Pharmacy Education (2023) 23(3) 136 - 150
https://doi.org/10.46542/pe.2023.233.136150

CONFERENCE ABSTRACTS

FIP Seville conference abstracts 2022

80th FIP World Congress of Pharmacy and Pharmaceutical Sciences in Seville, Spain, 18 to 22 September 2022

Drug delivery and manufacturing

Impact of viscosity and pH of dissolution medium on ethanol-induced dose dumping from sodium alginate matrix tablets

Nizar Al-Zoubi[1]*, Safwan Abdel Rahim[2], Heba Khader[1], Rahaf Alwaraydat[1], Faisal Al-Akayleh[3]

[1]*The Hashemite University, Zarqa, Jordan*

[2]*Applied Science Private University, Amman, Jordan*

[3]*University of Petra, Amman, Jordan*

** nzoubi@hu.edu.jo*

Background: The release kinetics of oral modified-release (MR) formulations can change if concomitantly ingested with alcoholic beverages. This may lead to unfavourable changes in the drug bioavailability. The FDA requests *in vitro* dissolution of MR products in the presence of 5%, 20%, and 40% ethanol in 0.1N HCl for 2 h. However, there is limited information on the ethanol effect under various physicochemical properties of the dissolution medium, such as pH and viscosity, which may vary considerably in gastric fluids.

Objectives: To investigate the influence of pH and viscosity of acidic dissolution media on ethanol-induced dose dumping from sodium alginate (SA) matrix tablets.

Methods: The swelling and disintegration of drug-free sodium alginate compacts and the release of metformin HCl from SA matrix tablets were evaluated in acidic media of different pH (1.2 and 4.5), viscosity (HPMC K4M 0-1% w/v) and ethanol concentration (0%, 10%, 20% and 40% v/v). The dissolution efficiency and the time to 50% release (t50%) were used as release parameters. The similarity factor (f2) was used to compare the release in different dissolution media with that in the reference medium (0% ethanol, 0% HPMC). ANOVA tests were performed to determine significant factors on release parameters.

Results: It was found that ethanol level affected drug release from SA matrix tablets and the swelling of SA compacts. The effect was highly dependent on the medium pH. Dose dumping occurred at a high ethanol level (40%) with almost complete release occurring within the first 15-30 minutes associated with rapid matrix disintegration. Increasing the viscosity of the dissolution medium by dissolving HPMC (0.25-1%) prevented dose dumping at a high ethanol level. However, the release profiles were different ($f2 < 50$) from that in the reference (0% ethanol, 0% HPMC). The disintegration time of SA compacts was increased by increasing the viscosity concentration of HPMC in the dissolution media containing a high ethanol level (40%). ANOVA tests showed significant effects of pH and concentrations of ethanol and HPMC in the dissolution medium on the release parameters.

Conclusions: The presence of ethanol at 40% concentration caused dose dumping associated with the rapid disintegration of SA matrix tablets in dissolution media of low viscosity. Increasing the viscosity of the dissolution medium prevented ethanol-induced dose dumping resulting, however, in different release profiles in comparison with the reference medium (0% ethanol, 0% HPMC).

Using sophorolipids for the prevention of microbial contamination on silicone-based medical devices

Ana Bettencourt*, Rita Mendes, Ana Paula, Isabel A.C. Ribeiro

Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisbon, Portugal

** abettencourt@campus.ul.pt*

Background: Biomaterials used in medical devices such as silicone can easily promote surface microbial colonisation and related biofilm-associated infections. Thus, preventing the microbiological contamination of the device surface is a potential way to favour its performance.

Methods: Sophorolipids (SLs) were biosynthesised by *Starmerella bombicola* and purified using an automated flash chromatography system. Compound identification was performed by HPLC-MS. Surfaces were functionalised by lactonic SLs adsorption or by covalent bonds (i.e. through carbodiimide crosslinker reaction) of acidic SLs obtained after alkaline hydrolysis. Further, antimicrobial activity was assessed by the minimum inhibitory concentration assay (MIC) and biofilm inhibition (i.e. crystal violet staining, SEM) against *Staphylococcus aureus*.

Results: Through the MIC assay it was observed that the acidic C18:1 deacetylated compound obtained from alkaline hydrolysis was not able to inhibit microorganism growth under the tested concentrations (MIC> 800 µg mL-1). The lactonic C18:1 and C18:0 diacetylated compounds showed a MIC of 50 µg mL^{-1} and 100 µg mL^{-1}, respectively. The *S. aureus* biofilm inhibition by sophorolipids-coated silicone showed a 98% inhibition of biofilm formation with L-C18:1 and L-C18:0 in concentrations from 3.0 to 0.1 mg mL^{-1}. The SEM imaging also showed moderate inhibition of the biofilm formation.

Conclusions: Tested SLs showed potential to prevent biofilm formation on silicone surfaces.

Harnessing artificial intelligence for drug carrier systems development

Safa Damiati[1*], Samar Damiati[2]

[1]Department of Pharmaceutics, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia.

[2]Department of Chemistry, College of Sciences, University of Sharjah, United Arab Emirates.

** safa.a.damiati@gmail.com*

Background: In response to COVID-19, the role of artificial intelligence (AI) in influencing the scope and pace of research in pharmaceutical sciences was evident. For instance, in pharmaceutical research, natural or synthetic polymers are widely used as drug carriers. In these systems, investigating the physical properties such as particle size is a key feature for particle stability. Studying such systems, however, usually require extensive laboratory experimentation, costs, and time. Therefore, utilising AI and machine learning technologies, in particular, for studying these systems can offer efficient and economical solutions.

Objectives: To study the potentials of machine learning in advancing drug carrier research by insilico optimisation of the conditions used to generate prospective polymeric vehicles for drug delivery.

Methods: Glass microfluidic chip and poly(lactide-co-glycolide) (PLGA) were used to form polymeric microparticles. Experimental data of the generation of PLGA particles were used to train a machine learning model using artificial neural networks (ANNs) to generate an insilico model that is capable of optimising the conditions used in the generations of uniform microparticles.

Results: Preliminary results showed that ANNs are excellent tools to study the particle size distribution of PLGA microparticles which is one of the most important physical properties of drug carriers. The developed ANN model provided highly accurate predictions for generating monodisperse drug particles using PLGA as a synthetic polymer.

Conclusions: Machine learning technologies are promising tools for studying drug carrier systems as they offer efficient, fast, and economical solutions. Future work will focus on developing the wider implementation of ANNs using different natural and synthetic polymers and various microfluidic systems.

Study of the effect of chitosan-glutathione nanoparticles on the redox state of breast cancer cells.

Laura Denise López Barrera, Roberto Díaz Torres*, Patricia Ramírez Noguera

Universidad Nacional Autónoma De México, Mexico

** diaztorres_r@cuautitlan.unam.mx*

Background: Oxidative stress is involved in various diseases, including breast cancer. It has been seen that in this pathology the redox state plays an important role in the progress and development of the disease and its late stages, it is associated with the development of resistance to chemotherapeutic agents, for which the use of nanoparticle systems (NPs) has been proposed to improve response. On the other hand, glutathione is the main antioxidant agent and is related to the early response of the cell to stressful stimuli, in the same way, it is used by antioxidant enzymes as a mechanism to counteract induced stress.

Objectives: This work aimed to evaluate the effect of the exposure of chitosan nanoparticles with glutathione on the activity of antioxidant enzymes in transformed cells of the mammary gland.

Methods: The nanoparticles were characterised in terms of glutathione content, zeta potential and size. Subsequently, they were exposed to MDA 231-MB cells to evaluate the effect on the activity of the enzymes glutathione peroxidase, glutathione reductase and catalase.

Results: The results showed that the exposure of cells to NPs increased the activity of enzymes such as glutathione peroxidase and catalase and reduced the activity of the enzyme GRX, which is directly related to the glutathione content of the cell. On the other hand, combined treatments with the antineoplastic doxorubicin showed a potentiated increase in the activity of these enzymes.

Conclusions: In conclusion, according to the results obtained, the activity of antioxidant enzymes is modified by the presence of chitosan nanoparticles with glutathione, in addition to being sometimes enhanced by combined exposure with doxorubicin. This could be useful given the cellular events that could be modified by alterations in the redox state, such as apoptosis and cell proliferation.

Flow cytometry as an analytical method of drug-induced apoptosis in 3d bio-printed melanoma cells

Lissinda Du Plessis*, Maryke de Villiers

Centre of Excellence for Pharmaceutical Sciences, North-West University, South Africa.

** Lissinda.DuPlessis@nwu.ac.za*

Background: In drug discovery and drug delivery research there is a constant need for viable and reliable alternatives for *in vitro* drug efficacy and toxicity testing. Cell-based assays are critical in the pre-clinical drug discovery and development process with the advancements of 3D bio-printed cell cultures, analytical assays are required to be adapted.

Objectives: The purpose of this study was to develop a flow cytometry-based analytical strategy for the quantification of apoptosis with Annexin V and PI staining in 3D bio-printed hydrogel melanoma cell scaffolds to be used in drug efficacy evaluations.

Methods: A flow cytometric analysis method of detecting apoptosis in 3D bio-printed scaffolds were validated by a series of tests involving specificity, precision and sensitivity utilising unstained beads, eight-peak fluorescence calibration beads, and unstained and stained A375 melanoma cells. After validation, bioprinting of melanoma cell-laden scaffolds was completed. Thereafter, scaffolds were de-gelated and treated with an anti-cancer drug, stained and subsequently evaluated.

Results: The precision and sensitivity of the analytical method showed satisfactory results inside the accepted criteria range therefore, the method was concluded to be suited for the analysis of 3D cell-laden hydrogel scaffolds. Following, the anti-cancer efficacy of etoposide was evaluated on the A375 melanoma cells cultured in 2D and 3D bio-printed scaffolds were analysed through the previously validated flow cytometric method and compared with a common fluorometrically based micro-plate reader method. Both analysis methods indicated that the etoposide had a significant effect on the viability of the cells at both concentrations (50 µM and 100 µM). Additionally, the analysis showed that the 3D bio-printed cells experienced the same amount of drug-induced apoptosis as the 2D cultured cells, but the flow cytometric method had shown more in-depth quantification of the efficacy of the evaluated drug on the cells.

Conclusions: It was concluded that the Annexin V/PI flow cytometry method can be used to detect and identify drug-induced apoptosis in 3D bio-printed hydrogel scaffolds and can therefore be used in drug efficacy evaluations. Other hydrogels and cells could also be evaluated for a more relevant and universal application of this method.

Print parameter optimisation for three-dimensional bioprinting of hydrogel scaffolds

Lissinda Du Plessis*, Monja Hibbert, Joe Viljoen

Centre of Excellence for Pharmaceutical Sciences, North-West University, South Africa

** Lissinda.DuPlessis@nwu.ac.za*

Background: In drug discovery and delivery research, there is a continuing need for valid and reliable alternatives for *in vitro* drug efficacy and toxicity studies, and consequently 3D bioprinting of tissue constructs has caught the attention of researchers within this field. The mechanical properties, bioactivity and printability of these tissue constructs/ scaffolds, however, remain a challenge. Various studies have focused on investigating alternative printers, biopolymers and cross-linking methods of the hydrogels/bio-inks and recent papers mainly focus on the final tissue constructs; nonetheless, few have focused on the performance of the printing process.

Objectives: This study aimed to systematically optimise the printing parameters required to successfully 3D bioprint a preselected computer-aided design model into a sustainable scaffold with a preformulated hybrid hydrogel. Pneumatic extrusion which utilises air pressure to extrude the bio-ink was used as 3D bioprinting technology.

Methods: The formulated hybrid hydrogel, which consisted of alginate, a naturally derived biomaterial and Pluronic F-127, a synthetic poloxamer, had to undergo characterisation studies which included determining the optimal concentration of alginate, the ionic cross-linking solution used, as well as the cross-linking method. The formulated hybrid hydrogel used as bio-ink underwent printing parameter optimisation with the BioX™ 3D bioprinter from Cellink® (Gothenburg, Sweden) with a HeartOS operating system. The selected bioprinting parameters for evaluation included nozzle size, printing speed, pneumatic extrusion pressure as well as temperature.

Results: A concentration of 6% (w/v) alginate formulated with a 46% (w/v) Pluronic F127 in a 2:1 ratio had an average porosity of 50.50±3.1%, a low degradation rate that can be attributed to the PF127 addition, and a rheological characterisation of a non-Newtonian fluid. Cross-linking this hybrid hydrogel with a 4% (w/v) calcium chloride proved successful. This was ascribed to Pluronic F127's chemical inertness, as it does not undergo ionic cross-linking, but entangles within the polymer chains formed from the alginate. Optimal printing parameters to yield a successful scaffold print were found to be a nozzle size of 27G, extrusion pressure of 70kPa and a printing speed of 30mm/s at 37ºC. The printed scaffold was submerged into a 4% (w/v) calcium chloride cross-linking solution for 24h. The swelling ratio of the printed scaffold was also determined optimal in 2% (w/v) calcium chloride at 37^{o}C.

Conclusions: Although the printing parameters were successfully optimised with the formulated hybrid hydrogel, high concentrations of PF127 could have a negative effect on cell proliferation if they are included in the bio-ink, therefore, a clear need for the design and development of alternative polymer support materials is needed.

Formulation design and characterisation of transungual patches enabled by itraconazole nanosponges for targeting onychomycosis: a strategical approach for antifungal drug resistance

Limce Thampi*, Ranik Kuriakose, Aswani Balakrishnan, PM Muhsena, Sona Grace Nelson, TM FathimaLulu, Veena Jayan

Chemists College of Pharmaceutical Sciences and Research, Kerala, India

** limcesunil.02@gmail.com*

Background: Onychomycosis is considered one of the cosmetic problems that lead to cellulitis in adults and diabetic patients, and causes permanent damage to the nail. Therefore, the need of developing optimised drug formulations to minimise drug resistance and to widen the spectrum of antifungal activity against highly drug-resistant fungal infections. Nanosponges have got a significant application in targeted controlled release.

Objectives: The objective is based on the concept of developing nanosponges of itraconazole enabled in adhesive transungual patches to overcome antifungal drug resistance. The eradication of onychomycosis is difficult to achieve due to the non-vascularity and low permeability of nails, which are made of keratin. Itraconazole is an alternative first-line therapy for dermatophyte infections. Patients with diabetes and immunodeficiency disorders are at high risk of onychomycosis. Due to the increase in untoward side effects and the highest risk of liver injury occurring in oral therapy, it is difficult to achieve the therapeutic activity of the drug, if it is in the conventional dosage form.

Methods: Nanosponges of Itraconazole-loaded transungual patches are designed. Drug-polymer interaction studies, size and texture were performed by using FT-IR, XRD, DSC and SEM. Nanosponges loaded Itraconazole transungual patches were prepared by solvent evaporation techniques using Ethylcellulose, Hydroxypropyl methylcellulose and Polymethyl Methacrylate. Polymers were dissolved in methanol and dichloromethane. Polyethylene glycol and diethyl phthalate is used as permeation enhancers and plasticiser respectively. The study is outlined by the application of the Analytical Hierarchy Process for the selection of best approaches for nail drug delivery using a

multicriteria decision-making tool. Optimisation of formulation and prediction of drug release, permeability and drug entrapment etc. from the dosage form are performed using DoE (Design of Experiments). *In vitro* diffusion studies and kinetic modelling were also performed.

Results: The alternative with the highest score of 0.49 was obtained for nail patches using AHP.The data obtained from the spectra of FTIR, XRD and SEM of all formulations shows that there is no interaction between the drug and polymers. The total amount of drugs released for the formulations (F01 to F12) was about 77.5% to 93%, observed at different time intervals for a period of 9 hrs, and the optimised formulations containing ethyl cellulose nanosponge are F03, F07 and F11. The in vitro release data obtained were fitted into various kinetic models. Correlation coefficients of controlled release tablets showed a higher correlation with zero order plots than Higuchi and first order with the mechanism of controlled release. The *in vitro* release profiles were expressed most fitly by zero-order release kinetics (R2= 0.9077).

Conclusions: The developed formulation is used as a promising tool for the treatment of Onychomycosis, and helps to avoid the surgical removal of nails with deeper drug release and drug retention in the nail cuticle. Patients who are suffered from onychomycosis are facing embarrassment and disfigurement in society and thereby designed patches can improve the quality of life. The manufacturing method employed is simple and easily adaptable to achieve safe levels of drugs in fragile populations.

Evaluating mechanical properties of paroxetine-loaded filaments to enable printability by fused deposition modelling

Sara Figueiredo[1*], Paula Rato[1], Fátima Carvalho[1], João F. Pinto[2], Ana Isabel Fernandes[3]

[1]LEF – Infosaude, Portugal

[2]iMed.Ulisboa – Instituto de Investigação do Medicamento, Universidade de Lisboa, Lisbon, Portugal

[3]Egas Moniz Center for Interdisciplinary Research (CiiEM), Egas Moniz School of Health & Science, Almada, Portugal

** sara.figueiredo@anf.pt*

Background: Three-dimensional printing (3DP) has been recently identified as an opportunity to make a significant technological leap over traditional pharmaceutical manufacturing processes, namely regarding the customisation of medicines. Fused deposition modelling (FDM), the most commonly used 3DP technique, involves the production of a drug-loaded filament, obtained previously by hot-melt extrusion (HME), which is then melted and continuously deposited on a surface, layer by layer, building the 3D-printed dosage form.

The successful integration of HME and FDM requires that both extrudability of the raw materials and printability of the HME filaments fabricated are attained, properties which are influenced by the mechanical, rheological and thermal properties of materials. Since the filament is pulled by the printer feeding gears towards the heated nozzle where it softens to allow the accurate deposition on the building plate, evaluation of their mechanical properties is of the utmost importance. These properties are influenced not only by the polymeric formulation composition and the processing parameters used but also by the storage conditions of the filaments.

Objectives: This work aims to evaluate the impact of the environmental conditions on the quality and printability of paroxetine-loaded polymeric formulations for integrated HME-FDM, by assessing the mechanical properties of the filaments.

Methods: PRX (30% w/w), HPC (54% w/w) and other excipients (16% w/w of a mixture made of CaP, MS and TEC in a 10:1:5 ratio). Filaments containing polymeric formulation were prepared by HME (Notzek Pro single screw extruder, Notzek). Filaments were stored in stability chambers (Fitoclima D1200PH, Aralab) and desiccator inside open plastic bags, and re-examined at pre-defined times. Mechanical properties, moisture content and feeding/printing performance of filaments were evaluated for the different time points and conditions of storage. Whenever possible, FDM 3D Printed tablets were manufactured (3D printer Delta WASP 20 40 Turbo 2, Wasp, Italy) with printing temperatures of 200ºC (extrusion) /50ºC (plate).

Results: Under high humidity conditions (>60%RH), filaments became successively more ductile due to moisture absorption. The increment of water content promoted a plasticisation of the filaments, which suffered significant deformation and were unable to feed the printer.

Under low humidity conditions (11% RH), filaments became stiffer allowing adequate feeding of the 3D printer head. These changes are the consequence of the water loss during storage, and they are more prominent after one week of the HME process.

Conclusion: This study corroborates that the successful integration of HME and FDM technologies is highly dependent on the mechanical properties of the filaments used in the production of 3D printed dosage forms, since they affect processability. In turn, it proves that these characteristics are greatly influenced by storage conditions which must be carefully controlled during the continuous manufacturing process. Complementary studies to speed up the printability of filaments should be explored.

Intestinal absorption of the prodrug olmesartan medoxomil is enhanced by OATP2B1-mediated uptake

Naomi Fukazawa*, Saki Noguchi, Tomohiro Nishimura, Masatoshi Tomi

Keio University, Tokyo, Japan

** naomi_fuka@keio.jp*

Background: Ester-prodrugs generally have a neutral charge at physiological pH and are orally absorbed via passive diffusion in the small intestine. Olmesartan medoxomil (OL-MX), an ester-prodrug of olmesartan (OL), is an oral approved-drug of angiotensin II receptor blockers (ARBs) and presents in the form of anions at intestinal pH. Organic anion transporting polypeptides (OATP) 2B1 is involved in the intestinal absorption of acidic molecules. OL-MX may be absorbed by uptake via OATP2B1.

Objectives: The purpose of this study was to show that the conversion of OL to be a substrate for OATP2B1, OL-MX, promotes intestinal absorption.

Methods: OL-MX uptake via OATP2B1 was measured in human OATP2B1/HEK293 and rat Oatp2b1/HEK293 cells and mock cells. The effects of the inhibitor on OL-MX uptake in Caco-2 cells and OL-MX transport across the rat intestine were evaluated. Also, OL-MX and OL were quantified by LC-MS/MS.

Results: OATP2B1-mediated uptake of OL-MX was significant with the Km value of 9.0 μM, but that of OL was not observed. Other non-prodrug types of ARBs including losartan and valsartan were also incorporated into the cells by OATP2B1. Human OATP2B1-mediated uptake and OL-MX uptake in Caco-2 cells were significantly inhibited by rifamycin SV and fluvastatin. Rat Oatp2b1-mediated uptake and rat intestinal permeability of OL-MX were significantly decreased by naringin. These results suggest that OATP2B1-mediated uptake contributes to improved absorption of OL-MX.

Conclusions: Our results indicate that OATP2B1 recognises OL-MX. OATP2B1 may be a useful target for prodrug approaches to improve intestinal absorption.

Extraction procedure of an extract rich in flavonoids from murta (*Ugni molinae*) and its nanoencapsulation in chitosan-tripolyphosphate

Alexander Gamboa[1*], Arturo Intiquilla[2], Lilian Abugoch[1], Cristian Tapia[1]

[1]*Universidad de Chile, Chile*

[2]*Universidad Nacional Mayor de San Marcos, Peru*

** agamboa@ciq.uchile.cl*

Background: Murta (*Ugni molinae*) is a berry rich in flavonoid compounds with potential use in preventing and treating inflammatory diseases, such as inflammatory bowel disease. However, the effectiveness of its use depends on it reaching its site of action in adequate concentrations, for which the use of nanocarriers can improve drug delivery at the colonic level.

Objectives: Establish the best mixture of solvents for the extraction from Murta lyophilised powder and subsequent encapsulation of the extract rich in flavonoids in chitosan-tripolyphosphate nanoparticles.

Methods: Three extraction solvent systems were tested: ethanol, methanol, and acetone in a 1:1 ratio with water. All the extracts obtained were lyophilised and subsequently characterised according to their antioxidant capacity (ABTS), percentage of flavonoids, weight yield and moisture content. On the other hand, the liquid extracts were encapsulated by ionic gelation of chitosan with tripolyphosphate. The obtained nanoparticles were characterised by particle size distribution, zeta potential, encapsulation efficiency and extract loading.

Results: The weight yields of the extracts obtained using the mixtures ethanol/water, methanol/water and acetone/water, 1/1 ratio were 32.2%, 32.1%, and 38.5%, respectively. The moisture content was 21.5%, 21.3%, and 23.0%, respectively. The antioxidant capacity of the mixtures was 36.4, 21.4 and 44.9 mmol/L Trolox equivalent, respectively. The highest content of flavonoids was 88.1% of the total chromatographic area. The extracts nano encapsulated in chitosan-tripolyphosphate showed the following Z-averages: 280.9 nm (ethanol/water), 237.1 nm (methanol/water), and 365.6 nm (acetone/water) from extract at 5%w/w.All the nanoparticles showed a positive Z-potential between 50.3 mV and 51 mV, and the encapsulation efficiency was 82.7%, 70.4% and 71.3%, respectively. The highest flavonoid loading was 2.2% with the ethanol/water (1:1) extract.

Conclusions: The preparation of nanoparticles with chitosan and tripolyphosphate allows the efficient encapsulation of an extract rich in flavonoids from Murta (*Ugni molinae*).

Additive manufacturing in the era of personalised therapy: a focus on solid dispersion coupled FDM 3d printing

Garba M. Khalid[1*], Nashiru Billa[2]

[1]*Department of Pharmaceutics and Pharmaceutical Technology, Bayero University, Kano P.M.B. 3011, Nigeria*

[2]*Pharmaceutical Sciences Department, College of Pharmacy, QU Health, Qatar University, P.O. Box 2713, Qatar*

** khalidgmk@gmail.com*

Background: Additive manufacturing (AD) appears to be ideal for the fit-for-purpose manufacturing of medicines in contrast to the several shortcomings associated with the conventional fit-for-all mass production that accounts for less than 50% of pharmacotherapeutic treatment/ management of diseases. This concept is revolutionising the way medicines are designed, manufactured, and utilised, especially medicines for children and elderly patients, as well as patients with special needs.

Method: In this review, the authors discuss the current trends in the application of additive manufacturing to prepare personalised dosage forms on demand, focusing attention on the relevance of coupling solid dispersion with Fused Deposition Modeling (FDM) 3D printing. The literature was searched from PubMed, Embase, and Web of Science using the following keywords: “additive manufacturing”, “3D printing”, “4D printing”, “fused deposition modelling”, “solid-dispersion”, “personalized therapy”, and “poorly soluble drugs”.

Results: Combining the two technologies could offer several advantages to improve the solubility, dissolution, and oral bioavailability of poorly soluble drugs in tandem with the concept of precision medicine and personalised dosing and to address the dilemma of commercial availability of FDM filaments loaded with Class II and/or Class IV drugs. However, in thermal treatment, especially for heat-sensitive drugs, regulatory and ethical obligations in terms of quality control and quality assurance remain points of concern. Hence, the need to advocate for a concerted effort between the scientific community, the pharmaceutical industries, the regulatory agencies, the clinicians and clinical pharmacists, and the end-users to address these hurdles.

Conclusion: The scholarly outputs employing FDM in conjunction with solid dispersion for 3D printing of dosage forms attest to the potential of this approach. Thus, significant inroads based on the coupling of the two technologies will improve the provision of personalised dosage forms.

Peptide hydrogels as a long-acting injectable drug delivery platform for HIV/AIDS

Garry Laverty[1*], Sophie Coulter[1], Sreekanth Pentlavalli[1], Emily Cross[1], Yuming An[1], Ralf Schweins[2]

[1]*School of Pharmacy, Queen’s University, Belfast, United Kingdom*

[2]*Large Scale Structures Group, Institut Laue – Langevin, Grenoble, France*

** garry.laverty@qub.ac.uk*

Background: Eradicating HIV/AIDS by 2030 is a central goal of the World Health Organisation. Key to addressing this challenge is overcoming patient medication adherence issues and complicated drug dosage regimens associated with existing therapies, including a commitment to daily intake of tablets. There is a need for a convenient and effective long-acting formulation to deliver drugs over a sustained period.

Objectives: The study aimed to develop an injectable *in situ* forming hydrogel implant for the delivery of a model HIV/AIDS antiretroviral drug, zidovudine, over 28 days using peptides that form hydrogels in response to phosphatase enzymes present in the subcutaneous/intramuscular space. The formulation is composed of a self-assembling ultrashort D or L-α peptide hydrogelator composed of phosphorylated (naphthalene-2-ly)-acetyl-diphenylalanine-lysine-tyrosine-OH (NapFFKY[p]-OH) to which zidovudine is conjugated covalently via an ester linkage and formulated as an antiretroviral-peptide injectable solution.

Methods: Zidovudine conjugated peptides were synthesised using solid-phase synthesis methods, purified using semi-preparative HPLC and lyophilised by freeze-drying. Peptide-drug formulations were characterised for their mechanical properties using oscillatory rheology (frequency, strain and time sweeps). The underlying structure of hydrogel fibre networks was studied using circular dichroism and small angle neutron scattering (SANS) at ILL, Grenoble. Cell toxicity was assessed using a combination of MTS, Live/Dead, LDH and haemolysis assays. Biostability was studied using a broad-spectrum protease, Proteinase K, providing an in vitro indication of biostability over 28 days. Cumulative drug release from hydrogels was assessed for 28 days in phosphate-buffered saline (pH 7.4) and drug release kinetics was modelled using KinetDS.

Results: Rheological analysis showed peptides demonstrated enzyme-instructed self-assembly, forming hydrogels within minutes in the presence of phosphatase enzyme. SANS demonstrated peptide gels closely fit model data for the flexible cylinder elliptical model and the presence of entangled gel fibres suggests there is a large component of gel stiffness/strength that can be controlled by external conditions e.g. the gelation process. This may allow a change in gelation or formulation parameters to optimise material specifications, most notably gel strength and therefore drug release kinetics, for long-acting drug delivery. D-peptides

were particularly promising as a long-acting drug delivery platform, displaying resistance to protease degradation for 28 days. Drug release, via hydrolysis of the drug-peptide ester linkage, was shown to progress under physiological conditions. Burst release from physically encapsulated zidovudine (79.3%, 72 hours) was reduced by >30% to 47.3% via chemical conjugation of zidovudine to the same D-peptide gelator (NapffkYG-OH). This followed zero-order drug release kinetics.

Conclusions: This work is a proof-of-concept for the development of a long-acting combined injectable in situ forming implant using a peptide hydrogel formulation strategy which will provide positive societal, health and economic impact. Future work will focus on improving the manufacturing upscale of our platform and in vivo safety and pharmacokinetics. We are also focusing on combining this platform with multiple more potent HIV/AIDS drugs, and contraceptives and studying their potential use in different diseases.

Release profiles and *in vivo* bioavailability in beagle dogs of rebamipide nanosuspension-loaded bilayer tablet

Beom-Jin Lee[1*], Gang Jin[1,2], Hai Ngo[1]

[1]*Ajou University, Suwon, South Korea*

[2]*Jilin Institute of Chemical Technology, Jilin, China*

** bjl@ajou.ac.kr*

Background: Novel bilayer matrix tablet loaded with immediate release (IR) nanosuspension and sustained release (SR) of rebamipide (RBM) was developed to be effectively delivered in a controlled manner for one day.

Objectives: Salt-caged RBM nanosuspensions (NSPs) was designed to overcome the pH-dependent poor water solubility of RBM in low pH condition using an acid-base neutralisation method and used for the IR layer.

Methods: The SR layer was prepared by using diverse SR polymers such as polyethylene oxide (PEO100,000 and PEO 5,000,000) and hydroxypropyl methylcellulose 4000 (HPMC 4000).

Results: The release profile of the optimised bilayer tablet having 50% IR and 50% SR layer of 300 mg RBM showed that the IR layer could rapidly disintegrate in pH 1.2 buffer solution within 2 h, reaching 50% of drug release from the tablet, followed by an extended drug release from the SR layer in pH 6.8 buffer over 24 hours.

Conclusions: Regardless of the well-designed bilayer tablet in a controlled manner, *in vivo* pharmacokinetic study in beagle dogs had no dose- and dosage form dependent *in vivo* bioavailability in beagle dogs as compared with IR RBM reference tablet (Mucosta 100 mg) due to the biopharmaceutical factors such as gastric residence time and absorption site dependency of RBM.

Investigation of nanoparticulate platforms for transdermal delivery of tolvaptan

Jyothsna Manikkath*, M Resvanulla

Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Karnataka, India

** jyothsna.manikkath@manipal.edu*

Background: Tolvaptan is an orally administered BCS class IV agent used for vasopressin antagonism in congestive heart failure. Its low solubility and permeability present issues such as variation in gastrointestinal absorption, food effect, low bioavailability and poor compliance.

Objectives: The objective of this work was to develop nanoparticulate carriers for investigating the transdermal route of delivery of this agent, to overcome the issues with oral delivery.

Methods: Three delivery platforms viz., transfersomes (ultra deformable lipid vesicles with phospholipid and Tween 80), invasomes (phospholipid vesicles with terpene and ethanol) and solid dispersions with PVPK30, were developed. The design of experiments was used for formulation development, with drug formulations were chosen based on the highest desirability value. The lipid carriers were prepared by thin-film hydration followed by size reduction using probe sonication. The solid dispersions were prepared by solvent evaporation. The lipid carriers were characterised by particle size, polydispersity index, zeta potential and entrapment efficiency. The solid state characterisation of the lyophilised formulations was undertaken by differential scanning calorimetry and Fourier transform infrared spectroscopy. The optimised formulations were comparatively analysed for *ex vivo* drug permeation across porcine skin.

Results: The optimised transfersomes and invasomes were found to have a particle size of 80-90 nm, polydispersity index <0.3, zeta potential ranging from -15 to -20 mV and drug entrapment efficiency >85%. Differential scanning calorimetry revealed the transition of the drug from crystalline to amorphous nature in the formulations. Fourier transform infrared spectroscopy indicated the presence of characteristic functional groups of drugs in the formulations, implying drug-excipient compatibility. The formulations tested for *ex vivo* permeation across porcine skin

demonstrated the highest flux from transfersomes, followed by invasomes when compared with passive diffusion of the drug from buffer alone. Solid dispersions showed enhanced drug solubility (2-fold from phosphate buffer pH 7.4) but lower permeation than via passive diffusion. The mass balance of passive diffusion of the drug was within the limits of 100+/-20%.

Conclusions: This work indicates the feasibility of employing nanoparticulate platforms viz transfersomes and invasomes for transdermal delivery of tolvaptan. This could be useful for bioavailability enhancement of the drug, and also for overcoming issues related to variable gastrointestinal absorption and food effect. *In vivo* studies will be required to confirm these effects.

Evaluation of nanocarriers for the encapsulation and antimicrobial activity enhancement of *Origanum vulgare* subsp. *hirtum* extract

Angelos Odyseakis, Tolis Panayi, Yiannis Sarigiannis, Evroula Hapeshi, Christos Papaneophytou, Elena Mourelatou*, Angelos Odyseakis

University of Nicosia, Nicosia, Cyprus

** mourelatou.e@unic.ac.cy*

Background: Resistance of bacterial pathogens to antibiotics is a worldwide threat causing high morbidity and mortality. This phenomenon is highly attributed to the overuse, and often unnecessary use, of antibiotics in patients, but also to the widespread use of antibiotics in food-producing animals, to treat and prevent infectious diseases. Hence, it is essential for public health protection, to seek alternatives to antibiotics for animal disease prevention, and fight the development of antibiotic-resistant bacteria. Natural products have attracted attention, as they demonstrate unique therapeutic properties and represent a major source of drug discovery due to their chemical and structural diversity. The key representor of natural products is herbal extracts (HEXs), which although demonstrate a better overall therapeutic action over individual plant constituents, they have low therapeutic value in clinical practice due to their poor stability, solubility, pharmacokinetics and bioavailability. Nanocarriers, which are systems in the nanoscale, expect to overcome the limitations and problems arising from the use of crude HEXs.

Objectives: In this study, an alternative strategy to conventional antibiotics for farm animals is proposed, comprising of HEXs with antimicrobial properties being encapsulated in biocompatible nanocarriers that will protect and deliver HEX to the animals' gut, thus enhancing their antimicrobial activity. This approach could lead to limited antibiotic usage in farm animals, thus decreasing the development of antibiotic resistant bacteria.

Methods: For this purpose, the authors developed lipidic and polymeric nanocarriers to entrap the ethanolic extract from *Origanum vulgare* subsp. *hirtum* (OEE), an oregano specie in Cyprus flora, to enhance its antimicrobial activity, through the protection of its ingredients and controlled release. Two different nanocarriers with encapsulated OEE were prepared and studied, specifically liposomes, consisting of L-α-Phosphatidylcholine, and chitosan nanoparticles. Liposomes were prepared using the *Mozafari* method, whilst chitosan nanoparticles using the ionic gelation method, avoiding in both cases the use of organic solvents. Probe sonication was used for size reduction to around 100nm. After preparation, the size, ζ-potential, polydispersity index (PDI), stability, encapsulation efficiency (EE), *in vitro* release and antimicrobial activity (AA) of OEE nanocarriers were studied and the suitability and efficiency of the two nanocarriers were evaluated and compared. Preparation conditions were optimised in response to size, stability and EE.

Results: Both nanocarriers were able of encapsulating OEE and control its release. Between the two, chitosan nanoparticles exhibited higher EE, more controlled release and improved AA. The latter is enhanced by the antimicrobial properties of chitosan, specifically its natural positive charge which leads to binding with negatively charged bacterial cell components, and their low pH (<6) which disrupts bacterial biofilms. On the other hand, liposomes had smaller particle sizes, better size distribution and an easier production method, but exhibited lower EE, faster release and lower AA compared to chitosan nanoparticles.

Conclusions: Nanocarriers can effectively encapsulate OEE, control its release and overall enhance its antimicrobial activity thus providing a high-potential alternative to conventional antibiotics. However, in vivo studies in broiler chickens are planned to take place to validate the results of in vitro experiments.

Biopharmaceutical properties of mucoadhesive powder platform for donepezil nasal delivery

Mirna Perkušić[1], Laura Nižić Nodilo[1*], Cvijeta Jakobušić Brala[2], Ivan Pepić[1], Jasmina Lovrić[1], Anita Hafner[1]

[1]*University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Pharmaceutical Technology, Zagreb, Croatia*

[2]*University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Physical Chemistry, Zagreb, Croatia*

** lnizic@pharma.hr*

Background: Donepezil hydrochloride (DH) is commonly used for the treatment of symptoms of Alzheimer's disease as an oral dosage form. Nasal DH administration provides the potential for direct and efficient DH delivery to the central nervous system while reducing its systemic side effects.

Objectives: This study aims to investigate the potential of spray-dried DH-loaded chitosan/mannitol microspheres for nasal DH delivery by relevant biopharmaceutical *in vitro* and *ex vivo* characterisation.

Methods: DH-loaded chitosan/mannitol microspheres were characterised in terms of DH *In vitro* release profile, *ex vivo* muco-adhesiveness using porcine nasal mucosa, *in vitro* biocompatibility with Calu-3 cells and DH permeability across Calu-3 cell monolayer. DH-loaded chitosan microspheres, pure DH powder and/or pure mannitol were used as controls where appropriate.

Results: Both the chitosan and chitosan/mannitol microspheres provided prolonged DH release in comparison to the pure DH powder. DH-loaded chitosan/mannitol microspheres showed prominent mucoadhesive properties. Calu-3 cells exposed to microsphere suspensions prepared at a chitosan concentration of 10–30 µg/mL retained viability above 90% same as the negative control. The Papp values of DH from chitosan/mannitol and chitosan microspheres showed 1.16 and 1.30-fold higher DH permeation in comparison to the DH solution, respectively.

Conclusions: Formulating DH as chitosan/mannitol microspheres resulted in prolonged drug release, increased muco-adhesiveness and enhanced drug permeation across the Calu-3 monolayer. Results on *in vitro* biocompatibility confirmed the formulation potential for safe and efficient DH nasal delivery.

Development of sprayable *in situ* gelling system for nasal donepezil delivery: Preliminary formulation and deposition studies

Mirna Perkusic[1*], Laura Nižić Nodilo[1], Cvijeta Jakobušić Brala[2], Ivan Pepić[1], Vesna Saršon[3], Maša Safundžić Kučuk[3], Dijana Zadravec[4], Livije Kalogjera[5], Anita Hafner[1]

[1]*University of Zagreb Faculty of Pharmacy and Biochemistry, Department of Pharmaceutical Technology, Zagreb, Croatia*

[2]*University of Zagreb Faculty of Pharmacy and Biochemistry, Department of Physical Chemistry, Zagreb, Croatia*

[3]*Jadran-galenski laboratorij d.d., Zagreb, Croatia*

[4]*Department of Diagnostic and Interventional Radiology, Sestre milosrdnice University Hospital Center, University of Zagreb, Zagreb, Croatia*

[5]*ENT Department, Zagreb School of Medicine; Sestre milosrdnice University Hospital Center, Zagreb, Croatia*

** mperkusic@pharma.hr*

Background: Due to the disadvantages of oral administration of the anti-dementia drug donepezil, nose-to-brain delivery attracts much attention as a more efficient and safe route of administration. Nasal drug administration enables delivery to the central nervous system via the olfactory nerve that innervates the olfactory region of the nasal cavity. To ensure targeted delivery to the olfactory region, nasal deposition studies should be implemented in the early phase of formulation development.

Objectives: The purpose of this study is to set the basis for the development of a sprayable *in situ*-gelling donepezil delivery system and to recognise the formulation and administration parameters with the highest impact on nasal deposition pattern.

Methods: Thermosensitive chitosan (CS) based formulations for donepezil nose-to-brain delivery were prepared using CS and β-glycerophosphate as the gelling agent. *In situ* gelling systems were characterised in terms of rheological properties, droplet size distribution (DSD), spray cone angle (SCA) and nasal deposition pattern by using a representative 3D-printed nasal cast.

Results: Thermosensitive CS based in situ gelling donepezil formulations have been successfully prepared. Formulations showed suitable rheological characteristics and thermo-gelling properties. Under the formulation and administration parameters employed, the appropriate window of DSD and SCA was reached. This resulted in a targeted deposition within the olfactory region.

Conclusions: Preliminary studies revealed crucial formulation and administration parameters that resulted in suitable

rheological properties and aerosol performance that paved the way for targeted nose-to-brain delivery.

Physicochemical stability of allopurinol, amitriptyline hydrochloride, amlodipine besylate, clindamycin hydrochloride, hydrocortisone, metronidazole, naltrexone hydrochloride, spironolactone, trimethoprim with sulfadiazine, and ursodiol extemporaneously compounded oral suspensions in suspendit base

Yashoda Pramar[1*], Richard Graves[1], Tarun Mandal[1], Levon Bostanian[1], Maria Carvalho[2]

[1]College of Pharmacy, Xavier University of Louisiana, New Orleans, United States

[2]PCCA Science, Professional Compounding Centers of America, Houston, United States

** yvpramar@xula.edu*

Background: Extemporaneously-compounded oral suspensions are commonly prepared to meet the individual needs of pediatric and geriatric patients. However, their formulation and stability are usually complex.

Objectives: Ten physicochemical stability studies were undertaken to determine the beyond-use-dates of the following oral suspensions: allopurinol 10 and 20 mg/mL; amitriptyline hydrochloride 1 and 5 mg/mL; amlodipine besylate 0.5 and 10 mg/mL; clindamycin hydrochloride 10 mg/mL; hydrocortisone 1 and 20 mg/mL; metronidazole 25 and 50 mg/mL; naltrexone hydrochloride 0.5 and 5 mg/mL; spironolactone 5 mg/mL; trimethoprim 20 mg/mL with sulfadiazine 100 mg/mL; and ursodiol 50 and 100 mg/mL. Microbiological stability studies were also undertaken for amitriptyline, hydrocortisone, and metronidazole. A contemporary suspending vehicle PCCA Base, SuspendIt™ was used for its organoleptic and thixotropic properties.

Methods: Samples were prepared and stored at two temperatures (5°C and 25°C) for the allopurinol, amitriptyline, amlodipine, hydrocortisone, metronidazole, naltrexone and ursodiol oral suspensions; and three temperatures (5°C, 25°C and 40°C) for the clindamycin, spironolactone, and trimethoprim with sulfadiazine oral suspensions. Chemical stability was tested and validated using high-performance liquid chromatographic assay at baseline, and subsequently at eight pre-determined time points. The physical stability was tested by monitoring the pH, viscosity and appearance of the suspensions over a period of six months.

Results: Most of the oral suspensions retained at least 90% of the initial concentration and underwent no significant physical changes.

Conclusions: An extended beyond-use-date of six months may be assigned to the allopurinol, amitriptyline, clindamycin, hydrocortisone, metronidazole, naltrexone, spironolactone, trimethoprim with sulfadiazine, and ursodiol oral suspensions in PCCA Base, SuspendIt when stored in the refrigerator or at room temperature. The amlodipine oral suspension should be stored in the refrigerator for a beyond-use date of three months.

Plasma-mediated covalent bonding of rhamnolipids onto pdms surface as a strategy to overcome vascular catheter-related infections

Dardouri Maissa[1], Ana Bettencourt[1], Victor Martin[2,3], Filomena A. Carvalho[4], Catarina Santos[5,6], Nuno C. Santos[4], Maria Fernandes[2,3], Pedro Gomes[2,3], Isabel Ribeiro[1*]

[1]Research institute for Medicines, iMed.Ulisboa, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

[2]BoneLab - Laboratory for Bone Metabolism and Regeneration – Faculty of Dental Medicine, U. Porto Rua Dr. Manuel Pereira da Silva, 4200-393 Porto, Portugal

[3]LAQV/REQUIMTE, U. Porto, Porto, 4160-007, Portugal

[4]Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Av. Prof. Egas Moniz, 1649-028 Lisbon, Portugal

[5]CQE Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1049-001, Lisboa, Portugal

[6]EST Setúbal, CDP2T, Instituto Politécnico de Setúbal, Campus IPS, 2910 Setúbal, Portugal

** iribeiro@ff.ulisboa.pt*

Background: Fighting bacterial biofilm formation on Poly(dimethylsiloxane) (PDMS)-based bloodstream catheters are of major concern to reduce the occurrence of related infections.

Objectives: In this study, rhamnolipids (RLs), glycolipid biosurfactants, namely an RLs mixture and purified di-RL (RhaRhaC10:0C10:0), were covalently bonded onto PDMS to penetrate active antibiofilm surfaces.

Methods: An Ultra High Performance Liquid Chromatography method couplet to Mass Spectrometry (UHPLC-MS) confirmed RLs presence as well as compound isolation after automated flash chromatography. PDMS surfaces underwent air-plasma treatment and carbodiimide reaction for RLs grafting, which was confirmed by the contact angle, Fourier Transform Infrared-Attenuated Total Reflection (FTIR-ATR)

and Atomic Force Microscopy (AFM) measurements. The antibiofilm activity towards different Gram-positive strains was evaluated by colony forming units (CFU) count and confocal laser scan microscopy. The biocompatibility of the materials was also evaluated.

Results: RLs were successfully bound onto PDMS and RLs mixture, and RhaRhaC10C10:0 functionalised specimens reduced biofilm formation over 2.3 log units against methicillin-sensitive *Staphylococcus aureus*. Moreover, a decrease of 1 log unit was observed against *S. epidermidis* and methicillin-resistant *Staphylococcus aureus*. Functionalised samples revealed cytocompatibility towards human dermal fibroblasts. No vascular irritation potential and hemocompatibility were observed.

Conclusions: The results revealed a synergy between the antimicrobial and the anti-adhesive properties of RLs, making these compounds good candidates for the improvement of the medical devices' antibiofilm properties.

The emergent need for novel antimicrobial strategies: can biosurfactants play that role?

Maissa Dardouri[1], Rita Mendes[1], Cristiana Pontes[1], Filomena Carvalho[2], Victor Martin[3,4], Catarina Santos[5,6], Nuno Santos[2], Maria Fernandes[3,4], Lidia Gonçalves[1], Ana Bettencourt[1], Pedro Gomes[3,4], Isabel Ribeiro[1*]

[1]Research Institute for Medicines, iMed.Ulisboa, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

[2]Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Av. Prof. Egas Moniz, 1649-028 Lisbon, Portugal.

[3]BoneLab - Laboratory for Bone Metabolism and Regeneration – Faculty of Dental Medicine, U. Porto Rua Dr. Manuel Pereira da Silva, 4200-393 Porto, Portugal.

[4]LAQV/REQUIMTE, U. Porto, Porto, 4160-007, Portugal.

[5]CQE Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1049-001, Lisboa, Portugal.

[6]EST Setúbal, CDP2T, Instituto Politécnico de Setúbal, Campus IPS, 2910 Setúbal, Portugal.

** iribeiro@ff.ulisboa.pt*

Background: Due to the increased antibiotic resistance and their reduced efficiency in penetrating biofilms, innovative preventive strategies are mandatory to block and control infections. Among those are medical device-related infections. In this context, finding solutions to improve the antimicrobial activity of biomaterials surfaces is a requirement.

Objectives: The herein-described work aimed to study different approaches that use antimicrobial biosurfactants towards the reduction of medical catheter bacterial colonisation. Medical grade poly-di-methylsiloxane (PDMS), a common material used in catheter production, was selected as an abiotic surface and subjected to different coatings/modifications to test their antibiofilm activity. Among the approaches applied were release strategies namely the release of sophorolipids (SLs) previously adsorbed onto the PDMS surface and rhamnolipids (RLs)-nanoparticulate systems. Moreover, contact kill release strategies through the covalent bond of RLs or SLs onto PDMS surfaces following different methodologies, were also evaluated.

Methods: The SLs mixture was produced by *Starmerella bombicola* and recovered by liquid-liquid extraction. Both SLs and (purchased) RLs mixtures were characterised by UHPLC-MS and purified by flash chromatography. Functionalisation of surfaces occurred either by biosurfactant adsorption or covalent bonding through the carbodiimide reaction in a PDMS surface previously activated by plasma or oxidation. Functionalised surfaces/materials were characterised through different methodologies, namely FTIR-ATR, contact angle, SEM, AFM and XPS. Antimicrobial/antibiofilm evaluation was performed by colony forming units count, crystal violet assay, CLSM and SEM imaging. Biocompatibility studies involved cytocompatibility evaluation towards suitable cell lines, and assays regarding hemocompatibility and irritation assessment were also performed.

Results: All systems tested revealed antimicrobial- and antibiofilm-activity-reducing biofilm formation on medical-grade PDMS. The covalent bonding of RLs-PDMS contact kill/ anti-adhesive approach was also active towards dual-species biofilms. With a free compound release strategy, a higher reduction in biofilm formation may be achieved. However, with a contact kill approach the anti-adhesive/ kill activity will be present for a longer time. Moreover, all the obtained functionalised systems were biocompatible under the tested conditions.

Conclusions: Overall, the use of biosurfactants towards PDMS surfaces improved antimicrobial activity with the tested approaches. The gathering of all the results made it possible to increase knowledge on the use of biosurfactants in catheter-related infection prevention, and within this context, their usage may become an option regarding the reduction of medical device-related infections.

Development of ibuprofen amorphous solid dispersion by hot melt impregnation process using design of experiments approach

Wiesław Sawicki[1*], Kamil Garbera[2]

[1]*Deparment of Physical Chemistry, Medical University of Gdańsk, Gdańsk, Poland*

[2]*Tarchomin Pharmaceutical Works "Polfa" S.A., Warsaw, Poland*

** wieslaw.sawicki@gumed.edu.pl*

Background: Amorphisation in most cases should increase the dissolution rate and enhance the performance of the drug. Hot melt impregnation (HMI) is a process of applying heat and mechanically created stress, to a mixture of processed raw materials and forcing it through a barrel in a form of powder.

Objectives: The study aimed to optimise the hot melt impregnation (HMI) process of ibuprofen to assure the possibly highest amorphisation rate.

Methods: The "*design of experiments*" (DoE) has been employed to prepare the experimental plan. The feeding rate, screw speed and ibuprofen content were chosen as the input variables. Each variable was tested on three levels (-1, 0, 1). For each combination in the design, ibuprofen crystallinity level was evaluated as an output variable. Optimal ranges of the input factors were established by Response Surface Methodology. In the given application, the blended raw material (a mixture of ibuprofen and neusilin) was transported through the extruder's barrel by co-rotating twin screws. The temperature applied to the barrel heated the raw material which melted substances with low-melting-point. The melted substances penetrated the open neusilin pores. The capillary effect is considered a driving force of this phenomenon. As a product of the described process, a fine free-flowing extrudate was obtained. Due to the different physical forms of the extrudate, the name "*hot melt impregnation*", instead of "*extrusion*" was adopted for this application. Differential scanning calorimetry (DSC) was used to carry out DSC runs of the pure active substance, excipients, physical mixtures and extrudates. About 2–5 mg of the sample was placed in a sealed aluminium pan with a pierced lid. All of the examined samples were heated at 10°C/min from 30°C to 200°C under a dry nitrogen atmosphere (nitrogen flow 30 ml/min). Observed thermal effects were quantified. For DoE and statistical analysis, MODDE Pro 12 software was used. Optimisation of the hot melt impregnation process for the development of amorphous ibuprofen solid dispersion was the aim of the design. The tested factors were feeding rate, screw speed and ibuprofen content

Results: All prepared extrudates were white to off-white free-flowing powders. From the presented result, an increase in screw speed caused the remaining crystalline ibuprofen to increase in size. The reduction of crystalline fraction in the sample was promoted by the elongation of residual time.

Conclusions: Hot melt impregnation process has been successfully optimised using the DoE approach. It has been proven that screw speed and ibuprofen content have a significant impact on the amorphisation rate. These findings will improve process yield, shorten process time and maximise predefined amorphisation rate.

The feasibility study of nanoparticles derived from *Glycyrrhizae radix* as a vaccine adjuvant for cancer immunotherapy

Yuno Suzuki[1*], Lisa Munakata[1], Daiki Omata[1], Akiko Inujima[2], Keiichi Koizumi[2], Ryo Suzuki[1,3]

[1]*Faculty of Pharma-Science, Teikyo University, Tokyo, 173-8605, Japan*

[2]*Institute of Natural Medicine, University of Toyama, Toyama, Japan*

[3]*Advanced Comprehensive Research Organisation, Teikyo University, Japan*

** marina.suzuki@pacificu.edu*

Background: Previously, we have reported that herbal medicines contain a lot of nanoparticles. In particular, we showed that Glycyrrhiza nanoparticles (Glycyrrhiza NP) derived from *Glycyrrhizae radix*, one of the major herbal medicines induced the activation of macrophages and the production of inflammatory cytokines such as IL-6 and TNF-α via NF-κB activation. Therefore, Glycyrrhiza NP would be applied to vaccine adjuvants.

Objectives: In this study, to evaluate the ability of Glycyrrhiza NP as vaccine adjuvants in cancer immunotherapy, we examined the effects of Glycyrrhiza NP on the maturation of dendritic cells, which are major antigen-presenting cells, and the immune response in vivo. In addition, we assessed the feasibility of Glycyrrhiza NP as a vaccine adjuvant for cancer immunotherapy.

Methods: The extracts of *Glycyrrhizae radix* were centrifuged, and then Glycyrrhiza NP was obtained. The average diameter of Glycyrrhiza NP was 158.5 nm. Glycyrrhiza NP was incubated with a murine dendritic cell line (DC2.4 cells) for 24 hr. After incubation, the expression of maturation markers (CD40, CD80 and CD86) on the surface of DC2.4 was measured. Mice (C57BL/6J, female, six weeks old) were subcutaneously injected with Ovalbumin (OVA) alone or the mixture of OVA and Glycyrrhiza NP on days -14 and -7. On day zero, splenocytes were collected from the mice. The splenocytes (2×10^6 cells/well) were incubated with the H-2Kb epitope peptide of OVA (SIINFEKL: SL8) for three days. After that, the concentration of IFN-γ in the supernatant of

splenocytes was measured by ELISA. In the experiment of antitumor effect, on day zero, an OVA-expressing murine lymphoma cell line (E.G7-OVA cells, 1 × 10^6 cells) was intradermally inoculated into the back flank of mice, and the tumour size was measured.

Results: The expression of maturation markers on DC2.4 cells was significantly increased by Glycyrrhiza NP. This suggests that Glycyrrhiza NP has an immune-stimulatory activity to induce dendritic cell maturation. Moreover, we evaluated the T cell responses after immunisation with OVA and Glycyrrhiza NP. When the splenocytes were stimulated with SL8, the production of IFN-γ in mice immunised with OVA and Glycyrrhiza NP was significantly higher compared with that with OVA alone. This result showed that Glycyrrhiza NP induced antigen-specific CD8+ T-cell responses. Next, we examined the induction of antitumor immunity by Glycyrrhiza NP. The immunisation with OVA and Glycyrrhiza NP completely rejected E.G7-OVA cells. This result suggested that Glycyrrhiza NP effectively induced antitumor immunity.

Conclusions: Glycyrrhiza NP showed anti-tumour effects by effectively inducing dendritic cell maturation and antigen-specific cellular immunity. Therefore, Glycyrrhiza NP could be an effective vaccine adjuvant for cancer immunotherapy.

Development and evaluation of an innovative approach using niosomes-based dissolving microneedles to deliver dual antioxidant drugs

Ahlam Zaid Alkilani*, Hadeel Abu Zour

Zarqa University, Zarqa, Jordan

** ahlamk@zu.edu.jo*

Background: Microneedles (MN) are minimally invasive, pain-free, micron-sized projection arrays, capable of piercing the skin's stratum corneum, overcoming its barrier properties to create a transport pathway for drug molecules. Niosomes are a type of vesicular nanocarrier exploited for enhancing the therapeutic efficacy of various drugs in clinical practice.

Objectives: The present study aimed to prepare niosomal formulations loaded with dual hydrophilic drugs; Ascorbic acid (AA) and Caffeine (CAFF) by thin film method then loading them into dissolving MN for dermal delivery.

Methods: High-performance liquid chromatography (HPLC) was used and validated according to the ICH guideline. AA and CAFF niosomes were prepared using the thin film method. The obtained niosomes were characterised by particle size, polydispersity index (PDI), zeta potential (ZP), drug encapsulation efficiency (EE), and *in vitro* release. Nine formulations were prepared to screen the effect of formulation and processing variables on niosomal characteristics. Different niosomal formulations were prepared using span and tween mixed with cholesterol.

Results: The S4 and S7 niosomal formulations showed the highest EE% of AA and CAFF (61.98±0.19%, 59.71±0.09%), (70.89±0.42%, 67.07±0.30%) respectively. The sizes of the niosomes were between (130.6-411.2)nm and ZP was around -35 mV. The formulations manufactured using the optimised composition S4, S7 and S9 proved to be stable for 2 months. The drug release study showed that 46.83± 6.43% and 70.71± 4.98% of AA and CAFF respectively were released from the S7 noisome and 54.94± 4.57%, 70.26± 9.45% from S4 in 5 hours. The formulations S4 and S7 were loaded to 60%PVP with 5%PEG 400 (H5) and 60%PVP with 7%PEG 400 (H6) dissolving microneedles. The cumulative amount of AA and CAFF respectively (Q) for different formulations was in the order as follows: M4 (PVP containing 7% PEG 400) > M2 (PVP containing 7% PEG 400) > M3 (PVP containing 5% PEG 400) > M1 (PVP containing 5% PEG 400). All formulations in this study were stable at room temperature for over two months, in terms of moisture content and percentage release.

Conclusions: In this study, we described a novel dissolving MN array, fabricated and loaded with AA and CAFF under simple conditions at room temperature without using any special conditions. A dermal delivery of AA and CAFF noisome loaded in dissolving MN was successfully prepared to provide a controlled release of AA and CAFF for five hours.

Doxorubicin-containing gold nanoparticles-anchored liposomes as delivery carriers: a quality-by-design strategy to optimise the surface functionalisation

Jazmín Torres[1,2,3], Francisco Prieto-Dapena[4], Marcela Longhi[1,2], Antonio M Rabasco[3*], Mónica García[1,2], María Luisa González-Rodríguez[3]

[1]Universidad Nacional de Córdoba. Facultad de Ciencias Químicas. Departamento de Ciencias Farmacéuticas. Ciudad Universitaria, Córdoba, Argentina

[2]Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET. Unidad de Investigación y Desarrollo en Tecnología Farmacéutica, UNITEFA. Ciudad Universitaria, Córdoba, Argentina

[3]Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Sevilla, Sevilla, Spain

[4]Departamento de Química Física, Facultad de Química, Universidad de Sevilla, Sevilla, Spain

** amra@us.es*

Background: Liposomes (Lip) exhibit high biocompatibility and are attractive carrier systems for delivering anticancer drugs. They can be functionalised with gold nanoparticles (AuNPs) and are useful in cancer nanomedicine for diagnosis

and treatment. Thermosensitive Lip constitutes an interesting alternative as a nanosystem capable of responding to thermal stimuli. A thermo-responsive lip can be obtained by using temperature-sensitive phospholipids as well as anchoring their surface with AuNPs because of their light-induced heating response. Surface functionalisation of Lip with AuNPs can affect their interfacial properties and their ability to carry and deliver the payload.

Objectives: This study aimed to apply a quality-by-design (QbD) strategy to rationalise the experimental design to anchor doxorubicin (Dox)-loaded Lip with AuNPs and evaluate their performance as carrier systems.

Methods: Thin-film hydration and transmembrane pH-gradient methods were used to prepare the Lip and for the Dox-loading, respectively. Selected variables: Lip-Dox: AuNPs ratio (3:8, 8:8 and 8:3 (v/v)), stirring time (1 min, 2 min and 3 min), temperature (4°C, 25°C and 42°C) and time of anchoring (0 h, 24 h and 48 h post-functionalisation) were subjected to experimental verification through variable-response correlation, using a Taguchi matrix design. Interfacial properties: hydrodynamic diameter (dH), polydispersity index (PDI) and electrokinetic potential (Z); and Dox loading efficiency (EE %) were evaluated. The data obtained were mathematically and statistically analysed, using the DOEpack 2000 software. Variables that presented adequate levels of statistical significance (p <0.001) were considered critically. Drug release studies toward different media (pH 7.4 and 5.1 at physiological and hyperthermal temperatures) were carried out for optimised liposomal dispersions.

Results: Non-functionalised AuNPs Dox-loaded Lip exhibited nano-scale sizes (355 nm), acceptable PDI values (< 0.46), and positive Z-potential (14 mV, after drug loading) because of the presence of di-methyl-di-octadecyl-ammonium bromide in the bilayer. This cationic surface of Lip allowed the anchoring with the negatively charged AuNPs by electrostatic interactions. Lip-Dox: AuNP ratio, stirring time and temperature significantly affected the interfacial properties of AuNP-Lip-Dox. Nanometric sizes (516 nm), acceptable PDI values (<0.4), positive Z-potential (9.85 mV) and EE % equal to (80% ± 2) were only achieved at 8:3 Lip-Dox:AuNPs ratio, 3 min of stirring time and 42°C. When the dH of the empty Lip (287 nm), Dox-loaded Lip and AuNPs-Lip-Dox were compared, it was observed that the higher the system complexities, the higher the sizes. Although the anchoring can destabilise the Lip bilayer, the Dox remained well-encapsulated, and no leakage was observed after functionalisation ((78% ± 2) and (80% ± 2) for Lip-Dox and AuNPs-Lip-Dox, respectively). The release of Dox toward both receptor media was controlled, and as expected, its release was triggered under hyperthermia conditions (light-induced heating), confirming the thermo-responsive behaviour of liposomal dispersions. The acid medium also promoted the Dox release, thus, it showed the AuNPs-Lip-Dox had a pH-sensitive responsiveness.

Conclusions: The QbD was a useful strategy to evaluate different parameters for surface functionalisation of Lip with AuNPs which can consequently affect their performances. Optimised conditions for the anchoring process were achieved, allowing the preparation of liposomal dispersions with thermos-responsiveness that exhibit promising properties for cancer nanomedicine and hyperthermia therapy.