (r123) (a fluorescent marker for P-glycoprotein-mediated drug efflux at cellular membranes) in phosphate buffered saline (PBS) (X1) were loaded into 5mL gas-tight syringes connected to syringe pumps. The microfluidic chip was immersed in a sonicator bath and lipid and buffer solutions injected onto the chip through opposing channels under sonication. Resulting liposomes were collected, centrifuged and resuspended in PBS (pH 7.4) before determining entrapment efficiency (70-80%). Dynamic light scattering and zeta-potential confirmed population size distribution of < 100 nm. Release of r123 from liposomes was characterised by in-vitro microdialysis. An in-house microdialysis probe (cut-off 12kDa) was developed and calibrated (19% recovery) prior to use. A glass vial containing 5 mLs of resuspended r123-entrapped liposomes was maintained at 37 °C. A probe was inserted into the vial and perfused with PBS at a flow-rate of 5µL/min. Micro-dialysate was collected over 36 hours and release-rate determined from high-performance liquid chromatography (HPLC) analysis of free r123 in the micro-dialysate samples.

Hydrodynamic flow focussing coupled with microdialysis is an efficient and high-throughput mechanism to develop and assess the in-vitro release of entrapped drugs from nanoparticulate delivery system. The methodology described herein will allow rapid on-line production of nanoparticulate delivery systems and in-vitro/in-vivo pharmacokinetic assessment in target cellular tissue types.

DRUG DELIVERY TO THE CENTRAL NERVOUS SYSTEM: QUALITY BY DESIGN THROUGH PHARMACOKINETIC MODELLING

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Effective quality by design (QbD), when implemented during any drug development process, should consistently ensure the maintenance of science and quality risk management to meet patient needs. The key to any successful implementation of QbD process is the translation of *in-vitro* observations to *in-vivo* performance.

The assessment of dose response *in-vitro* and *in-vivo* (lower species) relationships for central nervous system (CNS) targeted drugs, provides valuable information about the pharmacokinetics and pharmacodynamics *in-vivo*. Nanoparticle drug delivery systems provide an opportunity to specifically target and deliver therapeutic compounds and biologics to the CNS, with minimal biodistribution thereby optimising therapeutics regiments. However, targeted drug delivery to the CNS remains challenging with little consideration of the *in-vivo* performance of a delivery vehicle.
Coupling molecular modelling (early discovery) with physiologically-based pharmacokinetic modelling (pre-clinical and clinical) is a useful approach to link drug properties to *in-vivo* performance. In particular, physiologically based biodistribution models are useful tools and provide a well-validated approach to the extrapolation of *in-vivo* performance across species and provides a quantitative assessment of the extent of absorption, distribution, metabolism and elimination of compounds and vehicles.

This poster explores the design and optimisation of potential candidate compounds (and drug delivery vehicles) during drug development. The approach illustrates how pharmacokinetic modelling and simulations can integrate prior knowledge in a mechanistic network, and ultimately carry out target site identification, drug binding, biopharmaceutics and pharmacokinetic *in-vivo* distributional patterns followed by interspecies extrapolation to estimate a clinical drug concentrations.

CHARACTERISATION OF THE INCLUSION COMPLEXES OF LANSOPRAZOLE WITH CYCLODEXTRIN AND METHYLATED CYCLODEXTRIN

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Lansoprazole is a proton pump inhibitor used for gastric acid suppression. The compound shows poor solubility and acid instability; the degradation half-life is 0.5 hour at pH 5 and 18 hours at pH 7 (at 25°C) (Ekype et al., 1999). For effective novel buccal drug delivery formulations the drug must be released at a rate and extent that facilitates buccal absorption (Conway, 2007). Cyclodextrins (CD) are cyclic oligosaccharides with a hydrophilic outer surface and a central hydrophobic cavity allowing inclusion formation with guest molecules. Complexation has been applied to improve physicochemical properties of active pharmaceuticals including stability, solubility and permeability (Figueiras et al., 2009). Lansoprazole inclusion complexes were prepared with β-cyclodextrin (βCD) and methylated- β-cyclodextrin (MβCD) at different 1, 1:1 and 1:3 ratios in an alkaline hydroalcoholic solution and freeze dried. Their behavior was studied using Phase solubility, Fourier Transformation-Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC) and X-Ray Diffractometry (XRD) to characterise complex formation.

Solubility profiling, in phosphate buffer at pH 6.1 (representing saliva), resulted in an increase in solubility in all CD complexes when compared to lansoprazole alone. A 15 fold increase was observed for 1:1 lansoprazole:MβCD samples. FTIR showed decreased band intensities at 1578, 1476 & 1401 cm-1 suggesting complexation. DSC data identified characteristic peaks, particularly a sharp endothermic peak at 182.3°C, corresponding to the melting point of lansoprazole (Kristl et al., 2000). This was absent from all thermograms of the CD complexes, indicating successful inclusion of the drug. XRD data showed a change in the crystallinity of the drug on MβCD samples suggesting further evidence of the formation of an inclusion complex. The results suggested that true inclusion complexes were formed and that a 1:1 lansoprazole:MβCD complex may be suitable for development of a buccal drug delivery system in novel formulations.

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FORMULATION DESIGN – CONTROLLED RELEASE OF A GLP-1 ANALOGUE FROM PLGA MICROSPHERES

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Type II diabetes affects 2.8 million people in the UK, with an estimated further 1 million undiagnosed suffers. Type II diabetics struggle to control their blood sugar levels and therefore many currently have to inject at least twice daily. Consequently, there is a need for a dosage form that can deliver therapies such as a GLP-1 analogue over an extended period.

Research has shown that controlled release of GLP-1 analogues from PLGA matrices has been problematic. High burst release, low drug loading and unfavourable release rates are common problems that are encountered, the latter being critical as nausea and vomiting are well documented side effects of GLP-1 analogues.

Using Q Chip Ltd proprietary microencapsulation platform (Q Sphera), a GLP-1 analogue has been encapsulated within a PLGA matrix. This platform has engineered out class 2 solvents, extremes of temperatures and lengthy post processing procedures; whilst enabling continuous production and a high throughput of monodisperse microspheres that have high drug loadings and encapsulation efficiencies.

Drug release profiles were assessed *in vitro* and analysed using micro BCA assay and HPLC. Characterisation by SEM and light microscopy show that monodisperse spheres within size range of 30-50µm can be manufactured with <5%CoV. Investigations found that microspheres suspended at 60mg/mL could be delivered via a 27G 'pain free' needle.

We have developed GLP-1 analogue formulations with drug loading >10% that deliver a controlled burst release *in vitro* of less than 6µg/mg in 24hrs. The microspheres then deliver zero order sustained release for 5 wks at a therapeutic concentration. Varying the polymer composition, sphere morphology and process conditions leads to the extension of the release profile to 8 weeks while not having a deleterious effect on either the drug loading or zero order rate release kinetics.

INTERACTION OF NANOPARTICLES WITH EPITHELIAL CELLS: PARTICLE PROPERTIES, INFLUENCING TOXICITY, CELL UPTAKE AND TRANSPORT

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The interaction of nanoparticles with cells has been under extensive interest during recent years because they can play a vital role in gene and drug delivery. The work we present involves the interaction of nanoparticles with epithelial cells (Caco-2 cells). *In vitro* experiments are focused on the physicochemical properties of nanoparticles, cytotoxicity and several factors such as duration of incubation, particle size, particle concentration, chemical surface properties and temperature and how these factors can influence cellular uptake and transport. Different sizes and concentrations of aminated polystyrene nanoparticles (50, 100, 200 nm) were used in this study. The size and zeta potential of nanoparticles in biological buffer were determined using dynamic light scattering and zetasizing. Toxicity studies of 50 and 100 nm nanoparticles were also investigated by using MTS assay (can measure metabolic function), LDH assay (measure the membrane integrity as a function of the amount of cytopasmic LDH released into the medium) and TEER. Higher concentrations of 50 and 100 nm aminated polystyrene nanoparticles can induce higher cellular toxicity when compared to lower concentrations.

Uptake and transport polystyrene nanoparticles (50, 100, 200 nm; aminated nanoparticles {positively charged} and 100 nm carboxylated nanoparticles {negatively charged}) across Caco-2 cells was also investigated. This work found that cellular uptake and transport was increased by increasing the concentration of nanoparticles (100 µg/ml > 50 µg/ml > 25 µg/ml), decreasing the diameter of aminated nanoparticles (50 nm > 100 nm > 200 nm) and increasing the temperature to 37°C. In this study we found the uptake of nanoparticles was decreased (60%) at 4°C which suggests that the uptake of these particles through Caco-2 cells can be energy dependent endocytic process. Furthermore, uptake and transport studies across Caco-2 cells were also conducted to investigate the effect of two different functional groups at the surface of polystyrene nanoparticles. Uptake and transport of 100 nm aminated and carboxylated polystyrene nanoparticles during 4 hours (100 µg/ml) was compared and we found that carboxylated nanoparticles experience a higher degree of repulsion from the negative cell membrane and can have a higher transport across the cells compared to aminated nanoparticles. This may be due to aminated nanoparticles (positively charged) becoming entrapped at the cell surface and undergo higher uptake, rather than transport across the cells than negatively charged particles. We propose this occurs through electrostatic interactions.

These findings create a field which explores surface modifications of nanoparticles and how these can be used to assess new formulations for oral administration of therapeutic proteins or peptides.

NEW FORMULATIONS FOR ORAL DELIVERY OF AMBIENT DRIED LIVE BACTERIAL CELLS

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Live bacterial cells (LBC) used as live vaccines are attenuated bacterial pathogens that are able to replicate in the host, mimicking the route of entry of natural infection and thereby promoting protective immune responses. Genetic engineering can design LBC that induce an immune response to both the attenuated strain but also to a carried heterologous antigens, thus protecting against a wide range of infections. A new formulation of LBC would offer the potential for oral administration, maintaining stability without refrigeration, with a low-cost, simple, manufacturing process. Therefore, it is so attractive to the world health and vaccination program, to the distribution chain in less developed countries and to economic industrial mass-production processes.

Preservation of microorganisms by desiccation is required for long term storage. Dehydration can damage cells through osmotic and oxidative stress and denaturation of biomolecules. The aim of this study is to improve current technologies, producing a stable oral formulation of live bacteria that maximizes cell survival and retains metabolic activity by drying at ambient temperature. Our goal is to provide a thermo-stable formulation that enables storage at ambient temperature avoiding freeze drying.

Specific project objectives relate to the thermal drying process, use of a polymer carrier and protective agents to produce an oral formulation of live bacteria with maximum cell viability and stability. Strain dependent optimization of the pre-drying conditions, drying process and post-drying conditions is essential. Preliminary data focus on a set of experiments carried out to evaluate cell survival during a dehydration process using room temperature drying. These experiments were performed using a probiotic model bacterial strain dried onto various pharmaceutical grade surfaces and mixed in different protective agents, evaluating the survival ratio and stability for the different conditions.

INVESTIGATION INTO THE EFFECT OF DIFFERENT PHYSIOCHEMICAL PARAMETERS OF DRUGS ON RELEASE FROM POLY(2-HYDROXYETHYL METHACRYLATE) USING REGRESSION ANALYSIS

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A major drive currently within the pharmaceutical industry is to develop new and improved delivery systems in order to release drugs in a controlled manner. An example of this is the release of drugs from cross-linked polymer networks known as hydrogels. However, in order to control release it is important to consider the properties of the drug being eluted and not just those of the delivery system. In many research papers drugs appear to be selected on an arbitrary basis with reference to the drugs character as either hydrophilic or lipophilic. The influences of the physiochemical parameters that contribute to this overall character are often overlooked. In 1997, Lipinski developed an approach for estimating solubility and permeability of drugs based on different parameters known as the "Rule-of-five". Lipinski's methodical approach inspired this study and was applied to drug release by disentangling a drug's character into its various parameters, and observing each variable's ability to influence release(1).

Using poly(2-hydroxyethyl methacrylate) with 1%w/w EGDMA as the cross-linker, 13 drugs with varying physiochemical parameters were selected and their release profiles plotted. The time taken for the hydrogel to elute 50% of the total imbibed drug was calculated from each profile using the Power law model(2). Multiple linear regression analysis of the data collated was performed in order to determine the linear relationship of the explanatory variables (physiochemical parameters) and how they correlate maximally with the outcome variable (50% release time)(3).

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PRODUCTION OF HYDROGEN BONDED LAYER-BY-LAYER COATINGS FOR ENTERIC RELEASE OF PHARMACEUTICALS

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The aim this work is to produce an appropriate enteric coating that is able to deliver pharmaceuticals to a predetermined area of the gastro-intestinal tract. A Layer-by-layer approach has been selected as an attractive method to achieve this due to its tailorability and relative simplicity of production.

Layer-by-layer complexes are formed by alternately depositing two or more complementary polymers in solution, onto a solid surface. A range of molecular interactions can be taken advantage of, including ionic, covalent and hydrophobic effects. This work specifically looks at hydrogen bonding. The formation of alternating polymer layers can be carried out on a range of surfaces with applications in the fields of drug delivery, biomedicine, materials science and biosensors making it a valuable technique.

A hydrogen bonded system has been chosen due to its ability to form and dissociate under different pHs. Two appropriate polymers have been selected namely, poly(acrylic acid) (PAA) and methylcellulose (MC), due to their lack of toxicity and capability to form hydrogen bonds. At acidic pHs the polymers remain in their unionised state and so are able to hydrogen bond, forming the coating. An increase in pH causes ionisation of the carboxylic acid groups of the PAA, preventing hydrogen bonding from occurring and so initiates a breakdown of the complex. For an effective formulation these pHs need to be related to those found in the gastro-intestinal, previous work has already found the coating to be stable at solution pHs resembling the stomach.

The aim of this specific study is to analyse the dissolution of the multilayers to assess their suitability as an enteric coating. The target pH of dissolution is 4-5 relating to proximal sections of the small intestine. This release would act to deliver drugs to either have a local action in the small intestine or to protect acid sensitive drugs from the harsh environment of the stomach.

SUSTAINED DELIVERY OF ACTIVE BMP-2 FROM PLGA MICROSPHERES FOR BONE REGENERATION APPLICATIONS

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Abbreviations:
BMP-2: bone morphogenetic protein-2, PLGA: poly(D,L lactic co-glycolic acid), PEG: polyethylene glycol, HSA: human serum albumin, SEM: scanning electron microscopy, PBS: phosphate buffered saline, pNPP: para-nitrophenylphosphate, DMEM: Dulbeccos modification of Eagles medium, FCS: foetal calf serum.

We aimed to create BMP-2 loaded microparticles having control over both size and in-vitro release profile. We wanted to demonstrate that BMP-2 remained active during microparticle fabrication and after subsequent release. To this end, PLGA microparticles were specially formulated to incorporate a triblock copolymer to achieve sustained release. The response of C2C12 myoblast cells to BMP-2 causing them to express alkaline phosphatase was used as a marker for BMP-2 activity.

Microspheres were fabricated from PLGA (lactide:glycolyde 50:50) formulated with 10% PLGA-PEG-PLGA triblock copolymer and loaded with BMP-2 with HSA as a carrier. Microspheres were sized by laser diffraction and imaged by SEM before being suspended in PBS and incubated, rocking, at 37oC. PBS was replaced regularly for construction of a release profile by total protein measurement (bicinchoninic acid assay). Releasate samples were diluted 1:1 in DMEM (10% FCS) for C2C12 cell culture where any BMP-2 activity would be detected by a pNPP assay to detect alkaline phosphatase. These microparticles were also co-cultured with C2C12 cells determine the direct effect of them on the cells.

The microspheres were loaded with 1% (w/w) total protein (HSA: BMP-2 ratio 95: 5) and were 20-30 micron in diameter. Entrapment efficiencies were in the order of 51-76%. The addition of the triblock copolymer resulted sustained release of total protein over 30 days which equated to a clinically relevant dose of BMP-2. BMP-2 activity could be detected in the releasates up to day 13. Co-culture resulted in a linear dose response of microparticle mass against BMP-2 activity and there were associated changes in cell morphology.

We have demonstrated that biodegradable microparticles can deliver active BMP-2 at a sustained rate. We hope to use these microparticles in ex-vivo work using the embryonic chick femur model to investigate developmental control and defect repair.

FORMULATION, OPTIMIZATION AND EVALUATION OF DIMENHYDRINATE MEDICATED CHEWING GUM

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The purpose of the present research work is to formulate & optimize chewing gum containing an antiemetic agent for treatment of motion sickness using different techniques. To evaluate chewing gum for physicochemical properties, drug content, *In-vitro release*, in vivo study and stability study.

In present work the bitter taste of Dimenhydrinate was masked by using ion exchange resin.For that Tulsion 335, 339, 344. Kyron 134, Amberlite 88 used as ion exchange resin. The drug resin comlex was optimized for drug content, drug loading, effect of P^H, stirring speed, temperature and taste pannel evaluation.Then best optimized drug resin complex used for further study.The excipients used in formulation of medicated chewing gum include pharmagum M, xylitol, Mint Flavor, Neosorb, Aerosil, Magnesium stearate, Talc etc. The drug excipient compatibility study was done by using the DSC & FTIR. The medicated chewing gum containing Dimenhydrinate resin complex was formulated by using direct compression method and Optimization was by using stasease software. The formulations were characterized for IPQC test, in vitro drug release, chew out study and in vivo buccal absorpation study. The vitro drug release was determined by using chewing gum apparatus which is developed in R.C. patel college of pharmacy (Indian patent no 1355/MUM/2008). The PCP Disso software was used to determine drug release pattern and finally stability study was performed according to ICH guidelines.

Tulsion335 ion exchange resin was found to be most suitable resin. The DSC & FTIR shows that selected excipient was compatible with Dimenhydrinate. It is seen that as the concentration of the gum base is increased in the formulation, the drug release was decreased. The best optimized formulation shows that 90% drug release within 25 min and drug release profile follow the matrix pattern. The buccal absorption and chew out study indicate that maximum 70% drug get absorbed through the buccal. The 90 day's stability study shows that formulation was the most stable & has good in vitro release.

From above study it concludes that by changing the concentration of gum base in the formulation, the drug release can be controlled. Due to more buccal absorption the first pass metabolism is avoided and formulation is most suitable for the treatment of motion sickness.

POWDER FLOW PROPERTIES OF BULK LYOPHILISED FORMULATIONS

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Bulk freeze dried Active Pharmaceutical Ingredients (API's) have seen emerging automated aseptic lyophilisers for fill to finish operations developed. Implications of flow behaviour across the production line during processing or conveying of powders to dosing systems needs addressing. Tablets of freeze dried powder have been proposed, requiring uniform hopper feeding and reproducible filling of dies. Maintenance of activity would also be seen as a priority. This study has investigated powder flow properties of lyophilised mannitol and sucrose solutions as potential carrier agents for bulk freeze dried proteins. Five batches of lyophilised mannitol and sucrose powder were produced from solutions (1, 3, 5, 10 and 15% w/v) using a lyophilisation cycle consisting of -30℃ primary drying (32 hr), -20℃ secondary drying (4 hr), -75℃ condenser and under a constant vacuum of 20 Pascal. Powder flow and other properties were assessed using angle of repose, compressibility index, Hausners ratio, Karl Fischer, helium pycnometry, light microscopy and thermal analysis. Flow of the produced powder was by pharmacopeial definition very poor; with a significant positive correlation with the increase in solute concentration of the lyophilised solution. Significant* differences in both compressibility index and Hausners ratio were noted across the powder range, however angle of repose measurements conflicted with the flow when assessed through other methods. Lyophilisation was concluded to confer poor flow properties to mannitol and sucrose powders; however increases of the solute concentration of the initial feed solution did improve flow of the final product.

* $p < 0.0001$ One Way Anova.

PRONIOSOME AS AN APPROACH TO MANUFACTURING RESPIRABLE NIOSOMES FOR PULMONARY DELIVERY USING NEBULIZERS

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Pulmonary delivery of liposomes via nebulization is a well-established for prolonging the residence of liposome-entrapped drugs in the lung. However, the instability of liposomes in aqueous dispersions is a considerable problem. In this study we developed stable beclometasone dipropionate (BDP) proniosomes for generating niosome aerosols via nebulization using the air-jet nebulizer Pari LC Sprint and the vibrating-mesh nebulizers Aeroneb Pro and Omron MicroAir.

Span 60 and cholesterol (1:1) with BDP (5 mole%) were dissolved in chloroform and sprayed over sucrose particles (300-500 µm) in a rotary evaporator. Evaporation of chloroform resulted in formation of proniosomes which were hydrated with HPLC grade water (40°C) followed by hand shaking and sonication. Size and zeta potential of niosomes were anlayzed using photon correlation spectroscopy and electrophoretic mobility respectively. Entrapment efficiency (EE) of BDP was determined after separation of the unentrapped drug using 0.45µm cellulose acetate filters. Following nebulization, aerosol output was determined gravimetrically and drug output was analysed by UV-spectrophotometry. Aerosol droplet size and expected pulmonary deposition were determined using laser diffraction.

Niosomes were negatively charged (-36.2 ±3.3 mV) and in the nano-size range (202.5 ±9.5 nm) and the EE of BDP in niosomes was approximately 35%. Drug output using the Pari Sprint or Aeroneb Pro devices was approximately 80% whilst for the Omron it was around 70%. The volume median diameter was 3.06 ±0.15, 3.32 ±0.17 and 4.86 ±0.16 µm for the Pari Sprint, Aeroneb Pro and Omron respectively. The calculated "fine particle fraction" was similar for the Pari Sprint and the Aeroneb Pro devices (approximately 75%) whilst for the Omron nebulizer it was around 40%.
Overall, this study has shown that niosomes generated from proniosomes are "respirable" when aerosolized using air-jet or vibrating-mesh nebulizers.

ION PAIRING A BCS IV MODEL DRUG WITH AMINO ACIDS AS SOLUBILITY AND PERMEABILITY ENHANCERS.

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One of the biggest challenges that face the formulation scientists nowadays is the poor drug absorption across the intestinal membrane. Such challenges arise after the introduction of high through-put screening into the drug discovery cycle.

Many physiological factors such as pH, food content and surface area of the small intestine affect drug absorption. Besides the physiological factors, other physicochemical factors like particle size, lipophilicity, polymorphism and dissolution rate are believed to play a major role in drug absorption. The aim of this study was to investigate the effect of amino acids as a salt former to form new salts with a BCS IV model drug; trimethoprim (TMP) and study the effect of amino acid concentration on TMP permeability across caco-2 monolayer. In order to prepare new salts of the basic drug, two acidic amino acids namely; aspartic acid and glutamic acid were used for this study. Octanol/water partitioning study was used to evaluate the binding constant between the amino acid and TMP while caco-2 monolayer was used to evaluate the permeability profile of the drug, salt form and physical mix between the drug and excess amino acid.

Binding constant (K11Aq) of 2.72 M-1 was obtained for TMP upon using quasi-equilibrium transport model to double reciprocal plots of octanol-water distribution coefficient versus aspartic acid counter ion concentration. Interestingly, the apparent permeability (Papp) of TMP significantly increased upon salt formation and upon using excess amounts of the counter ion. Nevertheless, adding aspartic acid more than 4 times (x4) the molar concentration of TMP showed a significant drop in Papp and even became less than that of the free drug.

TMP is an actively transported drug across the intestinal membrane, therefore our future work will investigate whether ion pairing the drug with the amino acids would affect the gene profiling of caco-2 cells and whether it would affect the mechanism of absorption of TMP.

INSULIN LOADED CHITOSAN MICROPARTICLES FOR ORAL DELIVERY: PREPARATION AND OPTIMISATION STUDIES

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Around 346 million people across the globe currently suffer from diabetes. Insulin therapy via subcutaneous injections has poor patient compliance. Investigation into alternative non-invasiveadministration routes has gained prominence. The aim of the current study is to develop amicroparticulate system for effective oral delivery of insulin.
Chitosan-based microparticles were prepared by emulsification cross-linking technique [1,2]. Four batches with variable chitosan and glutaraldehyde concentrations were prepared i.e. 2%v/v and 4%v/v glutaraldehyde was added to Batch A & B and Batch C & D respectively, along with 1%w/v and 2%w/v chitosan in Batch A & B and Batch C & D respectively. Morphology, particle size, zeta potential and swelling index were analysed. Insulin was loaded onto microparticles via passive absorption technique. The insulin protection efficiency of microparticles against gastrointestinal enzymes was investigated. In-vitro release studies were performed in simulated-gastric (SGF; pH 2) and simulated-intestinal fluid (SIF; pH 6.8). [1, 2]

Micrographs from scanning electron microscopy depicted spherical microparticles. Particle mean diameter ranged between 54.45±0.43µm and 103.55±0.27µm. Zeta potential of the microparticles ranged between 8.73±0.59mV and 10.23±0.51mV which indicates incipient instability. This finding, however, is in agreement with the fact that chitosan is cationic in nature. Loading efficiency and loading capacity of insulin onto chitosan microparticles increased with an increase in chitosan concentration. Loading efficiency and loading capacity of insulin for batch A was shown to be 80.4±0.69% and 40.23±0.69% respectively while for batch C, they were 84.1±0.21% and 42.05±0.21% respectively. Protection of insulin from enzymatic degradation yielded inconclusive results. In-vitro release studies showed that only 3.21±0.01% and 3.11±0.14% of encapsulated insulin was released in the first two hours of its presence in the SGF, compared to a 16.04±0.01% and 16.87±0.06% insulin release in the first two hours in SIF for batches A and C respectively. Based on the preliminary data, chitosan microparticles of Batch C demonstrated more potential especially in loading efficiency and insulin release. Further evaluation studies ongoing.

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POLYMERIC VESICLES FOR ANTIBACTERIAL THERAPY

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Synthetic polymers can be very powerful tools in the field of bacterial infection. For instance, synthetic polymers have been shown to bind and sequester bacteria via multivalent ligand display,while at the same time avoiding selection pressure and resistance invoked in bacteria by antibiotics.1 Less explored has been the possibility of applying synthetic polymers to other mechanisms of bacterial infection. For instance, bacteria use sophisticated cell-cell communication systems such as Quorum Sensing (QS), which allow them to synchronise genetic reprogramming at the population level.2-4 Inhibition of QS is itself a potential further method of controlling bacterial infection.5 We have recently described the synthesis of novel linear polymers with the ability to both bind to the surface of Vibrio Harveyi while at the same time interfere with its AI-2 signalling network.6 These new materials open the pathway to the development of novel polymeric 'dualaction' antibacterial therapies. In this communication we wish to present our current efforts, involving synthetic chemistry, and biological assays, towards the development of polymeric vesicles with similar dual action properties and the potential to deliver standard antibiotics.

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MODIFIED SILICONE ELASTOMER VAGINAL GELS FOR SUSTAINED RELEASE OF ANTIRETROVIRAL MICROBICIDES

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Silicone elastomer gels (SEGs) are commonly used as medical device lubricants, personal (sexual) lubricants, and in a wide range of cosmetic applications. They are also being developed pharmaceutically for topical applications in an attempt to improve bioavailability and aesthetics. Recently, we demonstrated, enhanced and sustainable release of the HIV entry inhibitor maraviroc (MVC), over 48h from SEGs following vaginal administration in rhesus macaques compared with a 2.2% HEC gel. SEGs are very lipophilic, consisting of a lightly cross-linked silicone elastomer and cyclomethicone. In this study, we have evaluated second generation SEGs wherein the cyclomethicone component has been replaced with a low MW hydroxyl terminated polydimethylsiloxane. We hypothesized that these more hydrophilic HSEGs may improve the gel solubility of HIV microbicide candidates leading to enhanced drug release rates.

Continuous flow rheology was performed on HSEGs prepared with different MW hydroxyl terminated PDMSs (h-PDMS). *In vitro* release testing was performed on viscosity matched gels containing either 5% w/w MVC or FTC in both simulated vaginal fluid (SVF) or a solvent/water system. Solubility of MVC and FTC was determined in different MW h-PDMSs. Aqueous ingression studies of placebo HSEGs and SEGs were performed using a methylene blue solution over 48h.

The viscosity of HSEGs increased exponentially with ST-Elastomer 10 concentration. HSEG gel compositions that were viscosity-matched to the 80/20 SEG and 2.2% HEC gel (~50Pa.S) were chosen for *in vitro* release testing and water ingression studies. MVC and FTC release was significantly increased in both media from DMSS12 (low MW) HSEGs after 24h (MVC; 18mg (SVF), 21mg (IPA/water), FTC; 4mg (SVF), 14mg (IPA/water)) compared to the SEG (MVC; 3 mg (SVF), 12 mg (IPA/water), FTC; 2 mg (SVF), 9mg (IPA/water)). Release from high MW HSEGs was comparable to SEG in both media. Solubility of MVC and FTC in DMSS12 h-PDMS (MVC; 40mg/mL, FTC; 0.4mg/mL) was significantly greater than cyclomethicone (MVC; <5µg/mL, FTC; <1.1µg/mL). Aqueous ingression was observed by 6h for DMSS12 HSEG compared with no ingression with SEG and DMSS51 (high MW) HSEG.

The studies demonstrated that SEG hydrophilicity can be readily modified by substituting the cyclomethicone component with low MW h-PDMS. Increasing the number of hydroxyl groups significantly enhanced release and solubility of both model hydrophobic (MVC) and hydrophilic (FTC) microbicide compounds compared to the conventional SEG. The results highlight the potential of these second generation SEGs for vaginal delivery of HIV microbicides.

EFFECT OF HYPROMELLOSE SUBSTITUTION LEVELS ON SWELLING AND DISSOLUTION RATE OF FLURBIPROFEN MATRIX TABLETS

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Hypromellose, HPMC, is a hydrophilic polymer widely used for the preparation of hydrophilic tablet matrices. It is a partly O-methylated and O-(2-hydroxypropylated) cellulose and the degree and pattern of substitution groups will have an impact on the compaction and release properties of the formulation. It tends to swell in liquid media and form a gel layer barrier which retards the diffusion of the drug from the matrix network.

Flurbiprofen (Aesica, UK) was compressed with HPMC at 5%, 10% and 15% w/w (Methocel F4M Premium, E4M Premium & K4M Premium, Dow Chemical Co). The 13 mm compacts (300±3 mg) were fixed in PTFE holders [1]. Dissolution studies were carried out in phosphate buffer (900 mL, pH 7.2) using USP apparatus 2 at 75 rpm and 37°C, (n=3). Dissolution rate was determined from the gradient of release profiles using UV analysis at 247 nm. Swelling was determined by following the change in weight over time [2].

K4M Premium (2208) has a higher percentage of hydroxypropoxyl substitution and was associated with higher hydration rate of the polymer but a slow dissolution rate of flurbiprofen. This is due to the hydrophilic nature of hydroxypropoxyl groups which have the ability to form a gel layer more quickly than methoxy substituted groups, which are relatively hydrophobic in nature. The flurbiprofen release rate from matrix tablets containing F4M (2906) was quick but the matrix had a slow hydration rate in comparison to other HPMC variants, i.e. E4M (2910) and K4M (2208).

The release of a poorly water soluble drug from HPMC matrices is affected by the different substitution pattern.

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UTILISING MONTMORILLONITE K-10 AND LAPONITE FOR DELIVERY OF CIPROFLOXACIN

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Clays have been used since prehistoric times to heal wounds. Today they are also utilised as pharmaceutical excipients. Clays have negatively charged surfaces allowing adsorption of cations including drug molecules. Other research teams have adsorbed drugs onto clays though few have investigated the subsequent release and applied this to clinical problems. This study investigates new ways of using clays to deliver antibiotics. Adsorption of the antibiotic ciprofloxacin was optimised on montmorillonite K-10 (MMTK10) then applied to two grades of Laponite®. Ciprofloxacin release was measured to determine if modified release was obtained.

One gram MMTK10 was dispersed in 80ml deionised water and swollen for 2 hours, after which ciprofloxacin in 20ml 0.1M HCl was added. The effects of time (1-24 hours), pH (0.05-12.0) and initial ciprofloxacin concentration (0.625-10.00mg/ml) were investigated. Amount of ciprofloxacin adsorbed was determined using UV-Visible spectrophotometry. The mechanism of adsorption was determined through Infrared Spectroscopy and X-Ray Diffraction (XRD). Optimised methodology was applied to Laponites® RD (LRD) and WXFP (LWXFP). Release of ciprofloxacin was measured using UV-Visible spectrophotometry.

Longer adsorption periods yielded only small increases in adsorption. Maximum ciprofloxacin adsorption occurred at pH 8.0 when in its zwitterionic state. Higher concentrations resulted in greater adsorption.Infrared analysis showed the positively charged amino group of ciprofloxacin interacted with the clays, indicating cation exchange as the adsorption mechanism. Increased interlayer spacing, determined through XRD, showed ciprofloxacin was adsorbed between clay layers. MMTK10 adsorbed more ciprofloxacin than either Laponite. However, LWXFP released more ciprofloxacin while LRD released the least.

Ciprofloxacin was successfully adsorbed onto MMTK10. The optimised method used 500mg ciprofloxacin at pH 8.0 for two hours and produced reasonable adsorption on both Laponites®. Ciprofloxacin release was modified, with LWXFP releasing most. The results indicate these clays can be exploited for drug delivery purposes.

IN VITRO PERMEATION STUDY OF THE NOVEL PYRROLOBENZODIAZEPINE DIMER SJG-136 THROUGH A SILICONE MEMBRANE AND HUMAN SKIN

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Pyrrolobenzodiazepine (PBD) compounds were first obtained from *Streptomyces* species, and bind covalently within the minor-groove of DNA in a sequence-selective manner. SJG-136 is a synthetic pyrrolobenzodiazepine dimer in which two DNA-alkylating subunits are linked through an inert propanedioxyl ether. Biophysical and biochemical studies of SJG-136 have shown a remarkable affinity for DNA and potent cytotoxicity *in vitro*, and significant *in-vivo* activities against a panel of tumour xenografts in mice. The molecule successfully completed Phase I clinical trials and has recently entered into a Phase-II trial in ovarian cancer. A Phase II leukaemia trial will start later this year. The aim of this project was to establish the permeability characteristics of SJG-136 through silicone membrane and human skin, with a view to establishing the suitability of this agent for development as a topical agent for the palliative treatment of melanoma.

Studies were performed in DMSO using static, horizontal 'Franz'-type diffusion cells under occlusion. The receptor phase consisted of 20% ethanol in phosphate buffered saline. Known amounts of SJG-136 were applied to the membrane and samples were collected at different time intervals up to 50 hr followed by mass balance studies. The permeation profile of SJG-136 through silicone membrane and human skin indicated that permeation began within one hour. At the end of the study (50 hrs), 7.95±4.35 and 145.03±104.87 $\mu g/cm^2$ of SJG-136 were permeated from silicone and human skin which were 1.61±1.02 and 27.05±19.06% of the applied dose respectively. The mass balance study confirmed that 0.61±0.42 and 2.40±1.53% of the applied dose were present within silicone and human skin respectively.

In conclusion, it was found that the permeation and residence of SJG-136 was 18-times and 4-times greater in human skin compared to silicone membrane. The results suggest that SJG-136 has the potential to be developed as a topical agent against melanoma.

METALLIC HYBRID NANOPARTICLES FOR IMAGE GUIDED DRUG DELIVERY

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Magnetic iron oxide nanoparticles (MNPs) have become widely studied for biomedical applications such as MRI contrast agents. However, increasing safety concerns over the use of polymer coated MNPs has arisen, with Feridex® being withdrawn for use in clinics within the last 6 months. Gold is renowned for its chemical stability, biocompatibility and its unique surface plasmon resonance (SPR) properties. After gold coating MNPs, a rigid shell surrounds the iron oxide preventing degradation and free radical initiation which caused previous concern. Here, the potential of these hybrids as MRI contrast agents and photo thermal nano-heaters will be deduced. Consequently, model drug 6-Thiguanine (6-TG) will be conjugated to the particle surface. 6-TG is used clinically to treat leukaemia. Here the ability of these particles to act as a multifunctional chemotherapy will be eluded.

6-TG was attached to particles via gold-thiol datives bonds. Particles were characterised using ICP-OES, UV-Vis spectrometry, PCS and TEM. The magnetic coercivity and relaxivity was analysed using SQUID analysis and on a 1.5 Tesla clinical MRI. Drug conjugation was determined using UV-Vis spectrometry. Laser irradiation was carried out on samples in agar using a Nd:YAG pulsed laser. *In vitro* cytotoxicity and cellular uptake studies were carried out using BxPC-3.

UV-Vis and TEM confirmed nanoparticle formation with particles being 70 nm. After 6-TG conjugation the hydrodynamic radius increased from 150 nm to 230 nm. The concentration ratio of iron/gold/drug was 3/1/10 were in every 3mg of Fe_3O_4, 1mg of Au and 10 mg of 6-TG was present. SQUID and MRI analysis showed excellent magnetic properties with the T_2 relaxivity being comparable to Feridex®. After laser irradiation, large temperature increases inside short time spans was observed (ΔT=35°C, 90 sec). 6-TG-nanoparticle increased the cellular uptake after 4h and IC_{50} after 24 h compared with free drug, 21-fold and 10-fold respectively. This data highlights the unique potential of nano-hybrids. Further work is on-going to fully exploit the properties of these nanoparticles for thermo responsive drug delivery.

DEVELOPMENT AND CHARACTERIZATION OF ANTI-PARKINSON MUCOADHESIVE SPRAY-DRIED ROPINIROLE MICROSPHERES FOR BRAIN TARGETTING VIA THE NASAL ROUTE

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Nasal drug delivery is very promising, since drugs delivered intranasally can bypass the first pass hepatic effect and be quickly absorbed across the nasal epithelium, offering rapid therapeutic response (1). Moreover, the nasal route has been suggested for brain targeting (2,3). Mucoadhesive microspheres are spherical micro particles made from biocompatible and biodegradable polysaccharides such as chitosan. They are safe and capable of delivering drugs to the nasal cavity and have drawn much interest in recent years. Mucoadhesion is an attractive way of increasing the contact time of the drug with mucosa thus enhancing drug absorption(4).

In this study mucoadhesive microspheres consisting of ropinirole and chitosan glutamate for brain targeting through the nasal route has been developed using spray drying, aiming to improve the treatment of Parkinson and restlessness disease. Microspheres were prepared using the Büchi Mini spray drier B-290 and subsequently characterized in terms of yield, morphology and particle size distribution. The amorphous property of the spray dried microspheres was studied using X-ray diffraction and the loading efficiency of the drug was analysed using high performance liquid chromatography (HPLC). Finally, FTIR was employed to study the drug polymer interaction. Scanning electron microscopy showed that microspheres were spherical and had smooth surfaces. Laser diffraction showed that particle size was directly proportional to chitosan concentration and in the range of 2.38 -3.52 µm which is suitable for nasal deposition. The yields were between 64.66 and 67.8% and drug loading was 101.29 -104.96% depending on formulation. FTIR showed no interaction between the drug and chitosan and X-ray diffraction showed that ropinirole has changed from crystalline to amorphous form.

Overall, this study has shown that spray drying was appropriate to prepare mucoadhesive ropinirole microspheres that could potentially be applicable for nasal delivery.

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THE DEVELOPMENT OF A FLOW THROUGH DISSOLUTION TEST METHOD FOR THE ASSESSMENT OF FLUCLOXACILLIN RELEASE FROM TASTE MASKED MICROSPHERES

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The dissolution of a drug is a key physicochemical parameter that has a major impact on the bioavailability of that compound. A new method, flow through cell (FTC), has proved to be very versatile with advantages of operating pH gradients, achieving sink conditions for sparingly soluble drugs and is appropriate for micro-sized dosage forms, as it obviates sample aggregation. The aim of this work was to develop a robust dissolution method to be applied to the assessment of flucloxacillin release from microspheres during drug product development.

A flucloxacillin 500 mg hard gelatin capsule (HGC) disintegration test was used as a surrogate marker of dissolution for establishing an *Assumed IVIV relationship* [1]. An FTC, CE 7 Smart, equipped with a CY 7-50 pump and a Cell size of 22.6 mm (Sotax AG, Switzerland) was used. The system was operated in the closed loop mode at different flow rates using compendial and biorelevant dissolution media. Further, flucloxacillin dissolution was carried out at 4 and 8 mL/min with sampling done at 5, 10, 15, 30 and 60 minutes followed by HPLC analysis.

Flow rates of 2 to 8 mL/min revealed longer disintegration times compared to 16 to 32 mL/min. Disintegration in SGF_{sp} pH 1.6 at 4 and 8 mL/min were shown to be more predictive of *in vivo* HGC rupture (7 ± 5 minutes) [2]. Disintegration was faster in biorelevant media compared to compendial solutions and was more than likely due to ionic strength and surfactant effects. Dissolution studies show complete drug release after 15 minutes for both 4 and 8 mL/min flow rates, which correspond to linear velocities of 1 and 2 cm/min respectively, mimicking physiological hydrodynamic mixing rates equating to a linear rate of 1 cm/min in the FTC.

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A DESIGN OF EXPERIMENTS (DOE) STUDY EVALUATING THE PROPERTIES OF FREEZE-DRIED ORALLY DISINTEGRATING TABLET (ODT) FORMULATIONS

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The term; DOE is defined as a structured and organised method of determining the relationship between factors affecting a process and the output of that process. DOE can therefore be applied to pharmaceutical formulations in order to establish the role and relationship of formulation excipients in terms of the properties and performance of formulations. The ODT formulations prepared consisted of three excipients, named; Excipient A, B and C, and the effect of the excipients, both individually and interactively, on the properties of the formulations and resulting tablets were evaluated.The properties of the formulations/tablets which were investigated, included; formulation viscosity, tablet hardness, tablet disintegration time and *in vitro* oral retention time.

In terms of formulation viscosity, all three excipients exhibited a significant effect, where Excipients A and B revealed the most significant effects on formulation viscosity. All three excipients had a significant effect on tablet hardness, with Excipients A and C exhibiting the most significant influence. Tablet disintegration time and *in vitro* oral retention time were both significantly influenced by Excipient C.

The use of DOE in evaluating the properties of ODTs has shown to be an efficient and insightful method of understanding the role that each excipient has on the properties of the formulations prepared. Future work will involve formulation optimisation.

THE INFLUENCE OF POLYANIONS ON THE IN VITRO BIOCOMPATIBILITY OF NANO-SIZE POLYCATIONIC-PROTEIN COMPLEXES FOR PROTEIN DELIVERY

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Today, nanocarriers are widely explored for the delivery of many challenging therapeutic agents particularly proteins. We have shown in our previous work that a polycation, 15kDa polyallylamine (PAH) and its hydrophobically modified counterparts, palmitoyl grafted-PAH (Pa2.5), dimethylamino-1-naphthalenesulfonyl grafted-PAH (dansyl 10) and hydrophilic modified counterpart, quaternized palmitoyl-PAH (QPa) form polyelectrolyte complexes (PECs) with insulin. However, polycations generally are cytotoxic and methods to modify the cytotoxicity commonly involve complex, complicated chemical reactions. In this study, we aim to use a simple method, i.e. complexation of polyanion with PECs to enhance their biocompatibility. This method is devoid of complicated chemical reactions and organic solvents which could denature the protein. Insulin loaded or unloaded polycations, namely PAH, Pa2.5, QPa2.5 and Dansyl10 were mixed with polyanions, dextran sulphate (DS) or polyacrylic acid (PAA) in the presence of 100µM $ZnSO_4$ leading to spontaneous formation of polyanion-polycation-insulin complexes (APECs). After 2h at room temperature, the resulting APECs were incubated with CaCo2 cells for 24h. The cytotoxicity of the APECs was determined using MTT (3-4,5 dimethyl thialzol2,5 diphenyl tetrazolium bromide) assay while the haemocompatibility of the APECs was evaluated using haemolysis assay. Measurement of oxidative stress was studied by reactive oxygen species (ROS) assay. Based on IC_{50} values, the degree of toxicity of polycationic PECs was rated as PAH>Da10=Pa2.5>QPa2.5. For the hydrophobically modified APECs, the presence of PAA remarkably reduced the cytotoxicity compared to DS. This demonstrates that the type of polyanion has an impact on the overall cytotoxicity. More specifically, introduction of PAA was able to increase the IC_{50} of PAH by 2 folds, Pa2.5 by 14 folds, Dansyl10 by 16 folds and QPa2.5 by 2.5 folds. All APECs exhibited no significant ability to induce haemolysis at the overall concentration range (0.075-10mgml^{-1}) tested. The entire range of APEC formulations displayed no significant ROS production by CaCo2 cells. Future work will investigate the *in vitro* genotoxicity and immunotoxicity of these APECs in the cell models.

BIO-INSPIRED SILICA NANOPARTICLES AS DRUG DELIVERY AGENTS

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Silica nanoparticles are of great interest in many areas of materials science including as drug delivery vehicles. In particular structures such as SBA-15 and MCM-41 which are highly biocompatible, have a relatively large pore volume and provide a high surface area for drug molecule adsorption, which makes them attractive as drug delivery vehicles. They also have the potential to act as vehicle for targeted drug delivery by altering the release rate of drugs. These silica structures tend to be synthesised under harsh conditions however and do not fit with current drives towards green technology. We report upon the use of a bio-inspired method of fabricating silica which is time, energy and materials efficient to fabricate silica nanoparticles for drug delivery.

Changing the bio-inspired amine additive (DETA, TEPA, PEHA, PAH) during fabrication gave very different materials properties and release characteristics for the model drug calcein. Some polymeric amines (PAH) showed sustained release over 870 hours from model fitting compared with 48 hours for smaller molecular amines (DETA). The mass of calcein release was also found to be well correlated with the length of the amine additive chain. Other fabrication parameters were investigated including reaction time, silicon / nitrogen ratio in the reaction mix and concentration of reactants. The silicon / nitrogen ratio was found to influence the loading of calcein and its release without adverse effects on the yield of nanoparticles.

LIPOSOMAL-HYDROGEL FORMULATION AS OPHTHALMIC DRUG DELIVERY SYSTEM

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The design and synthesis of biocompatible and comfortable materials useful as basis of sustained drug dosage forms for ocular delivery is still under development. Topical beta blockers, in particular Timolol, are the first choice in medical management of primary open-angle glaucoma and ocular hypertension. In the particular research we have combine liposomes with hydrogels in order to investigate the release of drugs for ocular delivery. In this research, we explore the release profile of the model drug Timolol maleate (TM) from drug-in-gel systems and from drug-in-liposome-in-gel systems against PBS and BSS Plus solutions. The effect of liposome-membrane rigidity was evaluated by testing the 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) / Cholesterol, and by using a peptide hydrogel. The developed formulations were further characterized by AFM, CD, FT-IR, particle size, and zeta potential.

The mean particle size of the DSPC-Chol-TM liposome was found to be 200 ± 30 nm. The spherical nature and particle size of liposomes was confirmed by AFM studies. Liposome can be clearly seen as spherical particles with very high homogeneity and hydrogel forms fibers with diameter of 25 ± 7 nm. For the drug-in-gel system, the entire amount of TM was totally released in 24 h and from the DSPC-Chol liposomal formulations with the use of hydrogel was totally release in 1 week. In comparison, the release from the liposomal formulations without the use of hydrogel was 48 h. The release in PBS is slightly faster than in PSS Plus. A sustained release formulation of Timolol in DSPC liposomes containing cholesterol with the use of hydrogel was developed which could potentially be used for delivery of ophthalmic drugs such as antibiotics.

RESPONSIVE PROTEIN-POLYMER CONJUGATE THERAPEUTICS

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The site-specific delivery of therapeutic agents for diseases such as cancer is challenging[1]. Here, we explore how recombinant proteins can help tailor the properties of responsive polymeric systems for potential drug delivery applications[2]. The systems are designed to non-covalently encapsulate a high therapeutic load and display controlled release characteristics in response to appropriate stimuli. The proteins utilised in the systems have been designed to allow for the site-specific conjugation and should thus avoid issues (e.g. loss of biological activity) observed with random protein conjugation techniques.

An atom-transfer radical polymerisation (ATRP)[3] initiator was synthesised with a pyridyl disulphide end-group functionality for subsequent site-specific protein conjugation via a free cysteine residue. This macroinitiator was used in the polymerisation of thermoresponsive polymers using poly ((ethylene glycol) methacrylate) based monomers ($PEGMA_{246}$ and $PEGMA_{475}$). Gel electrophoresis was used to verify the formation of protein-polymer conjugates. The cloud-point behaviour of the protein-polymer conjugates was studied using ultraviolet-visible spectroscopy. Their hydrodynamic sizes were determined using atomic force microscopy (AFM) and dynamic light scattering (DLS).

Here, we demonstrate the ability to synthesize responsive systems for potential drug delivery applications, using recombinant proteins conjugated to thermoresponsive polymers. Polymers were 'grafted from' proteins using ATRP. Characterisation demonstrated the lower critical solution temperature (LCST) of the conjugates using $PEGMA_{246}$ was 24°C whereas; protein conjugated to $PEGMA_{246/475}$ underwent phase transition at 37°C. AFM and DLS confirmed conjugates were between 19-20 nm in size, and this increased to 28-45 nm in response to temperature. Molecular weight shifts in gel electrophoresis confirmed conjugate synthesis.

In summary, we have outlined the use of a controlled polymerisation technique for the careful design of 'smart' architectures for their potential application as drug delivery systems. We have demonstrated the ability to synthesize protein-polymer conjugates using a specific site on recombinant proteins and characterised these using various techniques to assess the responsive behaviour.

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EVALUATING IN-SITU GELATION USING TERAHERTZ AND DIELECTRIC SPECTROSCOPY

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Hydrogels have been intensively studied for drug delivery and tissue engineering applications. Of particular interest have been temperature induced sol-gel hydrogels which offer the possibility of *in situ* gel formation following injection. There is an increasing need to monitor the sol-to-gel transition process as it occurs, and potentially in *in vivo* situations. The main focus of this work has been to evaluate application of two physicochemical methods, Terahertz and Dielectric Spectroscopy, to characterise sol-to-gel transitions of hydrogels based on hyaluronic acid and methylcellulose.

The major consideration in applying terahertz spectroscopy to monitoring changes, in water rich environment of sol-to-gel transition systems, is the high absorbance of terahertz radiation by water. This has indeed proved the case, as changes observed in the time domain signal (a reduction in absorbance) were dominated by heating of the water rather than reflecting the gelation of the hydrogel.

Applying dielectric spectroscopy to the same hyaluronic acid and methylcellulose hydrogels show a distinct step change in conductance at 35°C, the temperature that corresponds to sol-to-gel transition assessed by other methods, including test tube inversion. The data obtained thus shows promise for the use of dielectric spectroscopy for *in situ* monitoring of the gelation process.

PREFORMULATION STUDY ON SILICA BASED SOL-GELS AS A CARRIER OF SODIUM IBUPROFEN SALT: EFFECT OF ACID OR BASE CATALYSIS ON DRUG INCORPORATION AND RELEASE

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A novel approach to sustained drug delivery is the use of silica based sol-gels as drug carriers. The aim of the present study was to evaluate the effect of the type and amount of catalyst used in the preparation of sol-gel derived xerogels on the incorporation and release of the selected drug, sodium ibuprofen.

Sol-gels were prepared from the hydrolysis and condensation of tetraethyl orthosilicate in the presence of varying amounts of an acid or basic catalyst, 0.1M hydrochloric acid or 0.1M ammonium hydroxide respectively. Drug loaded matrices were characterized using Scanning Electron Microscopy, Energy Dispersive Analysis, Differential Scanning Calorimetery and Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy. In vitro drug release measurements were carried out in phosphate buffer solution (pH 6.8).

Microporous and mesoporous matrices were obtained with acid and base catalysis respectively. The drug remained intact as sodium ibuprofen within ammonium hydroxide catalyzed sol-gels; catalysis with hydrochloric acid resulted in the protonation of the incorporated salt to ibuprofen. The amount of drug released at each sampling time was greatest from base catalyzed sol-gel systems. A reduced burst effect and a controlled release of ibuprofen was favoured from the microporous matrix that was catalyzed with the largest amount of 0.1M hydrochloric acid.

The type and amount of catalyst used in sol-gel preparation is a key parameter to control the release of the incorporated drug. The release of ibuprofen from the prepared samples was dependent on the degree of drug loading and the morphology of the xerogels.

STUDYING THE IN VITRO TRANSFECTION EFFICIENCY OF DIFFERENT DNA LIPOPLEXES

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Amongst the different gene delivery systems, cationic liposomes have been shown to be particularly useful in delivery of nucleic acids both in vitro and in vivo [1,2]. Several studies[3,4,5,6] have considered controlling parameters such as cationic lipid structure, chain length, vesicle size, lipid toxicity, lipoplex structure and topology in a bid to identify the key correlations between formulation and function. The aim of this research was to investigate the effects of such parameters on the transfection efficiency of a range of lipoplexes.

DNA lipoplexes composed of 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) as a helper lipid in combination with cationic lipids of 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) or 1,2-stearoyl-3-trimethylammonium-propane (DSTAP) or dimethyldioctadecylammonium (DDA) as well as combination of 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE) with DOTAP were prepared by lipid hydration[7]. In vitro studies were performed on a COS-7 cell line using the Luciferase assay for transfection and MTS assay for cytotoxicity.

Transfection efficiency of lipoplexes in the order of DOPE:DSTAP>DSPE:DOTAP≈ DOPE:DOTAP>DOPE:DDA shows efficiency of DOPE:DSTAP being significantly higher than the other formulations. In general, lipoplex size has been reported to affect the transfection efficiency with larger sized lipoplexes promoting higher transfection efficiency[4]. However, in this study DOPE:DSTAP which has the highest transfection efficiency, has the smallest vesicle size. In addition, decreasing the hydrocarbon chain length of the cationic lipids has previously been shown to increase the transfection efficiency[5] however, DOPE:DOTAP and DOPE:DSTAP have similar chain length but different transfection efficiency. DOPE:DDA with a lamellar structure has lower transfection efficiency compared to DOPE:DOTAP and DOPE:DSTAP which have hexagonal structures HII. Similar transfection efficiency of DSPE:DOTAP to DOPE:DOTAP suggest that DSPE might be a suitable replacement for DOPE to promote in vitro transfection efficiency.

In conclusion, this study shows parameters such as cytotoxicity and liposome structure clearly influence the transfection efficiency of lipoplexes.

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COCRYSTALLIZATION OF WATER INSOLUBLE ACTIVES BY USING HOT MELT EXTRUSION

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Hot melt extrusion (HME) was employed for the engineering of cocrystals of Carbamazepine – Saccharin blends (1:1 molar ratio). The extrudates were produced at different processing temperatures and screw speeds to investigate the effect of the continuous changes in the quality of the cocrystals. The extrudates were characterized using differential scanning calorimetry (DSC), hot stage microscopy (HSM), X-ray powder diffraction and X – ray photon electron spectroscopy (XPS). The obtained cocrystals showed increased crystallinity up to 90% compared to a prototype prepared by a precipitation process. XPS analysis revealed strong hydrogen bonding interactions between carbamazepine and saccharin functional groups. The dissolution profiles of the HME cocrystals demonstrated rapid dissolution rates.

EFFECT OF CYCLODEXTRINS ON RIBOFLAVIN SOLUBILITY AND CORNEAL PERMEABILITY ENHANCEMENT

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The effect of α, β, γ and HP-β-cyclodextrins on the aqueous solubility of riboflavin and its permeability through bovine cornea was investigated.

It was found that α-cyclodextrin (100 mg ml-1) and β-cyclodextrin (20-30 mg ml-1) offered a statistically significant enhancement on the aqueous solubility of riboflavin, whilst γ-cyclodextrin (10-100 mg ml-1) and HP-β-cyclodextrin (10-100 mg ml-1) did not significantly enhance the solubility of riboflavin.

The effect of cyclodextrins (30 mg ml-1) on the permeability of solubilised riboflavin through fresh and previously frozen bovine cornea was investigated. In the fresh corneas it was found that γ-cyclodextrin did not enhance permeability, but α-cyclodextrin, β-cyclodextrin and HP-β-cyclodextrin showed significant permeability enhancement.
Previously frozen corneas were shown to be more permeable to riboflavin compared to fresh cornea, supporting the hypothesis that freezing disrupts the epithelial cornea membrane, which offers the greatest barrier against aqueous drug formulations.

For previously frozen bovine corneas α-cyclodextrin and HP-β-cyclodextrin did not show any enhancement, β-cyclodextrin enhanced permeability whilst γ-cyclodextrin produced a reduction in permeability.

Fresh, whole bovine eyes were exposed to cyclodextrin solutions at the cornea and it was found that α, β, γ and HP-β-cyclodextrin (30 mg ml-1) all caused disruption to epithelium. A microscopic examination has revealed that cyclodextrins cause disruption to the epithelial cells which intensifies with increasing time of exposure. It has been shown by HPLC analysis that cyclodextrin solutions placed against the cornea are able to extract cholesterol and this is what is thought to be causing the observed epithelial disruption

PEGYLATED SILICA NANOPARTICLES FOR DRUG DELIVERY

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Nanoparticles with short chains of polyethylene glycol attached to their surface (PEGylated nanoparticles) have attracted a lot of attention because of their wide applications in drug delivery. It was demonstrated that PEGylation of particles facilitates their permeation through mucosal surfaces making them highly promising for nasal, pulmonary and vaginal drug delivery [1, 2].

Recently Irmukhametova et al. [3, 4] have reported a novel approach for synthesis of thiolated silica nanoparticles that were capable of adhering to mucosal surfaces. PEGylation of these nanoparticles was found to reduce their adhesive properties.

In the present study we have synthesised a series of thiolated nanoparticles using a range of aprotic solvents (dimethyl sulfoxide, dimethyl fromamide, N-methyl-2-pyrrolidone, tetrahydrofuran, dioxane, acetone and acetonitrile) and established that the nature of the medium determines the size of nanoparticles.
PEGylated nanoparticles (NPs) were synthesised by reaction of thiolated silica nanoparticles with methoxypolyethylene glycol (750, 5000) maleimide. PEGylation was confirmed by dynamic light scattering, transmission electron microscopy, Raman spectroscopy and thermogravimetric analysis. It was established that PEGylation prevents nanoparticles from aggregation and reduces the number of thiol groups on their surface which makes them promising as materials with high mucus-penetration ability.

The application of these PEGylated nanoparticles in drug delivery will be discussed.

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FORMULATION AND CHARACTERISATION OF INDOMETACIN SPRAY DRIED DISPERSIONS

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Solid dispersions are a promising strategy for improving the dissolution and oral bioavailability of poorly water soluble drugs. Reduction of particle size and the availability of drug in amorphous form can help to improve the dissolution of a poorly water soluble drug and hence, the bioavailability may significantly increase. Solid dispersions of poorly water soluble drugs are frequently prepared by the solvent evaporation method. However commercial applications of solid dispersions have been very limited due to manufacturing and stability issues. The main manufacturing issue is one of scale-up which can be overcome by the use of spray drying. The formation of interactions between drug and polymer in the dispersion has the potential to resolve stability issues. The aim of this study was to characterise the nature of interactions in solid dispersions and dissolution rate with the different polymers.

Solid dispersions of indometacin were prepared using polyvinylpyrrolidone (PVP), Eudragit L 100 or Eudragit S 100 by solvent evaporation method in 1:1 weight ratio using spray dryer. Spray dried dispersions were quantified for their drug and solvent contents using high performance liquid chromatography and thermogravimetric analysis. The physical state and drug:polymer interactions of solid dispersions and physical mixtures were characterised by differential scanning calorimetry (DSC), infrared spectroscopy, X-ray powder difraction (XRPD) and in vitro dissolution testing.

DSC and XRPD confirmed the formation of amorphous monophasic dispersions. The glass transition temperatures for PVP, Eudragit L 100 and Eudragit S 100 solid dispersions with indometacin were found to be 76.86 °C, 45.53 °C and 58.45 °C respectively. Dissolution studies revealed a statistically significant improvement in drug release at 95%CI for solid dispersions. After 30 minutes at least 75% drug release was observed from all solid dispersions. Therefore, formation of solid dispersions can increase the dissolution rate of indometacin.

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TOWARDS THE DEVELOPMENT OF A SIMPLE, ONCE-A-DAY DOSAGE FORM FOR THE DELIVERY OF INSULIN THROUGH THE NASAL ROUTE: FORMULATION, IN VITRO ASSESSMENT AND IN VIVO EVALUATION

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In previous work we have reported the preparation and characterisation of an *in situ* thermogelling formulation based on *N*-trimethyl chitosan chloride (TMC). The formulation, which has been designed for application as intranasal solution, forms a mucoadhesive gel within the timeframe of mucociliary clearance (MCC) to affect prolonged residence at mucosal surfaces. We now extend this work by assessing the capability of these hydrogels to act as controlled-release matrixes for insulin. Spectroscopic and analytical investigations (UV-Vis, fluorescence, IR, Raman and SDS-PAGE) have indicated that the incorporated insulin is in monomeric form and that it retains its structural integrity and conformational order over several days. Also, by recording the formulation-induced changes in transepithelial electrical resistance (TEER), we use Calu-3 monolayers as an *in vitro* probe of the potential permeation-enhancing capacity of the applied formulation. In parallel, we assess the cytotoxicity of each formulation by monitoring the essential viability of the Calu-3 monolayer substrates. We also report the results from *in vivo* (rat model) studies that have been designed to assess the effects of this thermosensitive intranasal formulation of insulin and TMC on MCC and also to monitor the hypoglycemic effect of formulations of the same therapeutic agent.

PROCHITOSOMES: NOVEL CHITOSAN-ENRICHED SPRAY-DRIED FORMULATIONS FOR PULMONARY DRUG DELIVERY

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Liposomes are promising for inhalation due to their ability to localise drug action in the lung (1). Because of the instability of liposomes, proliposomes have been introduced. Upon contact with the pulmonary fluids, proliposomes may generate liposomes in situ within the lung (1). Chitosan is a safe, biocompatible, biodegradable and mucoadhesive polymer which can enhance the interaction of the drug with the mucosal epithelium (2). Chitosomes are liposomes coated with chitosan (3).

This study aimed to develop prochitosomes which combine the advantages of chitosan and proliposomes. Prochitosomes were developed by spray drying an ethanolic dispersion of soya phosphatidylcholine (SPC), cholesterol, chitosan, mannitol and Salbutamol Sulfate (SS) to manufacture an inhalable dry powder formulation. Chitosan was included in concentrations ranging between 0.05 and 0.3 %. Prochitosome powders were subsequently hydrated with deionised water and the entrapment efficiency (EE) of SS was determined using high performance liquid chromatography (HPLC) and particle size, zeta potential and morphology were investigated using laser diffractions, electrophoretic mobility and scanning electron microscopy (SEM) respectively.

EE of SS entrapment was the highest (64.92%) for chitosomes produced from prochitosomes containing 0.3% chitosan and the lowest (62.1%) when using the lowest polymer concentration (i.e. 0.05%). SEM showed that prochitosomes were spherical, and following hydration, the zeta potential were increased by increasing chitosan concentration, having values between +8.4 and +23.6mV. The measured particle size following hydration was in the range of 2.8 – 3.4 µm.

Overall, this study has shown that spray drying was appropriate to produce novel mucoadhesive prochitosomes that upon hydration generated chitosomes that offered superior EE of SS. Thus prochitosomes could potentially be applicable for pulmonary drug delivery.

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COMPUTATIONAL PHARMACEUTICS – THE APPLICATION OF MOLECULAR MODELLING IN DRUG DELIVERY

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Computational pharmaceutics combines experimental approaches and computer modelling technique to explore the mechanisms of drug delivery and then develop novel drug delivery systems. Four examples will be discussed: siRNA delivery, salt formation, cyclodextrin complexes and solid dispersion.1-5 For siRNA delivery, molecular dynamics (MD) simulation can provide us a physical view of gene-polymer complexation. For salt formation process, the simulation results shows that hydrophobic interaction is very important in salt formation. For cyclodextrin-drug complexes, MD simulation can provide the structure, dynamics and energetics of cyclodextrin-drug complexes. For solid dispersion, MD simulation can help us to explore the physical state between polymer and drug, which strongly relate to the physical stability of solid dispersion. As these examples shown, molecular modelling is a powerful technique for the investigation of mechanism of drug delivery on the molecular level.

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DELIVERING PREDNISOLONE-POLYMER COMPLEX VIA QUICK DISSOVING FILM – A STEP TOWARDS THE DEVELOPMENT OF AN APPROPRIATE PAEDIATRIC FORMULATION

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Prednisolone is recommended for the treatment of acute asthma, but the lack of availability of suitable formulations could limit treatment compliance. The aim of this study was to develop prednisolone-polymer complexes with enhanced solubility and to incorporate this complex into orally disintegrating films to enable rapid drug delivery. The prednisolone- polymeric complexes were prepared using solvent evaporation and freeze drying techniques with a drug-polymer ratio of 1:1. Hydroxypropyl β-cyclodextrin (HP β-CD), hydroxypropyl methylcellulose 4 cps, and polyvinyl pyrolidone K30 (PVP K30) were explored as polymeric carriers. The aqueous solubility, dissolution profile, and solid-state characterisation using differential scanning calorimetry (DSC) of the complexes determined. The optimized complex was then incorporated into films prepared with polyvinyl alcohol (PVA), PVP K30 and glycerine using solvent casting technique. The weight variation, thickness, solid-state characterisation, in vitro disintegration and dissolution profiles of the films were then determined. The highest prednisolone solubility was seen with the prednisolone- HP β-CD complex prepared by freeze drying (1.82 mg/ml) followed by the same complex prepared by solvent evaporation (1.70 mg/ml). These solubilities were statistically significantly different to prednisolone powder (0.225 mg/ml) ($P<0.05$). The complexes prepared using HPMC and PVP did increase the solubility compared to pure drug but the increase was not statistically significant. DSC analysis of complexes revealed a reduction in area of the endothermic peak indicating the presence of amorphous drug. In comparison the DSC analysis of films did not show endothermic peak showing complete absence of crystalline drug. This may be due to presence of PVA and maltodextrin in film preventing the drug from crystallising out. The film was thin, uniform in weight and thickness, showing rapid disintegration of 55 sec with almost complete drug release within 3 min. The study revealed the incorporated drug-polymer complex have maintained the amorphous state and enabled rapid drug release.

FAST DISSOLVING FILM OF ALPRAZOLAM FOR ORAL TRANSMUCOSAL ADMINISTRATION

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The aim of the present study was the development of fast dissolving thin films of poorly soluble drug alprazolam which would enable quick drug release in the buccal cavity. The films were produced via a solvent casting technique using hydroxypropylmethylcellulose (HPMC 4cps and E5LV) and polyvinyl alcohol (PVA 4-88) as film formers with either maltodextrin or polyvinyl pyrrolidone (PVP K30) as a pore former and glycerin as a plasticizer. The films were evaluated for weight variation, thickness and drug content, solid state characterisation by differential scanning calorimetry (DSC), *in vitro* disintegration (static and normal), *in vitro* drug dissolution*, in vitro* drug release and ex*-vivo* drug permeation using pig buccal mucosa. All the films had acceptable physical attributes including consistent weight and thickness. The film prepared using HPMC 4cps with maltodextrin (P5) was found to be a promising formulation which showed a static disintegration time of less than 3 min, with 95% of drug dissolved in 2 min and a dissolution rate significantly faster than the drug powder ($p<0.05$). The DSC results revealed complete absence of an endothermic peak in the film showing presence of alprazolam in the amorphous state which potentially contributed towards increased dissolution from this film compared to the powder. The static disintegration test was able to detect changes in formulation and hence found to be more discriminatory compared to the normal disintegration test. The permeation profile of drug from film P5 was promising with approximately 60% of the applied dose permeated across the buccal mucosa within 6 h. The combination of HPMC 4cps with maltodextrin was found to be superior in terms of disintegration, drug dissolution and the drug permeation profile compared to HPMC 4cps with PVP K30 and HPMC E5LV with PVA 4-88. The prepared films were thin, light weight, easy to handle and could be administered easily to the buccal cavity.

TARGETED DELIVERY OF DRUGS VIA THE PepT1 TRANSPORTER

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PepT1 is a transmembrane proton-oligopeptide transporter that is mainly expressed in the brush border membrane of the small intestine. The primary function of PepT1 is to transport the di- and tri- peptides that are the result of the hydrolytic breakdown of dietary proteins in the GI tract. However, the low-affinity, high capacity of PepT1 has made it a key drug delivery target.[1] Known drugs which are transported by PepT1 include β-lactams, ACE inhibitors and nucleoside antivirals.[1]

Traditionally drugs that are targeted towards PepT1 are designed with the necessary transport characteristics in mind. However, our group has developed and patented hydrolysis resistant thiodipeptide 'carriers' which are PepT1 substrates.[2] These 'carriers' can be attached to structurally suitable, poorly absorbed drugs, to create PepT1 targeted prodrugs. This method has been validated both *in vitro* and *in vivo* and the first example of targeting large macrocyclic compounds towards the PepT1 transporter has been achieved.

Work by our group to extend the linker bond type from ether and ester[3], to hydroxyimine[4] has also been reported. The research reported in this paper aimed to improve our understanding of important structural features required by our thiodipeptide prodrugs for optimal transport. In particular, this research highlights the importance of spacer length on PepT1 transport.

The discovery that PepT1 is over-expressed in several cancer cells has also led to research being undertaken into targeted anti-cancer therapy, via our thiodipeptide carriers. This method also has the potential benefit of improving the oral activity of anti-cancer drugs such as Doxorubicin.

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LIPOSOME INCORPATION INTO POLYMER MATRIX SYSTEMS

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Liposomes are increasingly popular drug delivery systems with many possible applications[1]. Even though their application is growing, liposomes are subject to various stability problems. Liposomes can readily fuse, aggregate or sediment. Moreover, sterilisation and large-scale production are problematic [2]. These problems can be succumbed when incorporating liposomes into polymer matrix systems. These matrix systems can not only stabilise the liposomes but also steer the pharmacokinetics of the formulation.

Liposomes were formulated from dimethyldioctadecylammonium (DDA) or distearylphosphatidylcholine (DSPC) in combination with the immunostimulatory glycolipid trehalose 6,6' – dibehenate. (TDB) using the lipid film hydration method as described in Davidsen et al (2005) [3]. The formulations were prepared as either multilamellar vesicles (MLVs) or small unilamellar vesicles (SUVs) and were combined with ovalbumin adsorbed onto the liposome surface.
The stability of these formulations were investigated post freeze-drying and freeze thawing to assess any potential aggregation by measuring size and zeta-potential. Subsequent studies will investigate the role of cryoprotection and lyoprotection with liposomes. Following optimisation, the liposomes will then be incorporated in a polymer matrix system and their release from the matrix will be measured. Future work will involve the creation of a test-formulation which can then be used for further *in-vivo* release studies.

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DEVELOPMENT OF AN ORAL GABAPENTIN FORMULATION WITH A FLEXIBLE RELEASE PROFILE

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Gabapentin is a highly-soluble amino acid used to treat female menopause symptoms. Sustained release formulations are beneficial for this indication in that they are able to reduce the instance of hot flushes during sleep. The optimum plasma profile was not known in advance, so a drug product with a flexible *in vitro* release profile was developed based on minitablet cores, the release properties of which could be modified by the application of different functional coats.

Tablet cores containing 75 % gabapentin by weight were produced. The coatings evaluated were methacrylic acid (delayed release) and modified methacrylic copolymers (sustained release). The enteric coating eroded at or above pH 7.0. The release rate of the sustained release coat (EUDRAGIT® RL/RS) could be altered by changing the RL:RS ratio. By incorporating more EUDRAGIT® RS, the release rate is retarded. Uncoated tablet cores were tested for disintegration, hardness, friability, weight uniformity and thickness. Coated tablets and two prototype formulations containing known ratios of the different tablet types were tested for dissolution.

Dissolution profiles of the tablet cores coated with the different polymer systems show complete release after 15 min for the immediate release variant, after 30 min for the delayed release variant following exposure to a dissolution medium at neutral pH, and close to zero-order release over 6 hours for tablet cores treated with a sustained release coat. Dissolution profiles of prototype formulations containing known ratios of the different tablet types provide proof that this approach to formulation development works *in vitro*.

Using combinations of the different tablet types, it is possible to extend the release of gabapentin for up to 6 hours or more. The ability to tailor drug release has the potential to save costly and time consuming reformulation in the event that *in vivo* data suggest that a different profile is required.

DEVELOPMENT AND OPTIMISATION OF AN AGE APPRPRIATE SPIRONOLACTONE FORMULATION

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The objective was to produce an oral liquid dosage of Spironolactone, an existing antihypertensive drug from the group of potassium-sparing diuretics. To achieve this excipient selection would prove vital in overcoming the various hurdles which are present between the selection of the drug molecule to the production of the final formulation. Reformulation is carried out for a variety of reasons, in this case to develop a formulation which is more capable of targeting the paediatric age group. Age appropriate oral liquid formulations are much preferred when paediatrics may have problems swallowing tablets and capsules.

Problems in solubilising Spironolactone to the required dosage lead to the production of a suspension. Steps were taken to produce and elegant suspension successfully avoiding the common issues associated with suspensions such as poor homogeneity, caking following sedimentation and ostwald ripening. The application of surfactants to ensure a uniform particle size, prevent clumping and limit caking was investigated using laser diffraction equipment for particle size analysis, observation of the rate and volume of sedimentation, contact angle measurement and zeta potential analysis. A buffer system was optimised to regulate the pH of the formulation to the pH at which Spironolactone is most stable and the inclusion of a suspending agent was investigated for both improving the physical stability of the suspension and also to provide the formulation with desirable pseudoplastic flow properties. A HPLC method was also developed to assay the stability of the formulation in accelerated and long term storage conditions.

A suitable combination of surfactant and suspending agent concentrations was identified for use which prevents the sedimentation of the drug particles. This same combination also prevents particle clumping. In preventing sedimentation the problems associated with caking and also the homogeneity of the particles on re-suspension are eradicated. Ostwald ripening was also proven to be prevented with the inclusion of the suspending agent. The formulation is currently in the process of accelerated and long term stability analysis and has been shown to be stable after 1 month in storage.

EVALUATION OF INSULIN-LOADED SURFACTANT BASED BILOSOMES FOR ORAL DELIVERY

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The key challenges involved in delivering proteins such as insulin orally include conferring protection on its conformational matrix as they undergo transit through a hostile gastrointestinal tract, consisting of digestive enzymes and direct exposure to pH ranging from 1–7.5. A study previously conducted by Conacher and Mann [1] used drug entrapped non-ionic surfactant vesicles formulated with bile salts, these were shown to stabilize the membrane against the detrimental effects of bile acids in gastrointestinal tract [1].

A series of bilosomes and lipo/niosomes were prepared using insulin into four vesicle formulations :-
i) Phosphatidylcholine (PC):cholesterol (Chol):stearylamine (SA) (16:16:3µmol),
ii) Phosphatidylcholine:cholesterol:stearylamine:NaDeoxycholate (16:16:3µmol),
iii) Polyoxyethylene alkyl ether 92 (Brij^{TM} 92):Chol:SA (16:16:3µmol),
iv) Polyoxyethylene alkyl ether (Brij^{TM}) 92:cholesterol:stearylamine:NaDeoxycholate (16:16:3µmol). Characterisation used both HPLC and U.V techniques to quantify encapsulated insulin measurements and determine release profiles.

Results showed that entrapment of insulin in the bilayer structure of bilosomes/niosomes protected it against proteolytic activity of α-chymotrypsin, trypsin and pepsin *in vitro*, and further work will be conducted in this area to observe the effect of bile salts incorporated into the bilayer of this series of surfactant vesicles. Encapsulation efficiency of these formulations ranged from 47.10 ± 0.26% to 43.04 ± 0.29%, which was consistent with previous studies.
Liposomal formulations showed a rapid release profile, releasing 80% of the encapsulated insulin within one hour. Brij 92 formulations exhibited a more controlled release in simulated intestinal fluid over a 24 hour period as compared to liposomal formulations. This may be due to the fact that they have higher rigidity and order in packing versus PC based formulations, enhancing their resistance whilst their surface integrates with free bile salts. Consequently, the Brij 92 formulations (with and without integration of bile salts) may be a suitable vehicle for future work in the development of an oral delivery system for insulin.

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SUSTAINED RELEASE SOLID LIPID EXTRUDATES PROCESSED BY HOT MELT EXTRUSION

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The current study focuses on the development of sustained release matrices of Diclofenac-Na prepared by hot melt extrusion (HME). Compritol® 888ATO was used as a lipid matrix with different drug loadings (30 – 50%) an extruded at temperatures below or above the lipid melting point. Extrudates were analyzed using differential scanning calorimetry (DSC), hot stage microscopy (HSM), X-ray powder diffraction and Scanning electron microscopy (SEM). The drug was found to retain its crystallinity in the lipid matrices processed under different temperatures.

The extrudates were compressed to obtain tablets and the effect of the drug loading, compression force and the processing parameters on the dissolution profiles were examined. The results demonstrate that Compritol® 888ATO can be efficiently used for sustained release formulations of hydrophilic active substances.

EVALUATING THE RADIAL TENSILE STRENGTH OF AVICEL PH-101 TABLETS WITH TERAHERTZ SPECTROSCOPY

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Here, we use terahertz-pulsed spectroscopy (TPS) to develop the use of THz refractive index (RI) values, (as an established surrogate to density assessment [1]) to evaluate whether RI can be used to assess the radial tensile strength (RTS) of tablets.

Avicel® PH-101 was used to prepare tablets, compacted in a confined die on an instrumented tablet-press (Gamlen Tabletting, Nottingham). Two tablet batches were produced: One with a fill- weight of 200 mg, and compacted with forces between 110-460 Kg. The second batch had a fill- weight of 295 mg, and compacted between 85-430 Kg. Geometric measurements were made of the tablets 48 hours post-compaction and RI values were measured on a TPS-3000 (TeraView, Cambridge). The (RTS) [2] of each tablet was calculated, following diametric crushing-force determination on a PharmaTest PTB-311E.

As a surrogate measurement for density, RI is intrinsically sensitive to the effect of compaction-force. During consolidation, rearrangement and deformation of particles causes removal of voids and decreases porosity. The tablets displayed a linear response with increasing RI and compaction-force (R^2>98%). One might expect to in-directly correlate RI and RTS, as the results indicate RTS sensitivity to density. However, our apparent linear results have differing gradients with RTS against RI, so that the two batches converge at a RI=1.6 yet, at a RI=1.5 there is a 0.2 *MPa* difference in RTS. During compaction, pressure exertion by the punch-head is predominately conveyed axially to the powder. With decreased fill-weight this pressure is conveyed traversally to a greater extent, increasing consolidation at the die-wall [3]. Hence, tablets of lower fill-weight will endeavour to recover elastically, yet this may form internal integrity flaws and reduce RTS.

With knowledge of a batches fill-weight and compaction-force, a calibration curve will provide a means to assess the RTS of 100% of produced tablets, non-destructively.

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AN ANALYTICAL APPROACH ON THE ROLE OF CHOLESTEROL WITHIN BILAYER VESICLES

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Niosomes are an example of vesicles which are prepared from non-ionic surfactants including the addition of cholesterol to form bilayer vesicles. Cholesterol has been widely used in the formation of bilayer vesicles to aid formulations in terms of stability, increased bilayer fluidity and increased drug retention of the vesicles. The aim of this study was the utilisation of DSC and Langmuir to understand the influence of cholesterol used to form vesicles.

Niosome formulations were prepared by using a 5:4:1 blend of monopalmitoyl-glycerol (MPG), cholesterol (CHO) and dicetyl-phosphate (DCP) respectively by melting the surfactants and then homogenising to produce vesicles. The niosomes were then analysed by DSC as a liquid formulation to determine the gel-liquid transition phase. Additionally monolayer studies of the individual surfactants (MPG, CHO and DCP) and a mixture of surfactants in the ratio 5:4:1 of MPG:CHO:DCP respectively were carried out using a KSV mini trough Langmuir system equipped with a platinum Wilhelmy plate in an isolated area. The surfactants were analysed for surface collapse pressure and mean molecular area upon contraction of the hydrophilic barriers on the Langmuir system.

Results from the individual monolayer studies indicate that the ideal surfactant mixture of the 5:4:1 (MPG:CHO:DCP) should result in a mean molecular area of 29.19 A^2/molecule. The experimental value obtained results in a mean molecular area of 28.3 A^2/molecule showing a minimal 3% deviation from ideality. This data suggests that no surfactant is more dominant within the monolayer and that uniform monolayers are favoured with an even distribution of the surfactants. The implications of cholesterol packing into the spaces within surfactant monolayers can be confirmed by a DSC study carried out on the niosome formulation prepared which shows the removal or non-existence of a gel-liquid phase transition. Generally the gel-liquid phase transition is caused in the absence of cholesterol in vesicles by the hydrophobic tails of lipids or surfactants crystallising into a rigid phase and the presence of heat causes the tails to become flexible and cause this transition (Moghaddam et al, 2011; Taylor and Morris, 1995).

Cholesterol incorporation within the niosome formulation uniformly spreads and inserts itself between the lipids and surfactants as shown by the Langmuir study hence, preventing crystallisation of the hydrophobic tails of the other surfactants as proven by DSC.

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A VACCINE STUDY TO INVESTIGATE THE ROLE OF LIPOSOME SURFACE CHARGE IN THE IMMUNOGENECITY OF THE LATENT TUBERCULOSIS ANTIGEN, HYBRID56

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Sub-unit protein antigens in vaccine formulations are becoming more common due to their increased safety profile, however sub-unit antigens lack immunogenicity when administered alone but when formulated with adjuvants such as liposome delivery systems they are able to deliver a powerful immune response [1]. The aim of the present study was to investigate the role of liposome surface charge of adjuvants in effective delivery of protein antigens and also the subsequent immune response generated.

Liposomes were formulated from dimethyldioctadecylammonium (DDA) bromide or 1,2-distearoyl-*sn*-glycero-3-phospho-L-serine (DSPS) in combination with the immunostimulatory glycolipid trehalose 6,6' – dibehenate. Vaccine preparations were prepared by the lipid film-hydration method [2] with the liposomes adsorbing Ag85B-ESAT-6-Rv2660c (H56) antigen to a final concentration of 0.1 mg/ml (5 µg/vaccine dose). All mice were immunised intramuscularly into the left quadricep with the proposed vaccine (50 µl/dose) three times, with two week intervals between each immunisation.

Delivery of H56 with cationic DDA/TDB gave a Th_1 immune response as shown by high levels of interferon-γ, interleukin-2 (from H56-restimulated splenocytes) as well as enhanced antibody titres. Once restimulated with antigen, splenocytes were able to show enhanced proliferative responses (as shown by increased uptake of ^{3}H-thymidine) following DDA/TDB:H56 immunisation. The production of IL1β was analysed at the injection site with high concentrations being recorded for mice immunised with DDA.TDB:H56 therefore suggesting a role for the inflammasome in the immune response.

In contrast, mice immunised with DSPS/TDB:H56 showed immune responses with a Th_2 bias as shown by production of IL5 and IL10 and decreased levels of IFNγ, IL2, IL1β and antibody titres as compared to DDA/TDB:H56 immunised mice. In conclusion, these *in vivo* vaccine studies have shown that there is a charge-dependent trend in the immunogenicity of liposome vaccine formulations when combined with the anionic H56 antigen.

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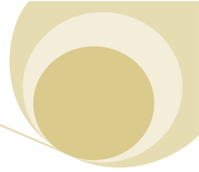

Delegate List

First name	Surname
Fadi	Abdulrazzaq
Adeola	Adebisi
Eilis	Ahern
Robert	Ahern
Yousef	Al-ebini
Ali	Al-khattawi
Terry	Allen
Tahir	Ansari
Basel	Arafat
Sohail	Arshad
Philip	Attwool
Raj	Badhan
Paul	Bahl
Katherine	Bamsey
Azzah	Bannunah
Joao	Barros
Russell	Beeching
Rory	Bell
Sagida	Bibi
Samuel	Bizley
Angels	Cano-Odena
Victoria	Capel
Woei Ping	Cheng
Rosalind	Chong
Michael	Cook
Helen	Cox
Martyn	Davies
Usha	Devi
Mahesh	Dhalwade
Timothy	Doody
Anna	Duszynska
Abdelbary	Elhissi
Amr	El-Shaer
Edmond	Ekenlebie
Kristina	Ferkova
Kevin	Fernandes
Francisco	FernandezTrillo
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Manjit	Kaur
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Vitaly	Khutoryanskiy
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Alireza	Mahboubian
Karl	Malcolm
Viraj	Mane
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Shilpa	Mistry
Namita	Masurkar
Behfar	Moghaddam
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Peter	Morrison
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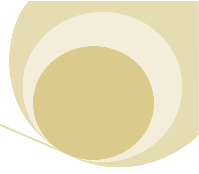
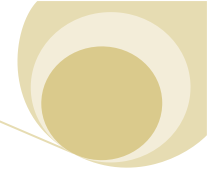
Dharmendra	Muttha
Hamde	Nazar
Huner	Omer
Yewandi	Oni
Wilson	Oguejiofor
Defang	Ouyang
Andrew	Parker
Viralkumar	Patel
Gayle	Pearson
Yvonne	Perrie
Bart	Pouls
Mark	Powell
Samuel	Pygall
Craig	Russell
Katie	Ryan
Harry	Schimanski
Saif	Shubber
Lauren	Shurety
Paul	Smith
Molly	Stevens
Enes	Supuk
Anil	Vangala
Jon	Veal
Paul	Verkey
Kapil	Vithani
Leon	Wall
Jitinder Singh	Wilkhu
Alexander	Wilkinson
Phil	Williams



Notes 1

Notes 2



Notes 3