Nordic POP
1st Annual Meeting

Oslo, Norway

14 -16 January 2019

NORDIC POP

UiO : Department of Pharmacy
University of Oslo

Venue

Holmenkollen Park Hotel

(Scandic Holmenkollen Park)

Kongeveien 26

0787 Norway

CONTENTS

PROGRAM

MONDAY 14 JANUARY

11:30 – 13:30 **REGISTRATION, MOUNTING OF POSTERS** (Saga Hall C)

11:30 – 12:30 **LUNCH**

13:30 – 13:45 **OFFICIAL OPENING** (Saga Hall AB)

Henrik Schultz, Head of Department of Pharmacy, University of Oslo
Per Morten Sandset, Vice-Rector for Research and Innovation, Univ. of Oslo
Jukka Rantanen, Leader of Nordic POP

EARLY AFTERNOON SESSION (SAGA HALL AB)

Chairs: Regina Scherließ and Henrik Schulz

13:45 – 14:30 **Kjetil Taskén**, Head and Director of Institute for Cancer Research, Oslo University Hospital

Precision Cancer Medicine, next generations of individualised treatment

14:30 – 15:00 **Maija Lahtela-Kakkonen**, University of Eastern Finland (Keynote, p 16)

Epigenetics regulation as anticancer targeting

15:00 – 15:15 **Tina Gulin-Sarfraz**, Åbo Akademi University (p 27)

Photonic interactions in vitro and in vivo between fluorescent tracer and nanoparticle-based scavenger for enhanced molecular imaging

15:15 – 15:30 **Yassir Al-Tikriti**, Uppsala University (p 28)

Amphiphilic drug molecules in oppositely charged microgels

15:30 – 16:00 **Hongbo Zhang**, Åbo Akademi University (Keynote, p 17)

Nanotechnology for Precision Medication

16:00 – 16:20 **COFFEE BREAK & POSTERS**

16:20 – 17:20 **For SAB members** (University and industry representatives): Meeting in the foyer. Lead by Jukka Rantanen.

LATE AFTERNOON SESSION (SAGA HALL AB)

Chairs: Leena Peltonen and Jarkko Ketolainen

16:20 – 16:35 **Jernej Štukelj**, University of Helsinki (p 29)

First of the kind – the apparent pH-solubility profile of an amorphous drug using the Single Particle Analysis (SPA) method

16:35 – 16:50 **Simon Bock**, University of Kiel (p 30)

Particle engineering for Dry Powder Inhalation (DPI): Modifying drug-to-carrier interaction in adhesive mixtures

16:50 – 17:05 **Anne Linnet Skelbæk-Petersen**, University of Copenhagen (p 31)

Effect of fragmentation on dissolution rate of paracetamol after tabletting

17:05 – 17:20 **Maja Szczepanska**, University of Gdansk (p 32)

Optimization of the coating process of minitablets in two different lab-scale fluid bed systems

TRANSPORTATION TO OSLO RÅDHUS (OSLO CITY HALL)

18:00 Buses leaving from the hotel to Oslo Rådhus (where doors open at 18:30)

NB! Remember to bring the invitation card!

WELCOME RECEPTION, OSLO RÅDHUS

19:00 – 20:30 Reception, drinks and finger food. NB! Cloakroom is obligatory

TUESDAY 15 JANUARY

EARLY MORNING SESSION (SAGA HALL AB)

Chairs: Karin Kogermann and Korbinian Löbmann

09:00 – 09:30 **Andrea Heinz**, University of Copenhagen (Keynote, p 18)

Protein-based biomaterials for cutaneous application

09:30 – 09:45 **Jostein Grip**, UiT The Arctic University of Norway (p 33)

Sprayable beta-glucan hydrogel as wound dressing: in vitro and in vivo evaluation

09:45 – 10:00 **Celia Pozo Ramos**, University of Tartu (p 34)

Evaluation of fibroblasts attachment and growth on electrospun fiber scaffolds

10:00 – 10:30 **Anna Ström**, Chalmers University of Technology (Keynote, p 19)

Rheological and mechanical study of peptide/cell constructs

10:30 – 11:00 **COFFEE BREAK & POSTERS**

LATE MORNING SESSION (SAGA HALL AB)

Chairs: Tiina Sikanen and Anders Østergaard Madsen

11:00 – 11:30 **Trine Grønhaug Halvorsen**, University of Oslo (Keynote, p 20)

Dried blood spots and mass spectrometry in analysis of protein biomarkers and biopharmaceuticals

11:30 – 11:45 **Line Laursen**, University of Southern Denmark (p 35)

Preparation, upscaling and lyophilization of soybean cochleates for subcutaneous administration

11:45 – 12:00 **Maximilian Karl**, University of Copenhagen (p 36)

Micromechanical Thermal Analysis (MTA) for Large and Small Molecule Samples

12:00 – 12:30 **Jukka Saarinen**, University of Helsinki (Keynote, p 21)

Non-linear optical imaging in pharmaceutical applications

12:30 – 12:45 **Josefine E. Nielsen**, University of Oslo (p 37)

Probing the structure of lipid bilayer and their interaction with antimicrobial peptides using x-ray and neutron scattering techniques

12:45 – 13:00 **Iiro Kiiski**, University of Helsinki (p 38)

Immobilization of membrane-bound enzymes on microreactors via fusogenic liposomes: An enabling tool for drug research

13:00 – 13:05 **GROUP PHOTO**

13:05 – 14:30 **LUNCH BUFFET (Hotel restaurant)**

EARLY AFTERNOON SESSION (SAGA HALL AB)

Chairs: Gøril Eide Flaten and Carsten Uhd Nielsen

14:30 – 15:00 **Per Larsson**, Uppsala University (Keynote, p 22)

Using molecular dynamics simulations to probe various aspects of pharmaceutical systems

15:00 – 15:15 **Mette Dalskov Mosgaard**, Technical University of Denmark (p 39)

Ex vivo intestinal perfusion model to determine mucoadhesion of microcontainers

15:15 – 15:30 **Irina E. Antonescu**, University of Southern Denmark (p 40)

Acamprosate is an inhibitor of the renal organic anion transporter 1

15:30 – 16:00 **Maria João Gomes**, University of Southern Denmark (Keynote, p 23)

Experiences with dissolution-/permeation-tests of Tadalafil

16:00 – 16:15 **Riikka Laitinen**, University of Eastern Finland (p 41)

Dynamic dissolution/permeability testing of co-amorphous glibenclamide mixtures using a PAMPA-membrane

16:15 – 16:30 **Caroline Alvebratt**, Uppsala University (p 42)

A dissolution-digestion-permeation assay to investigate the performance of advanced drug delivery systems

16:30 – 17:00 **COFFEE BREAK & POSTER SESSION**

LATE AFTERNOON SESSION (SAGA HALL AB)

Chair: Christel Bergström

17:00 – 17:30 **May Wenche Jøraholmen**, UiT The Arctic University of Norway (Keynote, p 24)

Localized delivery of natural polyphenols: an alternative to antibiotics in treatment of genital chlamydia?

17:30 – 18:00 **Hakon Hrafn Sigurdsson**, University of Iceland (Keynote, p 25)

Ear plugs as a drug delivery system to treat acute otitis media

19:30 - **CONFERENCE DINNER** (Saga Hall ABC)

WEDNESDAY 16 JANUARY

MORNING SESSION – WORK PACKAGE (WP) MEETINGS IN PARALLEL

The Work Package meetings are for all who are interested in the topics!

09:00 – 10:15

WP 1 BARRIERS (SAGA HALL A)	**WP 3 ENGINEER** (SAGA HALL C)	**WP 6 MODELLING** (SAGA HALL B)
WP leader: Carsten Uhd Nielsen, University of Southern Denmark	WP leader: Leena Peltonen, University of Helsinki	WP leader: Christel Bergström, Uppsala University

10:15 – 10.30 COFFEE BREAK (NB! Last chance to check out!)

10:30 – 11:45

WP 2 PRODUCTS (SAGA HALL A)	**WP 4 ANALYTICS** (SAGA HALL C)	**WP 5 SYNCHROTRON** (SAGA HALL B)
WP leader: Gøril Eide Flaten, UiT The Arctic University of Norway	WP leader: Tiina Sikanen, University of Helsinki	WP leader: Anders Ø. Madsen, University of Copenhagen

12:00 – 13:00 PLENARY UPDATE, AWARDS & CLOSING

13:00 – LUNCH (grab and go) and departure

14:00 – Guided tour to Holmenkollen (for those who signed up during registration). The guide will meet you at the Entrance to the Ski-museum at 14:00.

LIST OF PARTICIPANTS

Almarsdottir	Anna Birna	aba@sund.ku.dk	UCPH
Alopaeus	Julia	j.f.alopaeus@farmasi.uio.no	UiO
Al-Tikriti	Yassir	yassir.al-tikriti@farmaci.uu.se	UU
Alvebratt	Caroline	caroline.alvebratt@farmaci.uu.se	UU
Andersson	Yvonne	yvonne.andersson @sykehusapotekene.no	SAHF
Antczak	Alexandra Patricia	alant14@student.sdu.dk	SDU
Antonescu	Irina Elena	antonescu@sdu.dk	UiO
Azumah	Joseph	joseph.azumah@farmasi.uio.no	UiO
Bannow	Jacob	jacob.bannow@sund.ku.dk	UCPH
Barmpatsalou	Vasiliki Styliani	vicky.barmpatsalou@farmaci.uu.se	UU
Bauer-Brandl	Annette	annette.bauer@sdu.dk	SDU
Bergström	Christel	christel.bergstrom@farmaci.uu.se	UU
Berthelsen	Ragna	ragna.berthelsen@sund.ku.dk	UCPH
Bhattarai	Niina	niina.bhattarai@uef.fi	UEF
Bjerknes	Kathrin	kathrin.bjerknes @sykehusapotekene.no	SAHF
Bock	Frederik	rgh958@alumni.ku.dk	UCPH
Bock	Simon	simonbock@pharmazie.uni-kiel.de	Uni Kiel
Bohsen	Mette Sloth	bohsen@sdu.dk	SDU
Bor	Gizem	gizem.bor@sund.ku.dk	UCPH
Braathen	Frida	fridabra@student.farmasi.uio.no	UiO
Brandl	Martin	mmb@sdu.dk	SDU
Breiby	Bente Amalie	b.a.breiby@farmasi.uio.no	UiO
Brodin	Birger	birger.brodin@sund.ku.dk	UCPH
Christensen	Hege	h.s.christensen@farmasi.uio.no	UiO
Christiansen	Tina	tinak@sdu.dk	SDU
Dalskov Mosgaard	Mette	medmo@nanotech.dtu.dk	DTU
Davies	Nigel	nigel.davies@astrazeneca.com	AstraZeneca
Degerstedt	Oliver	oliver.degerstedt@farmaci.uu.se	UU
di Cagno	Massimiliano Pio	max.dicagno@gmail.com	UiT
Drustrup	Jørn	joern.drustrup@gmail.com	Novo Nordisk
Egeskov Meyer	Julie	jumey13@student.sdu.dk	SDU
Elíasson	Alex	aleli13@student.sdu.dk	SDU
Ervasti	Tuomas	tuomas.ervasti@uef.fi	UEF
Falavigna	Margherita	margherita.falavigna@uit.no	UiT
Fjellaksel	Richard	richard.fjellaksel@uit.no	UiT
Flaten	Gøril Eide	goril.flaten@uit.no	UiT

Foged	Camilla	camilla.foged@sund.ku.dk	UCPH
Forooqi Motlaq	Vahid	vahid.motlaq@farmaci.uu.se	UU
Frokjaer	Sven	sven.frokjaer@sund.ku.dk	UCPH
Gomes	Maria	mjgomes@sdu.dk	SDU
Govardhanam	Narayana Prakirth	gnarayan@abo.fi	ÅAU
Govender	Rydvikha	rydvikha.govender@chalmers.se	CTH
Granås	Anne Gerd	anngg@uio.no	UiO
Grip	Jostein	jostein.grip@biotec.no	UiT
Grohganz	Holger	holger.grohganz@sund.ku.dk	UCPH
Grove	Ivar	ivar.grove@farmasi.uio.no	UiO
Grønlien	Krister Gjestvang	k.g.gronlien@farmasi.uio.no	UiO
Gulin-Sarfraz	Tina	tgulin@abo.fi	ÅAU
Hagner Nielsen	Line	lihan@nanotech.dtu.dk	DTU
Halvorsen	Trine Grønhaug	trinegha@farmasi.uio.no	UiO
Hansen	Trond Vidar	t.v.hansen@farmasi.uio.no	UiO
Heinz	Andrea	andrea.heinz@sund.ku.dk	UCPH
Heinämäki	Jyrki	jyrki.heinamaki@ut.ee	UT
Hemmingsen	Lisa Myrseth	lisa.m.hemmingsen@uit.no	UiT
Hempel	Nele-Johanna	nele.hempel@sund.ku.dk	UCPH
Herzberg	Mikkel	mikkel.herzberg@sund.ku.dk	UCPH
Hiorth	Marianne	marianne.hiorth@farmasi.uio.no	UiO
Hirschberg	Cosima	cosima.hirschberg@sund.ku.dk	UCPH
Hirvonen	Jouni	jouni.hirvonen@helsinki.fi	UHEL
Holm	Tobias P.	tobias.holm@sund.ku.dk	UCPH
Holsæter	Ann Mari	ann-mari.holsater@uit.no	UiT
Hossain	Md Shakhawath	shakhawath.hossain@farmaci.uu.se	UU
Hrebikova	Barbora	barbora.hrebikova@sund.ku.dk	UCPH
Huang	Jianfei	jianfei.huang@sund.ku.dk	UCPH
Inngjerdingen	Kari Tvete	k.t.inngjerdingen@farmasi.uio.no	UiO
Jacobsen	Ann-Christin	acj@sdu.dk	SDU
Jensen	Nikolaj Sølvkær	Mgn684@alumni.ku.dk	UCPH
Jonassen	Helene	helene.jonassen@farmasi.uio.no	UiO
Jøraholmen	May Wenche	may.w.joraholmen@uit.no	UiT
Jørgensen	Jacob	jacob.r.joergensen@sund.ku.dk	UCPH
Kabedev	Aleksei	aleksei.kabedev@farmaci.uu.se	UU
Kamguyan	Khorshid	khokam@nanotech.dtu.dk	DTU
Karl	Maximilian	maximilian.karl@sund.ku.dk	UCPH
Karlgren	Maria	maria.karlgren@farmaci.uu.se	UU

Kauppinen	Anu	anu.kauppinen@uef.fi	UEF
Keemink	Janneke	janneke.keemink@farmaci.uu.se	UU
Ketolainen	Jarkko	jarkko.ketolainen@uef.fi	UEF
Kiiski	Iiro	iiro.kiiski@helsinki.fi	UHEL
Kissi	Eric	eric.of.kissi@gmail.com	UiO
Klitgaard	Mette	mette.jensen@sund.ku.dk	UCPH
Kneiszl	Rosita	rosita.kneiszl@farmaci.uu.se	UU
Knopp	Matthias	matthias.manne.knopp@sund.ku.dk	UCPH
Kogermann	Karin	kkogermann@gmail.com	UT
Korhonen	Ossi	ossi.korhonen@uef.fi	UEF
Kõrkjas	Arle	arlekorkjas@gmail.com	UT
Koskela	Jaana	jaana.koskela@helsinki.fi	UHEL
Kristensen	Maja Nørgaard	maja.kristensen@sund.ku.dk	UCPH
Kristensen	Søren	kristensen@sdu.dk	SDU
Kuba?ková	Jana	kubackovaj@gmail.com	UCPH
Kullenberg	Fredrik	fredrik.kullenberg@farmaci.uu.se	UU
Kuntsche	Judith	kuntsche@sdu.dk	SDU
Kutter	Jörg	jorg.kutter@sund.ku.dk	UCPH
Kvingan	Bendik Nikolai	bendiknk@student.farmasi.uio.no	UiO
Labba	Nils-Anders	n.a.labba@farmasi.uio.no	UiO
Lahtela-Kakkonen	Maija	Maija.Lahtela-Kakkonen@uef.fi	UEF
Laidmäe	Ivo	ivo.laidmae@ut.ee	UT
Laitinen	Riikka	riikka.laitinen@uef.fi	UEF
Lanno	Georg-Marten	georg.lanno@gmail.com	UT
Larsen	Natashia Mai Yde	natashia.larsen@sund.ku.dk	UCPH
Larsen	Tove	tove.larsen@farmasi.uio.no	UiO
Larsson	Anette	anettel@chalmers.se	CTH
Larsson	Per	per.larsson@farmaci.uu.se	UU
Laursen	Line Hedegaard	llaur14@student.sdu.dk	SDU
Leitzen	Sarah	leitzen@sdu.dk	SDU
Li	Zhuoxuan	zhuoxuan.li@sund.ku.dk	UCPH
Lid	Marthe	marthe.lid@farmasi.uio.no	UiO
Lund	Reidar	reidar.lund@kjemi.uio.no	UiO
Ly	Quyen	ngocquyenly@gmail.com	UiO
Löbmann	Korbinian	Korbinian.loebmann@sund.ku.dk	UCPH
Madsen	Anders Østergaard	a.madsen@sund.ku.dk	UCPH
Matilainen	Laura	laura.matilainen@orionpharma.com	Orion Pharma
Morsch	Flemming	qrz492@alumni.ku.dk	UCPH

Mulac	Alma	alma.mulac@farmasi.uio.no	UiO
Mullertz	Anette	anette.mullertz@sund.ku.dk	UCPH
Mustafa	Rawand	rmustafa@abo.fi	ÅAU
Nezvalova-Henriksen	Katerina	katerina.nezvalova.henriksen@sykehusapotekene.no	SAHF
Ngo	Huan Bao	huanbn@outlook.com	UiO
Nielsen	Josefine Eilsø	j.e.nielsen@kjemi.uio.no	UiO
Nielsen	Salli	sanie14@student.sdu.dk	SDU
Nielsen	Sidsel Strandgaard	sistr13@student.sdu.dk	SDU
Nielsen	Rasmus Blaaholm	rasmusbn@sdu.dk	SDU
Nielsen	Carsten Uhd	cun@sdu.dk	SDU
nilsson	niklas	niklas.nilsson@farmasi.uio.no	UiO
Nordström	Josefina	josefina.nordstrom@farmaci.uu.se	UU
Novakovic	Dunja	dunja.novakovic@helsinki.fi	UHEL
Nystedt	Helene Liepelt	heleneliep@gmail.com	UiO
Okeyo	Peter Ouma	peter.okeyo@sund.ku.dk	UCPH
Ostergaard	Jesper	jesper.ostergaard@sund.ku.dk	UCPH
Paaver	Urve	urve.paaver@ut.ee	UT
Palmelund	Henrik	Henrik.palmelund@sund.ku.dk	UCPH
Parlow	Julia	julia.parlow@farmaci.uu.se	UU
Pedersen	Maria Diana Læssøe	mariap@sdu.dk	SDU
Pedersen-Bjergaard	Stig	stigpe@farmasi.uio.no	UiO
Peltonen	Leena	leena.peltonen@helsinki.fi	UHEL
Petersson	Karsten	KAPDK@leo-pharma.com	LEO Pharma
Pihlaja	Tea	tea.pihlaja@helsinki.fi	UHEL
Poulsen	Jessie	jpoulsen@sdu.dk	SDU
Pozo Ramos	Celia Teresa	celia.t.pozo@gmail.com	UT
Rades	Thomas	thomas.rades@sund.ku.dk	UCPH
Ramakrishnan	Venkatasubramanian	venkatasubramanian.ramakrishnan@sund.ku.dk	UCPH
Ranta-aho	Sofia	sofira@uef.fi	UEF
Rantanen	Jukka	jukka.rantanen@sund.ku.dk	UCPH
Rautio	Jarkko	jarkko.rautio@uef.fi	UEF
Restan	Magnus	m.s.restan@farmasi.uio.no	UiO
Rodler	Agnes	agnes.rodler@farmaci.uu.se	UU
Rosenholm	Jessica	jerosenh@abo.fi	ÅAU
Saarinen	Jukka	jukka.saarinen@helsinki.fi	UHEL
Samuelsen	Anne Berit	a.b.c.samuelsen@farmasi.uio.no	UiO

Sande	Sverre Arne	s.a.sande@farmasi.uio.no	UiO
Scherließ	Regina	rscherliess@pharmazie.uni-kiel.de	Uni Kiel
Schultz	Henrik	henrik.schultz@farmasi.uio.no	UiO
Senniksen	Malte Bøgh	Malte.senniksen@sund.ku.dk	UCPH
Shuhaiber Clemensen	Anne Marie	annemarie.clemensen@sund.ku.dk	UCPH
Sigurdsson	Hakon Hrafn	hhs@hi.is	UI
Sikanen	Tiina	tiina.sikanen@helsinki.fi	UHEL
Sironi	Daniel	daniel.sironi@gmail.com	SDU
Skelbæk-Pedersen	Anne Linnet	apse@novonordisk.com	UCPH
Slita	Anna	aslita@abo.fi	ÅAU
Smistad	Gro	gro.smistad@farmasi.uio.no	UiO
Snorradóttir	Bergthóra	bss@hi.is	UI
Solberg	Rigmor	rigmor.solberg@farmasi.uio.no	UiO
Stein	Paul C	pcs@sdu.dk	SDU
Strachan	Clare	clare.strachan@helsinki.fi	UHEL
Ström	Anna	anna.strom@chalmers.se	CTH
Stukelj	Jernej	jernej.stukelj@helsinki.fi	UHEL
Støttrup Larsen	Anders	anders.larsen@sund.ku.dk	UCPH
Svendsen	Emilie Juanita	emilie.svendsen@gmail.com	SDU
Szczepanska	Maja	maja.szczepanska123@gmail.com	MUG
Sznitowska	Malgorzata	msznito@gumed.edu.pl	MUG
Tasici	Anca Maria	A.m.tasici@farmasi.uio.no	UiO
Tho	Ingunn	ingunn.tho@farmasi.uio.no	UiO
Tiihonen	Miia	miia.tiihonen@uef.fi	UEF
Tofiq	Maryam	maryam.tofiq@farmaci.uu.se	UU
Tyni	Jonna	jonna.tyni@uef.fi	UEF
Tzanova	Martina	marti_djen@yahoo.com	UiO
Tønnesen	Hanne Hjorth	h.h.tonnesen@farmasi.uio.no	UiO
van der Zwaan	Irès	ires.vanderzwaan@farmaci.uu.se	UU
Verma	Ajay	ajay.verma@abo.fi	ÅAU
Vermehren	Charlotte	charlotte.vermehren@sund.ku.dk	UCPH
Viidik	Laura	laura.viidik@gmail.com	UT
Vu	Hoang Uyen Mu	hovu14@student.sdu.dk	SDU
Wang	Junwei	junwei.wang@sund.ku.dk	UCPH
Wanselius	Marcus	Marcus.Wanselius@farmaci.uu.se	UU
Zhang	Hongbo	hongbo.zhang@abo.fi	ÅAU
Zheng	Hangsheng	hs_zheng@yahoo.com	UCPH
Zosen	Denis	denis.zosen@farmasi.uio.no	UiO

Ørbæk	Dorthe	dorthe.orbaek@sund.ku.dk	UCPH
Özliseli	Ezgi	eozlisel@abo.fi	ÅAU

Abbreviations:

CTH	Chalmers University of Technology
UiT	UiT The Arctic University of Norway
UCPH	University of Copenhagen
UEF	University of Eastern Finland
UI	University of Iceland
UiO	University of Oslo
SDU	University of Southern Denmark
UU	Uppsala University
UHEL	University of Helsinki
ÅAU	Åbo Akademi University
Uni Kiel	University of Kiel
MUG	Medical University of Gdańsk
UT	University of Tartu
SAHF	Hospital Pharmacies Enterprise South-Eastern Norway

Nordic POP (patient oriented products) is aiming at providing the scientific foundation for the next generation pharmaceutical products by strengthening the use of interdisciplinary approaches within Nordic pharmaceutical sciences.

Prescribing medicine today is based on a One Size Fits All principle. We have, however, a need for more personalized solutions in several critical therapy areas. The recent development within genomics and diagnostic field has enabled development of new innovative medicinal products relying on a combination of diagnostic tools and personalized dose. This approach is paving the way towards future health care system based on personalized medicines. Limited attention has been given to the personalized end-product requirements for optimal therapeutic performance. Complex dosing regimens will require advances in product design to enable precise administration of the most appropriate dose. Innovations in pharmaceutical product design are required to alleviate potential problems.

Pharmaceutical sciences is a broad area covering biology, chemistry and physics, as well as engineering disciplines. Nordic POP (patient oriented products) is aiming at providing the scientific foundation for the next generation pharmaceutical products by strengthening the use of interdisciplinary approaches within Nordic pharmaceutical sciences. More specifically, this proposal will deliver innovative patient oriented products; our approach is including new product design principles, novel methods for better understanding of the product performance, and a paradigm shift in the manufacturing of these products. With this approach, new type of personalized treatment strategies can be designed and the individual variation between patients can be taken into account.

Nordic POP will focus on publishing all the research findings in high impact scientific journals according to a research dissemination plan. This project will focus on mentoring younger scientists and aim for at least two new European Research Council (ERC) grants from this consortium. It is expected that Nordic POP will position this network in an absolute leading position in Europe within pharmaceutical sciences. At the educational level, the mobility of PhD students and senior staff in the HUB will be forming a basis for shared teaching activities.

Additionally, innovation and entrepreneurship will be in an active focus of the network this proposal will form a basis for new start-up companies. Finally, industrial dissemination will be an important part of the network activities with a specific goal of safeguarding the strong global position of Nordic pharmaceutical companies.

The research network is funded by NordForsk from the Nordic University Hub program (#85352 Nordic POP, Patient Oriented Products).

PROJECT LEADER

Jukka Rantanen, University of Copenhagen

PROJECT ADMINISTRATOR

Barbora Hrebikova, University of Copenhagen

PARTNER UNIVERSITIES AND LOCAL POINTS OF CONTACT

- Chalmers University of Technology (Gøteborg; Anette Larsson)
- UiT The Arctic University of Norway (Tromsø; Gøril Eide Flaten)
- University of Copenhagen (Jukka Rantanen)
- University of Eastern Finland (Kuopio; Jarkko Ketolainen)
- University of Helsinki (Leena Peltonen)
- University of Iceland (Reykjavik; Hakon Hrafn Sigurdsson)
- University of Oslo (Ingunn Tho)
- University of Southern Denmark (Odense; Martin Brandl)
- Uppsala University (Christel Bergström)
- Åbo Akademi University (Åbo/Turku; Niklas Sandler)

AFFILIATED UNIVERSITIES AROUND THE BALTIC SEA AND LOCAL POINTS OF CONTACT:

- University of Tartu, Estonia (Jyrki Heinämäki)
- Medical University of Gdansk, Poland (Malgorzata Sznitowska)
- University of Kiel, Germany (Regina Scherließ)
- University of Greifswald, Germany (Sandra Klein)
- University of Hamburg, Germany (Claudia Leopold)

Nordic POP is organised in the following 6 work packages:
(cWP: cross work package, i. e. cross-disciplinary activities):

WP1 BARRIERS

(e.g. cell lines, barriers, biological relevant models, animal models)
Leader Carsten Uhd Nilssen, University of Southern Denmark

WP2 PRODUCT

(Formulation strategies; e.g. amorphous, lipid, co-crystal etc.)
Leader Gøril Eide Flaten, UiT The Arctic University of Norway

WP3 ENGINEER

(e.g. additive manufacturing, continuous processing etc.)
Leader Leena Peltonen, University of Helsinki

cWP4 ANALYTICS

(broader sense analytical sciences)
Leader Tiina Sarinen, University of Helsinki

cWP5 SYNCHROTRON

(e.g. use of imaging, synchrotron)
Leader Anders Østergaard Madsen, University of Copenhagen

cWP6 MODELING & SIMULATION

(all type of computational work, big data etc.)
Leader Christel Bergström, Uppsala University

All work packages and their activities are open for those who are interested in the topic (in the broadest possible sense). It is possible to be active within several of the work packages.

Join the work package meetings on Wednesday to share ideas and have an impact on upcoming activities and plans!

Keynote speakers

Epigenetics regulation as anticancer targeting

Maija Lahtela-Kakkonen
School of Pharmacy, University of Eastern Finland, Kuopio, Finland

Brief CV

Maija Lahtela-Kakkonen is Adjunct Professor in Drug Design at School of Pharmacy, University of Eastern Finland, Kuopio, Finland. Her research is focused on *in silico* and *in vitro* models to identify novel regulators for epigenetic targets that are involved in histone acetylation. Especially, the focus is on the cancer research. Current funding comes from highly competitive source Academy of Finland. She has published over 65 papers.

Abstract

Epigenetics has important function in the development of age-related diseases such as cancer.[1,2] Acetylation/deacetylation of chromatin-interacting proteins is central to the epigenetic regulation of genome architecture and gene expression. Among the most promising epigenetic targets in drug development for age-related diseases is considered one family of anti-aging genes, the sirtuins. Sirtuins are class III Histone deacetylases (HDACs) that remove the acetyl groups; consequently, DNA binds tightly to histones silencing genes. Other epigenetic targets are bromodomain and extraterminal proteins (BETs) which bind to acetylated histones regulating transcription and helping to control cell cycle and proliferation. Both BETs and HDACs control several oncogenic drivers such as MYC which is implicated in the pathogenesis of many types of human tumors.[3] Inhibitors of HDAC and BET have individually observed to induce apoptosis in cancer cells, however, to date they appear to have limited efficacy as single agents.[4,5] The dual-targeting of HDACs and BETs is a novel promising strategy in cancer treatment and can give advantages over mono-targeting. In this talk the design of novel sirtuin regulators and the identification of the functional link between BETs and sirtuins[6,7] are presented. Novel knowledge about the interplay between epigenetic regulators together with knowledge of the genome can eventually be used in personalized medicine in cancer.

References

1. Roth et al. (2014)Oncogene 33:1609.
2. Filippakopoulos et al. (2014) Nat Rev Drug Discov 13:337
3. Poole et al. (2017) Genes 8:142
4. Mellini P et al. (2013) J Med Chem 56: 66815
5. Pérez-Salvia M et al. (2017) Epigenetics. 12:323
6. Kokkola T et al. (2015) Chembiochem 16:1997
7. Hytti M et al. (2016) Biochem Pharmacol 110-111:71

Nanotechnology for Precision Medication

Hongbo Zhang, Tenure Track Associate Professor

[1]*Department of Pharmaceutical Science Laboratory, Åbo Akademi University, Turku, Finland*

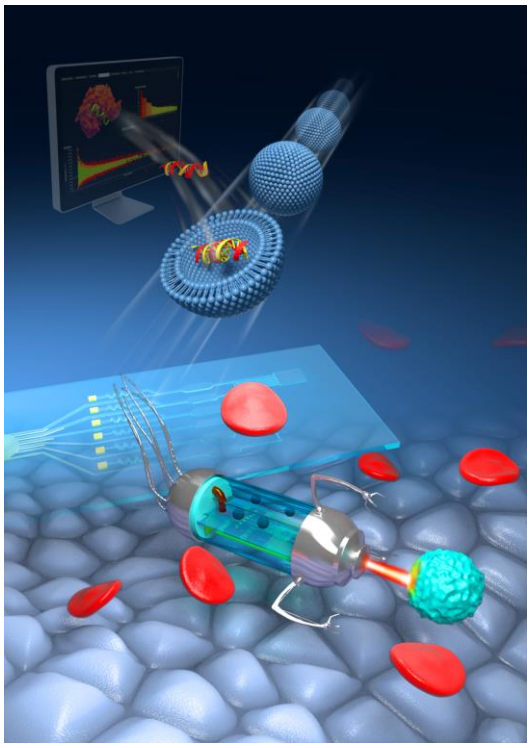
[2]*Ruijin Hospital, Shanghai Jiaotong University, Shanghai, China*

[3]*Turku Center for Biotechnology, Åbo Akademi University and University of Turku, Turku, Finland*

Brief CV:

Hongbo Zhang graduated his PhD from Faculty of Pharmacy, University of Helsinki in December 2012 and then he did three years postdoc in Harvard University supervised by Prof. David A. Weitz. He has a multidisciplinary background in pharmacy, nanotechnology, microfluidics, precision medication, bioimaging; and he knows the world leading technologies. In September 2016, he becomes a Tenure track Assistant Professor in Åbo Akademi University and established his own research group. In two years, the group has grown up to 20 people, with 40+ publications, including 18 impact factor 10+ publications. He has also been corresponding 13 research projects in Finland and in China. Due to his excellent performance, he became an Associate Professor in November 2018.

Abstract:



Nanobiotechnology is a novel discipline that indicate the merger of biological and nanotechnology. It is a multidisciplinary subject, which interplay between physics, materials science, synthetic organic chemistry, engineering and biology. To efficiently diagnostic and treat diseases, the understanding of detail mechanism of disease occur would be critical. The qualitative and quantitative analysis at the molecular scope in real time is highly demanded but very challenging to achieve. Nanobiotechnology is to solve the fascinating problems offered by biology through designing and synthesizing of specific nanomaterials and utilize specific techniques. Our research group focuses on the development of functional nanomaterials for drug delivery, imaging, and therapy etc. We are also specially interested in microfluidics for nanoparticle and microparticle fabrication, as well as for biological analysis. And we develop different kinds of scaffolds and hydrogels for tissue engineering, for example for wound healing and bone regeneration. We have many collaborators in China hospitals, and most of our projects start from a real clinical problem, and the clinical doctors are involved in the project.

ACKNOWLEDGMENTs

We acknowledge financial support from Academy of Finland (decision no. 297580), Jane and Aatos Erkko Foundation (grant no. 4704010) and Sigrid Jusélius Foundation (decision no. 28001830K1).

Protein-based biomaterials for cutaneous application

Andrea Heinz
Department of Pharmacy, University of Copenhagen, Denmark

Brief CV

Andrea Heinz works as an Assistant Professor in at the LEO Foundation Center for Cutaneous Drug Delivery, Department of Pharmacy of the University of Copenhagen in Denmark. Her research is focused on extracellular matrix proteins including their biosynthesis, structural assembly and turnover, which helps understanding aging and wound healing processes as well as the development and progression of cardiovascular diseases. In this context, her group has recently started developing protein-based biomaterials for wound healing, which are biocompatible show similar biomechanical properties as the natural proteins present in the extracellular matrix. These biomaterials are produced using electrospinning or cross-linking of protein or peptide solutions.

Abstract

Wound healing is a complex biological process, which may be impaired in pathological conditions such as diabetes, leading to the development of acute or chronic non-healing wounds. Thus, there is a great interest in the development of wound dressings that actively promote wound healing, for instance through the delivery of bioactive molecules. Our research aims to develop protein-based biomaterials for the use as wound dressings. Proteins such zein or the extracellular matrix protein elastin, which shows unique properties such as elasticity and biocompatibility, are used as building blocks of the biomaterials. Preparation techniques include electrospinning and cross-linking of elastin-based peptides by different cross-linking agents resulting in the formation of hydrogels. The materials are characterized physicochemically using a range of analytical techniques. The addition of bioactive peptides and growth factors allows for a stimulation of wound healing.

Rheological and mechanical study of peptide/cell constructs

Anna Ström
Department of Chemistry and Chemical Engineering, Chalmers, Sweden

Brief CV

Anna Ström is “docent” in Biopolymer technology at the Department of Chemistry and Chemical Engineering, Gothenburg, Sweden. Her research focus is on the understanding of how molecular aspects of polysaccharides links to their conformation, supramolecular assemblies and material properties. Knowledge that is of importance in several sectors as shown by her broad group of collaborators ranging from biomedicine, pharmaceutical technology, food and materials. She is further responsible for pharmaceutical technology courses at the BSc and MSc pharmacy program in Gothenburg. Anna has published 40 scientific papers, book chapters and conference proceedings, she is the inventor of 9 patents and is invited speaker at conferences and workshops.

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Abstract

Low back pain is a common global problem leading to suffering for individuals and burden on society due to sick leave and treatment costs. Current clinical treatment options are often invasive and do not address the underlying problem, believed to be degeneration of the nucleus pulposus (NP), a tissue region of the intervertebral disc. Stem cell therapy has been suggested as a potential route forward to regenerate the NP within the patient. Injection of stem cells requires however a gel that supports cell retention *in-vivo*, survival of cells and promotion of the NP phenotype. In this talk, I will share some data on the rheological and mechanical properties of two different types of gels, a self-assembled peptide gel (PuraMatrix) and a cryogel. The PuraMatrix is commercially available, and shown to bear promise as cell carrier or as scaffold in cell therapy in animal trials (1,2). Here, I present the impact of cell culture time and differentiation on the rheological and mechanical properties of the peptide gel. Further, I will quickly discuss biocompatibility and the ability to inject strain resilient cryogels.

References

1. Henriksson et al., Spine (2009) 34: 141–148
2. Hansson et al., Tissue and Cell (2017) 49: 35–44

Dried blood spots and mass spectrometry in analysis of protein biomarkers and biopharmaceuticals

Trine Grønhaug Halvorsen
Department of Pharmacy, University of Oslo, Norway

Brief CV

Trine Grønhaug Halvorsen is Associate Professor in Pharmaceutical analysis at the Department of Pharmacy, University of Oslo. Her main research area is with-in bioanalysis of drugs and protein biomarkers using LC-MS/MS. She focus on novel sampling materials for dried blood spot analysis of proteins and affinity based sample preparation of proteins and peptides from biological matrices. She has published approx. 50 papers in international journals, and contributed to two teaching books in Analytical Chemistry for Pharmacy students.

Abstract

The Dried Blood Spot (DBS) sampling technique has been around for decades, however, until recent years DBS was mainly used for newborn screening, and subsequent protein analysis was performed by immunometric assays. The combination of DBS with mass spectrometry (MS) for protein analysis mainly is in the explorative stage, but the interest is increasing. In the current talk an overview of DBS sampling for mass spectrometric based protein analysis will be given. Analysis of proteins is of importance both in treatment and follow-up of diseases as well as in drug development. Advantages and limitation of the DBS-MS approach will be discussed. Focus will be on recent progress including use of so-called dried matrix spots (DMS)[1], sampling of other biological matrices, and developments in DBS sampling technology such as novel sampling materials/devises[2-4]. Examples of use in both qualitative and quantitative protein analysis will be given.

References

1. Rosting *et al.* J Chromatogr B (2018)1077–1078: 44-51
2. Andersen *et al.* J Pharm Biomed Anal (2018) 156:239-246
3. Skjærvø *et al.* Analyst (2018) 143:3184-3190
4. Skjærvø *et al.* Talanta (2019) 195:764-770

Non-linear optical imaging in pharmaceutical applications from drug delivery to solid-state analysis

Jukka Saarinen
Division of Pharmaceutical Chemistry and Technology, University of Helsinki, Finland

Brief CV

Jukka Saarinen is currently working as a postdoctoral researcher in the Division of Pharmaceutical Chemistry and Technology at the University of Helsinki. His research is focused on non-linear optical imaging in pharmaceutical applications including cells, nanoparticle-cell interactions, dosage forms and solid-state analysis. He finished his doctoral degree in spring 2018 and his PhD thesis was entitled: "Non-linear Label-free Optical Imaging of Cells, Nanocrystal Cellular Uptake and Solid-State Analysis in Pharmaceutics". Currently he is employed by an Academy of Finland project, which is focused on label-free analysis of nanocrystal-cell interactions. In addition to non-linear optical imaging, his research also includes spontaneous Raman microscopy, especially in biological analysis. One of the publications he co-authored was awarded "Most Outstanding Pharmaceutical Research Article 2017/2018" by the Finnish Pharmaceutical Society. He has also authored book chapters about spectroscopic imaging.

Abstract

Non-linear optical imaging including coherent anti-Stokes Raman scattering (CARS) and second harmonic generation (SHG) have advantages that make these techniques desirable in pharmaceutical and biomedical analysis.[1,2] These techniques offer rapid, non-destructive, chemically- or structurally-specific, label-free, inherently confocal imaging. The signal in CARS is based on the probing of molecular vibrations, while structures lacking inversion symmetry, such as non-centrosymmetric crystals, provide SHG signals.[1,2] In this talk, principles of techniques are provided with examples of non-linear optical imaging in drug delivery and surface-sensitive solid-state analysis. Current drug development includes different types of nanoparticles and the fate of these particles in biological matrices needs to be monitored in the early pre-clinical phases of drug research. CARS offers much potential for this, as demonstrated with non-fluorescent nanocrystals.[3,4] On the other hand, in drug development, there is a trend to use different types of amorphous drug formulations, including co-amorphous salts, to improve poor water-solubility of drugs. To ensure that there is no solid-state conversion, which can potentially affect bioavailability, during the storage of amorphous formulations, suitable analytical techniques are required. Multimodal non-linear optical imaging with CARS and SHG offers solid-state-specific image analysis with high surface sensitivity.[5,6] As a conclusion, non-linear optical imaging holds plenty of potential in pharmaceutical applications and can be used in many phases during the drug development to ensure the safety and efficiency of drugs.

References

1. Strachan *et al. Int. J. Pharm.* (2011) 417:163-172
2. Evans and Xie. *Annu. Rev. Anal. Chem.* (2008) 1:883–909
3. Darville, Saarinen *et al. Eur. J. Pharm. Biopharm.* (2015) 96:338-348
4. Saarinen *et al*. *Biotechnology Journal* (2018) 1800413
5. Novakovic, Saarinen *et al. Anal. Chem.* (2017) 89:11460-11467
6. Ojarinta, Saarinen *et al. Eur. J. Pharm. Biopharm.* (2018) 132:112–126

Using molecular dynamics simulations to probe various aspects of pharmaceutical systems

Per Larsson
Department of Pharmacy, Uppsala University

Brief CV

Per Larsson has a PhD in Theoretical Biochemistry from Stockholm University, and then worked as a post-doctoral researcher at the University of Virginia, Charlottesville, USA, on simulations of so-called viral fusion peptides and how they interact with membranes. He is currently Assistant Professor at the Department of Pharmacy, Uppsala University. His research is focused on using in silico simulation methods to understand the molecular level details of the interplay between poorly soluble drugs and the intestinal environment, and how various enabling formulations, in particular lipid-based, work to modify drug solubility and absorption. More related to his post-doctoral work, he has also recently started to work on simulation approaches to understand how different peptides are transported across and interact with the intestinal cellular membrane. Within the context of the newly established Swedish Drug Delivery Forum, the particular aim of this research is to understand peptide permeability in the context of inter-individual intestinal differences and the use of permeability enhancers.

Abstract

Little is known about the dynamics of the intestinal lipoidal nanostructures present in the fasted and fed state, or after oral intake of lipid-based formulations. As a response to dilution upon water intake, digestion and absorption, restructuring of these solubilizing nanoaggregates will occur. This talk will circulate around simulation protocols applicable to complex and lipid-rich water-based systems with special emphasis on the rearrangement of lipoidal nanostructures in response to dilution, digestion and absorption, as well as the molecular level details of the lipid-based formulations themselves and drug-lipid interactions. The implications of results from simulations of amorphous drug-polymer systems will also be discussed, as well as modeling of the aggregation behavior of sodium caprate as a permeation enhancer, and future directions for molecular resolution studies of oral peptide drug delivery.

Combined Dissolution-/Permeation Testing for the Prediction of Oral Bioavailability of Tadalafil

Maria João Gomes
Institute of Physics, Chemistry and Pharmacy - University of Southern Denmark, Odense, Denmark

Brief CV

Maria Gomes is a post-doc researcher at the University of Southern Denmark, Odense, Denmark. During her PhD at University of Porto she has collected experience in blood-brain barrier and nanoparticulate carriers. Her current research is focused on development of innovative oral bioavailability tools for studying the dynamics dissolution-/permeation of poorly soluble drugs-based enabling formulations, while working on *in vitro* mimicking biological membranes. She currently also serves as an editorial assistant of the highly prestigious European Journal of Pharmaceutical Sciences.
She has published 11 papers and 2 book chapters and supervised 2 master students.

Abstract

Traditionally, for performance ranking of enabling formulations for poorly soluble (BCS II) drugs, solubility on the one hand and dissolution-rate on the other hand have been widely used. The predictive power of these approaches however is more and more being questioned. At the same time permeation testing of enabling formulations is gaining increasing attention for formulation development. Commonly used permeation setups have a relatively small barrier area, which is good enough to rank APIs with respect to permeability.
For formulations, where dissolution-rate or release-rate is expected the rate limiting step, classical set-ups do not allow the observation of the interplay between dissolution and permeation. We describe here the evolution from conventional steady-state flux scenarios to advanced interdependent dynamic dissolution / permeation setups. We summarize the efforts that have been made during the last years in our group to better mimic *in vivo* conditions. This has resulted in the development of a novel permeation setup, PermaLoop®.
To illustrate the impact of experimental parameters, tadalafil was selected as a poorly soluble model compound. Crystalline and amorphous tadalafil enabling formulations were prepared and assessed under different conditions. Biomimetic media, pH, membranes and volumes are among the tested variables of the dissolution-/permeation studies that have been performed, which will be addressed in this talk.

Localized delivery of natural polyphenols: an alternative to antibiotics in treatment of genital chlamydia?

May Wenche Jøraholmen

Drug Transport and Delivery Research Group, Department of Pharmacy, University of Tromsø The Arctic University of Norway

Brief CV

May Wenche Jøraholmen is Postdoctoral fellow in Drug Transport and Delivery Research Group at the Department of Pharmacy, University of Tromsø The Arctic University of Norway and is currently on a research stay at the Department of Clinical Science, Intervention and Technology (CLINTEC), Karolinska Institutet, Stockholm, Sweden. Her research is focused on the development of vaginal drug delivery systems for the localized treatment of vaginal infections. The project is funded by the Northern Norway Regional Health Authority (HelseNord).

Abstract

Chlamydia trachomatis is a major cause of sexually transmitted bacterial diseases. The hazard of increased antimicrobial resistance is a threat for the current oral antibiotics recommended for the treatment of this infection. In this talk, a novel approach in targeting chlamydia infections will be presented. The use of natural origin active ingredients, polyphenols, such as epicatechin (EPI) and resveratrol (RES), preserves available antibiotics while assuring equally efficient treatment. The combination of two delivery systems, one enabling the incorporation of poorly soluble polyphenols (liposomes) and another assuring the prolonged contact time at the vaginal site (hydrogels), represents a promising approach in optimization of localized therapy. Incorporation of polyphenols into liposomes allows fusion with bacterial cells, resulting in improved localized antimicrobial effect at lower doses. Liposomes containing EPI or RES were prepared by the modified film method and extruded to desired vesicle size[1]. Chitosan hydrogels were prepared by dissolving the polymer in the mixture containing 2.5 % acetic acid and 10 % glycerol (w/w), followed by 48 hour swelling[2]. Liposomes were mixed into hydrogels in the concentration of 10 % (w/w). To optimize the polymer concentration of the gels, the hardness, cohesiveness and adhesiveness of hydrogels were measured[2]. The mucoadhesive properties of the delivery system were determined by the detachment force[3] and amount of formulation retained on bovine vaginal tissue. The *in vitro* release studies were performed in the mimicked vaginal environment[4,5]. Anti-oxidative activities of EPI and RES were confirmed and preliminary antibacterial studies performed.

References

1. Jøraholmen *et al.* Eur J Pharm Sci (2015) 79:112-121
2. Hurler *et al.* J Pharm Sci (2012) 101:3906-3915
3. Hurler and Škalko-Basnet, J Funct Biomater (2012) 3: 37-48
4. Jøraholmen *et al.* Int J Pharm (2014) 472:94-101
5. Jøraholmen *et al.* Eur J Pharm Biopharm (2017) 113: 132-139

Ear plugs as a drug delivery system to treat acute otitis media

Hakon Hrafn Sigurdsson
Department of Pharmaceutical Sciences, University of Iceland

Brief CV

Hakon Hrafn Sigurdsson is Professor in Pharmaceutics at the Department of Pharmaceutical Sciences, University of Iceland. His research is mostly about drug delivery through biological membranes with focus on mucous membranes. Other projects include new therapeutic peptide to treat psoriasis.

Abstract

Essential oils are volatile oils derived from distillation of plant materials and have been known long for their antibacterial properties. Essential oils are not suitable in drug formulations due to variability in their composition. Their ingredients can also be irritant or allergenic. Thymol, which is the main ingredient in thyme oil extracted from *Thymus vulgaris* L., has broad antibacterial activity against major respiratory pathogens. A medical device, for example ear plugs that enable the administration of thymol vapor without allowing direct contact to the ear could be a potential new treatment for otitis media. The purpose of this project is to design earplugs intended for administration of Thymol to treat acute otitis media. The earplugs contain formulation that enhances the delivery of thymol in vapor form through the tympanic membrane *in vivo*. Antibacterial activity of selected combinations and types of devices have been tested against pneumococcal serotype 19F *in vitro* displaying promising results.

Short oral communications

Photonic interactions *in vitro* and *in vivo* between fluorescent tracer and nanoparticle-based scavenger for enhanced molecular imaging

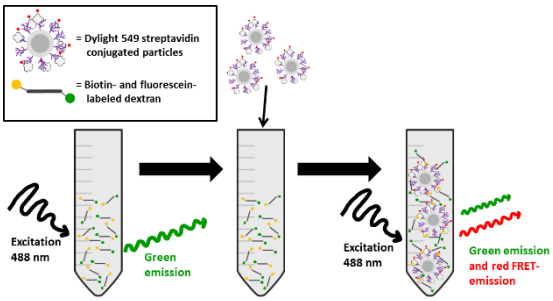
Tina Gulin-Sarfraz[1,2], Evgeny Pryazhnikov[3], Jixi Zhang[4], Jessica M. Rosenholm[1]* and Leonard Khiroug[3]*

1. Pharmaceutical Sciences Laboratory, Faculty of Science and Engineering, Åbo Akademi University, Turku, Finland
2. School of Pharmacy, University of Oslo, Oslo, Norway
3. Neurotar LtD, Viikinkaari 4, 00790, Helsinki, Finland
4. College of Bioengineering, Chongqing University, Chongqing 400044, China

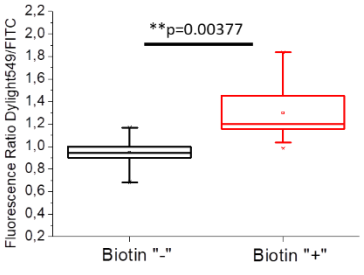
Introduction: Non-invasive imaging techniques are powerful diagnostic tools, applied in both academic research and clinics. The emerging field of molecular imaging has enabled visualization of molecular processes in living organisms and proved its efficiency for diagnosis of various diseases. Molecular imaging techniques require administration of a contrast agent prior to an imaging procedure to improve the quality of the generated images. These contrast agents are typically small molecules capable of crossing blood-tissue barriers, and thus easily accumulating in the tissues, especially if the blood-tissue barrier is disrupted. However, a major drawback is the failure to accurately differentiate between the signals originating from the contrast agent circulating in the blood stream and the signals originating from surrounding tissue. A method allowing for *in vivo* differentiation between these signals would allow for a radically improved contrast between tissue and blood.

Aim: To investigate the potential of silica nanoparticles to scavenge a specific tracer compound, and quench its fluorescence, directly in the blood circulation in mice.

Methods: The silica particle surface was functionalized with streptavidin, to specifically recognize and bind the biotin-labeled dextran (tracer model compound). The developed tracer-scavenger system was investigated under *in vitro* conditions to ensure that proper binding is taking place, as confirmed by FRET studies. *In vivo*, two-photon imaging was used to study the chemical and photonic interactions of the scavenger and the tracer directly in the blood circulation of mice, by detecting the change in the ratio of red and green fluorescence on individual nanoparticles.



Results: After intravenous injection of the tracer compound in mice, with subsequent injection of the scavenger, the interaction could be visualized in the cortical vessels. For comparison, tracer compound without biotin-labeling was utilized. A statistically significant shift in fluorescence ratio towards the scavenger fluorescence was observed, which demonstrates the physical and chemical interaction between tracer and scavenger *in vivo*.



Conclusion: This conceptual study highlights the possibilities for a novel method to remove and/or quench excess contrast media in the blood circulation prior to an imaging session; towards an efficient system for improving the contrast of the tissue area of interest, which consequently might increase the probability of early diagnosis of various diseases.

Amphiphilic drug molecules in oppositely charged microgels

Yassir Al-Tikriti , Per Hansson
Department of Pharmacy, Uppsala University, Sweden

Introduction

Cationic-amphiphilic molecules are an important group of active substances which are commonly used in cancer therapy, as antidepressants and antihypertensive agents. They have the ability to aggregate in a solution in a surfactant-like manner. The aggregation pattern can be quite different depending on the structure of the molecule[1]. Microgels, on the other hand, are crossed-linked polymer networks which are sensitive to environmental variables such as temperature, pH and ionic strength. The swelling/deswelling concept of the microgels is very important in drug release profile[2]. By this means, a large amount of drug of opposite charge can be loaded inside the microgels by ion exchange mechanism[3] to deliver it to the desired target inside the body[2,4].

Aim

In this project, we want to improve fundamental understanding of the mechanism of interaction between cationic amphiphilic drugs and oppositely charged microgels.

Method

Micromanipulator-assisted light microscopy was used to investigate single microgels during Amitriptyline loading ,as in figure 1, and release while the binding isotherm was determined by using µdiss profiler (pION). A single gel microscopy technique in a small liquid volume[5] will be used to investigate the distribution of the Amitriptyline inside microgels at intermediate levels.

Results

The deswelling of the polyacrylate microgels during the loading of Amitriptyline, in 10mM ionic strength, was investigated by Micromanipulator-assisted light microscopy as seen in figure 1.The microgel volume decreases gradually until the collapsed phase forms on the right side. In addition, the binding isotherm of the Amitriptyline with polyacrylate microgels was measured. The β ratio increases gradually until the micelles formation where the curve rises sharply until reach the plateau phase where all gels are fully collapsed.

Conclusion

The kinetics of loading a single gel affected by microgel size, amitriptyline concentration and the flow rate. Furthermore, there is a difference in phase behavior between the loading of a single microgel and the loading of a population of microgels. In the later, microgels have no intermediate phases and they are either fully collapsed or swollen.

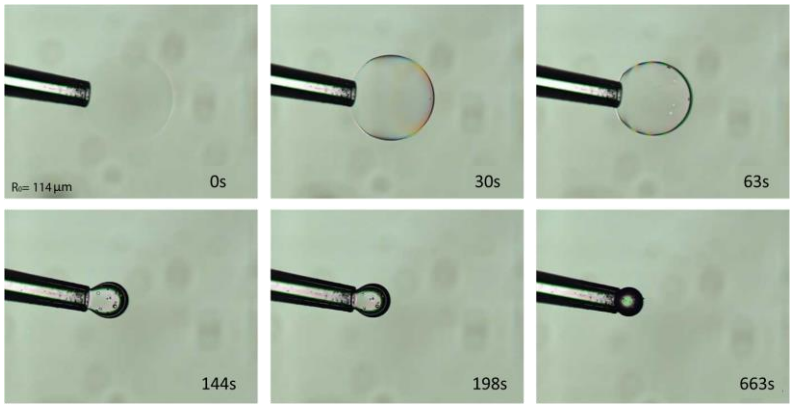


Figure 1. A single microgel with radius 114µm held by Micromanipulator, Deswelling kinetics in a flow of Amitriptyline.

[1] D. Attwood, *Advances In Colloid And Interface Science*, 1995, **55**, 271.
[2] PF. Kiser, G. Wilson and D. Needham, *J. Control. Release*, 2000, **68**, 9.
[3] P. Nilsson, P. Hansson, *J. Phys. Chem. B*, 2005, **109**, 23843.
[4] M. Malmsten, H. Bysell, P. Hansson, *Current Opinion In Colloid & Interface Science*, 2010, **15**, 435.
[5] C. Jidheden, P. Hansson, *J. Phys. Chem. B*, 2016, **120**, 10030.

First of the kind – the apparent pH-solubility profile of an amorphous drug using the Single Particle Analysis (SPA) method

J.Štukelj[1,2], S. Svanbäck[1,2], Clare J. Strachan[1], J. Yliruusi[2]
[1]University of Helsinki, Faculty of Pharmacy, Finland
[2]The Solubility Company Oy, Helsinki, Finland

Introduction

The measurement of amorphous solubility can be masked by crystallization taking place before the maximum supersaturation in solution is reached.[1] Direct measurement of amorphous solubility – drug supersaturation potential – not masked by crystallization, over biorelevant pH range would provide information for better drug formulation.

Aim

To use the image-based SPA method to measure the apparent solubility of amorphous model drug indomethacin over a biorelevant pH range and in biorelevant media.[2]

Methods

The amorphous form of indomethacin was prepared by quench cooling. Samples were characterized using XRPD and DSC. Using the SPA method, the apparent solubility of single particles (n≥10) of the amorphous indomethacin was measured in seven different USP buffers ranging from pH 1.2 up to pH 7.0. Moreover, the solubility was measured in three biorelevant buffers: FaSSGF, FeSSIF and FaSSIF. For comparison, all the measurements mentioned above were also conducted using the thermodynamically stable γ form of indomethacin (Fig. 1)

Results

Using the SPA method the apparent pH profile of amorphous indomethacin was obtained and plotted against the pH profile of the thermodynamically stable γ form (Fig. 2). The observed pKa values were 3.4 and 4.0 for amorphous and γ form, respectively. The amorphous to γ form solubility ratio of 31±10 was observed over the entire pH range, which is at least three-fold higher than any experimental amorphous to γ indomethacin solubility ratio previously reported. Moreover, amorphous to γ form solubility ratio in FeSSIF and FaSSIF, where surfactants are present above critical micellar concentration, was increased to 135 and 102, respectively.

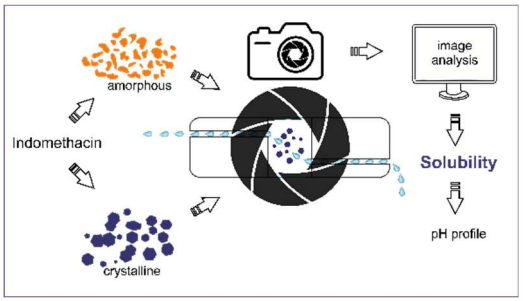


Figure 1. Simplified visualization of the experimental process.

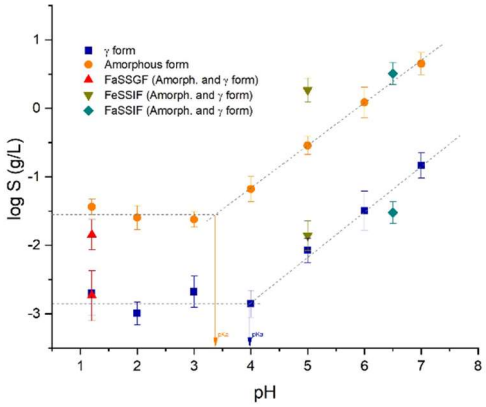


Figure 2. pH profile of amorphous and γ indomethacin.

Conclusion

The SPA method has proven a successful tool to measure, for the first time, the apparent pH profile of a drug in its amorphous form. Moreover, the method revealed that the amorphous to γ solubility ratio is most likely dependent on the concentration of surfactants in the solvent. Future research is needed to completely understand and establish this dependency.

References

1. Murdande S. et al. *Pharm Res*. (2010) 27: 2704-2714
2. Svanbäck S. Helsinki: University of Helsinki; 2016

Particle engineering for Dry Powder Inhalation (DPI): Modifying drug-to-carrier interaction in adhesive mixtures

Simon Bock, Regina Scherließ
Department of Pharmaceutics and Biopharmaceutics, Kiel University, Germany

Introduction

The pulmonary route is a well-established way to treat diseases of distinct genesis. The fine particle fractions (FPF, aerodynamic diameter < 5µm) from several DPIs with drug-carrier adhesive mixtures are considerably lower than the label claims and thus far less than the designated doses reach the target tissue [1]. Appropriate balancing of adhesion and dispersion forces are required for optimal performance, low adverse effects and thus for therapeutic success. The intensity of these forces depends on various parameters such as particle size, morphology and surface properties, but also on the inhaler device used [2]. The interplay of these variables is not yet completely elucidated. Therefore further fundamental investigations need to be conducted.

Aim

The aim of the work was to determine whether the deposition profile of an active pharmaceutical ingredient (API) can be influenced by modifying the surface of uniform, spherical DPI carrier particles with different amino acids.

Methods

Particle coating of MCC spheres (JRS Pharma, Germany) in a fluidized bed process using a Mini-Glatt (Glatt, Germany) was carried out with aqueous solutions of amino acids tyrosine, tryptophan or phenylalanine (Sigma Aldrich, Germany). Subsequently, the differently coated and non-coated carrier particles were each blended with 2 % salbutamol sulphate (SBS; Sigma Aldrich, Germany) as a well-tested model API using a high-shear mixer (Picomix®, Hosokawa, Germany) at predefined conditions (500 rpm, 2 x 60 sec.). Aerodynamic performance was evaluated using the Next Generation Pharmaceutical Impactor (NGI; Copley Scientific, UK) according to Ph.Eur. 9.2. As device the Novolizer® was used at a fixed flow rate of 78.3 L/min. HPLC analytics were carried out for drug quantification.

Results

Successful coating was confirmed by EDX and HPLC analysis. Aerodynamic assessment of the different interactive blends prepared with non-coated and coated carrier particles showed a shift of API deposition towards stages with smaller aerodynamic cut offs for coated carriers. The non-coated material led to a FPF of 14.6 % whereas the coating resulted in FPFs of 25.7 % (Tyr), 24.4 % (Trp) and 24.2 % (Phe), respectively (Figure 1).

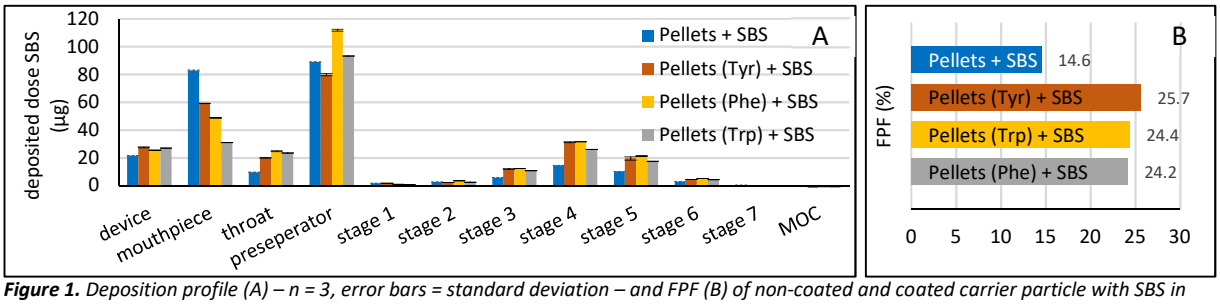


Figure 1. *Deposition profile (A) – n = 3, error bars = standard deviation – and FPF (B) of non-coated and coated carrier particle with SBS in the NGI.*

Conclusion

An adjustment of in vitro API deposition profile using interactive blends with chemically modified carrier particles was shown. Shift of deposition might be attributed to a change of physicochemical interaction between API and carrier surface which need to be verified by atomic force microscopy.

References

[1] A.H. de Boer, P. Hagedoorn, M. Hoppentocht, F. Buttini, F. Grasmeijer, H.W. Frijlink, Dry powder inhalation: Past, present and future, Expert opinion on drug delivery 14 (2017) 499–512.

[2] F. Grasmeijer, N. Grasmeijer, P. Hagedoorn, H.W. Frijlink, A.H. de Boer, Recent advances in the fundamental understanding of adhesive mixtures for inhalation, Current pharmaceutical design 21 (2015) 5900–5914.

Effect of fragmentation on dissolution rate of paracetamol after tableting

Anne Linnet Skelbæk-Pedersen [a,b], Thomas Vilhelmsen [a], Nikolaj Sølvkær [a,b], Jukka Rantanen [b].
[a] *Novo Nordisk A/S, Oral Pilot and Process Development, 2760 Måløv, Denmark.*
[b] *Department of Pharmacy, University of Copenhagen, 2100 Copenhagen, Denmark.*
Email: apse@novonordisk.com

PURPOSE

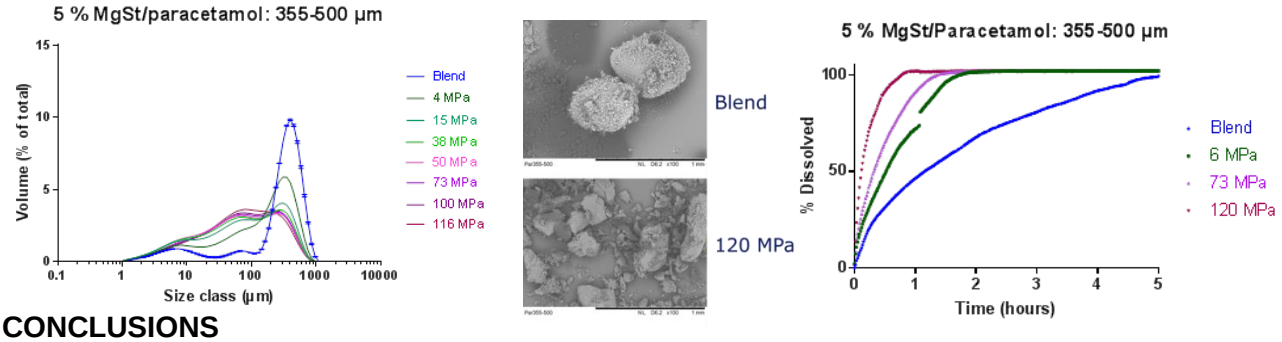
During the tableting process a material will undergo deformation. Powder deformation is the material property that has the largest impact on tablet formation and it can be divided into reversible (elastic) and irreversible (plastic/ fragmenting) deformation. A method for quantifying fragmentation after tableting was recently introduced.[1] The purpose of this study was to investigate the effect of fragmentation happening during tableting on the dissolution rate of paracetamol.

METHODS

Paracetamol was fractionated to 355-500 µm and blended with 5 % MgSt (<90 µm) and compressed into tablets (5 – 105 MPa) using 10 mm flat-faced punches. The compressed particles were recovered from the tablets by manual grinding and subjected to particle size distribution (PSD) analysis, scanning electron microscopy (SEM) and dissolution testing.

RESULTS

The compressed paracetamol particles were found to fragment extensively during tableting based on the PSDs and the fragmentation was found to mostly occur <50 MPa. This was supported by SEM images. Dissolution profiles of the compressed particles showed that fragmentation happening during tableting had a high impact on the dissolution rate, as higher pressures resulted in more extensive fragmentation leading to reduction in particle size and an increase in surface area.



CONCLUSIONS

By increasing the pressures applied during tableting, it was found that the dissolution rate increased markedly as a function of the degree of fragmentation.

ACKNOWLEDGEMENTS

Novo Nordisk A/S (Bagsværd, Denmark) is acknowledged for financing the PhD project of Anne Linnet Skelbæk-Pedersen (STAR program).

REFERENCES

[1] Skelbæk-Pedersen, Anne, et al. "Quantification of fragmentation of pharmaceutical materials after tableting." J. Pharm. Sci (2018), in press, doi: 10.1016/j.xphs.2018.10.040.

Optimization of the coating process of minitablets in two different lab-scale fluid bed systems

Maja Szczepanska[1*], Piotr Paduszynski[2], Malgorzata Sznitowska[1]
[1] Department of Pharmaceutical Technology, Medical University of Gdansk, Poland
[2] Research and Development Department, Polpharma SA, Poland

Introduction

In the contemporary pharmaceutical industry, the implementation of quality by design (QbD) principles becomes a necessity. The quality of drug may be designed and built into the product using statistical tool which is known as experimental design.[1] Minitablets (1-3 mm diameter) are a novel oral dosage form developed mostly for pediatric applications.[2] Coating the minitablets (MT) can be useful for obtaining taste masking properties or modifying drug release rate.

Aim

The aim of the study was to optimize the coating process of the MT with pantoprazole in two fluid bed systems using the QbD concept.

Method

Delayed pantoprazole release was achieved by coating the MT (3 mm, 18 mg) with an aqueous dispersion of Eudragit L 30D 55. Two apparatus for fluid bed coating were evaluated: Aircoater 025 (InnoJet) and 4M8-Trix (ProCepT). Optimization of fluid bed coating process for MT was executed by design of experiment (DoE). A 2^4 full experimental design was applied to describe the effect of formulation factors on characteristics of the product (Statistica 13). Four process parameters were chosen for monitoring: X1-inlet airflow rate (0.36; 0.27 m^3/min), X2-product temperature (27; 25˚C), X3-coating mixture flow rate (1.3; 0.4 g/min), and X4-spraying pressure (1.0; 0.7 bar). As a response values: Z1-film thickness [µm], Z2-uniformity of film coating [µm], and Z3-release of API after 130 min of dissolution test [%] were examined.

Results

It was observed that different process parameters were relevant in the optimization of both applied fluid bed systems. For 4M8-Trix parameter X1 had the most significant influence, whereas for coating in Aircoater 025 parameter X3 was a crucial parameter. A diverse dynamics air movement and geometry in both chambers may be a reason of these differences. The optimal process parameters for each fluid bed coater were also indicated.

Conclusion

The optimization of the MT coating process with QbD concept was carried out for the first time. Further investigation on the improvement of MT technology can become a direction for other research centers. Outcome of the research may accelerate development of modified release pediatrics drugs.

References

1. Tezyk M. *et al.* Drug Dev Ind Pharm (2017) 43: 1626–1636
2. Aleksovski A. *et al*. Drug deliv (2015) 12: 65-84

Sprayable beta-glucan hydrogel as wound dressing: *in vitro* and *in vivo* evaluation

Jostein Grip[a,b], Erik Steene[a], Rolf Einar Engstad[a], Ingrid Skjæveland[a], Purusotam Basnet[c,d], Nataša Škalko-Basnet[b], Ann Mari Holsæter[b,*]

[a]Biotec BetaGlucans AS, 9019 Tromsø, Norway
[b]Drug Transport and Delivery Research Group, Department of Pharmacy, Faculty of Health Sciences, University of Tromsø The Arctic University of Norway, 9037 Tromsø, Norway
[c]Women's Health and Perinatology Research Group, Department of Clinical Medicine, University of Tromsø The Arctic University of Norway, 9037 Tromsø, Norway
[d]Department of Obstetrics and Gynecology, University Hospital of North Norway,
Sykehusveien 5738, 9038 Tromsø, Norway.

Introduction

With an increased life expectancy, increased number of elderly patients as well as increased prevalence of diabetes are emerging globally, leading to an increase in prevalence of chronic wounds. Chronic or stalled wounds can, if left un-treated, lead to decreased patient life quality, amputation of affected limb or even death. Thus, there is a need for advanced wound healing products that can increase patient comfort, reduce healing time and lessen the economic burden on the healthcare system. β-1,3/1,6-glucan (βG) isolated from baker's yeast as an active wound healing ingredient. βG has in clinical studies shown great promise for treatment of chronic wounds (Zykova et al., 2014) and is currently available in a commercial gel-formulation (βG-Gel). A spray format could provide the advantages of multiple-use containers, minimal discomforts during application, easy and quick administration onto the wound, provide a big surface contact area of the formulation, and easier access to deeper wounds.

Aim

The aim of this study was to develop a sprayable hydrogel wound dressing with βG as active ingredient, targeting larger dry to low exuding hard-to-heal wounds, for treatment of chronic and burn wounds.

Methods

Using a central composite design, the sprayability and rheological properties of 15 different hydrogel formulations containing βG, carboxymethyl cellulose (CMC) and glycerol dispersed in different concentrations and ratios in distilled water, were characterized. One formulation (βG-Spray) and one carrier control (NoβG-Spray) formulation were selected for further testing investigating the *in vitro* toxicity on keratinocytes (HaCaT-cells), and moisture retention on a simulated wound model. Finally, the wound healing ability of the formulations was tested in an impaired excisional wound healing model in *db/db* diabetic mice (BKS.Cg-m Dock7^{m} +/+ Lepr^{db} /J).

Results

All the 15 prepared formulations were sprayable, thus the selection of formulation was based on the rheological properties, favoring the similar melting temperature as the βG-Gel. The selected spray formulation had an ability to both donate and absorb moisture in the simulated wound model. The result of the *in vivo* study showed a significantly improved wound closure rate in the βG-Spray group compared with the water control ($p < 0.05$) and the carrier control group receiving the NoβG-Spray ($p < 0.05$) between day 8 and day 16. The wound closure in the βG-Spray group was comparable to the commercial βG-Gel.

Conclusion

A novel advanced, sprayable, and easy to apply βG-formulation was developed, and the *in vitro* and *in vivo* results encourage further testing in humans, as improve clinical outcome seem attainable with this formulation when treating larger dry-to-low exuding hard-to-heal wounds.

References

Zykova, S.N., Balandina, K.A., Vorokhobina, N. V, Kuznetsova, A. V, Engstad, R., Zykova, T. a, 2014. Macrophage stimulating agent soluble yeast β-1,3/1,6-glucan as a topical treatment of diabetic foot and leg ulcers: A randomized, double blind, placebo-controlled phase II study. J. Diabetes Investig. 5, 392–399. doi:10.1111/jdi.12165

Evaluation of Fibroblasts Attachment and Growth on Electrospun Fiber Scaffolds

Celia Teresa Pozo Ramos[a], Georg-Marten Lanno[b], Liis Preem[a], Marta Putriņš[b], Ivo Laidmäe[a], Tanel Tenson[b], Karin Kogermann[a]
[a]Institute of Pharmacy, University of Tartu, Nooruse 1, 50411 Tartu, Estonia
[b]Institute of Technology, University of Tartu, Nooruse 1, 50411 Tartu, Estonia

Introduction

Electrospun fiber scaffolds are considered a novel strategy for wound healing therapy due to several characteristics such as the resemblance of the natural extracellular matrix, high surface area to volume ratio, tunable porosity, sufficient gas-exchange and possibility to include active substances into the fibers (1). All these notable physicochemical properties are known to promote the wound healing. However, it is important to study how differently designed structures of fiber mats interact with the living eucaryotic cells in order to understand their safety and suitability for wound healing applications.

Aim

The main aim of the study was to understand how the fiber mat structure affects the fibroblasts attachment and growth highlighting the relevance of fiber diameter and its porosity.

Method

Polycaprolactone (PCL) 15% was used to construct four different mats by electrospinning. Tetrahydrofurane:dimethylsulfoxide (THF:DMSO)(90:10) and acetic acid:formic acid (AA:FA) (30:10) were used as solvent systems. Drug-loaded (4% w/w) fiber mats with an antibacterial drug, chloramphenicol (CAM), were also prepared. Morphology of the mats was studied using scanning electron microscopy (SEM). Cell studies were performed using baby-hamster kidney (BHK-21) cells. Fiber mats were placed into 24-wellplates using cell crown inserts, where the cells were seeded ($\sim 10^5$ cells/well). After 48 h the mats were removed from the inserts, transferred into new media and MTS reagent was added. Absorbance of cell metabolism product formazan was measured after 1 h of incubation. Cell attachment on the fiber mats was confirmed by SEM.

Results

SEM confirmed that the mats with THF:DMSO consisted of porous microfibers (2.01±0.21 μm) while mats with AA:FA were non-porous nanofibers (0.45±0.11 μm). No large differences were observed in the fiber diameter after the drug-incorporation into the fibers. The amount of cells measured on the electrospun fiber mats was significantly higher on the THF:DMSO microfiber mats than on the AA:FA nanofiber mats. No significant differences were observed in the cell attachment between the drug-loaded and pure polymer porous microfiber mats (THF:DMSO).

Conclusion

Porous electrospun microfibers (THF:DMSO) allow better fibroblast attachment and growth compared to the non-porous electrospun fibers (AA:FA). Further studies will provide answers whether these can be correlated with the better *in vivo* wound healing properties highlighting the importance to understand the structure-activity relationships.

References

1. Hu X, Liu S, Zhou G, Huang Y, Xie Z, Jing X. 2014. Electrospinning of polymeric nanofibers for drug delivery applications. J Control Release 185:12–21.

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Preparation, upscaling and lyophilization of soybean cochleates for subcutaneous administration

L.H. Laursen[1], S. Kristensen[1], F. Steiniger[2], J. Kuntsche[1]

[1] Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, 5230 Odense M, Denmark
[2] Electron Microscopy Center, University Hospital Jena, Friedrich Schiller University, 07743 Jena, Germany

Introduction: Cochleates are phospholipid particles composed of tightly packed lipid bilayers. They can be prepared by mixing calcium ions with negatively-charged liposomes such as phosphatidylserine vesicles. Cochleates possess a much higher chemical and mechanical stability compared to liposomes, which makes them very interesting for drug delivery purposes. To facilitate the use of cochleates in the pharmaceutical industry, cheaper alternatives of phospholipids (natural lipids instead of synthetic lipids, calcium salts), and important process parameters (upscaling of preparation and lyophilization) are being investigated.

Aims: To investigate preparation of cochleates from soybean phosphatidylserine in larger scale by using a computer-controlled mixing device and to evaluate feasibility of freeze-drying of cochleate suspensions.

Methods: Cochleate dispersions were prepared in batch sizes from 2 – 40 ml by mixing sonicated liposomes prepared from soybean phosphatidylserine (94%, Lipoid KG) with a calcium chloride solution to reach a 1:1 and 1:2 lipid/Ca^{2+} molar ratio and a lipid concentration of 10 mg/ml. Selected cochleate dispersions containing 5% or 10% trehalose or 5% mannitol or without lyoprotectant were freeze-dried (Gamma 2-16 LSC, Martin Christ GmbH). The formulations were characterized by laser diffraction combined with polarization intensity differential scattering (LD-PIDS), small-angle X-ray scattering (SAXS), scanning electron microscopy (SEM), and thermogravimetric analysis (TGA).

Results: Preparation of cochleates in larger scale did not alter particle morphology, size distribution or structure (Fig. 1a). The lyophilized samples without or with mannitol as lyoprotectant could be easily redispersed in buffer within a few seconds, whereas the samples containing trehalose were more difficult to redisperse (>1 min). Interestingly, the highest water content was observed in the samples containing trehalose (Fig. 1b).

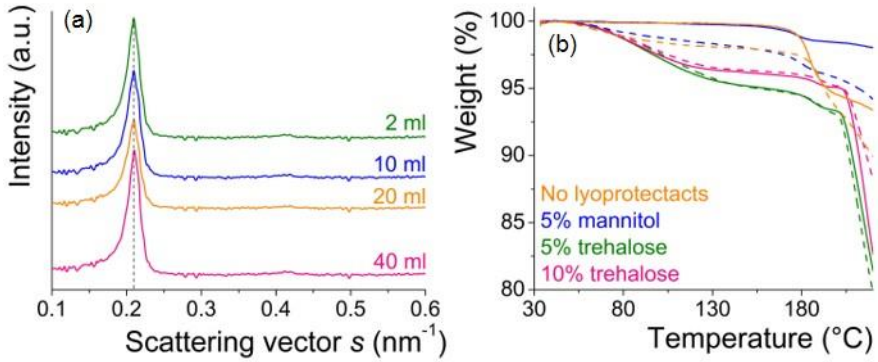


Figure 1. (a) SAXS diffractograms of soybean cochleates (1:1 lipid/Ca^{2+} molar ratio). (b) Residual water content analysis of lyophilizates measured using TGA; solid and dashed lines indicate 1:1 and 1:2 lipid/Ca^{2+} molar ratio, respectively.

Conclusions: Upscaling and lyophilization of the cochleate suspensions was generally feasible and the optimization of excipients in lyophilization and larger scale preparation should be investigated further.

Micromechanical Thermal Analysis (MTA) for Large and Small Molecule Samples

Maximilian Karl[1], Peter Emil Larsen[2], Jukka Rantanen[1], Anja Boisen[2], Thomas Rades[1]
[1]University of Copenhagen, Department of Pharmacy, Universitetsparken 2, 2100 Copenhagen, Denmark
[2]Technical University of Denmark, Department of Micro- & Nanotechnology, Ørsteds Pl., 2800 Lyngby, Denmark

Introduction

Thermal analysis is an important field in instrumental chemical analysis and comprises of many different conventional techniques. To complement and enlarge the analytical toolbox for use in material science, pharmaceutical analytics and general physico-chemical characterization, we have developed an instrumental thermal analysis technique based on microstring sensors. After the development process, which was reported earlier[1], we are now presenting the results of the initial measurement setup with a large variety of samples.[2]

Aim

Certain drawbacks are associated with conventional thermal analytical techniques such as a large sample size, long run times or lack of sensitivity. It is the aim of this novel technique to overcome these drawbacks and to create new fundamental insight into the physico-chemical nature of different thermal transitions.

Method

The SiN microstring sensors are cleanroom fabricated by depositing (LPCVD) SiN on a silicon wafer, followed by patterning using standard UV lithography, dry etching and a KOH etch. The sensors are placed on a custom build temperature stage inside a vacuum chamber. The change in frequency is measured with a laser-Doppler vibrometer (MSA-500, Polytec GmbH, Germany).

Results

The measurement setup has successfully been utilized to analyse a variety of samples, including small molecule drugs, polymers and proteins. Where applicable, the recorded thermal transitions where in good agreement with conventional techniques. As an example, the figure shows a typical MTA thermogram recorded from 1 ng of amorphous cimetidine.

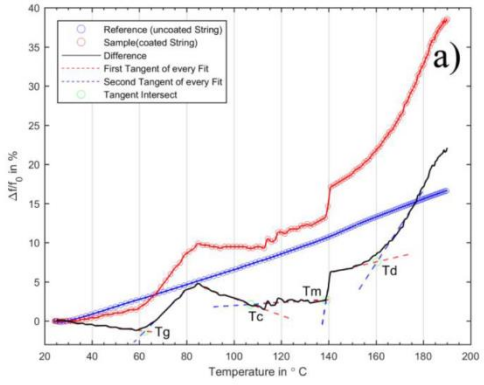


Fig. 1: Measurement example of amorphous cimetidine[2]

Conclusion

The results of this novel method highlight its potential for material characterization across many different branches of chemical sciences. Direct instrumental triplicates and high heating rates furthermore decrease the measurement time per sample and low sample amounts are specifically interesting for pharmaceutical- and chemical trace analysis.

References

1.Karl, M., Larsen, P., Rantanen, J., Boisen, A., Rades, T. *Micromechanical Thermal Analysis for Pharmaceutical Formulations.* Poster Presentation. Northern Pharma Meeting Odense (01/2018)
2.Karl, M., Larsen, P., Rangachara, V., Hwu, E., Rantanen, J., Boisen, A., Rades, T. (2018). *Ultra Sensitive Micro String Resonators for Solid State Thermomechanical Analysis of Small and Large Molecules*. Journal of the American Chemical Society. Just accepted Manuscript

☒ Poster

Probing the Structure of Lipid Bilayer and their Interaction with Antimicrobial Peptides using X-ray and Neutron Scattering Techniques

Josefine Eilsø Nielsen[1], Victoria Ariel Bjørnestad[1], Abdullah Lone[2], Tania Kjellerup Lind[3], Håvard Jenssen[2], Marité Cardenas[3] and Reidar Lund[1]

[1]Department of Chemistry, University of Oslo, Norway. [2]Department of Science and Environment, Roskilde University, Denmark. [3]Department of Biomedical Sciences and Bio Films, Malmö University, Malmö, Sweden.

Introduction

Antibiotic resistance is one of the biggest threats to global health, according to WHO. Antimicrobial peptides (AMPs) seem to be able to evade much of the bacterial resistance mechanisms and are therefore promising candidates for future antibiotics. Instead of blocking specific biochemical pathways as most available antibiotic agents today, most AMPs act physically on the cytoplasmic membrane itself.

Aim

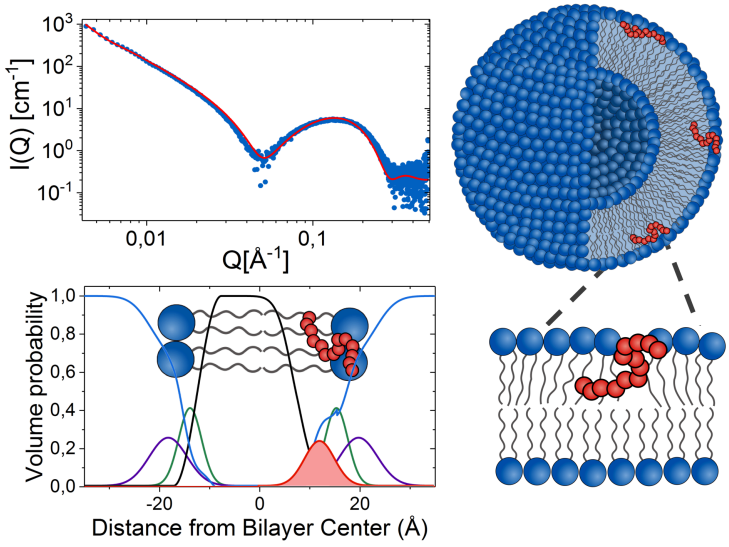
The precise microscopic mechanism for the disturbance of the membrane has not fully been proven but several theories has been suggested including membrane deformation and pore formation. Here we have used state of the art neutron and x-ray scattering techniques to investigate the microscopic mechanism of action of AMPs with model bacterial membranes.

Method

To study the structural interaction, we do static mall angle x-ray scattering (SAXS) together with detailed modelling. Further the peptides effect on the dynamical properties of the membrane is studied using time resolved small angle neutron scattering (TR-SANS).

Results

By using SAXS we have seen how some AMPs insert into the membrane, while others disintegrate the membrane in a detergent like manner. Further, we have studied how insertion of the peptide result in an increase in the dynamics of the phospholipids (5-fold increase) in the membrane.



Conclusion

When combining the results from the static and time resolved scattering methods, we gain novel understanding of the membrane effect of antimicrobial peptides. This knowledge brings us one step closer to understanding the microscopic mechanism of these antimicrobial peptides, which may provide important clues to help develop new antibiotics for the future.

1. *Nielsen J. E.; Bjørnestad V. A.; Lund R. 2018. Soft Matter, 14, 8750-8763*
2. *Nielsen J. E.; Lind T. K.; Lone, A.; Gerelli, Y.; Hansen, P. R.; Jenssen, H.; Cárdenas, M.; Lund, R. 2018. (submitted).*

Immobilization of membrane-bound enzymes on microreactors via fusogenic liposomes: An enabling tool for drug research

Iiro Kiiski[1*], Tea Pihlaja[1], Sanna Artes[1], Lauri Urvas[1], Ville Jokinen[2], Tiina Sikanen[1]
[1]Drug Research Program, Faculty of Pharmacy, University of Helsinki, Finland
[2]Aalto University, School of Chemical Engineering, Finland

Introduction

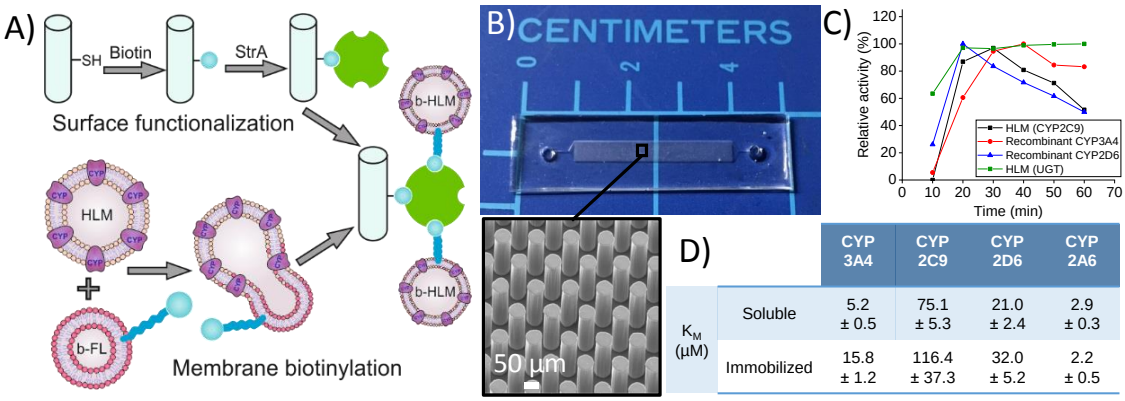
Immobilization of membrane-bound drug metabolizing cytochrome P450 (CYP) and UDP-glucuronosyltransferase (UGT) enzymes has puzzled researchers for decades. Enzyme immobilization on solid support structures would facilitate the construction of versatile through-flow immobilized enzyme microreactors for the purposes of, e.g., time-dependent inhibition assays or synthesis of metabolite standards. However, most conventional immobilization approaches result in diffusion-limited mass transfer (e.g. entrapment) or poor enzyme stability (e.g. covalent bonding via amino acid residues), which impair the enzyme affinity and/or stability and result in flawed enzyme kinetic determinations.

Aim

This work examines the possibility to use fusogenic liposomes for immobilization of membrane-bound proteins, such as the drug metabolizing CYP and UGT enzymes, on custom-designed microfluidic reactors.

Method

Human liver microsomes (HLM), containing all the major drug-metabolizing enzymes, were biotinylated with help of fusogenic liposomes to facilitate immobilization of HLMs onto a streptavidin-functionalized micropillar array (Figs. 1A & 1B) [1]. The micropillar arrays were fabricated in house by using off-stoichiometric UV-curable thiol-ene polymers, featuring ca. 133±13/nm^2 free surface thiols that facilitated further functionalization reactions (Figure 1A).



		CYP 3A4	CYP 2C9	CYP 2D6	CYP 2A6
K_M (µM)	Soluble	5.2 ± 0.5	75.1 ± 5.3	21.0 ± 2.4	2.9 ± 0.3
	Immobilized	15.8 ± 1.2	116.4 ± 37.3	32.0 ± 5.2	2.2 ± 0.5

Fig 1. A) Protocol for immobilizing lipid membranes (here, human liver microsomes (HLM)) on streptavidin-functionalized surfaces using fusogenic liposomes (FL). B) Photograph of the microreactor chip with a scanning electron microscopy (SEM) detail of the micropillar array. C) Relative activity of immobilized HLM and recombinant CYP enzymes over time. Activity determined by collecting 50 µL aliquots at a flow rate of 5 µL/min. D) Comparison of the isoenzyme-specific K_M values determined for immobilized and soluble CYP enzymes.

Results

Drug-metabolizing activity of the micropillar arrays was demonstrated with both CYP (Phase I metabolism) and UGT (Phase II metabolism) enzymes. Successful immobilization of recombinant isoforms was also demonstrated (Fig. 1C). The Michaelis constants (K_M) determined for the immobilized enzymes were in good agreement with those of soluble enzymes (Figure 1D), which suggests that our immobilization method ensures the preservation of the natural state of the membrane-bound enzymes.

Conclusion

An immobilized enzyme microreactor comprising all major drug metabolizing CYP and UGT enzymes was developed and qualified for drug metabolism research under flow conditions.

References

1. Kiiski, I. *et al.* Adv. Biosys. (2018) https://doi.org/10.1002/adbi.201800245

Ex vivo intestinal perfusion model to determine mucoadhesion of microcontainers

Mette D. Mosgaard[1], Sophie Strindberg[2], Lukas Vaut[1], Zarmeena Abid[1], Line Hagner Nielsen[1], Alina J. Andersen[1], Anette Müllertz[2] and Anja Boisen[1]

[1]The Danish National Research Foundation and Villum Foundation's Center for Intelligent Drug Delivery and Sensing Using Microcontainers and Nanomechanics (IDUN), Department of Micro- and Nanotechnology, Technical University of Denmark, Kgs. Lyngby, Denmark

[2]Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark

Aim

The purpose of this study was to develop a model for evaluating mucoadhesion of microcontainers (MC) and their orientation at the mucosal surface in the small intestine. MCs with different heights, shapes and materials were investigated to explore how these variations affected the mucoadhesion and orientation of the MCs.

Methods

Cylindrical MCs fabricated from SU-8 with a height of 100 µm were compared to: (i) cylindrical SU-8 MCs of 300 µm height, (ii) triangular shaped SU-8 MCs of 100 µm height and (iii) 100 µm high cylindrical MCs fabricated from polycaprolactone (PCL) (see images of the MCs in Fig. 1A-C). (1) All MCs were investigated in the *ex vivo* intestinal perfusion model. In the model, an intact piece of porcine small intestine was fastened to a tissue holder and placed in a chamber with controlled humidity (60.9 ± 11.5 %) and temperature (37.2 ± 0.9°C). After 15 min of washing with Fasted Simulated Small Intestinal Fluid (FaSSIF), a gelatin capsule loaded with MCs was placed at the beginning of the intestine and was allowed to dissolve. After dissolving the capsule, a flow of FaSSIF (1.55 mL/min) was initiated and continued for 30 min. After the 30 min, the tissue was opened and cut into pieces followed by microscopic examination under bright field and fluorescence conditions. The amount of MCs and their orientation were examined and counted on each piece of intestine.

Results

The distribution of MCs in the intestine provides an estimate of how mucoadhesive the different MCs were compared to each other. Figure 1 shows the MC distribution throughout the intestine as well as the amount of MCs that exited the intestine. The graphs indicate that the cylindrical SU-8 MCs are more mucoadhesive compared to the triangular MC and cylindrical PCL MCs. The triangular MC seems more likely to move down to the middle of the intestine and many of the PCL MCs exit from the intestine compared to the cylindrical SU-8 MCs. The height of the MCs seems to affect the mucoadhesion less than the shape and material. The orientation of the MCs in the mucus layer was as expected mainly controlled by the height of the MCs. The high MCs were primarily found lying sideways and the low MCs were mostly found with the bottom up or down.

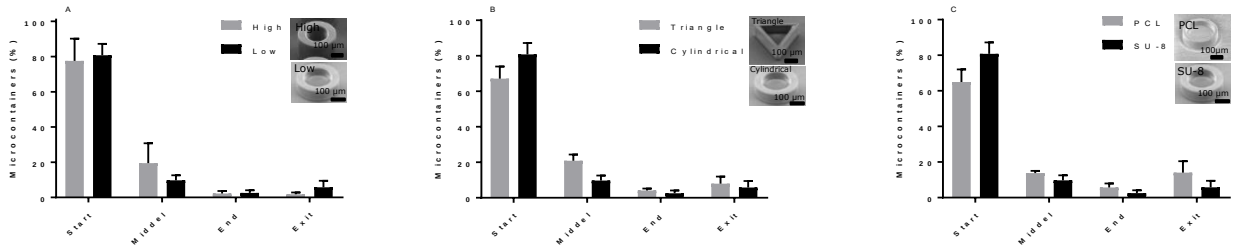


Figure 1: The graphs show the amount of microcontainers in percent located in the start, middle, end and exit of the porcine small intestine. A) Comparison between 300 µm (high) or 100 µm (low) high microcontainers. B) Comparison between triangular and cylindrical microcontainers. C) Comparison between microcontainers fabricated from SU-8 and polycaprolactone (PCL). Scanning electron microscope (SEM) images of all the different microcontainer types. Data is represented as mean±standard error of mean. n=4.

Conclusion

The developed model is very efficient when evaluating mucoadhesion and orientation of MCs. Shape and material of the MCs seem to have the biggest effect on the mucoadhesion of these microdevices.

References

1. Nielsen LH et al. Spatial confinement can lead to increased stability of amorphous indomethacin. Eur J Pharm Biopharm. 2012;81:418–25.

Acamprosate is an inhibitor of the renal organic anion transporter 1

Irina E. Antonescu[1*], Maria Karlgren[2], Maria L. Pedersen[1], Ivailo Simoff[3], Christel A.S. Bergström[2], Sibylle Neuhoff[5], Per Artursson[2], Bente Steffansen[4], Carsten Uhd Nielsen[1]

[1] Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Odense, Denmark
[2] Department of Pharmacy, Uppsala University, Uppsala, Sweden; [3] Uppsala University Drug Optimization and Pharmaceutical Profiling Platform (UDOPP), Department of Pharmacy, Uppsala University, Uppsala, Sweden, [4] LEO Pharma A/S, Ballerup, Denmark, [5] Certara UK, Simcyp Division, Sheffield, UK

Introduction

Acamprosate is a BCS class III, non-metabolized, anionic drug substance for which the excretion mechanism is not fully understood. Intravenous administration in rats of acamprosate together with probenecid, a known substrate and inhibitor of the organic anion transporter (OAT) 1 (SLC22A6) (1), decreased the renal clearance (Cl_R) of acamprosate in a dose-dependent manner (2). The hypothesis of the present study was that renal OATs expressed in the basolateral membrane of the proximal tubule epithelium (3) could be the carrier contributing to renal excretion of acamprosate and the reason for potential acamprosate-drug (*e.g.* probenecid) interactions.

Aim

To investigate if acamprosate interacts with OAT1 by measuring the concentration-dependent effect of acamprosate on the uptake of [^{14}C]-*p*-aminohippuric acid ([^{14}C]-PAH), a well-established substrate of OAT1 (4).

Method

The apical uptake of 0.5 µCi/mL [^{14}C]-PAH was measured for 5 minutes at 37°C and 100 rpm in the presence of 0 – 33 000 µM acamprosate in human embryonic kidney (HEK)293 cells transiently expressing OAT1 (Corning TransportoCells OAT1), or in a HEK293-Flp-in cell line stably expressing OAT1 (developed in-house). The uptake was in both series measured in parallel in mock-transfected HEK293 cells grown under similar cell culture conditions.

Results

A significant, time-dependent and saturable increase of [^{14}C]-PAH apical uptake was observed in the OAT1-transfected HEK293 cells compared to mock-transfected cells. The apical uptake of [^{14}C]-PAH in OAT1-transfected cells was decreased in the presence of acamprosate in a concentration-dependent manner, while no effect of acamprosate was observed in the mock-transfected cells. The mean (+/- SD) inhibition constant for [^{14}C]-PAH uptake (IC_{50}) was 1 042 ± 134 and 902 ± 20 µM in the transiently (n=3) and stably transfected (N=3, n=2) HEK293-OAT1 cells, respectively.

Conclusion

Acamprosate inhibits OAT1 *in vitro* at concentrations close to the maximum unbound plasma concentration of acamprosate, c_{max} = 154-768 µM (5), obtained after intravenous administration [333-2130 mg].

References

1.Nigam SK, Bush KT, Martovetsky G, Ahn SY, Liu HC, Richard E, et al. The organic anion transporter (OAT) family: a systems biology perspective. Physiol Rev. 2015;95(1):83-123.
2.Zornoza T, Guerri C, Polache A, Granero L. Disposition of acamprosate in the rat: influence of probenecid. Biopharm Drug Dispos. 2002;23(7):283-91.
3.Wright SH, Dantzler WH. Molecular and cellular physiology of renal organic cation and anion transport. Physiol Rev. 2004;84(3):987-1049.
4.FDA. In Vitro Metabolism and Transporter-Mediated Drug-Drug Interaction Studies - Guidance for Industry. In: CDER, editor. Silver Spring, MD: U.S. Department of Health and Human Services; 2017. p. 1-37.
5.Approval package for application number 21-431: Campral clinical pharmacology and biopharmaceutics review: Center for drug evaluation and research; 2004 [cited 2018 08 13]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/21-431_Campral_BioPharmr.pdf.

Dynamic dissolution/permeability testing of co-amorphous glibenclamide mixtures using a PAMPA-membrane

Marika Ruponen[1], Maiju Visti[1], Riikka Laitinen[1]
[1]School of Pharmacy, University of Eastern Finland, Kuopio, Finland

Introduction

Co-amorphous systems are an attractive alternative for enhancing the dissolution properties of poorly-soluble drugs and stabilizing them in the amorphous form[1]. However, the interplay between the drug dissolution rate, drug supersaturation and different co-formers on membrane permeability of the drug for co-amorphous formulations remains unexplored.

Aim

The aim was to simultaneously test dissolution and passive membrane permeability of the coamorphous combinations (1:1 molar ratio) of a poorly soluble drug glibenclamide (GBC) in combination with either serine (SER) or arginine (ARG). In addition, sodium lauryl sulfate (SLS) was included in the co-amorphous mixtures at two concentration levels (high (h) and low (l).

Method

The tests were conducted in phosphate buffer (pH 7.2) in side-by-side diffusion chambers, separated by a PAMPA (parallel artificial membrane permeability assay) membrane.

Results

Co-amorphous GBC mixtures with ARG and SLS had superior dissolution which was attributable to the strong intermolecular interactions formed between GBC and ARG. These formulations also had optimal permeability properties due to their high concentration gradient promoting permeation and possible permeation enhancing effect of the co-formers ARG and SLS.

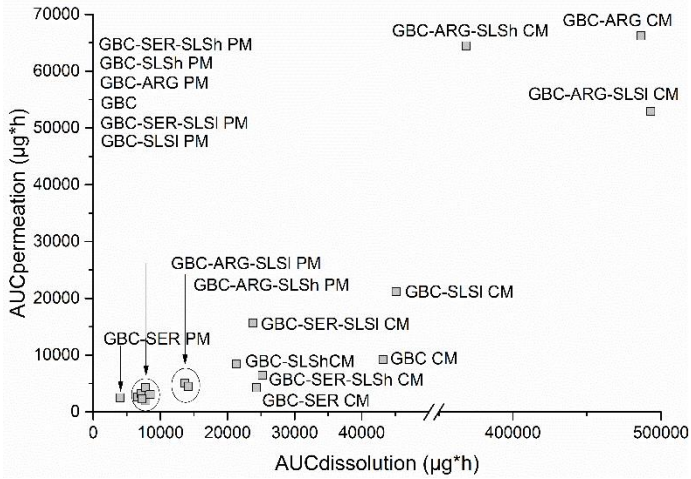


Fig. 1. *Area under curve (AUC, mean) values for permeation as a function of mean AUC-values for the dissolution for the crystalline and amorphous formulations. Note the axis break on the x-axis.*[2]

Conclusion

Simultaneous testing of dissolution and permeation through a PAMPA membrane may represent a simple and inexpensive tool for screening the most promising amorphous formulations in further studies.

References

1. Laitinen R et al. Int J Pharm (2017) 532: 1-12
2. Ruponen M et al. Eur J Pharm Biopharm (2018) 129: 247–256

A Dissolution-Digestion-Permeation Assay to Investigate the Performance of Advanced Drug Delivery Systems

Caroline Alvebratt[1*], Janneke Keemink[1], Khadijah Edueng[1], Ocean Cheung[2], Maria Strömme[2], Christel A.S. Bergström[1]
[1]*Department of Pharmacy, Uppsala University, Sweden*
[2]*Department of Engineering Sciences, Uppsala University, Sweden*

Introduction
A vast majority of the drugs under development suffers from poor aqueous solubility, resulting in low intestinal absorption. Many advanced drug delivery systems (ADDS) have been developed to circumvent this limitation. In these systems, the drug is presented in the intestine in a high-energy form as compared to the stable crystalline state, resulting in a higher apparent solubility and possibly supersaturated intestinal fluids. Commonly investigated ADDS are amorphous solid dispersions (ASD), carrier-based delivery systems and lipid-based formulations (LBFs) [1]. Although the benefit of various ADDS is compound dependent, experimental methods comparing the release and absorption of drugs from these ADDS head-to-head are limited.

Aim
To develop a real-time method that enables for studies of dissolution, digestion and absorption from various types of ADDSs.

Method
The µDiss Profiler™ with the µFLUX™ system was used for the dissolution-digestion-permeation studies. Caco-2 cell monolayers or artificial lipid membranes (GIT-0) were chosen as absorptive membranes and lucifer yellow was used to evaluate membrane integrity. Felodipine was selected as the model compound, and three different types of ADDS were studied; LBFs, an ASD and a drug-loaded mesoporous carrier. To protect the *in situ* UV-probes in the donor chamber from particle aggregation, a nylon filter was placed on the probes[2]. In addition, samples were taken from the donor compartment and analyzed using HPLC-UV. To allow for the digestion of the LBFs in the presence of Caco-2 cells, an immobilized lipase (Novozyme™) and a modified lipolysis buffer were used [3, 4].

Results
Different exposures of felodipine in the aqueous phase of the donor compartment were obtained during dissolution-digestion-permeation experiments (LBFs>Drug-loaded carrier>ASD). The permeation did not directly correlate to the exposure, and the highest permeation was determined for the ASD, followed by the LBFs and the drug-loaded carrier. This display the need for models that combine dissolution and permeation, as factors such as solubilization will influence the amount of drug available for absorption.

Conclusion
The method allows for studies of dissolution, digestion and absorption of felodipine from several types of ADDS, and is thereby a valuable addition to the currently available dissolution-permeation models.

References
1. Brouwers J. *et al.* J Pharm Sci (2009) 98: 2549-2572.
2. Alvebratt C. *et al*. AAPS PharmSciTech (2018): 1-7.
3. Keemink J. and Bergström C.A.S. Pharm Res (2018) 35: 74
4. Mosgaard M. *et al.* Eur J Pharm Biopharm (2015) 94: 493-500.

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The micellisation mechanism of Soluplus®: effect of temperature, concentration and disperse media

Julia Alopaeus, Ingunn Tho

School of Pharmacy, University of Oslo, Norway

Introduction

Soluplus® (SP) is an amphiphilic block co-polymer made up of polyvinyl caprolactam, polyvinyl acetate and polyethylene glycol, and is of interest in drug formulation, both as a solubiliser for poorly soluble drugs and for its capacity to form amorphous solid dispersions, e.g. in the shape of an oral film. In this study the self-assembly and micellisation mechanism of SP was studied with and without the effect of a poorly soluble drug, furosemide, through calorimetric analysis and the morphology of the formed micelles was inspected through transmission electron microscopy (TEM).

Methods

SP was dispersed in aqueous solutions (Milli-Q water, PBS pH 7.4 and HCl 0.1 M) in desired concentrations (0-20 % w/w). The size and morphology of SP micelles was examined by TEM with negative staining using uranyl acetate. The Microcal PEAQ-ITC System (Malvern Instrument Ltd., Malvern, UK) was used to determine CMC and ΔH for SP in water at 25 °C and 37 °C as well as in 25 °C with added furosemide to the receiving cell. The Gibbs free energy (ΔGmic) and the entropy of micellisation (ΔSmic) were further calculated from ΔHmic and CMC by the equation for free energy of micellisation. The change in heat capacity (ΔCp) associated with the sol-gel transition of the polymeric micelle solutions was studied using the Nano DSC Model 602000 (TA Instruments, Lindon, Utah, USA) for solutions.

Results and conclusions

The CMC of SP decreased with increasing temperature from 25 °C to body temperature as well as with addition of furosemide, as determined by ITC. Furthermore, the thermodynamic properties of the furosemide-Soluplus® micelles were influenced by temperature, pH and composition of the test medium showing increasing lower critical solution temperature from around 33 °C in PBS pH 7.4 to 36 °C in Milli-Q water and 38 °C in 0.1 M HCl.

References

Bouchemal, K.; Agnely, F.; Koffi, A.; Ponchel, G. A concise analysis of the effect of temperature and propanediol-1, 2 on Pluronic F127 micellization using isothermal titration microcalorimetry. Journal of colloid and interface science 2009, 338, 169-176

Taylor, M.; Tomlins, P.; Sahota, T. Thermoresponsive gels. Gels 2017, 3, 4.

Biopharmaceutical assessment of amorphous solid dispersions of the poorly soluble drug ABT-102 using PermeaLoop®

Alexandra P. Antczak[1], Daniel Sironi[1], Jörg Rosenberg[2], Martin Brandl[1]
[1]Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Odense Denmark
[2]Abbvie Germany GmbH & Co. KG, Ludwigshafen Germany

Introduction
PermeaLoop® is a novel small-scale dissolution/permeation set-up with a comparably large permeation area, deemed suitable for biopharmaceutical performance testing of advanced oral drug formulations such as amorphous solid dispersions (ASDs) [1].

Aim
So far, only a limited amount of data was collected with PermeaLoop®. The objective of the current study was therefore to explore the applicability of PermeaLoop® to various ASD formulations of the poorly soluble drug ABT-102. To this end, the protocol was refined by adding a pH shift in the donor compartment. The *in vitro* results were compared with *in vivo* bioavailability data from a dog study.

Method
Four different ASDs were prepared by hot-melt extrusion, and gelatin capsules containing the milled ASDs were tested in PermeaLoop® using two different dissolution protocols: one mimicking the fasted state (change from FaSSGF to FaSSIF-$V2_{mod}$) and one the fed state (FeSSGF + FeSSIF).

Results
In the fasted state protocol, precipitation of the weakly basic drug in the donor compartment was observed following the pH shift, and different plateau concentrations of apparently dissolved drug were obtained. The permeation rate was more or less constant over six hours for all formulations. Despite the different concentrations in the donor, no significant differences were observed neither for the steady state flux nor for the total amount permeated. Similarly, permeation in the fed state protocol occurred under steady state conditions with no difference between the formulations being detectable. In the fasted state the amount of apparently dissolved drug correlated fairly with the respective bioavailability. In contrast, no reasonable correlation between the permeated amount and the *in vivo* data was seen under the chosen experimental conditions.

Conclusion
The current study demonstrated that different dissolution protocols can be implemented in PermeaLoop®. Despite the high permeation area, no dynamic scenario was observed, i.e. the amount of drug dissolved in the donor compartment did not substantially decrease over the course of the experiment. A change in experimental parameters is suggested, such as using a different reference volume for scaling down the dose), to better reflect the dynamic nature of the *in vivo* process.

References
[1] Sironi, D., Rosenberg, J., Bauer-Brandl, A., Brandl, M. PermeaLoop™ a novel in vitro tool for small-scale drug-dissolution/permeation studies. (2018) J Pharm Biomed Anal, 156, pp. 247-251.

Development of alginate coated liposomes with potential of being used in treatment of dry mouth

Joseph Azumah[a*], Gro Smistad[a], Ørjan G. Martinsen[b], Marianne Hiorth[a].
[a] Department of Pharmacy, School of Pharmacy, University of Oslo, P.O. Box 1068 Blindern, 0316 Oslo, Norway
[b] Department of Physics, University of Oslo, P.O. Box 1048 Blindern, 0316 Oslo, Norway

Introduction
Saliva is vital for the health of the oral cavity (1), but many people suffer from dry mouth, which may be due to sicknesses, chemotherapy or medications side effects (2).There is a need to develop newer and better formulations that mimic the functions of saliva and have longer duration of action. Combinations of liposomes and polymer complexes as potential drug carriers, offer such an opportunity, since rehydrating agents can be incorporated. Also, liposomes coated with mucoadhesive polymers will give them the added advantage of longer residence time on the mucous membrane. The liposomes themselves may also mimic the glycoproteins of saliva.

Aim
The aim of the study was to evaluate whether alginate coated liposomes prepared by the one-pot method would be equally stable as those prepared by the conventional thin-film method. Furthermore, it was aimed to investigate whether dynamic vapor sorption (DVS) technique could be used to determine the time constants of both the polymers and the liposomes and consequently, their water vapor sorption capacities

Method
The alginate coated liposomes were prepared by either the conventional thin-film method or the one-pot method and extruded through 200nm polycarbonate membrane pore to reduce their sizes. The liposomes were characterized with respect to the size, zeta potential and water sorption capacities. The stability of the liposomes was tested over several weeks during storage in refrigerator. In order to visualize the liposomes, transmission electron microscopy (TEM) and Atomic force microscopy (AFM) pictures were taken.

Results
Size and zeta potential measurements showed that liposomes prepared by both methods were still stable after 11 weeks of storage. Liposomes from both preparation methods were successfully visualized with the TEM and AFM techniques. Results from the DVS analysis showed that it is possible to use time constants as a tool to compare water sorption capacities of different polymers or liposomes.

Conclusions
The one-pot method can be an alternative to the thin-film method to prepare alginate coated liposomes for delivery in the oral cavity intended to treat dry mouth.

Reference
1. Brosky MEJJSO. The role of saliva in oral health: strategies for prevention and management of xerostomia. 2007;5(5):215-25.
2. Guggenheimer J, Moore PA. Xerostomia: etiology, recognition and treatment. J Am Dent Assoc. 2003;134(1):61-9; quiz 118-9.

Studying the impact of polymeric excipients on the surface mobility of amorphous systems

J. Bannow[1], M. Karl[1], ET Hwu[2], P. E. Larsen[2], K. Löbmann[1], A. Boisen[2], T. Rades[1]

[1]University of Copenhagen, Department of Pharmacy, Universitetsparken 2, DK-2100, Copenhagen, Denmark
[2]Technical University of Denmark, Department of Micro- and Nanotechnology, Ørsteds Plads, 2800, Kgs. Lyngby, Denmark

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 surface mobility. A highly mobile surface layer has been shown to be directly responsible for fast crystal growth on free surfaces and to govern the overall recrystallization kinetics of molecular glasses.[1]
In this study, the influence of polymeric excipients on the surface mobility of amorphous solid dispersions (ASD) with low polymer content is investigated.

METHODS

The surface mobility of neat amorphous indomethacin (IMC, prepared by quench cooling) and an ASD of IMC in soluplus® was quantified by studying the flattening of a corrugated surface over time using a method originally developed by Mullins et al. (1959).[2] The ASD was obtained from a physical mixture of IMC and 5 wt. % soluplus®, which was extruded for 5 min at 170 ˚C in a twin-screw extruder (DSM Xplore® Micro Compounder).
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 + 30 ˚C). Atomic force microscopy was carried out using a Strømlingo™ advanced AFM kit to determine the height change of the embedded grating over time.

RESULTS

The decrease in height decrease of the sinusoidal grating embedded on the surface of amorphous IMC and an IMC ASD was measured using an AFM operated in tapping mode. Over an observation time of 5.6 hours, the grating height of the neat amorphous IMC decreased due to surface molecular mobility[3] losing 94 % of its initial height in this time period, whereas the soluplus® spiked sample retained 60 % of the initial height (Fig. 1). The obtained decay kinetics resulted in a decay constant log *K* of - 3.70 s^{-1} for the neat amorphous drug, compared to - 5.01 s^{-1} for the soluplus® containing sample. The observed difference in decay kinetics demonstrates the ability of already comparatively low amounts of a polymeric excipient (5 wt. %) to slow down molecular mobility on amorphous surfaces.

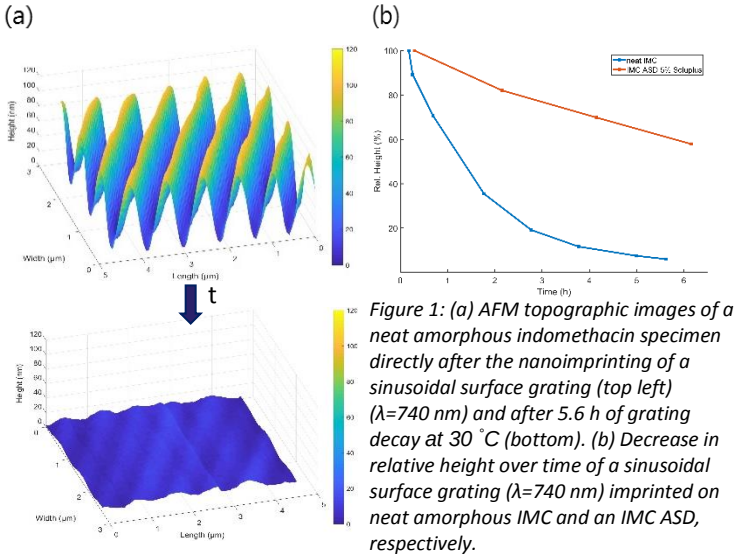


Figure 1: (a) AFM topographic images of a neat amorphous indomethacin specimen directly after the nanoimprinting of a sinusoidal surface grating (top left) (λ=740 nm) and after 5.6 h of grating decay at 30 ˚C (bottom). (b) Decrease in relative height over time of a sinusoidal surface grating (λ=740 nm) imprinted on neat amorphous IMC and an IMC ASD, respectively.

CONCLUSION

The described nanoimprinting of surface gratings and analysis using AFM enables fast and reproducible surface mobility measurements and was able to demonstrate the impact of low amounts of a polymeric excipient on the surface mobility of an IMC ASD.

REFERENCES

1. Yu, L., *Surface mobility of molecular glasses and its importance in physical stability.* Adv. Drug Deliv. Rev., 2016.
2. W.W. Mullins, J. Appl. Phys. 30, 77, 1959.
3. Brian, C.W. and L. Yu, *Surface self-diffusion of organic glasses.* J Phys Chem A, 2013.

Phloretin decreased lipopolysaccharide and antimycin A-induced cytokine release

Niina Bhattarai[1], Maria Hytti[1], Johanna Ruuth[2], Anu Kauppinen[1
[1]School of Pharmacy, University of Eastern Finland, Kuopio, Finland
[2]Department of Ophthalmology, University of Eastern Finland, Kuopio, Finland

Introduction

Age-related macular degeneration (AMD) is an eye-related disorder where primary abnormalities occur in retinal pigment epithelial (RPE) cells. Inflammation is linked to the pathogenesis of AMD. Phloretin is an anti-oxidative and anti-inflammatory compound present in the bark, leaves, and fruits of apple trees. It has inhibitory properties towards glucose transporters (GLUTs). It has been showed on mouse macrophages that phloretin attenuated the activity of transcription factor NF-κB and decreased the phosphorylation of MAPKs, while its structural analog, phlorizin did not alleviate inflammation. Phlorizin is almost structurally identical to phloretin, only one hydroxyl group of phloretin is changed to o-linked glucose in phlorizin.

Aim

To investigate the effects of phloretin on lipopolysaccharide (LPS) and antimycin A (Aa) -induced inflammation in human ARPE-19 cells.

Methods

Experiments were performed using spontaneously immortalized human retinal pigment epithelium cells (ARPE-19 cell line). Cells were pre-treated with phloretin for 1 h and exposed to LPS or Aa for additional 24 h. Cell viability was determined using the methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay. The concentrations of pro-inflammatory IL-6 and IL-8 cytokines were measured using the enzyme-linked immunosorbent assay (ELISA) technique.

Results

Phloretin was well-tolerated by ARPE-19 cells. LPS neither compromised the cell viability, and phloretin did not change the situation on LPS-treated cells, either. Aa diminished cell viability but phloretin did not reduce it further during Aa treatment. Instead, phloretin reduced the LPS and Aa-induced releases of IL-6 and IL-8.

Conclusion

Phloretin was not toxic to ARPE-19 cells and it reduced inflammation in two different stress conditions. Chronic inflammation is included in the pathogenesis of many age-related diseases, such as AMD, and its regulation would be highly beneficial.

Montmorillonite-surfactant hybrid particles for modulation of intestinal P-glycoprotein mediated transport

Rasmus Blaaholm Nielsen[1], Ariane Kahnt[2], Lieve Dillen[2], Koen Wuyts[2], Jan Snoeys[2], Ulla Gro Nielsen[1], René Holm[2], Carsten Uhd Nielsen[1]

[1]Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Odense, Denmark

[2]Janssen R&D, Johnson & Johnson, Beerse, Belgium

Introduction

P-glycoprotein (P-gp) plays an important role in the absorption of many drugs, as it effluxes substrates, which leads to lowered absorption of the substrate. This poses a challenge in the development of new drugs, as many lead compounds are avoided in drug discovery or even stopped during drug development, because they are P-gp substrates. P-gp is the most extensively studied efflux transporter and there is a vast interest to develop P-gp inhibitors. Three generations of small molecule P-gp inhibitors have been developed, but none have reached the market as viable P-gp inhibitors. Concurrently, pharmaceutical excipients, especially non-ionic surfactants, have been shown to inhibit P-gp *in vitro* and *in vivo*. However, large doses are required to observe P-gp inhibition *in vivo*, and optimisation is needed. Montmorillonite (MMT) is a layered clay material with a high surface area and distinct adsorptive properties, which makes it interesting in the formulation of nanomaterials for drug delivery. Montmorillonite-surfactant hybrid (MSH) particles containing the P-gp probe, digoxin, have been developed to enhance the inhibition properties of the non-ionic surfactant, polysorbate 20 (PS20). It is hypothesised that the association of PS20 and digoxin to the distinct adsorptive and mucoadhesive MMT layer surface will cause simultaneous release of digoxin and PS20. This will lead to intestinal mucosa-localised elevated concentrations of both PS20 and digoxin *in vivo*, which results in more effective inhibition of P-gp and increased absorption of digoxin.

Aim

To characterise MSH particles and investigate if MSH particles can increase digoxin exposure after oral administration.

Method

MSH particles were prepared by lyophilisation of suspensions of MMT and PS20 in a 1:1 ratio with low levels of digoxin (0.02-0.2 % w/w) to yield light, porous powders. MSH particles were characterised by scanning electron microscopy, thermogravimetric analysis, differential scanning calorimetry, X-ray powder diffraction, and low angle laser light scattering. To evaluate the ability of MSH particles to increase digoxin exposure *in vivo*, they were orally administered to Sprague Dawley rats.

Results

PS20 induces extrusion of water from MMT interlayer spaces, stabilisation of MMT aggregates in suspensions, and increasing clay interlayer distance as a result of PS20 intercalation. This confirms the strong interaction between PS20 and MMT in the solid and suspended MSH particle systems. Furthermore, MSH particles tended to increase digoxin exposure *in vivo*. Administration of 0.2 mg kg^{-1} digoxin and 0.5 g kg^{-1} PS20 as MSH particles compared to a simple solution led to a non-significant increase in $AUC_{0\text{-}6h}$ and C_{max} of 31 and 33 %, respectively. Furthermore, control digoxin-treated montmorillonite with no PS20 decreased $AUC_{0\text{-}6h}$ and C_{max} by 54 and 52 %, respectively, compared to digoxin administered alone, which suggests that MMT alone retains digoxin from absorption.

Conclusion

MSH particles tended to enhance the P-gp inhibition properties of PS20, which led to slightly increased digoxin exposure. There is a potential for MSH particles for oral drug delivery of P-gp substrate drug substances.

In Vitro In Vivo Correlation For Modified Release Subcutaneously Administered Insulins

Frederik Bock[a], Eva Lin[a,b], Claus Larsen[a], Henrik Jensen[a], Kasper Huus[b], Susan Weng Larsen[a], & Jesper Østergaard[a]
[a]Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark.
[b]Injectable Formulation Research, Novo Nordisk A/S, Novo Nordisk Park, DK-2760, Måløv, Denmark.

Introduction
The subcutaneous tissue resemble a gel-like matrix rather than bulk fluid and the local environment may significantly affect the performance of the formulation injected [1]. *In vitro* release models taking into account the structure and environment *in vivo may* have a better chance of predicting the *in vivo* behavior of subcutaneously administered injectables. In order take advantage of biopredictive *in vitro* release testing methods, the establishment of *in vitro in vivo* correlations (IVIVCs) should be pursued**.**

Aim
To explore the possibility of establishing an IVIVC utilizing a novel flow-through based *in vitro* release testing method for subcutaneously administered insulin formulations.

Method
The *in vitro* release setup consisted of a column-type continuous flow model containing a gel matrix (Sephadex) into which the insulin formulations were injected. At the outlet, medium was collected and analyzed by RP-HPLC. The release of insulin from Actrapid® (soluble human insulin), Insulatard® (isophane insulin) and Lantus® (insulin glargine) were compared and used in the pursuit of establishing the IVIVC utilizing *in vivo* data in man taken from the literature [2].

Result
The *in vitro* release method provided the correct rank ordering with respect to insulin release/disappearance. Non-linear time scaling (Fig. 1a) was used to establish an IVIVC for the modified release formulation (Fig. 1b). Actrapid® could not be incorporated into the correlation properly due to the *in vitro* release method missing a diffusion barrier, which is present *in vivo*.

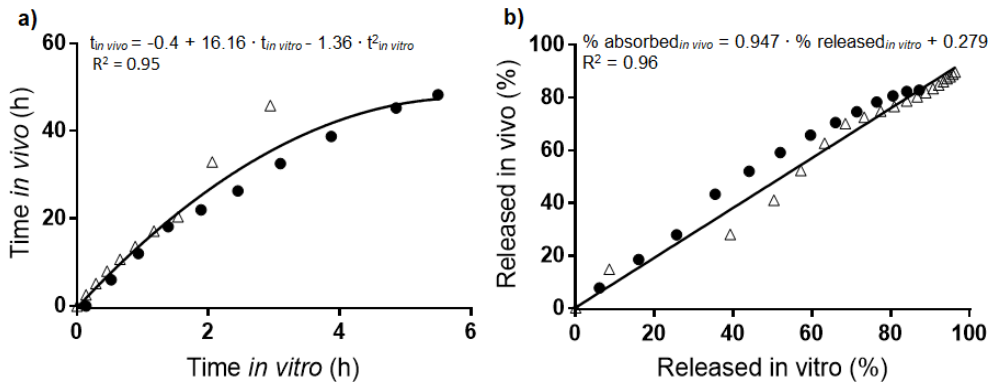


Fig. 1. a) Non-linear time scaling. b) Correlation between *in vitro* release and *in vivo* absorbed. Isophane insulin (Δ) and insulin glargine (●). The solid lines represent the fits attained.

Conclusion
The novel flow-through based method provided the same rank ordering on *in vitro* release and *in vivo* absorption for the three insulin products. A correlation between insulin release *in vitro* and insulin absorption *in vivo* was established for the modified release insulin formulations using non linear time scaling.

1. Kinnunen HM, Mrsny RJ. Journal of the Controlled Release (2014) 182: 22-32.
2. Luzio SD et. al. Hormone and metabolic research (2003) 35(7): 434-8

Co-existing colloidal phases of human duodenal aspirates: intra-individual variations and inter-individual differences in relation to molecular composition.

Mette Sloth Bohsen[1*], Philipp A. Elvang[1], Paul C. Stein[1], Annette Bauer-Brandl[1], Danny Riethorst[2], Joachim Brouwers[2], Patrick Augustijns[2] and Martin Brandl[1]

[1]*Drug Transport & Delivery, Dpt. Physics, Chemistry & Pharmacy, Univ. Southern Denmark, Odense DK 5230, Denmark.*

[2]*Drug Delivery and Disposition, Dpt. Pharmaceutical and Pharmacological Sciences, KU Leuven, Leuven 3000, Belgium.*

Aim

We investigated the ultrastructural pattern of colloidal phases in human duodenal fluids. Aspirates were collected from three volunteers in both fasted and fed nutritional states.

Methods

Analysis methods comprised the combination of asymmetric flow field-flow fractionation (AF4) and multi-angle laser light scattering (MALLS). Furthermore, dynamic light scattering (DLS) and diffusion-ordered NMR spectroscopy (DOSY-NMR) were employed as alternative analytical approaches for comparison.

Results

By AF4/MALLS, up to four, and in some cases up to five distinct co-existing fractions could be differentiated in the sub-micron size-range, which, in accordance with a previous study (Elvang et al., 2018), may be assigned to three main types of colloids, namely small bile salt micelles, intermediate size mixed bile salt/phospholipid micelles and large phospholipid aggregates/vesicles. Although more or less the same colloidal phases were found to co-exist in all aspirates, their prevalence was found to vary, both over time and between the three individual human volunteers. However, there could not be identified any uniform changes of patterns of colloidal phases over time. On the other hand, prevalence of specific colloidal phases was identified for aspirates of individual volunteers, which correlated reasonably well with the prevalence of certain lipid species in their molecular composition

Conclusion

The current study demonstrates the usefulness of AF4/MALLS in resolving ultrastructural patterns of complex colloidal media like human intestinal aspirates. Colloidal phase fluctuations could be followed by comparing the fractograms of aspirates sampled at distinct time intervals and they even could be related to molecular composition of the media. One should bear in mind that human intestinal fluids also contain structures outside the colloidal size range, which have been removed here by centrifugation.

References

Elvang, P.A., Jacobsen, A.-C., Bauer-Brandl, A., Stein, P.C., Brandl, M.
Co-existing colloidal phases in artificial intestinal fluids assessed by AF4/MALLS and DLS: A systematic study into cholate & (lyso-) phospholipid blends, incorporating celecoxib as a model drug (2018) European Journal of Pharmaceutical Sciences, 120, pp. 61-72.

HEXOSOMES BASED ON NEW OMEGA-3 MONOGLYCERIDES: NANOCARRIERS FOR DELIVERY OF NUTRACETUCALS AND THERAPUTIC AGENTS

Gizem Bor[1*], Xianrong Shao[1], Sabah Al-Hosayni [1], Heinz Amenitsch[2], Stefan Salentinig[3], Anan Yaghmur[1]

[1]Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen Ø, Denmark
[2]Elettra-Sincrotrone Trieste, Strada Statale 14, 34149 Basovizza, Trieste, Italy
[3]Laboratory for Biointerfaces, Empa, Swiss Federal Laboratories for Materials Science and Technology, Lerchenfeldstrasse 5, 9014 St. Gallen, Switzerland. *e-mail account: gizem.bor@sund.ku.dk

Introduction

The attractiveness of new omega-3 (*ω*-3) polyunsaturated fatty acid (PUFA) monoglycerides (MAGs) lies in the amphiphilic nature and the beneficial health effects as PUFA precursors in various disorders including cancer, pulmonary hypertension, and inflammatory diseases.[1,2] For exploring the potential therapeutic applications of these new amphiphilic lipids, particularly as main lipid constituents in the development of nanocarriers for delivery of drugs and PUFAs, it is of paramount importance to gain insight into their self-assembly behavior on exposure to excess water.

Aim

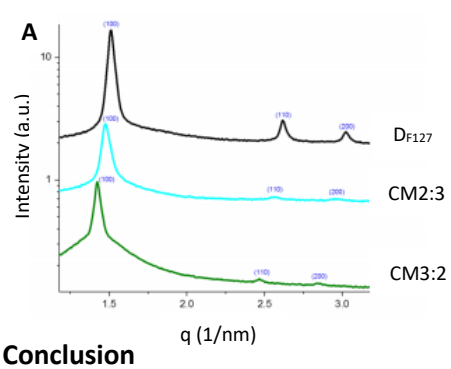
Our present work aim to investigate the influence of temperature, unsaturation degree and fatty acyl chain length on the self-assembled structures in both dispersed and non-dispersed states of MAG-EPA, MAG-DHA, and MAG-DPA by using synchrotron small-angle X-ray scattering (SAXS).

Method

In addition to SAXS, cryogenic transmission electron microscopy (cryo-TEM), nanoparticle tracking analysis (NTA), and zeta potential measurements were performed to gain insight into the structural and morphological features, size characteristics, and surface charge of the produced nanoparticles.

Results

In this study, we showed that MAG-EPA, MAG-DHA, and MAG-DPA tend to form a dominant inverse hexagonal (H_2) phase in excess water at 25 °C and a temperature-triggered structural transition to an inverse micellar solution (L_2 phase) is detected. As compared to monounsaturated monoglycerides with shorter fatty acyl chain lengths such as monoolein, ω -3 PUFA monoglycerides are more wedge-shaped amphiphiles and have, therefore, a propensity to form H_2 phase in excess water at room temperature (Fig 1). In addition, hexosomes stabilized by using the triblock copolymer F127 and the food-grade emulsifier citrem were investigated to gain insights into the effects of the stabilizer and temperature on the internal nanostructure.



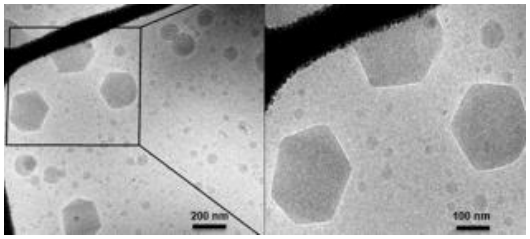


Fig. 1. (A) Structural characterization of three ultrasonicated MAGEPA-based dispersions stabilized by F127 and two dispersions prepared at two different citrem/MAG-EPA (CM) weight ratios: 3:2 and 2:3. **(B)** Cryo-TEM micrographs of the ultrasonicated MAG-EPAbased dispersion prepared at citrem/MAG-EPA (CM) weight ratio of 3:2. Scale bars: 200 nm and 100 nm.

Conclusion

In both dispersed and non-dispersed states, the beneficial health effects of this new family of *ω*-3 PUFA monoglycerides as well as the unique structural properties of their self-assemblies in excess water provide an efficient approach for the design of new platforms for delivering *ω*-3 PUFAs alone or in combination with nutraceuticals or therapeutic agents.

References

1. Yaghmur, A.; Al-Hosayni, S.; Amenitsch, H.; Salentinig, S. Langmuir 2017, 33, 14045-14057.
2. Shao, X.; Bor, G.; Al-Hosayni, S.; Salentinig, S.; Yaghmur, A. Phys. Chem. Chem. Phys. 2018, 20(37), 23928-23941.

Modeling the blood-brain barrier; Human Induced Pluripotent Stem Cells can be differentiated to High Resistance Monolayers with Cld-5 Expression at the Junctional Zones.

Goldeman C[1], Saaby L[1,2], Holst B[2], Hall V[3], Hyttel P[3] and Brodin B[1]

[1]Department of Pharmacy, University of Copenhagen, Denmark
[2]Bioneer A/S, Hørsholm, Denmark
[3]Department of Veterinary and Animal Science, University of Copenhagen, Denmark

The blood-brain barrier (BBB) functions to maintain a general brain homeostasis by regulating the transport of substances between blood and brain. The BBB consists of tightly connected endothelial cells, which restrict the passage of larger molecules and thus poses a challenge for development of drugs for brain diseases. By culturing endothelial cells in monolayers it is possible to investigate drug candidates for e.g. permeability properties across the BBB *in vitro*. A recent approach includes using human induced pluripotent stem cells (hIPSCs) which, contrary to animal *in vitro* models, eliminate species differences. The aim of this study was to generate and characterize *in vitro* barrier models derived from hIPSCs from different sources using a published protocol by Stebbins et al. (2015)[1] and to investigate the influence of co-culture with astrocytes (with or without the presence of retinoic acid (RA)), on tightness and expression of important genes and proteins in the endothelial cells. hIPSCs from different sources (Bioni010-C and WTSli024-A) were seeded and maintained on matrigel and mTesR1 media. When the cells reached 80 % confluence, cells were single cell seeded and after two days the media was changed to undifferentiated media. Endothelial cell media (with bovine platelet-poor plasma derived serum (PDS) and bovine fibroblast growth factor) w/o RA were added after six days followed by seeding on permeable supports, either in mono-, non-contact or contact co-culture (MC, NCC and CCC, respectively) with primary rat astrocytes. Endothelial media (with PDS) were added after 24h and the cells were used for experiments the following day. A positive claudin-5 immunostaining was obtained in mono-culture for Bioni010-C and WTSli024-A, both with and without the presence of RA and a high trans-endothelial electrical resistance (TEER) was obtained in all *in vitro* models upon addition of RA to the media (WTSli024-A: -RA: 22 $\Omega*Cm^2$, +RA: 316 $\Omega*Cm^2$; Bioni010-C: -RA: 220 $\Omega*Cm^2$, +RA: 6626 $\Omega*Cm^2$) . The presence of astrocytes had an inductive effect on TEER in co-culture models without the presence of RA (Bioni010-C: MC: 242 $\Omega*Cm^2$, NCC: 753 $\Omega*Cm^2$, CCC: 961 $\Omega*Cm^2$) and the presence of RA yielded even higher TEER in co-cultures (Bioni010-C: NCC: 7363 $\Omega*Cm^2$, CCC: 8055 $\Omega*Cm^2$). This indicates that the protocols published by the Shusta group are generally applicable for generating electrically tight monolayers expressing Claudin-5 at the junctional zones.

Further studies will elucidate the expression of a range of genes and proteins expresses at the brain endothelium, and the possible effects of astrocyte co-culture.

[1]Stebbins MJ, Wilson HK, Canfield SG, Qian T, Palecek SP, Shusta E V. Differentiation and characterization of human pluripotent stem cell-derived brain microvascular endothelial cells. METHODS. 2015.

Title: Manipulation of aspirin tablets to obtain paediatric doses

Contact data: J Brustugun[1], N Notaker[1 2], L H Paetz[1 2], I Tho[2], K Bjerknes[1].

1. Hospital Pharmacy Enterprises, South Eastern Norway. Oslo, Norway. jorgen.brustugun@sykehusapotekene.no.
2. Department of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Oslo, 0316 Oslo, Norway.

Background: Tablets can be crushed, split and suspended (i.e. manipulated) to obtain a fraction as the correct dose. This is frequently necessary when treating children, a group often lacking suitable drug forms. Manipulation in this manner could, however, be suspected to give rise to inaccurate doses. Does drug form or physicochemical drug characteristics decide dosing accuracy?

Purpose: The purpose of the study was to investigate factors affecting the dose accuracy obtained after manipulating four different tablets, two of which are commonly used in pediatric care, using validated UHPLC-analysis. The test substance was the slightly soluble aspirin (1:300), a substance with a pKa of 3.5.

Material and methods: Aspirin tablets: *Bayer Chewable* (81 mg), Bayer Healthcare LLC, *Dispersible Aspirin* (75 mg), Aspar Pharmaceuticals Ltd, *Disprin* (300 mg), Reckitt Benckiser, and *Aspirin*, (500 mg) Bayer. Instrument: UHPLC-system from Shimadzu Corp (Nexera, with Prominence DAD-detector). Analytical column: ACE Excel 2 µm C18-AR, 2.1 x 100 mm, (Advanced Chromatography Technologies Ltd.) Dosing accuracy study: Six tablets from each of the four formulations were dissolved in 10 mL water. After 3 minutes, samples (1 mL) were withdrawn. Dosing accuracy was recorded and compared between formulations. The pH of each tablet dispersed was recorded.

Results: The pH was 4.6 in dispersed *Dispersible Aspirin* and 5.0 in *Disprin*, both above the pKa-value of aspirin; the pH was 3.0 in *Bayer Chewable*, and 2.8 in *Aspirin*. Dosing experiments: For *Dispersible Aspirin* (75 mg) 98.7% (80.0–117.3%) of intended dose was found upon manipulation, for *Bayer Chewable* (81 mg) 9.3% (6.2–22.2%), for *Disprin* (300 mg) 45.7% (43.3-49.9%), and for *Aspirin* (500 mg) 3.4% (2.5-5.6%).

Conclusion: The dosing accuracy of *Dispersible Aspirin* (75 mg) and *Disprin* (300 mg) was markedly better than that of the other two formulations (*Bayer Chewable* (81 mg) and *Aspirin* 500 mg). Aspirin being more dissociated at the pH in suspended *Disprin* and *Dispersible Aspirin* (pH 5.0 and 4.6, respectively) correlated with favorable dosing accuracy. Other formulation differences could, however, also be important, as differences in dose accuracy between tablets with favorable pH was evident.

Using UV imaging to study flow through drug release from drug-eluting microparticles

Oliver Degerstedt, Fredrik Kullenberg & Hans Lennernäs
Biopharmaceutics, Department of Pharmacy, Uppsala University, Sweden

Introduction

Drug eluting microparticles are used worldwide in hospitals to treat intermediate stage hepatocellular carcinoma (HCC – primary liver cancer) in a procedure called transarterial chemoembolization. The polymer-based microparticles are loaded with the cytostatic drug doxorubicin (DOX) prior to intra-arterial and locoregional injection into patients. This treatment leads to a permanent embolization of the blood vessels feeding the tumour as well extended release of DOX in the liver tumour microenvironment. In recent years, a handful of different *in-vitro* methods have been developed to investigate both the loading and drug release from these formulations.[1-4] However, in many cases these methods are either based on USP2, USP4 or imaging type instrumentation. Very few methods attempt to combine these features into one single method.

Aim

To develop a flow through *in-vitro* method that also allows for real-time UV imaging of drug release from drug-eluting microparticles.

Method

The Surface Dissolution Imaging instruments (Sirius Analytical; pION) are traditionally used for studying dissolution properties of powder compacts or tablets (SDi2). We have modified the instruments flow cells by engineering inserts and novel sample holders (using 3D-printing) in order to allow for the analysis of the 300-500 µm sized formulations. Real-time UV imaging of the formulation and the loaded drugs was performed by the dual-wavelength capability of the SDi2.

Results

The initial insert prototypes containing nylon nets were successful at keeping the beads in front of the UV-imaging window without detrimental effect on the flow rate. The sub-mm sized formulations are studied at the visible wavelength (520 nm) meanwhile the release of various model compounds for DOX is investigated in the UV range: 255, 280 or 320 nm. Several versions of sample holders have been tested and more are under design and development. More extensive drug release experiments are currently being undertaken in order to collect a data set for comparison with results from our previously published in-house methods and *in vivo* release data from animals and human patients.[2,4]

Conclusion

A flow through drug release *in-vitro* method using UV-imaging has been developed. Improvements, optimisations and comparisons with established methods are ongoing.

References

1. Swaine, T. European Journal of Pharmaceutical Sciences (2016) 93: 351-359
2. Ahnfelt E. *et al*. Journal of Pharmaceutical Sciences (2016) 105: 3387-3398
3. Caine M. et al. Journal of Vascular and Interventional Radiology (2017) 28(2): 260-268
4. Ahnfelt E. *et al*. Journal of Controlled Release (2018) 292: 235-247.

Studying the effect of solubilizing agents on drug diffusion through the unstirred water layer (UWL) by localized spectroscopy

Massimiliano Pio di Cagno[1]*, Paul C. Stein[2]

[1] Department of Pharmacy, UIT the Arctic University of Norway, Norway

[2] Institute of Physics, Chemistry and Pharmacy, University of Southern Denmark, Denmark

Introduction

Hydroxypropyl-β-cyclodextrins (HPBCD) and phosphatidylcholine liposomes (Lip) have been extensively studied in the last decades as carrying agents for active pharmaceutical ingredients (API)[1]. It is still quite unclear if the use of solubilizing agent such as HPBCD and Lip actually increase drug bioavailability and therefore therapeutic outcome of medications. On this regard, it is crucial to better understand the interaction of drug and solubilizing agents with the unstirred water layer (UWL)[2].

Aim

To effectively monitor the transport of four API through an UWL in the presence of two types of solubilizing vectors (HPBCD and Lip) and evaluate the influence of solubilizers on UWL transport.

Method

Drug diffusion through UWL in the presence of HPBCD or, alternatively Lip, was monitored by localized UV-vis spectroscopy[3] and experimental data were analytically treated in order to extract all relevant parameters of transport: relative diffusivities, initial drug distributions, partitioning.

Results

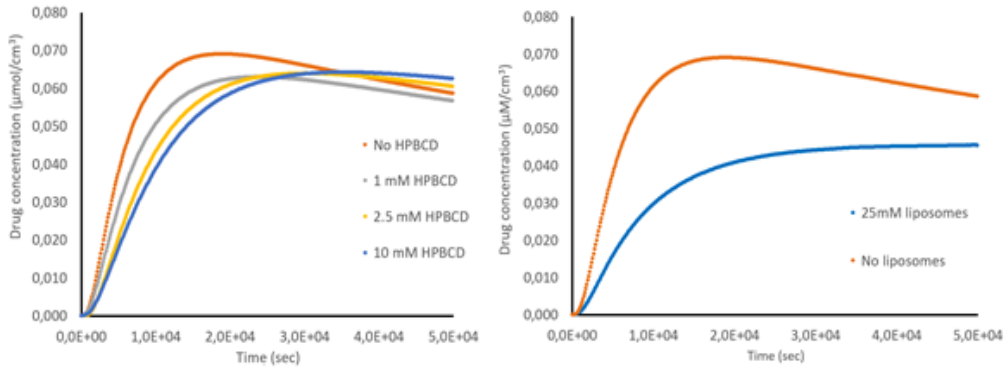


Fig. 1. Experimental data collected for hydrocortisone at growing concentration of HPBCD (left) and Lip (right) [4]

The diffusion profiles changed significantly (Fig. 1) when the concentration of HPBCD increased and in the presence of Lip. Computational data treatment (analytical approach) evidenced that both solubilizing agents reduced the drug transport through the UWL with a different mechanism. HPBCD induced a significant decrement in relative diffusivity for all drugs tested (to different extent). Differently, Lip mostly affected the initial drug distribution due to drug sequestration into the phospholipid bilayer.

Conclusion

With this work, we have demonstrated that both HPBCD and liposomes hamper the diffusion of API molecules through the UWL (below drug solubility) for all investigated compounds giving also a specific mechanistic explanation.

References

[1] R. Liu, Water insoluble drug formulations, second ed., CRS Press, Boca Raton, 2008.

[2] P.H. Barry, J.M. Diamond, Physiol. Rev. 64 (1984) 763-871.

[3] M.P. di Cagno, […], P.C. Stein, et al., Mol. Pharm. 15 (2018) 1488-1494.

[4] M.P. di Cagno and P.C. Stein, Eur. J. Pharm. Biopharm. (2018) *Under review*

Dissolution-/permeation studies of a poorly soluble drug “Compound A” using PermeaLoop®

Alex Elíasson[1], Elisabeth Feldhues[2], Iris Heep[2], Werner Hoheisel[3], Christopher Bland[2], Annette Bauer- Brandl[1], Martin Brandl[1]
[1]Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Odense Denmark
[2]Bayer Animal Health Germany GmbH, Monheim Am-Rhein, Germany, [3]Invite GmbH, Leverkusen, Germany

Introduction
PermeaLoop® is a novel small-scale dissolution/permeation set-up with a large permeation area, deemed suitable for performance testing of candidate-enabling oral drug formulations [1].

Aim
So far, only a limited amount of data was collected with PermeaLoop®. The objective of the current study was therefore to explore the applicability of PermeaLoop® and to fine tune the experimental parameters until dynamic conditions were met. For this purpose a suspension of proprietary drug Compound A from Bayer Animal Health was used.

Method
The suspension of Compound A was studied in PermeaLoop® using 4 different protocols to better reach dynamic conditions. The acceptor compartment contained 45 mL of SDS-solution of concentrations in the range from 0.9%-6%, the donor compartment contained 40 mL of Fasted state simulated intestinal fluid (FaSSIF). The drug dose was varied between 20 mg and 4 mg respectively. Sampling volumes from the acceptor compartment varied from minor to substantial fractions.

Results
In the 20 mg API 0.9% SDS concentration protocol the drug passing to the donor compartment quickly reached the saturation solubility; furthermore there was clear signs of precipitation in the donor compartment at the end of the experiment. An attempt to improve sink conditions was by adjusting the protocol to 6% SDS concentration in the acceptor compartment, which resulted in a substantial increase in flux. But still the flux slowed down after an hour run time. This was taken as an indication that the system was running out of dynamic conditions again. The dose was then lowered. The study of 4 mg API in 6% SDS acceptor medium resulted in comparable total amount of drug permeating as the 20 mg study showing that dissolution is not the rate limiting step. The donor concentration did not really decrease. The last study featured sampling of substantial fractions from the acceptor and replenishing with fresh 6% SDS-solution which resulted in a closer approximation to a dynamic scenario than the previous experimental setup(s).

Conclusion
Dynamic conditions can be achieved using PermeaLoop®, if the parameters are adjusted to ensure sink conditions in the acceptor and by ensuring that the “reservoir” of non-dissolved drug replenishing the dissolved drug in the donor stays within realistic limits.

References
[1] Sironi, D., Rosenberg, J., Bauer-Brandl, A., Brandl, M. PermeaLoop™ a novel in vitro tool for small-scale drug-dissolution/permeation studies. (2018) J Pharm Biomed Anal, 156, pp. 247-251.

Converting a batch based high-shear wet granulation process with a continuous dry granulation process; A demonstration with ketoprofen tablets

Tuomas Ervasti[1*], Krista Taipale-Kovalainen[1], Jarkko Ketolainen[1], Ossi Korhonen[1]
[1] School of Pharmacy, Promis Centre, University of Eastern Finland, Kuopio, Finland

Introduction

When a batch based manufacturing process of an existing tablet product is converted to a continuous process, several strategies for realization are available. Ideologically the most straightforward way would be to carry on with corresponding processing principles, for example to change a wet granulation (WG) batch process to a continuous WG process. However, in some cases choosing of dry granulation could be very inviting due to notably simplified manufacturing principles.

Aim

To demonstrate the chancing of a manufacturing process from high shear wet granulation (HSWG) to continuous roller compaction (RC) and study the effect on product performance.

Methods

Tablets were manufactured with both batch WG and continuous RC processes. Formulation, consisting of microcrystalline cellulose, hydroxypropyl methylcellulose, sodium lauryl sulfate, magnesium stearate and ketoprofen, was in other respects identical in both processes, except when manufactured with WG also a combination of glycerol, ethanol and water was used as a granulation liquid.

Results

Particle size of granules prepared with RC was a bit smaller as could be expected. They also presented somewhat higher angle of repose values indicating poorer flow properties. However, from both types of granules tablets could be successfully prepared. Dissolution revealed slightly faster API release for tablets prepared with the continuous RC process, but in both cases T80 was reached already after 15 minutes.

Conclusions

Tablet products from formulation based on batch HSWG process could successfully be manufactured also with a continuous RC process. Based on the results, concentration, as well as other properties of the tablets, were at acceptable level, proposing that direct conversion could be possible. However, optimization of formulation would be advisable to ensure processability also with higher production speeds.

Simulating the intestinal environment with the mucus-PVPA *in vitro* model: the impact of pH and simulated intestinal fluids on drug permeability

Margherita Falavigna[a], Mette Klitgaard[a,b], Erik Steene[c], Gøril Eide Flaten[a]

[a] Department of Pharmacy, University of Tromsø The Arctic University of Norway, Norway
[b] Department of Pharmacy, University of Copenhagen, Denmark
[c] Biotec Betaglucans AS, Tromsø, Norway

Introduction

Intestinal absorption of orally administered drugs can be influenced by food intake and regional variations. Therefore, it is central to evaluate the influence of these conditions on drug permeability when assessing the drugability of newly developed drugs and formulations[1]. *In vitro* permeability models able to handle the relevant intestinal conditions are thus important for screening purposes and formulation optimization.

Aim

To assess the impact of different pH conditions, fed and fasted state simulated intestinal fluids (SIFs) on drug permeability using the mucus-PVPA *in vitro* permeability model.

Method

Mucus-PVPA barriers were prepared as previously described[2]. The permeability of five model acidic/basic drugs was investigated at pH 5.5, 6.2 and 7.4. The permeability of one hydrophilic (calcein) and one lipophilic (ibuprofen) compound was studied in the presence of fed and fasted state SIFs (FeSSIF and FaSSIF, respectively).

Results

The mucus-PVPA barriers proved to be stable at all tested pH conditions, and the permeability of the five model drugs showed to be dependent on the specific pH, according to their physicochemical characteristics and following the pH partition hypothesis[3].

The barriers showed to be particularly stable in the presence of the fed state SIF (Fig. 1). The fed and fasted SIFs influenced the permeability of calcein and ibuprofen to a different extent (Fig. 1), dependent on the hydrophilicity/lipophilicity of the compounds, their degree of interaction with the mucus layer and on the content of bile salts and phospholipids present in the specific SIF.

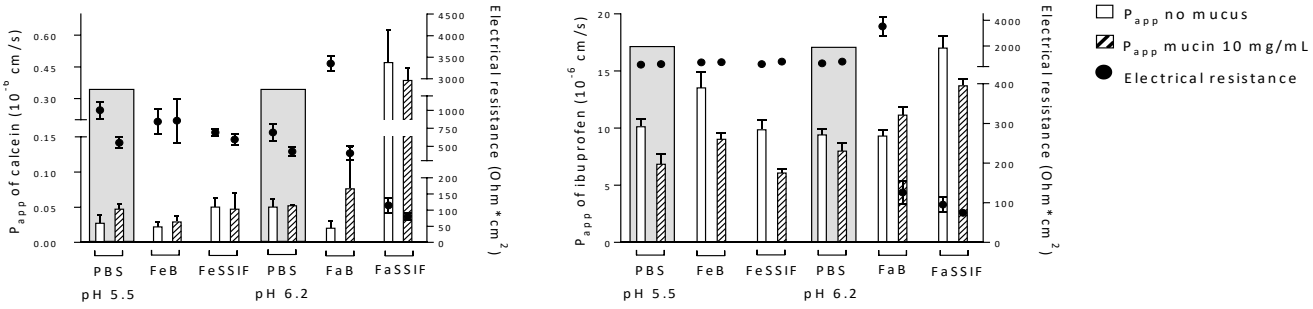


***Fig. 1**: Apparent permeability (P_{app}) in the presence of fed (Fe-) and fasted (Fa-) buffers (-B) and media (SSIF).*

Conclusion

This study highlights the impact that changes in pH and intestinal fluid composition have on drug permeability, and suggests the mucus-PVPA to be a suitable *in vitro* model for investigations regarding the impact that these different conditions, as well as the interaction with mucus, have on drug absorption.

References

1. Augustijns P. *et al.* Eur. J. Pharm. Sci (2014) 57: 322–332
2. Falavigna M. *et al.* Int. J. Pharm. (2018) 537: 213-222
3. Shore P.A. *et al.* J. Pharmacol. Exp. Ther. (1957) 119: 361-369

Confocal visualization of MSNs to assess permeability through the extracellular matrix of *Staphylococcus aureus* biofilms

Prakirth Govardhanam[1], Anna Slita[1], Didem Sen Karaman[1], Jessica Rosenholm[1]
[1]Pharmaceutical Science Laboratory, Faculty of Science and Engineering, Åbo Akademi University, Turku, Finland

Introduction

Around 80 % of all microbial infections in the human body are rooted to the formation of biofilms[1]. Prosthetic infections could also be caused at any point in the perioperative scenario, primarily by Staphylococcus species. The shielding effect of the extracellular matrix (EPS) on the outer layer of the biofilm necessitates a therapeutic selectivity to penetrate the EPS layer and to break down the biofilm from within[2]. This study focuses on the development of tailored mesoporous silica nanoparticle (MSN) -based structures, namely, nanospheres (MSP), and nanorods (MSR), jointly named as anti-biofilm nanoparticles (ab-NP).

Aim

To investigate the permeability through the EPS of the *Staphylococcus aureus* (*S. aureus*) biofilm by using confocal laser scanning microscopy (CLSM). Further, an antibacterial compound shall be loaded into the ab-NP to provide antibacterial activity from within the biofilm upon penetration.

Methods

The ab-NP are prepared with the incorporation of fluorescein isothiocyanate (FITC) into MSN matrix. FITC helps in visualizing the penetration of the nanoparticles through the biofilm by using CLSM. The physicochemical characteristics of developed ab-NP were confirmed by transmission electron microscopy, dynamic light scattering, and zeta–potential measurements. The *S. aureus* biofilm were cultured in confocal dishes for the convenience of imaging.

Results

The proposed ab-NP were successfully synthesized and characterized. According to the *in vitro* investigations, the penetration of the ab-NP through the EPS layer of the *S. aureus* biofilm has been established using Z-stack images acquired from CLSM and particle analysis using Image J. The results revealed the prominence of MSR at the bottom of the biofilm, whereas the MSP penetrates along all levels of the biofilm and more evenly compared to MSR (Fig1). PEI-functionalized MSR were successfully permeable through the EPS layer of the biofilm. PEI-functionalized MSP were successful in accumulating at the upper levels of the EPS layer of the biofilm (Fig1).

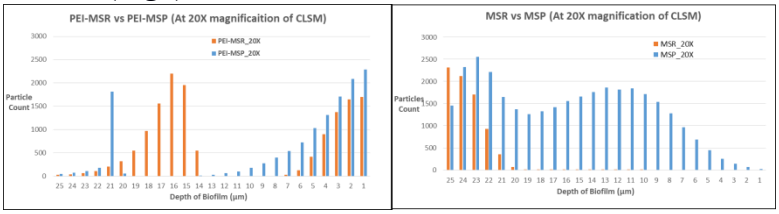


Fig1. Permeation analysis and comparison of Ab-NP (MSR vs MSP) with and without PEI-modifications.

Conclusion

Shape engineering of the MSN structures and the surface modifications of these structures have led to different levels of penetration into the EPS of *S. aureus* biofilms.

References

1. Sun, Fengjun, et al. Future microbiology 8.7 (2013): 877-886.
2. Fernandez-Moure, Joseph S., et al. Nanomedicine 12.11 (2017): 1319-1334.

Patient-centric Pharmaceutical Product Design and Manufacturing

Rydvikha Govender[*1,2], Anette Larsson[2], Susanna Abrahmsén Alami [1], Staffan Folestad[1]
[1]Pharmaceutical Technology and Development, AstraZeneca, Mölndal, Sweden
[2]Chemistry and Chemical Engineering, Chalmers University of Technology, Gothenburg, Sweden

Purpose:

Breakthroughs in molecular biology, diagnostics, genotyping and nanotechnology have provided the knowledge and technical capabilities for individualised therapies. However, designing individualised pharmaceutical products which facilitate accessibility to patients in an economically viable manner remains a challenge under the current paradigm of mass production. Mass customised precision pharmaceutics produced as subunits and assembled *via* manufacturing platforms into different configurations with tuneable characteristics could address these challenges. Firstly, we aim to understand patient requirements from pharmaceutical products as a consequence of biological, behavioural and environmental diversity. Secondly, we aim to explore the design of pharmaceutical formulations for oral delivery that could meet these needs and are suitable for mass customisation.

Methods:

Pharmaceutical formulations and promising manufacturing platforms are compared with regard to key prerequisites for predictable performance, manufacturability, scalability of function and ability to satisfy patient needs.

Results:

Patient needs determined from inter-individual variability lead to specific requirements from pharmaceutical products. Characteristics of manufacturing platforms for mass customisation and relevant product design criteria including the relationship between product unit dimensions and key characteristics such as uniformity and suitability for tailored drug release will be presented.

Conclusions:

This analysis serves to guide critical manufacturing and product design features for precision pharmaceutics and elicit suggestions for continued focus in this field.

Inositol and sucralose: promising new excipients in freeze-dried protein formulations

Tobias P. Holm, Helena Meng-Lund and Holger Grohganz

Department of Pharmacy, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark

PURPOSE

Excipients used as bulking agents and lyoprotectants in lyophilized protein formulations are mainly found in the group of saccharides and polyols. However, considering the vast differences in pharmaceutically relevant proteins, the practical choice of excipients is rather limited, as only a handful of well-described and approved sugars usually is a considered [1, 2]. Identifying other excipients, which are not traditionally used in freeze-drying, could lead to a broader range of suitable bulking agents or lyoprotectants.

METHODS

Freeze-drying was performed in 96-well plates. A screening freeze-drying study of 66 excipients identified promising excipients: inositol and N-acetyl-D-neuraminic acid could be potential bulking agents due to their crystalline nature, sucralose and maltotriose became amorphous and might be potential lyoprotectants. Mannitol and sucrose were included as reference standards. Bulking agent and lyoprotectant combinations were freeze-dried at solid content of 100 mg/ml at 5:5, 7:3 and 9:1 (m/m) ratio with three model proteins: human serum albumin, a fatty acid free human serum albumin and bovine serum albumin. Crystallinity was determined by X-ray powder diffraction, protein stability by intrinsic fluorescence, Tg' by modulated differential scanning calorimetry and cake appearance by scanning and subsequent image analysis [3].

RESULTS

The investigated combinations in general fulfilled several of the desired quality attributes, i.e. a crystalline or partly crystalline structure, a satisfactory cake appearance, and no apparent changes in protein structure after freeze-dying. The application of inositol and maltotriose led to results comparable with the established mannitol and sucrose. Sucralose was found not be generally applicable as lyoprotectant, which is in line with earlier findings of sucralose destabilizing some proteins in solution [4]. However, in combination with inositol, sucralose stabilized in all but one combination and provided good cake appearance. The expected crystallization of N-acetyl-D-neuraminic was prevented in the presence of a lyoprotectant, This resulted in freeze-dried cakes characterized by collapse, cracks and a glassy appearance thereby rendering the substance unsuitable as a bulking agent. However, no protein denaturation was observed and thus application as lyoprotectant might be possible.

CONCLUSION

Mixed results were obtained from the investigation of the four potential freeze-drying excipients. Although promising combinations were identified, no superiority to established combinations was found so far. Further studies, such as long-term stability assessment, of these and other combinations, as well as the investigation of different proteins, might nevertheless show a potential for the use of untraditional excipients for freeze-drying.

REFERENCES

1. Chang L, et al., Journal of Pharmaceutical Sciences. 2005;94(7):1427-1444.
2. Mensink MA, et al., European Journal of Pharmaceutics and Biopharmaceutics. 2017;114:288-295.
3. Trnka H, et al., Journal of Pharmaceutical Sciences. 2013;102(12):4364-4374.
4. Shukla N, et al., Archives of Biochemistry and Biophysics. 2018;639:38-43.

Microcontainers with functionalized lids for oral delivery of protein

Chiara Mazzoni[1,2], Jacob Mortensen[1,2], Rasmus Due Jacobsen[1,2], Jacob Rune Jørgensen[1,3], Anette Müllertz[1,3], Line Hagner Nielsen[1,2], Anja Boisen[1,2]

[1]The Danish National Research Foundation and Villum Foundation's Center for Intelligent Drug Delivery and Sensing Using Microcontainers and Nanomechanics (IDUN)

[2]Department of Micro- and Nanotechnology, Technical University of Denmark, Kgs. Lyngby, Denmark

[3]Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark

Aim: The aim was to functionalize SU-8 microcontainers loaded with the model protein, lysozyme. The functionalization was made by spray coating polymer lids of either poly lactic-co-glycolic acid (PLGA) and chitosan or PLGA and polyethylene glycol (PEG) (PLGA+chitosan or PLGA+PEG, respectively) onto the cavity of the loaded microcontainers. The lids have the function of enhancing protein absorption from the microcontainers. Furthermore, the coated microcontainers were investigated *in vitro* and *ex vivo* as an oral delivery system for proteins.

Methods: The cylindrical SU-8 microcontainers were fabricated by two-steps of photolithography with an inner diameter of 260 µm and a cavity depth of 270 µm (Fig 1). After fabrication, the microcontainers were loaded with lysozyme and the permeation enhancer sodium decanoate (C10) in the ratio 7:3 w/w using an embossing method. The loaded microcontainers were sealed and functionalized by applying polymeric lids onto the cavity of the devices using an ultrasonic nozzle in a spray coater system. The first lid was PLGA and on top of this either chitosan or PEG was applied. The functionalization was evaluated *in vitro* for morphology, drug release and mucoadhesive properties. These were coupled with *in vitro* and *ex vivo* studies using Caco-2 cells, Caco-2/HT29-MTX-E12 co-cultures and porcine intestinal tissue.

Results: The loading of the microcontainers resulted in approximately 3 µg of lysozyme in each microcontainer. After the spray coating, the thickness of each lid was measured and the PLGA layer was found to have a thickness of 14.0 ± 3.8 µm (Fig. 1A) followed by 12.2 ± 2.2 µm of chitosan (Fig. 1B), whereas the PEG lid had a thickness of 17.0 ± 5.6 µm (Fig. 1C). After spray coating of the lids, it was determined how fast the lysozyme in the microcontainers was released. PLGA+chitosan showed a slower release in buffer (Fig. 1D) and slower transport of lysozyme across cell cultures compared to PLGA+PEG or only PLGA. Microcontainers coated with chitosan or PEG demonstrated a three times stronger adhesion during *ex vivo* mucoadhesion studies compared to samples without coatings.

Conclusion: Altogether, functionalized microcontainers with mucoadhesive properties and tunable release for oral protein delivery have been developed and characterized.

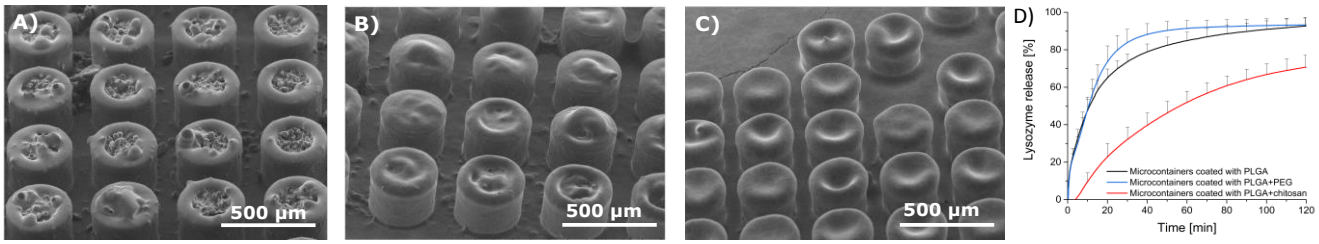


Fig. 1: SEM images of coated protein-loaded microcontainers either with a lid of A) PLGA B) PLGA and chitosan or C) PLGA and PEG. D) *In vitro* release of lysozyme from the coated microcontainers in buffer at pH 7.4. Data represent mean±SD, n=3.

The influence of particle size on microwave-assisted *in situ* amorphization

Nele-Johanna Hempel[1*], Matthias M. Knopp[2], Ragna Berthelsen[1], Korbinian Löbmann[1]
[1]Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark
[2]Bioneer:FARMA, Copenhagen, Denmark

Introduction

Amorphous drug delivery systems are a promising approach to overcome the poor aqueous solubility of many drugs in modern drug delivery.[1] However, due to their high internal energy, these systems are often too unstable during shelf life, which frequently limits their feasibility as drug delivery system. The microwave-assisted *in situ* formation of a polymeric glass solution constitutes a promising method to overcome long-term stability issues of amorphous dosage forms.[2] The drug is suggested to dissolve into the polymer at temperatures above the T_g of the polymer. Therefore, according to the Noyes-Whitney equation[3] particle size reduction should increase the dissolution rate of the drug into the polymer resulting in a higher degree of amorphization during similar microwaving times.

Aim

To investigate the influence of the drug particle size on the rate and degree of *in situ* amorphization using microwaves.

Method

Celecoxib (CCX) with two different particle sizes, i.e. small particles (d (0.5) =11.9 µm) and large particles (d (0.5) =137.8 µm), was used to prepare 100 mg compacts containing 30% (w/w) of drug, 69.5% (w/w) PVP K17 and 0.5% (w/w) magnesium stearate. After two weeks conditioning at 75% RH (ambient temperature), the compacts were microwaved in 60 seconds intervals for up to 600 seconds at 1000 W using a household microwave. Subsequently, the amorphous/crystalline fraction was quantified using transmission Raman spectroscopy.

Results

During the conditioning period, the polymer in the compacts absorbed water, which acts as an absorber for the microwaves and as a plasticizer for the polymer. Upon microwaving, up to 62% CCX was dissolved from the small particles into the polymer PVP after 600 seconds (Figure 1). In contrast to the small particles, the large particles only showed ~16% amorphization after 600 s of microwaving.

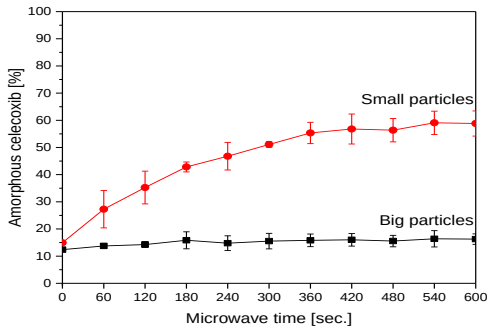


Figure 1 *Amorphization of celecoxib depending on particle size after microwaving with standard deviation (error bars)*

Conclusion

It could be shown that the particle size influenced the *in situ* amorphization capability using microwaves. Smaller particles led to a higher rate and degree of amorphization compared to larger particles.

Acknowledgement

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References

1. Hauss D. J. Adv. Drug Deliv Rev (2007) 59 (7): 667-676.
2. Doreth M. *et al.* Int J Pharm (2017) 519 (1): 343-351.
3. Noyes AA, Whitney WR. J Am Chem Soc (1897) 19(12):930-4.

Solvent effects on the crystal growth and morphology of theophylline and pyrazinamide – a combined experimental and computational approach

Mikkel Herzberg[1]*, Anders S. Larsen[1], Anders Ø. Madsen[1], Jukka Rantanen[1]
[1]Department of Pharmacy, University of Copenhagen, Denmark
*Mikkel.herzberg@sund.ku.dk

Introduction
Pharmaceutical compounds can typically form several organic crystal structures also known as polymorphs. The solvent used in the crystallization can have dramatic consequences for the outcome of this process: solvent selection affects the habit of the resulting crystals and solvent molecules can even be present in the crystals as solvates, because of voids in the molecular packing networks [1]. The crystallization process can therefore be controlled by careful selection of the solvent. A better understanding of solvent controlled crystallization is of great importance for the pharmaceutical industry, because the crystal structure is a key parameter influencing solubility, bioavailability and stability of drugs [2], while the morphology affects the processability.

Aim
To investigate the effect of various solvents on the crystallization of theophylline and pyrazinamide. Resulting crystals will be analyzed using different microscopic techniques, X-ray diffraction and the overall crystallization process will be addressed computationally using molecular dynamics (MD) simulations.

Method
Theophylline and pyrazinamide were dissolved in various solvents at 50°C to supersaturate the solutions. The solutions were then left at room temperature. After a few hours, crystals began to form. The crystals were investigated with optical microscopy and X-ray diffraction. MD simulations were performed using the GROMACS software package. Different faces of the crystal were simulated in order to determine their growth rate.

Results
This work is at a very early stage, but crystals of theophylline and pyrazinamide with varying size and shape could be isolated. The selected solvents will be implemented in the next phase for the computational MD simulation part to allow for prediction of the morphology based on solvent selection.

Conclusion
We plan to determine the relationship between the solvents used during the crystallization process of theophylline and pyrazinamide and the structure and morphology of the resulting organic crystals.

References
[1] J. Karpinska, A. Erxleben, P. McArdle, 17β-Hydroxy-17α-methylandrostano[3,2-c]pyrazole, Stanozolol: The Crystal Structures of Polymorphs 1 and 2 and 10 Solvates, Crystal Growth & Design 11(7) (2011) 2829-2838.
[2] S. Datta, D.J.W. Grant, Crystal structures of drugs: advances in determination, prediction and engineering, Nature Reviews Drug Discovery 3 (2004) 42.

Atomic layer deposition (ALD) based surface treatment of powders

Cosima Hirschberg [a], Nikolaj Sølvkær Jensen [a], Johan Boetker [a], Anders Østergaard Madsen [a], Tommi O. Kääriäinen [b, c], Marja-Leena Kääriäinen [c], Pekka Hoppu [c], Steven M. George [b], Matti Murtomaa[d], Changquan Calvin Sun [e], Jens Risbo [f], Jukka Rantanen [a]

[a] *Department of Pharmacy, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark*
[b] *Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309 USA*
[c] *NovaldMedical Ltd Oy, Telkäntie 5, 82500 Kitee, Finland*
[d] *Department of Physics and Astronomy, University of Turku, Finland*
[e] *Department of Pharmaceutics, University of Minnesota, 308 Harvard St. SE, Minneapolis, MN 55455, US*
[f] *Department of Food Sciences, University of Copenhagen, Rolighedsvej 30, 1958 Frederiksberg C, Denmark*

Email: cosima.hirschberg@sund.ku.dk

Introduction
Powder handling is a daily challenge in the pharmaceutical industry. The optimized functionality of each material is crucial for the successful production. In many cases, powder flowability can be a limiting factor for safe and robust production train.

Aim
This study explores the use of atomic layer deposition (ALD) as an approach for surface treatment of physicochemical diverse model powders.

Methods
Model excipients were chosen based on their diversity in particulate properties and coated with TiO_2 by using Atomic Layer Deposition (ALD) thin film coating method in a rotary particle ALD reactor. Different material characteristics (e.g. water-solid interactions, solid state composition) and bulk properties (e.g. powder flowability and static charging) were analyzed. Microcrystalline cellulose (MCC), croscarmellose sodium (Na-CC) and calcium hydrogen phosphate dihydrate ($CaHPO_4$) were used as model powders with different levels of crystallinity. All powders were coated with 5 ALD cycles of TiO_2. Additionally, Na-CC was coated with 20 ALD cycles of TiO_2.

Results
The success of the coating could be confirmed with X-ray photoelectron spectroscopy (XPS). The thin layer of TiO_2 did not affect the water sorption behavior and the solid state of the materials. The electrostatic charging was reduced by applying a thin conductive layer of TiO_2 on the sample and thereby the flowability was improved when compared with the flowability of both the uncoated starting material and a physical mixture of the material and TiO_2 powder.

Conclusions
The coating using atomic layer deposition improved powder flowability and did not affect the water sorption behavior. In the next phase, the compactability of the powder samples will be analyzed using a compaction simulator.

Acknowledgments
Innovation Fund Denmark funded this study; Project- High Quality Dry Products with Superior Functionality and Stability – Q-Dry; File No: 5150-00024B. Funding from NordForsk for the Nordic University Hub project for mobility during the project is acknowledged #85352 (Nordic POP, Patient Oriented Products).

Aggregation behavior of medium chain fatty acids studied by molecular simulation

Md Shakhawath Hossain[1], Staffan Berg[1], Christel A. S. Bergström[1], Per Larsson[1]

[1]Dept of Pharmacy and the Swedish Drug Delivery Forum (SDDF), Uppsala University, Uppsala Biomedical Center P.O. Box 580, SE-751 23 Uppsala, Sweden

Introduction

Medium chain fatty acids (MCFA) can be used to promote drug absorption by several different processes, including solubilization, increased membrane fluidity, and increased paracellular transport through the opening of the tight junction[1]. Therefore, understanding the aggregation behavior of the MCFAs is important.

Aim

To investigate critical micelle concentration (CMC) and aggregation behavior of four MCFAs (C_8, C_{10}, C_{12}, and C_{14}) as a function of both protonation state and the ionic strength of the buffer using coarse-grained molecular dynamics (CG-MD) simulations. We also measured the CMC of C_8, C_{10} and C_{12} in representative system conditions to validate our results.

Methods:

The MD simulations were performed using Martini force field[2] in Gromacs 2016.4[3] with a 30 fs time step for a total time of 1 μs. The length, width, and height of the box was 22×22×44 nm^3 respectively. For a number of simulations with C_{12} and C_{14}, a larger box of 44 nm^3 was used, since the aggregation occurs at much lower concentrations. The CMC of the MCFAs were also determined experimentally using the Wilhelmy or surface tension measurement method.

Results:

The CMCs from simulations for C_8, C_{10} and C_{12} were 1.8-3.5 fold lower than the respective CMCs determined experimentally, consistent with similar studies on other systems using the Martini force field. However, the variation, including rank order of the CMCs, MCFA aggregate numbers, sizes and morphology (Fig. 1) as a function of MCFA chain length, system pH condition and ionic strength is consistent with previous experimental observation, as well as with results in this study from the Wilhelmy method.

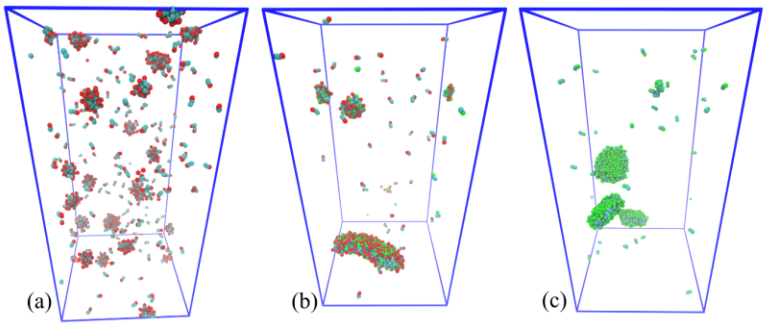


Fig 1. Snapshot of the simulations of 50 mM C_8 with 140 mM NaCl for the systems with (a) 100% deprotonated molecules, (b) 1:1 ratio of deprotonated and non-charged molecules and (c) 100% non-charged molecules

Conclusion:

This study suggests that the CG-MD Martini model is promising for studying colloidal systems including various MCFAs with careful validation for specific systems and research questions.

References:

1. Lindmark et al. J Pharmacol exp Ther. 1995;275:958–249 964, 2.Marrink et al. J Phys Chem B. 2004;108:750–760,
3. Abraham M, et al. GROMACS User Manual version 2016.4, 2016

Design of mucoadhesive films for buccal delivery of clotrimazole

J. Huang, J. Jacobsen, S.W. Larsen, A. Müllertz, H.M. Nielsen, H. Mu*

University of Copenhagen, Department of Pharmacy, Copenhagen, Denmark

Introduction

Clotrimazole (CTZ) is an antifungal drug used for the treatment of oral candidiasis infected by *Candida albicans*. However, oral delivery of CTZ is limited by its poor aqueous solubility and gastrointestinal intolerance. Buccal delivery of CTZ by retaining the drug in buccal mucosae may deduce its side effects and increase the bioavailability to treat oral candidiasis. Graphene oxide (GO), a single-layer of graphene with carboxylic groups, has been reported to increase the mechanical properties of chitosan (CS) film.[1] Moreover, the anti-*Candida albicans* effect of GO has been reported.[2] In this study, buccal formulations of CTZ were prepared using mucoadhesive excipients CS and sodium alginate (SA) incorporated with GO.

Aim

The aim of the present study was to evaluate the effect of excipients, especially GO, on the characteristics of buccal films and drug release profiles.

Method

The buccal formulation was prepared by mixing excipients and CTZ using a probe sonicator. Then a film casting of the obtained CS/SA/GO/CTZ composites was performed at 37 °C. Design of experiments (DoE) was applied to investigate the influence of GO concentration on the properties of CS/SA film, including mechanical strength, moisture absorption The mechanical properties of the films were evaluated by using a texture analyzer. The morphology of the films was observed by using scanning electron microscope (SEM). X-ray powder diffraction (XRPD) was applied to investigate the solid state of excipients in the buccal films. The release of CTZ from buccal films with different concentration of GO was investigated using Franz diffusion cell apparatus at 37°C. Before the release study, each film was covered with an impermeable backing layer to simulate one-way diffusion.

Results

The mechanical analysis of the buccal film with or without GO showed that the strength of the films was enhanced in the presence of GO. The drug was slowly released from buccal films without GO. When GO amount in the formulation increased from 0.05% to 0.1 wt%, the accumulated release of CTZ in 2 hours significantly increased from approximately 9.4% to 19.3 %. The result indicated that GO might interrupted the interactions between CS and SA so that the drug molecules could easily diffuse out of the polymer matrix. Moreover, the moisture absorption ability of the film increased as the amount of GO increased, thus could also contribute to the increased drug release.

Conclusion

The present study showed that GO had strong impact on the mechanical strength and moisture absorption ability of buccal films. Incorporation of GO into formulations could regulate the release of CTZ from the buccal films.

References

1. R. Justin *et al*. Carbohydr. Polym. (2014) 103 70-80.
2. R. Diez-Orejas *et al*. Colloids Surf., B (2018) 171 250-259.

High-throughput dissolution/permeation screening of enabling formulations: Testing a novel two-compartment microtiter plate approach

Ann-Christin Jacobsen[1], Martin Brandl[1], Annette Bauer-Brandl[1]

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

High-throughput formulation screening can alleviate the costly and challenging development of complex oral drug formulations e.g. amorphous solid dispersions (ASDs). Traditionally, the dissolution behaviour of solvent-casted films serves as surrogate to predict ASD performance. Recently, the predictive power of (apparent) solubility and/or dissolution for oral absorption performance has been questioned.

This study describes a high-throughput dissolution/permeation approach that simultaneously screens drug dissolution and drug permeation. Enabling simultaneous dissolution/permeation screening, the approach employs a novel two-compartment 96-well microtiter plate that comprises an integrated dialysis membrane. The predictiveness of the approach was tested on amorphous and crystalline tadalafil formulations with and without the polymer Soluplus® by comparing the in vitro results with in vivo bioavailability results (Krupa et al. 2016). As an alternative to solvent casting, freeze-drying from hydro-alcoholic solutions was used to obtain amorphous formulations.

The screening was conducted according to the following four steps: 1. Dispersion of the formulations, 2. Incubation of the two-compartment plate, where the integrated dialysis membrane separated the donor compartment containing the dispersed formulation from the acceptor compartment containing an acceptor medium, 3. Sampling from donor and acceptor compartment, where donor samples were centrifuged to separate dissolved from non-dissolved material and 4. Quantification of dissolved and permeated amount of tadalafil by UHPLC-UV. Three test parameters were varied to identify optimal screening conditions: dispersion medium (including biomimetic media), acceptor medium (including surfactants solutions) and incubation time (up to 6 hours).

The screening approach enabled the simultaneous determination of (apparently) dissolved and permeated tadalafil. Surfactant solutions used as acceptor medium increased tadalafil permeation across the dialysis membrane significantly as compared to aqueous buffer. Different surfactant solutions (all above CMC) had a similar effect on permeation promotion. Variations in the dispersion media noticeably affected the dissolved amount of tadalafil. In contrast, the permeated amount was not noticeably affected. Only in the case of freeze-dried tadalafil, the presence of biomimetic media containing bile salts and phospholipids significantly increased the permeated amount. Different incubation times yielded comparable results in terms of formulation ranking. Still, longer incubation times were found beneficial regarding the analytical sensitivity required for tadalafil quantification in the acceptor samples. The in vitro data reflected the outcome of the in vivo study in terms of bioavailability.

The high-throughput dissolution/permeation approach was found promising for formulation screening. Solubility/dissolution and permeation were investigated efficiently in a single experiment after optimization of experimental parameters (i.e. dispersion medium, acceptor medium, and incubation time). The presented case indicates an excellent correlation to preclinical bioavailability data.

References: Krupa, A., et al., 2016. High-Energy Ball Milling as Green Process to Vitrify Tadalafil and Improve Bioavailability. Molecular pharmaceutics 13, 3891-3902. DOI: 10.1021/acs.molpharmaceut.6b00688

Effect of adding an elastic, plastic or brittle material on the fragmentation behavior of lactose

Nikolaj Sølvkær Jensen[a,b], Anne Linnet Skelbæk-Pedersen [a,b], Thomas Vilhelmsen [a], Jukka Rantanen [b].

[a] *Novo Nordisk A/S, Oral Pilot & Process Development, 2760 Måløv, Denmark.*

[b] *Department of Pharmacy, University of Copenhagen, 2100 Copenhagen, Denmark.*

Email: qnjy@novonordisk.com

PURPOSE

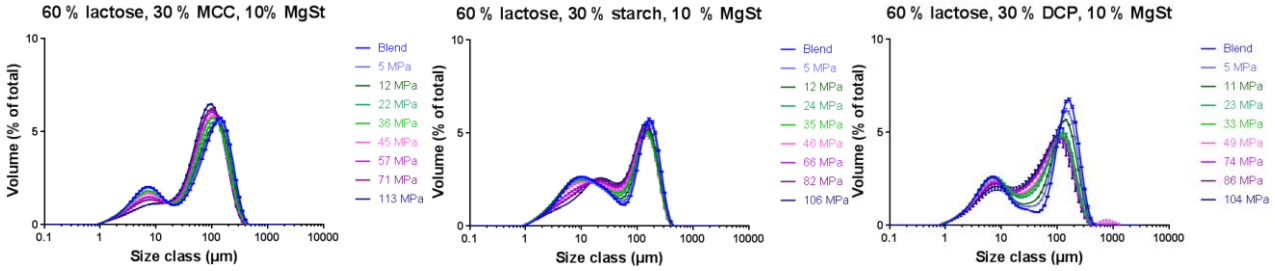
When compressing a powder bed into a tablet it will undergo deformation. Materials can be classified as elastic, plastic or fragmenting. Fragmentation and plastic deformation are irreversible deformations and are crucial for achieving sufficient tablet mechanical strength. Fragmentation has mainly been investigated in single-component and binary systems. The purpose of this study is therefore to systematically investigate the effect of the addition of a material with an elastic, plastic or brittle nature on the fragmentation behavior of brittle materials in a binary powder system.

METHODS

Four model compounds were selected with varying deformation properties (dicalciumphosphate/ DCP, lactose monohydrate/LMH, MCC and starch). The brittle material under investigation (LMH) was fractionated to 125-180 µm, whereas the second component (DCP, MCC and starch) was fractionated to 0-125 µm. The material under investigation (LMH) was blended with 10, 20 or 30 % of the second component, respectively, including 10 % MgSt and compressed into tablets (5 – 105 MPa) using 10 mm flat-faced punches. The compressed particles were recovered from the tablets by manual grinding, subjected to particle size distribution (PSD) analysis, and finally analyzed with both univariate and multivariate approaches.

RESULTS

It was found that materials with different deformation mechanism had a different impact on the fragmentation behavior of the brittle material (LMH). By adding the plastic MCC, the fragmentation of LMH decreased compared to addition of the elastic starch or the fragmenting DCP (see the figures). Furthermore, by adding the fragmenting component (DCP) to LMH increased the fragmentation the most (see figures). The increasing concentration of the second component was found to have a decreasing effect on the fragmentation of the brittle material (LMH).



CONCLUSION

Fragmentation behavior of brittle materials in binary mixtures can be altered by addition of elastic, plastic or brittle materials. Fragmentation of lactose monohydrate was influenced by the concentration and the deformation mechanism of the selected additional component.

Influence of proximity on the permeability enhancing effect of microcontainers for oral insulin delivery

Jacob R. Jørgensen[1,2], Morten L. Jepsen[2], Line H. Nielsen[2], Martin Dufva[2], Hanne M. Nielsen[1], Thomas Rades[1], Anja Boisen[2], Anette Müllertz[1]
[1]Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark
[2]Department of Micro- and Nanotechnology, Technical University of Denmark, Kgs. Lyngby, Denmark

Introduction

Permeation enhancers (PEs) are often used in the field of oral peptide delivery. The dilution effect caused by the large surface area of the small intestine might, however, decrease their effect. Microcontainers (MCs), capable of confining the absorptive area of peptide and PE by unidirectional release, could potentially help overcoming this issue, and have previously shown promising results in increasing absorption of small molecules.[1]

Aim

To validate the concept of increasing insulin permeability by confined co-locational release of insulin and PE, and to assess the influence of distance between the point of unidirectional release and the barrier.

Method

MCs, fabricated by photolithography in SU-8 on silicon chips, were filled with a powder mixture (1:1 w/w) of insulin and sodium caprate (C_{10}) by centrifugal force (Fig 1). Insulin transport was monitored by HPLC-UV across Caco-2 monolayers in Transwells® with different distances (0-2 mm) between the cells and the chip holding 625 microcontainers. Collective directional release from the MCs towards the monolayer was ensured for all distances. A solution of 0.1 mM insulin and 3 mM C_{10} (1:1 w/w), equivalent to the amounts filled in MCs, was used as control group. A combination of TEER measurements and confocal laser scanning microscopy was used to evaluate the integrity of the monolayers.

Results

Significant increases in insulin flux were achieved by release from all distances, compared to the solution of 0.1 mM insulin and 3 mM C_{10} (Fig 2). A drop in TEER value of 73 % was observed after release from microcontainers from 0.0 mm to the cells, and reversibility of this effect was evident upon 24 h subsequent incubation as an 86 % recovery of the initial TEER of the monolayer before the transport study. Confocal microscopy revealed local areas of cell damage after release from microcontainers from 0.0 and 0.2 mm, however, no monolayer deterioration was observed upon release from 0.5 and 2.0 mm.

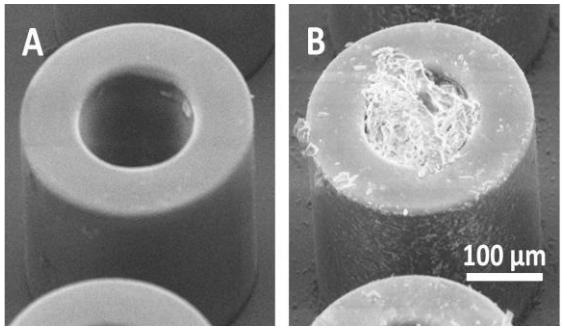


Figure 1: Left: empty MC, Right: loaded MC

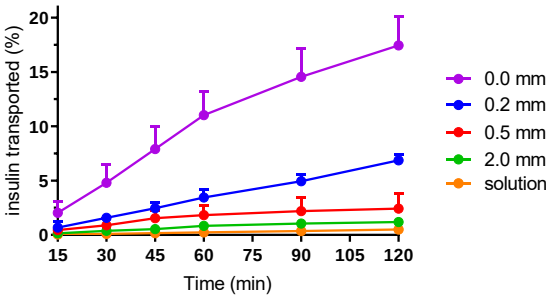


Figure 2: Transport profiles from MCs

1. Mazzoni, C. *et al. (2017) J. Controlled Release* **268,** 343–351

Energy barriers in micelles as a factor of drugs solubilization

Aleksei Kabedev*, Per Larson, Christel A.S. Bergström
Department of Pharmacy, Uppsala University, Sweden

Introduction

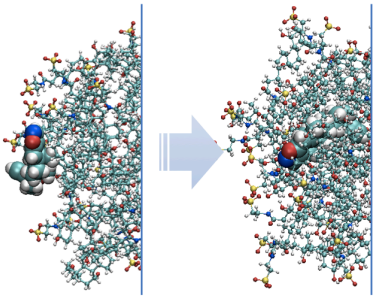
Poor aqueous solubility is affecting the bioavailability of the majority of modern drugs. The process for an active pharmaceutical ingredient (API) to be absorbed from the small intestine includes dissociation from the crystalline structure, dissolution in the gastrointestinal liquid, interactions with intestinal components such as lipids and bile salts (BS) and transport across the membrane. The latter parts of this process are influenced by micellular solubilization. Micelles consisting of bile salts associated with phospholipids (PL) and cholesterol are likely to attract the drug molecules, specifically hydrophobic ones, after an eventual collision. Once the API meet with such a micelle it can either stick to the surface of the micelle, penetrate inside the micelle or diffuse away as a result of thermal fluctuations. We propose that one of the main factors determining these scenarios is the energy barrier arising in the shell of micellular structures for various API's. Molecular dynamics (MD) computer simulations allow one to study both kinetics and thermodynamics of relevant processes for such energy effects.

Aim

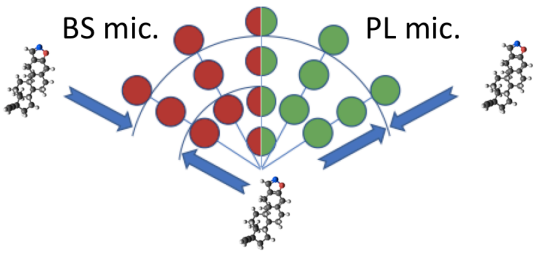
To investigate the contribution of the intra-micellular energy barriers to the solubilization of poorly water-soluble drugs using computational simulations. Further, to explore correlations between the solvation free energy differences for different API's and experimentally observed solubility values.

Method

Four API's were chosen for studies of their penetration through micelles built of either BS, PL or BS+PL (at various proportions). Umbrella sampling simulations were performed to get the potential of mean force (or free energy) profiles. In the laboratory we studied the solubility of the APIs via shake flask experiments with various mole fractions of PL/BS/PL+BS in water with a consequent HPLC analysis of the drug concentrations.



Barrier overcoming



Results

Current results from the work allow us to observe a significant difference in the barriers posed for the studied drugs within a pure PL, pure BS and combined PL+BS micelles. Regardless of their starting position (inside or outside the micelle), the API molecules end up at the same position with respect to the center of the micelle in case of PL, whereas for BS-containing micelles a difference of approximately 1 nm is observed. In the case of BS+PL the gap is even bigger, but also the transitions from one location to another are more likely to happen.

Conclusion

We have only performed preliminary simulations to date but the results thereof support our hypothesis that PL allows API to penetrate deep inside the micelle easier whereas the BS enables it to stick there.

Layer-by-layer coated microcontainers for colon-targeted oral drug delivery

Khorshid Kamguyan, Line Hagner Nielsen, Kinga Zor, Anja Boisen
Department of Micro and Nanotechnology, Technical University of Denmark

Introduction: Colon targeted oral drug delivery requires the drug carrier to overcome all the physiological obstacles in the gastrointestinal tract until reaching the colon and the drug can be released from the carrier [1]. Microcontainers have been introduced previously as new drug carriers, which are able to protect the drug from the harsh environment of the stomach and enhance bioavailability and absorption in the small intestine[2]. Moreover, polysaccharide based particle systems (e.g. chitosan, alginate, pectin) have been used previously for colon targeted drug delivery due to enzymatic biodegradation in the large intestine[3].

Aim: In this project, we have investigated the possibility of fabricating an enzyme-sensitive coating, stable at intestinal pH. For this matter, we implement a multi-layer polymeric complex fabricated from chitosan and alginate layer-by-layer assemblies, as enteric coating for microcontainers.

Method: Microcontainers were loaded with the model drug, indomethacin. The loaded microcontainers were spray-coated layer-by-layer with chitosan and alginate. Quartz crystal microbalance with dissipation monitoring (QCM-D) was employed to investigate the fabrication of the layer-by-layer assemblies of chitosan and alginate and disintegration of these assemblies at intestinal pH. Release from the coated microcontainers was then tested in medium with intestinal pH with a μ-Diss profiler to investigate the stability of the coating.

Results: Figure 1 shows the loaded microcontainers with indomethacin and layer-by-layer coatings of chitosan and alginate. QCM-D experiments show the mechanism through which inter-polymer complexes of chitosan and alginate are formed and investigation of disintegration in intestinal pH reveals the stability of the assemblies. SEM images from release study at intestinal pH (Figure 2) and data obtained by release studies gathered from experiments with the μ-Diss profiler confirms the stability of the coating in medium at intestinal pH.

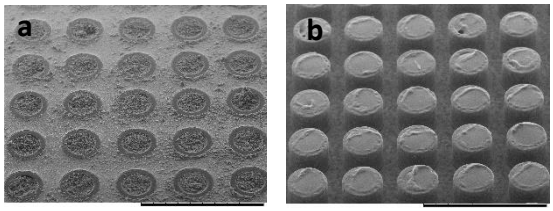


Figure 1- Microcontainers loaded with indomethacin (a) and coated with four layers of chitosan and alginate (b). Scale bar represents 1 mm.

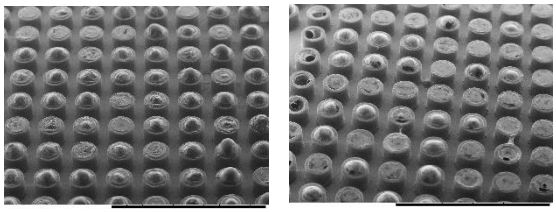


Figure 2- SEM images after release studies after 4 h in pH of 6.5 (a) and 7.5 (b). Scale bar represents 2 mm.

Conclusion: Microcontainers coated with layer-by-layer polymer assemblies as enteric coating show promising results for a new colon targeted oral drug delivery system.

References

1 Ashford, M., & Fell, J. (1994). Targeting drugs to the colon: delivery systems for oral administration. Journal of drug targeting, 2(3), 241-257.

2 Mazzoni, C., Tentor, F., Strindberg, S. A., Nielsen, L. H., Keller, S. S., Alstrøm, T. S., ... & Boisen, A. (2017). From concept to in vivo testing: Microcontainers for oral drug delivery. *Journal of Controlled Release*, *268*, 343-351.

3 Kosaraju, S. L. (2005). Colon targeted delivery systems: review of polysaccharides for encapsulation and delivery. Critical reviews in food science and nutrition, 45(4), 251-258.

Complete knockout of endogenous MDR1 (*ABCB1*) in MDCK wildtype and MDCK-hMDR1 cells by CRISPR-Cas9

Maria Karlgren[1,2], Ivailo Simoff[1], Maria Backlund[1,2], Christine Wegler[1,3], Markus Keiser[4], Niklas Handin[1], Janett Müller[4], Patrik Lundquist[1], Anne-Christine Jareborg[5], Stefan Oswald[4], Per Artursson[1,2]

[1]Department of Pharmacy, Uppsala University, Uppsala, Sweden
[2]Uppsala University Drug Optimization and Pharmaceutical Profiling Platform (UDOPP), Science for Life Laboratory, Uppsala, Sweden
[3] Cardiovascular Metabolic Diseases DMPK, AstraZeneca R&D, Mölndal, Sweden
[4] Department of Clinical Pharmacology, Center of Drug Absorption and Transport (C_DAT), University Medicine of Greifswald, Germany
[5] Department of immunology, genetics and pathology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden

Introduction
Madin-Darby canine kidney (MDCK) II cells transfected with transport proteins are commonly used for studying drug transport. However, endogenous transporters e.g. canine MDR1 (*ABCB1*) complicates the interpretation of transport studies.

Aim
The aim of this study was to establish MDCK cell lines lacking endogenous canine MDR1 (cMDR1) expression using the CRISPR-Cas9 gene editing technology.

Method
MDCK wildtype cells and MDCK cells stably transfected and overexpressing human MDR1 (hMDR1) were transfected simultaneously with three CRISPR-Cas9 vectors targeting three different regions of the canine *ABCB1* gene. After clonal selection and expansion, clones with complete cMDR1 knockout were identified by genomic screening. MDR1 protein was quantified and function was tested using Transwell© experiments and known probe substrates of MDR1.

Results
The genetic analysis revealed that clear homozygous indels occurred in five wildtype and in four MDCK-hMDR1 based clones. One wildtype based clone and one MDCK-hMDR1 based clone, both having a four-nucleotide deletion in exon 4 leading to a frameshift and a truncated protein, were selected for further studies. Protein quantification verified complete knock-out of cMDR1 and no compensatory up regulation of other efflux transporters could be detected. Functional studies using known MDR1 substrates verified absence of cMDR1 activity in the wildtype based clone with efflux ratios around 1 and preserved function of the hMDR1 transporter with efflux ratios ranging from 5 to 360 in the MDCK-hMDR1 based clone.

Conclusion
In conclusion, using CRISPR-Cas9 canine MDR1 has been successfully knocked out in both a MDCK wildtype and in a MDCK-hMDR1 cell line. These two new MDCK cell lines, both with identical deletions in the canine *ABCB1* gene and lack of cMDR1 expression represent excellent in vitro tools for use in drug discovery.

Docetaxel Liposomes – A Formulations Screening Study

Iselin Karlsen, Natasa Skalko-Basnet, Ann Mari Holsæter
Department of Pharmacy, University of Tromsø The Arctic University of Tromsø

Introduction

Docetaxel (DOC) is a potent anticancer drug with several limitations, including poor solubility and the reported serious side effects, attributed to either the drug itself or the solvent used. Thus, it is interesting to entrap the drug into liposomes in order to solubilize the drug and improve the therapeutic outcome.

Aim

The fist aim of this study was to establish a small-scale screening method for preparing and characterizing DOC-liposomes. Secondly, the established methods were applied to investigate the effect of lipid composition on the liposomal drug entrapment.

Methods

DOC-liposomes were made by the thin-film hydration method and size reduced by probe sonication. Centrifugation was used to remove crystallized DOC from the liposome dispersion. The amount of DOC was quantified by HPLC both in the total sample before centrifugation and in the supernatant containing the liposome dispersion after centrifugation. Liposome size and the zeta potential were determined using the Malvern Zetasizer Nanoseries ZS, and lipid recovery in the supernatant was determined by a phosphatidylcholine assay.

Results

When establishing the liposome preparation method, two formulations contained Soy phospholipids (SPC) and a combination of SPC and cholesterol (80:20 w/w) and a DOC:lipid ratio of 1:10 (w/w), were selected. The results obtained clearly demonstrated the reproducibility of the method and the negative effect of cholesterol on the DOC-entrapment (Table 1)

Table 1. Docetaxel-liposome characteristics for the plain SPC and the SPC-Cholesterol liposomes (n=3)

Liposome formulation	Liposome diameter (nm ± SD)	Poly disp. Index (AU ± SD)	Zeta Potential (mV ± SD)	DOC- entrapment (% ± SD)*	Lipid recovery (% ± SD)
SPC-DOC	83.7 ± 4.1	0.27 ± 0.02	-0.19 ± 0.14	103.3 ± 3.3	89.5 ± 2.2
SPC-CHOL-DOC	59.8 ± 3.3	0.21 ± 0.01	-2.14 ± 0.37	25.2 ± 3.0	94.7 ± 1.0

** Values adjustment for the lipid recovery in the supernatant after centrifugation using the Equation:* $DOC\ entrapment\ (\%) = \frac{Recovery\ of\ DOC\ (\%)}{Recovery\ of\ PC\ (\%)}\ x\ 100$

The formulation screening study involved 14 different liposomal formulations where the DOC entrapment varied between 20.2 and 114.6 %. The formulation containing 20% (w/w) positively charge DOTAP was the most promising with the highest drug entrapment, a diameter of 78.0 nm and a zeta potential of 76.3 mV. Thus, the SPC:DOTAP formulation was further investigated varying the concentration of DOTAP and DOC, and the results showed that a DOTAP concentration down to 10% (w/w) DOTAP gave the superior DOC-entrapment, and that the 1:10 (w/w) DOC:lipid ratio was the optimal with regard to obtaining high drug entrapment.

Conclusion

A small-scale probe sonication method for preparing DOC-liposomes was successfully established. Liposomes comprising the cationic lipid DOTAP showed best entrapment when screening different liposome formulations.

The *in vitro* lipolysis-permeation assay predicts *in vivo* performance of carvedilol administered with lipids in dogs

J. Keemink[1], L.C. Alskär[1], A. Parrow[1], P. Johansson[2], B. Abrahamsson[2], C.A.S. Bergström[1]

[1.] Department of Pharmacy, Uppsala University, Sweden
[2.] AstraZeneca R&D Mölndal, Sweden

Introduction
Lipid-based formulations (LBFs) are used to enhance intestinal absorption of poorly water-soluble compounds. Unfortunately, poor understanding of their *in vivo* performance limits the use of LBFs in commercial formulations. To better predict the behavior of LBFs *in vivo*, we recently developed a lipolysis-permeation method that captures the complex dynamics between digestion and absorption in the small intestine [1].

Aim
To explore the ability of the lipolysis-permeation setup to predict the performance of carvedilol predissolved in or co-administered with an LBF in dogs.

Methods
In order to evaluate the suitability of *in vitro* digestion medium used in the lipolysis-permeation setup (i.e. LBF digested with immobilized lipase in simulated intestinal fluids[2]), the solubility of carvedilol was determined in digestion medium and compared to solubility determined in dog intestinal fluids obtained after administration of blank LBF. Subsequently, *in vitro* lipolysis experiments were carried out in the lipolysis-permeation setup, using Caco-2 monolayers as an absorptive membrane, with (F1) carvedilol predissolved in LBF, (F2) carvedilol co-administered with a high dose of LBF and, (F3) carvedilol co-administered with a low dose of LBF. In addition, these formulations were administered to dogs and plasma concentration of carvedilol were determined. Distribution of carvedilol to the aqueous phase of the digestion medium and carvedilol permeated to the receiver compartment were used to predict the *in vivo* exposure of carvedilol in dog plasma.

Results
Carvedilol solubility in digestion medium upon *in vitro* lipolysis of blank LBF was similar to solubility in intestinal dog fluids sampled following administration of the LBF. Aqueous carvedilol concentrations in the digestion medium did not reflect the *in vivo* exposure in dogs. On the contrary, mass transfer of carvedilol across the Caco-2 monolayer during the lipolysis-permeation experiment correctly predicted higher exposure in dog plasma upon administration of F1 and F2 than F3.

Conclusion
Carvedilol transfer across the Caco-2 membrane in the lipolysis-permeation setup accurately reflected carvedilol exposure in dogs.

References
1. Keemink, J., Mårtensson E. & Bergström, C.A.S. A lipolysis-permeation setup for simultaneous study of in vitro digestion and absorption. Revised manuscript submitted,
2. Keemink, J. & Bergström, C. A. S. Caco-2 Cell Conditions Enabling Studies of Drug Absorption from Digestible Lipid-Based Formulations. *Pharm. Res.* 35, (2018).

Predicting oral absorption of lipid-based drug delivery systems using a novel combined lipolysis-permeation *in vitro* model (LipoPerm)

Mette Klitgaard[1], Anette Müllertz[1,2] and Ragna Berthelsen[1]
[1] Department of Pharmacy, University of Copenhagen, Denmark, [2] Bioneer:FARMA

Introduction

Many studies have reported poor *in vitro-in vivo* correlations (IVIVC) when trying to predict the oral performance of poorly water-soluble drugs formulated in lipid-based drug delivery systems (LbDDS). Several of these studies have pointed at the single compartment setup of the *in vitro* lipolysis models with no absorptive sink as a cause for the poor predictive power of the models. In a study by Siqueira *et al.* (2017) the authors were unable to correlate data from an intestinal *in vitro* lipolysis study of four self-nanoemulsifying drug delivery systems (SNEDDS) and an aqueous suspension with cinnarizine to a pharmacokinetic *in vivo* study in rats [1]. The study was used as a frame of reference in the evaluation of a novel combined lipolysis-permeation *in vitro* model, i.e. the LipoPerm model.

Aim

To improve the prediction of oral performance of LbDDS from *in vitro* models by designing and evaluating a combined lipolysis-permeation model.

Methods

The LipoPerm model was designed in an attempt to accommodate for the lack of a proper predictive combined lipolysis-permeation *in vitro* model by using and combining existing models. The aqueous suspension as well as the SNEDDS formulations described by Siqueira *et al.* were tested in the LipoPerm model. Each formulation was exposed to simulated digestive conditions in the regular pH-stat lipolysis model using simulated intestinal fluids and initiating digestion with addition of pancreatic extract of porcine origin. At four specific time-points (0, 15, 30, and 60 min) uninhibited digestion samples were transferred from the lipolysis vessel to Franz diffusion cells equipped with PermeaPad® barriers to study the permeation over a duration of 3 hours. To ensure sink conditions the acceptor medium consisted of phosphate buffered saline with 4 % (w/V) bovine serum albumin (pH 7.4). To evaluate the integrity of the barriers during the LipoPerm studies, calcein was added to the simulated intestinal medium in the lipolysis step and the permeation of cinnarizine from the formulations and calcein in the medium was evaluated simultaneously.

Results

The PermeaPad® barriers were able to maintain integrity when exposed to the digestive conditions and the LipoPerm setup was able to accurately predict the oral performance of four formulations by rank order. Furthermore, a decent multiple level C IVIVC could be obtained to the AUC, C_{max}, and T_{max} of the *in vivo* study.

Conclusions

A combined lipolysis-permation model, the LipoPerm model, was developed and successfully used to predict the oral performance of four tested formulations. Using the LipoPerm model, an IVIVC was achieved for the formulations where there previously was none.

References

[1] Siqueira SD, Mullertz A, Graeser K, Kasten G, Mu H, Rades T. Influence of drug load and physical form of cinnarizine in new SNEDDS dosing regimens: in vivo and in vitro evaluations. AAPS J. 2017;19(2):587-94.

Development of multi-layered polymeric nanofibrous structures with an ultrasound-enhanced electrospinning

A. Kõrkjas[1], I. Laidmäe[1,2], K. Kogermann[1], J. Hunnakko[3], H. J. Nieminen[4], A. Salmi[3], Kai Kronström[3], E. Hæggström[3] and J. Heinämäki[1]

[1]Institute of Pharmacy, Faculty of Medicine, University of Tartu, Estonia

[2]Institute of Biomedicine and Translational Medicine, University of Tartu, Estonia

[3]Electronics Research Laboratory, Department of Physics, University of Helsinki, Finland

[4]Medical Ultrasonics Laboratory, Department of Neuroscience and Biomedical Engineering, Aalto University, Espoo, Finland

Introduction

Multi-layered polymeric nano- and micro-fibrous structures have found uses in tissue engineering and drug delivery applications[1]. Ultrasound-enhanced electrospinning (USES) is a new continuous manufacturing technology to fabricate polymeric nanofibers[2]. In the USES, controlled high-intensity focused US bursts generate a liquid protrusion from the surface of a polymer solution, and the fibers are collected on a grounded plate (Fig 1).

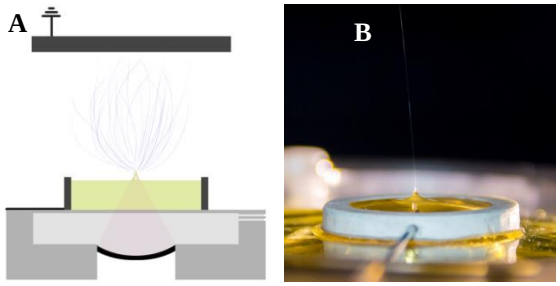


Fig. 1. Needleless ultrasound-enchanced electrospinning (USES): (A) Schematic and (B) ultrasonic fountain and nanofiber formation.

Aim

The aims of the present work were (1) to further develop the USES process in producing nanofibrous mats, and (2) to develop the multi-layered polymeric nanofibrous structures of a water-soluble polymer.

Method

An in-house USES method was used for fabricating the polymeric nanofibrous structures. Polyethylene oxide, PEO (Sigma-Aldrich Inc., St. Louis, U.S.A) (average MW 900,000) was used as a carrier polymer. Optical and scanning electron microscopy (SEM) were used for investigating the fiber size, orientation and separate fibrous layers in the nanomats.

Results

By using two different ultrasonic settings, USES enabled to fabricate the nanofibrous mats with two separate fiber layers with a boundary layer in between (Fig 2). USES allows the formation of the nanofibrous layers in a continuous manner.

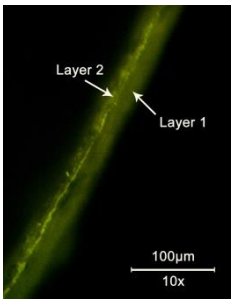


Fig. 2. Optical microscopy image (cross-section) of USES layered "sandwich" nano-fibrous mat. Arrows indicate different nanofibrous layers.

Acknowledgements

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References

1. A. Repanas, S. Andriopoulou, B. Glasmacher, J. Drug Delivery Sci. Technol. 31, 137 (2016).

2. H. Nieminen, I. Laidmäe, A. Salmi, T. Rauhala, T. Paulin, J. Heinämäki, E. Hæggström, A Nature Res. Journal, Scientific Reports 8, Article 4437 (2018).

Monitoring solid-state transformations in amorphous slurries *in situ* with low-frequency Raman spectroscopy

Jaana Koskela[1*], Sara J. Fraser-Miller[2], Joshua J. Sutton[2], Tiina Lipiäinen[1], Keith C. Gordon[2], Clare J. Strachan[1]

[1] Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland
[2] The Dodd-Walls Centre for Photonic and Quantum Technologies, Department of Chemistry, University of Otago, Dunedin, New Zealand

Introduction

Raman spectroscopy is an effective process analytical technique for monitoring both chemical and solid-state properties of pharmaceuticals as the measurements are fast, non-destructive and can be performed even in aqueous environments. Spectral changes associated with solid-state transformations can, however, be subtle and difficult to detect in mid-frequency Raman spectra (400 – 4000 cm^{-1}). Low-frequency Raman instrumentation is a recently available method that probes solid-state related lattice vibrations and can increase solid-state sensitivity. This technique may be suitable for *in situ* polymorph analysis of pharmaceutical slurries. Furthermore, low-frequency and mid-frequency Raman data can be obtained simultaneously. In slurries, where the drug is dispersed in an aqueous external phase, solid-state transformations are accelerated as the conversion can occur directly from the solid or via solution, making slurry studies useful for exhaustive polymorph and stability screening. The crystallisation behaviour in slurries can be complex: e.g. amorphous indomethacin (IND) has been shown to convert to the α- form at 25 °C, pH 1.2 and to the ε-, ζ- and η- forms at 5 °C, pH 1.2.[1] *In situ* low-frequency Raman spectroscopy could allow better understanding of the crystallisation behaviour of amorphous slurries, but has not yet been applied for this purpose.

Aims

1) To explore the potential of *in situ* low-frequency Raman spectroscopy for monitoring solid-state transformations in amorphous slurries
2) To gain a more insight into the solid-state conversions occurring in amorphous IND slurries

Methods

Amorphous IND was prepared by quench cooling, and amorphous IND slurries were prepared with a 30 mg/ml concentration in aqueous HCl solution (pH 1.2) and kept at 5 or 25 °C. Measurements were carried out with a home-built Raman spectrometer with a 785 nm excitation laser. Low- and mid-frequency spectral data were collected simultaneously allowing a direct comparison of the two regions. *In situ* measurements were conducted up to 24 h by collecting one spectrum per minute. Multivariate analysis methods were applied to follow the transformations.

Results

Solid-state transformations occurred faster with 25 °C samples when compared to 5 °C samples. Based on the analysis, at 25 °C the samples converted directly towards the α- form while at 5 °C the samples went through two transitions. However, the transitions at 5 °C were not complete, mixtures of more than one polymorph were always present with differences in the relative abundance observed.

Conclusion

Low-frequency Raman spectroscopy was suitable for monitoring IND transformations in slurries. It is a convenient *in situ* monitoring tool for analysing both chemical and solid-state properties of pharmaceutical slurries.

References

1. Surwase S. A. *et al*. Mol Pharmaceutics (2013) 10: 4472-4480

Application of microcontainers for targeting intestinal lesions found in inflammatory bowel disease

Maja Nørgaard Kristensen[1], Nazila Kamaly[2], Anja Boisen[2], Thomas Rades[1], and Anette Müllertz[1,3]
[1]Department of Pharmacy, University of Copenhagen, Denmark
[2]DTU Nanotech, Technical University of Denmark, Denmark
[3]Bioneer:FARMA

Introduction

Inflammatory bowel disease (IBD) is a chronic condition of the intestine that arises mainly due to a dysregulated immune system. Different drug compounds exist for the treatment of IBD, but most of the drug formulations are ineffective and side effects are often an issue [1]. The glucocorticoid, budesonide, is an example of a drug used in the treatment of IBD that is associated with severe side effects after long-term use [2].
Application of micrometre sized containers (microcontainers) as a new drug delivery system (DDS) that targets only the inflamed areas of the bowel could improve the treatment of IBD. Microcontainers are cylindrical containers that are open in one end, thus allowing filling of a drug compound [2]. The open end of the microcontainers is sealed with a suitable polymer lid, which can be dissolved in the gut.

Aim

To investigate the release profile of the model drug, budesonide, from poly(lactic-co-glycolic acid) (PLGA) coated microcontainers.

Method

Microcontainers were loaded with budesonide and coated with 0.5% (w/w) PLGA in dichloromethane (DCM) using an ultrasonic spray coater (Sono-Tek, USA). A small pilot study of the budesonide release and dissolution from these containers was performed with a µDISS Profiler™. Four chips with microcontainers (625 containers on each chip) were tested in phosphate buffer; for drug release, two chips were tested at pH 3, while the other two were tested at pH 7. These results will be used to optimize the DDS in order to obtain the desired release profile. The µDISS Profiler™ will be used to determine the solubility in different types of biorelevant media.

Results

Budesonide was successfully loaded into the microcontainers, and visualized with a scanning electron microscopy (SEM), (Fig. 1A). The PLGA coated microcontainers (Fig. 1B) did not release budesonide when investigated in phosphate buffer (both pH 3 and 7) for 13 h at 37 °C using a µDISS Profiler™. The PLGA coat had dissolved in small segments of each chip, however, budesonide was not released from these microcontainers. This may be attributed to the lack of solubility of budesonide in aqueous media and further investigations are ongoing to improve its release profile from the microcontainers.

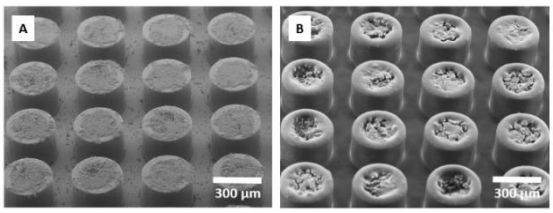

Figure 1. Scanning electron microscopy images of microcontainers loaded with budesonide (A), and further coated with 0.5% w/w PLGA in DCM (B). These microcontainers have dimensions from 200 to 300 µm.

Temporary conclusions and future perspectives

Polymeric microcontainers with biodegradable PLGA lids for the delivery of anti-IBD drugs were successfully fabricated and analysed by SEM. SEM experiments revealed successful dissolution of PLGA lids. Budesonide drug release from the containers was tested at two pH levels and current work is ongoing to optimise its release, in particular in both human and rat biorelevant media.

References

[1] Collnot E-M, Ali H, Lehr C-M. Nano- and microparticulate drug carriers for targeting of the inflamed intestinal mucosa. Journal of controlled release: official journal of the Controlled Release Society. 2012;161(2):235.
[2] Hussain A, Weigmann B, Collnot E-M, Kahn SA, Windbergs M, Lehr C-M. Budesonide loaded PLGA nanoparticles for targeting the inflamed intestinal mucosa – pharmaceutical characterization and fluorescence imaging. Pharmaceutical research. 2016;33(5):1085-1092.
[3] Nielsen LH, Melero A, Keller SS, Jacobsen J, Garrigues T, Rades T, et al. Polymeric microcontainers improve oral bioavailability of furosemide. International journal of pharmaceutics. 2016;504(1-2):98.

Oral delivery of oligonucleotides for local treatment of inflammatory bowel disease

Jana Kubačková[1], Ondřej Holas[1], Jarmila Zbytovská[1], Petr Pávek[1], Anette Müllertz[2]
[1]Faculty of Pharmacy, Charles University, Czech Republic
[2]Department of Pharmacy, University of Copenhagen, Denmark

INTORDUCTION

Inflammatory bowel disease (IBD) describes pathological conditions characterised by inappropriate and sustained activation of the mucosal immune system of the small intestine and/or colon[1]. Local treatment is preferred over systematic delivery often accompanied with undesired side effects. In IBD, phagocytic immune cells infiltrate the site of inflammation[2]. By delivering an anti-inflammatory acting miRNA oligonucleotide, production of proinflammatory cytokines by these immune cells would decrease, and the inflammatory process itself would be suppressed. However, delivery of instable negatively charged macromolecular oligonucleotides represents a considerable challenge[3]. Self-nanoemulsifying drug delivery system have been utilised to deliver a hydrophobic complex of oligonucleotides. Use of permeation enhancers, submicron droplet size and hydrophobic nature promotes the delivery to phagocytic immune cells through inflamed leaky epithelium of the intestine[2,4].

AIM

The current objective is to create a hydrophobic complex of an oligonucleotide with a cationic lipid. Further to load the complex into a (SNEDDS) and to characterise both systems.

METHODS

The complex was prepared by an extraction method, characterised by atomic force microscopy (AFM). Further it was loaded into SNEDDS, size and zeta potential were measured by dynamic light scaterring and laser Doppler electrophoresis respectively.

RESULTS

The complexes were prepared from a 20-nucleotide long oligomer and two cationic lipids dimethyldioctadecylammonium bromide (DDAB) and 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) with yield over 95% for molar ratio 1:60. The size of the complexes was estimated by AFM, 75-127 nm and 33- 62 nm, for DOTAP and DDAB complex respectively. Solubility of complexes was shown to be 200 nmol of complexed oligonucleotides per gram of SNEDDS preconcentrate. The size and zeta potential of dispersed SNEDDS show no significant difference between blank and loaded formulation, suggesting neutral charge of the complex.

CONCLUSION

The complexes of an oligonucleotide and cationic lipids were prepared and characterised. SNEDDS preconcentrate can be sufficiently loaded with the complexes and dispersed to form nanoemulsion.

REFERENCES

1. Podolasky, D. K. N. Engl. J. Med. (2002) 347: 417–429
2. Hua, S. *et al*.. Nanomedicine (2015)11: 1117–1132
3. Lam, *J. K. W. e*t al Mol. Ther. - Nucleic Acids (2015) 4: 1-20
4. Müllertz, A. *et al.* J. Pharm. Pharmacol. (2010) 62: 1622–1636

Preliminary experiences with release from 3D-printed core-shell tablets

Bendik N. Kvingan, Ingunn Tho
Department of Pharmacy, University of Oslo, Norway

Introduction
Drugs today are typically designed for the average patient. 3D-printing is a tool allowing preparation of dosage forms with personalized drug combinations, dose and release properties that are more tailored to the individual patient. 3D-printed objects may consist of horizontal layers building a wall or shell around a core with infill support structures. The thickness of the walls, the density of the infill and the height of the horizontal layers are adjustable parameters of the 3D-printed object. Designing a tablet for 3D-printing, these parameters would be interesting to explore with respect to drug release from a core-shell tablet.

Aim
To evaluate the effect of infill density, layer height and wall thickness on the release profile of FDM 3D-printed core-shell tablets based on PVA using a hydrophilic dye as model drug.

Method
Tablets with a core-shell structure with a diameter of 4 mm were designed in a CAD-software, sliced in Cura® and printed with a 3D printer from Ultimaker®. A 2^3 factorial design was applied investigating the design variables infill density, layer height and wall thickness. The tablets were printed in layers and loaded with the fluorescence marker 5(6)-carboxyfluorescein (5 mg/ml in ethanol) halfway through the print. For release studies each tablet was tested in 200ml phosphate buffer pH 6.8 under stirring, and samples were withdrawn every hour, and the quantified based on fluorescence. The Unscrambler® 9.8 was used to analyse the main effects and interactions of the data in a multivariate manner.

Results
The tablets showed a diversity in release profiles after 1, 2 and 3 hours respectively, with all tablets completely dissolved within 4 to 5 hours. Regression analysis revealed correlation between the variables, including some of their interactions, and the amount of released fluorescence marker at the given sample times. Wall thickness was found to have a significant influence on the amount released after 2 hours, whereas all variables and two interactions correlated with the amount released after 3 hours.

Conclusion
Infill density, layer height and wall thickness all had an effect on the release profile of the 3D-printed tablets, and affected the release in different ways throughout the dissolution process. Adjustment of these variables seems promising for customizing the release of 3D-printed core-shell tablets.

Ω3 PUFAs as protectants against paracetamol-induced neurodevelopmental toxicity

Nils A. Labba[1,2], Mussie G. Hadera[1,2], Ragnhild E. Paulsen[1,2]
[1]Neurobiology & Toxicology group, Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo, Norway
[2]PharmaTox Strategic Research Initiative, Faculty of Mathematics and Natural Sciences, University of Oslo, Norway

Introduction

Peripartal exposure to paracetamol in high doses or over long periods has in recent years been found to correlate with an increased incidence of ADHD in the offspring[1]. The metabolites of paracetamol are known to interact with the endocannabinoid and serotonergic systems, in addition to influencing BDNF expression[2]. Additionally, the CYP450 enzymes responsible for generation of NAPQI, the canonically hepatotoxic metabolite of paracetamol, have in recent years been found to be expressed in neural tissue[2]. The interactomes of paracetamol and its metabolites intersect the interactomes of the of marine-derived ω3 PUFAs and their corresponding metabolites at several junctions[2,3]. Peripartal deficiency in ω3 PUFAs has been found to correlate with a higher incidence of ADHD, with rescue studies indicating that supplementation ameliorates the deficiency effect[3]. Ω3 PUFA metabolites antagonize the effects of paracetamol-derived metabolites at three different junctions in the endocannabinoid system, in addition to both directly and indirectly counteracting the effects of paracetamol on the serotonergic system[2,3]. Ω3 PUFAs acts as a potent antioxidants, and their metabolites, the specialized pro-resolving mediators, act as potent anti-inflammatory and inflammation-limiting signal mediators[3]. Ω3 PUFAs have also been found to normalize damage-induced reduction in neuronal BDNF expression, in addition to eliciting effects similar to that of BDNF through its metabolites[3]. On the basis of these observations we hypothesize that paracetamol, in the context of early neurodevelopment, is eliciting subtle neurotoxic effects which ω3 PUFAs might be capable of protecting against.

Aim

Establish neurotoxic effects of paracetamol in the context of early neurodevelopment, and investigate whether ω3 PUFAs protects against these effects.

Methods

Employ viability assays, qPCR and long-term live-cell imaging on E17 chicken cerebellar granule neuronal culture exposed to paracetamol and ω3 PUFAs to investigate whether exposure changes cell viability, gene expression, gross morphology, neurite dynamics, and cell motility. Follow up on these results with agonists/antagonists of specific targets in order to attempt to establish mechanisms.

Results

Paracetamol has no effect on cell viability up to 1600µM over 72 hours, but reduces neurite length, neurite branch-point numbers, cell-body size and cell-motility in a dose-dependent manner from 100µM to 1600µM over the course of a week. Paracetamol also reduces cell-body count by up to 50% in a dose-independent manner over the course of a week. Paracetamol reduces expression of BDNF by 50% at 200µM, with 10µg/mL ω3 PUFA nanoemulsion reversing observed reduction.

Conclusion

Preliminary data suggests that paracetamol inflicts developmentally neurotoxic damage which can in part be reversed by ω3 PUFAs.

References

1. Masarwa R & Matok I, et al (2018) Prenatal Exposure to Acetaminophen and Risk for Attention Deficit Hyperactivity Disorder and Autistic Spectrum Disorder: A Systematic Review, Meta-Analysis, and Meta-Regression Analysis of Cohort Studies. Am J Epidemiol. doi: 10.1093/aje/kwy086
2. Candido KD & Knezevic NN, et al (2017) Pharmacology of Acetaminophen, Nonsteroidal Antiinflammatory Drugs, and Steroid Medications: Implications for Anesthesia or Unique Associated Risks. Anesthesiol Clin. DOI: 10.1016/j.anclin.2017.01.020
3. Dyall S (2015) Long-chain omega-3 fatty acids and the brain: A review of the independent and shared effects of EPA, DPA and DHA. Frontiers in Aging Neuroscience. DOI:10.3389/fnagi.2015.00052

Biofilm formation and antibiofilm activity testing of electrospun drug delivery systems (DDS) for infected wounds

Georg-Marten Lanno[1], Celia Teresa Pozo Ramos[2], Liis Preem[2], Marta Putriņš[1], Tanel Tenson[1], Karin Kogermann[1,2]

[1]Institute of Technology, University of Tartu, Estonia

[2] Institute of Pharmacy, University of Tartu, Estonia

Introduction

In wound care applications the electrospun DDS have several beneficial properties such as high surface area, porosity, oxygen permeability and possibility to include antibacterial agents. However, the electrospun polymeric matrix could be a preferred substrate for the bacterial biofilm formation. Therefore, it is important to understand whether the developed antibacterial electrospun DDS are able to prevent the biofilm formation.

Aim

The main aim was to investigate the bacterial attachment and biofilm formation on the differently designed electrospun fiber mats and understand the effect of different fiber structures. Hence to reveal possible antibiofilm activity of the antibacterial DDS.

Method

Porous fibers were prepared using electrospinning (ES) with tetrahydrofuran:dimethylsulfoxide (THF:DMSO) solvent system and nonporous acetic acid:formic acid (AA:FA) solvent system. Chloraphenicol (CAM) was used as a model antibacterial agent. Biofilm formation and antibiofilm activity testing on ES fiber mat from polycaprolactone (PCL) was performed using developed *in vitro* method.[1] Planktonic bacteria in each time point were always plated as controls in order to verify the growth of bacteria in each condition.

Results

E. coli biofilm formation on different fiber mats revealed that the ES fiber mats without any CAM provide good surfaces for biofilm formation, whereas the antibiofilm activity of CAM was seen in all drug-loaded fiber samples at all timepoints. Also, there are statistically significant differences in the colony forming unit values between differently designed fiber mats after 24 h. Less biofilm was formed on the porous microfibers (THF:DMSO system) than nonporous nanofibers (AA:FA system).

Conclusion

The biofilm formation and antibiofilm activity are affected by the structural differences between the porous and non-porous electrospun mats. The effect of structural properties on the release and antibacterial and antibiofilm activity of novel electrospun DDS needs to be understood in order to develop effective drug preparations for infected wounds.

References

1. Preem, L. et al Mol. Pharm. (2017) 14: (12), 4417–4430.

Achieving targeted release of freeze-dried probiotic strains by granulation, extrusion, spheronization and fluid bed coating

Natashia M. Y. Larsen[1], Aslihan Caglayan[1], Ibrahim Caglayan[1], Daniel Bar-Shalom[1,2], Anette Müllertz[1,2]
[1]Department of Pharmacy, University of Copenhagen, Denmark
[2]Bioneer:FARMA, University of Copenhagen, Denmark

Introduction: Many probiotic strains are sensitive to acid, bile and enzymes found in the human gastro-intestinal tract (GIT) making it a challenge to deliver viable probiotics to the ileum and/or colon by the oral route[1]. Freeze-drying is often used to obtain long term viability of probiotics resulting in irregular particles difficult to coat[2,3]. Additionally, it is a challenge to keep probiotic cells viable during down-stream formulation processes[3].

Aims: The first aim was to achieve targeted release of viable probiotics by pelletizing and coating freeze-dried bacterial strains using riboflavin as a marker for release. The second aim was to set up a dynamic 3-step *in vitro* model simulating the conditions in the human gastric, duodenum/jejunum and ileum compartments to evaluate targeted release of the formulation.

Methods: Granulation, extrusion and spheronization was used to obtain round uniform pellets from a dry mixture of freeze-dried probiotic powder, microcrystalline cellulose, dextrin, $CaCl_2$ and an alginate granulation solution. Fluid bed coating was used to apply Eudragit S100 to the pellets to obtain pH dependent targeted delivery. Targeted delivery of the probiotics was evaluated in the dynamic 3-step *in vitro* model by release of riboflavin quantified by HPLC. Additionally fluorescence-activated cell sorting (FACS) was used to assess survival of the probiotic cells at different time points.

Results: No significant difference in viability of the probiotic cells was found before and after pelletization. Quantification of riboflavin in the simulated gastric and duodenum/jejunum media showed < 5 % release (Fig. 1) indicating that the probiotic cells are protected against acid and bile. Riboflavin release significantly increased during incubation in simulated ileum medium indicating that Eudragit S100 polymer coatings can be used to obtain targeted delivery of probiotics.

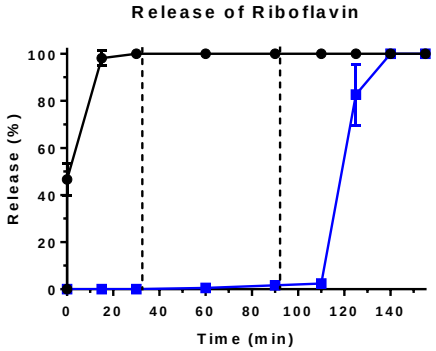

Fig. 1: Release of riboflavin from probiotic pellets. Pellets were incubated in gastric media from 0-30 min, in duodenum/jejunum media from 31-90 min and in ileum media from 91-150 min. *Black:* Control, *Blue:* Eudragit S100, 15 % WG. Data are represented as mean (±SD), n=3.

Conclusion: These findings show that freeze-dried probiotic strains can be formulated to improve their survival through the GIT and obtain targeted release.

References:

1. Ding & Shah, J. Food. Sci. 2007, 72(9), 446-450.
2. Carvalho, A. et al., Int. Dairy J. 2004, 14(10), 835-847
3. Morgan, C. A. et al., J. Microbiol. Meth. 2006, 66(2), 183-193

Identification and quantification of glucose degradation products (GDPs) occuring during heat-sterilization of glucose solutions

S. Leitzen[1], M. Vogel[1], T. Zapf[1], M. Brandl[2], J. Stingl[1]

[1]Federal Institute for Drugs and Medical Devices, Bonn (Germany)

[2]Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Odense (Denmark)

Introduction

Glucose degradation products (GDPs) formed during heat sterilization of glucose solutions exert cytotoxic effects and promote the formation of advanced glycation end products in the peritoneal cavity, which leads to a limitation of long-term application of continuous ambulatory peritoneal dialysis [1]. The composition and concentration of GDPs in glucose solutions after heat-sterilization must be known to evaluate their biological effects concerning parenteral administration.

Aim

This poster describes the identification and quantification of the α-dicarbonyl glucose degradation products glyoxal, methylglyoxal (MGO), glucosone, 3-deoxyglucosone (3-DG), 3,4-dideoxyglucosone-3-en (3,4-DGE) and 3-deoxygalactosone (3-DGal) by HPLC/MS-MS. For this purpose, the selected GDPs were converted with o-phenylenediamine (OPD) to give the respective quinoxaline derivatives, which were selectively monitored by HPLC-MS/MS [1].

Method

A series of dilutions with a volume of 100 µL in each Vial was produced containing concentrations from1 ng/mL to 1000 ng/mL. For the derivatization step a solution of 60 µg/mL OPD in Methanol was prepared. 100 µL of each GDP dilution were mixed with 100 µL of the OPD solution to a volume of 200 µL. Because of unforeseeable loss of sample constituents an internal standard is used. The standard that is used in this method is 2,3-dimethylquioxaline. 20 µL of the internal standard were added to each dilution of methylglyoxal in a concentration of 0.5 µg/mL. The derivatized series of dilutions of methylglyoxal with added internal standard was incubated at room temperature for 24h.

Results

The method's limit of detection (LOD) was set at 1 ng/mL and the limit of quantitation (LOQ) at 10 ng/mL, respectively. Linear calibration curves were performed at concentration levels between 1 ng/mL – 1000 ng/mL (R=0.99) [2,3].

Identification and quantification of GDPs methylglyoxal (MGO), glyoxal, 3-deoxyglucosone (3-DG), 3,4-dideoxyglucosone-3-en (3,4-DGE), glucosone, and 3-deoxygalactosone (3-DGal) are important tools to uncover and avoid high amounts of harmful substances, that will be administered parenteral. The present method validation suggests to stay in a working range between 1 ng/mL – 1000 ng/mL (R=0.99).

The results clearly show, that the analytes, built in the derivatization step, are stable at room temperature for at least 24 hours after preparation while standing in the dark.

Conclusion

The results suggest, that this method of simultaneous testing is a successful possibility to reliably determine identification and quantification of α-dicarbonyles via HPLC-MS/MS.

References

[1] Mittelmaier, S. *et al*; Journal of Chromatography B, 878 (2010) 877–882 ; doi:10.1016/j.jchromb.2010.02.004

[2] http://www.ich.org/products/guidelines/quality/quality-single/article/validation-of-analytical-procedures-text-and-methodology.html; last access: 30 November 2018

[3] www.kromidas.de/Uploads/Dokumente/ValidierunginderAnalytik.pdf; last access: 30 November 2018

Initial leuprolide acetate release from *in situ* forming implants as studied by UV-Vis imaging

Zhuoxuan Li[1], Huiling Mu[1], Henrik Jensen[1], Susan Weng Larsen[1], Jesper Østergaard[1]
[1] Department of Pharmacy, University of Copenhagen.

Introduction

A key challenge in the development of peptide and protein injectables is the lack of bio-predictive *in vitro* drug release testing approaches. A step toward more biorelevant, and possibly, biopredictive models include the introduction of a matrix emulating the subcutaneous (SC) matrix. Hyaluronic acid (HA), a component of human SC tissue matrix, has been proposed as a potential tissue surrogate [1]. UV imaging is currently explored as a tool for monitoring and quantifying drug release and diffusion processes in gel like systems [2]. To this end preliminary studies point to the suitability of UV-Vis imaging technique for detecting spatially and temporally resolved the occurrence of polymer precipitation of PLGA *in situ* forming implants [3].

Aim

Subcutaneous (SC) injection is an established route administration for long acting depot formulations of leuprolide acetate (LA). The objective was to obtain more details about the initial LA release from PLGA *in situ* forming implants and to assess the potential of UV-Vis imaging to characterize drug release from long-acting SC injectables.

Method

PLGA *in situ* forming implants contained 3% (w/w) of LA, 28% (w/w) of PLGA and 69% of NMP (w/w). An ActiPix D200 UV-Vis imaging system (CMOS chip size: 28 × 28 mm^2, effective pixel size: 13.5 × 13.5 μm^2) with a custom designed quartz cell was used for LA formulation imaging. HA matrix was prepared by adding 200 mg of HA in 20 ml of phosphate buffer (0.067 M, pH 7.4). A syringe pump was used to deliver 100 µl of the PLGA-NMP preformulations at the rate of 0.25 ml/min. Dual-wavelength imaging (n = 3) was employed using LEDs at 280 and 525 nm. Images were recorded at a rate of 1 frame per s at 34 °C for 3 h.

Results

Initially, imaging at 525 nm showed that the optically translucent LA depot allowed more light to reach the detector (low optical density). As the solvent diffused away from depots and polymer precipitated, an increasingly higher absorbance was observed, indicating rapid phase separation occurring during the first 5 min. The UV absorbance maps at 280 nm show the release of LA and subsequent diffusion in HA solutions. Based on a calibration curve, it is possible to quantify the concentrations of released LA in the vicinity of the depot. Depots exhibited similar in vitro release profiles in the triplicate tests. This shows that the method possesses good repeatability. Insert a comment to the effect of having the HA in the medium (relative to buffer and/or agarose).

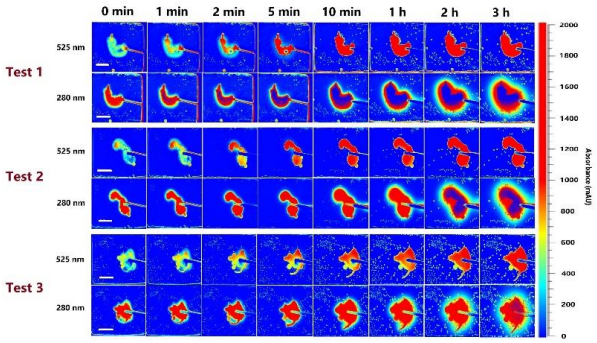

Fig 1: Absorbance maps observed by Vis imaging at 525 nm and UV imaging at 280 nm in 10 mg/ml HA solution with PLGA *in situ* forming implants containing 3 wt % LA (n = 3).

Conclusion

The results indicate that UV-Vis imaging is feasible for concomitant monitoring drug release and phase separation of in situ forming implants. Information on the PLGA precipitation, drug release and diffusion into the matrix can be acquired using this approach. Detection and quantification of initial LA release is possible, showing that the method is sufficiently sensitive even for long acting depots. Further development of the model is needed to follow LA release over prolonged time periods (~4 weeks) from in situ forming implants.

References

1. Kinnunen, M. et al. J. Control. Release (2015) 214: 94-102.
2. Østergaard, J. J. Pharm. Biomed. Anal. (2018) 147: 140-148.
3. Sun, Y. et al. J. Pharm. Biomed. Anal. (2017) 150: 95-106.

In situ formation of polymeric glass solutions: A comparison between conductive and radiant heating

Flemming Morsch[1*], Nele-Johanna Hempel[1], Matthias M. Knopp[2], Ragna Berthelsen[1], and Korbinian Löbmann[1]
[1]Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark
[2]Bioneer:FARMA, Department of Pharmacy, Copenhagen, Denmark

Introduction

Poor aqueous solubility of drugs intended for oral administration is a major challenge for the pharmaceutical industry. By increasing the drugs apparent aqueous solubility and dissolution rate, amorphous drug delivery systems constitute a suitable strategy to overcome this obstacle. However, the major shortcoming of amorphous drug delivery systems is their instability, leading to recrystallization of the drug [1]. The *in situ* formation of an amorphous solid dispersion is a promising approach to circumvent this problem. Here, a stable dispersion of crystalline drug and a polymer is prepared, which can subsequently be amorphized on demand. At temperatures above the glass transition temperature (T_g) of the solid dispersion (46°C), the molecular mobility increases and drug is able to fuse with the polymer [2]. This temperature threshold can either be overcome by microwave radiation, generating heat within the entire tablet by dielectric heating, or by conductive heating from an external heating source, e.g. an oven [3].

Aim

To investigate the influence of the heat transfer mechanism on *in situ* amorphization of drugs in a polymeric solid dispersion.

Method

Tablets of 100 mg, containing 30% (w/w) of celecoxib, 69.5% (w/w) PVP K17 and 0.5% (w/w) magnesium stearate were compacted with a single punch compaction simulator. The compacts were conditioned for two weeks at 75% relative humidity (RH) and ambient temperature. Subsequently, the compacts were heated in intervals of 1-10 minutes using a microwave oven (1000 W) or a preheated electrical furnace (100 °C). The amorphous content of celecoxib was quantified using transmission Raman spectroscopy.

Results and Discussion

The polymer absorbs water during the humid storage, which enables the absorption of the microwave irradiation, resulting in a high thermal conductivity. Furthermore, the pre-conditioning allowed some of the drug to dissolve into the polymer prior to heating, resulting in ~ 15% amorphization of the drug. Using the microwave oven, it was possible to achieve 62% amorphization of celecoxib after 10 min of microwaving. In comparison, the amorphous content (15 %) did not increase when using the furnace over a period of 10 min. Thus, the radiant (internal) heating of the compacts, using microwaves, appears to be necessary to create sufficient heat inside the compact to allow additional amorphization of the drug within the polymeric matrix. Hence, under the tested conditions, conductive heating using an electrical furnace was not a feasible approach for the amorphization of the drug.

Conclusion

The current study showed that radiant heating represents a more efficient mechanism of heating to obtain *in situ* amorphization, compared to conductive heating.

Acknowledgement

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References

[1] S. Baghel, *J. Pharm. Sci.*, vol. 105, no. 9, pp. 2527–2544, Sep. 2016.

[2] M. Doreth *et al.*, *Int. J. Pharm.*, vol. 519, no. 1–2, pp. 343–351, 2017.

[3] C. O. Kappe, *Angew. Chemie Int. Ed.*, vol. 43, no. 46, pp. 6250–6284, Nov. 2004.

Amphiphilic properties of drug molecules and their self-assembly in presence of phospholipids

Vahid Forooqi Motlaq, Magnus Bergström

Department of Pharmacy, Uppsala University, Sweden

Introduction

Many pharmacologically active compounds consist of amphiphilic molecules and possess many similar properties to ordinary surface active agents.[1-3] Amphiphilic drugs may be found within several classes of drugs including tranquilizers, analgesics, antibiotics, antidepressants, antihistamines, local anaesthetics, anti-inflammatory drugs and anticancer drugs. The amphiphilic nature of these molecules is believed to play a crucial role for their pharmacological activity as well as important properties related to toxicity and haemolysis.[2]

On the other hand, the self-assembly behaviour of amphiphilic drugs are expected to largely depend on the presence of additional surfactant or phospholipid components. While hydrophobic components are expected to be solubilized into the hydrophobic cores of micelles and bilayers, amphiphilic molecules may mix with surfactants and phospholipids and so largely influence the structural behaviour of self-assembled aggregates.[2]

Aim

Investigating the self-assembly properties of amphiphilic drugs in presence of surfactant, phospholipids and the effect of environmental variable on the process. Determining the influence of presence of amphiphilic drug on the structural deformation of bilayer membrane.

Method

Small angle scattering techniques (SLS, DLS, SANS and SAXS) are mainly utilized to investigate the self-associated aggregates and their correlations. By means of analysing data with various geometrical models for the self-associated aggregates, makes it is possible to generate detailed structural information, such as aggregation number, geometrical shape, flexibility and polydispersity of aggregates and complexes that are formed.

Result

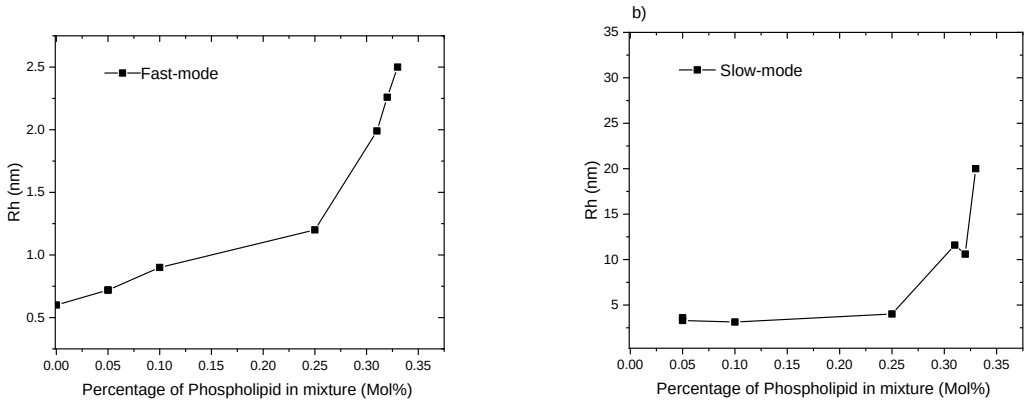


Figure 1: Hydrodynamic radii of two population of aggregates formed in mixture of Amitriptyline-DOPC (total concentration [0.16] M), measured with dynamic light scattering. a) Small size population (fast mode), b) Large size population (Slow mode).

References

1.Attwood, D., The mode of association of amphiphilic drugs in aqueous solution. *Advances in Colloid and Interface Science* **1995,** *55* (Supplement C), 271-303.
2.Schreier, S.; Malheiros, S. V. P.; de Paula, E., Surface active drugs: self-association and interaction with membranes and surfactants. Physicochemical and biological aspects. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **2000,** *1508* (1), 210-234.
3.Sosnik, A., Drug self-assembly: A phenomenon at the nanometer scale with major impact in the structure–biological properties relationship and the treatment of disease. *Progress in Materials Science* **2016,** *82* (Supplement C), 39-82.

Atropine loaded chitosan nanoparticles for transdermal delivery via Microneedle technique

Rawand A. Mustafa[1], Kuldeep Bansal[1], Didem Şen Karaman[2], Jessica M.Rosenholm[1]
[1]Pharmaceutical Sciences Laboratory, Faculty of Science and Engineering, Åbo Akademi University, Finland
[2]Department of Biomedical Engineering, Faculty of Engineering and Architecture, Izmir Katib Celebi University, Turkey

Introduction

Transdermal drug delivery systems (TDDS) have gained profound attention among scientists due to the advantages it holds over more preferred conventional routes of administration (e.g. oral). Despite several advantages of TDDS, poor permeation of drugs through skin limits the wide applicability of this approach. Recently, *drug loaded nano-carriers* demonstrate high penetration rate through skin and thus, they could be an alternative approach to overcome the skin barrier.

Aim

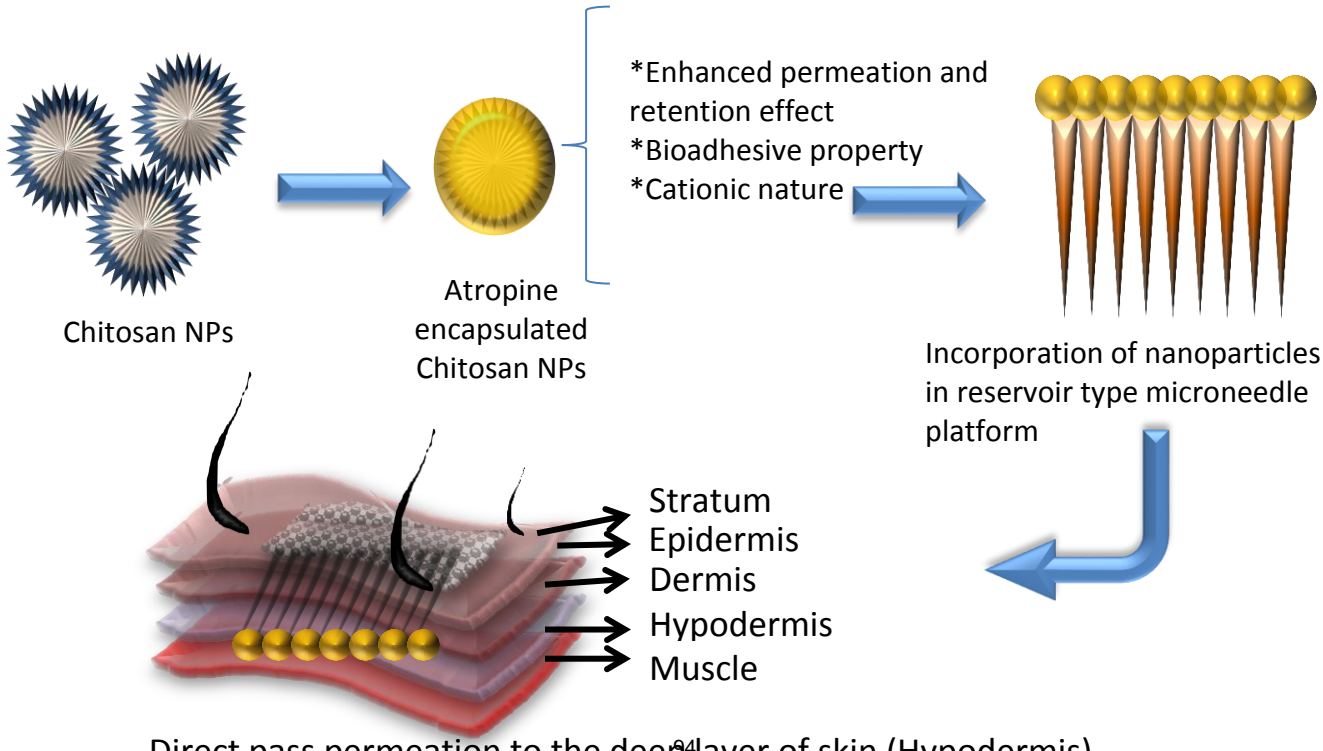
To design suitable transdermal carrier systems to deliver pharmacologically active substances using atropine as model drug, whose delivery is otherwise limited through other routes. The new dosage form could improve the bioavailability of incorporated drug(s); provide controlled drug release, reduce side effects and dosage frequency.

Method

Ionic gelation technique will be used for the fabrication of chitosan nanoparticles (NPs). Drug loaded NPs will be characterized for their loading efficiency, size, surface charge, morphology, skin permeation and cytotoxicity *in vitro.* Permeability and cytotoxicity studies will be carried out with the aid of Franz cell diffusion method and fibroblast cell lines as a model skin cells. Microneedles loaded with prepared nano carrier system will also be used to investigate their capability in increasing the penetration of drugs and/or particles through skin. *Ex vivo* testing (primarily pig skins) of prepared drug delivery systems will also be carried out.

Conclusion

The expected outcomes will be successful nanoformulation of atropine as model drug in chitosan NPs. Improved skin penetration is also envisioned as result of applying these nanoformulations on artificial and real skin models. Further permeability enhancement is expected upon incorporation of the nanoformulations into microneedles.



Etoposide transport in MDCKII-MRP2 cells is unaffected by P-gp expression and commonly used pharmaceutical excipients

Salli Nielsen, Anne Marijke Westerhoff, Lorraine Gaenaelle Gé, Krestine Lundgaard Carlsen, Maria Diana Læssøe Pedersen, Carsten Uhd Nielsen
Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Denmark

Introduction

Efflux transporters such as P-gp, BCRP, and MRP2 are limiting intestinal transport of various drug substances and contribute to poor absorption and low bioavailability of drugs[1]. Several non-ionic surfactants, which are excipients in drug formulations, have been shown to inhibit efflux transporters. Especially, for P-gp it has been shown that non-ionic surfactants decrease P-gp-mediated transport[2-3]. In contrast, little is known about the influence of non-ionic surfactants on MRP2-mediated transport.

Aim

Firstly, to setup a MRP2-specific cell-based assay and secondly to investigate the ability of non-ionic surfactants to inhibit MRP2-mediated transport *in vitro* in MDCKII-MRP2 cells.

Method

Transport studies across MDCKII-MRP2 cell monolayers were performed using ^{3}H-etoposide and ^{3}H-digoxin. 13 different non-ionic surfactants, including polysorbates (PS), cremophor EL, vitamin E-TPGS, and *n*-nonyl β-D-glucopyranoside, were investigated. Barrier function of the cell monolayers was investigated measuring TEER and transport of ^{14}C-glycine. The amount of isotope was quantified using liquid scintillation counting.

Results

In MDCKII-MRP2 cells, etoposide and digoxin had polarized transport in the secretory direction, with efflux ratios of 5.5 ± 0.7 and 18.5 ± 4.2, respectively. P-gp inhibitors such as valspodar and zosuquidar decreased digoxin transport, but did not affect etoposide transport. Furthermore PS20 decreased secretory transport of digoxin, but not of etoposide. Non-ionic surfactants did not alter MRP2-mediated etoposide efflux transport across the cell monolayers.

Conclusion

Etoposide transport across MDCKII-MRP2 cells is MRP2 and not P-gp dependent, and commonly used non-ionic surfactants did not decrease MRP2-mediated etoposide transport. These results suggest that etoposide transport in MDCKII-MRP2 cells is a model system to investigate MRP2 interactions, and that surfactants may not increase oral bioavailability of drugs through inhibition of MRP2 transport activity.

References

1. Glavinas H. *et al*. Curr Drug Deliv (2004) 1: 27-42
2) Seelig A., Gerebtzoff G. Expert Opin Drug Metab Toxicol (2006) 2: 733-752
3) Nielsen C. U. *et al*. Int J Pharm (2016) 513: 78-87

Are propofol emulsions stable when intravenously co-administered with remifentanil?

Niklas Nilsson, School of Pharmacy, University of Oslo and Sykehusapotekene Oslo, Rikshospitalet, Hospital Pharmacy Enterprise, Norway
Professor Dr. Ingunn Tho, *Personalized Dosage Form Design*, School of Pharmacy, University of Oslo, Norway
Dr. Kateřina Nezvalová-Henriksen, Sykehusapotekene Oslo, Rikshospitalet, Hospital Pharmacy Enterprise, Norway

Background
Propofol, a general anaesthetic, and remifentanil, an opioid analgesic, are used to both induce and maintain sedation. They often need to be administered simultaneously via the same venous catheter lumen. This predisposes to potential compatibility issues with undesirable consequences such as catheter obstruction and ultimately embolism. Propofol is a fat emulsion and available formulations differ considerably in fat composition. Diprivan® contains 100 % pure long chain triglycerides (LCT) whereas Propolipid® and Propofol-Lipuro® contain 50 % LCT and 50 % medium-chain triglycerides (MCT). The three formulations also differ in the type and amount of other excipients. There is no exhaustive information on all three propofol formulations.

Purpose
Our aim was to determine and compare emulsion stability of propofol formulations Propolipid®, Propofol-Lipuro® and Diprivan® when administered together with remifentanil.

Material and methods
To simulate Y-site compatibility, remifentanil (Ultiva®) 50 µg/ml was mixed in vials with 10 mg/ml concentrations of Propolipid®, Propofol-Lipuro® and Diprivan® respectively. The mixing ratios of remifenatnyl:propofol were1+1 and 10+1. Controls consisted of each propofol formulation analyzed separately. Analysis was conducted immediately after mixing and 4 hours later. Emulsion stability was determined by calculating the percentage of fat residing in globules larger than 5 µm (PFAT5), measuring pH and mean droplet diameter.

Results
None of the propofol formulations resulted in increased PFAT5 immediately after mixing with remifentanil in mixing ratios of 1+1 and 10+1. However, all formulations resulted in PFAT5 levels over what is acceptable 4 hours after mixing with remifentanil except for Propolipid and Diprivan in mixing ratio 1+1. No difference in mean droplet diameter was noticed and we did not see an association between the decreased pH that occurred and stability of the emulsions.

Conclusion
Remifentanil administered with propofol-formulations in the same intravenous catheter may lead to emulsion instability. If the infusion rate is slow, separate intravenous administration of these drugs should be considered.

Surface crystallization upon storage and dissolution visualized with multimodal nonlinear imaging

D. Novakovic[1], A. Isomäki[2], Sara J. Fraser-Miller[3], L. Peltonen[1], T. Laaksonen[4] and C. J. Strachan[1]
[1]Division of Pharmaceutical Chemistry and Technology, University of Helsinki, Finland
[2]Biomedicum Imaging Unit, University of Helsinki, Finland
[3]Department of Chemistry, University of Otago, New Zealand
[4]Laboratory of Chemistry and Bioengineering, Tampere University of Technology, Finland

Early stage crystallization of amorphous formulations typically starts at the surfaces of drug particles or dosage forms and can potentially hinder the desired dissolution performance. This work demonstrates the suitability and advantages of multimodal nonlinear optical imaging (combination of coherent anti-Stokes Raman scattering (CARS) and sum frequency generation (SFG) imaging in a single instrument) over the more established solid state analysis techniques in understanding storage[1] and dissolution[2] induced phase transformations.

Drug compacts composed of amorphous indomethacin powder were analyzed upon storage at 30°C/23%RH and 30°C/75%RH. Additionally, the same aged compacts were analyzed upon intrinsic dissolution testing at pH 6.8. Imaging was performed using the integrated Leica SP8 CARS microscope.

Phase transformations upon storage were detected at an earlier stage when compared to Raman microscopy and attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy. In addition, two coexisting crystalline forms as well as the amorphous form were detected simultaneously with nonlinear optical imaging, including at trace amounts.

Up to four crystalline forms and the amorphous form of indomethacin were imaged simultaneously upon dissolution of stored samples. Furthermore, the spatial distribution of these different solid-state forms was visualized. This analytical approach was more sensitive and information rich when compared to X-ray diffraction and ATR-FTIR spectroscopy.

In conclusion, multimodal nonlinear optical imaging is well suited for the analysis of multiple solid-state forms and their transformations, with several benefits:

(i) It is inherently surface specific allowing the detection of phase changes at the surface;

(ii) It allows the detection of multiple solid state forms present simultaneously;

(iii) Crystallization can be detected at an earlier stage than with conventional techniques;

(iv) The use of two nonlinear techniques simultaneously improves solid-state specificity compared to one technique alone.

References

[1] D. Novakovic, J. Saarinen, T. Rojalin, O. Antikainen, S. J. Fraser-Miller, T. Laaksonen, L. Peltonen, A. Isomäki and C. J. Strachan, Anal. Chem. 89, (2017).

[2] D. Novakovic, A. Isomäki, B. Pleunis, S. J. Fraser-Miller, L. Peltonen, T. Laaksonen and C. J. Strachan, Mol. Pharm. 15, (2018).

Thermomicromechanical understanding of hydrate-anhydrate transformations

Peter Ouma Okeyo[1, 2], Peter Emil Larsen[2], Thomas Rades[1], Jukka Rantanen[1], Anja Boisen[2]

[1]Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark

[2]The Danish National Research Foundation and Villum Foundation's Center for Intelligent Drug Delivery and Sensing Using Microcontainers and Nanomechanics (IDUN), Department of Micro- and Nanotechnology, Ørsted Plads, 2800 Kgs, Lyngby, Technical University of Denmark

PURPOSE

The aim of this study was two-fold: first, to investigate the use of a single crystalline particle as a resonator and second, to get a deeper understanding of the complex dehydration mechanism of an organic hydrate. By probing the mechanical properties of an individual drug particle through its resonant behaviour during heating and cooling cycles, we obtain information on both the intraparticulate mechanical processes and the different solid-state transformations that occur during dehydration.

INTRODUCTION

The majority of pharmaceuticals that are developed and marketed as solid dosage forms are prone to environment-induced stresses (temperature, humidity and pressure). This means that a thorough understanding of the physico-chemical and mechanical properties of these solid dosage forms is crucial in order to preserve the performance of the drug. It has been well documented that water can influence the stability, solubility and bioavailability of drugs. [1] There is also an increasing interest in understanding the dehydration of organic hydrates. Current, standard analytical techniques such as the differential scanning calorimeter (DSC), thermogravimetric analysis (TGA) and dynamical mechanical analysis (DMA) are used to investigate and monitor phase transformations of solid-state forms; however, these techniques require bulk amounts (in the mg range) of material for analysis. Therefore, we present a novel method that uses an individual particle of the crystalline drug as a resonator in order to track the dehydration of TP MH during heating-cooling cycles.

METHOD

The solid-state analytical methods DSC, TGA, and XRPD confirmed the solid-state form of TP to be TP MH (CSD ref code: THEOPH01). An individual particle of TP MH was actuated with a piezoelectric crystal, functioning as a micro cantilever, and a laser-Doppler vibrometer (LDV) was used to read out of the resonant behaviour. Temperature control was achieved using a Linkam hot stage. Dehydration of the model drug was measured in a linear temperature ramp from 25 °C to 90°C and back to 25 °C at a heating rate of 5 K/min. The mechanical spectra were obtained through Fast Fourier transformation (FFT) analysis on the displacement signal response to a chirping actuation (sampling time: 64 ms per point).

RESULTS AND DISCUSSION

DSC data showed a broad endothermic event (~55-80°C) and TGA revealed a weight loss of 9 % equivalent to the stoichiometric (1:1) water content of TP MH (Figure 1). The XRPD diffractogram of TP MH was in agreement with the calculated powder pattern (CSD ref code: THEOPH01). The LDV showed the first out of plane vibrational mode at 24 kHz (Figure 2). Initially, a decrease in the f_{res} (relative frequency shift = 9 %) [2] was observed during heating, indicating a softening of the crystal that destabilizes the crystal lattice during the loss of crystal bound water molecules (~25-68°C). From 68 °C, a dramatic frequency shift of the first resonance mode to 30 kHz was observed. By tracking the resonant behaviour of the system during the heating cycle, we obtain information both on the mass and mechanical properties of the system simultaneously.

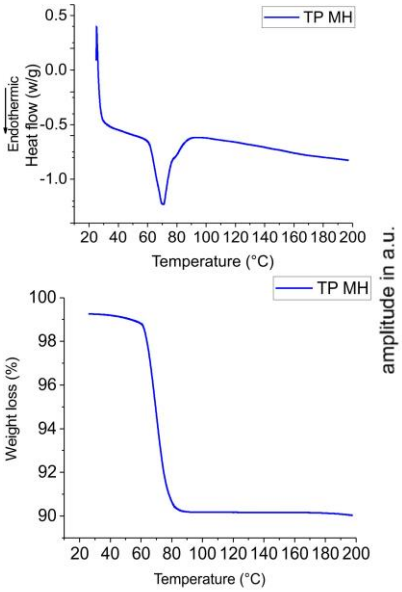


Figure 1. DSC and TGA thermograms showing water loss at ~55-90°C.

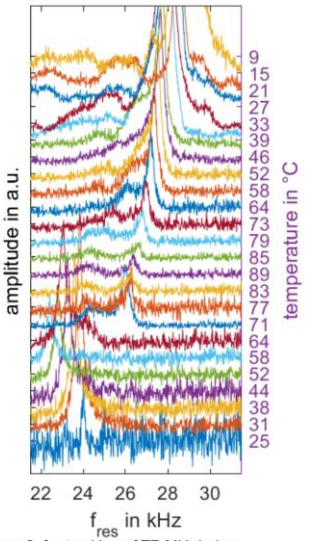


Figure 2. f_{res} tracking of TP MH during thermal cycling using a laser-Doppler vibrometer.

CONCLUSION

The observed results are in agreement with the DSC and TGA findings. While using an individual crystalline particle to characterize the dehydration behaviour of the drug, the LDV method offers the typical advantages of microtechnology, namely small sample sizes and fast analysis. Tracking the resonant behaviour provides information about the water loss and crystal lattice changes with a time resolution in the ms range. This gives a much more detailed view of the mechanical properties of a crystalline particle during the dehydration of TP MH to TP AH.

ACKNOWLEDGEMENT

We would like to acknowledge the Danish National Research Foundation (grant no. DNRF122), the Villum Fonden (9301) and the University of Copenhagen, Department of Pharmacy for the funding for this project.

REFERENCES

1. Byrn S. R., Pfeiffer R. R.; Stowell J. G. Solid-State Chemistry of Drugs, 2nd ed.; SSCI Inc., IN (1999).

2. Schmid S. et al, Fundamentals of Nanomechanical Resonators, Springer International Publishing, ISBN 9783319286914. (2016).

The effects of nanofibrillar cellulose on the water absorption capacity of polymeric composite films

Urve Paaver[1*], Karoliine Puusalu[1], Karin Kogermann[1], Jyrki Heinämäki[1]
[1]Institute of Pharmacy, University of Tartu, Tartu, Estonia

Introduction

Nanofibrillar cellulose (NFC) is produced via several hydrolysis and refining operations ending with a final high-pressure homogenisation of a softwood aqueous dispersion [1]. Due to a nano-scale fibril size and exceptionally high outer surface area, NFC has many unique material properties. NFC is composed of amorphous cellulose and it has a film formation ability, which makes it an interesting additive in pharmaceutical film coatings and thin films to modify the mechanical properties, water absorption and dissolution properties of the films [2,3].

Aim

The aim of this study was to investigate the effects of NFC on the physical solid-state, moisture absorption and mechanical properties of the moulded thin films of two synthetic water-soluble polymers, polyethylene oxide (PEO) and polyvinyl alcohol (PVA).

Materials and Methods

For film moulding, a 2.7-% aqueous dispersion of NFC (UPM Biofibrils AS 103, Finland) was diluted with purified water at the concentrations of 0.27% to 0.54% (NFC). These dispersions were mixed at different proportions with PEO (Sigma-Aldrich, Germany) 4% and 8% w/v, or polyvinyl alcohol (PVA, Mowiol®, Germany) 4% and 12% w/v water solutions. The moulded solutions were allowed to dry at 37°C, and the subsequent films were investigated with SEM (Zeiss EVO® 15 MA, Germany), optical microscope (CETI MagtexT), and texture analyzer (CT3 Ametek Brookfield, USA). The water absorption capacity was determined gravimetrically (APX-200, Denver instrument, Germany) and the thickness of the films was measured by digital caliper (IRONSIDE®, EU).

Results

The thin films of PEO and PVA with NFC differed by structure and transparency. Mechanical properties of the moulded films with NFC were also different as those of the reference films. The addition of NFC increased thickness and decreased the weight and mechanical strength of the films. The ability to swell increased as the amount of NFC in the films was increased (Fig. 1).

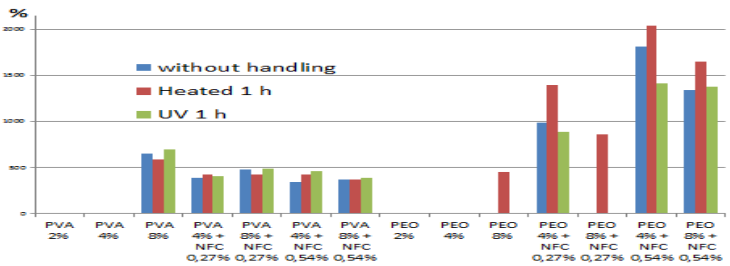


Fig. 1. *The water absorption (%) of PEO and PVA films with NFC after 1 h. The film samples were 1x1 cm in size (n=3). Some film samples were also exposed to high temperature (60 °C) or UV (254 nm) for 1 h prior to the water absorption test. The absence of column denote the films that dissolved or almost dissolved during the test. Key: PVA - polyvinyl alcohol; PEO - polyethylene oxide; NFC - nanofibrillar cellulose*

Conclusion: The inclusion of NFC in very small concentrations in the PEO and PVA films affects the film formation, enhances the water absorption, and modify the performance of the films.

Acknowledgements: This work is part of the national research grant projects IUT34-18 and PUT 1088. Estonian Ministry of Education and Research is acknowledged for financial support.

References:

1. Nechyporchuk, O., Naceur Belgacem, M., Bras, J. Ind Crop. Prod., 93, 2-25 (2016).
2. Xu, X., Liu, F. et al., ACS Appl. Mater. Interfaces, 5, 2999-3009 (2013).
3. Kolacović, R., Peltonen, L. et al., Eur. J. Pharm. Biopharm., 82, 308-315 (2013).

Rational Selection of Drug and Deep Eutectic Solvent Combination

Henrik Palmelund[1], Martin Andersson[2], Camilla Asgreen[1], Ben Boyd[3], Jukka Rantanen[1], Korbinian Löbmann[1]

[1]University of Copenhagen, Department of Pharmacy, Denmark
[2]Technical University of Denmark, Department of Chemical and Biochemical Engineering, Denmark
[3] Monash University, Monash Institute of Pharmaceutical Sciences, Australia

Introduction

A deep eutectic solvent (DES) is a mixture of two or more organic compounds that interact via hydrogen bonding and cause a melting point far below that of the individual components [1]. DESs have been used for various applications such as metal processing, biotransformation, and as medium for chemical synthesis [1]. Given their success as solvents in other fields, DESs have been suggested as promising solvents for poorly soluble drugs [2].

Aim

Establish an *ab initio* rank-order of different drug-DES combinations for rational drug-DES selection.

Method

The conductor-like screening model for realistic solvation method (COSMO-RS) was used for *ab initio* computational predictions of the relative solubility of 8 model drugs in the 6 different DESs (see table 1) with water as the reference solvent. The experimental solubility of drug in DES and water was determined using UV or HPLC.

Table 1. Molar composition of the studied deep eutectic solvents. Choline chloride (C), urea (U), glycerol (G), DL-lactic acid (L), betaine (B), water (W), and glucose (Glu).

DES abbrev.	Comp.1	Comp.2	Comp.3
CUW	1	2.1	0.4
CGW	1	2	0.2
CLW	1	0.9	0.9
BGW	1	2	1
CGluW	1	0.4	2
LGluW	1	0.2	1.2

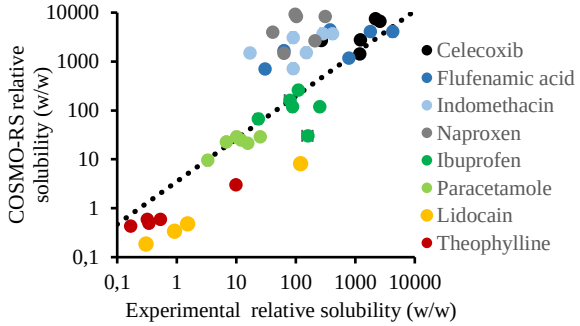


Figure 1. Predicted relative solubilities (with water as reference) of 8 drugs in 6 different DESs vs. the corresponding experimental relative solubilities. Slope = 1.09, offset 0.36, R^2 = 0.77

Results

A comparison of the experimental and predicted relative solubility increase of the investigated drugs gave a correlation with a good fit (see figure 1). This suggested that the COSMO-RS method can be used as an initial solvent screening to reduce the work load of experimental solubility screening. The largest experimental relative solubility was obtained for celecoxib, which was 12,360 fold more soluble in choline chloride-lactic acid-water (CLW) (18300 ± 0.0 µg/g) compared to its solubility in water (1.48 ±0.07 µg/g).

Conclusion

The relative solubilisation capacities could be predicted *ab initio* using COSMO-RS calculations. Hence, COSMO-RS may in future be used to reduce the experimental solvent screening for identification of the best DES for a given drug.

Acknowlegment

The authors acknowledge the Independent Research Fund Denmark [Grant No. 7017-00211B] for financial support.

References

1. Smith, E.L. et al. Chem. Rev. (2014) 114(21): 11060-11082
2. Morrison H.G. et al. Int. J. Pharm. (2009) 378: 136–139

Novel in vitro models for subcutaneous administration of drugs – Diffusion in polyelectrolyte gels

Julia Parlow, Agnes Rodler, Per Hansson
Department of Pharmacy, Uppsala University, Sweden

Introduction

Subcutaneous (SC) injection is a common route of administration in clinical medicine today, in particular for sensitive biopharmaceuticals with poor oral bioavailability.[1] However, for many biopharmaceutical formulations administered via the SC route the bioavailability is variable and incomplete, being in the range of 20-100 %.[2] Currently there is no standard accepted medium for simulation of the human SC environment.[3] Therefore, there is a need to develop and validate physiologically relevant *in vitro* models for subcutaneous administration of drug formulations.

Aim

The aim of this project is to study physicochemical aspects of the absorption of drug molecules after subcutaneous administration. The interactions of model drugs with the biopolymers present in the human extracellular matrix e.g. hyaluronic acid and collagen, will be investigated as well as the contribution of diffusion and convection to their transport.

Method

Hydrogels of cross-linked hyaluronic acid and collagen were synthesized and the diffusion of fluorescently labelled model peptides through these gel matrixes was investigated using confocal laser scanning microscopy (CLSM). Fluorescence recovery after photo bleaching (FRAP) is used to determine diffusion coefficients.

References

1. Turner, M. R. & Balu-Iyer, S. V. Challenges and Opportunities for the Subcutaneous Delivery of Therapeutic Proteins. J Pharm Sci 107, 1247–1260 (2018).
2. Kinnunen, H. M. et al. A novel in vitro method to model the fate of subcutaneously administered biopharmaceuticals and associated formulation components. Journal of Controlled Release 214, 94–102 (2015).
3. Leung, D. H. et al. Development of a Convenient In Vitro Gel Diffusion Model for Predicting the In Vivo Performance of Subcutaneous Parenteral Formulations of Large and Small Molecules. AAPS PharmSciTech 18, 2203–2213 (2017).

Mechanistic study of time-dependent cytochrome P450 inhibition using microfluidic immobilized enzyme assays

Tea L.M. Pihlaja, Iiro M.A. Kiiski, Tiina M. Sikanen
Drug research program, Faculty of Pharmacy, University of Helsinki, Finland

Introduction

Cytochrome 450 enzymes (CYP) catalyze the majority of drug elimination pathways in vivo. Determination the drug candidates' potency to induce time dependent (irreversible) inhibition of CYPs is of particular concern, because it requires synthesis of new enzymes to restore the CYP activity, which may lead to severe clinical adverse effects.

Aim

In this work, we developed a microfluidic concept for rapid discrimination between reversible and irreversible (time-dependent) inhibition.

Method

Immobilized CYP microreactors (Fig. 1A) were implemented according to a previously published protocol.[1] Briefly, a micropillar array featuring free surface thiols was made from thiol-ene polymer and functionalized sequentially with biotin, avidin, and biotinylated human liver microsomes.

Results

The CYP2C9 model activity was monitored by infusing 200 μM Luciferin-H (as the substrate) at a flow rate of 5 μL/min (corresponding to 5 min reaction time). Under/In these conditions, Luciferin-H metabolism followed Michaelis-Menten kinetics (Fig. 1B-C). The feasibility of the developed CYP microreactor for discrimination between reversible and irreversible CYP inhibition was demonstrated with help of known CYP2C9 inhibitors, sulfaphenazole and tienilic acid, respectively (Fig. 1D-E), and compared with conventional IC_{50} shift assay results (Fig. 1F).

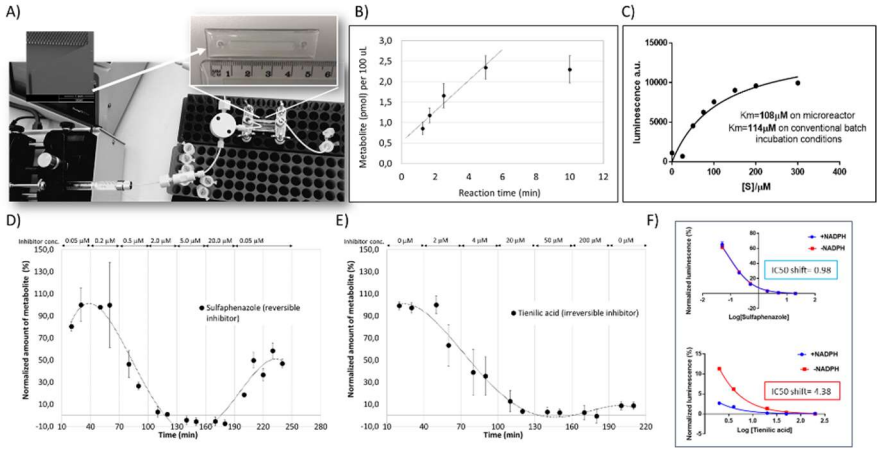


Fig. 1. A) A photograph of the CYP microreactor setup with two pump lines delivering the substrate (200 μM Luciferin-H) solution containing 0.2 mM NAPDH in Tris buffer (pH 7.5). B) The amount of metabolite (Luciferin) produced as a function of reaction time (adjusted via flow rate). C) Michaelis-Menten kinetics of the CYP2C9 mediated Luciferin-H dealkylation on the microfluidic array. D-E) Microfluidic TDI and F) conventional IC_{50} shift inhibition assays[2] of reversible (D) and irreversible (E) CYP2C9 inhibition caused by sulfaphenazole and tienilic acid, respectively. The threshold for irreversible inhibition in the IC50 shift assay is ≥1.5.

Conclusions

The developed microfluidic immobilized CYP assays were shown to be capable of predicting TDI in a high-throughput manner under flow conditions.

References

1. Kiiski, I. et al. Adv. Biosys. (2018) https://doi.org/10.1002/adbi.201800245
2. Berry and Zhao, Drug Metab. Lett. (2008) https://doi.org/10.2174/187231208783478407

Imaging of active pharmaceutical ingredient and excipient spatial distribution in medicated chewing gum by Raman spectroscopy

Jessie Poulsen[1,2], Kent A. Nielsen[2], Annette Bauer-Brandl[1]
[1]Deparment of Physics, Chemistry and Pharmacy, University of Southern Denmark, Denmark
[2]Fertin Pharma A/S, Nicotine Science Center, Dandyvej 19, 7100 Vejle, Denmark

Introduction

Presenting nicotine as chewing gum is challenging in relation to chemical stability during the product shelf life. The exact degradation mechanism has not yet been described for nicotine in medicated chewing gum. If the reaction mechanisms can be clarified by gaining knowledge on product homogeneity and mobility of excipients in the gum matrix over time, new formulations strategies leading to superior stability can be developed.

Aim

To investigate if Raman spectroscopy is a suitable method for visualizing nicotine and excipient distribution in a chewing gum formulation. Raman mapping has been used in numerous different pharmaceutical formulations to determine API distribution[1,2], but to the authors knowledge not in medicated chewing gum.

Method

Raman spectroscopy was carried out using a confocal Raman microscope. For all measurements a 50x objective was used. A 785nm laser was used for mapping and point spectra. For the mapping a spatial resolution of 2.8 µm was used. Acquisition of Raman spectra was obtained by Danish Technological Institute.

Results

Raman spectra were acquired from three different areas of each reference material (Fig. 1). The gum base showed some variations in the spectra depending on the location where the spectra were acquired, indicating that the gum base, as expected, is inhomogeneous. The distribution of reference materials in the mapped area is shown in Fig. 2.

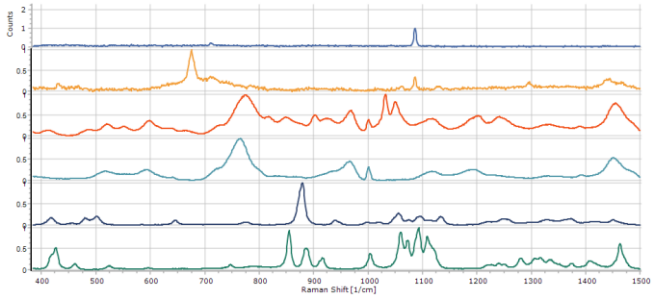


Fig.1. *Normalized Raman spectra of selected chewing gum reference materials. From top to bottom: calcium carbonate, gum base, NPR (nicotine polacrilex resin), polacrilex resin, sorbitol, and xylitol.*

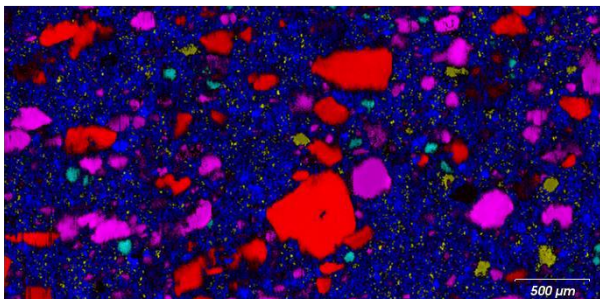


Fig. 2. *Map of chewing gum excipient distribution (2x4 mm cross section): calcium carbonate (yellow), gum base (blue), NPR (turquoise), sorbitol (red), and xylitol (purple). The black areas shows lack of fit from the selected reference materials.*

Conclusion

Imaging by Raman spectroscopy shows that the chewing gum is inhomogeneous. The majority of the surface area shows Raman spectra in agreement with the gum base (blue color, Fig. 2). The two bulk sweeteners (sorbitol and xylitol, respectively, red and purple color in Fig. 2) are located in separate domains of varying size and NPR (turquoise color in Fig. 2) is seen in separate domains of ~100 µm in diameter.

References

[1]Gala U. and Chauhan H. Expert Opin. Drug Discov. (2015) 10(2): 187-206
[2]Gordon K. C. and McGoverin C. M. Int. J. Pharm. (2011) 417: 151-162

Impact of different sterilization and disinfection methods on chloramphenicol-loaded electrospun fibrous matrices

Liis Preem[1], Ebe Vaarmets[1], Andres Meos[1], Indrek Jõgi[2], Marta Putriņš[3], Tanel Tenson[3], Karin Kogermann[1]
[1]Institute of Pharmacy, University of Tartu, Estonia
[2]Institute of Physics, University of Tartu, Estonia
[3]Institute of Technology, University of Tartu, Estonia

Introduction

Electrospinning is a technology gaining wide popularity for the production of novel drug delivery systems. Due to structural similarities of electrospun matrices to dermal extracellular matrix and other favorable properties, electrospinning is especially attractive for the development of wound dressings. High quality wound dressing should be sterile, although different treatments to achieve this could have an unpredictable impact on other functionality-related properties of the dressing.

Aim

To study the efficacy and effects of different sterilization and disinfection methods on drug-loaded electrospun fibrous matrices.

Method

Chloramphenicol-loaded electrospun fibrous matrices with two different polymeric carriers (polycaprolactone, PCL, alone or in combination with polyethylene oxide, PEO) were treated with UV-irradiation for 15-60 min on both sides, γ-irradiation with a dose of ∼50 kGy, *in situ* generated chlorine gas for 1 h or with low pressure argon plasma for 30-120 s on both sides. Sterility test based on the European Pharmacopoeia 9.6 instructions was implemented to evaluate the efficacy of different treatments. Drug content (high performance liquid chromatography, HPLC), morphology (scanning electron microscopy, SEM), solid state properties (fourier-transformed infrared spectroscopy, FT-IR), drug release profile (modified dissolution test) and mechanical properties (texture analysis) were analyzed to discover any differences between treated and untreated matrices.

Results

All treatments were able to reduce (UV-irradiation for 15 min, argon plasma) or eliminate (all other treatments) microbial contamination. Morphological changes were mostly not apparent, except for plasma-sterilization where some fractured fibers were present. All treatments reduced the amount of drug, whereas the loss was more prominent with PCL/PEO composite fibers. At the same time, no noticeable changes in FT-IR spectra occurred. Drug release profile was completely altered by plasma-sterilization as rapid release was seen from otherwise slow-release formulation. Also, some treatments could either increase or decrease the mechanical strength of the matrices.

Conclusions

The selection of optimal treatment method and time are essential for effective sterilization of electrospun matrices together with minimal loss of drug content and other changes in functionality-related properties.

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Development and in vitro characterizations of citrem based self – emulsifying drug delivery systems for oral delivery of insulin

V Ramakrishnan [1]*, Jingying Liu [1], Lasse Saaby [2], Anette Müllertz [1,2]
[1] Department of Pharmacy, Universitetsparken 2, University of Copenhagen, 2100, Copenhagen, Denmark; rlp880@alumni.ku.dk; [2] Bioneer: FARMA, Department of Pharmacy, Universitetsparken 2, University of Copenhagen, 2100, Copenhagen, Denmark

Introduction

Oral drug delivery is considered to be the most patient compliant, economical and simple route of administration. Due to high hydrophilicity, large size and easy target for enzymatic degradation limits the use of insulin for oral drug delivery.[1,2] SEDDS are an isotropic mixture of oil, surfactant, co-solvent and have shown great potential in oral delivery of therapeutic proteins and peptdies.[1]

Aim

The aim of this study was to investigate the potential of insulin-loaded SEDDS using citric acid esters (Citrem) as a co-surfactant to generate negatively charged droplets and thus inhibit/ limit proteolytic degradation of insulin.

Method

The formulations containing oil (Captex 300), surfactant (Labrasol) and co-surfactants (Citrem/Peceol/Maisine 35-1 and MAPC) were generated using quality by design (QbD) approach. To increase the lipophilicity of insulin, it was complexed with biocompatible surfactants like soy-bean phosphatidylcholine (SPC) or mono-acyl phosphatidylcholine (MAPC) and loaded into SEDDS **(Fig. 1)**. *In vitro* proteolysis was performed in biorelevant media in the presence of 10 µM α-chymotrypsin.

Results

The resulting QbD model had a R^2= 0.96 and Q^2= 0.88 for droplet size and R^2= 0.90 and Q^2= 0.66 for zeta potential. The formulations were within the size range of 117 to 355 nm and the zeta potential ranged from -27 to -34 mV. The *in vitro* proteolysis study showed that >50 % of intact insulin remained in the Citrem SEDDS after 30 mins, whereas it was only 30% in SEDDS without Citrem.

Conclusions

The study revealed that SEEDS containing Citrem were able to protect insulin better compared to the SEDDS without Citrem.

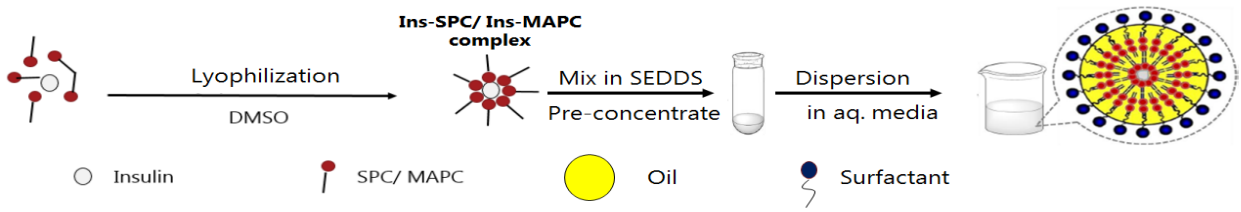


Fig. 1: Development of Insulin-phospholipid complex and loading in SEDDS

References

1. Li P, Fano M, Ullertz AM. Preparation and Characterization of Insulin – Surfactant Complexes for Loading into Lipid-Based Drug Delivery Systems. 2013;102(8):2689–98.

2. Cui F, Shi K, Zhang L, Tao A, Kawashima Y. Biodegradable nanoparticles loaded with insulin – phospholipid complex for oral delivery : Preparation , in vitro characterization and in vivo evaluation. 2006;114:242–50.

GLUT1 inhibitor, phloretin, downregulates inflammasome activation on human RPE cells with medium dependant effect

S. Ranta-aho[1], Hytti M[1], Bhattarai N[1], Ruuth J[2]. and Kauppinen A[1]

[1]School of Pharmacy, University of Eastern Finland, Kuopio, Finland
[2]Department of Ophthalmology, University of Eastern Finland, Kuopio, Finland

Introduction

One of the main functions of the retinal pigment epithelium is to transport glucose and other nutrients to the retina. At the retina, the glucose consumption is high but, like in diabetic retinopathy, hyperglycemia is harmful for the retina. At the diabetic retinopathy cytokines like IL-1b and growth factors like VEGF contribute to the development of diabetic retinopathy (Semeraro 2015).

Phloretin, a natural GLUT1 inhibitor present in apple has been observed to reduce the intracellular accumulation of glucose in human retinal pigment epithelium cells (ARPE-19).

Aim

The aim of these experiments was to test the effects of phloretin on inflammation in human retinal pigment epithelium cells. Special interest has been in inflammasome-mediated signaling.

Methods

Experiments were carried out using ARPE-19 cells cultured in high and low-glucose mediums. Our routinely used DMEM/F-12 ($C_{D\text{-}glucose}$ = 17.5 mM) as well as DMEM ($C_{D\text{-}glucose}$ = 25 mM) were high-glucose mediums in addition of which we also had DMEM-ng that was glucose-free. Cells were primed by IL-1a and the inflammasome signaling was activated using MG-132 (MG) + Bafilomycin A (BafA) that block proteasomes and autophagy, respectively. Cellular viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and the levels of IL-1β and VEGF from cell culture medium by ELISA method.

Results

At IL-1a+MG+BafA model, phloretin reduced the secretion of IL-1β in the DMEM/F-12 medium, but not in the DMEM from which DMEM-F12 is derived. The removal of glucose from the medium strongly compromised the cellular viability. Moreover, phloretin also reduced the secretion of VEGF in DMEM/F-12 and DMEM, which was visible also when the inflammasome was not activated.

Conclusion

Phloretin (0.1 mM) is able to prevent the inflammasome activation with the subsequent IL-1β secretion from human ARPE-19 cells cultured in their optimal medium. It also reduces the secretion of VEGF. Inhibition of inflammation and VEGF release are beneficial in diseases, such as age-related macular degeneration (AMD) and diabetic retinopathy (DR) but mechanisms still need further studies.

References:

Semeraro F, et al. J Diabetes Res. (2015) doi: 10.1155/2015/582060

Atropine loaded chitosan nanoparticles for transdermal delivery via Microneedle technique

Rawand A. Mustafa[1], Kuldeep Bansal[1], Didem Şen Karaman[2], Jessica M.Rosenholm[1]
[1]Pharmaceutical Sciences Laboratory, Faculty of Science and Engineering, Åbo Akademi University, Finland
[2]Department of Biomedical Engineering, Faculty of Engineering and Architecture, Izmir Katib Celebi University, Turkey

Introduction

Transdermal drug delivery systems (TDDS) have gained profound attention among scientists due to the advantages it holds over more preferred conventional routes of administration (e.g. oral). Despite several advantages of TDDS, poor permeation of drugs through skin limits the wide applicability of this approach. Recently, *drug loaded nano-carriers* demonstrate high penetration rate through skin and thus, they could be an alternative approach to overcome the skin barrier.

Aim

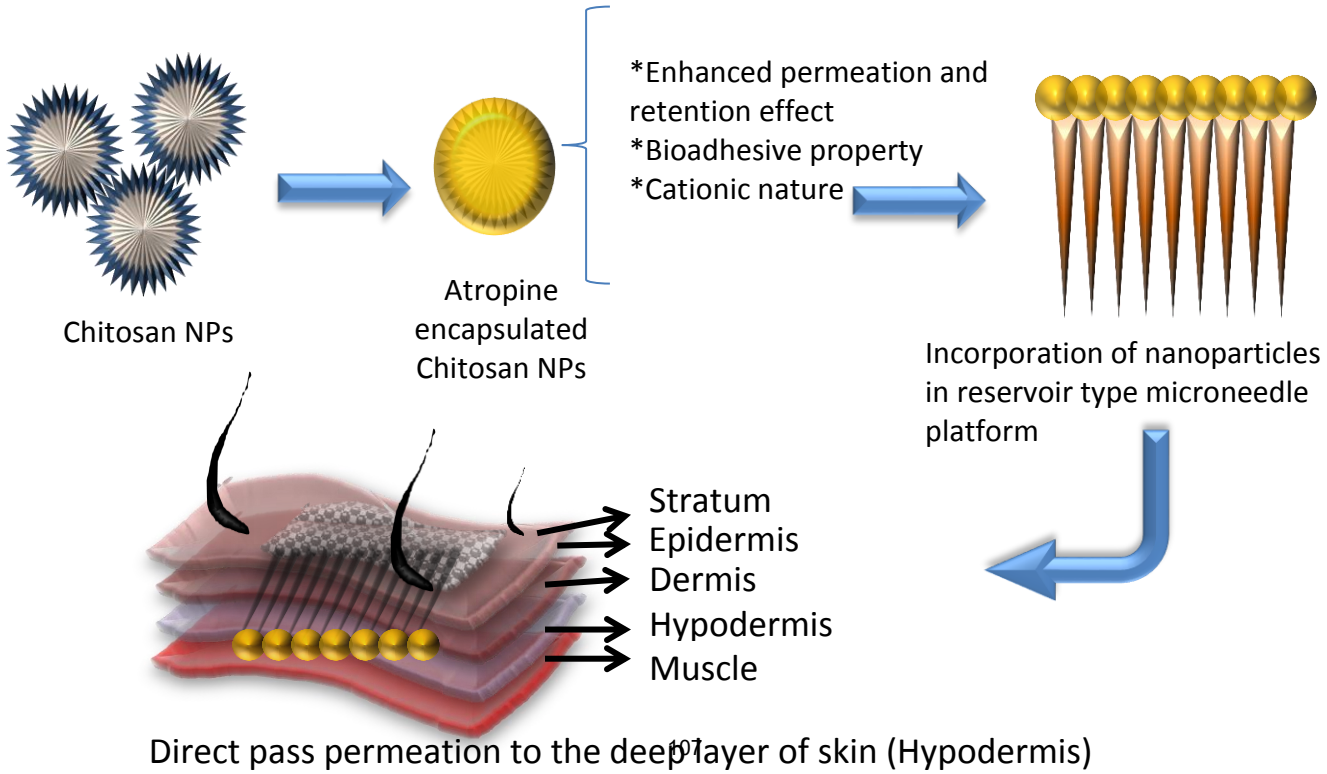
To design suitable transdermal carrier systems to deliver pharmacologically active substances using atropine as model drug, whose delivery is otherwise limited through other routes. The new dosage form could improve the bioavailability of incorporated drug(s); provide controlled drug release, reduce side effects and dosage frequency.

Method

Ionic gelation technique will be used for the fabrication of chitosan nanoparticles (NPs). Drug loaded NPs will be characterized for their loading efficiency, size, surface charge, morphology, skin permeation and cytotoxicity *in vitro.* Permeability and cytotoxicity studies will be carried out with the aid of Franz cell diffusion method and fibroblast cell lines as a model skin cells. Microneedles loaded with prepared nano carrier system will also be used to investigate their capability in increasing the penetration of drugs and/or particles through skin. *Ex vivo* testing (primarily pig skins) of prepared drug delivery systems will also be carried out.

Conclusion

The expected outcomes will be successful nanoformulation of atropine as model drug in chitosan NPs. Improved skin penetration is also envisioned as result of applying these nanoformulations on artificial and real skin models. Further permeability enhancement is expected upon incorporation of the nanoformulations into microneedles.



Fabrication and characterization of a Collagen-Hyaluronic acid based hydrogel towards an in vitro model for subcutaneous administration of drugs

Agnes Rodler, Julia Parlow, Per Hansson
Department of Pharmacy, Uppsala University, Sweden

Introduction

Subcutaneous (SC) drug delivery is becoming an increasingly preferred route of drug administration combining the benefits of robust formulations, self-administration and thus lower costs [1]. Whereas knowledge on both "ends" of SC-injection, formulation development and bioavailability studies, is advanced, there is still lack of mechanistic understanding of the events drug molecules face on their way through the interstitial space, often having lowered bioavailabilities as a consequence [2][3].

Aim

Our goal is to gain understanding of the interactions of drug molecules with the components of the interstitial space, starting from a macroscopic perspective down to a molecular level. We aim to identify the biophysical factors which impact drug transport through the subcutaneous matrix and to study to which extent they are size- or charge based.

Methodology and Results

Our in-vitro model prototype is a hydrogel composed from two of the most abundant components of SC-tissue, *type I* Collagen and Hyaluronic acid. Both were chemically modified and covalently cross linked by Thiol-Michael addition click reaction.
Rheology was used as tool to verify crosslinking as well as to adjust mechanical stability in dependence of the precursor concentrations in order to translate the hydrogel composition to a mesh-size similar to that of native SC-tissue. To probe structural homogeneity of- and transport in the gel, diffusion of fluorescently labelled molecules through the hydrogel matrix was captured by confocal laser scanning microscopy.

References

[1] M.R. Turner, S.V. Balu-Iyer, J. Pharm. Sci, 2018, 107, 5, 1247.
[2] W.F Richter, S.G. Bhansali, M.E. Morris, AAPS Journal, 2012, 14, 559.
[3] H.M. Kinnunen, R.J. Mrsny, J Control Release, 2014, 182, 22.

A microscopic approach to dissolution imaging

M. B. Senniksen[1,*], J. F. Christfort[2], J. Plum[1], A. Müllertz[1,3], T. Rades[1]

[1] **University of Copenhagen,** Department of Pharmacy, Universitetsparken 2, 2100 Copenhagen Ø, Denmark, *contact: Malte.senniksen@sund.ku.dk;
[2] **Technical University of Denmark,** Department of Micro- and Nanotechnology, Ørsteds Plads, 2800 Kgs. Lyngby, Denmark;
[3] **University of Copenhagen,** Bioneer:FARMA, Universitetsparken 2, 2100 Copenhagen Ø, Denmark

Introduction

An increasing number of drug candidates in the pipelines of the pharmaceutical industry have poor aqueous solubility [1], and therefore often suffer from low and inconsistent bioavailability. The dissolution rate of the drug candidates is an important property, traditionally determined in a regular dissolution setup, which operates with relatively large volumes [2]. Early development drug candidates are only available as a few milligrams of compound, leading to an increased need for simple and effective screening tools to investigate the dissolution in smaller volumes. The oCelloScope System™ is a video-microscopic setup creating a series of micrographs, as described by Fredborg et al. [3]. It has the capability of acquiring high resolution images of drug particles dissolving over time and has previously been applied to study precipitation from supersaturated systems [4].

Aim

To develop a dissolution method utilising image analysis of micrographs, as a means of determining dissolution rate of poorly water-soluble drugs, as well as determining particle sizes and habit.

Method

A total of five different model drugs were added to fasted state simulated intestinal fluid (FaSSIF, biorelevant.com), and their dissolution profiles assessed in the oCelloScope™ along with their particle sizes and habits. The investigated model drugs were; diazepam, fenofibrate, dipyridamole, indomethacin and felodipine. The dissolution tests were performed by spiking 20 µL of drug suspension into 180 µL of FaSSIF in the well of 96-well plate, where after images were acquired using the oCelloScope™.

Results

The dissolution images showed a clear decrease in particle area over time for all drugs. It was possible to rank the dissolution rates of the different compounds, by plotting the mean particle area against time between the replicates of each drug.

Conclusion

The oCelloScope™ has been revamped from an apparatus used to monitoring bacteria and other microorganisms to now additionally having the power to detect and segment objects in the size range of 0,5 µm – 1 mm. It shows great promise as a preformulation tool with a high throughput, and the possibility for using very low amounts of drug, for e.g. dissolution screening. However, the dissolution method has to be further validated before it can deliver the same quality of data as the current methods available.

References

[1] Pouton CW, Eur J Pharm Sci. (2006):278.287
[2] Andersson SBE, et al. J Pharm Sci. (2016);105(9):2864-72.
[3] Fredborg M, et al. J Clin Microbiol. (2013);51(7):2047-53.
[4] Christfort JF, et al. Mol Pharm. (2017);14(12):4154-60.

Sustainable Drug Discovery and Development with End-of-Life Yield (SUDDEN) – A Nationwide Effort to Foster Sustainability in the Pharmaceutical Sector

Jari Yli-Kauhaluoma[1], Tiina Sikanen[1], Henri Xhaard[1], Janne Martikainen[2], Mirella Miettinen[3], Mika Mänttäri[4], Mari Lundström[5], Sari Kauppi[6], and Mikael Sokero[7]

[1]Faculty of Pharmacy, University of Helsinki, Finland; [2]School of Pharmacy, University of Eastern Finland; [3]Law School, University of Eastern Finland; [4]Lappeenranta University of Technology, Finland; [5]School of Chemical Engineering, Aalto University, Finland; [6]Finnish Environment Institute; [7]Demos Helsinki, Finland

Introduction

The ecological risks associated with pharmaceutical emissions are an increasing concern both locally and globally.[1] The environmental signature of pharmaceuticals results from multiple emission sources including industrial raw material/drug production, incorrectly disposed pharmaceutical products, and human and veterinary use.[2] With societal transformations (urbanization, population growth and ageing), the Pharmaceuticals in the Environment (PiE) accumulate at an accelerated pace. While some emissions can be controlled by reinforcing regulatory actions (manufacture, farming) and raising awareness among drug consumers (disposal), the solution to the PiE originating from human and veterinary use appears the most complex and necessitates adaptation of new drug discovery and development strategies as well as new environmental risk assessment approaches. Recycling of the pharmaceutical packaging waste is also in its infancy.

Aim

To increase sustainability in pharmaceutical sciences and to foster sustainable development of pharmaceutical industry.

Method

SUDDEN is a Finnish multidisciplinary research initiative strategically designed to tackle the environmental hazards and sustainability challenges connected with drug design, production, consumption, and end-of-life.[3] The project is funded between 2019 and 2021 under the programme 'Keys to Sustainable Growth' by the Strategic Research Council of the Academy of Finland.

Results

SUDDEN project provides home for Nordic researcher exchange in the field of green and sustainable pharmacy in its affiliated research groups at the Faculty of Pharmacy, University of Helsinki, and the School of Pharmacy, University of Eastern Finland:

- Computational drug design and chemoinformatics (Dr. Henri Xhaard)
- Medicinal chemistry (Prof. Jari Yli-Kauhaluoma)
- Microfluidics-assisted environmental risk assessment (Dr. Tiina Sikanen)
- Pharmacoeconomics (Prof. Janne Martikainen)

Conclusions

Taking responsibility for the environmental impact of medicines calls of immediate actions and global collaborations. Nordic university hub could provide the needed scientific foundation and pave the way for the next generation green pharmaceutical products.

References

1. Executive Agency for Health and Consumers, Study on the environmental risks of medicinal products. Final report. 12 December 2013.
2. International Pharmaceutical Federation (FIP), Green pharmacy practice: Taking responsibility for the environmental impact of medicines. The Hague: International Pharmaceutical Federation; 2015.
3. https://www.aka.fi/en/about-us/media/press-releases/2018/src-adds-four-consortia-to-strategic-research-programme-keys-to-sustainable-growth/

The influence of liquid intake on the performance of an amorphous solid dispersion in rats

Daniel Sironi [1], Annette Bauer-Brandl [1], Martin Brandl [1], Jörg Rosenberg [2], Gert Fricker [3]
[1] University of Southern Denmark, Odense, Denmark
[2] AbbVie Deutschland GmbH & Co. KG, Ludwigshafen, Germany
[3] Heidelberg University, Heidelberg, Germany

Purpose:

In a previous study, an amorphous solid dispersion (ASD) of the poorly soluble compound ABT-869 dissolved in phosphate-buffered saline (PBS) only at room temperature but not at body temperature [1]. Yet, the formulation dissolved rapidly at all temperatures when water was employed as dissolution medium. High ionic strength of the medium was shown to negatively affect the dissolution of the ASD by inducing rapid precipitation on the surface. Therefore, the objective of this study was to test whether co-administration of high amounts of water would dilute the intestinal fluids sufficiently to enhance the performance of the formulation in rats.

Methods:

Sprague-Dawley rats were fasted overnight. Access to water was denied 30 min prior to dosing and was granted again 60 min after dosing. The lower part of a rat capsule made of gelatin (size 9) was filled with milled ASD and was administered using an appropriate applicator (Torpac Inc., Fairfield, NJ, USA). The first group of rats did not receive any further treatment whereas the second group additionally received 1.5 mL of water by oral gavage. The third group received 1.5 mL of an aqueous suspension of crystalline API by oral gavage instead of a capsule filled with ASD. Blood samples were collected over 8 h, and ABT-869 in the plasma was quantified by LC-MS/MS.

Results:

Surprisingly, the performance of the ASD was not better than that of a suspension of crystalline drug when the ASD was administered together with 1.5 mL of water. In both cases, flat plasma curves were obtained with late t_{max} values (3.7 ± 2.3 h and 4.7 ± 0.6 h, respectively). However, administration of the ASD without any water resulted in a 4-fold increase of the AUC with an early t_{max} value (1.3 ± 0.6 h).

Conclusion:

This phenomenon could be related to rapid precipitation in the stomach upon dissolution in a large volume of water, a protective layer formed by the gelatin capsule, and/or a general mismatch of dissolution rate and absorption rate. It is unclear whether this behavior is specific for this formulation. Yet, this finding emphasizes the relevance of the amount of liquid for the administration of enabling formulations.

Funding:
This work was funded by AbbVie Deutschland GmbH & Co. KG.

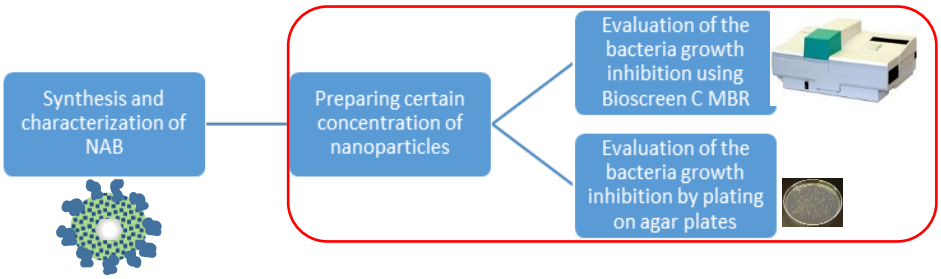
References:
[1] Sironi et al. 2018, J. Pharm. Biomed. Anal. 156, 247–251

Evaluation of core-shell structured nanoantibiotics on *E. coli* planktonic cultures

Anna Slita[1], Prakirth Govardhanam[1], Didem Sen Karaman[1], Jessica M. Rosenholm[1]
[1]Pharmaceutical Sciences Laboratory, Faculty of Science and Engineering, Åbo Akademi University, Finland

Introduction

Most known bacteria have developed resistance to at least one antibiotic since 1940, i.e., since antibiotics were developed[1]. According to WHO reports from 2014, there has been an alarming increase in the antibiotic resistant bacterial strains in most parts of the world. This study focuses on investigation of the antibacterial activity of so-called nanoantibiotics (NAB), composed of inorganic & organic constructs. For this purpose, the extent of *in vitro* bacterial growth inhibition caused by the produced NAB is investigated.



NAB are composed of cerium oxide (CeO_2) nanoparticles as anti-bacterial and anti-oxidant core, surrounded with a porous silica ($PSiO_2$) shell to eliminate the challenges of colloidal instability, and to act as a drug carrier. Chitosan (anti-bacterial agent)[3] is used for silica surface coating, to create CeO_2@$PSiO_2$@Chitosan nanocomposites. Capsaicin (anti-bacterial herbal extract) was loaded into the porous silica through solvent immersion method.

Aim

To investigate the *in vitro* antibacterial activity of the developed nanocomposite structure of NAB on ampicillin-resistant *E. coli* using turbidity measurements and colony counting techniques.

Methods

In the *in vitro* studies, the extent of time-dependent bacterial growth inhibition of *E. coli* caused by the produced NAB was investigated by plating bacteria after interaction with different amounts of NAB and by observing growth inhibition curves with the use of Bioscreen C MBR[2] for fixed time-points. As E. coli are mostly found in lower intestine, for evaluation of composed nanoparticles cytocompatibility, human epithelial colorectal adenocarcinoma cell line, CaCo2, was chosen.

Results

Bacterial growth inhibition results from *in vitro* tests showed significant growth inhibition by the improved design of NAB, compared with the pure cerium oxide cores and CeO_2@$PSiO_2$ composites. The designed NAB was shown to be not toxic for CaCo2 cell line.

Conclusion

The observed growth inhibition results from the *in vitro* experiments revealed that multiple antibacterial constructs in the designed NAB system help to improve its antibacterial activity and don't influence it's cytocompatibility.

References

1. Ventola, C. Lee. Pharmacy and Therapeutics 40.4 (2015): 277.
2. Oy Growth Curves Ab Ltd. 2002. Bioscreen. [ONLINE] Available at: http://www.bioscreen.fi/company.html. [Accessed 25 November 2018]
3. Chung, Ying-Chien, and Chih-Yu Chen. Bioresource technology 99.8 (2008): 2806-2814.

Probing passive transport with localized spectroscopy

Paul C. Stein[1*], Massimiliano P. Di Cagno[2]

[1]Institute of Physics, Chemistry and Pharmacy, University of Southern Denmark, Danmark
[2]Department of Pharmacy, UIT the Arctic University of Norway, Norway

INTRODUCTION

All active pharmaceutical ingredients (API) exhibit passive transport somewhere on their route from oral intake to their target in the human body. Even molecules, which are transported actively in or out of cells, will diffuse passively through diverse mucus layers. Parameters describing passive transport, *e.g.* diffusion coefficients in diverse media, are difficult to find and the permeability of the interfaces is often overlooked. Details of the transport through interfaces are badly known and seldom described.

METHODS

We have developed localized versions of UV-**v**is and NMR spectroscopy, which make it possible to investigate details of transport through the unstirred water layer, octanol and the water-octanol interface. The influence of complex formation with hydroxypropyl-β-cyclodextrin (HPβCD or CD) on the transport properties of ibuprofen (IBU) is used to illustrate the possibilities of these methods.

RESULTS

Binding of IBU to HPβCD: The binding IBU with HPβCD was investigated using ^{1}H-NMR spectroscopy. The biological less interesting R-isomer binds strongest to HPβCD.

Diffusivity: Following the diffusivity by localized UV spectroscopy showed the [IBU·CD] complex having the same diffusions constant as HPβCD alone (as obtained from NMR). This confirms that ibuprofen binds to the inside of the HPβCD cavity.

Distribution: The apparent octanol-water distribution of IBU·CD (d_{ow}) complexes was measured by localized NMR spectroscopy. The data show that $D_{ow} \approx 1/8$ for HPβCD.

Permeation: The transport of ibuprofen alone or in the presence of IBU·CD complexes through the octanol-water interface was investigated by localized NMR spectroscopy.

CONCLUSION

Time resolved localized spectroscopy yields information on concentrations as a function of space, time and species and permits thus the detailed investigation of transport parameters.

We applied the techniques to the investigation of the ibuprofen-HPβCD system showing on the one hand that the complexes move slower then ibuprofen alone and that there is less ibuprofen available for transport in the presence of HPβCD. On the other hand, the initial transport of ibuprofen through the water-octanol interface is faster in the presences of IBU·CD complexes.

REFERENCES

1. Bayir, E. bachelor report, SDU, Odense (2019).
2. Di Cagno, M.P. *et al.* Molecular Pharmaceutics (2011) 15: 1488-1494.
3. Fageeri, F. bachelor report, SDU, Odense (2019).
4. Stein, P.C. *et al.*, A. Pharmaceutical Research (2011) 28: 2140-2146

Amino acid transport in prostate PC-3 cells

Sidsel Strandgaard Nielsen[1], Maria Læssøe Pedersen[1], Carsten Uhd Nielsen[1]
[1] *Department of Physics, Chemistry and Pharmacy*, *University of Southern Denmark, Denmark*

Introduction

Sarcosine is involved in prostate cancer (PCa) progression. Levels of sarcosine are highly elevated during PCa progression[1] and sarcosine-related induction of genes involved with cell cycle progression has been found[2]. Sarcosine is therefore believed to be a potential oncometabolite rather than only a nonproteinogenic amino acid [3]. Recently, it was proposed that ^{11}C-sarcosine produces high-contrast images in prostate cancers presumably due to sarcosine uptake via the proton-coupled amino acid transporter PAT1 (SLC36A1) [4]. Sarcosine is a known substrate for PAT1, which is presumably important for proliferation of cells[5].

Aim

To investigate if PAT1 is expressed and functional active in PC-3 cells and if PAT1 is the cellular uptake mechanism for sarcosine uptake in prostate cancers.

Method

In vitro experiments were conducted using human prostate PC-3 cells seeded at a density of $5 \cdot 10^4$ cells/cm^2 in tissue-culture treated wells and cultured for 4-5 days. The uptake of ^{3}H-proline, ^{3}H-taurine, ^{14}C-glycine and ^{14}C- α–methylaminoisobutyric acid (MeAIB) was examined in the absence or presence of different unlabeled inhibitors/substrates. The amount of isotope inside the cells was quantified by a liquid scintillation counting, from which the uptake rate was calculated.

Results

The uptake rate of ^{3}H-proline was higher at pH 7.4 (5.5 fmol*min^{-1}*cm^{-2}) than at pH 5.5 (1.5 fmol*min^{-1}*cm^{-2}) and was linear as a function of unlabeled proline concentration (0-100 mM). ^{3}H-proline uptake was not inhibited by a surplus of unlabeled proline, 5-HTP (a known PAT1 inhibitor) or sarcosine and the absence of PAT1 protein in PC-3 cells was confirmed by western blot. Unlabeled sarcosine reduced the uptake of ^{14}C-glycine from an uptake rate on 8.1 pmol*min^{-1}*cm^{-2} to 1.7 pmol*min^{-1}*cm^{-2} and 5-HTP reduced the uptake rate to 3.5 pmol*min^{-1}*cm^{-2}. For ^{14}C-MeAIB the uptake rate was reduced from 7.4 pmol*min^{-1}*cm^{-2} to 1.7 pmol*min^{-1}*cm^{-2} in the presence of sarcosine and 3.6 pmol*min^{-1}*cm^{-2} in the presence of 5-HTP.

Conclusion

The present study finds no evidence for function or expression of PAT1 in PC-3 cells. Based on inhibition studies, sarcosine seems to interact with GlyT1 and SNAT2 or SNAT4 in PC-3 cells.

References

[1] Sreekumar A. *et al. Nature (2009)*, 457: 910-4
[2] Rodrigo M. A. M. *et al. Prostate (2018)*, 78: 104-12
[3] Cha Y. J. *et al. int J Clin Exp Pathol (2014)*, 15: 7824-33
[4] Piert M. *et al. J Nucl Med (2017)*, 58: 1216-23
[5] Heublein S. *et al. Oncogene (2010)*, 29: 4068-79

Citrem/lecithin dispersions for dermal administration

Emilie J. Svendsen[1*], Hoang U.M. Vu[1,2*], Jonathan Brewer[2], Judith Kuntsche[1]
[1] Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Denmark
[2] Department of Biochemitry and Molecular Biology, University of Southern Denmark, Denmark
** EJS and HUMV contributed equally*

Introduction: Lipid (nano)particles with a lyotropic liquid crystalline structure such as cubosomes and hexosomes are interesting drug carriers as they may provide advantages with respect to drug loading and release. Recently, formation of hexosomes (particles having an internal invers hexagonal phase) from Citrem/lecithin mixtures has been described [1]. So far, parenteral administration has been in focus, but dermal administration has not yet been investigated.

Aims: The aim of the study was to evaluate preparation parameters with respect to dispersion properties (size, morphology) and to investigate the effect of the dispersions on human stratum corneum (SC) lipid structure and thus their potential penetration enhancing effect.

Method: Dispersions of Citrem/lecithin (4:1 mass ratio, 50 mg/ml lipid) were prepared under different conditions (temperature, sonication time and power) in PBS pH 7.4. The samples were characterized by laser diffraction (size), cryo-TEM and small-angle X-ray scattering. Full-thickness human skin was treated with a DiD-labelled Citrem/lecithin (4:1 mass ratio, 50 and 20 mg/ml lipid) dispersions or liposomes (lecithin with 20 mol% cholesterol, 50 and 20 mg/ml lipid). After incubation for 4 h, the skin samples were cryo-sliced and cross sections were investigated by two-photon microscopy. Laurdan GP-analysis [2] was used to determine changes in SC lipid structure.

Results: All dispersions had a broad size distribution with a distinct fraction in the µm-size range. In good agreement with SAXS results, cryo-TEM indicates a diversity of structure with only a few particles having a well-ordered internal hexagonal structure (Fig. 1). Citrem/lecithin dispersions induced a more pronounced change in SC lipid fluidity than the liposomes and administration of higher concentrated formulations resulted in a larger effect. Accordingly, DiD penetrated deeper into the skin from Citrem/lecithin dispersions than from liposomes (20 mg/ml lipid).

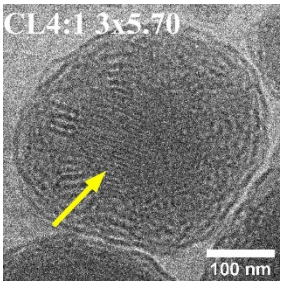


Fig 1: Hexagonal phase seen in a Citrem/lecithin dispersion, probe sonicated 3x5 min at 70% power

Conclusions: The results illustrate that even minor changes in composition (e.g. type or purity of lecithin) may result in distinct changes in particle morphology and homogeneity. The first experiments on skin appear interesting but more work is necessary to elucidate the impact of particle morphology and size of Citrem/lecithin particles with respect to their skin penetration enhancing effect.

References

[1] Azmi, I. D., et al. Journal of Controlled Release (2016), 239, 1-9.
[2] Bagatolli, L.A., et al., Methods in Enzymology (2003), 360, 481-500.

Rheological characterization of glyceraldehyde cross-linked chitosan hydrogels

Anca Maria Tasici[1*], Pierre Carmona[2], Sverre Arne Sande[1], Bo Nyström[3]
[1]Department of Pharmacy, University of Oslo, Norway
[2]Bioscience and Material/Agrifood and Bioscience, Research Institutes of Sweden
[3]Department of Chemistry, University of Oslo, Norway

Introduction

In general, orally administered drugs are expected to display a uniform absorption rate throughout the small intestine. However, some drugs have a narrow absorption window in the gastrointestinal tract. Increased residence time of the formulation in the upper intestine and a controlled-release system may be the solution for increased bioavailability of these drugs. Thus, a mucoadhesive cross-linked hydrogel could be used as matrix for controlled-release drug delivery system.

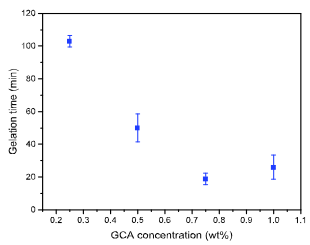
Aim

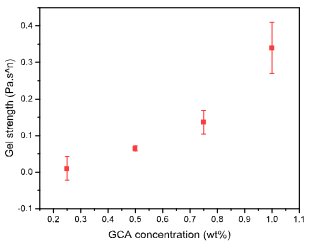
The aim of the project is to develop mucoadhesive films with a controlled and sustained release. The first step was to investigate the effect of cross-linker concentration, pH and temperature on gelation time, gel strength, and mesh size of chitosan hydrogels.

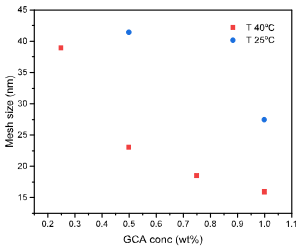
Method

A chitosan stock solution was prepared by dissolving chitosan in 1% v/v acetic acid. The pH of the chitosan solution was adjusted to 4.8 or 5.8 by adding 10M NaOH dropwise. A cross-linker stock solution was prepared by dissolving glyceraldehyde in MilliQ water. The cross-linker solution was added drop by drop and under magnetic stirring to the chitosan solution to obtain a final solution of 1%w/w chitosan and 0.25 to 1%w/w glyceraldehyde.
Oscillatory sweep experiments were carried out in an Anton Paar-Physica MCR 301 rheometer using a cone-and-plate geometry, with a cone angle of 1° and a diameter of 75 mm. This geometry was employed in all measurements. To prevent evaporation of the solvent, the free surface of the sample was covered with a thin layer of low-viscosity silicone. The measuring device is equipped with a temperature unit (Peltier plate) that gives an effective temperature control (±0.05°C) over an extended time for the temperatures (25 and 40°C) examined in this work.

Results







1. Gelation time is reduced by both increasing crosslinker concentration, pH and temperature.
2. The increase of cross-linker concentration has several effects on the properties of the gel network: values of fractal dimension parameter suggest that a tighter network is formed, the gel becomes stronger and the mesh size decreases.

Conclusion

By changing the formulation parameters, properties such as structure, strength, or tightness of the gel network can be modulated to develop functional hydrogels that can be used as drug delivery systems.

BET inhibitor (+)-JQ1 affects SIRT1 expression in two different breast cancer cells

Jonna Tyni[a], Tarja Kokkola[b], Marjo Huovinen[a], Minna Rahnasto-Rilla[a], Maija Lahtela-Kakkonen[a]

[a] School of Pharmacy, University of Eastern Finland, Yliopistonranta 1C, 70211 Kuopio, Finland
[b] Institute of Clinical Medicine, University of Eastern Finland, Yliopistonranta 1C, 70211 Kuopio, Finland

Introduction

Gene expression is controlled by various epigenetic processes, such as histone lysine acetylation. Histones are proteins that bind DNA tightly around themselves. When they are acetylated, the interaction between them and DNA weakens. Thus, histone lysine acetylation enhances gene expression. The acetylation process is controlled by many proteins, including sirtuins (SIRTs). SIRTs remove acetyl groups from histones and thus, prevent gene expression. Additionally, proteins such as and bromodomain and extraterminal proteins (BETs), can bind to acetylated histones and aid different transcription factors to interact with DNA and initiate gene transcription. As histone acetylation status affects also cancer development[1], both SIRTs and BETs are possible drug targets in cancer[2,3].

Aim

As both SIRTs and BETs affect gene expression through histone lysine acetylation status, there could be a functional link between them.

Methods

Hormone receptor negative breast cancer cells were exposed to DMSO (control) and five concentrations of (+)-JQ1 for 24 h. BET inhibitions effect on sirtuin 1 (SIRT1) expression and its activity was determined with western blotting. SIRT1 activity was determined indirectly by measuring the acetylation level of its target, p53.

Results

Previously, we have shown that BET inhibitor (+)-JQ1 increases SIRT1 expression in hormone responsive breast cancer cells (MCF-7 cells)[4]. In this study, we extended the research, and used breast cancer cells that lack hormone receptors (MDA-MB-231 cells). Opposite to our previous study with MCF-7 cells, (+)-JQ1 decreased SIRT1 expression in MDA-MB-231 cells. Additionally, the acetylation levels of SIRT1 substrate had no correlation to the changes in SIRT1 expression in MDA-MB-231 cells.

Conclusions

(+)-JQ1 affects SIRT1 expression, proposing that there can be an interplay between BETs and SIRTs. This interplay might open new starting points in developing treatments for SIRT1-related diseases. However, the effects differ between cancer types and thus, more studies are needed to determine the cancer types with most prominent responses.

References

[1] Gray SG, The BT. Histone acetylation/deacetylation and cancer: an "open" and "shut" case? *Curr Mol Med*. 2001;1(4):401-429.
[2] Chalkiadaki A, Guarente L. The multifaceted functions of sirtuins in cancer. *Nat Rev Cancer.* 2015;15:608-624.
[3] Fu LI, Tian M, Li X et al. Inhibition of BET bromodomains as a therapeutic strategy for cancer drug discovery. *Oncotarget*. 2015;6(8):5501-5516.
[4] Kokkola T, Suuronen T, Pesonen M et al. BET Inhibition Upregulates SIRT1 and Alleviates Inflammatory Responses*. Chembiochem*. 2015;16(14):1997-2001.

3D-printed polyethylene oxide gels with *in situ* UV-crosslinking

Laura Viidik[1*], Karin Kogermann[1], Jyrki Heinämäki[1], Ivo Laidmäe[1]
[1] Institute of Pharmacy, University of Tartu, Estonia

Introduction
Hydrogels are widely used in pharmaceutical sciences. Polymeric 3D-printed constructs for the incorporation and release of biologically active agents will further advance the use of hydrogels in wound healing settings. Many hydrogel-forming polymers, however, require additional irradiation for crosslinking to be applicable in such settings.[1] The crosslinking enables to control the mechanical robustness and active agent release properties of the preparations. It is of our interest to conduct crosslinking during 3D printing and reveal its advantages compared to post-process irradiation.

Aim
The aim of this study was to compare the influence of crosslinking during or after 3D printing to the mechanical properties of 3D-printed polyethylene oxide (PEO) / pentaerythritol triacrylate (PETA) constructs.

Method
An extrusion-based 3D printing system (System 30M, Hyrel 3D, USA) was used in printing 15%/10% PEO/PETA gels. The samples were UV-crosslinked either during or after 3D printing. The tensile strength of the 3D-printed samples was measured by a texture analyser (CT3 Ametek Brookfield, USA). The samples for a tensile test were 3D-printed using different printing speeds (0.5 mm/s; 1 mm/s; 1.5 mm/s).

Results
The tensile strength of 3D-printed PEO/PETA samples exposed to *in situ* UV-crosslinking was clearly different as the tensile strength of the samples UV-crosslinked after the printing. The dependency of the crosslinking time on the printing time must be taken into consideration.

Conclusions
In situ UV-crosslinking within a 3D printing process is a potential method for modifying the mechanical properties of 3D-printed PEO constructs. The *in situ* crosslinking gives also more flexible means to fine tune material properties (e.g. spatially and temporally controlled drug release).

Acknowledgements
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References
1. Hoffman. Adv. Drug Deliv. Rev., 18-23 (2012).

Improvement of in vitro antibacterial efficiency of thiamphenicol dry powder by increasing its dissolution rate

Junwei Wang[1*], Arshnee Moodley[2], William Couet[3], Jörg P. Kutter[1], Huiling Mu[1] and Mingshi Yang[1]
[1]Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark
[2]Department of Veterinary and Animal Sciences, University of Copenhagen, Copenhagen, Denmark
[3]INSERM, Université de Poitiers, Poitiers, France

Introduction

Lower respiratory infections is a major global health problem causing millions of deaths annually. By inhalation, antimicrobial agents can be directly delivered to the site of infection, which can afford a quick and effective treatment of the lung infection using a lower dose and minimal side effects when compared to oral administration[1]. Thiamphenicol (TAP) is reported to be effective against many respiratory pathogens including Methicillin-resistant Staphylococcus aureus (MRSA)[2]. However, it is poorly water soluble, which is one of the obstacles to formulate TAP into inhaled drug products.

Aim

The aim of this study was to formulate inhalable TAP dry powders with enhanced dissolution rate, and investigate whether a variation in the dissolution rate of TAP would result in different in-vitro antibacterial activity.

Method

Inhalable dry powders composed of TAP microcrystals (MDP) or nanocrystals (NDP) were prepared by using a wet ball milling method followed by spray drying. The size of ball-milled TAP particles, the morphology and solid state of inhalable TAP dry powders were characterized by using dynamic light scattering (DLS), scanning electron microscopy (SEM), laser diffraction (LD), X-ray diffraction (XRD), and differential scanning calorimetry (DSC), respectively. The in-vitro dissolution rates, antimicrobial susceptibility, and in-vitro antibacterial activity of the inhalable TAP dry powders were also evaluated using a modified time-kill assay..

Results

MDP and NDP were obtained by the spray drying process. XRD and DSC analyses showed that MDP and NDP possess the same crystalline form. NDP exhibited faster in vitro dissolution rate as compared to MDP and raw TAP. In addition, the in-vitro antibacterial efficiency of NDP and MDP were superior to raw TAP when the drug concentration was 2 times of minimum inhibition concentration of TAP.

Conclusions

This study showed that inhalable TAP dry powders with improved dissolution rates could be readily prepared by spray drying of micronized TAP. It also demonstrated that the in-vitro antibacterial efficiency of TAP could be improved with an increase in the dissolution rate of TAP.

References

1. Traini D, Young PM. Delivery of antibiotics to the respiratory tract: an update. Expert Opinion on Drug Delivery. 2009;6(9):897-905.
2. Maselli D, Keyt H, Restrepo M. Inhaled Antibiotic Therapy in Chronic Respiratory Diseases. International Journal of Molecular Sciences. 2017;18(5):1062.

Physiochemical aspects of subcutaneous administration of drugs

Marcus Wanselius[1], Susanna Abrahmsén-Alami[2], Christian Pedersen[3], Per Hansson[1]
1. Department of Pharmacy, Uppsala University, Uppsala 2. Astra Zeneca R&D Mölndal, Mölndal
3. Recipharm Pharmaceutical Development, Solna

Introduction

There are a growing amount of peptide and protein based pharmaceuticals with low oral bioavailability.[1] For these biopharmaceuticals the subcutaneous route are often a suitable alternative, but the bioavailability can vary greatly between the different pharmaceuticals and formulations. The basic process of absorption from the subcutaneous formulation to blood and lymph are quite well explored[1], but there are a lack of understanding of how the interactions between pharmaceuticals and the subcutaneous extracellular matrix (ECM) affects the bioavailability.

Aim

The aim of this project is to provide a mechanistic understanding of how pharmaceuticals (especially biologics) and excipients in drug formulations interact with the components of the ECM.

Method

Scattering methods such as SLS, DLS and SAXS are to be used together with NMR-diffusion to gain information about structure and size of interaction complexes. For quantification of interaction strength microgels consisting of cross-linked components of the ECM will be used. The interactions between these microgels and different biopharmaceuticals or excipients in solution will be investigated by a number of microscopy techniques. These includes visual light, fluorescence and confocal microscopy, also confocal Raman microscopy.

Initial research performed and expected outcomes

Similar microscopy experiments on microgels have been done within the research group before and a so called flow pipette technique combining micropipettes with microscopy have been developed.[2] This method gives reliable results of binding and release when looking at a single microgel, but it has the limitation of only being able test one microgel at a time. Therefore a method that makes it possible to test many gels at the same time to increase the efficiency is sought after. Such a method are currently being developed by combining microfluidics and microscopy.

The investigation of the interactions will provide the basis for the development of novel *in vitro* methods to model the behavior of subcutaneously administrated pharmaceutical products.

It will also provide the basis for development of new subcutaneous formulations with microgels as carriers.

References

1. Richter, W., Bhansali, S. and Morris, M. Mechanistic Determinants of Biotherapeutics Absorption Following SC Administration. *The AAPS Journal* **2012**, 14, 559-570.
2. Jidheden, C. and Hansson, P. Single Microgels in Core–Shell Equilibrium: A Novel Method for Limited Volume Studies. The Journal of Physical Chemistry B **2016**, 120, 10030-10042.

Preparation of nanoliposomes using a computer simulation based 3D-printed micromixer

Hangsheng Zheng*, Adam Bohr, Johan Peter Bøtker, Yongquan Li, Jukka Rantanen, Mingshi Yang
Department of Pharmacy, Faculty of Medicinal and Health Sciences, University of Copenhagen, Denmark
Email: hs_zheng@yahoo.com

Introduction

The size and size distribution of liposomes are critical quality attributes that determine the pharmacological effects of this kind of products. In the past decade many groups have demonstrated that the microfluidic hydrodynamic flow focusing (MHFF) method was superior to many conventional preparation methods with respect to the control of the size and size distribution of liposomes.[1-3] Recently, we utilized a computer simulation based design of 3-D printed micromixer (3DPM) to prepare nanoliposomes for drug delivery, which has shown some promising outcomes in terms of producing nanocrystals and polymeric nanoparticles. It is of interest to study the feasibility of utilizing this 3DPM (Fig 1) to produce liposomal formulations and compare the performance of the obtained liposomes with those obtained from an advanced technology such as MHFF.

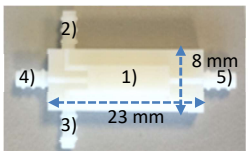

Fig. 1. *The picture of 3DPM, 1) mixing chamber, 2,3) two outer inlet channels, 4) one inner inlet channel, 5) one outlet channel.*

Aim

The aim of this study was to investigate the influence of the key process parameters of 3DPM and formulation variables on the size and size distribution of a model liposomal formulation.

Method

Soybean lecithin, cholesterol and vitamin E were dissolved in some alcohols such as methanol, ethanol and isopropanol to make a lipid solution. A phosphate buffer solution of pH 7.0 was used as an aqueous solution. The two solutions were fed into the 3DPM through the inlets. The effects of the total flow rate, the temperature, the flow rate ratio, the total lipid concentration, the type of alcohols to dissolve the lipids, the salt concentration in aqueous phase and the amount of cholesterol on the z-average diameter and polydispersity index (PDI) of the vesicles were investigated.

Results

The results indicated that the z-average diameters of the obtained nanoliposomes decreased with an increase in the total flow rate, and reached a plateau at around 200 µL/min. The PDI of the vesicles was lower than 0.178. In contrast, the z-average diameters of the vesicles increased with an increase in the flow rate ratio of aqueous solution to lipid solution. The minimum z-average diameters of the vesicles were obtained when the preparation was carried out at a temperature range of 20 – 40 °C. A striking increase in the z-average diameters and PDI was observed when the experiment was conducted above 40 °C. The z-average diameters and the PDI of the obtained vesicles increased with an increase in the total lipid concentration and the faction of cholesterol. The effect of salt concentration of the phosphate buffer solution as the aqueous solution seemed to be negligible. However, the type of the alcohols seemed to largely influence the size of the vesicles obtained, the ethanol gave better results. In the present study, nanoliposomes with z-average diameters of around 135 to 200 nm could be prepared controllably with narrow size distribution.

Conclusion

The study demonstrated the potential of computer simulation based 3DPM to produce lipid nanovesicles with controllable size.

References

1. Jahn A, et al. J Am Chem Soc (2004) 126:2674–2675
2. Jahn A, et al. Langmuir (2007) 23:6289–6293
3. Zook JM, Vreeland WN. Soft Matter (2010) 6: 1352-1360

Understanding nanoparticle design-toxicity relationship of mesoporous silica nanoparticles in the zebrafish model

Ilkka Paatero[1,3*], Eudald Casals[4], Rasmus Niemi[2,3], Ezgi Özliseli[4], Jessica M. Rosenholm[4], Cecilia Sahlgren[2,3,5,6*]

[1]Department of Cell Biology, Biozentrum, University of Basel, Basel, Switzerland, [2]Faculty of Science and Engineering, Cell Biology, Åbo Akademi University, Turku, Finland, [3]Turku Centre for Biotechnology, Åbo Akademi University and University of Turku, Turku, Finland, [4]Pharmaceutical Sciences Laboratory, Faculty of Science and Engineering, Åbo Akademi University, Turku, Finland, [5]Department of Biomedical Engineering, Technical University of Eindhoven, 5613 DR, Eindhoven, The Netherlands, [6]Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, The Netherlands

Introduction

Mesoporous silica nanoparticles (MSNs) have received extensive attention in the last decades as drug delivery systems due to its versatility. Surface functionalization of MSNs expands applicability as drug delivery platform by influencing dispersion stability, biocompatibility, biodistribution, drug release, efficacy and biodegradation[1]. Their design-toxicity relationship yet needs investigation in the more complicated biological systems relevant to human physiology to able to develop safer nanotherapies. Zebrafish model is widely used in toxicity studies due to its similar cardiovascular, nervous and digestive systems with mammals.

Aim

Zebrafish (*Danio rerio*) embryos were selected as a model system and their toxicity profiles of differently surface functionalized MSNs were studied.

Method

Amino(NH2-MSNs), polyethyleneimine(PEI-MSNs), succinic acid(SUCC-MSNs) or polyethylene glycol(PEG-MSNs) functionalized MSNs were synthesized under controlled conditions. Cellular viability and uptake of MSNs were determined *in vitro* by using C2C12 myoblasts. Chorion membrane intact embryos or dechoroniated embryos were incubated or microinjected with MSNs and toxicity was evaluated by viability and cardiovascular function.

Results

NH2-MSNs, SUCC-MSNs and PEG-MSNs did not show any lethality, whereas, 50 µg/ml PEI-MSNs induced 100% lethality 48 hours post fertilization (hpf). Dechoroniated embryos were more sensitive and 10 µg/ml PEI-MSNs reduced viability to 5% at 96 hpf. Cardiovascular toxicity was observed prior to lethality by stereomicroscope and confocal microscopy revealed that PEI-MSNs were penetrated to the embryos whereas PEG-MSNs, NH2-MSNs and SUCC-MSNs remained aggregated on the skin surface (Fig 1).

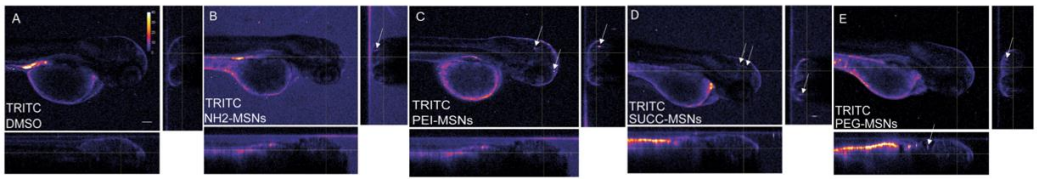

Fig. 1**. Confocal image of the uptake of the surface modified MSNs into embryo. 24hpf embryos were incubated for 48h with the highest tolerated dose of the MSNs. NH2- **(B)**, SUCC- **(D)** and PEG-MSNs **(E)**, and PEI-MSNs **(C)**. DMSO treated embryos were used as controls and showed background fluorescence (**A).

Conclusion

The results support that there is a requirement for careful analyses of toxicity mechanisms in relevant models and establish an important knowledge step towards the development of safer and maintainable nanotherapies.

References

1.Desai D. *et al.* Mesoporous Biomaterials (2014) 1,16-43, 2.Paatero I. *et al.* Scientific Reports (2017) 7, 8423, 1-13.