UKICRS Symposium 2013

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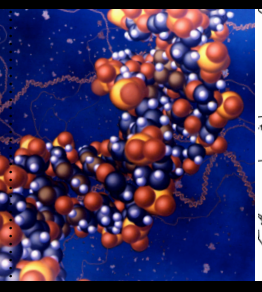
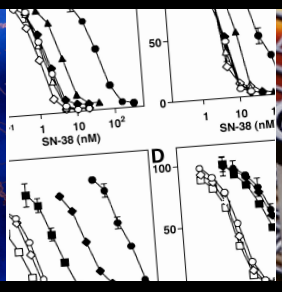


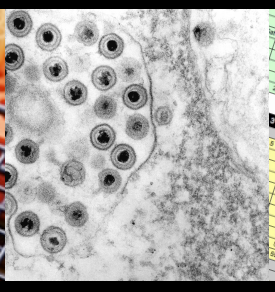
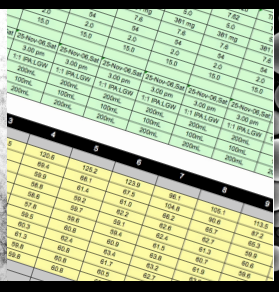
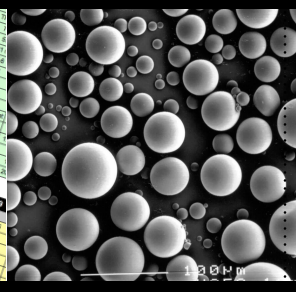

**Wednesday 15-16 April 2013**
Whiteknights Campus
The University of Reading
Reading, UK

# WELCOME!

A very warm welcome to the 2013 UKICRS Symposium! The committee are delighted to be hosting this year's event in the University of Reading. As with previous UKICRS symposia, this year's programme is aimed primarily at encouraging participation from UK postgraduate students, postdoctoral researchers and early stage industrialists working in the general fields of drug delivery and controlled release.

The symposium will present an excellent opportunity for networking. The 15 April afternoon session is geared towards industrial exhibitors, including Stable Micro Systems, NanoSight, Surface Measurement Systems, Caleva Process Solutions, Meritics and Fisher Scientific, who will be showcasing their products and technologies.

The scientific programme on 16 April includes two keynote lectures, as well as contributed talks and poster presentations from postgraduate students and postdoctoral researchers. We are delighted to welcome Prof Wim Hennink (Utrecht University) and Prof John Smart (University of Brighton) to the symposium as our keynote speakers. Both have an excellent reputation in their respective research fields and we are privileged to have them present their work to us.

We strongly encourage you to fully engage in the symposium, to ask questions, to discuss your research with your peers, and to make new friends and collaborators.

Enjoy the symposium!

Vitaliy Khutoryanskiy
2013 UKICRS symposium organizer

# PROGRAMME

| | |
|---|---|
| 8.30 am | Registration / Poster set-up (Palmer Building) |
| 9.30 am | **Vitaliy Khutoryanskiy**, *University of Reading* & **Woei Ping Cheng**, U*niversity of Hertfordshire* – Welcome & Opening Remarks |
| 9.40 am | **Wim Hennink**, *University of Utrecht* – Biodegradable polymers for protein delivery |
| 10.20 am | **Gayle Wilson**, *Keele University* – Targeted delivery of drugs via the PepT1 transporter |
| 10.35 am | **Nooshin Daveshpour,** *Queen's University Belfast* – Development and optimisation of a novel anti-inflammatory nanoparticle for the treatment of acute lung injury |
| 10.50 am | Tea / Coffee |
| 11.10 am | **Jit Wilkhu**, *Aston University* – Understanding the transit of bilosomes and subunit antigen via the oral route |
| 11.25 am | **Dolores Serrano Lopez**, *Universidad Complutense de Madrid* – Novel controlled release amphotericin B formulations: haemolytic and pharmacokinetic studies |
| 11.40 am | **Hamid Merchant**, *University College London* – Design and use of automatic pH control system to simulate the entire gastrointestinal pH in a conventional dissolution apparatus |
| 11.55 am | Poster Session 1 |
| 12.40 pm | Lunch |
| 1.25 pm | UKICRS Annual General Meeting |
| 1.40 pm | **John Smart**, *University of Brighton* – Buccal drug delivery systems |
| 2.20 pm | **Chen Chen**, *University of Cambridge* – Direct multi-nuclear magnetic resonance imaging studies of controlled drug release |
| 2.35 pm | **Giovanna Sicilia**, *University of Nottingham* – Reducible polymer DNA-hydrogel as a dual switchable release gate |
| 2.50 pm | **Samuel Bizley**, *University of Reading* – Hydrogen-bonded layer-by-layer enteric coatings for oral drug delivery |
| 3.05 pm | Tea / Coffee |
| 3.25 pm | Poster Session 2 |
| 3.55 pm | **Sukrut Somani**, *University of Strathclyde* – Evaluation of transferrin-targeted dendrimers for gene delivery to the brain |
| 4.10 pm | **Louise Harris**, *University of Sunderland* – Specific challenges and opportunities in formulating slow release products for farmed ruminants |
| 4.25 pm | **Fiona McCartney**, *University College Dublin* – Investigation of sugar esters as novel intestinal permeation enhancers |
| 4.40 pm | Close of meeting / Poster prizes |

# KEYNOTE SPEAKERS

# WIM HENNINK - UTRECHT UNIVERSITY

Prof. Wim Hennink is currently Science Director and Head the of Department of Pharmaceutics at Utrecht University. With a background in biochemistry Prof Hennink has progressed to work within the fields of biomaterials and drug targeting, with specific interest in biodegradable polymers.

Wim obtained his Ph.D. degree in 1985 at the Twente University of Technology on a thesis of biomaterials research topic. From 1985 until 1992 he had different positions in the industry. In 1992 he was appointed as professor at the Faculty of Pharmacy of the University of Utrecht. From 1996 on he is head of the Department of Pharmaceutics. His main research interests are in the field of polymeric drug delivery systems. He published over 350 papers and book chapters and is the inventor of 20 patents.

# JOHN SMART - UNIVERSITY OF BRIGHTON

Prof. Smart graduated in 1979 with a BSc (Hons) degree in Pharmacy, Brighton Polytechnic (now the University of Brighton), and completed his pharmacy preregistration training year with Cyanamid of Great Britain Ltd, Gosport and Portsmouth Area Hospitals. He then studied for PhD degree at the Welsh School of Pharmacy, UWIST before managing his own community pharmacy business for four years. In 1988 he was appointed Lecturer in Pharmaceutics, the School of Pharmacy and Biomedical Science, Portsmouth Polytechnic (now the University of Portsmouth), then became a Reader in Pharmaceutical Sciences (1997) and then Head of the School from August 1997 to September 2002. During this period the School flourished, scoring highly in the research assessment exercise and subject reviews. In February 2004 he returned to the University of Brighton as Head of the School of Pharmacy and Biomolecular Sciences and Professor of Pharmaceutical Sciences. He was on the Science committee of the Royal Pharmaceutical Society of Great Britain, was a member of the Executive committee of the Council of University Heads of Pharmacy, a member of the Modernising Pharmacy Careers work stream developing proposals for changes in pharmacy education for Medical Education England, a committee member of the South East Research For Patient Benefit grants, and is on the editorial boards of the International Journal of Pharmaceutics (Elsevier), Recent Patents on Drug Delivery and Formulation (Bentham Science), and Therapeutic Delivery (Future Science Group). He is currently chair of the Pharmacy Schools Council and sits on the English Department of Health Modernising Pharmacy Careers professional board. Prof. Smart's research interests are in drug delivery using bioadhesive materials, specifically hydrophilic macromolecules and lectins; the multifunctional properties of these materials being of particular interest. Target sites investigated within the body include the eye, mouth, oesophagus, stomach and colon. He has published over 80 research papers in this area. He is also led in the development of a research council UK-wide drug delivery research network.

# ORAL ABSTRACTS

# 1 BIODEGRADABLE POLYMERS FOR PROTEIN DELIVERY

**Wim E. Hennink**
Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands

The development of suitable protein delivery systems is one of the recent challenges in the pharmaceutical field. Nowadays proteins are generally administrated by intravenous injection or infusion but because of their poor pharmacokinetics, and repeated administrations are frequently required which is rather inconvenient for the patient and often associated with high costs when the administration has to be done in a clinical setting. Biodegradable polymers offer excellent opportunities for the development of systems that release proteins in a sustained manner. Proteins can be entrapped in biodegradable polymers based on aliphatic polyesters (e.g. poly(lactic acid-co-glycolic acid), PLGA) in the form of injectable micro- or nanospheres suitable for local and systemic administration, respectively. The protein is released in a in a continuous way mainly due to a combination of diffusion via pores present in the particles and degradation of the matrices [1]. As an alternative, proteins can be solubilised in aqueous solution of hydrophilic polymers which after administration form a hydrogel at the site of injection (in forming situ gelling hydrogels). Hydrogel formation is triggered y self-assembling processes and the protein is released by a combination of diffusion though the gel matrix and network degradation [2].

[1] M. Ye, S. Kim, and K. Park. Issues in long-term protein delivery using biodegradable microparticles, Journal of Controlled Release 146, 241-260, 2010.

[2] T. Vermonden, R. Censi, and W.E. Hennink. Hydrogels for protein delivery, Chemical Reviews 112, 2853-2888, 2012.

# 2 TARGETED DELIVERY OF DRUGS VIA THE PEPT1 TRANSPORTER

**Gayle Wilson** and Patrick Bailey
Faculty of Natural Sciences, Keele University, Keele, ST5 5BG, United Kingdom; Email: g.l.wilson@keele.ac.uk

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 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, easily creating 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 acheived.

Work on extending 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. Utilising PEG linkers has also been found to increase solubility, an issue of many drugs with bioavailability issues.

The discovery that PepT1 is over-expressed in pancreatic adenocarcinoma cell lines, AsPc-1 and Capan-2, has also led to research being undertaken into targeted anti-cancer therapy, via our thiodipeptide carriers[5]. Pancreatic cancer often has a poor prognosis, with only a 5% survival rate 5 years after diagnosis. This highlights the need for treatment options in this area.

[1] M. Brandsch, I. Knutter, E. Bosse-Doenecke J. Pharm. Pharmacol. (2008), 60, 543-585.
[2] P. D. Bailey, European Patent Office. (2005), WO2005067978, pp47.
[3] D. Foley, et al. Org. Biomol. Chem. (2009), 7(18), 3652-3656.
[4] D. Foley, P. D. Bailey, et al. Org. Biomol. Chem. (2009), 7(6), 1064-1067.
[5] D. E. Gonzalez, et al. Cancer Res. (1998), 58, 519-25.

# 3 DEVELOPMENT AND OPTIMISATION OF A NOVEL ANTI-INFLAMMATORY NANOPARTICLE FOR THE TREATMENT OF ACUTE LUNG INJURY

**Nooshin Daneshpour,** Michelle Greene, Shaun Spence, Francois Fay, Daniel McAuley, Adrien Kissenpfennig and Christopher Scott
School of Pharmacy, Queen's University Belfast, United Kingdom;
Email: n.daneshpour@qub.ac.uk

Acute Lung Injury (ALI) is a serious condition associated with significant morbidity, mortality and healthcare resource utilization. However, there is no effective treatment yet for acute lung injury. A novel biodegradable and biocompatible Sialic acid-coated PLGA nanoparticle (SA-NP) was prepared, using salting out method. SA-NP size (PS), polydispersity index (PDI) and zeta potential (ZP) was measured by dynamic light scattering. The stability study of the formulation was conducted at -80°C, 4°C and 25°C in PBS and plasma for duration of one week. The anti-inflammatory effect of SA-NPs was evaluated on peritoneal and bone-marrow derived macrophages in vitro, using Enzyme-linked immunosorbent assay (ELISA). Furthermore, the SA-NP was evaluated in vivo in murine ALI model, by measuring cytokine and chemokine concentration in Bronchoalveolar lavage fluid (BALF). The PS, PDI and ZP of SA-NP were measured at about 128 nm, 0.2 and -3 mV respectively. The formulation tested remained stable in PBS at all temperatures tested during the observation period. SA-NP exhibited potent anti-inflammatory effect in vitro by reducing the production of pro-inflammatory cytokine production such as Interleukin 6 (IL6) and Tumor necrosis factor-alpha (TNF-$\alpha$) from macrophages.The induction of TLR signaling by LPS, up-regulates the expression of Siglec-E in murine macrophages. The cell-surface engagement of the SA-NP with Siglec E induces receptor activation and induction of intracellular signaling, resulting in inhibition of the production of potent pro-inflammatory cytokines. There was a significant reduction regarding the cytokine and chemokine concentration in BALF in treated group with SA-NP compared to control group in vivo. In summary, it has demonstrated that SA-NP exhibit potent anti-inflammatory effects in vitro and in vivo.

# 4 UNDERSTANDING THE TRANSIT OF BILOSOMES AND SUBUNIT ANTIGEN VIA THE ORAL ROUTE

**Jitinder Singh Wilkhu,** David Anderson, Yvonne Perrie
[1] School of Life and Health Sciences, Aston University, Aston Triangle, Birmingham, United Kingdom; [2] Variation Biotechnologies INC, 1740 Woodroffe Avenue, Ottawa, Ontario
Email: jitinder@gmail.com

Whilst offering a range of advantages, few vaccines can be administered orally due to their degradation in the harsh gut environment and their poor uptake by appropriate target sites, namely M cells located in the Peyer's patches, which are responsible for secretory IgA and other mucosal responses1.Therefore within this work we have considered the use of bilosomes to enhance the protection and delivery of sub-unit vaccines and tracked their transit through the GI tract after oral administration. H3N2 antigen was radiolabelled with I-125 isotope and was then entrapped into bilosomes [5:4:1 ratio of Monopalmitoyl Glycerol: Cholesterol: Dicetyl Phosphate respectively at 6 µm] vesicles prepared via homogenisation melt method. Results show that the blood, spleen, kidneys and liver have trace amounts of antigen recovery with the majority of the antigen being located in the stomach, Small intestine, and the colon and cecum. Upon entrapping the antigen into bilosomes, significantly ($p < 0.05$) more antigen was located in the small intestine and significantly less was located in the colon and cecum. Of the dose administered, in general significantly ($p < 0.05$) more antigen (50.5%) was recovered when formulated with bilosome vesicles compared to the free antigen dose (38%). In terms of the antigen reaching the site of action, antigen recovery at the Peyer's patches and mesenteric lymph tissue show that the presence of the bilosome vesicles results in a higher recovery of antigen within the Peyer's patches with significantly higher ($p < 0.05$) levels of antigen within the mesenteric lymph tissue when delivered using bilosomes. In conclusion, there is an advantage of associating the H3N2 antigen with the bilosome vesicles as it increases the percentage of antigen recovered within the target site. Studies to consider if these differences translate into enhanced immune responses continue.

CLARK, M. A., JEPSON, M. A. & HIRST, B. H. 2001. Exploiting M cells for drug and vaccine delivery. Advanced Drug Delivery Reviews, 50, 81-106.
Mann, J. F. S., V. A. Ferro, et al. (2004). "Optimisation of a lipid based oral delivery system containing A/Panama influenza haemagglutinin." Vaccine 22(19): 2425-2429.

# 5 NOVEL CONTROLLED RELEASE AMPHOTERICIN B FORMULATIONS: HAEMOLYTIC AND PHARMACOKINETIC STUDIES

**D.R. Serrano[1],** L. Hernández[2], L. Fleire[1], I. González[3], A. Montoya[2], C. Luengo[1], M.P. Ballesteros[1], M.A. Dea[3,4], G. Miró[2], F. Bolás[4], J.J. Torrado[1]

[1] Farmacia y Tecnologia Farmaceutica, Facultad de Farmacia, Universidad Complutense de Madrid, Plaza Ramon y Cajal, 28040 Madrid, Spain, [2] Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense, Madrid, 28040 Madrid, Spain, [3] Departamento de Farmacia, Facultad de Ciencias de la Salud, Universidad Cardenal Herrera-CEU, Moncada, 46113 Valencia, Spain, [4] Departamento de Parasitología, Facultad de Farmacia, Universidad Complutense, Plaza Ramon y Cajal, 28040 Madrid, Spain.
Email: serrano_lopez85@hotmail.com

Amphotericin B (AmB) has a broad antifungal and antiparasitic spectrum. However, its use is limited by its severe nephrotoxicity and haemolytic effect. Novel controlled release formulations based on different encapsulation systems, drug aggregation state and particle size modifications have been developed in order to improve its efficacy/toxicity balance. The toxicity of the novel formulations was assessed by studying the kinetics of the haemolytic process which resulted to be an useful indicator of drug release too. Larger particle size and drug encapsulation into albumin carriers showed a reduction in AmB haemolytic toxicity. Pharmacokinetic studies were performed in beagles dogs with the least hemolytic formulations: poly-aggregated AmB and encapsulated particulate AmB formulations. Both formulations exhibited a very low hemolytic activity similar to the marketed gold standard liposomal formulation: AmBisome®. However, when it comes to the pharmacokinetic profile, both particulate formulations are markedly different from AmBisome®, being characterized by a faster disappearance from bloodstream resulting in a smaller area under the curve and large volume of distribution in tissues. This pharmacokinetic profile could be useful for the treatment of several diseases such as leishmanisis where the parasites are accumulated intracellularly in macrophages of the reticulo-endothelial system. Moreover, it was studied the effect of different parameters on AmB pharmacokinetics, playing a critical role its particle size and aggregation state, drug encapsulation, dose administered, anesthesia and dog's healthy state. In terms of haemolytic toxicity and pharmacokinetic profile, poly-aggregated AmB could be a cost-effective alternative for leishmania therapy.

# 6 DESIGN AND USE OF AUTOMATIC PH CONTROL SYSTEM TO SIMULATE THE ENTIRE GASTROINTESTINAL PH IN A CONVENTIONAL DISSOLUTION APPARATUS

**Hamid A. Merchant** and Abdul W. Basit
UCL School of Pharmacy, University College London 29-39 Brunswick Square, London WC1N 1AX, United kingdom; Email: hamid.merchant@ucl.ac.uk

Dissolution or drug release is one of the most important tests conducted in-vitro in oral drug delivery. A variety of dissolution testing techniques and approaches have evolved with time, the physiological relevance of the media used in the dissolution test has prime importance, in addition to the instrumentation. It is known that the physiological bicarbonate system resembles the pH, buffer capacity and ionic composition of the luminal fluids and has shown to provide improved predictions of the in-vivo behaviour of various enteric-polymers coated products. A difficulty, however, in the use of bicarbonate buffers is their progressive rise in pH due to loss of carbondioxide from the solution and approaches described previously to stabilise such buffer systems are often laborious and time consuming. Here, we present an automatic system which not only stabilises the physiological bicarbonate media and maintains a constant pH over time but also simulates the whole gastrointestinal environment. The desired pH across the gut was successfully achieved and maintained automatically by the system using physiological bicarbonate buffers. The formulations were successfully tested across the full range of gastrointestinal pH, simulating stomach, proximal and distal small intestine and the colonic environment without the need for any physical buffer change using this system. This showed a better prediction of the in-vivo behaviour of the investigated formulations. This new system offers a robust solution to dissolution testing employing physiological bicarbonate buffers and can be adapted to a conventional dissolution apparatus in any laboratory setting. The system provides a more realistic insight of the fate of the dosage forms in human gastrointestinal tract.

7

# BUCCAL DRUG DELIVERY SYSTEMS

**John Smart**
University of Brighton, United Kingdom

Drug delivery via the oral cavity has been developed to allow prolonged localised therapy and enhanced systemic delivery. The oral mucosa, however, while avoiding first-pass effects, is a formidable barrier to drug absorption, especially for biopharmaceutical products (proteins and oligonucleotides) arising from advances in genomics and proteomics. The buccal route, placing the formulation between the buccal mucosa and gingiva, is typically used for extended drug delivery, so formulations that can be attached to the buccal mucosa are favoured whereas the sublingual route is used for drugs requiring a more rapid onset of action. The bioadhesive polymers used in many buccal dosage forms to retain a formulation are typically hydrophilic macromolecules containing numerous hydrogen bonding groups. Newer bioadhesives have been developed and these include modified or new polymers that allow enhanced adhesion and/or drug delivery, in addition to site-specific ligands such as lectins. Over the last 30 years a wide range of formulations has been developed for buccal drug delivery (tablet, patch, liquids and semisolids) with only a few finding their way onto the market. Currently, this route is restricted primarily to the delivery of small lipophilic molecules that readily cross the buccal mucosa. However, this route could become a significant means for the delivery of a range of active agents in the coming years, if the barriers to buccal drug delivery are overcome. In particular, patient acceptability and the successful systemic delivery of large molecules (proteins, oligonucleotides and polysaccharides) via this route remains both a significant opportunity and challenge, and new/improved technologies may be required to address these.

# 8 DIRECT MULTI-NUCLEAR MAGNETIC RESONANCE IMAGING STUDIES OF CONTROLLED DRUG RELEASE

**C. Chen,** LF Gladden, MD Mantle
Department of Chemical Engineering and Biotechnology, University of Cambridge, CB2 3RA, United Kingdom; Email: mdm20@cam.ac.uk

The use of MRI as a tool in pharmaceutical dissolution research is now well established. The majority of the studies have used 1H MRI to acquire signals from water molecules within a pharmaceutical tablet during the dissolution process. In contrast, very few studies have investigated directly the behaviour of the active pharmaceutical ingredients (APIs), since the 1H signal from API is normally obscured by the huge 1H signal associated with the water based dissolution medium. In fact, due to the nuclear specific and non-invasive nature of MRI, the API can be tracked using signatory atoms it possesses. 19F is a very promising candidate for API screening due to its close relevance in the pharmaceutical industry and its high sensitivity in MRI experiments. Thus, MRI shows great potential in revealing the distribution and evolution of APIs under in vitro pharmacopeial dissolution conditions.

In this study, 19F MRI techniques were developed to directly image the drug release process of tablet formulations containing a fluorinated API. Co-registration of the quantitative 1H and 19F MRI enables the visualization of drug egress and water ingress into the polymer matrix simultaneously. It is therefore possible to comprehensively correlate drug release and drug mass transport with polymer matrix swelling and dissolution.

In summary, this work reports the first use of two-dimensional multi-nuclear (19F and 1H) magnetic resonance imaging (MRI) to study both dissolution media ingress and drug egress from a hydrophilic polymer matrix controlled release system in a USP-IV dissolution cell under pharmacopeial conditions. Spatial maps of water penetration, tablet swelling and dissolution, as well as the mobilization and distribution of the drug are quantified and visualized. Such comprehensive information is key for: (i) the correct interpretation of conventional drug dissolution profiles and (ii) the optimal design (QbD, Quality by Design) of controlled release formulations.

# 9 REDUCIBLE POLYMER DNA-HYDROGEL AS A DUAL SWITCHABLE RELEASE GATE

**Giovanna Sicilia,** Christine Grainger-Boultby, Nora Francini, Johannes P. Magnusson, Aram O. Saeed, Francisco Trillo-Fernandez, Sebastian Spain and Cameron Alexander, School of Pharmacy, Boots Science Building, University of Nottingham, University Park, Nottingham NG7 2RD, United kingdom; Email: paxgs3@nottingham.ac.uk

Recently the concept of DNA assembly has been expanded to construct biocompatible stimuli-responsive DNA hydrogels. The ability of DNA hybrid networks to undergo conformational changes based on DNA hybridization and strand displacement mechanisms has been widely analysed at the macroscopic level. However, in order to develop dynamic DNA based hydrogels capable of performing programmable logic operations, a better understanding of the switching capabilities at the microscopic scale is required to generate more flexibility to further applications as biosensors and/or drug/ gene delivery devices.

In the present study, we explore in more detail both the macroscopic and the microscopic phase transitions of a novel dual stimuli responsive polymer-DNA hydrogel that is cross-linked via DNA base pairing and disulphide bonds. By tailoring the swelling properties on disulfide bond disruption and toehold-mediated DNA strand displacement, the hydrogel can function as a programmable logic gate for multiplex detection and sensing or controlled release applications depending on the number of cross-linking units and on the selectivity of the DNA strands.

The hydrogel was synthesised via free radical polymerization. Gel formation and its capabilities to switch between mechanically distinct conformational states in response to a target DNA sequence and/or a reducing agent were evaluated by rheological studies. Microscopic morphological changes of the gel inner structure consequent to the exposure to the dual stimuli were analysed by cryo-scanning electron microscopy. Controllable release properties were investigated by fluorescence spectroscopy following the diffusion rates of FITC-Dextran 150 KDa from the gel matrix. Sensing and signalling abilities were evaluated by fluorescence microscopy. Fluorescent microparticles were chosen as signalling agents and their translocation through gels following exposure to different stimuli was examined.

In all cases directed changes in the cross-linking density caused by the addition of either a target DNA sequence or a reducing agent, allows control over pore sizes in the gel, enabling programmable release and transport of objects ranging from nano- to micro-scale. We conclude Solutol HS15 enhances the absorption of insulin and other macromolecules across epithelial and potentially in a broader sense most human cells as a consequence of changes within the phospholipid bilayer.

# 10 HYDROGEN-BONDED LAYER-BY-LAYER ENTERIC COATINGS FOR ORAL DRUG DELIVERY

**Samuel C. Bizley,** Adrian C. Williams and Vitaliy V. Khutoryanskiy
Reading School of Pharmacy, University of Reading, Whiteknights, PO Box 224, Reading RG6 6AD Berkshire, United Kingdom; Email: s.c.bizley@pgr.reading.ac.uk

The formation of interpolymer complexes (IPCs) between functional polymers has been widely utilised to develop multilayered materials through layer-by-layer (LbL) deposition. This approach involves the exposure of surfaces to alternating solutions of polymers that are able to form insoluble IPCs. Multilayered coatings can be deposited on a range of surfaces with potential applications in drug delivery, biomedicine, electronics, encapsulation and membrane technologies.
The aim of this work is to produce an ultrathin pH-responsive enteric drug coating on a solid drug loaded core with a layer-by-layer polymer complex outer shell to produce a formulation that will remain intact during passage through the stomach and will have release triggered in the small intestine in response to the change in environmental pH.

A hydrogen-bonded layer-by-layer system has been chosen to coat the cores due to its ability to form and dissociate in response to pH. Two appropriate polymers have been selected, namely poly(acrylic acid) (PAA) and methylcellulose (MC), due to their lack of toxicity and ability to form hydrogen bonded complexes. At acidic pHs, the polymers remain in their unionised state and so form interpolymer hydrogen bonds, resulting in a multi-layered coating. An increase in pH causes ionisation of the carboxylic acid groups of the PAA, preventing hydrogen bonding and so initiates a breakdown of the polymer complex.

Particulates containing drugs, specifically antibiotics, have been formulated into solid cores which have then been coated by the polymer combination following a layer-by-layer approach. A range of particle cores have been produced to maximise drug loading and recovery, including classic granule formulations (of various compositions), sodium alginate cores and gelatin constructs. In vitro testing was used to assess drug loading into the cores, stability under simulated gastric conditions and finally drug release in response to dissolution media pH, in addition to physical and chemical characterisation of the layer-by-layer complex itself.

# 11 EVALUATION OF TRANSFERRIN-TARGETED DENDRIMERS FOR GENE DELIVERY TO THE BRAIN

**Sukrut Somani,** David R. Blatchford, Christine Dufès
University of Strathclyde, Glasgow, United Kingdom; Email: sukrut.somani@strath.ac.uk

The treatment of cerebral disorders by gene therapy has been hindered by the presence of the blood-brain barrier that inhibits the entry of therapeutic DNA to the brain and by the lack of gene delivery systems able to efficiently cross this barrier. In order to remediate this problem, we propose to conjugate a highly efficient gene delivery system, polypropylenimine dendrimer, to transferrin, an iron transporter whose receptors are over-expressed on the blood-brain barrier. The objectives of this study are to evaluate the targeting efficacy of this novel transferrin conjugated gene delivery system in vitro and in vivo.

In vitro gene transfection efficiency of the transferrin-conjugated polypropylenimine dendrimer was determined on the cultured immortalized brain capillary endothelial cells. The cellular uptake of the transferrin-conjugated polypropylenimine dendrimer carrying plasmid DNA was observed after different durations of treatment by epifluorescence microscopy in vitro. After optimization of treatment duration, the same technique was utilized for comparison of transferrin-conjugated polypropylenimine dendrimer carrying plasmid DNA with the non-targeted dendrimer and naked DNA.

Transferrin-conjugated polypropylenimine dendrimer led to an enhanced in vitro transfection efficiency 1.2 times higher compared to non-targeted dendrimer. The cellular uptake of the transferrin-conjugated dendrimer carrying plasmid DNA reached its maximum after 120 minutes. Transferrin-conjugated dendrimer carrying plasmid DNA exhibited a higher cellular uptake compared to non-targeted dendrimer and naked DNA.

Transferrin conjugated polypropylenimine dendrimer showed an improved DNA uptake by brain capillary endothelial cells in vitro. This delivery system is therefore promising and should be further investigated.

# 12 SPECIFIC CHALLENGES AND OPPORTUNITIES IN FORMULATING SLOW RELEASE PRODUCTS FOR FARMED RUMINANTS

**Louise Harris**
The University of Sunderland, Sunderland SR1 3SD, United Kingdom;
Email: l.harris@sunderland.ac.uk

Slow release products, for Ruminants, are a must. Cows, sheep and goats are ruminants exploited profitably. Optimal performance is desired from these animals, as they are heavily relied on for their produce. They differ from monogastric animals by having a compartmental stomach, comprised of the Rumen; Reticulum; Osmasum and Abomasum. This means new applications/product designs have to be carefully selected. This area lacks in research and entrepreneurial design, due to the specialist approach required to resolve some of the issues surrounding the Rumen's biological processing of pharmaceuticals.
The choice of administration methods varies, depending on species, the deviation from release desired, the chemical release profile, dose required, the administering labour induced and cost of new products has to be kept minimal, appealing to the buyer – who will require it in large quantities due to the nature of faming on large scales.

This business is heavily dominated, by nutri-pharmaceuticals. Supplements are dominating the market, to improve animal production (milk yield, muscle mass and reproductive health); through an efficient supply of nutrients.
Rationale for delivery exploited: pH, rumen conditioning, addition of yeast, and adding organic sources as an alternative to cheap inorganic sources - for efficiency. The types of slow release seen are, intra-ruminal; reservoir, sustained, erosion / osmotic and post-rumen. Polymeric pH-dependent rumen-stable delivery systems have been developed and commercialized for other fields.
Furthermore, post-rumen delivery systems are needed that will protect the active ingredient from rumanal fermentation. These can pass through the rumen unchanged until they reach the GI tract Adsorption from here is more appropriate as it will then provide benefit to the animal and not the Rumen micro flora/ bacteria.

Other opportunities of systems, which in other fields have found success are, Hydrogel (biodegradable/non-biodegradable); Fullerenes; surfactants based carriers (hydrophobic components partition to the hydrophobic core of micelles) and emulsions.

# 13 INVESTIGATION OF SUGAR ESTERS AS NOVEL INTESTINAL PERMEATION ENHANCERS

**F. McCartney[1],** M. Rosa[2], I. Coulter[2], & D.J. Brayden[1]
[1] School of Veterinary Medicine, University College Dublin; [2] Sigmoid Pharmaceuticals, Invent Centre, Dublin City University; Email: fiona.mccartney@ucdconnect.ie

The aim was to investigate novel intestinal permeation enhancers (PEs) for poorly absorbed molecules, a major goal of the biotech industry. Permeation enhancers are added to oral drug formulations since intestinal absorption of peptides, proteins and macromolecules is difficult due to intestinal metabolism, poor membrane permeability and high intra-subject variability. They work using mechanisms related to boosting the paracellular and/or the transcellular routes across intestinal epithelia. While some PEs including carnitines and sodium caprate are in clinical trials, none have been approved as enhancers to date. Sucrose esters are non-ionic surfactants, consisting of sucrose and a fatty acid, and they are already established as safe emulsifiers and solubilisers in the food, cosmetics and pharmaceutical industry. A medium chain sucrose ester with a hydrophilic-lipophilic balance of 16 was studied. The permeation enhancement potential of the sucrose ester was tested using Ussing chambers. Isolated rat colonic mucosae were mounted and apical-side additions of concentrations ranging from 1.5mM to 10mM sucrose ester were tested. The transepithelial electrical resistance (TEER) and apparent permeability (Papp) of paracellular marker molecules, [14C] mannitol and FITC-Dextran 4kDa (FD4), were measured over 120 min. The transepithelial potential difference (PD, mv) and short circuit current (Isc, μA) were determined and TEER was calculated using Ohm's law. The tissue was voltage clamped to zero for 30 sec and switched to open circuit configuration for 3 sec. Apical addition of the sucrose ester showed a significant and concentration-dependent decrease in TEER associated with an increase in the Papp values of both [14C] mannitol and FD4. No major damage was seen in intestinal histology following exposure for 120 min. This data suggests that the tight junctions are being opened thereby allowing paracellular passage of the marker molecules. The sucrose ester may have potential as a non-toxic intestinal permeation enhancer for poorly absorbed molecules.

Co-funded by Science Foundation Ireland grant number 07/SRC/B1154 and Sigmoid Pharmaceuticals

# POSTER ABSTRACTS

# 14 GOLD NANOPARTICLE INCORPORATED LIPOSOMES FOR DETECTION OF PROTEINS FOR UTILISATION WITHIN A NOVEL IN VITRO ASSAY

**Sagida Bibi,** Afzal Mohammed and Yvonne Perrie
Aston University, Aston Triangle, Birmingham, B4 7ET, United Kingdom;
Email: s.bibi8@aston.ac.uk

The aim of this work is to establish the feasibility of utilising gold nanoparticles within liposomes as a means of absorbing the analyte of interest for a novel immune assay. ELISA assays are used ubiquitously as a means for measuring secreted proteins and antibodies of supernatants from cells, such released analytes then bind to the bottom of the coated plate. However by incorporating coated gold nanoparticles which have the ability to covalently bind proteins and antibodies; low level analytes can attach directly to the liposomes. This would allow a higher level of binding to occur and thus increase the sensitivity of the assay. 16 µM Phosphatidylcholine liposomes were prepared by dry film lipid hydration in which gold nanoparticles were incorporated into the bilayer. Characterisation of these modified vesicles for size and zeta potential was carried out alongside control liposomes. For three prepared batches it was found that there was no significant difference in size (6.73µM± 0.4 for gold incorporated and 7.71± 1.15 for the control formulations) although the zeta potential was slightly more negative than control liposomes (-10.02± 8.3 for control and -13.6±6.03 for gold incorporated liposomes). Morphological observations using light microscopy confirmed that neither liposome size nor morphology of the vesicles was affected. The nanoparticles themselves are not visible as they are 40nm and thus beyond the resolution of light microscopy. The liposomes were then incubated with BSA (bovine serum albumin) with conjugated FITC (Fluorescein isothiocyanate). Using confocal microscopy the associated BSA-FITC was assessed for both control and gold nanoparticle modified liposomes. Images obtain confirm a higher level of associated protein with liposomes containing gold nanoparticles in the bilayer. Infra-red spectrums of both samples also confirm that the presence of the gold nanoparticles does not cause any chemical changes with the liposomes and are simply physically incorporated within the external bilayer. Overall the results show that the incorporating coated gold nanoparticles into liposomes could increase the sensitivity of assays which assess low level analytes and thus offer a novel route for quantification of immune responses.

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This work was funded by TRANSVAC project number 228403

# 15 CONTROLLED SYNTHESIS OF LIMIT SIZE CATIONIC LIPOSOMES BY MICROFLUIDIC MIXING

**Elisabeth Kastner,** Randip Kaur, Alexander Wilkinson and Yvonne Perrie
Aston Pharmacy School, Aston University, Aston Triangle, Birmingham, B4 7ET, United Kingdom; Email: kastnere@aston.ac.uk

The size of liposomes significantly affects biodistribution and is tailored to their application; liposome size for efficient drug delivery varies due to constraints governing the penetration of the target tissue. Despite the great potential of liposomes as delivery vehicles and immune potentiators, large scale manufacturing of liposomes raises problems. Traditional liposome synthesis methods often require mechanical stresses like sonication, high-shear homogenisation or high pressures which are difficult to upscale. However, current requirements of the pharmaceutical industry necessitate the robust, reproducible and scalable production of size-uniform vesicles. The aim of this work was to determine whether a staggered herringbone micromixer was capable of producing limit size liposomes. Liposomes were synthesized using the automated Nanoassemblr (Precision NanoSystems, Inc). Streams of dissolved lipids and aqueous buffer were injected into separate chamber inlets, followed by rapid mixing of both streams due to the chamber design. The automated system allowed for controlled alterations in flow rates and flow rate ratios. Additionally the possibility of drug loading incorporated into the liposome synthesis process was investigated. Empty liposomes of 50 nm could be generated via controlled alterations in flow rate and flow rate ratios whilst retaining surface charge characteristics. Increased mixing rates are believed to contribute to the rapid increase in polarity of the medium driving nanoprecipitation reaction and liposome formation. Loading studies implied size and charge alterations based on molecular rearrangement with generally larger vesicle sizes compared to empty liposomes. Work presented emphasizes the potential of biomicrofluidics and process control by novel lab-on-a-chip based applications. Underlined by advantages of the automated microfluidic mixing device, we expect microfluidic mixing to become the preferred method of choice for reproducible and controlled bottom-up liposome synthesis of desired dimensions.

# 16 Regression of prostate tumours following intravenous administration of tumour-targeted gene therapeutic systems

**Majed Al Robaian,** Ker Yi Chiam, David R. Blatchford, Christine Dufès
University of Strathclyde, Glasgow, United Kingdon; E: majed.al-robaian@strath.ac.uk

Prostate cancer is one of the most commonly diagnosed cancers in men and remains the second leading cause of cancer-related deaths in industrial countries. To date, there is still no efficacious treatment for patients with advanced prostate cancer with metastases. New treatments are therefore critically needed for these patients. Gene therapy holds great promise for the intravenous treatment of prostate cancer. However, its use is currently limited by the lack of delivery systems able to selectively deliver therapeutic genes to tumours by intravenous administration. In this study, we demonstrated that new tumour-targeted therapeutic systems recognizing receptors specifically overexpressed on prostate tumours, were able to improve the in vitro therapeutic efficacy on PC-3, DU145 and LNCaP prostate cancer cells when compared to the non-targeted delivery system, by up to 100-fold in LnCaP cells. In vivo, the intravenous administration of the tumour-targeted therapeutic system encoding Tumour Necrosis Factor (TNF)α resulted in tumour suppression for 60% of PC-3 and 50% of DU145 tumours. When using a therapeutic DNA encoding Tumour necrosis factor-Related Apoptosis-Inducing Ligand (TRAIL), it also led to tumour suppression of 10% of PC-3 tumours. When using a therapeutic DNA encoding Interleukin (IL)-12, it resulted in tumour regression of 20% of PC-3 and DU145 tumours. The treatment was well tolerated, with no apparent signs of toxicity. These tumour-targeted therapeutic systems therefore hold great potential as a novel approach for the gene therapy of prostate cancer.

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# Novel Route of Administration for Aquasomes

**Fadi A. Abdulrazzaq,** Yvonne Perrie, and Deborah Lowry
Aston University, Birmingham, B4 7ET, United Kingdom; Email: abdulrff@aston.ac.uk

Aquasomes are recently developed 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. The purpose of this study is to show if aquasomes are able to prolong the release of BSA if administered by the pulmonary route. The hydroxyapatite solid cores were coated by mixing with a trehalose solution (0.1 M) for 1.5 hrs at 4oC followed by freeze-drying. The coated cores were then loaded by mixing with a BSA solution of 1 mg/mL for 1.5 hrs at 4oC followed by freeze-drying. Aquasomes were then characterized for size and zeta potential to confirm the coating and loading steps. The pMDI formulation was prepared by weighing 100mg of aquasomes (equivalent to 7.3mg BSA) into aluminium pMDI vials. The vials were then crimped with 30 μL valve and filled with approximately 10 mg HFA-134a. The in vitro aerosolisation of aquasomes was performed using the Next Generation Impactor (NGI) followed by In vitro release studies of aquasomes collected at stages 3 (2.82 μm) and 5 (0.94 μm) using Gamble's solution as the release medium. Zeta potential values were calculated after the coating (-1 ± 1) and loading (-11.6 ± 0.5) stages. An estimate of 60% of the delivered dose has a cut-off diameter of 2.82 μm. Release studies have shown that the steady release observed continues over the 6 hour study and the aquasomes with an aerodynamic diameter of 0.94 μm released 424 μg of BSA. Such results are promising for potential protein/peptide delivery via the pulmonary route using aquasomes. In conclusion, in vitro release studies of BSA show steady release from aquasomes with cut-off diameters of 2.82 μm. Further studies are being performed to optimize drug delivery over extended periods of release.

# 18 JANUS DENDRIMERS AS CONTROLLED MULTIDRUG DELIVERY SYSTEMS

**Aaron L. Acton**[1], Cristina Fante[2], Francesca Greco[2] and Wayne Hayes[1]
[1]Department of Chemistry, [2]Reading School of Pharmacy, University of Reading, Whiteknights, Reading, United Kingdom; Email: a.acton@pgr.reading.ac.uk

Polymer-drug conjugates are nano-sized assemblies that enable targeted delivery of highly potent yet frequently insoluble drugs to specific sites within the body by covalent conjugation of the drug to a hydrophilic polymeric backbone.1 In recent years, numerous polymer-drug conjugates carrying a combination of therapeutic agents attached to the same polymeric carrier have been developed.2 Such conjugates display an increased activity compared to administration of a combination of parent conjugates carrying a single agent.3 As yet, there are no combination delivery systems that enable exact control over the loading ratio of two different drugs to the same polymeric carrier. To this end, we propose a strategy to exploit the known advantages of using dendrimers for biomedical applications4 and have subsequently developed a series of novel dendritic delivery systems that enable exact control over the loading ratio of two different model therapeutic agents, which can be released at varying rates.

A series of PEG-based dendrimer combination delivery systems have been synthesised.5 The dendrimers involve first (G1) and second (G2) generation PEG-dendrons that incorporate amide branching linkages and two different model drugs attached to the dendron periphery through physiologically degradable carbonate and ester linkages. A combination of G1 and G2 dendrons were coupled together using [3+2] cycloaddition chemistries between alkyne and azide units to afford four Janus dendrimers with varied loading of each model drug (either 2 or 4) which can be released at different rates. Preliminary release studies were investigated in human plasma at 37 °C and the dendrimer-drug conjugates displayed release in varying concentrations dependent on the ratio of model drug loading. The dendrimers and their metabolites were non toxic as shown by cytotoxicity studies against HUVEC cells (human endothelial cell line) and haemolysis studies using red blood cells (RBCs) taken from human.

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# 19 PLURONIC-BASED IN SITU GELLING SYSTEMS FOR OCULAR DRUG DELIVERY

**Kosai Al Khateb,** Wing-Man Lau, Vitaliy Khutoryanskiy
Reading School of Pharmacy, University of Reading, Whiteknights, PO Box 224, Reading RG6 6AD, United Kingdom; Email: K.Alkhateb@pgr.reading.ac.uk

Inadequate ocular drug delivery results mainly from excessive pre-corneal loss, non-corneal absorption and poor corneal penetration. These factors greatly hinder corneal absorption of the drug and pose the challenge of enhancing ophthalmic dosage forms to achieve better drug delivery. Drug bioavailability can be improved by using in situ gelling systems which undergo a sol/gel phase transition triggered by the surrounding environment. Such systems have the advantage of enhancing the retention time of the instilled drug and, thus, improving its ocular absorption. Poloxamers (Pluronic®/Lutrol®) are triblock copolymers with a hydrophobic centre and two hydrophilic arms. They form thermo-reversible gelling systems that undergo a reversible sol/gel transition at certain "gelation" temperatures. With increasing temperature, Pluronics first form micelles which rearrange to form a gel structure above their gelation temperatures.

In the present work, the micellation and gelation behaviours of binary systems containing different concentrations of Pluronic F127 and Pluronic F68 were studied using differential scanning calorimetry (DSC), rheological measurements and dynamic light scattering (DLS). Two groups of samples were prepared: the first with 20% (w/v) Pluronic F127 and varying F68 concentrations, the second contained different concentrations of both polymers with the total system concentration fixed at 20% (w/v). The DSC study established that the micellation temperature (Tmf) of the first group (fixed F127 concentration) decreased with increasing concentrations of the relatively more hydrophilic Pluronic F68. On the other hand, the second group (fixed total system concentration) showed a fixed Tmf for all tested samples. In the rheological study, increasing F68 concentration led to an increase in gelation temperature (Tg) in the first group. The same behaviour was observed in the second group as F68 concentration was increased at the expense of F127. To further elucidate the gel formation process, micelle sizes were studied using DLS. It was observed that increasing the temperature was marked by an increase in the micellar diameter for each system. On the other hand, the micellar diameter was not affected by the composition of the binary system at any given temperature. Ofloxacin will be used as a model drug in order to study the feasibility of these systems in enhancing ocular drug delivery.

# 20 MULTIFUNCTIONAL EXCIPIENTS IN ODTS: INSIGHTS INTO NANOSCOPIC AND PHYSICO-MECHANICAL PROPERTIES

**Ali Al-khattawi[1,3],** Hamad Alyami[1], Bill Townsend[2,3], Xianghong Ma[2,3] & Afzal R Mohammed[1,3]

[1] Aston Pharmacy School, [2] School of Engineering and Applied Science, [3] Aston Research Centre for Healthy Ageing, Aston University, Birmingham, B4 7ET, United Kingdom;
Email: alkhaaaa@aston.ac.uk

The inclusion of excipient in formulations has seen an evolution from the traditional concept of inert component to the functional and essential constituent of pharmaceutical dosage forms. For compressed orally disintegrating tablets (ODTs), the informed choice of excipients is of prime importance as it determines essential product properties such as mechanical strength and fast disintegration [1]. To accelerate preformulation development of compressed ODTs, the work investigates the adhesive/cohesive interactions and nanoscopic features of multifunctional ODT excipients, MCC and mannitol. This helps to elucidate the main powder densification mechanisms responsible for optimum product functionality. Using AFM, nano adhesion forces were measured between pairs of particles by recording data from force-displacement curves. Each pair comprises one particle adhered on the AFM cantilever tip and another in a plate on the microscope stage. Surface topography images (100 $nm^2$-10 $\mu m^2$) and roughness data (Ra and Rq) were acquired from AFM. All AFM data were correlated to the bulk tablet properties obtained from traditional analytical techniques. The utilization of AFM capabilities to deliver topographical and adhesion information was valuable to understand the exact mechanisms for tablet formation. The study for the first time has concluded that a strong correlation exists between adhesive forces of particles and the resultant mechanical properties. MCC micro roughness was three times that of mannitol which explains the high hardness of MCC tablets as a result of mechanical interlocking. On the other hand, mannitol produced poor tablets due to fragmentation of surface crystallites during compression. Furthermore, AFM analysis has shown the presence of extensive nano fibril structures inhabiting long porous channels which further supports the use of MCC as a disintegrant. Overall, the study of few particles (<10 $\mu g$) using AFM potentially provides us with a significant tool to determine bonding mechanisms of excipients and drugs during compression to enhance the development of novel pharmaceutical formulations.

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# 21 STABILITY OF DRIED CAPSULES CONTAINING A MODEL OF PROBIOTIC DURING STORAGE

**H Albadran** [1], D Charalampopoulos [1] and V Khutoryanskiy [2]
[1] Department of Food and Nutritional Science, University of Reading, Whitenights, Reading, RG6 6AD United Kingdom. [2] School of Pharmacy, University of Reading, Whitenights, PO Box 224, Reading, RG6 6AD United Kingdom; Email: h.a.a.albadran@student.reading.ac.uk

Microencapsulation can be used to protect probiotic microorganisms from adverse environmental conditions, for example during storage in either dried form or in a liquid formulation, or during passage through the gastrointestinal tract. This study investigates the stability of freeze dried and fluid bed dried alginate/chitosan microcapsules containing a model of probiotic bacterium (Lactobacillus plantarum) during storage at different relative humidity and temperatures.
In order to encapsulate the cells, the extrusion technique was used which involved the extrusion of cell suspension in alginate (2% w/v in water); the microcapsules were then coated with chitosan (0.4% w/v in acetic acid). The coated microcapsules were then either freeze dried or fluid bed dried (at 35°C for 60 mins) and stored at 11% and 23% relative humidity at 30 and 37°C for up to 45 days. Two controls were used, free cells and free cells with 10% sucrose after freeze drying
After 45 days storing the viability of dried encapsulated bacteria and free cells with and without 10% sucrose that had R.H 11% at 30C were better than when R.H 11% and 23% at 37°C and R.H 23% at 30°C. However, the viability of the same dropped faster when R.H 23% at 37C. The viability of L. plantarum in the microcapsules that were dried using fluid bed drier had a loss of 0.63 log CFU/mL, while when using freeze drier caused more loss in the viability around 1.05 log CFU/mL. Moreover, the loss of viability was much faster when freeze drier was used either for dried encapsulated L. plantarum or as free cells. The reduction in the size of capsules using fluid bed drier (45%) was higher as compared to freeze drier (33%).
Using fluid bed drier as a technique to dry encapsulated bacteria gave better survival than using freeze drier even during storage; the physical characteristics of the microcapsules were better as well. In conclusion, the results suggest the potential use of fluid bed drier as a promising method to dry probiotic containing microcapsules.

# 22 EVALUATION OF MUCOADHESIVE INTERACTIONS BETWEEN HYDROPHILIC POLYMERS AND PORCINE GASTRIC MUCIN IN AQUEOUS MEDIA

**Y Albarkah,** R Green, V Khutoryanskiy
School of Pharmacy, University of Reading, Whiteknights, PO Box 224, Reading, RG6 6AD, United Kingdom; Email: y.a.s.albarkah@pgr.reading.ac.uk

Mucoadhesive drug delivery systems have attracted attention for their potential to optimize localized drug delivery by retaining the dosage form at the site of action. A number of studies had investigated the feasibility of using different polymers as mucoadhesives. Several factors such as the chemical nature of polymers, their molecular weight, concentration and environment (ionic content and pH) play very important role in adhesion of drug carrier systems to mucous membranes. In this work we have studied the colloidal properties of porcine gastric mucin in aqueous dispersions and its interactions with poly(acrylic acid)(PAA, 450 kDa), polyethylene oxide (PEO, 1000 kDa) and polyethylene glycol (PEG, 10 kDa).
Dynamic light scattering (DLS) was used to measure mucin particle size and its aggregation in the presence of polymers. Isothermal titration calorimetry (ITC) was used to determine the thermodynamic parameters of interactions between the mucin and the polymers. Turbidimetric titration was also used to follow aggregation of mucin particles in the presence of polymers.

It was established that porcine gastric mucin is a polydisperse colloidal system with bimodal particle size distribution (106±2 and 615±5 nm). The addition of PAA causes the aggregation of mucin particles and this interaction is an exothermic process at pH 3.0. The nature of this interaction is believed to be H–bonding formed between carboxylic functional groups of PAA and hydroxyl groups present in mucin. On the contrary the addition of PEG and PEO does not result in any aggregation of mucin, indicating a lack of specific interactions.

# 23 DEVELOPMENT OF NANOPARTICLES BASED APPROACH FOR TARGETING BASOPHILS AND MAST CELLS

**Walla Alelwani,** Franco H. Falcone and Snjezana Stolnik
School of Pharmacy, University of Nottingham, University Park, Nottingham, NG7 2RD, United Kingdom; Email: paxwa1@nottingham.ac.uk

Activation of mast cells and basophils by the clustering at least two high affinity receptors (FcεRI) by IgE bound to multivalent antigen induces signaling cascades leading to mediator release such as histamine (Bevil et al., 2006). The release of this mediator leads to inflammatory and allergic symptoms (Passante and Frankish, 2009). Here we describe the design of a nano-system as a potential drug carrier capable of targeting basophils and mast cells via FcεRI, which is restricted to these cell types. This will be performed via surface modification of model polystyrene nanoparticles (PS NPs) by attachment of two antibodies, IgE and IgG.

The aim of the present work is to: detect mediator release of rat basophilic leukemia (RBL-2H3) cells under various conditions in addition to defining the optimal condition for the degranulation assay which would suit both NPs colloidal stability as well as optimal mediator release; establish a formulation procedure for surface modification of PS NPs with antibody (BSA used as a model) that achieves colloidal stability; and Investigate basophils degranulation when PS NPs alone, NPs coated with IgE, DNP-BSA and NPs-DNP-BSA- IgE conjugate is applied to RBL-2H3 cells.

# 24 USE OF DVS-NEAR IR TECHNIQUE FOR MATERIAL CHARACTERIZATION

**Phil Attwool,** Manaswini Acharya, Steve Taylor, Majid Naderi, Dan Burnett
Surface Measurement Systems Ltd, United Kingdom

The combination of dynamic vapour sorption and near infrared spectroscopy ( DVS- NIR) has been considered a novel technique for studying various materials and making in-depth analysis into their sorption/desorption properties. The investigation under this technique would provide a better understanding of solid-state reactions and establish the relationship between the stability of materials and their structural changes. In this study, DVS- NIR studies were performed on Avicel, Spray Dried Lactose, Spray Dried Salbutamol Sulphate, and Nafion Membranes by using DVS, gravimetrically, an automated temperature and humidity controlled ultra-balance system coupled with a near infrared spectrophotometer (NIR). The results show that the use of gravimetric analysis together with NIR spectroscopy provides information about the dynamics of water interaction with various materials.

# 25 ENCAPSULATION OF AN P38 INHIBITOR IN NANOPARTICLES FOR PULMONARY DELIVERY.

**Bains, BK**[1], Farrow, SN[2], Scott, JC[1]
[1] Molecular Therapeutics, School of Pharmacy, Queens University, Belfast, United Kingdom;
[2] GlaxoSmithKline, Stevenage, Hertfordshire, United Kingdom; Email: b.bains@qub.ac.uk

p38 mitogen-activated protein kinase (MAPK) signalling is known to be increased in inflammatory diseases including asthma, chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS) and cystic fibrosis (CF). Current anti-inflammatory steroid based therapy is not effective in all patients with these diseases. The successful formulation and administration of a p38 MAPK inhibitor could potentially advance treatment options where there are sub-optimal responses to steroid therapy. In these situations on-going inflammation is a major driver of disease progression and morbidity.
We aim to prepare therapeutic agent entrapped poly(lactic-co-glycolic acid) (PLGA) nanoparticles using a single emulsion solvent evaporation method and the model p38 MAPK inhibitor SB203580. Oil in water emulsions (o/w) were prepared by dissolving PLGA polymer in dichloromethane. SB203580 dissolved in dimethyl sulfoxide (DMSO) was then added to this organic phase and pipetted drop wise into the aqueous phase containing polyvinyl alcohol (PVA) in MES buffer. The o/w emulsions were sonicated and stirred for 3 hours. PVA was removed from the nanoparticles using a sequential wash method. Particles where characterised for size and entrapment efficiency. To assess biological functionality, lipopolysaccharide (LPS) –stimulated murine bone marrow derived macrophages were cultured with SB203580, as either a free drug or entrapped in a PLGA nanoparticle. Down regulation of inflammatory cytokines tumor necrosis factor (TNF) $\alpha$ and interleukin 6 (IL6) was assessed using Enzyme-linked immunosorbent assay (ELISA).

We have demonstrated that a p38 MAPK inhibitor can be successfully entrapped and released from a polymer based nanoparticle system. Biological assessment revealed that the therapeutic nanoparticles successfully retained their functionality. Particles prepared by this method have the potential for the controlled release delivery of p38 MAPK inhibitor for a multitude of inflammatory based diseases.

# 26 EFFECT OF NANOPARTICLE SURFACE CHARGE ON INTERACTION WITH EPITHELIAL CELLS

**Azzah Bannunah,** Driton Vllasaliu and Snjezana Stolnik
Division of Drug Delivery and Tissue Engineering, School of Pharmacy, University of Nottingham, Nottingham, NG7 2RD, United Kingdom; Email: paxab9@nottingham.ac.uk

The interaction of nanoparticulates designed for drug delivery (eg polymeric nanoparticles, liposomes or emulsions) with cells is governed by the nature of their size, shape and surface characteristics. The surface properties, including charge, surface curvature, surface hydrophilicity/hydrophobicity and attachment of targeting moieties have extensively been exploited with the aim to provide opportunities for site-specific drug delivery.

The aim of the present work is to assess the effect of nanoparticle surface charge on their interaction with epithelial cells using an in vitro intestinal model based on Caco-2 (human colon carcinoma) cells cultured on permeable membranes. In this model, Caco-2 cells form polarised monolayers of differentiated cells with intercellular tight junction structures, presenting a physical and biochemical barrier to the passage of material such as macromolecules and nanoparticles2. Work found that cell uptake and transport of positively charged (aminated) and negatively charged (carboxylated) nanoparticles was concentration, size and temperature dependent. Cellular uptake and transport was increased as the concentration of applied nanoparticles increased. Cell internalisation and transport was studied for 50 nm and 100 nm nanoparticles. Cell uptake and transport were dependent on temperature, with notably larger uptake and transport observed at normal cell culture conditions 37°C compared to 4°C, hence indicating an energy-dependent process. Calculating nanoparticle transport efficiency (ratio of transported versus internalised nanoparticles), we found that negatively charged carboxylated nanoparticles are more efficiently transported across the cells compared to positively charged aminated nanoparticles. These findings show that size and surface characteristics of nanoparticles dramatically affect their interaction with epithelial cells. This work is therefore important in the field of oral drug delivery of biotherapeutics, where nanoparticle carriers have been suggested as drug carriers potentially crossing the mucosal barrier.

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# 27 ORAL FORMULATION OF AMBIENT DRIED LIVE BACTERIAL CELLS OFFERS NO LOSS IN VIABILITY DURING GASTRO-INTESTINAL DELIVERY

**J. Barros,** D. Charalampopoulos, V. Khutoryanskiy1 and A. Edwards
University of Reading, Reading, Berkshire, RG6 6AD, United Kingdom;
Email: j.barros@pgr.reading.ac.uk

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 and to carried heterologous antigens, thus protecting against a wide range of infections. A new formulation of LBC that would offer the potential for oral administration, maintain stability without refrigeration, with a low-cost, simple, manufacturing process would be very advantageous for the world health and vaccination program.

Preservation of microorganisms by desiccation is required for long term storage. Dehydration can damage cells through osmotic and oxidative stress and denaturation of biomolecules. Our aim is to develop a thermo-stable formulation that maximizes cell survival during gastro intestinal delivery and gives controled release of live bacterial vaccines to the small intestine enabling the appropriate immune response.

Preliminary studies have produced enteric formulations of live bacterial cells mixed in protective agents using an enteric polymer thin film surface. These gave high viable cell recovery after room temperature drying and after acidic environment challenge. Further optimisation to develop the 'ideal' oral vaccine or probiotic formulation shows proof of concept. Latest in vitro studies present a final oral dose form of LBC that offers protection from acid during gastric transit and release live cells to the small intestine with no loss in viability.

# 28 DEVELOPMENT OF PHOTOSENSITIVE LIPOSOMES FOR CONTROLLED DRUG RELEASE

**Pranav Bhujbal,** Qinguo Zheng, Yvonne Perrie
LHS, Aston University, Birmingham, West Midland, B4 7ET, United Kingdom;
Email: bhujbalp@aston.ac.uk

The facility to control triggered release from a "Cage" system remains a key requirement for novel drug delivery. There are ranges of triggers available including activation with specific wavelengths of light which can allow the controlled release of drugs and active moieties from systems such as liposomes. The main objective of this work is to design photosensitive liposomes by means of incorporation of a photosensitive lipid analogue – i.e., Bis azo phosphatidycholine into the liposome bilayer. Further, these photo-sensitive liposomes can be used for the triggered release of entrapped BSA-FITC (Bovine serum albumin conjugated with Fluorescence Isothiocyanate). So far, the synthesis of the photochromic phospholipid 1,2-bis (4-(n-butyl) phenylazo-4-phenylbutyroyl) phosphatidylcholine (Bis Azo PC) was done by the method of Sandhu et al. (1986). Briefly, an azobenzene-containing acid was prepared by condensation of 4-(n-butyl)nitrosobenzene with 4-(p-amino phenyl)butyric acid). Further, the azobenzene-containing acid was converted to phospholipid by a mixed anhydride acylation of L-α-glycerophosporylcholine using 4-(dimethylamino) pyridine as a catalyst. The Bis Azo PC was characterised by Nuclear magnetic resonance spectroscopy, IR and UV spectroscopy. The phase behaviour of Bis-Azo PC has been investigated by fluorescence spectroscopy. Bis Azo PC was reversibly photochromatic, isomerising on exposure to ultraviolet light to a cis isomer. Future work will investigate incorporation of this Bis Azo Phosphatidylcholine into a range of DPPC:Cholesterol liposomal formulations and characterise them. The aim is to demonstrate effective BSA-FITC retention and triggered release from liposomes which may have the potential of finding clinical applications as an intelligent drug delivery system. The advantage of this strategy is that the precise location and time of drug delivery could be controlled externally, which could potentially improve drug efficacy by minimizing drug dose to be administered and thereby the side effects.

Sandhu, S.S., et al., (1986). Biochimica et Biophysica Acta, 860, p.253-262

# 29 PREPARATION AND CHARACTERISATION OF NOVEL MEDICATED DRESSINGS FOR CHRONIC WOUND HEALING

**Joshua Boateng,** H.V. Pawar and John Tetteh
Department of Pharmaceutical, Chemical & Environmental Sciences, School of Science, University of Greenwich at Medway, Central Avenue, Chatham Maritime, ME4 4TB, Kent, United Kingdom; Email: J.S.Boateng@gre.ac.uk

The project aimed to develop and characterise lyophilised Polyox™ (POL) and carrageenan (CAR) wafers loaded with streptomycin (STP) and diclofenac (DLF) to target two phases of chronic wounds for effective healing.
Method: Wafers were prepared from gels containing POL and CAR in a 75/25 ratio (1% total polymer weight) and loaded with 30% w/w STP and 25% w/w DLF. Gels were freeze-dried using a cycle incorporating an annealing step (determined by differential scanning calorimetry). Wafers were characterised for morphology (SEM), mechanical strength and mucoadhesion (Texture analysis), swelling and in vitro and drug release properties.

Annealing helped to improve the porosity resulting in POL-CAR wafers that were adequately flexible, elegant in appearance and non-brittle in nature. Surface morphology of the blank (BLK) wafers showed a homogeneous, sponge-like circular and interconnecting network, whereas the drug loaded (DL) wafers showed porous polymeric strands with decreased porosity due to the entrapment of drug within the polymer network. Differences in swelling, mucoadhesion and in vitro drug release characteristics, could be attributed to differences in pore size and drug present in the wafers. DL wafers showed less swelling capacity compared to BLK though the DL wafers showed constant increase in swelling capacity. BLK wafers exhibited high stickiness, work of adhesion and cohesiveness in the presence of normal (2%BSA) and viscous (5% BSA) simulated wound fluid (SWF). A maximum of 81.37±3.81% of STP was released within 24 hours whilst a maximum of 73.58±3.77% DLF was released within 72 hours.

Wafers containing both STP and DLF have been formulated and optimised. STP can prevent as well as treat bacterial infection within a wound whilst DLF can target the inflammatory phase of wound healing to relieve pain associated with injury. The optimised wafer has the potential to achieve more rapid wound healing.

# 30 BIOADHESIVE POLYMERS AS AGROCHEMICAL FORMULANTS

**Brett L. Symonds**[1], Vitaliy Khutoryanskiy[1], Niall Thomson[2]
[1] Reading School of Pharmacy, University of Reading, Whiteknights, PO Box 224, Reading, RG6 6AD, United Kingdom; [2] Formulation Technology Group, Jealott's Hill International Research Centre, Bracknell, RG42 6EY, United Kingdom;
Email: b.symonds@pgr.reading.ac.uk

The world faces the significant challenge of securing a sustainable supply of food for a growing population. This challenge must be met with less arable land per person and with fewer resources. It is estimated that by 2050 the world's population will reach 9 billion and as a result arable land will shrink to 0.15 hectares per person from 0.23 in the year 2000.1,2 Agrochemicals increase efficiency of food production by minimising the impact of pests on crop yield. However, up to 90% of the sprayed product may not reach the target site.3 This reduced efficiency has been accredited to poor penetration through the plant cuticle, photolytic, hydrolytic, microbial degradation and wash-off due to rain.4

There exists a need to determine quantitatively the adhesive properties of agrochemical formulations to plant surfaces. This can be related to the formulation's property of rain fastness. Formulations with greater resistance to wash-off by rain are more desirable for several reasons; for example, they may require less frequent applications or less chemical to be applied. This project will identify methods for determining rainfastness and develop understanding of polymer functionality facilitates bioadhesion. In this contribution initial results are reported.

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# 31 CHARACTERISATION OF CLONIDINE HYDROCHLORIDE ORAL FILMS PREPARED BY THERMAL INKJET PRINTING

**Asma Buanz,** Claudia Christen, Simon Gaisford
UCL School of Pharmacy, University College London, 29-39 Brunswick Square, London WC1N 1AX, United Kingdom; Email: asma.buanz.11@ucl.ac.uk

Oral films (or orodispersible films) have gained considerable interest due to the benefits for both manufacturers and consumers (1). We have reported previously on successfully using thermal inkjet printing (TIJP) for personalised dosing with commercially available films as a substrate (2). The aim of this work was to investigate further the effect of the method of drug incorporation in films on their mechanical and physical properties. Oral films containing clonidine hydrochloride (CLN) were made by either TIJP or solvent casting (SC) from sodium carboxymethyle cellulose and polyvinyl alcohol. The drug was either mixed with the polymer solution before casting, or deposited onto cast plain films with TIJP using unmodified Hewitt Packard (HP) Deskjet 460 printer and HP black ink cartridge (modified as explained before2). CLN solutions of different concentrations corresponding to the required doses were printed and drug contents were measured with HPLC. Tests were developed for disintegration and dissolution while mechanical properties were tested with dynamic mechanical analysis (DMA). Dose uniformity was higher when the drug was jetted onto the films than when mixed before casting (coefficient of variance = 3.29±2.69% and 11.6±9.53%, respectively). No significant difference was observed for the in vitro release profiles. Nonetheless, upon exposing to high humidity (ramping from 0 to 95%) crystallisation was observed in SC films but not in TIJP films. In conclusion, TIJP appears to provide better dose uniformity and physical stability without much effect on the release properties of oral films.

1 Gaisford S., Verma A, Saunders M., Royall P. (2009) Int. J. Pharm., 380: 105.
2 Buanz A., Saunders M., Basit A., Gaisford S. (2011) Pharm. Res., 28: 2386.

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# NOVEL POLYMERS FOR THE MUCOSAL DELIVERY OF SIRNA

**Victoria Capel,** Martin C. Garnett, and Snow Stolnik,
University of Nottingham, Nottingham, NG7 2RD, United Kingdom;
Email: paxvc2@nottingham.ac.uk

RNA interference is a natural phenomenon mediated by short (21-23bp) RNA's (siRNA) which can be exploited to selectively silence genes that are involved in disease. This rapidly expanding area of research has led to studies with siRNA for a range of diseases such as HIV, respiratory viruses and cancer. Effective delivery remains the most significant barrier between the potential of siRNA technology and its success in clinic. Cationic polymers have been used to incorporate siRNA via electrostatic interactions into nano-sized polyplexes; their overall positive charge facilitating entry into the cell. Local delivery such as to the mucosal surfaces of the nasal passage and the lung, offers many advantages over systemic delivery due to the large surface area and extensive blood supply. In this work, a novel biocompatible polymer has been utilised to deliver siRNA to lung epithelial cells in vitro. A model housekeeping gene GAPDH was chosen as a target to evaluate gene silencing efficiency in 3 epithelial cell lines, differing in their ease of transfection. The rates of uptake of fluorescent siRNA polyplexes were followed using flow cytometry, and the uptake pathways were investigated by the use of a range of endocytosis inhibitors. We have been able to successfully transfect the H1299, A549 and Calu-3 lung epithelial cell lines, achieving gene silencing efficiencies of up to 80% in H1299 cells with minimal toxicity. The extent of polyplex uptake is dependent on cell line, with much higher rates in the rapidly dividing cell lines leading to higher gene silencing. The mechanism of polyplex uptake also appears to be cell line dependent and a combination of specific and non-specific endocytosis pathways. In conclusion, this novel polymer appears promising for the mucosal delivery of siRNA and is currently being considered for in vivo studies.

# 33 MULTILAYER ALGINATE-CHITOSAN MICROCAPSULES FOR THE ENCAPSULATION OF PROBIOTIC BIFIDOBACTERIUM – CHARACTERIZATION AND TESTING

**M.T. Cook**[1], D. Charalampopoulos[2], G. Tzortzis[3],V. Khutoryanskiy[4]
[1] Department of Chemistry, University of Reading, Reading, RG6 6AD, United Kingdom; [2] Department of Food and Nutritional Sciences, University of Reading, Reading, RG6 6AD, United Kingdom; [3] Clasado Research Services Ltd, Reading, Berkshire, RG6 6AD, United Kingdom; [4] School of Pharmacy, University of Reading, Reading, RG6 6AD, United Kingdom; Email: m.t.cook@reading.ac.uk

Many probiotic bacteria are unable to survive passage through the stomach if orally administered. This is due, in part, to the low pH of gastric fluid. Previous research has demonstrated the capability of alginate microcapsules containing probiotic cells, produced via an external gelation approach, to reduce the level of cell death associated with exposure to acidic solutions. In addition, chitosan has been shown to improve the level of survival further. These formulations can also display a degree of time and pH dependent release in the gastrointestinal tract. Our current research focuses on the production of multilayer alginate-chitosan capsules as a means of improving the survival and release of these bacteria further, with the aim or improving the effectiveness of orally administered probiotics. These multilayer coatings may be prepared simply by the exposure of an alginate matrix containing cells to chitosan and alginate, alternately. This exposure results in the formation of a coat, associated via electrostatics. These systems have been evaluated by several techniques, namely Biacore, texture analysis and confocal laser scanning microscopy. This characterisation allowed for better understanding of the coating process, and the confirmation of multilayer stability during exposure to gastric and intestinal solutions. In vitro testing of the capsules showed that the survival of probiotic cells in acid could be greatly improved by encapsulation into these systems. Multilayer-coated alginate microcapsules were also shown to be able to target the delivery of cells to simulated intestinal solution, whilst retaining them in simulated gastric solution.

Cook, MT; Tzortzis, G; Charalampopoulos, D; Khutoryanskiy, V, Layer-by-layer coating of alginate matrices for the improved survival and targeted delivery of probiotic bacteria after oral administration, Journal of Materials Chemistry B; 2013; 1, 52-60.

# 34 SYSTEMATIC INVESTIGATION INTO THE APPLICATION OF DUAL FUNCTIONAL POLYOX IN THE FORMULATION OF COMPRESSED ORALLY DISINTEGRATING TABLETS

**Eman Dahmash,** Cliff Bailey and Afzal Mohammed
Aston Pharmacy School, Aston University, Birmingham, B4 7ET, United Kingdom;
Email: dahmashe@aston.ac.uk

Orally disintegrating tablets (ODTs) owing to their ease in administration and higher patient concordance have acquired considerable recognition as solid dosage delivery systems. The use of direct compression as a method for developing ODTs offers several advantages including ease of manufacture and cost effective technology. However, achieving the desired tablets' properties for rapid disintegration (in less than 60s), excellent compressibility and good mouth feel still requires substantial excipients screening. Mannitol, a commonly used diluent displays a good balance between sweet taste, good mouth feel, aqueous solubility as well as rapid dispersibility due to its capillary action. Nevertheless, owing to its low compressibility, it has limited application in direct compression formulations. In this study, a systematic approach using mannitol and an increasing amount of a hydrophilic polymer, polyethylene oxide (POLYOX-N-10), as binder-disintegrant in a concentration ranging from 1% to 10% was studied to further enhance tablet properties. Following dry blending of the excipients, ODTs were compacted using low compression force of 5KN and a dwell time of 30s. Pre-compression results (angle of repose, compressibility index, Hausner ratio and particle size distribution) indicated good flow properties of the powder that was enhanced with the increasing concentration of POLYOX-N-10. Initial results on tablet properties demonstrated an increase in hardness with the increasing concentration of POLYOX-N-10, with an adverse affect on the disintegration time that escalated with the increase in hardens. It was noticeable form disintegration test that the tablets did not disintegrate into fragments but rather eroded slowly indicating a matrix forming material. Further investigations are underway to determine the effect of lower concentration of POLYOX-N-10 1-3% using higher compression force to obtain the balance between desirable disintegration time and hardness as well as investigating the effect of physical modification of the excipients on ODT properties.

# 35 FORMULATION OF DUAL MODALITY NANOPARTICLES TO ENHANCE TOBRAMYCIN EFFICACY IN CYSTIC FIBROSIS.

**Jill Deacon*,** Dr. Sharif Abdelghany#, Prof. Stuart Elborn*, Dr. Adrien Kissenpfennig*, Prof. Cliff Taggart*, Dr. Chris Scott
*Centre for Infection and Immunity, School of Medicine, Dentistry and Biomedical Sciences
#School of Pharmacy, Queen's University Belfast, United Kingdom;
Email: jdeacon02@qub.ac.uk

Cystic fibrosis (CF) patients aberrantly produce viscous mucus in their lungs, which acts as a penetrative barrier to antibiotic delivery and provides an ideal environment for bacterial colonisation. The aim is to develop a nanoparticle drug delivery system combining widely used CF therapeutics for an enhanced antibiotic effect. Tobramycin is an aminoglycoside antibiotic and the mucolytic DNase thins viscous secretions. For this study, it was hypothesised that alginate/chitosan nanoparticles (NPs) loaded with tobramycin and functionalised with DNase would enhance penetration of the tobramycin to the site of infection within the mucus.

Tobramycin loaded alginate/chitosan NPs were formulated by ionotropic gelation, prior to conjugation of DNase on their surface. NP formulation was optimised and CF sputum samples were tested with NPs to establish bactericidal effects against Pseudomonas aeruginosa. NPs were formed at around 500 nm, loaded with tobramycin 89µg/mg polymer and DNase 19µg/mg polymer. Activity of both modalities was confirmed against in vitro cultures of P. aeruginosa and by cleavage of DNA (plasmid and CF sputum samples). Treatment of CF sputum with DNase-tobramycin NPs resulted in reduced viscosity and increased bacterial killing in these samples. These novel alginate/chitosan NPs combining DNase and tobramycin are an effective antibacterial treatment in CF sputum. The incorporation of two widely used therapeutics into a single formulation provides promise in the future treatment regimen of CF patients.

# 36 THE ROLE OF DNA CHIPS IN FORMULATION DEVELOPMENT OF TRIMETHOPRIM

**Amr ElShaer,** Ayesha S. Rahman, Peter Hanson and Afzal R Mohammed.
Aston Pharmacy School, Aston University, Birmingham, B4 7ET, United Kingdom;
Email: elshamk1@aston.ac.uk

Oral drug delivery is considered the most popular route of delivery because of the ease of administration, availability of a wide range of dosage forms and the large surface area for drug absorption via the intestinal membrane. Nevertheless, drug biopharmaceutical properties besides the physiological and biological barriers reduce the bioavailability for orally administered drugs. Trimethoprim (TMP) was selected as a module drug for this study because it is believed to be a substrate for efflux transporters and digestive enzymes. The aim of the current work is to improve the physico-chemical properties of TMP via ion-pairing with amino acids and monitor the genetic changes that occur across the endothelial cells during the uptake of the drug.

The ion-pairs formed between aspartic acid and TMP showed low aqueous binding constant, which was tripled when glutamic acid was used. The improvement of the aqueous binding constant (K11aq) upon using glutamic acid resulted in a 3 fold increase in the permeability profile of TMP. Although ion-pairing TMP with glutamic acid had a negative effect on the partitioning capacities of the drug, the increased overall uptake suggest the involvement of active transporters rather than just passive diffusion. Genetic changes across Caco-2 showed that MRP7 is the major efflux protein that extrudes TMP from the cells while P-gp seems to have a minimal role in TMP efflux. Moreover, it was found that TMP is actively uptaken by organic cation transporters: OCTN1 which was over-expressed by 2.36 fold. The molar ratio of glutamic acid was found to affect the expression of OCTN1 as its expression reached 2.2 fold for 1:1 physical mixtures (PM) formulations and dropped to the basal state for 1:8 ion-paired formulations and this explains the higher uptake from 1:1 PM formulations. Interestingly, the microarray data revealed that CYP3A4 is involved in oxidizing TMP into intermediate molecules that binds to glutathione (2.5.1.18) to form a conjugate that could be eliminated easily. While the over-expressed CYP1A2 mediates the N-oxidation of the drug via monooxygenase (1.14.14.1). To conclude, microarrays could be used as a potential technique to investigate the endothelial uptake and metabolism not only for new chemical entities but for formulation development as well.

# 37 IN VITRO AND IN VIVO STUDIES OF PRE-CORNEAL RETENTION OF RIBOFLAVIN-LOADED MUCOADHESIVE POLYMERIC FILMS

**Peter W. J. Morrison**[1], Serzhan K. Seilkhanov[2], Marat N. Mussin[2],
Elvira K. Ozhmukhametova[2], Tolebai K. Rakhypbekov[2], Vitaliy Khutoryanskiy[1]
1 School of Chemistry, Food and Pharmacy, University of Reading, United Kingdom
2 Semey State Medical University, 103 Abai Street, Semey 071400, Kazakhstan;
Email: p.w.j.morrison@pgr.reading.ac.uk

Ocular bioavailability of drugs administered topically is typically very low because of a number of physiological barriers, including poor permeability of the cornea, tear and blinking reflexes as well as nasolachrymal drainage. One of the potential strategies to enhance the efficiency of ocular formulations is to improve their pre-corneal retention, which can often be achieved by the use of mucoadhesive polymers. In the present work we have developed a series of mucoadhesive polymeric films loaded with riboflavin, a drug that is used in corneal cross-linking procedure for the treatment of keratoconus. These films were based on combinations of poly(acrylic acid) and methylcellulose. The release of riboflavin from the films was studied in vitro and was found to correlate with the PAA/MC ratio in the films. The slowest film dissolution and drug release was observed for 100 % MC, whereas the fastest release was recorded for 100 % PAA films. PAA/MC blends exhibited intermediate release profiles. In vivo experiments on ocular retention of these films in rabbits have shown that these dosage forms can enhance pre-corneal retention of riboflavin. The maximal in vivo retention was achieved for the films containing 100 % of MC (for up to 1 hour), which is related to their slowest dissolution. However, because of the lack of mucoadhesive ability pure MC films were not convenient for ocular administration due to inefficient sticking to the cornea in the beginning of their application to the eye. The films composed of combinations of PAA and MC were found to be more suitable for application because of their mucoadhesive ability.

# 38 A CALORIMETRIC APPROACH: CONTINUOUS REAL TIME OBSERVATION OF INHIBITION OF CLOSTRIDIUM DIFFICILE BY PROBIOTICS

**MANSA FREDUA-AGYEMAN,** ABDUL BASIT, SIMON GAISFORD
UCL School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX, United Kingdom

Probiotics have a large market worldwide. It is claimed that ingesting them could have beneficial effects for man. One of the most frequent health claims is the reduction in duration of antibiotic-associated diarrhea mainly through the maintenance of colonization resistance against Clostridium difficile (Naaber et al., 2004). In this study, Isothermal microcalorimetry, which operates on the basic principle of measurement of heat, was used to study the antagonism of some probiotic strains against C. difficile.

Lactobacillus acidophilus LA-5®, Bifidobacterium animalis ssp lactis BB-12®, and Clostridium difficile NCTC 13565 were individually inoculated at 1x106 cfu/ml into 3 ml BHI Broth (Oxoid) supplemented with 0.1% w/v cysteine hydrochloride and 0.1% w/v sodium taurocholate in a sterile calorimetric glass ampoule. One species was also inoculated at 1x106 cfu/ml and mixed with 1 x106 cfu/ml of the other in the ampoules for co-culture studies. The sealed ampoules were transferred to a TAM 2277 (TA Instruments Ltd., UK) set at 37 ± 0.1oC. Data were captured with Digitam 4.1 and analysed with Origin (Microcal Software Inc.). The reference ampoule was loaded with 3 ml of sterile broth.

The power-time curves and heat outputs of the individual species were characteristic and could be used for identification of the organisms.
The antagonism test of the probiotic with Isothermal microcalorimetry demonstrated inhibition of C. difficile by competitive nutrition and by the extracellular products of the probiotic strains, which were sensitive to pH, and this correlated well with an agar diffusion assay.

The antagonism of probiotic strains L. acidophilus LA-5® and B. animalis ssp. lactis BB-12® against C. difficile NCTC 13565 was demonstrated in this work with Isothermal microcalorimetry.

Naaber et al., Journal of Medical Microbiology (2004), 53, 551-554.

# 39 IN VITRO PERMEATION STUDY OF A MODEL PYRROLOBENZODIAZEPINE MONOMER THROUGH SILICONE MEMBRANE AND HUMAN SKIN

**Tasnuva Haque[1],** Khondaker M. Rahman[2], David E. Thurston[2], Jonathan Hadgraft[1], Majella E. Lane[1]

[1] UCL School of Pharmacy, 29-39 Brunswick Square, London, WC1N 1AX, United Kingdom; [2] Institute of Pharmaceutical Sciences, King's College London, 150 Stamford Street, London SE1 9NH, United Kingdom; Email: ucnvtha@live.ucl.ac.uk

The pyrrolobenzodiazepines (PBD) represent a group of antibiotic antitumour agents. This group shows minimal side effects and a wide range of cytotoxic potency. Anthramycin (ANT) is the first PBD which was isolated from Streptomyces refuineus. ANT is effective against both solid and ascitic tumour type with a low nanomolar IC50. However, ANT could not be developed as a drug because of its cardiotoxic properties. Recently, a number of PBD-monomers have been developed that lack cardiotoxicity and can be developed clinically as anticancer agents. In this project, ANT has been used as a model PBD compound to evaluate and understand the permeation and penetration properties of PBD monomers in silicone membrane and human skin. Studies were performed in DMSO using static 'Franz'-type diffusion cells under occlusion. The receptor phase consisted of phosphate buffered saline. Known amounts of ANT were applied to the membrane and samples were collected at different time intervals up to 50hours followed by mass balance studies. The permeation profile of ANT through silicone membrane and human skin indicated that at the end of the study, 2.49±0.62µg/cm2 and 11.09±2.72µg/cm2 of ANT permeated in silicone and human skin, representing 0.50±0.05% and 2.58±0.68% of the applied dose, respectively. The mass balance study confirmed that 0.49±0.08% and 0.97±0.21% of the applied dose were present within silicone membrane and human skin, respectively. ANT was stable in DMSO as 96.17±19.41% of the given dose was recovered after 96 hours. In conclusion, the permeation and entrapment of ANT was about 4.5-times and 2-times greater in human skin compared to silicone. The results suggest that ANT may be used to understand the interaction of PBD compounds with silicone membrane and human skin. This information may be useful to develop further other potential PBDs as topical agents to treat actinic keratosis and melanoma.

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# SYNTHESIS AND IN VITRO ACTIVITY OF IFN-PEG-IFN

**A.P Herrington-Symes**[1, 2], J. Choi[2], S. Brocchini[1]
[1] Department of Pharmaceutics, UCL School of Pharmacy, London, United Kingdom; [2] PolyTherics Ltd, London, United Kingdom; Email: ucnvaph@live.ucl.ac.uk

Cytokine therapies are widely beneficial. Interferon $\alpha$-2 (IFN) is widely used to treat Hepatitis C infection. Due to the short half-life of IFN, PEGylated variants were developed and are currently first line therapies. While PEG-IFN is much more beneficial than IFN, there remain opportunities for improved versions of IFN. For example, clinically used PEG-IFNs are heterogeneous mixtures where the PEG is conjugated at different amino acid residues. Many of these 'positional isomers' have varied and diminished biological activity. To address heterogeneity, we have developed a site-specific method of PEGylation that exploits the selective conjugation chemistry of the two thiols from native disulfide bonds [1]. We are currently developing methodologies to utilize PEG as a scaffold to conjugate two therapeutic proteins together in an effort to improve efficacy. In this project we hypothesized that an IFN-PEG-IFN molecule would be more biologically active than PEG-IFN. We therefore have developed reagents that can undergo site-specific, disulfide bridging conjugation at each end of a PEG molecule. IFN-PEG-IFNs were prepared using 10 and 20 kDa PEG-di(bis-sulfone) reagents (1 eq.) after incubation for 5 h at 20 °C. Ion exchange and size exclusion chromatography were used to purify the IFN homodimers. It was found that these new IFN homdimers were successfully prepared in high purity (western blot). In an in vitro antiviral assay, PEGASYS® showed a specific activity of 2.42 MIU/mg. The 10 kDa IFN-PEG-IFN homodimer displayed the highest specific activity of 5.99 MIU/mg. 20 kDa IFN-PEG-IFN molecule displayed lower activity than PEGASYS®, possibly due to the steric hindrance of the larger PEG. All conjugates were stable in PBS for 4 weeks at 4 °C (SDS-PAGE). This work shows that the PEG can be used successfully as a scaffold to link two proteins together whilst retaining biological activity.

APH-S is grateful to UCL School of Pharmacy and PolyTherics Ltd for PhD funding.

[1] S. Balan et al., "Site-specific PEGylation of protein disulfide bonds using a three-carbon bridge.," Bioconjugate chemistry, vol. 18, no. 1, pp. 61–76, 2007.

# 41 CONTROLLED SYNTHESIS OF LIMIT SIZE CATIONIC LIPOSOMES BY MICROFLUIDIC MIXING

**Elisabeth Kastner,** Randip Kaur, Alexander Wilkinson and Yvonne Perrie
Aston Pharmacy School, Aston University, Aston Triangle, Birmingham B4 7ET, United Kingdom; Email: kastnere@aston.ac.uk

The size of liposomes significantly affects biodistribution and is tailored to their application; liposome size for efficient drug delivery varies due to constraints governing the penetration of the target tissue. Despite the great potential of liposomes as delivery vehicles and immune potentiators, large scale manufacturing of liposomes raises problems. Traditional liposome synthesis methods often require mechanical stresses like sonication, high-shear homogenisation or high pressures which are difficult to upscale. However, current requirements of the pharmaceutical industry necessitate the robust, reproducible and scalable production of size-uniform vesicles. The aim of this work was to determine whether a staggered herringbone micromixer was capable of producing limit size liposomes. Liposomes were synthesized using the automated Nanoassemblr (Precision NanoSystems, Inc). Streams of dissolved lipids and aqueous buffer were injected into separate chamber inlets, followed by rapid mixing of both streams due to the chamber design. The automated system allowed for controlled alterations in flow rates and flow rate ratios. Additionally the possibility of drug loading incorporated into the liposome synthesis process was investigated. Empty liposomes of 50 nm could be generated via controlled alterations in flow rate and flow rate ratios whilst retaining surface charge characteristics. Increased mixing rates are believed to contribute to the rapid increase in polarity of the medium driving nanoprecipitation reaction and liposome formation. Loading studies implied size and charge alterations based on molecular rearrangement with generally larger vesicle sizes compared to empty liposomes. Work presented emphasizes the potential of biomicrofluidics and process control by novel lab-on-a-chip based applications. Underlined by advantages of the automated microfluidic mixing device, we expect microfluidic mixing to become the preferred method of choice for reproducible and controlled bottom-up liposome synthesis of desired dimensions.

# 42 DEVELOPING SOLID PARTICULATE VACCINE ADJUVANTS - SURFACE BOUND ANTIGEN FAVOURING A HUMOURAL RESPONSE, WHEREAS ENTRAPPED ANTIGEN SHOWS A TENDENCY FOR CELL MEDIATED IMMUNITY

**Randip Kaur,** Daniel J Kirby, Yvonne Perrie
School of Life and Health Sciences, Aston University, Aston Triangle, Birmingham B4, United Kingdom; Email: kaurr3@aston.ac.uk

This present study compares the efficacy of microsphere formulations, and their method of antigen presentation, for the delivery of the TB sub-unit vaccine antigen, Ag85B-ESAT-6. Microspheres based on poly(lactide-co-glycolide) (PLGA) and chitosan incorporating dimethyldioctadecylammonium bromide (DDA) were prepared by either the w/o/w double emulsion method (entrapped antigen) or the o/w single emulsion method (surface bound antigen), and characterised for their physico-chemical properties and their ability to promote an immune response to Ag85B-ESAT-6. The method of preparation, and hence method of antigen association, had a pronounced effect on the type of immune response achieved from the microsphere formulations, with surface bound antigen favouring a humoural response, whereas entrapped antigen favoured a cellular response.

# 43 ORAL NANOMEDICINES FOR THE TREATMENT OF VISCERAL LEISHMANIASIS

**Maruxi Dea - Ayuela[1],** Spruhi Patel[2], Dolores Remedios Serrano-Lopez[3], Aikaterini Lalatsa[2]
[1] Departamento de Química, Bioquímica y Biología Molecular, Universidad CEU Cardenal Herrera, s/n, 46113-Moncada, Valencia, Spain; [2] Department of Pharmaceutics, School of Pharmacy, University of Hertfordshire, Hillside House, College Lane Campus, Hatfield, AL10 9AB, UK; [3] Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, Complutense University, 28040 Madrid, Spain; Email: a.lalatsa@herts.ac.uk

Visceral Leishmaniasis (VL), a protozoan parasitic infection endemic mostly in tropical countries, is fatal if left untreated accounting for 59,000 deaths annually1. Pentavalent antimonials remain low-cost treatments but their use is limited due to observed resistance, toxicity and long courses of parenteral administration. AmBisome, a liposomal formulation of Amphotericin B, has a long circulation half-life with reduced nephrotoxicity and is able to cure 95% of patients (single-course therapy) 2. However, several AmBisome ampoules are required for a single-course treatment making the cost of therapy refractive in developing countries and adverse-effects and temperature instability remain still issues 3. Miltefosine, an oral therapy licensed only in India, is administered for at least 28 days, resulting to poor compliance and relapses and results in an equivalent cost of therapy to single-dose therapy with Ambisome1. Thus, there is a need for novel oral cost-effective, safe therapies of VL. Buparvaquone, a hydroxynaphthoquinone antiprotozoal agent with known anti-Leishmaniasis activity in vitro (ED50: 0.005 μM against L. donovani), posesses poor oral bioavailability due to low aqueous solubility (< 300 ng mL-1) 4,5. Delivery strategies have focused on formulating Buparvaquone as a prodrug 6 or as a self-microemulsifying drug delivery system 7 with limited success in vivo. Novel solid-lipid nanoparticles (SLNs) and self-nanoemulsifying drug delivery systems (SNEDDs) were developed using a solvent evaporation method and phase-diagrams respectively from approved excipients for oral administration. Both nanoparticulate systems were characterised for particle size and morphology (SLNs <100nm, SNEDDs <400nm) and possess ideal size for enhancing oral absorption via the transcellular and lymphatic pathway, while they possessed a higher loading capacity for Buparvaquone (5 fold to literature values 5). Both systems illustrated significantly better stability in simulated gastric fluids compared to Buparvaquone alone 6. The in vitro efficacy in promastigotes and amastigotes is being assessed against L. Infantum, L.braziliensis, L.amazonensis 7.

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# 44 NANOSCALE CHARACTERIZATION OF THE EFFECT OF MULTIPLE CYCLES OF LYOPHILIZATION ON THE AGGREGATION OF AN IGG ANTIBODY ON ITS OWN; AND IN COLLABORATION WITH SUCROSE AND MANNITOL

**Mudasirmisra Lone,** Stephanie Allen, Clive J Roberts and Saul JB Tendler
Laboratory of Biophysics and Surface Analysis, School of Pharmacy, The University of Nottingham, NG7 2RD, United Kingdom; Email: paxml2@nottingham.ac.uk

The aggregation in biopharmaceutical formulations may be induced by the commonly employed process of lyophilization. As a consequence therapeutic potential may be reduced or lost. Moreover, aggregation also reduces shelf life and is considered to be a potential cause of immunogenicity. Here we investigate the effect of mutiple cycles of lyophilization (up to 7) on an antiglucose-6-phosphate dehydrogenase antibody with and without excipients (sucrose and mannitol alone, and in combination). Formulations at different molar ratios were analyzed for size characterization by dynamic light scattering (DLS), and atomic force microscopy (AFM) to identify and characterize different components in the lyophilized formulations. Differential scanning calorimetry (DSC) and ATR -Fourier transform infrared (FTIR) spectroscopy were also used to identify any structural alterations.

The propensity of IgG to form aggregates was shown to increase with successive lyophilization cycles, with and without lyoprotectants. IgG with sucrose alone more effectively resisted aggregation and this effect was more pronounced at higher molar concentration. This protection was enhanced in combination with mannitol. 1mg/ml of IgG formulated with 3.6mM sucrose and 3.6mM mannitol together provided the best protection. AFM images of lyophilized formulations revealed globular protein-like features of variable size. However, mean size of the agglomerates increased significantly with successive lyophilization. Samples that showed more protein aggregation had more crystalline features. DSC confirmed that the propensity of the unfolding of IgG increased during multiple cycles of lyophilization. However, the denaturation temperature (Tm) of IgG [Fab fragments] ~ 58°C remained unchanged. The unfolding of the Fc domain of IgG occurred at lower temperatures following multiple cycles of lyophilization. Sucrose at higher molar concentration provided protection against thermal denaturation both alone and in combination with mannitol. IgG lyophilized on its own conserved its amide bands I and II, suggesting that complete secondary structure collapse does not occur during multiple cycles of lyophilization. ATR-IR analysis demonstrated a significant change in the proportion of the different components within the amide band I. The β-sheet (main β-sheet structural component with parallel structure) of the protein increased during successive cycles of lyophilization of IgG, suggesting that this change is related to the increased aggregation observed.

# 45 SPECIFICITY AND INHIBITION OF SERINE PROTEASE PERMEATION ENHANCERS: A COMPARISON

**JENNIE LORD[1];** FRANCO H. FALCONE [1]; SARAH GAUNT[2] and SNOW STOLNIK[1]
[1] University of Nottingham, School of Pharmacy, University Campus, Nottingham, NG7 2RD
[2] Eminate Ltd, University of Nottingham, Sutton Bonington Campus, Sutton Bonington Leicestershire, LE12 5RD, United Kingdom; Email: paxjl6@nottingham.ac.uk

We have recently been interested by the absorption of nattokinase from the Japanese food Natto, across the Caco-2 model gut epithelium, as well as its cytotoxicity. Preliminary data reveals that nattokinase shows potential as a permeation enhancer at non-toxic concentrations. Other serine proteases have shown similar potential as permeation enhancers for improved oral drug therapy. Chymotrypsin, trypsin (mammalian serine proteases), subtilisin A and nattokinase (subtilisin-like serine proteases) have been described as having broad specificity for a large range of substrates, given their natural biological role to hydrolyse peptides and proteins. Nattokinase consumption has been linked to several health benefits, including a reduction in cardiovascular diseases, offering circumstantial evidence that nattokinase survives harsh gastro-intestinal conditions and is absorbed intact, retaining biological activity.

In the present study we carried out enzymatic activity assays of each of the aforementioned serine proteases, with several synthetic serine protease substrates. Our results describe the specificity of the serine proteases to hydrolyse certain substrates and the relative inhibition posed by serine protease inhibitors (serpins), endogenous to blood plasma. Results suggest that the serine proteases investigated have distinct affinities for each of the substrates. Chymotrypsin and subtilisin A showed a 2000- and 1000-fold increase, respectively, in specificity for the hydrolysis of peptide bonds adjacent to phenylalanine residues, compared to the specificity nattokinase had for the same region. Data also suggests that trypsin has 10-fold specificity for glycine-arginine bonds compared to valine-arginine bonds, while nattokinase shows a 4000-fold improvement in specificity for valine-arginine bonds over glycine-arginine bonds. Inhibition data suggests that subtilisin A is less inhibited by the presence of alpha-1-antitrypsin (A1AT) than the presence of alpha-1-anti-chymotrypsin (A1ACT). As expected, trypsin and chymotrypsin are completely inhibited by their respective inhibitors. Trypsin is unaffected by the presence of A1ACT whereas chymotrypsin is almost completely inhibited by A1AT. Nattokinase is inhibited to a lesser extent by A1ACT than by A1AT, and even less so by alpha-2-macroglobulin (A2M), which is also a less efficient inhibitor of trypsin and chymotrypsin.
Our results hope to demonstrate that the varied specificities displayed by each of the serine proteases, as well as their distinct inhibition by serpins, allows for further distinction to be made between these enzymes, particularly highlighting the advantage nattokinase may present as a permeation enhancer compared to other mammalian serine proteases.

# 46 INVESTIGATION OF THE ROLE OF A NEW GRADE OF HYDROXYPROPYL CELLULOSE IN THE FORMULATION OF ORALLY DISINTEGRATING TABLETS BY DIRECT COMPRESSION

**Affiong Iyire,** Clifford Bailey and Afzal Mohammed
School of Life and Health Sciences, Aston University, Birmingham, B4 7ET, Unites Kingdom;
Email: iyirear@aston.ac.uk

The development and optimisation of orally disintegrating tablet (ODT) formulations is gaining popularity because of the obvious advantages (ease of administration and high patient acceptance/ compliance) of this delivery system. To maximise drug loading without producing very large tablets, it becomes pertinent to employ excipients with multi-functionalities. Hydroxypropyl cellulose (HPC) has been reported for its multi-functionality as binder, disintegrant and mucoadhesive. The aim of this study was to investigate a new grade of HPC (HPC-SSL-SFP) to verify its functionality and determine its role/application in the formulation of ODTs. Pre-formulation characterisation of the powder included particle size analysis, flow properties, porosity/true density, wettability, differential scanning calorimetry (DSC) and scanning electron microscopy (SEM). Heckel's plots were generated from compacts containing 100% HPC at compaction force of 5-30 kN. Placebo tablets containing 0-10% HPC with mannitol as filler, were produced by direct compression at 10 kN compaction force; and tested for porosity, hardness, friability and disintegration time. Results showed HPC as a poor flowing fine powder with Heckel's mean yield pressure of 714 MPa-1 indicative of some plastic deformation on compaction. Surprisingly, compaction of pure HPC at compaction pressure of 10-30 kN produced very hard tablets that could not be broken by the tablet tester (hardness >520 N). However, contrary to expectation, the placebo tablets did not result in very hard compacts (maximum hardness of 57.67 ± 4.73 at 10% HPC concentration). As expected, there was a significant increase (ANOVA, $p<0.05$) in hardness and disintegration time, with decrease in friability, with increasing HPC concentration in the placebo tablets. But even at 10% HPC concentration, friability was >2%. Thus, although HPC showed binding properties, its use in ODT formulations will require addition of a disintegrant. Further studies will investigate and validate the mechanism of the hard HPC compact formation to determine the application of this characteristic in drug delivery system development.

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# PROTEIN CRYSTALS AS A FORM FOR PROTEIN DELIVERY

**NUNO MADEIRA DO O,** SNOW STOLNIK
University of Nottingham, School of Pharmacy, University Campus, NG7 2RD, Nottingham, United Kingdom; Email: paxnfm@nottingham.ac.uk

Delivery of protein based therapeutics is challenging. Recently, protein crystallisation has been attempted as a delivery method due to the possibilities it potentially offers, including avoidance of protein aggregation and high purity of the crystals obtained. Protein crystals also allow the possibility of several types of release profiles. In this work five different proteins – lysozyme, insulin, pepsin, chymotrypsinogen and thaumatin – were crystallised using Generally Regarded As Safe (GRAS) materials by means of a rotary solvent evaporation method.
This generated crystals with traditional morphology for all tested proteins over a period of 24h.

The effects of the crystallization buffer/method were analysed by means of Fourier Transform Infrared spectroscopy (FITR), liquid-state Nuclear Magnetic Resonance (lsNMR) and activity assays. The analysis compared the original amorphous powder with the crystals obtained and the results show that, in general, the proteins were only marginally affected by the whole process and that the proteins retained their biological activity. The crystallinity of the resulting material was assessed by X-ray powder diffraction, proving that the material obtained was of crystalline nature.

Surprisingly, the addition of the polyvinylpyrrolidone (PVP) into lysozyme and insulin crystals showed a reduction in particle size of obtained crystals by 90% for lysozyme and 70% for insulin, generating approximately 3.2μm and 8.3μm crystals, respectively. The structural analysis showed that the presence of PVP caused minor effects on the protein structure and that the biological activity of the fabricated small-sized crystals amounted to approximately 95% of the activity of the crystals prepared in the absence of the polymer

# 48 PREPARATION OF PH-RESPONSIVE POLYMERS FOR DELIVERY OF NUCLEIC ACIDS

**Teresa Matini,** Elena Gallon, Joaquin Sanchis, Maria J. Vicent, Paolo Caliceti, Stefano Salmaso, Cameron Alexander, Giuseppe Mantovani
School of Pharmacy, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom; Email: paxtm@nottingham.ac.uk

In the last years, a number of polymeric carriers have been developed to reduce limitations and improve the therapeutic potential of nucleic acid drugs. These systems need to be water-soluble, non-toxic and non-immunogenic as well as preserving the therapeutic activity of loaded drugs. Furthermore, they should exhibit functional groups able to interact, covalently or not, with the drug of interest, and they may also contain recognition motifs, which target disease-related antigens or receptors. Thus, in this work, new pH-sensitive polymersomes to promote long circulation, high specificity and retention in the heterogeneous, acid and hypoxic tumour environments have been engineered to carry and protect nucleic acids. The end-goal of the project is to deliver siRNA to reduce expression of the α1 Na+/K+ATPase transmembrane protein, abnormally overexpressed in many cancers [1]. The pH-sensitive polymersomes required for this study involved the synthesis of polymers featuring side-chain functionalities with pKa values in the 6-7 range, to exploit the reported lower pH in tumoral tissues. We prepared a class of diblock and triblock copolymers based on a series of functional monomers and chain-transfer agents: glycerolmethacrylate, PEG1900/3500-4-cyano-4-(phenylcarbono-thioylthio) pentanoic acid, 6-(1H-imidazol-1-yl)hexyl-methacrylate and 4-(1H-imidazol-yl)butyl-methacrylate. All polymerisations were carried out by reversible addition-fragmentation chain transfer (RAFT) polymerisation, following appropriately adapted reported methods. [2] In order to estimate the capability of these materials to respond to the changes in the pH in tumoral tissues, we evaluated their behaviour at different pH by 1H-NMR, Dynamic Light Scattering and TEM. All the block copolymers contain sequences that are hydrophobic and water-insoluble in their neutral form, but dissolve around pH ≈ 5.4 due to tertiary amine protonation. Polymersomes were prepared by 'pH-switch' method 2-7 which, according with the literature, might generate a narrower distributions of the particles.

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# 49 ENHANCING RIBOFLAVIN SOLUBILITY AND PERMEABILITY THROUGH BOVINE CORNEA USING HYDROTROPIC COMPOUNDS

**Peter W. J. Morrison,** Vitaliy Khutoryanskiy
Reading School of Pharmacy, University of Reading, Whiteknights, PO Box 224, Reading, RG6 6AD, United Kingdom; Email: p.w.j.morrison@pgr.reading.ac.uk

Keratoconus is a debilitating condition potentially leading to blindness, affecting around 1 in 2000 of the population, for some (~20%) the disease will progress to the stage where penetrating keratoplasty (corneal graft) becomes necessary. During the previous decade a procedure described by Wollensak et al1 has become routine in treating the condition and this involves riboflavin / UV 'A' corneal cross-linking. However, riboflavin has low aqueous solubility (0.08 ± 0.008 mg mL-1) and the current procedure requires removal of the corneal epithelium to enhance riboflavin penetration; clearly it would be beneficial if riboflavin could be delivered into the cornea without epithelial abrasion.

Generally, applying higher concentration drug formulation allows for better availability to the target tissue. Riboflavin aqueous solubility enhancement using hydrotropic compounds such as urea, nicotinamide, cucurbiturils (CB[6-8]), dendrimer(PAMAM G4) and α-, β-, γ-, HP-β-cyclodextrins were investigated. Riboflavin solubility in urea solutions (10 – 30 mg mL-1) were enhanced up to 0.180 ± 0.006 mg mL-1, in nicotinamide solutions (10 – 600 mg mL-1) up to 8.10 ± 0.36 mg mL-1, and in cyclodextrin solutions (10 – 30 mg mL-1) up to 0.19 ± 0.03 mg mL-1, CB[6] offered no enhancement to riboflavin solubility whilst CB[7] and CB[8] enhanced solubility up to 1.31 ± 0.05 mg mL-1 and 0.100 ± 0.006 mg mL-1 respectively. PAMAM G4 increased riboflavin solubility to 0.15 ± 0.01 mg mL-1 compared to riboflavin dissolved in deionised water (0.080 ± 0.008 mg mL-1).
Permeability of riboflavin dissolved in hydrotropic compound solutions through bovine cornea was examined and it was found that α-cyclodextrin, β-cyclodextrin and HP-β-cyclodextrin showed significant permeability enhancement, compared to the solubility of this drug in deionised water.2 There was no evidence of permeability enhancement using γ-cyclodextrin, urea, nicotinamide, cucurbiturils and PAMAM G4 dendrimer.

Cyclodextrins are reported to extract lipids from cellular membranes; our work using HPLC analysis and microscopy has shown that β-cyclodextrin and HP-β-cyclodextrin extract cholesterol from corneal epithelium, which could explain their permeability enhancing properties.

# 50 INVESTIGATION OF SUGAR ESTERS AS NOVEL INTESTINAL PERMEATION ENHANCERS

**F. McCartney** [1], M. Rosa [2], I. Coulter [2], & D.J. Brayden [1]
1School of Veterinary Medicine, University College Dublin; 2 Sigmoid Pharmaceuticals, Invent Centre, Dublin City University, Ireland; Email: fiona.mccartney@ucdconnect.ie

The aim was to investigate novel intestinal permeation enhancers (PEs) for poorly absorbed molecules, a major goal of the biotech industry. Permeation enhancers are added to oral drug formulations since intestinal absorption of peptides, proteins and macromolecules is difficult due to intestinal metabolism, poor membrane permeability and high intra-subject variability. They work using mechanisms related to boosting the paracellular and/or the transcellular routes across intestinal epithelia. While some PEs including carnitines and sodium caprate are in clinical trials, none have been approved as enhancers to date. Sucrose esters are non-ionic surfactants, consisting of sucrose and a fatty acid, and they are already established as safe emulsifiers and solubilisers in the food, cosmetics and pharmaceutical industry. A medium chain sucrose ester with a hydrophilic-lipophilic balance of 16 was studied. The permeation enhancement potential of the sucrose ester was tested using Ussing chambers. Isolated rat colonic mucosae were mounted and apical-side additions of concentrations ranging from 1.5mM to 10mM sucrose ester were tested. The transepithelial electrical resistance (TEER) and apparent permeability (Papp) of paracellular marker molecules, [14C] mannitol and FITC-Dextran 4kDa (FD4), were measured over 120 min. The transepithelial potential difference (PD, mv) and short circuit current (Isc, μA) were determined and TEER was calculated using Ohm's law. The tissue was voltage clamped to zero for 30 sec and switched to open circuit configuration for 3 sec. Apical addition of the sucrose ester showed a significant and concentration-dependent decrease in TEER associated with an increase in the Papp values of both [14C] mannitol and FD4. No major damage was seen in intestinal histology following exposure for 120 min. This data suggests that the tight junctions are being opened thereby allowing paracellular passage of the marker molecules. The sucrose ester may have potential as a non-toxic intestinal permeation enhancer for poorly absorbed molecules.

Co-funded by Science Foundation Ireland grant number 07/SRC/B1154 and Sigmoid Pharmaceuticals

# 51 STUDYING THE DIFFUSION OF SILICA NANOPARTICLES USING NANOSIGHT TRACKING ANALYSIS

**E. Mun,** A.C. Williams, V. Khutoryanskiy
Reading School of Pharmacy, Whiteknights, PO Box 224, RG6 6AD Reading, United Kingdom; E-mail: e.mun@pgr.reading.ac.uk

An understanding of the diffusion of nanoparticles in non-Newtonian fluids is of key importance in various applications. For example, it is essential to explore how nanoparticles are transferred into the blood stream or overcome the blood-brain barrier for designing nanoparticle-based drug delivery formulations to treat central nervous system disorders[1-3]. Additionally, diffusion is controlling factor in nanoparticles' deposition [4]. Biological gels line most tissues and organs in the human body and can act as selective barriers; these gels include mucus, extracellular matrix (ECM) and the biopolymer barrier within the nuclear pore [5]. Usually, the diffusion of small spherical particles is described by Stokes-Einstein equation which considers temperature, dynamic viscosity and diameter as the factors affecting their ability to diffuse. However, diffusion is a complex process that often cannot be described solely by this equation. In the present work, thiolated nanoparticles were synthesised in dimethyl sulfoxide, fluorescently labeled with Alexa Fluor 546, PEGylated with methoxypolyethylene glycol (750, 5000) maleimide, and their diffusion was studied using NanoSight Tracking Analysis (NTA) in four water soluble polymer solutions: hydroxyethyl cellulose (90,000 Da), poly(acrylic) acid (450,000 Da), poly(ethylene oxide) (1,000,000 Da) and polyvinylpyrrolidone (360,000 Da). NTA is a unique technique for size and diffusion studies which tracks each individual nanoparticle; it allows real time dynamic nanoparticle visualisation, particle-by-particle analysis, particle counting and sizing. The principle of measurement of NTA is based on Brownian motion. It was shown that one further crucial factor affecting the diffusivity of thiolated and PEGylated nanoparticles is the nature of their surfaces causing interactions between nanoparticles and macromolecules present in solutions. For example, PEGylation hampers the diffusion of nanoparticles in PAA while enhancing it in PEO solutions. In our work polymer solutions were considered as simple mimics of biological hydrogels, for example, mucus, that covers tissues and organs in the human body.

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# 52 A NOVEL SOLID DISPERSIONS TECHNIQUE FOR IMPROVED PH- TRIGGERED DELIVERY TO THE ILEO-COLONIC REGION OF THE GASTROINTESTINAL TRACT.

**Dharmendra K Muttha,** Richard J Singer, John G Fletcher
School of Pharmacy and Chemistry, Faculty of Science, Engineering and Computing.
Kingston University. London KT1 2EE, United Kingdom; Email: k0827841@kingston.ac.uk

Purpose: The Ileo-colonic region of the gastrointestinal tract has become interesting as a site for drug delivery due to the colonic disorders, low enzymatic activity and high retention time. pH responsive systems are a commonly used approach for ileo-colonic targeting. There are inherent problems with pH triggered site specific delivery. Recently researchers have reported failures of disintegration with enteric coated tablets in the colon. A low volume of free fluid in the colon can contribute to incomplete dissolution. The main objective of this study was to target the Ileo-colonic region with a novel solid dispersion technique using Eudragit S 100 as a co-polymer. The use of spray dried solid dispersions can improve the dissolution rate in the colon.
Methods: Solid dispersions of Indomethacin were prepared using polyvinylpyrrolidone (PVP) and Eudragit S 100 by solvent evaporation in different weight ratios using a spray dryer. Solvent content was determined by thermogravimetric analysis. The particle morphology was examined by scanning electron microscopy (SEM). The physical state and drug:polymer interactions of solid dispersions were characterised by differential scanning calorimetry (DSC), infrared spectroscopy and X-ray powder diffraction (XRPD). In vitro dissolution testing was carried out at pH 4.5, 6.2 and 7.2 phosphate buffers under sink conditions.

Results: DSC and XRPD confirmed the formation of amorphous solid dispersions. The glass transition temperature and solvent content for 1:1, 1:5, 1:7 and 1:9 drug polymer ratios were identified as 74.33±1.52 °C; 2.55±0.5 %w/w, 124±2.14 °C; 3.86±0.95 %w/w, 139±2.13 °C; 4.88±0.36 %w/w, and 142±3.65 °C; 6.82±0.51 %w/w respectively. SEM showed irregular and broken particles. Dissolution studies up to 45 min at pH 4.5, 6.2 and pH 7.2 for1:1, 1:5, 1:7 and 1:9 drugs:polymer ratio has shown as14.0±0.4 %, 86.85±5.7 %, 88.85±3.57 %; 28.4±4.3, 50.0±6.25 %, 93.1±2.62 %; 20.1±4.06, 55.64±3.78 %, 89.1±2.0 %; 7.95±1.73 %, 17.3±5.0 % and 83.26±8.68% respectively.

Conclusion: Solid dispersions of Indomethacin were produced with PVP and Eudragit S 100 at different ratios of drug and polymer. A maximum of 10% drug release was observed after 30 min at pH 6.2 and where as at least 50% of drug release occurred at pH 7.2. High glass transition temperature and molecular interactions between drug and polymer could prevent crystallization. Dissolution rate at pH 6.2 after 50 min can be reduced by increasing the particle size through optimizing the spray drying conditions.

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# 53 EXPLORING THE EFFECTS OF SODIUM CHLORIDE ON XANTHAN GUM AND HPMC HYDROPHILIC MATRICES

**Y. I. Oni** [1], L. Jasmani [2], W. Thielemans [2], A. Inchley [3], J. Burley [4], C. D. Melia [1]
[1] Formulation Insights, School of Pharmacy, University of Nottingham, Nottingham NG7 2RD, United Kingdom; [2] Chemistry and Chemical Engineering, University of Nottingham, Nottingham NG7 2RD, United Kingdom; [3] Boots Pharmaceutical Company, Nottingham, NG90 2PR, United Kingdom; [4] Laboratory of Biophysics and Surface Analysis, University of Nottingham, Nottingham, NG7 2RD, United Kingdom; Email: paxyo@nottingham.ac.uk

The influence of ionic environments on modified release dosage forms is important to our understanding of their drug release behaviour. Here we compare the responses of xanthan gum and HPMC in extended release matrices. Drug release from HPMC and xanthan gum matrices (8mm round flat-faced) were determined in dissolution media containing 0.0-2.0M NaCl at 37°C using USP I apparatus (100 rpm). Matrices contained 10-30%w/w polymer and 10% caffeine as the model drug. Early gel layer formation was investigated by confocal fluorescence imaging [2] using fluorescent-labelled xanthan gum (rhodamine B isothiocyanate) or Congo red. Xanthan matrices maintained extended release but exhibited significant changes in drug release profile. There was marked prolongation of release at very high NaCl concentrations (1M-2M NaCl). Confocal fluorescence images reveal no structural changes within the gel layer as a result of increased salt concentration, but showed compact gel layer morphologies at high NaCl concentrations. This suggests that drug release profiles do not arise from gross morphological differences in the gel barrier, but might arise from the widely reported random coil to rigid rod conformational transition in the xanthan molecule within this concentration range [3]. HPMC matrices showed salt sensitivity in lower polymer content matrices. These released drug rapidly at low salt concentrations (10%w/w polymer, >0.1M of NaCl), an expected result that reflects the water structuring activity known in HPMC [1]. Whereas xanthan gum matrices containing 10% w/w polymer exhibited extended release profiles in media up to 2M NaCl. In conclusion, xanthan gum matrices show extended release profiles that exhibit considerable resistance to dissolved ions over a wide range of salt concentration whereas HPMC showed sensitivity to dissolved salts, indicating the need for higher polymer loading into formulated matrices to achieve a sustained release tablet.

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# 54 INVESTIGATING THE IMPACT OF POLYMER BLENDS ON DRUG RELEASE FROM POLYMER MATRICES

**John Pollard**[1], Ali Rajabi-Siahboomi[2], Afzal Mohammed[1], Yvonne Perrie[1]
[1] Aston University, Aston Triangle, Birmingham, B4 7ET, United Kingdom
[2] Colorcon, Harleysville, PA, 19438, USA; Email: pollarj4@aston.ac.uk

This study characterises theophylline release from tablets formulated using two polymers commonly used for extended release (ER). The polymers of interest were cellulose-derived hypromellose (hydroxypropyl methylcellulose, HPMC), METHOCEL™ (K100LV and K100M), and the synthetic polyethylene oxide (PEO), POLYOX™ (WSR-1105 and WSR-Coagulant). Tablet formulations differed only in polymer grade. Tablets were evaluated for mechanical strength (hardness and friability), and release profiles were tested using USP apparatus II. The drug release mechanism was investigated in silico. Matrix tablets formulated with HPMC, using either K100LV (100% w/w) or K100M (100% w/w), showed a highly similar release profile to those prepared from K100LV/K100M (50: 50 % w/w), suggesting a single polymer formulation differs insignificantly in comparison with dual blend formulations. For PEO based matrices, release profiles of tablets prepared from WSR-Coagulant alone were notably different from those formulated using WSR-1105 or a 50:50 blend with WSR-Coagulant. For HPMC, viscosity and hardness did not appear to have a major influence on release rate: matrices formulated using K100LV and K100M gave no significant difference in release rates despite having large differences in viscosity (100 and 100,000 cPs, respectively) and hardness (46.5 - 122.2 N). Interestingly, for PEO, the rate of release was inversely proportional to polymer viscosity: WSR-Coagulant showed a notably slower rate of release than WSR-1105 (6,500 cPs at 1 % solution and 13,200 at 5 % solution, respectively). Moreover, the range of hardness from these tablets was similar (32.4 - 49.6 N). Data for both polymer platforms describes non-Fickian anomalous release with a strong correlation to first-order diffusion controlled release mechanisms. Furthermore, matrices formulated using PEO showed a stronger correlation to zero-order erosion release mechanism than HPMC-based matrices, particularly those containing WSR-1105 alone or as a 50:50 blend with WSR-Coagulant. For these matrices, the results of erosion testing confirmed that the erosion release mechanism plays more of an important role in drug release compared to HPMC-formulated matrices.

# 55 EVALUATION OF THE MECHANISM OF ACTION OF THE POLYGLUTAMIC ACID-DOPAMINE CONJUGATE - A POTENTIAL ANTIANGIOGENIC AGENTS

**Amit Kumar Rajora,** Cristina Fante, Helen Osborn, Francesca Greco
School of Pharmacy, University of Reading, Whiteknights Campus, RG6 6AD, United Kingdom, Email: id-A.K.Rajora@pgr.reading.ac.uk

Angiogenesis is the formation of new blood vessels from pre-existing vasculature. This is the central key step in tumour progression and metastasis. Hence, angiogenesis inhibition has become a therapeutic target for cancer therapy1. Dopamine (DA) is a neurotransmitter, which has recently been shown to display antiangiogenic activity2. However, DA has a short half-life (2-3 min) in blood circulation and some severe side effects, which have hindered its use as an antiangiogenic agent clinically. To overcome these limitations of DA, a polyglutamic acid-dopamine conjugate (PGA-DA) has been synthesised which has shown an extended antiangiogenic activity (active even after 24 hours) in comparison with the free DA3. In order to evaluate the mechanism of action of PGA-DA, a glutamic acid-dopamine conjugate (GA-DA) was synthesised by coupling of DA to Boc-L-glutamic acid 1-benzyl ester. The deprotection of the Boc (t-butyl group) was achieved using 20% triflouoacetic acid in dichloromethane at room temperature and benzyl group was deprotected using palladium on carbon (5% on wt/wt) to get the final product (GA-DA) with an overall yield of 60%. The identity of the product (GA-DA) was confirmed by 1H NMR, 13C NMR, IR and mass spectroscopy. Current studies are ongoing to determine the antiangiogenic activity of GA-DA and compare it with DA and PGA-DA.

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# 56 NOVEL POLYMERIC MATERIALS FOR SIRNA DELIVERY SYSTEMS

**Vanessa Loczenski Rose,** Andrzej Gallas, Stephanie Allen, Sebastiaan Winkler, Sanyogitta Puri and Giuseppe Mantovani
School of Pharmacy, Boots Science Building, University of Nottingham, NG7 2RD, United Kingdom; Email: paxvl@nottingham.ac.uk

Small interfering RNA (siRNA) therapeutics have great potential as a treatment paradigm for disease targets inaccessible by traditional small molecules drugs. siRNA via the RNAi pathway regulates gene expression by interacting with messenger RNA (mRNA) and can be used to selectively switch off genes. However, given its extreme instability in vivo and restricted cellular uptake, systemic delivery of siRNA requires complex formulations that prevent nuclease degradation and improve biodistribution.

Our current research focuses on the synthesis of a platform of polymers that have potential to complex, stabilise and ultimately deliver siRNA therapies systemically. Furthermore, the application of phosphonium and ammonium containing polymers is exploited. Polyphosphonium containing polymers are believed to be more efficient and less cytotoxic than the ammonium containing analogues [1]. The technique of controlled radical polymerisation has been employed to generate cationic, well defined polymers with tuneable size and architecture. The efficiency of polymers to completely bind siRNA is assessed through the use of a gel retardation assay. Furthermore, polymers are characterised by DLS and AFM and tested in biological assays to evaluate their effectiveness at gene knockdown in vitro.

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# 57 EFFECT OF RUBTURABLE COATING LEVEL ON LAG TIME OF PULSATILE RELEASE TABLET OF AMILODIPINE BESYLATE

**Dr. P.H.Sharma,** Padmashree Dr. D.Y. Patil
College of Pharmacy, Akurdi, Pune-411044, India; Email: pharmabrain20@yahoo.co.in

In the present studies, the effect of different coating level of rupturable layer on the lag time as well as release of the drug from pulsatile drug delivery system was studied. The pulsatile release tablets formulated using rupturable coating with the intention that the required amount of the drug should be released rapidly after the lag time. Amlodipin besylate taken as model drug, ethyl cellulose (10cp) as rupturable coating polymer and Ac-Di-Sol as swelling agent. The specific objective of the present research work is to assess effect of coating levels of rupturable layer on lag time. The Drug- Excipient compatibility study was carried out by FT-IR. Free film of ethyl cellulose film prepared by mercury substrate technique. The prepared film was evaluated for thickness and diffusion study. FTIR spectrum of drug and excipients were studied all the characteristic peaks of drug was found in the physical mixture of the spectrum. The dissolution study shows increase in coating level of rupturable layer prolong the lag time since time required to complete the disruption of the coating layer would be longer. A linear correlation was observed between coating levels and lag time. The lag time increases as the coating level of rupturable layer increases. The disintegration and dissolution time depend on the coating thickness as well as the amount of Ac-Di-Sol added. The coating level determined the lag phase of the formulation, where the coating level and the amount of Ac-Di-Sol in the tablet determined the burst effect and rapid drug release.

# 58 OVERCOMING PARKINSON'S DISEASES: DIRECT NOSE-TO-BRAIN DELIVERY OF AMANTADINE

**Shital Lungare[1],** Dr. Raj K. Singh Badhan[1], Dr. James Bowen[2]
[1] Aston University, Birmingham, [2] University of Birmingham, Birmingham, United Kingdom;
Email: lungares@aston.ac.uk

Treatments for Parkinson's disease face significant challenges in delivery to the due to the presence of blood brain barrier (BBB). The aim of this study was to develop and characterise an in-situ thermoresponsive polymer based gel system for possible exploitation of the intranasal olfactory route where the BBB is absent. Reaching the olfactory region in the nasal cavity and mucociliary clearance are the main hurdles from formulation point of view. Thermo-sensitive and pH sensitive polymers seem to be the answer to these problems. Formulation made with these polymers along with mucoadhesives will be liquid at room temperature and will turn into a viscous gel when comes in contact with the body either due to change in pH or temperature. We have successfully prepared the gels using cold method using amantadine, with Pluronic F127 as a thermoreversible polymer and carboxymethylcelluose (CMS) as a mucoadhesive polymer. Rheological characterisation confirmed the sol-to-gel phenomenon at desired temperatures. CMC increased the gelation temperature with increase in concentration, whereas amantadine lowered the gelation temperature with increase in concentration. After preliminary studies, 16 % of Pluronic F127 found to be a suitable concentration for the formulation to change from sol-to-gel at ambient nasal temperatures. Molecular, gene expression and drug transport studies using the nasal epithelial cell lines RPMI 2650 are also under investigation along with the development of an in-vitro olfactory-epithelial cell culture model to study axonal transport. Unlike the animal studies this model will be faster to use, efficient and also better representation of human olfactory system.

# 59 SELF-ASSEMBLY OF FATTY ACID NUCLEOSIDE ORGANOGELS FOR APPLICATIONS IN DRUG DELIVERY AND TISSUE ENGINEERING

**Kathryn J. Skilling** [1], Barrie Kellam [2], Tracey Bradshaw [2], Marianne Ashford [3] and Maria Marlow [1]

[1] Targeted Therapeutics and Next Generation Medicine Centre for Doctoral Training, School of Pharmacy, University of Nottingham, United Kingdom; [2] Centre for Biomolecular Sciences, School of Pharmacy, University of Nottingham, United Kingdom; [3] AstraZeneca, Alderley Park, Cheshire, United Kingdom; Email: paxks@nottingham.ac.uk

Gastric, oesophageal and pancreatic cancers are often inoperable; therefore systemic chemotherapy and palliative care are frequently utilised as treatment options. Targeted injectable polymer based therapies have recently been evaluated as an alternative treatment,1 as they are thought to improve overall survival and quality of life by minimising the systemic side effects associated with traditional chemotherapy. Organogels are semi-solid materials composed of low concentrations (<15%) of gelator molecules that self-assemble into solid aggregates in the presence of an appropriate solvent. Stabilised by weak chain interactions such as hydrogen bonding, Van der Waals forces and π-stacking,2 these aggregates are most often formed by uni-dimensional growth into fibres measuring a few nanometres in width and up to several micrometres in length. Our goal is to develop biocompatible organogels that can be administered as an injectable therapy that will form in-situ and thus localise chemotherapeutics within the tumour mass. Our design principle employs molecular subunits for the organogelator that are inherently non-toxic such as nucleosides and fatty acids. In the studies reported here, cytidine was conjugated to various long chain fatty acids (C12-C20), generating 2 libraries of compounds using: Chemical synthesis via selective acylation of the cytidine amine with the fatty acid; Enzymatic synthesis using Lipase B Candida Antarctica to catalyse the regio-selective esterification of the 5'-ribose hydroxyl group. These compounds formed organogels using organic solvents such as dimethyl sulfoxide, and ethanol in combination with water. Our data indicates that we require a high solvent ratio of at least 40% to form the organogel. Rheological measurements and CryoTEM analyses were performed to characterise these novel materials. The results indicate this class of long chain fatty acid conjugates show promise as intra-tumoral therapeutics and may also have further potential applications for use in drug delivery and tissue engineering.

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# NOVEL SERIES OF NO-HYBRIDS WITH A FUROXAN CORE

**Phillip Thomson,**
Chemistry Department School of Physical and Geographical Sciences
Keele University, Staffordshire, ST5 5BG, United Kingdom; Email: p.thomson@keele.ac.uk

Nitric oxide can be released in a controlled manner when locked in a 5 membered heterocyclic ring known as a furoxan or a 1,2,5-oxadiazole 2-oxide.
A novel series of symmetrical furoxans have been synthesised with various substituents positioned on each of the aromatic rings. Each compound in the series was monitored under various conditions to determine the rate of NO release. In addition all compounds have been tested against a range of ovarian cancer cell lines to determine any anti-cancer activity.

# 61 UNDERSTANDING THE TRANSIT OF BILOSOMES AND SUBUNIT ANTIGEN VIA THE ORAL ROUTE

**Jitinder Singh Wilkhu[1],** David Anderson[2], Yvonne Perrie[1]
[1] School of Life and Health Sciences, Aston University, Aston Triangle, Birmingham, UK,
[2] Variation Biotechnologies INC, 1740 Woodroffe Avenue, Ottawa, Ontario;
Email: jitinder@gmail.com

Whilst offering a range of advantages, few vaccines can be administered orally due to their degradation in the harsh gut environment and their poor uptake by appropriate target sites, namely M cells located in the Peyer's patches, which are responsible for secretory IgA and other mucosal responses1.Therefore within this work we have considered the use of bilosomes to enhance the protection and delivery of sub-unit vaccines and tracked their transit through the GI tract after oral administration. H3N2 antigen was radiolabelled with I-125 isotope and was then entrapped into bilosomes [5:4:1 ratio of Monopalmitoyl Glycerol: Cholesterol: Dicetyl Phosphate respectively at 6 μm] vesicles prepared via homogenisation melt method.

Results show that the blood, spleen, kidneys and liver have trace amounts of antigen recovery with the majority of the antigen being located in the stomach, Small intestine, and the colon and cecum. Upon entrapping the antigen into bilosomes, significantly ($p < 0.05$) more antigen was located in the small intestine and significantly less was located in the colon and cecum. Of the dose administered, in general significantly ($p < 0.05$) more antigen (50.5%) was recovered when formulated with bilosome vesicles compared to the free antigen dose (38%). In terms of the antigen reaching the site of action, antigen recovery at the Peyer's patches and mesenteric lymph tissue show that the presence of the bilosome vesicles results in a higher recovery of antigen within the Peyer's patches with significantly higher ($p < 0.05$) levels of antigen within the mesenteric lymph tissue when delivered using bilosomes. In conclusion, there is an advantage of associating the H3N2 antigen with the bilosome vesicles as it increases the percentage of antigen recovered within the target site. Studies to consider if these differences translate into enhanced immune responses continue.

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# 62 THE EFFECT OF LIPOSOME PREPARATION METHOD ON VACCINE RETENTION AT THE INJECTION SITE AND DRAINAGE TO THE LOCAL LYMPH NODES

**Alexander Wilkinson[1],** Randip Kaur[1], Dennis Christensen[2], Afzal Mohammed[1] and Yvonne Perrie[1]

[1] School of Life and Health Sciences, Aston University, Birmingham B4 7ET, United Kingdom; [2] Statens Serum Institut, Copenhagen, Denmark; Email: wilkina1@aston.ac.uk

A series of cumulative studies within our laboratory have identified a correlation between depot formation promoted by cationic liposomes (in combination with anionic antigen) and the efficacy to promote protective Th1 immune responses [1,2]. The aim of this research was to investigate two potential methods for entrapping antigen within liposomal adjuvants and the subsequent effect on the in vivo biodistribution of vaccine components. Liposomes were formulated from dimethyldioctadecylammonium (DDA) bromide or 1,2-distearoyl-sn-glycero-3-phospho-L-serine (DSPS) in combination with trehalose 6,6' – dibehenate (TDB). Liposomes were prepared by the lipid-film hydration (LH) method [3], dehydration-rehydration vesicle (DRV) method [4] or double emulsion (DE) solvent evaporation method [5] with a final lipid concentration of 1.98 mM and a TDB concentration of 0.25 mM respectively. These liposomes were formulated with either the cationic protein lysozyme or the anionic TB subunit antigen, H56 at an in vivo dose of 5 μg. Biodistribution studies have shown that irrespective of preparation method, cationic DDA/TDB have increased retention at the site of injection compared to anionic DSPS/TDB which have lower retention at this site due to their inability to electrostatically interact with negatively-charged interstitial proteins at the injection site. For all three preparation methods, this supported a strong 'H56 antigen depot' at the injection site, possibly due to the electrostatic binding of the anionic H56 antigen to the cationic liposomes. By varying the preparation method of liposome adjuvants in order to entrap the antigen within the delivery system (DE and DRV), this leads to significantly higher antigen drainage (compared to drainage of the delivery system) to the popliteal lymph nodes. This may possibly be due to increased protection of the antigen when incorporated within the liposome. Studies are ongoing in order to investigate the effect of preparation method on the immunogenicity of these vaccine formulations.

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# 63 SILENCING THE CENTRAL NERVOUS SYSTEMS: A GENE SILENCING APPROACH TO ENHANCING THE NEUROPHARMACOKINETICS OF CNS DRUG DELIVERY

**Manjit Kaur** and Dr.Raj K.S.Badhan, Aston University, Birmingham B4 7ET, United Kingdom; Email: kaurm12@aston.ac.uk

Drug delivery to the central nervous systems (CNS) is significantly hindered by the presence of the blood brain barrier (BBB) and blood cerebrospinal fluid barrier (BCSFB). The BCSFB is an interesting target for drug delivery as it possess enhanced perfusion rates and is a highly permeable when compared to the BBB, suggesting a possible approach to exploiting increased drug delivery to the CNS. The aim of this work is to modulate the expression of key drug transporters, which significantly influence the pharmacokinetics of drug disposition across the CNS. We utilised the recently developed immortalised rat choroid plexus cell lines, Z310, to examine the interaction of known phytochemical modulators of translational regulation of BCRP. Phytochemical modulators were demonstrated to posses high IC50 (hespertin: 106μM; quercetin: 293μM; naringenin: 454μM; chrysin: 75μM) and limited cytotoxicity. The expression of key pharmacokinetically relevant drug transporter proteins, namely BCRP and MRP1 were demonstrated through RT-PCR. Functional activity of the transporter proteins (BCRP and MRP1) was demonstrated through the use of a known fluorescent substrate (Hoechst 33342) in the presence and absence of 50μM of a known inhibitor (novobiocin) and two flavonoids (quercetin and chrysin). Significantly, exposure of chryin and quercetin to Z310 cells demonstrated up-regulation of gene expression for both BCRP and MRP1, suggesting flavonoids are potent inhibitors during short term incubation but exhibit up regulation over long term exposure. Work is on-going to examine the role of the nuclear hormone receptor Aryl Hydrocarbon Receptor (AHR) in regulating BCRP through the use of chemical modulators (flavonoids) along with gene silencing approached such as siRNA and microRNA.

# 64 IN VITRO PERMEABILITY ASSESSMENT OF RECENTLY DEVELOPED RAMIPRIL, LISINOPRIL AND SPIRONOLACTONE LIQUID FORMULATIONS

**Craig A. Russell**[1], Madjid Hussain[2], Qamar Nawaz[2], Amar. Nawaz[2], Yvonne Perrie[1] Afzal R. Mohammed[1].

[1] Aston University, Birmingham B4 7ET, United Kingdom; [2] Pharmaspec Ltd Birmingham, United Kingdom; Email: kaurm12@aston.ac.uk

Three liquid formulations were developed in order to provide age appropriate formulations of Ramipril, Lisinopril and Spironolactone with the aim of targeting the paediatric patient population. The three drugs in question were all listed in the 2010 EMEA priority list of off patent paediatric medications. Ramipril and Lisinopril are angiotensin converting enzyme (ACE) inhibitors and Spironolactone is an antihypertensive from the group of potassium-sparing diuretics.

The in vitro model for permeability used to assay the permeability of the drugs uses Caco-2 cells. The cells are grown on permeable membrane supports which create an apical and basal chamber inside each well of a six well plate. Once the cells are seeded onto the membrane inserts, they are cultured for 21 days with media changes every 2-3 days. Trans-epithelial electronic resistance (TEER) is used to ascertain confirmation of cell monolayer integrity and TEER measurements are carried out before and immediately after any experimental procedure to ensure the maintenance of cell monolayer integrity. Following addition of formulations to the apical chamber, transport media from the basal chamber is sampled at various time points and analysed via HPLC to measure the drugs transport as a factor of time. This allows for the calculation of the permeability coefficient or apparent permeability (Papp) of the drugs in each formulation. Papp is considered to be a reliable indicator for the expected in vivo drug absorption. It is generally accepted that; completely absorbed drugs have Papp >1x10-6cm/s. (LogPapp >-6) whereas, incompletely absorbed drugs have Papp <1x10-6cm/s. (LogPapp <-6).

In the case of each of the three formulations it was found that Papp values generated following the permeability assessment provided a good correlation with the data available for the expected in vivo drug absorption with Spironolactone indicating 100% absorption and Lisinopril and Ramipril indicating 50-60% and 20-30% absorption respectively.

# 65 CHITOSAN BASED NANOPARTICLES FOR ORAL ADMINISTRATION OF INSULIN: EVALUATION STUDIES IN CACO-2 CELLS

**Madhuri Dandamudi,** Elena Polycarpou, Mark Carew, Raid Alany, Anil Vangala
School of Pharmacy and Chemistry, Faculty of Science, Engineering and Computing, Kingston University. London KT1 2EE, United Kingdom; Email: k1170193@kingston.ac.uk

Around the world 346 million people are suffering from diabetes and the number is increasing drastically. Subcutaneous administration of Insulin is most common therapy for diabetes however, needle phobia and pain results in poor patient compliance. Developing an oral formulation for insulin is a challenge primarily because of its gastric instability and poor permeation across intestinal membrane. Previous reports have shown particulate delivery systems being effective in delivering insulin via oral route. The aim of the present study is to prepare and evaluate the potential of polymer based nanoparticles for oral delivery of insulin.

Nanoparticles were prepared using chitosan polymer and sodium tri polyphosphate (TPP) as cross linking agent via ionic gelation method. Characterization studies including morphology (scanning electron microscopy), particle size, zeta potential and loading efficiency have been evaluated. Insulin release profile from nanoparticles is performed in simulated gastric fluid and simulated intestinal fluid using dialysis membrane. Permeation studies were conducted using ussing's camber on Caco-2 cell monolayers which mimick intestinal membrane. Morphological analysis revealed that the insulin loaded nanoparticles were spherical structured with partial aggregation. Size of nanoparticles ranged between 203 ± 2.40 nm to 302 ± 9.38 nm with varying zeta potential of 26.16 ± 3.00 mV to 31.80 ± 2.47 mV. Loading efficiency of insulin was found to be 84.70 ± 1.20%, which is assayed at 214 nm using UV spectrophotometer. Insulin release studies had shown a sustained profile with maximum release of 43.97 ± 0.20% after 24 hour period. Insulin transport across caco-2 monolayers has been confirmed through the permeation studies using ussing's chamber. Decrease in resistance of cells (indicating permeation) is observed when formulation is placed on the apical side of the caco-2 monolayer (data to be presented on poster). With further studies ongoing, the current data suggests that chitosan-based nanoparticles enable successful insulin loading with effective permeation ability through the epithelial membrane, demonstrating the potential of these systems in delivering insulin via oral route.

# 66 QUANTIFICATION OF 2-OXOTHIAZOLIDINE-4-CARBOXYLIC ACID PERMEATED THROUGH EXCISED BOVINE SCLERA USING A NEW HPLC METHOD

**A. A. Al-Kinani[1],** A. Vangala[1], D. Naughton[2], B. Pierscionek[1, 2], G. Calabrese[1] and R. G. Alany[1, 3]

[1] School of Pharmacy and Chemistry, [2] School of Life Science, Kingston University London, Kingston upon Thames, London, United Kingdom, [3] School of Pharmacy, The University of Auckland, Auckland, New Zealand; Email: K0939984@kingston.ac.uk

There are over 18 million people worldwide suffering from cataract. Oxidative damage due to low level of glutathione is one of the main reasons for cataract formation. Recent work showed that cystine-glutamate exchangers are expressed in the lens nucleus which suggests that these exchangers could be targeted to increase the cysteine level and hence glutathione. It has been reported that 2-oxothiazolidine-4-carboxylic acid (OTZ) which is a cysteine prodrug can increase the cellular level of GSH. Currently there is no analytical method that can separate and quantify OTZ. The present study aims to develop and validate a stability-indicating HPLC method for the quantification of OTZ in simulated aqueous humour. The developed method was validated according to the FDA guidelines. Accuracy, precision, selectivity, sensitivity, linearity, lower limit of detection (LLOD) and lower limit of quantification (LLOQ) were the parameters considered in the method validation. The separation was achieved using the HPLC column (Kinetex 100×4.6mm); mobile phase consisted of ammonium acetate and acetonitrile with a flow rate of 1.5 mL/min and the detection wave length was set at 265 nm. Permeation of OTZ through excised bovine sclera was studied using a Franz diffusion cell apparatus; cell temperature was maintained at 35 ± 0.5°C and 400 μL samples were withdrawn from the receptor compartment at set time points and analysed using HPLC. The developed method was found to be accurate precise with LLOD and LLOQ of 100 ng/mL and 200 ng/mL respectively; method selectivity was confirmed by the absence of any interference of the analyte peak with the matrix peaks. The constructed calibration curve was linear in the range of 0.2 - 10 μg/mL. The method was used to quantify the scleral permeation of the OTZ. The flux and lag time were calculated and found to be 349.5 μg/cm2.hr and 0.65 h respectively. The developed method was found to be valid, accurate and precise. This method will be used in further studies to monitor and quantify the permeation of OTZ through excised bovine and porcine corneal and scleral tissue.

# NOTES


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