UKI CRS Symposium 2010

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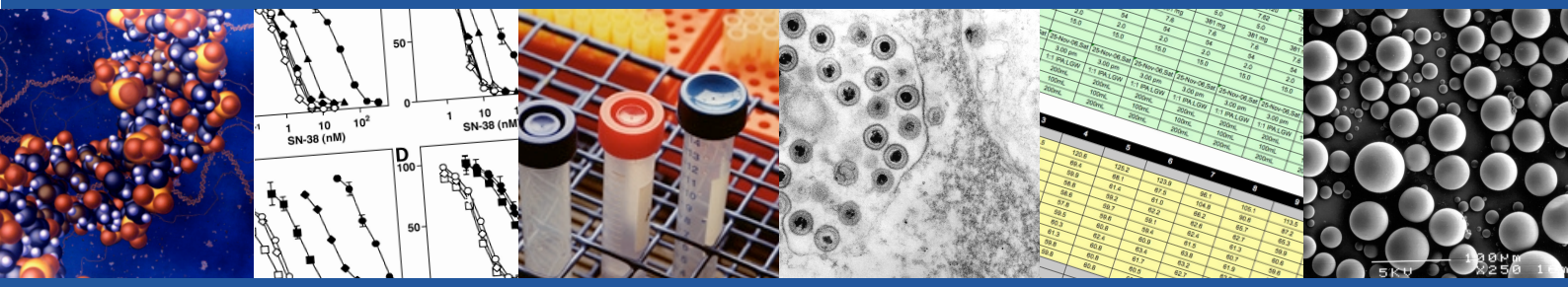

Controlled Release in Drug Delivery: Right Time, Right Place

Wednesday 14 April 2010

University of Hertfordshire
Fielder Conference Centre
Hatfield Business Park

WELCOME!

Dear delegates,

A very warm welcome to the 2010 UKICRS Symposium on 'Controlled Release in Drug Delivery: Right Time, Right Place'. This year we are back to our roots, focussing on various aspects in controlled release. We are delighted to have Prof Clive Wilson, a distinguished and excellent speaker addressing the conference on ocular drug delivery. This is followed by presentations from various top speakers in gastrointestinal tract delivery from academia and industry where Dr Martin Wickham looks at novel in vitro gastric model in assessing orally administered formulations and Dr Abdul Basit speaks on how to get his hand dirty on colonic delivery. Dr Emma Mc Connell and Dr Marina Levina provide their invaluable industrial views and experience on the challenges of investigating gastoretention in early development and improving 1st time right oral drug delivery using hydrophilic matrices. We are also fortunate to have Dr Peter Seville, an expert in pulmonary delivery to speak on spray-drying technology and Dr Ryan Donelly to present his latest research on hydrogel microneedles for transdermal delivery. Don't forget also 4 post-graduate presentations from our budding future scientists!

The current economic climate made us apprehensive about this year's symposium and we are delighted to have overwhelming support from delegates in the UK, Ireland and as far away as India. A big 'thank you' to you all!

Special thanks also go to our two exhibitors, Surface Measurement Systems and Micromeritics Ltd. Please don't forget to visit their stands and find out more about the companies and the products they offer.

This has been our 16th year organising the symposium - we are grateful for your support that keeps us going! We hope you will enjoy the talks, the poster and exhibition sessions, meeting new people, networking and exchanging fresh ideas and, of course, the food too!

Enjoy the symposium!

Woei Ping Cheng, Sam Pygall and Andrew Parker

(2010 UKICRS symposium organising committee)

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PROGRAMME

9.00 am	Registration & Coffee
9.45 am	**Prof Yvonne Perrie** (Aston University) Welcome & Opening Remarks
10.00 am	**Prof Clive Wilson** (University of Strathclyde) 'The outs and ins of ocular drug delivery'
10.45 am	**Dr Peter Seville** (Aston University) 'Better particles by design: using spray-drying technology to develop inhalable particles'
11.15 am	Coffee break
11.45 am	**Dr Martin Wickham** (University of East Anglia) 'The dynamic gastric model - A new in vitro model for the bio-relevant dissolution assessment of orally administered'
12.15 pm	Postgraduate talk 1
12.30 pm	Postgraduate talk 2
12.45 pm	Lunch and poster session
2.15 pm	**Dr Abdul Basit** (University of London) 'Drug delivery to the colon: it's a dirty job, but somebody has to do it'
2.45 pm	**Dr Ryan Donnelly** (Queen's University Belfast) 'Hydrogel microneedle arrays for transdermal and intradermal drug delivery'
3.15 pm	**Dr Emma McConnell** (Merck Sharp & Dohme) 'The challenges of investigating gastroretention in early development'
3.45 pm	Coffee break
4.00 pm	Postgraduate talk 3
4.15 pm	Postgraduate talk 4
4.30 pm	**Dr Marina Levina** (Colorcon) 'Improving "1st time right" oral drug delivery using hydrophilic matrices'
5.00 pm	Closing remark and poster prize

MAP & TRAVEL INFORMATION

Public Transport

By Rail.
Train services between Hatfield and London (Kings Cross) fastest journey time 22 minutes
Train services between Hatfield and London (Moorgate) fastest journey time 40 minutes
The Fielder Centre is 2 miles from the Hatfield station.
Taxis are available from the station forecourt.
Journey time approximately 5 minutes.
For information on train operators and times phone National Rail Enquiries on 0845 7484950.

Car Parking.
There is free parking at the Fielder Centre.

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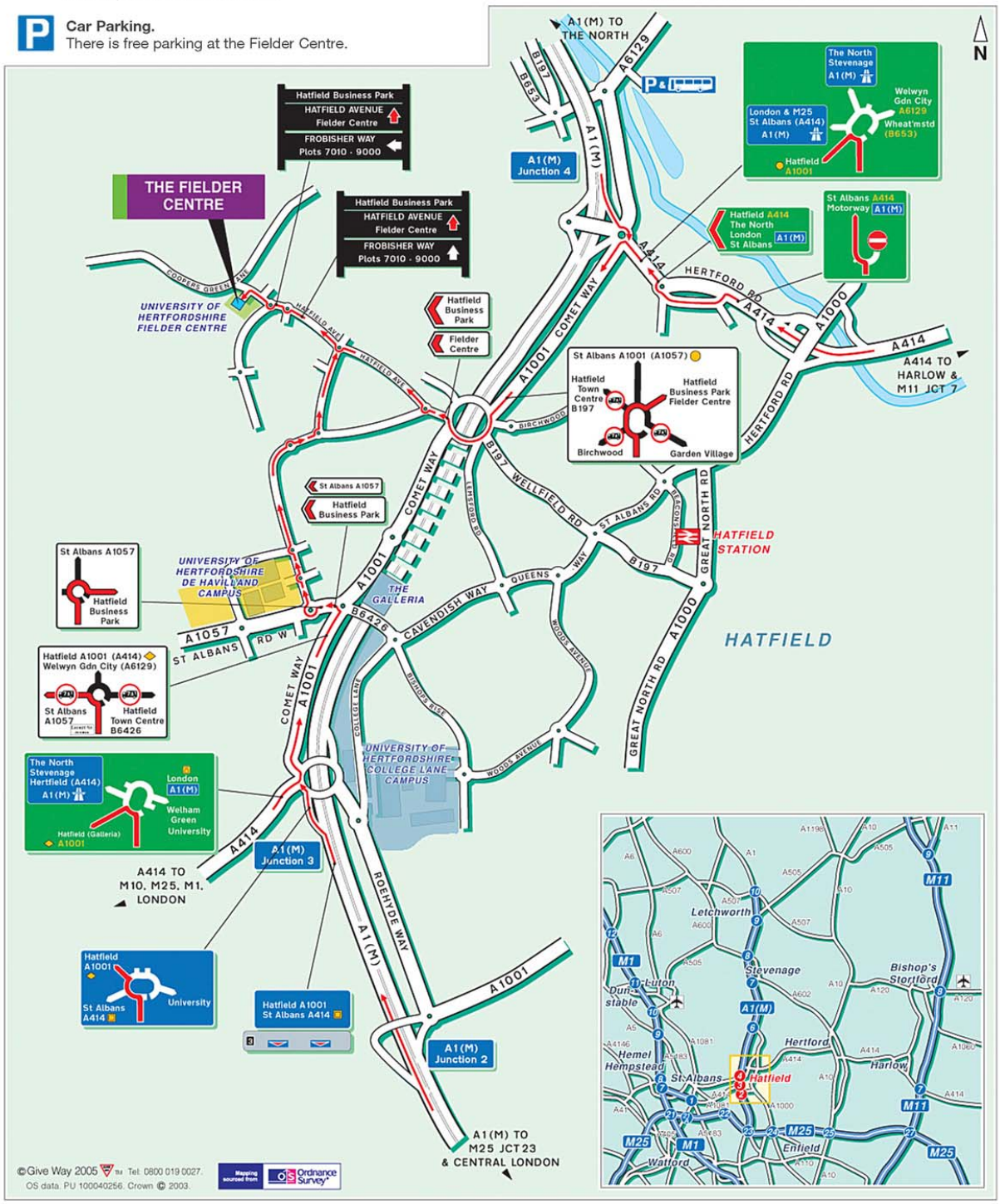

INVITED SPEAKERS

PROF CLIVE WILSON - UNIVERSITY OF STRATHCLYDE

'The outs and ins of ocular drug delivery'

Clive G. Wilson is the J.P. Todd Professor of Pharmaceutics in the Department of Pharmaceutical Sciences at the University of Strathclyde, Glasgow, Scotland. He received a Ph.D. (Drug Metabolism) in 1973 from the University of Surrey. He was a senior lecturer at the University of Nottingham where he maintains a honorary appointment in the Medical Physics Department. He also is an adjunct professor of Industrial Pharmacy at the University of Cincinnati. Dr Wilson's research has focused on the utilization of gamma scintigraphy in studying the behaviour of formulations in man. He is one of the pioneers in the application of imaging techniques in drug research, and has received the Amersham and Pfizer awards in recognition of this work. His key areas of interest are drug delivery to the eye and the transit absorption relationship in oral drug formulation. He has supervised more than forty Ph.D. students and has authored over 400 research papers, book chapters and reviews. The publications reflect his interest in imaging, physics, drug metabolism and pharmacokinetics. Dr Wilson is a member of the editorial boards of the European Journal of Pharmaceutics and Biopharmaceutics, and Drug Delivery and the editor of the Taylor & Francis; series in pharmaceutical technology. He is a member of numerous professional societies including British Society of Gastroenterology, Controlled Release Society, Association de Pharmacie Galenique, The European Vision & Eye Research Association, The British Pharmacological Society and the American Academy of Pharmaceutical Sciences.

DR PETER SEVILLE - ASTON UNIVERSITY

'Better particles by design: using spray-drying technology to develop inhalable particles'

Peter graduated with a BPharm from the Welsh School of Pharmacy, Cardiff in 1994, following which he undertook his pre-registration pharmacist placement at St Peter's Hospital, Surrey. He then spent some time as a basic grade pharmacist at the Royal Free Hospital, London, before returning to Cardiff University to commence a PhD investigating the development of solution pMDI systems. Subsequently, he embarked on a postdoctoral research position at Cardiff investigating pulmonary delivery of non-viral gene therapies.

Peter joined the staff at Aston University, Birmingham as a Lecturer in Pharmaceutics in 2002, and in 2008 was appointed as a Senior Lecturer. He heads up the Inhalation Technology Research Team with recent investigations focusing on use of spray-drying technology to develop DPI and pMDI formulations, with particular interests in the use of polymeric materials to generate powders that exhibit a modified drug release profile and the preparation of inhalable formulations of proteins and peptides. He has published several original research papers and has a patent in the area of pulmonary drug delivery. Peter also serves as a member of the Academy of Pharmaceutical Sciences Inhalation Focus Group steering committee, and has co-organised a number of symposia and conferences in the area of inhalation science.

DR MARTIN WICKHAM - UNIVERSITY OF EAST ANGLIA

'The dynamic gastric model - a new in vitro model for the bio-relevant dissolution assessment of orally administered'

Martin is the Model Gut Platform Leader at the Institute of Food Research, UK. Martins' first degree was in Biotechnology and his PhD studied the bio-physics of the human gut. Martin is a Biophysicist with over 15 years experience in modelling human digestion. His research interests are centred on understanding human gut physiology through the development of in vitro models of digestion, with particular emphasis on the interface between complex food and pharmaceutical systems and human response. He is a BBSRC/ Royal Society of Edinburgh Enterprise Fellow, and has an MBA from the University of Dundee. Martin also holds a visiting Professorship at the Pharmaco-Biological Department, University of Messina, Italy and an Adjunct Faculty Position at The New Zealand Institute for Plant and Food Research, NZ.

DR ABDUL BASIT - UNIVERSITY OF LONDON

'Drug delivery to the colon: it's a dirty job, but somebody has to do it'

Dr Abdul Basit holds the position of Senior Lecturer in Pharmaceutics at the School of Pharmacy, University of London. He is also a Visiting Professor in the Faculty of Chemical and Pharmaceutical Sciences at the University of Chile. He further holds an Honorary Lectureship in Gastroenterology at the Wingate Institute of Neurogastroenterology, Queen Mary College, University of London. Dr Basit read Pharmacy at the University of Bath and graduated in 1993 with first class honours. Following a short period with Pfizer in the UK, he undertook post-graduate studies in Pharmaceutics at the School of Pharmacy, University of London and was awarded a PhD in 1999. Dr Basit's research sits at the interface between pharmaceutical science and gastroenterology and is focused on oral delivery. Dr. Basit leads a large and multi-disciplinary research group and has published extensively. Dr Basit sits on the scientific advisory board of several pharmaceutical and healthcare companies and is on the editorial board of scientific journals. He is a frequent speaker at international conferences and is a consultant to the pharmaceutical industry. In recognition of his research achievements Dr. Basit was the recipient of the 2004 Young Investigator Award in Pharmaceutics and Pharmaceutical Technology from the American Association of Pharmaceutical Scientists (AAPS). He is the first scientist based outside of North America to receive this award.

DR RYAN DONNELLY - QUEEN'S UNIVERSITY BELFAST

'Hydrogel microneedle arrays for transdermal and intradermal drug delivery'

Ryan obtained a BSc (First Class) in Pharmacy from Queen's University Belfast in 1999 and was awarded the Pharmaceutical Society of Northern Ireland's Gold Medal. Following a year of Pre-Registration training spent in community Pharmacy Practice, he registered with the Pharmaceutical Society of Northern Ireland. He then returned to the School of Pharmacy in 2000 to undertake a PhD in Pharmaceutics. He graduated in 2003 and, after a short period of post-doctoral research, was appointed to a Lectureship in Pharmaceutics in January 2004.

Dr Donnelly's research interests are centred on transdermal and topical drug delivery. Recent work has included the design of novel dosage forms containing photosensitisers for Photodynamic Therapy (PDT) and Photodynamic Antimicrobial Chemotherapy (PACT) and the use of microneedle arrays to bypass the stratum corneum barrier. His microneedle research work is funded by BBSRC and EPSRC.

DR EMMA MCCONNELL - MERCK SHARP & DOHME

'The challenges of investigating gastroretention in early development'

Emma works as a Research Scientist in Formulation Development at Merck, Sharp and Dohme, focusing on developing oral drug delivery systems. Prior to this Emma held a post as a Research Fellow at The School of Pharmacy, University of London, working and publishing on various aspects of site-specific drug delivery and biopharmaceutics. This work was preceded by a PhD, also at the School of Pharmacy, investigating the targeting of vaccines to the colon, and an MPharm degree at Queen's University Belfast.

DR MARINA LEVINA - COLORCON

'Improving "1st time right" oral drug delivery using hydrophilic matrices'

Marina is Senior Manager, Product Development at Colorcon. She has 10 years of experience working with excipients, being responsible for all technical aspects of Colorcon's range of immediate and modified release products, including technical support and product development. Marina has experience in the production of tablets by both conventional and ultrasound-assisted compaction, and with the development of solid oral dose formulations for immediate- and modified-release applications.

She has published over 40 research abstracts and papers in the area of Pharmaceutical Technology and presented at various conferences and seminars. Dr Levina is a reviewer for the Journal of Pharmaceutical Sciences, European Journal of Pharmaceutics and Biopharmaceutics, AAPS PharmSciTech and Molecules; and is also part of the Editorial Advisory Board for Pharmaceutical Technology Europe. She regularly presents as an invited speaker at the European Continuing Education College Courses (London, UK). Marina acts in the capacity of Industrial Supervisor for Colorcon sponsored UK and Europe based MSc and PhD studies.

POSTER ABSTRACTS

1 ORAL DELIVERY OF SALMON CALCITONIN USING A NOVEL LIQUID EMULSION DRUG DELIVERY SYSTEM – LEDDS®

T Aguirre (1,2), SM Ryan (1), M Rosa (2), V Aversa (2), I Coulter (2) and DJ Brayden (1).
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Salmon calcitonin (sCT) is a polypeptide hormone composed of 32 amino acids. It is used in the treatment of osteoporosis, hypercalcaemia, and others bone related diseases. Establishing an oral delivery system for sCT would be of great importance because parenteral administration results in poor patient compliance and the nasal formulation has sub-optimal bioavailability. However, as for many other polypeptides, the oral bioavailability of sCT is limited due to enzymatic degradation and poor permeation across intestinal epithelial cells. The main objective of this work is to develop an oral delivery system for sCT employing the technology developed by Sigmoid Pharma Ltd. – Liquid Emulsion Drug Delivery System (LEDDS®). Four different LEDDS® formulations were prepared. Preliminary physicochemical characterization of sCT solutions with different components of LEDDS® was carried out using circular dichroism. In vitro bioactivity was assessed using the breast cancer cell line T47D, which over-expresses the calcitonin receptor and results in intracellular cAMP release upon activation. Results suggest that there are no relevant changes in the secondary structure of the peptide for the majority of excipients included in sCT formulations; however the CD spectra of sCT with one component showed an increase in β-sheet and α-helix profiles. sCT formulations retained approximately 100% in vitro bioactivity compared to native sCT, showing that the formulation process was not harmful to sCT. Overall, incorporation of sCT in to LEDDS® formulation may have a promising future.

2 SUPERCRITICAL FLUID TECHNOLOGY FOR THE PRODUCTION OF GASTRORETENTIVE FORMULATIONS

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Oral delivery of therapeutic compounds is widely recognised as the preferable route of administration. However, a significant number of therapeutics demonstrate narrow absorption window existing in the proximal section of the small intestine. Conventional oral delivery systems have a relatively short gastric residence time and travel past this window rapidly, limiting the amount of drug which is accessible for the absorptive surfaces in the gut. Bioavailability is consequently restricted. With the aim of improving bioavailability of orally administered therapeutics with narrow absorption window, the concept of gastroretention has been adopted, often coupled with controlled-release. Different gastroretention technologies have been exploited, including mucoadhesive systems, devices that rapidly increase in size, or density controlled systems i.e. floating delivery formulations that postpone gastric emptying and remain floating on the gastric media.

In the present study supercritical carbon dioxide technology is used to produce polymeric particles with a density lower than that of the gastric media; the main criteria of the system is to immediately float on the gastric fluid. This production technology exploits the ability of supercritical carbon dioxide to liquefy polymers, such as PLGA, and thus allow their processing into particulate formulation. Additionally it circumvents the need for organic solvents, typically used in conventional methods for particle preparation, and can therefore be considered a 'green technology'.

PLGA based microparticles were produced applying supercritical carbon dioxide technology based on the 'Particles from Gas Saturated Solutions' (PGSS) process in size ranges from 100 µm - 2 mm. These particles display reproducible porous morphologies, while density analysis reveals a size dependent relationship. The capacity of the particles to float on the stomach fluid has been confirmed using in vitro analysis. These initial studies indicate the potential of produced microparticles as a gastroretentive delivery system and warrant further studies.

3 ENHANCED GENE EXPRESSION OF POLYETHYLENIMINE BY SURFACE MODIFICATION WITH ARGININE, LYSINE AND LEUCINE

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Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde

Gene therapy has become a promising strategy for the treatment of many inheritable or acquired diseases that are currently considered incurable. However, the potential of gene therapy is currently limited by the lack of delivery systems able to efficiently carry therapeutic DNA to their site of action. In this study, we show that the grafting of arginine, lysine and leucine residues onto the surface of the cationic polymer polyethylenimine, chosen as a model non-viral gene delivery system, improves the gene expression and therapeutic efficacies of this delivery system on A431 epidermoid carcinoma and T98G glioma in vitro. Novel arginine, lysine and leucine-bearing polyethylenimine were able to condense DNA into particles with positive surface charges and average sizes inferior to 300 nm, attributes favourable for efficient gene delivery in vitro. The conjugation of arginine and lysine residues to polyethylenimine led to an improved transfection compared to the native polyethylenimine and DOTAP on both the tested cell lines, with improvements of up to 11.7 times following treatment with lysine-bearing polyethylenimine polyplex compared to DOTAP-DNA in A431 cells. The grafting of leucine to polyethylenimine improved the level of transfection only on T98G cells, by 4 times compared to DOTAP-DNA. The administration of therapeutic DNA complexed with lysine and leucine-bearing polyethylenimine led to an improved therapeutic efficacy compared to the unmodified polymer on both the cancer cell lines tested, by up to 51 times and 127 times respectively in A431 and T98G cells. Arginine-bearing polyethylenimine was more efficacious than the native polymer only on A431 cell line. These results show that the grafting of arginine, lysine and leucine to a model polyethylenimine delivery system is highly promising for improving transfection and therapeutic efficacies to cancer cells. The potential of these amino acid-bearing delivery systems will be further investigated.

4 SACCHARIDE FREE LYOPHILISED ORALLY DISINTEGRATING TABLET (ODT) USING NOVEL COMBINATION OF AMINO ACIDS

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Lyophilised orally disintegrating tablets (ODTs) have achieved a great success in overcoming dysphagia associated with conventional solid dosage forms. However, the extensive use of saccharides within the formulation limits their use in the treatment of chronic illnesses. The current study demonstrates the feasibility of using combination of proline and alanine as matrix supporting/disintegration enhancing agent to formulate zero saccharide ODTs. Proline was added to the formulation to promote the disintegration of the tablets because of its short wetting time while alanine was added as a crystallizing agent to enhance the stability of the tablets during freeze drying process.

The study used 5% gelatin as a binder and investigated the influence of inclusion of various ratios (100:0, 85:15, 70:30, 45:55, 30:70, 15:85, 0:100) of L-proline and L-alanine at total concentrations of 10%, 30%, 50%, and 70% w/w (total solid) on the thermal properties of the frozen formulations, formation of intact tablets after freeze drying and ODT characteristics in terms of disintegration time and mechanical properties. The optimised formulation was then used to investigate the effect of different freezing protocols such as flash freezing and annealing on sublimation rate, disintegration time and mechanical properties of ODTs. Additionally, the inner structural features imposed by the freezing protocol were studied using mercury porosimetry.

The results showed that inclusion of proline and alanine improved ODT properties when compared to their individual counterparts. The inclusion of alanine in the formulation at high concentration enhanced the mechanical properties of the ODTs without compromising the formation of intact tablets. On the other hand, proline promoted the disintegration by enhancing the wettability of the ODTs. Also the results showed that annealing induced morphological changes in the ODTs that not only allow faster sublimation rate but also shorter disintegration time.

5 CHARACTERIZATION OF THE GASTROINTESTINAL TRACT OF THE GUINEA PIG AND RABBIT FOR ORAL DRUG DELIVERY

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Laboratory animals such as guinea-pigs and rabbits are often used in drug delivery and research. However, basic information about their gastro-intestinal pH, fluid volume, and lymphoid tissue is not completely known. We have investigated these to ascertain the suitability of these animal models for pre-clinical studies.

The GI tract from six healthy male guinea-pigs (Dunkin-Hartley white; 460-524 g b.w., fed ad-libitum) and six healthy male rabbits (New Zealand white; 2.1-2.3 Kg b.w., fed ad-libitum) was studied. The GI tract was 423 cm long (stomach 8 cm, small intestine 267 cm, caecum 39 cm, colon 109cm) in the rabbit and 268 cm (stomach 5 cm, small intestine 148 cm, caecum 15 cm, colon 100 cm) in guinea-pig. A well defined appendix was found in rabbit (10cm long), which was not seen in the guinea-pig. Total wet mass in rabbit was 203g (75% water) – of which 43% were held in the caecum followed by 35% in the stomach. A similar situation was found in guinea-pig which had 65 g wet mass (79% water). In both animals, the gastric pH was higher in the fundus region compared to antrum (rabbit: 3.0 vs. 1.6, guinea-pig: 4.2 vs. 3.1) and the average pH in proximal small intestine was ~6.5 and rose to ~7.4 in distal parts. Colonic and caecal pH was in the range of 6.1-6.5. Lymphoid follicles were not seen in stomach; however were found in small and large intestines. Rabbit caecum did not have lymphoid follicles however they were present in guinea-pig caecum. Sacculus rotundus and appendix of the rabbit were, however, fully covered with lymphoid follicles, which were not present in guinea-pig.

We conclude that significant inter-species differences exist in the anatomy of the gut (pH profile, water content and lymphoid follicles); these differences should be considered when these animal models are used.

6 THE USE OF A VESICULAR DELIVERY SYSTEM IN THE ENHANCEMENT OF CISPLATIN BIOAVAILABILITY

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Lung cancer, the leading cause of cancer death, is mainly treated by cisplatin. Presently, it is administered as an intravenous infusion over 6-8 hours resulting in systemic exposure with dose-limiting severe and chronic side effects. The aim of this study was to assess the ability of non-ionic surfactant vesicles (NIVs) of cisplatin to enhance its in vivo pharmacokinetics and activity against cancer cells. The method involved preparing cisplatin-NIVs and determining drug loading, using an HPLC method modified from Lopez-Flores et al (2005), at different times post-production. The size and zeta potential of the vesicles were also measured to monitor their stability over time. The anti-cancer activity of cisplatin solution and cisplatin-NIVs were compared by assessing their cytotoxic effect on the in vitro proliferation of B16-F0 melanoma murine cell line using Alamar Blue assay. BALB/c mice, pre-inoculated with B16-F0, were administered a single dose of either formulation via intravenous injection then tissue drug levels were determined. The results demonstrated that high cisplatin loading in NIVs was achievable and that the vesicles were stable. Cisplatin-NIVs had significantly greater cytotoxicity on the proliferation of B16-F0 over free cisplatin ($p < 0.05$). In vivo results showed failure of free cisplatin to reach target tissues from a single dose. In contrast significant tissue uptake was observed from a single dose of cisplatin-NIVs, exploiting their uptake by the mononuclear phagocytic system preferentially in the lungs and in organs like the liver where metastases are common. In conclusion, NIVs can offer a suitable way of targeting cisplatin delivery; this could play a role in reducing exposure of other tissues to free cisplatin, which is currently a major clinical drawback. Further studies are underway to establish a safe and effective dose.

Lopez-Flores et al. (2005) Journal of Pharmacological and Toxicological Methods. 52: 366-372.

7 BIOLOGICAL AND PHYSICOCHEMICAL CHARACTERIZATION OF AMPHOTERICIN B-POLYMER COMPLEX

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Amphotericin (AmB) is a polyene antibiotic used for the treatment of systemic fungal infections and it is also highly effective against leishmaniasis, a neglected disease that often occurs in resource limited parts of the world. The main drawback for the clinical use AmB is its toxic side effects especially nephrotoxicity. Several lipid-based formulations have been developed to overcome the toxicity of AmB. Of these the most widely used is AmBisome®. Unfortunately its use is limited by high cost and in the case of leishmaniasis, use is also limited by the need of a cold chain.

We have shown that poly(methacrylic acid) sodium salt (PMA) can form a non-haemolytic and active complex with AmB. This strategy however involves the use of non-biodegradable polymer (PMA). Since the fate of the carrier in the body can be very important and may limit its use in more chronic conditions, we decided to examine the complexation of AmB with a biodegradable polymer, poly(α-glutamic acid) (PGA).

Non-covalent complexes of AmB and PGA (AmB-PGA) were prepared with loadings in the range of ~20.0 – 50.0%. This size of the complex was in the range of 125 –130 nm as determined by DLS. It had micelle like shape. The aggregation state of AmB was determined by the ratio of absorbance at 315-326:406 nm. This ratio was often above 2.5. The aggregation state of AmB plays an important role in the activity and toxicity of AmB. These AmB-PGA complexes were stable and non-haemolytic after storage at 4°C for 6 months and at 37oC for 30 days as a powder. The anti-leishmanial activity against intracellular amastigotes of L.major in differentiated THP-1 cells was in the range of EC50 0.077 ± 0.013 to 0.066 ± 0.006 μg/mL (AmB equivalent) which is similar to Fungizone® (0.06 ± 0.01 μg/ml) after an incubation period of 72 h at 34°C.

8 FORMULATING CARBOPOL BASED IN SITU GELLING SYSTEMS FOR OCULAR THERAPEUTICS

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Carbopol® is a polyacrylic acid lightly crosslinked with polyalkenyl ethers or divinyl glycol to produce a polymer that will hydrate in water, increasing the viscosity of a solution, or with an increase in pH, will form a gel. With their bioinert nature and easy dispersion in aqueous systems, there are a wide range of applications for Carbopol® polymers, including thickening agents for lotions, bioadhesion enhancers and controlled release tablets.

The in situ gelling behaviour of Carbopols® when exposed to certain areas of the human physiological system, such as rectal or ocular tissue, allow for the development of drug containing solutions that form gels when delivered to the target area, giving it a resistance to removal by bodily fluids, thus keeping the drug in place, and so prolonging it's therapeutic effect. For ocular drug delivery specifically, the gelation of a solution will prevent tear fluid from washing away the compound too rapidly.

In this present research, the gelation properties of several Carbopol® grades have been investigated, specifically the pH required to form a gel, and to subsequently break down the gel network, as well as the behaviour of drug-Carbopol® compounds when placed onto the surface of the cornea. It has been shown that rapid gelation of Carbopol® formulations takes place with an increase in pH, indicating a high suitability for ocular drug delivery. The stability of various concentrations with a subsequent increase in pH is also examined, allowing the generation of phase diagrams to describe the behaviour of different Carbopols®.

The in situ gelling is demonstrated by the application of Riboflavin/Carbopol® solutions to the surface of a pig's cornea. It is clearly observed that the solutions containing Carbopols® remain on the eye for a significantly longer duration, increasing the contact time between the cornea and the riboflavin.

9 OPTIMISING PRODUCTION AND PROPERTIES OF ALGINATE-CHITOSAN MICROCAPSULES

M Cook (1,2), G Tzortzis (3), D Charalampopoulos (1), V Khutoryanskiy (2)
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(2) Reading School of Pharmacy, University of Reading
(3) Clasado Ltd, Milton Keynes

The use of chitosan-coated calcium alginate beads as enteric targeting microcapsules has been reported extensively in the literature [1, 2]. Though this has been used as a method of bacterial delivery to the colon, it has been communicated in a relatively incomplete manner [3]. Our research aims to optimise the production of microcapsules for the purpose of bacterial encapsulation and to evaluate the possibility of making dry delivery forms.

Alginate-chitosan microcapsules were produced by extrusion of sodium alginate into calcium chloride solutions followed by coating with chitosan whilst wet or dry. The microcapsules were then dried to complete the process. The efficiency of coating was investigated using confocal laser scanning microscopy to visualise a FITC-labelled chitosan layer on the microcapsule. These microcapsules were then examined by gravimetric swelling studies in media of varying pH in order to investigate the effect of the different levels of acidity they may experience in vivo.

It was found that the microcapsules produced by the dry coating method with 30 minutes chitosan exposure gave the slowest swelling rate under the conditions used. However, the confocal images showed signs of deeper penetration of chitosan, possibly through capillaries produced upon drying. This would be disadvantageous as chitosan is anti-microbial and may affect the viability of encapsulated cells. The wet-coated microcapsules of all exposure times had a lesser effect on slowing the rate of swelling but did not exhibit deep penetration of chitosan. The chitosan-coated microcapsules were shown to be stable at the pH range associated with the stomach but slowly dissolved at pHs typical for intestinal tract.

Fernandez-Hervas, M.J., et al., Int. J.Pharm., 1998. 163(1-2): p. 23-34
Elzatahry, A.A., et al., J.Appl.Polym.Sci., 2009. 111(5): p. 2452-2459
Lin, J.Z., et al., J.Biosci.Bioeng., 2008. 105(6): p. 660-665

10 USING TRANSGLUTAMINASE INCORPORATED LIPOSOMES FOR TREATMENT OF LIVER CIRRHOSIS

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The school of Life and Health Sciences, Aston University, Birmingham

Liver fibrosis and its end stage disease cirrhosis are a major cause of mortality and morbidity around the world. There is no effective pharmaceutical intervention for liver fibrosis at present. transglutaminase (TG) is emerging as a well-characterized, multifunctional molecular element in numerous diseases and processes including chronic neurodegeneration, neoplastic diseases, autoimmune diseases, diseases involving progressive tissue fibrosis and diseases related to the epidermis of the skin (Griffin 2002). It can therefore be predicted that TG inhibitors could be used in the treatment of TG relevant human diseases such as liver cirrhosis. This study focuses on development of an efficient drug delivery system for site specific delivery of a novel group of inhibitors of enzyme transglutaminase to the liver with the aim of treating liver cirrhosis. Integrating TG inhibitors into liposomes as a potential carrier system and subsequently targeting liposomes into the liver reduces the large drug distribution, increases efficacy and eliminates the associated side effects of the drug. In view of the micro-anatomy of the liver, liposomes used for this purpose were small unilamellar vesicles (SUVs) to allow free passage through the endothelial fenestrations. 1,2-distearoyl-3-glyceryl-phosphatidylcholine (DSPC), phosphatidyl choline (PC), cholesterol (chol.) and phosphatidylserine (PS) were utilized as lipids at different molar ratios (as shown in Table 1). The size, surface charge and drug loading characteristics were measured to determine the most suitable formulations for in-vivo studies. Bio-distribution studies were then carried out in outbred mice after IV administration of the liposomes incorporating drug into the tail vein at preset time intervals. The liver, lungs, kidneys and spleen were collected and the bio-distribution of liposome-encapsulated enzyme inhibitor was monitored using H3 cholesterol in the formulations as tracers.

Table 1: Characteristics of liposome incorporating TG inhibitor

Formulations (molar ratio)	Size (nm)	Zeta potential (mV)	Drug loading (%)
PC: Chol: PS (12:1:4)	121.46 ± 5.10	-42.79 ± 1.58	46.50 ± 7.77
PC: Chol (16:1)	156.45 ± 7.84	-5.82 ± 0.92	75.00 ± 1.41
DSPC: Chol: PS (12:1:4)	139.88 ± 3.68	-49.99 ± 3.85	63.83 ± 7.18
DSPC: Chol (16:1)	129.7 ± 0.80	-6.82 ± 2.85	59 ± 11.31

There is no significant difference in terms of size and zeta potential when comparing between the two anionic formulations, as well as between the two neutral formulations (Table 1). However, there were substantial differences between anionic and neutral liposomes amongst different preparations tested. Phospholipids such as DSPC demonstrate considerably higher levels of drug loading compared to PC. Furthermore, the high transition temperature (57 ˚C) of DSPC may be employed to prepare liposomes which are resistant to plasma proteins and as a consequence could be more effective for in-vivo studies (Gregoriadis 1973). Regarding bio-distribution studies, the uptake of liposome by the liver was considerably higher than other organs. Therefore, it can be suggested that liposomes can be employed as a potential carrier system for site-specific delivery of TG inhibitors into the liver with the aim of treating liver cirrhosis.

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11 EFFECT OF CARBOXYMETHYLE-γ-CYCLODEXTRIN ON LYSOZYME

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Cyclodextrins have a wide range of applications in pharmaceutical industries. It is known that the inclusion of α-cyclodextrin phosphate in protein formulations have the advantage in refolding the processed proteins to their native three dimensional structure, which is important for their biological activity. In this study, carboxymethyl-γ-cyclodextrin was used to evaluate its influence on lyophilised lysozyme, as a model protein. During the preparation of lysozyme solution with carboxymethyl-γ-cyclodextrin (1:1) w/w, before freeze drying, a small precipitate was formed. The prepared solutions (with and without carboxymethyl-γ-cyclodextrin) were freeze dried using VirTis Benchtop Freeze Dryer. Visual inspection of the freeze dried preparation produced a cake which was slightly compromised. The formulated proteins were characterised for thermal stability using differential scanning calorimetry (DSC); for secondary structure using Fourier Transform Infra-Red (FT-IR), for protein content and for biological activity via enzymatic assay.

The DSC results for the freeze dried preparations with carboxymethyl-γ-cyclodextrin showed a very broad peak thus it was difficult to detect the apparent denaturation temperature (T_m) of the formulated protein compared to that of unprocessed lysozyme (T_m = 201˚C). The FT-IR data illustrated that the inclusion of carboxymethyl-γ-cyclodextrin with lysozyme led to major shifts for most of the IR bands by about 6 cm-1 compared to those of the control lysozyme. A significant reduction ($p<0.05$) of the protein content in freeze dried lysozyme with carboxymethyl-γ-cyclodextrin (77%) was produced as compared to the pure lysozyme (100%). Biological activity test showed a significant ($p<0.05$) reduction on lysozyme biological activity in the formulations containing carboxymethyl-γ-cyclodextrin. These results indicate that carboxymethyl-γ-cyclodextrin led to denaturation of the protein. Carboxymethyl-γ-cyclodextrin interact with lysozyme in different way compared α-cyclodextrin phosphate .Accordingly, the effect of carboxymethyl-γ-cyclodextrin could not be generalised as α-cyclodextrin phosphate that has a great stabilising effect on protein integrity and activity.

12 DENDRIMERS AS NANOCARRIERS OF A MODEL STEROID

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Dendrimers are hyper-branched polymers that have well-defined structures and offer a potential as carriers of poorly soluble drugs. Polyamidoamine (PAMAM) dendrimers are the most commercially available dendrimers and hence are extensively used in research. Dendrimers have been shown to be able to solubilize poorly soluble drugs and entrap therapeutic materials. In this study, we prepared and characterized G4 PAMAM dendrimers for entrapment of the antiasthma steroid beclometasone dipropionate (BDP).

A methanol solution of dendrimers was placed in a pre-weighed round-bottomed flask and solvent was removed by rotary evaporation. The flask was re-weighed to determine the weight of the dendrimers to which BDP was added to constitute 10% of the dendrimer's weight. The resultant mixture was solubilized in methanol followed by rotary evaporation to form a film of dendrimer-BDP onto which phosphate-buffered saline was added followed by stirring overnight. The dispersion was centrifuged to remove the unentrapped steroid. Size analysis of the dendrimers was performed using photon correlation spectroscopy and zeta potential was measured using laser doppler velocimetry. The entrapment efficiency (EE) of BDP was determined using high performance liquid chromatography (HPLC). Size analysis showed two peaks one having a median size of 7.53 nm ± 0.75 and the other a median size of 183.40 nm, indicating that some dendrimers have aggregated. The zeta potential was found to be neutral (-0.198 mV ± 1.04). The EE as determined by the percentage weight ratio of the steroid entrapped to the overall amount of steroid was 2.85% ± 0.22. However, when the EE was determined as the number of moles of BDP per number of moles of the dendrimers it was found to be 5.71% ± 0.74. This study has shown that the model steroid BDP was successfully associated with the polymeric structure of the G4 PAMAM dendrimers.

13 A STUDY ON THE EFFECT OF ELECTROLYTES ON THE PERFORMANCE OF A MODEL VIBRATING-MESH NEBULIZER

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Vibrating-mesh nebulizer is a recent type of inhalation devices that employ perforated plates which vibrate at high frequency to generate inhalable aerosols. The aerosol properties have been previously found to be dependent on fluid viscosity and surface tension. Recently we found that the presence of NaCl within solution markedly enhanced the performance of vibrating-mesh nebulizers. In this study, the effect of electrolyte concentration on vibrating-mesh nebulization was investigated using two model electrolyte solutions which are NaCl and NaI.

HPLC-grade water was used to prepare electrolyte solutions having 0, 0.1, 1 or 2% w/v concentrations. Solution (5 ml) was nebulized to "dryness" using an Aeroneb Pro nebulizer. The aerosol mass output was determined gravimetrically and the volume median diameter (VMD) of the droplets was measured using the Spraytec laser diffraction analyzer. The absence of electrolytes resulted in low output (58.60% ± 3.61) and large VMD (7.35 µm ± 0.03) of the aerosol. The output significantly ($P<0.05$) increased using either type of solution, being 92.25% ± 3.04, 90.47% ± 1.89 and 89.10% ± 1.05 for the NaCl 0.1%, 1% and 2% solutions respectively and 83.80% ± 4.82, 85.80% ± 1.31 and 92.73% ± 0.90 for the NaI 0.1%, 1% and 2% solutions respectively. For both types of solution, the VMD of the aerosols was decreased as the electrolyte concentration increased. The VMD was 6.32 µm ± 0.07, 4.32 µm ± 0.08 and 3.11 µm ±0.01 for the aerosols generated from the 0.1%, 1% and 2% NaCl solutions respectively. Smaller VMDs were obtained using NaI, being 6.69 µm ± 0.21, 3.14 µm ± 0.22 and 1.76 µm ±0.08 for the 0.1%, 1% and 2% solutions respectively. These differences are possibly attributed to that iodide ions may be present at the surface in higher concentrations. Overall, the type and concentration of electrolyte solution can be manipulated to control the aerosol output and droplet size.

14 FREEZE-DRIED LIPOSOMES FOR POTENTIAL APPLICATION IN ASTHMA

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Liposomes are suitable drug carriers in pulmonary delivery. Unfortunately, phospholipids are liable to hydrolysis and oxidation in aqueous dispersions. Freeze-drying has been suggested to prepare stable liposomes that can be rehydrated by addition of aqueous phase before administration. Inhalable antiasthma formulations in the market are designed either to relief the asthma symptoms (e.g. bronchodilators) or protect against asthma (e.g. steroids). In this study, freeze-dried liposomes containing both a bronchodilator and a steroid have been designed to have small size on rehydration and hence be potentially applicable for aerosol inhalation.

Soya phosphatidylcholine: cholesterol (1:1) multilamellar vesicles (MLVs) were prepared followed by probe-sonication to form small unilamellar liposomes (SUVs). Photon correlation spectroscopy was used to measure the size (Z-average) and polydispersity index (PI) of the liposomes. The surface charge (zeta potential) was analyzed using laser doppler velocimetry. SUV formulations including salbutamol sulphate (SS) and beclometasone dipropionate (BDP) were freeze-dried in absence of a cryoprotectant or using sucrose or trehalose as cryoprotectants in 5:1 w/w carbohydrate to lipid ratio. The Zaverage before freeze-drying was 73.80 nm ± 1.70 and the PI was 0.24 ± 0.00. When no cryoprotectant was included, the size of the rehydrated vesicles was increased by more than 30 times and the PI was increased by approximately 4 times, indicating that SUVs have aggregated and possibly reverted to MLVs. Sucrose was superior to trehalose as a cryoprotectant since the rehydrated vesicles were smaller and less polydispersed. The Zaverage and PI were respectively 97.15 nm ± 0.31 and 0.16 ± 0.01 when sucrose was included and 127.20 nm ± 0.79 and 0.23 ± 0.01 when trehalose was included. The zeta potential of the SUVs before freeze-drying was -7.87 mV ± 1.03 which was increased only slightly after freeze-drying and rehydration. Overall, stable liposomes including two anti-asthma drugs have been prepared for future investigations as inhalable aerosols.

15 GENOMICS EVALUATION OF DRUG PERMEABILITY FOR POORLY SOLUBLE MODEL DRUG

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The Caco-2 cell line is one of the most reliable and widely used in vitro systems for predicting gastrointestinal absorption. However most studies have investigated only the expression/down regulation of single transport systems with the majority of the work dedicated to the role of P-glycoprotein controlled network system. The aim of this study was to understand trancriptomic changes that occur during absorption studies by bringing out general indications of the significance of various transporters and the gene expression profile for a poorly water soluble drug candidate (indomethacin) when studied alone and as a solid dispersion.

Solid dispersion of indomethacin was prepared using melt fusion method using polyethylene glycol (mol wt 8000) as the carrier. Permeability studies were carried out using Caco-2 cells, (passage 70) obtained from American Type Culture Collection(ATCC) at passages 90-100. For the transport assay, cells were seeded into 6 well Transwell culture plate inserts (24mm, 4.7 cm^2) at a density of 2 $x10^5$ cells/cm^2. Total RNA was extracted and purified from Caco-2 cells using the Qiagen RNEASY Mini Kit according to the manufacturer's protocol.

Permeability studies revealed that formulation of solid dispersion system for indomethacin increased drug permeability across caco-2 cells 4 fold when compared to drug alone. Analysis of genome data comparing drug alone and solid dispersion against the control showed that 17,762 genes (47%) with a false discovery rate of 1% exhibited significant changes in expression profiling. Probing further into genes involved in transporter network systems demonstrated that indomethacin drug alone affected the expression of 26 ATP binding Cassette (ABC) transporter genes and 191 solute carrier transport (SLC) genes. On the other hand, analysis of gene expression changes upon reformulation as solid dispersion showed that 23 ABC and 191 SLC genes were significantly influenced. The results show that multiple transporter systems work in a dynamic environment to determine the absorption profiling of drug moieties. Systems biology approach provides a pragmatic approach to study global changes in expression changes and better explains the biopharmaceutics of drug absorption.

16 SYNTHESIS AND EVALUATION OF A NOVEL TOCOTRIENOL-BASED THERAPEUTIC SYSTEM FOR TUMOUR TARGETING

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Tocotrienol, a group of compounds present in vitamin E, has gained much attention in recent years for its tumour suppressive properties on cancer cells. Multiple pathways including anti- angiogenesis, p53 activation and apoptosis are among the proposed mechanism of action for the anti-cancer effect of tocotrienol. However, its therapeutic potential was hampered by the limited ability to reach tumours specifically after intravenous administration. In this study, the approach of entrapping tocotrienol in a tumour-targeted delivery system was investigated. Transferrin is an iron transporter whose receptors are often over-expressed in cancer cells due to high iron demand for tumour growth. The conjugation of transferrin to drug delivery systems appeared to be an attractive tool for selective receptor-mediated delivery of therapeutic drugs to tumours. The objectives of this study were therefore to prepare and evaluate the targeting and therapeutic efficacies of transferrin-bearing vesicles entrapping tocotrienol.

Tocotrienol was entrapped in Span 60 vesicles upon heating and probe sonication, prior to transferrin conjugation by cross-linking. Upon purification, vesicles were visualised and characterized for entrapment efficacy, transferrin conjugation efficacy, size and zeta potential. The therapeutic efficacy of this system was evaluated in vitro using an MTT assay and in vivo after intravenous administration to a murine xenograft model. In vitro results showed at least 80-fold improvement in therapeutic efficacy of tocotrienol when entrapped in vesicles. A further 2-fold improvement was observed when vesicles were targeted with transferrin. In vivo, marked tumour regression was observed in mice after administration of transferrin-bearing vesicles entrapping tocotrienol. In contrast, no significant advantage was observed in mice treated with control vesicles and free tocotrienol compared to untreated mice. In conclusion, the grafting of transferrin to tocotrienol-loaded vesicles improved the therapeutic potential of tocotrienol, leading to tumour regression of vascularised tumours after intravenous administration without visible toxicity.

17 INFLUENCE OF MICRONEEDLE GEOMETRY AND FORCE OF INSERTION ON SKIN PENETRATION

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Microneedle arrays are minimally invasive devices that can be used to by-pass the stratum corneum barrier and thus achieve enhanced transdermal drug delivery. Microneedles (MN) (50-900 μm in height, up to100 MN cm^{-2}) in diverse geometries have been produced from silicon, metal, carbohydrates and polymers using various microfabrication techniques.

The aim of this present study was to use optical coherence tomography (OCT) to extensively investigate, for the first time, the influence of MN geometry (MN height, and MN interspacing) and force of application upon the resultant penetration characteristics of soluble poly(methyl vinyl ether-co-maleic anhydride) MN arrays (9 MN/array) into neonatal porcine skin in vitro.

Using a custom designed spring-activated applicator, it was found that increasing the force used for MN insertion resulted in a significant increase in the depth of penetration achieved within neonatal porcine skin ($p < 0.01$). At a constant application force (11.0 N/array) it was found that, in each case, increasing MN height from 350, 600, to 900 μm led to a significant increase in the depth of MN penetration achieved ($p < 0.001$). Moreover, it was found that alteration of MN interspacing had no significant effect upon depth of penetration achieved, at a constant MN height and force of application ($p = 0.795$).

Importantly, this study has highlighted the effect that MN geometry and application force have upon the depth of penetration into skin. The successful use of OCT in this study could prove to be a milestone in the development for polymeric MN for widespread clinical use. To date, there has been no other method that can non-invasively visualise MN arrays whilst inserted into skin in vivo in real time. As such, this technique opens up the possibility to investigate MN penetration, dissolution, and the recovery of the skin on a patient-to-patient basis.

18 CUTANEOUS LEISHMANIASIS TREATMENT AND FUTURE THERAPEUTIC OPTIONS INVOLVING BUPARVAQUONE

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Cutaneous leishmaniasis (CL) is a parasitic skin disease with an estimated annual incidence of 1-1.5 million cases. Parasites are localised within dermal macrophages at the site of infection where papules develop and enlarge to form nodules that become lesions. It forms single or multiple ulcers which can last for 3 -18 months, leaving disfiguring scars. Current treatments are largely inadequate due to problems of varying efficacy, parenteral administration, toxicity and high cost. Development of successful topical treatment for CL has many advantages including lowered cost, limited side effects and improved patient compliance.

Buparvaquone (BPQ) is a potent antileishmanial with physicochemical properties suitable for topical delivery. Its topical delivery was evaluated from a range of gels (containing 1% BPQ and saturated BPQ gels). All BPQ gels contained 1% w/w hyaluronic acid (HA) as it has shown penetration enhancement properties as well as drug targeting in the lower skin layers. Dermal drug targeting is desirable as CL is found localised within the dermal macrophages. The ability of BPQ formulations to cross human and murine skin was tested in vitro using Franz diffusion cells. Topically applied BPQ formulations were developed using excipients acceptable for topical use (GRAS or FDA inactive ingredients). The BPQ formulations proved to be stable after four weeks at 25 °C and 40 °C. BPQ penetrated both mouse BALB/c and human full-thickness skin from all gels. The saturated BPQ gels (1.87 – 4.16 μg/cm²/h) showed higher permeation flux than 1% BPQ gels (0.93 – 2.68 μg/cm²/h). The saturated BPQ gel containing Transcutol P and Lauroglycol 90 showed higher percentages (1.69 ± 0.07%) of BPQ in the human dermal tissue than the remaining saturated gels containing urea and oleic acid, suggesting enhanced dermal delivery of BPQ. These results confirm BPQ is a potentially interesting candidate for future topical development in the treatment of CL.

19 DEVELOPING DISULPHIDE BRIDGING PEGYLATION FOR PROTEINS DURING FOLDING

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Proteins show great potential as medicines but their clinical use is limited by their short circulation half-life and immunogenicity meaning frequent dosing regimens, low patient compliance and poor clinical outcomes. PEGylation is one strategy being used to improve the efficacy of protein based medicines. Conjugated PEG increases a protein's molecular weight causing an increased circulation half-life. PEG also sterically shields the protein from exposure to antigenic determinants and proteolytic enzymes. For proteins that bind to receptors or to other proteins, steric shielding can also cause a reduction in biological activity although this does not always correlate with a decrease in clinical efficacy.

Non-specific PEGylation techniques target surface amine groups by inefficient reactions to produce heterogeneous mixtures that are difficult to purify, characterise and reproduce. Site-specific strategies tend to rely on recombinant modification of the protein. Our approach exploits the existence of native disulphide bonds. This involves the reduction of a native disulfide to liberate two sulphur atoms, followed by PEGylation using a bis-alkylation PEG reagent that inserts a three-carbon bridge to connect the two sulphurs of the original disulfide.

We have used our reagents to efficiently PEGylate leptin, which as an alpha helical barrel protein is structurally representative of many therapeutically used cytokines. Initially it was difficult to PEGylate leptin until we were able to find conditions that did not lead to aggregation. The single accessible disulphide in leptin links one helical barrel to another cysteine that is located in a part of the protein that lacks discernible secondary structure. Hence upon disulphide reduction there is a greater propensity for protein denaturation. A good yield of mono-PEGylated product (~55%) was obtained for leptin using 1.5 eq of our PEGylation reagent. Ion exchange and size exclusion chromatography were used to purify the mono-PEG conjugate. Digest data confirmed the site specificity of the reaction and the attachment of PEG to the disulphide containing fragments. We now aim to investigate the PEGylation of proteins during refolding from solubilised inclusion bodies.

20 EFFECT OF EXCIPIENTS ON FREEZE DRIED BOVINE SERUM ALBUMIN

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Producing stable and biologically active protein therapeutic drugs have been the main concern of scientists for the last 20 years. Accordingly, freeze drying technique was applied on bovine serum albumin (BSA), a model protein, using some stabilising excipients (Cremophor® EL and Pluronic® F-127) to assess their influence on protein integrity and stability. BSA was freeze dried using VirTis Benchtop Freeze Dryer-USA in absence and presence of excipients in concentration of 0.05%. The prepared formulations were characterised for stability employing Fourier Transform Infra-Red (FT-IR); UV spectroscopy; differential scanning calorimetry (DSC) and protein content. As a result, freeze drying of bovine serum albumin without excipient showed loss of biological activity and conformational integrity, this was confirmed by FT-IR, DSC, UV spectroscopy and protein content tests.

On the other hand, inclusion of cremophor® EL and pluronic® F-127 led to improve the physical appearance of freeze dried bovine serum albumin. FT-IR data showed that Cremophor® EL and pluronic® F-127 preserved the conformational integrity of the model protein. Moreover, DSC analysis illustrated that both used excipients increased the thermal stability of bovine serum albumin compared to unprocessed bovine serum albumin alone. The resulted apparent denaturation temperatures were 217.17±0.48°C, 223.03±0.99°C and 220.21±0.5°C for unprocessed BSA, freeze dried BSA with Cremophor® EL and freeze dried BSA with Pluronic® F-127, respectively. Excipients preserved protein content of freeze dried bovine serum albumin formulations compared to freeze dried bovine serum albumin alone. The overall results suggest that using Cremophor® EL and Pluronic® F-127 maintained the integrity of bovine serum albumin.

21 A COMPARATIVE STUDY ON THE EFFECT OF LIPOSOME SURFACE CHARGE AND MEMBRANE FLUIDITY ON THE ANTIGEN DEPOT-EFFECT

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One of the present notions by which liposomes are thought to act is via the 'depot-effect' whereby simultaneously administered antigen is held at the site of injection consequently exposing the necessary components of the innate immune system to antigen for a longer period. The aim of this study was to investigate the role of membrane fluidity, as determined by lipid transition temperature, in addition to the choice of cationic lipid on the depot-effect of the antigen and liposome.

A duel-radiolabelling method was used in which 3H-labelled liposomes and 125I-labelled protein were injected into Balb/c mice. Liposomes were formed using the lipid-film technique and comprised an 8:1 molar ratio of cationic or neutral lipid with the immunomodulator trehalose 6,6'-dibehenate (TDB). Prior to injection, the clinically relevant tuberculosis subunit protein antigen Ag85B-ESAT-6 (H1) was added to the liposomes. Tissue from the site of injection was removed on days 1, 4 and 14 post injection and the presence of 3H and 125I determined. All formulations were characterised for their vesicle size and zeta potential, in addition to their ability to adsorb antigen and their stability in conditions mimicking the in vivo environment.

Neutral liposomes composed of the lipid distearoyl-glycero-phosphatidylcholine (DSPC) were well retained at the injection site however antigen administered with the liposome drained rapidly. In contrast, rigid cationic liposomes such as those composed of dimethyldioctadecylammonium (DDA) were well retained at the injection site with their adsorbing antigen. Our results suggest that whilst liposome retention at the site of injection is affected by liposome bilayer fluidity, the retention of antigen at the injection site is more significantly affected by the surface charge of the liposome and therefore its ability to adsorb antigen. Liposomes composed of DDA are strongly immunogenic and are currently in clinical trials as adjuvants for TB vaccines.

22 SCREENING OF GLIBENCLAMIDE FOR TRANSDERMAL DEVELOPMENT BY PASSIVE AND IONTOPHORESIS

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In vitro iontophoretic transdermal delivery of glibenclamide across the pigskin was investigated. The experiment was carried out at three different donor drug concentrations, using cathodal iontophoresis (current density 0.5 mA cm^{-2}) along with the passive controls. For passive permeation, the steady state flux significantly increased with the donor drug concentration. In contrast, the permeation rate increased with concentration up to a certain level only in iontophoresis. At all concentration levels iontophoresis considerably increased the permeation rate compared to passive controls. The passive process followed a zero order profile whereas iontophoretic permeation was less linear. Competition by chloride ion is suggested to be the cause. Flux enhancement was highest at the lowest drug load and lowest at the highest drug load. The highest flux value obtained was 0.0603 μmol cm^{-2} h^{-1}. The target flux for glibenclamide was calculated to be 0.3933 μmol h^{-1}, which indicates the required permeation rate, could be met by iontophoresis using a much smaller application area.

23 EFFECT OF EXCIPIENTS ON LAG TIME OF PRESS COATED TABLETS AS PULSATILE DRUG DELIVERY FOR THE TREATMENT OF RHEUMATOID ARTHRITIS

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Oral controlled drug delivery systems are designed to deliver the drugs at a controlled and predetermined rate, maintaining their therapeutically effective concentration in systemic circulation for prolonged periods. On the other hand, for certain therapies a pulsatile drug release pattern, where the drug is released after well- defined lag time, exhibits significant advantages. Most of the body functions display circadian rhythms, e.g. heart rate, stroke volume, blood pressure, body temperature, gastric pH. The symptoms for a number of diseases, such as bronchial asthma, myocardial infraction, angina pectoris, hypertension, rheumatic diseases, etc. follow a circadian rhythm .

The dry-coated tablet with optimal lag time was designed to simulate the drug dosing time according to physiological needs. This press coated tablet was prepared with meloxicam in the core tablet & ethylcellulose powder (EC) with fine particles, in the whole outer shell. Along with this SDL and HPMC were mixed in different weight ratios with EC individually and the effect on the lag time of the drug release was investigated. The release profile exhibited a lag time followed by a rapid and complete release phase, where the outer shell ruptured or broke into two halves and the lag time was dependent on the weight ratios of EC/SDL or EC/HPMC in the outer shell.

The longest time lag of approximately 16 hr. was observed with 100% EC in the outer shell. Addition of SDL into the composition of the outer shell shortened the lag time to approximately to 10 mins. and to 4 hr. with HPMC in the outer shell. By changing the composition of the outer shell the peak plasma blood concentration can be matched with the early morning peak pain in rheumatoid arthritis. This type of formulation can be used for chronotherapy of rheumatoid arthritis.

24 BINDING STUDY OF FAB AND PEGYLATED-FAB USING BIACORE

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An IgG antibody consists of a variable (Fab) and a constant (Fc) region. While binding occurs on the Fab, the Fc region possesses other functions including recycling and effector functions [1]. There may be some applications where effector functions are not required and only the Fab function is required (e.g. antagonism). Unfortunately antibody fragments rapidly clear from circulation. PEGylation is a proven strategy to increase the circulation half-life of therapeutic proteins. We have developed a site-specific method of PEGylation that binds to the two sulfur atoms in a native disulfide [2]. This method of PEGylation is particularly appropriate for the accessible disulfide in Fabs. The aim of this study was to evaluate the binding characteristics of a PEG-Fab fragment by BIAcore.

The Fab was obtained by proteolytic digestion of bevacizumab, a clinically used IgG1 antibody that targets VEGF. Immobilised papain was used to cleave bevacizumab at its hinge region to produce the Fab fragment. The Fab was purified from the Fc by passing the digestion mixture over a protein A column with a recovery yield of ~86%. The Fab was then PEGylated using bis-alkylation PEGylation reagents and purified. The binding affinity of the purified Fab and PEGylated Fab were then examined using BIAcore methodology.

VEGF was immobilised onto a CM5 chip using routine amine coupling. To conduct a kinetic/affinity study, a low Rmax is required. Therefore, a R_{max} of 200 RU was set as a target for kinetic measurements using a low density of the ligand (VEGF) on the sensor surface [3]. Another chip was also prepared with a higher density of VEGF so that a higher Rmax of 2000 RU could be utilised for binding studies. Data were analysed with BIAcore evaluation software, applying a 1:1 binding model. The sensogram was fitted globally over the association and dissociation phase. Equilibrium dissociation constants (affinity) were then calculated from the rate constants ($KD = k_{off}/k_{on}$).

Results showed that the purified-Fab from several batches obtained over six months and purified (10 kDa) PEGylated-Fab maintained their binding affinity for VEGF. Preliminary kinetic results on the purified Fab showed a very fast association rate (kon) and a slow dissociation rate (koff) compare with the whole IgG. Therefore, lower affinity was observed. More studies are underway in an attempt to improve the low affinity of the Fab. Moreover, kinetic/affinity of the purified PEGylated-Fab will be studied.

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25 DEVELOPING AND EVALUATING SYNTHETIC HYDROGELS AS POTENTIAL MIMICS FOR MUCOSAL TISSUES

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Over the last decade, mucoadhesive polymers have been used widely in designing drug delivery systems to improve buccal, nasal, oral, ocular and vaginal administration of drugs, and considerable attention has been paid to the development of novel mucoadhesive materials. In most studies evaluating mucoadhesives in vitro the tissues of animals (cows, pigs, rabbits, dogs, guinea pigs, rats, mice, hamsters, etc.) were used. The variety of mucosal tissues, including ocular, oral, nasal and gastrointestinal among others, and different mucoadhesives in a variety of physical forms means that comparison and quantification of mucoadhesion is rarely straightforward. Therefore the ability to standardise a quantification of mucoadhesion against a consistent, reproducible and representative sample would be extremely advantageous. The diversity and non standardised nature of biological tissues has lead to the proposition of using synthetic mimics of mucosal tissues, which can be efficiently and reproducibly synthesised, as standardised test subjects. This also corresponds favourably to the 3R's principle to minimise the requirement for the use of animals in scientific testing.

In the present research we have developed a series of hydrogel materials and tested their applicability to mimic mucosal tissues in experiments evaluating the mucoadhesive properties of tablets based on hydroxypropylmethylcellulose (HPMC) and Carbopol® 934P. We studied how the nature of the monomers used (2-hydroxyethyl methacrylate, 2-hydroxyethyl acrylate, N-vinyl pyrrolidone, sorbitol methacrylate, N-acryloyl glucosamine, 3-acryloyl-a-glucofuranoside), the feed ratios and the degree of cross-linking can be varied to produce a hydrogel that mimics porcine buccal mucosa. The optimal hydrogel compositions have been identified. These hydrogels can potentially replace animal tissues in experiments on assessment of mucoadhesive properties of dosage forms.

26 OSMOTIC CAPSULES OF CEFUROXOME AXETIL – FORMULATION & EVALUATION

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Osmosis principle has been extensively reported for providing sustaining or controlling the release of active ingredient. Cefuroxiime axetil, a broad spectrum antibiotic possesses a very short half life and it has to be frequently administered to maintain the therapeutic level. An attempt has been made in this work to develop sustained release osmotic capsules by dip coating method. The body of capsule was dipped in coating solutions with different concentrations of cellulose acetate and ethyl cellulose and plasticizer like castor oil in the coating solution. After coating, the drug and the osmotic agent sodium chloride was filled in the capsules, content were plugged with molten beeswax, and orifice was drilled with screw. The cap portion of the capsules was fitted to the body portion to form osmotic capsules and evaluation was carried out by weight variation, content uniformity, in vitro release studies and kinetic evaluation. The weight variation and content uniformity was found to be within the specific limits. The results of the release studies showed that the release rate was sustained for a longer time from osmotic capsules with both semi permeable membranes. Ethyl cellulose was much more effective in slowing down the release than Cellulose acetate. The effect of concentration of plasticizer and coating thickness on release rate was also investigated in the work. It was observed that on increasing the plasticizer content and decreasing the coating thickness, the release rate was found to increase significantly in samples. The kinetic evaluation of the release data indicated that the release rate from the capsules was found to be diffusion controlled in nature and it tends to follow Higuchi model type release behavior. It can be concluded that by utilizing simple osmotic principle and suitable semi permeable membrane the release rate can be sustained with much ease.

27 NEW POLYMERIC FORMULATION OF AMPHOTERICIN B AS TREATMENT FOR VISCERAL LEISHMANIASIS

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Visceral Leishmaniasis (VL) is a parasitic disease that can be fatal without treatment. Currently available treatments have several disadvantages such as increasing resistance (Pentavalent antimonials), a prolonged 3-4 week treatment period and associated toxicity (Fungizone®), or a high cost and a cold chain requirement (AmBisome®). Since VL is prevalent in resource limited regions of the world, these are severe limitations. Often, it is difficult to treat patients over an extended period of weeks, and it is often impossible to maintain a cold chain. Cost is clearly a limitation that affects the provision of healthcare worldwide but especially in resource limited regions.

We are currently developing new a formulation of AmB that is based on its non- covalent complexation to a polymer rather than to lipids. Poly(methacrylic acid) (PMAA) is used directly to give a complex (AmB-PMAA) that is water-soluble and non-haemolytic. The use of the PMAA is designed to minimise the systemic distribution of AmB in order to reduce toxicity, while also providing a stable and cost effective formulation that does not require refrigeration.

The AmB-PMAA complexes can be reproducibly prepared with different AmB content (wt% ~20 to 50%). Complexes are easily soluble in water at 4 mg/mL and are stable in solution for over year at both 4 °C and 37 °C. Stability studies showed no change in solubility even after 20 freeze-thaw cycles. The AmB that is present within the AmB-PMAA complex displays similar aggregation and spectral shift properties as the AmB which is in AmBisome®, the liposomal gold standard for VL treatment. In contrast to Fungizone® or AmB alone, our AmB-PMAA complexes were not haemolytic to human red blood cells. The AmB- PMAA complexes also remained non-haemolytic after stability studies.

Using an acute mouse in vivo model of VL, the AmB-PMAA complexes displayed high and reproducible in vivo anti-leishmanial activity (> 90%, activity equivalent to AmBisome®) without being toxic in four independent experiments. The high in vivo activity was maintained even after the complex was stored as a solid for six months at 4 °C or for 27 days at 37 °C. Similarly, storage of the AmB-PMAA complex as a solution for 7 days at 37 °C did not affect the activity of the complex. These stability studies indicated that the complex is heat-stable. Further work is being undertaken to evaluate the stability of AmB- PMAA complexes.

28 A PARADIGM SHIFT IN ENTERIC COATING: ACHIEVING RAPID RELEASE IN THE PROXIMAL SMALL INTESTINE IN MAN USING A NOVEL

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It is a common misconception that enteric-coated products designed to release in the proximal small intestine disintegrate rapidly after emptying from the stomach. In vivo such products can take up to 2 hours to disintegrate in the human small intestine. This significant time delay has undesired clinical implications, especially for drugs that have an absorption window in the proximal small intestine or when a rapid onset of action is required. A novel double-coated enteric system comprising an inner layer of neutralised methacrylic acid copolymer (Eudragit® L30D-55) and organic acid, and an outer layer of standard Eudragit® L30D-55 was developed to achieve accelerated release in the proximal small intestine. Here we present a proof-of-concept study to investigate the in vivo performance of the novel double-coating system using gamma scintigraphy in human volunteers. Tablet cores were coated with a double-coated formulation consisting of an inner layer of EUDRAGIT® L30D-55 neutralised to pH 6.0 in the presence of 10% citric acid, and an outer layer of standard EUDRAGIT® L30D-55. A conventional single coating of EUDRAGIT® L30D-55 was also applied to tablets for comparison purposes, with the identical coating formulation and thickness as the outer layer of the double-coating. Eight fasted volunteers received the double-coated and single-coated tablets in a two-way crossover study. Tablets were radiolabelled and followed by gamma scintigraphy; the disintegration times and positions were recorded. After leaving the stomach, tablets coated with the single coating formulation showed a significant time delay before disintegration occurred in the mid to distal small intestine, with disintegration time of 66 ± 22 min post gastric emptying. The double-coated tablets disintegrated earlier at 28 ± 6 min post gastric emptying with consistent disintegration in the proximal small intestine. The accelerated in vivo disintegration of the double coating system can overcome the limitations of conventional enteric coatings.

29 THE USE OF PH 6.8 PHYSIOLOGICAL BICARBONATE BUFFER PROVIDES MORE REALISTIC AND DISCRIMINATIVE IN VITRO ASSESSMENT FOR ENTERIC-COATED FORMULATIONS COMPARED TO COMPENDIAL PHOSPHATE BUFFER

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The commonly used pH 6.8 compendial phosphate buffer for in vitro assessment of enteric-coated products does not resemble luminal fluids of the small intestine and consequently gives rise to poor in vitro in vivo correlations. A more representative in vitro medium, pH 6.8 physiological bicarbonate buffer, was developed here to evaluate the dissolution behaviour of enteric coatings. Tablets containing prednisolone were coated with the following enteric polymers: hypromellose phthalate (HP-50 and HP-55), cellulose acetate phthalate (CAP), hypromellose acetate succinate (HPMCAS-LF and HPMCAS-MF), methacrylic acid copolymers (EUDRAGIT L100-55, EUDRAGIT L 30D-55 and EUDRAGIT L100) and polyvinyl acetate phthalate (PVAP aqueous and organic). Dissolution of coated tablets was carried out using USPII apparatus in 0.1 M HCl for 2 h, and subsequently pH 6.8 phosphate buffer or a physiological bicarbonate buffer, which closely resembles the ionic composition and buffer capacity of the intestinal fluid. The bicarbonate media was evolved from pH 7.4 Hanks buffer by continuously sparging CO2 gas into the media to achieve pH 6.8 (modified Hanks buffer, mHanks). There was no drug release from the enteric-coated tablets in 0.1 M HCl for 2 h. Drug release in subsequent pH 6.8 phosphate buffer was rapid and each polymer had similar release profile with lag times of 5-10 min. However, slower and distinct dissolution behaviour was found between these enteric coating formulations in pH 6.8 mHanks buffer, with drug release lag times ranging from 25 – 60 min post acid exposure. The drug release results in mHanks buffer is akin to reported delayed post-gastric emptying disintegration time for enteric coated products in vivo. The use of pH 6.8 physiological bicarbonate buffer provides more realistic and discriminative in vitro assessment for enteric-coated formulations compared to compendial phosphate buffer.

30 IN SITU LYOPHILISATION FOR RELEASE ENHANCEMENT OF KETOCONAZOLE FROM HARD GELATIN CAPSULES

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Ketoconazole is a broad spectrum hydrophobic antifungal drug. It presents challenges for oral delivery due to its erratic bioavailability, and micronisation by controlled crystallization and complexation with β-cyclodextrin have been previously reported for its dissolution enhancement. The aim of this work was to enhance ketoconazole dissolution rate through co-lyophilisation with mannitol and SLS, and to perform physical characterisation of the lyophilised product.

A co-solvent system of 50 % v/v t-butyl alcohol, 40 % v/v distilled water and 10 % v/v 0.2 N HCL was used to dissolve 20 % w/v ketoconazole with 5 % w/v SLS and 10 % w/v mannitol. The resultant solution was frozen and lyophilised overnight. A corresponding ketoconazole/SLS/mannitol physical mixture was prepared for comparison. Dissolution performance of both ketoconazole formulations was conducted in USP II dissolution apparatus (37°C, 100 rpm, and 1000 mL 0.5 % w/v SLS aqueous solution) with analysis at 291nm. Physical characterisation was performed by XRPD (Bruker-AXS D8 X-ray powder diffractometer), DSC (Mettler Toledo DSC 822e) and ATR-FTIR (Jasco FT/IR – 4200 Fourier transform infrared spectrometer).

The lyophilised system showed significant dissolution enhancement in the first 30 minutes over the corresponding physical mixture (f_2 =24.72), raw drug (f_2 =35.04) and commercial tablet formulations (Nizoral®, f_2 =24.68). The corresponding DSC thermogram exhibited absence of ketoconazole, SLS and mannitol melting endotherms. XRPD lacked peaks and displayed a "hump" pattern suggesting formation of an amorphous solid dispersion. FT-IR analysis demonstrated shift and disappearance of some characteristic ketoconazole and excipients peaks in the spectrum of the lyophilisate highlighting drug/excipient interactions.

N Rasenack and BW Muller, Dissolution rate enhancement by in situ micronization of poorly water-soluble drugs. Pharm. Res., 19: 1894-900 (2002)

31 DEVELOPMENT OF A NOVEL PHASE SEPARATING NAIL LACQUER FOR THE TREATMENT ON ONYCHOMYCOSIS

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Topical treatment of onychomycosis (fungal nail disease) is desirable to avoid the systemic toxicity associated with oral antifungal treatment. However treatment of onychomycosis is lengthy, typically taking around one year to complete and the clinical cure rate is low. The polymers which are used are often hydrophobic / water insoluble preventing the lacquer being removed during bathing. If the hydrophobic polymer is unable to stabilise the drug when the lacquer solvent evaporates on application, the drug will crystallise out rendering it unavailable for absorption. The purpose of this study was to design an amorolfine nail lacquer containing a hydrophilic and a hydrophobic polymer, to observe whether phase separation of the two polymers in the could be induced, leading to a protective water hydrophobic upper layer and a drug stabilising hydrophilic layer next to the nail matrix. This type of system should allow improved drug delivery to the nail, whilst protecting the lacquer from being washed off, allowing it to be used over extended periods of time.

Polymeric films containing hydroxyl propyl methyl cellulose (HPMC), Eudgragit E100 and amorolifine were prepared. DSC results of combined polymer films indicated the presence of two separate glass transitions corresponding to those of the two polymers. This confirms that the two polymers exist as separate phases in the polymeric film. ATR-FTIR spectroscopy results confirmed a higher concentration of the more hydrophobic Eudragit E100 in the upper layer of the film. In addition when amorolfine was included it appeared at a higher concentration in the lower hydrophilic HPMC rich layer. These preliminary results indicate the feasibility of developing a phase separating nail lacquer system containing a drug stabilising hydrophilic layer next the nail tissue with a protective hydrophobic polymer coating. Future work will include in vitro diffusion studies across nail tissue and assessment of the water proof nature of the formulation.

32 THE EFFECTS OF ESTERIFIED SOLVENTS ON THE DIFFUSION OF A MODEL COMPOUND ACROSS HUMAN EPIDERMIS; AN ATR-FTIR SPECTROSCOPIC

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ATR-FTIR (Attenuated Total Reflectance FTIR) spectroscopy may be used to study the effect of penetration enhancers on absorption across human skin. One advantage of using this approach is that it allows the diffusion and partitioning of the drug in the membrane to be separated more easily than can be achieved with Franz diffusion cells. Mechanistic insight can therefore be gained into how the penetration enhancer exerts its effect which can facilitate and optimize formulation development. Furthermore, with use of chemometric data analysis the diffusion of several components and their effect on the membrane can be followed.

In this study the effect of three ester solvents on the transport of a model drug cyanophenol across human skin has been investigated. The esters examined, isopropyl myristate (IPM), isostearyl isostearate (ISIS) and dipelargonate propylene glycol (DPPG) are used in topical formulations and have differing structures and polarities allowing the effect of these structural differences on this class of enhancers to be investigated.

Typical Fickian diffusion behaviour was observed for CP, DPPG and IPM across human epidermis. Fickian behaviour was only observed for ISIS across stratum corneum, indicating that the hydrophilic epidermal layers interfere with the hydrophobic ISIS diffusion. These Fickian diffusion profiles could be modelled to obtain diffusion coefficients for each of the diffusing species. In all cases the solvents diffused more quickly than CP. DPPG produced the highest concentration of CP in the skin, with IPM exerting a greater effect than ISIS. ISIS produced a lower skin concentration of CP than when water was used as a solvent suggesting that ISIS, under these conditions, has a penetration retarding effect. Amongst the three esters the more polar DPPG is the best penetration enhancer for CP.

33 LIPOPLEX FORMULATION: EFFECT OF LIPID TRANSITION TEMPERATURE

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Recent studies have illustrated cationic lipids to be useful in nucleic acid delivery. However research has shown that cationic liposomes and lipoplexes may be influenced by the presence of electrolytes within the aqueous solvent can influence both the physicochemical properties and the in vivo efficacy (Perrie 2000, Kim 2004). In this work the influence of lipid transition temperature and salt effect on physicochemical properties of cationic liposomes and lipoplexes was investigated using a range of lipids with high and low transition temperatures. MLV and SUV liposomes composed of range of different lipids were prepared by hydration in dH2O or PBS. In all cases, DNA complexes were formed by mixing cationic liposomes with DNA at various concentrations. Their characteristics in terms of size and zeta potential were measured as well as DNA complexation.

For all formulations tested the rehydration media was shown to influence both the size and zeta potential. Most notable was the inability to prepare DSPE (1,2-distearoyl-sn-glycero-3-phosphoethanolamine) : DOTAP (1,2-dioleoyl-3-trimethylammonium-propane) liposomes in PBS. Additionally, rehydration of other formulations in PBS resulted in a decreased zeta potential compared to those prepared in dH2O. In terms of the size, whilst no significant difference in vesicle size of DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine) : DSTAP(1,2-stearoyl-3-trimethylammonium-propane) liposomes prepared in either of the two aqueous media, DOPE:DOTAP vesicles were shown to increase in vesicle size when formulated in PBS. These results suggest formulations which are rehydrated in PBS and containing a lipid with high transition temperature, do not follow the same trend as those of low T_C liposomes. In terms of DNA complexation, the presence of salts within aqueous media did not adversely influence the lipoplex ability to complex DNA.

Kim, A., Lee, E. H., Choi, S.-H. & Kim, C.-K. 2004. In vitro and in vivo transfection efficiency of a novel ultradeformable cationic liposome. Biomaterials, 25, 305-313.

Perrie, Y. & Gregoriadis, G. 2000. Liposome-entrapped plasmid DNA: characterisation studies. Biochim Biophys Acta, 1475, 125-32.

34 DEVELOPMENT OF NOVEL FORMULATIONS FOR PROTEIN DELIVERY TO THE LUNGS

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Amphiphilic polyelectrolytes have been widely investigated as hydrophobic drug solubilisers. Recently there are a few reports on the use of these systems as oral protein delivery systems due to their ability to spontaneously form nano-complexes with protein and protect the protein from in vitro enzymatic degradation. The aim of this study is to elucidate the use of two novel amphiphilic polyelectrolytes based on polyallylamine (PAA), 2.5% mole palmitoyl PAA (PA) and quaternised palmitoyl PAA (QPA) for pulmonary delivery of catalase, a model protein. Polymer and catalase (2 $mgmL^{-1}$ each) were prepared separately in pH7.4 Tris buffer and equal volume were added together. At the optimum polymer: catalase mass ratio of 1:1, nano-complexes with the hydrodynamic size of 225nm and 884nm and zeta potentials of +26.3mV and +0.02mV were formed by PA and QPA complexes respectively. The size and zeta potential for catalase was 248 nm and -2.17 mV. It is hypothesized that the complexation took place via electrostatic and hydrophobic interactions. The catalase enzymatic activity was determined using hydrogen peroxide as the substrate. PA increased the catalase activity by 1.5-fold while QPA decreased the activity by 1.4-fold. The difference observed might be due to the structure of the polymers which had an effect on its binding to the protein. The PA nano-complexes and free catalase (0.1% w/v) in pH7.4 Tris buffer were then spray-dried with 5%w/v of trehalose. For both complexes and free catalase, no significant difference in catalase activity was observed before and after spray drying. The scanning electron microscopy (SEM) images revealed that majority of the complex particles were in the region of 1-3μm while catalase particles were more than 10μm. This result corresponds to the particles found on the next generation impactor where smaller particles of Pa, catalase complexes were found in the later stages. In conclusion, the above results indicate the potential of PA for protein delivery to the lung.

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SITE-SPECIFIC PEGYLATION OF IFN-β-1B

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Interferon-β (IFN-β) is clinically used to treat multiple sclerosis (MS). Its therapeutic efficacy is compromised by a short in vivo half-life and a propensity to aggregate. Protein PEGylation has been used for a range of therapeutic proteins currently in the clinic to increase their blood circulation and therefore provide a longer duration of action. There still is a great need for efficient site-specific conjugation of the PEG to the protein. Of the methods for site-specific attachment of the PEG, we have developed the use of bis-alkylation reagents which selectively PEGylate proteins at the two sulfurs that make up a native disulfide.

We have site-specifically conjugated a 30 kDa PEG reagent to the naturally occurring disulfide bond present in IFN-β-1b (IFN-β). Mono-PEGylated IFN-β was purified by ion exchange and size exclusion chromatography. The bioactivity of the PEGylated IFN-β was analysed using an in vitro antiviral assay (EMCV-A549). Stability studies were conducted on mono-PEGylated samples to determine if the PEG remained bound to IFN-β-1b and to determine the rate of aggregation for PEGylated product in contrast to native product.

Our results show that disulfide PEGylation of IFN-β with 30 kDa PEG yielded ~70% of the desired mono-PEGylated product. Our PEG-IFN-β was more soluble and less prone to aggregation than the unPEGylated IFN-β. The in vitro antiviral activity of PEG-IFN-β was 12% of the unPEGylated form. This level of decrease in the in vitro activity is an expected feature of PEGylation and is due to the steric hindrance caused by the PEG.

These preliminary results demonstrate that disulfide PEGylation of IFN-β-1b is both feasible and practical to develop a disulfide bridged version of PEG-IFN-β.

36 EFFECT OF COILING CAPABILITIES OF POLY CAPROLACTONE (PCL) ON THE LOADING OF LANSOPRAZOLE-LOADED NANOPARTICLES

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There is increased interest in reformulation of licensed drug products into oral liquid formulations by the European Medicines Agency (EMEA), for use in the geriatric and paediatric patients. Lansoprazole is one such drug that has been listed as a potential as it is only available as a solid dosage form. By using the solvent displacement technique, the suitability of PCL at various ratios (5:1, 6:1 and 7:1) with Lansoprazole-loaded nanoparticles was studied. The formulations were characterised for particle size, zeta potential, polydispersity, and percentage of drug entrapped.

The investigation revealed interesting results. At the 5:1 ratio, nanoparticles displayed entrapment values of 85.5 ± 1.8%. Analysis of particle size measurement showed nanoparticles in the range of 271.6 ± 4.8 nm. Zeta potential measurement revealed an anionic surface (-15.8 ± 4.0 mV). However increase of polymer concentration to 6:1 and 7:1 resulted in the decrease of entrapment values to 80% and 56% respectively. This was also followed by an increase in particle size with 6:1 and 7:1 ratios exhibiting a particle size of 285.1 ± 13.4 nm and 308.7 ± 19.5 nm respectively. These trends can be explained on two factors. Firstly, PCL has relatively weaker coiling capabilities in comparison with other biodegradable polymers, and exists as long strands thus resulting in weaker polymer-polymer interactions, thus resulting in larger nanoparticles being produced with increasing polymer concentration. Additionally, the increase in size upon increase of polymer concentration potentially explains the reduction in drug entrapment. Increase in particle size occurs due to increase in viscosity of the organic phase upon increase of polymer concentration thereby increasing the resistance for the drug candidate to enter into the hydrophobic polymeric environment

This study has shown that lansoprazole can successfully be formulated as a nanoparticulate liquid oral delivery system, with the drug encapsulation efficiency being dependent upon the drug and polymer characteristics and ratios.

37 EFFECT OF DISSOLUTION MEDIA COMPOSITION AND IONIC STRENGTH ON DRUG RELEASE AND DISSOLUTION PROPERTIES OF HARD CAPSULES

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The influence of dissolution media composition, and ionic strength on the in-vitro shell dissolution and drug release properties of HPMC and gelatin hard capsules, after storage at 35% relative humidity for 24 hr was investigated.

Shell dissolution time of empty hydroxypropyl methylcellulose (HPMC) and gelatin capsules (size 0) was measured following the method described by Chiwele et al. Drug dissolution studies were then undertaken following the BP paddle method in a Caleva dissolution tester (Caleva Ltd, Dorset, UK) at 37°C and paddle speed 50 rpm using 900 ml of dissolution medium. In both studies, dissolution medium used was potassium phosphate buffer (KPB), sodium phosphate buffer (NaPB), both pH 7.4 (0.1-0.5 M), and 0.1 M HCl containing either KCl or NaCl (0.1-0.5 M).

Increase in K+ salt concentration and ionic strength hindered empty HPMC shell dissolution and slowed down the dissolution of gelatin. However, the increase in Na+ salt concentration did not affect the shell dissolution properties of the HPMC shells to the same extent. Drug dissolution tests for HPMC showed, < 4 % and 10 % drug release in 0.5 M KPB and NaPB respectively after 5 hours, complete release occurred in 0.1 M after 30 and 15 mins respectively. For gelatin, release rate decreased with increase in NaPB concentration, with complete release after 5 hours in 0.5 M, and after 1 hour in 0.1 M. In HCl, HPMC capsules gave a decreased drug release rate with increase in KCl and NaCl concentration, however, release from gelatin capsules was only affected by increase in NaCl concentration.

Shell dissolution and drug dissolution properties of HPMC and gelatin capsules differ from one another due to dissolution media composition and ionic strength. HPMC shells were affected by the ionic strength of all selected media, whereas gelatin capsules were influenced only by the presence of sodium ions.

Chiwele, I et al, Chem. Pharm.Bull. 48: 7 (2000) 951-956

38 CONTROLLING THE DISPOSITION OF GALLIUM COMPLEXES IN HUMAN SKIN USING IONTOPHORESIS

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The delivery of metals into the skin is problematic as inorganic molecules can form coordination complexes that alter the effective charge and molecular size of the metal and hence it's diffusivity. Ga(III) is a metallotherapeutic agent which is known to form coordination complexes in simple solutions resulting in poorly controlled topical delivery. The aim of this study was to determine the effect of coordination on the controlled delivery of Ga(III) and to attempt to modify this permeation using iontophoresis (ITP).

Ga(III) speciation was modelled using metal-ligand stability constants in silico to produce three different solutions containing Ga(III) as either 100% Ga^{3+} ions, a 100% negatively charged citrate complex (Ganeg) or a combination of positively charged hydroxide complexes (Gapos). These Ga(III) complexes were presented to full-thickness human skin sections in Franz diffusion cells (MedPharm, Guildford) and allowed to permeate for 65 h either passively or after 60 min of ITP pre-treatment (615 $\mu A/cm^2$). Ga(III) deposition within the skin was determined by tape stripping.

Gapos (3.0 ± 8.0 $\mu g/cm^2$) gave a ten-fold lower passive permeation rate compared to the Ga3+ ions (28 ± 5.0 $\mu g/cm^2$) and Ganeg (30 ± 5.0 $\mu g/cm^2$). However, the application of ITP changed this trend. The flux of Ga(III) was increased 9 and 800-fold from Ganeg and Gapos, respectively by ITP application, whereas free ion permeation was unaffected ($p > 0.05$, ANOVA) by ITP application. Ga(III) deposition within the skin was increased from 0.29 ± 0.26 mg/cm2 (passive) to 1.20 ± 0.25 mg/cm^2 (ITP) when applied as Gapos and from 0.71 ± 0.16 mg/cm^2 (passive) to 1.28 ± 0.27 mg/cm^2 (ITP) when applied as Ganeg.

The effect of ITP pre-treatment on Ga(III) coordination complex permeation and deposition in human skin was possibly due to reduced non-specific binding within the skin, however such a hypothesis requires further investigation.

39 INFLUENCE OF A PORE-FORMING AGENT ON SWELLING, NETWORK PARAMETERS AND PERMEABILITY OF POLY (ETHYLENE GLYCOL)-CROSSLINKED POLY (METHYL VINYL ETHER-CO-MALEIC ANHYDRIDE) HYDROGELS

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Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water or biological fluids. In the present study, the effect of a pore-forming agent, sodium bicarbonate ($NaHCO_3$) on the swelling kinetics, network parameters and bovine serum albumin (BSA) permeation was investigated. Hydrogels were prepared from aqueous blends of 15% w/w of poly (methyl vinyl ether-co-maleic anhydride) (PMVE/MA) and 7.5% w/w of polyethyleneglycol (PEG 10,000 Daltons) containing 0, 1, 2, or 5% w/w of $NaHCO_3$ were investigated for swelling and network parameters. In addition, the permeability of BSA through equilibrium swollen hydrogels containing different percentages of $NaHCO_3$ was also investigated. The ultimate aim of this research was to develop hydrogels for controlled delivery of proteins.

Aqueous gels of 15% w/w PMVE/MA and 7.5% w/w PEG containing different amounts of $NaHCO_3$ were cast into films and crosslinked for 24 hr at 80°C. Crosslinked films (1 cm^2) were swollen in phosphate buffer saline (PBS) at pH 7.4 for 24 h at room temperature and weighed at regular intervals. Network parameters, such as, equilibrium water content (EWC), molecular weight between crosslinks (Mc), and crosslink density (q) were determined by mathematical treatment of swelling results. A modified Franz-cell setup was used to investigate the diffusion of BSA across swollen hydrogels. In general, increase in $NaHCO_3$ content increased the swelling of hydrogels. For example, the % EWC was 731, 860, 1109 & 7536 % and the Mc was 8.26, 31.64, 30.04 & 3010 x 10^5 g mol^{-1} for hydrogels containing 0, 1, 2, and 5% w/w of $NaHCO_3$, respectively. Furthermore, the crosslink density (q) of the hydrogels decreased with increase in $NaHCO_3$ content. Permeation of BSA increased with increasing $NaHCO_3$ content in hydrogels. For example, the mean percentage cumulative permeation of BSA after 24 h was 14.71, 18.07, 33.56, and 63.05 % across hydrogels containing 0, 1, 2 and 5% w/w of $NaHCO_3$, respectively. In conclusion, increase in the content of pore-forming agent (i.e. $NaHCO_3$) in the hydrogels extensively affected the swelling properties of the hydrogels which, in turn, resulted in increased pore size (determined from M_c values) of hydrogels. In addition, a significant increase in the permeation of BSA was achieved. We are currently evaluating these systems for use as rate controlling membrane in sustained release drug delivery devices.

40 SOLID AND POCKETED MICRONEEDLE DEVICES FOR THE DELIVERY OF THERAPEUTIC PROTEINS INTO HUMAN SKIN

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Microneedle devices (MDs) painlessly perforate the external barrier layer of skin to create transient pathways for drug delivery. A diversity of MD designs are under development, each tailored for a proposed therapeutic application. This study aims to evaluate two methods of loading MDs for the delivery of a potent therapeutic protein into the dermal layer of human skin.

Pocketed and non-pocketed stainless steel MDs used in this study consisted of an array containing five individual microneedles, each with a length of 700 µm. Sulforhodamine (a model low molecular weight drug), β-galactosidase (a model protein) and fluorescent 100nm nanoparticles (a model nanoparticulate) were used to determine the loading capacity and delivery capabilities of the MDs. Non-pocketed MDs (NMDs) were drug loaded by a dip coating procedure and pocketed MDs (PMDs) were loaded by collection of a liquid formulation in an integrated reservoir. Excipients were used to modify formulations in order to increase loading efficiency.

Fluorescence microscopy revealed a relatively uniform layer of coating on NMDs for both sulforhodamine and fluorescent nanoparticles. Increasing the number of NMD immersions into a coating solution resulted in higher loading of these model compounds; quantitative analysis indicating that up to 1 µg of sulforhodamine can be coated on to a NMD containing five microneedles. Further, histological analysis confirmed delivery of the β-gal protein from a coated NMD into the epidermal and dermal regions of ex vivo human skin. Preliminary data indicates that PMDs can be successfully loaded with a liquid protein formulation following a single immersion.

Results indicate that molecules and macromolecules can be successfully loaded onto MDs for subsequent delivery into the epidermal/dermal layer of skin. Future studies will optimise the loading capacity of liquid formulations within the PMDs and investigate the resulting spatial and temporal deposition of proteins delivered by PMDs into skin.

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ARE ALL TOPICAL IBUPROFEN FORMULATIONS THE SAME?

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Topical ibuprofen is widely used for its analgesic and anti-inflammatory effects, especially in the elderly where it is advantageous over oral formulations due to the high incidence of adverse gastrointestinal events associated with chronic use of oral ibuprofen. There are a wide range of generic topical ibuprofen products available on the market, which to date have been viewed as interchangeable. The assumption has been that the rate of drug release and permeation through the skin from topical products of the same strength will be identical. The purpose of the present study was to compare the transdermal absorption of ibuprofen from a range of generic 5% and 10% ibuprofen gels using an in vitro test system.

The findings of this study conclude that there are statistically significant differences ($p<0.05$) in flux rate of ibuprofen across human skin in vitro within both the 5% formulation group and 10% formulations group. The data also shows that use of 10% formulations does not result in a doubling in either the flux rate or total amount of drug permeated compared to the 5% formulations.

The data illustrated that in topically applied formulations, the combination of excipients in the preparation had a considerable impact on the transdermal delivery of the drug. A simple view that the permeation of drug is a direct result of the concentration does not hold true. There are in reality a myriad of factors effecting release of drug from supposedly identical topical formulations, including degree of saturation, use of chemical penetration enhancers and viscosity of formulation. The data generated highlights the need to recognise that not all generic topical products are interchangeable and there is sufficient evidence to suggest that formulation design impacts on clinical efficacy.

42 INSIGHTS INTO THE ROLE OF MUCUS THICKNESS ON MUCOADHESION IN THE GASTROINTESTINAL TRACT

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Mucoadhesion in the gastrointestinal tract is a complex phenomenon and both formulation and physiological features need to be well understood and considered. Mucus thickness has been referred to play a role in this process, however no definitive influence has been established.

This study aimed to measure the mucus thickness along the gastrointestinal tract of pig, which closely resembles the human gastrointestinal physiological features. We also investigated the influence of mucus thickness on the mucoadhesion process on different sections of the gastrointestinal tract.

The mucus thickness of different regions of the gastrointestinal tract of three different pigs (100 kg) was fully measured by means of a histochemical method (hematoxilin/eosin) employing cryostat sections. Mucoadhesion was evaluated ex-vivo on different sections of porcine mucosa by tensiometry using a polyacrylic acid polymer (Carbopol 974P NF) compacts as a mucoadhesive material, both in a dry and swollen state. Mucus was found to be thicker in the stomach (body 67.9 ± 54.7 μm) than in the small and large intestine. Furthermore, mucus thickness increased from proximal to distal segments in both the small intestine (duodenum 25.9 ± 11.8 μm, ileum 31.0 ± 15.7 μm) and large intestine (caecum 19.4 ± 8.7 μm, ascending colon 31.9 ± 17.2 μm, descending colon 35.1 ± 16.0 μm and rectum 40.8 ± 12.5 μm). Dry Carbopol 974P NF compacts adhered more strongly to the stomach (0.285 ± 0.077 N) and colon (0.242 ± 0.062 N) than to the jejunum (0.165 ± 0.064 N) and ileum (0.134 ± 0.027 N). Swollen polymer compacts exhibited lower mucoadhesion than the dry forms in all sections analysed.

Mucus thickness plays a role on the mucoadhesion, thicker mucus provides deeper polymer chain diffusion and entanglements; however, other factors, such as mucus rheology, are also involved in the mucoadhesion process.

43 DEVELOPMENT OF BILOSOMES AS A CARRIER FOR ORAL VACCINE DELIVERY

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Oral vaccines offer significant benefits over traditional vaccines due to their ease of administration, improved patient compliance and potentially enhanced mucosal immune responses. Delivery systems can be employed to improve vaccine efficacy. For example bilosomes, which are lipid vesicles comprising bile salts (e.g. sodium deoxycholate), have been investigated as vaccine delivery systems (Conacher 2001). Inclusion of bile salts within the bilosome construct has a stabilising role after oral administration by preventing degradation with highly acidic conditions, and subsequently can act as the carriers for oral vaccines (Norris 1998). The aim of this work was to understand formulation factors that influence the physicochemical parameters of bilosome vesicles by measuring the effect of bilosome composition (monopalmitoyl phosphate (MPG), cholesterol (CHO), diacetyl phosphate (DCP) and bile salt) on the bilosome size, zeta potential and suspension pH.

A range of bilosome formulations were prepared by high shear homogenisation using different blends of MPG, CHO, DCP and bile salts. The lipids were heated to 120 °C / 10 minutes and while maintaining the melted lipid solution an emulsion was created by the addition of 5.2 mL 25 mM sodium bicarbonate buffer pH 7.6 (50°C) and homogenised for 2 min. While homogenising 0.55 mL of bile salt in 25 mM sodium bicarbonate buffer pH 9.7 was added and homogenised for a further 3 min. 0.28 mL of a peptide solution was then added and homogenised for a further 5 min. Upon cooling, the bilosome formulation was incubated for 2 hr with gentle shaking at 220 rpm. Vesicle size analysis was conducted using a sympatec helos particle sizer and zeta potential was measured in 1.5 mL double distilled water at 25 °C on a Zeta Plus Brookhaven Instrument.

The concentration of CHO, DCP and bile salt were all shown to influence vesicle characteristics. In terms of vesicle size, bile salt content was the major influencing excipient in the formulation: inclusion of bile salts within the vesicle constructs was shown to reduce vesicle size from 7.21 ± 0.05 μm for vesicles containing MPG, CHO and DCP only to 4.15 ± 0.05 μm when 200 mM sodium deoxycholate was added to the formulation. DCP and CHO ratios were shown to be critical in determining the pH of the suspension and the zeta potential of the bilosomes, overall increasing the DCP content resulted in decreasing pH and less negatively charged vesicles.

Average bilosome size should be between 5-6 μm to promote uptake and residence of the bilosomes within the Peyer's patches; vesicles of a smaller size are subject to lymphoid drainage (Eldridge 1990). MPG, cholesterol and DCP in a ratio of 5:4:1 respectively with the incorporation of bile salts at a concentration range of 70-110 mM produces vesicles in the required size range and optimum zeta potential for desired stability.

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44 MEDTHERM: A NOVEL STRATEGY FOR TRANSDERMAL DRUG DELIVERY

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Increasing the fluidity of lipids in the skin is an effective method to improve drug delivery. Traditionally, lipid fluidity is increased through the use of heat (Knutson 1985) or chemical agents (Barry 1987). At approximately 42˚C lipids in the SC undergo a solid-liquid phase change (Silva 2006, Gay 1994) increasing barrier mobility and reducing barrier function. Increases in lipid fluidity are proportional to temperatures applied (Park 2009); however prolonged high temperatures (> 45˚C) can result in burns. Reducing lipid transition temperatures using a strategy complimentary to heat application may provide a highly attractive means to improve drug delivery. Therefore, the aim was to investigate if simultaneous application of certain excipients to perturb the lipids and heat (together known as MedTherm) would influence skin barrier function.

Permeation of lidocaine through human epidermis was assessed after the application of four formulations. Two formulations generated heat, one with additional excipients (MedTherm) and one without, one formulation only contained the additional excipients and the final formulation acted as a vehicle control. Each formulation was applied above a lidocaine saturated gel, with samples taken every 5 min for 30 min for HPLC analysis.

After just 10 min significant increases ($p < 0.05$, t-test) in lidocaine permeation were observed compared to the control (0.16 ± 0.13 μg/cm^2). A 2.7-fold increase was observed using heat alone (0.43 ± 0.03 μg/cm^2), a 7.5-fold increase was observed using the additional excipients alone (1.22 ± 0.58 μg/cm^2), but when MedTherm was applied a 75-fold increase was observed (11.93 ± 5.79 μg/cm^2).

Simultaneous application of heat and additional excipients (MedTherm) acted synergistically to enhance the permeation of lidocaine by increasing lipid fluidity. Use of these additional excipients to reduce lipid transition temperatures coupled with moderate heat could significantly reduce the time required to reach a therapeutic level whilst increasing the safety of heat enhanced drug delivery.

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45 BILOSOME VACCINES: OPTIMISATION AND DEVELOPMENT OF A STABLE FREEZE-DRIED PRODUCT

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Freeze-drying is considered to be an effective approach that enhances the long-term stability of particulate vaccines during storage, by generating a dry solid product, through the removal of water under vacuum within the frozen formulation1. Here we assess and optimise a suitable freeze-drying protocol for the development of a stable freeze-dried bilosome product.

Bilosomes were prepared using the melt method technique and subsequently various concentrations of sucrose were added as a lyoprotectant. Three different rates of freezing; slow, medium and fast (i.e. -20°C, -70°C and liquid nitrogen, respectively), were tested, prior to drying. Subsequently, all bilosome preparations were subjected to primary drying (shelf temperature of -40°C) and then secondary drying (shelf temperature of +20°C). Each freeze-dried bilosome preparation was rehydrated to its original volume. Particle size and zeta potential was measured in filtered double-distilled water at 25°C using the Sympatec 2005 (Helos/BF) and the Brookhaven ZetaPlus, respectively.

Previously, the bilosome influenza vaccine formulation protected against fever and suppressed lung inflammation to extents comparable to the commercial vaccine given intramuscularly. Nevertheless, the wet bilosome product was not stable beyond 3 days, prompting efforts to develop a lyophilized product.

The rate of freezing, prior to freeze-drying, is shown to affect the stability of these empty bilosome systems. By applying a slow rate of freezing, the size of empty bilosomes significantly increased 2 hr after rehydration, with samples containing 100 and 200 mmol sucrose as a lyoprotectant. Larger particle sizes may be generated at -20°C due to the formation of large ice crystals forcing the particles together and subsequently causing aggregation. When these formulations were frozen in liquid nitrogen to give instant freezing, although the particle size was maintained, cracking of the freeze-dried product was visible. However, when frozen at -70°C (medium freezing) particle size is maintained and also a good freeze-dried product is generated.

46 A STUDY TO INVESTIGATE THE EFFECT OF EXTRUSION ON LIPOSOMES GENERATED FROM ETHANOL-BASED PROLIPOSOMES

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Liposomes are well established nanocarriers of small and large molecules. Unfortunately, liposome phospholipids are chemically unstable in aqueous dispersions. Proliposomes are stable phospholipid formulations which readily generate liposomes upon addition of aqueous phase and shaking. The production of liposomes in the nanosize range is advantageous in various drug delivery applications. In this study, liposomes entrapping salbutamol sulphate (SS) or ovalbumin (OVA) were generated from ethanol-based proliposomes. The resultant liposomes were extruded and characterised in terms of particle size, surface charge and entrapment efficiency of SS and OVA.

Soya phosphatidylcholine: cholesterol (2:1 mole ratio) liposomes were prepared from proliposomes followed by extrusion through 400 nm polycarbonate membrane filters. The size distribution of liposomes was measured using laser diffraction and surface charge (zeta potential) was analysed using laser Doppler velocimetry. The Entrapment efficiency of SS and OVA was determined using UV and bicinchoninic acid protein (BCA) assay respectively. The results indicated no difference in the size of liposomes in both formulations. Slight changes were observed in the zeta potential readings and were -5.82 ± 1.23 mV and -0.42 ±0.83 mV for the (OVA) and (SS) liposomes respectively. Entrapment efficiency of unextruded and extruded liposomes of SS was found to be significantly greater than for the OVA liposomes, being respectively 59.00% ± 5.18 and 41.30% ± 4.21 before extrusion and 43.02% ± 5.11 and 30.09 ± 4.23% after extrusion. The extruded samples also showed a slight difference in zeta potential readings, being -6.37 ± 0.80 mV and 0.59 ± 0.51 mV for the OVA and SS liposomes respectively. The results confirm the validity of the proliposome approach in the entrapment of small molecules (e.g. SS) and large molecules (e.g. OVA).

47 EVALUATION OF A CONTROLLED RELEASE (CR) MULTIPARTICULATE FORMULATION BY NEAR INFRARED (NIR) MICROSCOPY

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Spectroscopic imaging is a useful tool in the understanding of single unit and multi-unit dosage form performance. Multiparticulates allow: (i) dose flexibility, (ii) reduced risk of dose dumping and (iii) reduction in GI tract residence time variability [1]. However, multiparticulates are typically more complex to develop and require multifaceted scale-up, hence elucidating dosage form performance is critical. The utility of NIR microscopy stems from the ability to map rapidly large sample areas and the capability to (i) monitor chemical distribution, (ii) determine domain segregation and (iii) show spatial correlation which can be potential linked to macroscopic parameters This work describes the characterisation by near infrared (NIR) imaging of a sustained release (SR) multiparticulate formulation of MK-A.

Multiparticulates were manufactured using extrusion-spheronisation with subsequent spray coating. The samples were sectioned and analysed by NIR imaging. Chemical distribution was represented by colour-coded intensity maps. The chemical maps were compared pixel-by-pixel to generate a correlation chart. This indicated the spatial correlation of any two components in the formulation. Avicel and MK-A showed a positive correlation, indicating their distribution was spatially correlated. The association of MK-A and lactose was spectroscopically less evident, while the distribution of MK-A and PVP appeared to be poorly correlated. This information can be used to understand, for example, changes in dosage form performance on stability, or to predict the propensity for interactions within the dosage form affecting disintegration and dissolution performance.

This study shows how NIR spectroscopy can monitor multiparticulate manufacture through various processing stages, with potential use as a process analytical technology (PAT) tool. The microscopy approach on sectioned samples facilitated the acquisition of high quality maps of a dosage form illustrating: (i) the distribution of various components, (ii) their potential segregation in different domains and (iii) the spatial association of components.

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48 THE USE OF A NOVEL DICLOFENAC FORMULATION TO DETERMINE THE CORRELATION BETWEEN IN VITRO AND IN VIVO SKIN PERMEATION MODELS

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Due to the increasing cost, risk and ethical issues surrounding in-vivo testing on animals and humans it has been important within formulation development to incorporate in-vitro models as much as possible to predict the flux rates of an API through human skin to screen and optimise the efficacy of formulations. There are numerous limitations of in-vitro skin permeation models e.g. they are simplified generally non viable models of the complex human structure and exclude key factors such as metabolism and blood supply. However they can allow lead formulations to be selected for in-vivo testing reducing costs to the developer and speeding up the development process (1). The absorption patterns determined within in-vitro have been observed to parallel the patterns obtained in in-vivo (2). The quantitative agreement between the models has been observed to differ although in-vitro models have adequately distinguished compounds of low permeability from those of high permeability and ranked them in approximately the same order found in-vivo2. DermaSys® is a percutaneous drug delivery system developed by Futura Medical based on a novel combination of penetration enhancers. In this study the system was incorporated into novel NSAID, (diclofenac) formulations (pre-fix DCF100C1). The aim of the study was to compare the results obtained in an in-vitro experiment and in-vivo experiment of the DCF100C1 formulations with topical market comparators.

In order to investigate the correlation between in-vivo and in-vitro models the formulations DCF100C1 1%, 2.5% w/w and the commercial comparator Voltaren Emulgel® gel were assessed using an in-vitro epidermal membrane skin model and an in-vivo clinical trial. The in-vivo clinical trial was performed using open label randomised, single centre microdialysis study to compare the relative bioavailability of diclofenac in plasma after repeated administration. The method was a single centre, open label, five treatment, three period crossover (Youden square) design (3). In-vitro skin permeation of diclofenac was assessed through human abdominal epidermal membrane mounted in Franz diffusion cells, each formulation was applied at 10 mg/cm^2 and the Franz diffusion cells kept at a constant 35°C. Receiver fluid samples were taken at t=0,1,2,4,6,8,24 and 30 h and analysed for diclofenac content using HPLC.

Both assessment methods resulted in an increased permeation observed in the DCF100C1 formulations compared to the commercial comparator. Within the in-vitro model DCF100C1 2.5% w/w resulted in a significant increase ($p < 0.05$, t-test) in permeation (steady state flux 4-8 h) of diclofenac through the human epidermal membrane compared to the commercial comparator (greater than 20 fold enhancement). An increase in permeation was also observed in the DCF100C1 1.0% w/w compared to the commercial comparator but was not statistically different. The same trend was observed within the in-vivo study with the rank order of bioavailability being DCF100C1 2.5% > DCF100C1 1.0% > commercial comparator. The bioavailability for DCF100C1 2.5% demonstrated a 5 fold increase compared to the commercial comparator3. Both models demonstrated the trend of increased diclofenac absorption from the DCF100C1 formulations compared to the commercial comparator during the first three hours post-application indicating the potential for DCF100C1 to have a more rapid and effective onset of action. The data demonstrated a correlation between the two permeation assessment models. The in-vitro model ranked the formulations to be the same as observed within the in-vivo models demonstrating the developed DCF100C1 formulations to increase permeation compared to the commercial comparator.

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49 THE EFFECT OF A NOVEL PENETRATION ENHANCER (MEDNAIL®) ON THE IN VITRO NAIL PERMEATION OF ANTIFUNGAL AGENTS

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Onychomycosis is a fungal infection of the nail accounting for ~50% of all nail disorders and affects toenails substantially more than fingernails (1). Prevalence of onychomycosis has been estimated at 5% in Western countries and has continued to increase in recent decades. Treatment for onychomycosis may be topical or oral. The treatment times of the topical products are generally long (12 months for toenail infections) and the cure rates are low, therefore alternative drugs and formulations are being investigated. In comparison to the thin stratum corneum, the much thicker nail plate means a much longer diffusional pathway for drug delivery. In addition, in contrast to the elastic and pliable stratum corneum, the nail plate is dense and hard. MedNail® is a novel penetration enhancer consisting of sequential application of a reducing agent and an oxidising agent. It has been identified that the key oxidising and reducing agents are thioglycolic acid and urea hydrogen peroxide, respectively (2). It was subsequently identified that the sequential treatment of the nail with firstly, thioglycolic acid followed by urea hydrogen peroxide had the greatest effect on the permeation of the investigated drugs (3). The aim of this study was to demonstrate the effect of a novel penetration enhancer (MedNail®) on the permeation of two drugs, amorolfine and cyclopirox presented in two marketed nail lacquers (Loceryl and Penlac, respectively). An additional aim was to compare the effectiveness of terbinafine applied in a spray system using MedNail® with Loceryl and Penlac.

Initially a modified Franz cell was used, where full thickness human nail sections served as the barrier through which the drug penetrates into an agar filled chamber infected with dermatophytes. The two marketed products (Loceryl and Penlac) were applied once and after treatment of the nail surface with MedNail®. A second study was performed using a novel infected nail model where dermatophytes were incubated, grown into human nail and the ATP levels were used as biological marker for antimicrobial activity. During this second study the efficacy of the two marketed products (Loceryl and Penlac) was compared to the terbinafine spray system developed in house, using MedNail®.

During the initial study, MedNail®, was observed to enhance the effectiveness of both amorolfine and cyclopirox presented in Loceryl and Penlac, respectively, across full thickness nail sections, in vitro, after only one application. The in house terfinafine (MedNail®) spray system was significantly more effective (95% confidence interval, $P<0.05$) than both Loceryl and Penlac when applied to human cadaver nails. MedNail® resulted in complete kill in all assessed cells, and levels of ATP recovered from the nails (5.4 ± 3.2%, mean ± SD, of infected control levels) were equivalent to the negative control whereby no organisms were added to the nail (5.3 ± 1.6% of infected control levels). In comparison the MedNail® spray vehicle (i.e. containing no terbinafine), Penlac and Loceryl showed no statistically significant decrease ($p > 0.05$) in ATP levels recovered from the nails, compared to infected controls receiving no treatment.

This study clearly demonstrated that the benefit of a novel permeation enhancing system, MedNail®, which fundamentally alters the chemical structure of the nail not only enhances the efficacy of the existing topical formulations but increases the delivery and efficacy of terbinafine when applied ungually. Such a topically applied system has the possibility of overcoming the systemic side effects when terbinafine is delivered orally.

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50 ENHANCED TOPICAL DRUG DELIVERY THROUGH THE USE OF EUTECTIC/PENETRATION ENHANCER COMBINATIONS

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One of the most important factors when developing topical formulations is the ability of the API to permeate through the skin, and therefore any method of enhancing topical delivery would be beneficial. Methods of enhancing drug delivery include chemical enhancers and eutectic systems. Eutectic systems are mixtures of two API's that inhibit crystallisation and interact with each other to lower the melting point of the eutectic system so the combined melting point is less than the individual APIs (1). The eutectic system solubilises into lipids present within the skin at a faster rate, therefore enhancing topical API delivery and penetration through the skin in comparison with a non-eutectic formulation. Penetration enhancers also enhance topical API delivery by temporarily disrupting the barrier properties of the skin. The aim of this study was to investigate the combined effects of eutectic systems and penetration enhancers on API delivery through the skin barrier using a novel local anaesthetic formulation.

In order to first investigate the effects of eutectic systems enhancing API delivery through skin, scrotal full thickness skin was mounted in Franz diffusion cells (37°C) and three formulations, containing differing ratios of lidocaine and tetracaine eutectic mixtures solubilised in propellant, were applied. Receiver fluid samples were taken at pre-determined time points (0m,30m,1,2,3,4,5,6,24 & 28 h) and analysed via HPLC. One eutectic system was the re-optimised with the incorporation of different penetration enhancers and assessed in the in vitro skin permeation model described above.

The formulation with the highest average steady state flux for lidocaine and tetracaine was tetracaine:lidocaine (ratio 1:1) with a flux of 7.34 $\mu g/cm^2/h$, followed by tetracaine:lidocaine (ratio 1:3) (6.55 $\mu g/cm^2/h$). The formulation which provided the least flux was tetracaine:lidocaine (ratio 3:1) (2.74 $\mu g/cm^2/h$). However, no statistical significance was observed in the permeation of any of the eutectic systems ($p>0.05$, ANOVA). The formulations containing isopropyl alcohol, transcutol P and benzyl alcohol obtained the following flux 10.93, 7.34 and 7.20 $\mu g/cm^2/hr$, respectively. The flux for the control formulation was 6.18 $\mu g/cm^2/hr$ although again due to the variability in the data no statistical difference was observed ($p>0.05$, ANOVA). Although the difference was not significant there was a ca. 60% increase in the absorption of a eutectic local anaesthetic in combination with a penetration enhancer compared to the eutectic alone. Such data suggests that such delivery warrants further investigation.

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51 SYNTHETIC STUDIES TO PREPARE PEG-GLUTAMIC ACID ANALOGUES

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Protein therapeutics is associated with several limitations, such as short circulating half-life, immunogenicity, proteolytic degradation and low solubility. One of the strategies that has emerged to improve the efficacy of protein biopharmaceuticals is PEGylation. It is clinically proven that the covalent conjugation of poly (ethylene glycol) (PEG) to proteins can increase their circulation half-life. This provides a means to maintain a therapeutic dose of the protein between each dose. The influence that PEG has on the protein's properties is dependent on a number of factors including (i) the site on the protein where PEG is conjugated, (ii) the molecular weight of PEG and (iii) the morphology of the PEG molecule that is used.

We have recently developed PEG reagents that are capable of site-specific conjugation via bis-alkylation. We are able to control the site of PEGylation and the molecular weight of the PEG. We now wish to evaluate changes in PEG morphology.

To achieve this we are preparing a small family of branched PEG reagents derived from glutamic acid. The di-branched PEG reagents that are used clinically are derived from lysine. Our best bis-alkylation linkers possess functionality that require linkage to PEG through an amine group. Hence, the best way to make di- branched PEG reagents derived from a native amino acid is to use aspartic or glutamic acid. Utilising FMOC protected glutamic acid, the bis-activated N- hydroxysuccinimide (NHS) ester was obtained in 80% yield after purification by flash chromatography. While the material may still contain traces of the mono-NHS ester, this product is currently being used to couple with linear methoxy PEG compounds. These precursor branched PEG reagents will then be coupled to our bis-alkylation linker.

52 THE USE OF DIFFCORE™ TABLET TECHNOLOGY TO PROVIDE NOVEL DRUG RELEASE PROFILES IN-VITRO AND IN-VIVO

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DiffCORE™ is a fully industrialised proprietary modified release drug delivery technology owned by GlaxoSmithKline. A number of DiffCORE™ tablet formulations have been developed within GlaxoSmithKline and one has been launched in the USA in July 2009.

The dosage form consists of an immediate release (IR) or extended release (XR) matrix core containing the active ingredient. This core is surrounded by a functional barrier coating containing two apertures through which the drug is released. The apertures - typically 1-6 mm in diameter - are mechanically drilled into the top and bottom faces of the tablet. Drug release can be modified by varying the coat, apperture size and composition of the matrix core, creating unique drug delivery profiles with low variability.

There are three types/generations of DiffCORE™ technology that have been developed in order to optimise the pharmacokinetic profile depending on the drug substance properties. The 1st generation DiffCORE™ consists of an IR or XR matrix core surrounded by an insoluble coat with apertures, and is suitable for highly soluble drugs. The 2nd generation DiffCORE™ is similar to the 1st generation but uses an enteric coat. Therefore, within the stomach, drug is released via the apertures, whereas in the intestine the dissolving enteric coat results in an increase in drug release (via core erosion/diffusion) as a consequence of the increase in exposed core surface area. This compensates for a drug substance with a low solubility at intestinal pH. The 3rd generation DiffCORE™ is similar to the 2nd generation but comprises of a bilayer (IR and XR layer) matrix core. The presence of the IR layer allows for quick gastric release - accelerating initial drug absorption - followed by a sustained intestinal release. This is useful for those drugs that are highly soluble in gastric fluids, but only poorly soluble in intestinal fluids.

53 DYNAMIC SUPERSATURATED ANTIFUNGAL FORMULATIONS FOR TOPICAL DRUG DELIVERY

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The means to deliver antifungals are critical for effective prophylaxis and treatment of fungal infections such as tinea pedis, tinea cruris and intertrigo1, 2. Dry powders and particulate drug in suspension are strategies which have been used to treat topical infections. Powder formulations offers advantages over traditional dosage forms such as creams and ointments as no manipulation is required from the former, resulting in no transfer of infection to uninfected areas. However, powder formulations sometimes display low solubility and result in slow dissolution rates which would be sub-optimal for therapy. The formulation of an amorphous particle might improve the bioavailability of such antifungal formulations by increasing the dissolution rates under physiological relevant conditions3 resulting in faster drug penetration. The aim of this study was to prepare amorphous forms of clotrimazole in order to determine if increasing the rate and extent of dissolution of clotrimazole will result in supersaturated solutions capable of increasing the bioavailability and subsequent therapeutic activity of clotrimazole. Amorphous clotrimazole was prepared by rapid quench cooling from the melt, solvent evaporation and solid dispersion techniques. Saturated solubility of the prepared particles was measured in 0.1M citrate buffer pH 5.5 (physiological pH of skin's acid mantle) at 32°C. Amorphous particles dissolved rapidly to give maximum solubility values of 346.63±8.59 µg/mL, 270.66± 8.74 µg/mL and 375.19± 7.35 µg/mL for quench, solvent evaporation and solid dispersion (SD) respectively. This represented a supersaturation factor of 6 to 8 times the equilibrium solubility of microcrystallised clotrimazole (43.64 ± 2.35 µg/mL) ($p < 0.05$).Conclusions: Amorphophisation of clotrimazole resulted in improved dissolution and transient supersaturation for up to 30 min from quench and solvent evaporation particles and up to 50 min for SD particles under conditions mimicking powder deposition onto skin that could potentially improve the bioavailability and ultimately the efficacy of antifungal therapy.

54 DEVELOPMENT OF CYSTEAMINE POLYMERIC SYSTEMS FOR THE TREATMENT OF CORNEAL CRYSTALS IN CYSTINOSIS

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Corneal cystine crystal deposition is an early diagnostic feature of nephropathic cystinosis and leads to photophobia, blurred vision and corneal erosions. Current treatment is with topical cysteamine 0.55% eye drops, which can reduce crystal density. However, the recommended frequency of administration is hourly during the initial treatment phase followed by four to six times a day. It would greatly improve the quality of life of cystinotic patients, especially children to develop a slow release cysteamine ophthalmic preparation which reduces frequency of administration, improves patient compliance and bioavailability/efficacy of the drug. This can be achieved by formulating a gelling system which reduces the lachrymal drainage and increases drug ocular contact time.

After literature review and extensive pre-formulation studies, two polymers; polymer (A and B), (in combination or not) were further investigated. They were selected due to their compatibility with cysteamine, pH, their rheological behavior under different conditions, osmolarity, dropability and their resulting in vitro cysteamine drug release characteristics. In parallel, commercial ophthalmic preparations were used for bench marking. An iodimetric titration assay method was validated allowing accurate and reproducible measurement of cysteamine.

The top formulation containing 5% of polymer A (pH 7.4) maintained at 34°C and diluted with STF exhibited a viscosity of 56.32 +1.2 c.Pa at 20 rpm. The drop size of this formulation was 46.71+ 0.9µL and the osmolarity was 283 +0.6 mOsm/l. This result is in line with commercial gels or in situ gelling systems that were tested. For all tested polymers, in vitro release of cysteamine from Franz diffusion cell was slower compared with the bag dialysis, which allowed stronger discrimination between formulations. Thus, a good tolerability could be predicted. Future studies include the pharmacokinetic studies of cysteamine from the optimum formulations which will be evaluated in vivo, in cornea of New Zealand rabbits.

55 NON-VOLATILE SOLVENT EMULSION DIFFUSION: A NOVEL METHOD FOR FABRICATION OF LIPOSOMES

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Liposomes are phospholipid vesicles capable of encapsulating both hydrophilic and lipophilic compounds. Most of the methods currently available for the fabrication of liposomes either use a volatile organic solvent or use high shear, which can cause damage to macromolecular drugs. Here we report the formation of liposomes using a solvent emulsion diffusion method wherein the non-volatile water-soluble benzyl alcohol is diffused out of a pre-homogenised lipid emulsion into the aqueous continuous phase. The potential advantage of a pre-homogenised emulsion in this method is that it avoids homogenisation in the presence of the macromolecular drugs. Using this method various liposomal compositions comprising bovine serum albumin (BSA), as a model protein and an HIV gp140 plasmid were prepared.

Studies involved use of emulsions having different lipid compositions (neutral soyalecithin/cholesterol, anionic DMPC/DMPG/cholesterol, and cationic DMPC/SA/cholesterol), homogenisation times, surfactant concentrations and cholesterol contents. Correspondingly, liposomes from a size range of about 150nm to about 500nm could be generated. The presence of liposomes was confirmed using transmission electron microscopy (negative staining with uranyl acetate). Percentage association for BSA was measured using RP-HPLC for three batches the DMPC/DMPG based liposomes with different cholesterol contents. The percentage association ranged from about 18% to 52%. Cationic liposomes were used for complexing the HIV gp140 plasmid. The liposomes were also subjected to polyacrylamide gel electrophoresis (BSA) and agarose gel electrophoresis (plasmid) to confirm the integrity of the loaded macromolecule. wThis liposome fabrication method is capable of encapsulating macromolecules without inducing any significant degradation.

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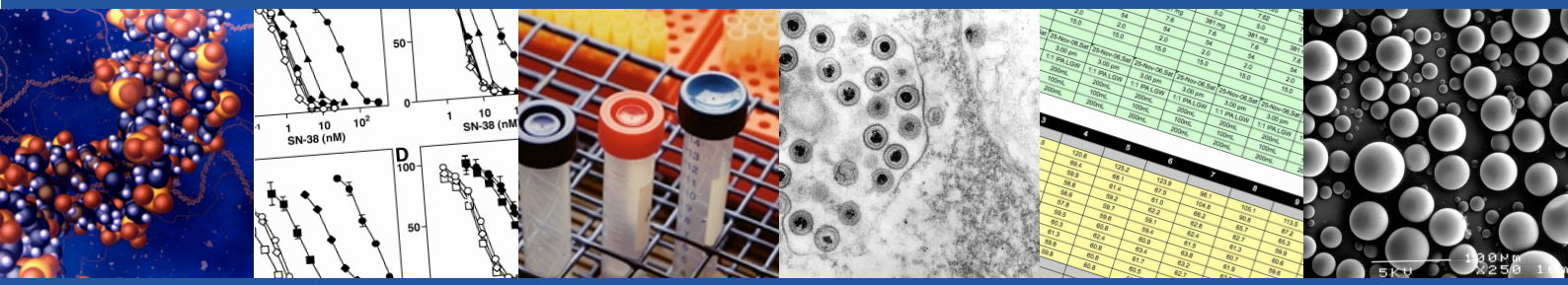
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