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Book of Abstracts

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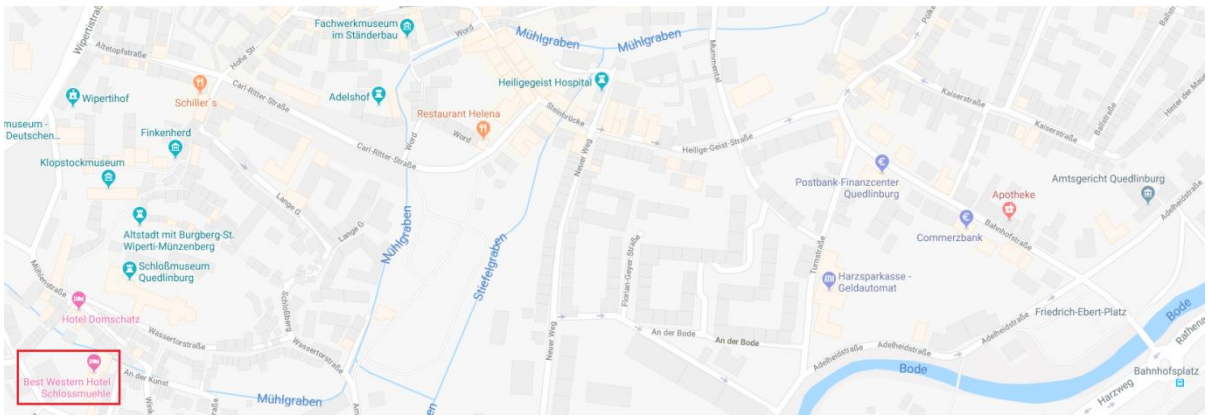

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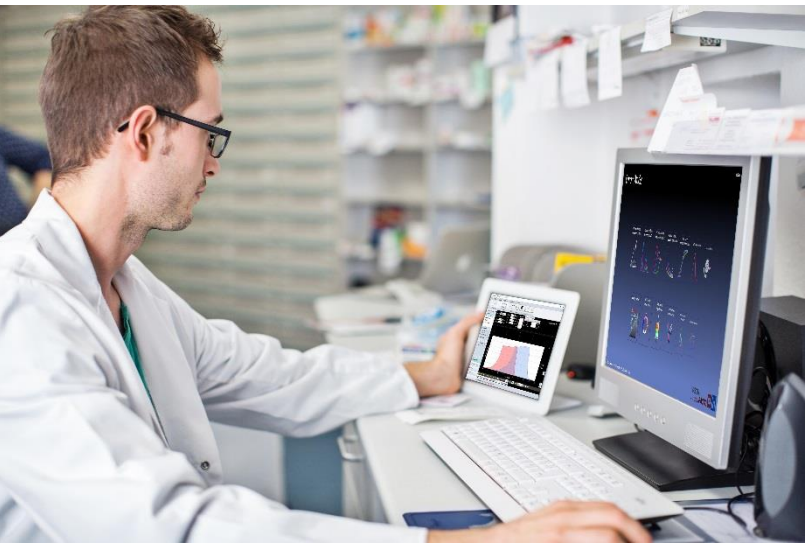

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Program

Sunday, 24 Feb.19	17:00	Arrival and Registration	
	19:00	Dinner	
Monday, 25 Feb. 19	From 7:00	Breakfast	
	8:20-8:30	Opening remarks	
	Session I:	**Process Development – CPC and Biopurification I** **Chair: Bettina Kattein**	
	08:30-08:50	Alina Mehl	Product purification and product analysis in mammalian cell cultivation
	08:50-09:10	Andreas Bauer	Extraction of astaxanthin from the microalgae Haematococcus pluvialis using a liquid-liquid chromatography column
	09:10-9:30	Angela Fromme	Flow regime map for different aqueous organic two phase systems used in a Centrifugal Partition Chromatograph
	09:30-09:50	Stefan Rauwolf	New affinity tags for non-functionalized silica
	09:50-10:10	Andreas Biselli	Adsorption as alternative purification technology in bio-refinery processes
	10:10-10:40	Coffee Break	
	Session II:	**Process Development – Biopurification II** **Chair: Sebastian Vogg**	
	10:40-11:00	Deborah Gernat	Off-flavor reduction in multicomponent aqueous food streams with zeolites: Selective Strecker aldehyde removal from alcohol-free beer
	11:00-11:20	Bastian Bartling	Membrane adsorber technology for GMP-conform manufacturing of bacterial polysialic acid
	11:20-11:40	Svenja N. Bolten	Substitution of heparin for purification of cytokines
	11:40-12:00	Jan Hübbers, Matthias Knödler	Impact of cultivation conditions on the chromatographic retention behavior of host cell proteins and target proteins in plants
	12:00-13:00	Lunch break	
	Session III:	**Process Development – Biopurification III** **Chair: Deborah Gernat**	
	13:00-13:20	Mohsen Fotovati	Extraction and isolation of valuable targets of saffron
	13:20-13:40	Jonas Lohr	Targeted production and purification of the antiviral peptides Labyrinthopeptin A1 and A2 by Quality-by-Design approaches
	13:40-14:00	Sebastian Vogg	Automated End-to-End Integrated Manufacturing of an Antibody
	14:00-14:20	Pia Gellermann	Production of recombinant fibrinogen gamma chain for 3D-Bioprinting and Tissue Engineering
	14:20-14:40	Catherine Mueschen	Purification of three halohydrindehalogenases and determination of their SMA Parameters
	14:40-15:10	Coffee Break	
	Session IV:	**Process Development – Continuous Chromatography** **Chair: Johannes Schmölder**	
	15:10-15:30	Mafalda G. Moleirinho	Evaluation of continuous chromatographic purification of extracellular vesicles
	15:30-15:50	Chantal Brämer	Monoclonal antibody purification using a self-established periodic counter-current chromatography device
	15:50-16:10	Carsten-Rene Arlt	Continuous fractionation of nanoparticles by magnetic field controlled multi-column chromatography
	16:10-16:30	Felix Funke	Continuous Centrifugal Extraction - A new Apparatus for Intensifying Extraction Processes
	17:00-19:00	City Tour	
	19:15	Dinner	

Tuesday, 26 Feb. 19	From 7:00	Breakfast	
	Session V:	**Theoretical aspects and fundamentals – Parameter Estimation** **Chair: Ivana Mutavdžin**	
	08:30-08:50	Ronald Colin Jäpel	Simplification of inverse SMA parameter determination using only gradient elution experiments
	08:50-09:10	Stefanie Gerlich	Efficient isotherm estimation using neural networks for applications in SMB process design and preparative chromatography
	09:10-9:30	Gabriela Sánchez Reyes	Modeling of anion-exchange chromatography: Influence of ligand densities on Donnan equilibrium and protein separation
	09:30-09:50	David Saleh	Industrial Application of Mechanistic Chromatography Modeling
	09:50-10:10	Alexander Gutzler	Chromatography Model Calibration with Bayesian Optimization
	10:10-10:30	Coffee Break	
	10:30-10:50	Patrick Adametz	Advantages of Membrane Adsorbers in chromatography processes
	Session VI:	**Theoretical aspects and fundamentals – Process Design** **Chair: David Saleh**	
	10:50-11:10	Franziska Hagemann	Conceptual performance modelling for novel affinity chromatography membranes
	11:10-11:30	Martin Leipnitz	Optimisation of resin selection by correlating structural properties with model parameters
	11:30-11:50	Nadia Galeotti	Influence of Mixed Electrolytes on HIC-Adsorption and Prediction of Chromatographic Elution Profiles
	11:50-12:10	Johannes Schmölder	Simultaneous identification of optimal chromatographic process concepts and operating conditions
	12:10-12:40	Coffee Break	
	Session VII:	**Theoretical aspects and fundamentals – Simulation** **Chair: Alexander Gutzler**	
	12:40-13:00	Xinghai An	Thermal Gradient in Preparative Liquid Chromatography
	13:00-13:20	Anna Christler	Online monitoring and real-time prediction of product quantity, purity and potency during chromatographic purification processes
	13:20-13:40	William Heymann	Error modeling in Chromatography and parameter confidence
	13:40-14:00	Jayghosh S. Rao	High Definition Simulation of Packed Bed Liquid Chromatography
	14:00-14:10	Closing remarks and nomination of SoCSS host 2020	

Session I: Process Development – CPC and Biopurification I

Chair: Bettina Kattein

8:30	Product purification and product analysis in mammalian cell cultivation	*Alina Mehl*
8:50	Extraction of astaxanthin from the microalgae *Haematococcus pluvialis* using a liquid-liquid chromatography column	*Andreas Bauer*
9:10	Flow regime map for different aqueous organic two phase systems used in a Centrifugal Partition Chromatograph	*Angela Fromme*
9:30	New affinity tags for non-functionalized silica	*Stefan Rauwolf*
9:50	Adsorption as alternative purification technology in bio-refinery processes	*Andreas Biselli*

Product purification and product analysis in mammalian cell cultivation

Alina Mehl[1], *Dörte Solle*[1], *Janina Bahnemann*[1], *Thomas Scheper*[1]

[1]*Institute of Technical Chemistry, Leibniz University Hannover, Hannover, Germany*

Mammalian cell culture has undergone a transformation from a research tool to a main innovation in biopharmaceutical production. In the last years a lot of therapeutics for the successful treatment of diseases were discovered and produced in mammalian cell culture processes. The most interesting product area which has grown significantly is the class of monoclonal antibodies. They are already approved as biopharmaceutical products for the treatment of cancer, rheumatic diseases, acute coronary syndrome or Crohn´s disease. For medical use in humans a high purity must be achieved and guaranteed. This results in the need for the optimization of the purification and high quality analytics.

A monoclonal antibody was produced in Chinese hamster ovary cells with a yield of about 3 $g \cdot L^{-1}$ of antibody in the supernatant. For product analysis a high performance liquid chromatography with a protein A column was used to determine the antibody concentration. For this analytical method various conditions such as buffer composition, pH value and flow rate were examined and optimal conditions were defined by design of experiments. The results were compared to a size exclusion column which was also used to measure the amount of aggregates in the supernatant. Furthermore fast protein liquid chromatography with a protein A column was used for product purification. This capture step for monoclonal antibodies was optimized and established. In addition other polishing chromatographic steps are going to be examined.

The aim after establishing the purification and the analytics of the monoclonal antibody is the implementation of a bioactivity test and further analysis of the product quality.

Extraction of astaxanthin from the microalgae *Haematococcus pluvialis* using a liquid-liquid chromatography column

Andreas Bauer[1]

[1]*Biothermodynamics, TU Munich, Freising, Germany*

Astaxanthin is a reddish carotenoid, used as a color agent in the aquaculture and poultry industry. Due to its antioxidant and health promoting effects, the demand for natural astaxanthin for the use in the cosmetic and nutrition industry has risen tremendously in recent years. The best source of natural astaxanthin is the microalgae *Haematococcus pluvialis* (*H. pluvialis*), which can accumulate up to 5 wt% astaxanthin when exposed to stress conditions (e.g. nitrate deficiency, high light intensity). The astaxanthin synthesis in *H. pluvialis* is accompanied by the formation of a resistant, thick cell wall, which impedes a direct extraction of the dye into a solvent. In the subsequent downstream processing, the algae broth is first harvested and dewatered through centrifugation, followed by a drying step, mechanically disrupted and finally astaxanthin is extracted with supercritical CO_2. For the simplification of the downstreaming it would be of interest to establish a direct extraction process of astaxanthin from the *H. pluvialis* broth into a solvent.

Solid support-free liquid-liquid chromatography (LLC), also known as countercurrent chromatography (CCC) or centrifugal partition chromatography (CPC) allows liquid-liquid extraction, simultaneously achieving both high selectivity and high sample loading capacity. In recent years, LLC has been increasingly used for the preparative isolation of valuable natural compounds from complex mixtures, e.g. plant extracts, marine organisms and microbial fermentation media. In LLC, the two phases of a biphasic solvent system are used as the mobile and stationary phases. The stationary phase is held in place during operation by application of a centrifugal field, and the mobile phase is pumped through it.

To allow a direct extraction of astaxanthin from *H. pluvialis*, it was taken advantage of the natural cell cycle. Exposing growth conditions to the *H. pluvialis* cysts causes a germination and a release of astaxanthin containing flagellated cells.

The germinated algae broth was injected into a CCC column, allowing a direct extraction of astaxanthin in high concentrations into a solvent (i.e. stationary phase).

Flow regime map for different aqueous organic two phase systems used in a Centrifugal Partition Chromatograph

Fromme A. [1], *Fischer C*[1]., *Schembecker G.* [1]

[1]*Laboratory of Plant and Process Design, TU Dortmund University, Dortmund, German*

Centrifugal partition chromatography (CPC) is a promising downstream technique to satisfy the increasing demand for high purity products. Due to the two liquid phases used, the operational mode of the CPC equals a multistage extraction but enables separation efficiencies comparable to packed-bed chromatography processes.

In CPC a centrifugal field is used to hold one liquid phase of a biphasic system stationary inside a rotor i.e. a chamber cascade, while the other phase is pumped through. The selection of the biphasic system and especially the polarities of the phases affect the solubility and the partitioning of solutes, resulting in a chromatographic separation. During operation, a high dispersion of the mobile phase at the chamber entrance promotes the mass transport and thus, the separation efficiency. Simultaneously, a good coalescence at the bottom is needed to maintain a high volume of the stationary phase providing a high capacity. In contrast to other separation techniques, the physical properties of the solvent system (density, viscosity and interfacial tension) play an important role in CPC separation efficiency, because of their influence on the fluid dynamics, and must not be neglected in the solvent system selection process.

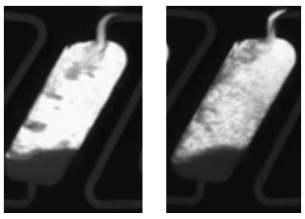

Figure 1: Observed flow patterns inside a CPC using two Arizona solvent systems

In Figure 1 the flow patterns of two Arizona phase systems are shown, recorded via an optical measurement system. To correlate the physical properties with differences in the fluid dynamics, the fluid dynamics of different aqueous organic two phase systems were classified by analyzing such flow videos. The illustrated Arizona D for example showed a low dispersed flow regime, whereas the mobile phase of Arizona Z was spread in many small droplets. Using the physical properties of the solvent systems, the operating parameters and the classified flow patterns a flow regime map in dependency of the Weber and the Eötvös number was developed. During the presentation, the flow regime map will be introduced and it will be shown how the map can be used for the characterization of the flow patterns in both operation modes of the CPC, the ascending and the descending mode.

New affinity tags for non-functionalized silica

Stefan Rauwolf[1], Sebastian Schwaminger[1], Sonja Berensmeier[1]

[1]Technical University of Munich, Bioseparation Engineering Group, Garching, Germany

The global market value for chromatography columns is expected to exceed more than US$ 3.3 billion by 2024 with affinity chromatography being the most commonly used techniques in protein purification. This technique is most convenient for high purity products such as recombinant proteins and antibodies. Many proteins are tagged with a high affinity peptide for a certain ligand. The functionalization of chromatographic materials is time and cost intensive and not as process stable than classical cheaper ion-exchange materials. To overcome this disadvantage, we develop affinity tags which can be fused to a target protein and selectively bind to non-functionalized technical surfaces such as silica.

In an earlier work, we showed the proof-of-principle for non-functionalized bare iron oxide nanoparticles (BIONs).

Here, we want to transfer these findings to another technical surface, silica. Silica is one of the most commonly used chromatographic materials and thus abundantly available at low costs. We studied static as well as dynamic interaction behaviour between single amino acids or short peptides and silica by LC/HPLC, peptide arrays and static binding capacity. For the binding we expect electrostatic interaction of positively charged amino acids to be strongest followed by hydrophilic H-bonding and very weak hydrophobic effects. Our findings based on retention times in chromatographic systems confirmed the very strong interaction with basic amino acids. However, hydrophilic H-bonding between amino acids and the silica surface are very weak in dynamic systems. These results play a very important role in understanding the fundamental interplay between biomolecules and silica in dynamic systems and the design of affinity tags for non-functionalized silica.

Adsorption as alternative purification technology in bio-refinery processes

Andreas Biselli[1], *Martin Leipnitz*[1], *Andreas Jupke*[1]

[1]*AVT Fluid Process Engineering, RWTH Aachen University, Aachen, Germany*

The key point for cost- and energy efficient bio-refinery processes is the downstream processing from aqueous solutions. Products like amino acids and di-carboxylic acids are currently purified using ion exchange chromatographic steps and crystallization steps.

Since ion exchange chromatography results in high salt loads, the recently started project “BioSorp” focuses on the hydrophobic adsorption of L-Lysin and itaconic acid, as conceivable products of a bio-refinery. A focus is laid on the investigation of activated carbons and recently developed hyper cross-linked polymers. Based on a sequential approach, model parameters will be determined and used for a model based investigation of the adsorption processes. Further, a model based process scale up will be conducted. A possible implementation of *in situ* product removal approaches will be evaluated in an interdisciplinary team.

In this presentation, first promising process alternatives are shown.

Session II: Process Development – Biopurification II

Chair: Sebastian Vogg

10:40	Off-flavor reduction in multicomponent aqueous food streams with zeolites: Selective Strecker aldehyde removal from alcohol-free beer	*Deborah Gernat*
11:00	Membrane adsorber technology for GMP-conform manufacturing of bacterial polysialic acid	*Bastian Bartling*
11:20	Substitution of heparin for purification of cytokines	*Svenja N. Bolten*
11:40	Impact of cultivation conditions on the chromatographic retention behavior of host cell proteins and target proteins in plants	*Jan Hübbers, Matthias Knödler*

Off-flavor reduction in multicomponent aqueous food streams with zeolites: Selective Strecker aldehyde removal from alcohol-free beer

D. Gernat[1], E. Brouwer[2], M. Ottens[1]

[1]Department of Biotechnology, Delft University of Technology, Delft , The Netherlands,

[2]Heineken Supply Chain, Zoeterwoude, The Netherlands

Aqueous food and beverage streams are chemically complex multicomponent systems. Their composition, particularly in volatile aroma compounds, is strongly related to their sensory properties and hence the perceived quality. If the flavor profile is distorted or imbalanced during processing, the result is the occurrence of sensory defects. A well-known sensory defect in biologically produced alcohol-free beers (AFBs) is the over-perception of wort aroma due the presence of high concentrations of Strecker aldehydes and absence of fruity ester compounds. Considering the sensitivity of the AFB matrix and the complexity of its aroma profile, the reduction of wort flavors requires high selectivity at mild operation conditions, desirably with low capital investment and operational costs.

Zeolites are readily available, inexpensive micro-porous materials that function as molecular sieves and are therefore excellent adsorbents to remove small volatile molecules from relative complex mixtures, without further altering the product composition and hence quality. Despite their already proven applications in other industries, their use in food processing has yet to be established.

In this work, the concept of wort flavor capture from AFBs on molecular sieves was investigated with the focus on the multicomponent interplay of aroma molecules as well as mass transfer (limitations) during the process. For this purpose, thermodynamic equilibrium data of selected zeolite adsorbents was directly determined in the real process stream. Consequently, the impact of different beer constituents on the thermodynamic model parameters was tested to determine process robustness and bottlenecks. To design an industrial scale process, the mass transfer of Strecker aldehydes during the capturing step was investigated in detail to describe the process by means of a mass transfer model. This will enable the design of a new processing operation, facilitating the production of flavor-controlled alcohol-free beers.

Membrane adsorber technology for GMP-conform manufacturing of bacterial polysialic acid

Bastian Bartling[1], *Daniel Boßmann*[1], *Sascha Beutel*[1], *Thomas Scheper*[1]

[1]*Institute of Technical Chemistry, Leibniz University Hannover, Hannover, Germany*

Polysialic acid (PolySia) is a homopolymer consisting of α-(2,8)- or α-(2,9)-linked N-acetylneuraminic acid monomers which is present in prokaryotic cells as well as vertebrates and humans. In the latter case, the polysaccharide participates in the development of the central nervous system and mediates cell-cell-interactions. Due to its natural properties and functions, polySia is a promising molecule for application in vaccine development. Furthermore, it provides potential for treatment of inflammatory neurodegenerative diseases such as age-related wet macular degeneration.

Biological compounds such as polySia intended for medical application have to fulfill high quality and purity standards according to mandatory regulations of good manufacturing practice (GMP). Reported production processes for polySia are based on single-use technology and use precipitation and adsorption methods for removal of impurities such as proteins, DNA and most importantly endotoxins. However, a recently developed production process includes harsh conditions which might affect the product quality or methods that do not comply to GMP-regulations.

The current work focuses on application of different chromatographic methods for GMP-conform production and purification of α-(2,8)-polySia from *Escherichia coli* K1. Therefore, experiments to optimize buffer, salt and pH-conditions for efficient separation of polySia were carried out using the strong anionic exchange membrane adsorber Sartobind® Q75. The anionic exchanger was then combined with hydrophobic interaction chromatography using Sartobind® Phenyl membrane adsorber. Both chromatographic methods were then implemented in an alternative manufacturing process and compared towards the common manufacturing process regarding product yield and quality.

Substitution of heparin for purification of cytokines

Svenja Nicolin Bolten[1], *Ursula Rinas*[1], *Thomas Scheper*[1]

[1]*Institute of Technical Chemistry, Leibniz University Hannover, Hannover, Germany*

Heparin is a highly sulfated polysaccharide. This glycosaminoglycan is involved in various important biological activities. The inhibition of the coagulation cascade to maintain the blood flow in the vasculature belongs to heparin's major biological purposes. These properties are employed in therapeutic drugs. Therefore, heparin is used in therapeutic applications as anticoagulant specially to cure and prevent thrombosis. These activities are associated with heparin's interaction to diverse proteins.

The structural heparin-protein interactions are not completely understood. An understanding of the heparin-protein interactions at the molecular level is not only advantageous in the therapeutic application but also in the biotechnological application since heparin is also used in downstream processing. The application of heparin affinity chromatography is successfully used for protein purification. While effective, heparin as an animal-based material is subject to strict quality controls and the validation of effective GMP implementation. Therefore, alternatives to the heparin affinity chromatography are extremely interesting to process developers.

The basic fibroblast growth factor (bFGF) was recombinant produced in *E. coli* in a fed-batch cultivation. The already established purification process of this cytokine consists of a cation exchange chromatography followed by the heparin affinity chromatography. Different animal-component free purification methods (resin columns and membrane adsorbers) were tested to replace the heparin affinity chromatography. The final aim is the establishment of an animal-component free purification method consisting of different chromatography techniques to purify heparin-binding proteins.

Impact of cultivation conditions on the chromatographic retention behavior of host cell proteins and target proteins in plants

Jan Hübbers[1], *Matthias Knödler*[1]

[1]*Fraunhofer IME, Aachen, Germany*

Compared to mammalian systems, the production of recombinant proteins in plants benefits from an increased product safety, a cost efficient upstream production and the potential for very-large-scale manufacturing. Numerous biopharmaceuticals, such as ELELYSO (Gaucher's disease) or the HIV monoclonal antibody 2G12, have been produced in plants and entered clinical trials. However, GMP regulations for whole transgenic plants were developed only recently and withhold the progress possible for the plant biotechnology sector due to uncertainties in batch-to-batch consistency when it comes to industrial applications. Even so, a precise characterization and identification of the critical quality attributes (CQA) of whole plant production platforms can inspires confidence. The downstream processing (DSP) of plant derived proteins remains challenging and cost-intensive since the removal of abundant host cell proteins (HCPs) such as ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is necessary after extracting the product from the plant tissue. Our work focused on the impact of different plant species and cultivation conditions on the binding behavior of HCPs and target proteins towards chromatographic resins. Possible reasons for a different adsorption/desorption behavior of HCPs or the target protein itself to chromatographic-resins can be altered post translational modifications which change the protein surface properties. Furthermore, the presence of plant-associated proteases in the feed stream during DSP may have an impact on the HCP composition and the target protein itself.

First, we analyzed the impact of different Tobacco species and cultivation setups on the binding behavior of several selected HCPs using anion exchangers. HCPs were purified via the same DSP routine and used for gradient elution experiments. The results were then compared to evaluate differences in the binding and elution behavior. Furthermore, we analyzed the impact of weather changes and harvest time point on the yield of a target protein after affinity chromatography.

Session III: Process Development – Biopurification III

Chair: Deborah Gernat

13:00	Extraction and isolation of valuable targets of saffron	*Mohsen Fotovati*
13:20	Targeted production and purification of the antiviral peptides Labyrinthopeptin A1 and A2 by Quality-by-Design approaches	*Jonas Lohr*
13:40	Automated End-to-End Integrated Manufacturing of an Antibody	*Sebastian Vogg*
14:00	Production of recombinant fibrinogen gamma chain for 3D-Bioprinting and Tissue Engineering	*Pia Gellermann*
14:20	Purification of three halohydrindehalogenases and determination of their SMA Parameters	*Catherine Mueschen*

Extraction and isolation of valuable targets of saffron

Mohsen Fotovati[1], *Mohammadreza Taheri*[2], *Alireza Ghasempour*[2], *Andreas Seidel-Morgenstern*[1,3]

[1]*Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany*

[2]*Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran*

[3]*Otto-von-Guericke University, Magdeburg, Germany*

Saffron is derived from the flower of Crocus Sativus and the most expensive spice in the world. In recent years, the pharmaceutical industries have recognized the importance of valuable components of saffron, most notably picrocrocin, various crocins and saffranal. These components can induce beneficial effects when applied as anti-depressants, anti-oxidants and in cancer therapy [1, 2, and 3]. Extraction and purification of these valuable ingredients have so far not been investigated applying engineering optimization methods.

Our contribution aims to design feasible and optimized methods for the extraction and purification of picrocrocin, crocins from Crocus Sativus. Combining experimental and simulation results, in a first project period we designed a multi-stage counter current solid-liquid extraction process. This process enables us to provide the feed for the planned development of subsequent chromatography and crystallization based processes for the isolation and purification of the target ingredients of saffron. In this direction we will discuss first evaluations of possible scenarios of chromatographic purification considering batch preparative chromatography and continuous chromatography exploiting stepwise gradients.

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Ref. [2] Papandreou M.A, Tsachaki M., Efthimiopoulos S., Cordopatis P., Lamari F.N, Margarity M.(2011). Behavioural Brain Research. 219: 197–204

Ref. [3] Bolhasani A., Bathaie S.Z., Yavari I., Moosavi-Movahedi A.A. and Ghaffari M.(2005). Asian J. Chem. pp 725-729.

Targeted production and purification of the antiviral peptides Labyrinthopeptin A1 and A2 by Quality-by-Design approaches

Jonas Lohr[1], *Jan-Hendrik Grosch*[1], *Antje Spieß*[1]

[1]*Technische Universität Braunschweig, Braunschweig, Germany*

Recently, peptide drugs have steadily increased to become the most important group of biopharmaceuticals behind monoclonal antibodies. While large biomolecules (> 10 kDa) usually offer several advantages such as high specificity and potency, their complex structure causes difficulties such as high production costs or low metabolic stability compared to small molecules (< 0.5-1 kDa). Medium-sized drugs like peptides (> 1 kDa and < 10 kDa), however, potentially combine the advantages of both. Peptide drugs offer comparable target specificity and potency to large biomolecules, but are smaller in size, more accessible and often metabolically and physically more stable.

Lantipeptides are a promising group of post-translationally modified peptides with a characteristic polycyclic structure. The majority of those lantipeptides is produced by morphologically challenging organisms. The lanthipeptides Labyrinthopeptin A1 and A2 produced by the filamentous bacterium *Actinomadura namibiensis* has already shown high potencies against retroviruses *in vitro* making them suitable candidates for future peptide drugs. However, high batch variations during cultivations as well as varying impurity profiles result in unreliable composition of the final product.

To react individually on variation within the cultivation and guarantee consequently process performance at any time, good understanding of the peptide properties, the up- and downstream processing is essential. Here, a systematic Quality-by-Design approach for the production of Labyrinthopeptins A1 and A2 is proposed in order to forecast metabolic profiles and broth composition depending on cultivation conditions and to adequately adjust the downstream processing. Structured analysis of the high number of potential variables is applied by Design of Experiments in combination with high-throughput process development. Mechanistic models of the purification process will help to assemble a flexible downstream processing to assure final product quality in given ranges. Thus, this integral process evaluation will help to develop model structures combining up- and downstream of lantipeptides providing reliable product quality and process performance.

Automated End-to-End Integrated Manufacturing of an Antibody

Sebastian Vogg[1], *Moritz Wolf*[1], *Fabian Feidl*[1], *Nicole Ulmer*[1], *Ruben Wälchli*[1], *Massimo Morbidelli*[1]

[1]*ETH Zurich, Zurich, Switzerland*

Recent years have witnessed an increased interest in continuous and integrated manufacturing of biopharmaceuticals. This is due to increasing cost pressure in the industry as well as diversifying pipelines asking for more flexible production concepts. With respect to upstream processing, perfusion cell cultures have emerged as the primary mode of operation, allowing more homogeneous product quality when operated at steady state. In downstream processing, multi-column capture processes such as the CaptureSMB were established to decrease cost of goods by increasing capacity utilization. Subsequent to protein A capture, a discontinuous feedstream is generally encountered for viral inactivation as well as polishing. Therefore, batch processes remain a suitable mode of operation. In this respect, frontal analysis is seeing increased interest as it enables simpler straight-through processing. While in a purely sequential operation, pooling between these unit operations removes process variations, integration requires the capability to respond to process disturbances to allow consistent process performance and product quality.

In this contribution, we present an automated end-to-end integrated platform for the production of an antibody. The process consists of a perfusion bioreactor, a continuous protein A capture step, which is followed by low-pH virus inactivation, and frontal and flow-through chromatographic steps for final polishing. Automated at-line HPLC systems equipped with protein A and size exclusion columns give insight into critical process parameters without the necessity of manual sampling. The platform is capable to respond to process disturbances, e.g. titer variation in the bioreactor harvest, by the introduction of a supervisory control layer enabling information exchange between the unit operations and adequate adjustment of process parameters.

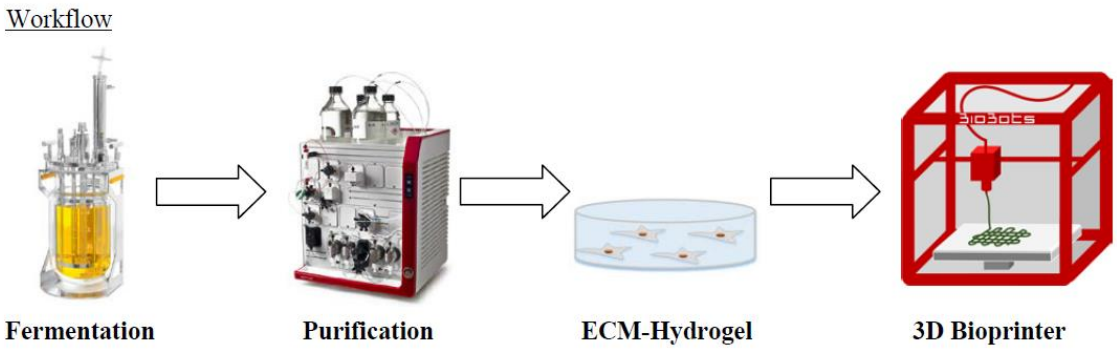
Production of recombinant fibrinogen gamma chain for 3D-Bioprinting and Tissue Engineering

Pia Gellermann[1], *Iliyana Pepelanova*[1], *Thomas Scheper*[1]

[1]*Institute of Technical Chemistry, Leibniz University Hannover, Hannover, Germany*

Natural proteins like fibrinogens are common in clinical research, where they are prized for their biocompatibility and bioactivity, which enables cell adhesion, as well as matrix remodeling by cells. Usually fibrinogen is sourced from blood sources, which bear the risk of pathogenic transmission and suffer from batch-to-batch product quality.

A recombinant production in *E.coli* circumvents these disadvantages by providing consistent quality in animal-free media. A fibrinogen gamma chain was cloned in *E.coli* under the control of the *lac* promotor. A producing clone was selected and cultivated at the 10 l scale. The protein was produced by inclusion bodies and the yield was 5 g/L. Purification of the target was performed by affinity chromatography (column and membraneadsorber). The FGG protein was tested for biocompatibility and cell adhesion in cell culture. It supported cell growth and its performance was indistinguishable from animal-derived gelatin. The collagen-mimetic protein represents a swift strategy to produce recombinant and human-based extracellular matrix proteins for various biomedical applications.



Purification of three halohydrindehalogenases and determination of their SMA Parameters

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Keywords**:** Downstream processing, Halohydrindehalogenases, steric-mass action model, chromatography

Purification of non-tagged recombinant proteins often is a challenging task and regularly requires a lot of experimental effort. Prediction of protein binding and elution can reduce this effort. The steric mass action model can be used for prediction of protein binding to anion exchangers. Protein structure and amino acid sequence are main impact factors on protein ligand interaction and therefore also on SMA parameters. In order to examine the influence of sequence varieties on SMA parameters, a high structural resemblance and low sequence identity is advantageous. Short chain dehydrogenases/reductases (SDRs) typically show a high structural resemblance despite low sequence identity. Halohydrindehalogenases (HHDHs) catalyze the dehalogenation of vicinal haloalcohols to corresponding epoxides and belong to the SDR superfamily.

Here we have expressed and purified three HHDHs (HheA2, HheB2 and HheC) for SMA parameter determination. HheA2 and HheC have similar structures despite a sequence identity of 33%. The structure of HheB2 differs more compared to HheA2 and HheC but still shows a high resemblance. Due to the high structural resemblance but low sequence identity, HheA2, HheB2 and HheC are suitable candidates as model proteins for determination of SMA parameters.

Session IV: Process Development – Continuous Chromatography

Chair: Johannes Schmölder

15:10	Evaluation of continuous chromatographic purification of extracellular vesicles	*Mafalda G. Moleirinho*
15:30	Monoclonal antibody purification using a self-established periodic counter-current chromatography device	*Chantal Brämer*
15:50	Continuous fractionation of nanoparticles by magnetic field controlled multi-column chromatography	*Carsten-Rene Arlt*
16:10	Continuous Centrifugal Extraction – A new Apparatus for Intensifying Extraction Processes	*Felix Funke*

Evaluation of continuous chromatographic purification of extracellular vesicles

Mafalda G. Moleirinho[1], *Ricardo J. S. Silva*[1], *Manuel J. T. Carrondo*[1], *Paula M. Alves*[1,2] *and Cristina Peixoto*[1]

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Extracellular vesicles (EVs) have recently been receiving increasing attention due to their potential as a novel therapeutic platform. EVs are naturally occurring nanoparticles and have been reported to play important roles in cell communication, acting as vehicles of genetic material and bioactive proteins. However, similarly to other complex biopharmaceuticals, there are still significant downstream purification challenges yet to be addressed. The current standard for the purification of a subset of EVs is differential centrifugation and density gradients. Although these methods have been used to purify other nanoparticles for vaccines applications at a commercial scale, it poses several limitations and has been gradually being replaced by other purification technologies such as chromatographic purification. In fact, recent developments in this field make use of different physicochemical properties of the EVs, such as size, charge or affinity. We report on the rational development of a continuous multi-column chromatography process aimed at the purification of EV supported by mechanistic modeling. Size-exclusion chromatography was evaluated for this purpose, as has been reported for purification of different sources of EVs, minimally altering their characteristics. The inherent potential of these class of processes to improve not only purification efficiency and economics but also product quality will be demonstrated.

Monoclonal antibody purification using a self-established periodic counter-current chromatography device

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Batch chromatography has several disadvantages, such as insufficient utilization of the resin capacity, high buffer consumption and discontinuity. Therefore and considering the high costs for downstream processing, a continuously working chromatography system was established. The basic principle of the setup is periodic counter-current chromatography (PCCC) which enables continuous operation by a sequential use of three circularly arranged columns. To achieve maximum productivity, the system is operated with membrane adsorbers instead of commonly used bed columns because in these mass transport is mainly due to convection.
In the course of work, the system has been extended to provide full functionality and flexibility, as well as the option of integrating a 4th membrane adsorber unit. The PCCC-system consists of four membrane adsorber units (4MA-PCCC). It was tested and optimized with the model proteins BSA and lysozyme.

With a global market size of 95 Billion USD in 2017, monoclonal antibodies (mAb) are the bestselling drugs on the market. They can be used for the treatment of several diseases e.g. autoimmune diseases or cancer. Therefore and in order to evaluate the potential of the PCCC-device, it was focused on affinity chromatography of a monoclonal antibody from CHO supernatant in the experiments. Protein A chromatography with Sartobind® Protein A membrane adsorber was optimized and transferred to the PCCC-device for monoclonal antibody capturing.

Continuous fractionation of nanoparticles by magnetic field controlled multi-column chromatography

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The size fractionation of nanoparticles on the basis of their size continues to be a major technical problem that has only been partially solved so far. Especially, a continuous classification of nanoparticles turns out as a great challenge. Thus, processes with meticulous demands in particle-size, such as targeted drug therapies, magnetic-particle hyperthermia or radiological imaging, are hard to realize due to economic reasons. Especially for particles below 1 µm there is no method that provides a multidimensional separation with narrow separation limits.

In this project, a magnetically controlled chromatography is used to achieve a size fractionation of nanoparticles. In this context, the interaction of three forces represents the relevant parameters for a successful separation: the hydrodynamic drag force, random diffusion force and the magnetic force. The method is implemented by means of a magnetisable chromatography matrix consisting of steel spheres which are magnetised with the aid of an external electromagnet. The presence of the chromatography matrix distorts the field to such an extent that bodies, like nanoparticles, are decelerated by the matrix. Since the effect of the magnetic force depends on size and shape of the nanoparticles, size fractionation can be achieved. We present first results of nanoparticle fractionation by magnetic chromatography and how the process will be advanced to continuous operation by applying a simulated moving bed principle, which is the actual state of the project.

Continuous Centrifugal Extraction – A new Apparatus for Intensifying Extraction Processes

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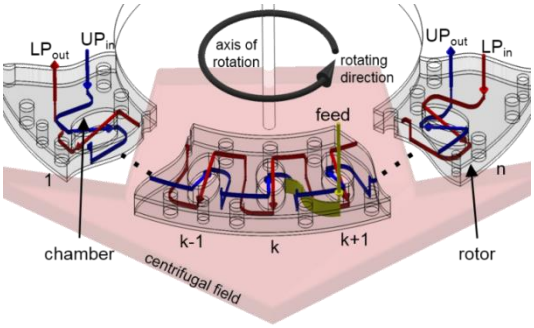
The rising demand for sustainable biochemical production increases the necessity of innovative downstream processes to become competitive with established processes.

A promising approach for sustainably purifying biotechnologically produced compounds like proteins is the application of phase systems with high biocompatibility like Aqueous Two-Phase Systems (ATPS). The major disadvantage of these phase systems is poor phase separation, which results in long phase settling times. The application of Continuous Centrifugal Extraction (CCE) overcomes this disadvantage by using a centrifugal field while supporting phase mixing at the same time.

The construction of the CCE is based on the Centrifugal Partition Chromatography (CPC), which uses a rotor that consists of several chambers in which one liquid phase is kept stationary using a centrifugal field. Contrary to CPC, in CCE, the chambers are interconnected with two ducts - one for each phase - and both phases are pumped continuously in counter-current direction through the rotor. A feed stream is applied in the middle of the chamber cascade and the components of the feed stream distribute, depending on their partition coefficient, in the upper (UP) or in the lower phase (LP), resulting in one extract phase at each end of the rotor. By the use of a rotor consisting of several chambers, a high number of separation steps can be achieved so that even a separation of components with very similar physicochemical properties is possible.



The working principle of the CCE, the visualization of the two-phase flow inside the CCE chambers, as well as the influence of volume flow, rotational speed and interval time on the fluid dynamics, will be presented.

Session V: Theoretical aspects and fundamentals – Parameter Estimation

Chair: Ivana Mutavdžin

8:30	Simplification of inverse SMA parameter determination using only gradient elution experiments	*Ronald Colin Jäpel*
8:50	Efficient isotherm estimation using neural networks for applications in SMB process design and preparative chromatography	*Stefanie Gerlich*
9:10	Modeling of anion-exchange chromatography: Influence of ligand densities on Donnan equilibrium and protein separation	*Gabriela Sánchez Reyes,*
9:30	Industrial Application of Mechanistic Chromatography Modeling	*David Saleh*
9:50	Chromatography Model Calibration with Bayesian Optimization	*Alexander Gutzler*

Simplification of inverse SMA parameter determination using only gradient elution experiments

Ronald Colin Jäpel[1]

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To simulate chromatographic retention, an equation is required that describes how strongly the molecule of interest binds to the stationary phase. The steric mass action (SMA) isotherm is the equation we use to simulate protein behavior on ion exchange chromatography columns. It contains three protein dependent parameters that have to be determined based on experimental data.

To fully utilize the isotherm this determination should take place within a modeling framework, so that subsequently the fitted parameters can be used to predict results of novel experiments. This approach is called inverse determination. However, previous inverse methods have either been computationally expensive or have required breakthrough experiments, which are experimentally expensive.

Here we propose a search algorithm that can identify the two non-steric SMA parameters keq (the equilibrium constant) and v (the characteristic charge) using only two gradient elution experiments and less than one hundred simulation steps. This facilitates the development and application of chromatography models.

Efficient isotherm estimation using neural networks for applications in SMB process design and preparative chromatography

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Preparative chromatography processes are conventionally operated in batch mode. The simulated moving bed (SMB) process, however, enables a continuous operation in which the separation efficiency can be improved. A counter-current flow of the stationary and the mobile phase is realized by periodically switching the ports of the inlets and the outlets of the individual columns. Operating parameters such as flow rates and switching time are typically designed using process models which are either based on the analogous true moving bed (TMB) process or directly consider the periodic nature of the SMB process. In all cases, model parameters need to be known a priori. While parameters describing the column packing can easily be determined, adsorption isotherm estimation usually involves time-consuming methods such as frontal analysis for example. The drawback of the frontal analysis and similar methods is that the isotherm model is fixed. In this work, a different approach is employed for the description of the adsorption behavior. A general isotherm model in form of a neural network is used in order to accelerate isotherm estimation. Here, a two-layer MLP (multi-layer perceptron) neural network (NN) with sigmoidal neurons is trained to calculate directly isotherm derivatives resulting in a higher flexibility since no specific isotherm model is assumed. The NN is trained by comparing the resulting calculated chromatogram with measured data. For a good estimation it is necessary that the experimental chromatograms contain sufficient information, that is covering the desired range of operating conditions. The choice of necessary experiments can be improved by applying design of experiments. Furthermore, the approach can easily be extended such that additional model parameters such as mass transfer coefficients can be estimated simultaneously. The resulting neural network for the isotherm model and estimated model parameters can then be used in the model based design of SMB processes.

Modeling of anion-exchange chromatography: Influence of ligand densities on Donnan equilibrium and protein separation

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Anion-exchange chromatography is an important tool in downstream processing of biopharmaceuticals. It is commonly used for the removal of fragments and/or aggregates of the target protein as well as for the depletion of nucleic acids, host cell proteins and viruses. Ligand density variations are known to influence the separation and affect process robustness. Mechanistic modeling can be an effective tool to understand these effects.

In this work, the application of a Donnan ion exchange (DIX) model to linear gradient elution (LGE) experiments was performed. This model considers differences in the concentrations of charged species between the resin and buffer phase due to the Gibbs–Donnan equilibrium. The distribution of charged species between the two phases is influenced by the ligand density of the stationary phase. The results show that by considering the Donnan effect, a global modeling of five Fractogel® EMD TMAE resins with different ligand densities is possible. Furthermore, the results demonstrate consistent accuracy of the model for three different proteins (mAb, fab fragments and BSA).

Industrial Application of Mechanistic Chromatography Modeling

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The simulation of chromatography processes is of increasing significance for the purification development of biopharmaceuticals. Experimental design, process optimization and risk assessment can be conducted *in silico*, leading to an increased process understanding while enabling savings in time and material [1, 2]. In this talk, the model calibration, scale up and *in silico* process characterization of an industrial cation exchange chromatography (CEX) step will be demonstrated.

The transport dispersive steric mass action model describing the monoclonal antibody polishing step considered multiple monomeric charge isoforms and high molecular weight (HMW) species. We simulated production scale runs on a 248 L column to reveal the scale-independent predictive power of the model. Here, the mechanistic model clearly outperformed the experimental scale down model (SDM) traditionally employed in late stage DSP development. Thus, the impact of critical process parameters on the purification outcome was investigated *in silico*. The increased process understanding helped us to explain deviations in consolidation scale experiments. Further, the found correlations may allow a significant reduction of the experimental effort in future process characterization studies.

This case study outlined the potentialities of mechanistic modeling for bioprocess development. On the other hand, we had to learn that the laborious and time-consuming model calibration represents a major bottleneck to the implementation of mechanistic modeling workflows in current development strategies. Thus, the second part of this presentation will focus on the possibility to predict adsorption isotherm parameters from structural information and protein surface properties. This approach could give first insights into the binding behavior of new biological entities before the early stage development starts. Therefore, our comprehensive study plan for building quantitative structure-activity relationship (QSAR) models for large biomolecules on CEX and mixed mode chromatography resins will be discussed.

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Chromatography Model Calibration with Bayesian Optimization

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Most mechanistic chromatography models contain parameters that are not accessible experimentally and which are therefore estimated by curve fitting. This method compares simulated chromatograms with their experimental counterparts. Non-accessible parameters in the simulation are then changed until the corresponding experiments' curves overlay. This constitutes a multivariate and non-linear optimization problem with local optima. Even though several computer algorithms can be used to facilitate such a task, model calibration is still a time-consuming process, involving experience and manual work.

With Bayesian Optimization, we introduce a new algorithm to the field of chromatography model calibration, that has previously mainly been used in machine learning applications. The algorithm works in two repeating steps. First, by utilizing all previously tested parameter sets and their corresponding objective values, a probabilistic approximation of the objective function is calculated. In a second step, this approximation is used to select the parameter set that is most likely to produce a good fit between experiment and simulation. This parameter set is then simulated, the result once again included into the approximation.

Bayesian Optimization can improve several aspects of currently used optimization algorithms. As each query to the objective function is associated with a simulation, the time per iteration is long. By reducing the number of iterations by up to factor 100, the algorithm minimizes calibration time. Currently used gradient descent algorithms often handle local minima poorly. By approximating the whole objective function, Bayesian Optimization investigates local minima while maintaining the ability to escape them. The result of the optimization in not only the best parameter set but also an approximation of the objective function. This approximation can be used for the calculation of confidence intervals.

Session VI: Theoretical aspects and fundamentals – Process Design

Chair: David Saleh

10:50	Conceptual performance modelling for novel affinity chromatography membranes	*Franziska Hagemann*
11:10	Optimisation of resin selection by correlating structural properties with model parameters	*Martin Leipnitz*
11:30	Influence of Mixed Electrolytes on HIC-Adsorption and Prediction of Chromatographic Elution Profiles	*Nadia Galeotti*
11:50	Simultaneous identification of optimal chromatographic process concepts and operating conditions	*Johannes Schmölder*

Conceptual performance modelling for novel affinity chromatography membranes

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Keywords: Membrane chromatography, affinity chromatography

Conventionally used porous particles in preparative affinity chromatography are limited by diffusive mass transfer into large particles (50-100 µm), resulting in low productivities (~70g/l.h). One strategy to enable a higher productivity of this unit operation, is the reduction of particle diameters. However, this leads to high pressure drop or consequently flat resin beds with large diameters causing problems in column packing, flow distribution and floor space. Therefore, a novel membrane based chromatographic media is investigated. Applying a high capacitive diffusive layer to the inner membrane surface, its specific surface can be significantly increased. Due to the reduced residence time, significantly higher productivities can be reached. To understand the optimal membrane and coating layer design, a model that correlates their characteristics with the overall process performance is developed. In order to obtain model parameters, as e.g. the mass transfer into the coated layer, experiments with conventional resin particles are performed. The general rate model is used to describe the transport of target molecules through the column. The model is fitted to the experimental data using the CADET software [1]. Results of the mass transfer in the pores of the resin particles are then transferred to the cylindrical shell of the porous membrane coating. By applying such a thin coating layer to a convective membrane structure, pressure drop and diffusive length can be reduced compared to conventional resins. An optimally designed coated membrane structure can combine high productivities (~ 550g/l.h) and a high accessible pore surface and thus binding capacity at low residence times.

[1] Leweke, S.; von Lieres, E.: Chromatography Analysis and Design Toolkit (CADET), Computers and Chemical Engineering 113 (2018), 274–294.

Optimisation of resin selection by correlating structural properties with model parameters

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Design of a chromatographic separation unit begins with the selection of well performing process materials for ad- and desorption processes. The chosen process materials (adsorbent and eluent) as well as the feed mixture form the so called chromatographic system. This selection of the chromatographic system is usually based on expert knowdledge or based on case studies of preselected adsorbents and eluents. Even though adsorbent and eluent influence the process performance strongly, the established methods are rather limited in selecting the best performing combination of process materials.

Model-based optimization approaches enable the possibility to add a predictive character to the evaluation and selection of chromatographic systems. In order to include these into model-based optimization approaches, mathematical correlations between adsorbent and the physical behavior depicting model parameters have to be developed.

In this contribution, first correlations between structural properties of polymeric resins with model parameters of a transport dispersive model are presented and evaluated in case studies. The separation of glucose and xylose is chosen as exemplary system, for which polymeric cation exchanger resins and the eluent water are used. Process influencing structural properties of these resins are the degree of cross-linking, ionic form and particle size.

Influence of Mixed Electrolytes on HIC-Adsorption and Prediction of Chromatographic Elution Profiles

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Proteins are often purified using chromatographic techniques in which the properties of the aqueous solvent are controlled by adding salts. The influence of the addition of single salts has been studied intensively, while much less is known on the effect of mixed salts. Therefore, a systematic study on the influence of single and mixed salts on protein adsorption was carried out using different model systems (see below). Unexpected synergetic behavior was observed.

The studied solutes are lysozyme, bovine serum albumin and poly(ethyleneglycol) (PEG). The salts are sodium chloride, ammonium sulfate, sodium sulfate, ammonium chloride and their mixtures. A 25 mM sodium phosphate buffer was used to adjust the pH value to 7. The adsorbent is Toyopearl PPG-600M, a mildly hydrophobic resin. Adsorption equilibrium isotherms were measured at 298 K with a fully automated liquid handling station and elution profiles were measured at 298 K with an Äkta chromatographic system.

A mathematical model is developed, that describes the influence of the salts on the adsorption. The model accounts for the influence of the different single ions and their cross-interactions. Using the model, adsorption isotherms can be predicted for different ionic strengths and different salts, including also salt mixtures. The model enables the identification of promising salt mixtures for chromatographic separations. Moreover, a simple equilibrium stage model was developed for the prediction of elution profiles. It enables the identification of suitable salt gradients for separations.

Simultaneous identification of optimal chromatographic process concepts and operating conditions

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Depending on the chromatographic separation problem at hand, various advanced operating concepts exist that can improve the performance beyond that of single column chromatography. The design and optimization of such processes is, however, complex. This holds in particular for the separation of multi-component mixtures that may contain more than one component of interest.

In this work, we present an optimization framework that automatically determines the optimum process concept for a given chromatographic problem. Simultaneously, process conditions such as the injection volume and cycle time are optimized. For this purpose, a superstructure is defined that incorporates numerous possibilities for the setup of chromatographic processes. This approach is highly useful in process development since there is no need to formulate individual models for every operating concept. A mixed-integer nonlinear programming (MINLP) approach is used to minimize an objective function based on e.g. specific productivity, eluent consumption, and recovery yield. In particular for complex multicomponent separation problems, this approach can identify sophisticated operating concepts that lead to chromatograms with unexpected peak shapes so that common fractionation algorithms fail here. Thus, a newly developed approach is required, capable of determining fractionation times for arbitrary chromatograms depending on the desired purity and value of the products of interest. The results are then fed back to the top-level optimization problem.

In this presentation, we will give an overview of the framework and demonstrate the capabilities of the tool for various case studies. Examples are given that demonstrate how different operating concepts are optimal depending on the contributions to the objective function, the superstructure, and the given separation problem at hand.

Session VII: Theoretical aspects and fundamentals – Simulation

Chair: Alexander Gutzler

12:40	Thermal Gradient in Preparative Liquid Chromatography	*Xinghai An*
13:00	Online monitoring and real-time prediction of product quantity, purity and potency during chromatographic purification processes	*Anna Christler*
13:20	Error modeling in Chromatography and parameter confidence	*William Heymann*
13:40	High Definition Simulation of Packed Bed Liquid Chromatography	*Jayghosh S. Rao*

Thermal Gradient in Preparative Liquid Chromatography

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Preparative chromatography has proven itself a powerful technology in various purification processes [1]. It is acknowledged that some potential strategies for improving process performance are gradient operations, e.g. solvent gradients, stationary phase gradients [2], etc. Nonetheless, researches on thermal gradients are rarely reported [3]. Temperature is highly interrelated with adsorption isotherm and transport rate thereby influencing process performance [4]. Hence, thermal gradients can be applied in retarded separations or difficult separations for optimizing productivity (in terms of cycle time) and purity (in terms of resolution). Our work is to simulate and experimentally validate the thermal gradient in these forced non-isothermal chromatographic processes. In the current stage, predictions from a simple equilibrium model will be presented. The experimental set-up is under installation and first experiments will be conducted in the near future.

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Online monitoring and real-time prediction of product quantity, purity and potency during chromatographic purification processes

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Regulatory agencies encourage pharmaceutical industry to implement Quality-by-Design approaches into manufacturing processes. We integrated a battery of online sensors into a chromatographic workstation for monitoring of loading and elution to be used as process analytical technology. Additional to the standard online detectors measuring UV/Vis absorption, pH and conductivity, ATR-FTIR, fluorescence, refractive index and light scattering sensors were implemented. These online measured signals are used as predictors in statistical models and were correlated with offline data for quantity, purity and potency to generate predictive models. Time and labour intense offline analytics were semi-automated to reduce both inter-assay variance and manual analytical workload. Efficient procedures for variable selection were needed and developed due to the large amount of data generated. The generated models enable controlled loading and product pooling based on the defined quality criteria. A major benefit of the system is that fractionation and offline analytics can be omitted after model set-up. Thus hold times between unit operations are reduced or even eliminated. Batch-to -batch variations can be detected in real time and batch failure is reduced by real-time process control. The extension of the system by mechanistic process models will further increase robustness and predictive power.

Error modeling in Chromatography and parameter confidence

William Heymann[1], *Eric von Lieres*[1]

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Once a chromatography model has been fit it is important to know how well defined the model fit is. Many parameters go into a model and some are better defined than others. For sensitive parameters, the more well defined a parameter is the more predictive a model is. To know how well defined your parameters are you must first know the impact of errors on your system. This particularly difficult in chromatography. Most of the errors in chromatography are not normal and randomly distributed. With typical chromatography errors such as pump delays, baseline drift, and pump flow rates are all systemic. To deal with this problem we present here a method to build an error model for chromatography and then using this error model along with Markov chain Monte Carlo (MCMC) for evaluating parameter credibility. The method presented here is based on the CADET-Match framework presented last year at SoCSS 2018 which uses the Chromatography Analysis and Design Toolkit (CADET) (Leweke et al., 2018). In this presentation we also compare parameter credibility obtained using an error model vs the standard assumed random and normally distributed error model.

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High Definition Simulation of Packed Bed Liquid Chromatography

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Packed bed chromatography is conventionally being simulated using methods such as the General Rate Model. These semi-empirical low-dimensional models provide quick and accurate solutions, but assume homogeneity in the packing structure and bead sizes. They also assume radial homogeneity within the beads, which allows reduction in the problem's dimension. In general, bead sizes in chromatography columns are not monodisperse unless carefully controlled. However, varying bead sizes and shapes, and the locations of differently sized beads within the column do affect the column performance. This, along with wall effects in miniaturized columns, also creates inhomogeneity along the radial column dimension. Conventional models cannot capture the effects of bead size, shape and distribution on the resulting chromatograms. Hence, we apply spatially resolved high definition simulations of chromatography columns for quantitatively studying these effects in detail. As spatially resolved chromatography simulations are extremely costly, the gained insights may ultimately be used to improve conventional models to obtain quick and more accurate results.

We use a space-time finite element method with a discontinuous Galerkin approach for time stepping to model packed bed chromatography columns. In our setup, a Stokes flow simulation is weakly coupled to an advection-diffusion-reaction simulation, both of which are solved using XNS, a fluid flow solver developed at CATS, RWTH Aachen. In this study, we perform mesh sensitivity analysis for various meshes in XNS and compare the results with a finely meshed setup solved with COMSOL. We then solve large-scale monodisperse and polydisperse packings and investigate individual bead loading for various positions and bead sizes. Finally, the large-scale simulation results are compared with results of the General Rate Model.

List of Participants

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