Graduate Program „Processing of poorly soluble drugs at small scale“ (µ-Props)

Coordinators

Prof. Dr. rer. nat. Heike Bunjes
Technische Universität Braunschweig
Institut für pharmazeutische Technologie
Mendelssohnstraße 1
38106 Braunschweig

Prof. Dr. rer. nat. Andreas Dietzel
Technische Universität Braunschweig
Institut für Mikrotechnik
Langer Kamp 8
38106 Braunschweig

Funded by

Niedersächsisches Ministerium für Wissenschaft und Kultur

Table of Contents

1 Cluster 1 – Chemical Synthesis and Downstream Processing

1.1 Developing metal-NHC complexes for pharmaceutical applications

Jessica Wölker (IMPC), Prof. Dr. Ingo Ott (IMPC); Project 1A

INTRODUCTION

N-heterocyclic carbene (NHC) metal compounds, especially the “Arduengo-type” metal complexes, have besides their important role in catalysis, recently started to play an important role as new possible anticancer agents. Although the most extensively investigated derivatives of this class of compounds contain gold(I) and silver(I) metal ions, rhodium(I) NHC complexes with 1,5-cyclooctadiene (COD), a halido ligand and pseudo halido ligand (X, Figure, left) have also shown a high potential for antitumor drug development.[1,2] Complexes of the type Rh(I)(NHC)(COD)Cl trigger antiproliferative effects against MCF-7, MDA-MB 231 (human breast adenocarcinoma) and HT-29 (colon carcinoma) cells and inhibit the enzyme thioredoxin reductase (TrxR)[1,2], which is overexpressed in tumor cells. Rhodium complexes were also proven to interact with DNA.[3,4]

Figure 1 shows newly synthesized rhodium-based complexes with a benzimidazole backbone or a halogen-containing phenylimidazole backbone.

Cytotoxicity, cellular uptake and solubility studies of the new complexes with variations in the X ligand and the backbone will provide new insights on the structure activity relationships (SARs).

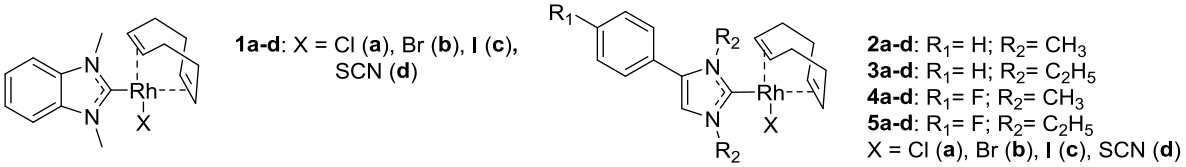
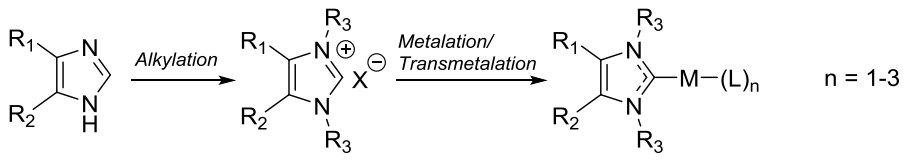


Figure 1: new series of rhodium(I) NHC complexes. left: Rh(I)(NHC)(COD)X with benzimidazole backbone; right: Rh(I)(NHC)(COD)X with phenylimidazole backbone.

RESULTS

To synthesize the N-heterocyclic carbene (NHC) metal compounds the reaction starts with the alkylation of the corresponding imidazole with iodomethane or iodoethane. To obtain the hexafluorophosphate imidazolium ion, an anion exchange of the imidazioliumiodide in methanole with KPF6 was necessary. This was just essential to form the S-thiocyanato-rhodium complexes. To receive the NHC-rhodium-complexes a metalation with silver-(I)-oxide and a following transmetalation with the corresponding goldchloride or rhodium(1,5-cyclooctadiene)dimer was performed. In Scheme 1 these steps are shown in an overview.



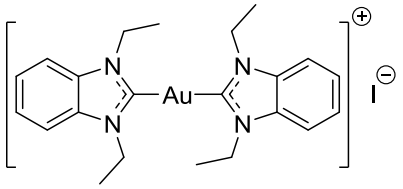
$R_1 = R_2$ = Benz
R_1 = Ph; R_2 = H
R_1 = *p*-F-Ph; R_2 = H

$R_1 = R_2$ = Benz;
R_3 = Met/Et, X = I, PF_6
R_1 = Ph; R_2 = H;
R_3 = Met/Et; X = I, PF_6
R_1 = *p*-F-Ph; R_2 = H;
R_3 = Met/Et; X = I, PF_6

$R_1 = R_2$ = Benz; R_3 = Et; M = Au(I);
L=Cl/ NHC
$R_1 = R_2$ = Benz; R_3 = Met; M = Rh(I);
L=COD + I/Br/I/SCN
R_1 = Ph; R_2 = H; R_3 = Met/Et; M = Rh(I);
L=COD +Cl/Br/I/SCN
R_1 = *p*-F-Ph; R_2 = H; R_3 = Met/Et; M = Rh(I);

Scheme 1: Overview of the synthesis of the NHC-metal-complexes.

All products were purified either by crystallization or chromatography on silica gel to get a very good purity for biological tests (antiproliferative assay, uptake-studies) and stability tests.
Our results in the stability tests showed that the NHC-gold-complex 6 shows an exceptional chemical stability in aqueous and polar environment at physiological temperature over several days. In highly apolar medium (chloroform) it undergoes some decomposition after extended periods (> 24 h).



6

Figure 2: Test compound **6** ([Di-(1,3-diethylbenzimidazol-2-ylidene)]gold(I) iodide), which was tested in stability experiments.

The antiproliferative assay showed in case of the NHC-rhodium-complexes that all synthesized complexes were antitumoractive in the micromolar range, whereas the metal free precursors were inactive. An increase of activity was achieved by changing the benzimidazole against a phenylimidazole backbone. Complexes 2a-d and 3a-d showed the most promising results with slightly higher activities due to ethyl side chains on the NHC nitrogens. They will be chosen for ongoing tests regarding the exploration of the structure-activity relationships (SARs).

ACKNOWLEDGMENTS

The financial support within a Georg Christoph Lichtenberg fellowship by the State of Lower Saxony (graduate program µ-Props, "Processing of poorly soluble drugs at small scale") is gratefully acknowledged.

REFERENCES

[1] Oehninger, L.; Küster, L.-N.; Schmidt, C. et al., A Chemical–Biological Evaluation of Rhodium (I) N-Heterocyclic Carbene Complexes as Prospective Anticancer Drugs. Chem. Eur. J. (2013), 19, 17871 – 17880.

[2] Oehninger, L.; Spreckelmeyer, S.; Holenya, P. et al., Rhodium (I) N heterocyclic carbene bioorganometallics as in vitro antiproliferative agents with distinct effects on cellular signaling. J. Med. Chem. (2015), 58, 9591−9600.

[3] McConnell, J. R.; Rananaware, D. P.; Ramsey, D. M.et al., A potential rhodium cancer therapy - Studies of a cytotoxic organorhodium (I) complex that binds DNA. Bioorg. Med. Chem. Lett. (2013), 23, 2527–2531.

[4] Kielkopf, C. L.; Erkkila, K. E.; Hudson, B. P. et al., Structure of a photoactive rhodium complex intercalated into DNA. Nature Struct. Biol. (2000), 7, 117-121.

PUBLICATIONS

POSTER PRESENTATIONS

1. Jessica Wölker, Ingo Ott Synthesis Optimization and HPLC-MS Method Development for Gold(I)-N-heterocyclic Carbene Complexes. Konferenz: SPhERe “Symposium on Pharmaceutical Engineering Research”, 19. – 20.10.2015, Braunschweig, Deutschland.

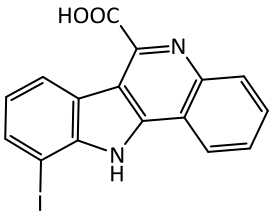
2. Jessica Wölker, Ingo Ott Rhodium(I) N-Heterocyclic Carbene Complexes as Possible Anticancer Drugs. Konferenz: ISBOMC16 “8th International Symposium on Bioorganometallic Chemistry”, 04. – 08.09.2016, Moskau, Russland.
3. Jessica Wölker, Ingo Ott Investigation of the Possible Anticancer Properties of Rhodium(I) N‑Heterocyclic Carbene Complexes. Tagung: DPhG Jahrestagung, 04. – 07.10.2016, München, Deutschland.
4. Jessica Wölker, Ingo Ott Synthesis and Biological Properties of Rhodium(I) N-Heterocyclic Carbene Complexes as Possible Anticancer Drugs. Tagung: 8. Jungchemikertagung, 09.05.2017, Braunschweig, Deutschland.
5. Jessica Wölker, Ingo Ott Investigation of Structure Activity Relationships of New Rhodium(I) N-Heterocyclic Carbene Complexes as Possible Anticancer Drugs. Konferenz: ISABC14 “14th International Symposium on Applied Bioinorganic Chemistry”, 07. – 10.06.2017, Toulouse, Frankreich.
6. Jessica Wölker, Ingo Ott Synthesis, Physicochemical and Biological Properties of Metal-N-Heterocyclic Carbene Complexes. Konferenz: SPhERe “Symposium on Pharmaceutical Engineering Research”, 06. – 08.09.2017, Braunschweig, Deutschland.
7. Jessica Wölker, Ingo Ott Synthesis and Biological Properties of Novel Rhodium(I) N-Heterocyclic Carbene Complexes as Possible Anticancer Drugs. Konferenz: EuroBIC14 “14th European Biological Inorganic Chemistry Conference“, 26. – 30.08.2018, Birmingham, United Kingdom.

1.2 2-Substituted indole-3-carbonitriles as new inhibitors of the protein kinase DYRK1A

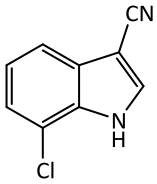
Rosanna Meine (IMPC), Prof. Conrad Kunick (IMPC); Project 1B

INTRODUCTION

Because its overexpression is associated with mental retardation in people with Down syndrome and with the development of Alzheimer's disease, the protein kinase DYRK1A represents an interesting target for the development of new inhibitors. [1] FALKE identified 11H-indolo[3,2-c]quinoline-6-carboxylic acids as potent inhibitors of DYRKA with KuFal194 (1) as the most potent and selective derivative. However, due to the large aromatic system this compound is very lipophilic, nearly insoluble in water and therefore not suitable for the potential use as a drug. [2] The aim of this work was to develop new DYRK1A inhibitors with improved solubility related to KuFal194 (1). By downsizing the ring system of KuFal194 the fragment 2a was created which showed improved physicochemical properties.



KuFal194 (1)
IC_{50} DYRK1A = 6 nM
logP = 4.22
$S_{kin., pH\ 7.4}$ = 5.28 µM



2a
IC_{50} DYRK1A = 3300 nM
logP = 2.53
$S_{kin., pH\ 7.4}$ = 116 µM

RESULTS

Docking studies revealed a potential binding mode in the ATP binding pocket of DYRK1A that led to possible modifications of the fragment 2a (Figure 1). The halogen substituent at position 7 forms a water mediated halogen bond to the hinge region and the nitrile group interacts with the conserved Lys188. Both structural elements are essential for the orientation within the host protein and were not changed.

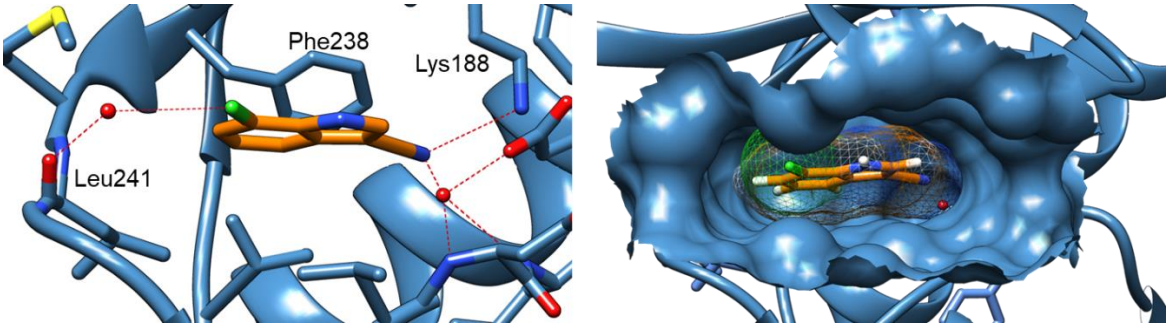
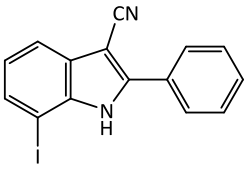


Figure 1: Possible binding mode of fragment 2a (orange) in the ATP binding site of DYRK1A (blue, PDB: 4YLJ).

By replacing the 7-chloro substituent with other halogens and introducing further substituents at positions 1 and 2 of the indole, new derivatives with improved potency were synthesized and structure activity relationships of the indole-3-carbonitriles were analyzed. New synthesis routes were developed based on three different methods for the introduction of the nitrile group at position 3 of the indole core.

For inhibition of DYRK1A activity a 7-iodo substituent was beneficial due to the water mediated halogen bond. An unsubstituted NH group was essential and in analogy to 1 an unsubstituted phenyl residue at position 2 led to the most potent inhibitors with 2b repre¬senting the derivative with the strongest DYRK1A inhibitory activity (IC50 = 10 nM). This compound also inhibited DYRK1A-mediated phosphorylation of SF3B1 in HeLa cells with submicromolar potency (IC50 = 320 nM). All compounds exhibited poor selectivity with comparable activity against the related kinases DYRK1B and CLK1.

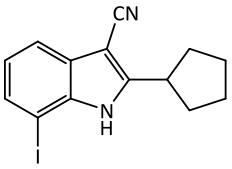


2b

IC_{50} DYRK1A = 10 nM

logP = 4.42

$S_{kin., pH\ 7.4}$ = 4.57 µM

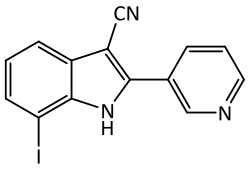


2c

IC_{50} DYRK1A = 70 nM

logP = 4.37

$S_{kin., pH\ 7.4}$ = 16.2 µM



2d

IC_{50} DYRK1A = 80 nM

logP = 3.89

$S_{kin., pH\ 7.4}$ = 10.0 µM

The physicochemical properties were evaluated by measuring the thermodynamic and kine¬tic solubility and by calculating the logP value. Compared to 1, 2b displayed neither reduced lipophilicity nor improved solubility. By replacing the 2-phenyl substituent of 2b with pyridin-3-yl (2d) or cyclopentyl residues (2c) the logP value was decreased and solubility was improved while the DYRK1A activity was only slightly diminished. Because of double digit nanomolar activity (IC50 = 70 nM) and a high fraction of saturated carbon atoms, especially 2c represents a promising structure for further optimisation of indole-3-carbonitriles as DYRK1A inhibitors with improved solubility.

REFERENCES

[1] Becker, W., Soppa, U., Tejedor, F. J.: DYRK1A: A potential drug target for multiple Down syndrome neuropathologies. CNS Neurol. Disord.: Drug targets 2014, 13, 26–33.

[2] Falke, H., Chaikuad, A., Becker, A., Loaëc, N., Lozach, O., Abu Jhaisha, S., Becker, W., Jones, P. G., Preu, L., Baumann, K., Knapp, S., Meijer, L., Kunick, C.: 10-Iodo-11H-indolo[3,2-c]quinoline-6-carboxylic acids are selective inhibitors of DYRK1A. J. Med. Chem. 2015, 58, 3131–3143.

PUBLICATIONS

1. Meine, R., Becker, W., Falke, H., Preu, L., Loaëc, N., Meijer, L., Kunick, C.: Indole-3-carbonitriles as DYRK1A inhibitors by fragment-based drug design. Molecules 2018, 23, 64.
2. Meine, R., Falke, H., Kötz, J., Schweda, S.I., Kunick, C.: 7-Iodo-1H-indole-3-carbonitrile. Molbank 2015, M869.
3. Meine, R., Blech, M., Lindhof, J., Kunick, C.: 7-Bromo-1-methyl-2-phenyl-1H-indole-3-carbonitrile. Molbank 2017, M941.

POSTER PRESENTATIONS

1. Meine, R., Loaëc, N., Meijer, L., Kunick, C.: 2-Substituted indole-3-carbonitriles as new DYRK inhibitors (Talk). Conference “DYRK1A, related kinases and human disease”, Saint Malo, France, 28.03. – 01.04.2017.
2. Meine, R., Loaëc, N., Meijer, L., Kunick, C.: 2-Substituted indole-3-carbonitriles as new inhibitors of the protein kinase DYRK1A with improved physicochemical properties (Poster). 2nd Braunschweig

International Symposium on Pharmaceutical Engineering Research (SPhERe), Braunschweig, 06.09. – 08.09.2017.

3. Meine, R., Loaëc, N., Meijer, L., Kunick, C.: 2-Substituted indole-3-carbonitriles as new DYRK1A inhibitors (Poster). DPhG Annual Meeting, Saarbrücken, 26.09. – 29.09.2017.

1.3 Predicting the solubility of drug candidates

Andreas Mecklenfeld (IfT), PD Dr.-Ing. Gabriele Raabe (IfT); Project 1C

INTRODUCTION

The knowledge of suitable solvents can greatly benefit the development of active pharma-ceutical ingredients. Molecular simulations can be interpreted as computer experiments to complement laboratory experiments. They not only enable the calculation of thermophysical properties [1] but also provide a detailed insight into a systems behavior on the molecular level (Fig. 1).
Relative solubilities can be estimated by computing the solvation free energies ΔGsolv of so-lutes in various solvents. The calculation of ΔGsolv is computational expensive, and commonly used workflows may result in ineffective simulation implementations or a lack of statistical precision of the results. On the other hand, the agreement between ΔGsolv results obtained from simulation and experiment can differ significantly, depending on the molecular model used to describe the solute and solvent compounds.

The goals of this project are

- the development of methods for improved statistical precision and reduced computational effort for the calculation of ΔGsolv
- a comparison of different molecular models to describe the solute-solvents interactions
- the adaptation of molecular models to improve their predictive capability with regard to solubility prediction of drug-like molecules.

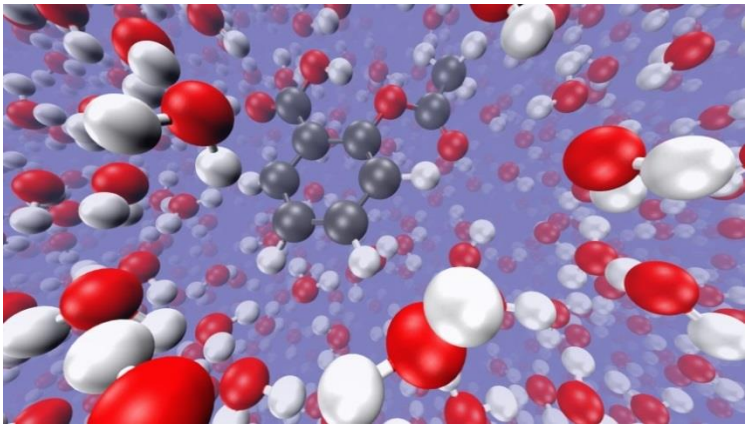


Figure 1: Snapshot from molecular simulation of acetylsalicylic acid (Aspirin) dissolved in water

RESULTS

We developed algorithms that allow for predefined statistical precisions and reduced computational cost for free energy calculations. These algorithms can be easily adapted to any free energy calculation workflow [2]. We found a strong correlation between the statistical variance and the ΔGsolv-specific simulation parameter vector λ that is used for scaling the inter-molecular solute/solvent interactions. Implemented into Python, our iterative method adapts the elements of vector λ. Simultaneously, the length of vector λ and by this the simulation effort is adjusted for the individual task. As both the statistical variances and the simulation effort can be significantly decreased, our algorithms make ΔGsolv simulations more applicable. At the same time, the agreement between simulation and experimental results is unaffected.

We have identified the determination of partial charges for the description of Coulomb inter-actions as a major impact factor on the ΔGsolv simulation results. Common molecular models use fixed charges, which can't consider polarization effects due to the change of the chemical environment caused by the dissolution of the solute. We studied the IPolQ-Mod [3] method for an implicit representation of polarization costs and found a good compatibility with the General Amber Force Field (GAFF) [4] model [5]. Applying IPolQ-Mod partial charges to add physical details to the model, we furthermore targeted solvation free energies for a variety of chemical classes for solutes and various solvents in a refitting process of selected model parameters. Our present refitting results demonstrate significantly improved agreements in ΔGsolv, with average deviations coming close to experimental uncertainties. An accurate description of solute/solvent interactions is an essential basis for future liquid phase simulation studies, e.g. of lipid dispersions used as carrier systems for poorly water-soluble compounds.

ACKNOWLEDGMENTS

This work was supported by grants of the µ-Props PhD project at the Center of Pharmaceutical Engineering (PVZ) at Technische Universität Braunschweig. Molecular simulations were performed with resources provided by the North-German Supercomputing Alliance (HLRN).

REFERENCES

[1] G. Raabe, Molecular Simulation Studies on Thermophysical Properties: With Application to Working Fluids, Springer, Singapore, 2017.

[2] A. Mecklenfeld and G. Raabe, "Efficient solvation free energy simulations: Impact of soft-core potential and a new adaptive λ-spacing method," Mol. Phys., vol. 115, 9-12, pp. 1322–1334, 2017.

[3] H. S. Muddana, N. V. Sapra, A. T. Fenley et al., "The SAMPL4 Hydration Challenge: Evaluation of Partial Charge Sets With Explicit-Water Molecular Dynamics Simulations," J Comput Aided Mol Des, vol. 28, no. 3, pp. 277–287, 2014.

[4] J. Wang, R. M. Wolf, J. W. Caldwell et al., "Development and Testing of a General Amber Force Field," J. Comput. Chem., vol. 25, no. 9, pp. 1157–1174, 2004.

[5] A. Mecklenfeld and G. Raabe, "Comparison of RESP and IPolQ-Mod Partial Charges for Solvation Free Energy Calculations of Various Solute/Solvent Pairs," J. Chem. Theory Comput., vol. 13, no. 12, pp. 6266–6274, 2017.

PUBLICATIONS

1. M. Schappals, A. Mecklenfeld, L. Kröger et al., "Round Robin Study: Molecular Simulation of Thermodynamic Properties from Models with Internal Degrees of Freedom," J. Chem. Theory Comput., vol. 13, no. 9, pp. 4270–4280, 2017.
2. A. Mecklenfeld and G. Raabe, "Efficient solvation free energy simulations: Impact of soft-core potential and a new adaptive λ-spacing method," Mol. Phys., vol. 115, 9-12, pp. 1322–1334, 2017.
3. A. Mecklenfeld and G. Raabe, "Comparison of RESP and IPolQ-Mod Partial Charges for Solvation Free Energy Calculations of Various Solute/Solvent Pairs," J. Chem. Theory Com-put., vol. 13, no. 12, pp. 6266–6274, 2017.

ORAL PRESENTATIONS

1. Mecklenfeld, A.; Raabe, G. Efficient Molecular Simulations of the Free Energy of Solva-tion; Jahrestreffen der ProcessNet-Fachgruppe Molekulare Modellierung; Frankfurt/Main, Germany, 2017.

2. Mecklenfeld, A.; Raabe, G. Comparison of RESP and IPolQ-Mod Partial Charges by Effi-cient Molecular Simulations of the Free Energy of Solvation; Twentieth Symposium on Ther-mophysical Properties; Boulder, CO, USA, 2018.

POSTER PRESENTATIONS

1. Mecklenfeld, A.; Raabe, G. Predicting the Solubility of Drug Candidates; SPhERe Sympo-sium; Braunschweig, Germany, 2015.
2. Mecklenfeld, A.; Raabe, G. Optimization of the Alchemical Pathway for Free Energy Simulations; Multiscale Modelling Conference; Manchester, England, 2016.
3. Mecklenfeld, A.; Raabe, G. Studien zu optimierten alchemischen Pfaden für freie Enthal-piesimulationen; Thermodynamik-Kolloquium; Kaiserslautern, Germany, 2016.
4. Mecklenfeld A.; Raabe, G. Improving Alchemical Pathway for Free Energy Calculations. 4th International Conference on Molecular Simulation (ICMS), Shanghai, China 2016.
5. Mecklenfeld, A.; Raabe, G. Comparison of RESP and IPolQ-Mod Partial Charges for Ac-curate Solvation Free Energy Calculations; Designing Forcefields in an Age of Cheap Com-puting; Sheffield, England, 2017.
6. Mecklenfeld, A.; Raabe, G. Comparison of RESP and IPolQ-Mod Partial Charges for Ac-curate Solvation Free Energy Calculations; SPhERe Symposium; Braunschweig, Germany, 2017.

1.4 Model based analysis of stability and robustness of continuous production of pharmaceutical ingredients

Xiangzhong Xie (InES), Prof. Ulrike Krewer (InES); Project 1D

INTRODUCTION

The stringent competition in pharmaceutical manufacturing industries necessitates the requirement of more efficient and reliable production processes. Mathematical models for upstream and downstream processes have attracted increased attention during the last decade. Moreover, model-based design tools, e.g., sensitivity and process analysis, can be implemented to provide a better understanding of the underlying principles, and model-based process design improves the quality and yield of products. However, the existence of model and parameter uncertainties might lead to suboptimal solutions or operating failures which are not acceptable from an economy and safety perspective. Therefore, it is necessary to perform research which: 1) gives connections between process system engineering tools and pharmaceutical processes, and 2) quantitatively address and alleviate the influence of uncertainties. In this project, we successfully combined process system engineering concepts with methods for uncertainty quantification for various pharmaceutical relevant processes. Moreover, our proposed framework guarantees efficient and robust model-based results. In Fig.1 we summarized the hierarchical structure of our model-based framework.

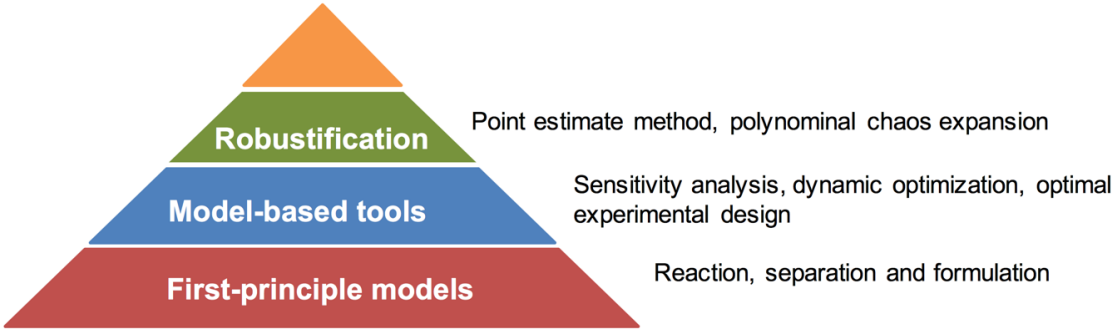


Figure 1: Structure of our model-based design framework.

RESULTS

In this section, we briefly summarize the achievements from the last three years. For all details regarding the implementation and results, we refer to the references listed below.

Sensitivity analysis

Global sensitivity analysis is used to provide quantitative measures of parameter uncertainties on process performances. Two global sensitivity measures, i.e., Sobol` indices and moment-independent sensitivities, are used in our studies. Here, the polynomial chaos expansion (PCE) is used to calculate the sensitivities as PCE is much more efficient than the traditional sample-based Monte Carlo simulations. Moreover, the significance of parameter correlations has also been addressed in our work. Sensitivity analysis has been implemented in [2], [4], [5], [7] and [8].

Optimal experimental design (OED)

Experiments are conducted to provide data for parameter estimation and model validation. Optimal experimental design optimizes the experimental conditions so that we can obtain maximum information and precise models with least experiment runs. Few but well-designed experiments are of vital importance in the field of pharmaceutical manufacturing as educts are limited and expensive.

Conventional OED strategies need reference parameter values in advance to compute local sensitivity. Practically, the lack of precise reference parameter values and system nonlinearities might lead to suboptimal design. In our work, we combined global parameter sensitivities with optimal experimental design to include uncertainties in the reference parameter values and to consider nonlinear effects adequately. Moreover, an efficient point estimate method (PEM) is used to alleviate the computational burden for global sensitivity analysis. The novel OED concept has been successfully applied in two case studies of pharmaceutical / (bio)chemical relevance [6].

Robust optimization

Pharmaceutical processes are optimized to achieve their best performance, i.e., high quality and productivity at low costs. However, parameter uncertainties might lead to suboptimal or even safety-critical model-based designs. Thus, it is necessary to design processes with the consideration of parameter uncertainties. In our work, we proposed an efficient and practical framework for robust optimization of pharmaceutical processes. The robustification concept has been successfully implemented for upstream and downstream processes as demonstrated in [2], [3], [4], [9] and [10].

ACKNOWLEDGMENTS

We acknowledge the funding from the Promotions program "µ-Props" by MWK Niedersachsen.

PUBLICATIONS

1. Schenkendorf R., Xie X., Krewer U., An Efficient Polynomial Chaos Expansion Strategy for Active Fault Identification of Chemical Processes. Computers & Chemical Engineering. 2018. In press.
2. Emenike, V., Xie, X., Krewer, U., Schenkendorf, R., Robust dynamic optimization of enzyme-catalyzed carboligation: a point estimate-based back-off approach, Computers & Chemical Engineering. submitted.
3. Xie, X., Schenkendorf, R., Robust Optimization of a Pharmaceutical Freeze-drying Process under Non-Gaussian Parameter Uncertainties, Chemical Engineering Science. submitted.
4. Xie, X., Krewer, U., Schenkendorf, R., Toward a Comprehensive and Efficient Robust Optimization Framework for (Bio)Chemical Processes, In Preparation.
5. Xie, X., Krewer, U., Schenkendorf, R., Efficient sensitivity analysis and interpretation of parameter correlations in chemical engineering. Reliability Engineering & System Safety. 2018. In press
6. Schenkendorf, R., Xie, X., Rehbein, M., Scholl, S., Krewer, U., The Impact of Global Sensitivities and Design Measures in Model-based Optimal Experimental Design, Processes, 6(4), 27, 2018.
7. Lin, N., Xie, X., Schenkendorf, R., Krewer, U., Efficient Global Sensitivity Analysis of 3D Multiphysics Model for Li-Ion Batteries. Journal of The Electrochemical Society, 165(7), pp. A1169-A1183, 2018.
8. Xie, X., Ohs, R., Spiess, AC., Krewer, U., Schenkendorf, R., Moment-independent Sensitivity Analysis of Enzyme-catalyzed Reactions with Correlated Model Parameters, IFAC-PapersOnLine, 51(2), pp.753-758, 2018.
9. Xie, X., Krewer, U., Schenkendorf, R., Robust Optimization of Dynamical Systems with Correlated Random Variables using the Point Estimate Method, IFAC-PapersOnLine, 51(2), pp. 427-432, 2018.
10. Xie, X., Schenkendorf, R., Krewer, U., Robust Design of Chemical Processes Based on a One-Shot Sparse Polynomial Chaos Expansion Concept. Computer Aided Chemical Engineering, 40, pp. 613-618, 2017.

11. Schenkendorf, R., Xie, X., Krewer, U., An Efficient Polynomial Chaos Expansion Strategy for Active Fault Identification of Chemical Processes. Computer Aided Chemical Engineering, 40, pp. 1675-1680, 2017.

ORAL PRESENTATIONS AND POSTERS PRESENTATIONS

1. Xie, X., Schenkendorf, R., Krewer, U., The effect of correlated kinetic parameters on (bio)chemical reaction networks. Jahrestreffen Reaktionstechnik 2018 (DECHEMA), Würzburg, Germany. May 7-9, 2018
2. Xie, X., Schenkendorf, R., Krewer, U., Robust Optimization of Dynamical Systems with Correlated Random Variables using the Point Estimate Method. 9th Vienna International Conference on Mathematical Modeling (MATHMOD), Vienna, Austria. February 21-23, 2018
3. Xie, X., Schenkendorf, R., Krewer, U., Moment-Independent Sensitivity Analysis of Enzyme-Catalyzed Reactions with Correlated Model Parameters. 9th Vienna International Conference on Mathematical Modeling (MATHMOD), Vienna, Austria. February 21-23, 2018
4. Xie, X., Schenkendorf, R., Krewer, U., Robust Design of Chemical Processes Based on a One-Shot Sparse Polynomial Chaos Expansion Concept. 10th World Congress of Chemical Engineering (WCCE10)/European Symposium on Computer Aided Process Engineering (ESCAPE 27), Barcelona,Spain, October 1-5, 2017.

1.5 Reactor design, reaction monitoring and Scale-Up for the continuous synthesis of poorly soluble API

Tobias Sauk (ICTV), Prof. Dr.-Ing. Stephan Scholl (ICTV); Project 1E

INTRODUCTION

In this project the continuous heterogeneous synthesis of selected diazolium salts (1,3-diethyl-1H-benzo[d]imidazol-3-ium iodide, 1,3-dimethyl-1H-benzo[d]imidazol-3-ium iodide and 1,3-diethyl-1H-benzo[d]imidazol-3-ium bromide) as precursors for metalorganic synthesis was successfully performed. In metalorganic synthesis the precursors act as ligands such as for the synthesis of gold-, rhodium- or silver-NHC-complexes [1, 2]. The precursors themselves originate from 1H-benzimidazole or other diazoles or derivatives by N-alkylation with various alkyl halides (e.g. methylene iodide, ethylene iodide, ethylene bromide) in the presence of a base (e.g. potassium carbonate).

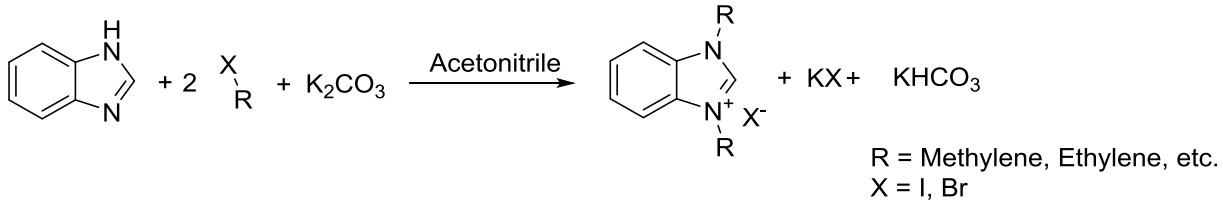


Figure 1: Reaction scheme of the synthesis of diazolium salts

RESULTS

As first step, the synthesis was transferred from round bottom flasks to a process automate and optimized in combination with an upscaling from 10 mL to 50 mL. The initial synthesis was performed under reflux conditions in acetonitrile. For optimization both, reaction monitoring (by HPLC measurements) as well as the control of process parameters (temperature, mixing), resulted in high yields (>90 %) as well as high purity (>98 %) and simultaneously in a decrease in overall process time.

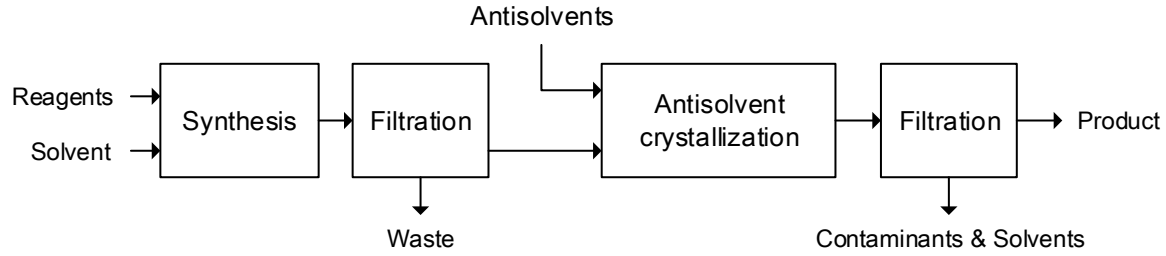


Figure 2: Block flow chart for synthesis and down-stream processing of various diazolium salts

The decrease of reaction time for instance for the methylation of 1H-Benzimidazole from 24 h to 3 h is a direct result of the adjusted parameters such as intense mixing, controlled temperature of 70 °C and high surface area of the solid base by using fine-grained potassium carbonate powder. In order to achieve high yield in both, synthesis and down-stream processing, and therefore maximize overall process yield, a complete revision of the down-stream-process was performed. The initial extraction of the product from a dried synthesis solution and filtration by silica gels was replaced by an antisolvent crystallization (mixture of hexane and ethylene acetate) as well as two filtration steps. Results indicate the feasibility of the synthesis and down-stream process for the presented as well as similar diazolium salts, whereas only small adjustments are needed due to differences in solubility of the precursors in the solvents.

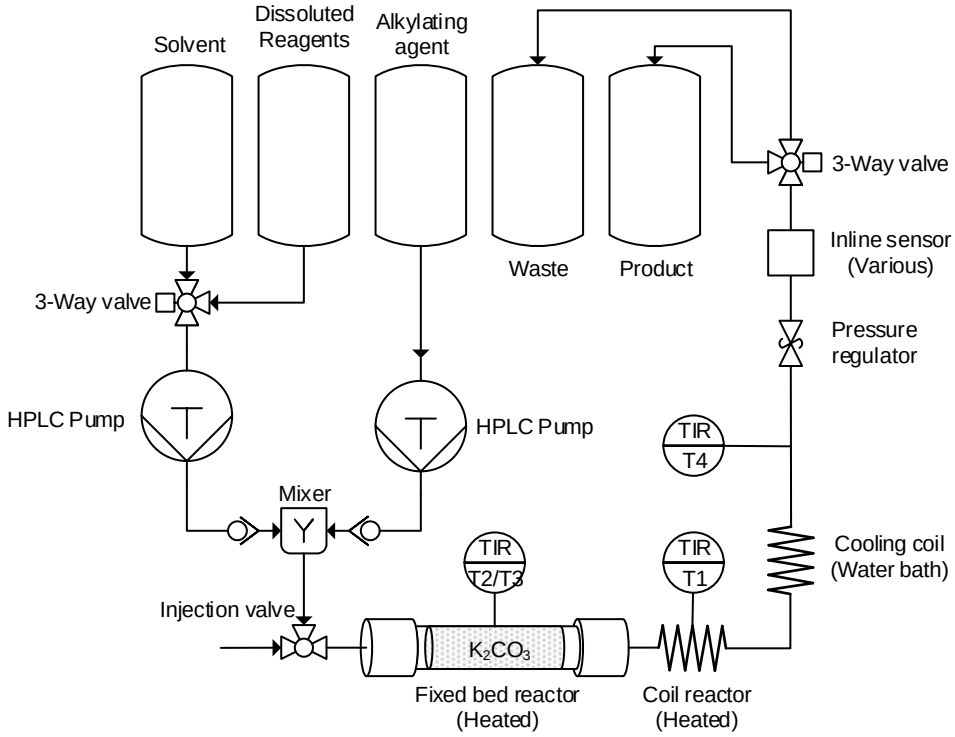


Figure 3: Process flow chart for the continuous N-Alkylation of diazoles

Based on studies of the solubility and in particular the reaction kinetics a continuous synthesis was developed. This is performed at 7 bar and temperatures of up to 150°C in an electrically heated fixed bed reactor filled with ground potassium carbonate to ensure a high surface area. Diazole and alkylating agent are prepared each as a separate solution of a specific concentration and fed by a HPLC-Pump. The solutions are mixed before entering the fixed bed reactor. Results indicate that the heterogeneous N-alkylation of diazoles can be performed in a continuous manner. Optimization is still required to ensure a high purity since elevated temperatures of 130°C lead to impurities. The impurities are assumed to occur due to decomposition of the alkyl halides. Furthermore investigations regarding alternative basic catalysts for heterogeneous synthesis are yet to be performed since Potassium carbonate takes an active role in the synthesis and decomposes to carbon dioxide and water resulting in a degradation of the fixed bed over time. This might result in fluctuation in conversion rates and residence time.

ACKNOWLEDGMENTS

This study was partially funded by the Niedersächsisches Ministerium für Wissenschaft und Kultur (MWK) in the joint research project µ-Props of the Center of Pharmaceutical Engineering (PVZ) of the Technische Universität Braunschweig – “Processing of poorly soluble drugs at small scale”.

REFERENCENS

[1] Oehninger et al., 2013, A chemical-biological evaluation of rhodium(I) N-heterocyclic carbene complexes as prospective anticancer drugs, Chemistry 19, 52, 17871–17880.

[2] Rubbiani et al., 2014, Cytotoxic gold(I) N-heterocyclic carbene complexes with phosphane ligands as potent enzyme inhibitors, ChemMedChem 9, 6, 1205–1210.

PUBLICATIONS

-

POSTER PRESENTATIONS

1. Sauk, T., Zindler, T., Briem, T., Wölker, J., Scholl, S., Ott, I.: Batch processing of N-alkylated 1H-Benzimidazole derivatives: Synthesis, Down-Stream processing and continuous approach, 2nd Symposium on Pharmaceutical Engineering Research (SPhERe), PVZ TU Braunschweig, Braunschweig, 06.-08.09.2017

1.6 Continuous Synthesis, Isolation and Purification of poorly soluble Protein Kinase Inhibitors

Moritz C. Rehbein (ICTV), Prof. Dr.-Ing. Stephan Scholl (ICTV); Project 1F

INTRODUCTION

Various potential APIs are derived from the structural class of Paullones [1,2]. The synthesis route of substituted Paullones is displayed in Figure 1. After an amide coupling reaction of A and ethyl succinyl chloride (i), resulting molecule 1 undergoes a Dieckmann-ester-condensation (ii) to yield 2. 2 is subsequently dealkoxycarbonylated at elevated temperatures (iii) according to Krapcho [3] to form the scaffold 3 [4]. Coupling 3 with different substituted phenyl hydrazines via Fischer Indole Synthesis (iv) yields substituted Paullones.

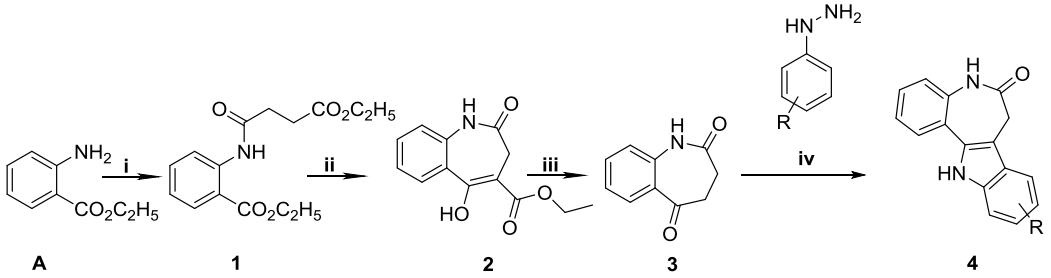


Figure 1: 5-step route for the synthesis of substituted Paullones

Paullones, but also their precursors are generally poorly water soluble which exhibits special challenges in synthesis and downstream processing. The presented project aims at the optimization and characterization of the synthesis process and subsequently on the development of a continuous flow synthesis. Special emphasis is given to the reaction kinetics and the selection of a suitable solvent.

RESULTS

After initial optimization of the synthesis route in batch mode towards reduced processing times, higher yield and purity, the establishment of continuous synthesis processes for reaction iii and iv has been targeted since these are the key steps for the formation of Paullones.

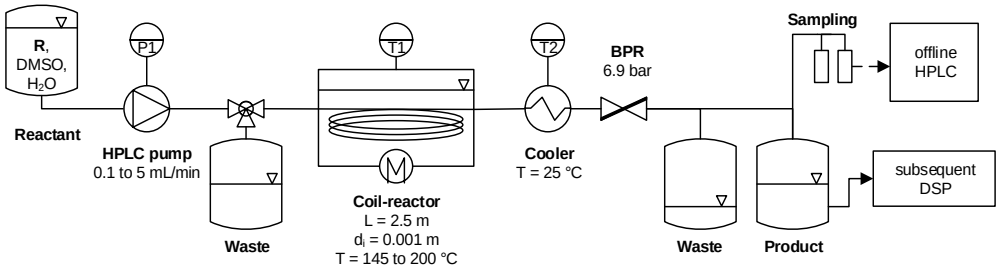


Figure 2: Reactor setup for the continuous high temperature synthesis of pharmaceutically relevant scaffold 3,4-dihydro-1H-1-benzazepine-2,5-dione (3)

Krapcho Dealkoxycarbonylation

Figure 2 shows a continuous process concept for the fast and effective dealkoxycarbonylation of reactant 2. The reactor design is based on batch kinetic data [5], and is able to operate under increased pressure and temperature compared to common batch lab equipment. The impact of process intensification using the continuous reactor at higher temperatures was predicted by extrapolation of the batch kinetics to higher temperatures. It could be proven by continuous synthesis experiments, that

reaction time can be significantly reduced from 3 h in batch at 150 °C to under 3 min at 200 °C in the continuous reactor.

In addition, different aprotic-polar solvents were rated towards their ability to serve as reaction medium. The rating was performed by simulation of the maximum reactant throughput possible. Simulations are based on solubility data for reactant 2 and the reaction rate achievable in the respective solvent. High boiling solvents DMSO, DMF and DMAc exhibit the best performance, which is an order of magnitude higher than that of low boiling aprotic-polar solvents, mainly due to the better solubility but also because of the higher reaction rate.

Fischer Indole (FI) Synthesis of Paullones

For the lab synthesis of Paullones via FI-synthesis, liquid acids like H_2SO_4 are utilized as catalysts. The usage of liquid acids shows drawbacks such as corrosiveness towards equipment, an additional neutralization step in the DSP and non-recyclability of the acid. In order to design a continuous process different solid or immobilized acids were investigated in batch experiments regarding their ability to catalyze the FI reaction. Amberlyst 15 H^+ and Silica-$AlCl_3$ were found to enable the formation of Paullone with high conversion rates and reaction times between 5 to 45 minutes in batch mode. A continuous reactor system was designed in which the solid catalyst acts as packing material in a continuous column reactor, enabling the flow synthesis of Paullones. Further investigation focus on the possibility for a serial, continuous synthesis process in which two flow reactions, Krapcho- and the FI-reaction, are coupled without intermediate isolation to provide different Paullones in a fast and efficient manner.

ACKNOWLEDGMENTS

This study was partially funded by the Niedersächsisches Ministerium für Wissenschaft und Kultur (MWK) in the joint research project µ-Props of the Center of Pharmaceutical Engineering (PVZ) of the Technische Universität Braunschweig – “Processing of poorly soluble drugs at small scale”.

REFERENCES

[1] N. Tolle and C. Kunick, “Paullones as Inhibitors of Protein Kinases”, Curr. Top. Med. Chem. (2011), 11, 1320-1332

[2] C. Schulz, A. Link, M. Loest, D. W. Zaharevitz, R. Gussio, E. A. Sausville, L. Meijer, C. Kunick, „Paullones, a series of cyclin-dependent kinase inhibitors: synthesis, evaluation of CDK1/cyclin B inhibition, and in vitro antitumor activity”, J. Med. Chem. (1999), 42, 2909-2919

[3] A. P. Krapcho, J. F. Weinmaster, J. M. Eldridge, E. G. E. Jahngen, A. J. Lovey, W. P. Stephens, “Synthetic applications and mechanism studies of the decarbalkoxylations of geminal diesters and related systems effected in dimethyl sulfoxide by water and/or by water with added salts”, J. Org. Chem. (1978) 43, 1, 138-147

[4] C. Kunick, „Synthese [b]-kondensierter azepindione durch Dealkoxycarbonylierung“, Arch. Pharm. Pharm. Med. Chem. (1991), 324, (9), 578-581

[5] M. C. Rehbein, S. Husmann, C. Lechner, C. Kunick, S. Scholl, “Fast and calibration free determination of first oder reaction kinetics in API synthesis using in-situ ATR-FTIR”, Eur. J. Pharm. Biopharm. 126 (2018) 95-100

PUBLICATIONS

1. M. C. Rehbein, S. Husmann, C. Lechner, C. Kunick, S. Scholl, “Fast and calibration free determination of first oder reaction kinetics in API synthesis using in-situ ATR-FTIR”, Eur. J. Pharm. Biopharm. 126 (2018) 95-100
2. R. Schenkendorf, X. Xie, M. C. Rehbein, S. Scholl, U. Krewer, “The Impract of global Sensitivities and Design Measures in Model-Based Optimal Experimental Design”, Processes (2018), 6, 27

ORAL PRESENTATIONS AND POSTER PRESENTATIONS

1. M. C. Rehbein, S. Husmann, J. Wolters, C. Lechner, C. Kunick, S. Scholl, Presentation: Batch-to-Conti Transfer of a Pharmaceutically Relevant Synthesis Utilizing in-situ ATR-FTIR. SPhERe “Symposium on Pharmaceutical Engineering Research”, 06.09 – 08.09.2017, Braunschweig, Germany
2. M. C. Rehbein¸ S. Husmann, J. Wolters, B. Ma, S. Kriese, C. Lechner, C. Kunick, S. Scholl. Poster: Continuous synthesis, isolation and purification of poorly soluble protein kinase inhibitors.
3. SPhERe “Symposium on Pharmaceutical Engineering Research”, 06.09 – 08.09.2017, Braunschweig, Germany

2 Cluster 2 – Biotechnological Production and Downstream Processing

2.1 Tailor-made production of labyrinthopeptin with Actinomadura namibiensis

Sebastian Tesche (ibvt), Prof. Rainer Krull (ibvt); Project 2A

INTRODUCTION

Due to their ability to produce a wide variety of bioactive substances, many filamentous microorganisms are of great interest to the industry. Several process parameters like mechanical stress by stirrer- and pneumatical-induced power input, dissolved oxygen, pH value, cultivation medium composition, osmolality, addition of (micro)particles and inoculum concentration influence the morphology and the productivity of these organisms [1]. Filamentous actinobacteria are of special interest for the development of new anti-infectives, because they do not only produce antibiotics but also a large number of other bioactive secondary metabolites. The strain Actinomadura namibiensis was chosen for cultivation studies with the objective to increase the productivity of labyrinthopeptin, a promising anti-infective secondary metabolite [2].

Figure 1: Culture of A. namibiensis on an agar plate.

RESULTS

The aim of the project is to increase the labyrinthopeptin productivity of A. namibiensis by influencing the morphology of the cells. Various cultivation strategies such as MPEC (micro particle enhanced cultivation) and SIEC (salt induced enhanced cultivation) are used to tailor the morphology. Automated image analysis and rheological measurements should be used to quantify the composition of the biomass during the cultivation process with different additives. To search for key parameters associated with high product concentration shaking flask experiments are performed. Subsequently, a scale-up to lab-scale bioreactors is carried out. In addition, a robust and scalable downstream processing will be developed to provide the target product labyrinthopeptin in larger quantities and high purity.

ACKNOWLEDGMENTS

The authors acknowledge financial support by the Ministerium für Wissenschaft und Kultur of Lower Saxony, Germany, for PhD scholarship in the joint research project Processing of poorly soluble drugs at small scale (µ-Props) within the Center of Pharmaceutical Engineering (PVZ) at the Technische Universität Braunschweig, Germany. M. Stadler, department Microbial Drugs and J. Wink, department Microbial Strain Collection at the Helmholtz Centre for Infection Research, Braunschweig, Germany, are thanked for their provision of the strain. The authors also thank L. Fillaudeau, Laboratoire d'Ingénierie

des Systèmes Biologiques et des Procédés (LISBP), Université de Toulouse, France, for giving the possibility to carry out image analysis in the laboratories of the GPE department.

REFERENCES

[1] Walisko R, Moench-Tegeder J, Blotenberg J, Wucherpfennig T, Krull R (2015). The taming of the shrew – controlling the morphology of filamentous eukaryotic and prokaryotic microorganisms. Adv. Biochem. Eng. / Biotechnol. 149, 1–27.

[2] Férir G, Petrova M I, Andrei G, Huskens D, Hoorelbeke B, Snoeck R, Süssmuth R D (2013). The lantibiotic peptide labyrinthopeptin A1 demonstrates broad anti-HIV and anti-HSV activity with potential for microbicidal applications. PLoS One, 8(5), e64010.

PUBLICATIONS

ORAL PRESENTATIONS

1. Tesche S, Rösemeier-Scheumann R, Krull R (2017). Improved production of labyrinthopeptin with Actinomadura namibiensis by salt-controlled modification of pellet morphology. 2nd Braunschweig International Symposium on Pharmaceutical Engineering Research – SphERe, TU Braunschweig, Germany
2. Tesche S, Rösemeier-Scheumann R, Krull R (2017) Strategies for increased production of labyrinthopeptin with the filamentous strain Actinomadura namibiensis. Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés (LISBP) INRA/INSA, Toulouse, France

POSTER PRESENTATIONS

1. Tesche S, Rösemeier-Scheumann R, Krull R (2017) Improved production of labyrinthopeptin by salt-controlled morphology of Actinomadura namibiensis. 2nd Braunschweig International Symposium on Pharmaceutical Engineering Research – SphERe, Proc. P.203, TU Braunschweig, Germany.
2. Tesche S, Krull R (2016) Tailored production of anti-infectives by Actinomyces. Braunschweig BRICS Opening Symposium, Poster No. 24, BRICS Building, Braunschweig, Germany.
3. Tesche S, Krull R (2016). Tailored production of anti-infectives by Actinomyces. DECHEMA Himmelfahrtstagung, New Frontiers for Biotech-Processes, Proc. P 3568, Koblenz, Germany.
4. Tesche S, Krull R (2015). Production of labyrinthopeptin with Actinomadura namibiensis. 1st Braunschweig International Symposium on Pharmaceutical Engineering Research – SphERe, TU Braunschweig , Germany.

2.2 Biochemical investigation of hyperforin-related prenyltransferases in Saccharomyces cerevisiae

Marco Grull (IPB), Prof. Ludger Beerhues (IPB); Project 2B

INTRODUCTION

Hypericum perforatum (Fig. 1), also known as St. John´s wort, is commonly used for the treatment of patients suffering from mild to moderate depression. It contains a number of pharmaceutically interesting constituents, with hyperforin (Fig. 2) identified as the major compound. Hyperforin is a hydrophobic as well as light, oxygen and temperature sensitive acylphloroglucinol derivative with several side chains derived from dimethylallylpyrophosphate (DMAPP) and geranylpyrophosphate (GPP) as MEP pathway products [1]. The biosynthetic pathway is not yet completely understood. As proposed, the biosynthesis of hyperforin starts with the formation of the core skeleton, PIBP (phlorisobutyrophenone), catalyzed by a type III polyketide synthase (2). Subsequent decoration of PIBP with prenyl and geranyl side chains is catalyzed by aromatic membrane-bound prenyltransferase (PT) enzymes (3, 4) [2]. The aim of this project was to characterize the enzymes and to transfer their coding sequences to S. cerevisiae.

Figure 1: Flowers of Hypericum perforatum

RESULTS

The first step is the biosynthesis of the hyperforin core skeleton PIBP, which is absent from yeast. Therefore, cDNAs encoding IBCL (1) and IBS (2) were cloned from H. perforatum, heterologously expressed in E. coli and functionally characterized. In S. cerevisiae harboring either episomal or genome-integrated HpIBCL and HpIBS, feeding isobutyric acid resulted in intracellular and extracellular detection of PIBP. Similarly, a PT named HpPT2 (3) was identified to prefer PIBP and GPP as major substrates. A second PT named HpPT6 (4) catalyzed the addition of a prenyl group from DMAPP to the HpPT2 product.

Both PT enzymes were further characterized regarding their preferred in vitro assay conditions, substrate specificities and kinetic parameters. For HpPT2, GPP as well as PIBP and 2-MBP were the preferred substrates among the acylphloroglucinol derivatives tested, using Tris buffer pH 7 - 8, $MgCl_2$ as divalent cation and 40 °C as incubation temperature for 10 min. The kinetic parameters were determined for PIBP and 2-MBP as well as GPP. HpPT6 mainly used 3-geranyl-X derivatives (X = PIVP, 2-MBP and PIBP) and DMAPP as substrates. The presence of $CaCl_2$ in Tris buffer pH 8 - 9 and 50 °C for 10 min were identified to be the preferred assay conditions and were used to determine the kinetic parameters for the 3-geranyl-X derivatives and DMAPP.

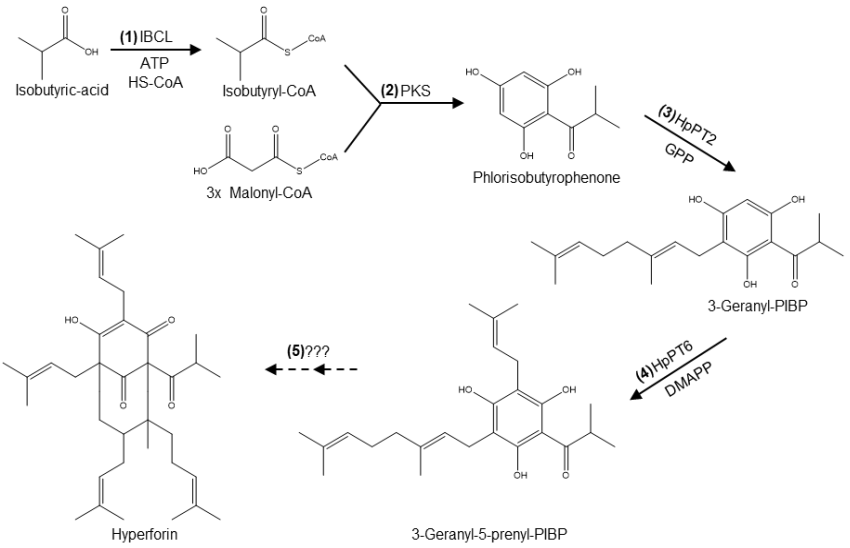


Figure 2: Enzyme cascade functionally expressed in S. cerevisiae and tested under in vivo and/or in vitro conditions

Our findings show that H. perforatum derived cDNAs encoding hyperforin biosynthetic enzymes can be functionally expressed in S. cerevisiae. A cascade consisting of four consecutive enzymes was identified and functionally expressed in yeast, enabling the generation of a hyperforin precursor molecule, i.e. 3-geranyl-5-prenyl-PIBP. This system may be a chassis for further research to complete (5) the hyperforin biosynthesis pathway in yeast, with a focus on the membrane-bound prenyltransferases.

ACKNOWLEDGMENTS

This work was supported by grants of the µ-Props PhD project and the joint research project SynFoBiA at the Center of Pharmaceutical Engineering (PVZ). Credit is also due to M. Gaid, B. Liu, T. Fiesel, T. Beuerle (IPB) and L. Ernst (Central NMR Laboratory).

REFERENCES

[1] U. Wölfle, G. Seelinger, and C. M. Schempp, “Topical Application of St . John ’ s Wort (Hypericum perforatum),” Planta Med, vol. 80, pp. 109–120, 2014.

[2] L. Beerhues, “Hyperforin,” Phytochemistry, vol. 67, pp. 2201–2207, 2006.

PUBLICATIONS

POSTER PRESENTATIONS

1. Grull M., Liu B., Gaid M., Fiesel T., Beuerle T., Ernst L., Beerhues L. Metabolic engineering of S. cerevisiae for the recombinant production of hyperforin precursor molecules. Konferenz: SPhERe “Symposium on Pharmaceutical Engineering Research”, 06.09 – 08.09.2017, Braunschweig, Deutschland.
2. Grull M., Liu B., Gaid M., Fiesel T., Beuerle T., Ernst L., Beerhues L. Metabolic engineering of S. cerevisiae for the recombinant production of hyperforin precursor molecules. Konferenz: „ProcessNet-Jahrestagung und 32. DECHEMA-Jahrestagung der Biotechnologen 2016. 12.09 – 15.09.2016, Aachen, Deutschland.

3. Grull M., Fiesel T., Beuerle T., Gaid M., Beerhues L. Expression of hyperforin-related prenyltransferases in yeast. Konferenz: SPhERe “Symposium on Pharmaceutical Engineering Research”, 19.10 – 20.10.2015, Braunschweig, Deutschland.
4. Grull M., Patz S., Fiesel T., Gaid M., Beerhues L. Expression of plant-derived prenyltransferases in yeast for the production of hyperforin and related compounds.
5. DECHEMA Summer school, “Quantitative Biology: Current concepts and tools for strain development”, 20.07 – 24.07.2015, Berlin, Deutschland.

2.3 Production of benzylglucosinolate in carrot callus suspension cultures

Elena Kurzbach (IPB), Prof. Dr. Ute Wittstock (IPB); Project 2C

INTRODUCTION

Glucosinolates are sulfur- and nitrogen-containing secondary metabolites found mainly in the plant order Brassicales. They contribute to the taste and flavour of common crucifer vegetables for example broccoli, cabbage and horseradish. Upon tissue disruption, glucosinolates degrade to isothiocyanates which serve as defence compounds against herbivores. In contrast to insects, humans benefit from the ingested glucosinolates due to the antimicrobial, antiviral, immuno-stimulative and anticarcinogenic effects of the isothiocyanates. There is a strong interest in obtaining individual glucosinolates and isothiocyanates for further pharmacological characterization. The structural diversity of the glucosinolates complicates the pure extraction from cruciferous plant material. Production of individual glucosinolates in a heterologous host represents an interesting alternative. Earlier studies proved the feasibility of engineering microbial hosts and plants for aliphatic, indolic and benzylic glucosinolate production [1,2,3,4]. In this study, we aimed at testing the suitability of transgenic callus suspension cultures of carrot (Daucus carota, Apiacae) as a scalable production platform for plant natural products using the simple glucosinolate benzylglucosinolate as a model compound. Its seven-step biosynthesis from phenylalanine requires transfer of six genes.

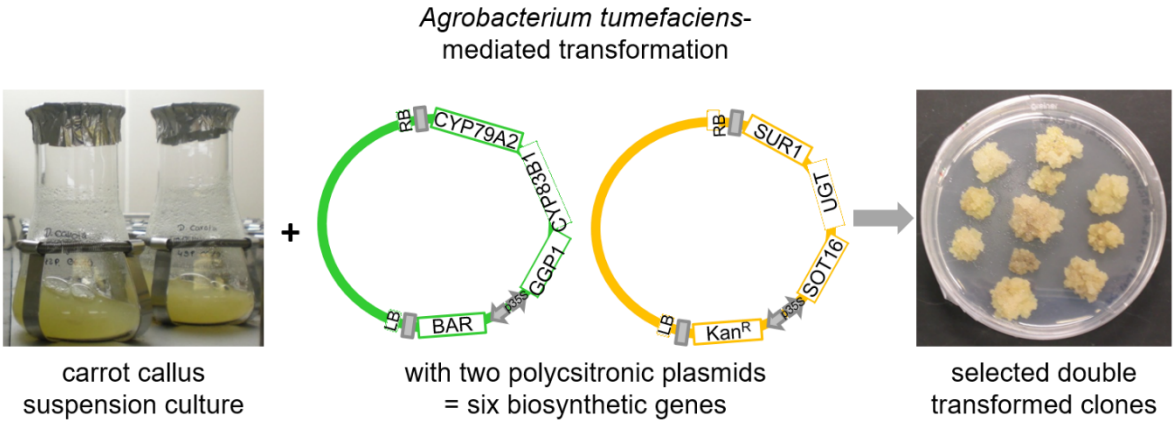


Figure 1: Multigene engineering in carrot, D. carota, to produce benzylglucosinolate naturally occurring in Brassicales. Carrot suspension cultures were transformed with two polycistronic constructs [1, 2] for expression of a total of six genes from Arabidopsis thaliana (thale cress) required for biosynthesis of benzylglucosinolate in a heterologous host.

RESULTS

The genes were transferred into carrot suspension cultures via Agrobacterium-mediated transformation with two polycistronic constructs [4,5] and positive clones selected based on resistance markers were further characterised (Fig. 1). Transformations yielded 255 putative transgenic clones. Viable clones were selected, and 79 clones were screened for benzylglucosinolate by HPLC-MS. The analysis identified sixteen clones as benzylglucosinolate producers. This provided proof-of-concept for generation of a benzylic glucosinolate in a non-cruciferous, scalable plant system. The five clones with highest benzylglucosinolate production were chosen for further characterisation and yield optimisation. This showed that individual clones differed largely with respect to growth, yield, accumulation of desulfoglucosinolate as an intermediate, and stimulation of production by sulfate supplementation.

ACKNOWLEDGMENTS

This work was conducted as part of the graduate program “Processing of poorly soluble drugs at small scale (µ-Props)” at the Centre of Pharmaceutical Engineering (PVZ). The scientific contributions by N. Elgahme, M. Strieker, E. J. Stauber and T. Beuerle are gratefully acknowledged.

REFERENCES

[1] Mikkelsen, M.D. et al. (2010) Molecular Plant, 3:751–759
[2] Pfalz, M. et al. (2011) The Plant Cell, 23:716–729
[3] Mikkelsen, M.D. et al. (2012) Metab Eng, 14:104–111
[4] Møldrup, M.E. et al. (2012) Plant Biotech. J., 10:435–442
[5] Geu-Flores, F. et al. (2009) Planta, 229:261–270

PUBLICATIONS

1. Wittstock, U., Kurzbach, E., Herfurth, A.-M., Stauber, E. J., 2016, Glucosinolate Breakdown. In: Advances in Botanical Research, Volume 80: Glucosinolates (Kopriva, S., ed.). Amsterdam, (Boston, Heidelberg, London, New York, Oxford, Paris, San Diego, San Francisco, Singapore, Sydney, Tokyo): Elsevier Academic Press, pp. 125–169

POSTER PRESENTATIONS

1. Kurzbach, E., Elgahme, N., Wittstock, U., Benzylglucosinolate production in carrot cell suspension cultures, Meeting of the section “Natural Products”, German Society for Plant Sciences (DBG), Meisdorf, 11.–13.09.2016.
2. Kurzbach, E., Elgahme, N., Strieker, M., Stauber, E. J., Wittstock, U., Sustainable production of the antiinfective benzylisothiocyanate from biosynthetic benzylglucosinolate, International Symposium on Pharmaceutical Engineering Research SPhERe, Braunschweig, 06.-08.09.2017.
3. Kurzbach, E., Elgahme, N., Strieker, M., Stauber, E. J., Wittstock, U., Sustainable production of the antiinfective benzylisothiocyanate from biosynthetic benzylglucosinolate, Annual Meeting of the German Society of Plant Biology (Botanikertagung), Kiel, 18.-21.09.2017.
4. Kurzbach, E., Strieker, M., Elgahme, N., Wittstock, U., Benzylglucosinolate production in transgenic callus suspension cultures of carrot, Meeting of the section “Natural Products”, German Society for Plant Sciences (DBG), Burg Warberg, 1.–3.10.2018

2.4 Discovery and development of drugs from fungal and bacterial sources

Zeljka Rupcic (HZI), Prof. Dr. Marc Stadler (HZI); Project 2D

INTRODUCTION

Confronted by the increasing number of drug candidates and the growing number of molecular targets relevant in human therapy, the Pharma industry needs to deliberate screening concepts and well-established strategies in an effort to maximize the efficiency in natural product based drug discovery. The screening process itself is often complex and requires the bioassay-guided isolation of active compounds, their structure elucidation and biological characterization.

A careful planning and development of methods and technologies that are essential to accelerate exploratory projects and preclinical development of drug candidates based on natural products is in the scope of the work presented herein, targeting the core objectives in discovery and development of new anti-infectives. These objectives includes, for instance, selection of potentially new compounds with novel bioactivities, structure-activity relationship around the core structure, optimization of downstream processing at large scale and formulation development. The ultimate aim was to evaluate parameters for different natural product classes from the different biogenetic origins and organism groups, in order to develop a general workflow.

RESULTS

The aims of the project can be divided into three parts, following the workflow in the natural product based drug discovery. The first part deals with an early stage compound discovery (I), the second is oriented towards determination of structure-activity relationships and hit-to-lead evaluation of selected compounds (II) while the third part covers the area of an early preclinical development (III).

In the scope of this work, both, fungal and bacterial secondary metabolites were evaluated, a downstream process for their purification was developed, and the strategy for the isolation of their secondary metabolites in a high purity was established.

(I) Six new compounds, thailanones A-F (1-6) and two known compounds, monocerin and deoxyphomalone (7-8) (Fig. 1) were obtained from cultures of Pseudobambusicola thailandica, a new species that represents a new genus, isolated from plant material in Thailand. The strain was chosen for further evaluation based on its nematicidal activity in the water agar plate assay. Secondary metabolites obtained from its culture were characterized as weak antihelminthics, and weak antifungal agents against Mucor plumbeus and Phellinus tremulae.

(II) Furthermore, two new erinacine derivatives, erinacine Z1 and Z2 (9-10), along with six previously identified cyathane diterpenoid derivatives (11-16), were isolated from Hericium sp. and studied for neurotrophic activity (Fig. 2). Additionally, three new corallocins A-C (17-19), together with hericerin (20) and [5-(2E)-3′,7′-dimethyl-2′,6′-octadienyl]-4-hydroxy-6-methoxy-1-isoindoline (21) were isolated from the fruiting bodies of H. coralloides (Fig. 3).

(III) The production of labyrinthopeptins in a stirring tank was established. As well, an effective method for the 1.2 and 4.2-fold increased amounts of the labyrinthopeptins A1 and A2 (structures not shown), respectively, obtained from the culture of the actinobacterium Actinomadura namibiensis, was developed.

Thailanone A (1) and thailanone D (4) showed moderate activity against *B. subtillis* and *M. plumbeus*, respectively. On the other hand, deoxyphomalone (8), known derivative structurally similar to thailanone A (1), was found to be active against *B. subtilis* and *M. plumbeus*.

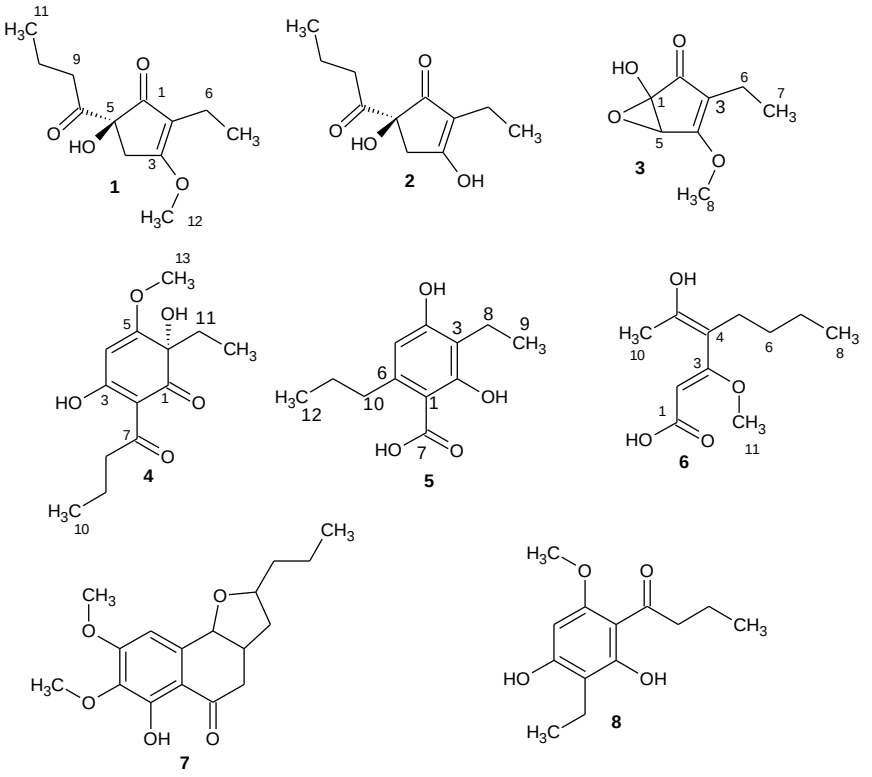


Figure 1: Chemical structures of thailanones A–F (1–6), monocerin (7) and deoxyphomalone (8)

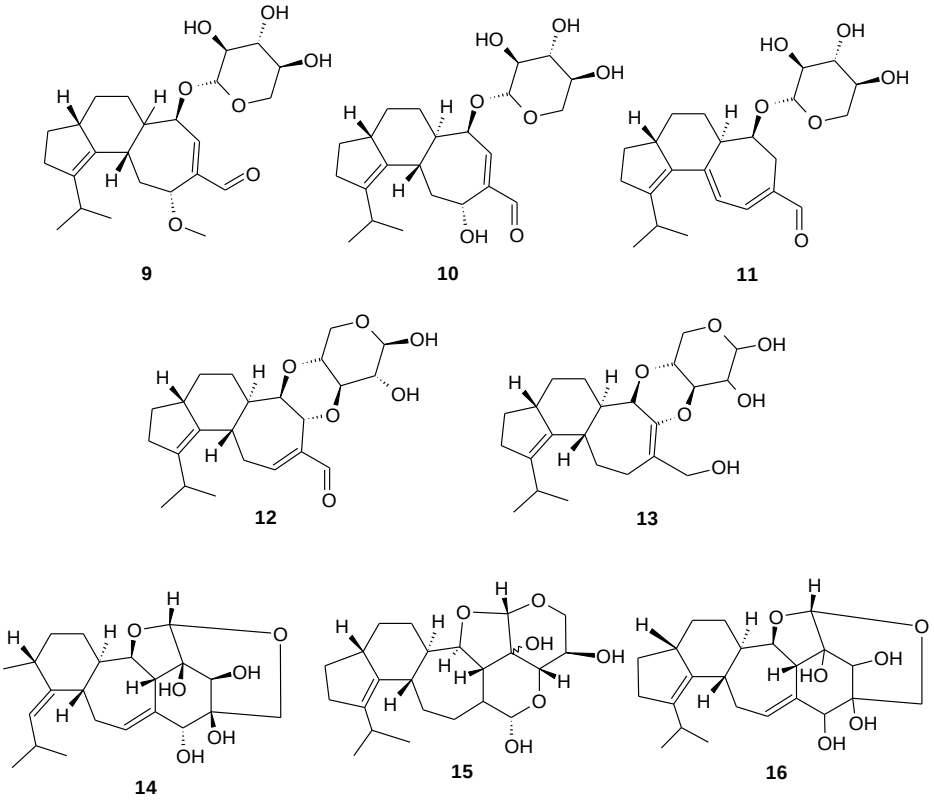


Figure 2: Chemical structures of erinacines Z1 (9) and Z2 (10), erinacine A, B, C, E, CJ 14.258 and erinacine F (11-16)

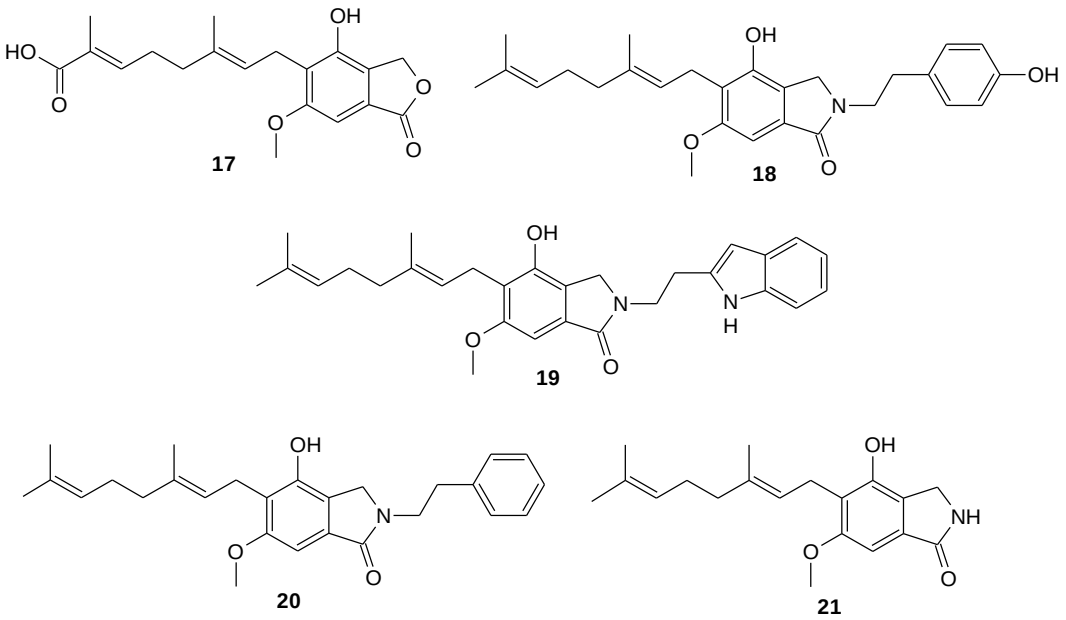


Figure 3: Chemical structures of corallocins A-C (17-19), hericerin (20) and [5-(2E)-3′,7′-dimethyl-2′,6′-octadienyl]-4-hydroxy-6-methoxy-1-isoindoline (21)

Since thailanones did not show strong nematicidal properties, the fungal strain itself can play an important chemo-ecological role in its natural habitat, e.g. protect against nematode predation. The restrictions of the current nematicides, because of their environmental, toxicological, or even sociological concerns, have prompted so called integrated pest management (IPM) development, where the soil-born plant pathogens are controlled non-chemically, by antagonistic microorganism. However, the alternative would be to test isolated compounds against other species of nematodes, for instance plant-parasitic nematode *Meloidogyne incognita*.

Regarding secondary metabolites from *Hericium* spp., they were tested for their neurotrophin inducing effects. For this purpose, immortalized PC12 cells derived from rat adrenal medullary tumor (pheochromocytoma) cells were used. Since Zhang et al. [1] reported that PC12 cells were not able to produce NGF themselves, human 1321N1 astrocytoma cells have to be used in addition.
Erinacines A (11), B (12), E (14), CJ14.258 (15) and Z1 (9) were found to act on 1321N1 cells by increasing the transcription of NGF, while erinacine C (13) increased transcription of both neurothophins as it was the case with corallocin C. However, erinacine Z2 (10) and erinacine E (14) did not show any significant activity. Corallocin A (17) and B (18) act on 1321N1 cells differently by increasing transcription of either NGF or BDNF, respectively. The mechanism on how neurotrophins, like NGF, are acting is still not quite clear although there are several publications indicating the possible patterns [1].
Since erinacine C, as well as other cyathane diterpenoids, are non-water soluble, choosing the suitable solvation system was of critical importance. To accomplish the latter, subsequent formulation experiments were conducted at the Institute of Pharmaceutical Technology (TU Braunschweig). Due to the low amount of the targeted substance, erinacine B was chosen as a model compound for preliminary experiments since it was a major accessible compound.
Since of their interesting, dual activity, labyrinthopetins have high probability to become a lead compounds in the drug development. Labyrinthopeptin A2 was found to be active *in vivo* in a spared nerve injury mouse model of neuropathic pain [2], while labyrinthopeptin A1 exhibited a broad antiviral activity.

Yields for both peptides were successfully increased, a 1.2-fold and 4.2-fold for A1 and A2, respectively. Titers of ca. 106 mg/L and 165 mg/L of 22 and 23, respectively, were obtained at 672 hours of the fermentation. However, a concurrent extensive process optimization is being conducted. The optimization can roughly be divided in the optimization of fermentation conditions (e.g. the production medium, a seed culture, and a time of the fermentation), downstream processing (bigger columns for the purification), or process parameters optimization, for instance design of the bioreactor and the stirrer, and the alteration of the stirring speed.

REFERENCES

[1] Z.J. Zhang, L.C.V. Cheang, M.W. Wang, G.H. Li, I.K. Chu, Z.X. Lin, S.M.Y. Lee “Ethanolic extract of fructus *Alpinia oxyphylla* protects against 6-hydroxydopamine-induced damage of PC12 cells in vitro and dopaminergic neurons in zebrafish”, Cellular and Molecular Neurobiology vol. 32(1), pp. 27-40, 2012.

[2] K. Meindl, T. Schmiederer , K. Schneider, A. Reicke, D. Butz, S. Keller, H. Güring, L. Vértesy, J. Wink, H. Hoffmann, M. Brönstrup, G-M. Sheldrick, R.D. Süssmuth RD “Labyrinthopeptins: a new class of carbacyclic lantibiotics”, Angewandete Chemie, vol 49, pp. 1151-1154, 2010.

PUBLICATIONS

1. Rupcic Z, Chepkirui C, Hernández-Restrepo M, Crous PW, Luangsa-ard JJ, Stadler M (2018) New nematicidal and antimicrobial secondary metabolites from a new species in the new genus, *Pseudobambusicola thailandica*. MycoKeys 33:1-23.
2. Rupcic Z, Rascher M, Kanaki S, Köster RW, Stadler M, Wittstein K (2018) Two new cyathane diterpenoids from mycelial cultures of the medicinal mushroom *Hericium erinaceus* and the rare species, *Hericium flagellum*. International Journal of Molecular Sciences, 19(3):piiE740.
3. Rupcic Z, Hüttel S, Kanaki S, Bernecker S, Stadler M (2018) Large scale production and downstream processing of labyrinthopeptins from the actinobacterium *Actinomadura namibiensis*. Bioengineering 5(2):42.
4. Wittstein K, Rascher M, Rupcic Z, Löwen E, Winter B, Köster RW, Stadler M (2016) Corallocins A–C, nerve growth and brain-derived neurotrophic factor inducing metabolites from the mushroom *Hericium coralloides*. Journal of Natural Products 79:2264-2269.
5. Schrey H, Müller FJ, Harz P, Rupcic Z, Stadler M, Spiteller P (2019) Nematicidal anthranilic acid derivatives from *Laccaria* species. Phytochemistry 160:85-91.

ORAL PRESENTATIONS

1. Rupcic Z, Wittstein K, Rascher M, Kanaki S, Köster RW, Bunjes H, Stadler M. Neuroprotective metabolites from Hericium. 11th International Mycological Congress. San Juan, Puerto Rico (2018).
2. Hernández-Restrepo M, Rupcic Z, Chrous P, Luangsa-Ard JJ, Stadler Marc. Bio-prospection of microfungi from Thailand. 11th International Mycological Congress. San Juan, Puerto Rico (2018).
3. Rupcic Z, Wittstein K, Rascher R, Köster RW, Stadler M. Nerve growth- and brain-derived neurotrophic factor inducing metabolites from *Hericium* spp. International Conference of the DGfM. Bernried, Germany (2016).
4. Rupcic Z, Wittstein K, Stadler M. Processing pf poorly soluble drugs at small scale- Production and processing of anti-infectives from microbial sources. Fungal Biodiversity and Biotechnology symposium. Mae Fah Luang University, Chiang Rai, Thailand (2016).

5. Wittstein K, Rupcic Z, Rascher M, Löwen E, Winter B, Köster RW, Stadler M. Novel antimicrobial and neurotrophic compounds from basidiomycetes. Fungal Biodiversity and Biotechnology symposium. Mae Fah Luang University, Chiang Rai, Thailand (2016).
6. Wittstein K, Rupcic Z, Rascher M, Löwen E, Winter B, Köster RW, Stadler M. Novel antimicrobial and neurotrophic compounds from basidiomycetes. CBS Spring Symposium "Fungi and Global Challenges". Amsterdam, Netherlands (2016).

POSTER PRESENTATIONS

1. Rupcic Z, Hüttel S, Bernecker S, Kanaki S, Stadler M. Optimization of the production and downstream processing of labyrinthopeptins from the actinobacterium *Actinomadura namibiensis*. 3rd European Conference of Natural Products. Frankfurt, Germany (2018).
2. Rupcic Z, Wittstein K, Rascher M, Kanaki S, Köster RW, Bunjes H, Stadler M. Neuroprotective metabolites from Hericium. 11th International Mycological Congress. San Juan, Puerto Rico (2018).
3. 3. Wittstein K, Rascher M, Rupcic Z, Mitschke N, Löwen E, Kellner H, Winter B, Köster RW, Stadler M. Neurotrophin inducing terpenoids from basidiomycetes of the family Hericiaceae. 30th Irseer Naturstofftage, Irsee, Germany (2018).
4. Rupcic Z, Wittstein K, Rascher M, Köster RW, Bunjes H, Stadler M. Production and processing of anti-infectives from microbial sources. SPhERe-Symposium on Pharmaceutical Engineering Research. Braunschweig, Germany (2017).
5. Rupcic Z, Wittstein K, Bunjes H, Stadler M. Production and processing of anti-infectives from microbial sources. 2nd PVZ Research Workshop. St. Andreasberg, Germany (2017).
6. Rupcic Z, Wittstein K, Rascher M, Löwen E, Winter B, Köster RW, Stadler M. Neuroprotective and antimicrobial metabolites from Hericium species. RGJ-Ph.D. Congress. Pattaya, Thailand (2016).
7. Wittstein K, Rascher M, Mudalungu CM, Helaly S, Richter C, Rupcic Z, Löwen E, Winter B, Thongbai B, Hyde KD, Matasyoh JC, Süssmuth R, Köster RW, Stadler M. New bioactive metabolites from cultures and fruiting bodies of basidiomycetes. PERCH-CIC Congress IX: 2016 "Chemistry in ASEAN Economic Community and Beyond", Pattaya, Thailand (2016).
8. Wittstein K, Rascher M, Mudalungu CM, Helaly S, Richter C, Rupcic Z, Löwen E, Winter B, Süssmuth R, Köster RW, Stadler M. Novel Antimicrobial and Neurotrophin-inducing Compounds from Basidiomycetes. Conference "Drug Innovation in Academia", DKFZ, Heidelberg (2016).
9. Helaly SE, Surup F, Wittstein K, Richter C, Föster B, Rupcic Z, Mudalungu CM, Matasyoh JC, Thongbai B, Chepkirui C, Decock C, Süssmuth RD, Hyde KD, Stadler M. Exploiting the secondary metabolome of tropical Basidiomycota. 28th Irseer Naturstofftage. Kloster Irsee, Germany (2016).

3 Cluster 3 – Micro-Precipitation for the preparation of nanoparticulate drug delivery systems

3.1 Processing of nanoparticles out of poorly water-soluble drugs by precipitation and drying using microsystems

Sebastian Melzig (iPAT), Prof. Dr.-Ing. Arno Kwade (iPAT); Project 3A

INTRODUCTION

It is estimated that nearly 90 % of newly developed active pharmaceutical ingredients (API) are poorly water-soluble and tend to show low bioavailability. Because of that, it is necessary to establish suitable galenic methods which increase bioavailability of these APIs. The production of API nanoparticles is a common method to enhance bioavailability of such APIs. Dissolution behavior of these APIs can be improved, as a direct consequence of increasing specific particle surface area. There are two general approaches to prepare API nanoparticles: top-down (e.g. milling) and bottom-up (e.g. precipitation). In particular, precipitation is of special interest for pharmaceutical industries because of the possibility to produce amorphous nanoparticles and the potential for continuous processing via microsystems. Furthermore, precipitation processes allow the avoidance of wear caused by milling media. API nanoparticles produced via precipitation commonly need to be transferred into dry powders to ensure long-term stability and to produce drug products with defined and appropriate properties for patients' needs.

Within this context, this project focused on investigating precipitation processes of API nanoparticles using a high-pressure microsystem. Furthermore, drying of API nanosuspensions was likewise under study with the aim of obtaining defined pharmaceutical powders with enhanced bioavailability through variation of matrix materials. The ultimate goal was to be able to process these drug-loaded powders in order to obtain pharmaceutical tablets and capsules (Fig. 1).

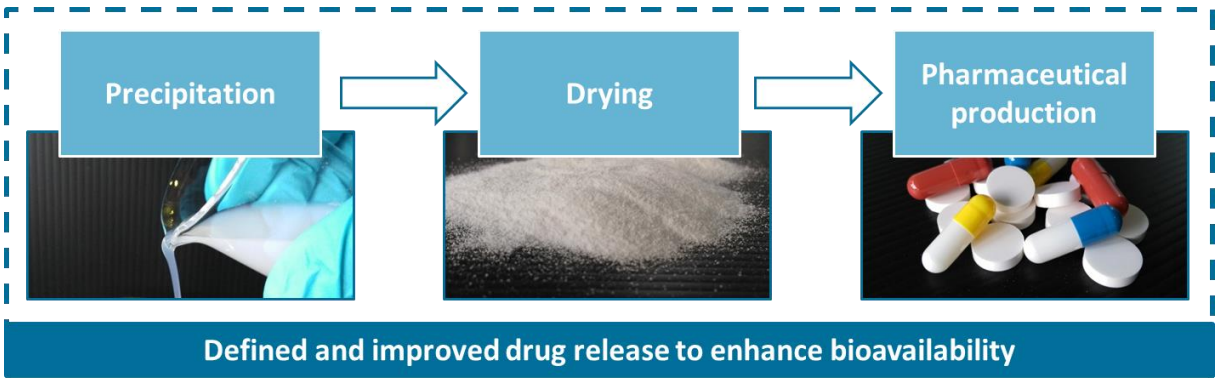


Figure 1: Process chain and the aim of project 3A

RESULTS

At the beginning of the project, a precipitation process was established using a high-pressure microsystem in order to synthesize API nanoparticles. In this context, fenofibrate and ibuprofen served as model drugs which both exhibit poor solubility. Fenofibrate nanoparticles were produced via classic antisolvent precipitation whereas a new precipitation method called "antisolvent melt precipitation" (AMP) was developed to synthesize ibuprofen nanoparticles. It was possible to manipulate the product properties (e.g. size, polydispersity and stability) by variation of process parameters (e.g. pressures and

temperature) and formulation parameters (e.g. drug content and stabilizer to drug ratio). This enabled the entire control of the synthesis in order to produce API nanoparticles with defined mean sizes in the range from 80 nm to 750 nm. The next process step was the transfer of the suspensions into a defined pharmaceutical powder via spray drying. Different matrix materials were used to produce granules with various release kinetics. On the one hand, soluble matrix materials (e.g. sugars and polymers) and, on the other hand, particulate matrix materials (e.g. silica nanoparticles) were chosen to design these granules. Additionally, a micro spray dryer was developed in close cooperation with the Institute of Microtechnology (IMT), TU Braunschweig, in order to engineer a selective device for the early stage development of specific pharmaceutical granules. Flow rates lower than 50 µl/min enables the reproducible production of granules and, by that, the screening of diverse formulations with a very low consumption of starting materials. The last process step transfers these previously manufactured granules, containing API nanoparticles, into solid dosage forms (tablets and capsules). The influence of the different granules and various pharmaceutical excipients on product properties (e.g. dissolution rate and mechanical properties) was investigated in order to design pharmaceutics with defined and improved drug release. To sum up, the established process chain, from the synthesis of API nanoparticles to the production of solid dosage forms, was successfully applied and facilitates highly defined control of product properties by process and formulation design.

ACKNOWLEDGMENTS

We want to thank Peter Pfeiffer, Institute of Material Science (IfW), TU Braunschweig, for taking SEM pictures and Manuela Handt, Institute of Pharmaceutical Technology (IPhT), TU Braunschweig, for carrying out DSC measurements. Furthermore, we are grateful about all students who have worked as a research assistant or have written their bachelor/master thesis in our project.

PUBLICATIONS

1. Melzig, S.; Niedbalka, D.; Schilde, C.; Kwade, A.: Spray drying of amorphous ibuprofen nanoparticles for the production of granules with enhanced drug release. Colloids and Surfaces A, vol. 536, p. 133-141, 2018.
2. Melzig, S.; Finke, J. H.; Schilde, C.; Kwade, A.: Formation of long-term stable amorphous ibuprofen nanoparticles via antisolvent melt precipitation (AMP). European Journal of Pharmaceutics and Biopharmaceutics, vol. 131, p. 224-231, 2018.
3. Further publications are in process

ORAL PRESENTATIONS

1. Melzig, S.; Schilde, C.; Finke, J. H.; Kwade, A.: Herstellung definierter pharmazeutischer Granulate aus gefällten Wirkstoffnanopartikeln. 8. SYMPOSIUM: Produktgestaltung in der Partikeltechnologie, 2017, Karlsruhe, Germany.
2. Melzig, S.; Schilde, C.; Finke, J. H.; Kwade, A.: Tailoring the release kinetics of nanosized API formulations by process chain design. Symposium on Pharmaceutical Engineering Research, 2017, Braunschweig, Germany.
3. Melzig, S.; Schilde, C.; Kwade, A.: Formulation strategies for the production of pharmaceutical powders out of precipitated API nanoparticles. Formula VIII, 2016, Barcelona, Spain.

POSTER PRESENTATIONS

1. Melzig, S.; Finke, J. H.; Schilde, C.; Kwade, A.: Synthesis of stable amorphous ibuprofen nanoparticles. World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, 2018, Granada, Spain.
2. Melzig, S.; Jürgens, F.; Lorenz, T.; Schilde, C.; Finke, J. H.; Dietzel, A.; Kwade, A.: Micro spray dryer as a selective device for the early stage development of defined pharmaceutical granules. PARMAT, 2017, Barcelona, Spain.
3. Melzig, S.; Möllhoff, J.; Schilde, C.; Finke, J. H.; Kwade, A.: Formulation strategies for the production of dry powders containing nanoparticles of poorly soluble APIs. European Conference on Pharmaceutics, 2017, Krakow, Poland.
4. Melzig, S.; Schilde, C.; Kwade, A.: Precipitation of poorly water-soluble drugs using microsystems and further drying while maintaining nanoparticular state of drugs. World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, 2016, Glasgow, Scotland.
5. Melzig, S.; Finke, J. H.; Schilde, C.; Kwade, A.: Precipitation of poorly water-soluble drugs using microsystems. Symposium on Pharmaceutical Engineering Research, 2015, Braunschweig, Germany.

3.2 Formulation and preparation of colloidal carrier systems for poorly soluble drugs by precipitation

Juliane Riewe (IPhT), Prof. Heike Bunjes (IPhT); Project 3B

INTRODUCTION

Many newly developed drugs lack sufficient water solubility. One option to formulate poorly soluble drugs is loading the drug into colloidal carriers like mixed micelles, liposomes or lipid nanoparticles. Although commonly produced by high pressure homogenization, precipitation out of water-miscible solvents is a further method to prepare solid lipid nanoparticles [1]. This technique has also been used to prepare lipid nanoemulsions, liposomes [2] and vesicles [3]. Precipitation occurs when a water-miscible organic solution of the lipid is combined with a surfactant-containing aqueous phase leading to supersaturation of the lipid and formation of stabilized nanoparticles. Formation of small particles requires a fast mixing of aqueous and organic solution. To ensure fast mixing, precipitation can be conducted in microsystems at more precise conditions. The aim of this project was to prepare small lipid nanoparticles with narrow particle size distributions. Production in a conventional batch set-up should be compared with micromixers of different designs with respect to resulting particle sizes and reproducibility.

RESULTS

A stirred volume of the aqueous solution in a beaker and a syringe pump controlling the volume flow of the lipid solution were parts of the batch set-up. Different segmented gas-liquid-flow microsystems were tested in cooperation with project 3C (Peer Erfle, Institut für Mikrotechnik). Besides, a high-pressure microsystem developed in the mikroPART project was used in cooperation with project 3A (Sebastian Melzig, Institut für Partikeltechnologie). The commercially available NanoAssemblr platform with herringbone structures inside the microfluidic cartridges represents a further microfluidic mixing principle (Fig. 1).

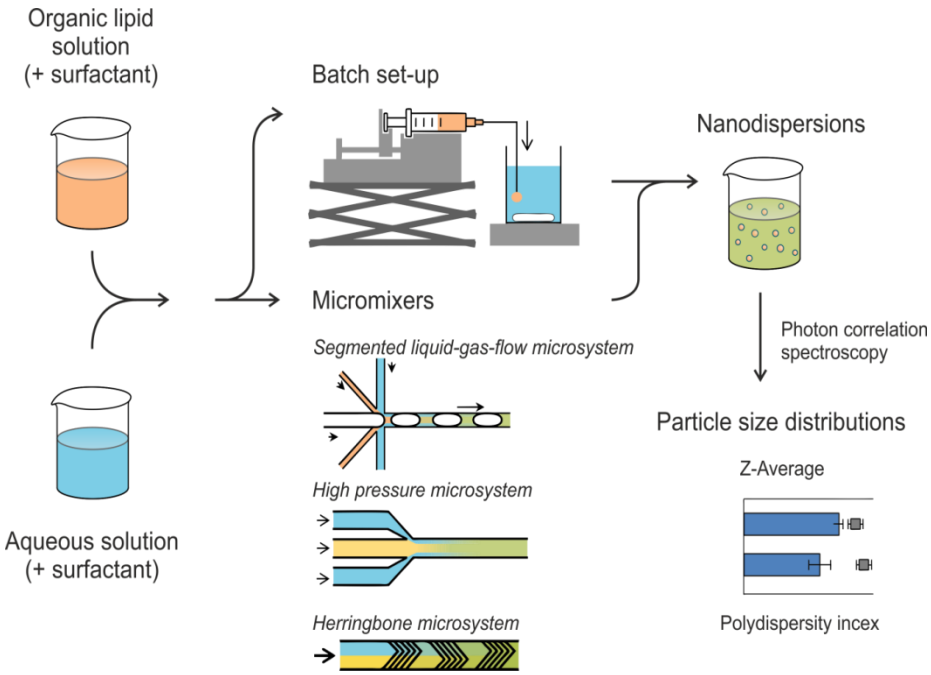


Figure 1: Preparation of nanodispersions in a batch set-up and in micromixers based on different mixing principles

Castor oil in combination with polysorbate 80 as surfactant and glycerol monooleate in combination with poloxamer 407 were chosen for precipitation in the different set-ups. Particle size analysis was performed by photon correlation spectroscopy which provided z average and polydispersity index (PdI) values. All set-ups were able to produce nanoparticles. The batch process led to dispersions with the lowest PdI values. Deposition of oil droplets onto the channel walls was observed in the segmented flow micromixer which might cause contamination with particles in the micrometer range. A reduction of the diameters of the organic solution inlet channels and processing of polysorbate 80 via the organic phase (castor oil nanoemulsions) could reduce but not completely eliminate fouling. The high pressure micromixer produced very small nanoparticles. However, they were associated with high PdI values for the castor oil nanoemulsions. An increasing total flow rate in the NanoAssemblr platform led to a reduction of the z-average values. At high flow rates similar effects as in the high-pressure micromixer were obtained: nanodispersions had low z-average values and castor oil nanoemulsions a high PdI value. Glycerol monooleate dispersions had relatively low PdI values in all set-ups.

ACKNOWLEDGMENTS

We acknowledge financial support by Boehringer Ingelheim Vetmedica and the Niedersächsisches Ministerium für Wissenschaft und Kultur within the graduate program (Niedersächsisches Promotionsprogramm) “μ Props – Processing of Poorly Soluble Drugs at Small Scale“.

REFERENCES

[1] Schubert, M.A. and Müller-Goymann, C.C. Solvent injection as a new approach for manufacturing lipid nanoparticles – evaluation of the method and process parameters, Eur. J. Pharm. Biopharm. 55, 125-131 (2003).

[2] Zhigaltsev, I.V.; Tam, Y.K.; Leung, A.K. and Cullis, P.R. Bottom-up design and synthesis of limit size lipid nanoparticle systems with aqueous and triglyceride cores using millisecond microfluidic mixing, Langmuir 28, 3633-3640 (2012).

[3] Esposito, E.; Drechsler, M.; Mariani, P.; Sivieri, E.; Bozzini, R.; Montesi, L.; Menegatti, E. and Cortesi, R. Nanosystems for skin hydration: a comparative study, Int. J. Cosmetic Sci. 29, 39-47 (2007).

PUBLICATIONS

1. Erfle, P.; Riewe, J.; Bunjes, H. and Dietzel, A. (2017): Optically monitored segmented flow for controlled ultra-fast mixing and nanoparticle precipitation. Microfluidics and Nanofluidics 21:1089. doi: 10.1007/s10404-017-2016-2

POSTER PRESENTATIONS

1. Riewe, J.; Erfle, P.; Melzig, S.; Kwade, A.; Dietzel, A. and Bunjes, H. Batch and microfluidic precipitation of lipids for the generation of lipid nanoparticles. 11th Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology (PBP) World Meeting, 19.03. – 22.03.2018, Granada, Spain.
2. Riewe, J.; Erfle, P.; Melzig, S.; Kwade, A.; Dietzel, A. and Bunjes, H. Precipitation of castor oil and glycerol monooleate nanoparticles – Challenges of microfluidic set-ups. 2nd Symposium on Pharmaceutical Engineering Research (SPhERe), 06.09. – 08.09.2017, Braunschweig, Germany.

3. Riewe, J.; Erfle, P.; Dietzel, A. and Bunjes, H. Microfluidic precipitation of nanoemulsions in comparison with batch processing. 2nd European Conference on Pharmaceutics, 03.04. – 04.04.2017, Kraków, Poland.
4. Riewe, J. and Bunjes, H. Preparation and characterization of precipitated lipid nanoparticles. 10th Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology (PBP) World Meeting, 04.04. – 07.04.2016, Glasgow, United Kingdom.
5. Riewe, J.; Erfle, P.; Dietzel, A. and Bunjes, H. Preparation of lipid nanoparticles by precipitation – Comparison of batch process and processing in a microsystem. Symposium on Pharmaceutical Engineering Research (SPhERe), 18.10. – 20.10.2015, Braunschweig, Germany.

3.3 Precipitation in micro systems

Peer Erfle (IMT), Prof. Andreas Dietzel (IMT); Project 3C

INTRODUCTION

Lipid nanoparticles can be used as carrier agents for lipophilic and poorly water-soluble drugs. Solvent injection is a relatively new and energy-saving process for the production of very small lipid nanoparticles with narrow size distributions. For the effective implementation of this process, a microfluidic system with a segmented gas-liquid flow for mixing different solutions is investigated in this sub-project. The lipid is dissolved in ethanol and precipitated in the micromixing system by adding an aqueous phase. A gas phase is additionally injected to separate the liquid flow into single plugs. The resulting two-phase flow, called Taylor-Flow, enables effective mixing and can be tuned continuously. By simply adjusting the gas volume flow rate, the sizes of the liquid plugs can be changed, whereby the mixing volume and the mixing time are adjusted. This allows varying the mean diameter of the nanoparticles. A new tree-like inlet structure was developed for the microfluidic system, which merges all phases at one point in the mixing system.

Deposits of particles on the channel walls (fouling) represent a major challenge in a continuous precipitation process in microfluidic systems. By modifying the material and the design of microfluidic systems as well as the formulations, the fouling can be suppressed continuous operation over longer periods becomes possible.

RESULTS

The microfluidic system was structured from a borosilicate glass wafer by femtosecond laser ablation to produce hydrophilic microchannels with an almost circular cross-section. The system comprises one intersection of all inlet channels where flow-focusing and gas-liquid segmentation occurs. Flow focusing merges the aqueous phase and the organic phase containing a solvent with a surfactant and a dissolved lipid. The Taylor-Flow appears by instant segmentation of the continuous flow during flow focusing and vortices in the individual segmented plugs induce ultra-fast mixing of the solutions. The sizes of the plugs and the mixing time of the solutions in the plugs were determined using optical methods and a specially developed algorithm. The plug was detected by automatic detection of the differences in brightness between the gas bubbles and the plugs. For the determination of the mixing quality, colored solutions were used for the individual phases, which turn into a uniform color after complete mixing. The algorithm overlays the images of detected plugs to form an averaged image for the entire channel. The progress of mixing of the solutions can be determined from the standard deviation of the brightness values. There is a direct correlation between the size of the plugs to the gas volume flow rate at constant liquid volume flow rate. The plug sizes and as a consequence also the mixing times can be adjusted over a wide range without destabilizing the Taylor flow. The good control of the plug sizes is reflected in the precipitation of the lipid nanoparticles. By adjusting the gas volume flow, the mean diameter of the lipid nanoparticles could be adjusted from 70 nm to 170 nm. The particle size is almost independent of the volume flow rate of the liquid phase as long as the ration of liquid and gas flow rates are maintained. To minimize fouling, the microsystem channel walls and geometries as well as the formulation of the solutions were adapted. With an improved ratio for the inlet channel diameters of the organic phase and other channel diameters, an improved and more centered inflow of the organic phase into the mixing channel and thus a reduced fouling was observed. With an annealing process, the roughness on the channel walls can be minimized, so that less surface sedimentation is generated. Furthermore, by adding a surfactant to the organic phase, the formation of agglomerates on the channel wall can be reduced.

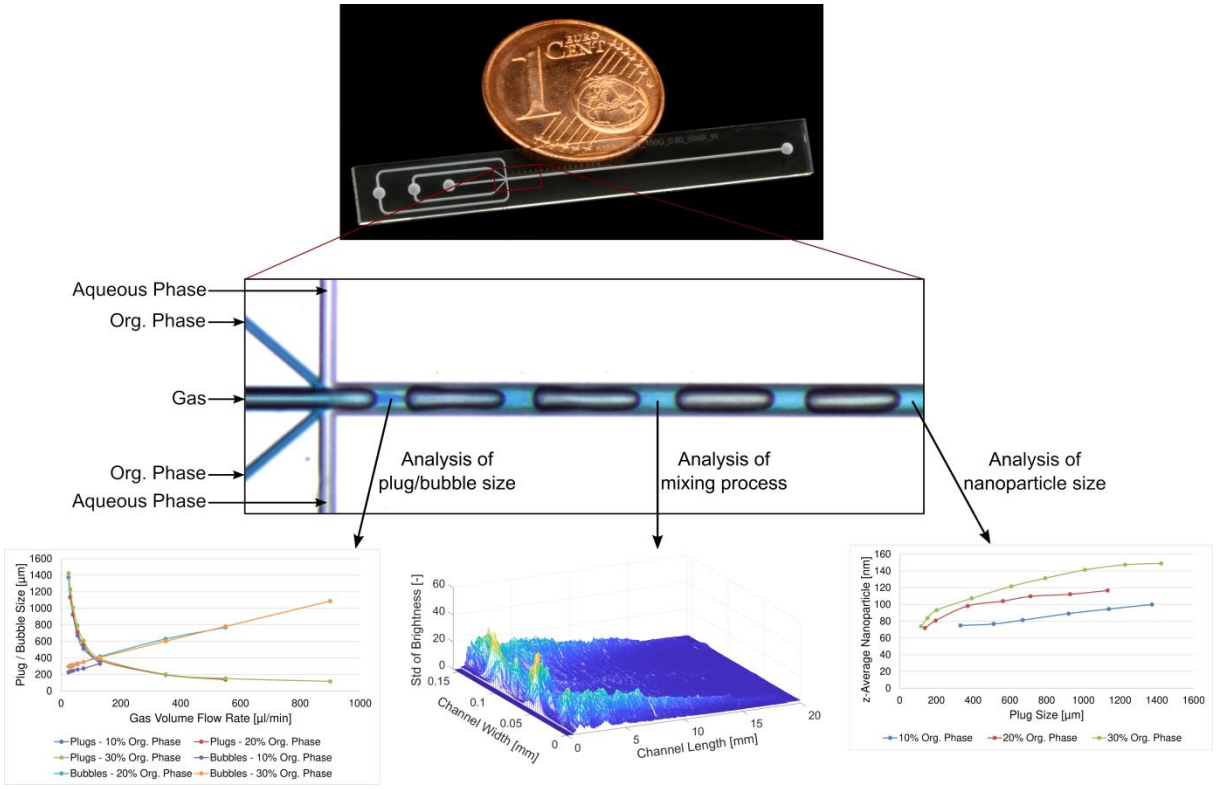


Figure 1: Microfluidic system with segmented multiphase flow and analysis of flow formation in the microsystem as well as the precipitation process.

ACKNOWLEDGMENTS

The financial support of the Niedersächsisches Ministerium für Wissenschaft und Kultur (MWK) and Boehringer Ingelheim Vetmedica within the graduate program “µ-Props – Processing of Poorly Soluble Drugs at Small Scale” of the federal state of Lower Saxony, Germany is duly acknowledged. Credit is also due to J. Riewe and Prof. H. Bunjes (Institut für pharmazeutische Technologie, TU Braunschweig, project 3B) for the development of the chemical composition of the lipid particles.

PUBLICATIONS

1. Erfle, P; Riewe, J; Bunjes, H; Dietzel, A (2017): Optically monitored segmented flow for controlled ultra-fast mixing and nanoparticle precipitation. Microfluidics and Nanofluidics 21:1089. doi: 10.1007/s10404-017-2016-2

POSTER PRESENTATIONS

1. Erfle, P.; Dietzel, A.: Characterization of the mixing process in microfluidic devices with segmented gas-liquid flow. Konferenz: SPhERe “Symposium on Pharmaceutical Engineering Research”, 06.09 – 08.09.2017, Braunschweig, Deutschland.

3.4 Microdevices for nanoparticle separation and analysis

Holger Bolze (MPI-BPC), Dr. Thomas P. Burg (MPI-BPC); Project 3D

INTRODUCTION

To combine the advantages of low-volume production of pharmaceuticals with the advantages of continuous processing, processes can be transferred to microfluidic systems. This transfer requires online methods for characterizing the product to react on changes of the process over prolonged production times. Sub-microliter online detectors capable of measuring important parameters without stopping the flowing liquid are therefore required. For this project, we have chosen to analyze lipid nanoparticles, which are used as carriers for poorly soluble drugs. To use the detector in the online analysis mode we needed additionally a microfluidic synthesis system which precipitated the particles by mixing of two liquids. Since the product of the mixing step contained also micron-sized particles, which could disturb the measurement, we had to add a microfluidic filter to remove them.

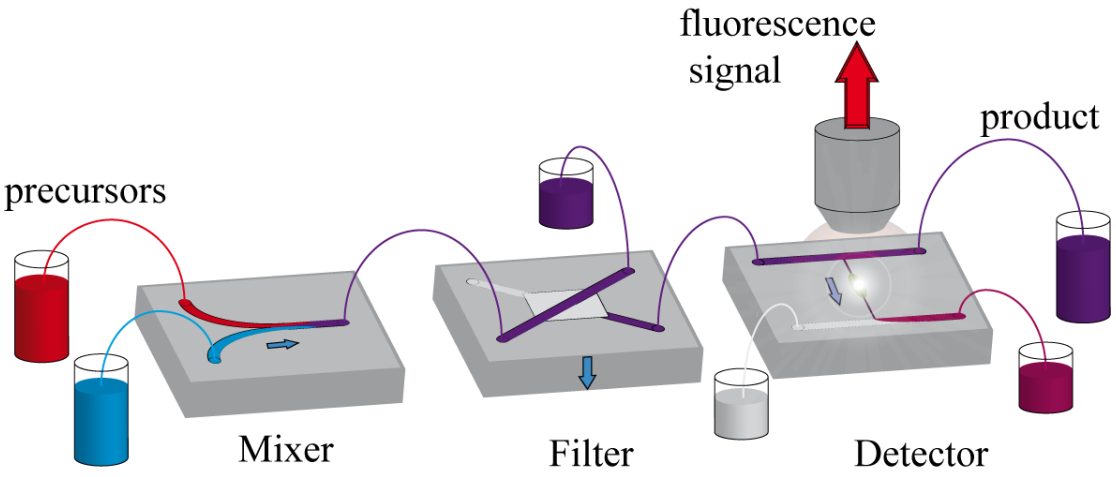


Figure 1: Online analysis of lipid nanoparticles

RESULTS

First, we screened the available detector concepts and chose a method for detecting single particles via fluorescence. This concept, adopted mostly from cytometry, directs a small fraction of the sample through a channel with a size of only 2 micrometers. A small portion of this channel was illuminated by a laser to excite the fluorescence, so that statistically each fluorescence signal contributes to a single particle. In comparison to bulk detection techniques, the method allows the detection of different particle populations and a direct concentration measurement by counting the particles. After establishing the detector with reference particles, we analyzed lipid particles which were colored by a dye enriched in the lipid particles. Under the approximation of an equal distribution of the dye over the whole lipid volume, a change in size should result in a change of intensity of the single particles. We tested the detection limits of the detector and established a detection window for particles of sizes between 100 and 500 nm. With this knowledge we combined our detector with the precipitation setup developed by P. Erfle. After changing the particle precipitation parameters, we were able to detect changes in the process by a change in intensity and the number of measured particles per minute. This result shows that changes in the microfluidic synthesis process can be monitored continuously by our method.

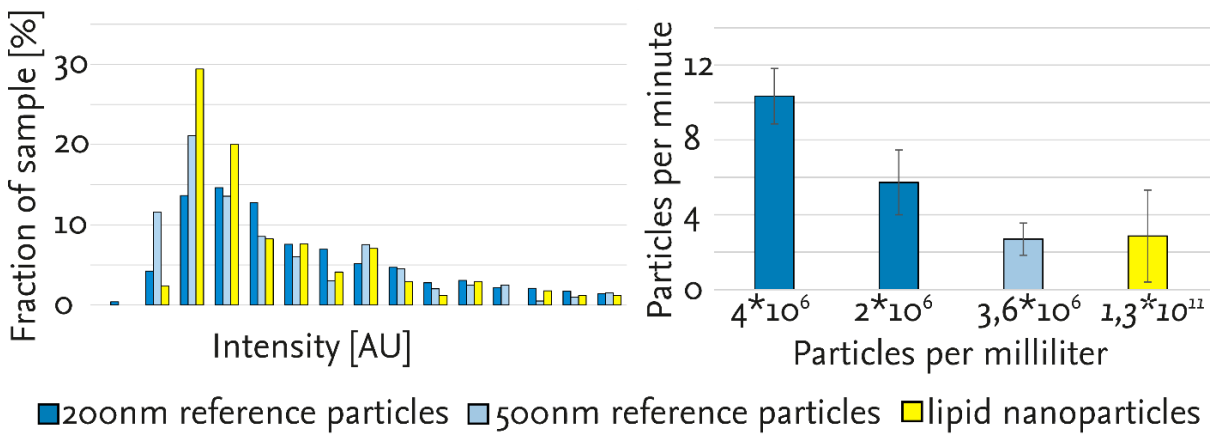


Figure 2: Analysis of fluorescence intensity of single lipid nanoparticles and reference particles

In addition to the characterization technique, we developed an ultrasonic treatment method for the fouling-free production of lipid nanoparticles in a microfluidic system. Without this treatment, the production in a single-phase microfluidic mixer broke down within minutes due to clogging. In addition to the expected nanoparticles, we also a fraction of micrometer-sized particles in the mixing product. This small fraction of waste particles was incompatible with our detection setup and, more importantly, would be inacceptable for in vivo applications due the risk of clogging blood capillaries. To remove these large particles, we designed and fabricated microfluidic cross flow filters with a low dead volume and combined these filters with our precipitation setup to enable the production of a constant flow of a pure nanoparticle suspension.

ACKNOWLEDGMENTS

This work was supported by grants of the µ-Props PhD program at the Center of Pharmaceutical Engineering (PVZ). Credit is also due to the Max Planck Institute for Biophysical Chemistry, Göttingen, J. Riewe and Prof. H. Bunjes (Institut für pharmazeutische Technologie, TU Braunschweig, project 3B) for the development of the chemical composition of the lipid particles, and P. Erfle and Prof. A. Dietzel (Institut für Mikrotechnik, TU Braunschweig, project 3C) for providing an advanced precipitation setup.

ORAL PRESENTATIONS

1. Bolze, H.; Riewe, J.; Bunjes, H.; Dietzel, A., Burg, T.P.: Herstellung von Lipidnanopartikeln in einem ultraschallunterstützten multilaminations Mikromischer. Conference: MikroSystemTechnik Kongress 2017, 23.10 – 25.10.2017, München, Germany

POSTER PRESENTATIONS

1. Bolze, H.; Riewe, J.; Bunjes, H.; Dietzel, A., Burg, T.P.: Microfluidic downstream processing of lipid nanoparticles produced by precipitation. Conference: NanoBioTech Montreux 2017, 12.11. – 15.11.2017, Montreux, Swiss.
2. Bolze, H.; Riewe, J.; Bunjes, H.; Dietzel, A., Burg, T.P.: Synthesis, downstream processing and characterization of lipid nanoparticles in a microfluidic system. Konferenz: SPhERe “Symposium on Pharmaceutical Engineering Research”, 19.10 – 20.10.2015, Braunschweig, Deutschland.

4 Cluster 4 – Crossing niological barriers by means of nanoscale formulations

4.1 Development and evaluation of nanoscale delivery systems to the brain – a microfluidic approach of a co-culture model of the blood-brain barrier (BBB)

S. Hinkel (IPhT), S. Reichl (IPhT), C. C. Müller-Goymann (IPhT); Project 4A

INTRODUCTION

The blood-brain barrier (BBB), mainly consisting of endothelial cells, pericytes and astrocytes, separates the central nervous system (CNS) and the blood circulation to provide vital homeostasis in the brain. In vitro models of barrier-forming cell lines are commonly used to study the permeability of new substances in the early phase of drug development. Therefore, it is essential to imitate cell environment preferably close to in vivo conditions to reduce drug failures and thus animal research, time and costs. This investigation is divided into two parts: The first one covers the optimization of a static mono-culture BBB model of a human immortalized cerebral endothelial cell line (hCMEC/D3) and the second part deals with the optimization and evaluation of microfluidic approach of the BBB. For this purpose, the novel cell culture platform DynaMiTES (Dynamic Micro Tissue Engineering System) is based on the idea of combining advantages of static cell culture insert systems with those of microfluidic devices for imitating flow conditions and shear stress [1; 2]. The DynaMiTES (Fig. 1) enables dynamic permeability studies as well as dynamic cultivation of various cell types. Furthermore, it combines online transendothelial electrical resistance (TEER) measurements, versatility in use and comparability with conventional insert systems such as Transwell® or ThinCert®.

Figure 1: Three-layered structure (A) and oblique view of the disassembled DynaMiTES with bottom, middle and top layer (B) as well as plan view of assembled DynaMiTES (left) and disassembled top layer (C)

RESULTS

In this study, a well-known static hCMEC/D3-cell culture insert system was characterized by proliferation (growth rate), morphology via light microscopy of the insert in top view and as cross section, permeation studies with sodium fluorescein (SoF), TEER-measurement via EVOM© device and immunofluorescence and polymerase chain reaction (PCR) of tight junction proteins (TJs). It was determined, that hCMEC/D3-cells form rather a weak barrier. For example, TEER-values ranged between 5 - 16 Ω·cm2 within 27 days (compared to the in vivo situation with TEER of ≥ 2000 Ω·cm2). For this reason, the static model was optimized by variation of different culture conditions. Therefore, cell and barrier properties in different commonly used media (DMEM, Ham's F 12, RPMI and M199), as well as specialized endothelial media (MCDB 131, ECGM MV 2) and serum free endothelial media (hESFM, SF ECM) were tested and compared with the conventionally used media (EGM-2). In addition, the influence of media supplements such as hydrocortisone and serum, of coating materials and of a co-cultivation with immortalized and primary astrocytes on TEER was examined. It was proven that cells grow well in serum free and in the other tested endothelial media. However, neither medium nor the variations did strengthen barrier function.

For the evaluation of dynamic cultivation of cell monolayers in DynaMiTES, the common cell line MDCK-I (Madin-Darby canine kidney-I), which shows particularly pronounced barrier properties, as well as the endothelial cell lines hCMEC/D3 mentioned above and cEND (murine cerebral endothelial cell line) were used. The effect of DynaMiTES cultivation on cell status was analyzed by cell viability, cell number and morphology and on barrier properties via TEER measurements and permeation studies. To enable the medium flow along the cell monolayer in DynaMiTES, it was necessary to change the cell orientation from conventional to inverted. Nevertheless, the platform can be easily integrated in existing work flows and protocols. Furthermore, short-term cultivation with a flow rate of 0.033 mL/min to 3.33 mL/min for 3 hours as well as 0.033 mL/min for elongated cultivation of 24, 48 and 72 hours had no negative impact on cell viability and cell morphology. Up to 24 h, flow conditions did not lead to a reduced cell count whereas for longer cultivation duration (48 – 72 h) the cell count was slightly reduced after DynaMiTES cultivation. In addition, hCMEC/D3 cells in DynaMiTES and 12-well plates showed comparable Papp data and TEER-values after 24 h indicating no effect on barrier properties. Subsequently, modification of cultivation conditions and their effect on the cells will be investigated. This study reveals that the application as dynamic cell culture model is feasible, yet requires further testing, wider usage and improvements.

ACKNOWLEDGMENTS

This study was funded by the Niedersächsisches Ministerium für Wissenschaft und Kultur (MWK) in the joint graduate program µ-Props - “Processing of poorly soluble drugs at small scale”.

REFERENCES

[1] Mattern, K. et al., 2018, European Journal of Pharmaceutics and Biopharmaceutics, Vol. 126., pp. 159-165.
[2] Beißner, N. et al., 2018, European Journal of Pharmaceutics and Biopharmaceutics, Vol. 126., pp. 166-176.

PUBLICATIONS

POSTER PRESENTATIONS

1. S. Hinkel, S. Reichl, C. C. Müller-Goymann, Influence of cell culture media on the growth behavior and barrier properties of hCMEC/D3 cells (poster), 1st Symposium on Pharmaceutical Engineering Research SPhERe, Braunschweig/Germany, 18.-20. October (2015)
2. S. Hinkel, K. Mattern, A. Dietzel, S. Reichl, C. C. Müller-Goymann, Optimization and Evaluation of the Cerebral Dynamic Micro Tissue Engineering System (Cerebral DynaMiTES) (poster), 2nd PVZ Research Workshop, (St. Andreasberg/Germany) 15.-16. February (2017)
3. S. Hinkel, K. Mattern, A. Dietzel, S. Reichl, C. C. Müller-Goymann, Evaluation of a novel Dynamic Micro Tissue Engineering System as blood-brain barrier model: Cerebral DynaMiTES (poster), 19th Barrier- and Transporter-Meeting, Bad Herrenalb/Germany, 15-17 May (2017)
4. S. Hinkel, K. Mattern, A. Dietzel, S. Reichl, C. C. Müller-Goymann, Evaluation of a novel cell culture platform for dynamic cultivation of barrier-forming cells (poster), 2nd Symposium on Pharmaceutical Engineering Research SPhERe, Braunschweig/Germany, 6.-8. September (2017)
5. S. Hinkel, K. Mattern, A. Dietzel, S. Reichl, C. C. Müller-Goymann, Evaluation of the Novel Cerebral DynaMiTES for dynamic cultivation of different barrier-forming cells (poster), 11th World Meeting on

Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Granada/Spain, 19-22. March (2018)

6. S. Hinkel, K. Mattern, A. Dietzel, S. Reichl, C. C. Müller-Goymann, Evaluation of a Novel Cell Culture Platform with Various Barrier Forming Cells for Dynamic Cultivation (poster), DECHEMA 3D Cell Culture conference, Freiburg/Germany, 5.-7. June (2018)

4.2 Alternatives to in-vivo testing: Micro manufactured organ-on-chip systems

K. Mattern (IMT), Prof. A. Dietzel (IMT); Project 4B

INTRODUCTION

The implementation of Organ-On-Chip (OOC) systems places new demands on material selection and tailor-made micro structuring processes. For the different tissue cultures used by different cooperating research groups, different designs and production processes are required. These requirements led to the development of two different microfluidic design and fabrication concepts: We used femtosecond laser ablation of glass as well as precision machining of polycarbonate to produce two unique, innovative systems for A) the cultivation and investigation of Langerhans islets (pancreas on chip) and B) the cultivation and investigation of epithelial cells and stromal cells (cornea on chip).

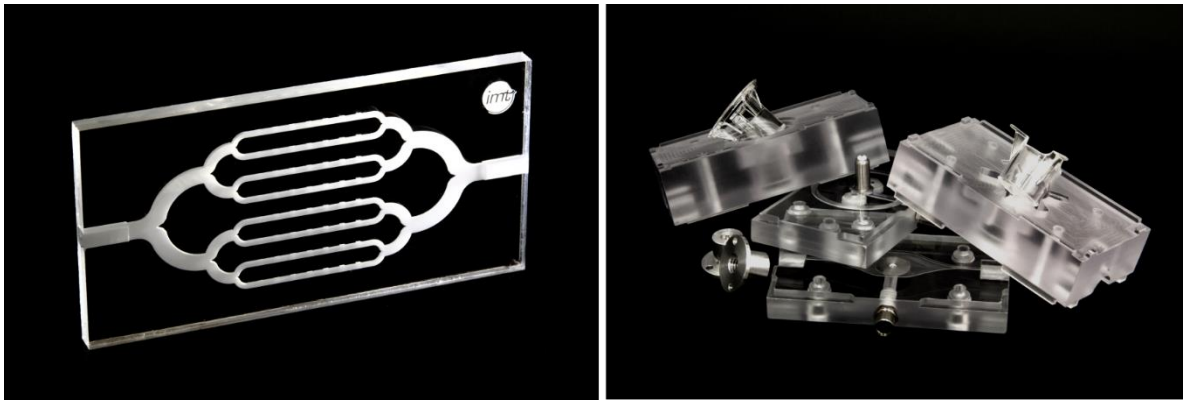


Figure 1: *Left:* Micro Perifusion System (MPS) for the cultivation and investigation of Langerhans islets. *Right:* DynaMiTES platform for the cell cultivation and investigation of barrier forming cell cultures like cornea tissue.

RESULTS

A) The present OOC / MPS system (Fig.1 left) was used to investigate critical characteristics for micro physiological experiments with isolated pancreatic islets. It could be shown that wells within a micro fluidic channel were capable of immobilizing isolated islands. This setup also enables sufficiently fast exchange of the test media without exposing the islands to potentially harmful shear stress. The transparent 3D geometries of the OOC were realized by femtosecond laser structuring of borosilicate glass (BOROFLOAT). The µm-scaled channels preserve a steep gradient between different media, which is a prerequisite for the generation of square wave stimulation during the tests. Glass as a material for the MPS has two advantages over polydimethylsiloxane commonly used for microfluidics. It has superior optical properties that allow fluorescence microscopy even during UV excitation and it is gas-impermeable, allowing measurements of oxygen consumption. This advantage could be illustrated by simultaneous multiparametric measurements of isolated pancreatic islets that respond to a sudden glucose concentration change.

B) The Dynamic Micro Tissue Engineering System (DynaMiTES) (Fig 1 right) was designed as a modular tool for dynamic cell cultivation and in vivo-like drug absorption measurements. For the intended three-hour test period, cell viability is not affected either by exposure to the used materials or to the fully assembled DynaMiTES. In addition, the material test showed that no corrosion is caused by the buffer solution over a period of three months. In addition, the materials enable autoclaving with moist, which is an essential prerequisite for multiple use. Therefore, the design can be regarded as cell-compatible and robust for lengthy experiments. A homogeneous loading of the tissue by shear stress

and homogeneous active agent concentrations after the injection could be confirmed by FEM flow simulations. The simulation of the electrical current showed that integrated electrodes allow a practically homogeneous load of the tissue. With the DynaMiTES a commercial transepithelial / endothelial electrical resistance measuring electronics can be used. The concept of compatibility with existing equipment promotes the transfer of this new, directly usable OOC platform to other laboratories.

ACKNOWLEDGMENTS

This work was supported by grants of the µ-Props PhD project and the joint research project SynFoBiA at the Center of Pharmaceutical Engineering (PVZ).

PUBLICATIONS

1. Schulze T, Mattern K, Früh E, Hecht L, Rustenbeck I, Dietzel A (2017) A 3D microfluidic perfusion system made from glass for multiparametric analysis of stimulus-secretioncoupling in pancreatic islets, Biomedical microdevices 19(3): 47.
2. Mattern K., Beissner N, Reichl S, Dietzel A (2018) DynaMiTES–A dynamic cell culture platform for in vitro drug testing PART 1–Engineering of microfluidic system and technical simulations, European Journal of Pharmaceutics and Biopharmaceutics 126:159-165.
3. Beissner N, Mattern K, Dietzel A, Reichl S (2018) DynaMiTES–A dynamic cell culture platform for in vitro drug testing PART 2– Ocular DynaMiTES for drug absorption studies of the anterior eye, European Journal of Pharmaceutics and Biopharmaceutics 126:166-176.

4.3 Plasma-induced modification of the dermal drug absorption

Monika Gelker (HAWK), Prof. Christel Müller-Goymann (IPhT), Prof. Wolfgang Viöl (HAWK); Project 4C

INTRODUCTION

Dermal drug delivery offers several important advantages over the more widely-used strategies of subcutaneous injection and parenteral administration: As a noninvasive, easy-to-use and often pain free method it promises improved patient compliance and avoids drug inactivation due to the first-pass-effect. To improve drug permeation through the stratum corneum, which constitutes the main barrier of mammalian skin, cold atmospheric plasma (CAP) is considered a prospective tool for permeabilization.

RESULTS

This project aimed to elucidate the mechanism of plasma-permeabilization by CAP produced through a µs-pulsed direct dielectric barrier discharge (µs-DBD) operated at a repetition frequency of 300 Hz with a power density of about 0.2 W·cm-2 [1]. For CAP-treatment, the plasma was ignited in a 1 mm air gap between the high voltage electrode enclosed by a ceramic dielectric and the stratum corneum. Due to the cylindrical geometry of the electrode, an area of 0.5 cm² was treated at the same time. By means of Franz-cell permeation experiments in combination with transepithelial electrical resistance (TEER) measurements, the overall effect of CAP-treatment on human skin barrier properties was studied. Additionally, electrochemical and redox reactions were employed to directly visualize patterns of permeabilized regions in treated stratum corneum and evaluate the production and permeation of oxidizing species formed in the plasma. Our results show a considerable permeabilizing effect of a filamentous µs-pulsed discharge (Fig. 1).

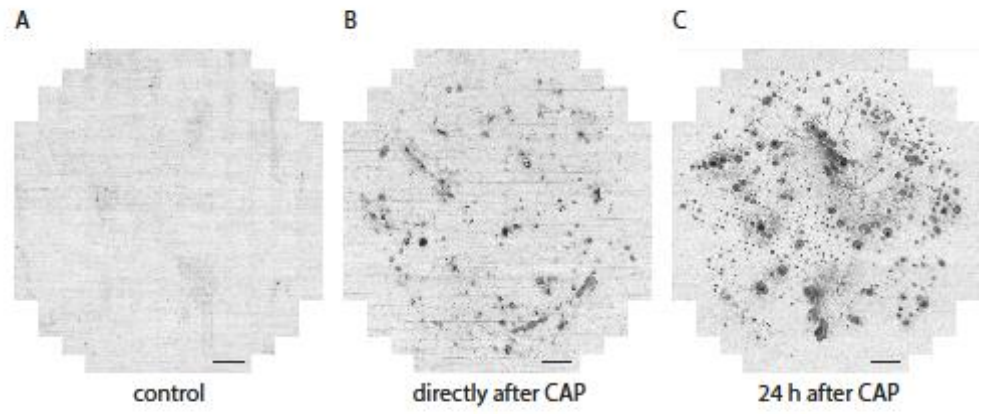


Figure 1: Electrolytic imaging of the formation of permeabilized regions in human stratum corneum treated with cold atmospheric plasma (CAP). (B/C) Darkened spots on a polished silver plate indicate localized current and small ion flux through treated stratum corneum samples. The pores are stable even 24 h after the plasma treatment (C). An untreated stratum corneum control is shown in A. The image size of each composite image is 10.5 mm × 10.9 mm (206 tiles). The size of the scale bar is 1 mm.

More specifically, permeation study results indicate that relatively small hydrophilic substances with Stokes' radii up to 1.4 nm are efficiently transported through human SC subsequent to 2x 90 s treatment with CAP produced by a µs-DBD. A moderate permeation of particles up to 6 µm in diameter is evidence for the occasional formation of large pores in the µm range. Plasma-permeabilization using CAP is a promising method for future application in dermatology and pharmacology. It potentially holds several advantages not only over established techniques of drug delivery, but also over other innovative methods to enhance dermal drug delivery (electroporation, sonoporation and many more), since recent studies have shown therapeutically beneficial effects [2–4], which may be used synergistically in future clinical applications.

ACKNOWLEDGMENTS

This research was funded by a Georg-Christoph-Lichtenberg grant to Monika Gelker through the PhD Program Processing of poorly soluble drugs at small scale, funded by Lower Saxony's Ministry of Science and Culture (MWK). Experimental equipment is funded by the Federal Ministry of Education and Research through the research project PlasBaWirk (FKZ: 03FH015IX5). Thanks are due to the Department of Trauma-, Orthopaedic- and Plastic Surgery of the University Medical Center Göttingen for continued help with the provision of donated skin.

REFERENCES

[1] J. Hirschberg, T. Omairi, N. Mertens, A. Helmke, S. Emmert, W. Viöl; Influence of excitation pulse duration of dielectric barrier discharges on biomedical applications; J. Phys. D: Appl. Phys. 46 (16) (2013) 165201.

[2] J.-H. Choi, Y.-S. Song, H.-J. Lee, J.-W. Hong, G.-C. Kim; Inhibition of inflammatory reactions in 2,4-Dinitrochlorobenzene induced Nc/Nga atopic dermatitis mice by non-thermal plasma; Sci. Rep. 6 (2016) 27376.

[3] T. Borchardt, J. Ernst, A. Helmke, M. Tanyeli, A.F. Schilling, G. Felmerer, W. Viöl; Effect of direct cold atmospheric plasma (diCAP) on microcirculation of intact skin in a controlled mechanical environment; Microcirculation 24 (8) (2017).

[4] A. Helmke, D. Hoffmeister, F. Berge, S. Emmert, P. Laspe, N. Mertens, W. Vioel, K.-D. Weltmann; Physical and Microbiological Characterisation of Staphylococcus epidermidis Inactivation by Dielectric Barrier Discharge Plasma; Plasma Process. Polym. 8 (4) (2011) 278–286.

PUBLICATIONS

1. M. Gelker, C.C. Müller-Goymann, W. Viöl; Permeabilization of human stratum corneum and full-thickness skin samples by a direct dielectric barrier discharge; Clinical Plasma Medicine 9 (2018) 34–40.

ORAL PRESENTATIONS

1. M. Gelker, C. Müller-Goymann, W. Viöl; Comparison of a µs-Pulsed and a ns-Pulsed DBD for Skin Permeabilization; 7th International Conference on Plasma Medicine; June 17th–22nd, 2018; Philadelphia, PA, USA
2. M.Gelker, C. Viöl, C. Müller-Goymann, W. Viöl; Modifikation der dermalen Barrierefunktion durch Atmosphärendruckplasmen; 5. Plasmamedizin-Workshop; September 13th–14th, 2017; Rostock, Germany

POSTER PRESENTATIONS

1. M. Gelker, C. Müller-Goymann, W. Viöl; Permeabilization of human stratum corneum and full-thickness skin samples by a direct dielectric barrier discharge; 2nd IST/IOT/HAWK Science-Day; January 9th, 2018; Braunschweig, Germany
2. M. Gelker, C. Müller-Goymann, W. Viöl; Modification of the Dermal Barrier Function by Cold Atmospheric Pressure Plasma; SPhERe (Symposium on Pharmaceutical Engineering Research); September 6th–8th, 2017; Braunschweig, Germany
3. M. Gelker, C. Müller-Goymann, W. Viöl; Modification of the Dermal Barrier Function by Atmospheric Pressure Plasma; 21. GD-Jahrestagung; March 20th–22nd, 2017; Munich, Germany

4. M. Gelker, C. Müller-Goymann, W. Viöl; Plasma-Induced Changes in Stratum Corneum Permeability; 18. Fachtagung für Plasmatechnologie; February 20th–22nd, 2017; Göttingen, Germany
5. M. Gelker, C. Müller-Goymann, W. Viöl; Plasma-Induced Modification of the Dermal Permeability; 6th International Conference on Plasma Medicine; September 4th–9th, 2016; Bratislava, Slovakia