SOCSS 2022 27-29 JUNE ETTLINGEN, KARLSRUHE

17TH INTERNATIONAL SEMINAR ON CHROMATOGRAPHIC SEPARATION SCIENCE

BOOK OF ABSTRACTS

Karlsruher Institut für Technologie

SoCSS 2022

OVERVIEW

MONDAY, JUNE 27

13:00 – 17:30	Workshops
17:30	Welcome Reception & Dinner (sponsored by Boehringer Ingelheim) Foyer & Restaurant Radisson Blu

TUESDAY, JUNE 28

8:30 – 17:15	Seminar Sessions 1-4
18:30	Conference Dinner (sponsored by Cytiva) Watt's Brasserie, Pforzheimer Str. 67, 76275 Ettlingen Bus transport: Meet at 18:00 in front of hotel

WEDNESDAY, JUNE 29

8:30 – 12:30	Seminar Sessions 5-6
12:30	Farewell Lunch

SCIENTIFIC PROGRAM

Session 1: Continuous processing and liquid-liquid chromatography

Page	Title	Presenter
	Welcome & opening	Organizing committee
(9)	Centrifugal partition chromatography: Optimization of long-term operability and implementation of additive manufacturing approaches	Felix Buthmann
(10)	Design of a twin-countercurrent purification (MCSGP) unit for the polishing of an oligonucleotide sequence	Ismaele Fioretti
(12)	Novel biphasic solvent system with bio-based and green solvents for the purification of hydrophobic biocomponents with liquid-liquid chromatography	Vanessa Buchweitz
(13)	Study of liquid-liquid chromatography operation in the nonlinear range of the solute distribution equilibria	Melanie Gerigk

Session 2: Membrane chromatography

	Online LC: How to get biologics faster to market [Sponsoring Talk]	Agilent
(17)	A capture step in antibody purification using a novel protein A coupled membrane adsorber prototype	Nils Gehrmann
(18)	Double flow-through application of membrane adsorbers for intensified purification of monoclonal antibodies	Fabian Schmitz
(19)	Novel hydrogel-based ion exchange membrane	Katharina Maria Thien
(20)	Development of an electrosorptive membrane chromatography process	Dennis Röcker

SCIENTIFIC PROGRAM

Session 3: Novel product types

Session 4: Processing of particles and polymers

SCIENTIFIC PROGRAM

Session 5: Mechanistic modeling I

Page	Title	Presenter
	Welcome & Opening	Organizing committee
(38)	An integrated multistep purification approach including online monitoring [Sponsoring Talk]	Novo Nordisk
(40)	Influence of salt selection onto cation-exchange chromatography of proteins	Thomas Fuchs
(41)	Development of a digital twin for a multimodal chromatography resin	Tim Ballweg
(42)	On the resurgence of colloidal chromatography models	Lena Enghauser
(44)	Mechanistic description of pH and ionic strength values in chromatography simulations	Alexander Gutzler

Session 6: Mechanistic modeling II

	SEC-MALS analysis of biotherapeutics: what it offers and what it demands [Sponsoring Talk]	Tosoh
(46)	Bayesian optimization using multiple directional objective functions allows the rapid inverse fitting of parameters for chromatography simulations	Ronald Jaepel
(47)	Integrated process model for the prediction of biopharmaceutical manufacturing chromatography and adjustment steps	Federico Rischawy
(49)	Method development for mechanistic modeling of mixed-mode antibody purification	Rudger Hess
(51)	Hybrid process modelling combining mechanistic equations with machine learning	Johannes Schmölder

BASF

We create chemistry

In times of rapid change, companies need to look at how their work not only adapts but also actively contributes to a better world. At BASF, every team and every individual has a role to play, and everyone contributes to our global mission: to provide innovative, clean and efficient solutions for a better tomorrow. The world needs solutions. We need you.

Are you up for the challenge?
Visit basf.com/career

avantor™

Entdecken Sie unsere Chromatographie-Webinare

Unsere informativen Webinare führender Experten bieten Ihnen Einblicke in die neuesten Chromatographie-Trends und nützliche Informationen für Ihre Applikationen. Hatten Sie noch keine Gelegenheit an unseren Webinaren teilzunehmen? Kein Problem, Sie können diese als Aufzeichnung jederzeit abrufen.

https://de.vwr.com/cms/webinar_chromatography

TUESDAY, JUNE 28
Session 1: Continuous processing and liquid-liquid chromatography

8:30	Welcome & opening	Organizing committee
8:40	Centrifugal partition chromatography: Optimization of long-term operability and implementation of additive manufacturing approaches	Felix Buthmann
9:00	Design of a twin-countercurrent purification (MCSGP) unit for the polishing of an oligonucleotide sequence	Ismaele Fioretti
9:20	Novel biphasic solvent system with bio-based and green solvents for the purification of hydrophobic biocomponents with liquid-liquid chromatography	Vanessa Buchweitz
9:40	Study of liquid-liquid chromatography operation in the nonlinear range of the solute distribution equilibria	Melanie Gerigk

Centrifugal Partition Chromatography: Optimization of long-term Operability and Implementation of Additive Manufacturing Approaches

Felix Buthmann, Philip Laby, Djamal Hamza, Jörg Koop, Gerhard Schembecker
Laboratory of Plant and Process Design, Department of Biochemical and Chemical Engineering, TU Dortmund University, Dortmund, Germany

The rising demand for effective and sustainable biochemical production increases the necessity of innovative downstream processes to provide the amounts of highly pure components required.

One of these promising downstream processes is Centrifugal Partition Chromatography (CPC). In contrast to conventional chromatography techniques, CPC uses a liquid as stationary phase. Thus, the separation is not based on solid-liquid equilibrium but on liquid-liquid equilibrium, allowing for higher volume specific loadings. From the liquid-liquid system used, one phase serves as stationary phase immobilized in the apparatus by centrifugal force, while the other phase is pumped through the former as the mobile phase (cf. Figure 1). The dispersion in each chamber is one of the decisive factors for the separation performance, but sufficient stationary phase retention is essential for the apparatus to work.

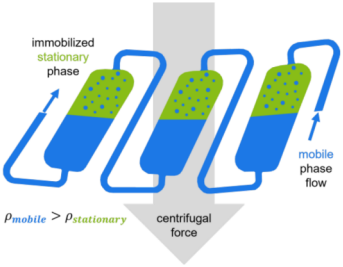


Figure 1: CPC operating in descending mode.

Due to non-complete coalescence of the dispersion at the chamber's outlet, however, stationary phase continuously drains from the apparatus, so-called *bleeding*. This limits the long-term operability of the chromatograph since efficient operation of the apparatus requires a constant phase volume ratio. Our goal is not only to prevent but actively counteract bleeding. Therefore, we developed a strategy of repeated redosing of stationary phase during operation.

Besides these results, we will show the advantages of 3D printed rotors in centrifugal partition chromatography. In addition to the complete transparency and thus decisively improved raw data acquisition, low costs, and an optimized prototyping workflow due to significantly shorter manufacturing times will also be highlighted in particular.

DESIGN OF A TWIN-COLUMN COUNTERCURRENT PURIFICATION (MCSGP) UNIT FOR THE POLISHING OF AN OLIGONUCLEOTIDE SEQUENCE

Ismaele Fioretti[1]
Thomas Müller-Späth, YMC ChromaCon[2]
Richard Weldon, YMC ChromaCon[2]
Sebastian Vogg, YMC ChromaCon[2]
Massimo Morbidelli, Politecnico di Milano[1]
Mattia Sponchioni, Politecnico di Milano[1]

[1] *Politecnico di Milano, Milan, Italy*

[2] *YMC ChromaCon, Zurich, Switzerland*

Oligonucleotides (ONs) are finding growing attention as a novel class of biopharmaceuticals due to the different clinical indications they are suitable for, as a result of their ability to regulate the gene expression[1-4]. The bottleneck in the manufacturing pipeline of these new substances is represented by the downstream processing, which is still anchored to discontinuous single-column chromatographic processes. This work aimed at developing a successful multicolumn countercurrent solvent gradient purification (MCSGP) with anion-exchange resins of a ssDNA sequence. The MCSGP is a continuous chromatographic process consisting of a complex system of valves and capillaries connecting two columns, whose positions are periodically switched to perform the simulated moving bed flow, opposite to the eluent stream direction[5,6]. The set-point for the MCSGP was obtained from an optimal batch chromatogram of the target product. Here, we exploited the competitive adsorption of the different species in the mixture to maximize the process performances. Therefore, after having obtained a good set-point in batch mode, we demonstrated, under comparative conditions, the potential of MCSGP in improving yield and productivity while reducing the consumption of buffer, in the direction of a lowered environmental footprint of the process, expressed in terms of process mass intensity (PMI).

References

1. Gold L. 1995. Oligonucleotides as research, diagnostic, and therapeutic agents. J. Biol. Chem.

2. Bilanges B, Stokoe D. 2005. Direct comparison of the specificity of gene silencing using antisense oligonucleotides and RNAi. Biochem. J. 388:573–583.

3. Chen X, Dudgeon N, Shen L, Wang JH. 2005. Chemical modification of gene silencing oligonucleotides for drug discovery and development. Drug Discov. Today 10:587–593.

4. Gooding M, Malhotra M, Evans JC, Darcy R, O'Driscoll CM. 2016. Oligonucleotide conjugates – Candidates for gene silencing therapeutics. Eur. J. Pharm. Biopharm. 107:321–340.

5. Aumann L, Morbidelli M. 2007. A continuous multicolumn countercurrent solvent gradient purification (MCSGP) process. Biotechnol. Bioeng. 98:1043–1055.

6. Aumann L, Morbidelli M. 2008. A semicontinuous 3-column Countercurrent Solvent Gradient Purification (MCSGP) process. Biotechnol. Bioeng. 99:728–733.

Novel Biphasic Solvent System with Bio-based and Green Solvents for the Purification of Hydrophobic Biocomponents with Liquid-Liquid Chromatography

Vanessa Buchweitz, Mirjana Minceva

Biothermodynamics, TUM School of Life Sciences, Technical University Munich, Freising, Germany

Liquid-Liquid Chromatography (LLC) combines the selectivity of liquid-solid chromatography with the high loading capacity of liquid-liquid extraction. The main feature of LLC is that both the stationary and mobile phase are liquid. The phases are prepared by mixing 3 or more solvents to obtain a pre-equilibrated biphasic solvent system. The separation is based on the different elution times which result from the different distribution of the target compounds and impurities in the biphasic solvent system (1). For the isolation of a compound with LLC a suitable biphasic solvent system is necessary.

Most used solvent systems in LLC contain environmentally unfriendly and/or harmful solvents, like hexane. In order to develop an environmentally friendly separation process for hydrophobic biocomponents with LLC these solvents will be replaced with bio-based and/or green solvents in this work. To determine if and at what compositions the mixtures of 3 or more solvents result in a biphasic system the liquid-liquid equilibrium (LLE) data is needed. For systems containing bio-based and green solvents this LLE data is mostly not available. Since the experimental screening of solvent systems is very time consuming the LLE data was first predicted using the thermodynamic model COSMO-RS. The obtained LLE data was used to predict the partition coefficient of the three hydrophobic target compounds (ß-Ionone, 2-Phenylethanol and Cembratrienol) in these systems. In order to estimate the accuracy of the predictions the partition coefficients in suitable solvent system compositions were determined experimentally with shake-flask experiments.

1. Morley R, Minceva M. Liquid–Liquid Chromatography: Current Design Approaches and Future Pathways. Annual review of chemical and biomolecular engineering. 2021;12:495-518.

Study of Liquid-Liquid Chromatography Operation in the Nonlinear Range of the Solute Distribution Equilibria

*Melanie Gerigk[1], Gergő Dargó[2], Árpád Könczöl[2], Simon Vlad Luca[1], Mirjana Minceva[1]**

[1]Biothermodynamics, TUM School of Life Sciences, Technical University of Munich, 85354 Freising, Germany

[2]RotaChrom Technologies LLC, H-1054, Budapest, Hungary

**Correspondence address: mirjana.minceva@tum.de*

Liquid-liquid chromatography (LLC) is a powerful separation technique with increasing popularity. Mostly, biphasic liquid systems composed of two to four solvents are used as mobile and stationary phases, in which separation is achieved through different distribution of the mixture components between the two phases. However, the operation at high feed concentrations and consequently in the nonlinear range of the distribution equilibria may lead to undesirable effects such as stationary phase loss due to the disturbance of the thermodynamic equilibria of the solvent system and change of the physical properties of the two phases (density, viscosity and interfacial tension), challenging the prediction of the elution profiles. In this work, the influence of the feed concentration on the elution profiles of the model component cannabidiol (CBD) was studied in three different LLC units ranging from 18.2 mL to 2.1 L volume. First, the CBD distribution equilibrium in the solvent system composed of *n*-hexane/methanol/water 5/4/1 (v/v/v) was determined by shake-flask experiments, showing a concave shape for the CBD distribution isotherm. Then, a series of pulse injections with CBD concentrations varying from 1 to 312 mg/mL was performed with the same solvent system in descending mode. The elution profiles were simulated using the equilibrium cell model and an anti-Langmuir-type of equation for describing the distribution equilibria isotherm. The model parameters were estimated from the CBD elution profiles using the peak fitting method [1, 2]. The model was validated and has shown to provide a good prediction of the CBD elution profiles in the entire concentration range.

[1] Martin and Synge 1941. Biochem. J. 35, 1358–1368.

[2] Seidel-Morgenstern 2004. J. of Chrom A. 1037, 255-272.

Rentschler Biopharma

SCHREIBEN SIE IHRE GESCHICHTE MIT UNS!

Laupheim, DE
Milford, USA
Stevenage, UK

1.100
Kollegen/-innen

Zukunfts-
sichere
Branche

Nachhaltig
wirksam

Familienunter-
nehmen

Rentschler Biopharma ist ein führendes Auftragsentwicklungs- und Produktionsunternehmen (CDMO) für Biopharmazeutika. Unsere Stärke: Die Bioprozessentwicklung und die Herstellung hochwertiger Biopharmazeutika sowie damit verbundene Beratungsleistungen einschließlich Projektplanung und regulatorische Unterstützung.

Was uns vereint, ist die Leidenschaft für das was wir tun: Nachhaltig Nutzen stiften.

WIR STELLEN EIN:
BTA/ CTA/ UTA/ Biotechnologen/ Chemiker,
aber auch Quereinsteiger wie Brauer/ Mälzer/
Lebensmitteltechnologen.

**BEWERBEN SIE SICH JETZT,
UM UNSER TEAM ZU VERSTÄRKEN:**

WWW.RENTSCHLER-BIOPHARMA.COM

Rentschler Biopharma SE
Erwin-Rentschler-Str. 21 · 88471 Laupheim · www.rentschler-biopharma.com

EY Entrepreneur Of The Year
Winner 2019

Deutschlands begehrteste Arbeitgeber

Keep Pace with Your Processes

Agilent 1260 Infinity II Prime Online LC System

Designed as a process analytical technology (PAT) tool for automated process monitoring, the 1260 Infinity II Prime Online LC System provides real-time data for greater control and faster understanding of your processes.

Agilent InfinityLab

Multiple detection options

Choose from a variety of detectors, including UV and single quadrupole LC/MS.

Versatile column handling

Apply high adaptability and temperature stability to the most complex analyses.

Reliable solvent delivery

Benefit from flow rates up to 5 mL/min within a pressure range of up to 800 bar.

Agilent 1260 Infinity II Online Sample Manager

Includes an external sampling interface to simplify connection between the analytical and process worlds in PAT applications.

Supports not only direct injections for real-time monitoring, but also vial sampling to retain process samples.

Automate your process analysis

Agilent Online LC Monitoring Software coordinates sampling and sample analysis, and provides all the tools required for process monitoring.

Advancing process quality

The 1260 Infinity II Prime Online LC system supports design, analysis, and control of processes through real-time measurement of CPPs and CQAs, contributing towards quality by design (QbD).

Learn more **www.agilent.com/chem/online-lc**

DE44318.3856944444

Published in the USA, May 11, 2021
5994-3552EN

Agilent

TUESDAY, JUNE 28
Session 2: Membrane chromatography

10:30	Online LC: How to get biologics faster to market [Sponsoring Talk]	Agilent
10:45	A capture step in antibody purification using a novel protein A coupled membrane adsorber prototype	Nils Gehrmann
11:05	Double flow-through application of membrane adsorbers for intensified purification of monoclonal antibodies	Fabian Schmitz
11:25	Novel hydrogel-based ion exchange membrane	Katharina Maria Thien
11:45	Development of an electrosorptive membrane chromatography process	Dennis Röcker

A capture step in antibody purification using a novel protein A coupled membrane adsorber prototype

Nils Gehrmann[1,2], *Florian Taft*[*1], *Rainer Hahn*[2], *Volkmar Thom*[1]

[1] *Sartorius Stedim Biotech GmbH, Goettingen, Germany*
[2] *University of Natural Resources and Life Sciences, Vienna, Austria*

A capture step using protein A as an affinity ligand is a widely used unit operation in the downstream processing of monoclonal antibodies (mAbs). Due to its high selectivity towards mAbs, a main application of protein A chromatography is the isolation of mAbs from cell-culture supernatant (CCS).

Packed porous beads are the most established separation matrices. However, mass transfer is strongly limited by diffusion into the beads resulting in long residence times and low productivities. Alternatively, ligands can be coupled to membrane adsorber matrices, where the mAb reaches the ligand by convection only, leading to high mass transfer rates and short residence times. However, in order to achieve acceptable levels of binding capacity for mAbs, the convective pore size of these purely convective media needs to be relatively small, leading to low membrane permeability, high pressure drops, limited scalability and high fouling propensity (see Fig. 1).

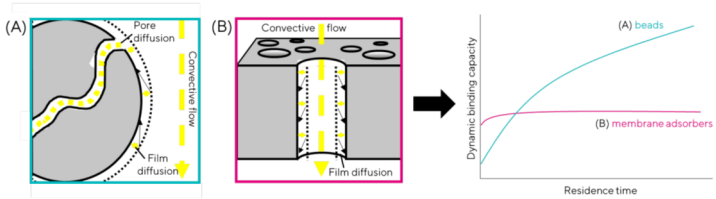


Figure 1: Schematic presentation of different principles of mass transfer in (A) beads and (B) membrane adsorbers and corresponding courses of dynamic binding capacity at varying residence times.

In this talk, a novel hydrogel-based protein A membrane adsorber prototype is introduced which offers fast mass transfer and high productivity by exhibiting short diffusional distances into a functionalized hydrogel. Convective pores remain large (~ 5 µm) leading to high permeability, low fouling propensity as well as full scalability. We will present its performance characteristics including binding capacities and levels of impurity removal investigated in cycling studies using various mAbs.

Double Flow-through application of membrane adsorbers for intensified purification of monoclonal antibodies

Fabian Schmitz[1,2], Thomas Kruse[1], Markus Kampmann[1], Mirjana Minceva[2]

[1]Coporate Research, Sartorius Stedim Biotech GmbH, Göttingen, Germany
[2]Biothermodynamics, TUM School of Life Sciences, Technical University of Munich (TUM), Freising, Germany

The increasing demand for monoclonal antibodies (mAbs) as one of the most promising biopharmaceutical products and the number of biosimilars on the market have resulted in the need for intensified continuous downstream processes. After product capture further purification is accomplished by anion exchange chromatography, cation exchange chromatography or hydrophobic interaction chromatography. However, mAb polishing requires at least two separate chromatography steps, resulting in high investment costs and a large footprint for required units. These steps are commonly performed with resin packed columns in bind and elute mode, a buffer- and time-consuming batch process. An alternative approach is the use of membrane adsorbers (MA) in flow-through mode, where the impurities instead of product bind on the stationary phase. MAs can be used with higher flow rates and to perform continuous operations to increase the productivity compared to resin-based bind and elute chromatography.

We developed a double flow-through process by combining two different MAs with a single-pass tangential flow filtration (SPTFF) prototype for continuous buffer exchange between the two MAs for continuous polishing. This application reduces the number of units, tanks and process steps and combines two chromatography steps into one single operation mode. During the operation in double flow-through mode impurities were removed to < 2 ppm of desoxyribonucleic acid and < 29 ppm of host cell proteins with a simultaneous high mAb yield. The obtained results offer great potential for the development of future purification processes based on flow-through operation and for process intensification.

Novel hydrogel-based ion exchange membrane

Katharina Maria Thien[1], Florian Taft[1], Volkmar Thom[1]*

[1]Sartorius Stedim Biotech GmbH, Göttingen, Germany

Ion exchange chromatography (IEC) prevails in research, analysis, and purification of proteins. In downstream processing of biomolecules IEC plays a crucial role, e. g. for aggregate removal. Advantages include high binding capacities compared to other purification methods such as protein A and adaptable buffer conditions, among others.

In this context beads are used as the standard matrix material due to their high binding capacities and good predictability. However, diffusion-limited mass transfer results in poor productivity of bead-packed columns. To circumvent this drawback, materials based on convective flow such as membranes or monoliths can be used. However, since these materials generally have comparatively low binding capacities a matrix material combining both main characteristics is used to develop a novel hydrogel-based ion exchange membrane.

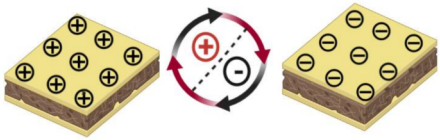
The performance of this membrane, which combines convective and diffusive mass transport, is investigated in the application as an ion exchanger. Therefore, characteristics such as binding capacities are studied depending on several aspects. On the one hand, membrane properties like surface area are varied to investigate their effects on separation. On the other hand, binding aspects such as buffer influence, due to a variation of pH and conductivity, as well as residence time dependency are considered. Finally, the aspects mentioned above are also studied for identically modified beads to classify the performance of the novel ion exchange membrane.

Development of an electrosorptive membrane chromatography process

Dennis Röcker

Technical University of Munich, TUM School of Engineering and Design, Bioseparation Engineering Group, Garching, Germany

Graphical Abstract:



Chromatography plays an essential role in separating and recovering biomolecules from biotechnological production media. While being unmatched in terms of selectivity and the resulting product purity, highly functionalized stationary phase materials and large amounts of elution media are required to separate the desired target species. This results in strong waste streams as well as time and cost-intensive processes. As an alternative to traditional chromatography, electrosorptive techniques introduce an innovative approach for reducing chemicals and enhancing process efficiency. Hereby, a unique opportunity for cost-efficient and sustainable processing is provided. This study presents the characterization and implementation of gold-coated flat sheet membranes as an innovative adsorbent in membrane chromatography. By applying an electrical potential, these membranes can be modulated in their surface charge and consequently their binding behavior towards charged target molecules. Membrane characteristics such as specific surface area, surface conductivity and charge, permeability as well as the current response were analyzed. In a two-electrode setup, the process principle was studied using chronoamperometry and cyclic voltammetry. At moderate overpotentials (up to ±1200 mV vs open circuit potential), the novel membranes display a potential dependant binding and release of the target molecule maleic acid. This study demonstrates that gold-coated membranes can be used as novel electrode material to control the adsorption of small organic molecules onto charged surfaces in a dynamic process.

TUESDAY, JUNE 28
Session 3: Novel product types

13:30	Analysis of amino acids with a dedicated liquid chromatography instrument solution [Sponsoring Talk]	VWR
13:45	Steric exclusion chromatography of lentiviral vectors using hydrophilic cellulose membranes	Jennifer Labisch
14:05	Overcoming challenges of mechanistic modeling development for a virus particle ion exchange chromatography step	Adrian Schimek
14:25	Acceleration of vaccine development by improvement of process understanding - analysis of the host cell proteome	Roxana Disela
14:45	Design of a pre-adsorption unit for purification of anti-malarial artemisinin from plant extract – Recent achievements and future challenges	Steffi Wünsche

Steric exclusion chromatography of lentiviral vectors using hydrophilic cellulose membranes

Jennifer Labisch[1,2], Meriem Kassar[3], Franziska Bollmann[4], Angela Valentic[3], Jürgen Hubbuch[3]

[1] Sartorius Stedim Biotech GmbH, Göttingen, Germany

[2] Institute of Technical Chemistry, Leibniz University Hannover, Hanover, Lower Saxony, Germany

[3] Karlsruhe Institute of Technology, Institute of Process Engineering and Life Sciences, Biomolecular Separation Engineering, Karlsruhe, Baden-Württemberg, Germany

[4] Marketing Separation Technologies, Sartorius Stedim Biotech GmbH, Göttingen, Lower Saxony, Germany

Enveloped viral vectors like lentiviral vectors pose purification challenges due to their low stability. A gentle purification method is considered one of the major bottlenecks for lentiviral vector bioprocessing. The transfer of methods developed for protein bioprocessing is unlikely due to the distinct bio- and physicochemical properties of the molecules. For enveloped viral vectors, a potential alternative to commonly performed chromatography methods is steric exclusion chromatography (SXC) as this method does not require any chemical interaction between the target species and the stationary phase. This allows for milder elution conditions and preserves viral activity. The mechanism of SXC relies on the depletion interaction of the viral particles with a hydrophilic cellulose membrane using polyethylene glycol.

In this talk, the successful identification optimal process parameters for steric exclusion chromatography to purify lentiviral vectors is presented. We will present lentiviral vector recoveries, impurity removal, and loading capacity. Moreover, we provide deeper insights into loading strategies and critical process parameters of SXC. We demonstrate that steric exclusion chromatography is a gentle purification method with high potential for fragile enveloped viral vectors as it yields high recoveries while efficiently removing impurities.

References

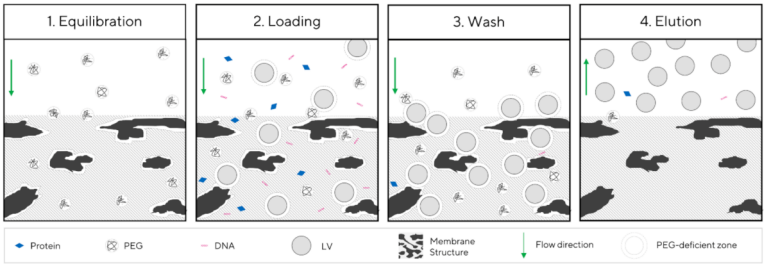
C. Perry, A.C.M.E. Rayat, Lentiviral vector bioprocessing, Viruses 13 (2021). https://doi.org/10.3390/v13020268.

J. Lee, H.T. Gan, S.M.A. Latiff, C. Chuah, W.Y. Lee, Y.-S. Yang, B. Loo, S.K. Ng, P. Gagnon, Principles and applications of steric exclusion chromatography, J. Chromatogr. A 1270 (2012) 162–170. https://doi.org/10.1016/j.chroma.2012.10.062.

K. Lothert, G. Sprick, F. Beyer, G. Lauria, P. Czermak, M.W. Wolff, Membrane-based steric exclusion chromatography for the purification of a recombinant baculovirus and its application for cell therapy, J. Virol. Methods 275 (2020) 113756. https://doi.org/10.1016/j.jviromet.2019.113756.

H.N.W. Lekkerkerker, R. Tuinier, Colloids and the depletion interaction, Springer Netherlands, Dordrecht, 2011. https://doi.org/10.1007/978-94-007-1223-2.

Graphical abstract:



Overcoming challenges of mechanistic modeling development for a virus particle ion exchange chromatography step

Adrian Schimek

ViraTherapeutics GmbH, Innsbruck, Austria

Mechanistic modeling supports the process development work by digital representation of process steps, based on the physical phenomena in the investigated system. [1,2] Such models have been successfully developed for antibody or virus-like particle (VLPs) process steps. [3,4] Ion exchange chromatography (IEX) monoliths are useful for purification of virus-based therapeutic products. In the presented work, the development of a mechanistic model to describe a purification step of an enveloped virus using an IEX monolith is shown. Limitations of the model and challenges in the development, especially regarding current analytical gaps, are presented and possible solutions provided. The SMA (steric mass action) isotherm and the CPA (colloidal particles adsorption) modeling approach are compared and evaluated for their ability to describe elution profiles. The determination of model coefficients is carried out by iterative parameter estimation using an error norm and genetic optimization algorithms.

[1] T. Hahn, T. Huuk, A. Osberghaus, K. Doninger, S. Nath, S. Hepbildikler, V. Heuveline, J. Hubbuch, Calibration-free inverse modeling of ion-exchange chromatography in industrial antibody purification, Eng Life Sci. 16 (2015) 107–113.

[2] N. Jakobsson, M. Degerman, E. Stenborg, B. Nilsson, Model based robustness analysis of an ion-exchange chromatography step, J Chromatogr A. 1138 (2007) 109–119.

[3] C.L. Effio, T. Hahn, J. Seiler, S.A. Oelmeier, I. Asen, C. Silberer, L. Villain, J. Hubbuch, Modeling and simulation of anion-exchange membrane chromatography for purification of Sf9 insect cell-derived virus-like particles, J Chromatogr A. 1429 (2016) 142–154.

[4] T.C. Huuk, T. Hahn, A. Osberghaus, J. Hubbuch, Model-based integrated optimization and evaluation of a multi-step ion exchange chromatography, Sep Purif Technol. 136 (2014) 207–222.

Acceleration of vaccine development by improvement of process understanding – analysis of the host cell proteome

Roxana Disela[1,2] r.c.disela@tudelft.nl, Daphne Keulen[1,2], Geoffroy Geldhof[2], Olivier Le Bussy[2], Martin Pabst[1], Marcel Ottens[1]

[1] Dept. of Biotechnology, Delft University of Technology, Van der Maasweg 9, 2629 HZ Delft, The Netherlands

[2] GSK Vaccines, Technical Research & Development – Microbial Drug Substance, Rue de l'Institut 89, 1330 Rixensart - Belgium

While regulatory agencies require stringent product quality and safety to be upheld in biopharmaceutical products, today's competitive biopharmaceutical market requires short process development times. The demand to accelerate especially the development of vaccines became obvious with the COVID-19 pandemic. By expanding process understanding with the use of process design tools the development time of the purification could be significantly shortened.
High throughput experimentation (HTE) provides an automated experimentation platform, which minimizes the amount of used samples and saves experimental time. In this approach, HTE is used to acquire experimental data to regress parameters used as inputs for a chromatographic mechanistic model with the objective to establish an *E. coli* vaccine purification process development platform for a recombinant subunit vaccine. To provide a generic process development strategy that can be applied to novel antigens, the focus lies on the description of the adsorption behavior of the impurities such as host cell proteins (HCPs) during the capture step. Therefore our approach focuses on the present impurities, in specific the HCPs (Figure 1). When using the same *E.coli* strain the knowledge regarding the host cell proteins could be transferred to a new product. The first step is the identification of HCPs. Over a thousand HCPs are identified in the *E.coli* harvest sample investigated by means of mass spectrometry based proteomics. A database containing the properties of these proteins can provide assistance in the decision on chromatography resins suited for the purification process of a new developed antigen.

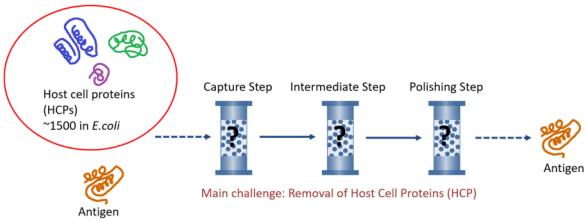


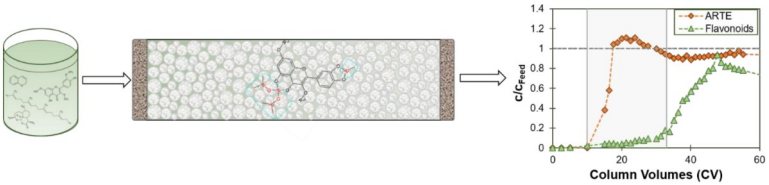
Figure 1 – Development of purification process for new antigen

Design of a pre-adsorption unit for purification of antimalarial artemisinin from plant extract – Recent achievements and future challenges

Steffi Wünsche[1], Moritz Nickel[1], Andreas Seidel-Morgenstern[1,2] and Heike Lorenz[1]

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

[2]Otto von Guericke University Magdeburg, Chair of Chemical Process Engineering, Magdeburg, Germany



The purification of a single target compound from a complex plant extract generally requires more than one purification steps. In the case of artemisinin (ARTE), obtained from *Artemisia annua*, the overall production process needs to be robust and cost-effective with a minimum of purification steps. It forms the base for effective treatments against malaria infections, a disease affecting over 200 million people, mostly in Africa, every year.

The starting point for the purification is a toluene extract containing ~6 $g \cdot L^{-1}$ dissolved substances with about 20 % of them ARTE. A two-step purification consisting of a pre-adsorption unit (PAU) and a final crystallization is currently under investigation. Flavonoids, a substance group of secondary metabolites, were identified to be critical and it was attempted to remove them by the PAU. A screening for suitable stationary phases was carried out and economical γ-Al_2O_3 was found to be a promising candidate. It can effectively bind the flavonoids while showing low affinity to ARTE. For the development of the PAU, static and dynamic adsorption tests were performed. The potential of the adsorbent to remove the flavonoids was approved. Next challenges will be the regeneration of the adsorbent and the design of the crystallization process.

TUESDAY, JUNE 28
Session 4: Processing of particles and polymers

15:35	Purification of a hydrophobic elastin-like protein towards scale suitable production of biomaterials	Sandra Haas
15:55	Model-based development of efficient chromatographic processes for the isolation of pure PEG homologs	Malvina Supper
16:15	Frequency-modulated dielectrophoretic particle chromatography	Jasper Giesler
16:35	Chromatographic classification and characterization of gold clusters and plasmonic nanoparticles	Lukas Gromotka
16:55	Bi-dimensional fractionation of rare earth compounds by magnetic field controlled chromatography	Laura Kuger

Purification of a hydrophobic elastin-like protein towards scale suitable production of biomaterials

Sandra Haas[1], Monika Desombre[1], Frank Kirschhöfer[2], Matthias C. Huber[3,4], Stefan M. Schiller[3,4], Jürgen Hubbuch[1,2]

[1] Institute of Process Engineering in Life Sciences, Section IV: Molecular Separation Engineering, Karlsruhe Institute of Technology (KIT), Fritz-Haber-Weg 2, 76131 Karlsruhe, Germany

[2] Institute of Functional Interfaces, Karlsruhe Institute of Technology (KIT), Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany

[3] Center for Biosystems Analysis, Albert-Ludwigs-University Freiburg, Habsburgerstr. 49, 79104 Freiburg, Germany

[4] Cluster of Excellence livMatS @ FIT, Freiburg Center for Interactive Materials and Bioinspired Technologies, University of Freiburg, Georges-Köhler-Allee 105, 79110 Freiburg, Germany

Elastin-like proteins (ELPs) are polypeptides with potential applications as renewable bio-based high-performance polymers which undergo a stimulus-responsive reversible phase transition. The ELP investigated in this manuscript – ELP[V2Y-15] – promises fascinating mechanical properties in biomaterial applications. Purification process scalability and purification performance are important factors for the evaluation of potential industrial scale production of ELPs. Salt-induced precipitation, inverse transition cycling (ITC) and immobilized metal ion affinity chromatography (IMAC) were assessed as purification protocols for a polyhistidine-tagged hydrophobic ELP showing low temperature transition behavior. IMAC achieved a purity of 86 % and the lowest nucleic acid contamination of all processes. Metal ion leakage did not propagate chemical modifications and could be successfully removed through size exclusion chromatography. The simplest approach using a high-salt precipitation resulted in a 60 % higher target molecule yield compared to both other approaches with the drawback of a lower purity of 60 % and higher nucleic acid contamination. An additional ITC purification led to the highest purity of 88% and high nucleic acid removal. However, expensive temperature dependent centrifugation steps are required and aggregation effects even at low temperatures have to be considered for the

investigated ELP. Therefore, ITC and IMAC are promising downstream processes for biomedical applications with scale dependent economical costs to be considered, while salt-induced precipitation may be a fast and simple alternative for large scale bio-based polymer production.

Model-based Development of Efficient Chromatographic Processes for the Isolation of Pure PEG Homologs

Malvina Supper[1] and Malte Kaspereit[1]

[1] Lehrstuhl für Thermische Verfahrenstechnik, Friedrich-Alexander Universität Erlangen-Nürnberg

The synthesis of polymers delivers products with pronounced distributions of molar weight (MW). However, size and size distribution are key parameters for product quality. Polyethylene glycol (PEG) is commonly used in pharmaceutical and medical applications, e.g. to influence bioavailability of proteins and drugs in the body. The use of pure homologs enables a precise adjustment of these product properties. Commercially, homologs of PEG are available only up to certain molar weights.

Chromatography is a promising separation method for the isolation of monodisperse PEGs from MW-distributed mixtures due to its separation power and scalability. A systematic model-based process development was used to identify optimal process concepts and operating conditions.

In a first step, investigations were performed at analytical scale and then transferred to semi-preparative scale, using a core-shell column (Kinetex C18, 100x21 mm, 100 Å, 5 um; Phenomenex). A modular setup with gradient pump, thermostat, fraction collector and coupled CAD/MS detection was implemented and controlled by a Python-based software. Obtained thermodynamic parameters are comparable to those derived from the analytical column. Optimized conditions for the preparative separations were chosen using a model-based approach and then validated in practice. Based on these conditions repetitive runs for polydisperse PEG samples delivered pure single homologs with different degrees of polymerization.

Current work focuses on model-based optimization of process performance parameters to ascertain quantitatively the separation limits. A trade-off between desired purity on the one hand, and productivity, solvent consumption and yield on the other hand was investigated.

Frequency-modulated dielectrophoretic particle chromatography

Jasper Giesler[1], Michael Baune[1], Jorg Thöming[1] and Georg Pesch[1]

[1]University of Bremen, Germany

Separating microparticles (< 10 µm) according to properties such as size, shape, or material is a field of recent research, since conventional separation techniques do not cover this size range. Therefore, novel separation techniques are required that are capable of addressing (multiple) properties of the particles. Dielectrophoresis is one candidate for these separations.
Dielectrophoresis is the movement of polarizable particles in inhomogeneous electric field. The dielectrophoretic force depends on several particle properties such as size, shape and material. Additionally, it is influenced by process parameters as the composition of the medium in which the targeted particles are suspended (water, air or oil) or the frequency and voltage of the applied electric field. Generally, particles will move towards field maxima (positive DEP) or away from them (negative DEP), depending on their relative polarizability.
In a recent publication we introduced frequency-modulated dielectrophoretic particle chromatography (DPC) (Giesler et al. 2020). Here, a particle suspension is injected into a carrier flow in a microfluidic device flowing over an electrode array. The electrodes generate an inhomogeneous electric field. In the approach, we modulate the field frequency by a function which leads to a periodic change of the frequency. Since strength and direction of the dielectrophoretic motion are frequency dependent for a variety of microparticles, this leads to different particle trajectories inside the channel and as a consequence to characteristic retention times for each particle type. In our lab we could achieve a separation of particles (size 2 to 6 µm) with respect to size and surface properties (Giesler et al. 2021). Additionally, we propose a setup to scale the throughput by several orders of magnitude (from ml/h to ml/min) (Giesler et al. 2022).

References

Giesler, Jasper, et al. "Polarizability-dependent sorting of microparticles using continuous-flow dielectrophoretic chromatography with a frequency modulation method." *Micromachines* 11.1 (2020): 38.
Giesler, Jasper, et al. "Separating microparticles by material and size using dielectrophoretic chromatography with frequency modulation." *Scientific Reports* 11.1 (2021): 1-12.
Giesler, Jasper, et al. - Manuscript under review. (2022)

Graphical abstract on page 2

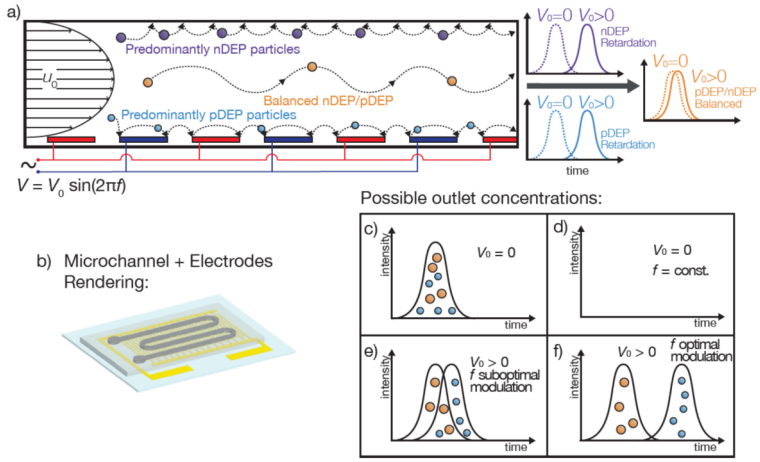


Figure 1 (a) Sketch of the DPC separation experiments. (b) Sketch of the DPC separation column. Meandering PDMS microchannel sealed by interdigitated electrodes on a glass chip. (c–f) Different possible outlet concentrations for DPC. (c) Without voltage, no retardation of the particles occurs, and both fractions elute at the same time. (d) When a voltage is applied and the frequency is fixed, the particles are trapped in the column due to DEP and will not exit the channel. If the frequency is modulated, a chromatographic separation occurs (e), which can be optimized by changing the frequencies and voltage (f). from (Giesler et al., 2020)

Chromatographic classification and characterization of gold clusters and plasmonic nanoparticles

L. Gromotka[1], M. Uttinger[1], C. Lübbert[1], W. Peukert[1]

[1] Institute of Particle Technology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany

Chromatography is a highly promising scalable technique for property classification of nanoparticles [1, 2, 3]. We herein report the classification and characterization of gold clusters and plasmonic nanoparticles by different chromatographic techniques. Glutathione (GSH) stabilized gold clusters with discrete, atomically precise structures (< 2 nm) are separated by interaction chromatography. By optimization of the mobile phase composition (acetonitrile and ionic strength), size-dependent interactions of individual clusters with the stationary phases are exploited in order to achieve a size separation (see left panel of **Error! Reference source not found.**). In contrast to the separation of gold clusters, larger plasmonic gold nanoparticles (5 nm – 80 nm) are separated by size-exclusion chromatography (SEC) where (attractive) interactions have to be strictly avoided since in ideal SEC the separation is solely governed by the size-dependent diffusion of nanoparticles into the pores of the stationary phase. The right panel of **Error! Reference source not found.** demonstrates the baseline separation of a multimodal nanoparticle dispersion for different injection volumes by SEC. Furthermore, we used various gold dispersions of different size to determine a calibration curve from which multimodal particle size distributions can be determined accurately over a broad size range.

With our work, we demonstrate the strength of chromatography for the classification of nanoparticluate systems ranging from small gold clusters to large plasmonic nanoparticles by tuning the interactions through modification of the stationary and mobile phases.

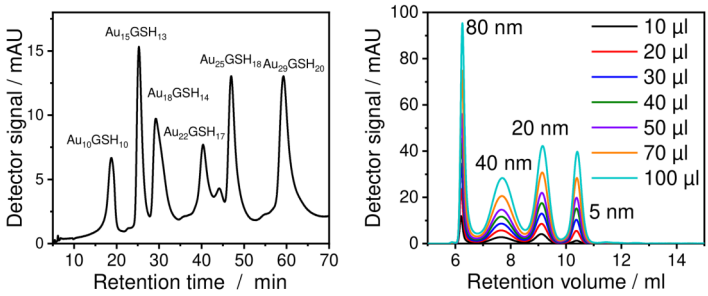


Figure 1: Chromatogram of a gold cluster mixture separated by interaction chromatography (left). Chromatograms of a mixture of different gold dispersions for various injection volumes (right).

References

[1] Hinterberger, V., Damm C., Guldi D., Peukert W., Purification and structural elucidation of carbon dots by column chromatography, Nanoscale 11 (2019) 8464-8474

[2] Süß S., Bartsch K., Wasmus C., Damm C., Segets D., Peukert W., Chromatographic property classification of narrowly distributed ZnS quantum dots, Nanoscale 12 (2020) 12114-12125

[3] Michaud V., Schilfarth F., Damm C., Platzer B., Haines P., Harreiß C., Guldi D., Spiecker E., Peukert W., Well-separated water-soluble carbon dots via gradient chromatography, Nanoscale 13 (2021) 13116-13128

Bi-dimensional fractionation of rare earth compounds by magnetic field controlled chromatography

Laura Kuger[1], Matthias Franzreb[1]

[1] Institute of Functional Interfaces, Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, Germany

Various paramagnetic rare-earth (RE) compounds are vital components determining the functionality of high-tech products. The market for RE containing parts has increased strongly as a result of the expansion of future technologies. Coincidently, manifold property dimensions and purity of such particle collectives are essential and quality-critical for the production and application of coatings, medical therapy or diagnostic systems, catalysts, etc. Diverse markets thus depend on highly productive, selective, and environmentally friendly processes for the separation of such particles from ores and complex end-of-life products.

Most RE particle fractionation methods provide only limited productivities and selectivities, particularly in the range of ultra-fine systems (< 10 µm) because of the complex superposition of forces. The fractionation of RE particles on industrial scale thus remains a relevant research task.

We use magnetic field controlled chromatography for a selective and efficient fractionation of paramagnetic particle systems. The technique is based on the competition between magnetic and hydrodynamic forces. Magnetic interactions evolve between the particles and a magnetizable matrix as a result of the application of an external magnetic field (see Figure 1); hydrodynamic forces are induced deliberately by adjusting the flow rate. Diverse particle collectives containing RE metals and luminescent RE doped were fractionated bi-dimensionally, specifically regarding particle size and magnetic susceptibility. High selectivities of $d_{25}/d_{75} > 0.65$ were attained while concurrently providing easy scalability. Furthermore, we demonstrated the feasibility of a process transfer to a continuously operating Simulated Moving Bed (SMB) chromatography system with low energy, space and solvent consumption, enabling space-time yields potentially relevant for industrial application.

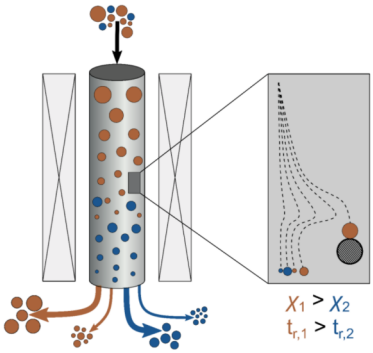


Figure 1: *Schematic principle of the magnetic field controlled chromatography technique. A particle collective containing particles of different magnetic susceptibilities χ_i and of different particle sizes passes a chromatography column, which is filled with a magnetizable matrix and surrounded by a magnetic field source. The trajectories of the particles differ in the vicinity of a matrix element depending on the magnetic susceptibility, particle size and other influencing factors (e.g. the initial position of the particle, local hydrodynamic influences and distortions, etc.). The differences in the trajectories ultimately result in characteristic retention times $t_{r,i}$, which are consequently utilized for a fractionation of the collective regarding magnetic susceptibility and particle size.*

WEDNESDAY, JUNE 29
Session 5: Mechanistic modeling I

8:30	Welcome & Opening	Organizing committee
8:40	An integrated multistep purification approach including online monitoring [Sponsoring Talk]	Novo Nordisk
8:55	Influence of salt selection onto cation-exchange chromatography of proteins	Thomas Fuchs
9:15	Development of a digital twin for a multimodal chromatography resin	Tim Ballweg
9:35	On the resurgence of colloidal chromatography models	Lena Enghauser
9:55	Mechanistic description of pH and ionic strength values in chromatography simulations	Alexander Gutzler

An integrated multistep purification approach including online monitoring

Florian Dismer, Anton Sellberg, Peter Tiainen
Downstream Technologies, Global Research Technologies, Novo Nordisk A/S

Niklas Andersson, Simon Tallvod, Prof. Bernt Nilsson
Department of Chemical Engineering
Lund University

Finding and developing new pharmaceutical compounds has become an increasingly complex task requiring production and screening of a large number of variants often including a multitude of multivalent molecular formats. Especially in the non-mAb world this puts a huge burden on pre-clinical process development timelines in order to cope with the large diversity in terms of physical and chemical properties of the variants. The presentation presented here targets reduction of lead times for both process development and variant production by combining a fully automated, closed modification and purification platform with online and very fast at-line analytics. The combination of both technologies could potentially result in a generic, scalable setup that can be used independent of the molecular format of interest and does not require affinity purification steps. Here a case study is presented to show an end-to-end purification platform combined with the analytical power of a diode-array detector for the production of an enzymatically modified peptide variant.

The automated platform is built on an ÄktaPure connected and controlled by an external software called Orbit[1,2]. The platform allows easy programming of end-to-end solutions along with the opportunity of adding extra essential functions like following chemical reactions, feed-forward control of process parameters and collection of critical information when needed. The end-to-end approach is based on connecting unit operations when, and only when, the protein is transported between them. The unit operations used can be seen as batch processes that are connected only when transferring the protein/peptide forward. One potential layout of such an end-to-end process could consist of a capture step followed by an enzymatic reaction or chemical modification, followed by one to two chromatographic polishing steps and a final buffer exchange.

Using such an approach in a generic fashion requires online or fast at-line analysis during and in-between steps in order to cope with process variations introduced by working with different molecular formats. Chemical and enzymatical reactions have to be monitored for completion, pooling criteria change depending on type and composition of the material to purify. A fast and non-invasive approach to analyse compositions of mixtures is the deconvolution of UV absorption spectra. This technique relies on the fact that the UV absorption spectrum of a mixture of proteins is a linear combination of the spectra of the single components of the mixture. When molecules are subjected to chemical modifications or when the composition of a mixture changes, these changes can be quantified by deconvolution of the UV spectrum of the mixture into the single components. Using external software to control purification and modification equipment adds the possibility of integrating analytical tools and advanced data processing and decision making into the automated workflow necessary for full automation.

[1]Andersson, N., Löfgren, A., Olofsson, M., Sellberg, A., Nilsson, B. and Tiainen, P. (2017), Design and control of integrated chromatography column sequences. Biotechnol Progress, 33: 923–930.

[2]Löfgren, A., Andersson, N., Sellberg, A., Nilsson, B., Löfgren, M. and Wood, S. (2018), Designing an Autonomous Integrated Downstream Sequence From a Batch Separation Process − An Industrial Case Study. Biotechnol. J., 1700691.

Influence of Salt Selection onto Cation-Exchange Chromatography of Proteins

Thomas Fuchs, Andreas Jupke;

Fluid Process Engineering (AVT.FVT), RWTH Aachen University, Forckenbeckstr. 51, 52074 Aachen, Germany

Ion Exchange chromatography (IEC) is widely applied in the downstream processing of proteins [1]. As chromatographic separation processes cover up to 70% of the manufacturing costs in the biopharmaceutical industry, the optimization of such unit operations is essential [2]. Hereby, the salt type used in the mobile phase is an important factor for the optimization of IEC steps [3].

In IEC processes, ions can be distinguished between counter-ions, which directly bind to the resin surface and displace bound proteins, and co-ions [4]. In case of cation exchange chromatography, counter-ions are cations and co-ions are anions. So far, most investigations regarding effects of salt types on protein retention focus on counter-ions [1,3,4]. Thereby, aforementioned effects of salt types on protein retention are distinguished between non-specific effects, if all proteins are affected in a similar way, or specific effects, if some proteins are influenced differently [5]. While the existence of specific effects is commonly agreed on, their significance on protein separation is still disputed [1,3,5,6].

Various approaches to account for comparability between ions with different valences are applied in literature when comparing protein retention times. Thereby, the influence of salt type on protein retention are investigated conducting different IEC experiments keeping either salt concentrations [6], overall ionic strengths [1] or the counter-ion strengths [5] constant.

In this contribution, the significance of counter-ion and co-ion selection on the cation-exchange chromatography of the three model proteins bovine serum albumin, lysozyme and α-chymotrypsin is investigated independently [7]. Hereby, the suitability of constant counter- or co-ion strength for the determination of salt effects of multivalent ions are compared. Moreover, protein specific effects are evaluated by their impact on protein separation.

References

[1] Liu R., and Shi Q., 2020 Chinese Journal of Chemical Engineering(7), pp. 1904–1910.
[2] Osberghaus A., Hepbildikler S., Nath S., Haindl M., Lieres E. von, and Hubbuch J., 2012 Journal of chromatography. A, pp. 54–65.
[3] Al-Jibbouri S., 2007 Journal of chromatography. A(1), pp. 57–62.
[4] Liu N., Yu L., and Sun Y., 2015 Journal of chromatography. A, pp. 44–50.
[5] Malmquist G., and Lundell N., 1992 Journal of Chromatography(627), pp. 107–124.
[6] Tugcu N., Song M., Breneman C. M., Sukumar N., Bennett K. P., and Cramer S. M., 2003 Analytical chemistry(14), pp. 3563–3572.
[7] Fuchs T., and Jupke A., 2022 Journal of Chromatography A (*submitted*)

Development of a Digital Twin for a Multimodal Chromatography Resin

Tim Ballweg; Modan Liu; Matthias Franzreb
Institute of Functional Interfaces, Karlsruhe Institute of Technology

In bioseparations, such as chromatography, bioinstructive adsorbents enable highly specific interactions with target molecules through nanoscale material structures and tailored surface functionalization by binding ligands to the polymer backbone of the resins. In the process development of chromatography steps, it would be beneficial to be able to predict ideal binding and elution conditions of specific target molecules. However, predicting these ideal conditions can often be challenging without using high throughput chromatography methods or design of experiment tools, especially for multimodal (MM) chromatography. In MM chromatography the ligands combine multiple types of binding mechanisms which leads complex correlations between pH or ionic strength of the buffer and the binding and elution of target molecules.

Here, a digital twin of MM chromatography resins with detailed information about the 3D structure of the adsorbent could help to predict the interaction with target molecules. Therefore, a workflow was developed that can generate molecular dynamic all-atom models of methacrylate-based chromatographic media. Details of the models like the type of monomer, the degree of crosslinking and the type of ligand of the resin as well as the porosity and the size of the polymer structure to be calculated can be set as input parameters. Thus, a large variety of different methacrylate-based adsorbent structures can be generated.

In future studies, the workflow will be used to develop a digital twin of a commercial MM chromatography resin by correlating the results of molecular docking simulations with experimental data of the interaction with small target molecules, like dyes or polypeptides.

On the resurgence of colloidal chromatography models

Tobias Hahn[1], Till Briskot[2], Thiemo Huuk[1], Lena Enghauser[1], and Jürgen Hubbuch[2]

[1] Cytiva, Karlsruhe, Germany [2] Karlsruhe Institute of Technology, Karlsruhe, Germany

Until a few years ago, stoichiometric models were favored when protein adsorption on any chromatography medium needed to be described. The derivation of such models is easy to understand, and they have very often delivered good results. The article by Brooks and Cramer (1992) that introduces the “Steric Mass Action” (SMA) model alone was cited over 500 times. When SMA was not sufficient, it was expediently expanded, but there were still many cases for which there was no adequate stoichiometric model: In the case of ion exchange (IEX) chromatography, low salt concentrations could not be accurately described, and in general, the lack of reference to measurable molecule and material attributes was criticized.

The product of the authors research was a colloidal model for IEX that is comparable to SMA in both the number of model parameters and the simulation speed. It provides deeper mechanistic insights on how resin properties, protein properties, and buffer conditions affect protein adsorption. Moreover, all model parameters are subject to physically meaningful limits which enables rapid parameter estimation and supports the use of homology models in model calibration.

The developed model was found to be capable of reproducing elution profiles that cannot be described by the traditional SMA model or extensions thereof. The nonlinearity at higher protein concentrations is described by a combination of steric surface blocking effects and electrostatic interactions between adsorbed proteins. While the mechanistic understanding of other chromatography modes is still limited, positive effects can be seen if the description of the steric effects is adopted. The underlying two-dimensional scaled-particle theory was included into models for hydrophobic interaction, mixed-mode, and affinity chromatography.

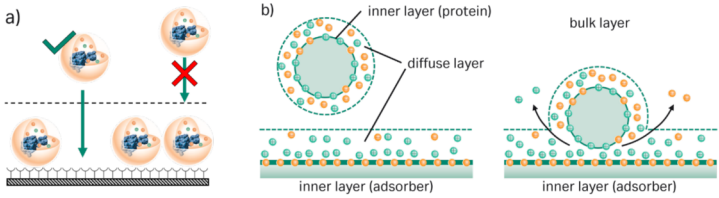


Fig.1: (a) Mechanistic interpretation of the protein adsorption on the available surface area.
(b) Schematic representation of the electrostatic interaction of the protein and the charged surface.

References

Briskot T, Hahn T, Huuk T, Hubbuch J. Protein adsorption on ion exchange adsorbers: A comparison of a stoichiometric and non-stoichiometric modeling approach. J Chromatogr A. 2021 Sep 13;1653:462397. doi: 10.1016/j.chroma.2021.462397

Briskot T, Hahn T, Huuk T, *et.al.* Analysis of complex protein elution behavior in preparative ion exchange processes using a colloidal particle adsorption model. J Chromatogr A. 2021 Sep 27;1654:462439. https://doi.org/10.1016/j.chroma.2021.462439.

Mechanistic description of pH and ionic strength values in chromatography simulations

Alexander Gutzler[1,2], *Tobias Hahn*[1]

[1]*Cytiva* [2]*Karlsruhe Institute of Technology*

In recent years, mechanistic chromatography modeling has become a staple for efficient bioprocess development. As most chromatography processes use a change in salt concentration or pH to elute protein from the chromatography column, accurate description of pH values and ionic strengths in buffers and their mixtures is important for modeling these processes. Many adsorption models include terms to account for changes in pH and ionic strength, so a good description of these is a prerequisite for a highly descriptive model.

We present a method to calculate with high accuracy the buffer ion compositions and pH values in an overall simulation of the chromatography process. The method uses the Henderson–Hasselbalch equation in combination with the Davies equation for calculating ion activities depending on the ionic strength and composition of the running buffer. To account for temperature changes, the method also includes a linear dependency of pKa values on temperature. The resulting pH values and ionic strengths serve as input parameters for the calculation of adsorption equilibria within the simulation.

This method improves the accuracy of descriptions of pH profiles and ionic strengths during chromatographic runs in which one or both conditions change, especially gradients. Models for these processes become more accurate, as nonlinearities in pH do not need to be “picked up” by unrelated parameters. Application across a wider pH range is also possible, as the model relies on a mechanistic description of pH rather than a linear extrapolation.

WEDNESDAY, JUNE 29
Session 6: Mechanistic modeling II

10:45	SEC-MALS analysis of biotherapeutics: what it offers and what it demands [Sponsoring Talk]	Tosoh
11:00	Bayesian optimization using multiple directional objective functions allows the rapid inverse fitting of parameters for chromatography simulations	Ronald Jäpel
11:20	Integrated process model for the prediction of biopharmaceutical manufacturing chromatography and adjustment steps	Federico Rischawy
11:40	Method development for mechanistic modeling of mixed-mode antibody purification	Rudger Hess
12:00	Hybrid process modelling combining mechanistic equations with machine learning	Johannes Schmölder

Bayesian optimization using multiple directional objective functions allows the rapid inverse fitting of parameters for chromatography simulations

Ronald Colin Jäpel[1], *Johannes Felix Buyel*[1,2]

[1]*Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Forckenbeckstrasse 6, 52074 Aachen, Germany*

[2]*Institute for Molecular Biotechnology, Worringerweg 1, RWTH Aachen University, 52074 Aachen, Germany*

The modeling of chromatographic separations can speed up downstream process development, reducing the time to market and corresponding development costs for new products such as pharmaceuticals. However, calibrating such models by identifying suitable parameter values for mass transport and sorption is a major, time-consuming challenge that can hinder model development and improvement. We therefore designed a new approach based on Bayesian optimization (BayesOpt) and Gaussian processes that reduced the time required to compute relevant chromatography parameters by up to two orders of magnitude compared to a multistart gradient descent and a genetic algorithm. We compared the three approaches side by side to process several internal and external datasets for ion exchange chromatography (based on a steric mass action isotherm) and hydrophobic interaction chromatography (a modified version of a recently published five-parameter isotherm) as well as different input data types (gradient elution data alone vs gradient elution and breakthrough data). We found that BayesOpt computation was consistently faster than the other approaches when using either single core or 12-core computer processing units. The error of the BayesOpt parameter estimates was higher than that of the competing algorithms, but still two orders of magnitude less than the variability of our experimental data, indicating BayesOpts applicability for chromatography modeling. The low computational demand of BayesOpt will facilitate rapid model development and improvement even for large datasets (e.g., >100 proteins) and increase its suitability for research laboratories or small and medium enterprises lacking access to dedicated mainframe computers.

Integrated process model for the prediction of biopharmaceutical manufacturing chromatography and adjustment steps

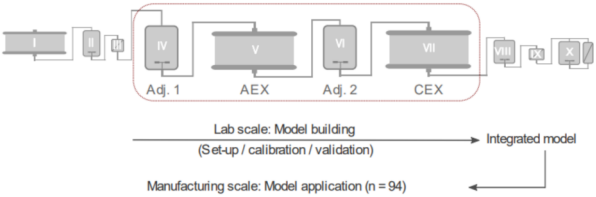
Federico Rischawy[1,2], Till Briskot[1], Adrian Schimek[2], Gang Wang[1], David Saleh[1], Simon Kluters[1], Joey Studts[1], Jürgen Hubbuch[2]

[1] DSP Development, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany

[2] Karlsruhe Institute of Technology (KIT), Institute of Engineering in Life Sciences, Section IV: Biomolecular Separation Engineering, Karlsruhe, Germany

A fundamental process understanding of an entire downstream process is essential for achieving and maintain the high-quality standards demanded for biopharmaceutical drugs. A holistic process model based on mechanistic insights could support process development by identifying dependencies between process parameters and critical quality attributes across unit operations to design a holistic control strategy.

In this study, state-of-the-art mechanistic models were calibrated and validated as digital representation of a biopharmaceutical manufacturing process. The polishing ion exchange chromatography steps (Q-Sepharose FF, Poros 50 HS) were described by a transport-dispersive model combined with the colloidal particle adsorption model. The elution behavior of four size variants was analyzed and included in the model. Titration curves of pH adjustments were simulated using a mean-field approach considering interactions between the protein of interest and other ions in solution. By including adjustment steps the important process control inputs ionic strength, dilution, and pH were integrated. The final process model was capable to predict online and offline data at manufacturing scale. Process variations at manufacturing scale of runs were adequately reproduced by the model. Furthermore, the process robustness against a 20% input variation of concentration, size variant and ion composition, volume, and pH could be confirmed with the model.


I
II
III
IV
V
VI
VII
VIII
IX
X
Adj. 1
AEX
Adj. 2
CEX
Lab scale: Model building
(Set-up / calibration / validation)
Integrated model
Manufacturing scale: Model application (n = 94)

Method development for mechanistic modeling of mixed-mode antibody purification

Rudger Hess[1, 2], Doil Yun[2], David Saleh[2], Till Briskot[2], Jan-Hendrik Grosch[2], Gang Wang[2], Thomas Schwab[2], Jürgen Hubbuch[1,]*

[1] Karlsruhe Institute of Technology (KIT), Institute of Engineering in Life Sciences, Section IV: Biomolecular Separation Engineering, Karlsruhe, Germany

[2] DSP Development, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany

Multimodal chromatography is a powerful tool in accelerating the industrial downstream purification of challenging biological formats as it can replace multiple orthogonal polishing steps. Unfortunately, the implementation of multimodal polishing into a generic downstream platform can be hampered by non-robust platform conditions leading to a time and cost intensive process development. In this work, we present a mechanistic modeling aided approach that could pave the way for an accelerated implementation of anionic mixed-mode chromatography (MMC) for downstream process development. A modified mechanistic isotherm model was calibrated using only three chromatographic experiments and was employed in the retention prediction of four antibody formats including a bispecific and fab variant at different pH environments. The chromatographic experiments were conducted using the anionic MMC resin Capto adhere at industrial relevant process conditions enabling a flow-through purification mode. An existing MMC isotherm model was successfully reduced to hydrophobic interactions in the linear isotherm range. The model reduction led to structural identifiability and allowed an analytical isotherm parameter determination. For each monomer species, three linear salt gradient elution experiments were performed during model calibration followed by an isotherm parameter uncertainty assessment. Lastly, each mechanistic model was validated with a set of step elution and isocratic elution experiments. This new straightforward modeling approach could facilitate the implementation of multimodal chromatography as a key unit operation of pharmaceutical downstream platform development, while

increasing the mechanistic insight to the multimodal adsorption behavior of complex biologics at industrial relevant process conditions.

HYBRID PROCESS MODELLING COMBINING MECHANISTIC EQUATIONS WITH MACHINE LEARNING

Jazib Hassan[1], Johannes Schmölder[1], Eric von Lieres[1]

[1] Research Center Jülich, Institute of Biotechnology 2, Wilhelm-Johnen-Str., 52425 Jülich, Germany.

Complex separation processes nowadays can be modelled with comprehensive mechanistic models [1]. However, even though state of the art chromatography simulation software is capable of computing and predicting multiple physico-chemical interaction mechanisms, the adsorption mechanisms of proteins in preparative chromatography are still not fully understood. This is because of the complex interplay of many physical and environmental factors involved. Moreover, for complex models, it also becomes challenging to design experiments to estimate the value of the model parameters.

In this work, a hybrid process modelling technique is proposed in which single component mechanistic adsorption models are replaced by several data-driven models. Adsorption is modeled with cubic splines, Gaussian Process Regression (GPR), and Artificial Neural Networks (ANN), while the transport through the column is still modelled mechanistically with partial differential equations. The training data (adsorption isotherm data) for the data-driven methods used in this study is either synthetically generated, using established mechanistic adsorption models like the Langmuir isotherm, or experimental isotherm data obtained from a published study [2]. The trained model for each method was then implemented in CADET [3].

References

[1] J. Diedrich, W. Heymann, S. Leweke, C. Kunert, W. Johnson, S. Hunt, B. Todd and E. von Lieres, "Multi-state steric mass-action model and case study on complex high loading behavior of mAb on ion exchange tentacle resin," Journal of Chromatography A, pp. 60-70, 2017.

[2] T. Pabst, J. Thai and A. Hunter, "Evaluation of recent Protein A stationary phase innovations for capture of biotherapeutics," Journal of Chromatography A, vol. 1554, pp. 45-60, 2018.

[3] S. Leweke and E. von Lieres, "Chromatography Analysis and Desing Toolkit (CADET)," Computers and Chemical Engineering, pp. 274-294, 2018.

cytiva

Mechanistic chromatography modeling

Replace lab experiments with computer simulations – get thousands of results in a few hours.

Go smarter. Go faster.

cytiva.com/modeling

Software
Columns
Services

THANKS TO OUR SPONSORS

PLATINUM

Agilent
Trusted Answers

novo nordisk®

Boehringer Ingelheim

avantor™
delivered by VWR™

cytiva

GOLD

TOSOH

BRONZE

Rentschler Biopharma

BASF
We create chemistry