Nordic POP Annual meeting 2020
2nd Nordic POP Annual meeting

Copenhagen, Denmark

7 – 9 January 2020

Conference Venue

Mærsk Tower

Faculty of Health and Medical Sciences,

University of Copenhagen

Blegdamsvej 3B

2200 Copenhagen N

Denmark

NORDIC POP

KØBENHAVNS UNIVERSITET
DET SUNDHEDSVIDENSKABELIGE
FAKULTET

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Table of abbreviations

AAU	Åbo Akademi University
CTH	Chalmers University of Technology
DTU	Technical University of Denmark
MUG	University of Gdansk
SDU	University of Southern Denmark
UCPH	University of Copenhagen
UEF	University of Eastern Finland
UHEL	University of Helsinki
UI	University of Iceland
UiO	University of Oslo
UiT	UiT The Arctic University of Norway
Uni Kiel	University of Kiel
UPP	Uppsala University
UT	University of Tartu

About Nordic POP

Nordic POP (patient oriented products) is a Nordic university consortium coordinated by the University of Copenhagen. It aims to provide the scientific foundation for the next generation pharmaceutical products - patient oriented products- by strengthening the use of interdisciplinary approaches within Nordic pharmaceutical sciences.

Prescribing medicine today is based on a One Size Fits All Principle. However, there is a strong need for more personalized solutions in several critical therapy areas. Recent development within genomics and diagnostic field have enabled development of new innovative medicinal products relying on a combination of diagnostic tools and personalized dose, paving the way towards future health care system based on personalized medicines, where a new type of treatment strategies, taking into account the individual variations between patients, can be designed.

Nordic POP aims to deliver innovative patient oriented products with the use of new product design principles, novel methods for understanding of the product performance, and a paradigm shift in the manufacturing of these products

We believe, that this could be reached by the the intensive use of interdisciplinary approaches within Nordic pharmaceutical sciences. Our strategy is, thus, based on working together and sharing the existing research infrastructures and expertise and on increasing mobility actions including senior scientific staff mobility and joint PhD programs between different participating units.

The key elements that Nordic POP will focus on are:

- Facilitating the mobility of PhD students and senior staff in the consortium; Publishing of the research findings in high impact scientific journals;
- Securing innovation and entrepreneurship within the network;
- Mentoring younger scientists and supporting the European Research Council (ERC) applications;
- Assuring industrial dissemination and safeguarding the strong global position of Nordic pharmaceutical companies;
- Positioning this network in an leading role in Europe within pharmaceutical sciences.

Nordic POP is an unique example of collaboration involving 10 Nordic partner universities and 5 affiliated Baltic universities.

Plenary program Nordic POP Annual Meeting 2020

Tuesday 7th January 2020

12:30- 13:00	**Registration, mounting of posters** Mærsk tower, 1st floor	
13:00 13:15	**Welcome and introduction** **Jukka Rantanen and Ragna Berthelsen, UCPH** *Niels Jerne auditorium*	
13:15- 14:00	**Plenary lecture:** Is the future going to happen? **Maja Horst,** Professor in Responsible Technology, DTU **Flemming Madsen**, Head of Department of Pharmacy, UCPH *Niels Jerne auditorium*	
14:00- 14:30	**WP1 (Barriers):** *In vitro* artificial permeability assays: a guide for users **Massimiliano Pio Di Cagno, UiO** *Niels Jerne Auditorium*	
14:30- 15:00	**WP2 (Product):** "Acoustic monitoring of an inhalation device" **Johan P. Bøtker, UCPH** *Niels Jerne Auditorium*	
15:00	**Afternoon break and poster presentation**	
16.00- 16.30	**WPX (upcoming): "**Patient perspectives on drug formulations" **Anna Birna, University of Copenhagen** *Niels Jerne Auditorium*	
16:40- 18:00	**WP1 student talks** **Chair: Carsten U. Nielsen, SDU** *Nielsine Nielsen Auditorium* **1. Oliver Hedge, UPP,** **1. Ann-Christin Jacobsen, SDU** **2. Margherita Falavigna, UiT** **3. Juri Timonen, UEF**	**WP2 student talks** **Chair: Gøril E. Flaten, UiT** *Niels Jerne Auditorium* **1. Henrik Palmelund, UCPH** **2. Xiaona Liu, UCPH** **3. Sybil Akua Okyerewa Obuobi, UiT** **4. Heidi Öblom, ÅBO**
18.00	**Opening Reception** Mærsk Tower, 15th floor lounge area	

Wednesday 8th January 2020

8.50-9.00	**Brief introduction day 2** **Andrea Heinz, UCPH** *Niels Jerne Auditorium*	
9.00-9.30	**WP 3 (Engineer):** "Printing technology for personalized dosing" **Mirja Palo, AAU** *Niels Jerne Auditorium*	
9.30-10.00	**WP 4 (Analytics):** „Surface crystallinity: detection, significance and control“ **Clare Strachan, UHEL** *Niels Jerne Auditorium*	
10.00	**Morning break and poster presentation**	
10:40 – 12:00	**WP3 student talks** **Chair: Leena Peltonen, UHEL** *Niels Jerne Auditorium* **1. Simon Bock, KIEL** **2. Mikkel Herzberg, UCPH** **3. Kuldeep kumar Bansal, UiO** **4. Anne Linnet Skelbæk-Pedersen, UCPH**	**WP 4 student talks** **Chair: Tiina Sikanen, UHEL** *Nielsine Nielsen Auditorium* **1. Jacob Bannow, UCPH** **2. Jacopo Zini, HEL** **3. Joseph Diab, UiT** **4. Mette Sloth Bohsen, SDU**
12:00 - 13:00	**Lunch**	
13:00 - 13:30	**Poster presentations**	

13.30 - 14.30	**Nordic POP status and WP session introduction** **Jukka Rantanen, UCPH** *Niels Jerne Auditorium*			
14:30 – 15:30	**WP 1 Meeting** *Niels Jerne Auditorium*	**WP 3 Meeting** *Nielsine Nielsen Auditorium*	**WP 5 Meeting** *room 13.1.41 /13.1.61*	Young Science excursion to Department of Pharmacy, UCPH (first year PhD students)
15:30 – 15:45	**Coffee break**			
15:45 – 16:45	**WP 2 Meeting** *Niels Jerne Auditorium*	**WP 4 Meeting** *Nielsine Nielsen Auditorium*	**WP 6 Meeting** *room 13.1.41 /13.1.61*	
18:30 – 23:00	**Conference dinner** *KUs festsal, Frue Plads 4, DK-1017 Kbh K*			

Thursday 9th January 2020

8.50-9.00	**Brief introduction day 3** **Johan Bøtker, UCPH** *Niels Jerne Auditorium*	
9.00-9.30	**WP 5 (Synchroton):** "An insight into small- and wide- angle X-ray imaging" **Barbara Berke, CTH** *Niels Jerne Auditorium*	
9.30-10.00	**WP 6 (Modelling and simulation):** "Physiologically based modeling - What can it do for you as a formulation scientist?" **Erik Sjögren, UPP** *Niels Jerne Auditorium*	
10.00-10.40	**Morning break and poster presentation**	
10:40 – 12:00	**WP 2/5 student talks** **Chair: Anders Ø. Madsen, UCPH** *Niels Jerne Auditorium* **1. Tobias Palle Holm, UCPH** **2. Nele-Johanna Hempel, UCPH** **3. Giulia Scapin, HEL** **4. Alma Akhmetova, UCPH**	**WP 6 student talks** **Chair: Christel Bergstörm, UPP** *Nielsine Nielsen Auditorium* **1. Aleksei Kabedev, UPP** **2. Patrick Sinko, UPP** **3. Shakhawath Hossain, UPP** **4. Troels Pedersen, UCPH**
12.00-12.20	**Voting break**	
12:20 – 13.00	**Plenary updates, awards and closing** **Jukka Rantenen and the Nordic POP 2020 organizing committee** *Niels Jerne Auditorium*	
12:40	**Lunch (grab and go) and departure**	

Keynote lecture

Maja Horst and Flemming Madsen: Is the future going to happen?

Flemming Madsen has extensive academic knowledge and experience combined with a broad technological and business-oriented understanding within life science and pharmaceutical science based on about 30 years of experience in academia and industry. He graduated from the Royal Danish School of Pharmacy and became PhD in pharmaceutical Physical Chemistry 1995. In 1998, he was appointed associate professor at the same institution. Left the academic world in 2000 to work in industry for Coloplast A/S, where he was engaged as a polymer senior scientist. For the latter part of that career, he worked on corporate venture funding and technological incubation as Head of Medical Delivery Systems. In 2010, Flemming returned to the University of Copenhagen as Head of Department of Pharmacy. A position he still holds, where he focuses on leading people who are smarter than himself. His greatest motivation comes from trying to facilitate the creation of the greatest possible impact of research and teaching within the field of pharmaceutical science.

Maja Horst is Professor of Responsible Technology at the Technical University of Denmark and Professor of Science Communication at University of Copenhagen. Her research is focused on responsible research and innovation, public engagement with science, management and communication of research. She has published widely, including the 2016 book *Science Communication: Culture, Identity and Citizenship* co-authored with Sarah R Davies. Maja Horst has also been experimenting with interactive science communication installations inviting citizens to discuss the social responsibility of emerging scientific fields. For this, she was awarded the Danish Science Minister's Communication Prize in 2009. She is also involved in policy advice, not least as member of the Danish Council for Research and Innovation Policy advising the Danish minister and parliament.

Is the future going to happen?

Yes – but…

Nordic POP is at the front edge of a technological push that promises to change health and medical care in the future. A technological push that is very much the core of what is also believed to be a new industrial revolution – "Industry 4.0". Futurists, technologists, politicians and quite a few others talk about "AI" as the saviour of humankind, of the "age of precision medicine" and of the convergence of "everything". A revolution driven by digitalization (AI and Big Data) and the convergence of various tech-resorts. A digital revolution that will make past versions of industry revolutions look like tiny waves in history, since this one is going to be an exponential jump into the future. We can hear that "AI" will not just challenge our democracy quietly – it will hit as an earthquake. We are told that therapeutic interventions of the future are individualised, accurate, wearable, sustainable, smart etc , and will change health care and human life. We are working on it ourselves. Intensively. Nevertheless, the future is a big "Perhaps". Fact is that when it comes to medicine we still live in the age of "imprecision medicine" and probably will for some time to come. Because reality brings about quite a few socio-regulatory-economic-ethical issues and considerations to take into account, which are not necessary technological "promoters". Sometimes the market, the futurists and the technologists also get it wrong.

Nevertheless, the times are a-changing. The future will happen. It is here already. However, there is more to the picture than meets the eye. The message here is that it might not hit us in the form of a "tsunami" as some advocate. Maybe more as bigger waves rolling against the shores of humankind. The plenary lecture tries to address some of the complications and issues that the technological push brings about, and why the exponential "jump" might be questionable. The lecture will also discuss the special role and responsibility of the universities in a responsible technological development, and hint at a unique opportunity for Nordic Universities to be the front-runners in this area. Provided that we manage a common agenda in the future. As is the case with Nordic POP.

ABSTRACTS

Workpackage 1: BARRIERS (Leader: Carsten Uhd Nielsen, SDU)

WP1 aims to design and apply *in vitro* barrier models resembling the variable barriers in the human body investigating barrier properties, transporters, solute carriers and enzymes. These barriers may be cell based mimicking epithelial or endothelial barriers. Or, they may be physico-chemical barriers estimating drug release, local concentrations or interactions with physiological constituents such as mucus or colloidal structures.

The design of these *in vitro* models will be based on physiological-based knowledge, computational design and simulation of flow patterns in restricted geometries, followed by the actual barrier investigations. In these models, the drug transfer across and within these barriers are characterized using diverse analytical tools, as well as high-resolution imaging and synchrotron-based methods.
Validity of these models will be preclinically tested by *in vivo* pharmacokinetic studies in animals. We aim to develop the future predictive barriers of pharmaceutical science.

Invited speaker talk:
Massimiliano Pio di Cagno, UiO: *In vitro* artificial permeability assays: a guide for users

Student talks:
Oliver Hedge, UPP,
Ann-Christin Jacobsen, SDU
Margherita Falavigna, UiT
Juri Timonen, UEF

Poster session:

1	Jonas Borregaard Eriksen, SDU
2	Ahmed Al-Ali,SDU
3	Jun Cai, UCPH
4	Astrid Subrizi, UEF
5	Leah Wright, UPP
6	Stephan Ta, SDU
7	Anna Slita, AAU
8	Rasmus Blaaholm Nielsen, SDU
9	Lisa Chinello, UHEL

In vitro artificial permeability assays: a guide for users

Massimiliano Pio di Cagno
Department of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Oslo

Brief CV
Dr. di Cagno is Associate Professor at the Department of Pharmacy of University of Oslo, section for Pharmaceutics and Social Pharmacy. His research interests vary quite a lot within the pharmaceutical field, and comprise solid state alterations to improve poor solubility of drugs, improve deliverability of drugs with nanomedicine and development of *in vitro* tools for drug permeability investigation (e.g. Permeapad®). Currently, he is working on new approaches for the parametrization of drug diffusivity in unstirred media.

Abstract
In vitro tools for drug permeability quantification (both cellular-based and artificial) are becoming more and more available and implemented at both academic and industrial level. They offer significant advantages in comparison to *ex vivo* and *in vivo* studies such as cost effectiveness, time effectiveness and ease of utilization. The aim of this presentation is to give a general overview of the most employed *in vitro* artificial methods for drug permeability investigations (e.g. PAMPA, PVPA, Permeapad®, AMI-system) developed and available, focusing on advantages and disadvantages of each methods. This presentation is intended to help the current (and future) users of these methods to correctly interpret empirical data obtained and to help them to maximize the experimental outcome, especially when enabling formulations are tested.

Alternative *in vitro* digestive agents improve *in vivo* relationship of lipolysis-permeation assay method with artificial membranes

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[2]The Swedish Drug Delivery Center, Department of Pharmacy, Uppsala University, Sweden

Introduction

Oral bioavailability of poorly water-soluble drugs can be improved through lipid-based formulation (LBF/SEDDS/LbDDS). In addition, food effects can be minimized through LBF, reducing the drug's susceptibility to the patient's gastrointestinal physiology. However, state-of-the-art *in vitro* methods such as the lipolysis assay show poor *in vitro in vivo* relationship (IVIVR) in many cases[1]. Novel *in vitro* methods are therefore required to reduce reliance on animal models. The lipolysis-permeation assay, which utilizes permeation across a cell monolayer in addition to lipolysis, is a promising method that has accurately predicted LBF ranking in several cases[2,3]. As cell culturing can be an expensive and time-consuming practice, use of artificial membranes could further benefit the development process of LBFs.

Aim

To explore the use of different digestive agents in lipolysis-permeation assays of LBFs, with artificial membranes based on lecithin in *n*-dodecane (LiDo) and PVDF filters.

Method

Representative LBFs of type IIIA-MC, IIIA-LC and IV were loaded with 80 mg/g fenofibrate and assayed in the ENA lipolysis-permeation system. Caco-2 cell monolayers as well as LiDo artificial membranes were used to separate the donor/lipolysis and receiver compartment. LBFs were dispersed in FaSSIF 10 minutes prior to addition of Novozyme 435 or porcine pancreatin. Samples were taken from the system over 70 minutes in total. Aqueous and solid phases of donor samples were separated *via* centrifugation and solubilized and precipitated amounts of fenofibrate was quantified using HPLC-UV. Mass transfer of fenofibrate across the membrane was also quantified by HPLC-UV.

Results

Significant differences were seen between formulations in the donor/lipolysis compartment in all cases, both in terms of solubilized (aq.) and precipitated fenofibrate (fig. 1). However, no difference between the formulations (in terms of fenofibrate mass transfer) could be observed with Novozyme 435. This ranking matches previously observed bioavailability rankings in a landrace pig model[4].

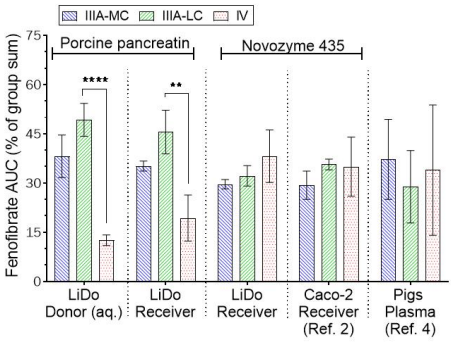

Figure 1 *Formulation ranking in different model systems. Data shown as area under the curve (AUC) of fenofibrate concentrations over time, normalized for comparison. Mean and standard*

Conclusion

Usage of Novozyme 435 instead of the commonly used porcine pancreatin as digestive agent in the lipolysis-permeation assay seems to improve the IVIVR when used with the LiDo artificial membrane.

Acknowledgement

This work was supported by European Research Council Grant 638965.

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Drug Permeability Profiling using the Permeapad® 96-well Plate

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[1] Drug Transport & Delivery Group, Department of Physics, Chemistry & Pharmacy, University of Southern Denmark,
Odense 5230, Denmark

Introduction

Commonly, permeability is determined using resource-intensive tissue or cell-based permeation models. Cell-free permeation models are generally accepted as cost and time effective alternatives for determining (passive) permeability (1). A recent addition to this tool-box is the phospholipid-based Permeapad® barrier (2). Using e.g. Franz-cells, this artificial barrier was found to be promising for determining passive permeability and particularly useful in early formulation development due to its high robustness against extreme pH values and aggressive additives (2, 3). Even though the usefulness of the Permeapad® barrier has been demonstrated, the limited throughput of devices such as Franz-cells hampers its use in drug discovery and early development. A novel 96-well plate format, the Permeapad® Plate, may resolve the challenge of limited throughput.

Aim

The aim of the study was to evaluate the Permeapad® Plate for passive permeability profiling. For this, the permeabilities of a set of drugs were determined and compared to published measures of oral absorption, such as human fraction absorbed (F_a) and in vitro permeability values obtained using other tools. Furthermore, the microscopic structure of the barrier was investigated.

Method

The Permeapad® Plate consists of a 96-well bottom and screen plate with the phospholipid-based barrier mounted between the plates' lower and upper compartments. The permeability of 14 model compounds including high- and low-absorption drugs, cationic, anionic, zwitterionic and neutral molecules, was determined by quantifying the compounds' transport over time, deriving the steady-state flux from the linear part of the cumulative curves and calculating apparent permeability (P_{app}). The obtained P_{app} values were compared to those obtained using: 1) the manually prepared Permeapad® in Franz-cells, 2) Caco-2 and 3) PAMPA. The membrane structure was investigated in a high-resolution digital light microscope.

Results

The Permeapad® Plate was found suited to distinguish high and low absorption drugs and yielded a hyperbolic correlation to F_a. Similar correlations have been shown for other permeation models such as the Caco-2 model (4). The P_{app} values obtained using the Permeapad® Plate were congruent with those determined using the manually prepared Permeapad® in a Franz-cell set-up. Furthermore, good to excellent correlations were seen with Caco-2 permeability (R^2=0.70) and PAMPA permeability (R^2=0.89). Microscopic investigation of the Permeapad® membrane revealed the development of phospholipid vesicles and myelin figures in aqueous environment.

Conclusion

The Permeapad® Plate is a promising tool for rapid and reproducible passive permeability profiling.

Acknowledgement

InnoMe GmbH, Espelkamp, Germany is acknowledged for providing prototype Permeapad® Plates and financial support towards a PhD-position for ACJ. Tina Christiansen is acknowledged for her excellent support with UHPLC-UV analyses.

References

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New combined *in vitro* lipolysis-permeation model predicts the *in vivo* absorption of fenofibrate from SNEDDS formulations

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[2]Department of Pharmacy, University of Copenhagen, Universitetsparken 2-4, 2100, Copenhagen, Denmark.

Introduction

The ability of lipid-based formulations, such as Self-Nanoemulsifying Drug Delivery Systems (SNEDDS), to increase the bioavailability of poorly water-soluble drugs after oral administration has received a lot of interest in the past decades. The lipid digestion in the gastro-intestinal environment influences the performance of SNEDDS, and it is of key importance to evaluate this[1]. *In vitro* lipolysis models have been extensively used to evaluate the impact of SNEDDS on drug solubilization during gastro-intestinal digestion[2]. However, the correlation between drug solubilization during *in vitro* lipolysis and *in vivo* absorption is not consistent, most likely due to the lack of an absorption step. For this reason, the aim of the present study was to combine *in vitro* lipolysis with the mucus-PVPA (Phospholipid Vesicle-based Permeation Assay) *in vitro* permeability model[3] to assess SNEDDS digestion and drug permeation simultaneously to improve the prediction of *in vivo* drug absorption.

Aim

To predict the *in vivo* absorption of fenofibrate from SNEDDS by combining *in vitro* lipolysis and *in vitro* permeation using the mucus-PVPA model.

Method: A SNEDDS (soybean oil 27.5 % w/w, Maisine 35-1 27.5 % w/w, Kolliphor RH-40 35 % w/w and absolute ethanol 10 % w/w) containing fenofibrate in different amounts (either at 75% or 150% of equilibrium solubility) was prepared according to a previously described method[4]; a super-SNEDDS solution$_{150}$, a super-SNEDDS suspension$_{150}$ and a SNEDDS$_{75}$ were produced. The three formulations were subjected to *in vitro* lipolysis (equal drug dose 0.48 mg/mL) while investigating their drug solubilization capacity. After 30 minutes the samples were added on top of the mucus-PVPA barriers to assess the permeation of fenofibrate. Biosimilar mucus[5] was added to the PVPA barriers to better mimic the intestinal mucosa.

Results

The ranking of the three SNEDDS, in terms of amount of drug solubilized in the aqueous phase after 30 minutes of lipolysis, was SNEDDS$_{75}$ > super-SNEDDS solution$_{150}$ > super-SNEDDS suspension$_{150}$, suggesting the potential ability of SNEDDS$_{75}$ to yield higher drug permeation. The super-SNEDDS solution$_{150}$ did, however, exhibit the highest fenofibrate *in vitro* permeation, a finding that correlated very well (Fig. 1; R^2= 0.99) with previous *in vivo* absorption data of fenofibrate in rats from the same SNEDDS[4].

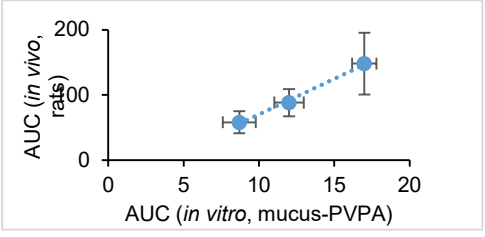

Fig. 1: *In-vivo-in-vitro correlation between in vivo AUC in rats[4] after administration of fenofibrate in SNEDDS and in vitro AUC using the mucus-PVPA barriers.*

Conclusion

The combination of *in vitro* lipolysis with the mucus-PVPA permeability model produced a remarkable correlation with *in vivo* plasma exposure of fenofibrate from SNEDDS.

Acknowledgement

The support of NordicPOP (project number: 85352), and COST Action UNGAP (project number: 16205) is greatly acknowledged.

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Boronated tryptophanes as boron carriers for BNCT treatment of malignant brain tumors

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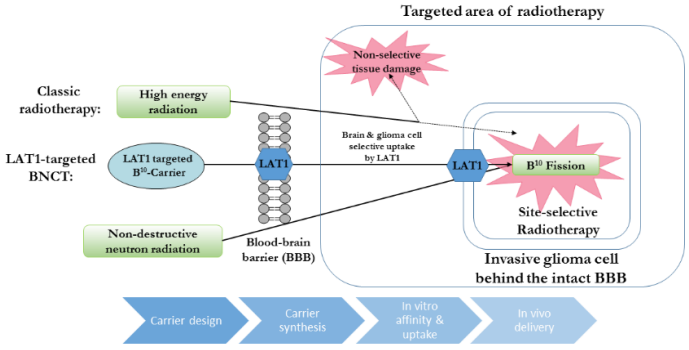
Introduction



Boron Neutron Capture Therapy (BNCT) is a unique type of radiation therapy that provides a way to selectively destroy malignant cells and spare normal cells. BNCT treats cancer with a non-invasive process. In BNCT, boron-10 (^{10}B) containing tumor seeking boron carrier (BC) is used to accumulate necessary amount of ^{10}B into cancer cells. Cancer cells are known to consume elevated amounts of amino acids and 4-borono phenylalanine (BPA) is one of the two clinically used BCs. L-type amino acid transporter subtype 1 has been shown to play a major role in the transport of BPA into tumor cells.[1,2]

Aim

To design and synthetize boron containing tryptophanes and to evaluate them as new BCs.

Methods

5-Borono tryptophane (5-BTrp) was synthetized starting from indole and glysine. Intermediates and the final product were purified using flash chromatography and High Performance Counter Current Chromatography (HPCCC) and identified by using MS, ^{1}H, ^{13}C and ^{11}B-NMR. Affinity to LAT1 was studied using a known radiolabeled LAT1-substrate [^{14}C]-L-leucine by incubating the LAT1 transfected HEK293 cells with [^{14}C]-L-leucine and studied BC. The radioactivity was measured by liquid scintillation counter. IC50 values of 5-BTrp and BPA were estimated to compare their potency.

Results

5-BTRp was synthetized using a multistep synthesis and characterized by NMR. Preliminary results of LAT1 affinity were promising and showed the 5-BTrp as a potent boron carrier for BNCT.

Conclusion

The synthetic pathway for 5-BTrp was feasible. Results from comparison of IC50 values of LAT1 affinities of BPA and 5-BTrp showed that boronated tryptophanes are working at least as well as clinically used BPA. Synthetized 5-BTrp was racemic mixture, and further studies are needed. However, the study proofs that boronated tryptophanes are highly promising group of boron carriers for BNCT.

References

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Dynamic Dissolution/Permeation: a Permealoop® Study on Dipyridamole Formulations

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2Departmentof Chemistry, Manufacturing and Control, Kobe Pharma Research Institute, Nippon Boehringer lngelheim Co., Ltd., 6-7-5, Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650-0047, Japan

Purpose

Obviously, an absorptive sink is useful to reveal the full extent and duration of super saturation during dissolution testing of enabling formulations [1]. Most dissolution/permeation tools have an area/donor volume ratio (A/V) far below the A/V observed in the intestine. In consequence supersaturation is expected to precipitate faster in these models than in vivo. Permealoop is an in vitro dissolution/permeation model with a higher area/donor volume ratio (A/V) [2].

Aim

The aim of this study was to determine the dissolution/permeation behaviour of dipyridamole formulations containing surface modifying agents using Permealoop and check for correlation with rat bioavailability studies, in comparison to the D/P model.

Methods

Granules with dipyridamole:fumaric acid ratios at 1:0, 1:0.5, 1:1 and 1:2 (m/m) were prepared by conventional wet granulation with mortar and pestle. The dissolution/permeation behavior of the granules and a physical mixture were studied on the current prototype of Permealoop and compared with results from Mizoguchi et al [3].

Results

The rank order of the dipyridamole absorptions of different formulations were shown to be the same as found in rat studies and with use of a D/P system [3]. The permealoop system and the D/P system had a very similar correlation with in vivo data. Despite the fact, that with Permealoop (in buffer) the supersaturation of the enabling formulations could be observed nicely, the cumulative permeation of dipyridamole was enhanced only about 2-fold compared to the D/P system, which likely is not enough to influence the supersaturation/ precipitation-interplay sufficiently.

Conclusion

This study has shown that the Permealoop (with buffer) provides similar in vitro in vivo correlation than the conventional D/P system (with FaSSIF), likely because the removal of drug from the donor chamber by permeation still is not high enough to observe the dynamic scenario between dissolution and permeation. A further optimization of the experimental conditions of Permealoop will be needed.

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Evaluation of P-glycoprotein efflux activity and cellular toxicity in MDCKII MDR1 cells in a one-pot assay

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Introduction

Currently, the interaction of pharmaceutical excipients with transporters and solute carriers is gaining increased attention. The interaction of the excipients with P-glycoprotein (P-gp) is easily screened in a calcein-AM efflux assay, where the calcein acetoxymethyl ester (calcein-AM, P-gp substrate) is loaded into cell cultures.[1,2] Intracellularly, calcein-AM can be converted by esterase to fluorescent calcein, which is a non-P-gp substrate.[1,2] Under P-gp inhibited condition, calcein-AM is entrapped in the cells and available for esterases, hence calcein levels increases resulting in a higher fluorescent signal. When screening excipients for their abilities to inhibit P-gp, they are used in different concentrations. At high concentrations of excipient, cellular toxicity might happen, and to understand the balance between interaction with transporters and cellular toxicity, other assays are needed. Therefore, CellTiter-Glo® Luminescent Cell Viability Assay (hereafter referred as CellTiter-Glo) was selected to investigate the toxicity of excipients in cell cultures. This assay is based on detection of the ATP levels as a biomarker of metabolically active cells.[3,4]

Aim

The aim of the present work was to investigate if calcein-AM efflux assay and CellTiter-Glo assay could be run consecutively in a one-pot reaction without affecting each other, and at the same time distinguish P-gp inhibition from altered cell viability.

Method

Four days after seeding MDCKII MDR1 cells in 96 well plates, the cells were exposed to increasing concentrations of non-P-gp inhibitors: mannitol, sodium dodecyl sulphate, and Triton X-100, and known P-gp inhibitors: quinidine, polysorbate 20, and polysorbate 80. Calcein-AM efflux assay was performed and the fluorescent responses were continuously recorded for 30 minutes at 37 °C. The cells were then re-equilibrated to room temperature for 10 minutes, before CellTiter-Glo assay was directly performed without a washing step, and the luminescence intensity was measured at 22 °C.

Results

Non-P-gp inhibitors did not affect the calcein fluorescent signals; whereas P-gp inhibitors enhanced the fluorescence time dependently. Interestingly, the luminescent responses from CellTiter-Glo performed after calcein-AM assays were similar to the responses from CellTiter-Glo performed alone (i.e. without calcein-AM) indicating that the presence of fluorescent calcein in the well did not affect the luminescence measurements.

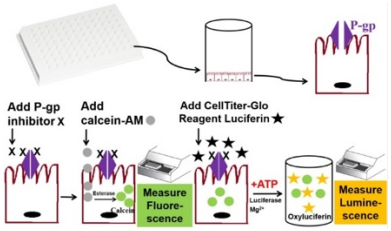

Figure 1, Schematic representation of performing calcein-AM assay followed directly by CellTiter-Glo assay in the same cell culture plate.

Conclusion

It was possible to performed calcein-AM and CellTiter-Glo in a one-pot assay, and this method may save time and reduce the cost of performing each assay separately, and simultaneously assists in distinguishing P-gp inhibition effects from toxic effects of the excipients.

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Wound Healing Peptidomics

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Introduction

Proteases, both endogenous and bacterial, have been described as major contributors to healing impairments, but the mechanistic implication of these enzymes are not yet fully understood. In Nature Communications1 and Scientific Reports2, a novel peptide-based mechanism by which bacteria modulate inflammation in wounds was described, and peptide fragments from thrombin were discovered that could serve as prospective biomarkers for inflammation and infection. Evidently, it is essential to more extensively investigate proteases and proteins/peptides in normal and impaired healing and to relate them to altered proteolytic activities.

Aim

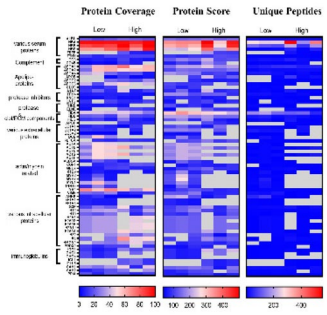
To discover the disease-state specific protein and/or peptide patterns in wounds that may be used for the development of diagnostic and prognostic biomarkers to target non-healing infected wounds, unresponsive to currently available wound care regimes, and may even lead to possible new drug targets.

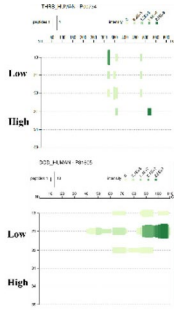
Method

We will study normally healing wounds and compare them with infected (non-healing) wounds. For this purpose, we will partially purify wound fluids by urea extraction and filtration, followed by analyses using SOS-PAGE, western blotting and LC-MS/MS peptidomic analysis.

Results

We have extracted peptides and filter out high molecular weight proteins from patient wound fluids, LC-MS/MS and data analysis were performed after sample purification. By comparing low- inflammation with high inflammation samples, we identified some specific peptide patterns that may serve as potential biomarker. Now, we incubate selected enzymes that involved in the wound healing process with citrate plasma samples to continue verifying the results.





Conclusion

It could be shown that there are big differences of proteins and peptide patterns between low- and high- inflammation patient samples through mass spectrometry. Selected examples of peptigrams that show different cleavage patterns are shown here and they may serve as potential biomarkers.

References

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Figure 1 *Heatmaps comparing the patients samples. (D)Selected peptigrams showing* (20 16.) *cleavage pattern differences*).

Lipid-based nanoparticles in vitreous humor: impact of diffusion and protein corona formation on the ocular pharmacokinetics of nanomedicines

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Introduction

Blinding eye disorders may be treated in the near future with breakthrough therapies based on nanomedicine. Nanomedicine is expected to improve drug delivery and targeting to ocular tissues, such as the retina, that are difficult to access with traditional pharmacological treatment. The treatment of retinal diseases is usually achieved via intravitreal drug administration, thus we deemed valuable to characterize the behavior of nanomedicines inside the vitreous gel.

Aim

To evaluate the mobility of lipid-based formulations in intact porcine vitreous and to characterize the vitreal protein corona surrounding the formulations.

Method

Lipid-based formulations (light-activated liposomes, rigid-membrane liposomes, hexosomes and nanostructured lipid carriers) with sizes 35-300 nm, with and without PEG, and anionic, neutral, and cationic charge were injected into intact porcine vitreous. Particle movement was captured on a spinning disk confocal microscope and particle trajectories were analyzed. The diffusion coefficients D_v in vitreous were calculated with the equation D_v=MSD/4t, where MSD is the mean square displacement and t is the time. The vitreal protein corona thickness and composition of anionic-light activated liposomes were determined using surface plasmon resonance and nLC-MS/MS, respectively. Both soft and hard corona were analyzed.

Results

Negatively charged formulations of any size up to 290 nm were freely mobile, and PEGylation did not affect their diffusion in vitreous. Neutral formulations were also freely mobile up to a size of 180 nm, however, larger liposomes had moderately restricted diffusion, which was improved by PEGylation. The mobility of cationic formulations was severely restricted by the vitreous, irrespective of their size. Adding PEG to these liposomes significantly improved their mobility. The protein corona thickness of anionic light-activated liposomes was 2.2 nm for the hard corona (HC) and 2.5-3.1 nm for the soft corona (SC). The same thickness was measured for non-PEGylated and PEGylated liposomes. We found clear differences in the protein composition of the two corona samples; none of the proteins that enriched in the SC were detected in the HC. However, within the same corona subsection, no difference was found between non-PEGylated and PEGylated liposomes.

Conclusion

The vitreal mobility of the tested formulations depended on the charge. The vitreous humor was only a weak barrier for the diffusion of negatively charged and neutral formulations, whereas it severely restricted the diffusion of cationic formulations. Our protein corona findings suggested that protein- specific rather than liposome-specific factors were drivers of protein adsorption on liposomes in the vitreous. Both methods evidenced that PEGylation did neither increase the vitreal mobility nor influence the protein corona formation of anionic-light activated liposomes.'

Acknowledgement

I would like to thank The Lundbeck Foundation for financial support (Grant No. R181-2014-3577).

Evaluation of capsaicin containing nanoantibiotics *versus* pure capsaicin on *E. coli* and *S. aureus* biofilms

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Introduction

Since antibiotics were discovered, bacteria have demonstrated the ability to develop resistance by many different mechanisms. According to WHO reports from 2014, there has been an alarming increase in the antibiotic resistant bacterial strains in most parts of the world. Our previous results showed that nanoantibiotics (NAB) created in our laboratory, composed of a cerium oxide core, mesoporous silica shell, capsaicin loading and chitosan coating, are effective against planktonic *E. coli*.[1] However, most of the pathogenic bacteria form biofilms during infections. Biofilms are communities of bacteria embedded within an extracellular polymeric substance matrix, which is formed by exopolysaccharides, proteins and DNA. It is an ecosystem, where each cell has its own role; they all experience different levels of nutrition, oxygenation and concentration of antibiotics. The latter is a problem especially in the deeper parts of the biofilm, since the dense matrix prevents effective treatment with molecular antibiotics. Appropriately designed delivery systems such as NAB could thus be utilized to circumvent this problem and enable efficient dosing of antibiotic drugs throughout the full biofilm matrix. Consequently, the next stage of studying NAB is to determine whether they are effective against biofilms of different species. In addition, our previous results of NAB efficiency against planktonic *E. coli* did not clearly show the contribution of the molecular antibiotic component of NAB – capsaicin. Hence, in the current follow-up study it is necessary to determine the actual efficient concentration of capsaicin against both *E. coli* and *S. aureus*.

Aim

To compare *in vitro* antibacterial activity of NAB and pure capsaicin on *E. coli* and *S. aureus* biofilms.

Methods

To investigate NAB efficiency on biofilms, MBEC-high-throughput assay[2] was performed. Equal biofilms formed on a plastic lid with 96 pegs in a 96 well plate with growth media overnight, were incubated with different concentrations of NAB and capsaicin. After different time points, the biofilms were sonicated and plated on agar plates to perform CFU counting. To determine the efficient concentration of capsaicin, biofilms were formed in 12 well plates and then incubated with different concentrations of capsaicin. To visualize the inhibitory effect, plating for CFU counting and Resazurin assay were applied.

Results

In MBEC-high-throughput assay no inhibitory effect of NAB against *E. coli* biofilms was detected in comparison with untreated bacteria.Resazurin assay and CFU counting method allowed us to determine the most efficient concentration of capsaicin against *E. coli* and *St. aureus* biofilms.

Conclusion

1. We cannot claim that NAB does not demonstrate any activity against *E. coli* biofilms, though we can suggest that the peg-lid set up is not appropriate for the NAB design.
2. The next step is to test different concentrations of NAB against biofilms with more appropriate methods than MBEC-high-throughput assay. These results will allow us to make conclusions about the benefits of NAB in comparison with pure capsaicin.

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Zosuquidar alters etoposide permeability across Caco-2 cell monolayers by P-glycoprotein inhibition in a concentration-dependent manner

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Introduction

P-glycoprotein (P-gp) lowers the transepithelial permeability and oral absorption of many drug substances. Zosuquidar is a potent competitive P-gp inhibitor, which increases oral absorption of P-gp substrates, but the dose dependency of this modulation has not yet been investigated. A previous study in rats (unpublished) showed that oral pretreatment with 20 mg/kg zosuquidar only increased oral etoposide bioavailability from 4.0 % to 34.5 %. Based on previous studies in bile cannulated rats[1], 20 mg/kg zosuquidar was assumed to elicit near-complete intestinal P-gp inhibition. Furthermore, etoposide bioavailability in *mdr1a* deficient rats was 92 %[2]. If P-gp was completely inhibited by 20 mg/kg zosuquidar, the etoposide bioavailability should be similar to that in *mdr1a* deficient rats. This discrepancy between etoposide bioavailability in zosuquidar-treated rats and in *mdr1a* deficient rats, raises the possibility that a zosuquidar dose of 20 mg/kg is too low to produce maximal inhibition of P-gp along the length of the intestine. To select zosuquidar doses for an *in vivo* dose-escalation study in rats, we investigated bi-directional etoposide permeability across Caco-2 cells in the presence of various zosuquidar concentrations.

Aim

The present study aimed to investigate the effect of various zosuquidar concentrations on the bi-directional permeability of etoposide across Caco-2 cell monolayers.

Method

The permeability of etoposide (50 µM) across Caco-2 cell monolayers grown on Transwell filters was assessed in the presence of 25 nM to 79 µM zosuquidar in both the basolateral-to-apical- (B-A) and apical-to-basolateral (A-B) direction at pH 7.4. Cell layer integrity was assessed by measurements of transepithelial electrical resistance before and after the experiment, and by ^{3}H-mannitol permeability. Etoposide was quantified by fluorescence-coupled HPLC. Apparent permeability of etoposide (P_{app}) was plotted as a function of the logarithmized zosuquidar concentrations, and IC_{50}-values of zosuquidar-mediated inhibition were estimated using non-linear regression.

Results

The permeability of etoposide across Caco-2 cell monolayers was highly polarised with an efflux ratio of 30.2±15.5. The efflux ratio decreased to 0.93±0.14 in the presence of 25 µM zosuquidar as the B-A P_{app} decreased 5-9-fold and the A-B P_{app} increased 3-6-fold. Derived IC_{50}-values for zosuquidar-mediated inhibition were 1.30 and 2.30 µM for B-A and A-B permeability, respectively.

Conclusion

Etoposide permeability across Caco-2 cell monolayers was highly polarized, and zosuquidar inhibited P-gp mediated efflux in a concentration-dependent manner.

Acknowledgement

The authors acknowledge Maria L. Pedersen for cell culturing.

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Effect of physicochemical characteristics of nanoparticles on uptake in MDCK cells.

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Introduction

Oral vaccine delivery holds significant potential for increasing patient compliance by elimination of unpleasant needle-based delivery and administration by trained medical professionals. Furthermore, enhanced immune cell activation mediated via induction of local mucosal immune tissues displays advantages for oral delivery. Nanoparticulate delivery systems are attractive due to their physical protection of antigens from gastric degradation and facilitation of intestinal uptake of their cargo.

Aim

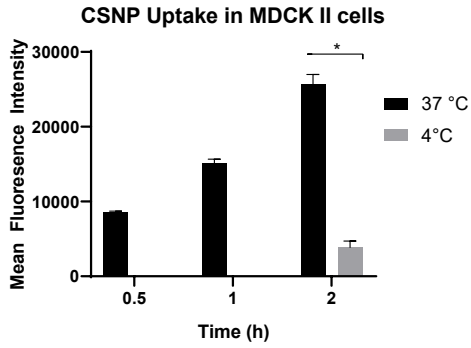
To decipher the endocytic pathway(s) involved in the intestinal uptake of chitosan, mesoporous silica and PLGA nanoparticles.

Methods

Chitosan nanoparticles (CSNP) were formulated via ionotropic gelation of chitosan solution with a sodium tripolyphosphate and FITC-OVA solution. PLGA nanoparticles (PLGA-NP) were formulated via a double-emulsion solvent evaporation method encapsulating FITC-OVA in the aqueous core. A modified oil-in-water emulsion method [1] was used to fabricate mesoporous silica nanoparticles (MSNP), and FITC-OVA was loaded via adsorption. Toxicity of all formulations against MDCK cells was assayed via a WST-1 reduction. Furthermore, quantification of cellular uptake was performed in MDCK cells.

Results

All formulations were synthesized in a size range of 120-160 nm with varied zeta potentials. Cellular viability revealed enhanced toxicity of cationic CSNP compared to anionic formulations. Further analysis of endocytic uptake demonstrated significant influence of active uptake mechanisms for CSNP and MSNP, with total particle uptake inhibited 2.6 and 1.6-fold, respectively, at 4 °C. Additionally, significantly enhanced magnitude of cellular uptake was apparent for CSNP compared with MSNP and PLGA-NP. Analysis of uptake in cells following knockdown of specific proteins is currently ongoing to delineate the endocytic pathway(s) involved.



Conclusion

Particle uptake efficiency in MDCK cells is highly influenced by surface charge, with significantly increased uptake observed with cationic CSNP, compared to anionic MSNP and PLGA-NP.

Acknowledgement

The author would like to thank the Australian Government Research Traineeship Program, Australian Government Endeavour Scholarships and Fellowships and the ARC Centre of Excellence in Bio-Nano Science and Technology for funding.

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Screening experimental conditions for *predictive* dissolution-/permeation-testing of the poorly soluble weak base dipyridamole by a PermeaLoop® prototype

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Introduction

In recent years, new molecular entities have trended towards poor aqueous solubility characteristics [1]. For this reason, various strategies for enabling formulations of poorly soluble drugs have been developed. With solubility being a key factor for oral drug absorption together with permeability, it is postulated that dissolution and permeation should be considered as an interplay rather than two separately key factors, especially when using enabling formulations for solubility enhancement [2]. PermeaLoop® is a promising in vitro tool for assessing the dissolution-permeation (D/P) interplay of poorly soluble drugs [3] and thus has been employed here for investigating the dissolution-/permeation-behavior of a poorly soluble weak base using dipyridamole as drug model.

Aim

To develop a set of experimental parameters suited to investigate the combined dissolution-/permeation-behavior of dipyridamole by PermeaLoop®.

Method

Solubility tests of excess dipyridamole in various media with/without additives were carried out in a preheated shaking water bath (37 ºC, 110 rpm) for 96 h. After 48 h and 96 h, aliquot samples were withdrawn and bench-top centrifugated (37 ºC, 14000 rpm) for 25 min. Supernatants were filtrated prior to quantification by reverse-phase HPLC. Combined dissolution/permeation studies of dipyridamole were performed with a PermeaLoop® prototype using PermeaPad® as permeation barrier. Over a period of 6 h, samples were withdrawn from both acceptor and donor reservoirs generating dissolution and cumulative permeation curves.

Results

The solubility of dipyridamole in the acidic FaSSGF was 1000-fold higher compared to the neutral phosphate buffer without additives. The addition of 2 % vitamin E TPGS in phosphate buffer (w/v) increased the solubility by a factor of 100, whereas the addition of 1 % HP-ß-CD (w/v) only yielded a 4-fold increase, thus phosphate buffer with 2 % vitamin E TPGS (w/v) was selected as acceptor. As donors, FaSSGF (pH 1.6), FaSSIF (pH 6.5) and citrate-phosphate buffer with/without bile compounds (pH 4.0) were selected to map the impact of pH and solubilizing additives on D/P. For all cases, the D/P experiments provided quantifiable amount in acceptor after a lag-time of 45 min. The cumulative amount of dipyridamole that permeated over time varied with pH and the presence/absence of bile salts/lipids. The amount of apparently dissolved dipyridamole in FaSSIF during D/P experiment evolved dynamically with a maximum at approx. 1 h.

Conclusion

The solubility of dipyridamole was highly pH-dependent, whereas the effect of solubilizing additives on solubility was moderate to negligible. Among the additives, phosphate buffer with 2 % vitamin E TPGS (w/v) showed to be the most promising acceptor medium. Comparison of D/P results from different donor media provided detailed mechanistic insights into the dynamic scenario of dissolution, permeation and bile salts/lipids partitioning.

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Workpackage 2: PRODUCT (Leader: Gøril Eide Faten, UiT)

Several current challenges in the drug development can be solved by identifying the optimal product design strategy. Within our consortium there are expertise within a broad range of different formulations strategies from nanomedicine to standard tablets. To utilize all of this the focus of WP2 is *"enabling formulations"***.**

There are three essential areas where the Nordic POP will aim for providing scientific excellence and new innovative solutions for drug delivery systems: biologicals, representing a category of protein/peptide based drugs that have rapidly gained momentum (specifically, this WP will focus on product design strategies aiming for non-invasive administration of large molecules), site specific drug delivery, covering more efficient and patient friendly products especially for patients with specific need, e.g., in pediatric and geriatric area (WP2 has a special focus on microbiota, mucoadhesion and nanotechnology),and poorly water soluble compounds, representing a major challenge in the drug development, which will be addressed by lipid-based and amorphous drug delivery systems.

Invited speaker talk:

Johan P Bøtker, UCPH: Acoustic monitoring of an inhalation device

WP2 student talks:

Henrik Palmelund, UCPH

Xiaona Liu, UCPH

Sybil Akua Okyerewa Obuobi, UiT

Heidi Öblom, ÅBO

WP2 part 2 students talks:

Tobias Palle Holm, UCPH

Nele-Johanna Hempel, UCPH

Giulia Scapin, HEL

Alma Akhmetova, UCPH

WP6/2 students talks

Troels Pedersen, UCPH

Poster session:

10 Camilla Kurth Ehlers,SDU
11 Amit Kumar Rajora, AAU
12 Sofia Lisina, AAU
13 Ye Liang, AAU
14 Wenhui Zhou, AAU
15 Laura Victoria Schulte Werning, UiT
16 Jingwen Liu, UCPH
17 May Wenche Jøraholmen, UiT
18 Christiane Færestrand Ellefsen, UiO
19 Bergthóra S. Snorradóttir, UI
20 Maria Hytti, UEF
21 Caroline Alvebratt, UPP
22 Teresa Quynh Hoa Pham Nguyen, SDU
23 Jeanette Frimand Hemmingsen, SDU
24 Stine Geisler, SDU
25 Julie Schmidt, SDU
26 Sonja Eileen Fiedler, SDU
27 Emilie Katrine Rasmussen, SDU
28 Randi Hamre Svendsen, UiO
29 Narayana Prakirth Govardhanam, AAU
30 Jenny Johannesson, UPP
31 Jyrki Heinämäki, UT
32 Lotte Ejskjær, SDU
33 Jana Kubackova, UCPH
34 Abishek Wadhwa, Anas Aljabbari, UCPH
35 Venkatasubramanian Ramakrishnan, UCPH

Acoustic monitoring of an inhalation device

Johan Boetker
Department of Pharmacy, University of Copenhagen, Denmark

Brief CV

Johan Boetker works as an Assistant Professor in the Manufacturing & Materials group, Department of Pharmacy at the University of Copenhagen, Denmark. His research is focused on image analysis, acoustics monitoring, finite element method simulations, solid state analysis, powder handling, 3D printing, multivariate data analysis and MATLAB® programming. These methods helps understanding pharmaceutical processes. In this context, he has recently started developing various programs that can either assess discoloration in pharmaceutical products using image analysis or assess flow rates based on changes in the acoustic signal. These programs are executed in the MATLAB® programming environment.

Abstract

Asthma is a medical condition, which is characterized as a chronic lung inflammation with sporadic attacks that can result in hospitalization and in extreme cases death. The asthma attacks can be largely prevented by better control of the asthma treatment. This may be achieved by correct inhaler dosing, medication adherence and tracking of the asthma symptoms. The Sonohaler company which is a spinout from the University of Copenhagen aims to develop a 3D printed acoustic add-on for commercially available inhalers and an associate app that can estimate the flowrate based on the acoustic signal from the 3D printed add-on. The acoustic signal processing is preliminarily done using multivariate data analysis and will later on be based on machine learning algorithms.

Influence of water of crystallization on the phase behavior of drugs and deep eutectic solvents

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[2]Monash Institute of Pharmaceutical Sciences, Monash University, Australia

Introduction: Deep eutectic solvents (DESs) are mixtures of hydrogen bond donors and acceptors which have a significantly lower melting point than the individual components. DESs have been proposed as promising drug carriers and/or solvents for drug compounds [1]. The components forming a DES are frequently carboxylic acids, sugars, polyols and amino acids, many of which are able to form hydrates [2] and hence, potentially have an impact on the drug loading capacity the DESs.

Aim: To investigate the influence of water of crystallization on the drug loading capacity and thermal phase behavior of ternary systems comprising DESs of betaine (anhydrate or monohydrate) and xylitol as well as the model drug acetylsalicylic acid.

Methods: The thermal phase behavior of physical mixtures of betaine anhydrate (AH), betaine monohydrate (MH), xylitol, and acetylsalicylic acid (ASA) were determined by DSC and hot stage microscopy.

Results: The binary mixtures of betaine AH-xylitol and betaine MH-xylitol showed identical deep eutectic phase behavior (T_m = 35°C) as seen in Figure 1A and 1B, respectively. The binary mixtures of betaine AH-ASA showed co-crystal formation in contrary to betaine MH-ASA, which showed a eutectic phase behavior (T_m = 35°C). The difference in binary phase behavior strongly affected the ternary phase behaviors of the two systems as seen in Figure 1. The ternary phases diagram with betaine AH shows a maximum drug loading of 10 wt% at the eutectic composition of the DES. On the other hand, for the betaine MH system drug loadings of up to 46 wt% were achieved, however, at betaine MH to xylitol ratios different from the DES ratio, indicating that there may be an optimal blend ratio between all three components, where the ternary interplay is maximized.

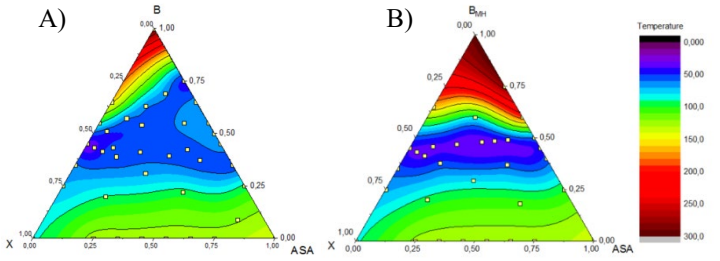

Figure 1. *Ternary phase diagram of A) Betaine AH, xylitol, and acetylsalicylic acid. B) Betaine MH, xylitol, and acetylsalicylic acid. Temperature scale indicating T_m from 0 to 300°C.*

Conclusion: The presence of water of crystallization significantly impacted the ternary phase behavior of the investigated DESs. As a result, different maximum drug loadings were obtained, indicating the importance of determining the ternary phase diagram for drug-DES combinations.

Acknowledgement
Independent Research Fund Denmark [Grant No. 7017-00211B] for financial support

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Design and evaluation of a self-emulsifying drug delivery system for pediatric use

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Introduction
There is growing interest in drug delivery to pediatric patients. Age-appropriate formulations need to be developed to maximize patient acceptability and maintain safety. Oral liquid dosage forms are favored by the young pediatric patients due to dose flexibility and ease of swallowing.
Amphotericin B (AmB), a poorly water-soluble drug, is used for pediatric patients with invasive fungal infections. However, the oral application of AmB is limited by its low oral bioavailability. Lipid-based drug delivery systems represent a popular approach for improving oral bioavailability of poorly water-soluble drugs as well as masking the unpleasant taste of drugs[1].

Aim: To increase the oral bioavailability of AmB in pediatric patients.

Methods: Pediatric-friendly ingredients originated from baby milk formula were selected to develop pediatric self-emulsifying drug delivery system (SEDDS) with low surfactant content. An AmB-lysophospholipid (LPC) complex (APC) was prepared by lyophilization of AmB and LPC at a molar ratio of 1:3. The selected optimal SEDDS loaded with APC (33 mg AmB in form of complex/ g SEDDS) was evaluated for self-emulsifying time, droplet size and drug precipitation upon dispersion in water (1:200, v/v). *In-vitro* lipolysis studies were performed to evaluate the impact of digestion on the drug solubilization under simulated pediatric gastro-intestinal conditions[2].

Results: A SEDDS containing 55% Akonino® oil, 25% Kolliphor® RH40, 15% Citrem® and 5% LPC was selected for efficient emulsification (<25 s), uniform droplet size (180.1±3.1 nm). After dispersion in water, the APC-loaded SEDDS formed a transparent yellowish emulsion with no visual precipitation within 6 h, indicating the emulsion is physically stable. Figure. 1 shows the percent of the AmB dose found in the aqueous phase during pediatric fed state *in-vitro* lipolysis. The APC-loaded SEDDS showed a significantly higher aqueous concentration than the APC without SEDDS, the AmB-loaded SEDDS and the AmB without SEDDS.

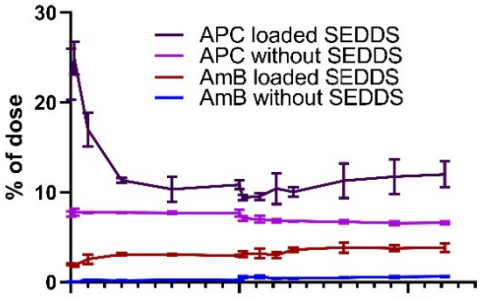

Fig. 1. Aqueous distribution of AmB during pediatric fed state in-vitro lipolysis of APC-loaded SEDDS, APC without SEDDS, AmB loaded SEDDS and AmB without SEDDS in lipolysis media.

Conclusion
An age-appropriate SEDDS was developed using pediatric friendly ingredients and showing good dispersion characteristics. APC-loaded SEDDS showed increased levels of AmB in the aqueous phase during *in-vitro* pediatric gastro-intestinal digestion studies, suggesting that the APC-loaded SEDDS can increase the bioavailability of AmB.

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Liposomal delivery of antibiotic loaded DNA nanogels against intracellular infections

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Introduction

Rising incidence of persistent and multi-drug resistant bacterial infections constitute a major global threat, with a large fraction of nosocomial and community acquired infections attributable to *Staphylococcus aureus*[1]. A significant percentage of the global population are notoriously persistent carriers due to the ability of *S. aureus* to escape immune response via host cell invasion[2]. Despite the efficacy of many antibiotics, high extracellular concentrations are required to achieve bactericidal activity due to low cellular drug accumulation[3].

Aim

Towards enhanced intracellular antibiotic delivery, self-assembled DNA nanostructures are exploited for the immobilization of antibiotics within liposomal vesicles.

Method

Liposomal hybrids were prepared via thin film hydration method and hydrated with vancomycin loaded DNA nanogels. The solutions were extruded, dialyzed and stored at 4°C. Dynamic light scattering and *in vitro* release kinetics was investigated. Finally, the intracellular uptake and bactericidal activity was examined in a macrophage infection model of *S. aureus*.

Results

The hybrids (Van_DNL) exhibited a significantly higher size of 225.20±1.91 nm and a spherical morphology. With a high entrapment of vancomycin (76.59±3.44 %), *in vitro* release of vancomycin was sustained over 24 h (57.84 ± 11.60 %) and responsive to lipases (84.97 ± 0.48 %) (Fig. 1a). Intracellular bactericidal activity was significantly higher than the free drug at low vancomycin doses (Fig. 1b) and confocal images revealed the intact hybrid within the Raw cells after 12 h (Fig. 1c).

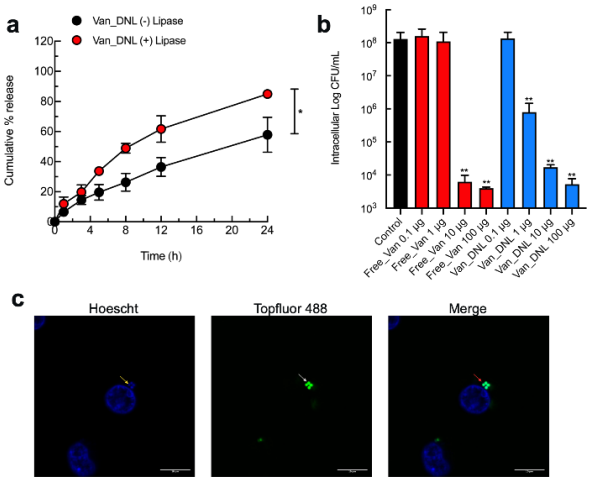

Figure 1 a) *In vitro* release of liposomal hybrids with (+) and without (-) lipases. b) *In vitro* intracellular antibacterial activity of the hybrids. c) Cellular uptake of Top_Fluor labelled Van_DNL after 12h. Cellular nuclei were stained with Hoescht.

Conclusion

By exploiting the high binding affinity between vancomycin and DNA nanostructures liposomal hybrids with higher encapsulation of vancomycin were fabricated. The hybrids demonstrated sustained release profiles that were enhanced in the presence of lipases. With a lower survival of *S. aureus* at low drug concentrations than the free drug, this approach provides a feasible platform for the delivery of antibiotics against persistent intracellular infections.

Acknowledgement

This project has received funding under the European Union's Horizon 2020 program Marie Curie Actions (grant No. 834811).

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Inkjet printing of medical cannabinoids enabling personalized and traceable drug products

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Introduction
Medical cannabinoids has gained increasing interest due to the possibility for helping a wider group of patients that have not benefitted from the products available on the market today. Medical cannabinoids are associated with highly individual dose requirements. Inkjet printing is a technique, which allows for precise deposition of a drug-loaded ink in a desired pattern making it suitable for manufacturing of personalized dosage forms.

Aim
The aim of the present study was to investigate the feasibility of utilizing inkjet printing for fabrication of flexible doses of lipophilic cannabinoids. The dosage form would, moreover, serve as a source of unique information as the drug-loaded ink would be deposited in an information rich pattern.

Methods
The suitability to use the marketed cannabinoid products, namely, Sativex® and Stenocare®, as a starting point for development of cannabinoid-containing printing inks was assessed by determining the Z-value, which has been described as a tool for predicting the printability of an ink[1]. Substrates for inkjet printing of cannabinoids were prepared by combining solvent casting and subsequent freeze-drying with slight modifications to the method previously reported by Iftimi et al[2]. An Epson XP-8500 desktop piezoelectric printer was used to imprint the prepared substrates with the cannabinoid-containing ink. The ink was deposited in a unique information-rich pattern (QR code).

Results
The ethanol and propylene glycol (PG) based product (Sativex®) was found to possess more suitable properties for inkjet printing with a Z-value of 6.3 compared to the oil-based product (Stenocare®) revealing a Z-value of 2.3. A Z-value in the range of 4-14 has been described as printable[1]. This would anticipate that an oil-based cannabinoid ink with a lower Z-value will form undesired satellite droplets during printing while an ethanol and PG-based ink as in Sativex® is expected to have stable jetting of the ink, which is required to produce reproducible dosage forms of high quality. A slightly modified Sativex® ink was, therefore, used to imprint the edible substrates with personalized doses of cannabinoids.

Conclusion
Cannabinoid-containing dosage forms were successfully printed into flexible doses by means of inkjet printing. Depositing the drug-loaded ink as an information rich pattern allowed incorporation of the desired information. Printing of such advanced dosage forms opens up multiple opportunities e.g. to incorporate personalized information for the patient and to trace the dosage form along the whole distribution chain.

Acknowledgement
Heidi Öblom acknowledges Suomen Kulttuurirahasto (Elli Turusen rahasto) for the personal PhD grant and NordForsk (the Nordic University Hub project #85352, Nordic POP, Patient Oriented Products) for funding the research visit to University of Copenhagen.

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Microwave-induced *in situ* amorphization of celecoxib using hydrates as a water source

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Introduction

Improving the aqueous solubility of poorly soluble small drug molecules is of great importance to ensure high bioavailability following oral administration. Amorphous solid dispersions (ASDs) are a well-known formulation principle used to increase the dissolution rate and the apparent solubility and, in some instances, to prolong supersaturation of these drugs [1,2]. Amorphization within the final dosage form just prior to administration, i.e. *in situ* amorphization, could potentially overcome the stability challenges normally associated with ASDs. *In situ* amorphization has been reported upon contact with dissolution media as well as induced through microwave irradiation - with water being the primary microwave absorbing, and in turn heating, material in the formulations and introduced by conditioning compacts at high relative humidity prior to microwave irradiation [3, 4, 5].

Aim

Enable complete microwave induced *in situ* amorphization of celecoxib (CCX) without the need for lengthy conditioning at high relative humidity, by utilizing inorganic salt hydrates as a water source for microwave absorption and subsequent heating.

Method

A series of CCX tablet formulations was prepared with the polymer PVP K12 at a 30:70 (w/w) drug-polymer ratio, with addition of 0.5% magnesium stearate. Sodium dihydrogen phosphate (SHP) di- and monohydrate were added to achieve 5.8% (w/w) water of crystallization in the final formulations and 200 mg compacts were prepared and subsequently microwaved at 900 W for 2-10 minutes in a laboratory microwave oven. Raman transmission spectroscopy and PLS modelling was utilized to quantify the amorphous content [5] and X-ray powder diffraction (XRPD) was used to confirm the drug amorphization. Differential scanning calorimetry and thermogravimetric analysis were used to determine the glass transition temperature and residual moisture content, respectively.

Results

Complete amorphization of CCX was achieved after 6 minutes of microwaving (Figure 1), by utilizing SHP di- and monohydrate as a source of water for microwave absorption. These findings were confirmed by XRPD showing only diffractions corresponding to the anhydrous SHP.

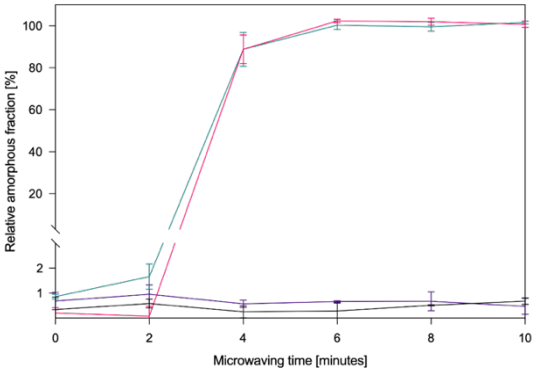

Figure 1 Amorphization kinetics of celecoxib. Celecoxib in PVP K12 (30% drug loading) with sodium dihydrogen phosphate (SHP) dihydrate (green), monohydrate (red), anhydrate (purple) and control without hydrate (black). Formulations microwaved at 900 W (n=3).

Conclusion

The current study showed the feasibility of using inorganic salt hydrates as excipients to eliminate a long conditioning step prior to *in situ* amorphization of poorly soluble drugs, achieving complete amorphization with a 30% CCX load.

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Plasmonic nanoparticles for *in situ* drug amorphization using laser irradiation

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Introduction

In situ amorphization is a promising approach to circumvent stability and downstream manufacturing issues of amorphous drug formulations. Using the approach, the crystalline drug can be formulated with a polymer using upstream manufacturing procedures, e.g. direct tableting, and the amorphous form can then be obtained inside the final dosage form, directly before administration [1]. *In situ* amorphization has been obtained so far upon immersion in water and through microwave-irradiation [1, 2]. The latter is suggested to be a temperature dependent process [2]. Upon laser irradiation, silver nanoparticles with plasmonic properties can generate heat [3] that could be sufficient for *in situ* amorphization.

Aim

To investigate *in situ* amorphization using laser irradiation and its influencing parameters, i.e. laser flux, exposure time to laser irradiation and drug loading.

Method

50 mg compacts containing 30 or 50 %wt celecoxib (CCX), 0.25 %wt plasmonic nanoparticles (pn), 69.25 or 49.25 %wt PVP 12 and 0.5 %wt magnesium stearate were irradiated with a laser at 808 nm wavelength. The laser flux was set to 1.12 and 1.31 W/cm^2 and the exposure time adjusted to reach complete amorphization.

Results

Figure 1 shows the relative residual crystallinity of CCX as a function of exposure time. With the higher laser flux, i.e. 1.31 W/cm^2, the rate of amorphization was faster and shorter exposure times were required to obtain complete amorphization. Additionally, with increasing exposure times, the degree of amorphization increased. Using a laser flux of 1.31 W/cm^2, complete amorphization was obtained after 180 sec and 210 sec of exposure to laser irradiation, for compacts containing 50 and 30 %wt CCX, respectively.

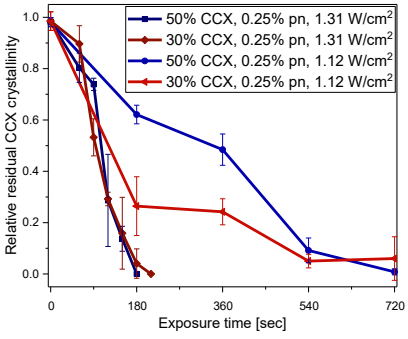

Figure 1: *Relative residual CCX crystallinity vs. exposure time [sec]. Mean ± SD (n = 3).*

Conclusion

Laser irradiation in the presence of small amounts of pn was found to be a promising approach within the field of *in situ* amorphization. Applying a higher laser flux resulted in faster rates of amorphization. Furthermore, the degree of amorphization increased with increasing exposure time to laser irradiation.

Acknowledgment

The authors would like to thank NordForsk for financial support (Nordic University Hub project #85352; Nordic POP) as well as the Independent Research Fund Denmark (Grant No. DFF-7026-00052B). Alexandra Teleki gratefully acknowledges funding from the Science for Life Laboratory.

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Freeze drying of PC3 and PNT2 extracellular vesicles for long term RT storage

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Introduction

Extracellular vesicles (EVs) are lipid bilayer vesicles released by cells and involved in cell-to-cell communications. EVs features potentially enable their application as carriers for targeted drug delivery and biomarker diagnostics[1]. However, exploitation of extracellular vesicles for clinical applications requires a formulation that is suitable for their administration and possesses adequate stability for storage and transportation. Thus, the possibility to generate a dehydrated form of EVs for storage and transport at room temperature would increase their usability in the pharmaceutical field. Therefore, freeze-drying of EVs may be considered as a procedure to generate a stable version of the vesicles, developed as carriers for targeted delivery.

Aim

Our aim is to develop a simple and effective scalable method to freeze-dry EVs for long term storage at room temperature in dry form and evaluate their stability and biological activity over the time.

Method

EVs derived from PNT2 and PC-3 cell lines were concentrated by differential centrifugation, purified by size exclusion chromatography and freeze-dried (FD) with different formulations. Before and after freeze-drying, EVs concentration and size, morphology, protein content, membrane integrity and RNA cargo were characterized by Nanoparticle Tracking Analysis, Transmission Electron Microscopy, Western Blot, flow-cytometry based Calcein AM-staining and RNA electrophoresis, respectively. Moreover, to further investigate the processing effect on EVs, a bio-photonic approach was chosen: Raman and FTIR spectra of freeze-dried EVs were compared with spectra of EVs stored at -80°C in order to evaluate any differences in their fingerprints. Finally, *in vitro* tests were performed to assess the functionality of FD PC-3 EVs.

Results

Different formulations preserved the size, morphology, RNA cargo and membrane integrity of EVs. However, only one formulation, containing sugars and glycine, preserved the overall protein quality, as determined by FTIR spectra. Thus, we chose this formulation for *in vitro* studies. FD EVs enhance cell proliferation with comparable performances of EVs stored at -80°C, used as controls.

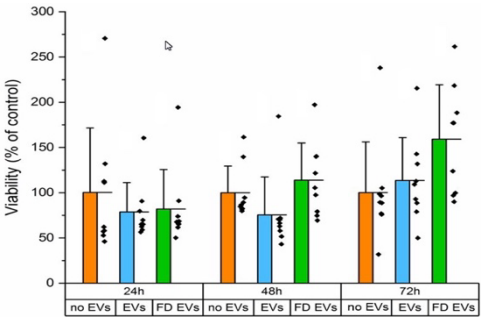

Figure 1 *In vitro proliferation assay: EVs and FD-EVs are able to enhance cell proliferation.*

Conclusion

EVs were successfully FD by using conventional lyo- and cryoprotectants, showing minimal alterations in their overall features compared to EVs stored at -80°C.

Acknowledgement

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Non-toxic protein-based microfibers for wound healing

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Introduction
Electrospun fibrous membranes have attracted a lot of attention in wound healing application due to the unique ability to mimic extracellular matrix (ECM) of healthy tissues. However, fabrication of such membranes often requires the use of toxic organic solvents and cross-linkers to obtain desired properties. Unfortunately, even after the evaporation and rinsing stages, the final product may still contain traces of toxic materials. In order to avoid this, biocompatible zein protein was selected as a self-assembling polymer soluble in aqueous ethanol[1]. Despite its potential benefits, the protein is difficult to electrospin continuously[2]. The obtained fibers often have ribbon morphology, weak mechanical strength and poor elongation. Zein fibers also have low water stability that leads to an instant loss of fibrous ECM-like structure resulting in film formation. Therefore, in this work we have attempted to overcome these drawbacks by using co-axial electrospinning and produce water-stable tubular shaped core-shell fibers that could potentially be used in wound healing applications.

Aim
Develop a protein-based microfibrous matrix free of toxic solvents and cross-linkers.

Method
Zein dissolved in aqueous ethanol was used as a core solution. 1% (w/w) of polyethylene oxide (PEO) or stearic acid (SA) of zein weight were added as hydrophilic and hydrophobic plasticizers, respectively. 1% (w/v) PEO dissolved in aqueous ethanol was used as shell solution. Electrospinning settings varied depending on the combination used. Prepared matrices were characterized for morphology, water stability, mechanical tests, and cell safety. Drug release profiles were studied on the selected combinations that were loaded with 5% (w/w) tetracycline hydrochloride (TCH) of zein weight.

Results
PEO had a profound effect on the electrospinning of zein fibers and improving morphology (Figure 1), water stability and mechanical properties due to the formation of hydrogen bonds. The hydrophilic character of PEO has considerably increased elongation of zein fibers and also led to a burst release of TCH.

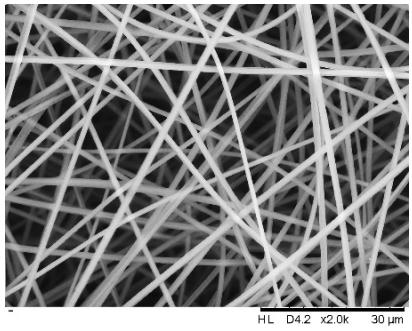

Figure 1 *Tubular-shaped morphology of zein fibers fabricated with PEO addition to the core and the shell solutions.*

Conclusion
Properties of zein fibers were successfully optimized without the use of toxic solvents and cross-linkers. The developed matrices could be potentially applied for wound healing purposes.

Acknowledgement
Authors acknowledge funding from LEO Foundation and NordForsk for the Nordic University Hub project #85352 (Nordic POP, Patient Oriented Products).

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Effect of material characteristics on residence time distribution in a continuous process

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Introduction
Continuous manufacturing has been gaining momentum in the pharmaceutical field during the past decade. One advantage for continuous processing is that the process scale-up can be achieved by either an increase in production time or by simply increasing the process throughput (feed rate). When throughput is increased, the time material undergoes mixing in continuous mixer is reduced. Thus, to ensure homogenization, the duration the material is processed should be estimated and controlled. The time an average particle resides within the blending unit can be determined from a probability density function commonly expressed as a residence time distribution (RTD). In this study, different model excipients with diverse powder characteristics were applied to explore the effect of material characteristics and the impeller speed of the mixer on the RTD in this specific production line by using a pulse tracer.

Aims
The aims of this study were to determine the RTD and mean residence time, using the continuous stirred tank reactor in series model to fit the RTD function by pulse tracer experiments applying non-invasive in-line process analytical tools.

Methods
Different mixtures of pregelatinized starch (STR) (PREGEFLO MI 20 A, Roquette), sorbitol (SBT), (Parteck SI, Merck Millipore), calcium hydrogen phosphate (CHP) (Emcompress Premium, JRS Pharma) and microcrystalline cellulose (MCC) (Avicel PH 102, FMC biopolymer) were systematically investigated. Experiments were conducted in a continuous mixing system (Modulomix, Hosokawa Micron, Doetinchem, The Netherlands) in Kuopio, Finland, with materials fed by individual screw feeders (K-Tron, KML-D5-KT20, Niederlenz, Switzerland). A pulse of 3 grams of paracetamol (Hangzhou Dayangchem CO. Ltd) was introduced as the tracer material. The tracer was detected using an in-line near infrared (NIR) spectroscopy setup (Specim, Oulu, Finland) with both unsupervised and supervised multivariate data analysis.

Results
The RTD of paracetamol could be detected using in-line NIR for all the diverse powder mixtures. Paracetamol was an ideal tracer material, as the in-line data obtained contained enough spectral information to measure the RTD and estimate the mean residence time, τ. Figure 1 presents an example of unsupervised kinetic profiling of paracetamol during an example tracer experiment. In this, the τ for all three repeated experiments with a powder mixture (1:1 w/w of MCC/CHP) was found to be within the same range (26.8±0.4 s, n=3).

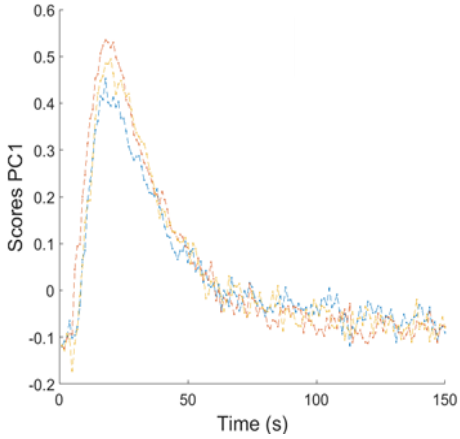

Figure 1. Residence time distribution concentration profiles of the tracer material (paracetamol) during continuous mixing process (n=3).

Conclusions
In-line NIR spectral data can be used for fast and efficient evaluation of powder flow and mixing behavior in a continuous production line. This allows for exploring a broad range of different materials based on the analysis of RTD.

Acknowledgements
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Synthesis of a novel Sorafenib prodrug and *in vitro* evaluation of Sorafenib

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Introduction

Sorafenib (Sf) is an important commercially available tyrosine kinase inhibitor used in the treatment of various cancers (1). Sf has been considered to be revolutionary in especially the treatment of hepatocellular carcinoma as it acts more specifically toward the malignant cells compared to traditional chemotherapy (2). However, the low aqueous solubility of Sf has resulted in its poor oral bioavailability (38-49%) and has restricted its further clinical applications (1).

This project focuses on improving the solubility and bioavailability of Sf by utilizing a phosphate prodrug strategy. In this project, a phosphonooxymethyl (POM) promoiety in the *N*-methylpicolinamide (*N*-MPA) position of Sf is used to improve the aqueous solubility (Figure 1). The drug will undergo bioconversion in the intestinal wall by alkaline phosphatases and the parent drug will be released (3).

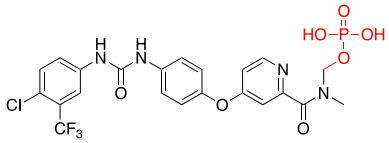

Figure 1 *Chemical structure of the desirable phosphonooxymethyl prodrug of Sorafenib.*

Aim

To improve the solubility of Sf by synthesizing a POM prodrug of Sf (Figure 1). If successful, an analytical method based on Ultra High-Performance Liquid Chromatography (UHPLC) for quantification and qualification will be developed to determine solubility- and potential permeation studies in a side by side set-up.

Method

NMR and MS were used to characterize the structures of intermediates within the synthetic routes. Flash chromatography and High-Performance Counter Current Chromatography (HPCCC) were used in purification of compounds. UHPLC was used to detect and quantify the amount of the parent drug in solubility- and potential permeation studies.

Results

The synthesis of the POM prodrug was not successful even using different synthetic approaches. However, two synthesized intermediates interestingly showed that the protons in the urea group were more acidic compared to the *N*-MPA group. Unfortunately, attempts to synthesize prodrugs located in the urea position was also unsuccessful. A qualitative and quantitative UHPLC method was developed. Solubility- and permeation studies of Sf can not be evaluated before these are obtained.

Conclusion

A POM prodrug of Sf located on the *N*-MPA group was not achieved. A method based on UHPLC for qualitative and quantitative analysis was developed. However, no conclusions on solubility and permeation studies can be confirmed before the desired compounds are obtained.

Acknowledgement

The authors would like to thank the Erasmus+ programme of the European Union and Nordic POP for financial support.

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Role of the Enhanced Permeability and Retention Effect and Cathepsins Levels On Polymer-drug Conjugates Activity

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Introduction

Polymer-drug conjugates (PDCs) are nano-sized drug delivery systems, in which one or more chemotherapeutic agent is covalently linked to a water-soluble polymer[1]. In addition, the anti-tumour activity of PDCs relies on two factors: (a) passive tumour accumulation via the enhanced permeability and retention (EPR) effect (b) drug release following a biological stimulus (e.g. enzyme (cathepsins) or pH)[2,3]. 19 PDCs have undergone in clinical evaluation so far. Clinical trials have shown evidence of tumour responses to PDC to various degrees, and some conjugates (e.g., PGA-paclitaxel) have progressed to Phase III trials[4]. Here, we retrospectively analyze data obtained from the literature concerning clinical trials carried out on PDCs in order to determine whether there is a connection between the clinical responses of various tumour types and the levels of enzyme expression/magnitude of the EPR effect in such tumour types.

Aim

To investigate the impact of the enhanced permeability and retention (EPR) effect and cathepsins levels on the activity of polymer-drug conjugates.

Method

The data were collected via systematic literature searches using search engines such as PubMed, Science Direct and Web of Science. No date restrictions were applied to the searches.

Results

We have analysed the data from the literature and examined the expression of enzymes (cathepsins) within various tumour types, the magnitude of the EPR effect in the various tumour types, and the clinical responses observed for PDCs. Breast, ovary and lung cancer types showed the highest response rates were observed (> 30%) both for cathepsin-activated PDCs and for all conjugates, independent of their activation mechanism. Marked heterogeneity was observed across the different tumour types with respect to the cathepsin content and the EPR effect. The high content of cathepsins were observed in lung and breast tumour types and the EPR-mediated accumulation was generally observed in all the tumour types, however the highest percentage responses were reported in breast, lung and ovarian tumours which have shown the highest percentage clinical response to PDCs. This correlation suggests that the cathepsin content and the EPR effect have a major role on the activity of PDCs.

Conclusion

The highest percentages of clinical responses to PDCs were observed for lung cancer, breast and ovarian cancer that were also found to express high levels of the cathepsin enzymes and profound EPR effect. This finding is in line with other studies[5] and in agreement with that concluded by others (e.g., ref 5). Moreover, this study also suggests that careful patient selection, in the form of pre-screening for enzyme content and the EPR effect, would be a rational approach for the further development and clinical application of PDCs. This could ultimately result in a more consistent efficacy of this drug delivery system in the clinical setting.

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Freeze-drying of 3D HepG2 cell spheroids with nanofibrillated cellulose for long-term room temperature storage

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Introduction

In the recent decade, the approaches of manufacturing of drugs and therapeutic systems have significantly advanced and new strategies based on Quality by Design (QbD) have emerged, paving the new pathways for the development of formulations and production processes. In this research, for the first time, the *QbD approach* is implemented *for the design and optimization of freeze dryable biomaterial formulations of nanofibrillated cellulose (NFC) hydrogel with 3D cell spheroids.* The aim is to reach a Design Space of formulations to be applied into the Freeze-drying (FD) of 3D HepG2 cell spheroids for long-term stability and storage in dry form at room temperature. Here we report our first results of successful screening of freeze-dryable formulations for the FD of 3D cell spheroids.

Aim

Our aim is to screen and optimize formulations and the freeze-drying cycle of 3D cell spheroids for long-term storage at room temperature.

Methods

3D HepG2 cell spheroids were cultured in NFC hydrogel for seven days[1] and then freeze-dried after the addition of chosen excipients. Appearance, pH, glass transition temperatures (Tg'), residual water contents and osmolarities of the formulations were assessed before and after FD. Freeze-dried 3D cell spheroids were stored up to seven days, after which they were reconstituted and their morphology and viability were evaluated by live/dead cell viability assay, fluorescence staining and scanning electron microscopy (SEM).

Results

Potential freeze-dried formulations of NFC were characterized and used for the FD of 3D cell spheroids. The reconstitution of the freeze-dried 3D cell spheroid formulations was successful, and esterase activities (used as cell viability marker) were detected even after seven days of storage.

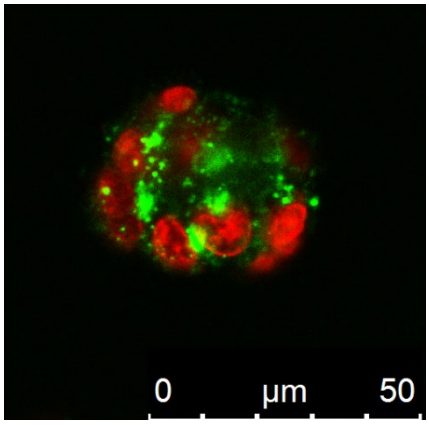

Figure 1 *Esterase activity of FD and reconstituted 3D HepG2 cell spheroid.*

Conclusion

NFC formulations show potential lyoprotective features for the FD and storage of 3D cell spheroids.

Acknowledgement

The Independent Academy of Finland BioEVa (n:o 315409) grant; UPM GD-96 project, Erasmus Exchange program and NordicPOP fundings.

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Development and investigation of double – layered suppositories as potential drug delivery systems

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Introduction

To date, suppositories as a traditional dosage form has not lost its significance as a drug delivery system (DDS). Knowledge of pharmaceutical factors affecting the effectiveness of suppositories have laid the foundation for their modernization and the creation of double – layered suppositories. In such suppositories, chemical incompatibility between two or more drugs can be avoided by its physical separation and, it is possible to combine layers with different drug release patterns (slow release with immediate-release)[1].Currently, peptides of synthetic and natural origin play an important role as bioregulators that have a pronounced geroprotective, antitumor effect, and so forth[2]. Due to the low tissue permeability and bioavailability as well as problems associated with absorption during the transdermal route of administration, the use of peptide drugs in clinical practice is limited[3]. A rectal route of administration may be considered as an alternative for the delivery of substances of peptide nature in the form of suppositories. Therefore, the development of double-layered suppositories in which it is assumed that API1 (located in the outer layer) will facilitate the penetration of API2 (contained in the inner layer) is crucial.

Aim

Development and investigation of double – layered suppositories as potential DDS for the treatment of prostate diseases.

Methods

Determination of the uniformity of weight of double-layered suppositories was carried out in accordance with the State Pharmacopoeia (SP) XIV (Russia) General Pharmacopoeia Monograph (GPM) 1.4.2.0009.15 on 20 units of dosage form. Disintegration time test was determined on ERWEKA PM 30 tester in accordance with the SP XIV (Russia) GPM 1.4.2.0010.15. The ERWEKA SSP tester was used for measuring the melting point estimation of suppositories.

Results

A mold for production of double-layered suppositories using 3D printing was developed. The composition (Figure 1) for obtaining double-layered suppositories is proposed. The manufactured double-layered suppositories comply with requirements of the SP XIV (Russia) in terms of general appearance, uniformity of weight, disintegration time test and melting point estimation.

Conclusion

Undertaken studies have shown the prospect of using double-layered suppositories in the treatment of prostate diseases (as a model). As a next step, pharmacological studies are required to evaluate the feasibility of the DDS.

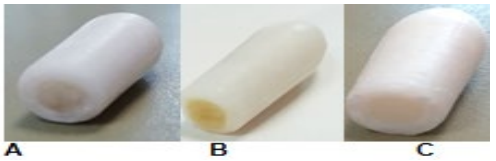

Figure 1 Double-layered suppositories. Outer layer Witepsol H15 and W35 (1:1)+API 1, inner layer – Suppocire NAI 25A and NAS 50 (1:1) (A), Cocoa

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Acetylglucosamine Sensitizes TRAIL-Induced Apoptosis through Activating Death Receptor 5 in Non-Small Cell Lung Cancer Cells

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Introduction

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a potential anti-cancer agent due to its selective toxicity[1]. However, nearly half of non-small cell lung cancers (NSCLCs) are resistant to TRAIL-induced apoptosis[2]. Therefore, there is a need for the development of novel adjuvant therapeutic agents combinating with TRAIL. Death receptor (DR) activation is a crucial upstream event to initiate TRAIL-induced apoptosis. A mechanism that modulates TRAIL signaling in tumors through DR O-glycosylation was previously discovered[3]. Acetylglucosamine (GlcNAc), a type of monosaccharide derived from chitosan, is involved in a series of physiological regulatory activities as a precursor of O-GlcNAcylation. However, little is known about the effect of GlcNAc on the receptor O-glycosylation and TRAIL-induced apoptosis.

Aim

To develop a novel adjuvant agents with TRAIL, the effect of GlcNAc was evaluated for TRAIL-induced apoptosis in NSCLC.

Method

Thirty NSCLC clinical samples were used to detect the expression of DR 4 and 5. After GlcNAc and TRAIL co-treatment, DR expression was determined by qPCR and western blotting. Cycloheximide was used to detect the protein half-life to further understand the correlation between GlcNAc and the metabolic rate of DR. Non-reducing SDS-PAGE was used to detect receptor clustering, and the localization of DR was visualized by immunofluorescence under a confocal microscope. Furthermore, a co-immunoprecipitation assay was performed to analyze the formation of death-inducing signaling complex (DISC). O-linked glycan expression levels were evaluated following DR5 overexpression and RNA interference mediated knockdown.

Results

The clinical samples expressed higher levels of DR5 than DR4, and GlcNAc co-treatment improved the effect of TRAIL-induced apoptosis by activating DR5 accumulation and clustering, which in turn recruited the apoptosis-initiating protease caspase-8 to form DISC, and initiated apoptosis. Furthermore, GlcNAc promoted DR5 clustering by improving its O-glycosylation.

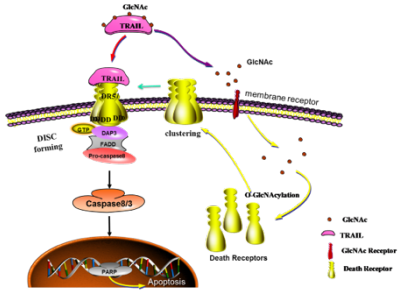

Figure 1 *The potential molecular mechanism of GlcNAc sensitizing cancer cells to TRAIL induced apoptosis.*

Conclusion

This research uncovered the molecular mechanism by which GlcNAc sensitizes cancer cells to TRAIL-induced apoptosis, thereby highlighting a novel effective agent for TRAIL-mediated targeted therapy in TRAIL-resistant NSCLC.

Acknowledgement

The author would like to thank the National Natural Science Foundations of China for financial support (Grant No. 81401899, 81472411).

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Ilamycin E, a natural product of marine actinomycete, inhibits triple-negative breast cancer partially through ER stress-CHOP-Bcl-2

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Introduction

Breast cancer is the most commonly diagnosed cancer and the leading cause of cancer death among women in the worldwide1. Triple-negative breast cancer (TNBC) has a poor clinical outcome. The antitumor efficacy of Ilamycins, natural products with anti-tuberculosis activity isolated from deep sea-derived Streptomyces atratus, in TNBC has not been investigated, and the mechanisms remain elusive2.

Aim

To investigate the anti-tumor activity and explore the mechanism of Ilamycin E in triple-negative breast cancer.

Method

Three different subtypes of breast cancer cell lines and an immortalized breast cell line were used for the cytotoxicity analysis. SRB assay, DNA synthesis assay, Cell cycle analysis, Cell apoptosis analysis, and lots of Western blotting analysis were performed to explore the main mechanism of Ilamycin E for its anti-tumor activity.

Results

We founded that Ilamycin-E, but not -F, decreases cell viability, inhibits G1/S cell cycle progression, and promotes apoptosis in the TNBC cell lines HCC1937 and MDA-MB-468. Ilamycin E promotes apoptosis via activation of endoplasmic reticulum (ER) stress, increasing the expression of CHOP, and down-regulating the expression of anti-apoptotic protein Bcl-2. Depletion of CHOP or overexpression of Bcl2 significantly rescued Ilamycin E-induced apoptosis.

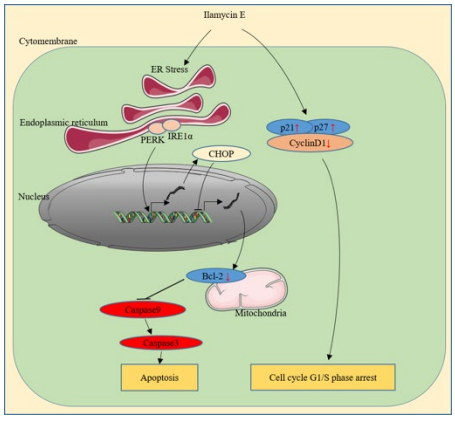

Figure 1: Working model of Ilamycin E's anti-tumor activity

Conclusion

In summary, we showed that Ilamycin E inhibits TNBC cell growth and induces apoptosis through activating the intrinsic apoptotic pathway. Ilamycin E effectively suppressed cell survival at least partially via the ER stress/CHOP/Bcl-2 axis, providing a potential therapeutic agent for breast cancer treatment.

Acknowledgement

This work was supported by grants from National Natural Science Foundation of China, the Seed Fund Program of Shanghai University of Medicine & Health Sciences and the Scientific Research Foundation for Talented Scholars of Hubei University of Medicine.

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Electrospinning chitosan-nanofibers using the needle-free Nanospider™ technology; Optimization of the polymer solution

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Introduction

Nanofibers have recently gained a lot of interest as wound dressings suitable for both acute and chronic wounds[1]. Chitosan is a natural biopolymer that is known to have good antimicrobial properties in combination with low toxicity and good biocompatibility, making it an interesting polymer for the formation of nanofibers used for wound healing[2]. Electrospinning is the most commonly used method to produce nanofibers from polymer solutions which allows several adjustments to the desired nanofiber characteristics[3]. The needle-free electrospinning technique allows faster production of the nanofibers compared to the traditional needle-spinning setup. Several parameters are known to influence the electrospinning parameters, mainly the solution composition, spinning parameters and environmental parameters such as temperature and humidity[4]

Aim

The aim of this study was to obtain highest possible concentrations of chitosan in the spinning solutions while maintaining both the spinability of the solutions and properties of the obtained nanofibers.

Method

Polymer solutions containing different concentrations of chitosan, hydroxypropylmethylcellulose (HPMC), polyethylene oxide (PEO), acetic acid, water and ethanol were prepared and spun applying the needle-free Nanospider™ technology. The polymers were dissolved in water acidified with acetic acid and three different concentrations of ethanol (50%, 60% and 70%). HPMC and PEO were the preferred co-polymers. An increasing concentration of chitosan (5-30%) was investigated together with the effect of ethanol on the spinning process. Solution characteristics such as conductivity, surface tension and viscosity were measured prior to electrospinning. Field Emission Scanning Electron Microscopy (FE-SEM) was used to confirm the successful spinning of nanofibers.

Results

Most of the prepared solutions were able to form nanofibers, but with an increasing concentration of chitosan higher concentrations of ethanol were needed to maintain spinability. The surface tension decreased with increasing ethanol concentrations, whereas the conductivity was dependent on both the chitosan- and ethanol concentration. Successful electrospinning of the solutions was confirmed by FE-SEM and the diameter of the fibers found to be 80 ± 11 nm, 86 ± 14 nm and 91 ± 7 nm for fibers successfully spun in 50, 60 and 70% ethanol, respectively. No increase in the diameter could be observed with an increasing chitosan concentration.

Conclusion

Fibers containing chitosan were successfully spun using the needle-free Nanospider™ technology. The spinability could be maintained at all chitosan concentrations investigated, but higher ethanol concentration (60-70%) was needed at higher chitosan concentrations.

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The influence of polymer addition on the dissolution behaviour of a co-amorphous system

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Introduction

Co-amorphous systems have been developed as an approach to address the poor water solubility challenge of many drugs [1]. However, the dissolution rate of co-amorphous systems can in some cases be too fast, leading to extreme super-saturation during contact with aqueous media, followed by precipitation and thereby loss of the dissolution advantage [2]. Therefore, it seems promising to add precipitation inhibiting and/ or release rate-modulating polymers into co-amorphous systems to optimize their dissolution behaviour.

Aim

To investigate the influence of addition of polymeric excipients on the dissolution and super-saturation behaviour of co-amorphous systems.

Method

Carvedilol (CAR), L-aspartic acid (ASP) and hydroxypropyl methylcellulose (HPMC) were chosen as the model drug, the co-former, and the polymer, respectively. Spray drying was used to prepare CAR-ASP co-amorphous systems (molar ratios of 1:1, 1:1.5 and 1:2), CAR-HPMC amorphous solid dispersions (with CAR weight fractions of 67.8%, 60.4% and 54.4%) and CAR-ASP-HPMC ternary systems. In the ternary systems, the weight fraction of HPMC was 10% of the total powder mass, and the molar ratios of CAR and ASP were at 1:1, 1:1.5 and 1:2, respectively. Powder dissolution tests under non-sink conditions were conducted in pH 7.2 phosphate buffer at 37℃.

Results

Compared with the crystalline drug, pure amorphous CAR showed no improvement in dissolution behavior (Figure 1). In binary systems, all CAR-ASP co-amorphous systems showed a significantly increased dissolution rate and achieved drug super-saturation, however, followed by precipitation. More gradual increases in dissolved drug concentration were observed using the CAR-HPMC systems. CAR-ASP-HPMC ternary systems showed a faster initial dissolution compared with CAR-HPMC systems and an improvement of precipitation phenomena compared with CAR-ASP systems.

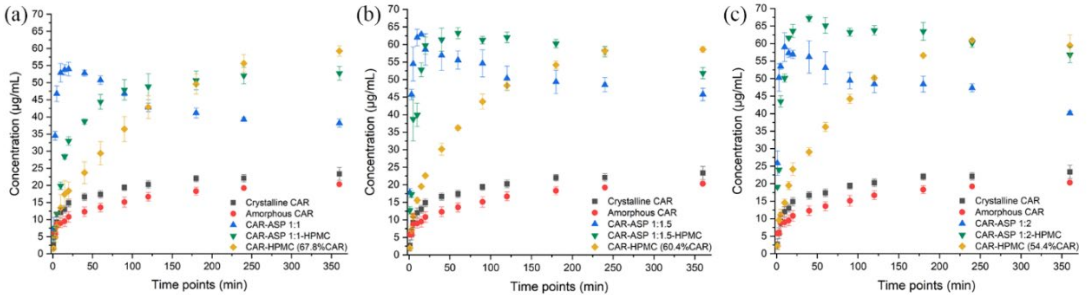

Figure 1 Powder dissolution profiles of CAR-ASP, CAR-HPMC and CAR-ASP-HPMC systems.

Conclusion

The addition of HPMC improved the dissolution behaviour of co-amorphous systems, by reducing the extremely fast initial dissolution rate of the co-amorphous systems and maintaining super-saturation for a longer period.

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Liposomes in chitosan-based drug delivery systems for vaginal therapy

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Introduction

To achieve the efficient and safe local vaginal therapy of bacterial infections, there is a need for a delivery system that is optimized for vaginal administration. Liposomes have the ability to incorporate poorly soluble substances, while chitosan is known for its excellent mucoadhesive properties as well as an intrinsic antibacterial effect. The combination of the two delivery systems represents a novel and promising approach in optimization of localized vaginal therapy. The optimization of liposomes in chitosan-based delivery systems was determined under the simulated vaginal conditions, and the antibacterial susceptibility of the delivery systems was tested.

Aim

The aim of this study was to explore the potential of chitosan by optimizing the following chitosan-based mucoadhesive drug delivery systems; liposomes-in-hydrogel and chitosan-coated liposomes.

Method

Liposomes were prepared by the thin film hydration method followed by vesicle size reduction by sonication. The liposomes were characterized in respect to their vesicle size, polydispersity and zeta potential. Liposomal suspensions were incorporated in chitosan hydrogels[1] resulting in the liposomes-in-hydrogel formulations, which were further assessed for their texture properties in the presence and absence of mucin, vaginal fluid and semen fluid simulants. Liposomes were also coated[2] with chitosan (0.01, 0.03, 0.1, and 0.3 %, w/v, respectively). The mucoadhesive properties of chitosan-coated liposomes (0.1 and 0.3 %, w/v) were determined through their binding efficiency to mucin compared to non-coated liposomes. An antibacterial susceptibility test of both chitosan-coated liposomes and chitosan hydrogel (0.1 and 0.3 %, w/w) was performed on selected bacterial strains of *Staphylococcus spp.*

Results

Liposomes were in the desired size range (200 nm). The incorporation of liposomes in chitosan hydrogels improved the texture properties of hydrogels, even in the presence of vaginal fluid simulants, indicating that liposomes-in-hydrogel is suitable for vaginal administration. An increase in vesicle size and zeta potential of chitosan-coated liposomes indicated a successful coating. The mucoadhesive properties of chitosan-coated liposomes were slightly increased when the higher concentrations of chitosan were used in the coating. Further, the antibacterial effect of chitosan-coated liposomes (0.03, 0.1 and 0.3 %, w/v) and chitosan hydrogels (0.1 and 0.3 %, w/w) on *Staphylococcus epidermidis and Staphylococcus aureus* was successfully confirmed.

Conclusion

The mucoadhesive properties of chitosan makes it a good excipient for vaginal drug delivery systems, improving the residence time at vaginal site. The antibacterial effect of chitosan depend on the type of the bacteria as well as the formulation.

Acknowledgement

We are grateful to Chitinor (Tromsø, Norway) for providing chitosan.

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Polysaccharide based Particles

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Introduction

The fruiting bodies of mushrooms are sources of biodegradable polysaccharide material that may have potential in drug delivery systems. The fungal cell wall contains several types of polysaccharides forming a complex and rigid network[1,2]. Amongst the different types of polymers found, there is special interest in the -glucans due to their potential abilities to activate macrophages through the dectin-1 receptor. Fungal -glucans are typically composed of (1→3)-and/or (1→6)-linked -d-glucose monomers. However, their fine structure, including the nature of the backbone and side chains, polymer length and distribution of side chains, varies and may affect the activity. In addition, it appears that this activity is restricted to polysaccharides formulated as particles[3]. Thus, in order to determine the activity of isolated fungal polysaccharides, there is a need to develop a method on how to prepare particles from the material.

Aim

To isolate fungal cell wall polysaccharides and develop a small scale method for preparation of particles from characterized fractions.

Methods

Polysaccharide fractions are isolated from lyophilized fungal fruiting bodies by extraction with water and diluted sodium hydroxide after several steps of pre-extraction and enzymatic treatment, in order to remove lipid- and alcohol-soluble material. Particles are prepared by controlled precipitation in liquid, by spray drying or by milling. Particle size is characterized by laser diffraction. Size and morphology are visualized by scanning electron microscopy (SEM).

Results

Water insoluble -glucans are isolated by extraction with hot alkali. Water-soluble fungal polymers tend to comprise -glucans, -mannans or -galactans. Particles are formed by milling with an ultra turrax dispersed in water or ethanol and cooled by an ice bath.

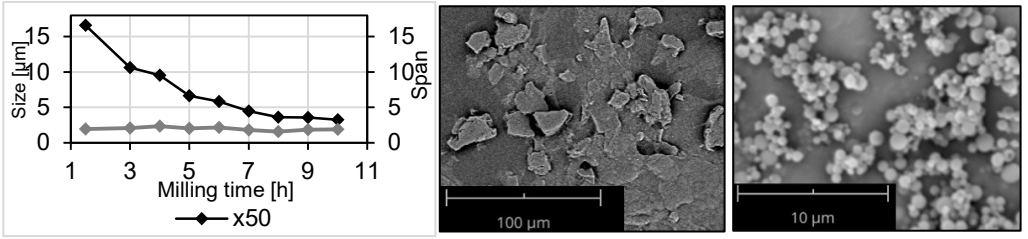

Figure 1. *Left: Milling of the water insoluble extract of Albatrellus ovinus in water; middle: SEM picture of the water insoluble extract of Albatrellus ovinus in ethanol, milled for 3 h; right: water-soluble fraction from baker's yeast, spray dried by Büchi Mini Spray Dryer B-290*

Conclusion

The method to be selected depends largely on material composition, solubility and behavior. Particles of soluble fractions can be prepared by spray drying, while the insoluble fractions have to be reduced in size by a milling process.

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Transdermal patches for hand osteoarthritis

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Introduction

Hand osteoarthritis (HOA) is a common disease, associated with pain and disability [1]. At present there are no disease modifying drugs available for this condition. Recent discoveries, starting with the identification of common genetic variants associated with increased risk for severe hand OA [2] and subsequent functional analyses [3-5] have indicated a central role for retinoic acid in the development of hand osteoarthritis. The genetic risk variants are associated with decreased bioavailability of retinoic acid [2, 3, 5]. The gene expression of the most common form of retinoic acid is also reduced in osteoarthritic joints, and apparently baseline expression of retinoic acid is particularly low in hand joints compared with knee or hip joints [5]. Applying retinoic acid or increasing retinoic acid concentrations ameliorates experimental arthritis *in vitro* and in animal studies [4, 6, 7]. This has led to the current study hypothesis that increasing the bioavailability of retinoic acid in hand joint tissues might affect the hand osteoarthritis process [8].

Retinoic acid derived drugs are widely used but are associated with considerable toxicity.

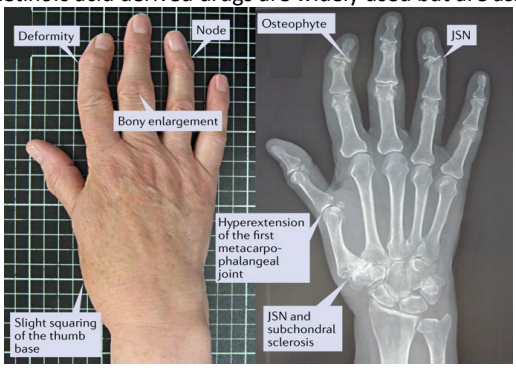

Figure 1 *Characteristic features of HOA*

Another and more feasible way of increasing the bioavailability of retinoic acid in hand joint tissues would be to block the breakdown of retinoic acid by inhibiting the CYP26 pathway, the main catabolic pathway of retinoic acid. A number of CYP26 inhibitors (RAMBAs) have been investigated in other diseases and have shown an ability to increase tissue concentrations of retinoic acid and even to ameliorate surgically induced osteoarthritis in pig knuckles [4, 9, 10]. Some RAMBAs are small molecules that could be suitable for transdermal application into hand joint tissues.

Aim

The project aim is to develop a transdermal patch, a multilayered patch that will be made up of an impermeable layer, the drug layer, and a release liner. The drug will have to have effective controlled drug release, and be compatible with the other layers in the patch.

Designing a medicinal patch for local treatment of osteoarthritis by increasing the amount of retinoic acid in the joints

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Sulforaphane decreases Inflammation in retinal pigment epithelial cells suffering from mitochondrial damage

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Introduction

The retinal pigment epithelium (RPE) is a single-cell layer at the back of the retina that has vital importance for the upkeep and survival of photoreceptors. Dysfunctional RPE cells, suffering from high oxidative stress levels and chronic inflammation are a key factor in the development of age-related macular degeneration (AMD) – the leading cause of blindness in the elderly. Previous research has shown that mitochondria are damaged in the RPE of AMD patients.[1] Damaged mitochondria can cause aberrant ROS production, an activation of inflammation, and apoptosis. More than 80% of AMD patients today have no viable treatment option, making it critically necessary to find compounds that can prevent mitochondrial damage-associated inflammation and oxidative stress in RPE cells. One possible candidate might be Sulforaphane, an activator of Nrf2, a master regulator of cell survival responses to endogenous and exogenous stressors.[2]

Aim

To investigate the effect of Sulforaphane on inflammation in RPE cells with mitochondrial damage.

Method

An immortal RPE cell line and induced pluripotent stem cell-derived RPE cells (iPS-RPE) were pretreated with different concentrations of Sulforaphane for 18 hours before exposure to Antimycin A, an inhibitor of complex III of the electron transport chain. Antimycin A exposure causes a loss of mitochondrial membrane potential and significant toxicity in RPE cells.[3] After 24 hours of Antimycin A exposure, medium samples were collected and the levels of secreted IL-6 and IL-8, as well as, lactate-dehydrogenase were measured. Cellular viability was determined with the MTT assay.

Results

Antimycin A caused a robust increase in IL-6 and IL-8 levels secreted by RPE cells. Pretreatment with Sulforaphane, dose-dependently decreased the release of the pro-inflammatory cytokines. Sulforaphane did not significantly increase toxicity caused by Antimycin A exposure. Results were comparable in both types of RPE cells studied.

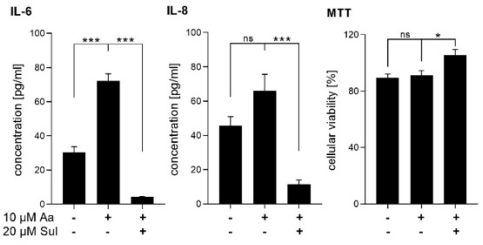

Figure 1 *Effect of Antimycin A exposure with and without a 1h pretreatment with Sulforaphane on inflammation and cellular viability in cultured human RPE cells.*

Conclusion

Sulforaphane is anti-inflammatory in RPE cells suffering from mitochondrial damage. Further experiments will confirm the role of Nrf2 in the observed anti-inflammatory effects.

Acknowledgement

The authors wish to thank the North-Savo Cultural Foundation for their support. Professor Katriina Aalto-Setälä is acknowledged for generating the original iPSC line.

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Physicochemical stability of lipid-loaded mesoporous magnesium carbonate studied by ^{1}H nuclear magnetic resonance spectroscopy

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Introduction

Solidification of lipid-based formulations (LBFs) have been introduced as a way to reduce issues relating to e.g. costly manufacturing associated with liquid formulations. Different techniques of varying complexity have been used to produce the solid LBFs, including adsorption of LBFs onto mesoporous carriers such as the silica-based Neusilin[1]. However, incomplete desorption of LBFs during storage has been observed for these formulations[2]. There is therefore a need to develop alternative carrier-based solidification systems.

Aim

To evaluate the physicochemical stability of a lipid-loaded mesoporous magnesium carbonate (MMC) during long-term storage using ^{1}H nuclear magnetic resonance spectroscopy (^{1}H NMR).

Method

A medium-chain triglyceride, Captex 355, was loaded into 20nm pore sized MMC in a 1:1 w/w ratio (CAP-MMC) using physical adsorption. One batch was prepared for each time point, and samples were analysed immediately after preparation, and after 1, 2, and 3 months of storage under dry conditions (<5%RH/25^{o}C). Pure Captex was used as reference. Any lipolytic products formed were extracted, and were analysed using solution ^{1}H NMR. The relative molar percentage of each component was then determined based on equations developed by Nieva-Echevarría *et al*[3].

Results

An initial decrease in triglyceride content, and subsequently an increase in free fatty acids and monoglycerides, was observed in the CAP-MMC compared to the Captex alone (Fig. 1). This is likely a consequence of basic ester hydrolysis of the triglycerides when incorporated into the MMC, catalyzed by water adsorbed on the surface of the MMC.

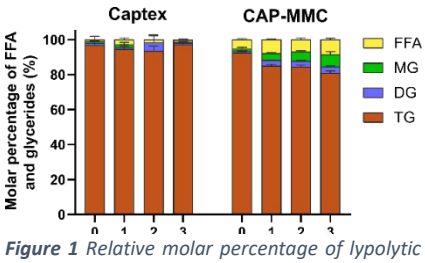

Figure 1 *Relative molar percentage of lypolytic components in Captex and CAP-MMC stored during three months determined using ^{1}H NMR (n=3). FFA: Free fatty acids, MG: Monoglycerides, DG: Diglycerides, TG: Triglycerides.*

Conclusion

^{1}H NMR was used to study changes in CAP-MMC occurring during storage, displaying an initial degradation of the triglycerides. To what extent the changes in lipolytic composition affects the in vivo performance of the lipid-loaded MMC is yet to be evaluated.

Acknowledgement

The research received financial support from the Erling Persson Family Foundation (Grant No. XX).

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Biopharmaceutical assessment of amorphous solid dispersions of ABT-102

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Introduction

Pharmaceutical industry is struggling with a large fraction of poorly water-soluble drug compounds, which necessitates enabling formulations to overcome the inherent restricted bioavailability of these compounds. For this reason, increasing attention is being given to *in vitro* predictive tools potentially allowing for a performance ranking of different formulations at an early stage of drug development. The PermeaLoop®, a novel tool for small-scale combined dissolution-/ permeation-testing, has earlier been demonstrated promising in this respect[1]. It has an increased area-to-volume ratio that may secure a dynamic interplay between dissolution and permeation, where dissolution is the rate-limiting step, a scenario, which *in vivo* exactly is the case for BCS Class II compounds.

Aim

To investigate the combined dissolution-/permeation performances of ABT-102 amorphous solid dispersions (ASDs) *in vitro* using the PermeaLoop®.

Method

The combined dissolution and permeation of ABT-102 amorphous solid dispersions (melt-extrudates, formulations A, B, and F) were examined, using the PermeaLoop® setup. The cumulative amount of drug appearing in the acceptor over time was quantified by HPLC, and the flux profiles compared to *in vivo* oral absorption data.

Results

Our PermeaLoop® results indicate that the flux generated by formulations A and B was similar, while the flux arising from formulation F was superior to both, formulations A and B. This correlated to some extent with *in vivo* bioavailability, deviating with only formulation B. Furthermore, PermeaLoop®-results obtained with a variant of formulation F, containing 10% (m/m) drug content, appear to indicate an inverse correlation between drug load and the *in vitro* permeation of ABT-102.

Conclusion

The *in vitro* flux of the ASDs partially correlated with *in vivo* results, though the results indicate an inverse correlation between the drug load and the *in* vitro permeation.

In order to investigate this phenomenon further, the impact of varying doses of a given formulation (formulation F) is currently being tested.

Acknowledgement
We would like to thank NCE Formulation Sciences at AbbVie GmbH & Co. KG for support in terms of permission to work in their laboratories, providing the ASDs as well as scientific discussions.

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Design, preparation, characterization and stability of docetaxel-containing liposomes

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Introduction

The cancer burden is rising, and WHO reported a cancer incidence of 18.1 million and 9.6 million deaths from cancer worldwide in 2018.[1] Thus, the need for improved therapy options is increasing. Docetaxel (DOC) is an anticancer drug with well-described clinical potential in treatment of e.g. breast-, lung- and head- and neck cancers. However, the marketed product has limited stability and contains excipients (polysorbate 80 and ethanol) observed to cause allergic reactions.[2] Therefore, an alternative i.v. DOC-formulation is desired. Liposomes were judged a suitable delivery system, not only to overcome the stability and to avoid the side-effect causing excipients, but also to help dissolve this poorly water-soluble drug.

Aim

The aim of the study was to investigate the impact of lipid composition and PEGylation on the liposome characteristics; size, PDI, DOC-entrapment and zeta potential, as well as storage stability and drug release.

Method

The liposomes were prepared by the thin-film method with SPC as the main lipid. VPGs were formed by dual asymmetric centrifugation (DAC) using the SpeedMixer™ DAC 150.1 FVZ-K.[3] The VPGs were diluted with water in the same machine to form liposome dispersions. Unentrapped drug was removed by centrifugation (3000 rpm, 20 min, 25 °C) and DOC remaining in the supernatant quantified by HPLC. The liposomes size, PDI and zeta potential were measured using the Zetasizer. The drug release was first studied using Franz diffusion cells, but later by using a dialysis bag setup.

Results

DOC-liposomes suitable for i.v. administration was obtained using standard DAC-settings; 3500 rpm, 30 min to form VPGs and 3500 rpm for 3 min when diluted to form liposome dispersions. The liposomes size was around 200 nm with a PDI < 0.15. Of the two PEGs applied; DSPE-PEG750 and DSPE-PEG2000, only the later gave a significant increase in liposomes size, and this increase was more extensive when positively charged DOTAP (10% w/w) was added; 280 nm and 355 nm, respectively. SPC-liposomes with DOTAP as well as the two PEGylated without DOTAP showed best drug entrapment directly after preparation. But, after 4 weeks of storage more than 50% of the drug was released from these liposomes, whereas the DOTAP containing PEGylated liposomes retained most of the drug (> 90%) after 4 weeks. Preliminary data from drug release studies shows that the final set-up using dialysis bags gave the more reliable results and was easier than the Franz diffusion set-up. Apparently, both DOTAP and PEG give a slower drug release.

Conclusion

The effect of PEGylation was found to be highly sensitive to the liposomes charge, and appeared to improve the drug entrapment positively only for the neutral SPC-liposomes at the time of production. However, the positively charged DOTAP-PEG750-liposome formulation was most promising concerning stability and the drug entrapment seen after 4 weeks of storage.

Acknowledgement

The author would like to thank Nordic POP for financial support.

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In vitro biopharmaceutical characterization of acyclovir and its co-crystals with organic acids

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Introduction

The majority of new drug compounds are classified as poorly soluble chemical entities. A standard formulation of such a drug compound will in most cases not result in sufficient systemic exposure and therefore different strategies need to be considered, e.g. the synthesis of different salts. A more recent strategy is the formation of co-crystals, which are multicomponent crystalline solids, which may improve physicochemical and biopharmaceutical properties such as solubility, dissolution rate, and bioavailability. This has been demonstrated by Bruni et al. (2013), who successfully prepared co-crystals of the well-known antiviral drug, acyclovir, using glutaric and fumaric acid as co-formers.[1] In order to investigate such formulations for their ability to improve bioavailability, parameters such as solubility, intrinsic dissolution rate (IDR) and permeability can be determined.

Aim

To investigate if co-crystallization improves the biopharmaceutical characteristics of acyclovir by determining solubility, intrinsic dissolution rate (IDR) and *in vitro* permeation.

Method

The acyclovir co-crystals were prepared by dry grinding of acyclovir and glutaric acid or fumaric acid, respectively, (molar ratio 1:1) for 3 hours at room temperature, using a Retsch MM2000 ball mill. The co-crystals were characterized by differential scanning calorimetry and powder X-ray diffraction. The solubility of pure acyclovir and physical mixtures of acyclovir and co-formers as well as the co-crystals, were determined at 37°C. The IDR was determined as well. Both the solubility and IDR were determined in three different media; 0.1 M HCl, water and PBS pH 7.4, and acyclovir was quantified by UV-spectrophotometry or HPLC-UV. The permeability was investigated for the respective solutions using the PermeaPad® 96-well plate. The cumulative permeated amount of acyclovir was quantified by HPLC-UV.

Results

The solubility of acyclovir was found to be increased in 0.1 M HCl compared to water and PBS pH 7.4 as expected by the molecular structure of the molecule. The IDR also increased correspondingly as expected.[2]The presence of glutaric acid increased the solubility of acyclovir in 0.1 M HCl significantly while only a small increase was observed in PBS pH 7.4 and water. The presence of glutaric acid also showed an increased IDR of acyclovir in 0.1 M HCl. The presence of fumaric acid didn't change the solubility or the IDR of acyclovir in any of the media. The apparent permeability value of acyclovir was not changed in the presence of glutaric acid or fumaric acid.

Conclusion

It can be concluded that both the solubility and IDR of acyclovir are increased in acidic medium. In this medium, the presence of glutaric acid also led to a higher solubility and IDR of acyclovir. The permeability of acyclovir remained unchanged in the presence of any of the co-formers.

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Synthesis of a novel sulfenamide prodrug of sorafenib and *in vitro* evaluation of sorafenib

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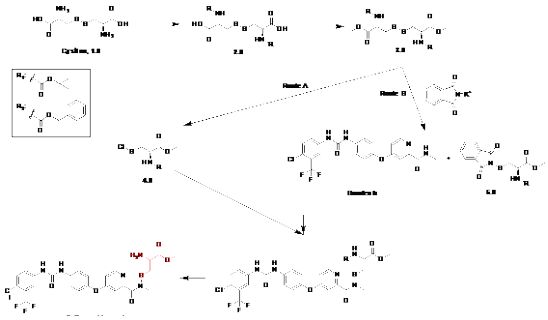
Introduction

Sorafenib, a chemotherapeutic agent, is commercially available in Denmark as an oral tablet formulation of the tosylate salt for treatment of renal cell carcinoma, hepatocellular carcinoma and thyroidal cancer[1]. In the biopharmaceutical classification system, sorafenib is classified as a BCS class II drug with high permeability and poor solubility[2]. Bioavailability studies show a wide difference in the relative bioavailability after oral drug administration compared to an oral solution. The relative bioavailability varies from values of 38-49% Absolute bioavailability has not been determined. Furthermore, high fat meals were reported to decrease the bioavailability by 29%[3]. An attempt to overcome this problem would be the synthesis of a novel sulfenamide prodrug of sorafenib, thereby increasing the solubility of the administered compound.

Aim

To synthesize the sulfenamide prodrug of sorafenib and perform *in vitro* biopharmaceutical tests.

Method



Scheme 1: The general plan for synthesis of a sulfenamide prodrug.

A synthetic route for the synthesis of a sulfenamide prodrug with cysteine as the promoiety was planned (**scheme 1**). Cysteine was protected, and the disulfide bridge was cleaved, forming either a phthalimide or chlorinated intermediate, which should work as a transferring group for the promoiety. Sorafenib was deprotonated at the desired amide position by a strong base thereby enabling the attachment of the promoiety.

In vitro studies of sorafenib included development of HPLC methods compatible for studies in Finland and Denmark, respectively. Solubility studies of sorafenib in water and FaSSIF and FeSSIF both with and without 1 %(m/V) TPGS surfactants were performed at 37°C. Measurements were made after 16, 24 and 96 hrs. The partitioning coefficient will be measured. Permeability studies of Sorafenib in both FaSSIF and FeSSIF media with TPGS surfactants in the donor media will be performed.

Results

Synthesis of a novel sulfenamide prodrug of sorafenib with the synthetic route planned for this study was not successful in the given time of the study. It is regarded as highly unlikely that the prodrug can be synthesized by this method. Solubility, logP value and results of permeation studies are not ready at this point but most likely will be for the poster under the pre-requisite that the HPLC methods are compatible with the different media types.

Conclusion

A sulfenamide prodrug cannot be synthesized with the synthetic route proposed in this study. A different route of synthesis must be planned for potential future studies.

Acknowledgement

The author would like to thank Nordic POP for financial support during this project.

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PermeaPad® Plate for development of buccal formulations containing nicotine

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Introduction

It is common knowledge that smoking causes serious health problems. Smoking is a significant cause of morbidity and mortality causing approximately 6 million deaths each year[1]. Nicotine replacement therapy (NRT) is a way to quit smoking. For this reason, more effective nicotine formulations for NRT are desired. Developing new formulations is connected to high costs and time-consuming experiments. PermeaPad® plate is a 96-well plate permeation set-up that comprises the biomimetic barrier PermeaPad® and was developed for high throughput screening. By that, the plate offers a possibility to test new NRT formulations in the early development with reduced time consumption and costs.

Aim

The aim of this study was to investigate if the PermeaPad® plate can be used in formulation development of buccal formulations. For this, the apparent permeability (P_{app}) of nicotine from different solutions of nicotine in buffer and the mouth spray formulation 'Quick Mist' was investigated.

Method

A set of permeation experiments was conducted using the PermeaPad® plate set-up where the concentrations and pH varied. In more detail, to investigate if pH influences the P_{app} of nicotine, donor solutions with 1 mg/mL and a pH range of 6.4 to 9.0 was used. To investigate if the donor concentrations influences the P_{app}, three donor concentrations (0.1, 1 and 7 mg/mL) were investigated at pH 6.8 and 9. In all experiments the acceptor was 0.1 M phosphate buffered saline (PBS) pH 7.4. Donor solutions were prepared in either 0.1 M PBS pH 6.8 and 8 or in borate buffered saline pH 9. For the experiments concerning 'Quick Mist', the 'Quick Mist' formulation was diluted in either 0.1 M PBS pH 6.8 or highly purified water (pH 8.9) to achieve 1 mg/mL nicotine. All permeation experiments were carried out at 36 ± 0.5 °C under shaking (300 rpm). Acceptor samples were taken every hour for 5 hours. Donor samples were taken at 0 and 5 hours.

Results

At a constant donor concentration of 1 mg/mL, nicotine flux and P_{app} increased when pH was increased. When varying the donor concentrations (0.1, 1 and 7 mg/mL), the nicotine flux increased but the P_{app} stayed constant at both tested pH values. From the experiments with 'Quick Mist', it was observed that the P_{app} at pH 6.8 and 8.9 showed the same P_{app} value as seen with the respective nicotine solutions at pH 6.8 and 8.9.

Conclusion

The PermeaPad® plate can be used in the early development of buccal formulations. This has been demonstrated for the effects of formulation, concentration and different pH values on drug permeation. Such *in vitro* experiments reduce the time and cost needed.

Acknowledgment

I would like to thank Tina Christiansen and Cecilie Hjorth Ruberg for the help in the laboratory. I would also like to thank for the support I received from all the nice people at Fertin Pharma A/S.

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High throughput microplate-based dissolution-/permeation-study of commercial nano-/micro-formulations of poorly soluble Fenofibrate.

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Introduction

Permeability and solubility are very important parameters in discovery and development of oral drugs. Many new drug candidates are BCS class II drugs, i.e. they show decent permeability, but poor solubility properties. Typically, these drugs are investigated on models like Caco-2, PAMPA or PVPA assays. But PermeaPad® Plate and PlainPlate can be used to investigating the permeability too. The PermeaPad® Plate uses the PermeasPad® membrane and the PlainPlate uses a dialysis membrane. Permeapad® is an artificial barrier consisting of lipids and a support sheet. PermeaPad® is designed to imitate the membrane in the gastrointestinal tract and to make it possible to efficiently and reliably investigate the passive permeability of drugs. PermeaPads® is a more cost effective and easy in use compared to other similar models [1].

Aim

The aim of the current study was to investigate the dissolution-/permeation-behavior of two commercial formulations of the poorly water soluble drug fenofibrate across a biomimetic barrier and a dialysis membrane in 96-well plate format (PermeaPad® plates and PlainPlates). A nanoparticle-based and a microparticle-based capsule formulation of fenofibrate were employed and different donor media were tested.

Method

For the study, high throughput screening with two compartment 96-wells PermeaPad® plates and PlainPlates was used, with the bottom compartment used as donor and the upper compartment used as acceptor. Suspensions of fenofibrate tablets and capsules, respectively, were freshly made. The permeation for both formulations was tested in three donor media: phosphate buffer, FaSSIF and FeSSIF [2]. Acceptor medium was phosphate buffer with 1% Vitamin E TPGS. Samples were taken from the acceptor after 8 and 24 hours. For quantification of the amount fenofibrate permeated, UHPLC with UV/VIS-detection was used.

Results

Clear differences in the cumulative amount fenofibrate permeated were observed: The tablet formulation with the nano particles of fenofibrate yielded a larger amount of permeated drug, than the capsule formulation with the micro particles. The same pattern was seen for both the barriers. But in general, more drug was permeating across the dialysis membrane than the PermeaPad® barrier. Despite the substantial increase in apparent solubility in FaSSIF and FeSSIF, for the PlainPlate there was no significant difference in cumulative amount permeated in the different media, whereas for the PermeaPad® plate, there was slightly more permeated at 8 hours in phosphate buffer as compared to the biomimetic media.

Conclusion

The tablet formulation containing the nano-particles yielded a higher amount of fenofibrate permeated than the microparticle-containing capsule formulation irrespective of barrier and donor medium used.

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Manipulation of tablets vs compounded mixture – How should we adjust the prednisolone dose in paediatric care? Outline of an ongoing Master project

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Introduction
Children require individualization of drug dosage to a larger extent than adults, as dosing is often weight-based. This can be difficult to achieve when fixed dose preparations, such as tablets, in many instances are the only licenced option available. Furthermore, children often require lower doses than adults, but frequently there is a lack of licenced drug with paediatric dose. Because of this, it might be necessary to manipulate the dosage form, e.g. dispersing tablets in water and extracting a partial dose. Manipulation of drugs is to a great extent not an evidence based practice, and the accuracy that can be obtained from different tablet types, through different manipulation methods, is largely unknown.
Extemporaneous compounding of mixtures for children by the hospital pharmacy represents an alternative to the bedside *ad hoc* manipulation of drugs, but comes with its own challenges concerning cost, stability and quality control. As of now, formulations may also vary between pharmacies. However, a joint European paediatric formulary is underway[1], an effort that could improve the quality of extemporaneous formulations for children in Europe.
Prednisolone is a substance that is manipulated in the paediatric hospital ward. It is low in content, and has low in aqueous solubility. It is also available as an extemporaneously compounded mixture from the hospital pharmacy.

Aim: To investigate the dose accuracy and dose precision attained after manipulation of two brands of prednisolone tablets – and to identify a *best method* of manipulation. This method will be compared with results using pharmacy compounded suspensions to establish the optimal way of adjusting the dose of prednisolone in paediatric care. Results from the manipulation of prednisolone will be compared with results from previous studies of ASA and warfarin, moving us closer towards establishing factors of importance to predict the appropriateness of manipulation of tablets in general.

Method : Tablets from each strength (2.5 mg, 5 mg, 20 mg) of the two different prednisolone tablets available are dispersed in water using different methods of manipulation. A partial dose (10% or 20%) is then withdrawn and analysed by UHPLC-UV. As an alternative manipulation method, tablets will also be split and dispersed in oral syringes. The dosing accuracy and precision of the two tablets will be compared with each other, with compounded mixtures and with the results from ASA and warfarin sodium.

Results: Previous studies have investigated the ability of various tablet types and manipulation methods to give accurate fraction doses from tablets containing the slightly soluble substance acetylsalicylic acid[2] (ASA) and the much better soluble warfarin sodium[3]. For the latter substance, acceptable results were attained regardless of tablet type and manipulation method. However, the opposite was the case for ASA. Dose accuracy varied markedly between tablet types and manipulations methods. The working hypothesis is that prednisolone being poorly soluble will show similar results to ASA.

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Development and evaluation of mesoporous silica nanocomposites as nanoantibiotics against *Escherichia coli* in *Drosophila melanogaster*

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Introduction': The rise of antimicrobial resistance (AMR) as a top ten global health threat in 2019 according to the WHO has led the path for alternative strategies of treatment against bacterial infections. Recent studies have revealed the potential of antibacterial nanoparticles to treat bacterial infections by inducing synergistic antibacterial activity by different combinatorial therapy approaches.

Aim: This study focuses on the development and investigation of the antibacterial activity of a mesoporous silica (MSN)-based nanocomposite structure denoted a "nanoantibiotic" (NAB). The antibacterial potential of NAB was evaluated through its *in vitro* cytotoxic effect and bacterial growth inhibition against ampicillin-resistant *Escherichia coli* (Amp-res *E. coli*). *Drosophila melanogaster* was used to evaluate *in vivo* localization and antibacterial activity of NAB in its digestive tract, with the prospect of using this model to simulate human intestinal diseases in future studies.

. **Method**

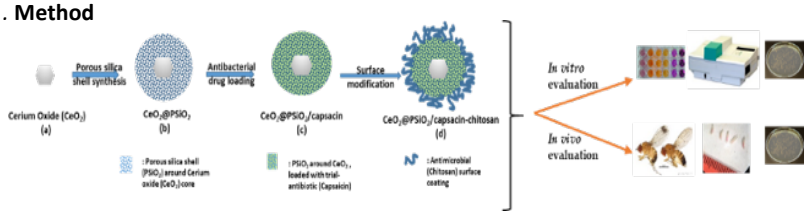

Figure 1. Development as well as in vitro and in vivo evaluation of NAB

NAB are composed of cerium oxide (CeO_2) nanoparticles as antioxidant core with a porous silica ($PSiO_2$) shell. Unstable cerium oxide cores are stabilized by the coating of porous silica shells, while simultaneously introducing drug carrying capacity. Exploiting this fact, capsaicin, was loaded into the porous silica shell and the NAB was surface coated with chitosan as another antibacterial construct and pH-responsive drug release. *In vitro* studies included (a) cytotoxicity tests of NAB and its components on human epithelial colorectal adenocarcinoma cells, (b) growth inhibition of Amp-res *E. coli* by NAB and its components through turbidity measurement and colony counting. *In vivo*, (a) the localization of NAB was evaluated by replacing capsaicin with DiI (fluorescent dye) to visualize NAB/DiI in the intestines of the larvae of *D. melanogaster,* while (b) the antibacterial activity of NAB was evaluated by feeding Amp-res *E. coli*-infested food to larvae and treating with NAB-supplemented food. Later, the larvae were processed for plating and colony counting.

Results: Materials characterization confirmed the core-shell structure of NAB. Capsaicin release from NAB in two different buffers over a period of 24 hours was observed to be pH controlled by chitosan as hypothesized. Bacterial growth inhibition results from *in vitro* tests for 24 hours showed significant growth inhibition by the composite design of NAB, especically in comparison with CeO_2. The *in vivo* study showed efficient localization of NAB/DiI in the intestines of *D. melanogaster* larvae after 2 hours of feeding. The synergistic *in vivo* antibacterial activity of NAB was significant in comparison with free capsaicin after 2 hours of treatment.

Conclusion: The observed growth inhibition results from *in vitro* experiments revealed that multiple antibacterial constructs in the NAB system improves its antibacterial activity in comparison with its components. The visualization and effective antibacterial activity of NAB from the *in vivo* results suggests the possibility of exploring NAB functionality against human intestinal diseases in the animal model.

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Development of Pickering-stabilized lipid-based drug delivery systems

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Introduction

A majority of new drug candidates are poorly water-soluble, which is associated with low absorption after oral administration.[1] Many strategies to enable oral delivery of such drug compounds have been developed, one approach being lipid-based formulations (LBFs). Recently, emulsions stabilized by solid particles at the oil-water interface, so-called Pickering emulsions, have gained increased interest.[2] Advantages of these formulations include high storage stability and possibilities of controlled and targeted drug release. Here, we study the potential to stabilize LBFs with silica nanoparticles to develop Pickering-stabilized lipid-based drug delivery systems.

Aim

To develop Pickering-stabilized lipid-based drug delivery systems suitable for oral delivery of poorly water-soluble drugs.

Method

Long-chain LBFs type I, II and IIIa loaded with a poorly water-soluble model compound (fenofibrate) were used as oil-phase and silica suspension as the water-phase. Stearic acid was added to the oil-phase to explore synergistic emulsion stabilization with silica nanoparticles. Oil-in-water (o/w) emulsions were produced by ultrasonication followed by high-pressure homogenization. Droplet size of produced emulsions was measured by dynamic light scattering. Emulsion morphology of Pickering-stabilized LBF type II was imaged by cryo-scanning electron microscopy (cryo-SEM).

Preliminary Results

All three LBF types I, II and IIIa were successfully stabilized by stearic acid and silica nanoparticles with submicron droplet sizes of produced Pickering emulsions type II and IIIa (Fig. 1a). Cryo-SEM of Pickering-stabilized LBF type II showed silica particles at the oil-water interface (Fig. 1b).

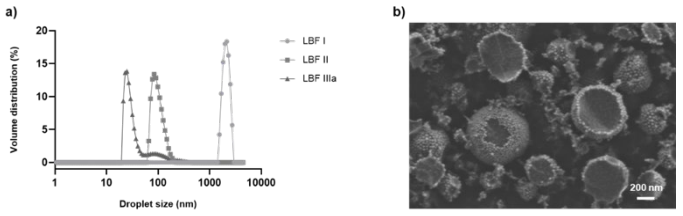

Figure 1. Droplet size distribution of Pickering-stabilized LBF type I, II and IIIa (a) and corresponding cryo-SEM of LBF type II (b).

Conclusion

Pickering-stabilized LBF type I, II and IIIa loaded with a poorly-water soluble compound have been developed. Future work will focus on *in vitro* characterization (digestion, release and stability) of these formulations.

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Coherent Anti-Stokes Raman Scattering (CARS) microscopy imaging of berberine-loaded liposomes

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Introduction

Berberine (BBR) is a quaternary isoquinoline alkaloid extracted from native plants. BBR has been shown to have anti-inflammatory, anti-diabetic and anti-atheroclerosis effects[1]. Oral route is the most convenient way for administration of drugs. However, BBR has very low oral bioavailability because of a poor aqueous solubility and permeability, multidrug resistance protein efflux and metabolic stability[2]. Such limitations associated with a poor oral bioavailability of BBR could be overcome by nanoformulating BBR to liposomes. The liposomes ranging from 100-500 nm in size have an unique ability to enhance the permeability of drugs across the enterocyte barriers and to stabilize drugs[3].

Aim: To investigate ethanol-injection and film hydration methods for generating BBR-loaded liposomes, and to gain understanding of the formation and internal structure of such liposomes. The liposomes are ultimately intended for the oral treatment of hypercholesterolemia.

Method

BBR (Sichuan Weikeqi Biological Technology Co., Ltd., China) was used as an active ingredient, and hydrogenated soy phosphatidyl choline (HSPC), distearoyl phosphatidylglycerol (DSPG), alpha-tocopherol (TP), and sodium deoxycholate (SDC) were used as liposome forming agents. The BBR-loaded liposomes were prepared by an ethanol-injection method and thin film-hydration method. Liposomes were characterized by Zetasizer NANO ZSP (Malvern instrument Limited, UK) and Coherent Anti-Stokes Raman Scattering (CARS) microscopy (Leica TCS SP8 CARS, Germany).

Results: The BBR-loaded liposomes prepared by an ethanol-injection method presented the average size ranging from 82.3 nm to 133.6 nm. The corresponding liposomes prepared by a thin film-hydration method exhibited as multivesicular, multilamellar and unilamellar vesicles (Figure 1). The polydispersity index (PDI) values for the liposomes obtained with both fabrication methods were less than 0.3 suggesting that the liposomes were very homogenous in size.

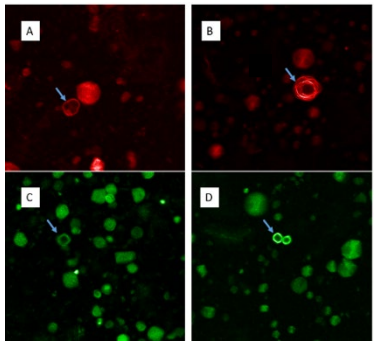

Figure 1. *CARS micrographs of BBR-loaded liposomes fabricated by thin film-hydration.*

Conclusion

An ethanol-injection and thin film-hydration are feasible methods for generating BBR-loaded liposomes. The active-loaded liposomes with an uniform size and PDI can be fabricated.

Acknowledgement

This study was funded by the Nordic POP researcher network, Estonian national research projects (IUT34-18 & PUT1088), and the EduShare and Erasmus Plus Programme.

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Comparative bioavailability study of solid dispersions of celecoxib with mono- and diacyl phospholipids

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Introduction

Poor aqueous solubility of new drug candidates is one of the major challenges in the pharmaceutical development pipeline. Solid phospholipid dispersions are a recent enabling formulation approach to increase the aqueous solubility of such drugs. The difference between diacyl-phospholipid and monoacyl-phospholipid (i.e. lyso-phospholipid) used as matrix in solid phospholipid dispersions has previously been studied *in vitro*. Here, monoacyl-PL formulations performed best in terms of increase in the (apparent) aqueous solubility of the drug and drug dissolution rate (1). However, an *in vitro* dynamic permeation study showed no difference between the two types of phospholipids (2).

Aim

The aims of the study were to investigate: a) if there is a difference in bioavailability when preparing solid phospholipid dispersion with monoacyl-phospholipid or diacyl-phospholipid, respectively, b) to investigate how different drug-to-phospholipid ratios affect the bioavailability of celecoxib (CXB).

Method

Solid phospholipid dispersions with different CXB-to-phospholipid ratios were prepared by rotary evaporation. The different CXB-to-phospholipid ratios tested was: CXB:PC 1:50, CXB:PC 1:100, CXB:PC 1:250, CXB:L-PC 1:50, CXB:L-PC 1:100 and CXB:L-PC 1:250. The formulations were dispersed in water before administration and the rats were administered a dose corresponding to 10 mg/kg body weight of CXB. Plasma samples were withdrawn over a period of 30 h and analyzed by UPLC-UV.

Results

The pharmacokinetic parameters were obtained from the plasma concentration profiles: maximum plasma concentration (C_{max}), the time to reach the maximum concentration (t_{max}) and the area under the curve ($AUC_{0\text{-}30\ h}$) by a non-compartmental analysis. The AUC showed no significant difference between the two types of phospholipid in accordance with the dynamic *in vitro* permeation study (2).

Conclusion

It can be concluded that no significant difference in the *in vivo* absorption of CXB was seen between the formulations with the two types of phospholipid.

Acknowledgement

The author would like to thank Janssen Pharmaceutics for conducting the *in vivo* study.

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SNEDDS for targeted oligonucleotide delivery to inflamed intestinal tissue

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Introduction
Inflamed intestinal tissue in inflammatory bowel disease is a result of inappropriate and sustained activation of the mucosal immune system of the small intestine and/or colon[1]. The inflammation is accompanied by leaky intestinal epithelium, altered mucus layer, increased concentration of positively charged proteins and infiltration of the immune cells[2]. In therapy, local approaches are preferred over conventional systematic treatment in order to minimize undesired side effects. Self-nanoemulsifying drug delivery systems (SNEDDS) have potential to deliver an anti-inflammatory acting oligonucleotide (OND) and thus to target inflamed intestinal tissue. SNEDDS based on medium-chain fatty acids are known to enhance absorption through tight junction opening[3]

Aim
To evaluate transport of OND and impact on transepithelial resistance (TEER) of formulations of OND encapsulated in tight-junction opening SNEDDS on a Caco-2 cell in presence and absence of the Compound 48/80 (C48/80), an inhibitor of tight-junction opening

Methods
A fluorescently labelled non-specific OND was complexed with a cationic lipid (1,2-dioleoyl-3-trimethylammonium-propane, DOTAP) (OND-CL) to increase its lipophilicity[4]. Subsequently, it was loaded into SNEDDS containing medium-chain fatty acids. Size and zeta potential were measured by dynamic light scattering and laser Doppler electrophoresis, respectively. The Caco-2 cells were pre-treated with C48/80 and subsequently incubated with OND-CL loaded in SNEDDS dispersed in a buffer, blank SNEDDS (SN) and the cationic lipid dissolved in SNEDDS both dispersed in a buffered solution of OND, and a buffered solution of OND. TEER values were monitored every 30 minutes, samples from the basolateral compartment were taken at specific time points and evaluated fluorometrically.

Results
Size of the tested formulations ranged between 180 and 215 nm, zeta potential was ~ - 9mV. All treatments containing SNEDDS showed permeation of OND into the basolateral compartment unlike in the case of the OND solution. The highest amount of OND of 6,9±0,7 pmol was found after treatment with the OND-CL in SNEDDS. C48/80 lowered and delayed (60 min) the transport of OND-CL, however, there was no significant difference observed in the case of SN in presence and absence of the inhibitor. The inhibitor had a significant impact on TEER values after the pre-treatment, 286±55 and 475±61 Ohm.cm^2, for non-inhibited and inhibited samples respectively. TEER values of cells treated with SNEDDS dropped to ~ 50% of respective initial values after 30 minutes and stayed unchanged till the end of the experiment.

Conclusion: SNEDDS disperse into droplets of submicron size with negative surface charge. These properties fulfil the requirement for passive targeting to the inflamed intestinal tissue. The inhibitor C48/80 modulates opening of tight junctions and suggests their role in transport of OND through Caco-2 monolayer. This experiment sets a solid basis for further investigations that can shed more light on mechanism of interactions of OND with intestinal epithelium in healthy and pathological conditions.

Acknowledgement: This work was supported by Czech Ministry of Education and Sports, project no. SVV 260 401 and Czech Grant Agency, project. No.GA17-06841S.

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The chain length of novel lipidoids in LPNs used for intracellular delivery of siRNA is crucial for *in vitro* transfection potential and immunogenicity

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Introduction: Small interfering RNA (siRNA) based anti-inflammatory therapeutics have promising potential through highly specific cellular gene silencing. (**Fig. 1**) However, intracellular delivery remains a major hurdle due to the large molecular weight and polyanionic backbone of siRNA. We designed a novel siRNA delivery system based on lipidoid-polymer hybrid nanoparticles (LPNs) exploiting the merits of both lipid- and polymer-based delivery systems [1, 2] (**Fig. 2**). The lipidoid compound with five alkyl chains of 12 carbon length ($L_{5\text{-}12}$) used for LPNs displays high transfection potential and low cytotoxicity, but it activates human toll-like receptor 4 (hTLR4) and is immunogenic [3]. We hypothesized that by systematically varying the chain length of L_5, it may be possible to identify compounds with high transfection potential that are devoid of any immunogenicity.

Aim: To synthesize an array of L_5 analogues with different chain lengths ($L_{5\text{-}12}$, $L_{5\text{-}14}$, $L_{5\text{-}16}$) and determine transfection efficiency, cytotoxicity, and immunogenicity of the resulting LPN formulations.

Method: The novel L_5 lipidoids were synthesized by Aza-Michael addition of acrylamide tails to a polyamine backbone and then purified by column chromatography. L_5-based LPNs were formulated by double emulsion solvent evaporation method [1]. Subsequently, the *in vitro* gene silencing and relative cell viability was evaluated by flow cytometry and MTT assay, respectively, after incubating H1299 meGFP cells with LPNs. Finally, immunogenicity of LPNs was evaluated by measuring activation of murine Bone-Marrow Dendritic Cells (BM-DCs) in the presence of LPNs.

Results: We observed that $L_{5\text{-}12}$ mediated significantly higher gene silencing, as compared to $L_{5\text{-}14}$ and $L_{5\text{-}16}$. Of the L_5 analogues, $L_{5\text{-}16}$ displayed the least influence on cell viability, while $L_{5\text{-}12}$ displayed the largest influence on cell viability. All LPNs induced BM-DC maturation, evident from upregulation of the maturation markers CD40, CD80 and CD86. Lower BM-DC activation was measured for $L_{5\text{-}14}$ and $L_{5\text{-}16}$-based LPNs. $L_{5\text{-}12}$-based LPNs was the least tolerated formulation, as it leads to the lowest cell viability of BM-DCs.

Conclusion: $L_{5\text{-}14}$ and $L_{5\text{-}16}$ are promising candidates for further development of siRNA-loaded LPNs due to their improved safety, as compared to $L_{5\text{-}12}$.

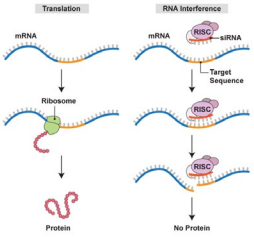

Figure 1 siRNA-mediated RNAi.

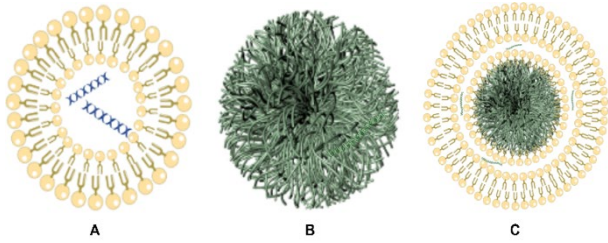

Figure 2 (A) Lipid-based NPs, (B) Polymer-based NPs, (C) LPNs.

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A novel approach to improve bioavailability of poorly water-soluble drugs

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Introduction

Poor aqueous solubility of small molecule APIs is one of the main challenges in oral drug delivery as it often leads to a low and/or varied absorption. Different formulation approaches including amorphous solid dispersions (ASD) and lipid-based formulations, such as self-nanoemulsifying drug delivery systems, SNEDDS, have been successfully utilized to overcome the aqueous solubility barrier, and further, enhance drug bioavailability. However, limited or no studies have been reported where the benefits of SNEDDS and ASD are combined to improve the bioavailability of poorly water-soluble drugs. This was tested using an *in vitro* lipolysis model, and a pharmaco-kinetic (PK) study in rats.

Aim': To investigate the novel combination of SNEDDS and ASD to maximize the dissolved dose and subsequently improve bioavailability of the model drug ritonavir.

Method: The equilibrium solubility (S_{eq}) of ritonavir was determined in the SNEDDS (27.5% w/w Soybean oil, 27.5% w/w Maisine®CC, 35% w/w Kolliphor®RH40 and 10% w/w Ethanol) without and with addition of Kollidon®VA64 at 1, 3 and 4% using HPLC-UV. The supersaturation capacity of the SNEDDS (SuperSNEDDS) without and with Kollidon®VA64 were determined by sonicating and heating the loaded SNEDDS to 60°C for 1 and 3 hours, respectively. The solubility of ritonavir in Kollidon®VA64 was determined using DSC. The maximum amount of ASD that dissolved in the selected SuperSNEDDS was determined and labelled as SuperSNEDDS+ASD. Relevant controls were developed: SNEDDS with 90% S_{eq} of ritonavir (Conventional SNEDDS), SuperSNEDDS with physical mixture of Kollidon®VA64 and crystalline ritonavir (SuperSNEDDS-PM), and an aqueous suspension of crystalline ritonavir. The ability of the four formulations to solubilize ritonavir during *in vitro* lipolysis was determined using a two-step model simulating rat digestion, including a 30 min gastric step, followed by a 60 min intestinal step.

Results

S_{eq} of ritonavir in the SNEDDS was 9.3±0.6 mg/g and the maximum degree of supersaturation was 250% of S_{eq} with a stability of 48 hours. Adding ASD to the SuperSNEDDS enabled an increase in drug load to 275% of S_{eq}, being stable for 1 month. The *in vitro* lipolysis (Fig. 1) show that significantly ($p< 0.05$) more ritonavir was solubilized for SuperSNEDDS+ASD compared to the SuperSNEDDS-PM. There was no difference between ritonavir solubilized for SuperSNEDDS+ASD and the conventional SNEDDS, however, it needs to be noted that the former uses almost three times less lipid than the latter.

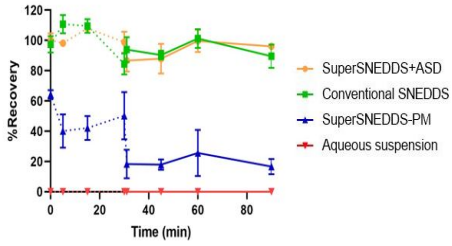

Fig.1: % Recovery-time profile of ritonavir in different formulations during two-step in vitro lipolysis. (mean±SD)

Conclusion

Using the model drug ritonavir, the addition of an ASD to a SuperSNEDDS increases the degree of drug supersaturation and the stability of the formulation. Further, the SuperSNEDDS+ASD was able to keep the drug solubilised during *in vitro* lipolysis when compared to the controls. This indicates a great potential for the novel formulation strategy of combining SuperSNEDDS and ASD.

Workpackage 3 ENGINEER (Leader: Leena Peltonen, UHEL)

Pharmaceutical manufacturing and processing has been identified as a rate-limiting step in translating new and innovative product ideas into new medicinal products. A key in enabling modern pharmaceutical production will be the implementation of additive manufacturing and continuous manufacturing principles: continuous manufacturing also leads to accelerated production processes, flexibility of scale, smaller facilities with lower costs, lower consumption of energy and materials, lower amount of waste production and improved process reliability and flexibility.

In the WP3, we focus on both traditional powder based processing and additive manufacturing preferably in a continuous mode using 2D/3D printing, electrospinning/electrospraying, and microfluidics based production platforms.

Invited speaker talk:
Mirja Palo: Printing technology for personalized dosing

Students talks:
Simon Bock, KIEL
Mikkel Herzberg, UCPH
Kuldeep Kumar Bansal, UiO
Anne Linnet Skelbæk-Pedersen, UCPH

Poster session:

36	Elle Koivunotko, UHEL
37	Junnian Zhou, AAU
38	Guido Teia, UiO
39	Farshid Vijeh, UCPH
40	Navid Hossein, UiOi
41	Shno Asad, UPP
42	Maria Malk, UT
43	Laura Viidik, UT
44	Kelli Randmäe, UT
45	Vili-Veli Auvinen, UHEL
46	Rydvikha Govender, CTH

Printing technology for personalized dosing

Mirja Palo

Pharmaceutical Sciences Laboratory, Faculty of Science and Engineering, Åbo Akademi University, Finland

Brief CV

Mirja Palo is a pharmaceutical scientist who has been working as a researcher and a teacher in pharmaceutical technology at Åbo Akademi University (Finland) since 2012. She received her PhD in pharmaceutical sciences from Åbo Akademi University and the University of Tartu (Estonia) in 2017. Her research interests are focused on the applications of printing technology in pharmaceutics, the development of individualized drug delivery systems, and the characterization and quality control of dosage forms.

Abstract

Printing technology is an emerging fabrication approach that provides various possibilities for the preparation of drug delivery systems (DDSs). The printed DDSs aim to provide higher flexibility in the pharmaceutical manufacturing, while maintaining high accuracy and controllability of the process and ensuring the safety of the drug treatment. As a rule, the two- (2D) or three-dimensional (3D) printers manufacture printed products according to a pre-defined digital design. Thus, the flexible design of printed systems allows meeting the personalization needs of drug treatments and tailoring the drug dosing at high precision. The examples on the use of printing technologies that are of potential interest in personalization of drug products and future manufacturing are presented. The advantages and limitations related to the individualized drug dosing by printing are emphasized. Furthermore, the manufacturing of multicomponent DDSs by combinational methods is discussed.

Additive manufacturing in respiratory drug delivery: Dispersing aids in Dry Powder Inhalation (DPI) formulations

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Introduction

From printed tablets with controlled or targeted drug delivery to tissue engineering, surgery training, implants or prostheses, additive manufacturing (AM) has already proven to be a key technology for next generation medical applications. In respiratory drug delivery, 3D-printing (3DP) has not yet been extensively evaluated, except in e.g. rapid prototyping of inhaler devices or respiratory airway replica.[1] The fabrication of tailor-made structures with intricate details potentially allows fundamental investigations of particle-particle interactions. Concerning dry powder inhalation (DPI) formulations utilising drug-carrier interactive mixtures, the use of additive manufacturing techniques could lead to an improved and controllable dispersion and aerosolisation and thus to a better therapeutic effect.

Aim

The aim of the work was to determine to which extent the deposition profile of DPI formulations with different carrier size can be influenced by various 3D printed dispersing aids.

Methods

Different dispersing aids (DA) were printed using a Form 2® 3D-printer (Formlabs, USA). Two types of lactose carriers (InhaLac 120 and InhaLac 230, Meggle, Germany) were each blended with 2 % salbutamol sulphate (SBS; Lusochimica S.p.A., Italy) as a model active pharmaceutical ingredient (API) using a high-shear mixer (Picomix®, Hosokawa, Germany; 500 UpM, 2 x 60 sec). Size 3 HPMC capsules filled with 20 mg of the respective formulation and a DA were inserted into a capsule based Twister® device (Aptar Pharma, France). The deposition profiles were evaluated with the next generation pharmaceutical impactor (NGI; Copley Scientific, UK) according to Ph.Eur. 9.4 using HPLC analysis for drug quantification.

Results :

Whereas the utilisation of DA in formulations containing Inhalac 230 had no determinable effect on the fine particle fraction (FPF), the deposition profile of the interactive mixture containing InhaLac 120 shifted towards stages with lower aerodynamic cut-offs. In particular, the use of a complex structure such as a rolling-knot led to an increase of the FPF (Figure 1).

Conclusion

Both the type of carrier used in DPI formulations as well as the usage of dispersing aids manufactured with an additive manufacturing technique influenced the FPF. This approach may contribute to a better understanding of particle-particle interactions in aerosols in the future.

FPF, %	Inhalac 230	Inhalac 120
Blank	9.69 ± 1.85	8.47 ± 1.60
C1	10.59 ± 0.94	8.68 ± 1.34
C2	9.83 ± 1.33	8.74 ± 2.23
C3	10.41 ± 0.60	10.22 ± 0.61
R	10.15 ± 1.79	12.87 ± 1.32

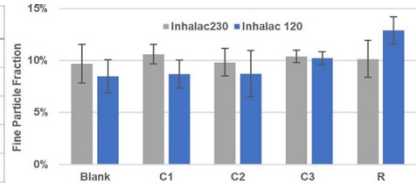

Figure 1 *Comparison of FPF < 5 µm obtained by NGI deposition profiles (n=3) utilising various dispersing aids compared to Blank (C = cube, R = rolling knot; 1 = small, 2 = medium, 3 = large)*

Acknowledgements: The authors would like to acknowledge Ingunn Tho (University of Oslo), Halvor Tramsen and Leonard Siebert (Kiel University) for help regarding the 3DP and Aptar Pharma for providing the Twister® device.

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Nanoscale characterization of the solvent effects on crystal surfaces

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Introduction

Solvents can dramatically impact the properties of molecular crystals. A better understanding of these solid-liquid interfaces would be of great importance for the pharmaceutical industry as it affects processability, bioavailability and stability of related products[1]. Atomic force microscopy (AFM) provides a method for investigating such systems with nanoscale precision by probing surfaces with a sharp cantilever, while a laser-based feedback system records movement of the cantilever.

Aim

To characterize the effect of solvents on molecular crystal surfaces using (AFM).

Method: Paracetamol was crystallized in water-ethanol mixtures containing 0 %, 20 % or 40 % v/v ethanol. A crystal from the 0 % batch was placed in a liquid cell and the force-distance curves were measured with an AFM. The force-distance curves were obtained in a grid on the surface, so when indentation or adhesion force were extracted, maps of the surface properties could be created. Then, the solvent was changed to 20 % and 40 % ethanol and back to 0 % again. Molecular dynamics (MD) simulations were performed to investigate the intermolecular structure in the solid-liquid interface.

Results: The chemical force maps showed a reverse relationship between indentation and adhesion force as shown in Figure 1. The indentation has previously been linked to amorphous domains on the surface[2], while adhesion force is routinely correlated with hydrophobicity[3]. Microscale amorphous domains were also observed in the presence of ethanol, which seemed to disappear when the solvent was changed back to pure water solution. This effect could be replicated in our MD simulations.

Conclusion

Atomic force microscopy can be used to monitor changes in surface properties when a molecular crystal is exposed to different solvents. Our results show that hydrophobicity and crystallinity are inversely related and that microscale surface amorphous domains form in the presence of ethanol and disappear, when we change back to pure water.

Acknowledgement

The authors would like to acknowledge the Independent Research Fund Denmark [Grant No. 8022-00154B] for financial support for this project.

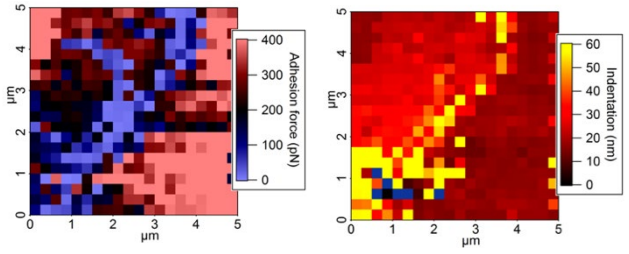

Figure 1 *Chemical force map visualizing hydrophobicity (left) and crystallinity (right) obtained using atomic force microscopy.*

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Facile methodology of nanoemulsion preparation using oily polymer for delivery of poorly soluble drugs

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Introduction

Aqueous solubility of an active pharmaceutical ingredient is a determining factor that has a direct impact on formulation strategies and overall bioavailability. Fabrication of nanoemulsions of poorly soluble drugs is one of the widely utilized approaches to overcome this problem. However, the applicability of nanoemulsions is limited due to stability problems. Nanoemulsions, could undergone Ostwald ripening along with flocculation and coalescence followed by creaming/sedimentation. The solubility of oil in the continuous phase is the main factor for Ostwald ripening, which could be slowed down by addition of a hydrophobic component into the oil[1]. In addition, expensive and tedious preparation methods of nanoemulsions is also a limiting factor in scale-up and preparation of a pseudoternary phase diagram is often needed to determine the optimum ratio of oil, surfactant, co-surfactant and water. Oxidation or rancidity of oil phase during storage is an another stability problem, and these factors altogether limit their clinical translation.

Aim

In this study, we have attempt to circumvent the above-mentioned hurdles associated with nanoemulsion by utilizing polymer as an oil phase instead of conventional oils.

Method

Polymer was synthesized using renewable δ-Decalactone monomer under mild reaction conditions, which is clear colorless viscous liquid[2]. A simple nanoprecipitation method was utilized to fabricate nanoemulsion using pluronic F-68 as surfactant. Five different hydrophobic drugs were used to establish the capability of nanoemulsion formulation towards enhancing the aqueous solubility. Long-term stability, haemolysis and cytotoxicity assays were performed to establish the toxicity and stability profile of prepared formulations.

Results

The prepared nanoemulsions with sizes less than 200 nm were capable to enhance the aqueous solubility of the drugs by 3 to 10 times compared to the well-established Pluronic F-68 micelles. No phase separation and significant changes in size and drug content was observed with PDL nanoemulsions after high-speed centrifugation and three months of storage at two different temperatures (20 °C and 50 °C). PDL nanoemulsions were found to be non-heamolytic up to concentrations of 1 mg/mL and the cell cytotoxicity studies on MDA-MB-231 cells suggests a concentration- and time-dependent toxicity, whereas the PDL polymer itself induced no cytotoxicity.

Conclusion

The results from this study clearly indicates that the PDL polymer has a tremendous potential to be utilized as oil phase to prepare stable nanoemulsions via a facile methodology, ultimately favoring clinical translations.

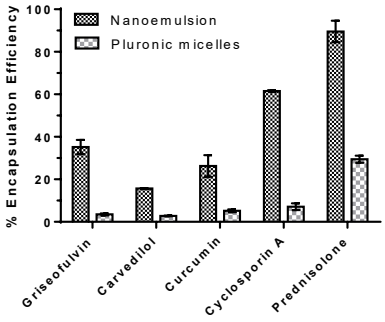

Figure 1. Percentage encapsulation efficiency of drugs in nanoemulsion and pluronic micelles. N=3 ± SD

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The effect of deformation on water ingress into tablets

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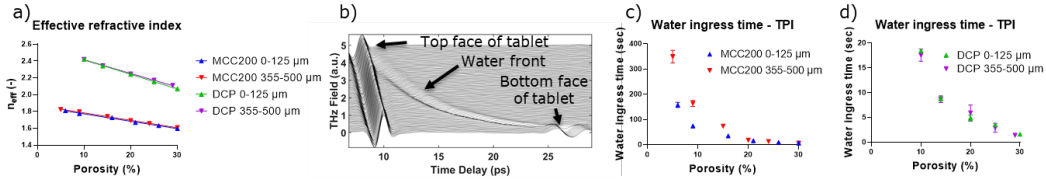
Introduction:
Materials undergo deformation during the tableting process. Powder deformation is the material property that has the largest impact on tablet formation and can be divided into reversible (elastic) and irreversible (plastic/fragmenting) deformation. A method to quantify fragmentation after tableting was recently introduced[1], proving that particles may fragment extensively during compression depending on their predominant deformation behavior and initial particle size.[1,2] The predominant deformation behavior is expected to impact the microstructure of tablets which has been found to affect liquid transport into tablets, thereby impacting tablet disintegration and performance.[3]

Aim:
The aim of this study was to investigate the effect on water ingress into tablets of plastically deforming microcrystalline cellulose (MCC) and fragmenting di-calcium phosphate (DCP).

Method: Two size fractions (0-125 and 355-500 µm) of MCC and DCP were compressed into tablets with porosities ranging from 5 to 30% (n=3). The tablets were subjected to terahertz time-domain spectroscopy (THz-TDS) and terahertz pulsed imaging (TPI). THz-TDS and TPI were used to obtain the effective refractive index (n_{eff}) and the time for water to ingress through the tablets, respectively.

Results: A linear relation between effective refractive index and tablet porosity was observed (Figure a), which is in accordance with previously reported results.[4] Waterfall plots were generated from the TPI measurements (Figure b). The time for water to ingress from the bottom to the top face of the tablet was determined and plotted as a function of porosity (Figures c,d). It was observed that tablets of larger MCC particles resulted in longer water ingress time compared to smaller MCC particles (Figure c). On the contrary, no difference was observed between different sized DCP particles (Figure d). This is expected to be caused by the low fragmentation degree of MCC, resulting in different particle size after compression of the two initial size fractions, whereas DCP fragments to approximately the same particle size after compression regardless of initial particle size.[2] Finally, lower porosity resulted in longer water ingress time and amplified the differences between small and large MCC particles.



Conclusion: The degree of fragmentation was found to impact water ingress into tablets and it would therefore be relevant to link water ingress to the effect of deformation on disintegration and dissolution of tablets containing active pharmaceutical ingredient.

Acknowledgement
Novo Nordisk A/S (Denmark) is acknowledged for financing the PhD project (STAR program).

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Effects of Freeze-drying on Physiochemical properties of Nanofibrillated Cellulose

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Introduction

Nanofibrillated cellulose (NFC) hydrogel is a biocompatible biomaterial, which can be used for 3D cell culturing and controlled drug release[1, 2].To increase storability and transportability of NFC one potential method is freeze-drying (FD). FD is a desiccation method in which samples are first frozen and then dried under vacuum and it is a common practice for protein drugs[3]. However, FD is a stressful process and excipients are required to protect active ingredients. We evaluated the effects of FD on NFC and screened the optimal excipients to preserve the properties of the sample. We hypothesized that the nanofibrillated cellulose hydrogel can be freeze-dried with the correct excipients.

Aim

To preserve rheological and physiochemical properties of nanofibrillated cellulose after freeze-drying, storage in room temperature and reconstitution.

Methods

NFC hydrogel was mixed with excipients, such as trehalose, glycine and DMSO. Appearance, pH, glass transition temperatures (Tg') and osmolarities of the formulations were studied before and in addition of the previous ones also residual water contents after FD. Successfully freeze-dried formulations were chosen for rheological analysis.

Results

We observed no changes in physicochemical properties of formulations containing different concentrations of sugars, glycine and nanofibrillated cellulose before and after freeze-drying. On the contrary, high DMSO concentrations in the formulations resulted in collapsing of the freeze-drying cake. However, by increasing trehalose concentration in DMSO and nanofibrillated cellulose formulations, freeze-drying was possible. Residual water contents were less than 1%, but the they could be adjusted by the excipients.

Conclusion

Nanofibrillated cellulose with needed excipients can be successfully freeze-dried and reconstitute after storage at RT. Potential of these formulations is further studied for preservation and controlled release of small molecules and proteins.

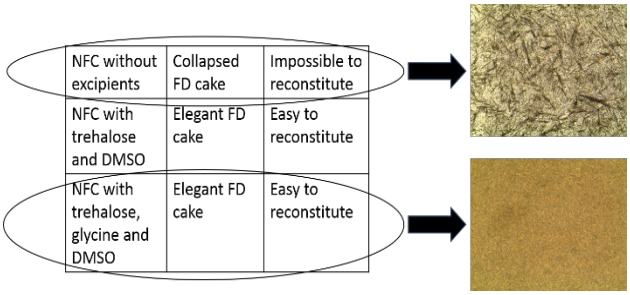

Figure 1 *Light microscopy images of different freeze-dried NFC formulations.*

Acknowledgement

Academy of Finland BioEva protect (n:o 315409). EU-Erasmus exchange program, UPM-GD96 project

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The lipid nanoparticles (LNPs)-based delivery of protein kinase A and its effects on chemoresistance in breast cancer cells

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Introduction

Lipid nanoparticles (LNPs) are the most excellent candidates for nucleic acid delivery. However, whether these systems can be used to deliver bioactive enzyme into cells, of which the enzyme functions directly to catalyze the specific substrate, needs to be further investigated. We want to set up a simple and high-reproducible method to quickly estimate the biological effects of active enzymes in cells. Here, we reported to exploit commercial lipid nanomaterials Lipofectamine™ CRISPRMAX™ to deliver protein kinase A (PKA) and assess its biological effects on cancer cells for the first time. This strategy will facilitate the screening of suitable target enzymes/proteins for construction of new drug delivery system or complex nanoparticles.

Aim

To investigate whether the specific lipid nanomaterials can guide the delivery of active enzyme (i.e. protein kinase A) and its effects on the drug resistance in breast cancer cells.

Method

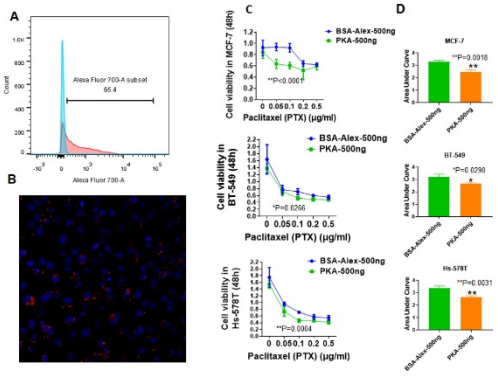
Active PKA (with specific activity 0.5 unit/μg) is ordered from Sigma. Briefly, 500ng enzyme or positive control (BSA-Alex Fluor 680) is encapsulated by 0.5μl Lipofectamine™ CRISPRMAX™ (Invitrogen) for 8, 000 cells in one 96-well. Flow cytometry is used to analyze the positive rate of positive control group in BT-549 cells.

Results

Day 4 after the transfection of lipid nanomaterials-coated BSA-Alex Fluor 680, we can detective a high positive cell rate (65.4% in BT-549) (Figure 1A,B). After active PKA enzyme transfection by this strategy, the chemoresistance of PTX is attenuated in three cell lines (Figure 1C,D).

Conclusion

The Lipofectamine™ CRISPRMAX™ can be exploited to deliver the active enzyme (i.e. PKA) successfully. Active PKA enzyme delivery into cells can attenuate chemoresistance of PTX.



Acknowledgement

The authors would like to thank Nordic POP and the Sigrid Jusélius foundation (Grant No. 28001830k1) for financial support.

Fig.1 Establishment of lipid-nanomaterial-based enzyme delivery. *Flow cytometry (A) and confocal microscope image of transfection efficiency in BT-549 cells day 4 after transfection of BSA-Alex Fluor 680. Cell viability by cell proliferation reagent WST-1 (Roche) (C) and Area under cure (AUC) (D) analyses of breast cancer cells with Paclitaxel (PTX) adding 12h after PKA transfection by liposome. Two-way ANOVA analysis was used in (C), unpaired two tailed t test analysis was used in (D).*

Evaluation of mechanical properties of in-house produced filaments for 3D printing

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Introduction

Fused Deposition Modeling (FDM) is a 3D printing technology that allows printing of objects through the deposition of molten material using a print head, nozzle, or another printer technology (1). The FDM technique has potential applications in pharmaceutical field as it shows promise in providing tailored formulations that fits the specific needs of the patient (Personalized medicine). Of prime interest to the development of 3D printing is the production of flexible and durable filaments (2). We, therefore, focused on producing in-house filaments by hot melt extrusion (HME). However, in order to reliably print these filaments, it is necessary to have insight into their mechanical properties. In view of this, we have developed and 3D-printed different rigs to characterize the mechanical properties of in-house filaments.

Aim

The purpose of this study was to develop two different rigs that could be used to evaluate the mechanical properties of suitable in-house filaments for 3D printing, and to compare them with the commercial acrylonitrile butandiene styrene (ABS) filament.

Method

Physical mixtures of polymers were made from a thermoplastic polymer polyvinyl caprolactam-polyvinyl acetate-PEG graft copolymer (Soluplus®); PVP-PVA graft copolymer (Kollidon® IR) and PEG 6000. These blends were extruded by HME using a single screw extruder (Filabot EX2®) into filaments. In-house rigs were designed using Tinkercad® and CAD® software, and they were printed with ZMorph printer (White PLA filament) or Ultimaker 3 (Natural PLA filament). The mechanical properties of the filaments were evaluated using a texture analyzer (TA-XT2i®) fitted with the printed rigs.

Results

Two in house rigs were designed to evaluate the mechanical properties of filaments for 3D printing, and to mimick the forces that the filament might be subjected to during printing with two different printers using different tests: the three point bending test and longitudinal compression test. The printed tools were able to show the mechanical properties of ABS as force-displacement curves (Figure 1). Hence, an initial trial of the in-house produced filaments (Soluplus®/Kollidon® IR/PEG 6000) was performed, and a comparable profile was obtained indicating that the filaments should be promising for printing.

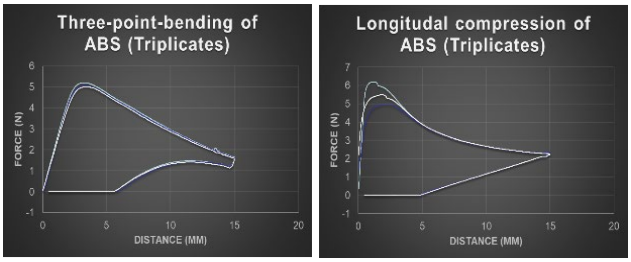

Figure 1 *Mechanical force-displacement profile of ABS obtained with two different 3D printed rigs.*

Conclusion

The two developed 3D printed rigs showed that they could be used to screen a large variety of in-house produced filaments. Selection of those that are suitable for printing.

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Development of polymer based electrospun nanofibers for wound healing

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Introduction

Wound healing is a complex biological process; a wound is the disruption of normal anatomic structure and function of the skin that can be acute or have non-healing chronic complications. Therefore, there is a great interest for the development of wound dressings that actively promote wound healing, for instance, biocompatible and elastic nanofibers made of novel synthetic polymers mimicking the extracellular matrix to accelerate cell attachment and, thus, wound healing.[1] Furthermore, delivery of anti-microbial peptides or other antimicrobial agents to the wound might augment the wound healing process through the reduction of bacterial infections.[2]

Aim

Production and characterization of polymer-based electrospun nanofibers for wound healing.

Method

The polymer RESOMER® LRP t 7046 was dissolved in binary mixtures of chloroform/dimethylformamide (DMF) or acetone/DMF[3] prior to uniaxial electrospinning. The fibers were electrospun using solutions with 12% w/v to 16% w/v polymer. Needle voltages of 5.0 kV to 7.9 kV, relative humidity of 45% and tip to collector distances between 11 cm to 18 cm were used. The diameter, bead formation and morphological characteristics of the collected nanofibers were analyzed using scanning electron microscopy (SEM) using an accelerating voltage of 15 kV. Thermal stability of the samples was analyzed using thermogravimetric analysis (TGA), and the samples were heated up to 450 °C at a constant heating rate of 20 °C min^{-1}. Contact angle and swelling capacity measurements will be performed for further characterization.

Results

Randomly oriented nanofibers with diameters of about 630 nm to 1020 nm for acetone/DMF and about 400 nm to 1100 nm for chloroform/DMF were obtained (Figure 1). TGA curves of the polymer show two stages of weight loss, which can be attributed to the remaining solvent and the main weight loss between 200 °C and 355 °C because 96% of the weight of the polymer consists of polylactic acid (PLA) and of only 4% polyethylene oxide. High hydrophobicity and low water absorption capacity are to be expected as well.

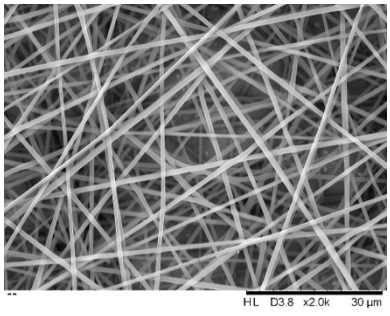

Figure 1 *SEM image of electrospun nanofibers of 15% (w/v) polymer in chloroform/DMF (5:5).*

Conclusion

The lowest concentration samples in both cases cause the formation of multiple beads. On the other hand, more concentrated solutions resulted in the production of fibers with the higher diameter. The most important factors that are affecting morphology of nanofibers are solution parameters such as concentration and solvents.

Acknowledgement

The author would like to thank Evonik (Darmstadt, Germany) for providing RESOMER® LRP t 7046.

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Functionalization of SiO_2-coated SPION with ligands targeting biomarkers for IBD

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Introduction

Superparamagnetic iron oxide nanoparticles (SPION) are promising for theranostics.[1] However, due to the lack of scalable and reproducible synthesis methods, few SPION-based medicinal products have reached the clinics. Flame spray pyrolysis (FSP) is an industrially established synthesis process that allows large scale manufacture of SPION. We have developed a single-step flame process for encapsulating SPION with a thin SiO_2 coating[2,3] that increases their biocompatibility and facilitates further functionalization with e.g. targeting ligands by click chemistry. We are developing a theranostic platform using these nanoparticles for the diagnosis and treatment of inflammatory bowel disease (IBD). Thus, our ongoing work focuses on investigating the global proteomes of commonly used *in vitro* and *in vivo* experimental models of acute IBD in order to find appropriate targets, which subsequently will enable higher SPION selectivity when functionalizing the particles.

Aim

To identify and quantify the proteins present in rodent and Caco-2 cell models for IBD and to develop SPION functionalized with ligands that target these IBD biomarkers.

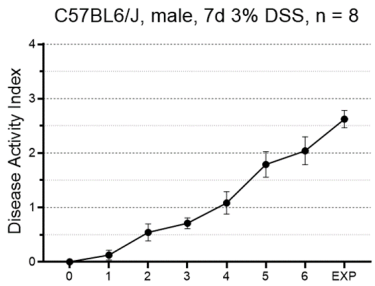

Figure 1. DAI of the mice treated with DSS in their drinking water.

Methods

SiO_2 coated SPION were made by FSP.[2,3] Magnetic hyperthermia performance of particles was measured. The *in vitro* model for the global proteomic study included filter-grown Caco-2 cells exposed to either DSS or a mixture of inflammatory inducing agents (IL-1β, LPS, TNF-α, IFN-γ). The treatments were applied during 24 h before the filters were excised and prepared for proteomic analysis. The *in vivo* model consisted of a total of 16 mice, where 8 of them were treated with 3% DSS in their drinking water for 7 days. Biopsies were collected from the small intestine and the distal and proximal part of the colon.

Results

SPION with a crystallite size of 15 nm and encapsulated with a nanothin (2-3 nm) silica coating were produced by scalable FSP and exhibited hyperthermia in an alternating magnetic field. All animals treated with DSS were ill as verified by determination of disease activity index (DAI; Fig. 1). Results from global proteomics are under processing.

Conclusions

FSP enables large scale manufacture of SPION and close control over particle properties. The SiO_2 coated SPION will be further functionalized with appropriate ligands to increase selectivity of the particles to target IBD.

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The impact of a lattice design on the 3D-printability of polyethylene oxide hydrogels

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Introduction

The implementation of a computer-aided design (CAD) and 3D-printing as a new manufacturing method is steadily rising in popularity in the pharmaceutical industry. The present combined technology enables to produce a wide variety of objects effectively and fast, and using various polymeric materials and methods. Despite of an increasing interest in pharmaceutical 3D-printing, the use of hydrogels in 3D printing is still very limited. For example, the application of polyethylene oxide (PEO) hydrogels is very under-research in this area to date. Hydrogels have good biocompatibility, thus making them suitable for the use in pharmaceutical and medical applications.[1]

Aim

To design and evaluate the impact of different lattice designs on the 3D-printability of PEO hydrogels intended for pharmaceutical applications.

Method

For preparing PEO hydrogels, 1.5 g of PEO (M ~900 000, Sigma-Aldrich, USA) was dissolved in 10 ml of purified water. Pentaerythrityl triacrylate (PETA) was used in 10:1 (PEO to PETA) ratio as a photosensitive substance in order to provide more stable hydrogels. PEO/PETA gels were printed using an extrusion-based 3D-printing system (System 30M, Hyrel 3D, USA). The printed lattices were either ultraviolet (UV)-crosslinked during printing, after printing or not at all. Six different lattices were designed using a Solidworks premium 2018 SP 3.0 program. The lattices used either triangle or honeycomb base patterns, varying the printer head moving pattern or pattern design density. The 3D-printed lattices developed were compared by their weight and dimensions.

Results

All lattice design patterns were 3D printable with a PEO hydrogel tested. However, all 3D-printed lattices were found to have some deformations. Furthermore, it was found by visual inspection that PEO polymer tended to spread out especially in the lower layers of the lattices. The microscopy and micrometer measurements of the PEO/PETA lattices showed that the pores in a smaller-design pore-sized structure decreased perceptually more than the pores of the lattices with larger-design pore sizes. The different crosslinking treatments studied did not affect the 3D-printability of lattices based on PEO polymer hydrogels.

Conclusion

It is possible to 3D-print all designed lattices with 15% PEO/PETA hydrogel. The 3D-printed lattices with larger pores resembled their theoretical design models more than the corresponding lattices with smaller pores.

Acknowledgement

This study is funded by the Nordic POP researcher network and the Estonian national research projects IUT34-18 and PUT1088.

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Preparation and stability of 3D-printable hot-melt extruded indomethacin filaments

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Introduction

In order to use 3D printing in generating personalized drug delivery systems (DDSs), suitable polymeric formulations need to be designed. In the 3D printing by fused deposition modelling (FDM), hot-melt extruded (HME) filaments are an essential intermediate product for the 3D-printing of DDSs.[1]

Aim

The aim of this study was to prepare and characterize 3D-printable HME filaments loaded with indomethacin (IND). The effects of different storage conditions (temperature and relative humidity, RH) on the physical solid-state properties of the filaments were studied.

Method

Physical powder mixtures of IND, arabic gum (ARA) and polycaprolactone (PCL) in different ratios were prepared manually with a mortar and pestle. The filaments were extruded using a Filabot EX2 (Filabot, USA) single-screw hot-melt extruder. Suitable extrusion temperature and speed was defined for each formulation. Preliminary 3D-printing tests were done by using FDM type 3D printer (System 30M, Hyrel, USA). The HME filaments were stored at 40 °C / 75% RH or in a refrigerator temperature (3-8 °C / 0% RH). Solid-state characterisation was performed using Fourier-transform infrared (FTIR) spectroscopy (IRPrestige 21, Shimadzu Corp., Japan) and X-ray powder diffraction (Bruker D8 Advance, Bruker AXS, Germany).

Results

The formulation screening enabled the incorporation of 20%, 30% and 40% (w/w) of IND into the HME filaments, which differed from each other mainly by the surface roughness. For further analysis and printing tests, the HME filaments with 20% IND were selected (a ratio of PCL, ARA and IND was 7:1:2, respectively). The present HME filaments were yellowish in colour, mechanically strong and flexible, and they had a uniform filament diameter and smooth outer surface. These filaments were successfully 3D-printed into a cylindrical model tablet. During storage at elevated temperature and RH, the filaments changed in colour from light yellow to brown, also showing some melting. IND loaded in the filaments remained in a crystalline form after a HME process. After 3D printing, however, IND was found to be in an amorphous form. A short-term storage of the filaments at 40 °C / 75% RH and at 3-8 °C / 0% RH resulted in the formation of crystalline IND.

Conclusion

HME is a suitable method for preparing the filaments intended for the 3D printing of DDSs by FDM. Further studies on the thermal behaviour, homogeneity and drug release of such filaments and printed DDSs are being conducted.

Acknowledgement

This study is funded by the Estonian national research projects IUT34-18 and PUT1088, and by the Nordic POP (patient-oriented products), a Nordic University Hub funded by NordForsk (Project number: 85352).

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Electrospinning and characterisation of fibrous antimicrobial peptide-loaded systems

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Introduction

Skin and wound infections are a major problem for the society due to increased healthcare costs and a significant burden to patients. As microbial biofilms are one of the main reasons for the development of non-healing skin wound infections, novel treatment strategies are seeked in order to fight against them. Antimicrobial peptides (AMPs) eradicate the infections more effectively due to their specific mechanisms of action which is usually related to the direct damage of bacterial plasma membranes and/or penetration within the bacterial cytoplasm to access intracellular targets and cause rapid bacterial cell death. However their formulation into suitable delivery systems and testing their suitability for the wound treatment needs more investigations in order to provide safe and effective products.

Aim

The main aim was to find suitable electrospinning conditions and concentrations for an antimicrobial peptide (AMP) and test its drug release behaviour from hydrophobic polymer matrix.

Method

Different solvents and polymers were tested for electrospinning. Hydrophobic polymer was selected and electrospun together with an hydrophilic AMP into a fiber mat. Mats with three different concentrations of AMP were prepared in order to understand the effect of AMP concentration on electrospinnability. AMP-loaded fiber mats with different concentrations allow also comparing their activity and safety in the next phases of the study. The morphology of the electrospun fiber mats was studied using scanning electron microscopy (SEM). Overall drug content within fiber mats and its release analyses, with high performance liquid chromatography (HPLC), were used for initial characterisation. Drug release tests were carried out in a phosphate buffer saline (PBS) at pH 7.4 for 24 h.

Results

Addition of AMP did not cause any major changes for electrospinning process. Furthermore, AMP did not have any negative effect on the fiber morphology since all fibers had smooth surface and were homogenous in size. Mean fiber diameter changed in the presence of different AMP concentrations. Mean fiber diameter was approximately 3.5 ± 0.7 µm for the pure hydrophobic polymer and 1.4 ± 0.3 µm for 1% AMP-loaded fiber mats. Fiber diameter decreased together with the AMP concentration increase. Content analyses revealed that no AMP degradation occurred during electrospinning nor during the drug release testing. Interestingly, only small amount of AMP was released from hydrophobic fiber mats after 24 h. The more AMP was loaded into the fibers the higher was the amount of drug released.

Conclusion

AMP can be successfully electrospun into a hydrophobic polymer fiber mat. Selected solvent nor electrospinning conditions do not cause any chemical degradation of AMP. Further testing in relevant eukaryotic and bacterial cell studies will be performed in order to understand the antimicrobial efficacy and safety of the AMP-loaded fiber mats.

Acknowledgements

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3D printed capsules for sustained drug release from nanocellulose hydrogel

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Introduction

The recent advances in biocompatible nanocellulose hydrogels and the new possibilities of printing specifically designed drug capsules were combined to create three rapid prototype products whose possibilities as dosage release capsules were evaluated in this study. Traditionally in pharmaceutical hydrogel applications, the release rate of the drug is controlled by the concentration of the loaded drug and other active ingredients [1]. Here, the same effect was achieved via controlled geometry. The capsules sustain the drug release by limiting the surface area of the anionic CNF hydrogel exposed to the outside. The idea of our devices differ from other drug release devices as the release is fundamentally controlled by the hydrogel, which facilitates a sustained release profile, and in addition the release is modulated via the geometry of the outer pharmaceutically non-active but biocompatible capsule.

Methods

The 3D printed capsules were filled with formulated placed in glass bottles with phosphate buffered saline and kept at 37 °C under constant shaking for 3 weeks. At chosen time points, 1 ml of sample was collected from each bottle and replaced with fresh buffer. All experiments were performed in triplicate. The concentrations of nadolol and metoprolol from these *in vitro* release tests were analyzed with Ultra performance liquid chromatography.

Results

During the three-week release study, a sustained release profile was obtained for both model compounds with all three PLA capsule designs as shown in figure 1.The small design with the smallest surface area sustained the release the most for both model compounds. The large design had less effect on sustaining the release, and the tube design sustained the release the least, as expected. Each capsule released the model compounds rapidly during the first hour, after which the release rate became more even. For the metoprolol tube design, the highest total released fraction of 96.4% was reached after 14 days.

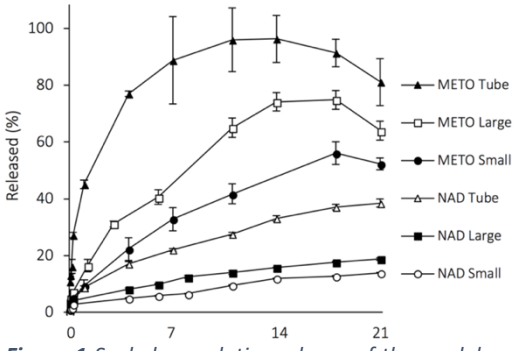

Figure 1 *Scaled cumulative release of the model compounds metoprolol (METO) and nadolol (NAD) from the three capsule designs (Tube, Large, and Small) carrying anionic cellulose nanofiber hydrogel drug formulations (mean ± S.D., n = 3).*

Conclusions

The hydrogel filled PLA capsules are suitable as sustained release platforms without the use of further excipients. The diameter of the capsules release channel can be modified effortlessly resulting in several adjustable parameters for sustained release.

Acknowledgements

The authors acknowledge and thank the University of Helsinki for co-operation and for providing access to their laboratories and screening instrumentation.

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High Content Solid Dispersions for Dose Window Extension in Fused Deposition Modelling

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Introduction
Fused deposition modelling (FDM) of pharmaceutical dosage forms holds considerable potential for flexible dosing. However, demonstrated solutions for delivering high dose drugs at acceptable dosage form sizes (e.g. to aid swallowability) are lacking (1,2,3,4). The purpose of our study is to use high drug content solid dispersions to extend the current FDM dose window in order to i) accommodate pharmaceutically relevant doses of drugs of varying potencies at acceptable dosage form sizes and ii) enable enhanced dose flexibility via modular dosage form design concepts (5).

Aim
To investigate: 1. uniformity of drug content in small volume FDM-printed units at high drug contents up to ~50% w/w API and 2. dispensing precision at small print volumes.

Method
FDM was used to generate small volume discs (2-40 mm^3) from melt-extruded feedstocks based on 10% to 50% w/w felodipine in ethyl cellulose. Drug content was determined in both the extrudates and the 3D printed discs by UV spectroscopy and dispensing precision from printed disc mass. Raman spectroscopy was used to confirm the solid-state form of the drug prior to FDM.

Results
Our principal finding of mean drug content in printed units within 5% of target values for payloads as high as 42% w/w API translates to a greater than eight-fold extension of the dose window relative to existing studies for standard-sized dosage forms. Furthermore, the drug was maintained in amorphous form prior to FDM.

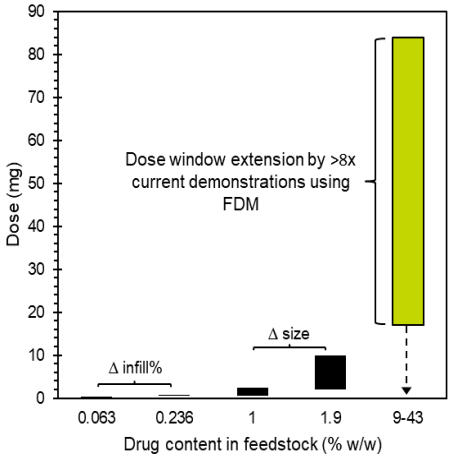

Figure 1 *Extension of FDM dose window using the high drug content approach*

Conclusion
These findings enable preservation of the design flexibility of FDM by extending its applicability to drugs of varying potencies and solubilities, beyond the low-dose applications already exemplified. Consequently, improved clinical relevance regardless of dose, is facilitated.

Acknowledgement
This study is funded by AstraZeneca & a Swedish Foundation for Strategic Research grant.

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Workpackage 4 ANALYTICS (Leader: Tiina Sikanen, UHEL)

Precise, sensitive, and selective (molecular) analysis is the basis for monitoring of the quality of pharmaceutical products. To ensure safety and efficacy of drug products, a variety of in-process and end product analyses as well as in vitro and in vivo analysis of drug compounds and their biological and chemical degradation products is needed. With the promise of improved throughput, microfluidics and miniaturized sample preparation and separation devices are emerging to supplement the well-established chromatographic separaration systems spectroscopic techniques.

WP4 aims to provide both new analytical methods (using established instrumentation) and novel analytical tools (new instrumentation) ensure high quality drug products and to provide us with new insights into relevant complex processes and molecular interactions including but not limited to membrane transport, formulation pathways, and production line.

Invited speaker talk:

Clare Strachan: Surface crystallinity: detection, significance and control

Students talks:

Jacob Bannow, UCPH

Jacopo Zini, HEL

Joseph Diab, UiT

Mette Sloth Bohseni

Poster session:

47	Margrét Þorsteinsdóttir
48	Minna Rahnasto-Rilla, UEF
49	Juulia Järvinen, UEF
50	Jixiang Wang, AAU
51	Yong Guo, AAU
52	Niklas Nilsson,UiO
53	Lorenz Göschl,UiT
54	Laura Hellinen, UEF

Surface crystallinity: detection, significance and control

Clare J. Strachan
Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, Finland

Brief CV
Clare Strachan is an Associate Professor at the Faculty of Pharmacy, University of Helsinki where she heads the PharmSpec Lab and Formulation and Industrial Pharmacy Unit. Her group's research focuses on the application of spectroscopy and imaging for pharmaceutical understanding and optimization during different stages of the drug lifecycle (e.g. manufacturing, storage, administration and drug delivery). Spectroscopic methods include infrared, Raman, CARS and other forms of non-linear optical imaging, and these are used in particular to understand and optimise pharmaceutical solid-state behaviour.

Abstract
Understanding, optimisation and control of solid-state behaviour is essential in the development of solid dosage forms, in particular with poorly water-soluble drugs due to potential therapeutic implications. Disorder in pharmaceutical solids, whether desired or otherwise, is widespread but its characterisation and prediction remains challenging in some respects. In particular, surface crystallinity and its changes and control during, processing storage and dissolution remain relatively poorly understood, even though surface crystallinity can critically pharmaceutical performance.
This talk explores the phenomenon of surface crystallinity and crystallisation, with a particular focus on its detection using spectroscopic imaging (including non-linear optical imaging). The interplay between surface crystallinity, and its control, with storage and dissolution will also be considered.

Probing the Surface Mobility of Supersaturated Amorphous Solid Dispersions by Atomic Force Microscopy

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Introduction

The poor physical stability of amorphous systems remains one of the main challenges in current formulation development. In this context, a growing number of publications are focusing on surface-related properties, in particular on the surface mobility. A highly mobile surface layer was shown to be directly responsible for fast crystal growth on free surfaces and governs the overall recrystallization kinetics of molecular glasses.[1]
In this study, the influence of different concentrations of the polymeric excipient Soluplus on the surface mobility of amorphous indomethacin (IMC) is investigated.

Aim

To quantify the effect of low amounts of Soluplus on the surface molecular mobility of amorphous IMC.

Method

The surface mobility of neat amorphous IMC and Soluplus-spiked amorphous IMC was quantified by studying the nanoscale flattening of an initially corrugated surface over time.[2] Amorphous thin films of neat IMC and Soluplus-spiked IMC were prepared by heat drying. As a first step, IMC and the corresponding amount of Soluplus were dissolved in a mixture of aceton:ethanol (80:20 V/V) followed by solvent evaporation on a microscopic cover glass at 175 ˚C. Master gratings for the embedding of the surface grating were derived from conventional DVDs (λ = *740 nm)* and imprinted on the amorphous surface at 70 °C (T_g of IMC + 30 °C). Topographic images of the amorphous thin films were acquired using a Strømlingo™ advanced atomic force microscope to monitor the height decrease over time of the sinusoidal amorphous surface gratings.

Results

The resulting height decay over time was fitted with an exponential function ($h=h_0\,exp(-Kt)$) with K being the decay constant. As shown in Figure 1, amorphous thin films spiked with 1 % (w/w) Soluplus exhibited a significant decrease of the resulting K value (-4.8 s^{-1}) when being compared with neat amorphous IMC (-3.9 s^{-1}). Increasing polymer concentrations resulted in a further decrease of surface mobility.

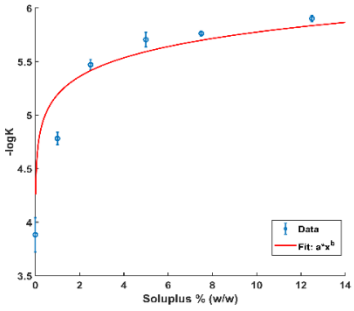

Figure 1. *Influence of different Soluplus concentrations on the decay constant K. Data obtained at 30 ˚C and 32 % rH, shown as mean ± SD.*

Conclusion

It could be demonstrated that already low amounts of the polymeric excipient Soluplus were able to slow down the surface mobility of amorphous IMC, providing insights into the stabilization effect of polymeric excipients in amorphous systems.

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Raman and FTIR spectra of extracellular vesicles reveal the efficiency of different purification protocols

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Introduction:
Extracellular vesicles (EVs) are a heterogeneous group of nanometer-sized membrane-derived vesicles released by most of the cell type in different bio-fluids. Evs, thanks to their ability to interact with cellular membranes have recently received considerable attention to their potential role as drug carrier and as biomarkers and prognostic indicators in the surveillance of a variety of diseases. Although vast knowledge on the subject of EVs has accumulated over the years, there are still fundamental issues associated with the correct approach to EVs isolation and characterization.

Aim:
The aim of the present study was compare the current purification and characterization methods and find a univocal and reproducible method to obtain and chracterize pure Evs.

Method:
EVs derived from two different cell lines, PC-3 and PNT2, were purified with ultra-filtration (UF), differential centrifugation (DC), density gradient centrifugation (DG), size exclusion chromatography (SEC) and salting out (SO) or a combination of two methods. Subsequently, Evs samples were characterized by the EVs biomarker expression, their Particle-to-Protein (Pa/Pr) ratio obtained by NTA and BCA protein kit and they Lipid-to-Protein (L/Pr) spectroscopy ratio obtained by FTIR and Raman spectroscopy

Results:
SEC was indicated as the most efficient purification methods by three different characterization protocols: P/L Raman, L/Pr FRIT and by expression of specific EVs biomarker, however, Pa/Pr identify DC +CG as most profitable purification method. These differences can be explained with the lack of sensitivity of the methods used for particle and protein quantification. On the other hand, FTIR and Raman spectra provided a fingerprint influenced by the bio-chemical composition of the sample, thus, it is possible not only gather information on the purity of the by L/P within one measurement but also gain information of the overall quality of the samples.

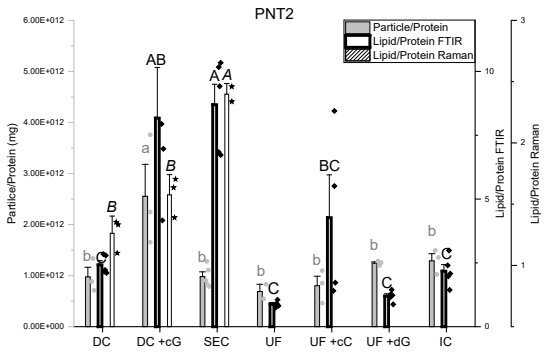

Figure 1; *Particle-Protein, spectroscopy Lipid-Protein ratii of PNT2 (A) derived EVs. Capitol, lowercase and italic characters represent rispectivly groups L/Pr FTIR Pa/Pr and L/Pr Raman with a statistical significant difference.*

Conclusion:
EVs can be fruitfully purified by SEC and characterized by spectroscopic techniques as FTIR and Raman. Specifically, Raman spectroscopy is able to reveal different features of the EVs preparation and reveal the overall quality of the EVs sample.

Acknowledgement:
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Metabolomics Coupled with Pathway Analysis Reveal the Metabolic Fingerprint in Ulcerative Colitis

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Introduction

Inflammatory bowel disease (IBD) is a chronic, relapsing inflammatory disorder in the gastrointestinal tract that affects up to 0.5% of the population of the Western world. The two major forms of IBD, Ulcerative Colitis (UC) and Crohn's Disease (CD), are characterized by a dysregulated mucosal immune response triggered by several genetic and environmental factors in the context of host-microbe interaction. This overwhelming complexity of IBD makes it ideal for metabolomic studies to unravel the disease pathobiology and to improve the patient stratification strategies toward personalized medicine.

Aim

To explore the mucosal metabolomic profile in UC patients, and to pinpoint the metabolic signature of IBD.

Methods

Colon mucosa biopsies were collected from 18 treatment-naive UC patients at the debut of the disease (inflamed mucosa), 10 UC patients in deep remission, and 14 healthy subjects. Metabolomic analysis of the colon biopsies was performed by a combined gas chromatography coupled to time-of-flight mass spectrometry (GC-TOF-MS) and ultra-high performance liquid chromatography coupled with mass spectrometry (UHPLC-MS) analyses. In total, 177 metabolites from 50 metabolic pathways according to Kyoto Encyclopedia of Genes and Genomes (KEGG) database were identified.

Results

The relative abundance of 60 and 46 metabolites were altered in UC treatment-naïve patients compared with healthy controls and with UC remission patients respectively. The most prominent changes among the study groups were in lysophosphatidylcholine (LPC), acyl carnitine, and amino acid profiles. Several pathways were identified as the most perturbed according the integrated pathway analysis. These pathways ranged from amino acid metabolism (such as tryptophan metabolism, and alanine, aspartate and glutamate metabolism) to antioxidant defense pathway (glutathione pathway). Furthermore, the pathway analysis revealed a disruption in the long and short chain fatty acid (LCFA and SCFA) metabolism, namely linoleic metabolism and butyrate metabolism.

Conclusion

The mucosal metabolomic profiling revealed the main metabolic signatures in active UC, and reflected the homeostatic disturbance in the gut. It seems that the microbiota is heavily involved in altering several metabolic pathways in the colon mucosa. This highlights the importance of integrating IBD-omes compartments by system biology approaches to identify key drivers of pathogenesis which prerequisite personalized treatment.

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Submicron and micron particles forming in aqueous medium upon dispersion of amorphous solid dispersions of the poorly soluble drug ABT-102

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Introduction

Amorphous Solid Dispersions (ASD's) are promising wrt increasing the bioavailability of poorly soluble drugs, that are becoming more prevalent in the pharmaceutical industry in recent years [1]. Exploring the supramolecular assemblies forming during dispersion of ASDs in aqueous media may contribute towards a better mechanistic understanding of improved drug performance.

Aim

To investigate the supramolecular assemblies emerging when dispersing different concentrations ASD's, containing the poorly water-soluble drug molecule ABT-102, in aqueous media.

Method

Asymmetric Flow Field-Flow Fractionation (AF4) coupled with Multi-Angle Laser Light Scattering (MALLS) was employed to identify and characterize sub-micron assemblies present in the supernatant upon bench-top centrifugation [2]. In addition, the micrometer sized populations in the supernatant, pellet and total samples prior to centrifugation were analyzed with Single Particle Optical Sensing (SPOS) to get a full representation of the supramolecular assemblies. Furthermore, Ultra High-Performance Liquid Chromatography (UHPLC) was utilized to quantify ABT-102 in the supernatant and samples prior to centrifugation.

Results

AF4/MALLS fractograms and size data indicate that three supramolecular assemblies emerged in all ASD dispersions. Triplicates made on three different days revealed a high reproducibility with the AF4/MALLS set-up. The concentration of ABT-102 was 200 times higher in samples prior to centrifugation compared to supernatants. SPOS results indicate micrometer particles below 20 µm in diameter are emerging.

Conclusion

Supramolecular assemblies forming in aqueous medium upon dispersion of ASD's were successfully and reproducibly identified and characterized with AF4/MALLS, SPOS and UHPLC.

Acknowledgements
This study is partly based upon work carried out under COST Action 16205 UNGAP, supported by COST (European Cooperation in Science and Technology) as well as Nordic POP (Patient Oriented Products), a Nordic University Hub funded by NordForsk (Project number: 85352).

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Design of experiments for optimization of quantitative UPLC-MS/MS clinical diagnostic assay

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Introduction

Tandem mass spectrometry coupled to ultra-performance liquid chromatography (UPLC-MS/MS) is an excellent analytical method with ultimate selectivity and sensitivity needed for quantification of biomarkers in biological matrices. The UPLC-MS/MS is a two-stage process, liquid introduction and analyte ionization. The goal is to transfer the analyte from condensed phase to gas phase and maintain conditions that are compatible for both the LC and the MS. This involves many experimental factors which need to be simultaneously optimized to obtain maximum selectivity and sensitivity at minimum retention time. Optimization of experimental conditions for UPLC-MS/MS methods is usually performed by changing one-factor-at-time (OFAT) experiments. However, a much more effective optimization strategy for discovering important experimental factors and to optimize the responses is to utilize design of experiment (DoE). DoE is a systematic approach where all relevant experimental factors are studied simultaneously according to predefined plan.

Aim

The aim of this study was to illustrate that method development can become much more efficient by utilizing the chemometric approach design of experiments (DoE).

Method

DoE was used for optimization of an UPLC-MS/MS assay for simultaneous quantification of 2,8-dihydroxyadenine (DHA), a biomarker for APRT deficiency, adenine, adenosine, inosine, hypoxanthine, xanthine and the drugs allopurinol, its active metabolite, oxypurinol, as well as febuxostat in human plasma and urine. A fractional factorial design was used for experimental screening to reveal the most influential factors. When multi-levels qualitative factors were included in the screening experiments D-optimal design was applied. Significant factors were studied via central composite design and related to sensitivity, resolution and retention time utilizing partial least square (PLS)-regression.

Results

A specific UPLC-MS/MS assay for simultaneous quantification DHA and the main purine metabolites was optimized efficiently utilizing the chemometric approach, DoE. There was a strong interaction effect between several variables, indicating that these variables cannot be independently controlled to obtain optimal conditions. The assay has been used to measure both plasma and urine concentration of the analytes in samples from healthy controls and APRT patients, before and after treatment. DHA was not detected in plasma from healthy controls. Highly significant changes were observed in the urinary excretion of DHA and adenine with drug therapy.

Conclusion

Optimization of the UPLC-MS/MS assay by DoE, was performed with only a fraction of experiments compared to what would have been required by changing one separate factor at a time (COST) approach. Our data suggest that the UPLC-MS/MS assay will greatly facilitate clinical diagnosis of patients with the rare kidney stone disorder APRT-deficiency. At the same time the clinical assay can be used for monitoring pharmacotherapy and treatment adherence among these patients.

Effects of Ellagic acid on Sirtuin 6 and its anti-tumorigenic activities

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Introduction

Sirtuin 6 (SIRT6), a member of Sirtuin family (SIRT1-7), regulates distinct cellular functions such as genome stability, DNA repair, and inflammation related diseases.[1-2] SIRT6 functions as a histone H3 lysine 9 (H3K9) deacetylase affecting gluconeogenesis, increasing mitochondrial respiration, and inhibiting glycolysis. Most cancer exhibit an altered metabolism with increased glycolysis, also known as the Warburg effect, which is important for rapid tumor growth. SIRT6 activation prevents this glycolytic shift. Overall, SIRT6 can act as a tumor suppressor, and an increase in SIRT6 activity or protein levels contributes to tumor development, indicating a promising strategy for cancer prevention. Recently, we demonstrated that anthocyanidins in berries induce the catalytic activity of SIRT6.[3]

Aim

In this study we explored the effects of Galloflavin and Ellagic acid, the most common polyphenols in berries, on SIRT6.

Method

We investigated SIRT6 deacetylation using HPLC and immunoblotting assays. The expression levels of SIRT6, glycolytic proteins and cellular metabolism were studied in Caco2 cells.

Results

Ellagic acid increased the deacetylase activity of SIRT6 by up to 50-fold whereas Galloflavin produced approximately 10-fold maximal activation. Galloflavin and Ellagic acid showed anti-proliferative effects on Caco2 cells. The compounds also upregulated SIRT6 expression whereas key proteins in glycolysis were downregulated. Galloflavin decreased glucose transporter 1 (GLUT1) expression, whereas Ellagic acid affected the expression of protein dehydrogenase kinase 1 (PDK1).

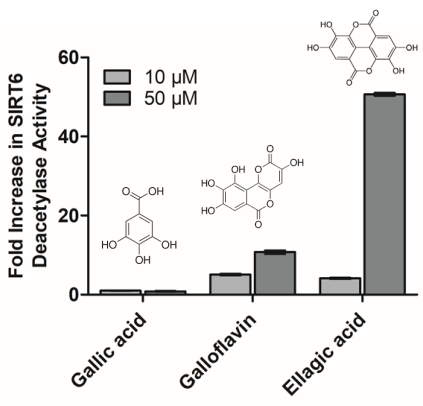

Figure 1. Galloflavin and Ellagic acid stimulate SIRT6.

Conclusion

The results of this study demonstrated that Galloflavin and Ellagic acid affected SIRT6 activity and the expression of proteins associated with cancer development. Thus, Galloflavin and Ellagic acid may be important for the prevention of cancer.

Acknowledgement

The authors thank Academy of Finland (grant no. 315824 and grant no. 269341), Orion–Farmos Research Foundation, the Foundation of Saastamoinen, Finnish Cultural Foundation for financial support.

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L-type amino acid transporter (LAT1) targeted prodrug of Palbociclib for improved drug delivery across the blood-brain barrier and to invasive glioma cells

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Introduction

Palbociclib is a cyclin dependent kinase 4/6 inhibitor, which has been approved in 2015 for treatment of breast cancer[1]. Preclinical studies have demonstrated that Palbociclib is potential drug to be used in the treatment of a variety of tumors including breast cancer and glioma[2]. However, previous studies have shown that Palbociclib is a substrate for P-glycoprotein (P-gp) and breast cancer resistant protein, which restrict the brain exposure of Palbociclib and effective drug concentration cannot be achieved in invasive tumor cells protected by the intact blood-brain barrier (BBB).

Aim

Our overall objective was to design, synthetize and evaluate a novel amino acid prodrug of Palbociclib with improved L-type amino acid transporter (LAT1)-mediated glioma cell delivery. LAT1 is highly expressed at the BBB and in various cancer cells and is therefore a potential drug target.

Methods

In vitro concentration-dependent uptake studies of Palbociclib and prodrug were performed by incubating the MCF-7 breast cancer cells with 10-400 µM of studied compound at room temperature for 15 minutes. The samples were analyzed by HPLC. Affinity studies were performed by using a known radiolabeled LAT1-substrate [^{14}C]-L-leucine by incubating the cells (MCF-7) for 5 min with [^{14}C]-L-leucine (0.157 µM) and studied drugs with concentrations of 10 µM and 100 µM. The radioactivity was measured by liquid scintillation counter. *In vitro* efflux studies were carried out in MDCKII-MDR1 cells and MDCK wild type cells with calcein-AM assay. Studied concentrations for Palbociclib and its prodrug were 1 µM, 10 µM and 100 µM, and for known P-gp inhibitor Elacridar 0.1 µM, 1 µM and 10 µM and substrates Verapamil 100 µM and MK-571 50 µM. P-gp substrate calcein acetoxymethyl ester (calcein-AM) was added to achieve the final concentration of 2 µM. The cellular fluorescence was measured by Victor Multilabel Counter.

Results

Concentration-dependent uptake of Palbociclib and its prodrug in MCF-7 cells was at a nanomolar range. For Palbociclib V_{max} of cellular uptake was 0.39±0.02 nmol/min/mg of protein, while V_{max} value for the prodrug was 10.79±3.12 nmol/min/mg of protein. In affinity studies in MCF-7 cells prodrug showed some reduction of [^{14}C]-L-leucine uptake indicating slight affinity for LAT1. In Calcein-AM assay in MDCKII-MDR1 cells and MDCK-wt cells, Palbociclib (100 µM) showed almost ten-times greater fold change compared to control, which suggests that Palbociclib is an efflux substrate. Compared to control, 10 µM Palbociclib showed also some affinity to P-gp, but 1 µM concentration did not show any difference. Prodrug itself did not show any difference compared to control.

Conclusion

The *in vitro* results of the novel prodrug are promising. The prodrug showed over 25 times greater cell uptake compared to Palbociclib. Furthermore, prodrug did not demonstrate any efflux affinity, which may improve, together with LAT1-mediated targeted uptake, the brain exposure of prodrug and lead to higher drug concentration in glioma cells compared to Palbociclib itself. Due to our preliminary studies it can be assumed that cell uptake for the prodrug may be LAT1-mediated. To deepen the knowledge of LAT1-mediated transport of the prodrug, further *in vitro*, *in situ* brain perfusion and *in vivo* pharmacokinetic studies are planned.

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Ag-Doped Ultrathin Surface-Imprinted Fluoroprobe for Direct, Rapid and Effective Detection of Trace Oxytetracycline in Real Samples

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Introduction

Molecularly imprinted fluoroprobes have shown great promise in environmental or food quality monitoring due to its high stability, selectivity and sensitivity.[1] Nevertheless, molecularly imprinted fluoroprobes still have some inevitable problems remain to be addressed, especially with regards to their slow response and low detection efficiency.[2] As known, the recognition sites in thin walled imprinted polymers are shallow, which facilitate the infiltration of target molecule into the imprinted sites. Ag nanoparticles contribute to promote the mechanism of energy transfer or the mechanism of charge transfer, thereby the excited state of fluorescent molecules returns to the ground state by contacting with the quencher, accelerating the efficiency of fluorescence quenching.[3] Therefore, we conjecture that the Ag nanoparticles doped ultrathin surface-imprinted fluoroprobes will significantly improve the fluorescence detection rate during molecular identification.

Aim

To investigate the influence of the introduction of Ag nanoparticles on the detection rate of ultrathin surface-imprinted fluoroprobes.

Method

After preparation of SiO_2, the Ag nanoparticles are formed on the surface of SiO_2 by reduction. Meanwhile, the fluorochrome (AOXB, 30 mg), template (OTC 0.1 mmol), functional monomers (AFBPA, 0.2 mmol), MAA (0.2 mmol) and cross-linking (EGDMA, 0.4 mmol) were dissolved in acetonitrile (20 mL), and Ag-doped ultrathin surface-imprinted fluoroprobes (SiO_2/Ag@FMIPs) were prepared by precipitation polymerization. Subsequently, the fluorescence detection performance was measured using fluorescence spectrophotometer.

Results

Compared with the SiO_2@FMIPs, fluorescence detection of the OTC with SiO_2/Ag@FMIPs, the fluorescence quenching could rapidly reach to equilibrium within 150 s, and saved 300 s (Figure 1). This is attributed to the fact that the SiO_2/Ag@FMIPs is doped with Ag particles on the basis of thin-layer imprinted layer, further increasing the fluorescence detection rate.

Conclusion

It proved that SiO_2/Ag@FMIPs could promote the mechanism of energy transfer or the mechanism of charge transfer due to the electronic cloud around it, and accelerating the efficiency of fluorescence quenching.

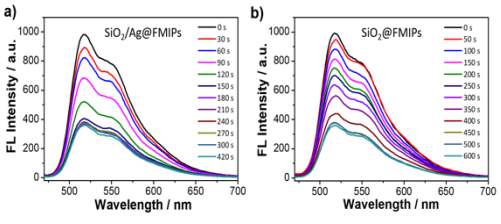

Figure 1 *Dynamic fluorescent analysis detection time of SiO_2/Ag@FMIPs and SiO_2@FMIPs to OTC solutions (300 nM).*

Acknowledgement

The author would like to thank Sigrid Juselius Foundation (28001830k1) and Academy of Finland (328933) grants.

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Pharmacokinetics of mifepristone by oral administration in beagle dogs

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Introduction

Mifepristone has a chemical structure similar to progesterone. It is a highly effective anti-progestin drug that can act on different phases of the menstrual cycle with a long half-life. Researchers are exploring to use it for a routine contraception. The researcher has conducted preliminary research on the clinical effects of mifepristone once a week orally [1], which suggested that it is hopeful to develop mifepristone into oral contraceptive methods once a week. In vitro and in vivo studies have shown that mifepristone has a good therapeutic effect on the treatment of uterine fibroids [2], endometriosis [3], endometrial cancer [4], etc., however, suitable doses and protocols are still needing further investigation. Before clinical trials, preclinical studies on new indications for mifepristone contraception are needed to evaluate their safety and effectiveness.

Aim

To study the pharmacokinetics of mifepristone with single administration in beagle dogs.

Method

Female beagle dogs with 8-12 kg were subdivided into three groups: 1, 3 and 10 mg/kg mifepristone, by means of intragastric administration, respectively. The plasma samples were collected before and 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h, 48 h, 72 h and 96 h after administration. Phoenix WinNonlin software was used to analyze the blood concentration data derived from beagle dogs after administration of mifepristone, and calculate the main pharmacokinetic parameters.

Results

There were significant differences in t1/2 value and MRT between 1 mg/kg group, 3 mg/kg group and 10 mg/kg group ($P < 0.05$). No significant difference between 3 mg/kg group and 10 mg/kg group ($P>0.05$) was observed. There were significant differences in AUClast and AUCInf values between the three groups ($P< 0.05$). No significant difference in Tmax values between the three groups ($P >0.05$) was observed. Bioavailability in 10mg/kg group (52.6%) was significant higher than that of 1mg/kg group (32.5%) or 3mg/kg group (27.6%) ($P<0.05$).

Conclusion

It is shown the characteristics of non-linear pharmacokinetics and drug accumulation of mifepristone in vivo.

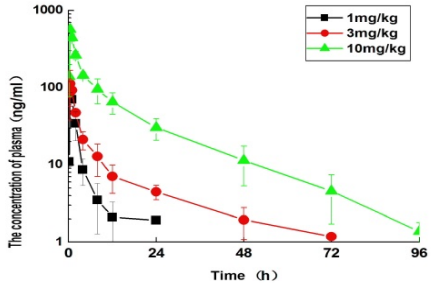

Figure 1 *Plasma concentration-time of mifepristone after oral administration in beagle dogs*

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Physical compatibility of three drugs and total parenteral nutrition used at the neonatal intensive care unit (NICU)

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Introduction

The newborn patient at NICU receives numerous drugs and most of them are administered intravenously. In addition, these patients receive an uninterrupted supply of parenteral nutrition. Because of few intravenous access ports, drugs and parenteral nutrition have to be co-administered via the same intravenous (i.v.) catheter line. This gives rise to potential undesirable consequences, such as precipitation or fat droplet growth due to destabilization. Precipitates and droplets with a diameter of 5 µm or more have an increased risk of being entrapped in small capillaries where they may cause embolism.
There is a lack of data on compatibility between drugs and parenteral nutrition in the pediatric population.

Aim

The aim of the study was to obtain compatibility information when mixing total parenteral nutrition (TPN) with three drugs commonly used at NICU.

Method

The TPN admixture Numeta G13E (Baxter, UK), was tested with three drugs (paracetamol, fentanyl and vancomycin, respectively). The drugs were mixed with Numeta 1+1 (drug+TPN) and in addition the most extreme mixing ratios estimated as clinically relevant in the lines (drug > TPN, TPN > drug). Subvisual particle counting, visual examination and measurements of turbidity and pH were used to assess potential precipitation[1]. Emulsion stability was investigated by estimating the percentage of fat droplets > 5 µm (PFAT5)[2], mean droplet diameter and pH measurements[1].

Results

All three drugs showed no sign of precipitation after mixing with TPN. Paracetamol mixed with Numeta G13E in the ratio 1+10 resulted in a small increase in PFAT5 after 4 hours contact time and immediately as well as after 4 hours for the ratio 3+2. Vancomycin mixed with Numeta G13E in the ratio 1+1 resulted in a small increase in PFAT5 immediately after mixing and for the ratio 1+2 after 4 hours contact time.

Conclusion

None of the drugs showed a clinically relevant sign of incompatibility after mixing with TPN.

Acknowledgement

The authors would like to thank Vigdis Staven Berge and Camilla Tomine Østerberg for their help and support.

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Development and validation of fast online-SPE method coupled to high-resolution mass spectrometry for the detection of stanozolol-N-glucuronides in urine samples

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[2] Department of Pharmacy, University of Tromsø – The Arctic University of Norway, Tromsø, Norway
[3] Institute of Applied Synthetic Chemistry, Technical University of Vienna, Austria

Introduction
Stanozolol is still the most commonly used illicit anabolic-androgenic steroid in professional sports[1]. Therefore, accurate and fast analysis and long detection windows are of high interest in the field of anti-doping analysis. Conventional steroid analysis is based on the enzymatic cleavage of steroid phase-II metabolite conjugates and the following analysis of remaining parent molecules and phase-I metabolites as their trimethylsilyl - derivatives with gas chromatography-tandem mass spectrometry[2]. However, many studies have shown that the direct analysis of steroid phase-II conjugates using liquid chromatography-tandem mass spectrometry (LC-MS/MS), is a highly promising approach for the detection of unknown steroid metabolites. Most published methods either use a solid-phase-extraction (SPE) as sample preparation with following LC-MS/MS analysis or a direct, so-called dilution-and-shoot approach for the detection of stanozolol-glucuronides. The new approach in this work is to combine the advantages of solid-phase extraction (SPE) with a fast and simple dilute-and-shoot LC – high-resolution tandem mass spectrometry (HRMS) method.

Aim
The present work aimed to develop and validate a fast, but accurate and sensitive online-SPE-LC-HRMS method for the analysis of stanozolol-N-glucuronides in human urine samples.

Method
As an extraction column, an Accucore Phenyl-Hexyl, 10 x 3 mm column with 2.6 µm particle- and 80 Å pore size was used, which was installed upstream to a two-position, six-port UHPLC valve. This valve allows the online switching between pre-trapping of the samples and the following analytical measurement. As an analytical column, a Kinetex EVO C-18, 100 x 2.1 mm column with 2.6 µm particle- and 100 Å pore size was used. Measurements were performed on a Vanquish Horizon UHPLC+ System coupled to a Q-Exactive Orbitrap high-resolution mass spectrometer. The method introduced in this work was validated for qualitative and semi-quantitative purposes according to the International Standard for Laboratories[3].

Results
This fully validated procedure includes virtually no sample preparation; a limit of identification (LOI) of 75pg/ml, high accuracy (3.06% – 7.80%), precision (87.06% - 102.13%) and sensitivity was achieved. Furthermore, good linearity (> 0.99) and robustness, as well as no carry-over effects, could be observed.

Conclusion
In the present study, we introduce a novel, highly functional analytical method for the analysis of stanozolol-glucuronides for doping control analysis. Sample preparation is reduced to diluting the sample with water and adding an internal standard solution. We established a very simple approach for installing an automatic online solid-phase extraction coupled with UHLPC-HRMS/MS.

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Biochemical and cellular screening methods to evaluate hit compounds for dry age-related macular degeneration

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Introduction
Dry age-related macular degeneration (AMD) does not currently have treatment and the patient number is increasing rapidly as the population ages. The functions of retinal pigment epithelial cells located in the posterior eye are compromised in dry AMD, for instance, the protein clearance is disturbed leading to cellular distress. The outcome of the advanced disease is sight loss. Our preliminary data showed that activity of a cytosolic enzyme is elevated in dry AMD cells in vitro. The inhibition of this enzyme was able to prevent the loss of autophagy in AMD cells. Further characterization and screening of the enzyme inhibitors is needed to evaluate this pathway in dry AMD.

Aim
The aim of this project was to set up biochemical and cellular screening assays to investigate enzyme inhibitors against dry AMD.

Methods
Microscale thermophoresis was utilized to determine compound binding to the target enzyme (Kd values). The hit compound potencies (IC_{50} values) were determined by monitoring the formation of fluorescent metabolite in the presence of compound and recombinant enzyme. The unbound partition coefficients (Kp_{uu}) of the enzyme inhibitors were determined with previously published methods[1,2]. Intracellular target engagement was evaluated with cellular thermal shift assay[3].

Results
The Kd values varied between 5-37 nM and the IC_{50} values were within the range of 2-11 nM. Cellular thermal shift assay confirmed, that 3 out of 5 compounds bound to the target protein, whereas two compounds did not cause shift in the aggregation curve. These two compounds displayed lower unbound partition coefficients (Kp_{uu}) compared to the other three studied inhibitors.

Conclusion
Biochemical methods revealed only minor differences among the hit compound potencies. The cellular thermal shift assay showed differences in the intracellular target engagement of the compounds, which was explained by their differences in the cellular kinetics.

Acknowledgement
The author would like to thank NordForsk (Nordic POP project 85352), Markku Juslin grant, Finnish Cultural Foundation and the Academy of Finland for financial support.

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Workpackage 5 SYNCHROTRON (Leader Anders Østergaard Madsen, UCPH)

Innovation within design and manufacturing of complex drug delivery systems (DDS) calls for high-end imaging techniques. Of special interest is the capability of studying these systems in operando – and to study the DDS behavior *in situ*, e.g. as a function of time, pressure, humidity, temperature and pH, and in both equilibrium and non-equilibrium situations, and to map out chemical composition as well as physical properties of these structures. For such studies, the large-scale facilities offer special advantages.

The high flux and hard X-rays from the synchrotron translates directly into higher spatial and temporal resolution. Neutrons offer the advantage of non-destructive and bulk analysis in combination with unique isotopic information that yields differing elemental sensitivity to that of X-rays. This WP will provide next generation solutions for the visualization of the structure of biological systems and pharmaceutical products.

Invited speakers talk:

Barbara Berke: An insight into small- and wide- angle X-ray imaging

An insight into small- and wide- angle X-ray imaging

Barbara Berke
Department of Physics, Chalmers University of Technology, Göteborg, Sweden

Brief CV
Barbara Berke works as Postodoctoral Researcher in the Liebi Resarch Group, at the Department of Physics of Chalmers University of Technology in Göteborg, Sweden.
Her research is focused on controlled alignment of anisotropic materials and the study of structural changes in hierarchical systems. Her current project is related to the development of the ForMAX beamline at MAX IV, which will be a combined SAXS and imaging beamline, in particular aimed at the Swedish forest industry. Therefore, cellulose based materials are of high interest in complex sample environments. Amongst others, 3D printing, electrospinning and materials in flow are studied by SAXS.

Abstract
Small angle X-ray scattering is an essential tool to study structures on the nanometer scale. Using this technique, we can gather information, for example, on particle shapes and sizes; atomic or molecular arrangements; crystalline or amorphous structures; grain or crystallite sizes; etc.
Laboratory sources can be used to get an overview of a sample but using synchrotron radiation allows us to perform 2D or 3D scans, which enables structural characterization with good spatial resolution. These options can be beneficial for, amongst other things, studying homogeneity of a sample, or gain information on local orientation in complex environments. Deeper understanding of the structural properties can be crucial for paving the way for further research, since this knowledge can be used to make materials with tailored characteristics.
In this presentation, I will introduce you to small angle X-ray scattering and give an insight on what it can be used for by showing examples from various fields and applications.

Workpackage 6 MODELLING AND SIMULATIONS (Leader Christel Bergström, UPP)

The accuracy and applicability of computational tools to model and simulate complex systems (ranging over biological systems, chemical engineering to production lines) has increased significantly during the last decade as a result of the increased computational power available to handle big data.

This WP brings together a critical mass with computational competences spanning over a wide range, from classical multivariate data analysis (MVA) and molecular dynamics (MD) simulations (mechanical/quantum) over discrete element, finite element and finite volume methods to physiology-based pharmacokinetic (PBPK) modeling. Our mission is to enable mechanistic insights into complex processes (membrane transport, formulation pathways, production line), visualize molecular interactions (important for crystallization, permeation, solubilization etc.), and facilitate e.g. 3D representation, images as well as templates, for e.g. 3D cell culture production or 3D printed dosage forms.

Invited speakers talk:

Erik Sjögren: Physiologically based modeling - What can it do for you as a formulation scientist?

Student talks:

Aleksei Kabedev, UPP

Patrick Sinko, UPP

Shakhawath Hossain, UPP

Poster session:

55	Joni Petteri Järvenpää, UEF
56	Fabrizio Clarelli, UiT
57	Jonna Tenhunen, UEF
58	Mahlet Tamirat, AAU
59	Katarzyna Krzemińska, MUG
60	Juri Timonen, UEF
61	Bianka Jacyna, MUG
62	Patrick Sinko, UPP

Physiologically based modeling - What can it do for you as a formulation scientist?

Erik Sjögren[1,2]
[1] Department of Pharmacy, Uppsala University, Sweden
[2] Pharmetheus, Sweden

Brief CV

Bio: Assoc. Prof. Sjögren graduated in pharmaceutical sciences at Uppsala University in 2004. He defended his doctoral thesis in 2010 at the department of Pharmacy, Uppsala University, with focus on mechanistic analysis in hepatic drug disposition and pharmacokinetic assessments. He was appointed Associate Professor in Biopharmaceutics in 2016 and has published over 50 scientific papers. Assoc. Prof. Sjögren joined Pharmetheus in 2017 as a senior consultant with responsibilities in mechanistic and physiologically based modelling & simulation, e.g., PBPK, PBBM and QSP, for prediction, translation and analysis across all phases of drug development

Abstract

Physiologically based modeling has the potential to translate in vitro and pre-clinical information, on the active pharmaceutical ingredient and drug delivery system, to clinically relevant endpoints, such as exposure and peak concentration in plasma. This is achieved by defined links, interactions and dependencies between organism and drug which are individually described with their distinct features. The approach has traditionally been used to predict or assess drug exposure effects due to physiological changes or variability but more frequently also for applications related to drug product performance. Establishment of physiologically based biopharmaceutics models (PBBM) facilitate for rational and physiology informed drug development and can also be applied in development of IVIVCs/IVIVRs and support when defining acceptance criteria of critical manufacturing parameters.

Molecular dynamics simulation as a tool to understand micelles-drug interactions in small intestine

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[1] Department of Pharmacy, Uppsala University, Sweden;
[2] The Swedish Drug Delivery Center, Dept of Pharmacy, Uppsala University, Sweden.

Introduction
Integration of the active pharmaceutical ingredient (API) into micellular or vesicular formations can lead to both positive and negative effects in terms of drug bioavailability. On the one hand, the drug molecules can avoid self-aggregation and crystallization. On the other hand, placement in the center of micelle can also complicate transfer from the intestinal fluid to the membrane cell. Molecular Dynamics (MD) simulations were performed to study the free energy profiles of the drug-micelle and drug-membrane interactions. A choice of a technique for specific tasks in the field is still unclassified.

Aim
The general aim was to compare different computational approaches for studying interactions of small molecules with bile and phospholipidic micelles and cell membranes.

Method
Molecular dynamics simulations were used to study the interactions of molecules with micelles and with membrane. Free energy profiles were analyzed for micelle-to-water and micelle-to-membrane transfers. Micellular structures were formed either via free self-assembly from random starting configurations or from the pre-defined placement of the molecules in a spherical shape with fixing certain degrees of freedom. Danazol, Probucol and Sodium Caprate were chosen to represent small molecules. We utilized umbrella sampling (US) and free energy perturbation (FEP) methods. Micelles were formed from bile salts (BS), phospholipids and mixtures of BS and PL.

Results
Spherically organized micelles served better for comparison of the free energy profiles under similar conditions, and also provided more detailed information on the internal energy barriers of the micelles. Profiles for the randomly aggregated micelles required less resources and in some cases are more relevant to experimental data. US method was found most suitable for detailed interaction analysis, but at the cost of relatively high computational resources. FEP is a relatively fast and computationally cheap method, but was found to be not accurate if less than 4 points were used for the interpolation. Free energy profiles that were obtained from pulling the small molecule from the micelle straight to the membrane have shown that the energy barrier is lowered as compared to micelle-water-membrane transfer. In other words, micelles serve as a shuttle and provide an environment for low energy cost transfer of drug molecules to the membrane.

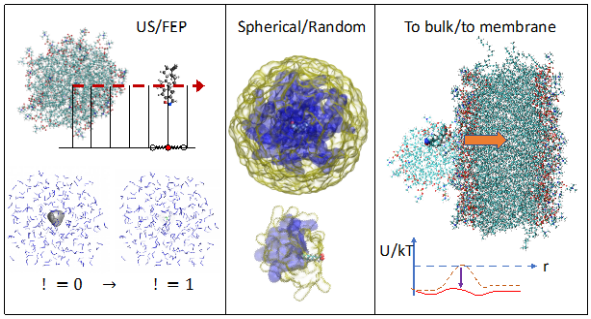

Figure 1 *Graphical abstract of the comparison performed in the study: umbrella sampling and free energy perturbation methods; pre-defined and random formation of micelles; various molecule transfers.*

Conclusion
Different combinations of techniques were used to computationally study the interactions of drug molecules with several types of micelles as well as a cellular membrane; all served different purposes and were beneficial when combined. These techniques allowed a mechanism of the transfer between the micelle and membrane to be proposed.

Acknowledgement
This study was funded by the European Research Council (grant number 638965).

Particle-Size and Dose Affect Passive Diffusion Flux Through an Ultra-Thin, Large-Area Poly(dimethyl siloxane) Membrane

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Introduction

It is critical to understand how disintegrating and dissolving formulations interact with the gastrointestinal (GI) environment, including the effect of particle size and dose on flux through the GI membrane. Sinko PD et al. introduced a dissolution apparatus with an artificial membrane that has high passive permeability for organophilic/lipophilic compounds in 2019. The ultra-thin large area membrane (UTLAM) diffusion cell using polydimethylsiloxane (PDMS) overcomes some traditional limitations of the USP II apparatus, such as having absorption capability, improved particle resuspension, and more homogeneous bulk fluid hydrodynamic performance.

Aim

We present a convection-diffusion-flux model that is particle size and dose-dependent to investigate dissolution phenomenon within the aqueous boundary layer at the membrane surface.

Method

Non-pre-saturated suspension experiments were conducted with three particle-size distributions (PSD), $r_{50\ volume\text{-}mastersizer}$ = 6.9µm, 27.1µm, 138.6µm at doses (0.2, 1, 10, and 40mg/mL). An 11.2 ± 0.5µm thick, 25.2cm^2 (44.4cm^2 total) PDMS UTLAM was fastened between two mesh supports in the diffusion cell; the first support traps particles at the surface of the membrane and the second allows particles to flow freely across the membrane surface. The diffusion experiments were performed at 37°C, stirred at 150RPM using a hydrofoil impeller, between pH 1.9-2.0 in the donor and pH 12.5 in the receiver, with ibuprofen as a model weak acid. An ImageJ routine in parallel with a Malvern Mastersizer 2000 measured mass/volume/number particle size distributions. Donor and receiver samples were analyzed with an Agilent 1100 HPLC.

Results

The effect of particles dissolving in the hydrodynamic boundary layer resulted in a significant increase in flux over the maximum solution flux for the smallest and medium size distributions of ibuprofen. There was better theoretical agreement at higher doses when particle confinement effects were accounted for using the Wang-Brasseur (Sherwood number) dissolution model. In deep mesh experiments, particles larger than the ABL length experienced flux enhancement due to physical entrapment at the membrane surface.

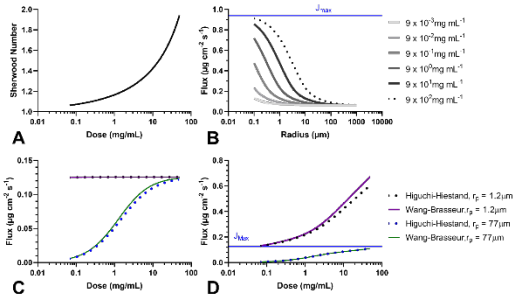

__Figure 1__ Theoretical Estimates and Comparisons of the Higuchi-Hiestand dissolution model and the Wang-Brasseur model for Ibuprofen. __A)__ Sherwood Number as a function of the total dose mass. __B)__ Effect of particle radius on flux at different doses. __C)__ Higuchi-Hiestand and Wang-Brasseur dissolution models in the tradition film model for flux with small and large particle radii. __D)__ Higuchi-Hiestand and Wang-Brasseur dissolution models in the convection-diffusion-flux model with small and large particle radii.

Conclusion

Dissolution occurs in the Aqueous Boundary Layer (ABL) and has significant effects on the flux, thus micronization of particles and/or large doses contribute to "shorting-out" the ABL. High doses can induce enhanced dissolution via the confinement effect.

Acknowledgement

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Investigation of transient permeability enhancers interaction with bile composition and intestinal cell membrane

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Introduction

To increase the absorption of poorly permeable drugs through the intestinal membrane, co-administration with transient permeability enhancers (PEs) has been found to be a promising approach.[1] Among the different transient PEs, medium chain fatty acids enhancers such as sodium caprylate, caprate and salcaprozate sodium (SNAC; a derivative of caprylate) are proved to be the most efficient for poorly permeable molecules. However, an improved understanding of the fate of the PEs, particularly their interaction with bile composition and the cell membrane, is warranted to better understand interindividual variability observed after oral administration.

Aim

To quantitively determine the free transient PE monomers that interact with the cell membrane in the presence of intestinal fluid. Also, to investigate how different transient PEs interact with the model cell membrane at the molecular level.

Method: Coarse-grained molecular dynamics (CG-MD) simulations were performed with systems containing i) fasted and fed state bile composition of five healthy volunteers[2] and (ii) fasted and fed state simulated intestinal fluids (FaSSIF and FeSSIF, respectively). The simulations were performed for 3 μs with box size of 19 nm x 19 nm x 22 nm. Thereafter, 100mM of three different PEs (caprylate, caprate and SNAC) were added at the end of each intestinal fluid simulation and the simulations were run for additional 1 μs. Next, we placed the simulation box in between two patches of model cell membranes and performed another 6 μs simulation. The total box size of the latter system was 19 nm x 19 nm x 50 nm. The simulation results of PEs (dispersed in FaSSIF and FeSSIF) interaction with membrane were contrasted to experimental data obtained by Quartz Crystal Microbalance with Dissipation (QCM-D) monitoring of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (POPC) membranes flushed with PE-containing FaSSIF and FeSSIF.

Results: Th CG-MD simulations showed that the added PEs became part of the mixed micelles, formed pure PE micelles or remained as free monomers. The free PE monomers interacted with the membrane. Caprylate and caprate were found to be incorporated into the membrane while SNAC were mainly adsorbed at the membrane surface (Fig 1). We estimated the total amount of PE monomers inserted or adsorbed at the membrane surface. It was found that 1.7-2.6 times more PEs were incorporated or adsorbed with fasted than fed state fluids. The final changes in *f,* at the QCMD experiments also indicated higher amount of PE translocated to the membrane with fasted than fed state fluids.

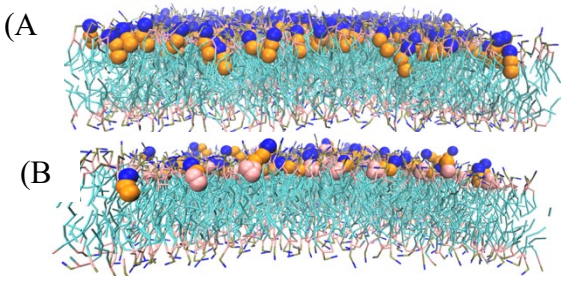

Figure 1: Inserted and adsorbed transient permeability enhancer (PEs) at the membrane surface (A) Caprate and (B) SNAC.

Conclusion

Bile composition can influence membrane interaction of the PEs. Membrane incorporation of caprylate and caprate suggested that transmembrane perturbation is a key mechanism by which caprylate and caprate increase permeability.

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Simulations of microplastics' transport across cell membranes

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Introduction

Plastics are very commonly used materials that have been in everyday life since the 1950's, but proper recycling has just started a few years ago therefore making plastic debris a widespread problem.[1] As plastic debris ends up in nature they begin degrading down into smaller micro- and eventually nanosized particles. These particles have been discovered in, e.g., tap water and human feces.[2,3]
Although plastic polymers are mostly inert, their degradation products, additives or absorbed contaminants can cause negative impacts on humans and the environment.[4,5] Smaller, nanometer sized nanoplastics, are potentially more dangerous than larger microplastics, because they are more likely to absorb into, or stay in the human body or cells longer. [5] Proportionally larger surface area of the nanoplastics can also more easily absorb other more toxic substances.
More research into the health effects of microplastics and their ability to transport in the human body is needed because the majority of the microplastics studies focus on marine life and the prevalence of plastics in the environment with far fewer studies on humans and health effects.[4,5]

Aim

The purpose of this study is to investigate how different types of nanosized microplastics can pass through cell membranes using molecular modelling methods.

Methods

Molecular modelling is a common method in early stage drug development. With the aid of molecular modelling we are able to study multiple different types of nanosized plastics quickly, safely and affordably. Molecular dynamics is used to simulate the transport of various microplastics through cell membranes. We have selected five common plastics for setting up the system: PET, PP, PE, PS and Nylon.

Conclusions

While the studies are still ongoing, we have gained some insight into the efficacy of the modelling methods for the study of microplastics. It seems that the methods are applicable for these small sized molecules as well. Preliminary results are indicative that certain types of small plastics could be able to pass through the cell membranes. In the future we will study larger systems and compare the dynamics of different plastics.

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A new mathematical approach to predict optimal antibiotic dose levels

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Introduction

The emergence and spread of antibiotic resistance is a major threat for global public health. Despite the magnitude of the problem, we have limited knowledge how to employ antibiotics in a way that maximizes treatment efficacy while minimizing resistance. Antibiotic treatment may be compromised by the sporadic appearance and selection of drug resistant mutants during therapy. Identifying optimal dosing strategies for treating bacterial infections that minimize the risk of resistance is difficult, and improving dosing guidelines usually requires long and costly investigations. Previously, we demonstrated that a drug target binding pharmacodynamic model that links bacterial population biology with chemical reaction kinetics has high predictive and explanatory power for antibiotic pharmacodynamics. Here, we extend the model to incorporate several distinct molecular mechanisms of resistance to explore the how these mechanisms may affect the risk of acquired resistance during treatment. To test our model, we use the action of Ciprofloxacin on *e.Co/i.*

Aim

We aim to build a new model that can help accelerate drug development by predicting optimal dosing and preserve the efficacy of existing antibiotics by predicting optimal treatment for possible resistant mutants.

Method

We introduce a new model based on a system of partial and ordinary differential equations to describe how the drug binds to their targets and how is the response of a bacterial population to different treatments. The central assumption encoded in the model is that the bacterial growth declines (and/or bacterial kill rate increases) as the fraction of target molecules within the bacterium that are bound by antibiotic increases.

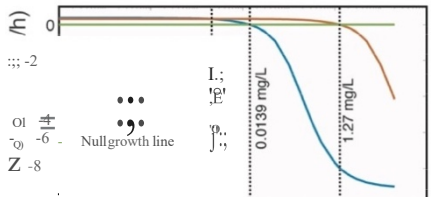

Figure1: Simulation of 18 h of exposure. The blue line represents the wild-type strain and the red line a resistant strain. The 1st vertical line indicates where the resistant strain becomes more fit than the wild-type, the 2nd indicates the MIC of the wild-type,

Results

We find how bacterial growth and death rates depend on the number of bound target molecules fitting ciprofloxacin experimental killing curves. We simulate the net growth rate of the bacteria as function of the drug concentration, with an MIC of 0.0139 mg/L for a wild type strain. Adding a theoretical resistant strain, we predict the antibiotic concentrations at which resistance would be selected. In this way, we find a mutation selection window to minimize the formation of resistance. In addition, we show how this selection window changes with the time.

Finally, we predict the relative overexpression of antibiotic target molecule content from time-kill curves, finding a good agreement with experimental measures of the target overexpression.

Conclusion

Here, we present a novel pharmacodynamic model that allows in silica quantitative predictions of ranges of antibiotic concentrations that should be avoided. We can establish a quantitative link between biochemical mechanisms and bacterial susceptibility. Also, we are able to predict mutation selection window and tailor therapy to prevent and suppress resistance to antibiotics.

Investigating Sirtuin 6 with molecular dynamics simulations

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Introduction

Sirtuins are histone deacetylases that regulate many biological pathways and gene expression. Sirtuin 6 (Sirt6) is one of the nuclear sirtuins and it has recently gained interest as a possible target in cancer and metabolic diseases.[1] In our previous study we introduced natural color pigments that were Sirt6 activators.[2] The binding sites of these activators are unknown and we would need information about them. The activators increased the substrate binding and we hypothesized that the activators might stabilize the substrate-binding loops' movement. Other possibility is that the activators affect the substrate binding area in another way. We need to explore conformational changes in Sirt6 to understand how the activators could affect the binding of the substrate.

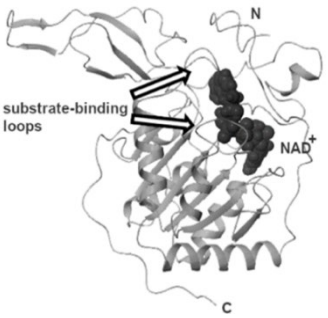

Figure 1 *Sirt6 structure (grey) with cofactor NAD⁺ (black).*

Aim

The aim of this study was to investigate the structural motions of Sirt6 with molecular dynamics (MD) simulation (Figure 1). Special emphasis was on the movement and interactions of substrate-binding area.

Methods

Three 200 ns MD simulations for Sirt6 and its cofactor nicotinamide adenine dinucleotide (NAD^+) were performed with GROMACS 2016.5[3] using Amber ff99SB-ILDN[4] force field. The RMSF of backbone atoms was calculated to examine movements of Sirt6. Additionally, hydrogen-bonding and salt bridges of the substrate-binding area were analyzed.

Results

The results suggested that substrate-binding loop backbone is quite stabile; the maximum movement observed was ~2 Å. Hydrogen-bonding and salt bridges between residues Glu189 and Arg220 in the two substrate-binding loops were observed frequently. Additionally, some hydrogen bonds between the substrate-binding loops and N-terminal loop were present. The N-terminal loop also moved towards the substrate-binding cavity.

Conclusion

The substrate-binding loops were stable and thus, our activators probably do not have great effect on this movement. However, these results suggest that the substrate-binding loops interact together, and that the N-terminal loop can move towards the substrate-binding area. These interactions and movements might hinder substrate from binding. MD simulations of Sirt6 with activators will be performed in the future to investigate if the activators affect the N-terminal loop movement and the interactions of substrate-binding area.

Acknowledgement

The authors would like to thank CSC – IT Center for Science, Finland, for computational resources. The study was supported by Academy of Finland (grant number 315824).

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Structural characterization of the Δ^{746}ELREA^{750} EGFR deletion mutation – a molecular dynamics simulation study

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Introduction

Epidermal growth factor receptor (EGFR) is a tyrosine kinase receptor crucial in multiple cellular processes involving cell proliferation and differentiation. Increased EGFR activity due to gene over-expression and/or activating somatic mutations is however often associated with various human cancers.[1] Exon 19 deletion mutations, the most common being Δ^{746}ELREA^{750}, account for nearly 50% of all EGFR activating mutations. Δ^{746}ELREA^{750} is widely linked with non-small cell lung cancer, which comprises 85% of lung cancer, the leading cause of cancer-related deaths worldwide.[2,3]

Aim

The goal of this study is to investigate the structural impact of the Δ^{746}ELREA^{750} EGFR mutation.

Method

Molecular dynamics simulation was performed on wild-type and Δ^{746}ELREA^{750} forms of apo active, ATP-bound active and apo inactive EGFRs for 100 ns. The simulation data was analyzed to assess the effect of the mutation on local structure, domain conformation and key interactions.

Results

Simulations of the active EGFR structure showed that ΔELREA constrains the β3-αC loop of EGFR where the mutation sequence is situated. Consequently, the adjacently located αC helix is stabilized in the 'αC-in' active conformation (Figure 1), which would extend the duration the activated stated. Furthermore, a catalytic salt bridge between K745 and E762, which is critical for kinase activity was more stable in the deletion mutant than the wild-type EGFR. Additionally, better interactions were recorded between the mutant kinase and ATP, the natural ligand of EGFR, as compared to the wild-type EGFR. Simulations of the inactive kinase on the other hand uncovered an ΔELREA induced inward movement of the αC-helix, a movement required to attain the active state conformation. The observations from the simulations were consistent with the effects of tyrosine kinase inhibitors on lung cancer cell lines of ΔELREA and wild-type EGFR, where greater inhibition by active kinase conformation recognizing drugs was reported for the mutant than the wild-type EGFR.

Conclusion

The simulations revealed that ΔELREA exerts its activating effect in a dual manner: by stabilizing the active EGFR conformation and by promoting a conformational shift from the inactive towards the active EGFR state.

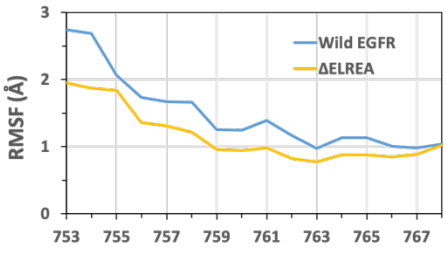

Figure 1 *Cα atom root-mean square fluctuations of the residues of the αC helix during the 100 ns simulation.*

Acknowledgment

The author would like to thank the ISB doctoral school for the research funding and the CSC IT Center for Science for the computing resources.

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Stability studies of water-sensitive antibiotics suspended in self-emulsifying drug delivery systems for ocular administration

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Introduction

Self-emulsifying drug delivery systems (SEDDS) are water-free dosage forms consisting of an isotropic mixture of oils and surfactants. Following dilution with the aqueous media (lacrimal fluid) a fine oil-in-water emulsion is created at the site of administration [1]. Vancomycin (V) and cefuroxim (C) are antibiotics freely soluble but unstable in aqueous solutions and no ophthalmic dosage forms are commercially available [2, 3].

Aim

The objective of the project is focusing on the development of ocular self-emulsifying suspensions of the water-unstable drugs. In this study, the chemical stability of V and C suspended in SEDDS of various composition, was tested.

Method

SEDDS carriers were obtained by dissolving surfactants: Cremophor EL, Tween 20 or Span 80 in Miglyol oil (5% m/m). V or C (particle size < 25 μm) was suspended (1-5% m/m) in SEDDS and the suspensions were stored at 4°C, 25°C and 40° for up 90 days and HPLC analysis was performed. 5% aqueous solution of V or C was used as a reference. Moreover, microscopic observation of drug-loaded SEDDS before and after reconstitution with water was carrried out. In resulting emulsions and Zeta potential and oily droplets size distribution was determined .

Results

In contact with water drug particles suspended in SEDDS dissolve rapidly with no solid particles left undissolved. Individual systems differed slightly regarding certain features (reconstitution time, mean droplet size), although they all exhibited similar general properties. The samples showed negative zeta potential range of -40mV - -50mV and the mean droplet size from 20 μm to 30 μm. These parameters did not change significantly during storage.

Developed SEDDS provided V and C stability for the entire time of the study, even at 40°C, whereas in aqueous solution met the requirements for stability only when stored at 4°C (results at 25°C for V showed losses more than 10% of drug in water after 30 days).

Conclusion

The developed formulations proved to be physically and chemically stable over storage and seem feasible to serve as the effective carriers for water-sensitive drugs. The microbiological activity studies of the formulations are in progress.

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Virtual screening as a tool for the identification of novel painkillers against transient receptor potential cation channel, subfamily A, member 1, TRPA1

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Introduction

Neuropathic pain (NeP) can induce chronic pain which lowers person's health and increases visits in healthcare providers.[1] TRPA1 (Transient receptor potential cation channel, subfamily A, member 1) is a protein which functions as a detector for noxious chemical agents. It has been associated with NeP and other pain conditions making it a potential drug target for painkillers.[2,3] Compounds pinosylvin and resveratrol have been studied to function as antagonists for TRPA1[3]. This inspired us to study a library of 3-phenylcoumarins which share similar chemical structure with these antagonists. These studies are carried out with the collaboration of immunopharmacology research group in University of Tampere.

Aim

The overall aim was to identify novel inhibitors for TRPA1. The binding site of TRPA1 antagonists is unknown, therefore various binding sites were examined to get a new information about the binding of antagonists.

Methods

Homology model was created with Discovery Studio Client 2018. Molecular docking was performed with Schrödinger Maestro version 11.7. The computational capacity and licenses support provided by CSC-IT Center for Science, Finland, is acknowledged.

Results

The homology model of TRPA1 was created utilizing a structure of TRPA1 determined by electron cryomicroscopy[2]. TRPA1 homology model was used to discover the potential drug candidates by docking a library of 3-phenylcoumarins into TRPA1. The most potent compounds formed hydrogen bonds with Ala 628, Asn 660, Leu 654, Leu 663 and Met 692.

Conclusion

The study resulted in discovery of three promising 3-phenylcoumarin compounds as a TRPA1 antagonists based on their docking scores. These compounds have been synthetized and will be validated with *in vitro* studies in near future.

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Effect of API on alginate polymer behavior in solutions

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Introduction

Alginate is used as a thickener, stabilizer and gelling agent in diverse fields of industry[1]. In biotechnology, food, cosmetic and pharmaceutical industries sodium salt of alginic acid are mainly used due to its high biocompatibility and low cost[2,3]. Alginate are polysaccharides mostly derived from brown algae but they are also produced by bacteria. It is a charged and linear copolymer consisting of (1–4) linked b-D-mannuronic acid (M) and a-Lguluronic acid (G), whose ratio varies with the alginate source. The physical properties of the alginate depend on the M/G ratio as well as the distribution of the M and G units along the alginate chain[1,4].

Aim

To determine whether the API affects the alginate polymer chains behavior in solutions.

Method

Four types of polymer solutions were prepared. The solutions contained 0.3-1.4% (w/w) of sodium alginate alone or with an additive one of the APIs (arginine, glycine, dihydroxyacetone). As a solvents it were used a phosphate buffers, for both ionic strength and pH. After preparation the viscosity of the solutions were measured.

Results

Viscosity of the 24 prepared solutions were measured and their relative viscosity were calculated. From these results the overlap concentration of polymer coils (C*) was determined for each kind of tested solution. Obtained data for the sodium alginate solutions and these which contained also one of the APIs were compared.

Conclusion

It is shown that in the tested concentration range of alginate solutions there is no significant changes in relative viscosity values for solutions contained APIs in concentrations of 2% for amino acids and 5% for dihydroxyacetone. It was also found that the overlap concentration of polymer coils in tested solutions do not differ significantly between each type. these results showed that tested APIs did not have significant impact on the alginate polymer behavior in solutions.

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Comparing Standard and Emerging Caco-2 Cell Based Permeation Assays using Levich Hydrodynamics and a Rotating Membrane Diffusion Cell.

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Introduction

Replicating key aspects of the oral dissolution and absorption process in vitro are fundamental to measuring a realistic response for the formulation of interest. Much of the attention into in vivo relevant in vitro testing goes towards designing the aqueous medium, with less significant attention to the absorption capabilities. What is further lacking in the current in vitro test methods are the critical hydrodynamic, physical, and mechanical parameters that are present in the human gastrointestinal tract (GI), including a robust understanding of the intrinsic nature of drug permeation through cell monolayers. The aqueous, cell, and cell substrate component permeability can be determined using the rotating disk equations developed by Levich in 1962 (Eq. 1). This type of analysis enables improvement of existing and newly created assays toward true in vivo relevance.

$$\frac{1}{P_{eff}} = \left[\frac{\mu/\rho^{\frac{1}{6}}}{0.62D_{eff}^{\frac{2}{3}}}\right]\omega^{-1/2} + \frac{1}{P_{mem}} \quad (1)$$

Aim

We aim to measure the intrinsic permeability of Caco-2 cells for 4 permeation markers which interrogate both the active and passive transport pathways as well as characterize the dynamic viscosity of a medium used to test lipid based formulations. We compare the different resistances to diffusion in the commercially available µFlux system and the newly developed Enabling Absorption (ENA) device.

Method

Caco-2 cells were cultured on standard track etched polycarbonate transwell membranes from Corning for 21-35 days using passage numbers 95-105 for permeation experiments. A rotating membrane diffusion cell is rotated at 50, 100, and 150RPM and the measured effective permeability is plotted against the inverse square of the rotational speed. The intrinsic permeability of the membrane is calculated from the extrapolation to the y-intercept. The intrinsic permeability used to calculate the effective hydrodynamic resistance in the p Ion µFlux and the ENA device.

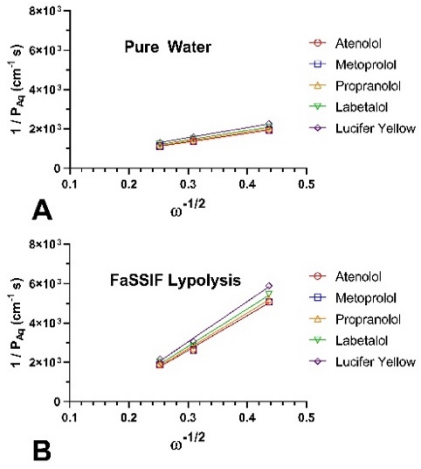

***Figure 1**: Stirring significantly affects aqueous diffusion layer permeability under laminar flow. Atenolol (passive para-), Metoprolol (high passive trans-), propranolol (high passive trans-), Labetalol (active, p-gp efflux), and Lucifer yellow (cell barrier function) permeability was predicted for simple and complex buffer systems, which are more common in modern in vivo relevant in vitro methods.*

Results

The FaSSIF based lipolysis medium's viscosity is very shear sensitive in the range of stirring for all three devices. The lipolysis medium is both shear thinning and shear thickening.

Conclusion

While this work is in its early stages however, it is apparent from the early data that the dynamic viscosity should be a standard part of characterizing new biorevelant mediums. The range of shear rates produced a new or standard device should also be considered when using or designing a new in vitro protocol.

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Workpackage X:

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Patient perspectives on drug formulations

Anna Birna Almarsdóttir works as a Professor of Social and Clinical Pharmacy at the Department of Pharmacy of the University of Copenhagen in Denmark. Her research spans the areas of Medicines Use, Clinical Pharmacy, and Pharmaceutical Policy. Within clinical pharmacy the focus is on clinical pharmacy services and medication safety (in the primary, secondary and tertiary health care sectors); within medicines use the focus is on adherence to medicines from the patient perspective and how policies affect adherence; and within pharmaceutical policy the focus is on regulatory decision making about medicines and the pharmacy profession.

Charlotte Vermehren is part-time Associate Professor of Clinical Pharmacy at the Department of Pharmacy of the University of Copenhagen, but her full-time position is as Head of The Medicine Unit, Department of Clinical Pharmacology, Copenhagen University Hospital in Denmark. Her research focuses on deprescribing, polypharmacy, personalized medicine and drug/patient safety. Within these areas the main focus is on medication review among geriatric patients in collaboration with the General Practitioners and Municipalities where there is also a considerable focus on patient involvement and Health Care Professionals attitudes toward deprescribing. Charlotte was previously a formulation scientist focusing on the development of new drug formulations and drug delivery systems.

Patient perspectives on drug formulations

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Abstract

Formulation research is rapidly developing exemplified by novel types of formulations, increased personalization of medicines use, innovative medical devices in conjunction with drug delivery, and continuous patient monitoring of physiological and chemical parameters to improve dosing. In addition, there is a general tendency for increased focus on the development of patient-centered formulations for complex patients, e.g. geriatric and pediatric patients. The development of formulations that are age-appropriate and acceptable to these patients is of outmost importance in order to improve adherence, disease management and clinical outcomes.

In this presentation the aim is to cover 1) Why the patient perspective on formulations is an important and upcoming research field; 2) What already is known about patient perspectives on drug formulations; and 3) How this knowledge translates into new research on the patient perspective on drug formulations. Social and clinical pharmacy researchers have experience in working with the patient perspective on medicines, they have expertise in the methods used in the field non-adherence and capacities to develop and improve current data collection and analysis methods; whereas formulation scientists have the insight into the methodology surrounding the development of novel formulations. This means that there will be a need for closer collaboration between these two researcher types to use their synergies to understand the patient perspective on drug formulations.

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