Current trends in pharmaceutical preformulation and manufacturing process design

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The XXIX Symposium of the
Finnish Society of Physical Pharmacy

Thursday 8th of February

8:30 - 9:00 Registration
9:00 Opening of the Symposium
   
   
   Jaana Koskela, Chair of the Society 2017

9:05 Novel developments in the formulation of amorphous drugs and solid dispersions
   
   Thomas Rades, University of Copenhagen, Denmark

09:40 High-throughput screening platform for selection of amorphous solid dispersion formulations
   
   Ruzica Kolakovic, Janssen Pharmaceutica, Belgium

10:15 Coffee break, poster session and exhibition

11:00 On-demand inkjet-printed personalized multi-layered dosage forms
   
   Erica Sjöholm, Åbo Akademi University, Finland

11:20 Single particle analysis (SPA) - Image-based method for rapid and accurate solubility screening
   
   Sami Svanbäck, University of Helsinki, Finland

11:55 Atomic pair distribution function (PDF) analysis to assess amorphous organic compounds
   
   Detlef Beckers, Malvern Panalytical

12:10 Lunch, poster session and exhibition

13:20 Toos for biophysical characterisation
   
   Timo Saarela, Hosmed Oy

13:35 Formulation and manufacturing of nanomedicines using microfluidics
   
   Adam Bohr, University of Copenhagen, Denmark

14:10 Coffee break, poster session and exhibition

14:50 From material characterization to optimal processing
   
   Pirjo Tajarobi, AstraZeneca, Sweden

15:25 Drivers for continuous tablet manufacturing in pharmaceutical industry
   
   Satu Lakio, Orion Pharma, Finland

16:00 Annual meeting of the Society of Physical Pharmacy

18:00 Symposium dinner at Trattoria Sogno
The cellZscope from nanoAnalytics is a device for measuring TEER; the transepithelial/endothelial impedance of cell layers under physiological conditions. It is computer-controlled and allows automated, long-term monitoring experiments in the incubator with up to 24 different cell cultures simultaneously or 24 different exposures. It also measures $C_{CL}$: Cell Layer Capacity.

Baker Ruskinn provide solutions for cell biology, stem cell and regenerative medicine, including accurate and stable anaerobic chambers, hypoxia workstations, and precise oxygen regulation in culture media. Sci-tive is the flexible Hypoxia system with many sizes and shapes. InvivO$_2$ is single or dual chamber Hypoxia Workbench - latest technology.
Friday 9th of February

9:30 Opening of the second day

09:35 Implementation and examples of pharma OSD continuous manufacturing
   Cait Boyd, GEA Group, Belgium

10:10 Significance and measurement of residence time distributions in continuous manufacturing
      Ossi Korhonen, University of Eastern Finland, Finland

10:45 The influence of co-monomers applied in the SFPP course on the electrokinetic potential of thermosensitive microparticles for controlled drug delivery
      Witold Musial, Wroclaw Medical University, Poland

11:05 Coffee break, poster session and exhibition

11:50 A continuous and controlled pharmaceutical freeze-drying technology for unit doses
      Thomas De Beer, Ghent University, Belgium

12:25 Biophysical tools for characterizing protein self-association
      Petteri Heljo, Novo Nordisk, Denmark

13:00 Closing of the symposium
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Editorial

For the 29th time the Finnish Society of Physical Pharmacy brings together professionals from academia, industry and regulatory authorities to interact and discuss about ongoing research and trends in physical pharmacy. Our aim was to arrange a valuable symposium with topics reflecting the current trends in the field. Thus, we are happy and honored to have gathered experts with knowledge about the recent advancements in preformulation and manufacturing process design.

This year’s commentary article “Are we measuring too much?” is written by Anne Juppo, Professor in Industrial Pharmacy at the University of Helsinki. In the article she shares the history about the development of the analytical technologies during the past decades. Furthermore, she also advises on to reflect on why we are doing certain measurements – are we doing the tests just because it is nice to know?

Wishing you interesting reading moments and a nice symposium!

Henrika Wickström  
Editor in Chief - Polymorfi 2017

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From the chairman

The world is moving faster, and it has become ever more important to get new and better products to the market faster and more efficiently. Preformulation is the key for a new drug candidate to enter a successful drug development, and finding an appropriate formulation and process in a cost effective fashion is crucial. As these topics are both contemporary and interesting, the 29th Annual Symposium of the Finnish Society of Physical Pharmacy seeks to examine the latest trends in preformulation and manufacturing process design.

The purpose of the Society is to promote knowledge of physical pharmacy in Finland and internationally, and to enable broad sharing of knowledge and collaboration between academic and industry professionals. Thus, the presentations and talks in the symposium aim to cover a broad range of inspiring topics from novel material sparing analytical methods in determining physiochemical properties of compounds to continuous manufacturing designs.

I would like to thank the participants, speakers, poster presenters and sponsors for your contributions. You and your active participation makes this symposium valuable. We hope that all participants will have inspiring and productive discussions during the symposium, and that together we make this a lively event.

Wishing you all a pleasant symposium!

Jaana Koskela
Chairman of the Finnish Society of Physical Pharmacy 2017
Lecture abstracts
Amorphous solid dosage forms are one of the most promising formulation strategies to overcome the limited bioavailability of many poorly water soluble drugs. However, the industrial application of amorphous solid dosage forms is still rather limited. This is likely to be due to an insufficient understanding of the physico-chemical properties of amorphous solid dispersions including their physical stability, as well as due to the lack of predictive in vitro models. In this presentation, methods to predict amorphous drug stability and drug–polymer solubility will be discussed. We will then focus on alternatives to polymers in the formulation of amorphous solid dispersions and finally on the question how both the in vitro and in vivo performance of amorphous solid dispersions is influenced by the use of amorphous solid dispersions.

About the presenter
Since March 2012 Professor Thomas Rades is the Research Chair in Pharmaceutical Design and Drug Delivery in the Department of Pharmacy, University of Copenhagen. Before that he has been the Chair in Pharmaceutical Sciences at the National School of Pharmacy, University of Otago, New Zealand from 2003 – 2012. In 1994 he received a PhD from the University of Braunschweig, Germany for his work on thermotropic and lyotropic liquid crystalline drugs. After working as a Research Scientist in the Preclinical Development and Formulation at F. Hoffmann-La Roche in Basel, Switzerland, he became a Senior Lecturer in Pharmaceutical Sciences at Otago in 1999 and since 2003 held the Chair in Pharmaceutical Sciences in Otago. Professor Rades has developed an international reputation for his research in the physical characterization of drugs and solid dosage forms as well as in vaccine delivery using nanoparticulate systems (both polymeric and lipid based). He has published more than 350 papers in international peer reviewed journals as well as 18 book chapters, 11 patents and 3 books.

Professor Rades is an Editor of the Journal of Pharmaceutical Sciences and the European Journal of Pharmaceutics and Biopharmaceutics and an Associate Editor of the Journal of Pharmacy and Pharmacology.

He holds an honorary doctorate of Åbo Akademi University, Finland, a visiting professorship at the Department of Medicine at the University of Adelaide, Australia and an honorary professorship at the University of Otago, New Zealand. He is an Eminent Fellow of the Academy of Pharmaceutical Sciences (UK), a Fellow of the New Zealand Institute of Chemistry and a member of the College of Fellows of the Controlled Release Society.

Professor Rades has successfully supervised more than 60 PhD students (completed) with currently supervising or co-supervising 12 PhD students. For his undergraduate and postgraduate teaching he was awarded the New Zealand Tertiary Teaching Excellence Award for Sustained Excellence (2005).

His research interests include The solid state of drugs and dosage forms, and Nanoparticles as delivery systems for drugs and vaccines. Research in both areas aims to improve drug therapy through appropriate formulation and characterisation of medicines and to increase understanding of the physico-chemical properties of drugs and medicines. It combines physical, chemical, and biological sciences and technology with analytics to optimally formulate drugs and vaccines for human and veterinary uses.

Current research projects include: Co-amorphous drug delivery systems, Solubility of drugs in solid polymers, lipid based drug delivery systems
High-throughput screening platform for selection of amorphous solid dispersion formulations

Ruzica Kolakovic

*Janssen Pharmaceutica, Pharmaceutical Companies of Johnson & Johnson, Belgium*

Vast majority of new active pharmaceutical ingredients (APIs) entering development pipeline from discovery possess insufficient water solubility and/or low dissolution rate. These cause challenges in achieving desired bioavailability.

Conversion of crystalline API to its amorphous form is often taken as approach to achieve desired bioavailability based on higher solubility, faster dissolution rate, and enhanced oral bioavailability of amorphous APIs compared to their crystalline counterparts. Over the last decade the production of stabilized amorphous drugs via amorphous solid dispersions ASDs has become an increasingly popular formulation strategy and the success of this strategy is reflected in the significant number of marketed amorphous products (2,4).

Variety of formulation variables can affect the development of ASDs with drug and polymer physicochemical properties, selection of polymeric excipients, the definition of the drug polymer ratio, addition of surfactants being some of them. A number of research laboratories have been developing their own screening methodologies, either based on experimental data or based on theoretical fundamentals with one common goal – choosing the right formulation composition which will ensure both desired bioavailability and physical stability of ASD over intended shelf-life.

Selecting the optimal formulation can take a vast amount of time, can be extremely costly and importantly can require significant amount of API which is not readily available in early phase of development. In order to reduce development time and cost, improve success rate and minimize API consumption, Janssen Pharmaceutica has developed automated high throughput screening platform for selection of ASD formulations. Such a screen is usually performed using 5-7 polymers and 2 additives and their combinations. 7 - 43 formulations can be tested simultaneously. The amorphous solid dispersions are produced in 96 well-plate by low volume film casting method resulting in amorphous films containing 25 - 75 μg of API. The produced films are evaluated for their dissolution rate by small scale two-stage dissolution method. Stability of the formulations is assessed as well by exposing films to stressing conditions (elevated temperature and/or relative humidity) and assessing crystallization propensity by cross-polarized imaging.

Use of automated high throughput screening platform allows selection of 2-5 formulations for further scale-up with minimum time and API input (1 week and 1.5 g of API).
References


About the presenter

Ružica Kolakovic obtained her PhD in Pharmaceutical Technology from the University of Helsinki where she studied use of nanofibrillar cellulose as novel excipient in drug delivery. She worked as a postdoctoral scientist at Åbo Akademi in Turku, Finland focusing on application of printing as a technique for production of drug delivery systems. In 2014 she joined Janssen Pharmaceuticals (Johnson & Johnson) in Belgium as scientist in preformulation group where she is responsible for developability assessment of compounds entering development pipeline. She is focused on defining the early drug development strategy through in depth understanding of the compound properties and targeted product profile.
One of the key physicochemical properties determining the developability of a drug is solubility. Currently in solubility measurement, there appears to exist a discrepancy between throughput, substance consumption and accuracy [1, 2].

While solubility is generally determined from bulk solutions after long incubation times, the diffusion layer dissolution rate model implies that solubility, as the rate limiting factor of dissolution, can be rapidly extracted from dissolution rate data of individual particles.

Automated image-based single-particle analysis (SPA) could substantially simplify data acquisition and processing, reducing sampling steps and operator contact with potentially hazardous substances. It could also allow for compounds of high value or scarce availability, such as in drug development, to be reliably analyzed. The non-specific and non-interfering nature of image analysis further entails several potential advantages.

We have applied the SPA method to measure the equilibrium solubility of drugs, excipients, pesticides and sugars. The correlation with gold standard “shake-flask” equilibrium solubility values is high. In addition, the SPA method has been used to measure the apparent solubility of salts, polymorphs and amorphous drug forms.
Formulation and manufacturing of nanomedicines using microfluidics

Bohr Adam

Department of Pharmacy, University of Copenhagen, Denmark

Microfluidics has seen immense advances for analytical applications and is currently experiencing increasing popularity for the formulation and manufacturing of nano/micro systems intended for drug delivery. Nanopharmaceutical products have to some degree been limited by their production scalability and cost, and microfluidics-based systems may overcome these challenges and allow for the mass commercialization of nanopharmaceutical products.

This talk will focus on the application of microfluidic devices for preparing nanoparticulate pharmaceutical products, giving examples of different types of devices and nanoparticle systems, and the characteristics and performance of the resulting nanoparticles. Moreover, the application of 3D printing for fabrication of microfluidics devices and the application of in silico design and modelling for microfluidic systems will be discussed.

About the presenter

Adam Bohr received his PhD degree in biomedical engineering in 2013 from University College London. From 2013-2015 he worked as a Postdoctoral researcher at the Institut Galien, University of Paris-Sud and from 2015-16 he worked as a Postdoctoral researcher at the Department of Pharmacy, University of Copenhagen. In 2016 he was appointed as Assistant Professor at the Department of Pharmacy, University of Copenhagen, Denmark. His research interests are in formulation and manufacturing of drug delivery systems, in particular within the area of nanomedicine. This includes the formulation of polymeric drug-loaded nano- and microparticles for oral and pulmonary delivery for the treatment of infections and inflammation using novel polymers and drugs with low bioavailability. This also includes manufacturing of drug delivery systems and drug delivery devices using microfluidics and 3D printing. He has 31 publications in international, peer-reviewed journals (h-index 12, determined from Google Scholar), as well as 3 patents.
During processing of new drug compounds material related problems are quite common. This has an immediate impact on the work within product development, as well as on manufacturability during scale-up and large scale production. A possible early identification of descriptors for material related issues would save both time and resources. This talk will focus on the material characterization methods and predictive models typical for direct compression, roller compaction and wet granulation processes.

**About the presenter**

Pirjo Tajarobi (née Luukkonen) is a pharmacist and received her Ph.D. in the Pharmaceutical technology from the University of Helsinki (Finland) in 2001. She joined AstraZeneca Mölndal, Sweden 2001 and she has worked in the area of Product Development, PAT and Material Science. Currently Pirjo is working as an Associate Principal Scientist in the Drug Product Manufacture. She is an Associate Professor both at the University of Helsinki and Åbo Akademi University in Finland. She is an expert in the areas of high-shear wet granulation, continuous manufacturing, and powder flow characterization. She has published >20 peer-reviewed papers in these areas.
Continuous manufacturing (CM) can offer significant advantages over batch manufacturing of solid dosage forms. These benefits include enhanced cost and quality aspects but CM also provides tools for accelerating product development which can decrease the time-to-market for new drug products. CM also offers more flexible manufacturing of tablets and thereby better enables personalized medicine concept than traditional batch manufacturing does. Thus many pharma companies are taking steps towards CM. However, succeeding with CM requires not only significant investments on infrastructure but also change in mindset, ways-of-working, new skillset and expertise.

About the presenter
Satu Lakio is Senior Development Manager and Scientific Manager in Continuous Manufacturing at Orion Pharma, Finland. She got her PhD degree from University of Helsinki, Finland and her PhD research covered real time monitoring of pharmaceutical powder processing. Dr Lakio did her postdoctoral period at Monash University in Melbourne, Australia focusing on inhalation powder research. She has previously worked in several positions at academia and as an Associate Principal Scientist at AstraZeneca in Gothenburg, Sweden. She also has long history of working in community pharmacies. Dr Lakio holds an adjunct professorship in University of Helsinki (Pharmaceutical technology) and she has supervised several under and post graduate as well as PhD students over the years. Her research covers pharmaceutical powder characterization and processing all the way from inhalation powders to coated tablets including Process Analytical Technologies (PAT). Currently her main focus area is continuous tablet manufacturing.
Traditionally the pharmaceutical industry has manufactured their drugs in batch processes. In recent years, there has been a shift to explore continuous manufacturing for oral solid dosage drugs. The drivers of this move to continuous manufacturing fall under two main areas: lean manufacturing to remove non-value added steps in a process and Six Sigma to use process understanding to improve quality and efficiency.

Using the ConsiGma™ Continuous Tabletting Line, we explored how time to market was affected by moving to a continuous wet granulation process. It was found that commercial manufacturing could begin over 1 year earlier due to a reduction in scale up steps.

For a continuous direct compression process the ConsiGma™ CDC-50 was used to investigate the effect of lot to lot raw material variation on the system. After the raw material lot change the loss in weight feeders adjusted the feed factor to adapt to the change in raw material bulk density which in turn reduced variation in the final product.

Invited Lecture

Implementation and Examples of Pharma OSD Continuous Manufacturing

Cait Boyd

GEA Group, Belgium

About the presenter

Cait Boyd has a background in chemical engineering and started her career as a process engineer focusing on the scale up of emulsions. In 2012 she joined GEA and shortly after transitioned from engineering into sales of pharmaceutical powder processing equipment in the US market. In 2016 she moved to Belgium and is currently a sales support and business development manager focusing on pharma OSD continuous manufacturing.
The current manufacturing methods of drug products are very old-fashioned in comparison with the other mass production industries like food, chemical, pulp and paper, oil, mining, etc. Drug manufacturing based mostly on batch production. The main disadvantage of batch production is that it is very time consuming due to the multiple quality checks between unit operations. Also the scale up of batch production is often problematic from the R&D to production scale. Now Pharma industry has also started a transition from batch production to the continuous production. One of the driving forces for the continuous production is many new guidelines (like QbD and PAT guidelines) from regulatory bodies which emphasizes that the quality of the drug product cannot be “tested in” but it has to be “build in” with the aid of PAT-tools. The main advantages of the continuous manufacturing of drugs are a smaller footprint of the production site, smaller equipment, less material in process at the time, basically no scale up since the production scales up by time instead of the dimensions of production equipment as in the batch process, and production runs in the steady state most of the time whereas for example in batch mixing is a transient process. All of these enhance the efficiency and lower the costs of drug manufacturing. Challenges are how to handle out of spec situations, how to feed accurately low content ingredients. Also some existing drug and excipient grades are very cohesive and thus difficult to feed accurately. Finally how to synchronize the production rates of unit operation in series in order to avoid material overfill and/or run-out. For successful implementation of continuous manufacturing the key parameters are residence time distributions and mass hold-ups in each unit operations under different process and formulation conditions. Based on these parameters, powder stream can be monitored and controlled along the serial unit operations. Also they enable the traceability of powder in out of spec situations.

The presentation will cover the introduction of continuous PROMIS tabling line, the performance of unit operations, the residence time distributions and mass hold-ups of key unit operations and practical pros and cons observed during experiments.
About the presenter

Ossi Korhonen has graduated as a M.Sc. from the Department of Pharmaceutics, University of Kuopio, 1997. He received Ph.D. from the same University, 2004. Title of his thesis was "Starch Acetate as a novel tablet excipient for extended oral drug delivery". He got the title of Docent in Pharmaceutical Technology, 2013. He has visited as a post-doc at the School of Pharmacy, University of Connecticut, Storrs, USA in Mike Pikal’s Lab between 2004-2005. The topic of research conducted during the post-doc year was "The Stabilization of small molecule amorphous drugs". Since then he has been in different teaching and project positions in the School of Pharmacy, University of Eastern Finland. He has supervised 4 Ph.D.s, 43 M.Sc., 22 Baccalautare thesis. Currently he is supervising 5 Ph.D. students. He has published 43 papers and is the author of one book chapter. His main research topics are continuous manufacturing of tablets, Stabilization of small molecule, low soluble amorphous drug and development and optimization freeze-drying formulations and processes. He has an excellent knowledge of physical pharmacy, formulation and process optimization in solid dosage forms, Design of Experiment, multivariate analysis, spectroscopy, and thermal analysis.
A Continuous and Controlled Pharmaceutical Freeze-Drying Technology for Unit Doses

Thomas de Beer

Laboratory of pharmaceutical process analytical technology, Ghent University, Belgium

Driven by growing needs in the biopharmaceutical market and regulatory pressure, a continuous and controlled freeze-drying technology for unit doses to preserve biopharmaceuticals has been developed. Such continuous process allows a more efficient, cheaper, greener and controllable manufacturing method compared to traditional batch production systems, offering competitive advantages and business opportunities.

Pharmaceutical freeze-drying (lyophilization) is a low-temperature drying process in which aqueous solutions of heat-labile biopharmaceuticals are converted into solids with sufficient stability for distribution and storage. Similar to all manufacturing processes of drug products (solids, semi-solids and liquids), conventional pharmaceutical freeze-drying is generally accomplished using batch processing that is considered time-consuming, costly, non-flexible and lacking robust quality control and real-time release.

Four major industrial drivers are demanding a more efficient and better controllable pharmaceutical freeze-drying technology for unit doses: cost-cutting, regulatory pressure, a fast growing biopharmaceutical market and an ageing population requiring more personalized medicines.

The continuous and controlled freeze-drying technology, developed following the principle of model based design, offers clear advantages over current batch production such as cost reduction (up to 50%), track-and-trace product quality control, and a significant reduction of processing time (> 40 times faster, e.g. 1 hour instead of 5 days at a vial level), reduced need for clean room and a substantial sustainability gain.

About the presenter

Thomas De Beer graduated in pharmaceutical sciences in 2002 at the Ghent University in Belgium. He obtained his PhD at the same university in 2007. For his PhD research, he examined the suitability of Raman spectroscopy as a Process Analytical Technology tool for pharmaceutical production processes. Within his PhD research period, he worked four months at University of Copenhagen in Denmark, Department of Pharmaceutics and Analytical Chemistry (Prof. Jukka Rantanen). After his PhD, he was an FWO funded post-doctoral fellow at the Ghent University (2007-2010). Within his post-doc mandate, he worked 9 months at the Department of Pharmacy, Pharmaceutical Technology and Biopharmaceutics from the Ludwig-Maximilians-University in Munich, Germany (Prof. Winter and Prof. Frieß). In February 2010, he became professor in Process Analytical & Technology at the Faculty of Pharmaceutical Sciences from the University of Ghent. His research goals include bringing innovation pharmaceutical production processes (freeze-drying, hot-melt extrusion, continuous from-powder-to-tablet processing etc.). More specifically, Prof. De Beer contributes to the development of continuous manufacturing processes for drug products such as solids, semi-solids, liquids and biologicals (continuous freeze-drying of unit doses).
One possible form of instability for protein structured pharmaceuticals is aggregation, where protein molecules bind covalently or non-covalently together to form larger self-associated entities. The resulting aggregates tend to be biologically inactive, and they have also been suspected of being capable of eliciting the immune response, meaning that their formation is often considered an undesired instability event. However, controlled protein self-association can also be used to adjust the pharmacokinetic profile of a protein product, when the availability of bioactive monomers in plasma is affected by the rate of dissociation of the self-associated state at the injection site. Furthermore, the physicochemical stability of protein oligomers can be significantly better than that of monomers.

One of the aims of biophysical characterization is to predict how protein monomers interact with one another. Protein self-association tendency can be probed by trying to induce structural alterations within the molecule, for example by heating or freezing the formulation, or subjecting it to mechanical stress. Individual amino acid residues can also be exchanged at key locations of the peptide backbone if these residues are expected to contribute to the overall aggregation profile.

The complexity of the parameters affecting self-association makes it necessary to employ several complementary analytical techniques in order to understand the factors which govern protein aggregation. Biophysical screening attempts to form an estimate of real-life shelf stability, and it is often used to weed out unstable molecules during lead candidate selection step of drug development.

This talk will give a brief introduction to protein self-association and unwanted aggregation, and describe some of the most common biophysical characterization tools used to study these. The aim is to provide an overview of the field rather than drilling deep into individual techniques and phenomena.

This is abstract template for abstract book Polymorfi in Symposium of Physical Pharmacy. Abstract can be 1-2 pages long.

Graphical work in abstract should fit to one column. If possible, send the image separately or at least make sure that word is not compressing images. Do not draw, edit or plot images in Word, images are not good enough for a print.

About the presenter

Petteri Heljo finalized his PhD thesis with the title “Comparison of disaccharides and polyalcohols as stabilizers in freeze-dried protein formulations” at the University of Helsinki in 2013 under the supervision of Prof. Anne Juppo. He spent the next two years as a postdoc at Roche (Switzerland) investigating peptide – excipient interactions, before moving to work as a Senior Research Scientist at Novo Nordisk (Denmark). His current research interests include high-throughput sterile formulation workflows and novel particle analysis techniques.
On-demand Inkjet-printed Personalized Multilayered Dosage Forms

Erica Sjöholm

Pharmaceutical Sciences Laboratory, Åbo Akademi University, Åbo, Finland

Producing flexible and tailored drug delivery systems according to a patient’s need instead of manufacturing mass-oriented drug delivery systems in a ‘one size fits all’ manner, has gained interest in recent years [1]. Printing techniques have emerged as potential technologies for on-demand manufacturing. Inkjet printing with short manufacturing time that enables precise deposition of an ink onto a given carrier matrix (substrate) is a potential new solution to produce tailored drug delivery systems at the point of care [2].

The main research focus of this work was to study the feasibility to deposit drug-loaded ink onto orodispersible polymer films by a 2D printing approach using inkjet printing to produce prednisolone containing multilayered personalized dosage forms. An off-the-shelf office inkjet printer was selected as a quick and economical manufacturing method that potentially could be used in a hospital setting. A schematic illustration of the process steps can be seen in Figure 1.

In the 15% (w/w) HPC film solution, hydroxypropyl cellulose (HPC) (Klucel–EXF PHARM, kindly provided by Ashland, Germany) served as film-forming agent and ethanol (Etax A, Altia, Finland) served as solvent.

Figure 1. A schematic illustration of the workflow for manufacturing printed multilayered dosage forms.

Figure 2. An Epson XP-760 desktop inkjet printer was used in this study for printing prednisolone containing multilayered dosage forms.
Dispersible films were cast to a wet thickness of 200 µm with a film-casting knife (Film Applicator MULTICATOR 411, Erichsen, Germany). Dried film sheets were used as printing substrates.

Prednisolone (≥ 99% Sigma-Aldrich, China) was used as a model drug. An ethanol-based ink solution consisting of 25 mg/ml prednisolone, 20% (v/v) distilled water and 1% (v/v) red food color was prepared. All water used was purified by Millipore SA-67120 from Millipore, Molsheim, France.

An Epson XP-760 desktop inkjet printer (Figure 2) was used to print the prepared drug ink onto the dried HPC orodispersible films according to the pattern set in the computer-aided design (CAD) (1x2 cm rectangle). A total of four different multilayered dosage forms were produced consisting of 5, 10, 15, and 20 printed drug layers with a fresh HPC layer cast after every fifth printed drug layer.

The multilayer dosage forms (n=3) were immersed in distilled water and drug content was measured using PerkinElmer Lambda 25 UV-Vis spectrophotometer (Singapore). As reference prednisolone ink was printed on copy paper.

The drug ink could successfully be printed on the casted HPC film and re-casted after five printed drug layers. The reference drug amounts printed on copy paper were slightly higher than the obtained amounts for the multilayer dosage forms. For the 2 cm² 5, 10, 15, and 20 drug printed layers on copy paper the drug amounts achieved were 0.3, 0.7, 1.0, and 1.3 mg, whereas the drug amounts achieved for the printed 2 cm² multilayered dosage forms were 0.3, 0.6, 0.9, and 1.2 mg respectively (Figure 3).

Low standard deviation (± 0.0 mg for drug printed on copy paper and in the range of ± 0.00-0.05 mg for the multilayered dosage forms) and good linearity (R² = 1 for drug ink printed on copy paper and R² = 0.9984 for the multilayered dosage forms) indicates that aimed personalized doses can be produced utilizing this method.

In conclusion, the desktop inkjet printer was successfully used to accurately imprint HPC films with prednisolone. Multiple printing layers could be used to increase the final drug content. In this study, one drug was investigated, however by printing various drugs between the casted film layers multilayer multidrug formulations could be obtained. Overall, inkjet-printed orodispersible multi-layered dosage forms appear as a promising new approach to enable personalized dosing.

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References


The influence of co-monomers applied in the SFPP course on the electrokinetic potential of thermosensitive microparticles for controlled drug delivery

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Various thermosensitive polymers, synthesized in the course of surfactant free precipitation polymerization, may be obtained, due to high number of possible co-monomers, cross-linkers, and solvents proposed for the process. One of the most interesting thermosensitive polymers with high applicative potential is poly-N-isopropylacrylamide, (pNIPA) and its variation may be applied as drug carriers and medical devices[1,2]. The microparticles may be characterized i.a. by electrokinetic potential, which is significant for the stability of colloidal dispersions.

The aim of the work was evaluation of the influence of variable co-momers on the electrokinetic potential of polymeric microparticles synthesized with the use of N-isopropylacrylamide (NIPA).

The poly-NIPA particles were prepared in an aqueous solution with redox initiators, and in some cases accelerator. The components were dissolved in 900 mL of distilled water heated up to 70 °C in a four-necked round bottom flask equipped with magnetic paddle stirrer, reflux condenser, thermometer, and nitrogen gas inlet. The reaction vessel was kept at 70 °C for 6 h with respective mixing. After the reaction was terminated, the mixture was cooled to room temperature. Obtained polymer solutions were purified via dialysis for 15 days at room temperature. The water was stirred and changed every 24 h. The purified products were frozen and freeze-dried by Chris Alpha 1–2 LD (Osterode am Harz, Germany) for 32 h.. Dynamic Light Scattering (DLS) Zeta Sizer Nano device of Malvern Instruments was used to measure values of zeta potential in water dispersions of the synthesized polymers as a function of temperature. Zeta potential was measured in capillary cell type DTS-1070. Data of zeta potential were averaged from three replicates using ZetasizerNano Software, Version 7.11.

Figure 1. Electrokinetic potential of NIPA-derivatives – polymeric microparticles synthesized with various co-momers and initiators at 18 °C.
The electrokinetic potential of synthesized microparticles was between -32.10 mV and 47.97 mV. The values depended on temperature of the sample, and substrates used in the course of the synthesis (Figures 1 and 2). The values obtained for the samples at lower temperature are smaller in absolute numbers, comparing to the samples assessed at increased temperature. This indicates, that the differences in size, resulting from the temperature changes may influence the stability of the colloid.

The further evaluation of the structures in comparison with the size changes may give pronounce information on the colloidal stability of structures presumed for thermally triggered drug delivery.

References


Poster presentations
PCL-Gelatin nanofibers incorporating two antibiotics loaded MSNs: A potential wound dressing

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Wound healing is a challenging process for patients who suffer from severe burns or diabetic ulcers. The healing becomes more problematic when the wound becomes infected. Electrospun nanofibers are becoming highly considered for developing wound dressings due to high-surface area, micro-porosity, and the ability of loading antimicrobial agents or required biomolecules. In this project, PCL (polycaprolactone) or PCL/Gelatin (70:30 or 50:50) were used in an acidic solvent system to develop electrospun nanofiber mat. The carboxyl modified mesoporous silica nanoparticles (C-MSNs), unloaded C-MSNs or loaded with polymyxin B and vancomycin (ABs^C-MSNs), were obtained by adding suspension of N-MSNs in DMF to stirring solution of succinic anhydride in DMF under N2 atmosphere. The Prepared C-MSNs were used to obtain dual antibiotics laoded C-MSNs (Abs^C-MSNs). Abs^C-MSNs were then mixed with gelatin and PCL and used for electrospinning.

![Image](https://via.placeholder.com/150)

**Figure 1.** AAPTMS (N-(2-aminoethyl)-3-aminopropyltrimethoxy-silane) was used for amination of B-MSNs. Carboxyl modified mesoporous silica nanoparticles (C-MSNs) were obtained by adding suspension of N-MSNs in DMF to stirring solution of succinic anhydride in DMF under N2 atmosphere. The Prepared C-MSNs were used to obtain dual antibiotics laoded C-MSNs (Abs^C-MSNs). Abs^C-MSNs were then mixed with gelatin and PCL and used for electrospinning.

increased hydrophilicity and degradability of nanofibers. The antibacterial assays against P. aeruginosa and S. aureus showed high antibacterial efficiency of PCL/Gelatin nanofibers (70:30 and 50:50) incorporated with ABs^C-MSNs (2.5% and 5%). However, PCL nanofibers incorporated with ABs^C-MSNs (2.5% and 5%) showed mild antibacterial effects. Also low antibacterial effects were obtained for PCL or PCL/Gelatin nanofibers incorporated with 1% ABs^C-MSNs. All types of the studied electrospun formulations showed high biocompatibility via MTT and hemolysis assays.
Engineering of MSN-based structures to enhance anti-biofilm activity

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The study focuses on the development of tailored mesoporous silica nanoparticles (MSN)-based structures, namely, nano spheres (S-MSN), and nano rods (R-MSN) by using inorganic and organic constructs, for the in vitro treatment of Staphylococcus aureus (S. aureus) biofilm. These developed S-MSN and R-MSN, jointly named as anti-biofilm nanoparticles (AB-np), have been investigated for their antibacterial activity through surface modifications and natural antibacterial compound loading. In the further stages of the study the better penetrating MSN-based structures are going to be employed to design species-specific AB-np with proper surface modification strategies.

Figure 1: Schematic of the step-by-step synthesis of S-MSN and R-MSN (Blue circle indicates the present stage of the study) [* in the next stages of the study, PEI-AMP conjugation is performed]

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 biofilm by using confocal laser scanning microscopy (CLSM). The porous structure of the MSN enables it to be utilized as a drug carrier. The surface modification of the AB-np is performed using poly ethylene imine (PEI) to enhance the penetrative capability of the AB-np through the extracellular polymeric substance (EPS) layer of the S. aureus biofilm and improve the loading capacity of MSN for the natural antibacterial compound, dehydroabietic acid (DHA), an abietane-type diterpene, to eliminate the biofilm from within upon penetration. The schematic of the AB-np developed are as shown in Figure 1.

The physicochemical characteristics of developed AB-np were confirmed by checking the shape, size, surface modification and drug loading efficiency through, transmission electron microscopy (TEM), dynamic light scattering (DLS), Zeta-potential and UV/Vis spectrophotometry measurements, respectively. The S. aureus biofilm were cultured in confocal dishes for the convenience of imaging. The in vitro tests were performed by incubating AB-np and its predecessors ((a), (b) and (d) in Figure 1) at 100 µg/ml in the confocal dishes for 24 hours. The penetrative capability of AB-np through S. aureus biofilm were investigated by resazurin cell viability assay, CLSM, and Image J.

The proposed AB-np were successfully synthesized and characterized. The AB-np will be enhanced and proceeded on to the next stage for the preparation of species-specific AB-np. According to the in vitro investigations the penetration of S-MSN and R-MSN through S. aureus biofilm has been established through Z-stack images acquired from CLSM and upon particle analysis using Image J.
The particles were visualized at different layers of the biofilm and abundantly at the bottom and the center of the biofilm. The cell viability assay established the eradication of the biofilm with DHA-loaded S-MSN (d) in Figure 1. Although, the DHA loaded in S-MSN was ~11µg/ml, compared to the positive control (100µg/ml of DHA) the assay produced comparable results in these two samples.

The observed in vitro results reveal the successful multimodal nature of the MSN to accommodate dye (FITC), drug (DHA), and surface modification (PEI) to provide enhanced antibacterial activity against S. aureus biofilm in vitro. The obtained results have represented R-MSN and S-MSN rather uniquely. The results revealed the prominence of R-MSN at the bottom of the biofilm, whereas the S-MSN penetrates along all levels of the biofilm and more evenly compared to R-MSN. PEI functionalization improved the penetration of R-MSN through the biofilm, whereas PEI functionalization of S-MSN prevented the penetration of S-MSN particles and accumulated at the upper level of the biofilm. The resazurin cell viability assay revealed the antibacterial efficiency of the DHA-loaded S-MSN and comparable behavior with respect to DHA alone. Hence, PEI functionalized R-MSN particles were successful in penetrating through biofilm, abundantly. PEI functionalized S-MSN particles were successful in accumulating at the upper levels of the biofilm, abundantly.

**Manufacturing Films for Individualized Dosing**

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Background: Intra- and inter-individual variability in drug response is the premise for individualized therapy, which requires the design of the pharmaceutical product to be tailored to the characteristics of diverse patients to optimise health outcomes. One well studied product feature requiring individualization is the dose strength. Current pharmaceutical mass production can produce some dose variants of solid oral dosage forms but lacks the flexibility required to individualize dosing to the extent required to fully meet patient needs. Delivering flexible dosing via subdivision of a manufactured product to different sizes requires a uniform distribution of the drug prior to subdivision.

To investigate the suitability of different film manufacturing techniques to individualize dose strength in polymeric films.

Drug-loaded polymeric films containing 5% & 15% w/w carbamazepine (CBZ) in ethyl cellulose were prepared by a) hot melt extrusion and melt pressing and b) solvent casting using 95% ethanol. 4mm sections of the film were evaluated for carbamazepine content by UV after dissolution in 95% ethanol.
Carbamazepine exhibited reproducible, narrow drug distribution in hot melt extruded and melt pressed films relative to solvent casting, therefore films produced by this method can be subdivided in order to reliably and accurately tailor the delivered dose. However, this technology, like others in individualization, must overcome manufacturability challenges to facilitate patient access.

Figure 1. CBZ content in 4mm sections of hot melt extruded and melt pressed films at 5% and 15% drug loading. Results shown as mean ±SD, n=5.

Figure 2. CBZ content in 4 mm sections of solvent casted films at 5% and 15% drug loading. Results shown as mean ±SD, n=5.

References
Application of ultrasound-enhanced electrospinning for fabricating drug-loaded polymer nanocomposites

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Electrospinning (ES) is an emerging nanotechnology method for fabricating nanocomposites for therapeutic agents. In traditional ES (TES), a polymer solution is generated from a capillary toward a grounded metal collector plate by applying high voltage between the capillary and the plate [1,2]. The morphology and diameter of TES nanofibers depend on the intrinsic properties of the solution, type of polymer, conformation of polymer chain, viscosity, elasticity, electric conductivity, as well as on the polarity and surface tension of the solvent [3].

A novel ultrasound-enhanced ES (USES) provides an orifice-less ES technique that employs US for creating nanofibers [4]. In this technique, high-intensity focused US bursts are used to generate a liquid protrusion with a Taylor cone from the surface of a drug-polymer solution. When the drug-polymer solution is charged with a high negative voltage, a nanofibers jet from the tip of the protrusion is generated and lead to an electrically grounded collector at a constant distance.

The objectives of the present study were (1) to compare the TES and USES techniques in fabricating drug-loaded polymer nanofibers, and (2) to investigate the physico-chemical and pharmaceutical properties of nanofibers.

The nanofibers were fabricated with TES (ESR-200Rseries, eS-robot®, NanoNC, South Korea) and with an in-house USES method (Figure 1). The USES method is described in more detailed elsewhere [4]. To modulate fiber diameter, specific ultrasonic parameters (frequency, pulse repetition frequency and cycles per pulse) were applied during spinning.

Figure 1. A. Schematic of an ultrasound-enhanced electrospinning (USES) setup. B. Photograph of an USES device.
Figure 2. Scanning electron microscopy (SEM) images of traditional electrospun (TES) and ultrasound-enhanced electrospun (USES) nanofibers. Key: (A,B) TES nanofibers (magnification 2,500x and 10,000x); (C,D) USES nanofibers (2,500x and 10,000x).

Polyethylene oxide, PEO (average Mw 900,000) and chitosan (medium molecular weight) (Sigma-Aldrich Inc., St. Louis, U.S.A) were used as carrier polymers. A diluted mixture of aqueous acetic acid and formic acid solution (3% w/v) were used with both polymers for TES and USES experiments. Theophylline anhydrate (Sigma-Aldrich Inc., St. Louis, U.S.A) was used as a water-soluble model therapeutic agent. The fiber size, size distribution and morphology of nanofibers were studied with scanning electron microscopy (SEM) and optical microscopy. ImageJ software was used to measure the size of nanofibers. Physical solid-state properties were investigated by means of vibrational spectroscopy (FTIR), X-ray diffraction (XRD), and differential scanning calorimetry (DSC).

In general, TES produces thinner fibers compared to USES technique but lacks the possibility to modulate fiber thickness in wide range during an active ES process (e.g., change in voltage resulted in 11.6 nm change in diameter) (Figure 2).

In contrast fiber diameter can be modulated in USES by changing cycles per US pulse (duty factor). Changing the cycle number from 400 to 700 produced fibers with diameters of 402 nm and 555 nm accordingly (the other parameters frequency and pulse repetition frequency were kept constant). Figure 3 shows the average diameter of both TES and USES nanofibers (reference is also made to Figure 2).

In conclusion, USES enables to modulate fiber thickness in real time, thus allowing more flexible way to produce nanofibers compared to TES. Both methods can be applied for the aqueous-based fabrication of non-woven drug-loaded nanofibrous systems.

Figure 3. The average diameter of traditional electrospun (TES) and ultrasound-enhanced electrospun (USES) nanofibers (n = 3).

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Bioadhesive nanofibrillated cellulose films for drug release

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Bioadhesive materials have been gaining increasing interest due to inherently unstable drug compounds [1]. Newly emerged peptide drugs are unable to cross biological barriers without being exposed to heavy enzymatic activity present in the GI-tract and liver, therefore resulting in poor bioavailability. Mucoadhesive formulations are generally designed to prolong GI-tract retention, however, local drug delivery systems can enhance bioavailability by avoiding metabolic pathways, such as first-pass metabolism. Oral mucosa functions as a biological barrier, which has been used in site specific delivery of local oral diseases.

In addition to industrial applications, nanofibrillar cellulose (NFC) has been investigated in biomedical and pharmaceutical sciences, such as a scaffold that promotes three-dimensional cell culture or a drug-releasing matrix [2,3]. NFC fiber properties have several advantages to act as a functional biomaterial, e.g. inherent similarity to collagen fibers [2], great modification capabilities, high water content, pseudoplastic and thixotropic properties. Additionally, NFC is considered as a safe, biocompatible and non-toxic biomaterial [4]. In this study, we have fabricated bioadhesive films with the use of NFC and anionic type nanofibrillar cellulose (ANFC). Mucin, pectin, and chitosan were investigated as mucoadhesive components to evaluate film mucoadhesive properties with texture analysis. Solid state characteristics and drug release properties of the films were examined with the use of metronidazole, an antibacterial drug compound used to treat periodontal diseases.

We observed that the bioadhesive properties of NFC could be enhanced by incorporating mucoadhesive components into the film. This indicates potential local drug delivery systems for site specific medication of oral disease or to bypass metabolic routes to increase bioavailability.

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References

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Influence of surface chemistry on adsorption and confinement of drug in porous silicon

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Drug delivery using PSi as dissolution enhancing or payload protecting carrier material has been a widely studied application for the past 15 years [1,2]. By adsorbing small organic molecules into relatively small pores, where the average pore diameter is only 10–20x the size of the adsorbent molecule [3,4], porous materials become an effective tool for crystal engineering, which can be used to control e.g. specific polymorph nucleation within the porous matrix or even the complete suppression of the crystalline state. From drug delivery standpoint, the ability to prevent the formation of crystalline structure through physical confinement leads to an effective method of keeping the adsorbed drug molecules in a disordered, amorphous-like state for prolonged periods, while enhancing the aqueous solubility and permeability, is a considerable advantage [4].

Here, the effect of hydrophobic and hydrophilic PSi surface chemistry (THCPSi and TOPSi, respectively) was studied with regard to the molecular dynamics of the adsorbed drug, ibuprofen, using thermal analysis and variable temperature solid-state NMR because the selected mesopore size enabled the presence of both a nanocrystal- and an amorphous-like phase concurrently inside of the mesopores [3,5]. Also, the effects of different parameters such as drug concentration and the loading solvent dielectric constant and (a)protic nature were studied for finding optimal loading parameters.

The obtained results show that the drug loading appears most effective when adsorption occurs from solvent with low permittivity, such as CHF. However, after ca. 75% pore filling, corresponding 80% (w/w) drug payload, rapid accumulation of drug begins on the particle external surfaces, blocking further adsorption into the pores.

Interestingly, thermal analysis indicates that nucleation of ibuprofen nanocrystallites within the confinement of the pores begins already at low payloads of ca. 200 mg/cm³ (~20 % (w/w)). This separation of the nanocrystalline phase and an amorphous-like phase becomes more evident with larger drug payloads, with 40–50 % of the confined drug in the amorphous phase. Utilizing the selectivity for molecular mobility between direct MAS and CP MAS ¹³C NMR measurements, this presence of two distinct populations of drug molecules with differing mobilities was also confirmed.

The results suggest that the often Langmuir-like appearance of the drug payload-solution concentration is more coincidental, as the drug molecules appear to cluster rapidly within the pores. Hence, the extent of the interactions between the pore walls and the drug molecules appears limited to only the immediate drug molecules. However, this interaction is readily visible with ¹H NMR as shown in Figure 1. The surface silanol groups provided by TOPSi are able to break the ibuprofen tendency to form dimeric structures and bind the molecules to the pore walls through hydrogen bonds. While ibuprofen is a poor model drug considering crystal engineering, the ability to affect through simple surface modifications to the ordering of the drug mol-
ecules within the confined space prompts interesting possibilities. Combined with the complex fir-tree like wall structure inherent for mesoporous Si, these features could be exploited in e.g. polymorph selection and stabilization.

The adsorption of drug molecules within PSi can be optimized to yield high confined drug payloads. The interactions between the drug molecules and the pore walls characterized with NMR spectroscopy indicate that while the extent of the interactions appears limited, the possibilities for using PSi as a crystal engineering platform for polymorph screening warrants further investigation.

References

Figure 1. Schematic representation of possible interactions near the pore walls with 1H MAS spectra of hydrophobic THCPSi and hydrophilic TOPSi.
Multimodal imaging of surface solid-state transformations

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Surface and bulk regions of amorphous materials differ in molecular mobility resulting in different behavior with regard to susceptibility for solid-state transformations, for instance. Surface molecules with higher mobility result in higher crystallization rates compared to molecules in the bulk. Crystallization on the free surfaces may affect critical properties such as dissolution. Nonlinear optical imaging is a relatively novel approach that provides both chemical and solid-state specificity. By simultaneously combining second-order (sum frequency generation (SFG) and second harmonic generation (SHG)) and third-order non-linear phenomena (coherent anti-Stokes Raman scattering (CARS)) differentiation between different solid-state forms of the drugs can be performed with greater confidence.¹ The aim of this study was to combine SFG/SHG and CARS imaging to monitor surface solid-state transformations. Additionally, the effect of different levels of surface crystallinity on the dissolution behavior was monitored.

The amorphous indomethacin was prepared by quench cooling the melted gamma form of indomethacin (Orion, Finland). Pulverized amorphous indomethacin was compressed into tablets that were stored at 30°C at two different humidities: 23% RH and 75% RH. A Leica TCS SP8 CARS microscope was used for obtaining spectra and imaging. Tablet surfaces were analyzed on days 0, 1, 2, 5, 7 and 22. The intrinsic dissolution rates of fresh and stored samples were measured using a channel flow system similar to the one described by Peltonen et al.²

The nature and level of surface crystallinity was dependent on storage conditions. Over the 22-day period, tablets stored at 30°C/23% RH crystallized mostly to the gamma indomethacin form whereas the tablets stored at 30°C/75% RH crystallized predominantly to the alpha indomethacin form. The crystallization was, however, not exclusive to only one of the polymorphs. Similarly, several small regions of tablets stored at 30°C/75% RH generated a CARS signal corresponding to the gamma form. Furthermore, storage induced surface solid-state changes were confirmed by having different intrinsic dissolution rate profiles.

The combination of two nonlinear imaging techniques was successfully used to study surface crystallization in amorphous indomethacin tablets. Such surface crystallization can affect dissolution behavior.

References


Preparation of ibuprofen-arginine salt by spray drying from water

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Co-amorphous formulations are amorphous homogenous single-phase systems comprising of two or more low molecular weight compounds [1]. For example, co-amorphous drug-amino acid systems have been shown to enhance both physical stability and dissolution properties of amorphous drugs. These effects have been especially significant with mixtures possessing strong interactions, such as salt formation, between the components.

In the present study, a co-amorphous mixture of IBU and ARG was prepared with an up-scalable method (spray drying). Our aim was to produce a stable co-amorphous mixture (1:1 molar ratio) with enhanced dissolution properties and to investigate whether a salt would form between the components. Additionally, we aimed to conduct spray drying from aqueous solution without the use of organic solvents or solubilizers, such as surfactants.

Due to the solubilizing effect of ARG, dry IBU-ARG (1:1) powder (moisture content ~2.8%) could be obtained by spray drying from water, although the yield remained rather low (~34%). The resulting material was X-ray amorphous, and the temperature modulated DSC revealed a single Tg, which indicates the formation of a homogenous single-phase system. The Tg-value of the spray dried IBU-ARG mixture (82.8 ± 1.93 °C) was also significantly higher than the theoretical Tg (-10.2 °C) calculated with the Gordon-Taylor equation, which suggests strong interactions between IBU and ARG. The FTIR spectrum analysis revealed the interaction to be salt formation.

The cumulative dissolved amount of IBU from the amorphous salt was higher than from the crystalline drug and from the 1:1 physical mixture throughout the study, but the difference between the physical mixture and spray dried mixture was statistically significant only at 30 and 60 min time points (Fig. 1). The spray dried mixture remained amorphous at least for one year in dry conditions, but in the presence of high humidity, liquefaction occurred.

Figure 1. The cumulative dissolved amount of ibuprofen (IBU) in HCl buffer (pH 1.2) from crystalline drug (CD), from IBU-arginine (ARG) physical mixture (PM), and from spray dried (SD) IBU-ARG mixture. The first four hours have been enlarged.
Based on this study, spray drying from aqueous solutions seems to be a feasible up-scalable technique for the preparation of co-amorphous mixtures, if the co-former solubilizes the drug adequately. With this technique the amorphous form of very low Tg drugs may be stabilized at least in the presence of strong interactions between the components, and also the dissolution properties may be enhanced.

References


Electrospinning of nanofibrillar cellulose reinforced nanofibers for pharmaceutical applications

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Nanofibrillar cellulose (NFC) is an interesting novel nanomaterial potential for a wide range of technical, biomedical and pharmaceutical applications. NFC has several unique material properties for an excipient use in both pharmaceutical immediate-release and controlled-release dosage forms. NFC is non-toxic, has a high surface area, and possesses excellent mechanical strength that could be used to improve the mechanical properties of solid dosage forms [1,2]. NFC absorbs a tremendous amount of water and readily forms a gel which makes it a suitable candidate to be used also as a wound protective material [2].

Figure 1. Aqueous dispersion of 2.7% biofibrils form a continuous network of hydrated fibrils. The material is isolated from Pinus sylvestris and Picea abies or Betula sp.

The main objectives of the present study were to investigate the effects of NFC on the formation and physicochemical properties of electrospun composite nanofibrous mats and the corresponding moulded thin films.

NFC was purchased as a 2.7-% aqueous dispersion from UPM Biofibrils AS 103, UPM-Kymmene Corporation, Finland (Figure 1). Polyethylene oxide, PEO (mw 900,000, Sigma-Aldrich) and polyvinyl alcohol, PVA (mw 125,000, Mowiol®, Sigma-Aldrich) were used as a water-soluble polymer in nano-fibrous mats and thin films. Alginate (ALG) was studied as a native origin fibrous carrier material. Purified water was used as a main solvent.

For electrospinning (ES) and film moulding, aqueous solutions of both PEO (4% and 8% w/v) and PVA (Mowiol®) (4% and 12% w/v) were prepared. NFC was incorporated in the polymeric solutions at different concentrations ranging from 0.1 to 0.6% (w/v). The ALG solutions (1-3% w/v) were prepared by dissolving the material in purified water and used in different combinations with PEO. The nanofibers were prepared using an ESR200RD robotized ES system (NanoNC, South-Korea) at an ambient room temperature and relative humidity (RH). The flow rate of the solution was 0.1, 0.5 and 1.0 ml/h, and the voltage applied...
9-16 kV. The distance between the spinneret and the fiber collector was 10-15 cm. The thin films were prepared by a solvent evaporation method in polytetrafluoroethylene (Teflon®) molds. The molded solutions were allowed to dry for at least 24 hours at an ambient room temperature and RH.

The fiber size, size distribution, surface morphology and thickness (nanomats and thin films) were studied with a high-resolution optical (CETI MagtexT) and scanning electron microscopy, SEM (Zeiss EVO® 15 MA, Germany). ImageJ software was used to measure the size of nanofibers. The thermal properties were studied by means of differential scanning calorimetry, DSC (DSC823e, Mettler Toledo AG, Switzerland). The mechanical properties of nanofibrous mats were tested with a Brookfield Texture Analyzer CT3 (U.S.A).

Figure 2 shows the SEM and optical microscopy micrographs of the desiccated aqueous dispersion (2.7%) of NFC. After desiccation, a fragile NFC film was formed.

As seen in Figure 3, the 1:1 ratio of NFC (0.135% w/v aqueous dispersion) and PEO (8% w/v) solution in ES produced the nanofibers with a uniform structure. The mean diameter of the fibers was less than 200 nm, and the fiber diameter was dependent on the voltage used.

When NFC was added to the PEO solution, the electrospun fibers decreased compared to that obtained with the pure PEO fibers. Increasing the NFC concentration resulted in the decrease in the diameter of the composite nanofibers. The higher concentration of NFC impaired the formation of nanofibers, and consequently, several beads were detected on the nanofibrous platforms. The corresponding composite thin films of NFC and PEO/PVA were transparent and uniform in structure (Figure 4).

Figure 2. SEM and optical microscopy images of the desiccated aqueous dispersion of nanofibrillar cellulose (NFC).
In conclusion, NFC can be successfully electrospun with PEO, PEO+ALG and PVA to obtain composite nanofibrous platforms for e.g., drug delivery applications. The corresponding composite free films can be fabricated with a simple moulding method. The concentration of NFC will greatly affect the formation and performance of the present nanofibrous mats and thin films. Further studies will reveal the storage stability, swelling and dissolution behavior of the present composite platforms.

Acknowledgements

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References


coherent anti-Stokes Raman scattering and electron microscopy (C-CARS-EM) method allows insights into nanoparticle uptake that would not be available with either of these techniques alone.

RAW 264.7 cells grown on glass bottomed gridded Petri dishes (Mattek) were incubated with glibenclamide nanosuspensions (250 µg/ml) for 6 h. Cells were fixed (2% glutaraldehyde in sodium cacodylate buffer) and imaged with Leica TCS SP8 CARS microscope. 633 nm He/Ne laser was used for bright field (BF) imaging the grids on the dish. Z-stack CARS images were recorded and CARS spectrum from the inside of the cells was measured. Cells were flat-embedded in plastic for microtome sectioning the area selected based on the grid markings and BF images [1]. 120 nm sections were imaged with Jeol JEM-1400 TEM (80 kV) using Gatan Orius SC1000B bottom mounted CCD-camera.

3080 cm⁻¹ peak was used to image the uptake of crystals by cells in a chemically-specific label-free manner. There was a good correlation in the observed particle localization inside the cells between CARS and TEM images. TEM could be used to visualize the subcellular localization of the nanocrystals, while the drug crystals were confirmed as such by CARS.

C-CARS-EM imaging of drug nanocrystal uptake in cells with CARS and TEM was successfully achieved. Both techniques had their inherent benefits. CARS is chemically-specific with easy sample preparation, whereas TEM has better spatial resolution capable of showing fine structural details of the particles. TEM images revealed that most of the nanoparticles were located in membrane bound vesicles.

References


Exploring mesoporous silica nanoparticles as carriers for poorly soluble drugs in orodispensible films

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Fast dissolving orodispensible films (ODF) are rapidly gaining interest especially to solve the problems encountered in the administration of drugs to pediatric and elderly patients. To be able to deliver poorly soluble drugs with ODFs, solubility enhancers are often required. Various substances have been incorporated as enhancers into ODFs to increase the absorption of drugs through the mucosa. However, they are associated with irritation, membrane damage, and toxicity, which thereby limits their use. Biocompatible nanoparticles as carriers for poorly soluble drugs have been shown to be promising in improving the solubility of drugs. In the last two decades, mesoporous silica nanoparticles (MSNs) have been extensively used in drug delivery applications. MSNs possess unique properties,
such as high specific surface area, high pore volume and appropriate pore sizes in the molecular range, ordered pore structures and silanol groups on their surfaces. These features make MSNs optimal drug carriers. Immediate and controlled release MSN-based drug delivery systems have been developed for oral, transdermal and intravenous administration of compounds with poor stability or solubility.

In this study, we explored MSNs as carriers for the delivery of prednisolone in fast dissolving polyvinyl alcohol orodispersible films. In detail, two different methods (i.e. pre-incorporation and dip-coating) were used to accommodate MSNs into the ODF matrix. Furthermore, mechanical properties of the ODFs were investigated to evaluate the resulting changes of ODF due to the incorporation of the MSNs carrier. In addition, the drug loading efficiency was investigated. Lastly, the drug release properties of the optimized the ODF formulation was investigated in simulated saliva fluid (SSF).

The results revealed that the MSN incorporation strategies have a significant impact on the mechanical properties of the ODF. The thickness of ODF significantly decreases when the number of immersions is increased (>5 times) in the dip-coating process, whereas pre-incorporation of MSN into the polymeric matrix increased the thickness and improved the mechanical properties of ODF. The even distribution of the incorporated MSNs on ODF was shown with atomic force microscopy (AFM). The drug (i.e. prednisolone) loading on MSN was carried out prior to incorporation of the MSN into ODF. The archived drug loading degrees was confirmed by UV-VIS spectrophotometry and the highest drug loading was achieved with the pre-incorporation method. The drug release studies show that 90 % of the drug content releases in the first 2 minutes whereas only less than 40% of the content is released out when the drug is incorporated without particle loading.

Consequently, in this study we have evaluated the potential of MSN as a drug carrier within ODF to be used for precise dosing and fastening the dissolution of the drug content from ODF.

References


Characterization of mesoporous silica nanoparticles as vectors for siRNA delivery

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Gene therapy using siRNA molecules is nowadays considered as a promising approach. For successful therapy, development of a stable and reliable vector for siRNA is crucial. Non-viral and non-organic vectors like mesoporous silica nanoparticles (MSN) are associated with lack of most viral vector drawbacks, such as toxicity, immunogenicity, but also generally a low nucleic acid carrying capacity. To overcome this hurdle, we here modified the pore walls of MSNs with surface-hyperbranching polymerized poly(ethyleneimine) (hbPEI), which provides an abundance of amino-groups for loading of a larger amount of siRNA molecules via electrostatic adsorption. After loading, the particles were covered with a second layer of pre-polymerized PEI to provide better protection of siRNA inside the pores, more effective cellular uptake and endosomal escape. To check transfection efficiency of PEI covered siRNA/MSNs, MDA-MB 231 breast cancer cells stably expressing GFP were used. We demonstrate that PEI-coated siRNA/MSN complexes provide more effective delivery of siRNAs compared to unmodified MSNs.

Thus, it can be concluded that appropriately surface-modified MSNs can be considered as prospective vectors for therapeutic siRNA delivery.

Figure 1: Schematic representation of: (A) MSN (B) the construction of cleavable organic linkers on MSN pores surface through hyperbranched PEI with loaded NA; (C) the NA loading, particle surface grafting of exterior PEI.
Determining the Solubility of Polymorphs in Biorelevant Media using Image-Based Single Particle Analysis (SPA) Method

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To investigate the applicability of the image-based SPA for measuring the solubility of different polymorphic and amorphous forms of indomethacin in biorelevant media.

Three different solid-state forms of indomethacin: stable γ, metastable α and unstable amorphous form; and five different buffers: HCl pH 1.6, HCl pH 1.6 with NaCl (34.2 mM), Fasted State Simulated Gastric Fluid (FaSSGF) pH 1.6, acetate buffer pH 5.0 and Fed State Simulated Intestinal Fluid (FeSSIF) pH 5.0, were used in this study.

First, the set-up was calibrated using 10 compounds, with a solubility range spanning over 7 orders of magnitude, against their respective aqueous shake-flask solubility values (R²=0.959). Afterwards, the solubility of indomethacin solid-state forms was individually measured from single particles (n>5) in each of the buffers using the image-based SPA method. Individual measurements were approximately 15 minutes long. Mann-Whitney statistical tests were used to determine if the image-based SPA method was able to differentiate between different solid-state forms of indomethacin based on their solubility values.

Figure 1 The solubility of indomethacin’s solid-state forms in solvents with pH 1.6 and pH 5.0, respectively, measured with the SPA method.

Mann-Whitney test results showed that single particle solubility values were statistically different (α=0.05) between any two forms in a given solvent. The ratio of α to γ solid-state form solubility was 3.33±0.47 in all of the solvents used. The solubility ratio of amorphous to γ form was very similar in HCl (28.7) and FaSSGF (29.8) buffers, the lowest in acetate (18.6) and the highest in FeSSIF (229.6) buffer. When comparing solubility of the same form in related solvents (same pH), the biggest increase was detected for the amorphous form between FeSSIF and acetate buffer (49.7). The increase was also present, though much smaller, for α (5.63) and γ (4.03) forms. Practically no increase in solubility appeared between FaSSGF and HCl buffer.
The Image-Based SPA method is capable of differentiating between polymorphic forms of indomethacin based on the solubility values of their single particles. Moreover, using the method, a quick estimation of the drug behaviour in the gastrointestinal tract can be achieved, and the effects of surfactants on drug solubility studied.

References

Hot-melt extrusion of 3D printable isoniazid formulations

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The use of three-dimensional (3D) printers to manufacture flexible and tailored drug delivery systems is an emerging area of interest in pharmaceutical research since the precise and additive nature of the process potentially could solve the unmet need for personalized medicine (1,2). The aim of the present study was to produce 3D printable feedstock material possessing properties relevant for oral dosage forms and compare how the subsequent fused deposition modeling (FDM) 3D printing affects the final dosage form.

A Thermo Scientific Process 11 (USA) co-rotating twin-screw hot-melt extruder was used to produce 3D printable feedstock materials (filaments) that subsequently were printed into model geometries using a MakerBot Replicator 2 FDM 3D printer. Highly water-soluble isoniazid (TCI America, USA) served as a model drug and polymers with relevant pharmaceutical properties were included in the formulations. Ten different hot-melt extruded formulations containing various polymer and excipient combinations and a drug loading of 30% (w/w) were prepared. The printability in an FDM printer of the manufactured filaments was evaluated. Formulations were furthermore analyzed regards to mechanical and solid-state properties e.g. by utilizing a texture analyzer (TA-XT2i, Texture Technologies, Hamilton, MA, USA) and x-ray diffraction. In vitro drug release was carried out in phosphate buffer pH 7.45.

Extruded filaments were evaluated for their printability in a MakerBot 2 Replicator 3D printer. Six formulations were successfully 3D-printed, the other formulations were discarded either due to unsuitable filament diameter or mechanical properties leading to an unsuccessful loading of the filament into the print-head (Figure 1). Mechanical testing of the produced HME filaments showed a fairly good correlation to the printability of the filaments, revealing that filaments that in the 3 point bend test failed to bend 1.5 mm or more were too brittle to feed into the printer.

Solid-state analysis revealed that the drug was present in a crystalline form in all of the hot-melt extruded formulations. Due to the high water-soluble nature of the drug, a complete drug release could be achieved for all hot-melt extruded as well as the printed formulations. The 3D-printed geometry that was designed to have the same geometrical outlines as the filaments showed similar in vitro drug release as the corresponding hot-melt extruded filaments, indicating that 3D printing at elevated temperatures does not seem to affect the drug release behavior (Figure 2). 3D-printed tablets (8x2.5 mm), however, showed a more sustained drug release profile due to the difference in surface area compared to the filaments. This indicates that 3D printing may be used to tailor the drug release profile simply by changing the shape of the printed dosage form. The drug-release may furthermore be altered by changing the composition of the extruded filaments. In this study, 3D-printed and hot-melt extruded filaments showed 80% isoniazid release within 11-85 minutes depending on the polymer and excipient composition of the formulations.
In conclusion, 3D printable formulations containing isoniazid were successfully prepared by means of hot-melt extrusion. In terms of printability, diameter and mechanical properties of the extruded filaments were crucial. Relevant pharmaceutical polymers for oral drug delivery were used to produce 3D printable dosage forms, where the release properties could be varied from immediate to more prolonged depending on the polymer composition used. 3D-printed model geometries printed with the same surface area showed similar drug release profiles as the hot-melt extruded filaments used as feedstock material for the printing process, indicating that the 3D printing process itself at elevated temperatures do not affect the drug release for the formulations studied.

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References


Tissue penetrance and toxicity investigations of differently functionalized mesoporous silica nanoparticles in the zebrafish model to understand chemical design-toxicity relationship

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Mesoporous silica nanoparticles (MSNs) have been receiving extensive attention in the last decades as drug delivery systems due to its versatile design flexibility and uniform mesopores for drug molecule loading and release. Surface functionalization of MSNs expands applicability as drug delivery platform by influencing dispersion stability, biocompatibility, biodistribution, drug release, efficacy and biodegradation [1]. However, their design-toxicity relationship needs to be investigated in the more complicated biological systems relevant to human physiology to able to develop safer nanotherapies. In this study, zebrafish (Danio rerio) embryos were selected as model system and their toxicity profiles of differently surface functionalized MSNs were studied. Zebrafish model is widely used in toxicity studies and has similar cardiovascular, nervous and digestive systems with mammals. Chorion membrane intact embryos or dechoroniated embryos were incubated or microinjected with amino (NH2-MSNs), polyethylenimine (PEI-MSNs), succinic acid (SUCC-MSNs) or polyethyleneglycol (PEG-MSNs) functionalized MSNs and toxicity was evaluated by embryo viability and cardiovascular function. Different surface functionalizations of MSNs resulted as different surface charges, which is the key parameter for the interactions of nanoparticles with the biological membranes, and therefore influencing cellular internalization, biodistribution, residence time, immune system and toxicity.

NH2-MSNs, SUCC-MSNs and PEG-MSNs did not show any lethality, whereas, 50µg/ml PEI-MSNs induced 100% lethality 48 hours post fertilization (hpf). Dechoroniated embryos were more sensitive and 10µg/ml PEI-MSNs reduced viability to 5% at 96 hpf. Dechoronation increased the sensitivity for PEG-MSNs and SUCC-MSNs, but not NH2-MSNs. 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 (Figure 1). Direct exposure of inner organs was tested by microinjecting NH2-MSNs and PEI-MSNs which were demonstrated the lowest and highest toxicity previously, displayed similar toxicity indicating that functionalization affects the toxicity profile by influencing penetrance through biological barriers. The data reveals that functionalization critically determines penetrance of biological barriers and resulting toxicity, and emphasize the
need of careful assessments of nanoparticles on the physiological functionality of tissues and organs in addition to evaluation of gross mortality. The results support that there is the requirement for careful analyses of toxicity mechanisms in relevant models and establish an important knowledge step towards the development of safer and maintainable nanotherapies.

Figure 1. Confocal microscopy images of zebrafish embryos show MSN uptake with different surface functionalization from aqueous medium into embryo.

References


When the Society of Physical Pharmacy was founded in 1988 it felt like a dream come true. For a M.Sc. student doing the last year in M.Sc. studies, it was mind-blowing to learn about polymorphs, solid-state properties and physicochemical characterisation of APIs, excipients and products. We were introduced the techniques like X-ray powder diffraction and differential scanning calorimetry (DSC). Before that pharmaceutical technology was very empirical and the first techniques really opened our mind for molecular interactions and physical phenomena in pharmaceuticals.

Since then, the number and level of solid state and particle analytical technologies has increased markedly. This is demonstrated with two example areas. When spectroscopic technologies like NIR came to use, the concept of process analytical technologies were introduced to pharmaceutical processes and understanding of APIs, formulations and processes increased one step further. Novel spectroscopic technologies have been introduced to pharmaceutics with increasing tempo in last decades, like FTIR, solid-state NMR, Raman, CARS, tera-hertz spectroscopy and time-gated Raman. As another example, the area of DSC has expanded by introducing the high-performance DSC, StepScan, modulated temperature DSC and for biomolecules techniques like micro-electromechanical systems (MEMS)-DSC, infrared (IR)-heated DSC, gas flow-modulated DSC (GFMDSC), parallel-nano DSC (PNDSC), pressure perturbation calorimetry (PPC) and self-reference DSC (SRDSC) (1).

It seems that the development of analytical technologies has almost become a meaning and area of research itself (and a business). Do we remember what we want to do with these technologies? How can these techniques do better medicines for patients? Are we losing the understanding of the meaningful parameters for APIs and formulations since we can measure everything, and we do that just to make it sure? As a research scientist we always recommend that one should investigate a phenomenon by measuring with more than one analytical method, but how much is enough? Are our methods really relevant or just nice to know and looking good for the publications?

Further, more and more process data are obtained from our instrumented manufacturing equipment. The systematic study design and statistical and multi-variate modelling of our data has given us more understanding of our pharmaceutical processes and systems. However, the amount of data is enormous, processing data of in-line measurements combined with analytical in-line and other data gives a huge challenge how to store, handle and process the data to get the valuable information. The human brain might come to a limit in its capacity to handle these multidimensional data spaces. Pharmaceutical companies have started to use advanced artificial intelligence in handling and processing the data. Utilisation of AI has been successful in the pharma industry to improve drug discovery and development processes.
of augmented reality sounds like science fiction or entertainment, but it is already in use in handling the video data of pharmaceutical manufacturing area to investigate the line clearance in GMP areas in real time.

Also, in silico tools are already widely used in drug discovery and they will be also utilised even more in formulation development. In silico tools based on structural informatics will give insight on solid state landscape and assist us in material design and predict the material performance. The prediction capacity of solid state properties with these tools is improving (2).

What will be the needs of physicochemical characterisation of pharmaceuticals in future? Should we start to prepare for processing of pharmaceuticals in space in gravity free areas? Should we start to study the handling and behaviour of powders in those conditions and develop the flowability and compressibility tests to be used in space? This has already been started by the mining industry being understandingly first in line and some research in pharmaceutical production in space has already been done (3).

Physicochemical characterisation of raw materials will be emphasized even further when continuous processing will increase in order to ensure smooth processes, especially powder flow in production lines. What will be the drug forms used in future? Will the use of biological drugs switch the focus from solid dosage forms towards parenterals and liquid systems? The physicochemical characterisation of biologicals is challenging and requires a different portfolio of techniques than low-weight molecules that we are used to work with. Thus, the topics of the Physical Pharmacy Symposium in 2047 are impossible to predict.

The aim of this article is to provoke critical thinking and discussion of what we are doing and why, and to present wild guesses what might be needed in future. A deeper and true understanding of our APIs, excipients, formulations and processes for the benefit of patients should be our common goal. The use and easier access to synchrotrons, for example, will definitely improve understanding of our systems. A wise use of the techniques available combined with know-how in pharmaceutical materials and processes will be the success factors even for coming decades.

References


PhD and MSc theses – 2017
Currently, there is no major discovery of an effective cure to restore the function of an injured heart, despite the existing and developing therapies. While existing options ameliorate the care of myocardial infarction (MI) and heart failure patients, cardiac stem cell therapy has only recently shown positive results in clinical trials, and thus there is an urgent medical need to develop advanced therapeutic entities to reverse this disease burden. The employment of biomaterials as potential therapeutics for MI is at the pre-clinical stage. Particulate systems are arising as a promising tool to provide minimally invasive treatment, an important aspect to take into account for clinical translation and patient compliance.

Porous silicon (PSi) and spermine-acetalated dextran (AcDXSp) are emerging biomaterials for applications in varying biomedical fields. Drug delivery is one of these fields benefiting from the materials’ properties, such as biocompatibility, biodegradability, customized particle preparation, surface functionalization, simple yet efficient drug loading, and tunable release of the therapeutic cargos. Therefore, the aim of this thesis was to develop multifunctional PSi and AcDXSp platforms for targeted drug delivery to and imaging of the ischemic heart.

Initially, the biocompatibility of PSi-based carriers of different sizes and surface chemistries was evaluated.

Secondly, three different PSi-based nanosystems were developed, functionalized with a metal chelator for radiolabeling and three different peptides (atrial natriuretic peptide (ANP) and two other heart-homing peptides), with the aim to screen the targetability of the nanoparticles to the ischemic heart. All the nanosystems showed no toxicity up to 50 µg/mL concentration, and cell–nanoparticle interaction studies in cardiomyocytes and non-myocytes revealed a preferential cellular interaction with ANP-functionalized nanoparticles in both the cell types, through the natriuretic peptide receptors (NPRs) present at the cell surface.

Thirdly, the ANP-PSi functionalized nanoparticles were PEGylated in order to improve the colloidal stability and enhance the circulation time. Upon labeling with radioisotope Indium-111, the ANP-PSi nanoparticles displayed a preferential accumulation and selectivity towards the endocardial layer of the ischemic heart. In vivo delivery of a cardioprotective small drug molecule from the ANP-PSi showed attenuation of the extracellular signal-regulated kinase pathway that is involved in the hypertrophic signaling of the injured heart. Lastly, and in parallel, the development of functionalized and dual-loaded AcDXSp nanoparticles for potential application in cellular reprogramming was proven successful, by utilizing acidic pH-triggered drug delivery of the two poorly water-soluble cargos. The incubation of non-myocytes with ANP-functionalized AcDXSp nanoparticles showed therapeutic modulation of key signaling pathways involved in the direct fibroblast reprogramming into cardiomyocytes.
Overall, PSi and AcDXSp-based (nano) particulate systems were developed, bringing new insights about potential therapeutic advances in the applicability of imaging and targeted delivery of relevant pharmacological molecules to the ischemic heart with a minimally invasive therapeutic approach.

Melanin Binding and Drug Transporters in the Retinal Pigment Epithelium: Insights into Retinal Drug Delivery

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Diseases affecting the posterior of the eye (retina, choroid) are difficult to treat since there are protective ocular barriers. As the population ages, the number of patients with sight threatening diseases is expected to grow rapidly. Currently, the treatment of retinal diseases involves frequent intravitreal injections, but many patients suffer from diseases for which even today there is no effective treatment. The retinal pigment epithelium (RPE) is a single cell layer in the posterior of the eye. The RPE protects the eye from xenobiotics and therefore it affects drug entry into the ocular tissues. It also represents an important drug target since its functions are compromised in some retinal diseases, such as age-related macular degeneration.

One aim of this study was to quantify the drug transporting proteins on the RPE surface. These transporters might significantly influence ocular pharmacokinetics (PK), but the published literature is based on qualitative data, complicating the prediction of their functionality. In addition, the localization of the transporters in the RPE is mostly unknown. Thus, another aim was to quantify the transporters separately from the apical and basolateral surfaces of the RPE. The localization is important when predicting PK parameters since a drug can enter the RPE from either side, depending on the administration route. The clinical significance of the efflux transporters was evaluated with simulation models.

RPE is heavily pigmented, i.e. it contains melanin polymer packaged inside intracellular organelles, melanosomes. Melanin binds many drugs, thereby affecting their ocular PK. Since melanin binding can prolong a drug’s action, which would be desirable for many retinal drugs, melanin targeting is an interesting targeting approach. However, many aspects of melanin binding are far from clear. One aim of this study was to develop an isolation method for RPE melanosomes so that they could be used in in vitro pigment binding assays. An additional aim was to assay melanin binding with a small compound library to compare melanin binding under similar study conditions.

These experiments revealed that multidrug-resistance associated proteins (MRPs) are most likely involved in drug efflux in the RPE. Most of the studied drug transporters remained below the detection limit, indicating that passive permeation may be the most prominent permeation mechanism by which many drugs cross the RPE. The simulations indicated that efflux proteins hindered the intracellular drug accumulation in the RPE regardless of the localization of the efflux proteins. This finding highlights the importance of RPE efflux proteins for modifying a drug’s PK properties. Therefore, substrates of efflux proteins should be avoided when the RPE is targeted in drug discovery programs. We devised an isolation method that
isolated intact and functional melanosomes for further in vitro studies. We also classified 33 compounds based on their melanin binding into low, intermediate and high binders. It was demonstrated that melanin binding and plasma protein binding do not correlate. These two parameters can be used as selection criteria in drug discovery programs aiming to target the pigmented ocular tissues from the systemic circulation. This study provided insights into the role of transporters and melanin binding for retinal drug delivery.

Improving the Palatability of Minitablets for Feline Medication

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Product acceptability and administration of drugs on a free choice basis are key factors determining the success of peroral veterinary medication, and are particularly important when treating chronic diseases of companion animals. Veterinary medicines found unpalatable and repulsive in odour, taste or form will result in refused voluntary intake. Particularly with cats, the issues related to product unacceptability are difficult to overcome, and specific tailor-made peroral dosage forms for cats are currently lacking. Both animal treatment compliance and owner treatment bonding are essential for successful veterinary drug therapy. Product dosing should be simple and easy, and it should be performed without any complications. The present study was undertaken to investigate feline peroral drug therapy and to develop novel feline-specific minitablets with increased palatability supporting the safe, simple, flexible and convenient drug treatment of cats.

The acceptability of minitablets was evaluated in a new feline behavioural test setting with domestic pet cats. The minitablets, developed with a focus on target species characteristics, were found to be more acceptable than non-favoured food. However, improvements in minitablet odour and/or taste were required.

Feline-specific flavours of non-natural origin together with a model substance having a bitter taste were investigated in the minitablet formulations. For a pharmaceutical industry point of view, synthetic flavours are considered more suitable over natural substances. In the present study, amino acids such as L-leucine and L-methionine, and thiamine hydrochloride, were considered as suitable candidates for feline minitablet formulations.

New flavoured polymer coating formulations for feline medication purposes on minitablets were developed. Feline-specific synthetic flavours and their mixtures were incorporated in the aqueous film coatings of the polymethacrylate copolymer of Eudragit® E. The film coatings containing meat flavours of 2-acetylpyridine and 2-acetylthiazole in small concentrations were found the most applicable for minitablet coating and taste-masking purposes.

Atomic layer deposition (ALD) was investigated as a novel ultrathin-coating method for pharmaceutical minitablets and for taste masking applications. The ALD thin coating, however, did not provide effective taste masking (with the coating levels studied) for the bitter tasting minitablets composed of heterogeneous excipients.

In conclusion, the present results support the more cost-effective product development of palatable feline peroral medication. It is evident that pet and owner compliance as well
as drug treatment efficacy and safety can be increased with the feline-specific products introduced here. The present results are also likely to be applicable for other veterinary target species, such as pet dogs. However, minitablets as dosage forms would additionally be suitable for humans.

Development of Thin Film Formulations for Poorly Soluble Drugs

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Polymeric thin films are used to achieve a systemic and local drug effect via the buccal, sublingual, ocular, vaginal and transdermal routes. Thin films have many advantages such as convenient administration, the potential for tailored personalized medication and low unit dose cost. For example as the population ages, many elderly patients experience problems swallowing tablets and thus thin films are interesting alternatives to oral dosage forms. Thin film formulations usually consist of drug, polymers and plasticizers. For example, the choice and qualities of the polymer e.g. its molecular weight, can influence the film’s properties such as its mechanical strength, drug release rate and disintegration time. Thin films are usually manufactured by solvent casting or solid extrusion methods. The in vitro evaluation of thin film formulations includes physical, and mechanical testing.

The overall aim of this work was to formulate a poorly soluble drug perphenazine (PPZ) into a thin polymeric film where the drug would exist in an amorphous form. The specific aims were: (1) to develop a spraying method to manufacture thin polymer-drug films with good mechanical and drug release properties and to optimize manufacturing conditions by the Design of Experiments, (2) to observe in real time the physical changes occurring in the films during drug dissolution by applying a novel multi-parametric surface plasmon resonance method (MP-SPR), (3) to explore the mechanical and physical properties of the thin films during storage under different conditions of temperature and humidity.

It was found that a pneumatic airbrush can be used to manufacture thin polymer-PPZ-films with good mechanical and release properties. The systematic evaluation of the effect of the formulation and process variables revealed that the amounts of drug and the film thickeners (polyvinylpyrrolidone (PVP), Soluplus®) were the two main variables affecting the mechanical properties of the films. Moreover, it was found that the amount of PVP enhanced the dissolution rate of drug and the release of drug followed a square root of time kinetics. The MP-SPR method was utilized for the first time to acquire real-time information about the physical changes occurring in the films during drug dissolution. In addition, this technique can be used to study and optimize drug release from thin drug delivery systems. The physical stability of thin films was evaluated under three different storage conditions with different methods. High temperature and humidity were found to induce drug crystallization especially in binary phase systems. Instead, in low temperature and dry condition, the drug remained amorphous. Crystallization of the drug was found to have an impact on the films’ mechanical properties and the in vitro drug release from the films.
In conclusion, well designed, wide-ranging studies are crucial in the manufacture of drug delivery systems. Temperature and relative humidity can destabilize amorphous drug formulations during manufacturing and storage, and thus it is important to control these parameters. In addition to traditional methods, new techniques, such as MP-SPR, can be exploited to monitor the changes occurring in thin films during drug dissolution.

**Early discovery approaches of biofilm inhibitors from naturally-inspired sources and insights into biofilm models**

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Biofilm formation complicates diagnosis and treatment of bacterial infections. Bacterial biofilms can be defined as structurally organized communities of bacterial cells embedded in a matrix of extracellular polymeric matrix (EPS). The majority of bacteria exist as biofilms in most natural environments. Biofilm bacteria are highly tolerant to antimicrobials and host immune responses. Conventional antibiotics are inefficient in the treatment of biofilm-associated infections, especially those occurring in hospitalized patients and associated with the use of medical devices. Moreover, in vitro laboratory methods that have been designed for growing of planktonic bacteria and evaluation of antimicrobials against them are not applicable for biofilms. Therefore, alternative methods and models have been developed for investigation of biofilms and testing of antimicrobials against biofilm-growing bacteria. However, so far, the repertoire of existing antibiofilm agents is extremely limited and thus, there is a great need for the discovery and development of novel anti-biofilm compounds. In that context, the primary aim of this thesis project was to identify biofilm inhibitors from naturally-inspired sources. Towards this goal, 3570 compounds were screened for biofilm inhibition. Screening campaigns were designed to explore different strategies aimed at the discovery of anti-biofilm leads with bactericidal or non-bactericidal effects. In one direction, two synthetic flavan derivatives as well as the D-tryptophan and the β-cyclohexyl-L-alanine derivatives of (+)-dehydroabietic acid (DHA) were identified as anti-biofilm leads. These leads were characterized as desirable antimicrobials that displayed both antibacterial and anti-biofilm activity in contrast to conventional antibiotics. They were able to prevent biofilm formation and eradicate pre-formed biofilms at micromolar concentrations. A second discovery strategy allowed the identification of two flavone derivatives as Quorum Sensing Inhibitors (QSIs). As opposed to the leads identified by the first strategy, these leads did not display any bactericidal activity but interfered with biofilm formation and maturation. Furthermore, given the relevance of biofilm models for drug discovery, a comparative methodological study was also performed. Efficacy testing of conventional antibiotics in prevention of biofilm formation was conducted in two distinct biofilm models, microtiter well plates (MWP) and drip flow reactor (DFR), classified as closed and open systems, respectively. The goal was to investigate if the choice of model affects the experimental outcome. The comparative study revealed that biofilms grown under continuous flow of nutrients displayed significantly higher antimicrobial tolerance than those grown in the absence of flow. Altogether, this thesis project led to the identification of anti-biofilm leads, which can serve as starting points for
further optimization towards more potent biofilm inhibitors that can be used either as alternatives to conventional antibiotics or as adjunctive agents in combination with conventional antibiotics or other antimicrobials. Given the complexity of biofilms, it is increasingly understood that no single strategy will be sufficient for biofilm control. Thus, complementary strategies aimed at interfering with biofilms in different mechanisms could offer a promising solution. Further, when selecting the best anti-biofilm compounds, activity of the most promising compounds needs to be confirmed using different biofilm models, as the choice of biofilm model was shown to have a profound impact on the experimental outcome.

Design and development of personalized dosage forms by printing technology

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The development of tailored dosage forms provides a wide range of possibilities for meeting the needs of individual drug therapy. The personalized dosage forms improve the safety of drug treatment by decreasing the risk of overdosing and adverse reactions. Conventional drug preparations with fixed dose strengths are generally produced in large industrial scale. However, the tailored dosage forms for individual patients could be manufactured in small batches with specific materials, drug content and release profile. Therefore, alternative fabrication methods, such as printing technology, are being investigated for the customization of the dosage forms. Printing technology is a flexible method for the on-demand production of drug preparations with variable doses at the point-of-care. The thesis was aimed at investigating the feasibility of two-dimensional (2D) printing technology for the fabrication of personalized dosage forms. In the 2D printed dosage forms, a pharmaceutical ink is typically deposited and solidified on a planar carrier substrate according to a predefined pattern. The dosing accuracy and reproducibility of the inkjet-printed formulations could be controlled on the single droplet scale. Furthermore, tailoring the properties and the composition of the formulations allows obtaining drug delivery systems (DDS) with controlled drug release profiles and/or with multiple active pharmaceutical ingredients (APIs). The versatility of 2D printing technology was demonstrated by preparing printed formulations either by inkjet or flexographic printing on planar edible substrates with different types of pharmaceutical inks. The printed formulations and their components were analyzed to allocate the crucial aspects in the development process and to improve the knowledge about the physicochemical properties, in vitro performance and stability of the printed APIs. The printability of the inks and the specific printing parameters were closely related to the rheological properties of the drug solutions. The solid state of the printed APIs was dependent on the ink composition, the ink incorporation capacity of the substrates, and the physicochemical properties of the APIs. Solid state analysis of the final dosage forms showed that the APIs were distributed uniformly in a crystalline or molecularly dispersed state. Furthermore, the flexographically prepared solid nanoparticulate systems exhibited an enhanced in vitro drug release due to the spatial distribution of the crystalline nano-suspension inks. The high dosing precision of the inkjet printing process was ensured by the stable jetting of the drug solutions.
However, the dosing of nanosuspensions by flexographic imprinting was less accurate mainly because of the format of the ink transfer system. The dosing flexibility of the inkjet-printed pharmaceuticals could be regulated by adjusting the printing resolution or the physical size of the dosage units. Furthermore, the implementation of non-destructive attenuated total reflectance Fourier transform infrared spectroscopy with multivariate data analysis showed high applicability for the quantification of printed pharmaceuticals. In addition to edible commercial substrates, the suitability of gelatin-based electrospun fiber matrices as carrier substrates for the fabrication of printed dosage forms was studied. Moreover, drug-loaded electrospun fiber mats were produced by stabilizing the amorphous state of a poorly water-soluble drug within the inner structure of these fibers. The use of drug-loaded fibrous substrates presented a unique approach for the preparation of dual DDS, where an API was inkjet-printed on the drug-loaded matrices that contained another API. The analysis of the designed combination DDS showed that both drugs exhibited an independent release behavior. The thesis presents an extensive overview on the main aspects of the development of personalized dosage forms by 2D printing technology. The research improves the understanding of the key factors for successful tailoring and manufacturing of the printed dosage forms, elaborates on the quality control aspects of the printing process, and provides an insight into the essential properties and the performance of the printed pharmaceuticals.

Insights into particle formation and analysis

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This thesis consists of two parts, particle formation and analysis. In the first part, particle formation in microfluidic devices and in devices employing supercritical fluids is investigated, and in the second part, essential issues in analytical methods for determining drug release and solid-state properties are addressed.

Microfluidic technology was employed to produce microcapsules for protein formulations. The microcapsules were produced with a biphasic flow to create water-oil-water double emulsion droplets with ultrathin shells. All the particles were found to be intact and with a particle size of 23 - 47 µm. The encapsulation efficiency of bovine serum albumin in the microcapsules was 84%. This study demonstrates that microfluidics is a powerful technique for engineering formulations for therapeutic proteins.

A new, robust, stable, and reproducible method based on expansion of supercritical solutions using carbon dioxide as a solvent was developed to produce nanoparticles. The method, Controlled Expansion of Supercritical Solution (CESS), uses controlled mass transfer, flow, pressure reduction, and particle collection in dry ice. CESS offers control over the crystallization process as the pressure in the system is reduced according to a specific profile. Controlled pressure reduction keeps the particle growth and production process stable. With CESS, we produced piroxicam nanoparticles, 60 mg/h, featuring narrow size distribution (176 ± 53 nm).
The Lyophilic Matrix (LM) method was developed for investigating dissolution rates of nanoparticles, powders, and particulate systems. The LM method is based on its ability to discriminate between non-dissolved particles and the dissolved species. In the LM method, the test substance is embedded in a thin lyophilic core-shell matrix. This permits rapid contact with the dissolution medium while inhibiting dispersion of non-dissolved particles without presenting a substantial diffusion barrier. By minimizing method-induced effects on the dissolution profile of nanopowders, the LM method overcomes shortcomings associated with current dissolution tests.

Time-gated Raman spectroscopy was applied for solid-state analysis of fluorescent powder mixtures. A setup with a $128 \times (2) \times 4$ CMOS SPAD detector was used for the quantitative analysis of solid-state forms of piroxicam. Time-gating provides an instrumental method for rejecting the fluorescence signal. This study demonstrated that traditional PLS analysis of time-gated Raman spectra resulted in mean RMSE of 4.1%. The time-gated Raman spectroscopy method shows potential for relatively routine quantitative solid-state analysis of photoluminescent pharmaceuticals.

Imitation of biologically relevant oxidation reactions by titanium dioxide photocatalysis: Advances in understanding the mimicking of drug metabolism and the oxidation of phosphopeptides

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Redox reactions play an important role in human physiology and pathophysiology. For example, oxidative stress and free radical-mediated oxidation of proteins and lipids are implicated in several diseases such as Alzheimer’s and Parkinson’s disease. Oxidation reactions belong also to the most important phase I metabolism pathways of drugs, which can give rise to pharmacologically active or toxic metabolites. The established methods for in vitro drug metabolism studies, e.g. methods using hepatocytes, human liver microsomes (HLMs), and recombinant enzymes, are relatively time-consuming and expensive. Thus, the potential of several nonenzymatic oxidation methods, such as those based on metalloporphyrins, electrochemistry (EC), and Fenton reaction, have been explored for metabolism studies. However, new methods need to be developed to enable rapid production of drug metabolite standards and since none of the above nonenzymatic methods allow comprehensive prediction of phase I drug metabolism.
The titanium dioxide (TiO2) photocatalysis method was developed and applied to evaluate the effect of phosphorylation of tyrosine on the oxidation of (phospho)peptides with the same sequence but different phosphorylation states. The results obtained using ultra-high-performance liquid chromatography – mass spectrometry (UHPLC-MS) show that nonphosphorylated tyrosine was the amino acid most susceptible to hydroxyl radical-initiated oxidation, but oxidation of tyrosine was in most cases inhibited by its phosphorylation.

The feasibility of TiO2 photocatalysis for imitation of in vitro phase I HLM metabolism of small drug molecules was studied using UHPLC-MS and compared with the electrochemically assisted Fenton reaction (EC-Fenton) and EC. TiO2 photocatalysis, EC-Fenton, and EC imitated 44%, 31%, and 11%, respectively, of the in vitro phase I HLM metabolites of four model compounds. As TiO2 photocatalysis proved most feasible for the imitation of in vitro phase I HLM metabolism, its feasibility for imitation of in vitro phase I HLM metabolism of five anabolic steroids was also examined. TiO2 photocatalysis was able to imitate over half of the hydroxylation and dehydrogenation metabolites, but its imitation of the metabolites resulting from combinations of these reactions was considerably poorer.

To enable even more rapid experiments to study biologically relevant oxidation reactions, TiO2-photocatalysis was simply integrated with desorption electrospray ionization (DESI)-MS by using the same TiO2-coated glass wafer for photocatalytic reactions and DESI-MS analysis. This new method enabled high-throughput investigation of photocatalytic oxidation reactions, as demonstrated using 12 model compounds, and imitation of several drug metabolism reactions of three model compounds studied in more detail.

In conclusion, TiO2 photocatalysis proved a feasible method for oxidation of compounds with different polarities. TiO2 photocatalysis cannot predict drug metabolism comprehensively, but offers a potential method for rapid, simple, and inexpensive study of oxidation reactions of biomolecules and imitation of several drug metabolism reactions. Preparative scale synthesis of oxidation products by TiO2 photocatalysis is likely an alternative application of the method, but this remains to be demonstrated.
Designing drugs that are selective is crucial in pharmaceutical research to avoid unwanted side effects. To decipher selectivity of drug targets, computational approaches that utilize the sequence and structural information of the protein binding pockets are frequently exploited. In addition to methods that rely only on protein information, quantitative approaches such as proteochemometrics (PCM) use the combination of protein and ligand descriptions to derive quantitative relationships with binding affinity. PCM aims to explain cross-interactions between the different proteins and ligands, hence facilitating our understanding of selectivity.

The main goal of this dissertation is to develop and apply field-based PCM to improve the understanding of relevant molecular interactions through visual illustrations. Field-based description that depends on the 3D structural information of proteins enhances visual interpretability of PCM models relative to the frequently used sequence-based descriptors for proteins. In these field-based PCM studies, knowledge-based fields that explain polarity and lipophilicity of the binding pockets and WaterMap-derived fields that elucidate the positions and energetics of water molecules are used together with the various 2D / 3D ligand descriptors to investigate the selectivity profiles of kinases and serine proteases.

Field-based PCM is first applied to protein kinases, for which designing selective inhibitors has always been a challenge, owing to their highly similar ATP binding pockets. Our studies show that the method could be successfully applied to pinpoint the regions influencing the binding affinity and selectivity of kinases. As an extension of the initial studies conducted on a set of 50 kinases and 80 inhibitors, field-based PCM was used to build classification models on a large dataset (95 kinases and 1572 inhibitors) to distinguish active from inactive ligands. The prediction of the bioactivities of external test set compounds or kinases with accuracies over 80% (Matthews correlation coefficient, MCC: ~0.50) and area under the ROC curve (AUC) above 0.8 together with the visual inspection of the regions promoting activity demonstrates the ability of field-based PCM to generate both predictive and visually interpretable models. Further, the application of this method to serine proteases provides an overview of the sub-pocket specificities, which is crucial for inhibitor design. Additionally, alignment-independent Zernike descriptors derived from fields were used in PCM models to study the influence of protein superimpositions on field comparisons and subsequent PCM modelling.
Polyamine Analogues as Anticancer Agents

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Polyamines are low molecular weight aliphatic polycations, found in all kingdoms of life. At physiological pH, they are positively charged, enabling them to interact with negatively charged cellular constituents, such as nucleic acids, phospholipids and some acidic protein motifs. It is recognized that polyamines play an important role in fundamental cellular processes such as replication, transcription, translation, cell proliferation and differentiation. Moreover, polyamine metabolism is often dysregulated in cancer, thus it has been, and still is, an attractive target for the development of anticancer drugs. The main focus of this thesis was to expand our knowledge in polyamine analogues as potential anticancer agents and to clarify their effects on polyamine metabolism in detail.

This thesis comprises of three original studies. In the first study, we developed a simple and straightforward synthesis of polyamine analogues such as diacetylated polyamines, N1,N8-diAcSpd and N1,N12-diAcSpm, which are known as early stage cancer biomarkers. In the second study, the effects of the anticancer agent, triethylenetetramine (TETA), on polyamine metabolism were investigated. Biological assays with TETA were performed to evaluate its effects on cell proliferation, polyamine metabolism and uptake in comparison to other Cu(II) chelators, D-penicillamine and tetrathiomolybdate in DU145 prostate cancer cells. The results of this study revealed that TETA is a multitargeting drug and its anticancer effect is not only attributable to its property as a selective Cu(II) chelator but also due to its significant effects on polyamine and energy metabolism.

In the third study, the catabolic pathways of N-alkylated polyamine analogues, N,N’-bis-(3-ethylaminopropyl)butane-1,4-diamine (DESpm), N-(3-benzyl-aminopropyl)-N’-(3-ethylaminopropyl)butane-1,4-diamine (BnEtSpm), N,N’-bis-(3-benzylaminopropyl)-butane-1,4-diamine (DBSpm) and their variably deuterated counterparts were tested in vitro with recombinant enzymes. Deuteration retarded the total reaction rate and changed the preferred cleavage site of both enzymes participating in polyamine catabolism i.e. spermine oxidase (SMO) and acetylpolyamine oxidase (APAO). BnEtSpm was found to be the most cytotoxic of the evaluated analogues in the tested cancer cell lines, whereas in mouse embryonic fibroblasts, DBSpm exhibited the highest cytotoxicity. Our findings showed that the analogues’ antiproliferative efficacies correlated with the induction of SMO. As a result of this study, we undertook targeted polyamine analogue deuteration to demonstrate that the kinetic isotope effect could be applied to redirect analogue catabolism to SMO and APAO. Moreover, total hydrogen peroxide generation by both catabolic enzymes was decreased with the deuterated analogues as compared to the parent non-deuterated analogues. Unexpectedly, their efficacies remained almost the same regardless of deuteration of the analogue, indicating that analogue catabolism plays only a minor role in their antiproliferative action in those cell lines where basal APAO and SMO activities are low.
Design and Evaluation of Nanoparticle-Based Delivery Systems: Towards Cancer Theranostics

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The design, characterization and applicability of nanoparticle (NP)-based delivery systems intended for cancer theranostics, are presented in this thesis. Mesoporous silica nanoparticles (MSNs) have been widely established as biocompatible and efficient carriers of hydrophobic molecules, such as drugs for in vitro and in vivo tumor targeting. Although their intracellular delivery and cargo release have been demonstrated, knowledge of the underlying drug release mechanisms still remain unclear. For future control and prediction of these parameters, which from a clinical perspective are imperative to all drug delivery systems (DDSs), the release of hydrophobic cargo from MSNs is studied. In simple aqueous solvents, cargo release is strongly associated with nanocarrier degradation, whereas in media mimicking intracellular conditions, where lipids or hydrophobic structures are present, the physicochemical properties of the cargo molecule itself and its interactions with the surrounding medium are the release-governing parameters. For comparison, the relationship between intracellular cargo release and degradation of poly(alkylcyanoacrylate) (PACA) nanocarriers is also investigated, for which the release is found to be dependent on the biodegradation of the carrier. The influence of NP monomer composition on intracellular delivery and the role of different endocytosis pathways are also assessed. This thesis moreover presents a novel multifunctional composite NP for combined optical imaging, tracking and drug delivery. The used approaches include creation and optimization of core-shell nanostructures of photoluminescent (PL) nanodiamonds (NDs) encapsulated within mesoporous silica shells that allow tuning of the composite NP size and loading of hydrophobic cargo molecules. Through subsequent surface engineering, efficient passive uptake by endocytosis, followed by intracellular release of cargo, is achieved and displayed by optical fluorescence imaging. The approaches presented in this thesis are highly interdisciplinary, placed at the meeting point between chemistry, physics, engineering, biotechnology and pharmaceutical sciences, and provide a basis for the rational design and evaluation of NP-based DDSs, intended for cancer theranostics, mainly by intravenous (IV) administration.
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